1983

Hypothalamic And Peripheral Noradrenergic Responses To Changes In Baroreceptor Input In Conscious Rats With And Without Renal Nerves

Kaushik Parbhubhai Patel

CANADIAN THESES ON MICROFICHE

I.S.B.N.

THESES CANADIENNES SUR MICROFICHE

National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

Canada

HYPOTHALAMIC AND PERIPHERAL NORADRENERGIC RESPONSES TO CHANGES IN BARORECEPTOR INPUT IN CONSCIOUS RATS WITH AND WITHOUT RENAL NERVES

by

Kaushik Parbhubhai Patel

Department of Physiology

Submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

Faculty of Graduate Studies

The University of Western Ontario

London Ontario

July, 1983

increase in arterial pressure and normalized the elevated NE turnover in the hypothalamus and skeletal muscle seen after ADN transection. In the acutely hypotensive group the significantly increased NE turnover seen in the PH was abolished after renal denervation; however, renal denervation did not change NE turnover in the peripheral organs of acutely hypotensive animals. The data in renal denervated rats subjected to acute hypotension suggest a dissociation between changes in hypothalamic and peripheral noradrenergic activity. Removal of renal nerves in the acutely hypertensive animals caused a significant decrease in NE turnover in the hypothalamus, suggesting that removal of renal nerves unmasked an inhibitory influence of "loaded baroreceptors" on the noradrenergic activity in the hypothalamus. It is suggested that there is an inhibitory influence from the baroreceptors and an excitatory influence from the renal afferents to the noradrenergic systems in the hypothalamus. In addition, these findings suggest that hypothalamic and peripheral noradrenergic activity are not tightly linked, as dissociation of these two systems can be produced under certain conditions.

ABSTRACT

A role for central and peripheral noradrenergic systems has been implicated in the baroreflex control of arterial pressure. Most of the information in this regard is derived as a byproduct of studies examining noradrenergic mechanisms in various models of experimental hypertension. However, it is not clear if the altered noradrenergic systems in the hypertensive models are the cause or an effect of the hypertensive process. In addition, it is not clear how changes in the hypothalamic noradrenergic activity are related to changes in peripheral sympathetic activity.

To determine the relationship between hypothalamic and peripheral noradrenergic activity, the first series of experiments examined the changes in hypothalamic and peripheral noradrenergic activity in response to changes in baroreceptor "input" to the central nervous system (CNS) in conscious Wistar rats. The change in baroreceptor input to the CNS was achieved by four methods 1) transectioning of aortic depressor nerve (ADN) to remove a specific baroreceptor input to the CNS, 2) acute hypotension for 60 min produced by infusion of nitroprusside, 3) acute hypotension by hemorrhage for 60 min, and 4) acute hypertension for 60 min by infusion of phenylephrine. An index of norepinephrine (NE) turnover was used to assess the noradrenergic activity by measuring the decline in endogenous concentration of NE after inhibition of tyrosine hydroxylase, the rate limiting enzyme in the synthesis of

NE, by α-methyltyrosine. NE was measured using either fluorescence assay or High Performance Liquid Chromatography in combination with an electrochemical detector after extraction with alumina.

In the study with ADN transection, there was a significantly elevated arterial pressure and heart rate that at 3 days was associated with an increased NE turnover in the hypothalamus, midbrain, medulla, skeletal muscle, and kidney but not in duodenum. In acute studies 60 min of hypotension produced a reflex increase in heart rate and an increased NE turnover in the posterior hypothalamus (PH) and all the peripheral organs examined (skeletal muscle, kidney and duodenum). In the baroreceptor loaded group there were no significant changes in NE turnover in the hypothalamus or the peripheral organs examined. The studies in baroreceptor "unloaded" rats suggest the presence of an inhibitory influence from the baroreceptors on the noradrenergic activity in the hypothalamus and peripheral organs.

Recently there has been electrophysiological and neuroanatomical evidence to suggest that baroreceptor information and afferent renal fibers may interact at the level of the hypothalamus. To determine the nature of such an interaction, in the second part of this project the effect of renal denervation on hypothalamic and peripheral noradrenergic responses to changes in baroreceptor input were examined. Renal denervation prevented the normal

increase in arterial pressure and normalized the elevated NE turnover in the hypothalamus and skeletal muscle seen after ADN transection. In the acutely hypotensive group the significantly increased NE turnover seen in the PH was abolished after renal denervation; however, renal denervation did not change NE turnover in the peripheral organs of acutely hypotensive animals. The data in renal denervated rats subjected to acute hypotension suggest a dissociation between changes in hypothalamic and peripheral noradrenergic activity. Removal of renal nerves in the acutely hypertensive animals caused a significant decrease in NE turnover in the hypothalamus, suggesting that removal of renal nerves unmasked an inhibitory influence of "loaded baroreceptors" on the noradrenergic activity in the hypothalamus. It is suggested that there is an inhibitory influence from the baroreceptors and an excitatory influence from the renal afferents to the noradrenergic systems in the hypothalamus. In addition, these findings suggest that hypothalamic and peripheral noradrenergic activity are not tightly linked, as dissociation of these two systems can be produced under certain conditions.

Dedicated to: "Ba" and "Bapuji"

ACKNOWLEDGEMENTS

To Dr. R.L. Kline I would like to express my sincere gratitude. His high scientific standards and relentless pursuit for the truth will remain as my inspirational goal.

I wish to thank Dr. P.F. Mercer for his advice and constructive criticism and the rest of my advisory committee for their helpful suggestions.

I would like to thank Dr. K.Y. Chow for her invaluable technical assistance throghout this project.

I would also like to thank Dr. J. Ciriello and Dr. M. Knuepfer for their valuable assistance in experiments presented in Chapter 5 and Chapter 8 respectively.

I am grateful to the Ontario Heart Foundation for their financial support.

Finally, I would like to thank my family for their continued support and patience throughout, and Rekha, who I met during this project and made it all worthwhile.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

As early as 1867 Ludwig gathered enough information to write a complete monograph about blood pressure and its regulation. Of the various hypotheses that attempt to explain the mechanisms for the control of arterial pressure and the development of elevated arterial pressure, one hypothesis that has been prominent, even before the standard measurement techniques for blood pressure were widely available, was that the nervous mechanisms were involved in the establishment of arterial hypertension. At the turn of this century the nervous system was thought to play a crucial role in the regulation of blood pressure. However, some fifty years later, the neurogenic concept fell out of grace mainly due to failure of sympathectomy (Smith, 1948), ganglionic blockers (Frew et al., 1949; Kleinerman et al., 1958; Celiva et al., 1959), and reserpine(a drug that depletes norepinephrine from noradrenergic terminals) (Moyer, 1959) to control elevated blood pressure. Furthermore, evidence of hyperresponsiveness of blood vessels from hypertensive patients to vasoconstrictor stimuli favoured the "vascular abnormality" concept (Doyle et al., 1955),which led to the idea of the non-neurogenic mechanisms being involved in elevated arterial pressure (Conway, 1963).

At the same time, the "neurogenic hypertension" concept suffered yet another blow in that elevated arterial pressure in baroreceptor deafferented animals was not of a large magnitude and the arteriolar medionecrosis of malignant hypertension never developed (Crout, 1959). In addition, other animal models of hypertension were easily developed by administration of desoxycorticosterone (DOC) plus salt (reviewed in Genest 1973), by renal arterial stenosis (Goldblatt 1934), or by wrapping the kidney (Page, 1939). This led to the concept of "humoral hypertension".

Nevertheless, in the past ten to fifteen years the concept of the involvement of noradrenergic mechanisms in the control of arterial pressure and the etiology of hypertension has been revitalized (see reviews by Chalmers, 1975; Folkow, 1974; Julius et al., 1974; de Champlain, 1977; Frohlich, 1977; Chalmers, 1978; NIH Hypertension Task Force Report, 1979; Brody et al., 1980; Abboud et al., 1982). Both empirical evidence of the success of antiadrenergic drugs in the treatment of hypertension and studies on experimental animal models of hypertension tend to support such a proposal. However, it is not clear whether the proposed sympathetic hyperactivity in the hypertensive models results from some dysfunction at the level of the peripheral nervous system or whether it is a secondary manifestation of a primary dysfunction elsewhere along the reflex arc regulating arterial pressure. In addition, there have been relatively few attempts to tie

together observations of changes in the central noradrenergic activity to changes in the peripheral component of the sympathetic nervous system. Furthermore, it is not clear if the changes in noradrenergic activity observed in hypertensive models are a cause or an effect of the hypertensive process.

Using experimental results in conjunction with computer analysis, Guyton et al. (1974) have proposed that the kidneys act as a servocontroller of long-term arterial pressure. In other words, renal function in relation to renal perfusion pressure establishes the "set-point" around which chronic blood pressure will be regulated. Combining this hypothesis with the idea of sympathetic involvement in the hypertensive disease, Coleman et al. (1975) have logically proposed that excessive nervous input to the kidney could produce a chronically elevated arterial pressure. Consistent with this hypothesis, renal denervation has been shown to delay the development of genetic hypertension (Liard et al., 1977; Kline et al., 1978; Dietz et al., 1978; Kline et al., 1980; Winternitz et al., 1980; Diz et al., 1981; 1982) and DOCA-salt hypertension (DOCA for deoxycorticosterone acetate) (Katholi et al., 1979; 1980), and reverse the elevated arterial pressure associated with one- and two-kidney, one-clip Goldblatt hypertension (Katholi et al., 1982a; 1982b) and one-kidney Grollman hypertension (Katholi et al., 1982a) in rats. However, Katholi et al. (1982a; 1982b) have shown

that the decrease in arterial pressure after renal denervation in one- and two-kidney, one-clip Goldblatt hypertension was not due to a pressure natriuresis and diuresis. They found that renal denervation normalized a previously elevated plasma NE concentration and proposed that increased activity of renal afferent fibers may be responsible for the enhanced peripheral sympathetic tone in these animals.

There are several lines of evidence to indicate that renal afferent nerves may participate in cardiovascular regulation. Electrical stimulation of renal afferent nerves has been shown by several investigators to produce changes in systemic arterial pressure (Ueda et al., 1967; Aars et al., 1970; Calaresu et al., 1976) and renal denervation has been shown to alter the concentration of NE in hypothalamic sites known to be involved in the regulation of cardiovascular variables (Calaresu et al., 1981a). An interaction between renal afferents and baroreceptor afferents is suggested by the studies showing that renal afferent fibers project to hypothalamic sites known to influence baroreceptor reflexes (Ciriello et al., 1980), and that a majority of single units in the hypothalamus which respond to stimulation of afferent renal fibers also respond to electrical stimulation of arterial baroreceptor afferent fibers (Calaresu et al., 1981b). Recently, Faber et al. (1983) showed that intact baroreceptors can mask renal nerve-dependent arterial pressure changes produced by acute

renal artery stenosis, suggesting an interaction between baroreceptors and afferent renal nerves. In one- and two-kidney, one-clip Goldblatt hypertensive rats, renal denervation decreased arterial pressure and normalized both the increased concentration of NE in the hypothalamus and blood (plasma) (Katholi et al., 1982a; 1982b; Winternitz et al., 1982). Therefore, it is conceivable that removal of afferent renal fibers may affect noradrenergic mechanisms in the hypothalamus which in turn may be involved in modulating the neurohormonal control of the circulation and body fluids.

It should be noted, however, that most of the information to date has been obtained from studies on various models of experimental hypertension where the cause and effect relationship between changes in noradrenergic activity and arterial pressure are not clear. In the present study experiments were performed in normotensive conscious rats that were subjected to changes in arterial pressure and removal of ADN (aortic depressor nerve). The purpose of the present study was two fold: 1) to investigate the relationship between hypothalamic and peripheral noradrenergic activity in normotensive rats subjected to changes in arterial pressure and removal of aortic baroreceptor nerves, and 2) to examine the possible interaction between arterial baroreceptors and renal afferent information at the level of the hypothalamus as measured by changes in noradrenergic activity.

The Historical Review which follows will concentrate on reviewing the evidence for the involvement of hypothalamic and peripheral noradrenergic systems in the regulation of arterial pressure and the role of renal nerves in the regulation of arterial pressure.

CHAPTER 2

HISTORICAL REVIEW

2.1 Evidence for the involvement of hypothalamic and peripheral noradrenergic systems in the regulation of arterial pressure

2.1.1 Hypothalamus and arterial pressure

2.1.1.1 Neurophysiological and Neuroanatomical evidence

One of the earliest studies to suggest the involvement of supramedullary structures in the control of cardiovascular variables was done by Karpus et al. (1909). They were able to elicit cardioacceleration and an elevation of arterial pressure by stimulation of the hypothalamus. For the following half a century there was a general belief that medullary sites and not supramedullary structures were involved in regulation of arterial pressure. Such a hypothesis was supported by reports that transection of the brainstem at the pontomedullary junction did not alter arterial pressure, whereas a transection at the level of the obex decreased it (Alexander, 1946). Nevertheless, there were reports by various investigators (Kabat et al., 1935; Magoun et al., 1938; Hess, 1957) that stimulation of the different sites within the hypothalamus caused either a

pressor or a depressor response. Later Manning (1965) presented the first evidence for the involvement of higher centres in baroreceptor reflexes. He made large lesions just ventrolateral to the Nucleus Tractus Solitarius (NTS) in cats. These lesions did not significantly impair cardiovascular reflexes to bilateral carotid occlusions or sciatic nerve stimulation. Normal pressor responses to hypothalamic stimulation were also intact. Supramedullary decerebration prevented all these responses, indicating that higher control centres exist and are an integral part in the maintenance of cardiovascular reflexes.

Since these reports, there have been several electrophysiological studies to suggest further that cardiovascular afferents have an influence on the hypothalamus. Thomas et al. (1972) reported that units in the posteromedial hypothalamus were responsive to electrical stimulation of the carotid sinus afferent nerve. Spyer (1972), reported that single unit activity in the hypothalamic depressor area (anterior hypothalamus) could be affected by carotid sinus nerve stimulation, and Dreifuss et al. (1976), Yamashita (1977), and Calaresu et al. (1980) have reported electrophysiological data supporting the existence of functional connections between carotid sinus afferents and the supraoptic and paraventricular nuclei of the hypothalamus. Taken together these data provide electrophysiological evidence for direct functional connections between the baroreceptor afferents and the

hypothalamus. There has also been a large body of evidence to suggest that the hypothalamus is involved in the control of the autonomic nervous system (for review see Mancia et al., 1981). The present account cannot possibly endeavour to review all the neurophysiological studies that have attempted to investigate the pathways followed by cardiovascular afferents to the hypothalamus and the integration by the hypothalamus of various cardiovascular reflexes. The details of such studies have been extensively reviewed elsewhere (for detail see Calaresu et al., 1975, Spyer, 1981, Mancia et al., 1981).

These electrophysiological studies have been supported by neuroanatomical evidence of ascending projections from the NTS (the site for primary synapse of baroreceptor afferents (Crill et al., 1968; Biscoe et al., 1970)) to the hypothalamus (Sakumoto et al., 1978, Ricardo et al., 1978). Sakumoto and coworkers used a combination of retrograde neuronal tracing techniques (horseradish peroxidase) and fluorescence histochemistry to identify noradrenergic cell bodies and connections between A1 and A2 catecholamine cell groups in the brainstem and the medial anterior hypothalamus. The lateral and periventricular hypothalamus receive noradrenergic fibers from many additional locations in pontine and medullary regions. Recently Swanson et al. (1983) have shown that the A2 cell group from the NTS projects to the paraventricular nucleus of the hypothalamus via a primarily noradrenergic pathway. In addition, they

have also reported a massive projection of A1 cell group to the vasopressinergic regions in both the paraventricular nucleus and supraoptic nucleus. The A1 cell group is known to receive non-noradrenergic projection from the noradrenergic A2 cell group region of the NTS (Swanson et al., 1983). These data substantiate the existence of connections between the NTS and the hypothalamus, supporting the reports concerning hypothalamic involvement in the regulation of baroreceptor reflexes.

At the same time that advances were made in neurophysiology and neuroanatomy there were parallel advances in the mapping of central catecholamine cell bodies and their terminal networks. Since the original work of Dahlstrom et al. (1964), Fuxe (1965), and Anden et al. (1966) there have been a large number of papers dealing with this subject (reviewed in Hökfelt et al., 1978, Moor et al., 1979 and recently by Chalmers et al., 1981) which give additional important information concerning the distribution of catecholamines in the brain and spinal cord. There is now general agreement that cell bodies of the central noradrenergic system are found predominantly in the brainstem, with concentrated projections of these cell bodies to the hypothalamus and reticular formation, and minor projections to the cerebral cortex and basal forebrain structures. Other axons descend in the spinal cord (Chalmers et al., 1981). From these studies it is evident that the pathways followed by the noradrenergic neurons are

strikingly similar to the central pathways involved in cardiovascular regulation (Korner, 1971). As a consequence of these neuroanatomical studies plus numerous other biochemical, physiological and pharmacological studies it is postulated that the noradrenergic systems in various medullary and hypothalamic sites may be involved in the regulation of cardiovascular parameters (Chalmers 1975, Haeusler 1975, Antonaccio 1977).

The rest of this section will concentrate on the evidence that the noradrenergic mechanisms in the hypothalmus are involved in the regulation of blood pressure.

2.1.1.2 Noradrenergic mechanisms and arterial pressure

Electrical stimulation of the posterior hypothalamus elicits a rise in blood pressure along with a host of other somatic and autonomic effects, all of which together have been termed the "defence reaction" (Folkow et al., 1966). Folkow et al. (1966) have reported that chronic stimulation of the posterior hypothalamus can produce a sustained elevated arterial pressure. However, recently Buñag et al. (1979) have reported that chronic hypothalamic stimulation in awake rats fails to induce hypertension. But it should be noted that there was greater brain damage in the study by Buñag et al. (1979) compared to the study by Folkow et al. (1966). Nevertheless there is general

agreement that acute electrical stimulation of the posterior hypothalamus produces an increase in arterial pressure (Mancia et al., 1981). Recent evidence suggests that the pressor responses resulting from stimulation of the hypothalamus may be due to the release of NE in the hypothalamus. The posterior hypothalamus contains a high density of noradrenergic nerve terminals which originate mainly from cell bodies located in the locus coeruleus (Audēn et al., 1966, Palkovits et al., 1974). Stimulation of the locus coeruleus causes a pressor response which is reduced by lesions of the posterior hypothalamus (Przuntek et al., 1973). Electrical stimulation of the posterior hypothalamus causes a rise in arterial pressure and enhances the release of NE from the posterior hypothalamus (Philippu et al., 1970), while the rise in arterial pressure is prevented by local application in the posterior hypothalamus of α-adrenoceptor blocking drugs (Philippu et al., 1974). The pressor response is further potentiated by superfusion of the hypothalamus with desipramine, an inhibitor of the reuptake of released NE by nerve endings (Philippu et al., 1971). Conversely, perfusion of the hypothalamus with bretylium, an inhibitor of NE release from nerve endings, reduced the effects of electrical stimulation of the posterior hypothalamus on blood pressure (Przuntek et al., 1971). Destruction of noradrenergic nerve endings in the hypothalamus by prior treatment with 6-hydroxydopamine did not affect the basal arterial pressure but reduced the rise

in pressure elicited by electrical stimulation of the posterior hypothalamus (Przuntek et al., 1971). Furthermore, direct application of NE to the posterior hypothalamus by superfusion of this area causes an increase in arterial pressure which is also potentiated by desipramine (Phillippu et al., 1973).

The pressor response observed by stimulation of the posterior hypothalamus can be attributed to activation of α-receptors in the posterior hypothalamus, since it is inhibited by α-receptor blockade. Interestingly, α-receptor stimulation of the anterior hypothalamus causes a depressor response (Philippu et al., 1974). It should be noted that NE applied to a localized area such as the posterior hypothalamus causes a pressor effect while application of NE to the anterior hypothalamus causes hypotension and bradycardia (Struyker-Boudier et al., 1974). It appears that the noradrenergic mechanisms in the anterior hypothalamus may have an opposing function to the norandrenergic mechanisms in the posterior hypothalamus in terms of controlling arterial pressure.

More recently, using a push-pull cannula in the anterior and posterior hypothalamus of the anesthetized cat (Sinha et al., 1980) and conscious rabbits (Philippu et al., 1981), it has been reported that the superfusate from the posterior hypothalamus of animals which were subjected to hypotension had higher concentrations of NE than before

hypotension and the animals which were subjected to elevated blood pressure had enhanced release of NE in the anterior hypothalamus. From these results Philippu and co-workers (1981) have postulated that noradrenergic mechanisms in the anterior hypothalamus are concerned with eliciting a depressor response while trying to counter an elevated arterial pressure. Conversely the noradrenergic mechanisms in the posterior hypothalamus are involved with the pressor response to counteract a fall in arterial pressure.

Consistent with such an hypothesis, Wijnen et al. (1978) have demonstrated an increased turnover of NE in paraventricular nucleus of the hypothalamus in response to a decrease in arterial pressure by controlled hemorrhage or administration of guanethidine (depletes NE from terminals by promoting release). Contrary to these findings, experiments by Haeusler et al. (1975) reported that partial depletion (80% in hypothalamus and 45% in medulla and pons) of central noradrenergic content by injection of 6-hydroxydopamine into the lateral brain ventricle of rats did not affect the fall in arterial pressure elicited by bilateral electrical stimulation of the sinus nerves. These results indicate that central noradrenergic neurons may not be necessary for baroreceptor reflex. However, since the destruction of central noradrenergic neurons was known to be incomplete it is conceivable that transmission through the reflex arc may have been maintained by the residual noradrenergic neurons.

The studies described above are a summary of the evidence for the role of noradrenergic mechanisms in the regulation of arterial pressure obtained from "normotensive animals". In addition, there has been a large body of literature published dealing with the possible role of the central noradrenergic mechanisms in experimental hypertension. The reader is referred to review articles by Chalmers (1975), Haeusler (1975), and Antonaccio (1977) for in depth coverage of this topic.

The next section will concentrate on the evidence regarding the role of noradrenergic mechanism of the hypothalamus in the etiology and maintenance of experimental hypertension.

2.1.1.3 Hypothalamic noradrenergic mechanisms in various models of experimental hypertension

2.1.1.3.1 Baroreceptor Deafferentation

The arterial baroreceptor reflex is a homeostatic mechanism which serves to regulate arterial pressure through a negative feedback loop to minimize changes in arterial pressure (review by Korner, 1979). This negative feedback mechanism depends on the presence of inhibitory afferent and excitatory efferent limbs of the reflex arc. The primary inhibitory afferents consist of carotid sinus nerves and

aortic depressor nerves (Korner, 1971; Spyer, 1981), which make their first synapse in the NTS (Crill et al., 1968; Spyer, 1981). "Neurogenic hypertension" is produced either by peripheral sinoaortic denervation (Krieger, 1964) or lesioning the NTS (Doba et al., 1973), thus reducing the inhibition of tonic bulbospinal vasomotor activity. As observed above, there is a great similarity between the central noradrenergic pathways and neurons subserving central cardiovascular control, implying a crucial role for noradrenergic mechanisms in control of arterial pressure. This concept is supported by results obtained in "neurogenic hypertension" where it has been demonstrated that sinoaortic denervation in rabbits produces an increased turnover of NE in the hypothalamus (Chalmers et al., 1972). The increase in noradrenergic activity in the hypothalamus and the observation that midcollicular decerebration abolished hypertension produced by NTS lesion (Doba et al., 1973) is consistent with the concept that baroreflex function is not mediated purely at the medullary level, but in fact utilizes neural loops involving higher centers like the hypothalamus (Manning, 1965). In addition, 6-hydroxydopamine administered centrally prevents neurogenic hypertension produced by sinoaortic denervation (Chalmers et al., 1972). Doba et al. (1974) have made similar observation in the NTS lesioned rats. Similar to the sinoaortic denervated and NTS lesioned rats, lesioning of the aortic depressor nerve (ADN) alone, also causes an increased arterial pressure (Ciriello

et al., 1980) in rats. Furthermore, lesions of the paraventricular nucleus of the hypothalamus in ADN transected rats prevents (Zhang et al., 1983) and reverses (Zhang et al., 1982) the "neurogenic hypertension" produced by bilateral aortic nerve transection. Since there is a very dense noradrenergic innervation of the hypothalamus (paraventricular nucleus), it is plausible that noradrenergic mechanisms of the hypothalamus (paraventricular nucleus) play an integral role in the neurogenic control of arterial pressure (Swanson et al., 1983). These results taken together suggest an integral role for noradrenergic mechanims of the hypothalamus in neurogenic control of arterial pressure.

2.1.1.3.2 DOCA-salt Hypertension

Administration of deoxycorticosterone acetate (DOCA) and substitution of 0.9% NaCl for drinking water to drink is known to produce hypertension (reviewed in Genest, 1973). In this model of hypertension there has been some evidence for the role of central noradrenergic mechanisms in the control of arterial pressure. Nakamura et al. (1971) have demonstrated a decreased turnover of NE in the hypothalamus and have suggested that this is a primary cause for the elevated arterial pressure in this model of hypertension. This suggestion is supported by the fact that ganglionic blockade abolishes the hypertension but not the decreased

turnover of NE in the hypothalamus and medulla (Nakamura et al., 1971). In addition, intraventricular administration of 6-hydroxydopamine prevents the induction and the development of DOCA-salt hypertension (Finch et al., 1972, Hauesler et al., 1972). However, it is of interest to note that Hauesler et al. (1972) have demonstrated that a similar 6-hydroxydopamine treatment in the chronic stage of DOCA-salt hypertension will not normalize arterial pressure. This evidence suggests that noradrenergic mechanisms in the central nervous system are involved in the initiation of an elevated arterial pressure in this model of hypertension, but not the maintenance of hypertension.

2.1.1.3.3 Renal Hypertension

Support for the hypothesis that hypothalamic noradrenergic mechanisms are involved in the hypertensive process in the one-kidney, one-clip Goldblatt model of hypertension comes from the recent findings that NE content (De Quattro et al., 1978, Eide et al., 1980) and tyrosine hydroxylase activity (Eide et al., 1980) of the hypothalamus are increased 3 weeks after renal artery clipping in uninephrectomized rats. Eide et al. (1980) found no changes in the hypothalamic NE content or tyrosine hydroxylase activity in the two-kidney, one-clip hypertensive rat, despite elevation in blood pressure comparable to that seen in the one-kidney model. This suggests that the elevated

hypothalamic NE concentration observed in the one-kidney model is not a compensatory response to increased blood pressure. Wintернitz et al. (1982) have also demonstrated an elevated concentration of NE in the hypothalamus of one-kidney, one-clip Goldblatt hypertensive rats one week after renal artery clipping.

In contrast to these studies Petty et al. (1977) found a reduction in the NE concentration of the peri- and paraventricular, anterior and posterior hypothalamic nuclei 3 days after renal artery clipping (one-kidney, one-clip Goldblatt model). In a subsequent study, Petty et al. (1979) reported a similar decrease in tyrosine hydroxylase activity in the peri- and paraventricular and posterior hypothalamus. No changes in NE concentration and tyrosine hydroxylase activity were present in animals studied 7 days or 4 weeks after clipping. Similarly, Wijnen et al. (1980a) using the two-kidney one-clip model of Goldblatt hypertension showed a transient (3 days) decrease in NE turnover in two nuclei of the anterior hypothalamus (nucleus intersitialis striae terminalis and paraventricular nucleus). This increase was no longer present at 3 and 5 weeks after the clipping of the kidney.

Although the discrepancies between the studies quoted above are difficult to resolve, all these studies allude to noradrenergic mechanisms being involved in the hypertensive process of the Goldblatt renal hypertensive rats. Further

support for this concept comes from the experiments dealing with intervention of the central neural mechanisms with a treatment of 6-hydroxydopamine or lesioning of periventricular tissue surrounding the anteroventral third ventricle (AV3V) or ventromedial-median eminence regions of the hypothalamus. Such interruptions of central neural mechanisms have been reported to prevent the development of hypertension in renal hypertension (Dargie et al., 1976; Brody et al., 1978; Johnson et al., 1981). In addition, posterior hypothalamic lesions, areas with relatively rich noradrenergic innervation (Moor et al., 1979), have been shown to lower blood pressure in the chronic established phase of hypertension in the one-kidney one-clip Goldblatt model (Bunag et al., 1976), again alluding to the hypothesis that noradrenergic mechanisms of the hypothalamus may be involved in renal hypertension.

2.1.1.3.4 Spontaneously Hypertensive Rats (SHR)

A vast number of studies have been done to measure changes in the metabolism of NE in the CNS of SHR, however the age of SHR studied and the choice of normotensive controls have remained a major problem (Patel et al., 1981). Initially, rats of normotensive outbred Wistar strains were generally used as controls. Recent comparisons have been performed between the SHR and Wistar Kyoto (WKY) as a "control". Patel et al. (1981) have suggested that better

comparisons can be made within the SHR at various ages before and after the development of hypertension.

The strongest evidence for the involvement of central catecholaminergic mechanisms in the hypertensive process of SHR comes from Haeusler et al. (1972) who demonstrated that central administration of 6-hydroxydopamine during the prehypertensive stage interrupts the development of hypertension in the SHR. Administration of 6-hydroxydopamine centrally in mature hypertensive animals has little effect on the level of arterial pressure (Finch et al., 1972). This suggests that central catecholaminergic systems are somehow involved in initiating or triggering the hypertension in the SHR rat but that they do not play a significant role in the maintenance of the elevated blood pressure.

Evidence for altered noradrenergic mechanism in the hypothalamus of SHR is provided by Nagaoka et al. (1977) who reported that the activity of tyrosine hydroxylase and dopamine β-hydroxylase in the hypothalamus of 3 week old SHR was not different from that of 3 week old WKY rats; however, at a later stage (5-8 weeks of age) of spontaneous hypertension, tyrosine hydroxylase and dopamine β-hydroxylase activity in the hypothalamus were higher than in WKY controls. These results suggest an enhanced synthesis of NE in the hypothalamus at a later stage of spontaneous hypertension. The results of Patel et

al. (1981) agree with the results above qualitatively, in that, they observed no significant change in NE turnover between SHR and WKY at 5 weeks of age. However, there was an increased turnover of NE in the hypothalamus of SHR at 9 weeks of age compared to 5 weeks of age while comparing within the SHR strain. These measurements were made in gross brain areas making interpretations difficult. Studies performed by Wijnen et al. (1980a) using discrete brain regions showed a decrease in the activity of noradrenergic neurons in the anterior hypothalamus during the onset of the development of the hypertension. It was spectulated that the lower activity in a noradrenergic hypothalamic inhibitory system during the onset of hypertension, might be related to enhanced sympathetic nerve activity and thus the cause of hypertension. Whereas, alterations in the activity of the noradrenergic systems in the hypothalamus from 5 to 9 weeks of age might be seen as possible compensatory change in activity to counteract the rise in blood pressure in the SHR (Okomoto et al., 1967; Wijnen et al., 1980b; Patel et al., 1981). However, Wijnen et al. (1980b) did not have a measure of sympathetic nervous system (SNS) activity. In addition, Ciriello et al. (1983) have shown recently that lesioning the paraventricular nucleus of hypothalamus (PVH) (area rich in noradrenergic innervation) in the SHR delays the hypertensive process (at 5 weeks of age) but does not prevent it, again alluding to the involvement of hypothalamic noradrenergic mechanisms in the initiation of

an elevated pressure in this model of hypertension.

In summary, the results obtained with destruction of central noradrenergic pathways would support the concept of increased noradrenergic activity in the CNS as a contributing factor in the early stages of spontaneous hypertension (Haeusler et al., 1972). However, the reduced NE turnover in the brain (specifically the hypothalamus) which may be associated with hypertension and the finding that destruction of central noradrenergic pathways prevent the development of hypertension are hard to reconcile. Furthermore it is not clear if the altered noradrenergic activity in the hypothalamus of hypertensive rats is the cause or the effect of the hypertensive process, or related at all.

2.1.2 Peripheral Sympathetic System and arterial pressure

2.1.2.1 Sympathetic Nervous System (SNS) in normotensive rats

The first evidence for a role of catecholamines in the control of arterial pressure was provided by Oliver et al. (1895) when they demonstrated that the active ingredient in the adrenal medulla could raise arterial pressure. Later Von Euler in 1946, identified NE as the neurotransmitter of the sympathetic nerves. Since then there has been a great

advance in our knowledge concerning the pharmacology, physiology and biochemistry of the sympathetic nervous system (specifically containing NE), (Axelrod, 1973).

The baroreceptor reflex for the regulation of cardiovascular homeostasis uses the peripheral sympathetic system as one of the primary neurogenic pathways to control peripheral resistance and cardiac output (Chalmers 1975; Korner, 1979; Abboud, 1982). This reflex arc presumably uses noradrenergic neurons in spinal cord, brainstem and hypothalamus (Chalmers 1975). Studies examining the reflex changes in peripheral sympathetic activity in response to changes in arterial pressure have been relatively few in the recent past (Kezdie et al., 1968; Echtenkamp et al., 1980; Takimoto et al., 1981). De Jong et al. (1975) have reported that electrical stimulation and application of micro-injection of NE in the NTS (primary site of baroreceptor input) of the brain stem results in a reduction in arterial pressure, heart rate and, presumably, in efferent sympathetic impulse flow. In addition Ectenkamp et al. (1980) have demonstrated a reflex reduction in sympathetic efferent impulse flow in response to an increased firing along the carotid sinus nerve in the monkey. Conversely, sectioning the baroafferent sensory nerves (Krieger 1964) or lesioning the NTS (Doba et al., 1974) interrupts the reflex arc and thus causes an increase in peripheral resistance which is a result of elimination of inhibitory input from baroreceptors. Chalmers et al. (1971)

have shown an increased activity of tyrosine hydroxylase, the rate-limiting enzyme in NE biosynthesis in thoracolumbar cord (a site for preganglionic sympathetic outflow), suggesting an increased neuronal activity in this region of the sinoaortic denervated rabbits. Similarly De Quattro et al. (1969) have shown an increased turnover of NE in the heart and adrenal glands of sinoaortic denervated rabbits. Alexander et al. (1974) have demonstrated regional hemodynamic patterns that are consistent with the turnover studies, indicating activation of sympathetic nervous system. Subsequently a number of studies have shown an increased noradrenergic activity in rats after baroreceptor denervation by sectioning the carotid sinus and aortic depressor nerves (Alexander et al., 1976; Alexander et al., 1980). Furthermore regional resistance changes in the mesenteric and skeletal muscle reported by Touw et al. (1979) also agree with the concept of increased vasoconstrictor tone in animals with removal of major baroreceptor input (ADN). Finally, Doba et al. (1974) have shown that 6-hydroxydopamine administered centrally prevents the exaggerated peripheral sympathetic response observed after NTS lesion.

Taking all these data together it has been suggested that changes in the noradrenergic activity in the central structures, possibly the hypothalamus (see section 2.1.1.2 of Historical Review), may be responsible for the activation of peripheral sympathetic activity.

2.1.3 Relationship between hypothalamic and peripheral noradrenergic mechanisms

The evidence in normal animals that stimulation of various areas of the hypothalamus rich in NE can cause either vasopressor or vasodepressor responses, increases or decreases in the heart rate, and interaction with baroreceptors suggests a direct relationship between changes in hypothalamic and peripheral noradrenergic mechanisms (Przuntek et al., 1971; Philippu et al., 1971). This suggestion is further supported by the observation that application of NE directly in various hypothalamic areas produced responses in the SNS similar to those observed following electrical stimulation of the same areas (Philippu et al., 1974). In addition, Folkow et al. (1966) have reported that chronic stimulation of the posterior hypothalamus can produce a sustained hypertension. However, more recently Buñag et al. (1979) have failed to reproduce the results reported by Folkow and co-workers. On the other hand depletion of catecholamines in the hypothalamus with 6-hydroxydopamine reduces the effect of the electrical stimulation of the hypothalamus (Przuntek et al., 1973). Overall these studies suggest a direct relationship between noradrenergic mechanisms in the hypothalamus and peripheral organs.

In theory, functional changes in the SNS in hypertension could result from altered activity at any

changes in renal vascular vasomotor tone could cause a shift in the renal function curve and cause chronic arterial hypertension (Cowley et al., 1979).

In the past, many experiments which have shown an effect of the renal nerves on sodium and water excretion have been interpreted as indicating a direct action of the nerves on tubular processes (Di Bona 1982). However it is only recently that direct assessment of tubular function by micropuncture techniques has been used to study the role of renal nerves in tubules (Gottschalk, 1979). A depression of proximal reabsorption of sodium, water and other solutes has been shown to occur following denervation in kidneys in which the glomerular filteration rate and renal blood flow were the same before and after denervation (Bello-Reuss et al., 1975; Takacs et al., 1978; Di Bona et al., 1981). An increase in proximal tubular reabsorption has been reported to occur after low-level direct or baroreceptor reflex stimulation of renal sympathetic nerves in the rat (Di Bona et al., 1977; Di Bona et al., 1981). It is now an accepted fact that renal nerves have a direct action on the proximal tubule to influence sodium and water excretion and thus renal function (Di Bona 1982).

There is ample evidence to show that renal nerves can also effect renin release (see review Di Bona, 1982). Direct electrical stimulation of renal nerves or activation of renal nerves under a variety of conditions such as

activity were not obtained in that study. More recently Nakamura et al. (1981) have demonstrated a decrease in the dopamine β-hydroxylase activity and NE content in the posterior hypothalamic nucleus with an elevated plasma level of epinephrine and dopamine β-hydroxylase one week after sino-aortic baroreceptor denervation in rats. It is of interest to note that even though dopamine β-hydroxylase activity was increased, the plasma level of NE was not changed (Nakamura et al., 1981). Generally the results from studies in neurogenic models of hypertension suggest a direct relationship between changes in hypothalamic and peripheral noradrenergic activity.

In contrast to these findings Nakamura et al. (1971) and Van Ameringen et al. (1977) have reported a reciprocal relationship between central noradrenergic activity and peripheral noradrenergic activity in DOCA-salt hypertensive rats. Nakamura et al. (1971) observed a decreased turnover of NE in the hypothalamus and medulla with a concomitant increase in NE turnover in the heart. Similarly Van Ameringen et al. (1977) has shown a decreased NE turnover in the brainstem with an elevated NE turnover in the intestine. Furthermore it is suggested that the primary change was at the bulbar level since neither ganglionic blockade (Nakamura et al., 1971) nor cervical cord section (Van Amerigen et al., 1977), which abolished the hypertension and the increase in peripheral sympathetic activity (i.e. NE turnover), had any effect on the NE turnover in the brain.

Similarly a reciprocal relationship between central and peripheral noradrenergic mechanisms has been suggested to exist in SHR (Yamori et al., 1972 Okomoto et al., 1967). More recently, Patel et al. (1981) supported this contention with a systematic comparison of NE turnover in brain and peripheral organs of SHR and two normotensive control strains (WKY and Wistar) at various ages (5 weeks - prehypertensive stage; 9 weeks - developmental stage; 18 weeks - established hypertensive stage). They demonstrated that at 5 weeks, the turnover of NE was significantly lower in the cortex and significantly higher in the kidney and skeletal muscle of SHR. By 9 weeks, within SHR, NE turnover had increased significantly in hypothalamus and brainstem, while decreasing significantly in kidney and duodenum. No such changes were seen in these regions of normotensive WKY or Wistar rats at 5 and 9 weeks of age. These data support the hypothesis that decreased central noradrenergic activity may be responsible for increased noradrenergic activity in peripheral organs of young SHR (5 week old), which in turn may have initiated or contributed to the development of hypertension. Changes in turnover of NE in peripheral organs between 5 and 9 weeks in SHR suggest compensatory responses to increasing arterial pressure or changing central noradrenergic activity; however, similar changes in NE turnover were not seen between 9 and 18 weeks, although pressure continued to increase (Patel et al., 1981). As with other hypertensive

models, it is not clear if the altered noradrenergic activity in the central and peripheral structures of the SHR are the cause or the effect of the hypertensive process.

On the other hand Tanaka et al. (1982) have demonstrated in one-kidney, one-clip Goldblatt hypertension, an increased turnover of NE in the aorta, mesenteric artery, and left ventricle; however, there was no change in the turnover of NE in the brain areas of hypothalamus, midbrain and medulla. In addition, they found no changes in central or peripheral noradrenergic activity in the two-kidney, one-clip Goldblatt hypertension. It is clear from reviewing the pertinent literature that there is no definite consistent relationship between the central and peripheral noradrenergic mechanisms in the various models of experimental hypertension.

In normotensive rats, Wijnen et al. (1978) have demonstrated an increased turnover of NE in specific nuclei of the hypothalamus in response to a decrease in arterial pressure; however no measure of peripheral sympathetic activity was reported. On the other hand Takimoto et al. (1981) showed an increased tyrosine hydroxylase activity in the peripheral organs in response to hypotension in anesthetized rabbits but did not look for changes in the central nervous system.

In conclusion, it appears that it is not clear how the changes in the noradrenergic activity in the hypothalamus

are related to changes in peripheral sympathetic system. Furthermore it is still not clear whether the changes in noradrenergic mechanisms in various hypertensive models are a cause or effect of the hypertension.

2.1.4 Interaction between nonneural mechanisms and noradrenergic mechanisms

Many studies have provided strong evidence that there are large numbers of vasopressor agents such as vasopressin and angiotensin II which are endogenously secreted to participate in the stabilization of arterial pressure in response to various cardiovascular challenges (Cowley et al., 1980). This section will examine the interaction between two major vasopressor agents, vasopressin and angiotensin II and noradrenergic mechanisms.

2.1.4.1 Vasopressin

Recently there has been increasing evidence that the neurohypophysial hormone vasopressin, is not only involved in the concentration of urine but may participate also in the regulation of the cardiovascular system (Cowley 1982). Vasopressin has been identified in the paraventricular and supraoptic nuclei of the hypothalamus (for review see Swanson et al., 1983). Neurophysiological evidence for the involvement of the supraoptic and paraventricular nuclei in

cardiovascular regulation has been provided by electrophysiological studies. Electrical stimulation of the buffer nerves or selective baroreceptor, chemoreceptor or atrial stretch stimulation has been shown to alter unit activity in the paraventricular and/or supraoptic nuclei (Calaresu et al., 1980; Yamashita 1977; Kannan et al., 1978; Koizumi et al., 1978). In addition, Calaresu et al. (1980) demonstrated that carotid sinus nerve stimulation affected activity in almost half of the identified units recorded from the paraventricular nucleus. This suggests that possibly baroreceptor information is responsible for vasopressin release. These studies together indicate the involvement of vasopressin from the paraventricular and supraoptic nuclei of the hypothalamus in the regulation of arterial pressure (see review by Swanson et al., 1983). Furthermore, it has been suggested that vasopressin participates in the regulation of arterial pressure by modulating neurotransmission in various brain areas (Tanaka et al., 1977). Tanaka et al. (1977) have reported that NE turnover is increased in the hypothalamus, thalamus and medulla oblongata in response to administration of vasopressin into the cerebral ventricles.

Vasopressin has also been shown to modify the activity of the peripheral sympathetic system by augmentation of the vasoconstrictor effect of peripheral noradrenergic activity by its action on either ganglia, sympathetic terminals, or sympathetic adrenergic receptors (Bartelstone et al., 1965;

Commarato et al.,1969; Gerke et al., 1977). Recent studies using receptor blockade have demonstrated that in addition to having a direct effect on vascular smooth muscle cells, vasopressin could interact with pre- and post junctional adrenergic sites, enabling it to facilitate both the indirect and direct effects of the adrenergic agonist (Gerke et al., 1976; Erker et al., 1977).

Although the major sites of vasopressin action and the specific interaction between noradrenergic mechanisms and vasopressin remain unclear, data suggest that vasopressin is involved in the control of the cardiovascular system and that these alterations can occur at plasma concentrations of vasopressin that are well within the observed physiological range (Cowley et al., 1982).

2.1.4.2 Angiotensin II

There is a growing body of evidence that angiotensin II affects the function of peripheral noradrenergic nerves and of the adrenal medulla (Ferrario et al., 1972; Zimmerman 1978). Similarly there has been a focus on the action of angiotensin II on the central noradrenergic mechanisms (Severs et al., 1973; Antonaccio, 1977). The observation that depletion of central noradrenergic neurons with reserpine blocks the hypertensive effect of intraventricular angiotensin suggests that the pressor action of angiotensin may be mediated by noradrenergic neurons in the CNS (Sweet

et al., 1971). In addition, the central pressor effect of angiotensin II is blocked by α-antagonists (Camacho et al., 1981). Administration of renin or angiotensin II into the cerebrospinal fluid stimulates NE turnover in specific hypothalamic nuclei (Ganten et al., 1980; Sumnars et al., 1983) and Fukyama (1973) has shown that intraventricular administration of angiotensin II reduces the gain of the baroreceptor reflex. These results taken together show that brain regions rich in norepinephrine are influenced by central angiotensin II injections and indicate that central noradrenergic mechanisms may be involved in mediating the central angiotensin II pressor response. However it should be recognized that in these studies the doses of angiotensin are in the pharmacological ranges.

Yet other studies refute this hypothesis that angiotensin is involved centrally in mediating noradrenergic activity. Two separate groups of researchers, Coleman et al. (1981) and Zerbe et al. (1981), have demonstrated that endogenous angiotensin has no influence on the baroreceptor function and angiotensin II is not essential for the release of catecholamines in response to hemorrhage, respectively. In summary it is not clear how angiotensin II interacts physiologically with central and peripheral noradrenergic systems in controlling arterial pressure.

2.2 Evidence for the involvement of renal nerves in the regulation of arterial pressure

2.2.1 Role of efferent renal nerves in normal pressure regulation

One of the major functions served by the kidney is the control of body fluid homeostasis. Guyton and coworkers (1974) have suggested that the renal function curve, a graphical representation of the steady state relationship between the urinary output and arterial pressure provides the basis for understanding the relationship between renal function and long-term arterial pressure regulation. Such relationships were initially reported under acute conditions in isolated kidneys (Selkurt 1951; Guyton et al., 1967) and have since been reported under chronic conditions in humans (Murray et al., 1978), dogs (De Clue et al., 1978) and rats (Norman et al., 1978). Guyton et al. (1974) have suggested that a change in the slope or the position of the renal function curve along the pressure axis dictates the "set" level of chronic arterial pressure around which arterial pressure would be regulated (Guyton et al., 1974).

It is now known that numerous factors, both intrinsic and extrinsic to the kidney, can operate to alter the relationship between arterial pressure and urine output, that is, cause shifts in the renal function curve (Guyton et al., 1969; 1974; 1976). It is conceivable that the

efferent sympathetic nerves could alter the position and slope of the renal function curve (Coleman et al., 1975). Such a contention is supported by anatomical, physiological and pharmacological studies.

Anatomical studies have reported extensive noradrenergic innervation of afferent and efferent arterioles (reviewed in Barajas et al., 1978), juxtaglomerular apparatus (Gorgas 1978; Barajas et al., 1981) and proximal and distal tubules (Di Bona 1977). For further details refer to reviews by Barajas (1978; 1981) and Di Bona (1982). Generally, the anatomical evidence suggests that there is innervation of all parts of the nephron and so it is conceivable that the sympathetic nervous system is capable of altering renal function by its action on any one or all parts of the nephron (Di Bona, 1982).

It has been demonstrated that electrical stimulation of efferent renal nerves causes constriction of renal arterioles by activation of sympathetic receptors (Auckland 1976). Similarly activation of renal nerves has been produced by a variety of physiological manoeuvers as well (see reviews by Donald et al., 1980; Coleridge et al., 1980; Di Bona, 1982). It is suggested that changes in renal nerve activity would change blood flow which consequently changes the sodium and water excretion by the kidney and thus the renal function (Di Bona 1982). Such

changes in renal vascular vasomotor tone could cause a shift in the renal function curve and cause chronic arterial hypertension (Cowley et al., 1979).

In the past, many experiments which have shown an effect of the renal nerves on sodium and water excretion have been interpreted as indicating a direct action of the nerves on tubular processes (Di Bona 1982). However it is only recently that direct assessment of tubular function by micropuncture techniques has been used to study the role of renal nerves in tubules (Gottschalk, 1979). A depression of proximal reabsorption of sodium, water and other solutes has been shown to occur following denervation in kidneys in which the glomerular filteration rate and renal blood flow were the same before and after denervation (Bello-Reuss et al., 1975; Takacs et al., 1978; Di Bona et al., 1981). An increase in proximal tubular reabsorption has been reported to occur after low-level direct or baroreceptor reflex stimulation of renal sympathetic nerves in the rat (Di Bona et al., 1977; Di Bona et al., 1981). It is now an accepted fact that renal nerves have a direct action on the proximal tubule to influence sodium and water excretion and thus renal function (Di Bona 1982).

There is ample evidence to show that renal nerves can also effect renin release (see review Di Bona, 1982). Direct electrical stimulation of renal nerves or activation of renal nerves under a variety of conditions such as

hemorrhage, constriction of the thoracic vena cava and mild sodium depletion can produce an increase in renin release from the kidney (Di Bona, 1982). Evidence is available to support the view that renal nerves may affect renin release by: 1) producing changes in the degree of constriction of the renal arterioles through activation of a renal vascular receptor; 2) changing glomerular filtration rate and thus filtered sodium load to the macula densa; and 3) stimulation of the juxtaglomerular apparatus directly (reviewed in Davis et al., 1976; Reid et al., 1978; Fray, 1980; Di Bona, 1982).

In summary, electrical or physiological stimulation of renal sympathetic nerves directly, reflexly (physiologically) or centrally (electrically) can significantly alter renal hemodynamics, glomerular filtration rate, renin release from juxtaglomerular apparatus and tubular reabsorption (Di Bona, 1982). On the basis of these results it is apparent that sympathetic activity is capable of altering renal function and consequently the long-term level of arterial pressure (Guyton et al., 1974; Coleman et al., 1975).

2.2.2 Role of afferent renal nerves in arterial pressure regulation

The functional significance of the afferent signals that originate from the kidney is as yet unclear. Various

different receptor groups have been identified in rats (Astrom et al., 1967; Recordati et al., 1978), cats (Beacham et al., 1969; Calaresu et al., 1978), dogs (Uchida et al., 1971; Francisco et al., 1980), and rabbits (Niijima 1975). Receptors that respond to mechanical distortion of the structures surrounding the nerve terminal have been termed "renal mechanoreceptors" (Recordati et al., 1978). Such receptors are selectively sensitive to changes in arterial perfusion pressure, compression of the kidney, or alterations in venous and ureteral pressure. Nonmechanical stimuli, such as the infusion of chemical substances and renal ischemia (Astrom et al., 1967) activate renal afferent fibers through a second set of receptors called "renal chemoreceptors" (Recordati et al., 1978; 1980). Recordati et al. (1978; 1980) have classified the chemoreceptors into two groups. R1 renal chemoreceptors are stimulated by renal ischemia as produced by renal artery occlusion, systemic hypotension, renal vein occlusion, or systemic asphyxia (Recordati et al., 1978). The R2 receptors are activated by changes in the chemical environment of the renal interstitium as produced by changes in the excretory function of the kidney; i.e. the passage of ions from the renal pelvis across the pelvic epithelium (Recordate et al., 1980).

Electrophysiological studies have demonstrated that stimulation of afferent renal nerves evokes responses in neurons of various hypothalmic nuclei presumably involved in

cardiovascular regulation of sodium and water balance in cats (Ciriello et al., 1980), and rats (Knuepfer et al., 1980). In addition Calaresu et al. (1981b) have reported that 75% of the hypothalamic units that respond to renal afferent stimulation also respond to afferent carotid sinus nerve stimulation. In agreement with these results, was the finding that electrical stimulation of afferent renal nerves elicited changes in systemic cardiovascular variables, suggesting the existence of cardiovascular reflexes originating from renal receptors. In support of such a hypothesis Aars et al. (1970) showed that electrical stimulation of renal afferents produced a reflex decrease in cervical sympathetic efferent nerve activity and systemic blood pressure. Similar decreases in pressure have also been observed by other investigators in dogs (Ueda et al., 1967). In addition, Kostreva et al. (1981) have demonstrated that renal afferents, activated by increases in renal venous pressure, can reflexly inhibit contralateral renal and cardiopulmonary sympathetic efferent nerve activity, decrease contralateral renal vascular resistance, and decrease right ventricular contractile force, without altering heart rate. Furthermore, they observed two types of renal afferent fibers. The majority were activated in response to renal vein occlusion and were not tonically active. However, there were a few afferent fibers that were tonically active and they decreased their activity during renal vein occlusion. These data agree with Calaresu et

al. (1978) who suggested three types of afferent fiber groups (Aβ, Aδ and C) with the majority of the fibers being unmyelinated C fibers that are tonically active. The cardiovascular reflex responses reported above are different from those described by Calaresu et al. (1976) who showed that stimulation of the central end of a sectioned renal nerve in the anesthetized cat produces an elevated arterial pressure and tachycardia. The conflicting results have been attributed to different preparations used, species difference, anesthestic effects, and parameters of stimulation, since selective stimulation of chemo or baro receptors could be expected to elicit different results.

Although the physiological significance of renal chemo- and mechanoreceptors remains to be determined, it is conceivable that they participate in reflexes arising in the kidney and affect cardiovascular and renal mechanisms important in the regulation of arterial pressure, regional vascular resistance, and body sodium and water balance (Calaresu et al., 1978; Kostreva et al., 1981; Di Bona et al., 1982).

2.3 Interaction between renal afferents and noradrenergic mechanisms in the hypothalamus and peripheral sympathetic system

As pointed out in the previous section many manipulations within the kidney have been shown to affect

afferent renal nerve activity. However, it is not clear whether the renal afferents influence the noradrenergic mechanisms in hypothalamic areas implicated in cardiovascular regulation (see section 2.1.1.2 of Historical Review) or the peripheral sympathetic activity. There is a growing body of electrophysiological evidence to suggest that renal afferent information reaches the hypothalamus. Ciriello et al. (1980) have reported that electrical stimulation of the afferent renal nerves of the cat causes excitation of 96% of the spontaneously active units recorded from in the hypothalamus. These units were observed primarly in the preoptic area, lateral hypothalamus and paraventricular nucleus. Similarly Knuepfer et al. (1980) have reported that stimulation of renal afferent nerves in the rat produces changes in the unit activity of neurons in the AV3V region. These findings suggest that renal afferent fibers are capable of influencing neurons in the hypothalamus. Combining this concept with the idea that baroreceptor mechanisms use the same hypothalamic neurons as a part of the reflex arc (see Historical Review Section 2.1.1.2) it is conceivable that the two modes of information interact at the level of the hypothalamus. The first piece of evidence for such an interaction was provided by Calaresu et al. (1981b). They demonstrated that the majority (75%) of hypothalamic neurons responding to stimulation of the afferent renal nerves also respond to stimulation of carotid sinus nerve. In addition, Mahoney et al. (1978) have

reported that stimulation of renal afferent nerves in the rat resulted in decreased vasomotor tone in hindlimbs, mesenteric and renal vascular beds, and that this response was abolished by lesioning of the AV3V region (Brody et al., 1980). Further evidence that changes produced by afferent renal nerve stimulation may be mediated via hypothalamic neurons comes from studies reporting that stimulation of afferent fibers elicits efferent sympathetic responses with reflex latency period comparable to known somatosympathetic reflexes which are modulated at central levels (Calaresu et al., 1978). All these studies considered together suggest a hypothesis that the afferent renal nerves may have an excitatory effect on the hypothalamic neurons and thereby the peripheral sympathetic nervous system. Such an effect of afferent nerves on central and peripheral noradrenergic mechanisms imply that it may be important in the regulation of arterial pressure.

Renal nerves and their possible role in the development and maintenance of arterial hypertension has received much interest in recent years (Di Bona, 1982). However, it is not clear what relative roles both renal efferent and afferent fibers may play in the hypertensive process.

The first study to suggest that renal nerves may trigger the onset of hypertension was reported by Ratner, (1953). He reported that bilateral renal denervation prevented the neurogenic hypertension induced by sino-aortic

deafferentation in rabbits. Since then renal denervation has been shown to delay the development of genetic hypertension (Liard et al., 1977; Kline et al., 1978; Winternitz et al., 1980; Diz et al., 1981), DOCA-salt hypertension (Katholi et al., 1980) and reverse the elevated arterial pressure associated with one- and two-kidney, one-clip Goldblatt hypertension (Katholi et al., 1982a;1982b) and one-kidney Grollman hypertension (Katholi et al., 1982a) in rats. The decrease in arterial pressure after renal denervation in one- and two-kidney, one-clip Goldblatt hypertensive rats was not mediated by alterations in sodium intake or excretion, water intake or excretion, creatinine clearance or plasma renin activity (Katholi et al., 1981). In addition, it has been reported that renal denervation in one-clip, one-kidney Goldblatt hypertensive rat normalized both elevated plasma NE concentration (Katholi et al., 1981) as well as increased concentration of NE in the hypothalamus (Winternitz et al., 1982). These data suggest that renal denervation with interruption of renal afferent fibers can modulate noradrenergic activity in the hypothalamus, leading to a decrease in peripheral sympathetic activity and arterial pressure.

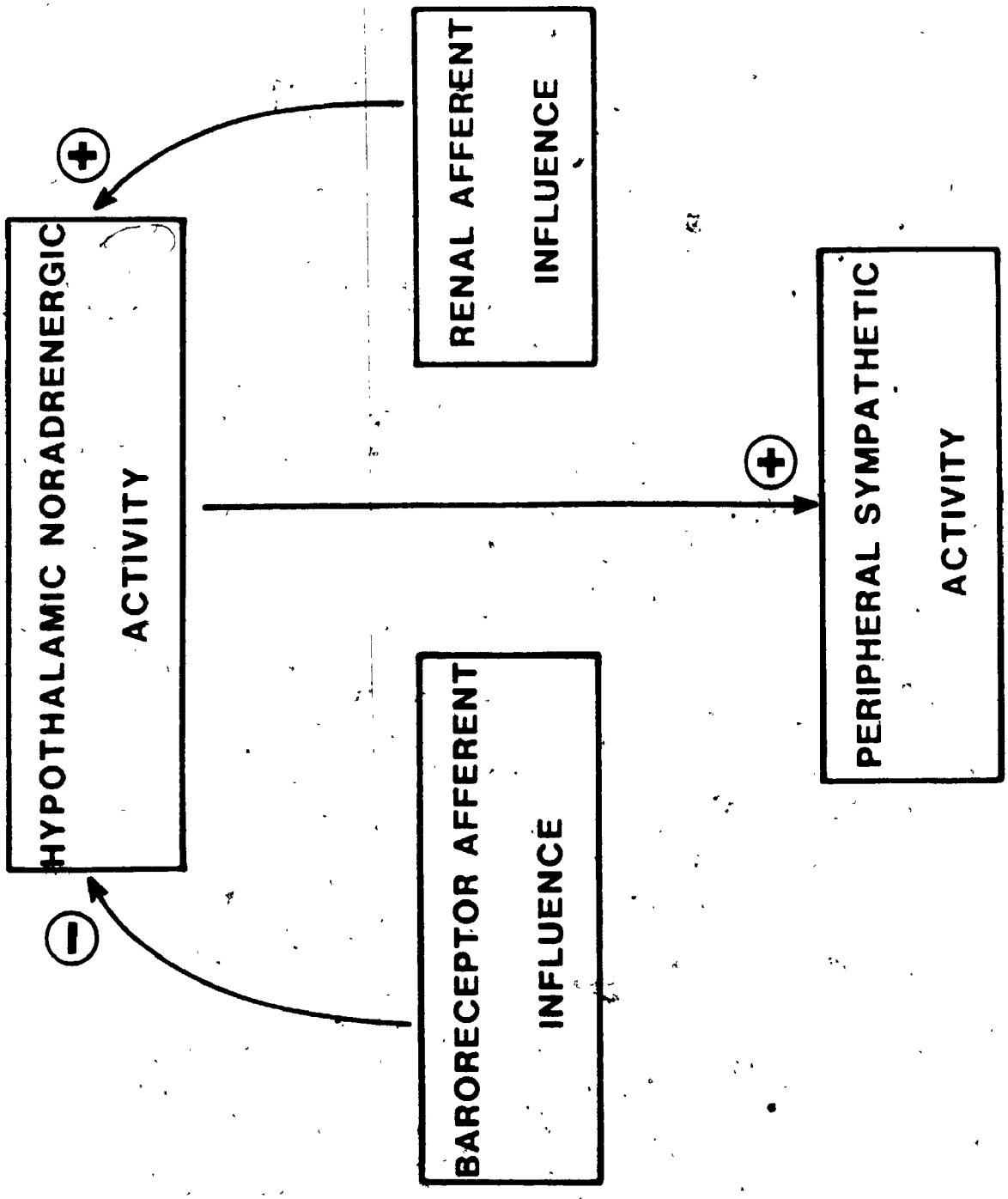
Recently Faber et al. (1983) have shown that activation of renal afferent nerves by acute renal artery stenosis causes an increased sympathetic activity and an elevation of arterial pressure. However, blocking the renin-angiotensin system with captopril prevents the sympathetic and arterial

pressure response to renal artery stenosis. Since baroreceptors were intact in these animals, baroreflex mechanisms may have obscured renal nerve-dependent cardiovascular changes. Subsequent experiments in baroreceptor deafferented (sino-aortic denervated) rats demonstrated that captopril no longer blocked the sympathetic and arterial pressure response to renal artery stenosis and that renal denervation (of the occluded kidney) prevented the response (Faber et al., 1983). They concluded that renal afferent nerves were important in the cardiovascular responses observed in response to acute renal artery stenosis.

Surmised from the review of the pertinent literature to date, it would seem reasonable to propose that there is an interaction between an inhibitory influence from baroreceptors and excitatory influence from renal afferents at the level of the hypothalamus. This interaction may involve the hypothalamic noradrenergic system. This interaction may also be important in modulating peripheral sympathetic activity and neurohormonal responses related to cardiovascular adjustments and to the control of body fluids. A working model of such an hypothesis is shown in Figure 1.

FIGURE 1

Proposed interaction between baroreceptor afferents and renal afferents in the regulation of noradrenergic activity at the level of the hypothalamus and the peripheral sympathetic system.


HYPOTHALAMIC NORADRENERGIC ACTIVITY
RENAL AFFERENT INFLUENCE
BARORECEPTOR AFFERENT INFLUENCE
PERIPHERAL SYMPATHETIC ACTIVITY
+
+
−

CHAPTER 3

OBJECTIVES

The aim of this research is to test the hypotheses developed in Chapter 2. The experiments can be divided into two major groups of several related projects. The first group of experiments examined the relationship between changes in noradrenergic activity in the hypothalamic structures and changes in sympathetic outflow. The second group of experiments investigated the interaction between inhibitory influences from baroreceptor afferents and excitatory influences from renal afferents on the noradrenergic activity at the level of the hypothalamus. The specific objectives of the studies presented in this thesis are:

1) To examine hypothalamic and peripheral noradrenergic response to surgical removal of aortic arch baroreceptor input by transection of ADN.

2) To investigate hypothalamic and peripheral noradrenergic response to changes in arterial pressure ("loading and unloading baroreceptors") in conscious rats.

3) To determine the effect of renal denervation on the noradrenergic response to removal of ADN and to changes in arterial pressure.

4) To investigate the effect of electrical stimulation of afferent renal nerves on hypothalamic and peripheral noradrenergic activity.

CHAPTER 4

GENERAL METHODS AND MATERIALS

This Chapter deals with the rationale for the use of the NE turnover technique in this project as well as the assumptions involved in using this technique. Secondly, the measurement of NE concentration using a fluorescence assay and High Performance Liquid Chromatography (HPLC) in combination with an electrochemical (EC) detector after alumina extraction are described. The fluorescence assay has been used in Dr. R. L. Kline's laboratory for a number of years now and is well established (for details see Patel, 1980). The HPLC in combination with an EC detector assay was developed to increase sensitivity and specificity of NE measurement during the progress of this project. The initial studies were performed using the fluorescence assay (studies in ADN transected rats) and the rest of the project was done using HPLC in combination with EC. The specificity and detection limits of the HPLC with EC assay are also discussed. Thirdly, this section illustrates the histology of the anterior and posterior hypothalamic sections used for analysis of NE turnover. Specific measurements and techniques, such as arterial pressure measurement or surgical techniques, are described in detail in the relevant Methods sections of subsequent chapters.

4.1 Measurement of Turnover of Norepinephrine (NE)

4.1.1 General Comments

Over the last 20 years there has been a remarkable increase in our knowledge about the biochemistry and pharmacology of noradrenergic neurons. During that same time several techniques have been devised to evaluate sympathetic nerve function. The next section will concentrate on some of these techniques and the interpretation of results obtained with these techniques.

Plasma NE levels are often measured in hypertensive animals and man to assess sympathetic tone, as circulating NE is mainly derived from transmitter released from noradrenergic nerve endings supplying blood vessels (Reid et al., 1975; Kopin et al., 1978). An increased sympathetic drive, with a concomitant rise in arterial pressure leads to the inference that the two are related. Such an inference is supported by Yamaguchi et al. (1979) who have demostrated a direct relationship between stimulation of the complete sympathetic outflow from the spinal cord of pithed rats and the increases in plasma NE concentration and arterial pressure. However this technique for measuring sympathetic activity has two major shortcomings. 1) A generalized sympathetic nerve activity is measured with this technique so it would be very difficult to know how the sympathetic nerve activity was affected in specific vascular beds. 2)

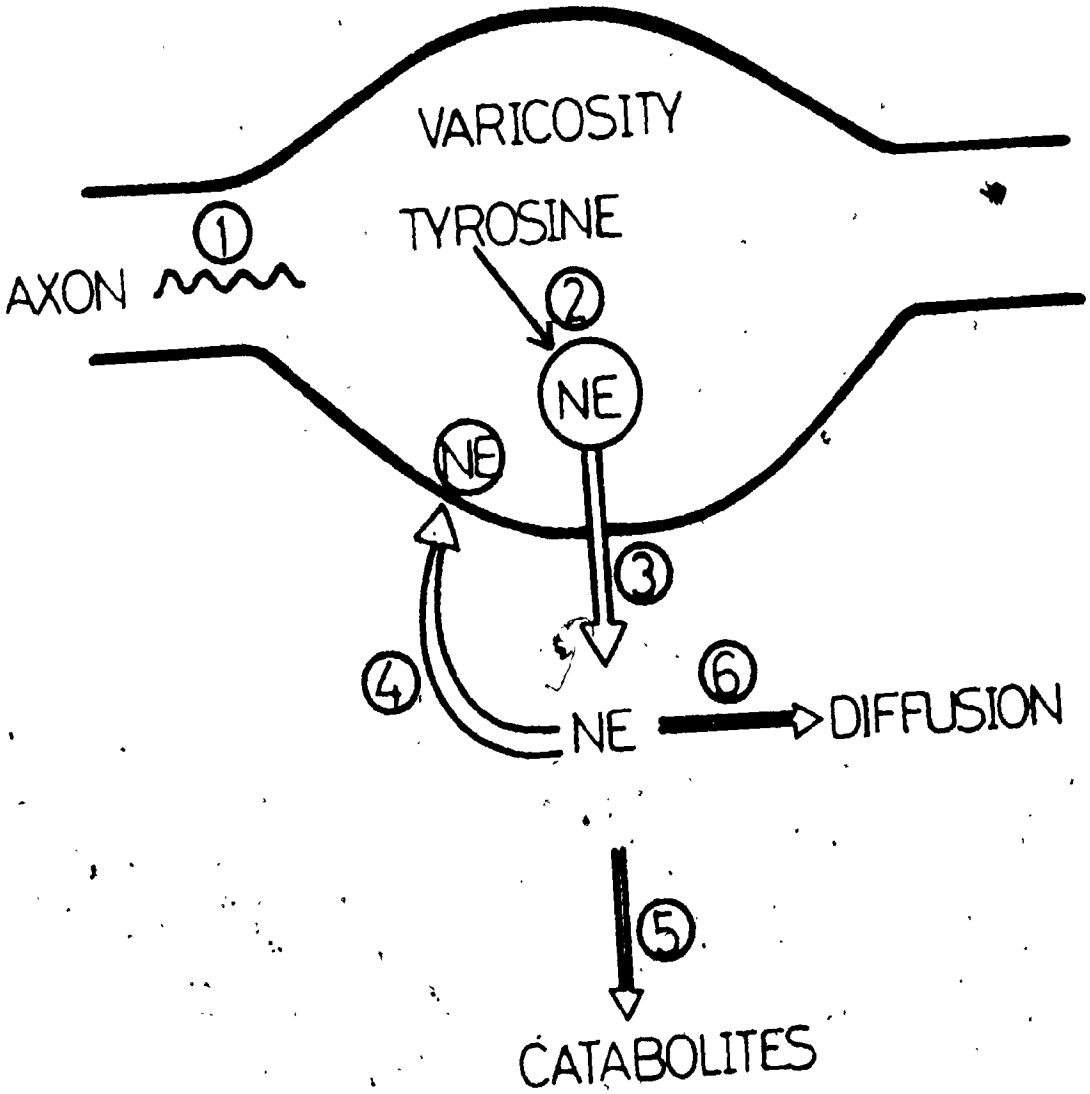
It is unlikely that there would be a simple relationship between the amount of transmitter measured and the constrictor tone it evokes because many factors, particularly the mechanism of termination of the action of NE and receptor sensitivity, influence the relationship, and these factors vary in different vessels and under different circumstances (Brodie, et al., 1966; Blaschko, 1973). Estimation of the plasma NE is currently the best method available for assessing levels of sympathetic tone in man, but it should be recognized that significant changes in sympathetic nerve and vascular smooth muscle interaction could occur without detectable changes in plasma NE concentration.

In animals more invasive techniques have been devised to measure the dynamic function of the noradrenergic neuron. Figure 2 shows a schematic representation of the multifactorial equilibrium that exists in noradrenergic neural tissue.

The effector organ is under the influence of a "resting secretion" of NE from postganglionic sympathetic nerves. This resting secretion consists of two components: 1) spontaneous random discharge of packets or quanta of NE which are far too small to cause a response of the effector organ (Blaschko, 1973), and 2) release of relatively large amounts of NE as a result of impulse traffic from the central nervous system, which maintain tonic activity,

FIGURE 2

Schematic representation of the various processes at a noradrenergic nerve terminal. 1) propagation of action potential in presynaptic terminal; 2) synthesis of NE from tyrosine; 3) release of NE; 4) neuronal uptake of released NE; 5) catabolism of released NE; 6) overflow of unchanged NE to circulation.


VARICOSITY
TYROSINE
1
AXON
2
NE
NE
3
4
NE
6
DIFFUSION
5
CATABOLITES

particularly in the vascular smooth muscle. Since endogenous concentrations of NE in the tissues are maintained at a fixed level characteristic of each individual organ, it appears that there is a dynamic balance between the rates of synthesis of NE and its disappearance (Brodie et al., 1966; Tozar et al., 1966).

A measurement that describes either the rate of production or decline of NE in noradrenergic tissue is referred to as the turnover of NE (Brodie et al., 1966). This turnover of NE is influenced by five factors: 1) rate of synthesis (strongly influenced by frequency of electrical impulses), 2) storage of NE within nerve terminals, 3) catabolism, 4) uptake into the nerve terminal, and 5) diffusion away from the site of release (see Figure 1). Change in any one of these factors could cause an alteration in the turnover of NE. Synthesis, uptake and catabolism of NE are under the influence of electrical impulse; that is, a change in the electrical impulse traffic is accompanied by a change in synthesis, uptake and catabolism, thus maintaining a steady balance between synthesis and disappearance of NE (Brodie et al., 1966; Tozar et al., 1966; Blaschko, 1973). Therefore the turnover of NE can conceivably be used as a method of indirectly assessing sympathetic nerve activity provided all other factors are constant (Brodie et al., 1966; Tozar et al., 1966; Sharman, 1981).

The turnover of NE is estimated using different methods based on the conversion of radio-labelled precursor (tyrosine or dopamine) to labelled NE (Udenfriend et al., 1963; Gordon et al., 1966; de Champlain et al., 1969), the decline in specific activity of labelled NE over time (Costa et al., 1966; Kopin, 1978), and the rate of decline in tissue NE concentration after inhibition of tyrosine hydroxylase (Brodie et al., 1966). The various techniques give roughly the same results (Brodie et al., 1966; Tozar et al., 1966; de Champlain et al., 1969; Sharman, 1981). However isotopic procedures require evidence that the labelled ^{3}H -NE is evenly distributed throughout the stores of endogenous amine (Brodie et al., 1966; Sharman, 1981). For further details about the various techiques used for measurement of catecholamime turnover refer to Sharman (1981).

The next few paragraphs will concentrate on the nonisotopic method using a tyrosine hydroxylase inhibitor, as this was the technique used in this study and it is among the best measurement techniques to access noradrenergic activity to date (Salzman et al., 1979; Sharman, 1981).

When the synthesis of NE is inhibited at the rate-limiting step (i.e. tyrosine to dopa stage with α-methyl-tyrosine), the NE content in sympathetically innervated organs decreases exponentially with time (Carlsson et al., 1963; Spector et al., 1965; Sharman,

1981). Several studies indicate that the frequency of neural impulses plays an important role in the depletion of NE from a sympathetically innervated organ after inhibition of synthesis (Tozar et al., 1966; Grabowska et al., 1976). Since no new NE is formed, continuous tonic release is sufficient to lead to gradual depletion of NE (Bhagat, 1967). Thus it seems acceptable to use this technique of measurement of NE turnover as an index of sympathetic nerve activity (Brodie et al., 1966; Grabowska et al., 1976; Patel, 1980). The validity of this technique for measuring turnover of NE in sympathetically innervated tissue is based on the following assumptions:

1) α-methyl-tyrosine completely inactivates tyrosine hydroxylase. Since α-methyl-tyrosine is a reversible and competitive inhibitor (Nagatsu et al., 1964) the effect would be expected to last only as long as adequate concentrations of inhibitor were present in the tissues. This can be achieved by administration of several doses of the inhibitor during the experiment to maintain a maximal block. Once inhibition was maximal, increased amounts of inhibitor would not be expected to hasten the rate of NE depletion since the latter would depend on the rate of NE utilization. Such was found to be the case (Spector et al., 1965).

2) α-methyl-tyrosine does not release the catecholamines or change their catabolic rate. Spector et al. (1965) have demonstrated that α-methyl-tyrosine does not

release the catecholamines or alter their rate of inactivation.

3) The inactivation of tyrosine hydroxylase is rapid. The rapidity of the blockade is evident from the fact that the regression line for NE concentration versus time intercepts the vertical axis at the point which describes the initial concentration of the NE (Brodie et al., 1966).

In spite of the limitations of this technique for measuring the turnover of NE, several investigators have demonstrated that NE turnover rates in brain and heart are similar to those observed by radio-labelled isotope methods (Udenfriend et al., 1963; Brodie et al., 1966; Costa et al., 1966; Iversen et al., 1966; Neff et al., 1968; Sharman, 1981). The fact that estimates of NE turnover made by different methods have given comparable results suggests that the same process is being measured by the different approaches to the problem. It has been well documented that the process being measured by these various techniques is directly related to electrical activity in the neurons examined (Salzman et al., 1979; Sharman, 1981). Such evidence is provided by studies showing increased NE turnover in nerve terminals in response to electrical stimulation of the nerve, in vivo and in vitro as well as in experiments designed to increase sympathetic outflow (e.g. stress) (Salzman et al., 1979; Sharman, 1981). In addition, the technique using decline in NE concentration after inhibition of synthesis has been used by several

investigators in the various models of hypertension to evaluate sympathetic nerve activity e.g. de Champlain et al. (1969); Van Amerigen et al. (1977) and, Nakamura et al. (1971) in DOCA-salt hypertension, Yamori (1972), Louis et al. (1969), and more recently by Patel et al. (1981) in SHR, where increased sympathetic outflow has been demonstrated by electrophysiological techniques (Judy et al., 1976).

Recently Patel et al. (1981) used this technique to measure changes in turnover of the various peripheral organs of SHR. The results of changes in the turnover of NE observed in the peripheral organs of the SHR agreed with the results of Touw et al. (1980) who reported similar changes in neurogenic vasoconstrictor tone of peripheral vasculature in the same model of hypertension at 9 weeks of age. These results suggest that NE turnover can be a good indicator of peripheral sympathetic activity.

In the CNS it has also been possible to demonstrate increased NE turnover in response to increased neural activity (Salzman et al., 1979; Sharman, 1981). As in the periphery, increased impulse flow (via electrical stimulation) through central noradrenergic neurons resulted in increased NE turnover in the brain regions in which the nerve terminals are located (Grabowska et al., 1976; Salzman et al., 1979). Thus this technique for measurement of NE turnover should provide a valid index of mean

noradrenergic neuronal activity in the CNS as well as the peripheral sympathetic nervous system.

4.1.2 Procedure

The decline in endogenous NE was measured following inhibition of NE synthesis with the tyrosine hydroxylase inhibitor, (DL)-α-methyl-p-tyrosine (Spector et al., 1965) using the method originally described by Brodie et al. (1966) and more recently by Van Ameringen et al. (1977) and Patel et al. (1981). The methyl ester HCl salt of the inhibitor (Aldrich Chemical Co., Milwaukee, Wis., U.S.A.) was dissolved in saline (60 mg/ml) and administered at a dose of 300 mg/kg (i.p.) every 4 hours. For the rest of this thesis, the tyrosine hydroxylase inhibitor will be referred to as α-methyltyrosine. In the initial studies (Chapter 5) tissue NE was determined at different time intervals after inhibition of tyrosine hydroxylase. A plot of NE concentration as a percent of the initial value on a log scale versus time gave a straight line. Linear regression analysis gave a correlation coefficient greater than 0.81 for all organs examined. Since it was demonstrated that there was a single exponential relationship for the disappearance rate of NE in the various tissues after inhibition of tyrosine hydroxylase, in subsequent chapters I used a single time interval in combination with zero time controls to infer the turnover of

NE. This procedure has been suggested and used by several investigators (Wijnen et al., 1978; Anden et al., 1980; Sole et al., 1980; Wijnen et al., 1980; Persson et al., 1981). Further details pertaining to each particular chapter are provided in the respective methods sections.

The rest of this section will concentrate on the details of fluorescence assay for NE (which was well established in Dr. R. L. Kline's laboratory when this project was begun), and the NE assay using HPLC in combination with EC detection (which was made operational during the progress of this project).

4.1.3 Fluorescence Assay for NE

4.1.3.1 General Comments

The technique used for assaying tissue for NE concentration was essentially the trihydroxyindole (THI) method described by Anton et al. (1962). Modifications were adopted from revised methods described by Sipes (1969) and Shellenberger et al. (1971). Briefly, the assay involves tissue homogenization in perchloric acid, adsorption of catecholamines onto activated aluminum oxide, extraction and oxidation. Norepinephrine is thereby isolated and oxidized at a slightly acidic pH to form its quinone derivative, noradrenochrome. In alkaline solutions, the quinone

undergoes intramolecular rearrangement to form the cyclized trihydroxyindole, noradrenolutine. The fluorescent noradrenolutine derivative is stabilized by the addition of a reducing agent and readjustment to a slightly acidic pH. The actual procedure is described in detail below.

4.1.3.2 Chemicals and Reagents

Chemicals:

All chemicals used were reagent grade (Fisher unless otherwise stated).

Acetic acid (CH_3COOH, glacial)

Aluminum oxide (AL_2O_3, alumina, activity grade 1, Woelm Pharma, ICN Nutritional Biochemicals)

Ethylenediaminetetracetic acid, disodium salt (EDTA)

Hydrochloric acid (HCl, concentrated)

Iodine (resublimed)

L-Norepinephrine bitartrate monohydrate (M.W. 337.2, Sigma)

Nitric acid (HNO_3, concentrated)

Perchloric acid (70%)

Potassium dihydrogen phosphate (KH_2PO_4)

Potassium iodide (KI)

Sodium hydrogen phosphate (Na_2HPO_4)

Sodium hydroxide (NaOH, pellets)

Sodium metabisulfite ($Na_2S_3O_5$)

Sodium sulfite (Na_2SO_3)

Trizma base (Tris-(hydroxymethyl)-aminomethane, Sigma)

Reagents:

All solutions were prepared using deionized glass-distilled water (GDW). All glassware was thoroughly washed in 30% nitric acid and rinsed with deionized GDW.

Homogenizing solution: 34.5 ml of 70% perchloric acid (approx. 11.6 N) was diluted to 1 litre with GDW. 1.0 g Sodium metabisulfite and 0.5 g EDTA were then added. The solution was kept refrigerated.

HCl (2.0 N): 167.0 ml of concentrated HCl was diltued to 1 litre with GDW.

Aluminum oxide: 250 g of alumina was placed in a 2 liter cylinder and washed with flowing tap water for 12 h such that there was constant mixing and the lighter particles were washed away. Two liters of 2.0 N HCl were prepared by diluting 334 ml of concentrated HCl with GDW. 500 ml of 2.0 N HCl were added to the washed alumina in a 1 liter beaker, placed on a magnetic stirrer and heated at 90-100 C for 45 min in a fume hood. The beaker was removed from the heat, the alumina allowed to settle for 2 min, and the supernatant decanted and discarded.

250 ml of 2.0 N HCl were then added to the alumina and the mixture heated at 70 C for 10 mih. The supernatant was

decanted and discarded. This was repeated once. 500 ml of acid were then added and the mixture heated at 50 °C for 10 min. The supernatant was again discarded and the alumina washed in flowing distilled water to pH 3.4. The alumina was dried in an oven at 250-300 °C and stored in a desiccator.

Tricine solution: 0.525 N NaOH was prepared by dissolving 21.0 g of NaOH pellets in GDW and adjusting the volume to 1 liter. To this were added 17.9 g of Trizma base and 25.0 g of disodium EDTA.

Perchloric acid (0.05 N): 4.3 ml of 70% perchloric acid were diluted to 1 liter with GDW.

Sodium hydroxide (5.0 N): 20.0 g of NaOH pellets were dissolved in GDW and the volume adjusted to 100 ml.

Phosphate buffer (0.1 N): 4.27 g of Na_2HPO_4 and 9.52 g of KH_2PO_4 were dissolved in GDW and the volume adjusted to 1 liter. 9.0 g of EDTA were added and the pH adjusted to 7.0 with 5.0 N NaOH.

Iodine reagent (0.1 N): 4.0 g of potassium iodide and 1.0 g of iodine were dissolved in GDW and the volume adjusted to 80.0 ml. The solution was stored in an amber bottle under refrigeration and filtered through glass wool before use.

Alkaline sodium sulfite (2.5%): 25% sodium sulfite was prepared by dissolving 25.0 g of Na_2SO_3 in 100 ml of GDW. Just before use, 1.0 ml of this mixture was added to 9.0 ml of 5.0 N NaOH and thoroughly mixed.

Acetic acid (0.02 M): 1.2 ml of glacial acetic acid was diluted to 1 liter with GDW.

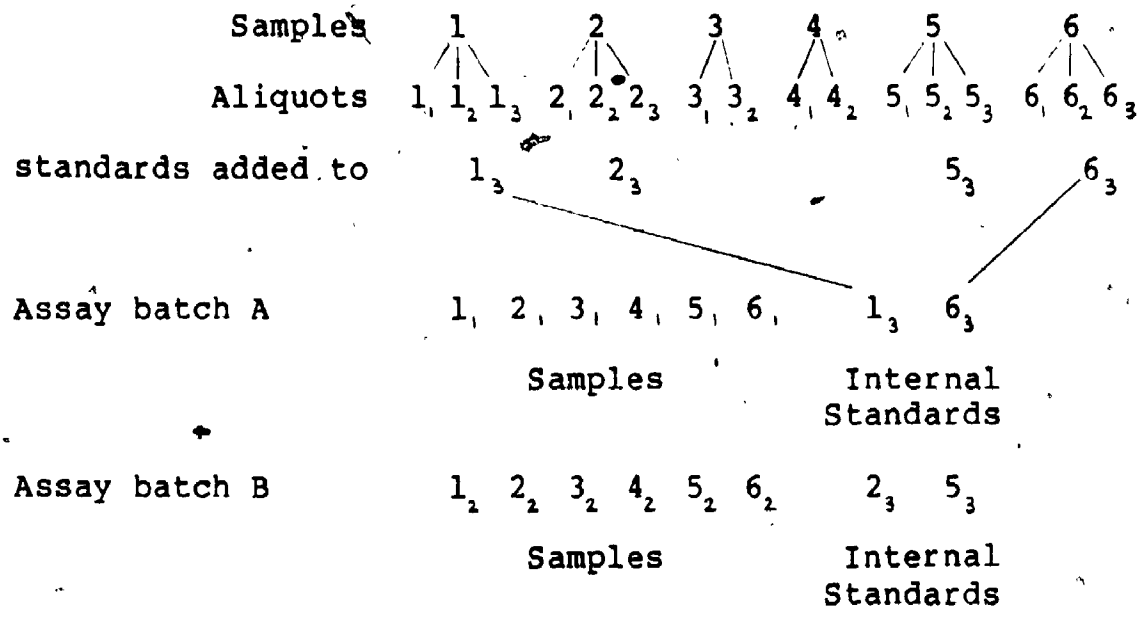
Norepinephrine stock solution (100 µg/ml): 19.9 mg of L-norepinephrine bitartrate monohydrate were dissolved in 100 ml of 0.01 N HCl. The solution was kept refrigerated. New stock solutions were prepared at least once per month.

4.1.3.3 Homogenization procedure

Peripheral tissues: The left kidney, a section of the intestine (duodenum) and a piece of skeletal muscle (from hind limbs), were quickly removed from each rat and placed in 10 ml of chilled homogenizing solution in polycarbonate centrifuge tubes (28 x 100 mm). The tissues were then homogenized for 30 sec using a Polytron homogenizer (Brickmann Instruments, Model PT 10-35), at a speed control setting of 5 for kidney and intestine and 7 for skeletal muscle. The tubes were then spun in a refrigerated centrifuge (Sorvall Model RC-2) for 15 min at 4 C and 30,000 x g. Two seperate 3.0 ml aliquots were taken from the clear supernatant fluid and placed in clean polypropylene tubes (17 x 100 mm), capped and stored at

-75 C. These duplicates were then assayed in different assay batches as described below. A third aliquot of 3 ml was taken from some samples to serve as an internal standard. An assay batch consisted of 6 samples and two internal standards.

Example:



A standard dilution of NE (200 ng/ml) was prepared by diluting 0.1 ml of NE stock solution (100 µg/ml) to 50 ml with 0.02 M acetic acid. A total of 40 ng (0.2 ml of NE dilution, 200 ng/ml) of NE were added to each of the internal standard tubes. The tubes were capped and stored at -75 C as above.

Brain tissues: Brain tissues were treated similarly except that 3.5 ml of homogenizing solution were used and 1 ml samples were placed in each of the assay tubes and appropriate internal standard tubes.

4.1.3.4 Extraction procedure

The extraction of NE was performed in groups of 8 tubes at a time (1 assay batch) consisting of 6 tissue samples and two corresponding internal standards. On the day of extraction, the tubes were removed from the freezer, 3 ml and 5 ml of homogenizing solution added to the peripheral and brain tissue samples respectively, and the solution allowed to thaw. Each tube then had a total of 6 ml of homogenizing solution. Activated alumina (300 mg) was added to each tube and thoroughly mixed. Approximately 4.0 ml of tricine solution were added and, after mixing, the pH was carefully adjusted to 7.80 $\pm$ 0.05 with a drop-wise addition of tricine. The tubes were then placed on an inversion shaker and mixed gently at slow speed for 20 minutes.

After the catechols were bound to the alumina the tubes were spun in a clinical centrifuge (International Model CL) at 6,000 r.p.m. for 5 min and the supernatant solution aspirated and discarded. The alumina was then washed 2 times with 10 ml aliquots of GDW, centrifuging and discarding the wash water each time. After the last wash, the catechols were eluted from the alumina by adding 3.0 ml of 0.5 N perchloric acid and shaking vigorously for 20 min on a horizontal shaker (Eberbach). The tubes were then spun in a refrigerated centrifuge for 10 min at 10°C and 30,000 x g. A 1.0 ml aliquot of the supernatant solution from each tube was placed in a borosilicate glass tube

(13 x 100 mm), covered with Parafilm and stored at -75 C. From one of the tissue samples in an assay batch an additional aliquot was taken to serve as the tissue blank (To) during the oxidation procedure.

4.1.3.5 Oxidation procedure

The oxidations were performed in groups of 9 tubes at a time consisting of 6 tissue samples, 2 internal standards and a tissue blank (To). The tubes were removed from the freezer and allowed to thaw. Phosphate buffer (1.5 ml) was added to each tube and mixed thoroughly. For the oxidation of NE 0.2 ml of iodine reagent was added, the tube shaken briefly, and the reaction timed for exactly 2 min. The oxidation was then stopped by adding 0.5 ml of freshly prepared alkaline sodium sulfite, shaken and timed for exactly 2 min. Glacial acetic acid (0.4 ml) was then added to bring the pH to 4.4 - 4.8. It was thus possible to react 8 samples in the oxidation process at 15 sec intervals in one batch. The tissue blank (To) was prepared by addition of the reagents in reverse order, i.e. acetic acid, followed by alkaline sodium sulfite, then iodine reagent. This procedure prevents oxidation of the catecholamines.

After oxidation or "reverse" oxidation, all tubes were placed in a boiling water bath for 4 min, as heating of the acidified solution enhances the development of fluorescence, then cooled in an ice bath for a further 4 min. Tubes were

then removed from the ice, covered with Parafilm and allowed to reach room temperature. Previous studies using this technique have shown fluorescence intensity to remain stable for 20-30 min after oxidation (Anton et al., 1962). Fluorescence intensity was read on an Aminco-Bowman spectrophotofluorometer (American Instrument Co.), fitted with an off-axis ellipsoidal condensing system and an RCA 1P-28 photomultiplier tube. The excitation and emission wave lengths used for NE were 388 mμ and 488 mμ, respectively. The tissue blank was read between each tissue sample.

4.1.3.6 Calculation of NE concentration in the tissue

The concentration of NE (ng/ml) in the samples was determined by first subtracting the percent transmission (% T) of the tissue blank (To) from that of the tissue sample and then multiplying by the meter multiplier setting to obtain the relative fluorescence (RF) of sample. The corresponding NE content in 3 ml was then calculated by the formula:

$$\text{NE ng} = \frac{\text{RF sample} \times 40}{\text{RF internal standard}}$$

where: 40 = the standard amount of NE added in ng

RF sample = the relative fluorescence of the sample

RF internal standard = the mean of the differences in relative fluorescence (RF) between the RF of each internal standard and the RF of its corresponding sample

Total NE (ng) present in the tissue sample was calculated by multiplying the value obtained from the previous calculation by an appropriate conversion factor to correct for dilution due to water content of the tissue. A correction factor of 77 to 79% (water content) was used in all the brain tissues, intestine and skeletal muscle as the water content did not change in these tissues after injection of tyrosine hydroxylase inhibitor. However, in the kidney, there was an indication that water accumulated over time after the injection of tyrosine hydroxylase inhibitor (Table 1). Therefore, from each of the rats the right kidney was weighed when wet and again after having dried to constant weight in an oven at 60 °C. The water content of each right kidney was subsequently used as a correction factor in NE concentration calculations for the corresponding left kidney. The total NE (ng) was then divided by the wet tissue weight to obtain the [NE] in ng/g.

TABLE 1

Water Accumulation in the Kidney Over Time After Injection of Tyrosine Hydroxylase Inhibitor

	Time after the 1st Injection of Tyrosine Hydroxylase Inhibitor				
	0 hr	2 hr	4 hr	6 hr	8 hr
% H O Content	77.4±0.2	82.3±1.6	82.7±1.1	83.7±0.8	84.3±0.3

Sample: From 9 week old Wistar rats.

Note: The zero time value was obtained from rats that were not injected with the tyrosine hydroxylase inhibitor.

n = 3 - 6 at each time period

A sample calculation is as follows:

500 mg of tissue homogenized in 10 ml of solution.

RF sample = 2.10

RF internal standard = 1.40

Total volume = 10.00 ml + 0.39 ml

(correction for water content) = 10.39 ml

$$\text{Total NE content in 3.0 ml sample} = \frac{2.10 \times 40 \text{ ng}}{1.40} = 60 \text{ ng}$$

$$\text{Total NE present in sample} = \frac{60 \times 10.39}{3} = 207.8 \text{ ng}$$

Tissue NE concentration in ng/g wet weight =

$$\frac{207.8}{0.5 \text{ g}} = 415.6 \text{ ng/g}$$

4.1.3.7 Determination of Assay Variability

The specificity of the fluorescence assay for NE has been investigated in detail and discussed by previous workers (Anton et al., 1962; Shellenberger et al., 1971). In addition we have determined that there was no interference of tissue extracts with the measurement of NE by assaying denervated kidneys devoid of NE (3 days after surgical stripping and application of alcohol to the renal vessels). These samples yielded fluorescence values that were not significantly different from tissue blanks obtained by reverse oxidation. In the present study standard curves for NE concentration (ng/ml) versus relative fluorescence

(RF) were prepared by oxidizing samples containing known amounts of NE, using three different stock solutions of NE (100 µg/ml). A plot of NE concentration versus RF was constructed (n = 36) and the regression line calculated using linear regression analysis. A sample curve is illustrated in Figure 3. The linear range from 1 ng/ml to 5 ng/ml covered all the tissue samples encountered throughout the study.

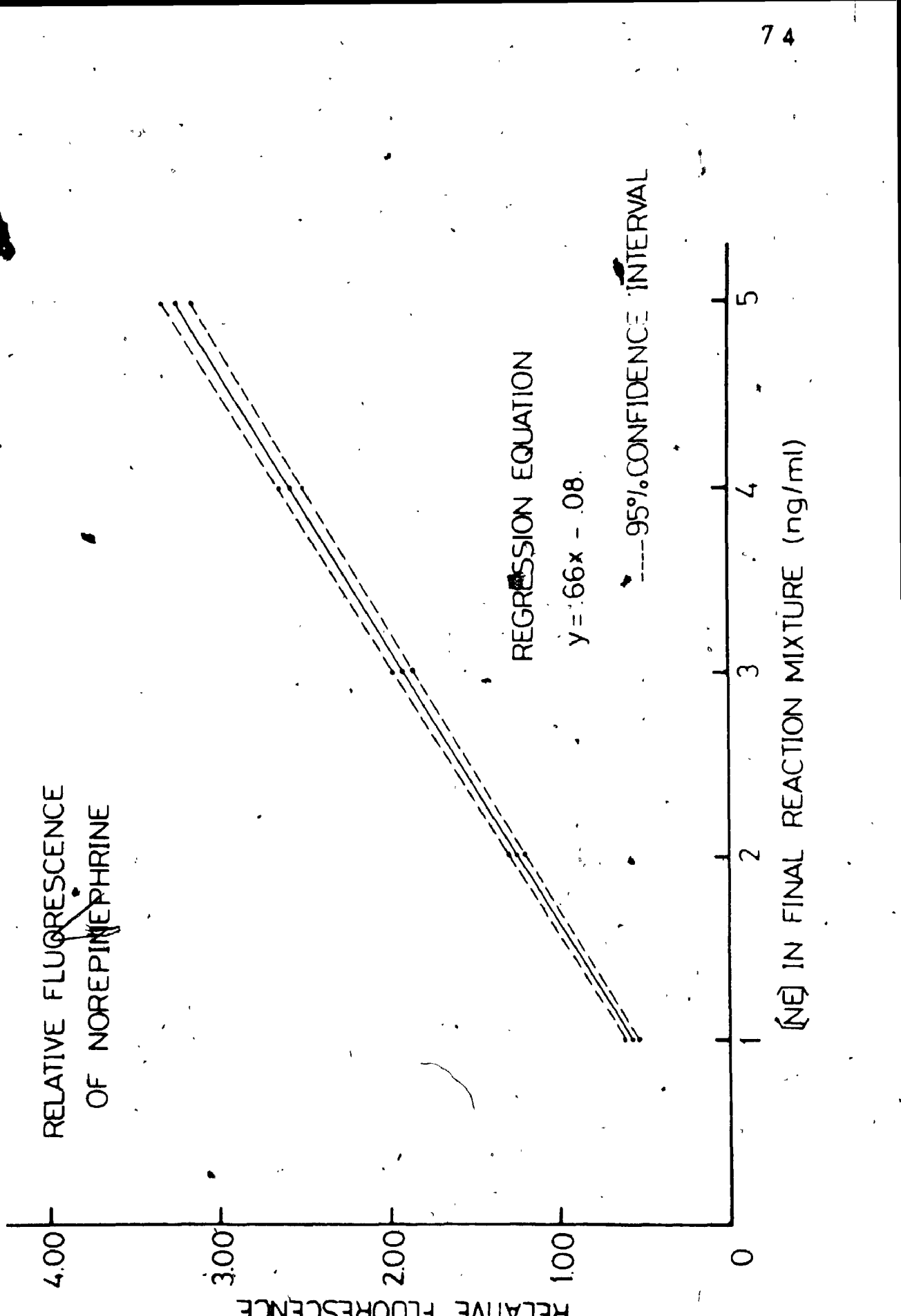
The variability of the assay procedure over a long time period was determined by two separate procedures: 1) The efficiency of the extraction procedure was determined by measuring the percent recovery. Percentage recovery was determined by comparing the value of an internal standard and the value obtained by oxidation of the same amount that did not go through the extraction procedure, and was found to be approximately 70%. This is comparable to the percent recovery in previous studies done in this laboratory (Stuart et al., 1979). 2) To check the consistency of the oxidation and reading procedures, new regression lines were generated at various times during a period of a year by the method described above. At no time were there significant differences between the slopes or intercepts of regression lines calculated for standard curves. The coefficient of variation (calculated from internal standards) between all the assays performed among organs was less than 20%, with the variation within an assay of less than 5%. Furthermore the majority of the duplicate samples, corrected for percent

FIGURE 3

Typical standard curve of NE concentration versus relative fluorescence. Two additional curves were obtained one month and four months after the original curve. Curves were derived using linear regression analysis. Using a t-test for linear regression, there was no significant difference between the slopes of these curves. The Y-intercepts of the latter curves fell within the 95% confidence interval for the Y-intercept of the original curve.

	Regression Equation (Y =)	Correlation Coefficient
Original Curve	0.66x - 0.08	0.99
Curve obtained 1 month later	0.66x - 0.01	0.99
Curve obtained 4 months later	0.66x - 0.08	0.99

n = 36 for each curve

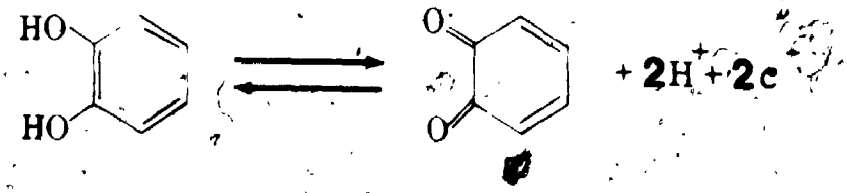

RELATIVE FLUORESCENCE OF NOREPINEPHRINE
REGRESSION EQUATION
y = .66x - .08
---95% CONFIDENCE INTERVAL
RELATIVE FLUORESCENCE
4.00
3.00
2.00
1.00
0
1
2
3
4
5
(NE) IN FINAL REACTION MIXTURE (ng/ml)

recovery, were within 5% of the mean for that sample (determined from 50 random samples).

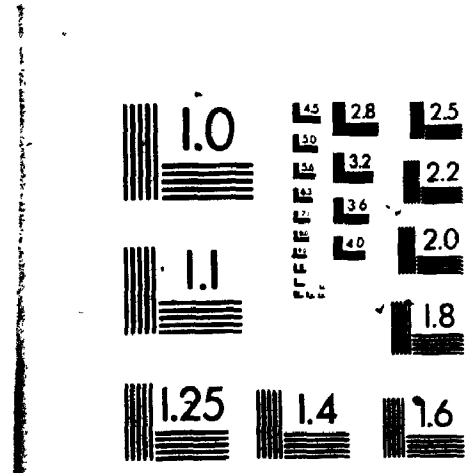
4.1.4 Determination of NE by High Performance Liquid Chromatography (HPLC) in combination with Electrochemical Detection (EC)

4.1.4.1 General Comments

The second technique used for assaying tissue NE after extraction on activated aluminum oxide was essentially derived from the original work by Keller et al. (1976), known as the HPLC in combination with EC (pioneered by Kissenger et al. (1973)). Briefly, the assay involves tissue homogenization in perchloric acid, adsorption of catecholamines onto alumina oxide, extraction with perchloric acid and injection of each sample on the HPLC. The catecholamines are quickly and efficiently separated by the HPLC. The separated catechols then pass a sensitive electrochemical detector. All catechol derivatives are readily oxidized at a glassy carbon electrode, to generate two protons and two electrons.



2



This anodic oxidation is used analytically to measure the rate at which electrons are transferred across the electrode-solution interface, in other words, the anodic current. The instantaneous current is directly proportional to the number of molecules coming into contact with the interface per unit time and can, therefore, be used to determine the concentration of the reactant in the neighboring solution. The compound of interest (identified by retention times of standards) is quantified by calculating the height of the peak using a Hewlett Packard 3390A integrator, and their actual amount determined by using a known amount of artificial catecholamine dihydroxybenzylamine (DHBA) included in each sample as an internal standard (for details of the calculation see section 4.1.4.6). The actual procedure is described in detail below.

4.1.4.2 Chemicals and Reagents

This is an extension of the list of chemicals and reagents presented in Section 4.1.3.2.

Chemicals:

Potassium Phosphate (KH_2PO_4 monobasic) HPLC grade

Octyl sodium sulphate (Kodak)

Methanol HPLC grade

3,4,-dihydroxybenzylamine hydrobromide (DHBA, Sigma)

Reagents:

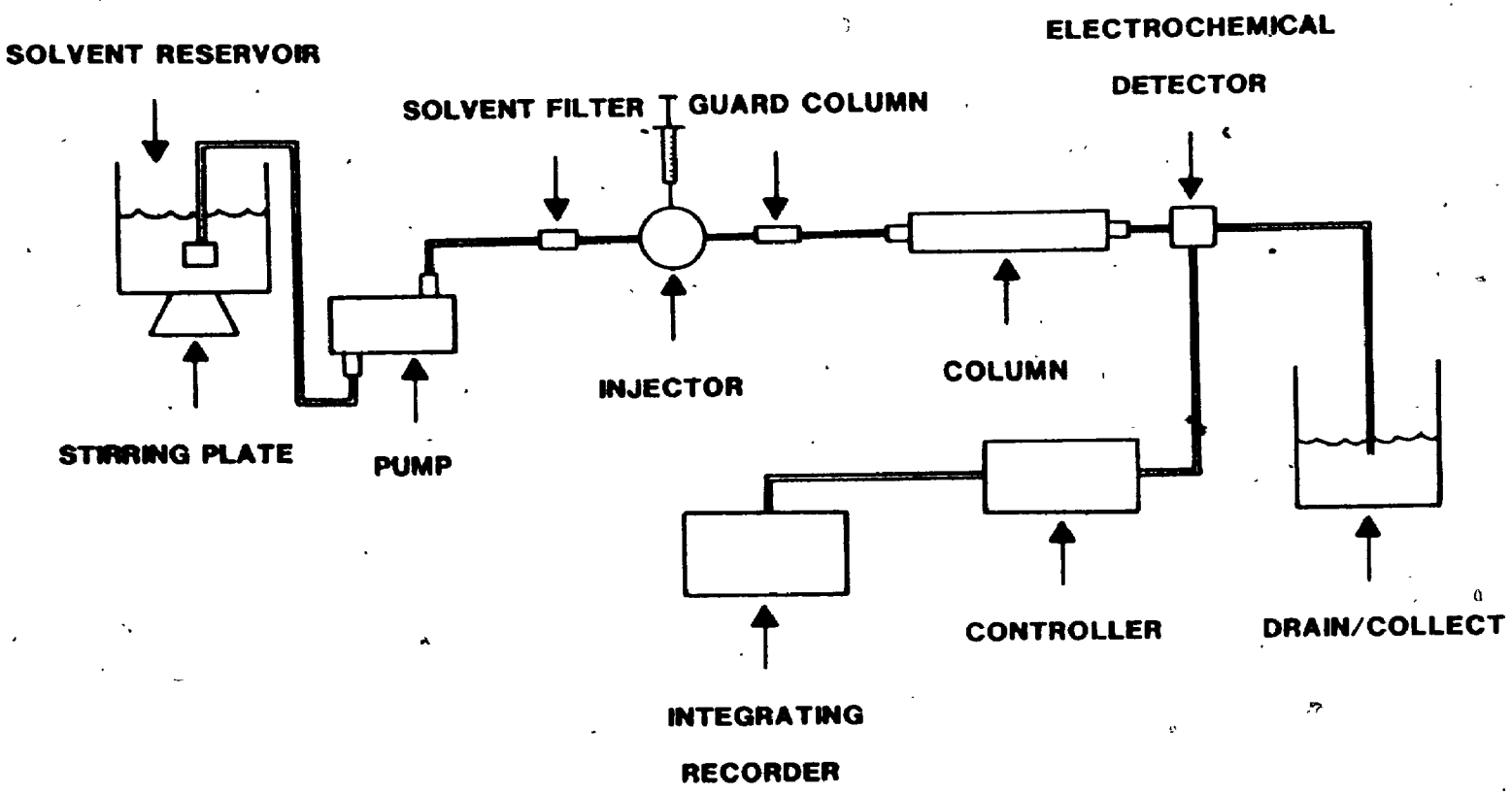
Phosphate buffer: 15.0 g of KH_2PO_4, 0.75 g of octyl sodium sulfonate, and 0.1 g of sodium EDTA were dissolved in GDW after which 300 ml of methanol was added and the volume adjusted to 2 liters. The pH was adjusted to 4.0 and the solution was then filtered through a 0.5 µm organic Millipore filter (FH, 0.5 µm, Millipore Corp., Bedford, Mass.). Degassing of solvents was achieved by continuous stirring of the solution in the reservoir feeding the pump (Figure 4).

Standard stock solution: Stock solution of norepinephrine (100 µg/ml) and dihydroxybenzylamine hydrobromide (100 µg/ml) was made by dissolving 19.9 mg of L-norepinephrine bitartrate monohydrate and 15.8 mg of dihydroxybenzylamine hydrobromide in 100 ml of 0.01 N HCl. The solution was aliquoted into 5 ml vials and stored at -70 C in the freezer. Each 5 ml vial was thawed and kept in the refrigerator prior to use. Solution from such a 5 ml vial was not used if the vial had been refrigerated for longer than 1 month.

Dihydroxybenzylamine stock: 15.8 mg of dihydroxybenzylamine hydrobromide were dissolved in 100 ml of 0.01 N HCl. The solution was packaged the same way as standard stock solution described above.

Figure 4

Solvent reservoir for element; stirring plate to keep the solvent degassed; Pump with damping coil to limit pumping pulsations; solvent filter; injector for on-line injection of 0 to 2 ml sample; Guard Column with uBondapack C18 packing same as the actual column for filtering possible contamination of sample; Column for separation of catecholamines - µBondapack C18; Electrochemical detector for measuring the oxidation of catecholamines; controller, potentiostat-amplifier; Integrating recorder which converted the signal from the controller into a chromatographic trace and measured the area or height of each peak in the chromatograph.



SCHEMATIC DIAGRAM OF HPLC WITH AN ELECTROCHEMICAL DETECTOR AND INTEGRATING RECORDER.

4.1.4.3 Apparatus

The chromatographic system consisted of a 2 liter conical glass flask used as a solvent reservoir with stir bar, a Model M45 dual piston pump, a Model U6K injector and a 6.25 mm x 30 cm μBondapak C18 column (all from Waters Assoc., Milford, Mass., U.S.A.) (Figure 4). The detection device consisted of a detector block (TL-8A Bioanalytical Systems) and a controller similar to the amperometric controller (Model LC-2A) from Bioanalytical Systems (West Lafayette, Ind., U.S.A.), made in the Department of Physiology, University of Western Ontario. The detector consisted of glassy carbon electrodes with a flow cell defined by the 0.127 mm gasket. The electrode potential was maintained at 0.8V versus a silver-silver chloride reference cell. The signal generated by the detector was converted by a Hewlett Packard 3390 integrator to a chromatographic trace (Figure 4).

4.1.4.4 Homogenization procedure

The homogenization procedure used was similar to the one used in the fluorescence assay described previously with the exception that different volumes were used for some of the tissues. Only the differences in the homogenization procedure will be highlighted. Kidney and skeletal muscle tissues were homogenized in 6.5 ml of solution; intestine and brain were homogenized in 3.5 ml solution. A 6.0 ml

aliquot for kidney and skeletal muscle and a 3.0 ml aliquot for intestine and brain tissues were taken from the clear supernatant fluid and placed in clean polypropylene tube (17 x 100 mm), capped and stored at -75 °C.

4.1.4.5 Extraction procedure

The extraction of NE was performed in groups of 16 tubes at a time (1 assay batch) consisting of 14 tissue samples and two external standards. On the day of extraction, the tubes were removed from the freezer and 3 ml of homogenizing solution added to intestine and brain tissue samples, and the solutions allowed to thaw. Each tube then had a total of 6 ml of solution. The standards consisted of 6 ml of homogenizing solution with an addition of 100 ng each of NE and DHBA. A 100 ng of DHBA was also added to each sample to control each sample for loss of NE during the extraction process. The samples were then extracted using a procedure identical to the one outlined in Section 4.1.3.3.

4.1.4.6 Injection Procedure and Calculation of NE Concentration in the Tissue

The tubes containing extracted catecholamines in perchloric acid were removed from the freezer and allowed to thaw. Calibration standards and samples (50 ml) were injected onto the HPLC column using a Hamilton microsyringe

with a stainless-steel needle. The calibration standards were injected first and response factors were calculated and averaged over the two standards. The response factor (R) is calculated as a ratio of peak height of 100 ng DHBA to peak height of 100 ng NE in the calibration standards. This factor (R), obtained for each batch of 14 extracted samples was then used to calculate the NE content of the samples, as illustrated below:

$$\text{Amount of NE in sample prior to extraction} = \frac{\text{peak height of NE in sample} \times R \times 100^*}{\text{peak height of DHBA in sample}}$$

$$R = \frac{\text{peak height of NE in calibration standard}}{\text{peak height of DHBA in calibration standard}}$$

* = 100 ng of DHBA was added to each sample prior to extraction

Total NE (ng) present in the tissue sample was calculated by multiplying the value obtained from the previous calculation by an appropriate conversion factor to correct for dilution due to water content of the tissue. See Table 1. The total NE (ng) was then divided by the wet tissue weight to obtain the [NE] in ng/g. Calculation based on a 6.0 ml sample from 6.5 ml of homogenate or a 3.0 ml sample from 3.5 ml of homogenate were made using the following formulae:

6.0 ml sample:

$$\frac{\text{NE in 6 ml sample} \times (6.5 + (0.77 \times \text{wt.}))}{6} = \frac{\text{Total ng}}{\text{wt}} = \text{ng/g}$$

3.0 ml sample:

$$\frac{\text{NE in 3 ml sample} \times (6.5 + (0.77 \times \text{wt.}))}{3} = \frac{\text{Total ng}}{\text{wt}} = \text{ng/g}$$

4.1.4.7 Specificity and Detection Limits

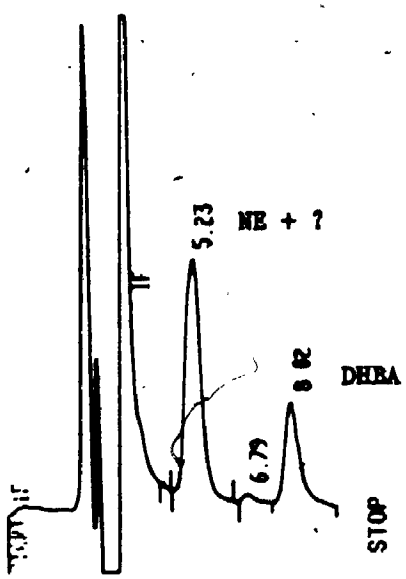
The catecholamine assay using HPLC with electrochemical detection involves a separation of the catecholamines by reverse phase chromatography (Keller et al., 1976) and consequent detection by a highly sensitive electrochemical detector introduced by Kissinger et al. (1973). The usefulness of HPLC with EC detector in the determination of catecholamines has been amply demonstrated by a number of laboratories (Kissinger et al., 1973; Refshange et al., 1974; Adams, 1976; Borchardt et al., 1978; Christensen et al., 1979). It is known that separation of catechol groups and maximum detector response are affected by changes in solvent pH (Moyer et al., 1978). In the present study I observed an optimum solvent pH of 4.0 for the separation of the peaks observed in this study. The kidney tissue seemed to produce the most complex chromatograph seen in this study. An unknown substance co-chromatographed with NE at solvent pH of 5.0. However at a solvent pH of 4.0, separation of the unknown substance and NE peak was

satisfactory (Figure 5). Although Figure 5 does not show a chromatogram at pH 4.0, Figure 7C shows a clear separation of the unknown peak from NE peak at pH 4.0.

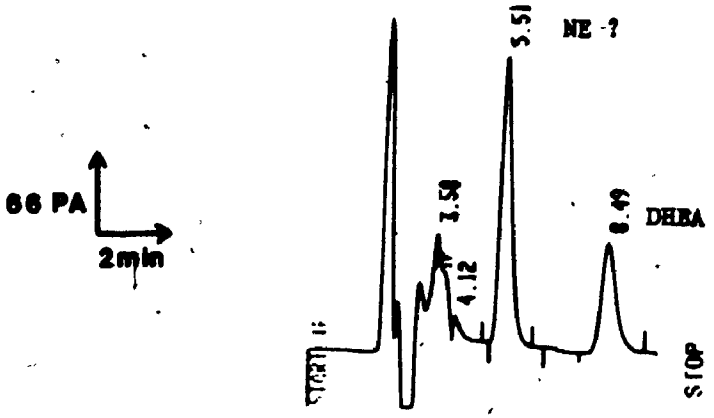
To enhance the resolution of catecholamines and particularly to effect baseline separation between closely chromatographed peaks, the technique of ion pairing was used (Moyer et al., 1978). Sodium octanesulfonate was used since it was demonstrated to provide the best separation by Moyer et al. (1978). The potential difference for oxidation was set at 0.8 V since in my system it provided the maximum detector response at this potential for NE (Figure 6). A sample tracing of the separation of catecholamines in a standard is provided in Figure 7A. Examples of the separation seen for extracts of brain and peripheral tissues are shown in Figure 7B, 7C, 7D, 7E. The specificity of this assay for NE has been investigated in detail and discussed by previous workers (Horvath et al., 1976; Molnar et al., 1976; Moyer et al., 1978; Sasa et al., 1979). In addition we have determined that there was no interference of tissue extracts with measurement of NE by assaying surgically denervated kidneys and chemically denervated (6-hydroxydopamine) intestine and skeletal muscle, which are devoid of NE. These samples yielded no peaks on the chromatograph which corresponded with the NE peaks in the chromatographs of samples taken from animals with intact innervation.

FIGURE 5

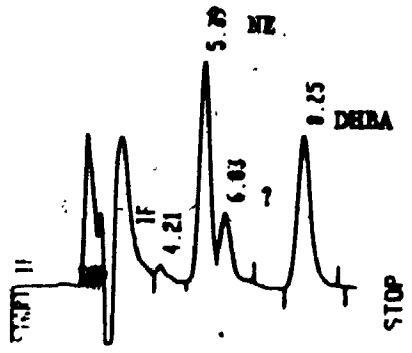
Effect of pH on the separation of NE + (unknown) in the kidney sample. pH of phosphate buffer was adjusted by addition of HCl. Solvent flow rate, 1.5 ml/min. 100 µl of the same sample (with addition of 1 ng DHBA) was injected at each different solvent pH. The numbers at the zenith of each peak represents the retention time of the peak. PA represents pico-amps.



PH = 5.8



PH = 5.1



PH = 4.4

FIGURE 6

Signal response generated by electrochemical detector versus potential difference (P.D.) across the cell. The phosphate buffer was at pH 4.0. Solvent flow rate, 1 .5 ml/min. 1 ng of NE in 100 ul was injected at each different P.D. Area represented in $\times 10^6$ area counts (1 area count = 0.125 uV-sec).

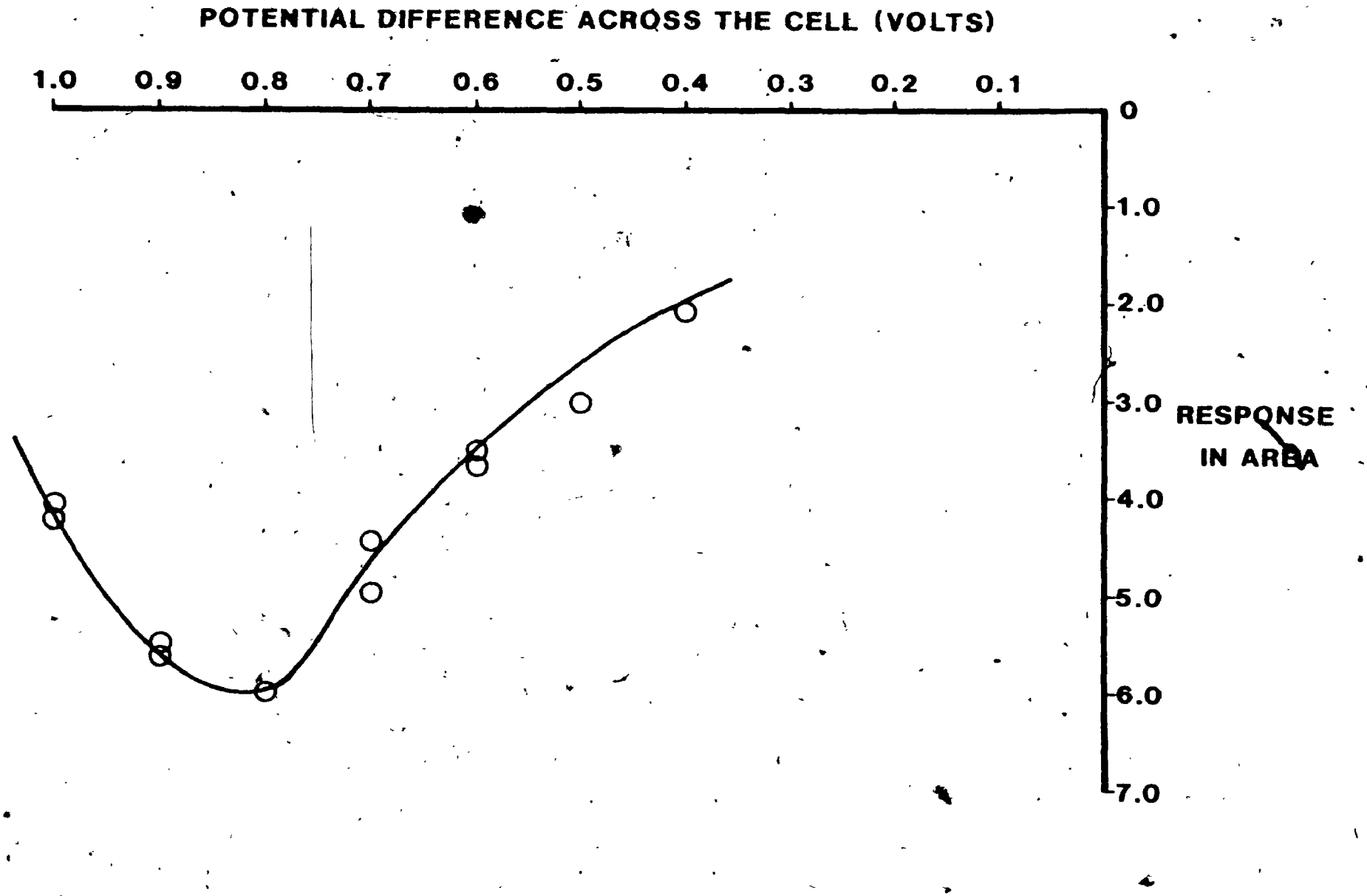

POTENTIAL DIFFERENCE ACROSS THE CELL (VOLTS)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
RESPONSE
IN AREA

FIGURE 7

Examples of typical chromatograms:

A Typical chromatogram of stock solution after extraction with alumina. Final 50 µl of injection solution containing 1.75 ng NE, DHBA and Dopamine (DA).

B Typical chromatogram for hypothalamic tissue (anterior) sample 1.75 ng DHBA in 50 µl of injection volume.

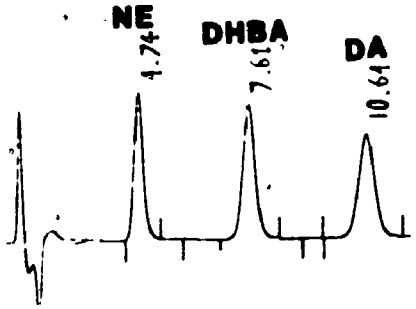
C Typical chromatograph for kidney sample 1.75 ng DHBA in 50 µl injection volume.

D Typical chromatograph for muscle sample with 1.75 ng DHBA in 100 µl injection volume.

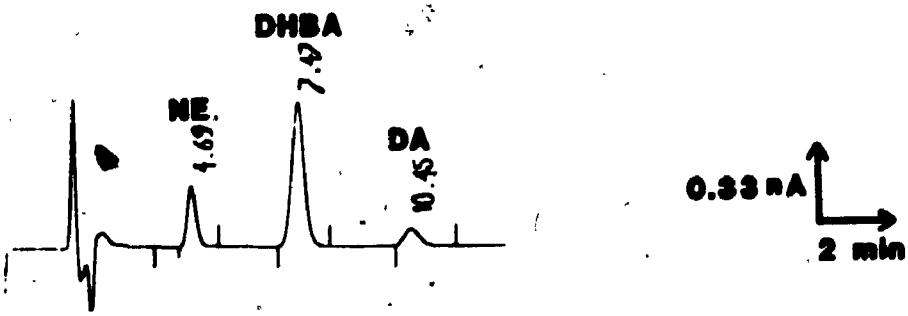
E Typical chromatograph for intestine sample 1.75 ng DHBA in 100 µl injection volume.

The numbers at the zenith of each peak represents the retention time of the peak. nA represents nano-amps.

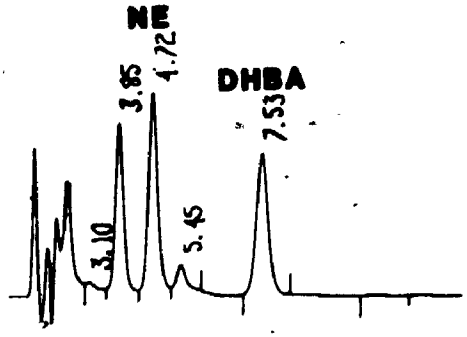
7A


NE
4.74
DHBA
7.61
DA
10.61

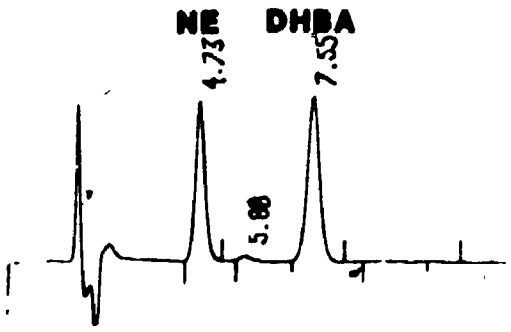
7B

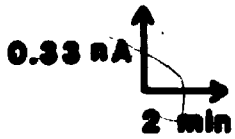

DHBA
7.49
NE
4.69
DA
10.45
0.33 nA
2 min

7C

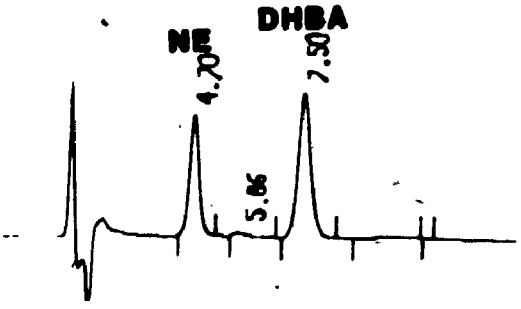

NE
4.72
3.85
DHBA
7.53
3.10
5.45

7D





7E



Standard curves for NE (pg to ng) versus current (pico-amps) were prepared by injecting known amounts of NE. A plot of NE concentration versus current (pico-amps) was constructed (n = 24) and the regression line calculated using linear regression anlaysis. A sample curve is illustrated in Figure 8. The linear range from 50 pg to 2000 pg covered all the tissue samples encountered throughout the study. Similar standard curves were constructed for other catecholamines including DHBA, which also showed linearity over a wide range. Thus DHBA could be used as an internal standard for each sample (Figure 9) (Moyer et al., 1978). In addition, standard curves were constructed using various tissue homogenates. A plot of NE added to the tissue sample versus the NE calculated with the use of DHBA as an internal standard showed a linear relationship in heart, kidney, hypothalamus and skeletal muscle tissue homogenates (Figure 10). Therefore, NE and DHBA were extracted in a constant ratio over a wide range of concentrations. The linear range from 0 to 200 ng added to the sample covered all the tissue samples encountered throughout the study.

The variability of the assay procedure was determined by calculating the coefficient of variation. The coefficient of variation (calculated from injection of known amount of NE) for 13 separate injections was 3.6%. The largest coefficient of variation calculated for two injections (highest value and lowest value) was 9.6%.

FIGURE 8

Typical standard curve of NE concentration versus current (pico-amps). Curve was derived using linear regression analysis. n = 24

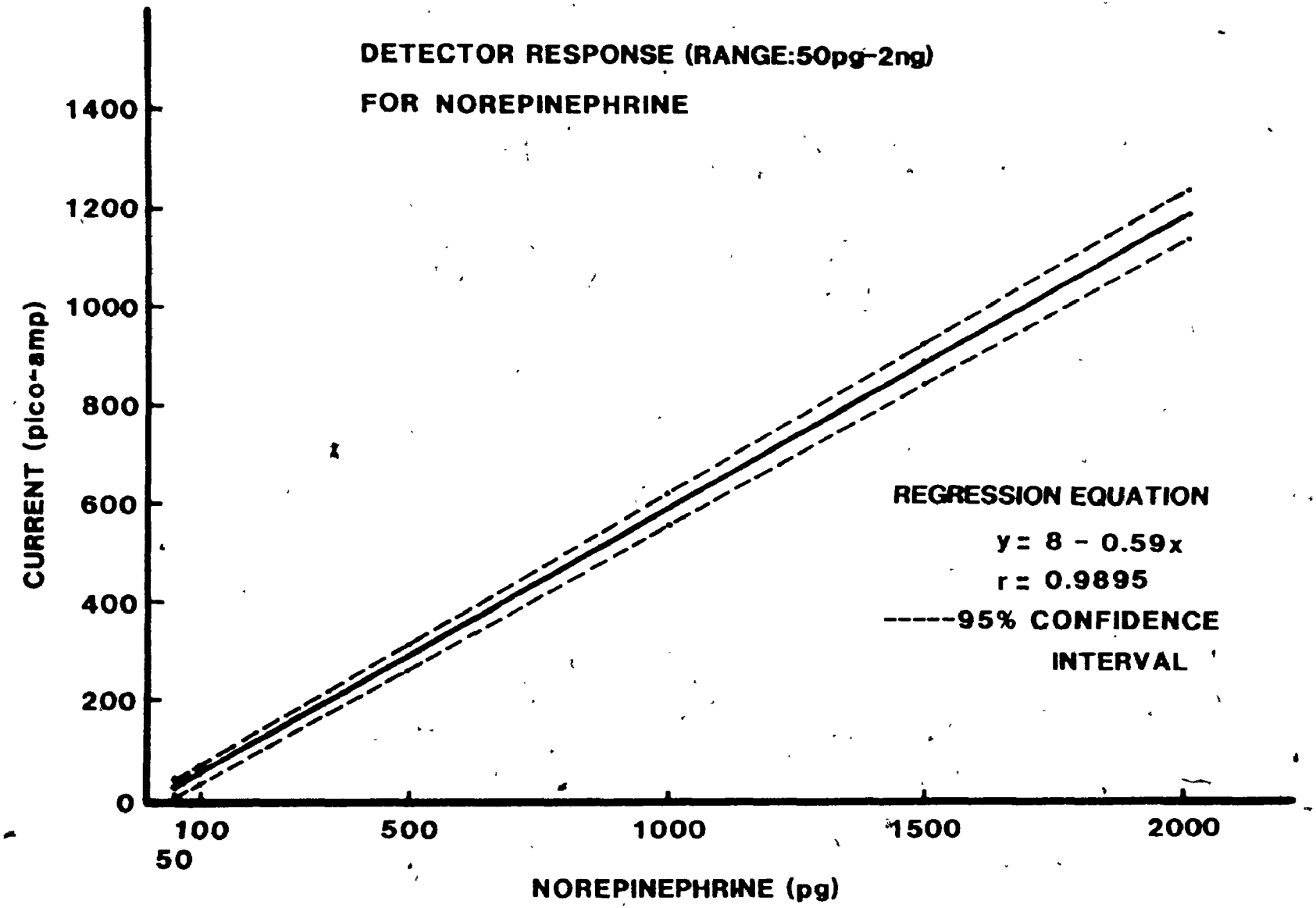

DETECTOR RESPONSE (RANGE:50pg-2ng)
FOR NOREPINEPHRINE
CURRENT (pico-amp)
1400
1200
1000
800
600
400
200
0
50
100
500
1000
1500
2000
NOREPINEPHRINE (pg)
REGRESSION EQUATION
y = 8 - 0.59x
r = 0.9895
-----95% CONFIDENCE
INTERVAL

FIGURE 9

Standard curves of NE, Epinephrine, DHBA and DA. Curves were derived using linear regression analysis.

Substance	Regression Equation (Y =)	Correlation Coefficient
Norepinephrine	10.80 + 0.60x	0.99
Dopamine	-11.40 + 0.51x	0.99
Epinephrine	- 9.40 + 0.45x	0.97
Dihydroxybenzylamine	7.70 + 0.46x	0.98

n = 11 for each curve

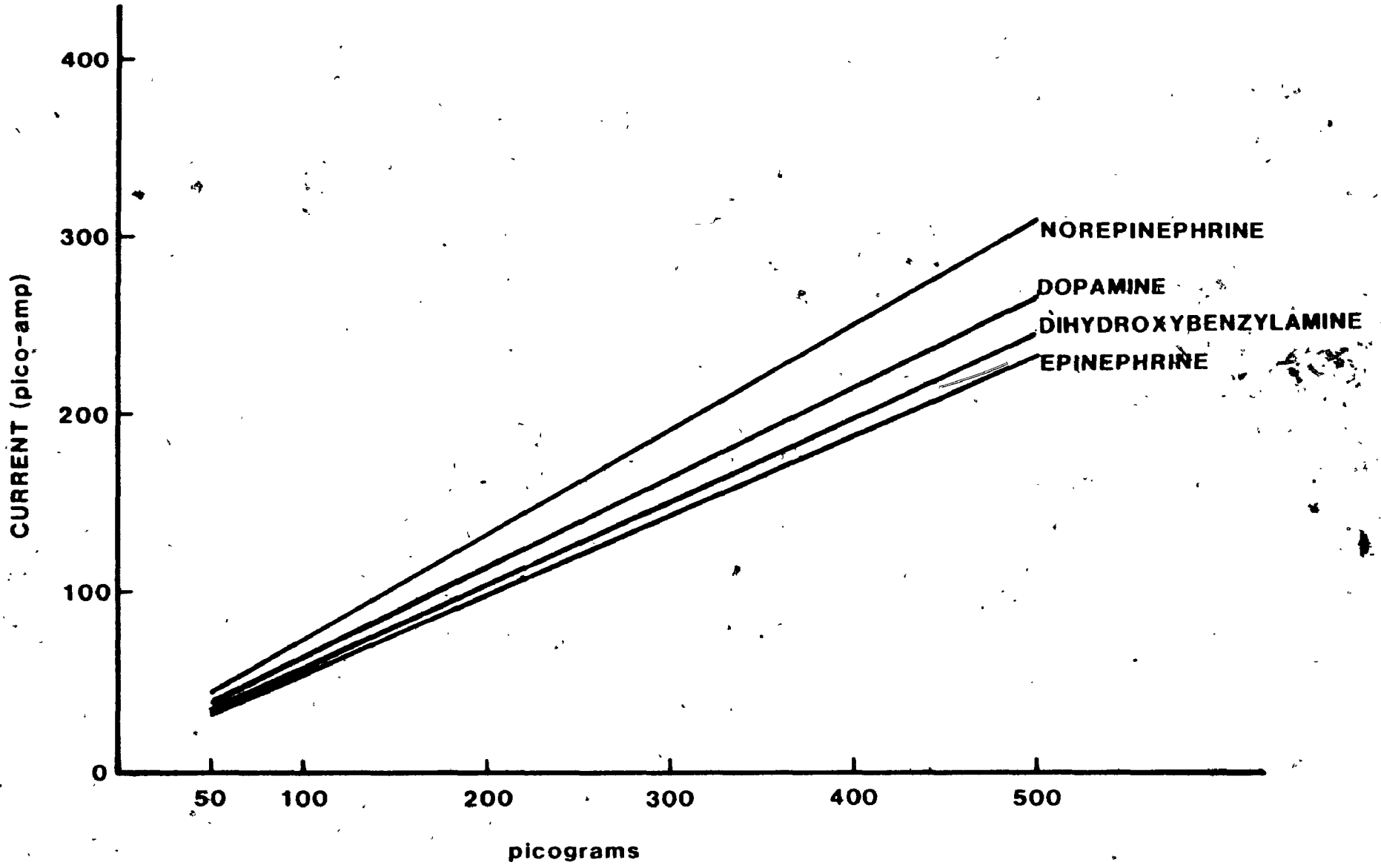
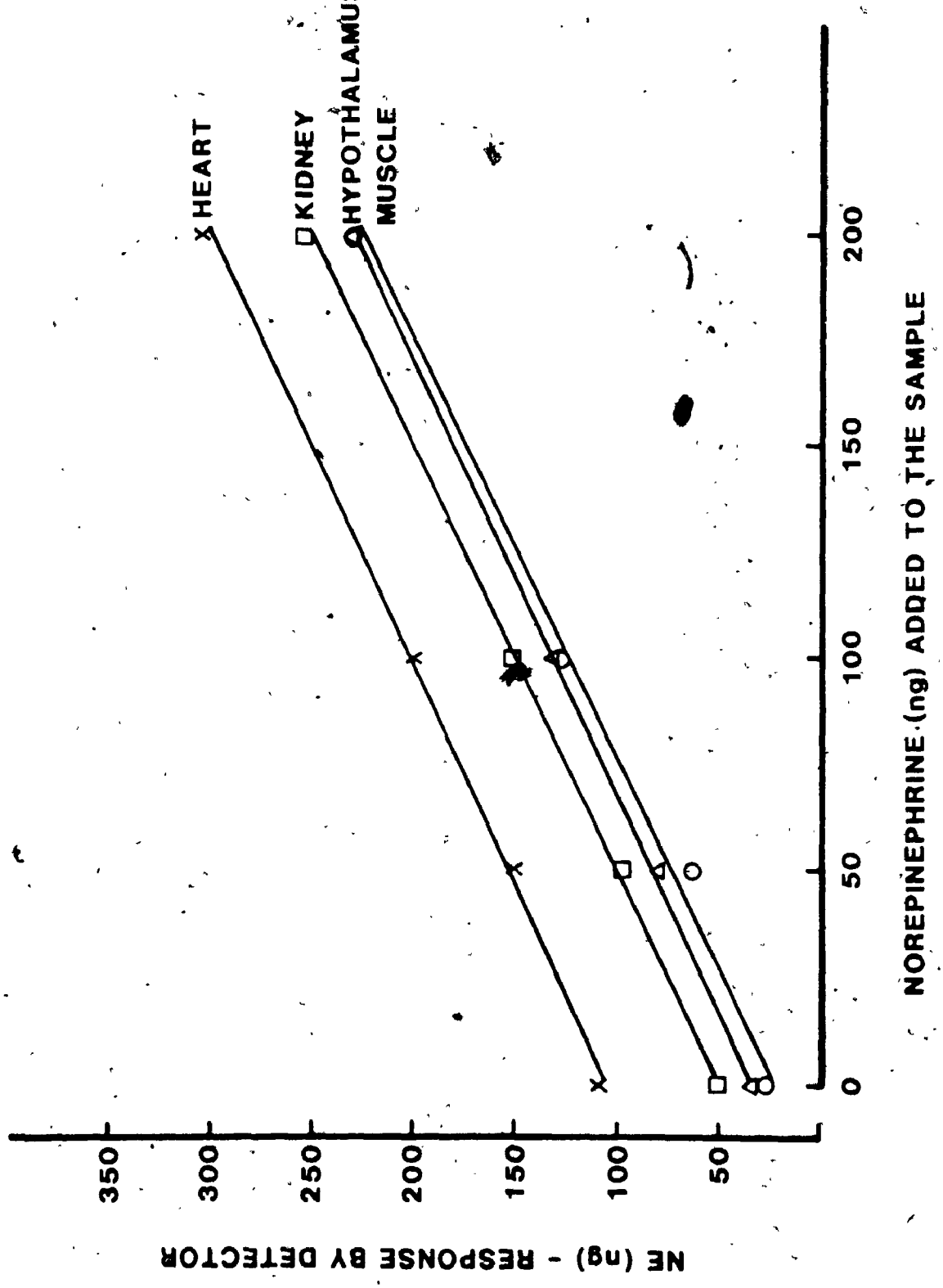

400
300
200
100
0
CURRENT (pico-amp)
NOREPINEPHRINE
DOPAMINE
DIHYDROXYBENZYLAMINE
EPINEPHRINE
50
100
200
300
400
500
picograms

FIGURE 10

Standard curve of NE calculated from addition of known amounts of NE and DHBA to heart, kidney, hypothalamic and muscle samples.

Tissue	Regression Equation (Y =)	Correlation Coefficient
Heart	106 + 0.99x	0.99
Kidney	52 + 0.51x	0.99
Hypothalamus	32 + 0.45x	0.99
Muscle	128 + 0.46x	0.99

n = 4 for each curve


HEART
KIDNEY
HYPOTHALAMUS
MUSCLE
NE (ng) - RESPONSE BY DETECTOR
350
300
250
200
150
100
50
0
50
100
150
200
NOREPINEPHRINE (ng) ADDED TO THE SAMPLE

4.2 Analysis of Data

The data were analysed with appropriate statistical analyses in each section of this study. For details of the statistical analyses used, refer to the Methods section of the relevant chapter. Statistical significance was inferred at $P < 0.05$ and the data were expressed as a mean $\pm$ standard error of the mean.

4.3 Histology

The hypothalamus is defined (Jacobson, 1972) as follows: The ventral and medial region of the diencephalon forming the wall of the ventral half of the third ventricle, delineated from the thalamus by the hypothalamic sulcus, lying medial to the internal capsule and subthalamus, continuous with precommisural septum anteriorly and with the mesencephalic midbrain tegmentum and central gray substance posteriorly. Its ventral surface is marked by, from front to back: 1) the optic chiasma, 2) the unpaired infundibulum, and 3) the paired mamillary bodies.

In the initial studies the "hypothalamus" was defined as the area from the optic chiasm to the mamillary bodies rostrocaudally, from corpus callosum to the ventral surface dorsoventrally, and laterally to the edge of the optic tract (Chapter 5).

In the subsequent experiments the hypothalamus was defined more discretely and was further divided into anterior and posterior hypothalamus. In these studies the hypothalamus was defined as the tissue from the optic chiasm to the mamillary bodies rostrocaudally, from the dorsal aspect of the third ventricle (~5 mm from the ventral surface) to the ventral surface dorsoventrally, and laterally to the edge of the internal capsule. The hypothalamus was further divided equally into anterior and posterior sections by a coronal transection.

To determine the variability in the dissection procedure 6 brains fixed with 10% formalin were sectioned as stated above. The hypothalamic tissues were frozen and sectioned at 50 um, mounted and stained with thionin for histological verification of the borders for the anterior and posterior hypothalamus. Figure 11 and 12 give the extent and variability in the coronal, sagittal, and horizontal sections made to obtain the anterior and posterior hypothalamic tissues. Evidence of the reproducibility of the dissection procedure is suggested by the mean (±S.D.) of weights for each of the hypothalamic tissues.

Anterior hypothalamus 61.2 ± 6.2 mg

Posterior hypothalamus 64.4 ± 11.1 mg

n = 12

Appendix 1 shows coronal sections midway through the anterior and posterior hypothalamic tissues showing the

FIGURE 11

Sagittal section of rat brain showing the location of transverse and horizontal cuts made to obtain the anterior and posterior hypothalamic sections. The stippled area represents the extent of variation of cuts histologically verified in 6 animals. Number in the upper right corner represents distance in mm from the midsagittal section. Sagittal section from de Groot (1959).

AHA: Anterior hypothalamic area; ARH: Arcuate nucleus of the hypothalamus; DMH: Dorsomedial nucleus of the hypothalamus; MM: Medial mamillary nucleus; PH: Posterior nucleus of the hypothalamus; PMD: Dorsal premamillary nucleus; POA: Lateral preoptic area; PVH: Paraventricular nucleus of the hypothalamus; RE: Reuniens nucleus of the thalamus; SC: Suprachiasmatic nucleus; VMH: Ventromedial nucleus of the hypothalamus

L0.2

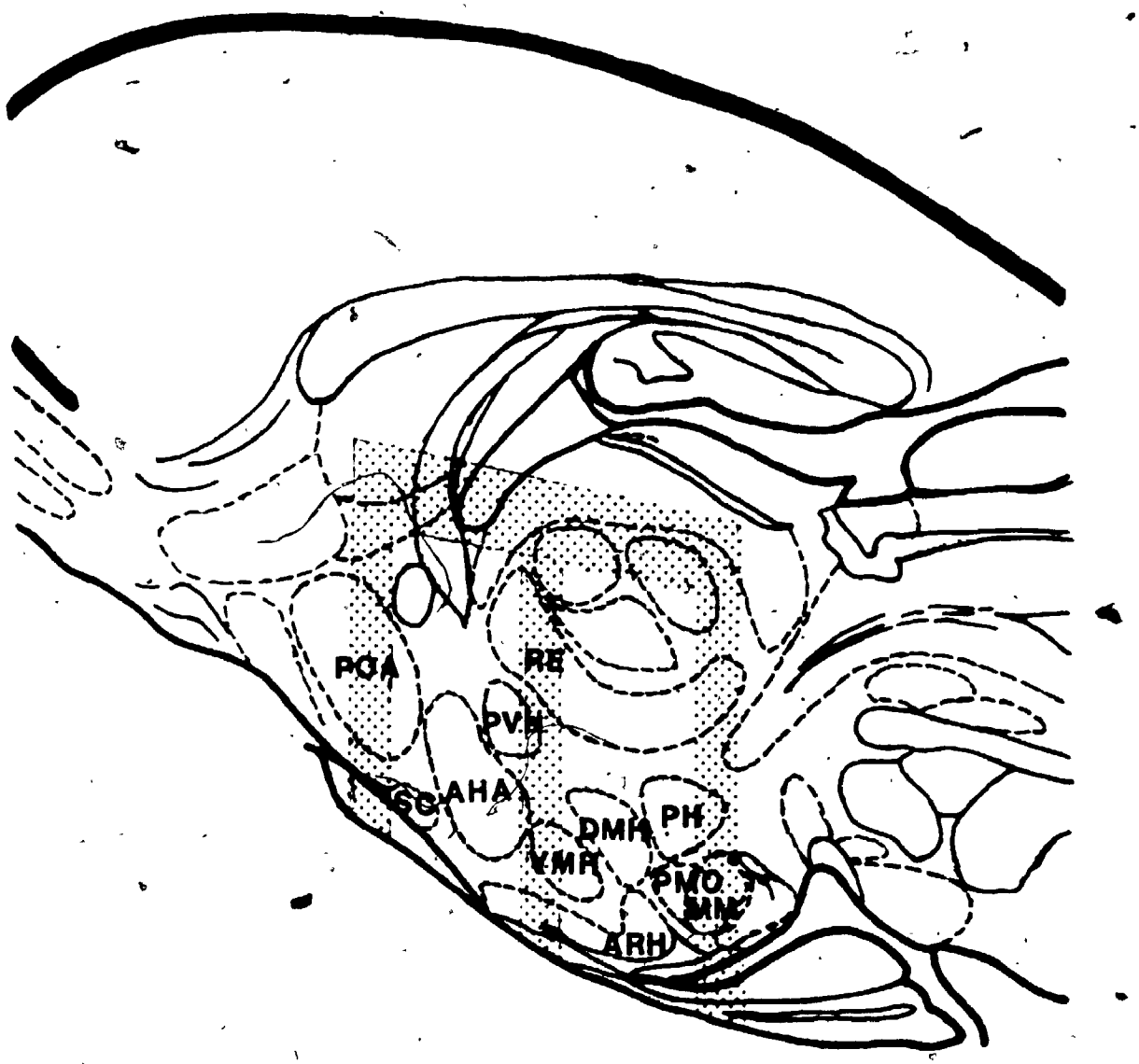
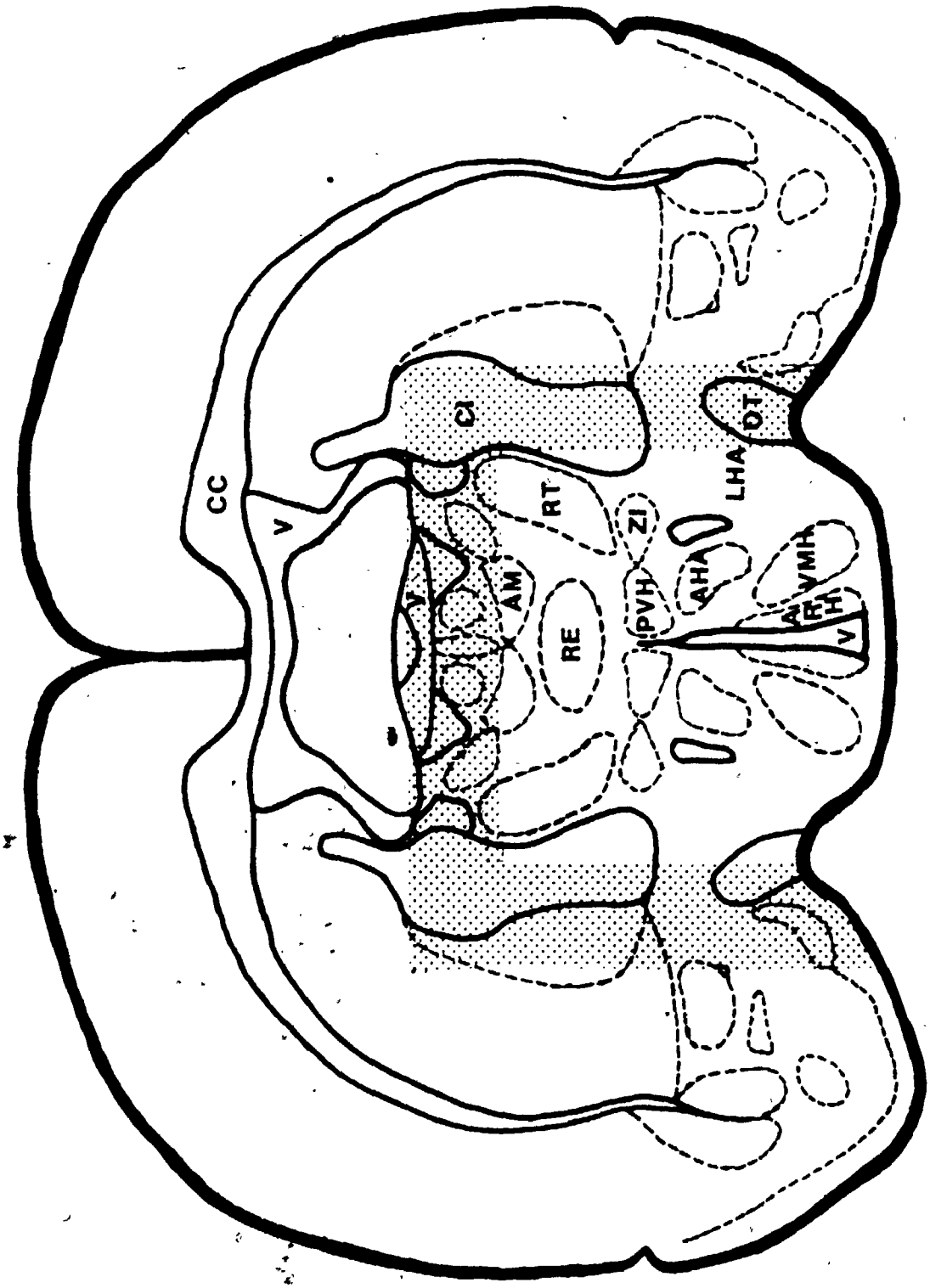


FIGURE 12

Coronal section of the rat brain showing the extent and variation of sagittal and horizontal cuts (n = 6). Format as in Figure 11. Number in the upper left corner indicates rostrocaudal distance in mm from Bregma. This coronal section was at the level at which anterior and posterior hypothalamic sections were separated. Coronal section from Pellegrino et al. (1981).

AHA: Anterior hypothalamic area; AM: Anteromedial nucleus of the thalamus; ARH: Arcuate nucleus of the hypothalamus; CC: Corpus callosum; CI: Internal capsule; LHA: Lateral hypothalamic area; OT: Optic tract; PH: Posterior nucleus of the hypothalamus; RE: Reuniens nucleus of the thalamus; RT: Reticular nucleus of the thalamus; V: Ventricle; ZI: Zona incerta



0.2

various nuclei that are present in these sections.

CHAPTER 5

Noradrenergic response in brain and peripheral organs after ADN transection

5.1 Introduction

The lowering of blood pressure in hypertensive man and animals by administration of drugs which interfere with the physiological action of NE have proved to be the main indirect evidence indicating the involvement of noradrenergic mechanisms in the control of arterial pressure (de Champlain et al., 1975). Some direct evidence has been provided by the reports of hyperactivity of the peripheral sympathetic system in various models of experimental hypertension (Chalmers, 1975). In fact most of the information implicating noradrenergic mechanisms in the control of arterial pressure has been obtained as a by product of studies in various models of experimental hypertension. However, it is not clear whether the sympathetic hyperactivity in various models of experimental hypertension results from a dysfunction at the level of the peripheral nervous system, or a secondary manifestation of a primary dysfunction elsewhere along the blood pressure regulating reflex arc. One reason for the uncertainty is that previous studies have been concerned with the problems of central nervous system (CNS), peripheral components of the sympathetic nervous system (SNS), and effector organs

(heart and vascular system) separately. There have been relatively few attempts to tie together observations of changes in the noradrenergic activity in the CNS to changes in the peripheral component of the SNS. Furthermore, altered NE metabolism has been shown using the turnover of NE in brain and peripheral organs in various hypertensive models (Chalmers, 1975; Antonaccio, 1977); however, there are no systematic studies (studies examining CNS and SNS simultaneously) of NE turnover in normotensive rats subjected to changes in baroreceptor input. Therefore, it is not clear if the changes in turnover of NE observed in hypertensive models are a cause or an effect of the hypertension.

Recent evidence suggests that there may be functional differences between aortic and carotid baroreceptor reflexes in the overall control of arterial pressure, as bilateral transection of the ADN produces a sustained increase in arterial pressure (Ito et al., 1979; Ciriello et al., 1980a) while carotid sinus denervation does not chronically elevate arterial pressure (Krieger, 1964; Ito et al., 1978; Ciriello et al., 1980a). These findings suggest that ADN input to the CNS may be preferentially baroreceptor mediated. In addition, Sapru et al. (1979) have shown that the aortic depressor nerve is not chemosensitive. Therefore, in this study I examined the changes in the hypothalamic and peripheral noradrenergic activity in response to surgically removing a specific baroreceptor

input to the CNS (Sapru et al., 1977; Ito et al., 1979; Ciriello et al., 1980a). by transectioning the ADN bilaterally in the rat.

5.2 Methods

Studies were conducted using male Wistar rats (Biobreeding Laboratories, Ottawa, Canada) weighing 350-400 g. The animals were assigned randomly to one of two groups and placed in individual cages in a room with a controlled 12 h light cycle and maintained at 20-22 °C. Food and water were available ad libitum.

Arterial pressure was measured in the conscious rat using an indirect tail-cuff method with the rat placed in a temperature-controlled restraining device (Narco Biosystems). Pressure was recorded on a Grass Model 7 polygraph using a Grass piezoelectric microphone connected to a balloon plethysmograph and a 7P8 preamplifier. The pressure at which the pulsations appeared during the slow release of pressure from the occlusive tail-cuff was taken to be the arterial pressure. Pressures obtained by this method have been found to correlate well with those obtained by direct arterial cannulation (Pfeffer et al 1971). In our own laboratory the tail cuff pressure is closer to mean arterial pressure than systolic pressure. At least three good recordings of an individual rat's pressure were obtained over a 5-10 minute period and averaged to represent

the arterial pressure on that day. Heart rate was obtained by counting arterial pulsations on the blood pressure tracing (for 5 sec) and expressed as beats per minute.

In one group of rats (n = 32) under ether anesthesia, the ADN and cervical sympathetic nerves were approached by a ventral midline incision in the neck, dissected free from the vagus nerves for approximately 1 cm and cut bilaterally. The carotid arteries were also stripped of all connective tissue. In the other group of rats (n = 31), the vagus, ADN and cervical sympathetic nerves were exposed in the neck, but were not damaged (sham operation). The animals were given postoperative care and were allowed to recover from the anesthesia.

5.2.1 Measurement of disappearance rate of tissue norepinephrine concentration after inhibition of tyrosine hydroxylase

After a postoperative survival period of either three or thirteen days, the disappearance rate of NE in brain and peripheral organs was determined using the technique described in Chapter 4, in both ADN-transected and sham-operated rats. The rate of decline of endogenous levels of NE was measured following inhibition of tyrosine hydroxylase with α-methyltyrosine (300 mg/kg, i.p. every 4 h). Rats with a survival period of three days were killed by cervical dislocation at 0, 4, and 8 h after inhibition of

tyrosine hydroxylase, and the brain, left kidney, a section of the duodenum, and a piece of skeletal muscle from the hindlimb (anterior and posterior femoral muscles) were quickly removed. The sample from skeletal muscle represents a homogeneous tissue in terms of noradrenergic innervation to vasculature (Rhodin, 1967); whereas, noradrenergic innervation to the kidney is involved in three functions 1) vasoconstriction, 2) renin release, and 3) sodium reabsorption (De Bona, 1982). The duodenum represents yet another major vascular bed, the mesenteric circulation, which may respond differently to changes in baroreceptor input (Kirchheim, 1976) compared to skeletal muscle and kidney.

The brain was further divided into three areas: the medulla (from 1 mm caudal to approximately 4 mm rostral to the obex), the midbrain (extending from the rostral end of the medulla to just caudal to the mammillary bodies) and the "hypothalamus" (from the optic chiasm to the mammillary bodies rostrocaudally, from the corpus callosum to the ventral surface dorsoventrally, and laterally to the edge of the optic tract). Similarly, in rats after a postoperative period of thirteen days NE concentration in the hypothalamus, kidney, and skeletal muscle was determined at 0 and 8 h after inhibition of tyrosine hydroxylase.

The tissue samples were weighed, homogenized in perchloric acid, and the homogenates were immediately frozen at -75 °C. NE was assayed using alumina extraction and a

fluorescence assay as previously described (Chapter 4). All samples were corrected for percent recovery using internal standards and results were expressed as ng/g wet weight.

In three rats, ganglionic blockade was used to produce hypotension in order to examine the effect of removal of baroreceptor inhibition on the disappearance rate of NE in brain areas and peripheral organs of sham-operated and ADN-transected rats. Hexamethonium chloride (10 mg/kg, i.m., Sigma Chemical Co.) was administered every 2 h and NE concentration was determined at 6 h after inhibition of tyrosine hydroxylase.

5.2.2 Data Analysis

The rate of decline of tissue NE was determined by expressing the tissue concentration of NE at different time intervals after inhibition of tyrosine hydroxylase as a percent of the mean initial value. Data points (mean $\pm$ S.E.) were plotted on a log scale and a best fit line was calculated using linear regression analysis. Correlation coefficients greater than 0.90 were obtained for all organs except the kidney ($r = 0.83$) and muscle ($r = 0.81$) of the ADN transected group. Differences between slopes of lines obtained for sham and experimental groups, which were interpreted to represent a change in NE turnover, were tested for statistical significance using a t-test. Half-life values were calculated from the regression

equations. Comparisons between mean percentages for 8 h values was done using Duncan's multiple range test (Duncan, 1955) and a P value of less than 0.05 was considered to indicate statistical significance.

5.3 RESULTS

5.3.1 Arterial pressure and heart rate after ADN transection

For the two days immediately following surgery, both systolic arterial pressure and heart rate were significantly elevated in rats with ADN transection (Figure 13, 14). On the other hand, in the group of rats that were observed for thirteen days, only arterial pressure remained consistently elevated while heart rate tended towards control values. On day 12, arterial pressure was 124 $\pm$ 3 mmHg and 140 $\pm$ 3 mmHg, while heart rate was 341 $\pm$ 7 beats/min and 352 $\pm$ 5 beats/min, for the sham-operated and ADN-transected groups, respectively. Blood pressure and heart rate did not differ significantly between the two groups prior to the ADN operation.

5.3.2 NE concentration in brain and peripheral organs

Endogenous concentration of NE in brain and peripheral organs at "0 time", 3 days after sham or ADN surgery are

FIGURE 13

Effect of ADN transection on arterial pressure. Arrow indicates day of either ADN transection (n = 24) or sham operation (n = 24). Values are means $\pm$ S.E.

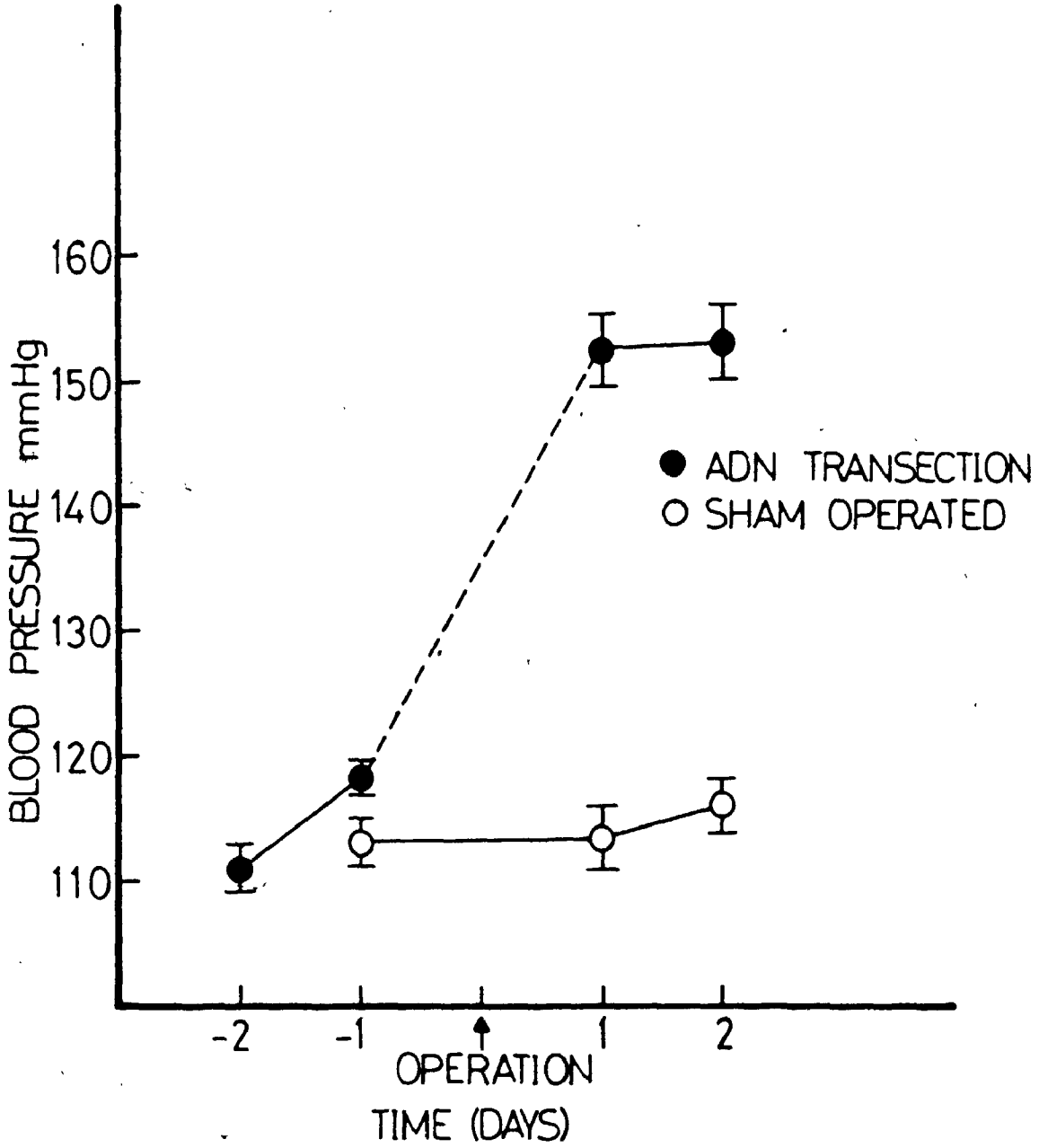
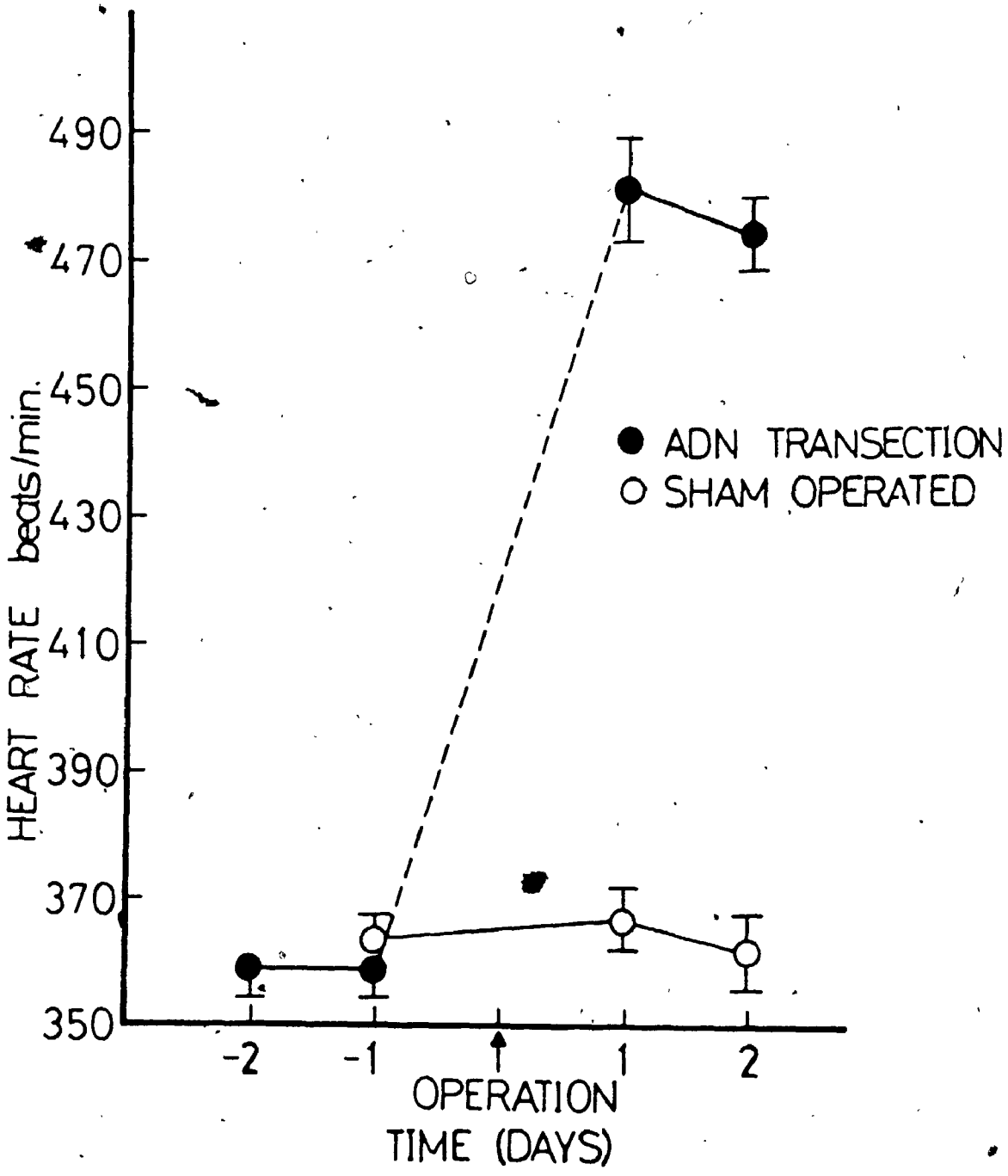

160
150
140
130
120
110
BLOOD PRESSURE mmHg
ADN TRANSECTION
SHAM OPERATED
-2
-1
1
2
OPERATION
TIME (DAYS)

FIGURE 14

Effect of ADN transection on heart rate. Arrow indicates day of either ADN transection (n = 24) or sham operation (n = 24). Values are means ± S.E.


490
470
450
430
410
390
370
350
HEART RATE beats/min.
ADN TRANSECTION
SHAM OPERATED
-2
-1
1
2
OPERATION
TIME (DAYS)

summarized in Figure 15. Compared to control the NE concentration in the hypothalamus and midbrain was significantly higher in rats with ADN transection. However, there was no significant difference in NE concentration in the medulla between the two groups. In peripheral organs, the only difference noted was significantly lower NE concentration in the kidney of ADN transected rats. For "0 time" values at 13 days, there were no significant differences in NE concentration between the two groups for any organ examined (Table 2), although the values for hypothalamus tended to be higher.

5.3.3 Disappearance rate of norepinephrine in brain and peripheral organs after inhibition of tyrosine hydroxylase

Figures 16, 17, 18 shows that three days after ADN transection, the disappearance rate of NE was increased significantly in all three brain regions examined (indicated as a significant difference between slopes of regression lines for the two groups of rats). The half-life of NE in rats with ADN transection when compared to values obtained for sham-operated animals was 2.7 vs 5.0 h in the hypothalamus, 3.0 vs 4.9 h in the midbrain, and 3.1 vs 5.2 h in the medulla. In sham-operated rats (n = 3) treated with hexamethonium, the disappearance rate of NE in brain areas tended to increase, but no effect was observed in

TABLE 2

Norepinephrine concentration in tissues 13 days after either ADN transection or sham operation

Organ	Sham operation	ADN transection
Hypothalamus	846 ± 34	969 ± 59
Kidney	179 ± 11	178 ± 11
Skeletal Muscle	71 ± 5	70 ± 3

Mean norepinephrine concentration given as ng/g wet weight ± S.E. in animals killed at 0 time.

n = 4 for each group.

FIGURE 15

Endogenous concentration of NE (±S.E.) in brain and peripheral organs of rats 3 days after either ADN transection or sham operation.

* $p < 0.05$ compared to sham operated group.

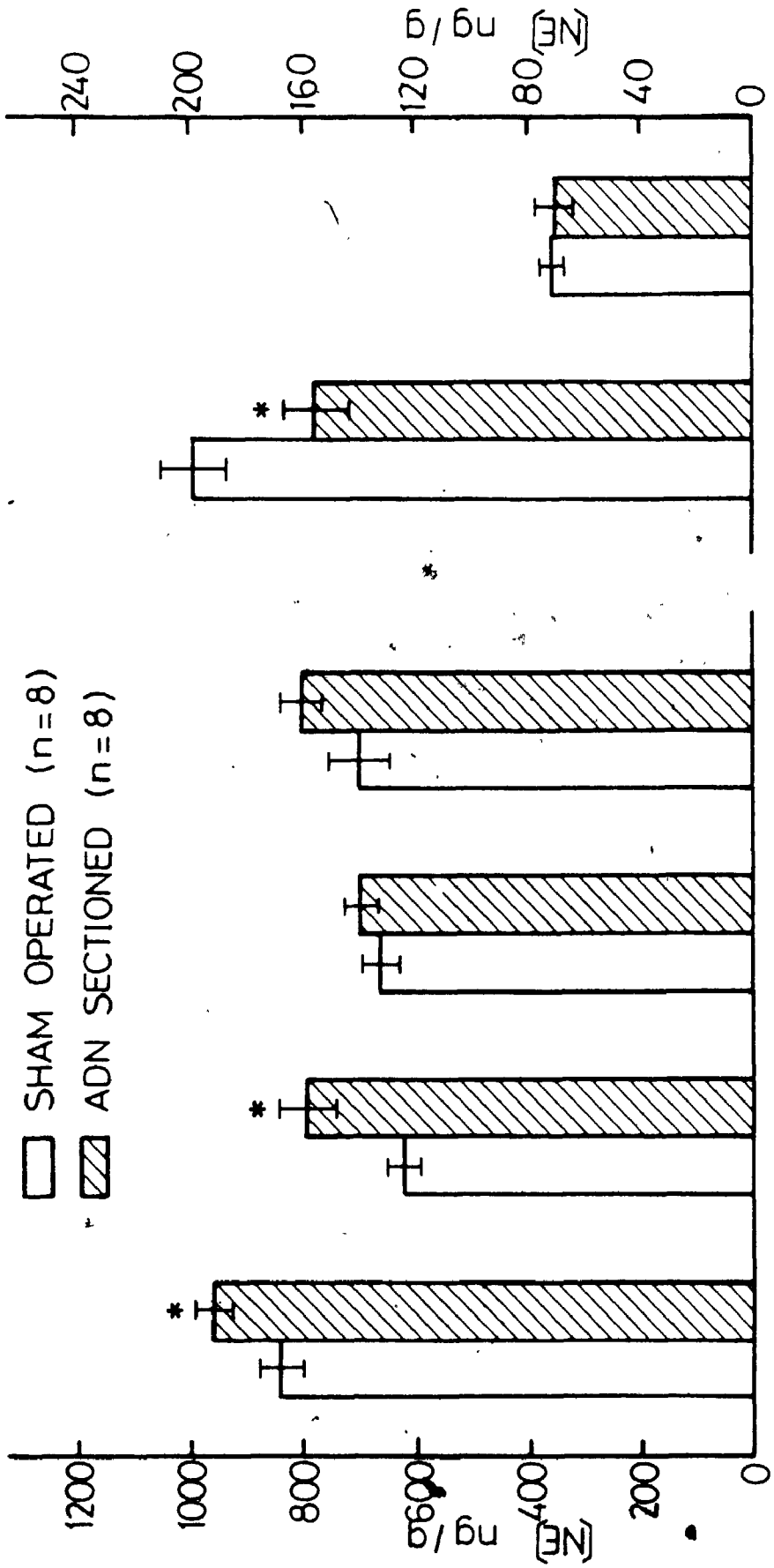

SHAM OPERATED (n=8)
ADN SECTIONED (n=8)
(NE) ng/g
1200
1000
800
600
400
200
0
HYPOTHAL.
MIDBRAIN
MEDULLA
INTESTINE
(NE) ng/g
240
200
160
120
80
40
0
KIDNEY
MUSCLE
*

FIGURE 16

Disappearance rate of NE in hypothalamus of rats 3 days after either ADN transection or sham operation. Each point represents NE concentration expressed as a percent (±S.E.) of initial concentration (n = 5-6 for each point). Slope of regression line for hypothalamus from ADN transected rats is significantly ($P < 0.01$) different from control. See text for half-life values.

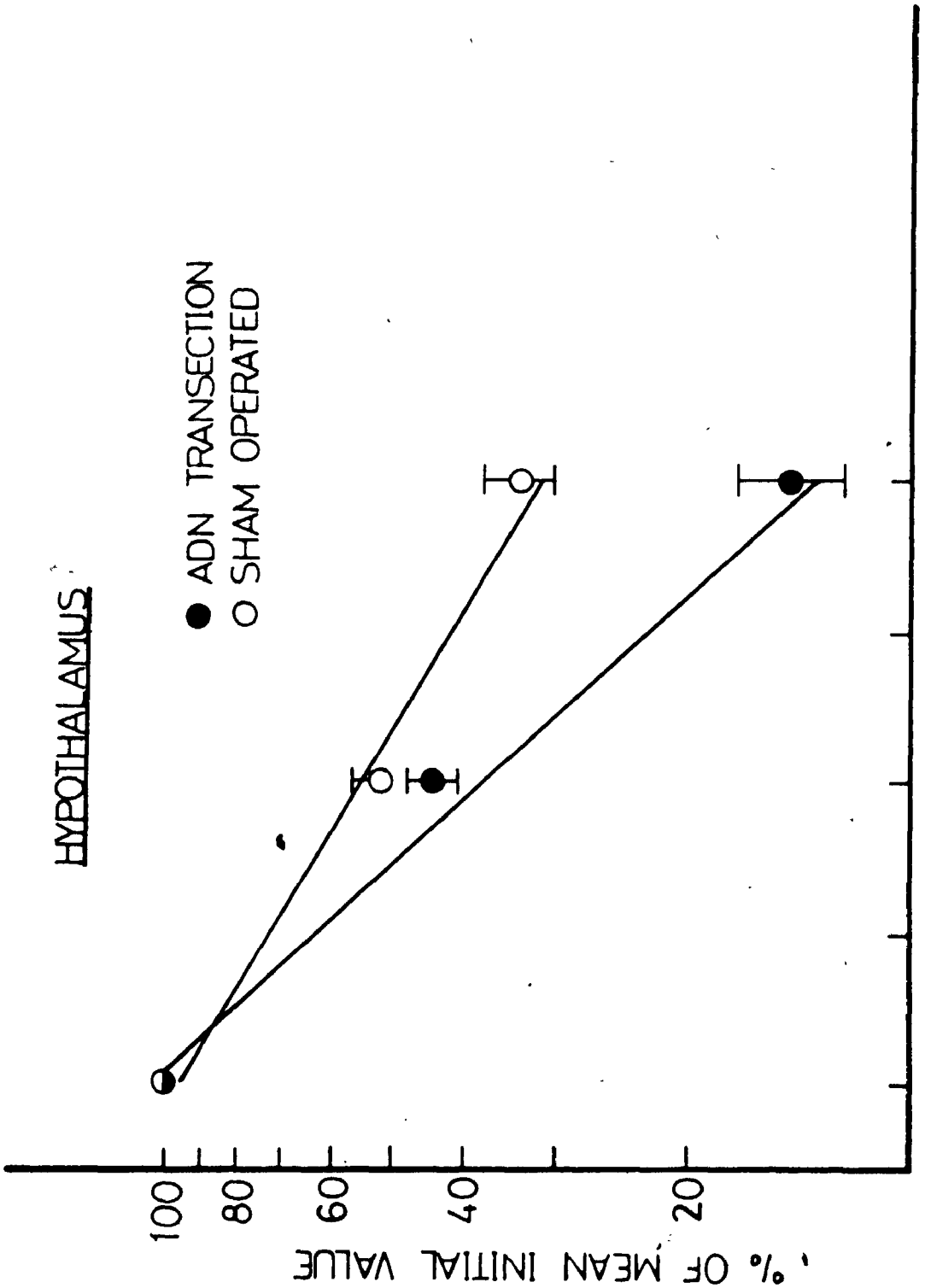

HYPOTHALAMUS
● ADN TRANSECTION
○ SHAM OPERATED
% OF MEAN INITIAL VALUE
100
80
60
40
20
0
2
4
6
8
TIME (HR)

FIGURE 17

Disappearance rate of NE in midbrain of rats 3 days after either ADN transection or sham operation. Format as in Figure 16. Slope of regression line for midbrain from ADN transected rats is significantly ($P < 0.01$) different from control. See text for half-life values.

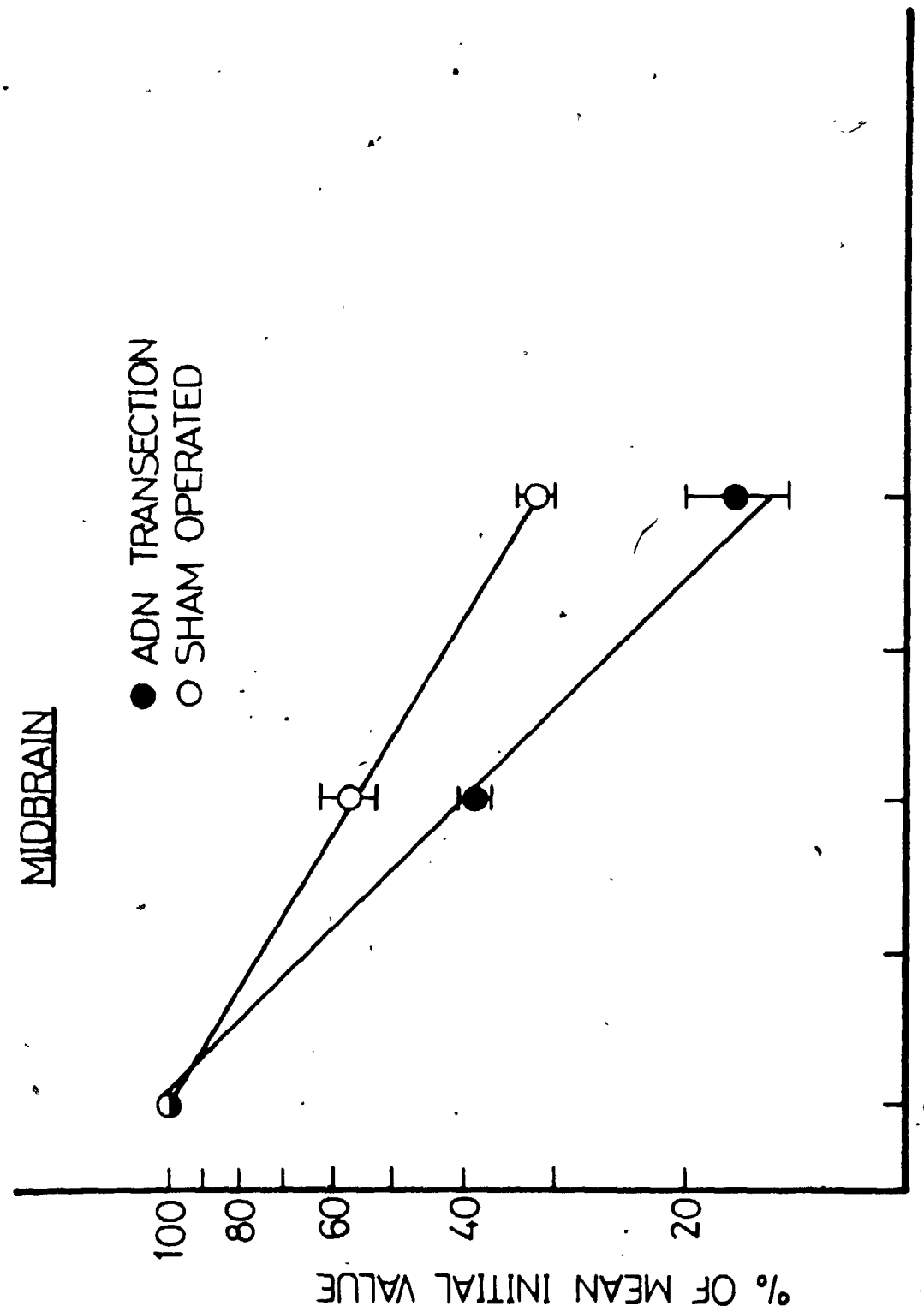
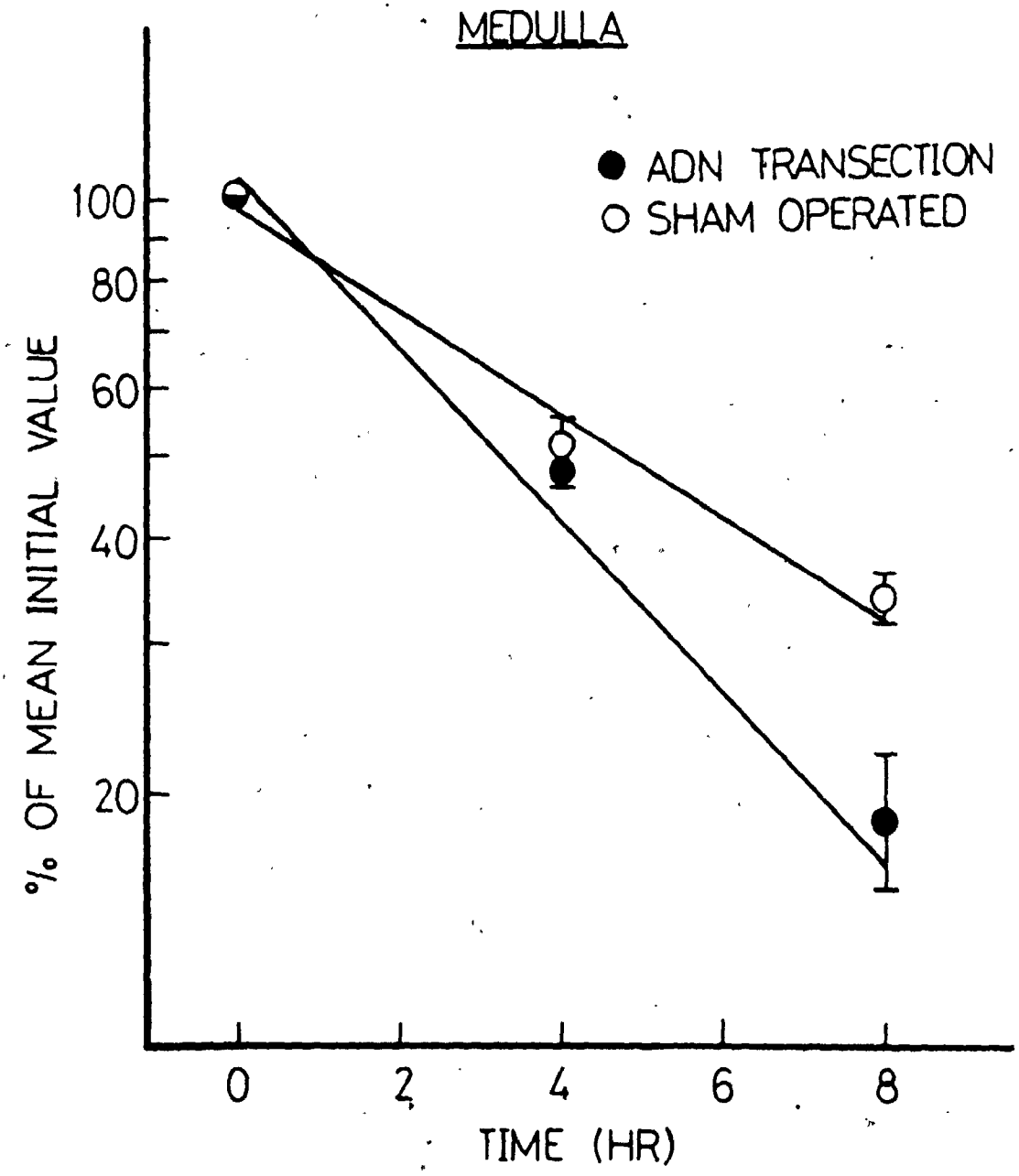

MIDBRAIN
● ADN TRANSECTION
○ SHAM OPERATED
% OF MEAN INITIAL VALUE
100
80
60
40
20
0
2
4
6
8
TIME (HR)

FIGURE 18

Disappearance rate of NE in medulla of rats 3 days after either ADN transection or sham operation. Format as in Figure 16. Slope of regression line for medulla from ADN transected rats is significantly ($P < 0.01$) different from control. See text for half-life values.


MEDULLA
● ADN TRANSECTION
○ SHAM OPERATED
100
80
60
40
20
% OF MEAN INITIAL VALUE
0
2
4
6
8
TIME (HR)

ADN-transected rats (n = 3). Due to the relatively few animals examined, no statistical comparisons were done in these hexamethonium treated animals, although approximate half-life values were calculated. The half-life of NE for sham and ADN groups respectively was 3.8 and 3.4 h in the hypothalamus, 3.4 and 3.1 h in the midbrain and 4.0 and 3.6 h in the medulla.

The disappearance rate of NE in peripheral organs is shown in Figures 19, 20, 21. Significant differences between the slopes of regression lines obtained in sham and ADN-transected rats were found for kidney and skeletal muscle, but not for duodenum. The half-life of NE decreased dramatically in skeletal muscle, changing from 9.0 h in controls to 2.5 h in rats with ADN transection. Half-life decreased in kidney from 5.5 to 4.0 h, while there was no change in the duodenum (4.5 vs 4.4 h). Hexamethonium treated rats showed a decreased disappearance rate of NE in peripheral organs as would be expected following blockade of sympathetic nerve activity.

In animals studied at 13 days after ADN transection, tissue NE concentration determined 8 h after inhibition of tyrosine hydroxylase was expressed as a percent of the initial concentration, and the results were used to infer changes in NE turnover (Figure 22). Included in this figure are the 8 h values obtained 3 days after ADN transection, also expressed as a percent of initial concentration, for

FIGURE 19

Disappearance rate of NE in kidneys of rats 3 days after either ADN transection or sham operation. Format as in Figure 16. Slope of regression line for ADN transected rats is significantly ($P < 0.01$) different in the kidneys of ADN transected rats compared to controls. See text for half-life values.

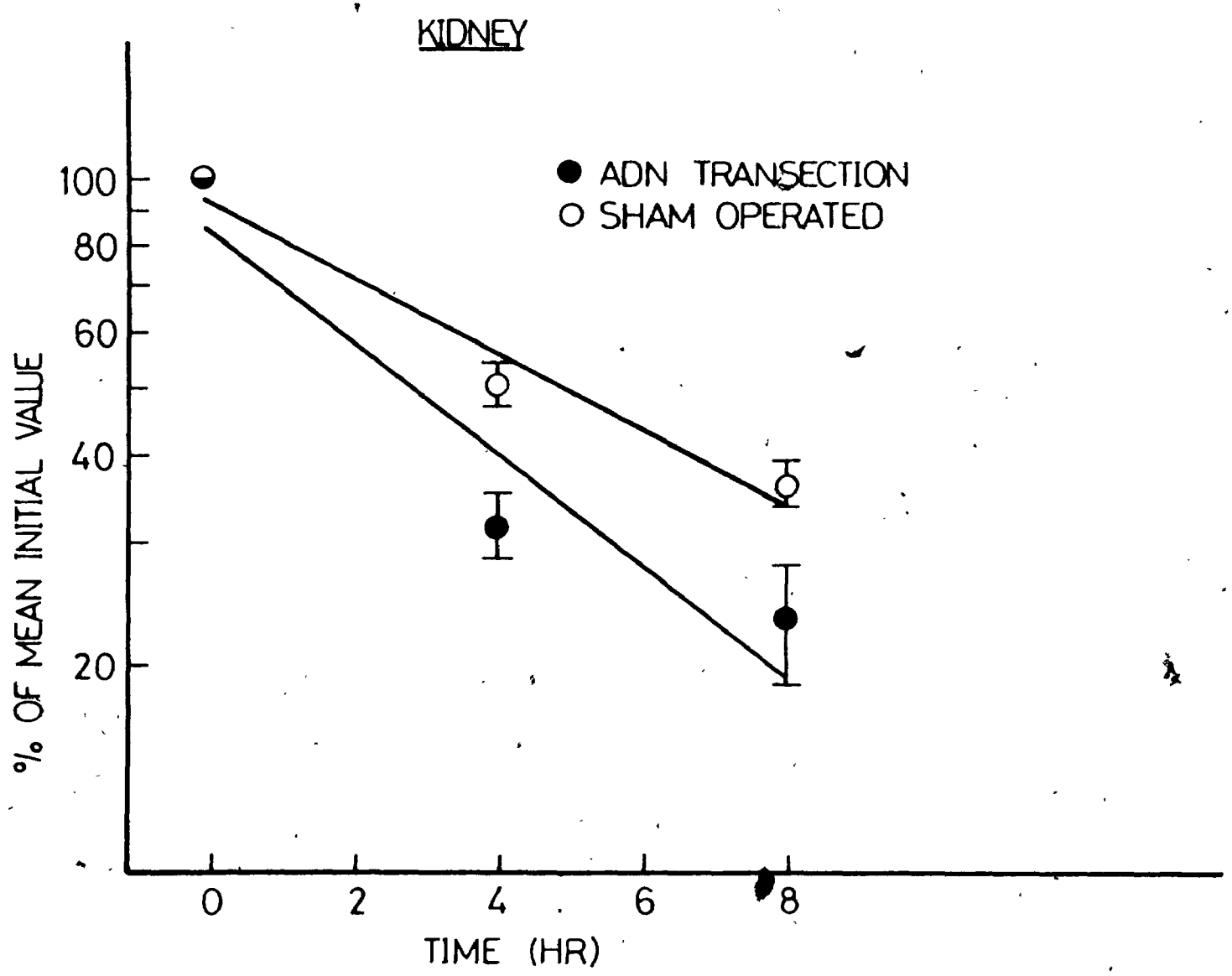

KIDNEY
● ADN TRANSECTION
○ SHAM OPERATED
100
80
60
40
20
% OF MEAN INITIAL VALUE
0
2
4
6
8
TIME (HR)

FIGURE 20

Disappearance rate of NE in intestine of rats 3 days after either ADN transection or sham operation. Format as in Figure 16. Slope of regression line for ADN transected rats is not significantly different in the intestine of ADN transected rats compared to controls. See text for half-life values.

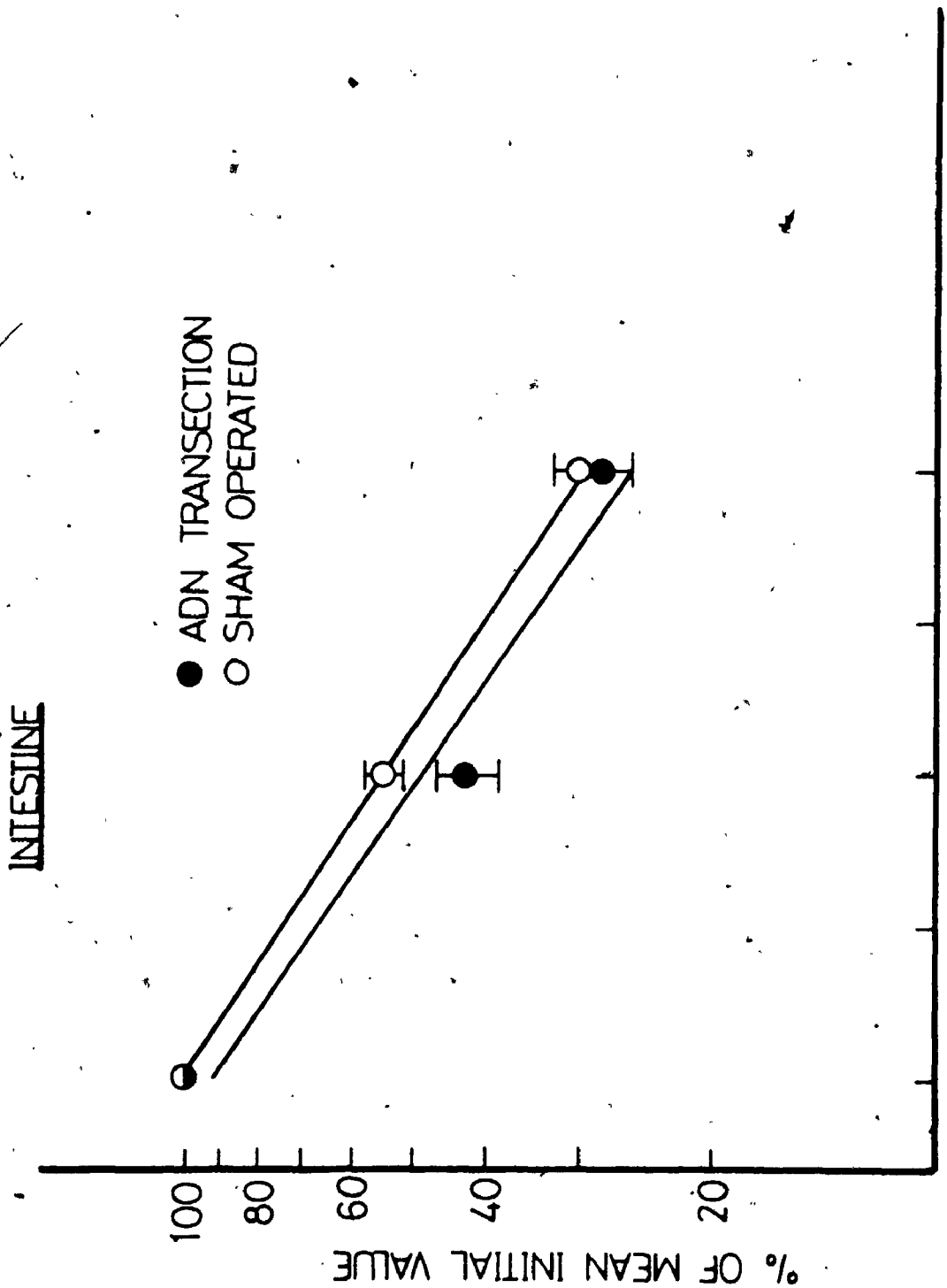
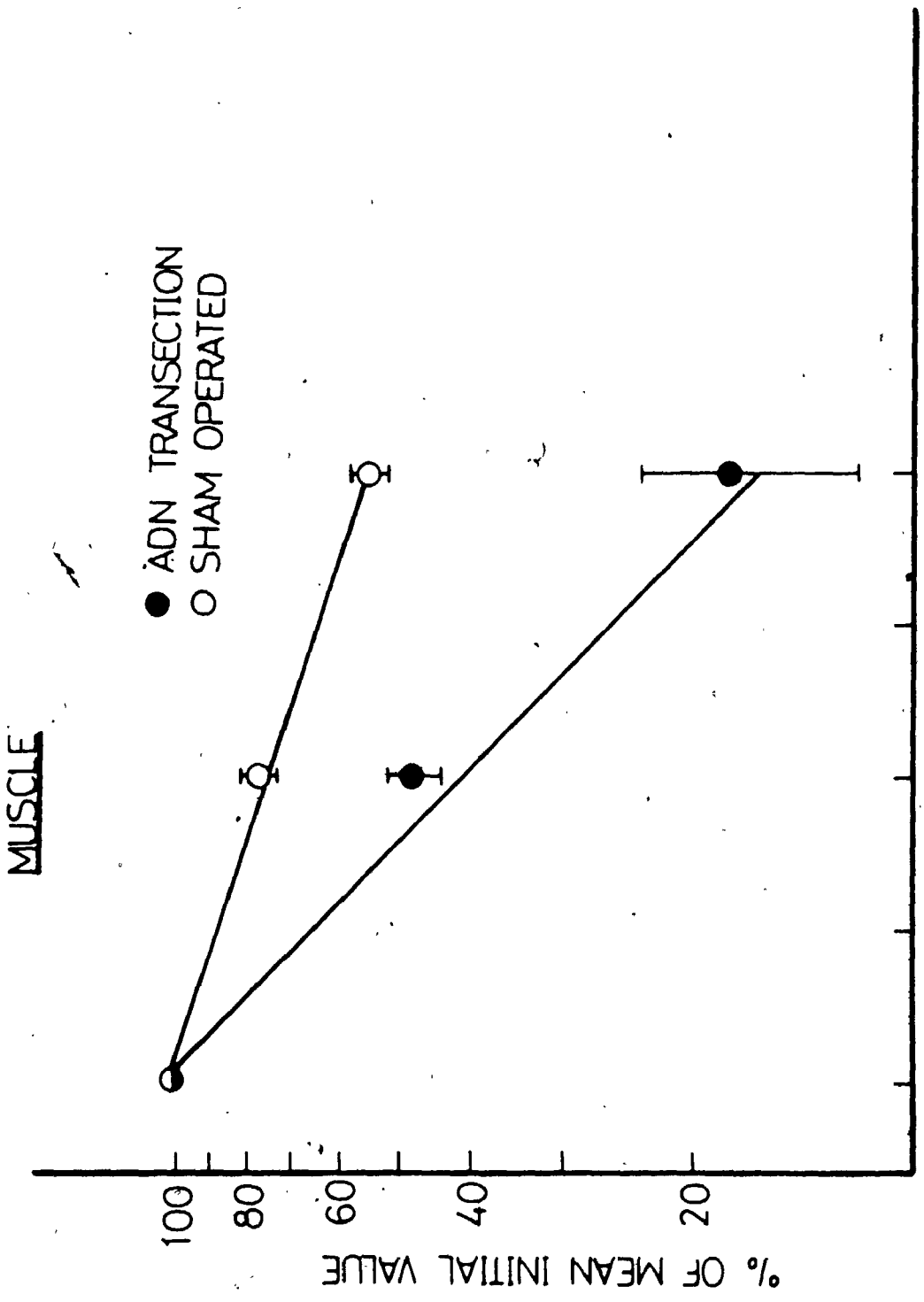

INTESTINE
● ADN TRANSECTION
○ SHAM OPERATED
% OF MEAN INITIAL VALUE
100
80
60
40
20
0
2
4
6
8
TIME (HR)

FIGURE 21

Disappearance rate of NE in skeletal muscle of rats 3 days after either ADN transection or sham operation. Format as in Figure 16. Slope of regression line for ADN transected rats is significantly ($P < 0.01$) different in the skeletal muscle of ADN transected rats compared to controls. See text for half-life values.


MUSCLE
● ADN TRANSECTION
○ SHAM OPERATED
% OF MEAN INITIAL VALUE
100
80
60
40
20
0
2
4
6
8
TIME (HR)

comparison of the two studies. At 13 days, ADN-transected animals had slightly, but significantly lower NE levels in the hypothalamus 8 h after α-methyltyrosine when compared to sham-operated animals; however, no significant differences were found in muscle and kidney.These results suggest that although NE turnover remained slightly increased in the hypothalamus, there was no evidence for increased turnover of NE in muscle and kidney at this time .

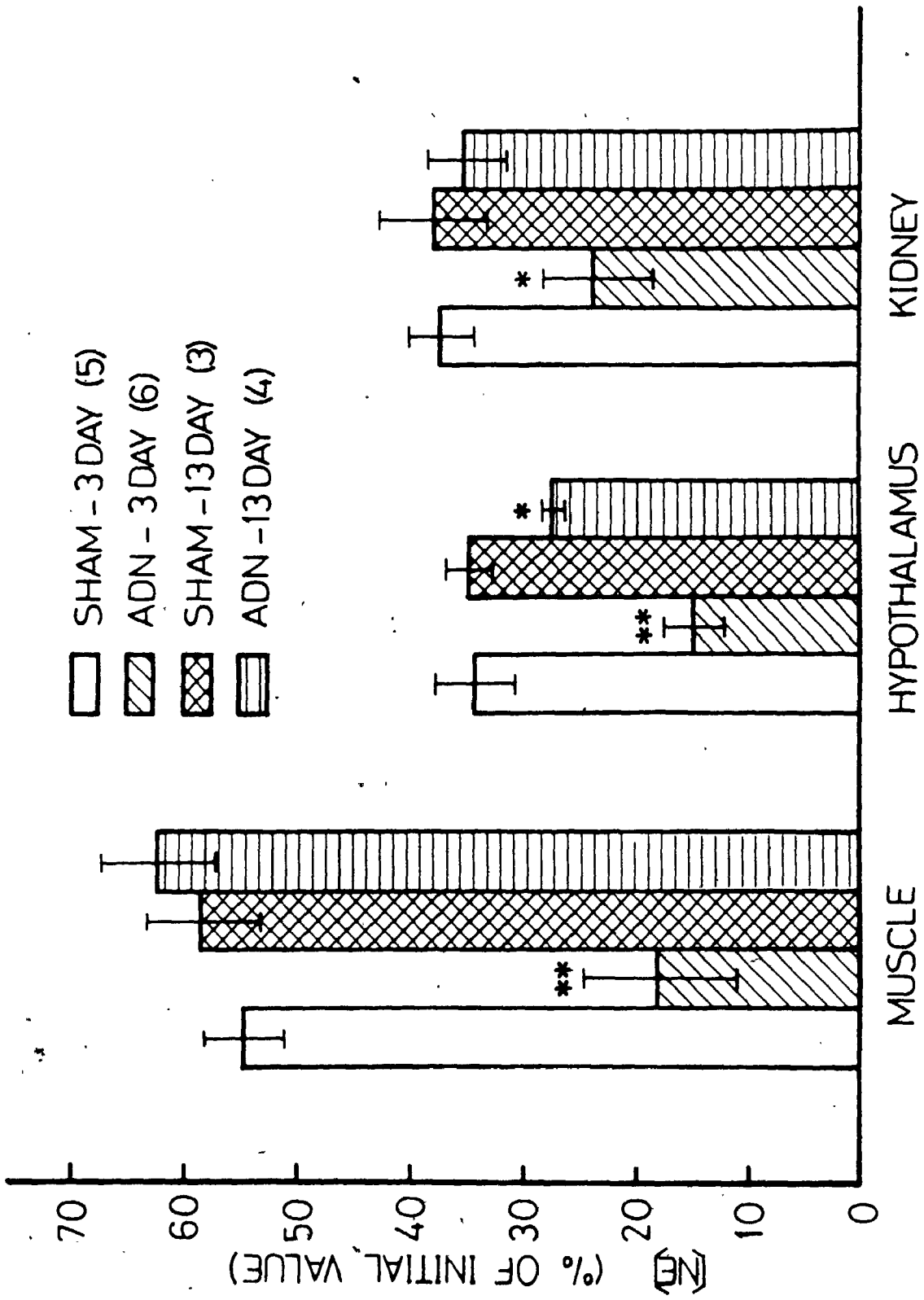
5.4 DISCUSSION

Surgical removal of specific baroreceptor afferents by transection of ADN and cervical sympathetic nerves in the rat produced an elevated arterial pressure and heart rate, which at three days was associated with an increased rate of disappearance of NE after inhibition of tyrosine hydroxylase in the hypothalamus, midbrain, medulla, skeletal muscle, and kidney, but not in the duodenum. At 13 days, arterial pressure was still elevated but heart rate was normal, and there was no evidence of altered turnover of NE in the peripheral organs examined (skeletal muscle and kidney).

These data suggest that the early elevation of arterial pressure following ADN transection is due to increased sympathetic activity, while the elevated pressure at two weeks is maintained by factors other than increased sympathetic activity. It may be argued that ADN transection does not produce chronically elevated arterial pressure as

FIGURE 22

Norepinephrine concentration 8 h after α-methyltyrosine, expressed as a percent (±S.E.) of initial concentration. Values were obtained from rats at 3 or 13 days after either ADN-transection or sham operation. *P < 0.05. **P < 0.01, compared to respective sham-operated control.


SHAM - 3 DAY (5)
ADN - 3 DAY (6)
SHAM - 13 DAY (3)
ADN - 13 DAY (4)
MUSCLE
HYPOTHALAMUS
KIDNEY
[NE] (% OF INITIAL VALUE)
0
10
20
30
40
50
60
70
*
**
*
**
*

there is evidence that total baroreceptor deafferentation produces only labile arterial pressure in dogs (Cowley et al., 1973) and rats (Norman et al., 1981) and that the elevated arterial pressure observed may be related to the technique used to measure pressure (tail-cuff method) (Norman et al., 1981). This explanation would not be inconsistent with our findings at 13 days, as turnover of NE, determined with animals housed in their home cages, is apparently normal in peripheral tissues. On the other hand, if arterial pressure is elevated only during the actual measurement of blood pressure it might be expected that heart rate would also be elevated (Alexander et al., 1980). This was not observed, as the heart rates were similar to those measured in sham-operated animals at 13 days. Similarly, Ciriello et al. (1980a) reported elevated arterial pressure and normal heart rate 85 days after ADN transection in rats, in which arterial pressure was measured by a chronic indwelling catheter in the femoral artery. Therefore, although the data are not conclusive, the available observations suggest that ADN transection produces a mild chronic hypertension in rats (Fink et al., 1980b).

In recent years, several reports suggest the participation of central noradrenergic neurons in the normal control of arterial pressure and in the development of hypertension in various experimental models (Chalmers, 1975; Antonaccio, 1977). The data reported in this study support the suggestion of a role for noradrenergic pathways in the

hypothalamic and brainstem regions in producing an elevated arterial pressure 3 days after removal of baroreceptor information carried by the ADN. Although the disappearance rate of NE as measured in this study can be influenced by several factors involved with storage, release, and uptake of the neurotransmitter, it is probable that the changes observed were related to alteration of activity of central noradrenergic neurons and peripheral sympathetic fibers. Similar results have been reported after sino-aortic denervation (de Quattro et al., 1969), and suggest that the increased activity of central noradrenergic pathways after removal of ADN inhibitory effects facilitates a rise in arterial pressure by increasing sympathetic outflow to cardiovascular effector organs (Doba et al., 1974). It seems reasonable to suggest that in the ADN model, the increased turnover of NE in the brain is the cause of the increased activity in sympathetic nerves. This suggestion is supported by the observation that the disappearance rate of NE in the brain tended to increase during prolonged hypotension produced by autonomic blockade with hexamethonium in control animals, and did not increase further in animals with ADN section.

Although it is difficult to assign functional roles to changes in NE concentration in brain regions, the results of this study are similar to those reported by Chalmers et al. (1979). In sino-aortic denervated rats, NE concentration of specific brainstem nuclei and hypothalamic

areas was significantly increased when compared to control rats 7 days after the operation, but was unchanged 28 days later. Similar differences were found for changes in tyrosine hydroxylase activity. On the contrary, phenylethanolamine N-methyltransferase activity in the hypothalamus was not changed at 7 days, but was decreased at 28 days. They concluded that noradrenergic mechanisms in the brain were responsible for initiating the increased arterial pressure after baroreceptor denervation, but that adrenergic neurons were responsible for maintaining the increased arterial pressure. However there was no measure of peripheral noradrenergic activity in these studies to demonstrate that sympathetic activity was actually increased at these times. Our data support the idea that removal of baroreceptor input to the CNS activates noradrenergic mechanisms which in turn initiate the rise in arterial pressure and heart rate via an increased sympathetic activity; however, if adrenergic neurons are involved in maintaining the elevated pressure the mechanism apparently does not involve the peripheral sympathetic nervous system. Similar conclusions concerning the time-course of the involvement of the sympathetic nervous system after sino-aortic denervation in rats have recently been reported (Alexander et al., 1979). Alexander et al. (1979) have shown an elevated plasma catecholamine concentration and dopamine-β-hydroxylase activity during the first 1-3 weeks after sino-aortic denervation but not later.

Finally, the finding of a differential effect of ADN transection on the disappearance rate of NE in peripheral organs is interesting in light of the nonuniform distribution of sympathetic activity to vascular beds during activation of baroreceptor reflexes (Kirchheim, 1976). In the present study, the disappearance rate of NE in skeletal muscle was increased four fold, significantly increased in kidney, but not changed in the duodenum. These changes agree well with a report by Touw et al. (1979) in which aortic baroreceptor deafferentation produced significant, neurally mediated increases in vascular resistance in kidney and hindlimbs but not in the mesenteric circulation. Similarly, bilateral lesions of the NTS show differential changes in vascular resistance, with large changes in skeletal muscle and moderate changes in kidney and mesenteric circulation (Snyder et al., 1978). In the latter study, however, no attempt was made to determine the contribution of neural components to the changes in vascular resistance.

In conclusion, I have described alterations in the disappearance rate of NE in brain and peripheral organs following surgical removal of a specific baroreceptor influence by ADN transection in the rat. The data indicate that within three days after removal of the inhibitory effects of the ADN on CNS produces elevated arterial pressure and heart rate presumably as a result of increased sympathetic activity initiated by increased noradrenergic

activity in the hypothalamus and brainstem. On the other hand, two weeks after denervation, the disappearance rate of NE was normal in peripheral organs but arterial pressure remained elevated. The exact mechanism by which the arterial pressure remains elevated is unknown; however, it seems there is little contribution to the sustained hypertension from peripheral noradrenergic pathways. It may well be that the increased NE turnover in the hypothalamus at 2 weeks after ADN transection was due to the perpetual loss of ADN afferent input to the CNS, and other mechanisms are responsible for returning the peripheral noradrenergic activity back to normal.

CHAPTER 6

Changes in hypothalamic and peripheral noradrenergic activity in response to acute changes in arterial pressure in conscious rats

6.1 Introduction

There is a large body of evidence to suggest the involvement of the sympathetic nervous system in nearly all models of experimental hypertension (Abboud, 1982). Similarly, alterations in central noradrenergic mechanisms have been described and linked to enhanced sympathetic activity in several forms of experimental hypertension (see Historical review). Most of the information concerning the role of central and peripheral noradrenergic pathways in the control of arterial pressure has been obtained by studying either the effects of acute and chronic sino-aortic denervation (De Quattro et al., 1969; Chalmers et al., 1979) or lesions of the nucleus tractus solitarii (Doba et al., 1974), the primary site of termination of cardiovascular afferent fibers (Cottle et al., 1964; Crill et al., 1968; Biscoe et al., 1970). In the last chapter it was demonstrated that 3 days after ADN transection in the rat, arterial pressure is elevated concomitant with an increased activity of noradrenergic neurons in the hypothalamus and brainstem, as well as an increased sympathetic activity to peripheral organs, particularly skeletal muscle. Taken together, the data obtained from these and other studies

suggest that the augmented sympathetic activity which results from the release of baroreceptor inhibition (Doba et al., 1974; Laubie et al., 1979) may be mediated by excitation of central noradrenergic neurons. However all of these studies which report changes in noradrenergic activity have also reported an elevated arterial pressure. Therefore it is not clear if the altered noradrenergic activity observed in the central structures in these studies is the cause or the result of the hypertension. Furthermore, even though a major baroreceptor reflex was removed in the ADN sectioned rats all the other baroreceptor reflex mechanisms were intact. Thus the altered noradrenergic mechanisms in these studies cannot be attributed totally to the removal of aortic baroreceptors alone.

The present study was done to investigate the cause and effect relationship between noradrenergic mechanisms in the hypothalamus and sympathetic output to kidney, intestine and skeletal muscle in response to changes in arterial pressure (baroreceptor input) in normotensive conscious rats. The turnover of NE was measured in the anterior and posterior hypothalamus (known depressor and pressor areas respectively, (see Historical review)) as well as peripherally in the kidney, intestine and skeletal muscle in response to acute changes in arterial pressure induced by either infusion of nitroprusside (vasodilator) or phenylephrine (vasoconstrictor), or controlled hemorrhage.

6.2 Methods

Studies were done using male Wistar rats (Canadian Breeding Laboratories, St. Constant, Quebec or Woodlyn Farms, Guelph, Ontario) weighing 250-325 g. The rats were allowed at least one week to acclimatize to the laboratory animal facility after delivery from the breeder. A 12-hour-light cycle and 20-22 °C room temperature was maintained in the animal housing area. The rats were allowed water and food ad libitum. All experiments were performed between 10 a.m. and 2 p.m. to standardize the effects of circadian rhythm.

One day prior to the experiment, rats were anesthetized with methoxyflurane anesthesia (Pitman-Moore, INC., Washingtion Crossing, N.J.) and were implanted with femoral arterial and venous cannulae which were exteriorized through the skin in the back and plugged. On the day of the experiment, mean arterial pressure was recorded on a Grass model 7 polygraph in conscious, freely moving rats by attaching the arterial cannula to a Statham pressure transducer by a length of Tygon tubing. Arterial pressure was recorded for one hour prior to, and during the experiment. Heart rate was determined using a tachograph triggered by the arterial pulsations.

6.2.1 Measurement of tissue NE concentration after inhibition of tyrosine hydroxylase as an index of NE turnover

Animals were randomly assigned to four experimental groups: 1) saline-infused, 2) nitroprusside-infused, 3) hemorrhaged, 4) phenylephrine-infused. One group of animals with no drug treatment served as zero time controls. Thirty minutes before infusion of drugs, saline or hemorrhage, all rats in the experimental groups received α-methyltyrosine (300 mg/kg i.p.).

A decrease in blood pressure was maintained for 60 min by infusion of sodium nitroprusside (20-40 ug/kg.min i.v., Sigma Chemical Co.) through the venous cannula using a Harvard infusion pump. Arterial pressure was also decreased by withdrawing blood via the arterial cannula to maintain the arterial pressure of the rat at a level at least 30 mmHg below control level (7.1 $\pm$ 0.8 ml total volume removed). An acute hypertension was maintained for 60 min by infusion of phenylephrine hydrochloride (10-15 µg/kg.min i.v., Sigma Chemical Co.). The control rats received a saline infusion (10-20 µl/min) comparable in volume to the drug infused animals for the same period.

In all experimental groups, 90 minutes after α-methyltyrosine administration, the rats were killed by cervical dislocation. The brain, left kidney, a piece of skeletal muscle from the hindlimb of all animals and a

section of the duodenum in some animals (hypotensive groups only) were quickly removed. The brain was dissected on an iced glass plate to remove the hypothalamus, which was defined for this study as the tissue from the optic chiasma to the mammillary bodies rostrocaudally, from the dorsal aspect of the third ventricle to the ventral surface dorsoventrally (~5 mm from the ventral surface to the third ventricle), and laterally to the edge of the internal capsule. Note that this is a smaller section than the one presented in the previous Chapter. For further detail refer to the Histology in Chapter 4. The hypothalamus was then further divided equally into anterior and posterior sections by a coronal transection. Tissue samples were weighed, homogenized in cold perchloric acid, and centrifuged at 4.0 °C for 15 minutes at 30,000 x g; the supernatants were frozen at -75 °C. NE was assayed using an alumina extraction followed by an HPLC separation and finally electrochemical detection. Dihydroxybenzylamine (DHBA Sigma) was used as the internal standard in each tissue sample to quantify the amount of NE in each sample (see Chapter 4).

6.2.2 Data analysis

All data are reported as mean ± SE. Differences in the turnover of NE were inferred by comparing the values for tissue concentration of NE 90 minutes after α-methyltyrosine as a percent of the average tissue concentration of NE obtained from zero time controls. A low percentage of NE

remaining 90 min after inhibition of tyrosine hydroxylase would imply a high turnover of NE in that tissue (Brodie et al., 1966; Salzman et al., 1979; Sharman, 1981). Comparisons between the 90 min values as a percent of zero time controls for control groups and the experimental groups were done using the Duncan's Multiple Range test (Duncan, 1955). A $P < 0.05$ was considered to indicate statistical significance.

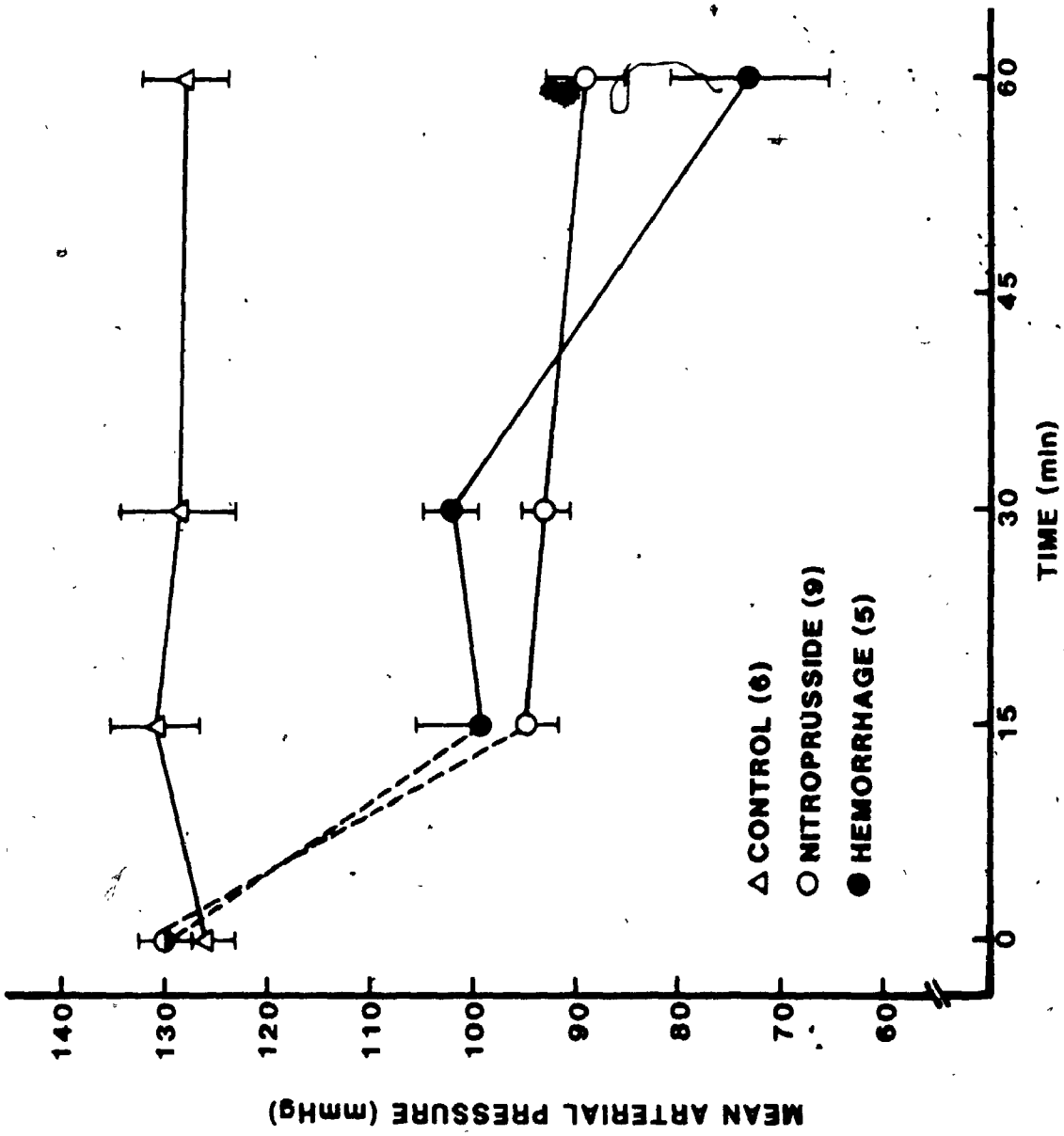
6.3 Results

6.3.1 Arterial pressure and heart rate

The control, saline-infused groups had no significant change in arterial pressure and heart rate during the 60 minutes of infusion (Figure 23, 24). Mean arterial pressure decreased by an average of 35 mm Hg initially and remained an average 38 mm Hg below control mean arterial pressure during the 60 min of nitroprusside infusion (Figure 23). Heart rate changed initially by an average of 100 beats/min and remained an average of 60 beats/min above control level throughout the hypotensive period (Figure 24). Similarly arterial pressure decreased by an average of 32 mm Hg initially and remained an average of 40 mm Hg below control mean arterial pressure during the 60 min period of hemorrhage (Figure 23). In response to such a change in arterial pressure there was an initial increase of

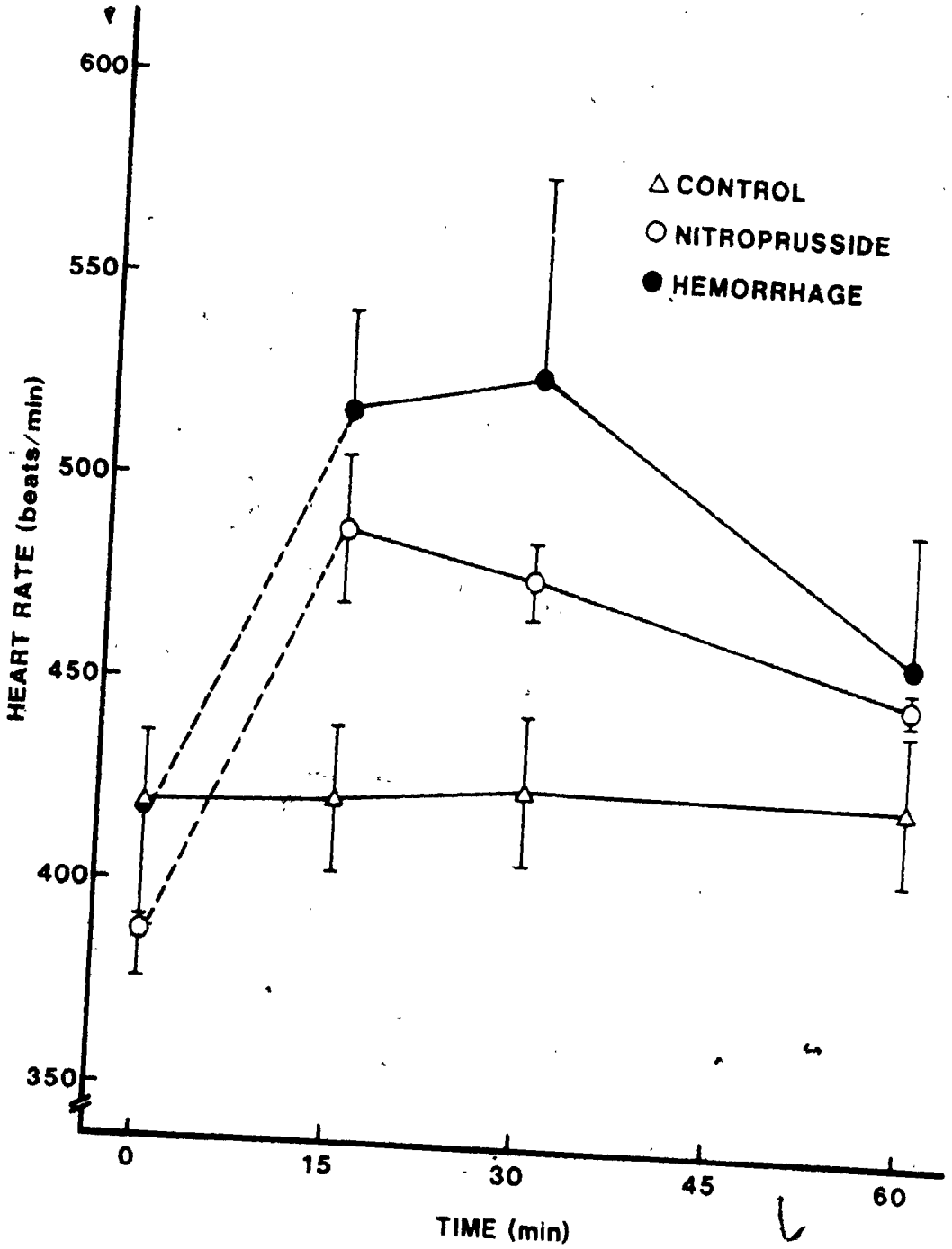
FIGURE 23

The effect of i.v. infusion with sodium nitroprusside (20-40 µg/kg.min) or controlled hemorrhage on arterial pressure in conscious rats. The zero time values represent the arterial pressure before the acute hypotensive period. The control group received an infusion of heparinized saline comparable in volume to the nitroprusside-infused group (10-20 µl/min). Numbers in parentheses represent number of animals/group. The values represent the mean ± S.E.


MEAN ARTERIAL PRESSURE (mmHg)
140
130
120
110
100
90
80
70
60
0
15
30
45
60
TIME (min)
△ CONTROL (6)
○ NITROPRUSSIDE (9)
● HEMORRHAGE (5)

FIURE 24

The effect of i.v. infusion with saline (n = 6) or sodium nitroprusside (20-40 μg/kg.min) (n = 5) or controlled hemorrhage (n = 4) on heart rate. Format as in Figure 23. The values represent the mean ± S.E.


600
550
500
450
400
350
HEART RATE (beats/min)
△ CONTROL
○ NITROPRUSSIDE
● HEMORRHAGE
0
15
30
45
60
TIME (min)

100 beats/min in heart rate which was sustained by an average of 50 beats/min above control during the 60 min experimental period (Figure 24). Phenylephrine infusion produced an average increase of 50 mm Hg in arterial pressure initially and the pressure was maintained an average of 24 mm Hg higher than the control mean arterial pressure for 60 min infusion period (Figure 25). Heart rate decreased initially by an average 100 beats/min and remained an average of 90 beats/min below control heart rate throughout the acute hypertensive period (Figure 26). The reciprocal relationship between the changes in blood pressure and heart rate illustrated an operational baroreceptor reflex arc in all the groups examined in this Chapter and the next Chapter (Figure 27). It should be noted that there was no significant difference between the various groups in terms of their arterial pressure at the start of the turnover study. In addition, α-methytyrosine administration had no significant effect on arterial pressure.

FIGURE 25

The effect of i.v. infusion with phenylephrine hydrochloride (10-15 µg/kg.min) on arterial pressure. Format as in Figure 23. The values represent the mean ± S.E.

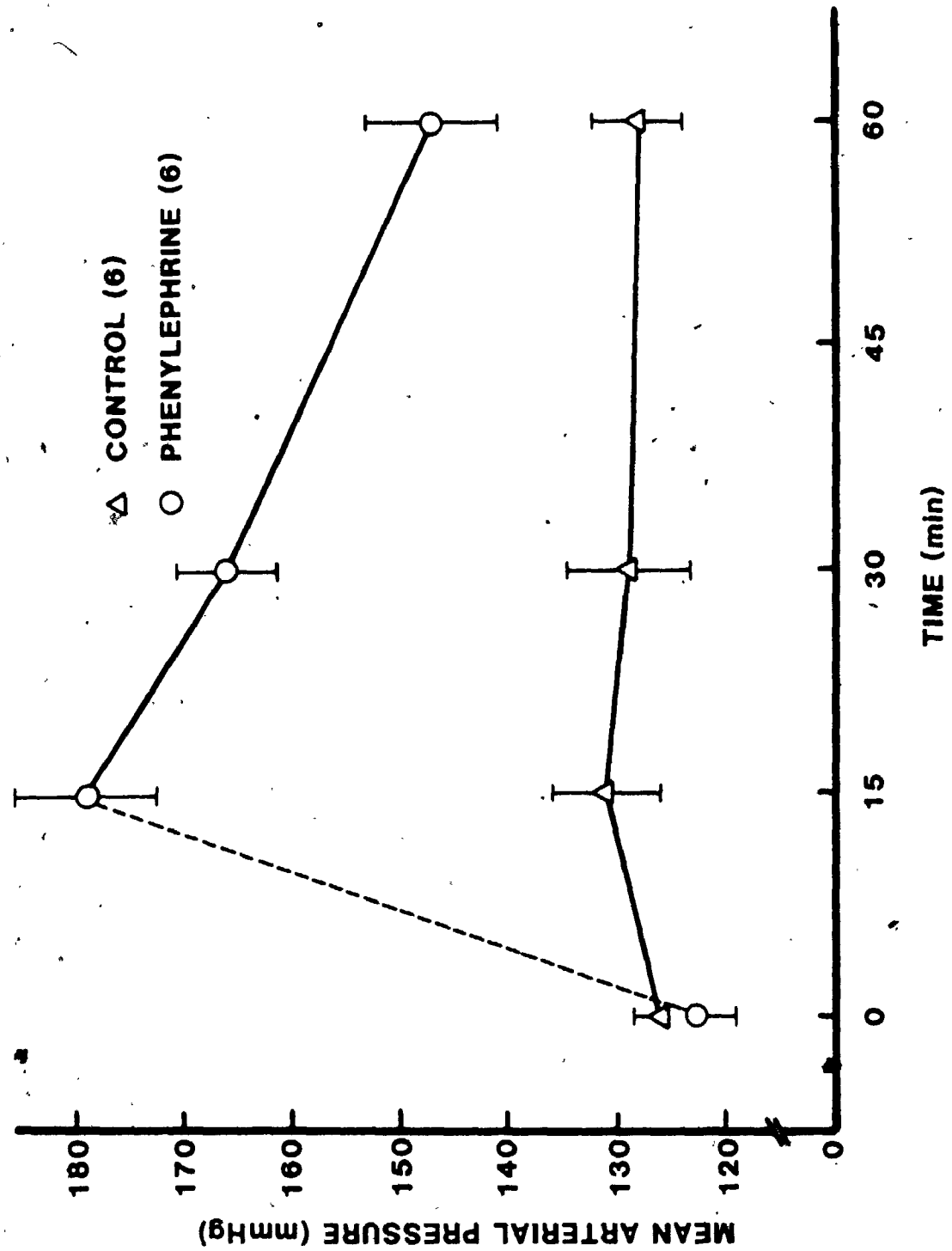

MEAN ARTERIAL PRESSURE (mmHg)
180
170
160
150
140
130
120
0
0
15
30
45
60
TIME (min)
△ CONTROL (6)
○ PHENYLEPHRINE (6)

FIGURE 26

The effect of i.v. infusion with phenylephrine hydrochloride (10-15 µg/kg.min) on heart rate. Format as in Figure 23. The values represent the mean ± S.E.

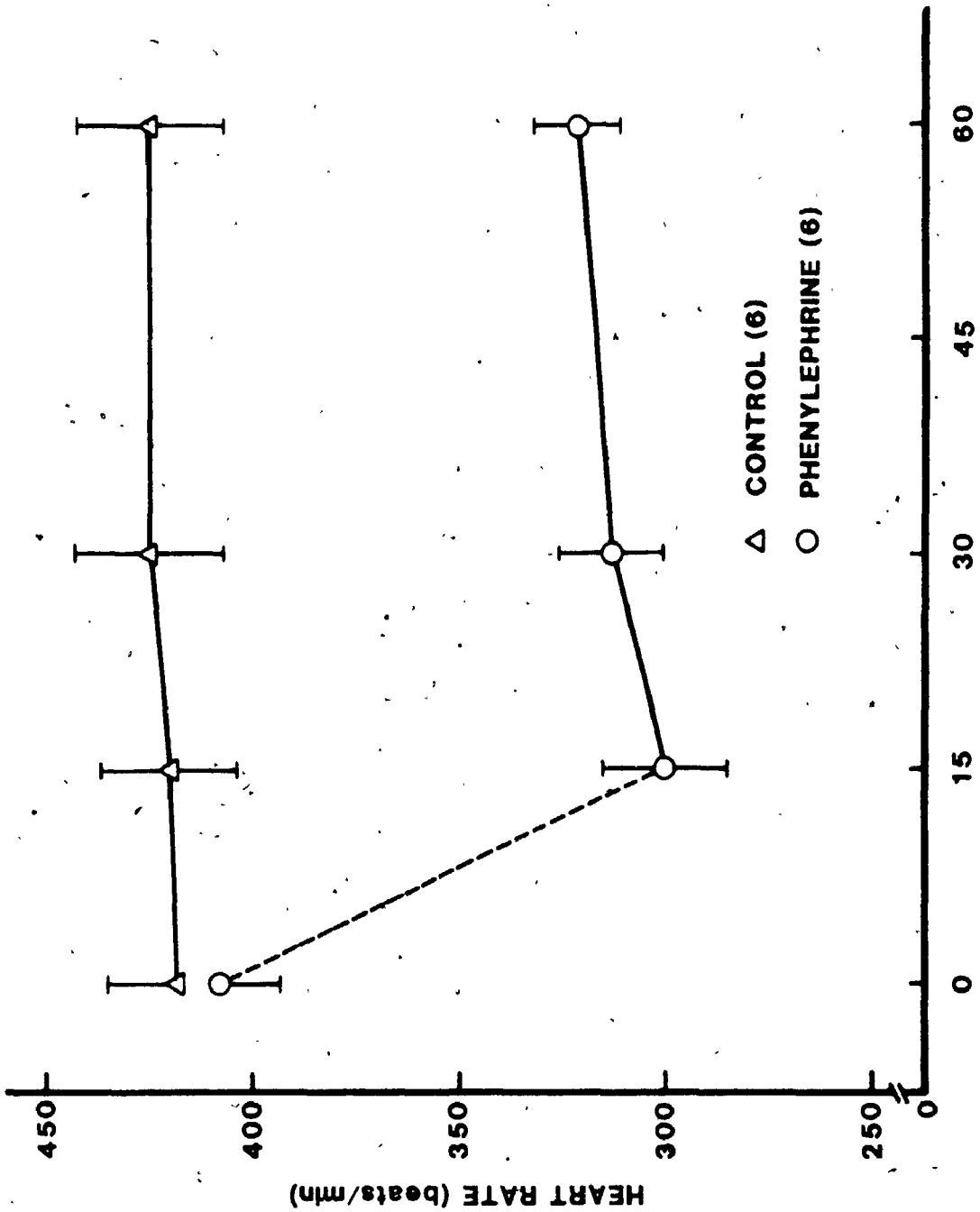
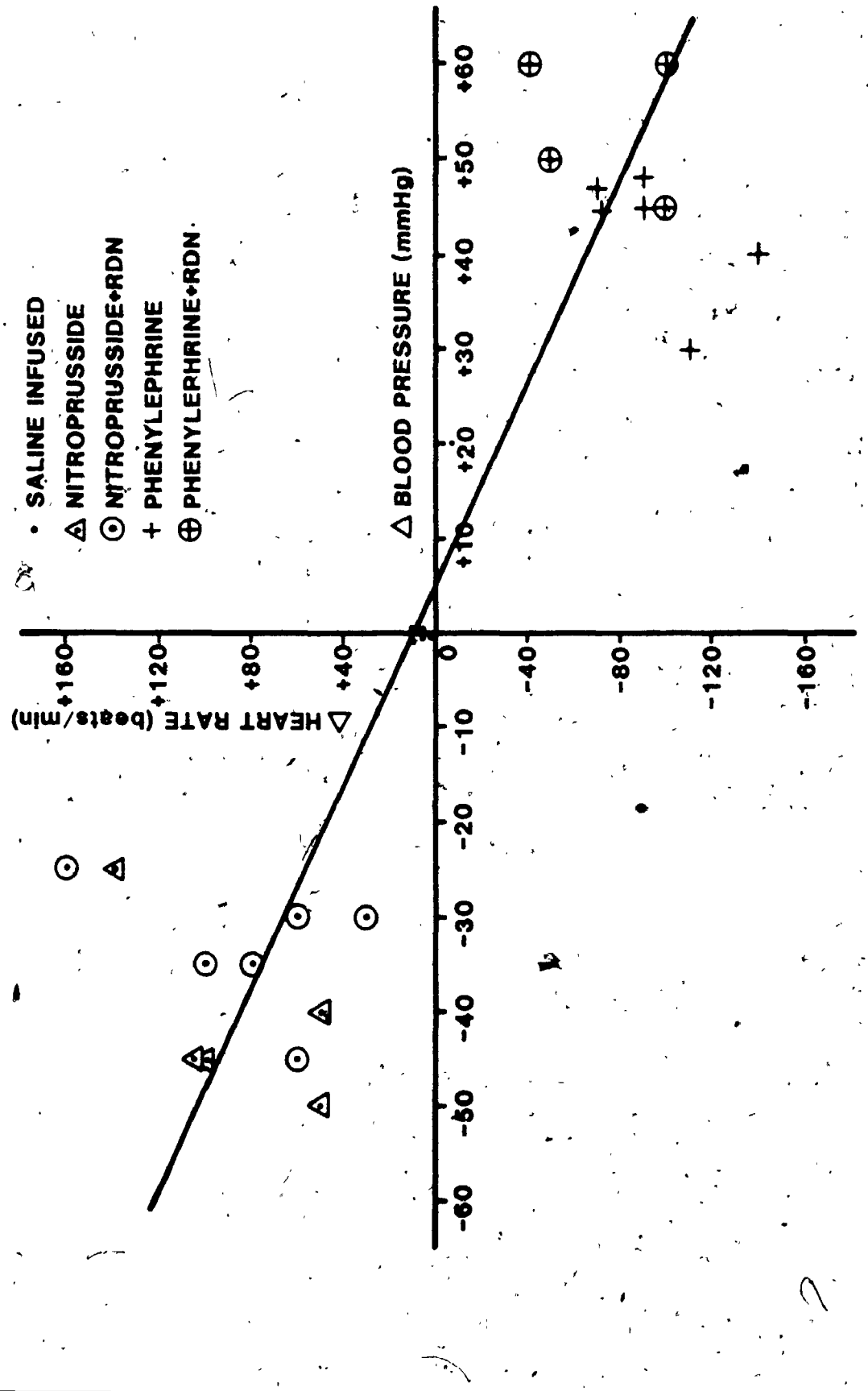

HEART RATE (beats/min)
450
400
350
300
250
0
0
15
30
45
60
TIME (min)
△ CONTROL (6)
○ PHENYLEPHRINE (6)

FIGURE 27

Relationship between changes in arterial pressure and changes in heart rate in rats with and without renal nerves. RDN-represents animals that had bilateral renal denervation. These data points were taken 30 min (halfway) through the infusion period of saline or nitroprusside or phenylephrine.


• SALINE INFUSED
△ NITROPRUSSIDE
⊙ NITROPRUSSIDE+RDN
+ PHENYLEPHRINE
⊕ PHENYLERHRINE+RDN
△ BLOOD PRESSURE (mmHg)
▽ HEART RATE (beats/min)
+60
+50
+40
+30
+20
+10
0
-10
-20
-30
-40
-50
-60
-40
-80
-120
-160
+40
+80
+120
+160

6.3.2 Measurement of tissue NE concentration after inhibition of tyrosine hydroxylase as an index of NE turnover

6.3.2.1 Effect of acute hypotension on noradrenergic activity

There was a significantly increased turnover of NE in the anterior hypothalamus of hemorrhaged animals and a tendency toward a similar increase in nitroprusside-infused animals compared to saline-infused controls (Figure 28). In the posterior hypothalamus there was a significantly higher turnover of NE in the nitroprusside-infused and hemorrhaged groups compared to saline-infused controls (Figure 28). The turnover of NE was significantly increased in kidney, intestine, and skeletal muscle of both hemorrhaged and nitroprusside-infused groups, consistent with a reflex increase in sympathetic activity (Figure 29). There were no significant differences between the nitroprusside-infused group and the hemorrhaged group in all the tissus examined.

6.3.2.2 Effect of acute hypertension on noradrenergic activity

There was no significant change in the turnover of NE in the anterior or posterior hypothalamus of the phenylephrine-infused groups compared to saline-infused controls (Figure 30). There was no significant change in

FIGURE 28

Effect of acute hypotension on concentration of NE remaining 90 min after α-methyltyrosine, expressed as a percent (±S.E.) of initial concentration in anterior and posterior hypothalamus. Values were obtained from saline-infused (control), nitroprusside-infused and hemorrhaged rats. Initial concentration of NE (ng/g wet wt.) in the anterior hypothalamus was 1264 ± 35 and 809 ± 39 in the posterior hypothalamus (n = 6). Numbers in the bars represent number of animals/group. *$P < 0.05$, compared to control animals.

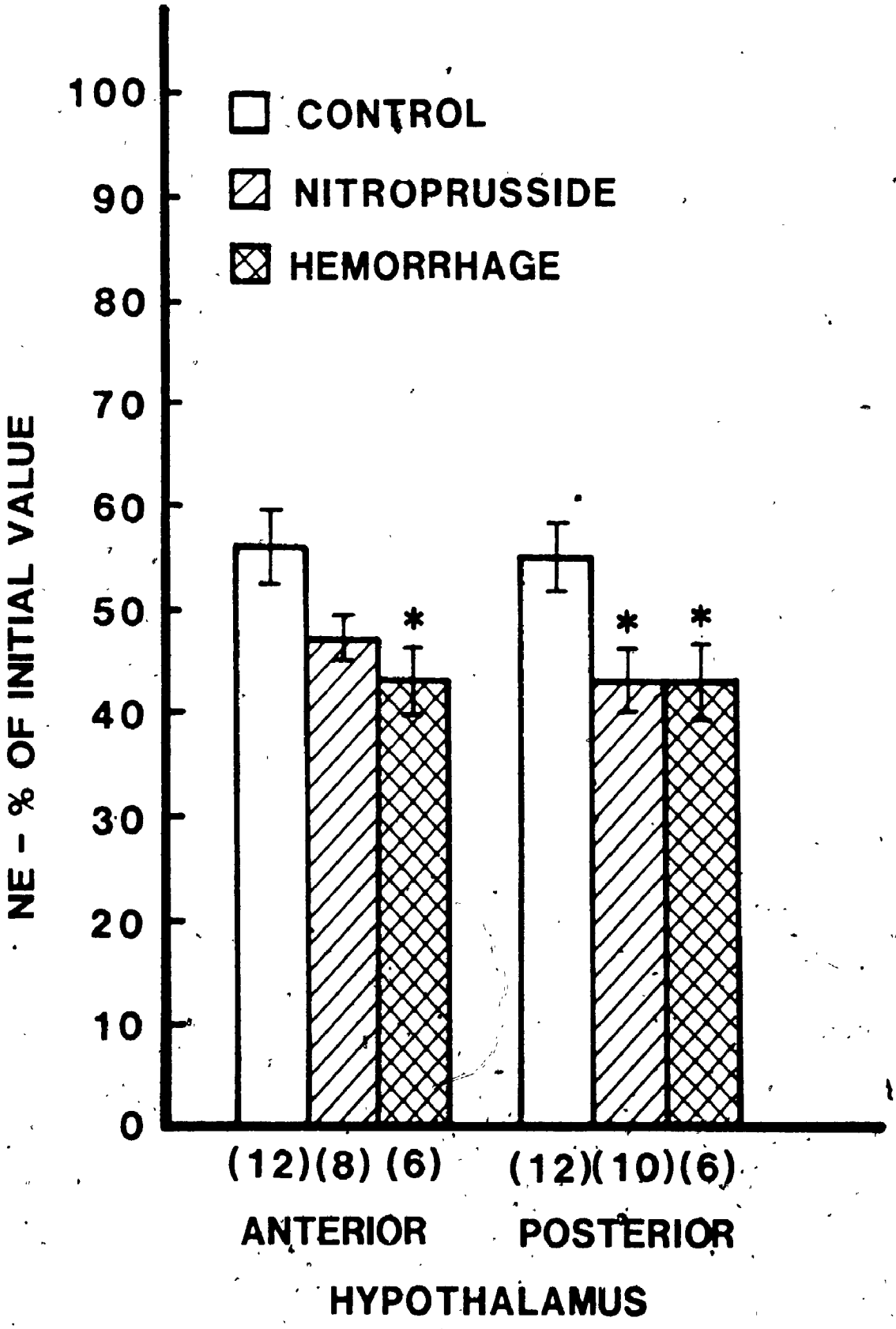

100
90
80
70
60
50
40
30
20
10
0
CONTROL
NITROPRUSSIDE
HEMORRHAGE
NE - % OF INITIAL VALUE
*
*
*
(12)(8) (6)
(12)(10)(6)
ANTERIOR
POSTERIOR
HYPOTHALAMUS

FIGURE 29

Effect of acute hypotension on concentration of NE remaining 90 min after α-methyltyrosine, expressed as a percent (±S.E.) of initial concentration in kidney, intestine and skeletal muscle. Format as in Figure 28. Initial concentration of NE (ng/g wet wt.) in the kidney was 213 ± 12, 917 ± 43 in the intestine and 66 ± 3.2 in the skeletal muscle (n = 6). *$P < 0.05$ compared to control group.

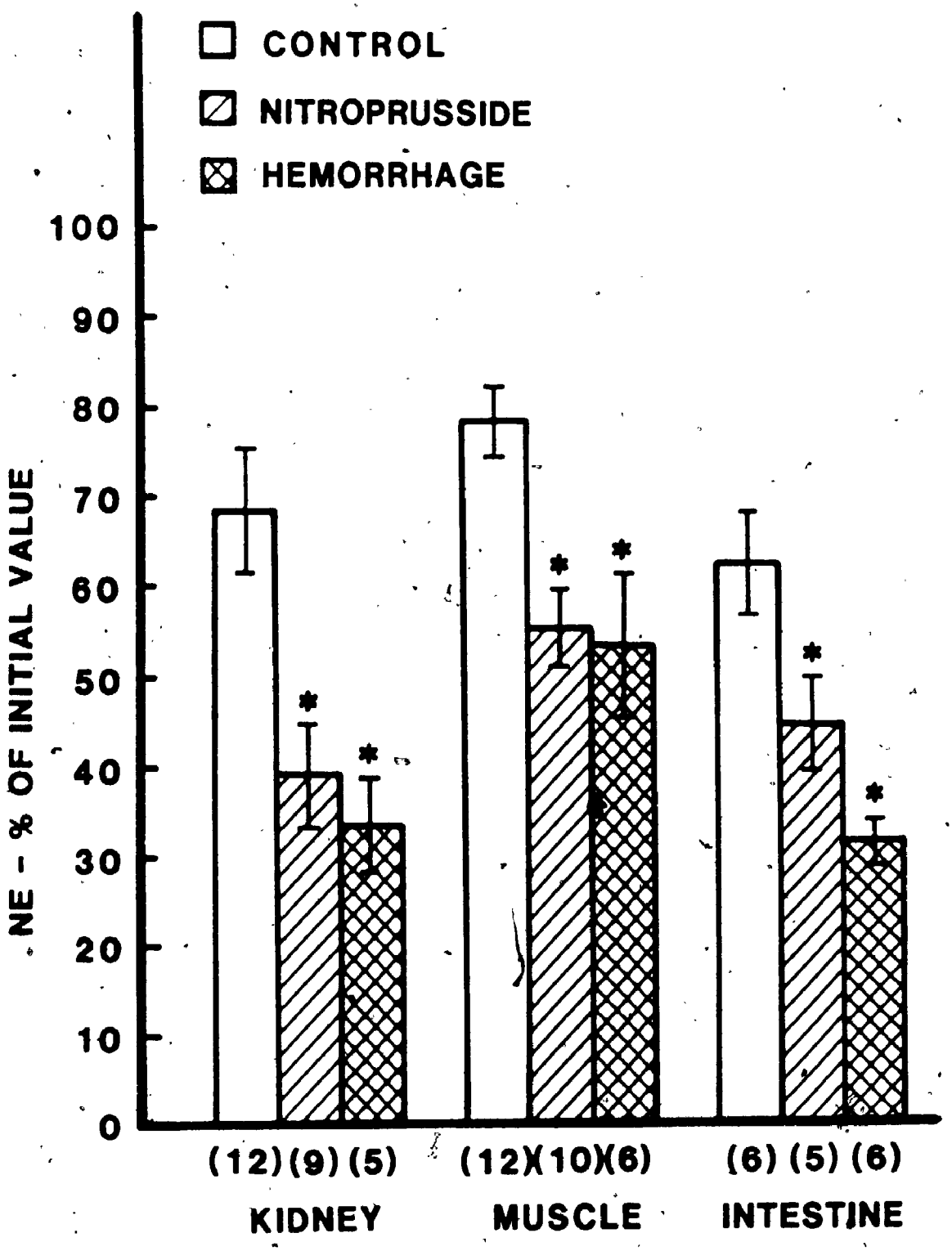
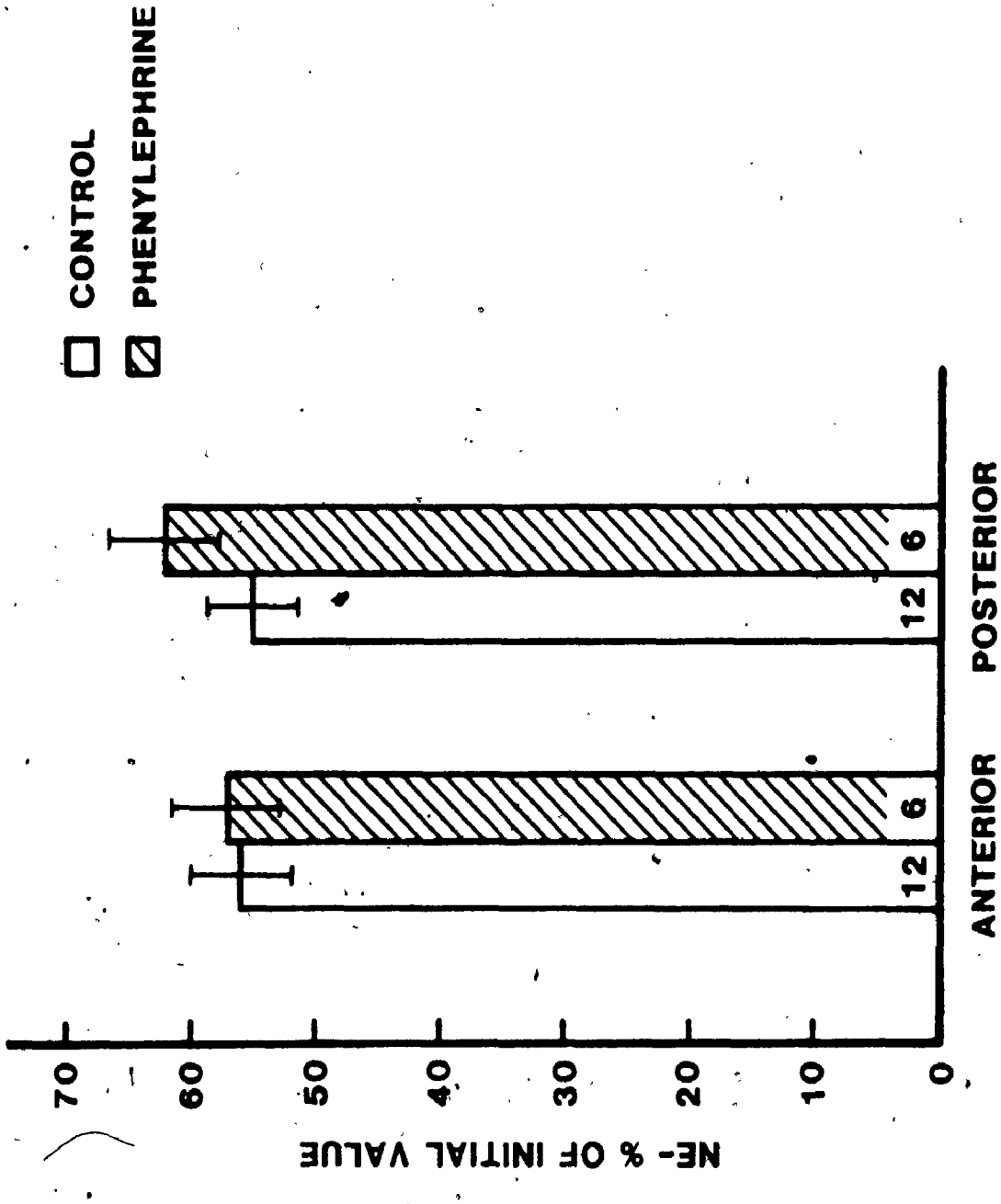

CONTROL
NITROPRUSSIDE
HEMORRHAGE
NE - % OF INITIAL VALUE
100
90
80
70
60
50
40
30
20
10
0
*
(12) (9) (5)
KIDNEY
(12) (10) (6)
MUSCLE
(6) (5) (6)
INTESTINE

FIGURE 30

Effect of acute hypertension on concentration of NE remaining 90 min after α-methyltyrosine, expressed as a percent (±S.E.) of initial concentration in anterior and posterior hypothalamus. Values were obtained from saline-infused (control) and phenylephrine-infused rats. Format as in Figure 28.


CONTROL
PHENYLEPHRINE
NE- % OF INITIAL VALUE
70
60
50
40
30
20
10
0
12
6
12
6
ANTERIOR
POSTERIOR
HYPOTHALAMUS

the turnover of NE in kidney, skeletal muscle and intestine in response to an elevated arterial pressure (Figure 31).

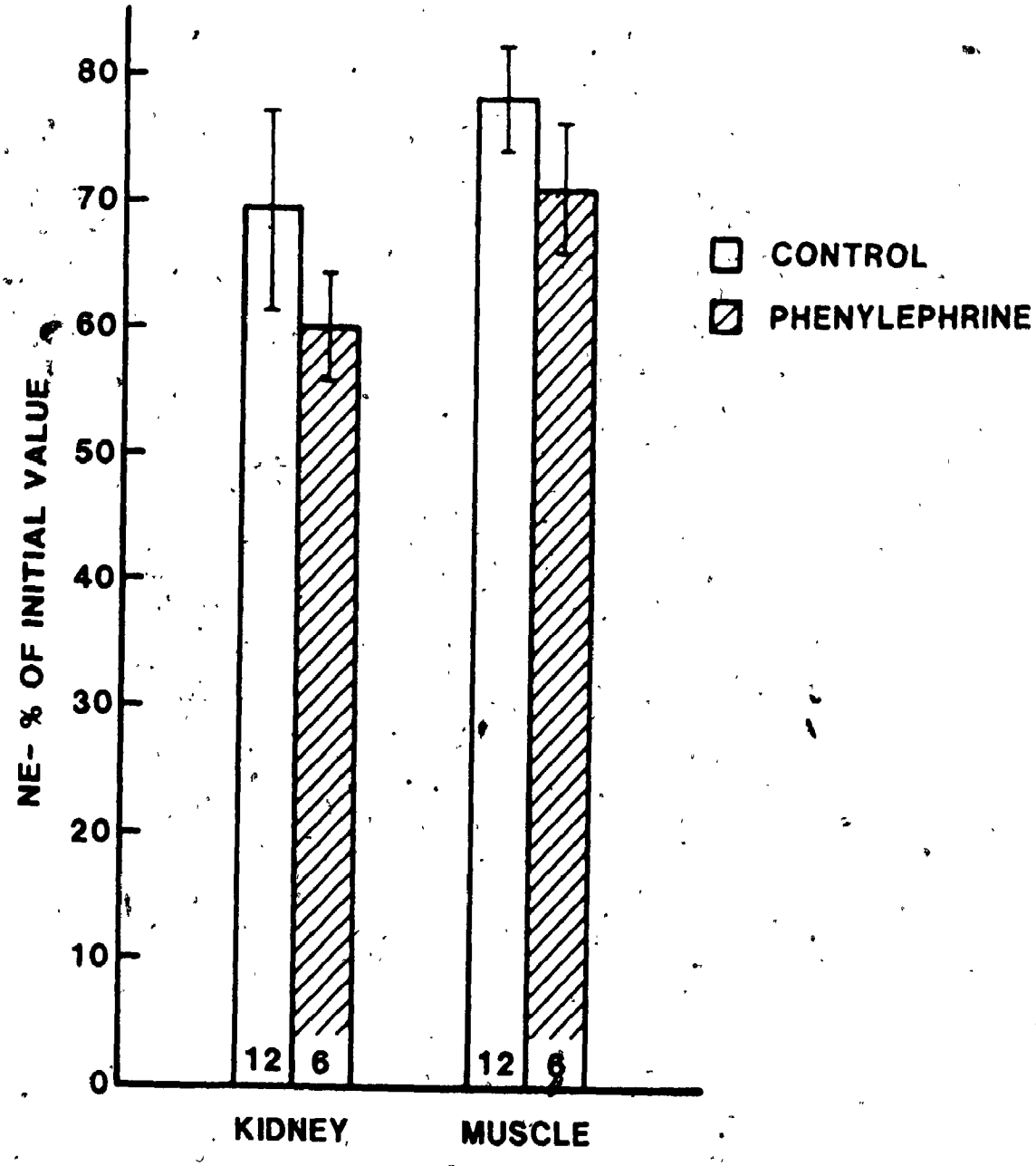
6.4 Discussion

The vasoactive drugs nitroprusside and phenylephrine elicited the expected depressor and pressor responses, respectively, and resulted in an increase (nitroprusside) or decrease (phenylephrine) in heart rate throughout the 60 min infusion period, suggesting that the blood pressure change elicited a compensatory reflex response indicative of an operational baroreflex arc. A similar reflex response in the heart rate was observed in response to hemorrhage. The vasoactive drugs were used to artificially manipulate the peripheral resistance as the action of these agents has been reported to be mediated by a direct action on vascular smooth muscle rather than via alteration in sympathetic neuronal function (Eckstein et al., 1962; Verhaeghe et al., 1976).

This study demonstrated for the first time that an acute decrease in arterial pressure in conscious rats by either nitroprusside infusion or hemorrhage caused a significant increase in the turnover of NE in the posterior hypothalamus as well as an increased NE turnover in all the peripheral organs examined. These results suggest that there is a direct relationship between changes in the noradrenergic activity in the posterior hypothalamus and

FIGURE 31

Effect of acute hypertension on concentration of NE remaining 90 min after α-methyltyrosine, expressed as a percent (±S.E.) of initial concentration in kidney and skeletal muscle. Format as in Figure 30.


80
70
60
50
40
30
20
10
0
NE- % OF INITIAL VALUE
12
6
12
6
KIDNEY
MUSCLE
CONTROL
PHENYLEPHRINE

peripheral sympathetic activity. It is conceivable that elevated noradrenergic activity in the hypothalamus may be responsible for the increased peripheral sympathetic activity.

In the literature, similar observations of increased peripheral sympathetic activity have been reported by Takimoto et al. (1981) who measured an elevated tyrosine hydroxylase activity in various cardiovascular tissues, and increased sympathetic efferent impulse flow (measured by recording from pre- and postganglionic sympathetic nerves) in response to nitroprusside- induced hypotension in anesthetized rabbits.The same group also observed a two-fold increase in plasma catecholamines indicative of an overall increase in sympathetic activity; however this group did not attempt to measure changes in the CNS. On the other hand Wijnen et al. (1978) observed an increased turnover of NE in specific nuclei of the hypothalamus in conscious rats subjected to a controlled hemorrhage or by the administration of guanethidine (depletes NE from terminals); however these investigators did not provide a measure of peripheral sympathetic activity.

Nevertheless all these findings are consistent with observations reported in the previous Chapter made in animals that had specific baroreceptor input to the central nervous system removed surgically by transection of the aortic depressor nerve (ADN). ADN transection caused a significant increase in arterial pressure and NE turnover in

the hypothalamus, kidney and skeletal muscle, suggesting that surgical removal of an inhibitory input to the central nervous system by denervating aortic baroreceptors caused an excitation of noradrenergic activity in the hypothalamus and peripheral organs. It is of interest to note that in contrast to the observation of increased NE turnover in all peripheral organs in the acutely hypotensive animals in this study, in the ADN transected rats in the previous study there was a differential change in turnover of NE in the peripheral organs. One possible explanation for the difference could stem from the fact that in the acutely hypotensive rats all of the arterial baroreceptors (ADN and carotid sinus) are "unloaded", whereas in the ADN transected rats only the aortic baroreceptor are surgically removed but the other major arterial baroreceptors are actually loaded due to an elevated arterial pressure. Such conflicting afferent baroreceptor information in the ADN transected rat may be responsible for the different response observed in the peripheral sympathetic system.

In recent years, several reports suggest the participation of central noradrenergic neurons (hypothalamic) in the normal control of arterial pressure (Chalmers, 1975; Antonaccio, 1977). Similar to the ADN transected rats, Chalmers et al. (1979) have reported an increased turnover of NE in the hypothalamus and spinal cord of sinoaortic denervated rats. In addition, Sinha et al. (1980) have demonstrated an increased release of NE in

the superfusate of the posterior hypothalamus (obtained by means of a push-pull cannula) during a fall in blood pressure elicited by infusion of nitroprusside or hemorrhage in anesthetized cats. Subsequently Philippu et al. (1981) have reported an increased release of endogenous catecholamines in response to hypotension produced by nitroprusside infusion in conscious rabbits. Furthermore, application of NE to the posterior hypothalmus evokes an excitatory sympathetic response which can be blocked by adrenoceptor blocking drugs (Philippu et al., 1973; Antonaccio et al., 1975). All these studies considered together suggested 1) the presence of an inhibitory influence of baroreceptors on the noradrenergic activity in the hypothalamus, and 2) an excitatory role for the noradrenergic activity of the posterior hypothalamus in the initiation of sympathetic tone in the periphery.

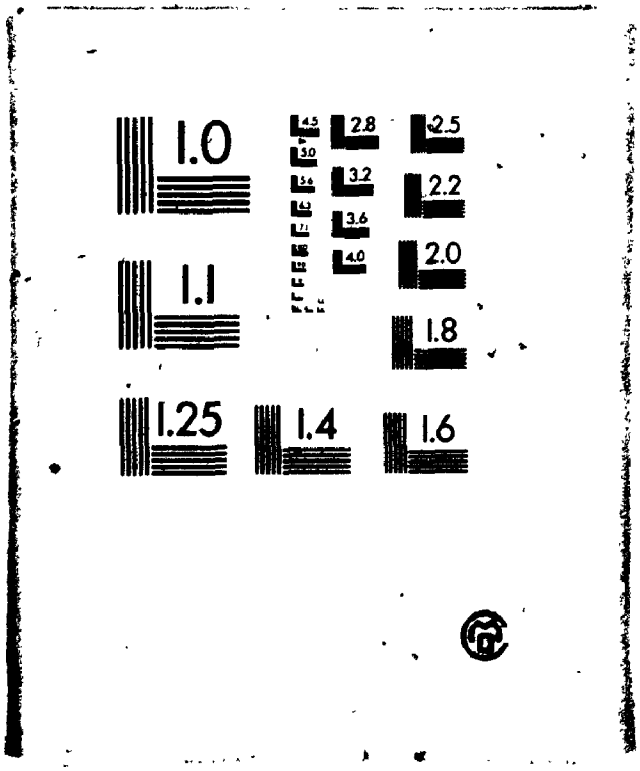
Such an hypothesis would predict a direct relationship between the noradrenergic activity in the hypothalamus and peripheral organs. Consistent with this hypothesis, when a plot was made of NE turnover in the hypothalamus versus NE turnover in the muscle using data from acutely hypotensive rats and ADN transected rats it was observed that there was a significant correlation between the two variables (Figure 32). However, it is recognized that such a correlation does not mean a conclusive cause and effect relationship.

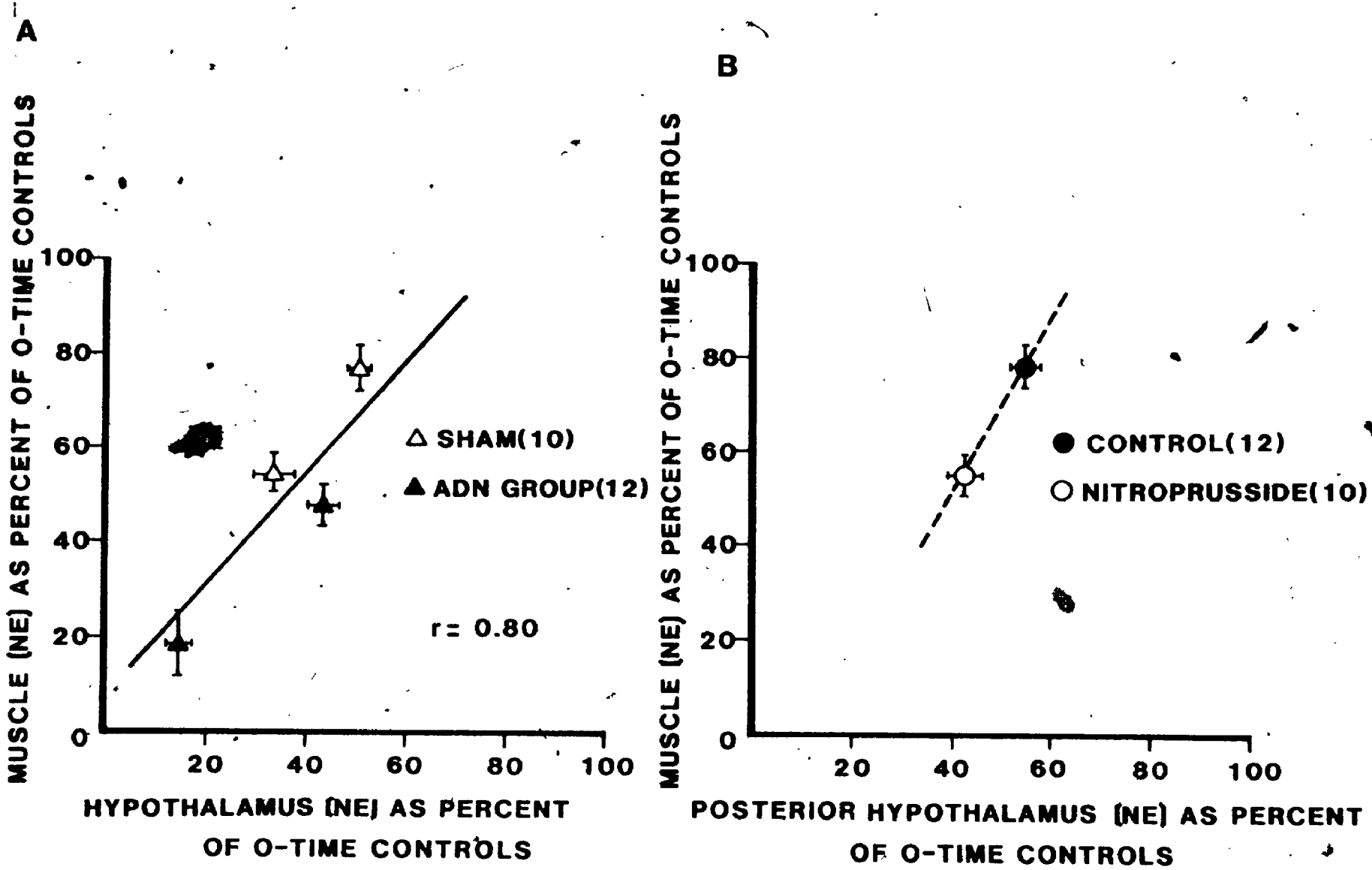
FIGURE 32

A) Relationship between index of NE turnover in hypothalamus versus index of NE turnover in skeletal muscle in rats with and without ADN. The index NE turnover is represented as the percent of NE left 4h and 8h after inhibition of tyrosine hydroxylase with α-methyltyrosine in the two groups.

B) Relationship between index of NE turnover in posterior hypothalamus versus index of NE turnover in skeletal muscle in rats with saline-infusion and nitroprusside infusion. Values represent percent of NE (±S.E.) left 90 min after inhibition of tyrosine hydroxylase.

3




A
MUSCLE (NE) AS PERCENT OF O-TIME CONTROLS
100
80
60
40
20
0
20
40
60
80
100
△ SHAM(10)
▲ ADN GROUP(12)
r= 0.80
HYPOTHALAMUS (NE) AS PERCENT
OF O-TIME CONTROLS
B
MUSCLE (NE) AS PERCENT OF O-TIME CONTROLS
100
80
60
40
20
0
20
40
60
80
100
● CONTROL(12)
○ NITROPRUSSIDE(10)
POSTERIOR HYPOTHALAMUS (NE) AS PERCENT
OF O-TIME CONTROLS

In contrast to these results, previous observations in SHR (Yamori 1972, Patel et al., 1981) and DOCA-salt (Nakamura et al., 1971; Van Ameringen et al., 1977) hypertensive rats have suggested a reciprocal relationship between central and peripheral noradrenergic mechanisms. However, in these latter studies it is not clear if the changes observed in noradrenergic mechanisms in hypertensive models are a cause or effect of the hypertension. Although studies with DOCA-salt and SHR models of hypertension used similar hypothalamic sections as in the present study, it is plausible that one explanation for the controversy could be that the changes occuring in various small nuclei within relatively large brain sections may be opposing one another under these various different situations. Secondly, the changes observed in the present study were due to a relatively short term perturbation (60 min) whereas the results from SHR and DOCA-salt study were obtained after long term perturbation lasting several weeks. Furthermore there is a controversy as to the proper control for the SHR (Patel et al., 1981), making it difficult to assess if the changes observed in noradrenergic activity in SHR are real or merely attributable to genetic differences. In addition, de Champlain et al. (1967) have reported a reduced retention and storage of norepinephrine in the peripheral organs of DOCA-salt hypertensive rats, suggesting that changes observed in the turnover of NE in the peripheral organs may be independent of the change reported in the brain. On the other hand van Ameringen et al. (1977) have shown that the

central change in noradrenergic activity persists after normalizing both arterial pressure and sympathetic activity by spinal cord transection in DOCA-salt hypertensive rats. The latter results would suggest that the central noradrenergic mechanisms in this model are involved in the initiation of change in the peripheral noradrenergic activity.

In the present study there was a significant increase in NE turnover in the anterior hypothalamus of the hemorrhaged group compared to saline-infused controls; however, no such change was observed in the nitroprusside infused group. Furthermore there was a tendency toward a greater increase in the turnover of NE in the peripheral organs of the hemorrhaged group compared to the nitroprusside-infused group. One possible explanation for these observations may be that the extra afferent information (because of volume loss) in the case of hemorrhaged animals would cause a greater sympathetic activity. Such a contention is conceivable since Svensson et al. (1979) have reported that reflex mechanisms involved in conserving intravascular volume also uses the noradrenergic mechanisms of the hypothalamus and medulla to activate the efferent sympathetic activity.

According to the hypothesis presented above, loading the baroreceptors by elevating the arterial pressure should result in a decreased turnover of NE in the posterior hypothalamus and the peripheral organs. However there was

no significant difference in NE turnover in all the tissues examined from the acutely hypertensive group when compared to the saline- infused controls. Similarly, Takimato et al. (1981) were unable to elicit a reduction in tyrosine hydroxylase activity in either sympathetic ganglia or vasculature in response to an increase in arterial pressure produced by infusion of phenylephrine in anesthetised rabbits. These results suggest that there was little sympathetic tone in the peripheral organs under resting conditions. Similarly results from another project (unpublished) showed that the turnover of NE did not decrease further in peripheral organs of animals that were treated with hexamethonium (a ganglionic blocker) prior to phenylephrine infusion (Appendix 2). This would suggest that the turnover of NE observed in the peripheral organs of phenylephrine infused group have a very minor component if any from sympathetic nerve activity.

It should also be noted that according to Philippu and coworkers (Philippu et al.; 1979; 1981) the acutely hypertensive group should have showed an increased turnover of NE in the anterior hypothalamus. No such response was observed in the present study. This controversy between my study and the literature remains unresolved. A possible explanation may be that the two techniques (push-pull and turnover of NE) being used may be sampling different areas of the hypothalamus in terms of size. In the present study the tissue referred to as anterior and posterior

hypothalamus are relatively large compared to the areas perfused during push-pull by Philippu and co-workers. Consistent with results from the present study, Sole et al. (1980) also found an increased turnover in both anterior and posterior hypothalamus in response to ischemia of the left ventricle. In addition, Moore et al. (1979) describe the anatomy of the noradrenergic terminals in the hypothalamus as neurons transversing the entire hypothalamus with varicosities along the entire length. In light of such evidence it is conceivable that an increased activity in the noradrenergic neurons would cause an increased NE turnover in both anterior and posterior hypothalamus simultaneously.

In conclusion the results in this study support and extend the observation from the previous Chapter suggesting the presence of an inhibitory influence from the baroreceptors to the noradrenergic adtivity in the posterior hypothalamus and a direct relationship between changes in hypothalamic and peripheral noradrenergic activity (Figure 32).

CHAPTER 7

Effect of renal denervation on the noradrenergic response to removal of ADN and acute changes in arterial pressure

7.1 Introduction

Renal nerves and their possible role in the control of arterial pressure have been an area of much interest in recent years. Several studies have shown that an enhanced noradrenergic influence on the kidney, produced by chronic intrarenal infusion of norepinephrine, may lead to chronically elevated arterial pressure (Katholi et al., 1977; Cowley et al., 1979). In addition, renal denervation has been shown to delay the development of genetic hypertension (Liard, 1977; Kline et al., 1978; Winternitz et al., 1980; Diz et al., 1982) and DOCA-salt hypertension (Katholi et al., 1980), and reverse the elevated arterial pressure associated with one- and two-kidney, one-clip Goldblatt hypertension (Katholi et al., 1982a; 1982b) and one-kidney Grollman hypertension in rats (Katholi et al., 1982a). As renal function has been suggested to be a primary determinant of arterial pressure the abundance of evidence demonstrating an influence of renal sympathetic nerves on renal vascular resistance, renin release, and sodium reabsorption lends further support to the idea that renal nerves may contribute to the overall control of arterial pressure, especially in those instances when

sympathetic activity is increased (see Historical Review).

Although most of the work in this area has been directed towards understanding the physiology of efferent renal nerves, there is a growing interest in the possible influence of afferent renal nerves on the mechanisms controlling water balance and arterial pressure. Recordati (Recordati et al., 1978; 1980) has demonstrated the presence of several types of renal receptors, and several investigators have reported cardiovascular effects produced by electrical stimulation of afferent renal fibers (Ueda, 1967; Astrom et al., 1968; Aars et al,, 1970; Calaresu et al., 1976). In addition, recently it has been shown that renal denervation decreased arterial pressure in one and two-kidney, one-clip Goldblatt hypertensive rats, while normalizing a previously elevated concentration of NE in the hypothalamus and an increased plasma level of NE, suggesting a normalization of an increased peripheral sympathetic activity (Katholi et al., 1982a; Winternitz et al., 1982; Katholi et al., 1982b). The depressor effect of renal denervation was not mediated by alterations in sodium intake or excretion, water intake or excretion, creatinine clearance or renin activity (Katholi et al., 1981). It has been suggested that the depressor effect of renal denervation is secondary to alterations in hypothalamic noradrenergic activity produced by removal of elevated activity in renal afferent fibers, with a resultant decrease in peripheral sympathetic activity.

Recent studies have shown that afferent renal fibers project to hypothalamic sites known to influence neurohormonal control of the circulation (Knuepfer et al., 1980; Calaresu et al., 1981b). In addition, Calaresu et al. (1981b) have shown that a majority of single units in the hypothalamus and medulla which respond to stimulation of afferent renal fibers also respond to electrical stimulation of arterial baroreceptor afferent fibers. Therefore, it is conceivable that removal of afferent renal fibers may affect the baroreceptor control of sympathetic outflow.

In the previous two chapters I have demonstrated an increased NE turnover in the hypothalamus and peripheral organs in response to ADN transection (Chapter 5) and acute hypotension (Chapter 6). These preparations would seem to be ideal to test the hypothesis that afferent renal fibers play a role in determining the activity of hypothalamic and peripheral noradrenergic systems in animals with decreased baroreceptor input to the CNS. The present study examined the effect of renal denervation on changes in noradrenergic response to removal of baroreceptor input to the CNS.

7.2 Methods

7.2.1 Renal Denervation in ADN transected rats

7.2.1.1 Effect of renal denervation on the arterial pressure response to ADN transection

Studies were done using male Wistar rats weighing 250-300 g. In the first study 16 animals were randomly assigned to either a sham renal denervated or renal denervated group and placed in individual cages. Arterial pressure was measured using the tail cuff technique (described in Chapter 5). Renal denervation was performed 3 days prior to ADN transection. Tail cuff pressure was measured for 12 days following the ADN transection.

7.2.1.2 Denervation Technique

Surgical procedures for ADN transection are described in detail in the Method section of Chapter 5. For renal denervation, animals were anesthetized with either sodium pentobarbital (60 mg/kg, i.p.; Somnotal, M.T.C. Pharmaceuticals, Hamilton, Canada) or methoxyflurane anesthesia. The abdominal viscera were retracted to expose the kidneys. The renal arteries and veins were isolated and stripped free of connective tissue, after which 95% alcohol was applied to the vessels. In this laboratory it has been

reported previously that this technique reduces renal NE concentration by over 90% by 3 days after surgery (Kline et al., 1978). A fluorescence assay for NE or HPLC with EC was used to verify that renal denervation was successful. Sham-renal denervation surgery consisted of exposing the kidneys and vessels, but the vessels were not isolated and alcohol was not applied.

7.2.1.3 Measurement of tissue NE concentration after inhibition of tyrosine hydroxylase as an index of NE turnover

To determine whether renal denervation affected the noradrenergic response to ADN transection, the decline in tissue concentration of NE after inhibition of tyrosine hydroxylase was used as an index of NE turnover in hypothalamus and skeletal muscle. Fifty rats were divided randomly into one of four groups 1) Sham ADN + sham renal, a control group for both renal and ADN surgery 2) ADN + sham renal, an experimental group to study the effects of ADN transection, 3) Sham ADN + renal, an experimental and control group to study the effects of renal denervation, 4) ADN + renal, an experimental group to study the possible interaction between ADN transection and renal denervation. Renal denervation was done in all animals 3 days before ADN surgery. Arterial pressure (tail cuff) was measured before and after renal surgery and on Day 1 and 2 after ADN

surgery. The turnover study was done on Day 3 after ADN surgery, since I measured the changes in noradrenergic activity in brain and peripheral organs previously at the same time period (see Chapter 5). Rats within each group were further randomly divided into two subgroups, which would be used as 0-time controls for each of the four groups, and for 8 h values after inhibition of tyrosine hydroxylase with α-methyltyrosine, respectively. The use of 0-time and 8 h values can be justified on the basis that I have used this method in control and ADN-transected rats and have demonstrated linear decay curves between 0 and 8 h when data were expressed as semi-log plots of percent NE concentration remaining vs time (see Results section Chapter 5). At 0 and 8 h the rats were killed by cervical dislocation and the tissue samples were removed and processed as described in the Methods section of Chapter 5.

7.2.1.4 Data Analysis

All data are reported as mean ± S.E. Data obtained over time were analyzed using an appropriate ANOVA. Post hoc comparisons between selected means were done with Duncan's multiple range test when initial ANOVA indicated statistical differences between treatments. Comparisons involving only two means within or between groups were done using a t-test. Differences in the turnover of NE were inferred by plotting the values for tissue concentrations of

NE 8 h after α-methyltyrosine as a percent of the average tissue concentration of NE in respective 0-time controls. A low percentage of NE remaining 8 h after inhibition of tyrosine hydroxylase would imply a high turnover of NE in that tissue (see Chapter 4). Comparisons between these 8 h values were done using Duncan's test. A $P < 0.05$ was considered to indicate statistical significance.

7.2.2 Renal denervation and acute changes in arterial pressure

Studies were done using male Wistar rats weighing 250-325 g. The protocol of this experiment was identical to the one followed in the Methods section of Chapter 6 with one exception, renal denervation was performed 3 days prior to the day ADN were sectioned. Renal denervation was performed using the surgical technique described in Section 7.2.1.2 of this Chapter.

7.2.2.1 Measurement of tissue NE concentration after inhibition of tyrosine hydroxylase as an index of NE turnover

Animals were randomly assigned to three experimental groups: 1) saline-infused and renal denervated 2) nitroprusside-infused and renal denervated, and 3) phenylephrine-infused and renal denervated. A group of

animals with no drug treatment but with bilateral renal denervation served as zero time controls for renal denervated groups. It should be noted that respective control groups with intact renal nerves were used from the previous chapter, since the experiments in the present study and previous chapter were performed during the same time period.

The changes in arterial pressure with vasoactive agents were induced under the same protocol stated in the previous chapter. Similarly the rest of the protocol for the measurement of index of NE turnover in the various tissues was identical to the one described in Chapter 6.

7.2.2.2 Data analysis

All data were reported as mean $\pm$ S.E. Comparisons between the 90 min values as a percent of zero time control for renal innervated and the corresponding renal denervated groups were done using the Duncan's Multiple Range test (Duncan 1955). Again it should be noted that appropriate renal innervated control groups from the previous Chapter were used. A $P < 0.05$ was considered to indicate statistical significance.

7.3 RESULTS

7.3.1 Renal denervation in ADN-transected rats

7.3.1.1 Effect of renal denervation on the arterial pressure response to ADN transection

Renal denervation 3 days prior to ADN transection completely prevented the rise in arterial pressure normally seen after cutting the ADN (Figure 33). Heart rate responses were transient and variable, being increased by 53 $\pm$ 11 and 31 $\pm$ 9 beats/min in the sham-renal denervated and renal denervated groups, respectively, on Day 1 but returning to control levels by Day 4.

The changes in arterial pressure during the turnover study are summarized in Figure 34A. Arterial pressure on Day 2 after ADN transection was increased significantly only in the rats with ADN transection and sham renal denervation (Figure 34A). In addition, rats which received renal denervation and sham ADN transection had a significantly lower tail cuff pressure when compared to the surgical control group.

FIGURE 33

Effect of renal denervation on the arterial pressure response to ADN transection. Arrows indicate time of renal surgery and ADN transection. Both groups had the ADN cut bilaterally. Numbers in parentheses are numbers of animals/group. Values are means $\pm$ S.E. F-ratio = 51.77 (1,14) $P < 0.01$.

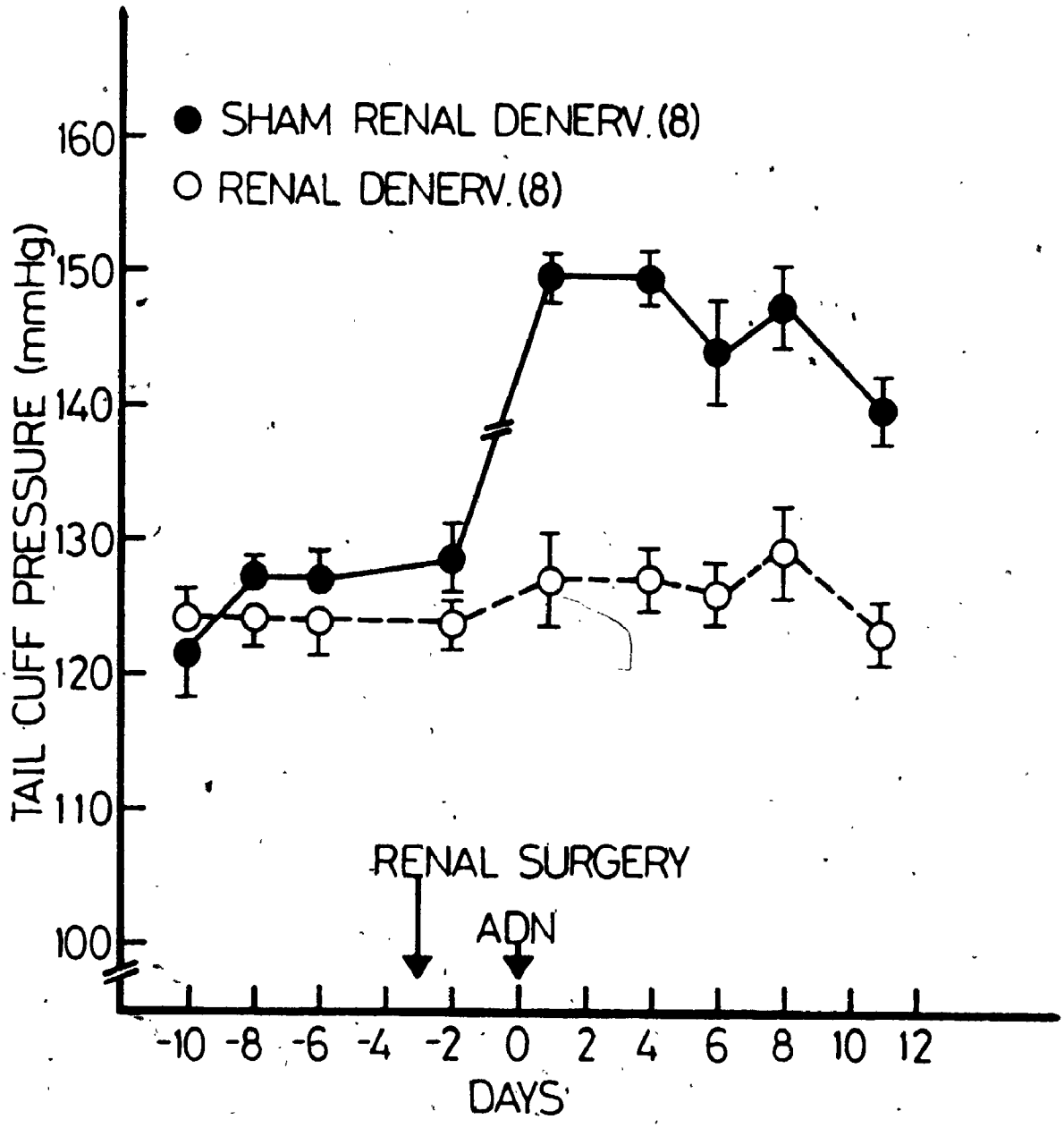
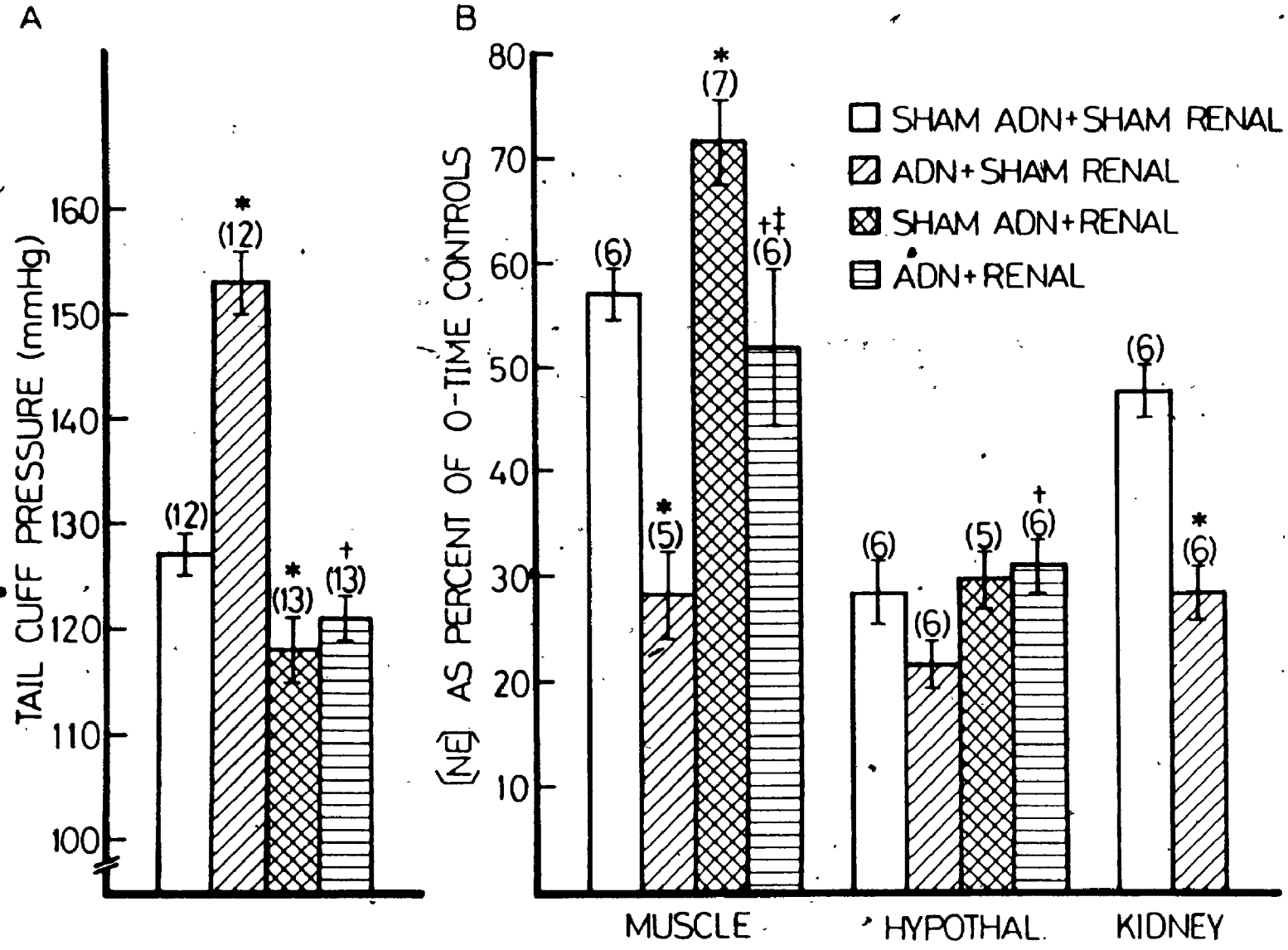

SHAM RENAL DENERV.(8)
RENAL DENERV.(8)
TAIL CUFF PRESSURE (mmHg)
160
150
140
130
120
110
100
RENAL SURGERY
ADN
-10 -8 -6 -4 -2 0 2 4 6 8 10 12
DAYS

FIGURE 34

Effect of renal denervation on arterial pressure and index of NE turnover after ADN transection.

A) Tail cuff pressure on Day 2 after ADN surgery.

B) Norepinephrine concentration 8h after α-methyltyrosine, expressed as a percent (±S.E.) of 0-time controls for each group. Values were obtained at Day 3 after ADN surgery. Numbers in parentheses are numbers of animals/group. *$P < 0.05$, compared to Sham ADN + Sham renal; +$P<0.05$ compared to ADN + Sham renal; ǂ$P < 0.05$ compared to Sham ADN + Renal (Duncan's test).

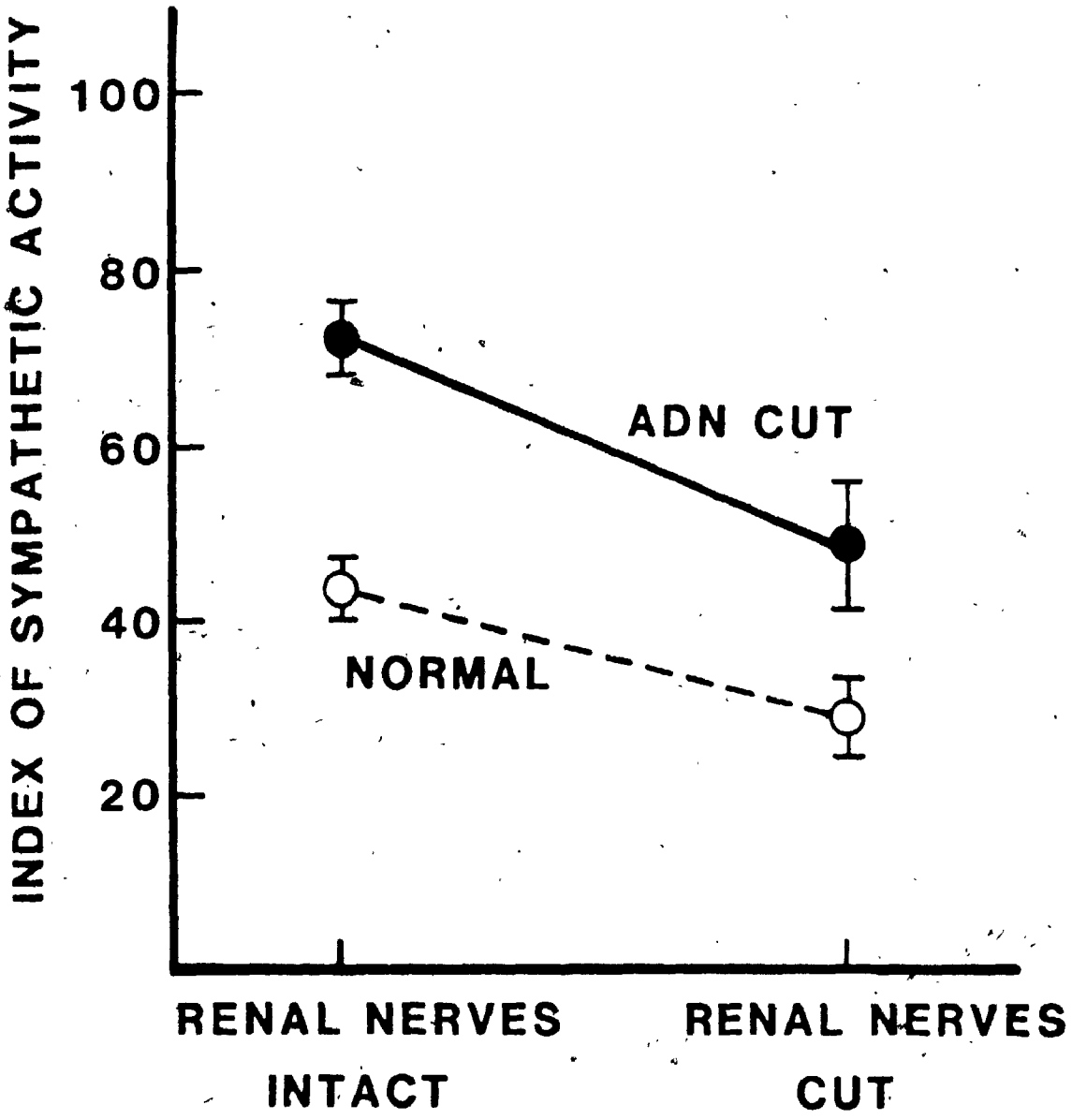

A
B
TAIL CUFF PRESSURE (mmHg)
100
110
120
130
140
150
160
(12)
(12)
(13)
(13)
[NE] AS PERCENT OF 0-TIME CONTROLS
10
20
30
40
50
60
70
80
(6)
(5)
(7)
(6)
(6)
(6)
(5)
(6)
(6)
(6)
SHAM ADN+SHAM RENAL
ADN+SHAM RENAL
SHAM ADN+RENAL
ADN+RENAL
MUSCLE
HYPOTHAL.
KIDNEY

7.3.1.2 Effect of Renal Denervation and ADN transection on Index of NE Turnover

On day 3, the data imply that NE turnover was increased significantly in skeletal muscle of rats with ADN transection + sham renal denervation when compared to all of the other groups (Figure 34B). Rats with only renal denervation had a significantly larger percentage of NE remaining in skeletal muscle 8 h after α-methyltyrosine when compared to the surgical control group. Rats with ADN transection + renal denervation had a significantly lower percentage of NE remaining in skeletal muscle when compared to the sham ADN + renal denervated group, but were not different when compared to the surgical control group. This relationship is shown more clearly in Figure 35. Note that the lines are essentially parallel, suggesting that renal denervation did not prevent the response to ADN transection. In the hypothalamus there was a significant difference between the two ADN transection groups; however, the ADN + sham renal group was not significantly different from the surgical control group (Duncan's least significant difference = 7.7 for $P < 0.05$; actual difference = 6.7). In addition, there was no significant difference between the sham ADN + sham renal denervated group and sham ADN + renal denervated group in terms of NE turnover in their hypothalamus. Only data for the sham renal groups is given for turnover of NE in kidney, as renal denervation reduced kidney NE concentration in this and other experiments to

FIGURE 35

Relationship between the presence or absence of renal nerves and sympathetic activity to skeletal muscle in rats with and without ADN transection. The index of sympathetic activity was calculated as the percent of NE "used" during an 8 hour period after inhibition of tyrosine hydroxylase with α-methyltyrosine.


INDEX OF SYMPATHETIC ACTIVITY
100
80
60
40
20
ADN CUT
NORMAL
RENAL NERVES INTACT
RENAL NERVES CUT

less than 10% of normal. The data suggest that NE turnover was increased significantly in the kidneys of rats with ADN transection + sham renal denervation.

The reciprocal relation between arterial pressure recorded on Day 2 and the index of NE turnover in skeletal muscle measured on Day 3 suggested a possible correlation between these two variables. Figure 36 shows the results of such a plot and the best fit line calculated by linear regression analysis. There was a significant correlation between the turnover of norepinephrine in skeletal muscle and arterial pressure under the conditions of this experiment.

7.3.2 Renal denervation and acute changes in arterial pressure

7.3.2.1 Arterial pressure and heart rate

The renal-denervated group receiving saline-infusion had no significant changes in arterial pressure and heart rate during the 60 minutes of infusion (Figure 37, 38). Mean arterial pressure decreased by an average 31 mmHg initially and remained an average of 42 mmHg below control at the end of the 60 min infusion of nitroprusside (Figure 37). Heart rate increased initially by an average

FIGURE 36

Correlation between index of NE turnover in skeletal muscle and arterial pressure measured using the tail cuff technique. The x-axis plots the concentration of NE as a percent of 0-time control 8 hour after α-methyltyrosine administration. The relation between this variable and NE turnover is shown on the figure. n = 24

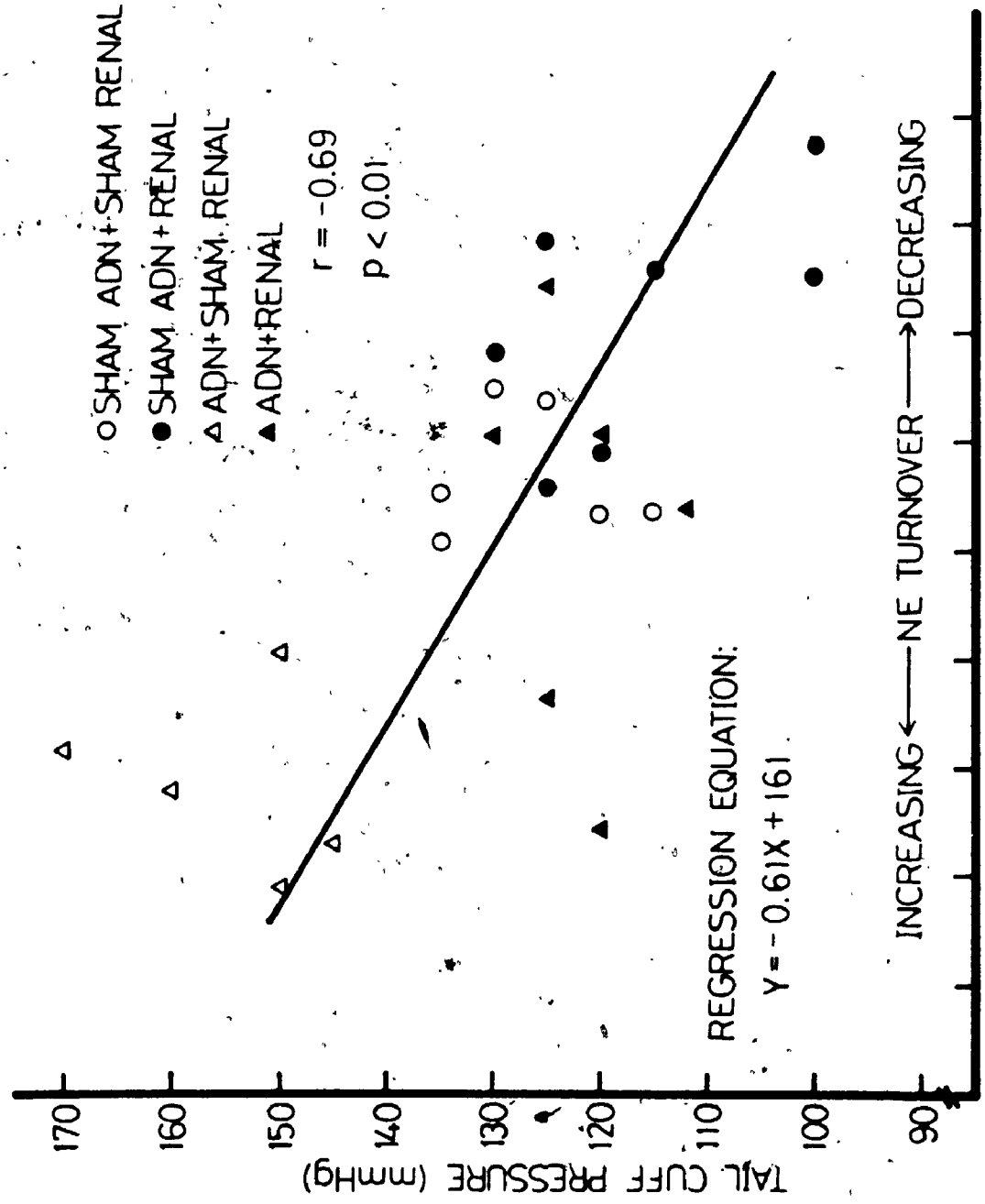

SHAM ADN+SHAM RENAL
SHAM ADN+RENAL
ADN+SHAM RENAL
ADN+RENAL
r = -0.69
p < 0.01
REGRESSION EQUATION:
Y = -0.61X + 161
TAIL CUFF PRESSURE (mmHg)
170
160
150
140
130
120
110
100
90
0
10
20
30
40
50
60
70
80
90
INCREASING ← NE TURNOVER → DECREASING
(NE) AS PERCENT OF 0-TIME CONTROLS

FIGURE 37

The effect of i.v. infusion with sodium nitroprusside (20-40 µg/kg.min) or phenylephrine (10-15 µg/kg.min) or saline on arterial pressure in rats with bilateral renal denervation. The zero time values represents the arterial pressure before the infusion period. The control group received an infusion of heparinized saline comparable in volume to drug infused groups. Numbers in parentheses represents number of animals/group. The values represent the mean $\pm$ S.E..

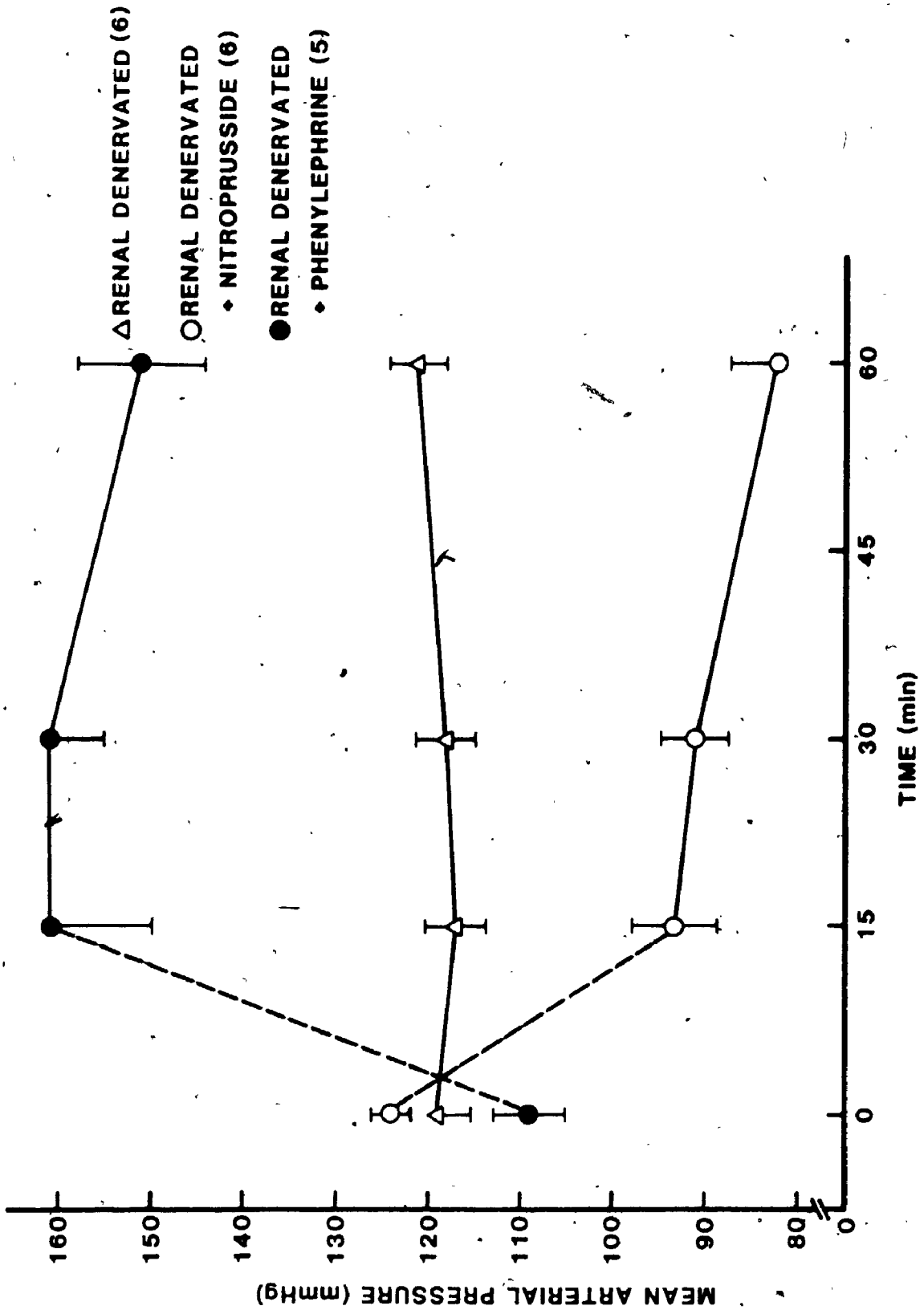
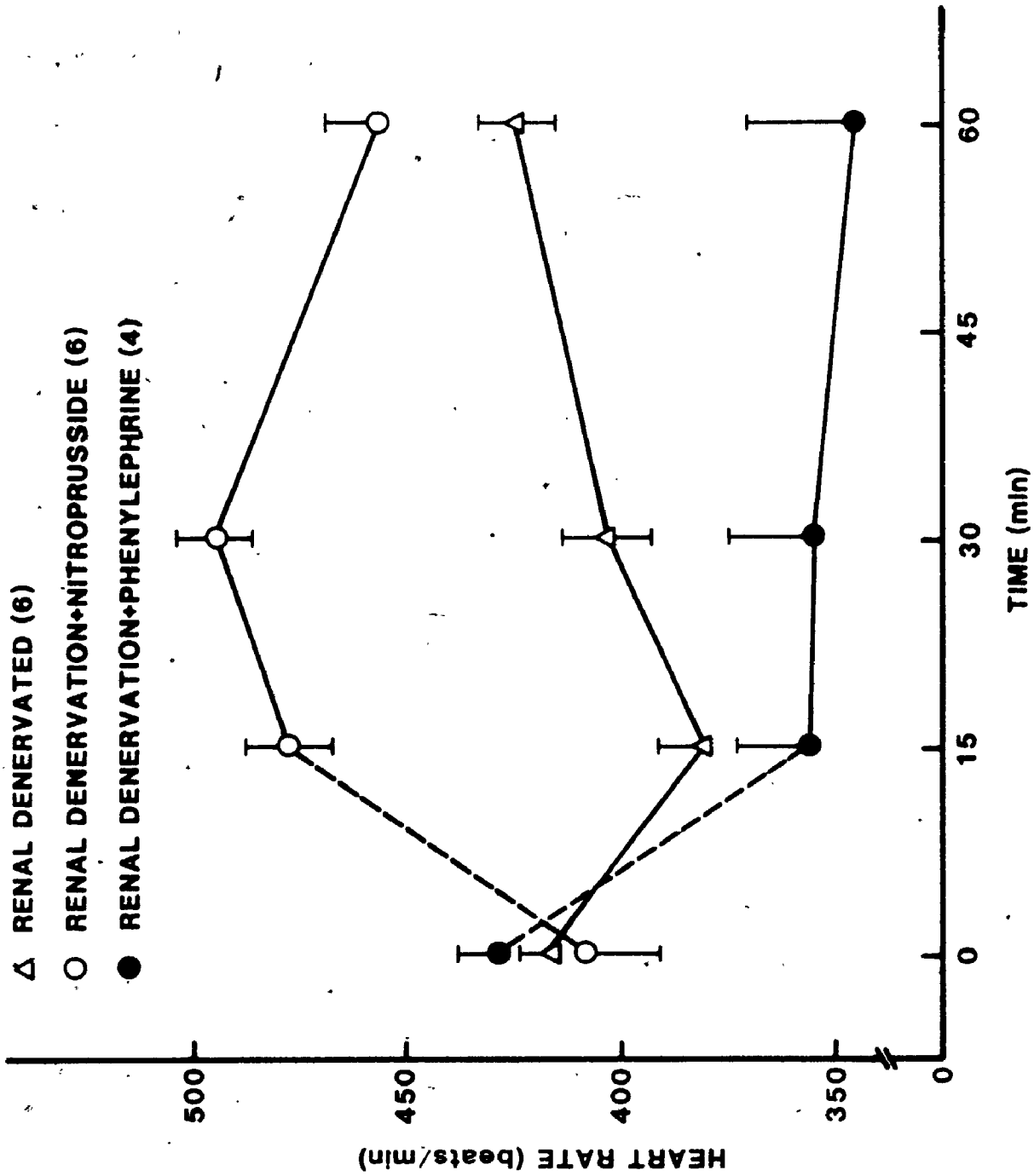

ΔRENAL DENERVATED (6)
○RENAL DENERVATED
◆ NITROPRUSSIDE (6)
●RENAL DENERVATED
◆ PHENYLEPHRINE (5)
MEAN ARTERIAL PRESSURE (mmHg)
160
150
140
130
120
110
100
90
80
0
0
15
30
45
60
TIME (min)

FIGURE 38

The effect of i.v. infusion with sodium nitroprusside (20-40 µg/kg.min) or phenylephrine (10-15 µg/kg.min) or saline-infused on heart rate in rats with bilateral renal denervation. Format as in Figure 37.


Δ RENAL DENERVATED (6)
○ RENAL DENERVATION+NITROPRUSSIDE (6)
● RENAL DENERVATION+PHENYLEPHRINE (4)
HEART RATE (beats/min)
500
450
400
350
0
0
15
30
45
60
TIME (min)

of 70 beats/min and remained an average of 50 beats/min above control level throughout the hypotensive period (Figure 38). Phenylephrine infusion produced an average increase of 55 mmHg in arterial pressure initially and the pressure was maintained an average 40 mmHg higher than the control mean arterial pressure for the 60 min infusion period (Figure 37). Heart rate decreased initially by an average 70 beats/min and remained an average of 90 beats/min below control heart rate throughout the hypertensive period (Figure 38).

It should be noted that the changes in arterial pressure produced in the renal denervated groups in this study during infusion of nitroprusside or phenylephrine were not significantly different from the corresponding groups with intact renal nerves in the previous Chapter. The reciprocal relationship between the changes in blood pressure and heart rate in renal denervated groups illustrated an operational baroreceptor reflex arc in all the groups studied.

7.3.2.2 Norepinephrine Concentration

There were no significant differences in the zero time value for NE concentration in the various tissues from the renal denervated animals and the animals with intact renal nerves from the previous Chapter (Table 3). The concentration of NE in the denervated kidneys was below the

TABLE 3

Norepinephrine concentration (ng/g) in various organs of intact and renal denervated rats killed at time 0.

	Norepinephrine (ng/g)	
	Intact Renal Nerves	Renal Denervated
Anterior Hypothalamus	1264 ± 35	1318 ± 85
Posterior Hypothalamus	809 ± 39	691 ± 48
Kidney	213 ± 12	----
Intestine (Duodenum)	917 ± 43	788 ± 61
Skeletal Muscle	66 ± 3.2	67 ± 2.4

3 days after renal denervation.

Mean norepinephrine concentration expressed as ng/g wet wt. ± SE.

n = 6 for each group.

detection limits of the assay used in the present study (5-10 ng/g), indicating better than 95% depletion of renal NE.

7.3.2.3 Measurement of tissue NE concentration after inhibition of tyrosine hydroxylase as an index of NE turnover

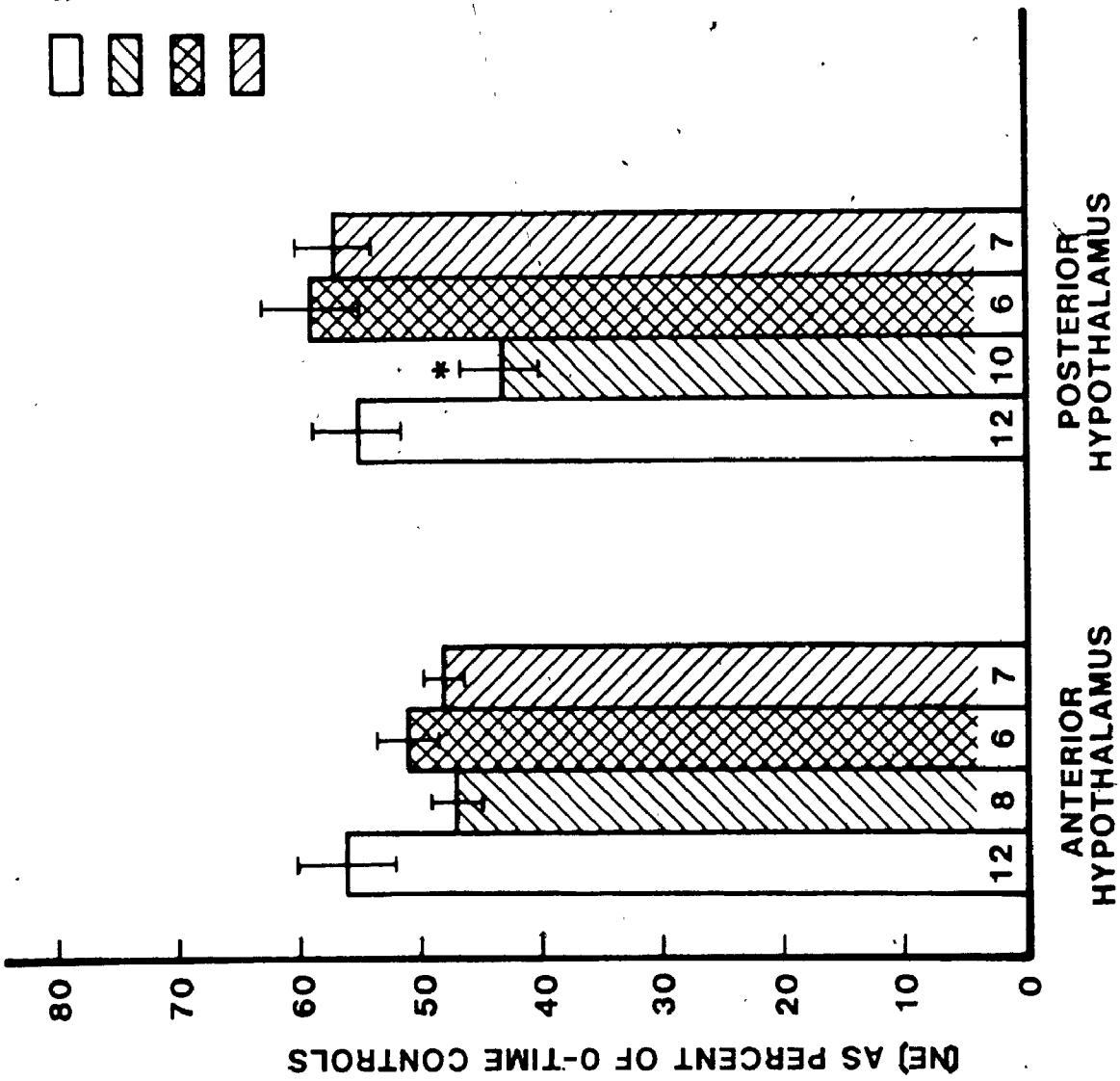
The renal innervated groups are used from the previous Chapter since the experiments from the previous Chapter and present experiments were done using the same protocol and during the same time period.

7.3.2.3.1 Effect of acute hypotension on noradrenergic activity in renal denervated rats

There was a significantly lower concentration of NE in the posterior hypothalamus of the nitroprusside-infused group compared to saline-infused controls as noted in the previous Chapter; however, in this experiment this effect was abolished in rats with renal denervation (Figure 39). No significant changes were seen in the anterior hypothalamus. Renal denervation by itself did not produce any significant changes in NE turnover in the hypothalamic sections examined. The turnover of NE was significantly increased in kidney, intestine, and skeletal muscle of the nitroprusside-infused group, indicative of reflexly

FIGURE 39

Effect of renal denervation on turnover of NE in the hypothalamus of conscious rats subjected to acute hypotension with nitroprusside. NE concentration 90 min after α-methyltyrosine is expressed as a percent (±S.E.) of appropriate 0-time controls for each group. Numbers in the bars represent number of animals/group. *$P < 0.05$ compared to saline-infused controls.


SALINE-INFUSED
NITROPRUSSIDE
NITROPRUSSIDE+ RENAL DENERVATED
SALINE-INFUSED+ RENAL DENERVATED
(NE) AS PERCENT OF 0-TIME CONTROLS
0
10
20
30
40
50
60
70
80
12
8
6
7
ANTERIOR HYPOTHALAMUS
12
10
6
7
POSTERIOR HYPOTHALAMUS
*

increased sympathetic activity (Figure 40). Changes in the peripheral organs of nitroprusside-infused animals persisted after renal denervation.

7.3.2.3.2 Effect of acute hypertension on noradrenergic activity in renal denervated rats

There was no significant change in the turnover of NE in the anterior or posterior hypothalamus of the phenylephrine-infused group compared to saline- infused controls as noted in the previous Chapter (Figure 41); however, in animals with renal denervation there was a significantly decreased turnover of NE in the anterior and posterior hypothalamus of the phenylephrine-infused group (Figure 42). In addition, phenylephrine infusion in renal denervated rats did not have a significant effect on the turnover of NE in the skeletal muscle.

7.4 DISCUSSION

These experiments were performed to investigate the possible interaction between renal nerves and the noradrenergic responses that were produced by ADN transection (described in Chapter 5) and acute changes in arterial pressure (described in Chapter 6). The hypothesis being tested was presented in the Historical Review after reviewing the relavent literature (Figure 1). This

FIGURE 40

Effect of renal denervation on the turnover of NE in the peripheral organs of conscious rats subjected to acute hypotension with nitroprusside. Format as in Figure 39. *$P < 0.05$ compared to saline-infused controls; +$P < 0.05$ compared to saline-infused, renal denervated animals.

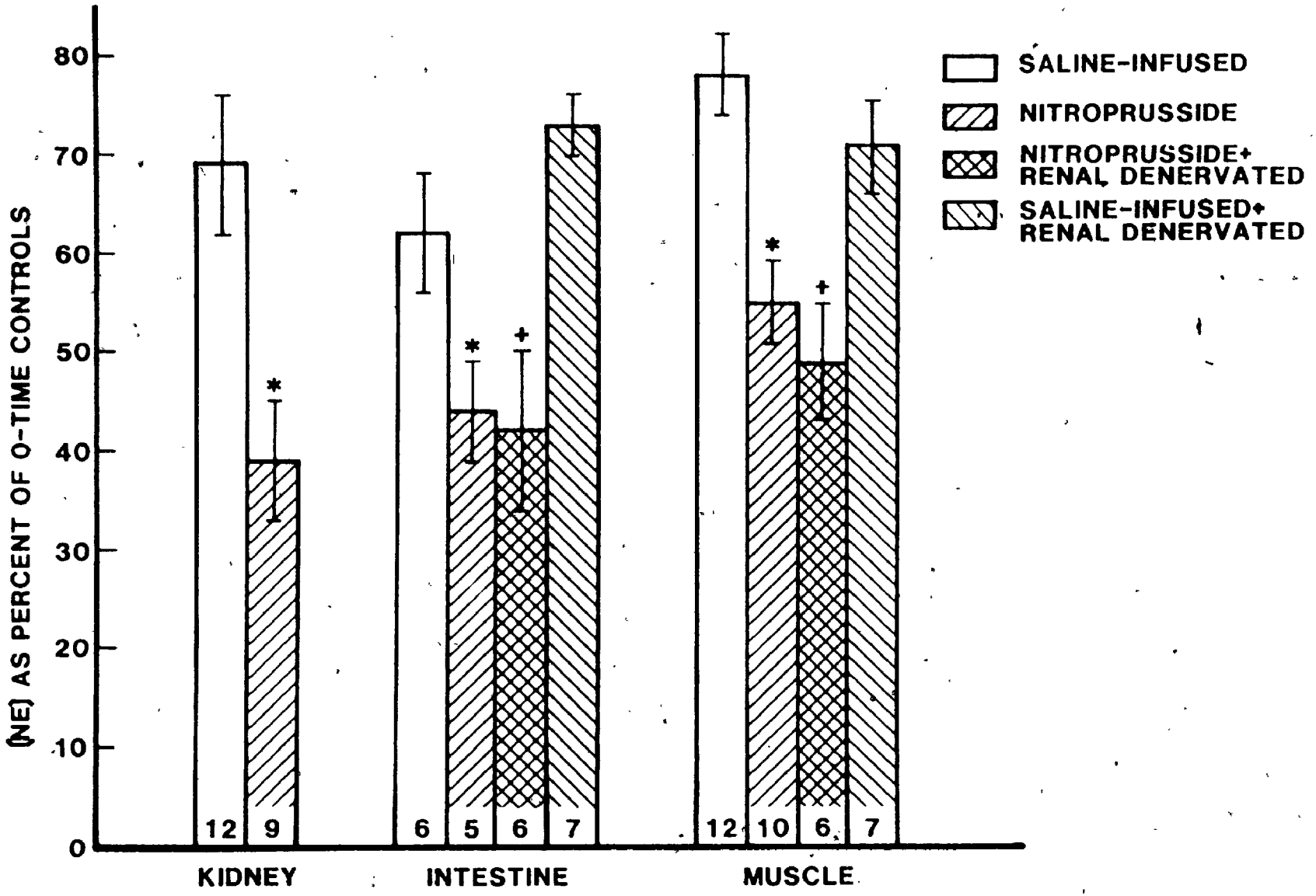

(NE) AS PERCENT OF 0-TIME CONTROLS
0
10
20
30
40
50
60
70
80
12
9
6
5
6
7
12
10
6
7
*
*
+
*
+
KIDNEY
INTESTINE
MUSCLE
SALINE-INFUSED
NITROPRUSSIDE
NITROPRUSSIDE+ RENAL DENERVATED
SALINE-INFUSED+ RENAL DENERVATED

FIGURE 41

Effect of renal denervation on turnover of NE in the hypothalamus of conscious rats subjected to acute hypertension with phenylephrine. Format as in Figure 39. *$P < 0.05$ compared to saline-infused renal denervated animals.

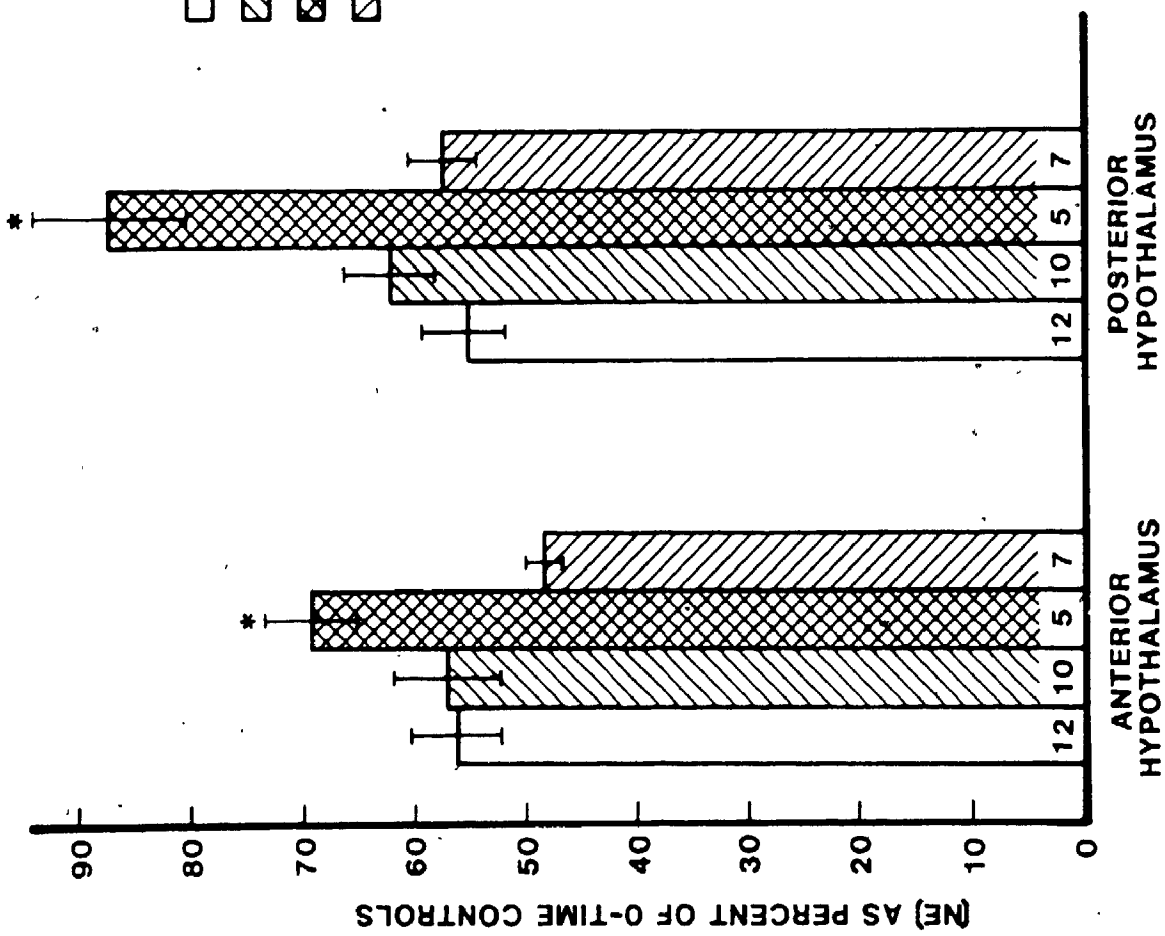
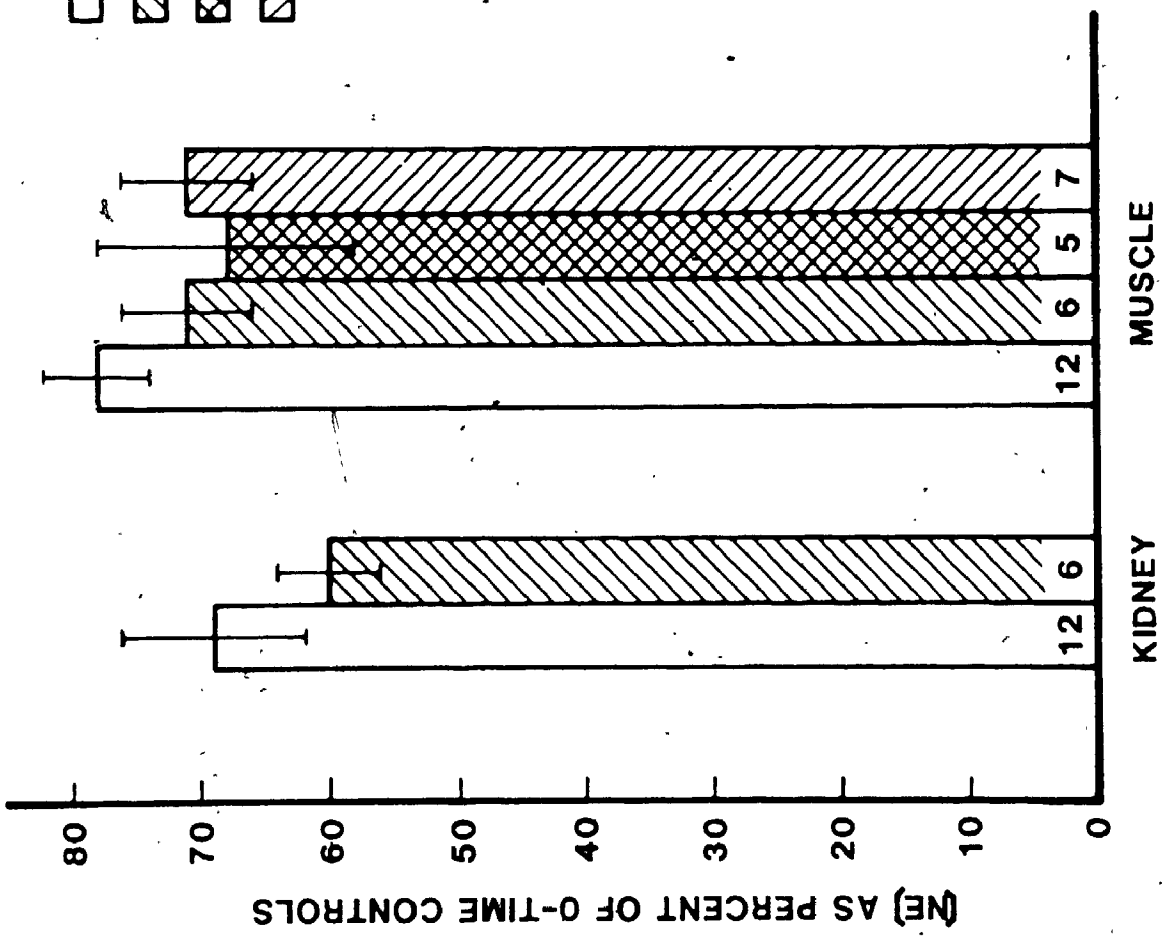

SALINE-INFUSED
PHENYLEPHRINE
PHENYLEPHRINE+ RENAL DENERVATED
SALINE-INFUSED+ RENAL DENERVATED
(NE) AS PERCENT OF 0-TIME CONTROLS
0
10
20
30
40
50
60
70
80
90
ANTERIOR HYPOTHALAMUS
POSTERIOR HYPOTHALAMUS
12
10
5
7
*
*

Figure 42

Effect of renal denervation on the turnover of NE in the peripheral organs of rats subjected to acute hypertension with phenylephrine. Format as in Figure 39.


SALINE-INFUSED
PHENYLEPHRINE
PHENYLEPHRINE+ RENAL DENERVATED
SALINE-INFUSED+ RENAL DENERVATED
(NE) AS PERCENT OF 0-TIME CONTROLS
0
10
20
30
40
50
60
70
80
KIDNEY
MUSCLE
12
6
12
6
5
7

hypothesis proposes that there is an interaction between an inhibitory influence from baroreceptors and an excitatory influence from renal afferents at the level of the hypothalamus. This interaction may involve the hypothalamic noradrenergic system. The noradrenergic activity in the hypothalamus may in turn be important in modulating peripheral sympathetic activity and consequently arterial pressure (see Historical Review).

7.4.1 Renal denervation and ADN transected rats

Bilateral transection of the ADN has been shown to result in an increased NE turnover in the hypothalamus as well as in kidney and skeletal muscle (as shown in this Chapter and Chapter 5) with a concomitant increase in arterial pressure, consistent with the hypothesis presented above. While there is controversy as to whether baroreceptor deafferentation leads to chronically increased arterial pressure (Cowley et al., 1973), there are several lines of evidence suggesting that there is increased peripheral sympathetic activity, at least for the first few days after ADN transection, as demonstrated in this Chapter (also see Chapter 5, and Touw et al., 1979).

Of particular interest is the fact that renal sympathetic nerve activity appears to be increased after ADN transection (Touw et al., 1979; Fink et al., 1980; Chapter 5). According to the prediction made by Guyton et

al. (1974), increased renal sympathetic nerve activity could produce increased arterial pressure as a result of neurally-mediated changes in the ability of the kidney to excrete urine. This change in the excretory ability of the kidney would be expressed as a change in the shape or position of the renal function curve and consequently arterial pressure (see Historical Review). Therefore this preparation of ADN transected rats would seem to be ideal to test the effect of renal denervation on the control of arterial pressure.

The results in the first study clearly demonstrated that renal denervation, done 3 days before ADN transection, completely prevented the arterial pressure response to cutting the ADN. Subsequently we have also shown that cutting the renal nerves reversed the arterial pressure response to cutting the ADN (Kline et al., 1983). The response was probably not due to elimination of an influence of the renin-angiotensin system on arterial pressure in these rats, as acute administration of captopril showed that there was little if any difference between the contribution of the renin-angiotensin system to the maintenance of arterial pressure in rats before and after ADN transection (Kline et al., 1983). In addition, adrenergic blockade with phentolamine decreased the arterial pressure of ADN transected rats to the same level as that in normal rats treated with the blocking agent (Kline et al., 1983). These results, which are in agreement with those of Fink et

al. (1980b) obtained one month after aortic baroreceptor denervation in rats, further support the idea that the elevated arterial pressure in the ADN transected rats is primarily due to increased α-adrenergic activity.

If the effects of renal denervation were due solely to the removal of efferent sympathetic fibers, then pressure diuresis and natriuresis should have occurred. Kline et al. (1983) have demonstrated that no such changes in fluid balance occurred after renal denervation in rats with bilateral ADN transection. It is possible that due to the time periods of collection and errors inherent in this type of metabolic study, that transient differences in urinary sodium and volume may have occurred, but if anything, both groups with ADN transection tended to excrete less urine volume and sodium after ADN transection than before (Kline et al., 1983). The decrease in urine output, plus the marked decreases in food and water intake in rats with ADN transection reported by Kline et al. (1983) is similar to that observed by Werber et al. (1981). These data would suggest that the decrease in arterial pressure after renal denervation in ADN transected rats may not be due to loss of efferent sympathetic fibers but possibly afferent renal nerve fibers.

From results in recent studies, Katholi et al. (1982a; 1982b) have proposed that increased activity of renal afferent fibers may be responsible for the enhanced

noradrenergic activity in the hypothalamus and peripheral sympathetic tone in one-kidney, one-clip Goldblatt model of hypertension. The above observations taken together with evidence for involvement of afferent renal nerves in the control of arterial pressure (see Historical Review) makes it conceivable that removal of afferent renal fibers may affect the baroreceptor control of sympathetic outflow.

To test this hypothesis, we used the turnover of NE in skeletal muscle as an index of sympathetic activity in ADN transected rats. As reported in Chapter 5 and in the present study, sectioning the ADN caused a significant increase in NE turnover in skeletal muscle; however, the turnover of NE in skeletal muscle was entirely normal in rats with combined renal nerve and ADN transection. This observation is similar to that reported by Katholi et al. (1982a; 1982b) for one- and two-kidney, one clip Goldblatt hypertensive rats, who used plasma NE concentration as an index of sympathetic activity. Thus, the elimination of enhanced sympathetic activity explains both the blood pressure response and the failure to see pressure diuresis and natriuresis (Kline et al., 1983) in ADN transected rats with renal denervation.

The exact mechanism whereby renal denervation produces these effects is not clear; however, three observations should be noted: 1) renal denervation alone significantly decreased the turnover of NE in skeletal muscle

(Figure 34B), 2) ADN transection in renal denervated animals did increase the turnover of NE in skeletal muscle when compared to the renal denervated control group and 3) renal denervation abolished the increased NE turnover in the hypothalamus after ADN transection. These results suggest that the removal of afferent renal fibers does not prevent the noradrenergic response in the peripheral organs to ADN transection, but that afferent renal fibers may have a tonic influence on hypothalamic noradrenergic mechanisms and on sympathetic outflow to skeletal muscle. That this effect may be important in the control of arterial pressure, at least over the time period during which these experiments were done, is illustrated in general by the significant correlation obtained between the index of NE turnover in skeletal muscle and arterial pressure, and specifically by the complete prevention of the arterial pressure response to ADN transection in renal denervated rats (Figure 36). A similar correlation between peripheral sympathetic activity and blood pressure in one- and two-kidney Goldblatt hypertensive rats was shown by Katholi et al. (1982a; 1982b), however, these investigators did not report data for animals which received renal denervation alone. Since renal denervation alone showed an effect on the NE turnover in skeletal muscle in the present study, a similar change in the renal denervated group in Katholi's experiments would have changed the conclusion inferred by the authors. In that, they would not have suggested an interaction between

the presence of renal nerves and the peripheral noradrenergic response in one-kidney, one-clip Goldblatt hypertensive rat, but rather an effect of renal denervation and an effect of the clip in the uninephrectomized rats.

Changes in the turnover of NE in the hypothalamus showed that there was a significant difference between the turnover of NE in the two groups of ADN transected rats, illustrating an effect of renal denervation. It is suggested that this effect may be due to elimination of tonic excitatory information carried by afferent renal fibers. Such a tonic activity in the renal afferent fibers under basal conditions has been previously reported (Calaresu et al., 1978; Kostreva et al., 1981) in anesthetized cats and dogs. The results in this study are congruent in principle with those reported by Winternitz et al. (1982) who showed that renal denervation decreased arterial pressure in one-kidney, one-clip Goldblatt hypertensive rats, while normalizing a previously elevated concentration of NE in the hypothalamus. Both of these studies suggest that the increased noradrenergic activity in the hypothalami of hypertensive rats is renal nerve dependent, possibly involving afferent renal fibers.

Considering the data overall, it seems that there is an inhibitory influence on the noradrenergic activity in the hypothalamus from the baroreceptors (Chapter 5, 6) and an excitatory influence from renal afferents which is

consistent with the hypothesis presented above (Figure 1). However, it is not clear what relative roles both renal efferent and afferent fibers may play in the peripheral noradrenergic response to ADN transection if any.

7.4.2 Renal denervation and acute changes in arterial pressure

To further investigate the hypothesis proposing an interaction between baroreceptor afferents and renal afferents on hypothalamic and peripheral noradrenergic activity, in this section of the study I examined the noradrenergic activity in the hypothalamus and peripheral organs in animals with and without renal nerves subjected to changes in arterial pressure. The removal of renal nerves abolished the noradrenergic response of the posterior hypothalamus to hypotension, but did not change the elevated NE turnover in the peripheral organs. These results suggest that hypothalamic and peripheral noradrenergic changes can be dissociated under these conditions. These results agree qualitatively with the results obtained in ADN transected rats with renal denervation, in that, renal denervation did not alter the peripheral noradrenergic response induced by transecting the ADN, nor did it alter the peripheral noradrenergic response to acute hypotension.

The results obtained for the hypothalamus of ADN transected rats and rats subjected to acute hypotension

agree with the results of studies in one-kidney, one-clip Goldblatt rats (Winternitz et al., 1982). All these studies concur that renal denervation normalizes increased noradrenergic activity in the hypothalamus of rats in response to ADN transection (Chapter 5) acute hypotension (Chapter 6) and renal artery stenosis by application of a clip (Winternitz et al., 1982). However, the results in studies with one-kidney, one-clip Goldblatt rats differ somewhat in terms of the peripheral response to renal denervation. Katholi et al. (1982a) showed that renal denervation abolished the elevated plasma NE concentration and concluded that renal nerves were responsible for the elevated plasma NE concentration in the one-kidney, one-clip Goldblatt rats. As pointed out previously the group with renal denervation alone was missing in their study, and the results of this group are crucial to their inferred conclusions, which differ from the conclusions drawn in the present study. For example, if renal denervation by itself decreases plasma NE concentration by eliminating the NE entering the plasma from the kidney, then there would be a decrease in plasma NE concentration after renal denervation in the control group similar to the decrease observed in the renal denervated one-kidney, one-clip Goldblatt hypertensive rat.

The data in the present study suggest that the relationship between changes in the noradrenergic activity in the posterior hypothalamus and peripheral sympathetic

activity can be dissociated under the conditions of the study in this Chapter. In other words, there is no direct relationship between changes in noradrenergic activity in the posterior hypothalamus and peripheral sympathetic activity. Nevertheless, as pointed out by several investigators (Haeusler 1975; Chalmers, 1975; Antonaccio, 1977), NE may play an important modulating role in regions which receive indirect input (hypothalamus) from the peripheral vascular receptors. Therefore, it is conceivable that changes observed in noradrenergic activity in the hypothalamus may be responsible for subtle changes in total sympathetic activity which are obscured in the present study by overriding subhypothalamic baroreflex mechanisms. This suggestion is plausible considering the fact that medullary structures possess all the circuitry for an operational baro-reflex arc (Calaresu et al., 1975; Spyer, 1981).

If baroreceptor afferents have an inhibitory influence on the noradrenergic activity in the hypothalamus, then loading the baroreceptors by elevating the arterial pressure should result in a decreased turnover of NE in the posterior hypothalamus and an increased NE turnover in the anterior hypothalamus according to Sinha et al. (1981). No such effect was observed in animals with intact renal nerves (Chapter 6). However, in animals without renal nerves the turnover of NE in the hypothalamus decreased significantly during acute hypertension suggesting that renal denervation permitted the expression of the inhibitory influence arising

from the afferents of loaded arterial baroreceptors. It should be noted that contrary to opposing responses observed by Sinha et al. (1980) in anterior and posterior hypothalamus in response to changes in arterial pressure, the present study showed no such distinction between anterior and posterior hypothalamus.

The effect of renal denervation on hypothalamic noradrenergic activity may be caused directly by elimination of excitatory information carried by afferent fibers or indirectly by removal of efferent renal nerves. The latter effect could be attributed conceivably to the angiotensin II formed from neurally released renin (see Section 2.1.4.2 of Historical Review). However, recent work by Winternitz et al. (1982) showed that renal denervation in one-kidney, one-clip Goldblatt hypertensive rats normalized hypothalamic NE concentration without affecting plasma renin activity or renal renin activity (Katholi et al., 1981). In addition, acute hypertension produced by phenylephrine does not elevate plasma renin activity (Levy et al., 1977; Kelton et al., 1980) yet renal denervation in these animals significantly altered hypothalamic noradrenergic activity. Consistent with such a hypothesis Faber et al. (1983) have reported that acute renal artery stenosis in conscious rats causes an increase in arterial pressure of baroreceptor deafferented animals, despite interruption of the renin-angiotensin system with captopril. However, renal denervation prevents the increase in arterial pressure in

response to acute renal artery stenosis in these baroreceptor deafferented rats (Faber et al., 1983). Therefore, in the present studies, the results suggest that renal denervation influences hypothalamic noradrenergic responses to changes in baroreceptor input directly by removal of an excitatory influence from afferent renal fibers.

The excitatory influence from renal afferents may represent a tonic input from the kidneys (Calaresu et al., 1978; Kostreva et al., 1981) or perhaps an activation of renal afferents in response to the effects of nitroprusside and phenylephrine infusion. The former suggestion is probably not correct as renal denervation had no significant effect by itself in the control animals, at least under the conditions in the present study. The latter possibility is reasonable given the fact that a variety of stimuli, including alterations in renal arterial pressure, ischemia, renal venous occlusion, uretral occlus-ion, compression of the kidney and changes in the ionic composition of the pelvic urine, have been shown to produce alteractions in renal afferent nerve activity (Recordati et al., 1978; Recordati et al., 1980; Gottschalk, 1979). Niijima (1972) has shown that infusion of small amounts of NE in anesthetized rabbits caused an increase afferent renal nerve discharge rate comparable to phenylephrine infusion in the present study. Conversely lowering arterial pressure by hemorrhage produced an increase in afferent renal nerve

firing rate in anesthetized rats which would be comparable to acute hypotension by nitroprusside (Recordati et al., 1978). Furthermore, Faber et al. (1983) have shown that acute renal artery stenosis in rats with baroreceptor deafferentation induces an elevated arterial pressure which is not dependent on renin-angiotensin system but can be abolished by renal denervation. They suggest that activation of renal afferent nerves induces changes in sympathetic nervous system activity related to cardiovascular changes. It should be noted that captopril (angiotensin II synthesis blocker) prevents the increase in arterial pressure in response to acute renal artery stenosis in baroreceptor intact rats. Therefore baroreceptor deafferentation is necessary to unmask the effects of afferent renal nerves on the arterial pressure response to acute renal artery stenosis. This is yet another piece of evidence to support the hypothesis that there is an interaction between baroreceptor and renal afferent information to control arterial pressure.

CHAPTER 8

Effect of afferent renal nerve stimulation on noradrenergic activity in the hypothalamus and peripheral organs

8.1 Introduction

Several investigators have reported that electrical stimulation of renal afferent nerves produces a change in systemic arterial pressure (Ueda et al., 1967; Aars et al., 1970; Calaresu et al., 1976). Presently there is disagreement among investigators as to whether the response to stimulation is pressor or depressor. These apparent discrepancies have been attributed to differences in anesthesia, species used, or the stimulation parameters used (Calaresu et al., 1976). Nevertheless electrophysiological studies demonstrating that stimulation of renal afferent nerves produces changes in the electrical activity of neurons in several regions of the hypothalamus (Ciriello et al., 1980; Kneupfer et al., 1980) provide evidence to suggest that changes produced by renal afferent nerve stimulation are mediated via supramedullary structures.

In the previous Chapter I provided evidence for an excitatory influence from renal afferents on the noradrenergic activity in the hypothalamus. Winternitz et al. (1982) have provided indirect evidence suggesting that activation of renal afferents by clipping the renal artery causes an activation of the noradrenergic mechanisms in the

hypothalamus. However, there is no direct evidence to suggest that activation of renal afferents causes an increased noradrenergic activity in the hypothalamus.

In order to investigate more directly the proposed relationship between activity in renal afferents and noradrenergic mechanisms in the hypothalamus, in the present study, I examined the noradrenergic activity in the hypothalamus in response to electrical stimulation of renal afferent nerves for a period of 60 min in conscious rats. In addition, I also examined the effect of renal afferent nerve stimulation on peripheral sympathetic activity in the contralateral kidney, intestine, and skeletal muscle.

8.2 METHODS

Studies were done using male Wistar rats weighing 250-325 g. One day prior to the experiment, rats were anesthetized with methoxyflurane anesthesia and were implanted with femoral arterial and venous cannulae, exteriorized through the skin in the back and plugged. At this time a stimulation cuff was placed on the renal nerves (see surgical procedure below) in all rats used in this study. On the day of the experiment, mean arterial pressure was recorded on a Grass model 7 polygraph in conscious, freely moving rats by attaching the arterial cannula to a Statham pressure transducer by a length of Tygon tubing. Arterial pressure was recorded for one hour prior to, and

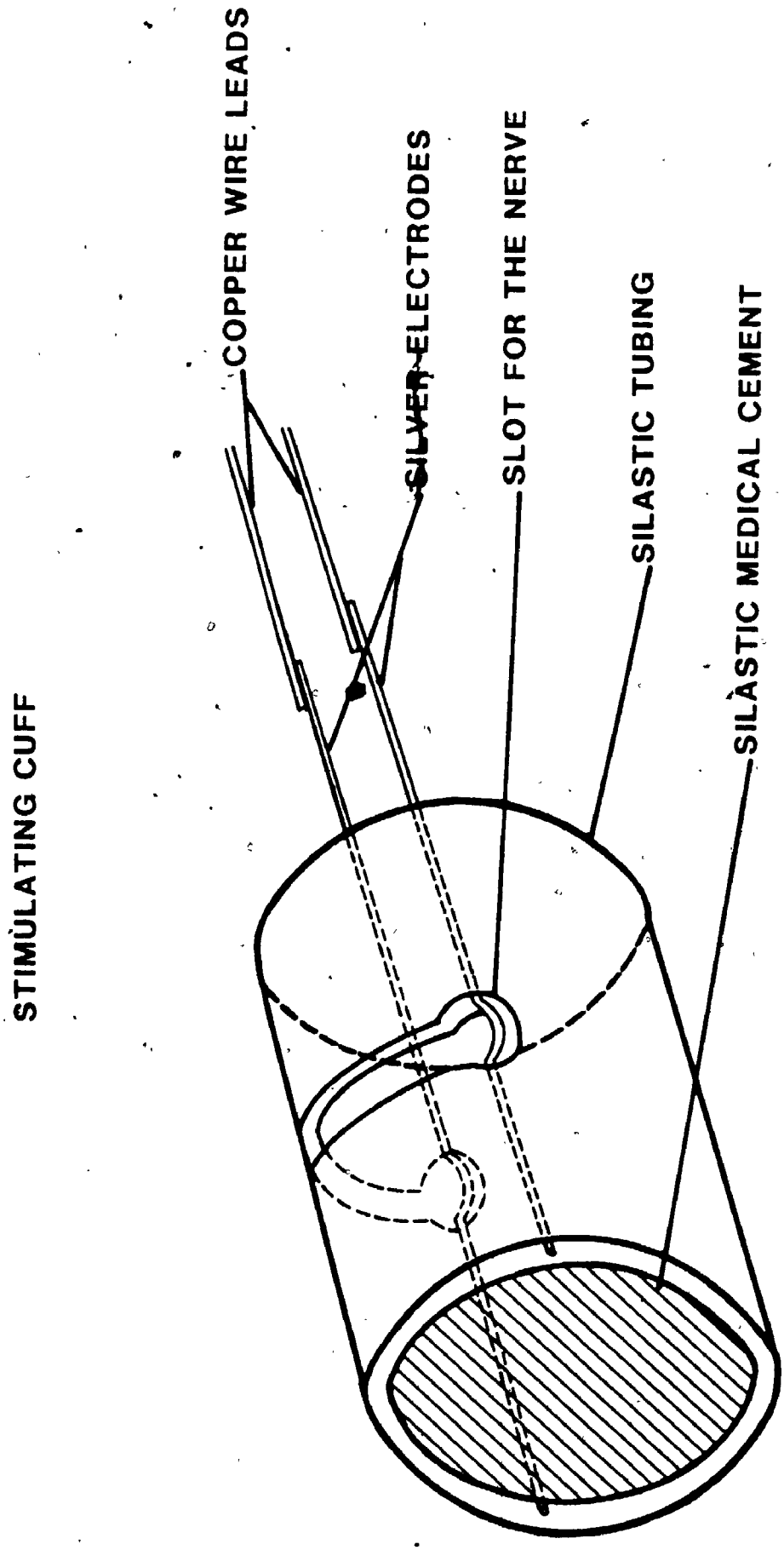
during the NE turnover experiment. Heart rate was determined using a tachograph triggered by the arterial pulsations.

8.2.1 Construction of stimulating cuff

A miniature bipolar electrode cuff was constructed for chronic stimulation (60 min) of the proximal end of the cut renal nerve in awake animals. The cuff was constructed from a short section (1.5-2.0 mm) of Silastic tubing (0.76 mm x 1.65 mm, Dow Corning). Two silver wires (0.127 mm diameter) were inserted through the wall of the tubing crossing through two openings made with an 18 gauge needle in the middle of the tubing. After soldering the two silver leads (0.5-0.8 mm long) to two pieces of Teflon-insulated 36 AWG copper wire (Bioflex wire, Cooner Wire Sales), both open ends of the tubing were sealed with Silastic Medical cement (Dow Corning). Therefore the only exposed wire was in the two openings made by the passage of the 18 gauge needle. See Figure 43. A cut in the tubing was made to extend from one opening to the other. Such a cut allowed the nerve to be inserted so that the nerve crossed from one opening to the other and therefore, in contact with the exposed silver wires.

Figure 43

Diagram of a stimulating cuff with copper wire leads. Slot in the Silastic tubing is to place the renal nerve. For details of the construction and implanting of the stimulating cuff refer to the text.


STIMULATING CUFF
COPPER WIRE LEADS
SILVER ELECTRODES
SLOT FOR THE NERVE
SILASTIC TUBING
SILASTIC MEDICAL CEMENT

8.2.2 Surgical procedure for implanting the stimulating cuff

The abdomen was opened via a ventral midline incision and abdominal viscera retracted to expose the left renal artery and vein under methoxyflurane anesthesia. A branch of the left renal nerve was isolated between the coeliac ganglion and the adrenal vein under light microscope (Nikon, Japan). The nerve was tested using a bipolar stainless steel electrode with a five second period of electrical stimulation (20 Hz, 3-5 V, 0.1 msec duration). Blanching of the kidney during stimulation verified that the nerve innervatd the kidney. After identifying a branch of the renal nerve it was placed in the miniature stimulating cuff described above. The cuff was filled with a non-conductive fluid (Medical Fluid, Dow Corning) and the nerve was stimulated again to demonstrate that the nerve was in contact with the silver electrode. The distal section of the nerve was crushed and tied to the base of the stimulating cuff. Electrical stimulation of the nerve was repeated to varify that no visible changes in the colour of the kidney occurred. Thus it was possible to stimulate the afferent renal nerve fibers selectively. The wires from the cuff were tied to the dorsal abdominal muscle wall and then passed subcutaneously to the back where they were exteriorized through the skin and connected to an electrical socket. Before closing the abdominal wound, the cuff was covered with petroleum jelly to further insulate the silver

electrodes and prevent current spread. Animals were allowed to recuperate for 18-24 hours after surgery. I would like to add that Dr. M. Knuepfer was instrumental in the construction and implanting of the stimulating cuff.

8.2.3 Measurement of tissue NE concentration after inhibition of tyrosine hydroxylase as an index of NE turnover

Animals were randomly assigned to two groups: 1) sham-afferent renal nerve stimulated, 2) afferent renal nerve stimulated. Thirty minutes before stimulation, all rats received α-methyltyrosine (300 mg/kg i.p.).

The electrical socket was connected to the Grass S48 stimulator via a plug in order to deliver electrical stimuli to the afferent renal nerve. Monophasic, square-wave pulses (20 Hz, 1.0 msec duration, 2-10 V) were used to stimulate the renal nerves. These pulses were delivered in trains (100 msec duration and 4 trains per sec) using a Grass S48 stimulator in line with a Grass SIU 5 Stimulus Isolation Unit. Stimulation parameters were set so as to maintain at least a 10 mmHg increase in the arterial pressure of the stimulated group during the 60 min of stimulation. Stimulation parameters did not exceed the threshold stimulation necessary for behavioural changes such as moving about and licking the abdomen. In other words, the voltage was increased to produce a blood pressure response, however

if the voltage used elicited a behavioural response, the voltage was immediately reduced below the threshold voltage required to elicit behavioural response.

In all animals, 90 minutes after α-methyltyrosine administration, the rats were killed by cervical dislocation. The anterior and posterior hypothalamus from the brain, the right - kidney, a piece of the hind limb skeletal muscle, and a piece of the duodenum were removed and analysed for NE concentration using the HPLC in combination with EC procedure described in Chapter 4.

8.2.4 Data Analysis

All data are reported as mean ± S.E. Differences in the turnover of NE were inferred by comparing the values for tissue concentration of NE 90 minutes after α-methyltyrosine. Similar comparison of NE concentrations after inhibition of tyrosine hydroxylase between various experimental groups has been used to compare noradrenergic activity (Sole et al., 1978; Andén et al., 1980; Persson et al., 1981).

8.3 RESULTS

8.3.1 Arterial pressure and heart rate

Typical examples of acute responses to stimulation of afferent renal nerve on arterial pressure are shown in Figure 44. The control group, that had the operation for implanting the stimulating electrodes on renal nerves had no significant changes in arterial pressure and heart rate during the 60 minutes of sham stimulation (Figure 45, 46). Arterial pressure was significantly increased in the rat with renal afferent nerve stimulation compared to sham-stimulated rats during the 60 min of stimulation (Figure 45). Arterial pressure increased by an average of 10 mmHg initially in the rats with afferent renal nerve stimulation and remained elevated above control by an average of 13 mmHg at the end of the 60 minutes of stimulation. Heart rate increased by 37 beats/min initially and remained elevated by at least 23 beats/min during the stimulation in the stimulated group, however these differences were not statistically significant (Figure 46).

8.3.2 Measurement of tissue NE concentration after inhibition index of NE turnover

There was no significant difference in the concentration of NE after α-methyltyrosine in the anterior

FIGURE 44

Typical pressor responses to electrical stimulation of proximal end of a cut renal nerve in two different concious rats. Monophasic, square-wave pulses (20 Hz, 1.0 msec duration, 1-3V) were used. Trains of 100 msec duration and 4 trains per sec were delivered.

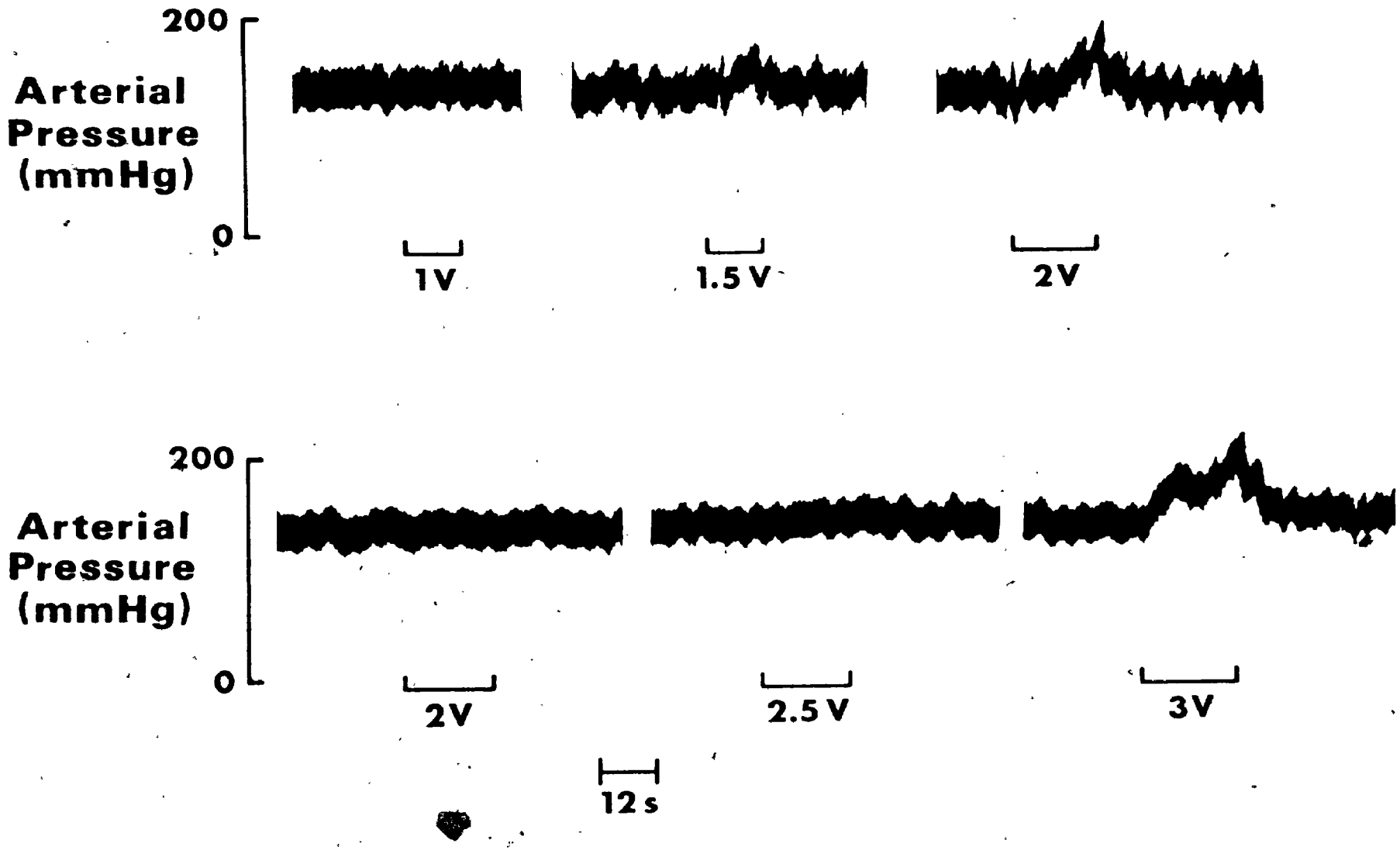
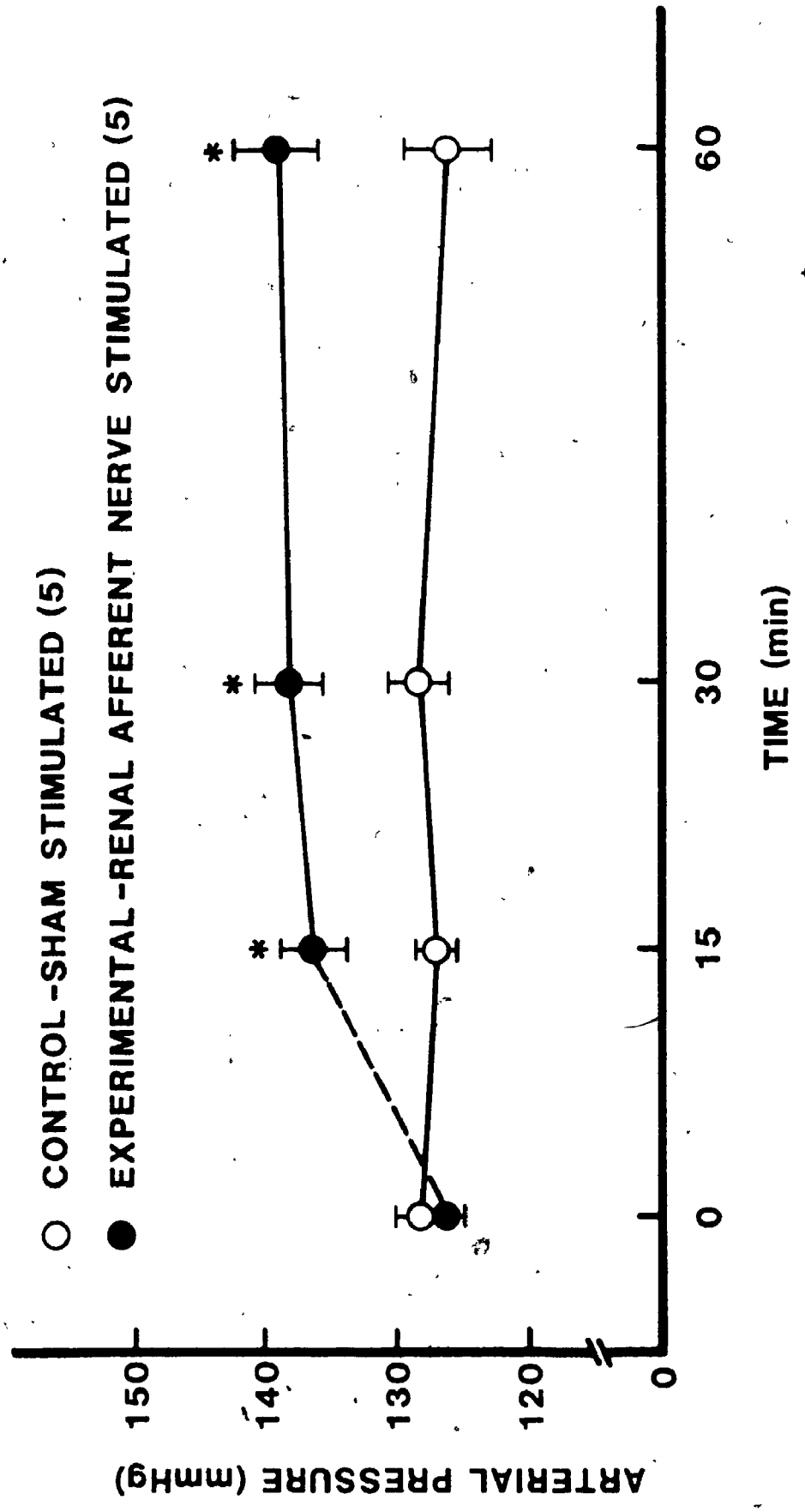

200
Arterial Pressure (mmHg)
0
1V
1.5 V
2V
200
Arterial Pressure (mmHg)
0
2V
2.5 V
3V
12 s

FIGURE 45

The effect of electrical stimulation of the proximal cut end of the renal nerves for 60 min on arterial pressure in conscious rats. The zero time values represent the arterial pressure before the stimulation was started. The values represent the mean ± S.E. Number in parentheses represents number of animals/group. F-ratio = 21.2 (1,39) *P < 0.01.


○ CONTROL-SHAM STIMULATED (5)
● EXPERIMENTAL-RENAL AFFERENT NERVE STIMULATED (5)
ARTERIAL PRESSURE (mmHg)
150
140
130
120
0
0
15
30
60
TIME (min)
*
*
*

or posterior hypothalamus of the group which received stimulation of afferent renal nerves compared to the sham-stimulated group where the afferent renal nerves were not stimulated (Figure 46). However, in the peripheral organs there was a significant decrease in the concentration of NE in the skeletal muscle of renal afferent nerve stimulated group compared to sham-stimulated group after inhibition of tyrosine hydroxylase (Figure 47). In addition, there was a tendency for a greater decline in concentration of NE in the intestine of the afferent renal nerve stimulated group compared to the sham-stimulated group 90 min after α-methyltyrosine. There was no significant difference in the concentration of NE in the contralateral kidney between the two groups.

8.4 Discussion

This study demonstrated for the first time that stimulation of afferent renal nerves in conscious rats produces an acute rise in arterial pressure. At higher stimulation voltages behavioural responses (such as moving about and licking the abdomen) were observed. In this study the stimulation parameters were maintained at levels below threshold values for such behavioural response as stated above. Contrary to the results in this study, Aars et al. (1970) and Mahoney et al. (1978) have reported a decrease in arterial pressure in chloralose/urethane

FIGURE 46

The effect of electrical stimulation of proximal cut end of renal nerve for 60 min on heart rate. Format as in Figure 45.

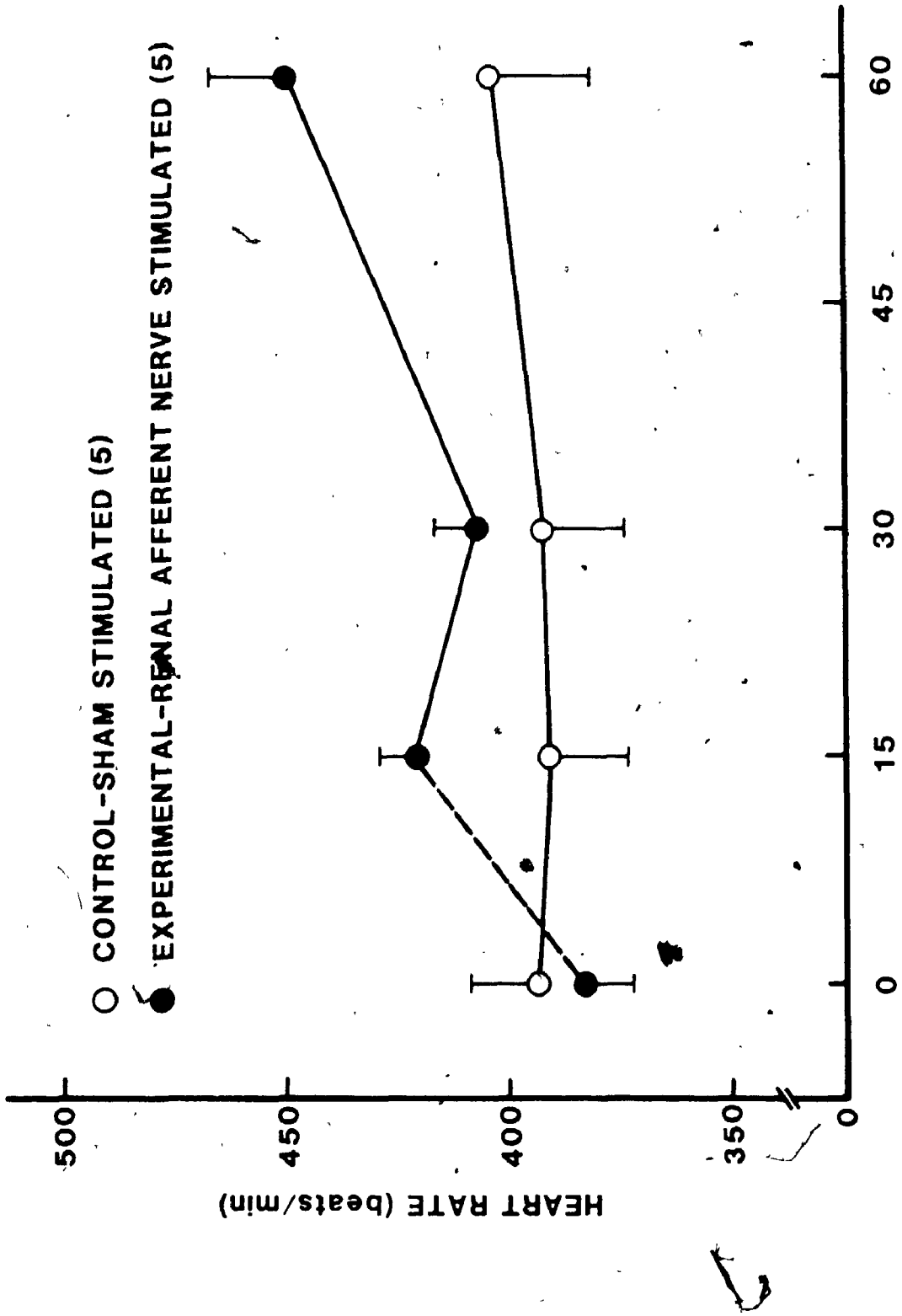

○ CONTROL-SHAM STIMULATED (5)
● EXPERIMENTAL-RENAL AFFERENT NERVE STIMULATED (5)
HEART RATE (beats/min)
500
450
400
350
0
0
15
30
45
60
TIME (min)

FIGURE 47

Effects of stimulation of afferent renal nerves on the disappearance of NE in the hypothalamus following inhibition of NE synthesis. The values represent concentration of NE (±S.E.) remaining 90 min after α-methyltyrosine. Numbers in the bar represent number of animals/group. *$P < 0.05$ compared to sham-stimulated controls.

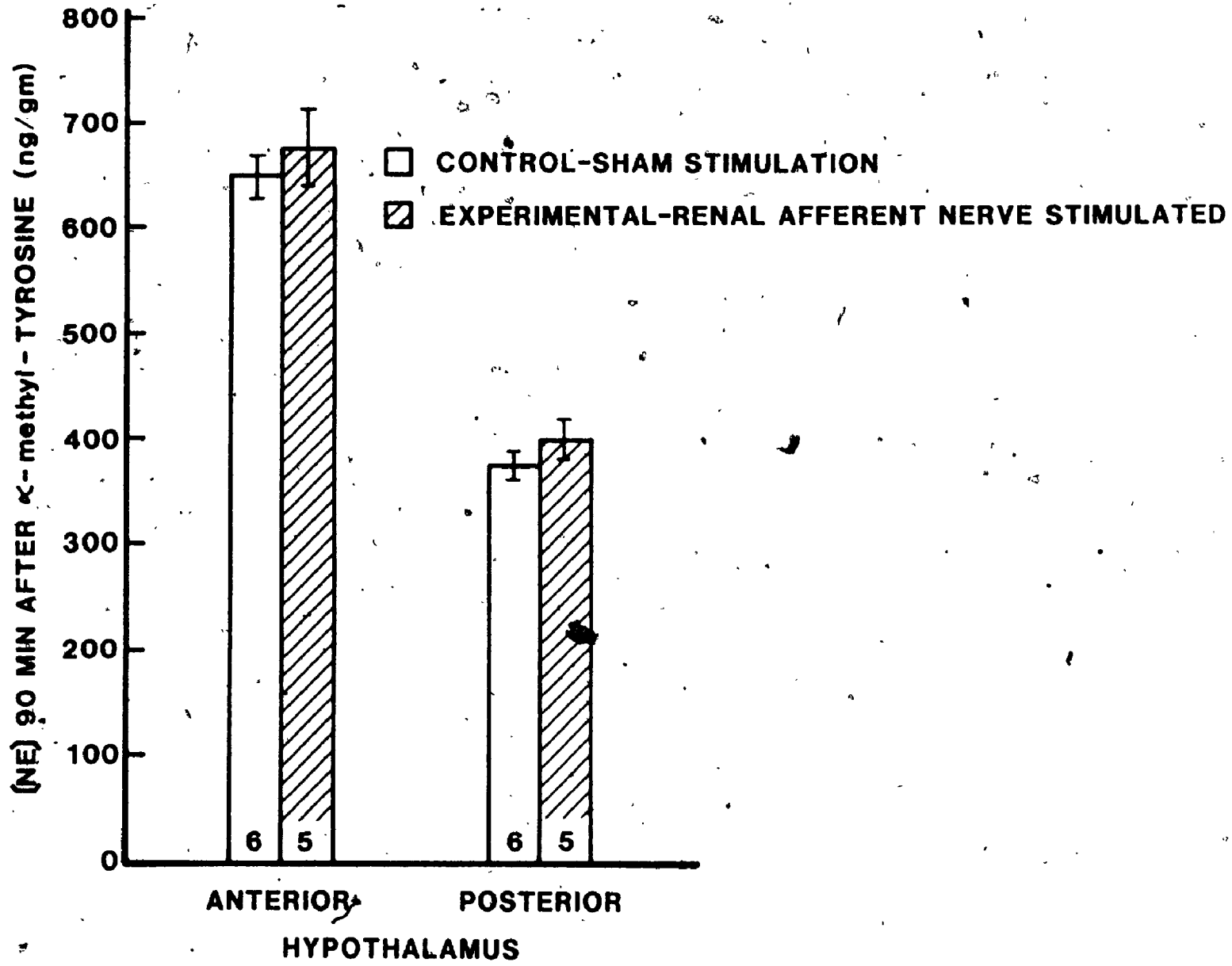
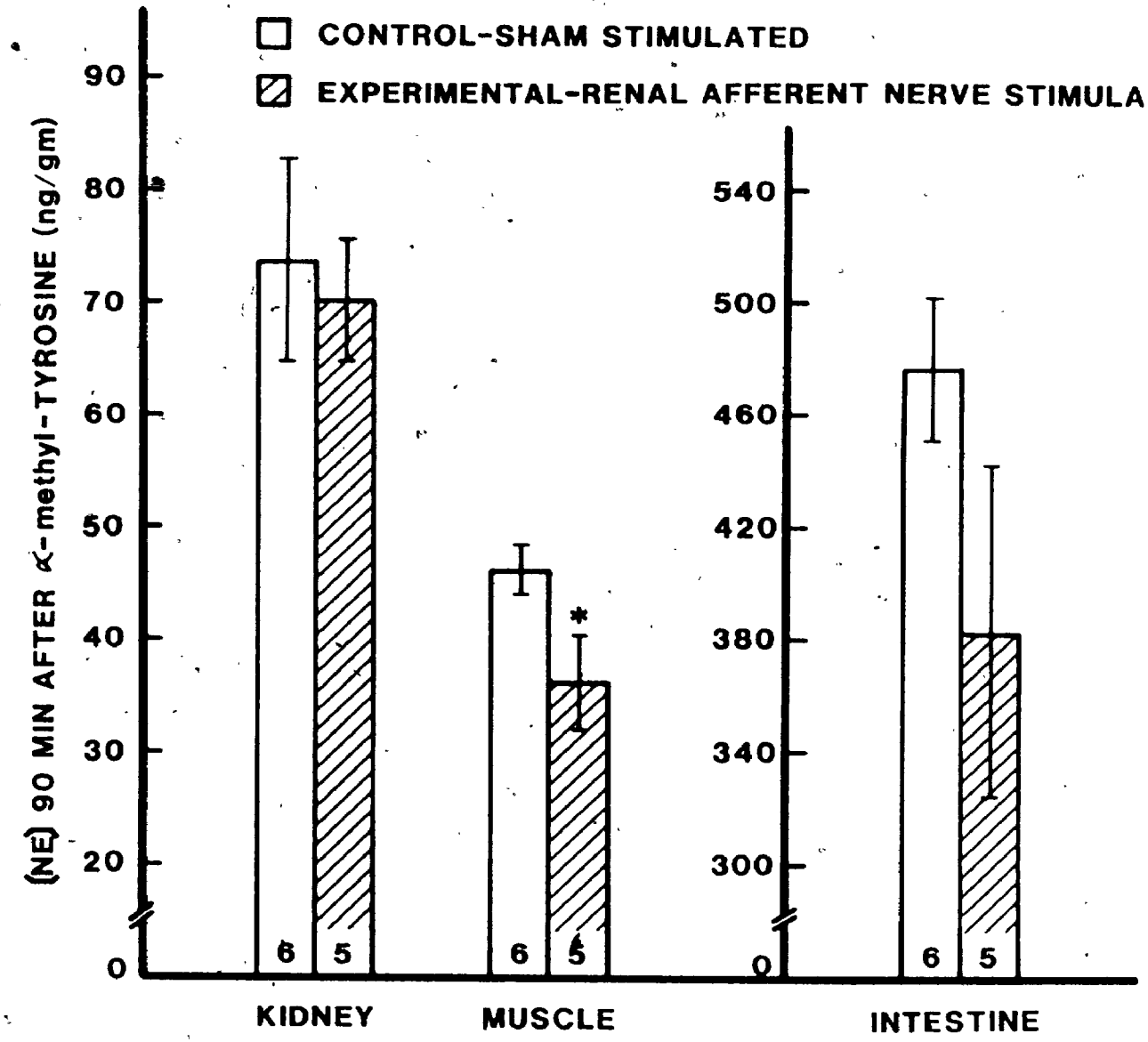

800
700
600
500
400
300
200
100
0
(NE) 90 MIN AFTER α-methyl-TYROSINE (ng/gm)
CONTROL-SHAM STIMULATION
EXPERIMENTAL-RENAL AFFERENT NERVE STIMULATED
6
5
6
5
ANTERIOR
POSTERIOR
HYPOTHALAMUS

FIGURE 48

Effect of stimulation of afferent renal nerve on the disappearance of NE in the controlateral kidney, intestine and skeletal muscle following inhibition of NE synthesis. The values represent concentration of NE (±S.E.) left 90 min after α-methyltyrosine. Format as in Figure 47. *P < 0.05 compared to sham-stimulated controls.


CONTROL-SHAM STIMULATED
EXPERIMENTAL-RENAL AFFERENT NERVE STIMULATED
(NE) 90 MIN AFTER α-methyl-TYROSINE (ng/gm)
90
80
70
60
50
40
30
20
0
540
500
460
420
380
340
300
0
6
5
6
5
6
5
*
KIDNEY
MUSCLE
INTESTINE

anesthet-ized rabbits and a decrease in vascular resistance in various vascular beds in pentobarbital anesthestized rats respectively. The different responses may be attributed to the effects of anesthesia and stimulation parameters. It is conceivable that during the anesthesia certain reflex pathways are exacerbated while others are obscurred (Farnebo et al., 1979; Leenen et al., 1981).

In this study I did not observe any effect of afferent renal nerve stimulation on the noradrenergic activity in the hypothalamus. According to the hypothesis presented in Chapter 2, stimulation of renal afferents would be expected to cause an increased NE turnover in the hypothalamus. Failure to observe an increased NE turnover in the hypothalamus could possibly be explained by the fact that electrical stimulation of renal afferent nerve may be stimulating fibers carrying opposing types of information and consequently the effect is cancelled out in terms of noradrenergic activity at the level of the hypothalamus. Such a contention is conceivable since stimulating the afferent renal nerves is comparable to activation of both mechano- and chemo-receptors in the kidney (Recordati et al., 1978; 1980). Alternatively another explanation for the lack of change in noradrenergic activity in the hypothalamus might be that the significantly elevated arterial pressure (Figure 45) may be initiating an inhibitory influence from the baroreceptors and possibly cancelling the excitatory influence of the electrically

stimulated renal afferent fibers. Such an explanation is supported by the results of Faber et al. (1983), who showed that acute renal artery stenosis did not produce an increase in arterial pressure in rats treated with captopril to interrupt the renin-angiotensin system, however when renal artery stenosis was performed in baroreceptor denervated animals treated with captopril, the stenosis did produce an increase in arterial pressure. They concluded that baroreflexes may obscure renal afferent nerve-dependent cardiovascular changes. Yet another possibility for the lack of changes in the hypothalamus in response to stimulation of renal afferent fibers is that the stimulation of the renal afferent nerves may have opposing effects in discrete areas within the hypothalamus, and therefore changes may be obscurred by the examination of the large hypothalamic sections used in the present study.

Peripherally however, there was a significant increase in the index of NE turnover in the skeletal muscle. Similarly there was a trend towards an increase in the intestine. These results are in agreement, qualitatively, with the results that showed a significant decrease in NE turnover in the skeletal muscle in rats with bilateral renal denervation compared to innervated controls (Chapter 7). In other words, according to the results in this study one would predict that removing the renal afferent nerves in rats, would cause a decrease in the turnover of NE in the skeletal muscle, which was observed in Chapter 7.

These results considered together with the blood pressure data from this study suggest that increased afferent renal activity is responsible for an exaggerated sympathetic outflow to the skeletal muscle and an elevated arterial pressure. It is conceivable that increased afferent renal nerve activity causes an increased sympathetic outflow to the whole circulatory system except the kidneys (since no significant difference in NE turnover was observed in the contralateral kidney), thus maintaining an arterial pressure for adequate renal perfusion pressure without compromising renal blood flow.

CHAPTER 9

General Discussion

Central and peripheral noradrenergic mechanisms play an important role in the regulation of arterial pressure (Chalmers, 1975; Antonaccio, 1977; Mancia, 1981; Abboud, 1982). However, the relationships between changes in the central (hypothalamic) noradrenergic systems and peripheral noradrenergic systems are not clear. In the initial experiments of this study it was demonstrated that removal of baroreceptor input either by transection of the ADN or by a decrease in arterial pressure caused an increased turnover of NE in the hypothalamus and peripheral organs. These results suggest an inhibitory influence of baroreceptors on the noradrenergic activity in the hypothalamus and a positive correlation between changes in the hypothalamic noradrenergic activity and peripheral sympathetic activity and provide support for the hypothesis presented in Figure 1 of the Historical Review.

However, subsequent experiments in animals which underwent renal denervation showed that there was an increased turnover of NE in the periphery in response to acute hypotension although there was no change in noradrenergic activity in the hypothalamus. These results show a dissociation between changes in noradrenergic activity of the hypothalamus and changes in peripheral sympathetic activity. On the basis of these results the

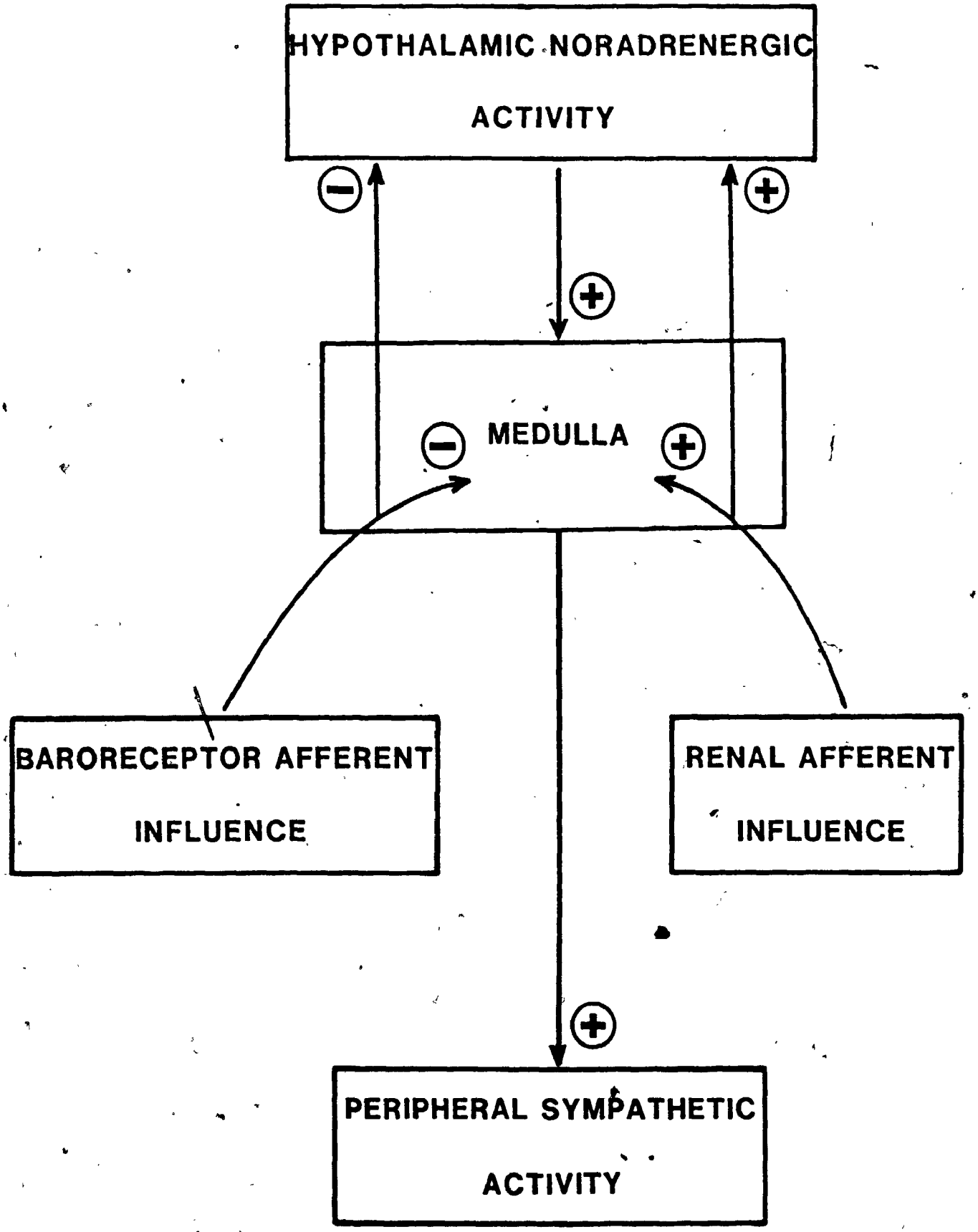
following model is proposed (Figure 49). This figure illustrates a hierarchical control of peripheral sympathetic activity. It is conceivable that medullary mechanisms were overriding the influence of hypothalamic noradrenergic activity modulating normal sympathetic outflow, since medullary structures possess all the circuitry for an operational baroreflex arc (Calaresu et al., 1975; Spyer, 1981) in renal denervated animals subjected to acute hypotension.

Nevertheless, as pointed out by several investigators (Haeusler, 1975; Chalmers, 1975; Antonaccio, 1977) NE may still play an important modulating role in regions of the CNS which receive indirect input (i.e. hypothalamus) from peripheral vascular receptors. Noradrenergic activity in the hypothalamus may modulate the ongoing activity in the subhypothalamic regions responsible for baroreflex control. Thus changes in noradrenergic activity in the hypothalamus may produce subtle changes in total sympathetic activity by utilization of medullary structures known to be involved in baroreflex sympathetic activity (Figure 49).

The results of studies in renal denervated rats suggest that the afferent renal nerves exert an excitatory influence on the noradrenergic activity in the hypothalamus providing further support for the model presented in Figure 49. Thus a reduction in noradrenergic activity in the hypothalamus due to removal of renal afferent fibers may be involved in

FIGURE 49

Hierarchical control of peripheral sympathetic activity. There is an inhibitory influence of baroreceptor input and an excitatory influence of renal afferent input on the noradrenergic activity of the hypothalamus relayed via the medulla. Although the noradrenergic activity in the hypothalamus can exert its effects on peripheral sympathetic activity via the medulla, a differential input from baroreceptor afferents and renal afferents can cause a dissociation between hypothalamic and peripheral noradrenergic activity.


HYPOTHALAMIC NORADRENERGIC ACTIVITY
MEDULLA
BARORECEPTOR AFFERENT INFLUENCE
RENAL AFFERENT INFLUENCE
PERIPHERAL SYMPATHETIC ACTIVITY

modulating the neurohormonal control of circulation. As pointed out recently (Yates, 1982), this would not be an unexpected role of renal afferent fibers, given the dependence of the renal circulation on arterial pressure for maintenance of blood flow. In addition, the results of the experiment with renal afferent nerve stimulation complement this hypothesis, since stimulation increased sympathetic outflow to the circulatory system, except the kidneys, thus maintaining an arterial pressure without compromising renal blood flow.

CHAPTER 10

Summary and Conclusions

The objectives of this research were: 1) to examine the relationship between changes in noradrenergic activity in the hypothalamus and changes in sympathetic outflow, and 2) to investigate the interaction between influences from baroreceptor afferents and influences from renal afferents as noradrenergic activity at the level of the hypothalamus. The results of this project can be summarized as follows:

1) There was an increased noradrenergic activity in the hypothalamus, kidney and skeletal muscle in response to surgical removal of aortic arch baroreceptor input by transection of the ADN.

2) There was an increased noradrenergic activity in the hypothalamus and all peripheral organs examined in response to acute hypotension ("unloading baroreceptors") in conscious rats.

3) There was no significant change in noradrenergic activity in hypothalamus and peripheral organs in response to acute hypertension ("loading baroreceptors") in conscious rats.

4) Renal denervation abolished the hypothalamic noradrenergic response in both ADN transected rats and acutely hypotensive rats. Renal denervation also abolished the elevated arterial pressure and increased NE turnover in peripheral organs in response to ADN transection in rats, but not the increased NE turnover

in the peripheral organs of acutely hypotensive rats.

5) Renal denervation unmasked a decreased turnover of NE in the hypothalamus of the acutely hypertensive rats.

6) Finally, direct electrical stimulation of afferent renal nerves failed to show any significant changes in hypothalamic noradrenergic activity, however there was an increased NE turnover in the skeletal muscle and an increased arterial pressure.

In conclusion, these results suggest that there is an inhibitory influence from the baroreceptors and an excitatory influence from the renal afferents to the noradrenergic systems in the hypothalamus. These results provide biochemical evidence for an interaction between renal afferent information and baroreceptor information at the level of the hypothalamus. In addition, these findings suggest a dissociation between hypothalamic and peripheral noradrenergic activity under acute conditions in renal denervated rats.

REFERENCES

Aars, H.S. Akre (1970). Reflex changes in sympathetic activity and arterial blood pressure evoked by afferect stimulation of the renal nerves. Acta. Physiol-Scand.d 78:184-188.

Abboud, F.M. (1982). The Sympathetic System in Hypertension State-of-the-Art Review. Hypertension 4:suppl II:208-225.

Adams, R.N. (1976). Probing Brain Chemistry with Electroanalytical echnique. Anal. Chem., 48:1126A-1138A.

Alexander, N., and De Quattro, V. (1974). Regional and systemic hemodynamic patterns in rabbits with neurogenic hypertension. Circ. Res. 35:636.

Alexander, N., McClaskey, J., and Moronde, R.F. (1976). Elevated plasma dopamine beta hydroxylase activity in rats with neurogenic hypertension. Life Sci. 18:655.

Alexander, N., and Velasquez, M. (1979). Indices of sympathetic activity in hypertensive rats. In: Catecholamine: Basic and Clinical Frontiers. (E. Usdin, I. Kopin, and J. Barchas, eds.). New York: Pergamon. vol. II, pp. 903-905.

Alexander, N., and Velasquez, M.T. (1980). Blood-pressure and plasma catecholamines in arterial baroreceptor-denervated rats. In: Arterial baroreceptors and hypertension. (Peter Slight ed.). Oxford University Press, New York, Toronto.

Alexander, R.S. (1946). Tonic and reflex functions of medullary sympathetic cardiovascular centres. J. Neurophysiol. 9:205-217.

Andēn, N.E., Dahlstrom, A., Fuxe, K., Larsson, K., Olson, L., and Ungerstedt, V. (1966). Ascending monoamine neurons to the telencephalon and diencephalon. Acta Physiol. Scand. 67:313-326.

Andēn,N.E., and Grabowska-Andēn, M. (1980). Influence of H_2-Receptor Blocking Agent Metiamide on the Clonidine-Induced Changes in the Brain Catecholamine Turnover. J. Neur. Trans. 47:175-180.

Anton, A., and Sayre, D. (1962). A Study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. J. Pharmacol. Exp. Ther. 138:360.

Antonaccio, M.J. (1977). Neuropharmacology of central mechanisms governing the circulation. In: Cardiovascular Pharmacology (M.J. Antonaccio ed.). New York, Raven Press, pp. 131-165.

Antonaccio, M.J., and Halley, J. (1975). Inhibition of centrally-evoked pressor responses by diazepam: Evidence for an exclusively supramedullary action. Neuropharmacology 14:649-658.

Astrom, A., and Crafoord, J. (1967). Afferent activity recorded in the kidney nerves of rats. Acta Physiol. Scand. 70:10-15.

Astrom, A., and Crafoord, J. (1968). Afferent and efferent activity in renal nerves of cats. Acta Physiol. Scand. 74:69-78.

Aukland, K. (1976). Renal blood flow. In: International review of physiology: Kidney and urinary tract physiology II vol 2 (K. Thurau ed.). University Park, Baltimore, pp. 25-79.

Axelrod, J. (1973). The fate of noradrenaline in the sympathetic neurone. Harvey Lect. 67:175-197.

Barajas, L. (1978). Innervation of the renal cortex. Fed. Proc. 37:1192-1201.

Barajas, L. (1981). The juxtaglomerular apparatus: anatomical considerations in feedback control of glomerular filteration rate. Fed. Proc. 40:78-86.

Bartelstone, H.J., and Nasmyth, P.A. (1965). Vasopressin potentiation catecholamine actions in dog, rat, cat and rat aortic strip. Am. J. Physiol. 208:745-762.

Beacham, W.S., and Kunze, D.L. (1969). Renal receptors evoking a spinal vasomoter reflex. J. Physiol. (Lond.) 201:73-85.

Bello-Reuss, E., Colindres, R.E., Pastoriza-Munoz, E., Mueller, R.A., and Gottschalk, C.W. (1975). Effects of acute unilateral denervation in the rat. J. Clin. Invest. 56:208-217.

Bhagat, B. (1967). The influence of sympathetic nervous activity on cardiac catecholamine levels. J. Pharmacol. Exp. Ther. 157:74-80.

Biscoe, T., and Sampson, S. (1970). Field potentials evoked in the brainstem of the cat by stimulation of the carotid sinus, glossopharyngeal, aortic and superior laryngeal nerves. J. Physiol. (London). 209:341-358.

Blaschko, H. (1973). Catecholamine biosynthesis. Brit. Med. Bull. 29:105.

Borchardt, R.T., Hegazi, M.F., and Schowen, R.L. (1978). Determination of O-Methylated Metabolites and Catecholamines Using High-Performance Liquid Chromatography and Electrochemical Detection. J. Chromatogr. 152:255-259.

Brod, J., Fencl, V., Hejl, Z., Jirka, J., and Ulrych, M. (1962). General and regional haemodynamic patterns underlying essential hypertension. Clin. Sci. 23:339-349.

Brodie, B.B., Costa, E., Dlabac, A., Neff, N.H., and Smookler, H.H. (1966). Application of steady state kinetics to the estimations of synthesis rate and turnover time of tissue catecholamines. J. Pharmacol. Exp. Ther. 154: 493-498.

Brody, M. Fink, G., Buggy, J., Haywood, J., Gordon, F., and Johnson, A. (1978). The role of the anteroventral third ventricle (AV3V) region in experimental hypertension. Circ. Res. 43:(suppl 1):I-2.

Brody, M.J., and Johnson, A.K. (1980). Role of the anteroventral third ventricle region in the fluid and electrolyte balance, arterial pressure regulation and hypertension. In: Frontiers in Neuroendocrinology (L. Martin and W.F. Ganong). New York, Raven Press, pp. 249.

Brody, M.J., and Zimmerman, B.G. (1976). Peripheral circulation in arterial hypertension. Prog. Cardiovasc. Dis. 18: 5:323-340.

Buñag, R.D., and Eferakeya, A.E. (1976). Immediate hypotensive after-effects of posterior hypothalamic lesions in awake rats with spontaneous, renal or DOCA hypertension. Cardiovasc. Res. 10:663.

Buñag, R.D., and Riley, E. (1979). Chronic Hypothalamic Stimulation in Awake rats fails to induce hypertension. Hypertension 1:498-507.

Calaresu, F.R., and Ciriello, J. (1980). Projections to the hypothalamus from buffer nerves and nucleus tractus solitarius in the cat. Am. J. Physiol. 239:R100-R109.

Calaresu, F.R., and Ciriello, J. (1981a). Altered concentration of catecholamines in the hypothalamus of the rat after renal denervation. Can. J. Physiol. Pharmacol. 59:1274-1277.

Calaresu, F.R., and Ciriello, J. (1981b). Renal afferent nerves affect discharge rate of medullary and hypothalamic single units in the cat. J. Auton. Nerv. Syst. 3:311-320.

Calaresu, F.R., Faiers, A.A., and Mogenson, G.J., (1975). Central neural regulation of heart and blood vessels in mammals. Prog. Neurobiol. 5: Part 1 pp. 1-35.

Calaresu, F.R., Kim, P., Nakamura, H., and Sato, A. (1978). Electrophysiological characteristics of reno-renal reflexes in the cat. J. Physiol. (Lond) 283:141-154.

Calaresu, F.R., Stella, A., and Zanchetti, A. (1976). Haemodynamic responses and renin release during stimulation of the afferent renal nerves in the cat. J. Physiol(Lond) 255:687-700.

Camacho, A., and Phillips, M.I. (1981). Seperation of drinking and pressor responses to central angiotensin by monoamines. Am. J. Physiol. 240:R106-R110.

Carlsson, A., Carrodi, H., and Waldeck, B. (1963). -Substituted dopa-cetamide as an inhibitor of catechol-O-methyl-transferase and of the enzymatic hydroxylation of aromatic amine acid in catecholamine metabolism. Helr. Chem. Acta 46:2270.

Chalmers, J.P. (1975). Brain amines and models of experimental hypertension. Circ. Res. 36:469-480.

Chalmers, J.P., Blessing, W.W., West, M.J., Howe, P.R., Costa, M., and Furness, J.B. (1981). Importance of new catecholamine pathways in control of blood pressure. Clin. Exp. Hypertens. 3(3):393-416.

Chalmers, J.P., Petty, M.A., and Reid, J.L. (1979). Participation of adrenergic and noradrenergic neurones in central connections of arterial baroreceptor reflexes in the rat. Circ. Res. 45:516-522.

Chalmers, J.P., and Reid, J.L. (1972). Participation of central noradrenergic neurons in arterial baroreceptors reflexes in the rabbit. Circ. Res. 31:789-804.

Chalmers, J.P., and Wurtman, R.J. (1971). Participation of central noradrenergic neurones in arterial baroreceptor reflexes in the rabbit. Circ. Res. 28:480-491.

Celiva, F.S., Harvis, J.F., and Lyons, R.H. (1959). Peripheral resistance in hypertension following the abolition of local sympathetic tone. Circulation 19:564.

Christensen, H.D., and Blank, C.L. (1979). The Determination of Neurochemicals in Tissue Samples at Subpicomole Levels. In: Biological/Biomedical Applications of Liquid Chromatography II. (Hawk, G.L. ed.). Marcel Dekker, New York.

Ciriello, J., and Calaresu, F.R. (1980). Hypothalamic projections of renal afferent nerves in the cat. Can. J. Physiol. Pharmacol. 58:574.

Ciriello, J., Kline, R.L., Zhang, T.X., and Caverson, M. (1983). Paraventricular nucleus lesions alter the development of spontaneous hypertension. Fed. Proc. 42:1193.

Ciriello, J., Palmer, E.M., and Calaresu, F.R. (1980a). Arterial pressure and heart rate in the rat after section of the aortic depressor or carotid sinus nerve. Proc. Can. Fed. Biol. Soc. 23:103.

Coleman, T.G., Cowley, A.W., and Guyton, A.C. (1975a). Experimental hypertension and the long-term control of arterial pressure. In: Cardiovascular Physiology. (A.C. Guyton and C.E. Jones eds.). University Park Press, Volume 1. pp. 259-297.

Coleman, T.G., Manning, R.D., Norman, R.A., and De Clue, J. (1975). The role of the kidney in spontaneous hypertension. Am. Heart J. 89:94-98.

Coleman, T.G., Selgado, M., Bengis, R., Davis, M., and Dent, A. (1981). Baroreceptor reflex in the rat after converting enzyme inhibition or angiotensin receptor blockade. Clin. Exp. Pharmacol. Physiol. 8:79-82.

Coleridge, H.M., and Coleridge, J.C. (1980). Cardiovascular afferents involved in regulation of peripheral vessels. Ann. Rev. Physiol. 42: 413-427.

Commarato, M.A., and Lum, B.K. (1969). Cardiovascular interactions of amphetamine and ephedrine with norepinephrine and with vasopressin. Eur. J. Pharmacol. 7:125-134.

Conway, J. (1963). A vascular abnormality in hypertension: A study of blood flow in the prearm. Circulation 27:520.

Costa, E., and Neff, N.H. (1966). A study of the control of catecholamine synthesis. Fed. Proc. 25:259.

Cottle, M.K. (1964). Degeneration studies of primary afferents of 9th and 10th cranial nerves in the cat. J. Comp. Neurol. 122:329-343.

Cowley, A.W., Jr. (1982). "Vasopressin and Cardiovascular Regulation" In: Cardiovascular Physiology IV. International Review of Physiology Volume 26 (A.C. Guyton and J.E. Hall ed.). University Park Press.

Cowley, A.W., Liard, J.F., and Guyton, A.C. (1973). Role of baroreceptor reflex in daily control of arterial blood pressure and other variables in the dog. Circ. Res. 32:564-576.

Cowley, A.W., and Lohmeier, T.E. (1979). Changes in renal vascular sensitivity and arterial pressure associated with sodium intake during long-term intrarenal norepinephrine infusion in dogs. Hypertension 1:549.

Cowley, A.W., Switzer, S.J., and Guinn, M.M. (1980). Evidence and quantification of the vasopressin arterial pressure control system in the dog. Circ. Res. 46:58-67.

Crill, W., and Reis, D. (1968). Distribution of carotid sinus and depressor nerves in cat brainstem. Am. J. Physiol. 214:269-276.

Crout, J.R. (1959). Sympathetic and adrenal medullary factors in hypertension. In: Hypertension: The First Hahnemann Symposium on Hypertensive Disease. (J.H. Moyer ed.) Philadelphia, Saunders, pp. 159-166.

Dahlstrom, A., and Fuxe, K. (1964). Evidence for the existance of monoamine-containing neurons in the central nervous system. 1. Demonstration of monoamines in the cell bodies of brainstem neurons. Acta Physiol. Scand. 62:1.

Dargie, H., Franklin, S., and Reid, J. (1976). The sympathetic nervous system in renovascular hypertension in the rat. Br. J. Pharmacol. 56:365P.

Davis, J.O., and Freeman, R.H. (1976). Mechanisms regulating renin release. Physiol. Rev. 56:1-56.

De Champlain, J., Krakoff, L., and Axelrod, J. (1967). Catecholamine metabolism in experimental hypertension in the rat. Circ. Res. 20:136.

De Champlain, J., Meuller, R.A., and Axelrod, J. (1969). Turnover and synthesis of norepinephrine in experimental hypertension in rats. Circ. Res. 25:285-290.

De Champlain, J., and van Ameringen, M.R. (1975). Adrenergic mechanisms and the aetiology of hypertension. In: Central Action of Drugs in Blood Pressure Regulation. (D.S. Davis and J.L. Reid ed.) University Park Press, Baltimore.

De Clue, J.W., Guytou, A.C., Cowley, A.W., Coleman, T.G., Norman, R.A., and McCaa, R.E. (1978). Subpressor angiotensin infusion, renal sodium handling, and salt-induced hypertension in the dog. Circ. Res. 43(4):503.

De Groot, J. (1959). The rat forebrain in stereotaxic coordinates. Verh. K. Ned. Akad. Wet., B. Natuurkd. 2:1-40.

De Jong, W. (1974). Noradrenaline: central inhibitory control of blood pressure and heart rate. Eur. J. Pharmcol. 29:179.

De Quattro, V., Eide, I., Myers, M., Eide, K., Kollock, R., and Whigham, H. (1978). Enhanced hypothalamic noradrenaline biosynthesis in Goldblatt I venovascular hypertension. Clin. Sci. 55:109.

De Quattro, V., Nagatsu, T., Maroude, R., and Alexander, N. (1969). Catecholamine synthesis in rabbits with neurogenic hypertension. Circ. Res. 24:545-555.

Di Bona, G.F. (1977). Neurogenic regulation of renal tubular sodium reabsorption. Am. J. Physiol. 233:F73-F81.

Di Brna, G.F. (1981). The role of the renal sympathetic nerves in the control of renal function. In: New trends in arterial hypertension (M. Worcel, J. Bonvalet, S. Langer, J. Menard, and J. Sassard, ed.). INSERM Symposium No 17, Elsevier/North Holland, Amsterdam, pp. 225-240.

Di Bona, G. (1982). The function of the renal nerves. Rev. Physiol. Biochem. Pharmacol. 94:76-157.

Diz, D.I., Nasjletti, A., and Baer, P.G. (1981). Effect of renal denervation on development of hypertension, plasm renin and urinary excretion of kallikrein, PGE and sodium in the New Zealand genetically hypertensive rat. Fed. Proc. 40:435.

Diz, D.I., Nasjletti, A., and Baer, P.G. (1982). Renal denervation at weaning retards development of hypertension in New Zealand genetically hypertensive rats. Hypertension 4:361-368.

Dietz, R., Schomig, A., Haebara, H., Maun, J.F., Rascher, W., Luth, J.B., Grunherz, N., and Gross, F. (1978). Studies on the pathogenesis of spontaneous hypertension in rats. Circ. Res. 43(Suppl. 1):98-106.

Doba, N., and Reis, D.J. (1973). Acute fulminating neurogenic hypertension produced by brainstem lesions in the rat. Circ. Res. 32:584-593.

Doba, N., and Reis, D.J. (1974). Role of central and peripheral adrenergic mechanisms in neurogenic hypertension produced by brainstem lesion in rats. Circ. Res. 34:293-301.

Donald, D.E., and Shepherd, J.T. (1980). Autonomic regulation of peripheral circulation. Ann. Rev. Physiol. 42:429-439.

Doyle, A.E., and Black, H. (1955). Reactivity to pressor agents in hypertension. Circulation 12:974.

Dreifuss, J.J., Harris, M.B., and Tribollet, E. (1976). Excitation of phasically firing hypothalamic supraoptic neurons by carotid occlusion in rats. J. Physiol. (Lond) 257:337-354.

Duncan, D.B. (1955). Multiple range and multiple F test. Biometrics 11:1-42.

Echtenkamp, S., Zucker, I.H., and Gilmore, J.P. (1980). Characterization of high and low pressure baroreceptor influences on renal nerve activity in primate Macaca fasicularis. Circ. Res. 46:726-730.

Eckstein, J.W., and Abboud, F.M. (1962). Circulatory effect of sympathomimetic amines. Am. Heart J. 63:119-135.

Eide, I., Myers, M.R., De Quattro, V., Kolloch, R., Eide, K., and Whigham, M. (1980). Increased hypothalamic noradrenergic activity in one-kidney, one-clip renovascular hypertensive rats. J. Cardiovasc. Pharmacol. 2:833.

Erker, E.F., and Chan, W.Y. (1977). The site and mechanism of phenoxybenzamine potentiation of the pressor response to oxytocin and vasopressin: In vivo and isolated aortic strips studies. J. Pharmacol. Exp. Ther. 202:287-293.

Faber, J.E., and Brody, M.J. (1983). Renal nerve-dependent hypertension following acute renal artery stenosis in the conscious sinoaortic deafferented rat. Fed. Proc. 42:3404.

Farnebo, L-O., Hallman, H., Hamberger, B., and Jonsson, G. (1979). Catecholamines and hemorrhagic shock in awake and anesthetized rats. Circ. Shock 6:109-118.

Fernandez, B.E., Dominquez, A.E., Videl, N.A., and Taquini, A.C. (1974). Renal denervation and catecholamine of the central nervous system. Neuroendocrinology 15:338-345.

Ferrario, C.M., Gildenberg, P.L., and McCubbin, J.W. (1972). Cardiovascular effects of angiotensin mediated by the central nervous system. Circ. Res. 30:257-262.

Finch, L., Haeusler, G., and Thoenen, H. (1972). Failure to induce experimental hypertension in rats after intraventricular injection of 6-hydroxydopamine. Br. J. Pharmacol. 44:356-357.

Fink, G.D., and Bryan, W.J. (1980). Renal vascular resistance and reactivity in the neurogenic hypertensive rat. Am. J. Physiol. 239:F474-F477.

Fink, G.D., Kennedy, F., Bryan, W.J., and Werber, A. (1980b). Pathogenesis of hypertension in rats with chronic aortic baroreceptor deafferentation. Hypertension 2:319-325.

Folkow, B. (1974). The neurogenic component in spontaneously hypertensive rats (SHR): A survey. In: The nervous system in arterial hypertension (S. Julius, and M.D. Esler, ed.). Charles C. Thomas, Springfield, Illinois, pp. 3-16.

Folkow, B., Gothberg, G., Lundin, S., and Ricksten, S. (1977). Structural "resetting" of the renal vascular bed in spontaneously hypertensive rats (SHR). Acta Physiol. Scand. 100:270.

Folkow, B., Hallback, M., Lundgren, Y., Silvertsson, R., and Weiss, L. (1976). The importance of adaptive changes in vascular design for the establishment and maintenance of primary hypertension as studied in man and in spontaneously hypertensive rat. In: Spontaneous hypertension, (K. Okamoto, ed.). Springer-Verlag, New York, pp. 37-40.

Folkow, B., Hallback, M., Lundgren, Y., and Weiss, L. (1972). The effects of "immunosympathectomy" on blood pressure and vascular "reactivity" in normal and spontaneously hypertensive rats. Acta Physiol. Scand. 84:512.

Folkow, R., and Rubinstein, E.H. (1966). Cardiovascular effects of acute and chronic stimulations of the hypothalamic defence area in the rat. Acta Physiol. Scand. 68:48-57.

Francisco, L.L., Hoversten, L.G., and Di Bona, G.F. (1980). Renal nerves in the compensatory adaptation to ureteral occlusion. Am. J. Physiol. 238:F229-F234.

Fray, J.C. (1980). Stimulus-secretion coupling of renin. Circ. Res. 47:485-493.

Frew, J.L., and Rosenheim, M.L. (1949). Labile neurogenic component of hypertension: Comparison of effects of tetraethyl-ammonium bromide and rapidly acting barbiturate. Clin. Sci. 7:217.

Frohlich, E.D. (1977). The adrenergic nervous system and hypertension, state of the art. Second Pan-American Symposium on Hypertension. In: Mayo-Clin. Proc. 52:361.

Fukyama, K. (1973). Central modulation of baroreceptor reflex by angiotensin. Jpn. Heart J. 14:135-139.

Fuxe, K. (1965). Evidence for the existance of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. Acta Physiol. Scand. 64(247):37.

Ganten, D., Unger, T., Rascher, W., Fuxe, K., Hokfelt, T., and Agnati, L. (1980). Peptidergic and catecholaminergic mechanisms in central blood pressure control. Contrib. Nephrol. 23:93-104.

Genest, J. (1973). Relationship of steroid hormones to hypertension: Present state of art. In: Mechanisms of Hypertension. (M.P. Sambhi ed.) Excerpta. Med. Int. Cong. Ser. 302:142-147.

Gerke, D.C., Frewin, D.B., and Frost, B.R. (1976). The influence of adrenergic nerves on the response of blood vessels in the rabbit ear to 2-phenylanine-8-lysine vasopressin(octapressin). Aust. J. Exp. Biol. Med. Sci. 54:467-474.

Gerke, D.C., Frewin, D.B., and Frost, B.R. (1977). The synergistic vasocontrictor effect of octapressin and catecholamines on the isolated rabbit ear artery. Aust. J. Exp. Biol. Med. Sci. 55:737-740.

Goldblatt, H., Lynch, J., Hanzel, R.F., and Summerville, W.W. (1934). Studies on experimental hypertension. In: The production of persistent elevation of systolic blood pressure by means of renal ischemia. J. Exp. Med. 59:347.

Grabowska, M., and Anden, N.E. (1976). Noradrenaline synthesis and utilization control by nerve impulse flow under normal conditions and after treatment with alpha-adrenoreceptor blocking agents. Naunyn. Schmiedebergs. Arch. Pharmacol. 292:53-58.

Gordon, R., Spector, S., Sjoerdsma, A., and Udenfriend, S. (1966). Increased synthesis of norepinephrine and epinephrine in the intact rat during exercise and exposure to cold. J. Pharmacol. Exp. Ther. 153:440.

Gorgas, K. (1978). Innervation of the juxtaglomerular apparatus. In: Peripheral neuroendocrine interaction (R.E. Coupland and W.G. Forssman, ed.). Springer, Berlin, Heidelberg, New York, pp. 144-152.

Gottschalk, C.W. (1979). Renal nerves and sodium excretion. Ann. Rev. Physiol. 41:229-240.

Guyton, A.C., Cowley, A.W., Young, D.B., Coleman, T.G., Hall, J.E., and De Clue, J.W. (1976). Integration and control of circulatory function. In: Cardiovascular Physiology II (A.C. Guyton, and A.W. Cowley, ed.). University Park Press, Baltimore, pp. 341-385.

Guyton, A.C., and Coleman, T.G. (1969). Quantitative analysis of the pathophysiology of hypertension. Circ. Res. 24(Suppl. I):1-14.

Guyton, A.C., Coleman, T.G., Cowley, A.W., Manning, R.D., Norman, R.A., and Ferguson, J.D. (1974). A systems analysis approach to understanding long-range arterial blood pressure control and hypertension. Circ. Res. 35:159-176.

Guyton, A.C., and Coleman, T.G. (1967). Long-term regulation of the circulation: Interrelationships with body fluid volumes. In: Physical Bases of Circulatory Transport: Regulation and Exchange (E.B. Reeve and A.C. Guyton, ed.). W.B. Saunders Co., Philadelphis, pp. 179-201.

Guyton, A.C., Coleman, T.G., and Granger, H.J. (1972). Circulation: Overall regulation. Ann. Rev. Physiol. 34:13.

Haeusler, M.D. (1975). Cardiovascular regulation by central adrenergic mechanisms and its alteration by hypotensive drugs. Circ. Res. 36 and 37:Suppl. 1:223-228.

Haeusler, G., Finch, L., and Thoenen, H. (1972). Central adrenergic neurons and the intiation and development of experimental hypertension. Experientia 28:1200-1203.

Haeusler, G., Gerold, M , and Thoenen, H. (1972). Cardiovascular effects of 6-hydroxydopamine injected into the lateral brain ventricle of the rat. Naunyn. Schmiedebergs. Arch. Pharmacol. 24:211-228.

Haeusler, G., and Lewis, P. (1975). The baroreceptor reflex and its relations to central adrenergic mechanisms. In: Recent advances in hypertension vol. 2 (P. Milliez, and M. Safer, ed.). Laboratories Boehringer Ingelheim, Reims, pp. 17-26.

Hallback, M., and Folkow, B. (1974). Cardiovascular responses to acute mental "stress" in spontaneously hypertensive rats. Acta Physiol. Scand. 90:684.

Haywood, J.R., Patel, N.P., Mahoney, L.T., Touw, K.B., Johnson, A.K., Corry, R.J., and Brody, M.J. (1979). Afferent renal nerves in the pathogenesis of experimental hypertension. Fed. Proc. 38:883 (Abstr).

Hess, W.R. (1957). The functional organization of the diencephalon. New York, N.Y. Grune and Stratton. pp. 4-58.

Hökfelt, T., Eide, R., Fuxe, K., Johansson, O., Ljungdahl, A., Goldstein, M., Luft, R., Efeudic, S., Nilssou, G., Terenius, L., Gunten, D., Jeffcoate, S., Rekfeld, J., Said, S., Pavez de la Mora, M., Possaui, L., Rapia, R., Teran, L., and Palacios, R. (1978). The hypothalamus. In: Research publications: Association for research in nervous and mental disease, vol. 56 (S. Reidilin, R.J. Baldessarini, and J.B. Martin, ed.). Raven Press, New York.

Horvath, C., Melander, W., and Molnar, I. (1976). Solvophobic interactions in liquid chromatography with nonpolar stationary phases. J. Chromatogr. 125:129-156.

Ito, C.S., and Scher, A.M. (1978). Regulation of arterial blood pressure by aortic baroreceptors in the unanesthetized dog. Circ. Res. 42:230-236.

Ito, C.S., and Scher, A.M. (1979). Hypertension following denervation of aortic baroreceptors in unanesthetized dogs. Circ. Res. 45:26-34.

Iverson, L.L., and Glawinski, J. (1966). Regional studies of catecholamines in the rat brain. II. Rate of turnover of catecholamines in various brain regions. J. Neurochem. 13:671-682.

Jacobson, S. (1972). Hypothalamus and Autonomic Nervous System. In: An Introduction to the Neurosciences. (B.A. Curtis, S. Jacobson and E.M. Marcus eds.) W.B. Saunders Co., Philadelphia, London, Toronto.

Johnson, A., Buggy, J., Finke, G., and Brody, M. (1981). Prevention of renal hypertension and of the central pressor effect of angiotensin by ventralmedial hypothalamic ablation. Brain Res. 205:255.

Johnson, J.A., Davis, J.O., and Witty, R.T. (1971). Effects of catecholamine and renal nerve stimulation of renin release in the non-filtering kidney. Circ. Res. 29:646-653.

Judy, W.V., Watanebe, A., Henery, D., Besch, H., Murphy, W., and Hockel, G. (1976). Sympathetic nerve activity - role in regulation of blood pressure in spontaneously hypertensive rat. Circ. Res. 38:(suppl II)21.

Julius, S., and Esler, M.D. (1974). In: The Nervous System in Arterial Hypertension. (S. Julius and M. Esler eds.). Charles C. Thomas, Springfield, Illinois.

Julius, S., Pascual, A., and London, R. (1971). Role of parasympathetic inhibition in the hyperkinetic type of borderline hypertension. Circulation 44:413-418.

Kabat, W., Magoun, H.W., and Ranson, S.W. (1935). Electrical stimulation of points in the forebrain and midbrain. The resultant alterations in blood pressure. Archs. Neurol. Psychiat. 34:931-955.

Kannan, H., and Yagi, K. (1978). Supraoptic neurosecretory neurons: Evidence for the existance of converging inputs from carotid baroreceptors and osmoreceptors. Brain Res. 145:385-390.

Karpus, J., and Kreidl, A. (1909). Cited by: Calaresu et al. (1975).

Katholi, R.E., Carey, R.M., Ayers, C.R., Vaughan, E.D., Yancey, M.R., and Martin, C.L. (1977). Production of sustained hypertension by chronic intrarenal norepinephrine infusion in conscious dogs. Circ. Res. 40 (Suppl. I):118-126.

Katholi, R.E., Naftilan, A.J., and Oparil, S. (1979). The effect of renal denervation on the development of DOCA-salt hypertension in the rat. Clin. Res. 27:467A (Abstr).

Katholi, R.E., Naftilan, A.J., and Oparil, S. (1980). Importance of renal sympathetic tone in the development of DOCA-salt hypertension in the rat. Hypertension 2:266-273.

Katholi, R.E., Winternitz, S.R., and Oparil, S. (1981). Role of the renal nerves in the pathogenesis of one-kidney renal hypertension in the rat. Hypertension 3:404-409.

Katholi, R.E., Winternitz, S.R., and Oparil, S. (1982a). Decrease in peripheral sympathetic nervous system activity following renal denervation or unclipping in the one-kidney, one-clip Goldblatt hypertensive rat. J. Clin. Invest. 69:55-62.

Katholi, R.E., Whitlow, P.L., Winternitz, S.R., and Oparil, S. (1982b). Importance of renal nerves in established two-kidney, one-clip Goldblatt hypertensive rat. Hypertension 4:369-373.

Kelton, T.K., and Campbell, W.B. (1980). The pharmacological alteration of renin release. Pharmacol. Rev. 32:81.

Keller, R., Oke, A., Mefford, I., and Adams, R. (1976). Liquid chromatographic analysis of catecholamines routine assay for regional brain mapping. Life Shi. 19:995-1004.

Kendrick, E., Oberg, B., and Wennergren, G. (1972). Vasoconstrictor fibre discharge to skeletal muscle, kidney, intestine and skin at varying levels of arterial baroreceptor activity in the cat. Acta Physiol. Scand. 85:464-476.

Kezdie, P., and Gellar, E. (1968). Baroreceptor control of postganglionic sympathetic nerve discharge. Am. J. Physiol. 214:427-435.

Kirchheim, H.R. (1976). Systemic arterial baroreceptor reflexes. Physiol. Rev. 56:100-176.

Kissinger, P.T., Refshange, C.J., Dreiling, R., Blank, L., Freeman, R., and Adams, R.N. (1973). An electrochemical detector for liquid chromatography with picogram sensitivity. Anal. Lett. 6:465-477.

Kleinerman, J., Sancetta, S.M., and Hackel, D.B. (1958). Effects of high spinal anesthesia on cerebral circulation and metabolism in man. J. Clin. Invest. 37:285.

Kline, R.L., Kelton, P.M., and Mercer, P.F. (1978). Effect of renal denervation on the development of hypertension in spontaneously hypertensive rats (SHR). Can. J. Physiol. Pharmacol. 56:818-822.

Kline, R.L., Patel, K.P., Ciriello, J., and Mercer, P.F. (1983). Effect of renal denervation on arterial pressure in rats with aortic nerve transection. Hypertension.

Kline, R.L., Stuart, P.J., and Mercer, P.F. (1980). Effect of renal denervation on arterial pressure and renal norepinephrine concentration in Wistar-Kyoto and spontaneously hypertensive rats. Can. J. Physiol. Pharmacol. 58:1384-1388.

Knuepfer, M.M., Mohrland, J., Shaffer, R., Gebhart, G., Johnson, A., and Brody, M. (1980). Effects of afferent renal nerve (ARN) stimulation and baroreceptor activation on unit activity (UA) in anteroventral third ventricle (AV3V) region (abst). Fed. Proc. 39:387.

Koizumi, K., and Yamashita, H. (1978). Influence of atrial stretch receptors on hypothalamic neurosecretory neurons. J. Physiol 285:341-358.

Kopin, I.J. (1978). Measuring turnover of neurotransmitters in human brain. In: Psychopharmacology. A Generation of Progress (M. Lipton, A. Di Mascio, and K. Killam eds.). Raven Press, New York. pp. 933-942.

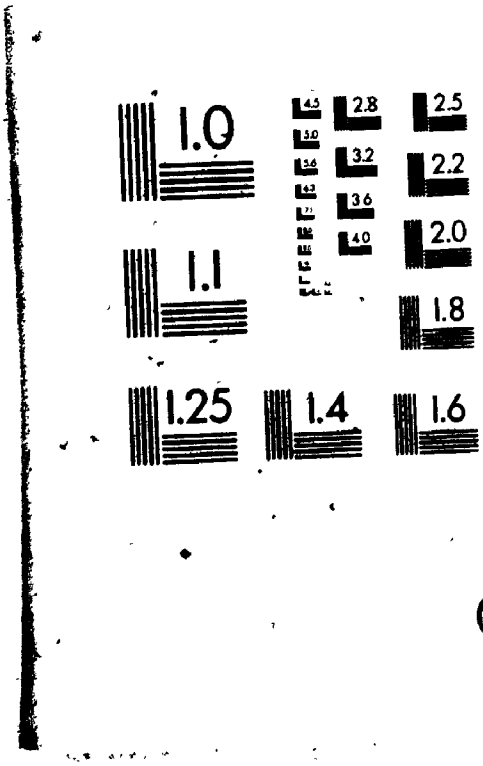
Kopin, I.J., Lake, C.R., and Ziegler, M. (1978). Plasma levels of norepinephrine. Am. Int. Med. 88:671-680.

Korner, P.I. (1971). Integrative neural cardiovascular control. Physiol. Rev. 51:312-367.

Korner, P.I. (1976). Problems of integrative cardiovascular control. Proc. Aust. Physiol. Pharmacol. Soc. 7:35-48.

4 4

OF / DE



Korner, P.I. (1979). Central nervous control of automatic cardiovascular function. In: HANDBOOK OF PHYSIOLOGY. Section 2: Circulation Volume 1: The Heart. Am. Physiology. Soc. Chapter 20. (R.M. Berne and N. Sperflakis eds.).

Korner, P.I., Shaw, I., West, M.J., and Oliver, J.R. (1972). Central nervous system control of baroreceptor reflexes in the rabbit. Circ. Res. 31:637-652.

Korner, P.I., and Uther, J.B. (1969). Dynamic characteristics of cardiovascular autonomic effects during severe arterial hypoxia in the unanestetized rabbit. Circ. Res. 24:671-687.

Kostreva, D.R., Seagard, J.L., Costaner, A., and Kampine, J.P. (1981). Reflex effects of renal afferents on the heart and kidney. Am. J. Physiol. 241: R286-R292.

Krieger, E.M. (1964). Neurogenic hypertension in the rat. Circ. Res. 15:511-521.

Krieger, E.M. (1970). Time course of baroreceptor resetting in acute hypertension. Am. J. Physiol. 218:486.

Laubie, M., and Schmitt, H. (1979). Destruction of the nucleus tractus solitarii in the dog: comparison with sinoaortic denervation. Am. J. Physiol. 236:H736-H7743.

Leenen, F., and Provoost, A. (1981). Maintenance of blood pressure by β-adrenoceptor mediated renin release during different forms of anesthesia in rats. Can. J. Physiol. Pharmacol. 59:364-370.

Levy, S.B., Holle, R., Hsu, L., Lillely, J.J., and Stone, R.A. (1977). Physiologic and pharmacologic influence on adrenergic regulation of renin release. Clin. Pharmacol. Ther. 22:511-518.

Liard, J.F. (1977). Renal denervation delays blood pressure increase in the spontaneously hypertensive rat. Experientia 33:339-340.

Louis, W.J., Talsei, R., Spector, S., and Sjoerdsma, A. (1969). Studies on the spontaneously hypertensive rat. Circ. Res. 24(25):(suppl. 1)193.

Ludwig, C. (1867). Quoted In: The Control of the Circulation of Blood. (R.J. McDowell, ed.). Dawson and Sons Ltd., London. 1938 & 1956.

Magoun, H.W., Ranson, S.W., and Hetherington, A. (1938). Descending connections from the hypothalamus. Archs. Neurol. Psychiatr. 39:1127-1149.

Mahoney, L.T., Haywood, J.R., Packwood, W.J., Johnson, A.K., and Brody, M.J. (1978). Effect of anteroventral third ventricle (AV3V) lesions on regional hemodynamic responses to renal afferent nerve stimulation (abstr). Circ. Res. 58(Suppl II):II-68.

Mancia, A., Donald, D.E., and Shepherd, J.T. (1973). Inhibition of adrenergic outflow to peripheral blood vessels by vagal afferents from the cardiopulmonary region in the dog. Circ. Res. 33:713-721.

Mancia, G., and Zanchetti, A. (1981). Hypothalamic control of Autonomic Function. In: Handbook of the hypothalamus. Volume 3. Part B. (P.J. Morgane and J. Panksepp eds.). Marcel Dekker, Inc., pp. 147-202. New York and Basel.

Manning, J.W. (1965). Cardiovascular reflexes following lesions in medullary reticular formation. Am. J. Physiol. 208:283-288.

Molnar, I., and Horvath, C. (1976). Reverse-phase chromatography of polar biological substances: Seperation of catechol compounds by high-performance liquid chromatography. Clin. Chem. 22: 9:1497-1502.

Moor, R., and Bloom, F. (1979). Central catecholamine neuron systems: Anatomy and physiology of norepinephrine and epinephrine systems. Ann. Rev. Neurosci. 2:113-168.

Moyer, J.H. (1959). Comparison of blood pressure responses and symptomatic effects of mecanylamine and currently available quarternary ammonium compounds. In: Hypertension in the First Hahnemann Symposium on Hypertensive Disease. (H. John ed.). Philadelphia. Saunders, pp. 446-450.

Moyer, T., and Jiang, N. (1978). Optimized isocratic conditions for a analysis of catecholamines by high-performance reversed-phase paired-ion chromatography with amperometric detection. J. Chromatogr. 153:365-372.

Murray, R.H., Luft, F.C., Block, R., and Weyman, A.E. (1978). Blood pressure responses to extremes of sodium intake in normal man. Proc. Soc. Exp. Biol. Med. 159:432.

Nagaoka, A., and Lovenberg, W. (1977). Regional changes in the activities of aminergic biosynthetic enzymes in the brain of hypertensive rats. Eur. J. Pharmacol. 43:297.

Nagatsu, T., Levitt, M., and Udenfriend. S. (1964). Tyrosine hydroxylase: The initial step in norepinephrine biosynthesis. J. Biol. Chem. 239:2910.

Nakamura, K., Gerold, M., and Thoenen, H. (1971). Experimental hypertension in the rat: Reciprocal changes of norepinephrine turnover in the heart and brainstem. Naunyn Schmiedebergs Arch. Pharmacol. 268:125.

Nakamura, K., Gerold, M., and Thoenen, M. (1970). "Experimental hypertension in the rat: Reciprocal changes of norepinephrine turnover in heart and brainstem". Jpn. J. Pharmacol. 20:605.

Nakamura, K. and Nakamura, K. (1981). Influence of sino-aortic receptor denervation on catecholamines, catecholamine-synthesizing enzymes and choline acetyltransferase activity in the brainstem nuclei of the rat. Jpn. J. Pharmacol. 31:95-105.

Neff, N.H., and Costa. E. (1968). Application of steady-state kinetics to the study of catecholamine turnover after monoamine oxidase inhibition or reserpine administration. J. Pharmacol. Exp. Ther. 160:40-47.

NIH Hypertension Task Force Report. (1979). Volume 4, U.S. Department of Health, Education and Welfare. Public Health Service, NIH Publication No. 79-1626.

Niijima, A. (1975). Observation on the localization of mechanoreceptors in the kidney and afferent nerve fibers in the renal nerves in the rabbit. J. Physiol. (Lond) 245:81-90.

Niijima, A. (1972). The effect of efferent discharges in renal nerves on the activity of arterial mechanoreceptors in the kidney in rabbit. J. Physiol. (London). 222:335-343.

Norman, R.A., Coleman, T.G., and Dent, A.C. (1981). Continuous monitoring of arterial pressure indicates sinoaortic denervated rats are not hypertensive. Hypertension 3(1):119-125.

Norman, R.A. Enobakhave, J.A., De Clue, J.W., Douglas, B.H., and Guyton, A.C. (1977). Renal function curves (RFC) in Goldblatt and spontaneously hypertensive rats. Fed. Proc. 36:531.

Norman, R.A., Enobakhave, J.A., De Clue, J.W., Douglas, B.H., and Guyton, A.C. (1978) Renal function curves in normotensive and spontaneously hypertensive rats. Am. J. Physiol. 234:R98.

Okamoto, K., Nosaka, S., Yamori, Y., and Matsumoto, M. (1967). Participation of neural factors in the pathogenesis of hypertension in the spontaneously hypertensive rat. Jpn. Heart J. 8:168.

Oliver, G., and Schafer, E.A. (1895). The physiological effect of extracts of the suprarenal capsules. J. Physiol. (Lond) 18:230-279.

Page, I.H. (1939). The production of persistent arterial hypotension by cellophane perinephritis. J. Am. Med. Assoc. 113:2046.

Palkovits, M., Brownstein, M., Saavedra, J., and Axelrod, J. (1974). Norepinephrine and dopamine content of hypothalamic nuclei of the rat. Brain Res. 77:137-149.

Patel, K.P. (1980). Noradrenergic mechanisms in brain and peripheral organs of normotensive and spontaneously hypertensive rats (SHR) at various ages. MSc. Thesis. University of Western Ontario, London, Canada.

Patel, K.P., Kline, R.L., and Mercer, P.F. (1981). Noradrenergic mechanisms in the brain and peripheral organs of normotensive and spontaneously hypertensive rats at various ages. Hypertension 3:682-690.

Persson, B., and Svensson, T.H. (1981). Control of Behaviour and Brain Noradrenaline Neurons by Peripheral Blood Volume Receptors. J. Neu. Trans. 52:73-82.

Petty, M., and Reid, J. (1977). Changes in noradrenaline concentration in brain stem and hypothalamic nuclei during the development of renovascular hypertension. Brain Res. 136:376-380.

Petty, M., and Reid, J. (1979). Catehcolamine synthesizing enzymes in brainstem and hypothalamus during the development of renovascular hypertension. Brain Res. 163:277-288.

Pfeffer, J.M., Pfeffer, M.A., and Frolich, E.D. (1971). Validity of an indirect tail-cuff method for determining systolic arterial pressure in unanesthetized normotensive and spontaneously hypertensive rats. J. Lab. Clin. Med. 78:957-962.

Philippu, A., Demmeler, R., and Rosenberg, G. (1974). Effects of centrally applied drugs on pressor responses to hypothalamic stimulation. Naunyn-Schmiedebergs Arch. Pharmacol. 282:389-400.

Philippu, A., Dietl, H., and Eisert, A. (1981). Hypotension alters the release of catecholamines in the hypothalamus of the conscious rabbit. Eur. J. Pharmacol. 69:519-523.

Philippu, A., Dietl, H., and Sinha, J.N. (1979). In vivo release of endogenous catecholamines in the hypothalamus. Naunyn-Schmiedebergs Arch. Pharmacol. 308:137.

Philippu, A., Heyd, G., and Burger, A. (1970). Release of noradrenaline from the hypothalamus in vivo. Eur. J. Pharmacol. 9:52-58.

Philippu, A. Przuntek, H., Heyd, G., and Burger, A. (1971). Central effects of sympathomimetic amines on the blood pressure. Eur. J. Pharmacol. 15: 200-208.

Philippu, A., Rosenberg, W., and Przuntek, H. (1973). Effect of adrenergic drugs on pressor responses to hypothalamic stimulation. Naunyn-Schmiedebergs Arch. Pharmacol. 278:373-386.

Pomeranz, B., Birtch, A.G., and Barger, A.C. (1968). Neural control of intrarenal blood flow. Am. J. Physiol. 215:1067-1081.

Przuntek, H., Guimaraes, S., and Philippu, A. (1971). Importance of adrenergic neurons of the brain for the rise of blood pressure evoked by hypothalamic stimulation. Naunyn-Schmiedebergs Arch. Pharmacol. 271:311-319.

Przuntek, H., and Philippu, A. (1973). Reduced pressor response to stimulation of the locus caeruleus after lesion of the posterior hypothalamus. Naunyn-Schmiedebergs Arch. Pharmacol. 276:119-122.

Ratner, M.Y. (1953). Effect of kidney denervation on experimental neurogenic hypertonia. Bull. Exp. Biol. Med. 36:28-33.

Recordati, G.M., Moss, N.C., Genovesi, S., and Rogenes, P.R. (1980). Renal receptors in the rat sensitive to chemical alterations of their environment. Circ. Res. 46:395.

Recordati, G.M., Moss, N.C., and Waselkov, L. (1978). Renal chemoreceptors in the rat. Circ. Res. 43:534.

Refshange, C.J., Kissinger, P.T., Dreiling, R., Blank, L., Freeman, R., and Adams, R. (1974). New High Performance Liquid Chromatographic Analysis of Catecholamines. Life Sciences. 14:311-322.

Reid, I.A., Morris, B.J., and Ganong, W.F. (1978). The renin-angiotensin system. Ann. Rev. Physiol. 40:377-410.

Reid, J.L., Zavin, J.A., and Kopin, I.J. (1975). Central and peripheral adrenergic mechanisms in the development of deoxycorticosterone saline hypertension in rats. Circ. Res. 37:569.

Ricardo, J.A., and Koh, E. (1978). Anatomical evidence of direct projections from the nucleus tractus solitarius to the hypothalamus, amygdala and other forebrain structures in the rat. Brain Res. 153:1-26.

Sakumoto, T., Tohyama, M., Satoh, K., Kimoto, Y., Kinugasa, T., Tanizowa, O., Kurachi, K., and Shimizu, N. (1978). Afferent fiber connections from lower brain stem to hypothalamus studied by the horseradish peroxidase method with special reference to noradrenaline innervation. Exp. Brain Res. 31:81-94.

Salzman, P., and Roth, R.H. (1979). Role of impulse flow in the short-term regulation of norepinephrine biosynthesis. Prog. Neurobiol. 13:1-60.

Sapru, H.N., and Krieger, A.J. (1977). Carotid and aortic chemoreceptor function in the rat. J. Appl. Physiol. 42(3):344-348.

Sasa, S., and Blank, C. (1979). Simultaneous determination of norepinephrine, dopamine, and serotonin in brain tissue by high-pressure liquid chromatography with electrochemical detection. Anal. Chem. Acta 104:24-45.

Selkurt, E.E. (1951). Effect of pulse pressure modification on renal hemodynamics and electrolyte and water excretion. Circulation 4:541-551.

Severs, W.B., and Daniels-Severs, A.E. (1973). Effects of angiotensin on the central nervous system. Pharmacol. Rev. 25:415-449.

Sharman, D.F. (1981). 2. The turnover of Catecholamines. In: Central Neurotransmitter Turnover. (C.J. Pycock and P.V. Taberner ed.). 20-58.

Shellenberger, M., and Gordon, J. (1971). A rapid, simplified procedure for simultaneous assay of norepinephrine, dopamine and 5-hydroxytryptamine from discrete brain areas. Analy. Biochem. 39:356-372.

Sinha, J.N., Dietl, H., and Philippu, A. (1980). Effects of a fall of blood pressure on the release of catecholamines in the hypothalamus. Life Sci. 26:1751-1760.

Sipes, I.G. (1969). Studies on the pharmacology and peripheral mechanisms of action of fenfluramine hydrochloride. Ph.D. Thesis, University of Pittsburg.

Snyder, D.W., Doba, N., and Reis, D. (1978). Regional distribution of blood flow during arterial hypertension produced by lesions of the nucleus tractus solitarii in rats. Circ. Res. 42:87-91.

Sole, M.J., Nasir, M.N., and Lixfeld, W. (1980). Activation of brain catecholaminergic neurons by cardiac vagal afferents during acute myocardial ischemia in the rat. Circ. Res. 47:166-172.

Spector, S., Sjoerdsma, A., and Udenfriend, S. (1965). Blockade of endogenous norepinephrine synthesis by α-methyl-tyrosine, an inhibitor of tyrosine hydroxylase. J. Pharmacol. Exp. Ther. 147:86.

Spyer, K.M. (1972). Baroreceptors sensitive neurons in the anterior hypothalamus of the cat. J. Physiol (Lond) 224:245-257.

Spyer, K.M. (1981). Neural organisation and control of the baroreceptor reflex. Rev. Physiol. Biochem. Pharmacol. 88:24-124.

Struyker Boudier, H.J., Smeets, G.M., Brouwer, G.M., and van Rossum, J.M. (1974). Hypothalamic alpha adrenergic receptors in cardiovascular regulation. Neuropharmacol. 13:837-846.

Stuart, P.J., Kline, R.L., and Mercer, P.F. (1979). Norepinephrine concentration in kidneys of normotensive Wistar-Kyoto and spontaneously hypertensive rats. Can. J. Physiol. Pharmacol. 57:438.

Sumners, C., and Phillips, M.I. (1983). Central injection of angiotensin II alters catecholamine activity in rat brain. Am. J. Physiol. 244: R257-R263.

Svensson, T.H., and Thoren, P. (1979). Brain noradrenergic neurons in the locus caeruleus: inhibition by blood volume load through vegal afferents. Brain Res. 172:174-178.

Swanson, L.W., and Sawchenko, P.E. (1983). Hypothalamic Integration: Organization of the Paraventricular and Supraoptic Nuclei. Ann. Rev. Neurosci. 6:269-324.

Sweet, C.S., Kadowitz, P., and Brody, M.J. (1971). Arterial hypertension elicited by prolonged intravertebral infusions of angiotensin II in the conscious dog. Am. J. Physiol. 221:1640-1645.

Takacs, L., Bencsath, P., and Szalay, R. (1978). Decreased proximal tubular transport capacity after renal sympathectomy. In: Proceedings VII th International congress of nephrology (S.Karger ed.). Basel, pp. 553-558.

Takimoto, G.S., and Weiner, N. (1981). Effect of nitroprusside-induced hypotension on sympathetic nerve activity, tyrosine hydroxylase activity and [H] norepinephrine uptake in rabbit vascular tissues. J. Pharmacol. Exp. Ther. 219:420-427.

Tanaka, M., de Kloet, E.R., de Wied, D., and Versteeg, D.H. (1977). Arginine -vasopressin affects catecholamine metabolism in specific brain nuclei. Life Sci. 20:1799-1808.

Tanaka, T., Seki, A., Fujii, J., Kurihara, H., and Ikeda, M. (1982). Norepinephrine turnover in the cardiovascular tissues and brain stem of the rabbit during development of one-kidney and two-kidney Goldblatt Hypertension. Hypertension 4:272-278.

Thomas, M,R., and Calaresu, F.R. (1972). Responses of single units in the medial hypothalamus to electrical stimulation of the carotid sinus nerve in the cat. Brain Res. 44:49-62.

Touw, K., Fink, G., Haywood, J., Shaffer, R., and Brody, M.J. (1979). Elevated neurogenic vasoconstrictor tone is the mechanism of hypertension in rats with aortic baroreceptor deafferentation. Fed. Proc. 38:1232.

Touw, K.B., Haywood, J.R., Shaffer, R., and Brody, M. (1980). Contribution of the sympathetic nervous system to vascular resistance in conscious young and adult spontaneously hypertensive rats. Hypertension 2:408-418.

Tozar, T.N., Neff, N.H., and Brodie, B.B. (1966). Application of steady state kinetics to the synthesis rate and turnover time of serotonin in the brain of normal and reserpine treated rats. J. Pharmacol. Exp. Ther. 153:177.

Uchida, Y., Kamisaka, K., and Ueda, H. (1971). Two types of renal mechanoreceptors. Jpn. Heart J. 12:233-241.

Udenfriend, S., and Zaltzman-Nirenberg, P. (1963). Norepinephrine and 3,4-dihydroxyphenylethylamine turnover in guinea pig brain in vivo. Science 142:394-396.

Ueda, H., Uchida, Y., and Kamisaka, K. (1967). Mechanism of the reflex depressor effect by kidney in dog. Jpn. Heart J. 8:597-606.

Van Ameringen, M.R., De Champlain, J., and Imbeault, S. (1977). Participation of central noradrenergic neurons in experimental hypertension. Can. J. Physiol. Pharmacol. 55:1246.

Verhaeghe, R.H., and Shephered, J.T. (1976). Effect of nitroprusside on smooth muscle and adrenergic nerve terminals in isolated blood vessels. J. Pharmacol. Exp. Ther. 199:269-277.

Vollmer, R.R., and Bush, E.N. (1982). Role of neurally released renin during sodium nitroprusside hypotension in conscious rats. Fed. Proc. 41:1669.

Von Euler, U.S. (1946). A specific sympathomimetic ergone in adrenergic nerve fibers (sympatlum) and its relation to adrenaline and noradrenaline. Acta. Physiol. Scand. 12:73-97.

Werber, A.H., and Fink, G.D. (1981). Cardiovascular and body fluid changes after aortic baroreceptor deafferentation. Am. J. Physiol. 240:H685-H690.

Wijnen, H.J., De Kloet, R., Versteeg, D., and De Jong, W. (1980a). Noradrenaline concentration and turnover in nuclei of the hypothalamus and the medulla oblongata at two stages in the development of renal hypertension in the rat. Brain Res. 198:411-417.

Wijnen, H.J., De Kloet, E.R., Versteeg, D.H., and De Jong, W. (1978). Differences in regional brain catecholamine metabolism after decrease in blood pressure. Life Sci. 23:2587-2592.

Wijnen, H., Spierenberg, H.A., De Kloet, E.R., De Jong, W., and Versteeg, D.H. (1980b). Decrease in noradrenergic activity in hypothalamic nuclei during the development of spontaneous hypertension. Brain Res. 184:153-162.

Winternitz, S.R., Katholi, R.E., and Oparil, S. (1980). Role of the renal sympathetic nerves in the development and maintenance of hypertension in the spontaneously hypertensive rat. J. Clin. Invest. 66:971-978.

Winternitz, S.R., Katholi, R.E., and Oparil, S. (1982). Decrease in hypothalamic norepinephrine content following renal denervation in one-kidney, one-clip Goldblatt hypertensive rat. Hypertension 4:369-373.

Yamaguchi, I., and Kopin, I.J. (1979) Plasma catecholamine and blood pressure responses to sympathetic stimulation in pithed rats. Am. J. Physiol. 237(3):H305-h310.

Yamashita, H. (1977). Effect of baro- and chemoreceptor activation on supraoptic nuclei neurons in the hypothalamus. Brain Res. 126: 551-556.

Yamori, Y. (1972). Organ difference of catecholamine metabolism in spontaneously hypertensive rats. In: Spontaneous Hypertension (K. Okamoto, ed.). Igaku Shoin Ltd. pp. 59.

Yamori, Y. (1974). Interaction of neural and nonneural factors in the factors in the pathogensis of spontaneous hypertension. In: The nervous system in arterial hypertension (S. Julius, and M.D. Esler, ed.). Charles, C. Thomas, Springfield, Illinois, pp. 17.

Yamori, Y., Nakada, T., and Lovenberg, W. (1976). Effect of antihypertensive therapy on lysine incorporation into vascular protein of the spontaneously hypertensive rat. Eur. J. Pharmacol 38:349.

Yates, F.E. (1982). Outline of physical theory of physiological systems. Can. J. Physiol. Pharmacol. 60:217-248.

Zhang, T.X., and Ciriello, J. (1982). Lesions of paraventricular nucleus reverse the elevated arterial pressure after aortic baroreceptor denervation in the rat. Soc. Neurosci. 12:120.17.

Zhang, T.X., Ciriello, J., and Caverson, M. (1983). Lesions of the paraventricular nucleus (PVH) prevent the elevated arterial pressure and heart rate after aortic baroreceptor denervation in the rat. Fed. Proc. 42:1192.

Zerbe, R.L., Feurstein, G., and Kopin, I.J. (1981). Effect of captopril on cardiovascular, sympathetic and vasopressin response to hemorrhage. Eur. J. Pharmacol. 72:391-395.

Zimmerman, B.G. (1978). Actions of angiotensin on adrenergic nerve endings. Fed. Proc. 37:199.

APPENDIX 1

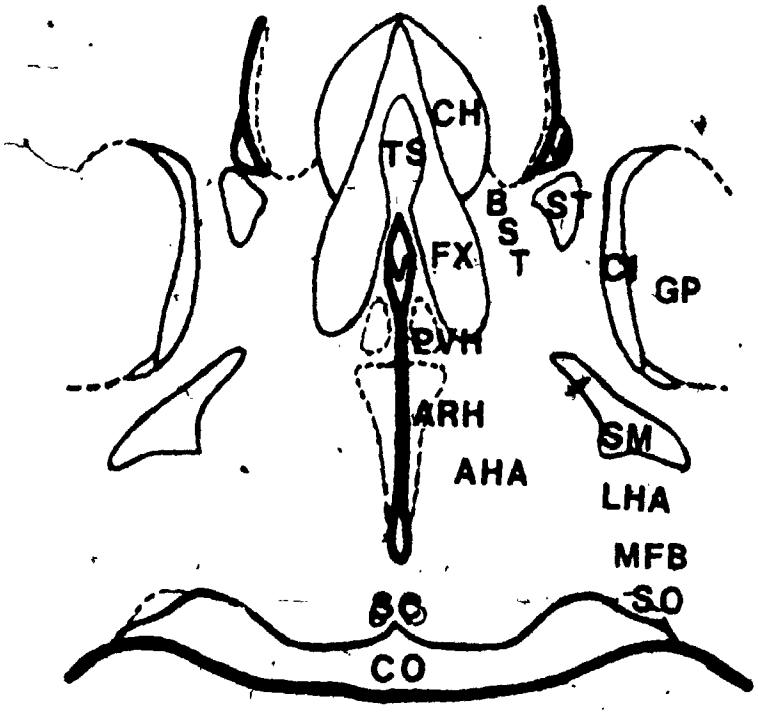
TOP: Coronal section midway through the tissue termed "anterior hypothalamus" in this study.

BOTTOM: Coronal section midway through the tissue termed "posterior hypothalamus" in this study.

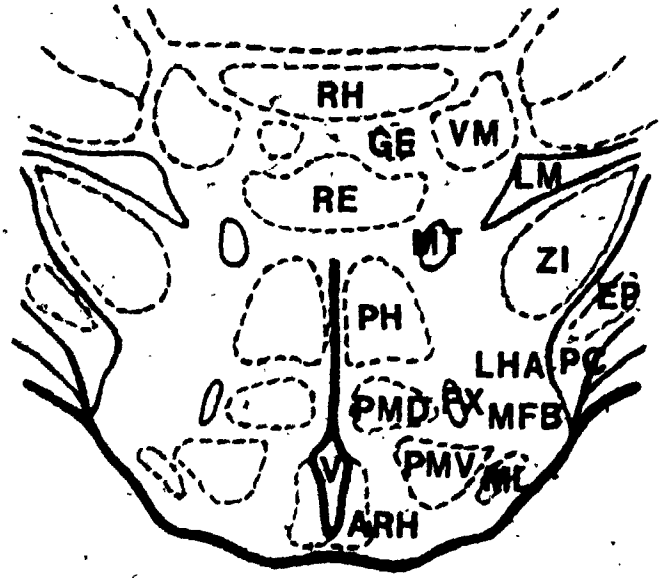
Number in the upper left corner of each diagram indicates rostrocaudal distance in mm from Bregma. Sections from Pellegrino et al. (1981).

AHA: Anterior hypothalamic area; ARH: Arcuate nucleus of the hypothalamus; BST: Bed nucleus of stria terminalis; CH: Hippocampal commissure; CI: Internal capsule; CO: Optic Chiasm; EP: Entopeduncular nucleus; FX: Fornix; GE: Nucleus gelatinosus thalami: GP: Globus pallidus; LHA: Lateral hypothalamic area; LM: Medial lemniscus; MFB: Median forebrain bundle; ML: Lateral mamillary nucleus; MT: Mamillothalmic tract; PC: Cerebral peduncle; PH: Posterior nucleus of the hypothalamus; PMD: Dorsal premamillary nucleus; PMV: Ventral premamillary nucleus; PVH: Paraventricular nucleus of the hypothalamus; RE: Reuniens nucleus of the thalamus; RH: Rhomboid nucleus of the thalamus; SC: Suprachiasmatic nucleus; SM: Stria medullaris thalami; SO: Supraoptic nucleus of the hypothalamus; ST: Stria terminalis; TS: Nucleus triangularis septi; V: Ventricle; VM: Ventral nucleus of thalamus; ZI: Zona incerta.

1.2

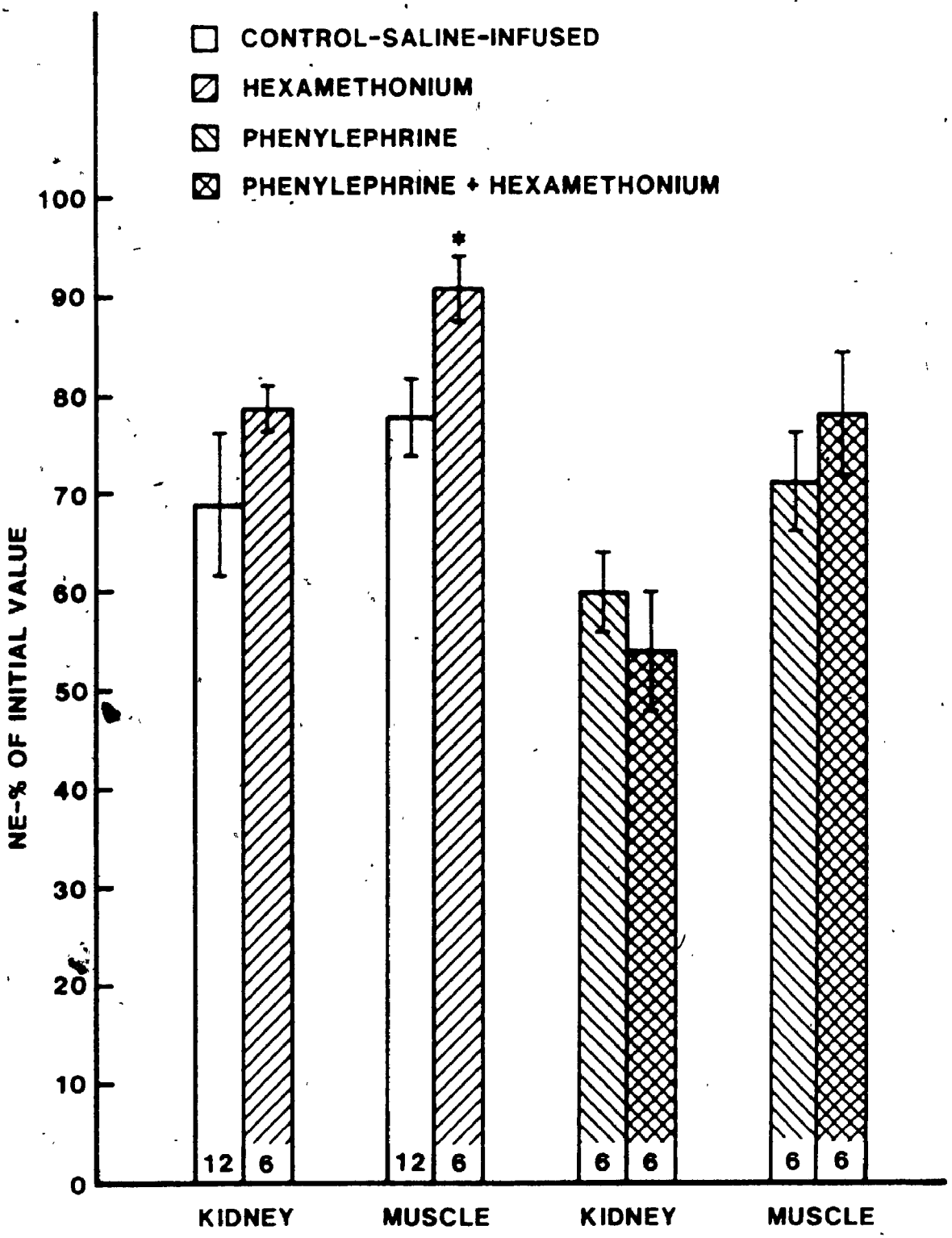

CH
TS
BST
ST
FX
GP
PVH
ARH
SM
AHA
LHA
MFB
SO
OC
CO

-1.0


RH
GE
VM
LM
RE
MT
ZI
EP
PH
LHA
PC
PMD
FX
MFB
PMV
V
ARH

APPENDIX 2

Norepinephrine concentration 90 min after α-methyltyrosine, expressed as a percent (±S.E.) of initial concentration in kidney and skeletal muscle. Values were obtained from saline-infused, saline-infused + hexamethonium treated (30 mg/kg), phenylephrine-infused (10-15 μg/kg.min) and phenylephrine-infused + hexamethonium treated rats. Numbers in the bars represent number of animals/group. *$P < 0.05$, compared to respectively infused controls.


CONTROL-SALINE-INFUSED
HEXAMETHONIUM
PHENYLEPHRINE
PHENYLEPHRINE + HEXAMETHONIUM
NE-% OF INITIAL VALUE
100
90
80
70
60
50
40
30
20
10
0
*
12
6
12
6
6
6
6
6
KIDNEY
MUSCLE
KIDNEY
MUSCLE

END

16 03 84

FIN