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Effects Of Opiates And Opioid Peptides In The Hypothalamus On Feeding And Temperature In The Rat

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EFFECTS OF OPIATES AND OPIOID PEPTIDES IN THE

HYPOTHALAMUS ON FEEDING AND TEMPERATURE IN THE RAT

by

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Department of Pharmacology and Toxicology

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

August, 1983

-opium, which the Creator himself seems
to prescribe, for we often see the
scarlet poppy growing in the corn
fields, as if it were forseen that where-
ever there is hunger to be fed there
must also be pain to be soothed-"

Oliver Wendell Holmes
Address to the
Massachusetts Medical Society
May 30, 1860

Abstract

When injected subcutaneously into rats, morphine stimulated food intake after a period of depressed motor activity. This ingestion was always accompanied by hyperthermia. Because of the nature of this type of administration, the site of action of the drug cannot be readily ascribed to any one of the diverse systems involved in ingestion. To circumvent this problem, morphine was administered to a specific brain location, the ventro-medial hypothalamus (VMH). This site is associated with feeding regulation and could provide some indication of the central component of opiate-induced feeding behaviour. Accordingly, the objectives of the investigation were to establish whether dose-dependent feeding can be induced by administration of opiates and opioid peptides into the VMH of rats; if this response involves stereospecific opiate receptors; how this effect is mediated; which class of opiate receptor is involved; and whether feeding and core temperature are interdependent phenomena. In addition, the ability of various other sites in the rat brain to respond to morphine injection by increasing ingestion was surveyed.

Male Sprague-Dawley rats were implanted stereo-taxically with a stainless steel guide cannula, directed.

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to the particular site under investigation. During the studies, animals received pre-weighed quantities of rat chow pellets and their temperatures were monitored by rectal probe.

Opiates injected into the VMH stimulated feeding in a dose-dependent fashion. Morphine and levorphanol were effective, as was the quaternary opiate, morphine methiodide. Following injections, these narcotic agonists elicited prolonged feeding which was preceded by a lengthy latent period. This contrasted with the vigorous, but brief feeding produced by noradrenaline.

As the non-analgesic analogue of levorphanol, dextrorphan, was inactive, the opiate "feeding" receptor appeared to exhibit specificity. The effects of morphine and its quaternary analogue were reversed by subcutaneous injection of the opiate antagonist, naloxone. Pre-treatment with naloxone administered into the VMH did not diminish morphine-induced feeding as effectively, and did not alter feeding due to the quaternary compound. Codeine, which is a weak ligand at opiate receptors, and codeine methiodide did not induce ingestion. Moreover, morphine, a μ-opiate receptor agonist, but not ketocyclazocine or phencyclidine (κ- and σ-opiate receptor agonists, respectively), was able to influence food intake after VMH injection.

Opioid peptides also elicited feeding, which was not suppressed when naloxone was administered into the VMH. Ingestion following injection of the synthetic enkephalin, D-Ala^2-D-Leu^5-enkephalin (DADLE), a δ-receptor ligand, was rapid in onset and ended within an hour. β-Endorphin-induced feeding was similar to that observed following morphine.

The feeding effects were not always accompanied by changes in temperature. Morphine and β-endorphin could induce a prolonged hyperthermia which corresponded in time to heightened feeding activity. Levorphanol and certain doses of DADLE, however, elicited food intake without altering temperature, while morphine methiodide injection elicited prolonged feeding, but only a brief period of hyperthermia. Small doses of β-endorphin elevated temperature without affecting ingestion.

Noradrenaline may mediate the feeding response to opiates, since the α-adrenergic antagonist, phentolamine, but not the β-adrenergic antagonist, propranolol, blocked ingestion due to morphine and DADLE. Additionally, the α_2-adrenergic antagonist, yohimbine, increased food intake. Dopamine, apomorphine, haloperidol, 5-HT and methysergide were ineffective. Phentolamine induced hyperthermia by itself and when given in conjunction with morphine.

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In a comparison of various brain sites, the most vigorous feeding appeared to occur following injection of morphine into the VMH and became weaker at more lateral, ventral and caudal sites.

Acknowledgements

There are several people whose assistance enabled me to complete this thesis.

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1. Introduction

1.1. Feeding: Hypothalamic Regulation

Food intake is a parameter used to measure objectively
the very subjective state known as hunger. Feeding is a
complex phenomenon involving numerous brain and peripheral
loci. It can be influenced by various components such
as taste, smell, activity level, temperature, emotionality
and various visceral factors. Subtle alterations in the
constituents of the plasma including carbohydrates,
lipids and amino acids play a crucial part in the complex
feedback regulation of energy balance and food intake
(Myers, 1974); for example, sensors may monitor levels
of circulating carbohydrates in the plasma (Mayer, 1953),
or blood-borne factors from fat depots (Andersson, 1971).
A variety of agents can be introduced which, acting at
many sites and by differing mechanisms of action, are
able to affect food intake. This further demonstrates
that the system for regulation of feeding is both
extensive and complex.

Early research into food intake focussed attention
on the hypothalamus when it was established that
ventromedial hypothalamic damage caused hyperphagia and
obesity while lateral hypothalamic destruction led to

aphagia (Anand and Brobeck, 1951; Brobeck, Tepperman and Long, 1943; Hetherington and Ranson, 1940; Smith, 1927, 1930; for review see Stevenson, 1969). Accordingly, electrical stimulation of the lateral hypothalamus elicited spontaneous feeding, while stimulation of the ventromedial region tended to inhibit feeding (Brobeck et al., 1943; Delgado and Anand, 1953; Smith, 1956). While these sites are still believed to be involved in food intake regulation, they are presently thought to be an intimate part of a much larger circuitry.

The hypothalamus is traversed by numerous neural pathways; the monoamines, noradrenaline, dopamine and serotonin, as well as acetylcholine are all available. Although there is some evidence that 5-hydroxytryptamine (5-HT, serotonin) and dopamine may be involved in food intake in rats (Booth, 1968; Goldman, Lahr and Friedman, 1971; Hoebel, 1977; Myers and Yaksh, 1968; Singer, Sanghvi and Gershon, 1971; Slangen and Miller, 1969), the reliable, vigorous responses evoked by injection of noradrenaline into certain hypothalamic sites strongly implicate its involvement in ingestive processes (Booth, 1968; Leibowitz, 1970; Slangen and Miller, 1969). Noradrenaline's involvement in central control of feeding in rats has been reaffirmed through knowledge of anatomical location (Leibowitz, 1978), specificity (Booth, 1968;

Slangen and Miller, 1969), increased release during feeding periods (Martin and Myers, 1975) and decreased feeding following noradrenaline depletion (Ahlskog and Hoebel, 1973; Richardson and Jacobowitz, 1973; Rossi, Zolovick, Davies and Panksepp, 1982). The effects of cholinergic substances on feeding is not clear; acetylcholine and carbachol, given into the lateral cerebral ventricle or preoptic-anterior hypothalamus of rats did not stimulate food intake (Avery, 1971; Myers and Yaksh, 1968) although food intake was increased after application of crystalline carbachol to the lateral hypothalamus (Sciorelli, Poloni and Rindi, 1972).

1.2. Temperature: Hypothalamic Regulation

The hypothalamus has emerged additionally as a prime centre for temperature regulation. From earlier studies involving lesioning and stimulation, the anterior hypothalamus was considered to provide defense against heat while the posterior hypothalamus was implicated in heat production in defense against the cold (Anderson, Grant and Larsson, 1956; Hemingway, 1963; Hemingway, Forgrave and Birzis, 1954; Ranson, 1940).

From available evidence, the pre-optic anterior hypothalamus (POAH) now appears to be the area most

sensitive to the temperature-altering effects of biogenic monoamines. Small doses of 5-HT (0.05 to 1.0 µg) injected into this area in the rat produced a rise in temperature (Crawshaw, 1972), although 5-HT given intraventricularly had a hypothermic effect (Myers and Yaksh, 1968). Hypothalamic or intraventricular injections of noradrenaline into rats did not give as consistent changes. In various studies conducted at room temperature, doses of noradrenaline caused a fall in core temperature (Avery, 1971; Cantor and Satinoff, 1976; Lomax, Foster and Kirkpatrick, 1969), a rise in core temperature (Lomax et al., 1969; Myers and Yaksh, 1968) or a biphasic response (Feldberg and Lotti, 1967; Veale and Wishaw, 1976). Dopamine, presumably acting at specific dopamine receptors in the pre-optic anterior region, caused a rapid fall in temperature (Cox, Kerwin and Lee, 1978; Cox and Lee, 1977). Acetylcholine or cholinomimetics also can modify rat core temperature, although their effects are variable. Applications of cholinergic substances, by the ventricular route (Myers and Yaksh, 1968) or directly to the anterior hypothalamus (Avery, 1970, 1971) caused a long-lasting hyperthermia. Hypothermia, however, has been observed in restrained rats after administration of carbachol to the rostral hypothalamic area (Kirkpatrick and Lomax, 1970; Lomax and Jenden, 1966).

Other brain sites, however, may be involved in thermoregulation. The posterior hypothalamic area was able to alter core temperature in response to very subtle alterations in the ratio of calcium to sodium ions at this site (Myers and Brophy, 1972; Myers and Veale, 1971). A significant capacity for both autonomic (Lipton, 1973; Lipton, Dwyer and Fossler, 1974; Lipton and Trzcinka, 1976; Stuart, Kawamura, Hemingway and Price, 1962) and behavioural (Lipton, 1968; Roberts and Martin, 1977; Satinoff and Rutstein, 1970; Satinoff and Shan, 1971) temperature control still persisted after POAH lesions. Furthermore, control by anatomically separate sites, distinct from the POAH, of certain individual autonomic and behavioural responses has been demonstrated (Lipton et al., 1974; Roberts and Mooney, 1974; Satinoff and Shan, 1972).

1.3. Opiates: Receptors

The initial identification of specific opiate receptors proved difficult, although their existence was suspected for a long time. Goldstein and his colleagues (Goldstein, Lowney and Pal, 1971) were the first to use stereospecificity as the criterion for opiate receptor binding in mouse-brain homogenates. Although only 2% of total binding was stereospecific, they were able to

distinguish high-affinity, readily-saturable binding sites from non-specific sites. By using modifications of Goldstein's procedure, several groups (Pert and Snyder, 1973; Simon, Hiller and Edelman, 1973; Terenius, 1973) were able to report stereospecific opiate binding which represented a major portion of total binding (50-90%).

From these beginnings, research has progressed rapidly. Much evidence has accumulated suggesting that these binding sites represent receptors to which opiates must bind in order to produce their responses. They have been found in man (Hiller, Pearson and Simon, 1973) and in all vertebrates, but not invertebrates, which have been studied (Pert, Aposhian and Snyder, 1974). The distribution of these sites within the central nervous system was found to be neither homogenous nor random. High concentrations were located within the limbic system (except for the hippocampus), the medial thalamus and throughout the hypothalamus. The lowest concentrations were in the cerebellum, the spinal cord, many of the cerebral hemispheric gyri and white matter area (Hiller et al., 1973; Kuhar, Pert and Snyder, 1973).

The characteristics of the specific binding have been studied. A good correlation existed between binding affinities of a homologous series of ketobemidones and analgesic potencies (Wilson, Rogers, Pert and Snyder, 1975),

and there was an excellent correlation between binding of a series of opiate agonists and antagonists to receptors in the guinea pig ileum and the concentration of them required to inhibit electrically-induced contractions of this same tissue (Crease and Snyder, 1975).

There is extensive evidence that opiate binding sites exist peripherally as well as in the central nervous system. They have been located in such smooth muscle tissues as the ileum (guinea pig), vas deferens (mouse), nictitating membrane (cat) and ear artery (rabbit) (Ronai and Berzetei, 1978), and, perhaps, in skeletal muscle (frog) (Frank, 1975).

Various studies have indicated that there is probably more than one type of opiate receptor. Martin and his colleagues (Gilbert and Martin, 1976; Martin, Eades, Thompson, Huppler and Gilbert, 1976) were the first to propose, from their studies on chronic spinal dog preparations, that there were three subclasses of opiate receptors. These were designated μ, κ and σ after the prototypic agonists morphine, ketocyclazocine and SKF 10,047 (N-allylnormetazocine), respectively. In vitro studies on rat brain, guinea pig ileum, and rat and mouse vas deferens have further distinguished a site with some selectivity for enkephalins, which has been designated δ (Chang and Cuatrecasas, 1979; Lord, Waterfield, Hughes

and Kosterlitz, 1977) and a site with selectivity for
β-endorphin, which has been labelled ε (Wuster, Schulz
and Herz, 1979). Enkephalins have some μ affinity, and
β-endorphin binds also to μ and δ receptors (Chang,
Cooper, Hazum and Cuatrecasas, 1979; Wuster et al.,
1979).

1.4. Opiates: Endogenous Ligands

With the existence of opioid binding sites confirmed,
the search for endogenous ligands began. The first
reports of such activity came simultaneously from two
laboratories. Both Hughes (1975) and Terenius and
Wahlstrom (1975) reported the presence of opioid activity
in aqueous extracts of animal brain. Goldstein and
colleagues (Teschemacher, Opheim, Cox and Goldstein,
1975) subsequently reported the presence of opioid activity
in extracts of bovine pituitary glands. In 1975, Hughes
and colleagues,(Hughes, Smith, Kosterlitz, Fothergill,
Morgan and Morris, 1975) isolated two pentapeptides from
pig brain that acted like morphine on the electrically-
stimulated guinea pig ileum. These were named methionine
(met)-enkephalin and leucine (leu)-enkephalin. These
researchers also noted that the sequence of met-enkephalin
was present at position 61-65 in the structure of β-
lipotropin, a 91 amino acid peptide isolated from

pituitary glands (Li, Barnafi, Chretien and Chung, 1965).
Subsequently, opioid activity was found to exist in
various fragments of β-lipotropin, the most important
of these being the 31 amino acid peptide β-endorphin
at position 61-91 (Cox, Goldstein and Li, 1976). Quite
recently, the existence of a new 17 amino acid opioid
peptide, dynorphin, has been revealed (Goldstein, Fischli,
Lowney, Hunkapiller and Hood, 1981; Goldstein, Tachibana,
Lowney, Hunkapiller and Hood, 1979). This potent com-
pound contains the sequence for leu-enkephalin at its
N-terminal.

Many biochemical and histochemical studies have
indicated that the enkephalins and β-endorphin are neuro-
transmitters. Subcellular fractionation studies have
shown that the peptides are concentrated in the synapto-
somal fraction of brain homogenates (Osborne, Hollt and
Herz, 1976; Simantov, Snowman and Snyder, 1977). However,
neurons that contain enkephalins exist separately from
those that contain β-endorphin (Bloom, Battenberg,
Rossier, Ling and Guillemin, 1978), and their distributions
differ. In brief, enkephalin-containing neurons are
present throughout the entire rat central nervous system,
from cerebral cortex to spinal cord, including the
pituitary. The limbic system, hypothalamus, central
gray and reticular formation are heavily innervated with

enkephalinergic neurons (Rossier and Bloom, 1972; Sar,

Stumpf, Miller, Chang and Cuatrecasas, 1972; Uhl,

Kuhar and Snyder, 1978). Peripherally, enkephalins can

be located in many regions including the intestinal tract

and adrenomedullary cells (Linnoila, DiAugustine,

Miller, Chang and Cuatrecasas, 1978; Polak, Bloom and

Sullivan, 1977; Schultzberg, Lundberg, Hokfelt, Terenius,

Brandt, Elde and Cuatrecasas, 1978). β-Endorphin is

distributed in the hypothalamus, septum, midbrain, medulla,

pons and pituitary (Rossier, Vargo, Minick, Ling, Bloom

and Guillemin, 1977).

1.5. Opiates: Feeding

There are several lines of evidence supporting the

possibility that opioids may be involved in the regulation

of food intake. A frequently used technique involves

peripheral injection of an opiate antagonist, generally

naloxone, and measurement of resulting changes in feeding.

If endorphinergic activity has a physiological role in

the control of feeding, then suppressing this activity

should disrupt feeding. Holtzman (1974) was the first to

show that naloxone, given systemically, could reduce

food intake in food-deprived rats. Subsequent studies have

confirmed this (Brands, Thornhill, Hirst and Gowdey, 1979;

Brown and Holtzman, 1979; Frank and Rogers, 1979).

Food intake due to night feeding, tail pinch, 2-deoxy-D-glucose and insulin has been shown by some investigators to be reduced by systemic naloxone (Lowy, Maickel and Yim, 1980; Ostrowski, Rowland, Foley, Nelson and Reid, 1981; Sewell and Jawaharlal, 1980). Studies with naloxone must, however, be interpreted with caution since it may have effects not mediated by opiate receptors (Sawynok, Pinsky and Labella, 1979).

More direct evidence comes from altered food consumption following administration of opiate agonists. Upon repeated systemic administration, various opiates were found to increase ingestion in rats (Kumar, Mitchell and Stolerman, 1971; Thornhill, Hirst and Gowdey, 1976, 1978a). This ingestion is stereospecific, since levorphanol but not dextrorphan stimulated it (Thornhill, Hirst and Gowdey, 1979). Moreover, β-endorphin injected directly into the ventromedial hypothalamus stimulated increased food intake in free-feeding rats (Grandison and Guidotti, 1977). Further enhancing a possible association between feeding and central endorphins was the finding that genetically obese rodents have higher levels of the opioid peptides in their pituitaries than related, non-obese strains (Margules, Moisset, Lewis, Shibuya and Pert, 1978).

1.6. Opiates: Core Temperature

Morphine and heroin have well-established effects
on thermoregulation when given systemically in the rat.
Low doses produce hyperthermia (Gunne, 1960; Herrmann,
1942; Martin, Pryzbylik and Spector, 1977; Thornhill et
al., 1976). Higher doses evoke hypothermia or a biphasic
response in which hyperthermia is preceded by hypothermia
(Gunne, 1960; Herrmann, 1942; Martin et al., 1977; Oka,
Nozaki and Hosoya, 1972; Thornhill et al., 1976).

Administration of opiates into brain sites can also
alter temperature. Injection of morphine into the
rostral hypothalamic area, pre-optic anterior hypothalamus
or paraventricular area elicited hypothermia (Cox, Ary,
Chesarek and Lomax, 1976; Lotti, Lomax and George, 1965)
and hyperthermia was produced by application of morphine
to the pre-optic anterior hypothalamus, or mammillary nu-
clei (Cox et al., 1976; Lotti et al., 1965; Martin and
Papp, 1979). Opioid peptides including the enkephalins, a
synthetic enkephalin derivative (FK.33-824), and β-
endorphin applied to the lateral ventricles or pre-optic
anterior hypothalamus caused hyperthermia at low doses,
while large doses lowered body temperature (Blasig,
Bauerle and Herz, 1979; Ferri, Reina, Santagostino, Scoto
and Spadaro, 1978; Holaday, Law, Tseng, Lo and Li, 1977;

Holaday, Lo and Li, 1978). The alterations in core temperature which are produced by opiates are affected by both restraint and changes in ambient temperature (Blasig et al., 1979; Martin and Morrison, 1978; Martin et.al., 1977; Paolino and Bernard, 1968; Trzcinka, Lipton, Hawkins and Clark, 1977)..

Naloxone is able to antagonize the thermogenic effects of morphine and the hypothermic effect of β-endorphin (Bloom, Segal, Ling and Guillemin, 1976; Holaday et al., 1978; Martin et al., 1977; McGilliard, Tulanay and Takemori, 1976; Thornhill et al., 1978a).

1.7. Objectives

As mentioned above, systemic injections of opiate agonists and antagonists can alter, food intake in rats, probably by affecting central control mechanisms. The VMH was identified as a brain locus involved in feeding regulation and containing a high concentration of opiate receptors. In initiating the direction of study for this work, it was hypothesized that if endogenous opioids were involved in the central regulation of feeding, then the VMH likely would be an important area of action. This consideration was, in fact, supported by the preliminary report of Grandison and Guidotti (1977), who indicated that β-endorphin applied to the VMH of satiated rats could

promote feeding.

Although few studies have measured simultaneously the pattern of feeding and temperature changes produced by the opiates, Thornhill et al. (1976, 1978a) found that repeated subcutaneous injection of low doses of morphine or heroin into rats caused vigorous feeding, accompanied by pronounced hyperthermia. It was decided that thermal changes could be monitored readily within protocols directed towards feeding studies, and so these were incorporated. This addressed the question of the independence or interdependence of feeding behaviour and thermoregulation in opiate-treated animals.

The objectives of this investigation were, then, to establish whether dose-dependent feeding could be induced by administration of opiates and opioid peptides into the VMH of rats, if this response involved stereospecific opiate receptors, how this effect was mediated, and which class of opiate receptor was involved. Moreover, this study included an evaluation of whether feeding and core temperature changes were interdependent, as information from subcutaneous injections suggested, or if the receptors responsible for these two effects were separable. An extension from this theme was a survey of the ability of various other sites in the rat brain to respond to morphine

injection with increased ingestion. This was conducted
in order to determine how specific the VMH site was for
this behaviour.

2. <u>Materials</u>

Several of the drugs used in these studies were made available through the kindness of Mr. R.A. Graham, Chief, Scientific Services, Department of National Health and Welfare, Ottawa. These included: morphine sulphate (May and Baker), codeine phosphate (British Drug Houses Chemicals Ltd.), levorphanol tartrate monohydrate (Hoffman-LaRoche Ltd.), dextrorphan tartrate monohydrate (Hoffman-LaRoche, Ltd.), ketocyclazocine hydrochloride (Sterling-Winthrop Research Institute), apomorphine hydrochloride (McFarlan, Smith) and phencyclidine hydrochloride (#277239).

Naloxone hydrochloride was provided by Endo Laboratories, New York; haloperidol by McNeil Laboratories Ltd.; phentolamine hydrochloride by CIBA-GEIGY, Canada Ltd.; and prazosin hydrochloride by Pfizer Company Ltd. The generosity of these companies is gratefully acknowledged.

Noradrenaline hydrochloride and dopamine hydrochloride were purchased from Sigma Chemicals; propranolol hydrochloride from Ayerst Laboratories; yohimbine hydrochloride from Aldrich Chemicals; 5-hydroxytryptamine (5-HT, serotonin creatinine sulphate) from British Drug Houses Chemicals, Ltd.; methysergide bimaleate from

Sandoz, Basel; D-Ala^2, D-Leu^5-enkephalin (DADLE) from Biosearch, California and β-endorphin from Beckman Chemicals.

The synthesis of morphine methiodide and codeine methiodide was developed and carried out by Dr. M. Hirst, Department of Pharmacology and Toxicology, University of Western Ontario. For details of preparation, see Appendix.

Structures of all opiates and opioid peptides used are illustrated in Fig. 1.

FIGURE 1

Structures of opiates and opioid peptides used in the following studies.

Tyr-Ala-Gly-Phe-Leu

D-ALA²,D-LEU⁵ ENKEPHALIN

Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr
Glu-Gly-Lys-Lys-Tyr-Ala-Asn-Lys-Ile-Ile-Ala-Asn-Lys-Phe-Leu
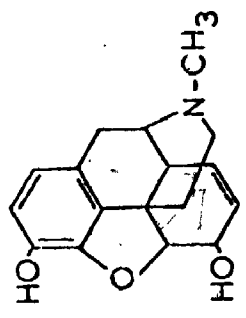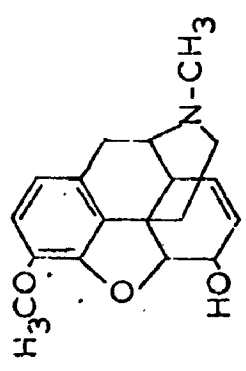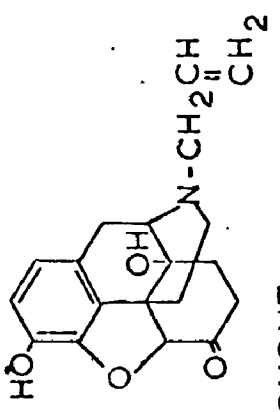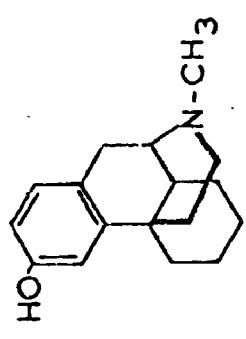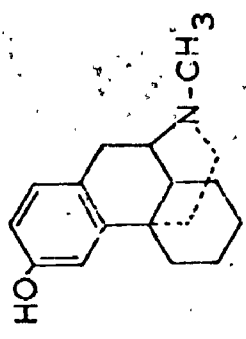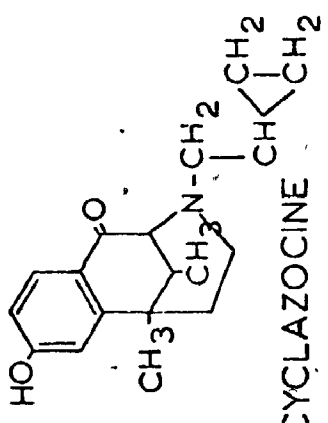
β-ENDORPHIN

NALOXONE

KETOCYCLAZOCINE

PHENCYCLIDINE

CODEINE

DEXTRORPHAN

CODEINE
METHIODIDE

MORPHINE

LEVORPHANOL
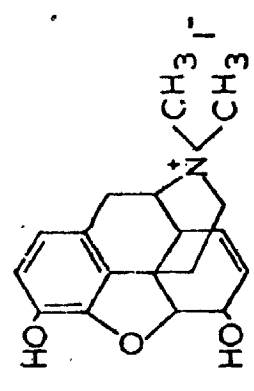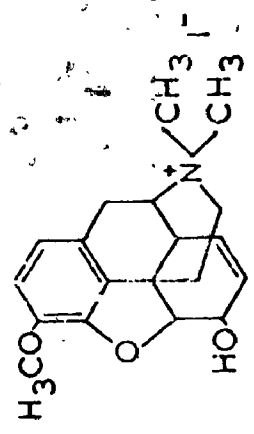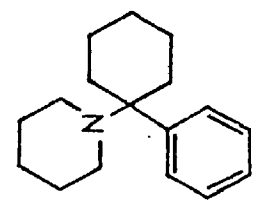
MORPHINE
METHIODIDE

3. Methods

3.1 Studies involving application of drugs to specific brain sites

3.1.1. General

Male Sprague-Dawley rats weighing over 270 g prior to surgery were employed in these studies. Animals were housed individually in standard metal cages and maintained with ad lib water and food pellets (Purina). Their room was kept on a fixed 12 hour light-dark cycle (lights on 0800 to 2000 h) at 24.0 ± 2.0º C. Rats were kept at least five days in their home cages before stereotaxic surgery was begun.

3.1.2. Stereotaxic Surgery

Rats were anaesthetized with 3.5 mg/kg Equithesin (200 ml propylene glycol, 50 ml absolute ethanol, 4.8 g sodium pentobarbital, 21.3 g chloral hydrate, 10.6 g magnesium sulphate, and distilled water to 500 ml total volume). The animals were then placed in a stereotaxic frame (Kopf Instruments), ear bars inserted, and head adjusted so that the interaural line was 5 mm below the level of the upper incisor bar. The skull was exposed and bregma located. Co-ordinates were taken from the atlas of Pellegrino, Pellegrino and Cushman (1979), with bregma

used as reference point. A small hole was drilled in the skull at the appropriate location, the dura was cut and the cannula was lowered. All cannulae were constructed from 23 gauge hypodermic tubing (Small Parts, Inc., Miami, Fla.). Each cannula was anchored to the skull with three stainless steel jeweller's screws (Watch Material Co., Montreal) and dental acrylic cement (Caulk Repair Material, L.D. Caulk Co.). When animals were not being injected, an obdurator pin of the same length as the cannula and fashioned from stainless steel wire (0.03 cm diameter, Small Parts, Inc., Miami, Fla.) was inserted. Following surgery, rats were injected intramuscularly with 0.1 ml Pen-Di-Strep [procaine penicillin G (130,000 I.U./kg) and dihydrostreptomycin (170 mg/kg)] and then allowed six to ten days to recover.

3.1.3. Experimental Procedure for Studies Involving Feeding and Temperature Measurements

Throughout the study animals had access to as much food and water as they required. At intervals during the period of recovery from surgery and during the morning of trial days, animals were handled frequently and the injection cannula was occasionally lowered through the implanted cannula, but no injection was made. This was done to familiarize the animals with the experimental procedure and reduce stress.

All trials were performed in the home room with at least one rest day intervening. On intermediate, non-trial days animals were observed but otherwise undisturbed. In the morning of a trial day, animals were weighed and occasionally handled. Between 1215 and 1245 h initial injections were made. Injections into the brain were administered over 20 to 40 seconds through 30 gauge hypodermic tubing (Small Parts, Miami, Fla.) which extended 0.5 mm beyond the permanent cannula. This injection cannula was joined by PE 10 polyethylene tubing (Intra-medic) to a 10 µl syringe (Hamilton Co.). Drugs were applied to the brain in a volume of 0.5 µl sterile, pyrogen-free saline (Abbott Laboratories, Montreal) unless otherwise specified. All trials were performed in the home room and rats were only removed from their cages when weighed and injected.

A pre-weighed quantity of rat chow pellets (Purina) was provided after injection and was changed at hourly intervals during the study. Paper towels were placed under each cage to catch any spillage and the towels were replaced in conjunction with the food pellets. The weight of remaining pellets plus spillage at the end of each hour subtracted from the original weight of pellets indicated the weight of food consumed per hour.

Core temperatures were recorded just prior to the first injection and at hourly intervals thereaf̶̶ while the animal remained in the home cage. Temperatures were measured by insertion of a lubricated, pre-calibrated rectal probe (Yellow Springs Instruments 423) 6 cm beyond the anus and allowance of up to 20 seconds for the Yellow Springs 44TA tele-thermometer to equilibrate.

3.1.4. Stereotaxic Co-ordinates for the Ventro-medial Hypothalamus

In these studies, injections were made into the ventromedial hypothalamus (VMH). From the atlas of Pellegrino et al. (1979), with bregma as reference point, cannulae were positioned according to the following co-ordinates: +0.4 mm anterior, -0.5 mm lateral and to a depth of 8.3 mm from the surface of the brain.

3.1.5. Alterations in Feeding and Temperature Following VMH Application of Morphine Sulphate and Noradrenaline Hydrochloride

For this study, six rats with VMH cannulae were divided into two equal groups. One group received morphine (2.7, 5.3 and 10.6 nmole) while the other group received noradrenaline (10, 20 and 40 nmole). A control trial day where 0.5 µl saline was injected intervened between each drug trial day. Drug doses were given according to a Latin Square design. The groups were crossed over so that

all animals received both drugs at all doses.

3.1.6. Effect of Procaine Hydrochloride Applied to the VMH on Feeding and Temperature

Five rats from the six used for the above study (Section 3.1.5.) received procaine (110 nmole) injected into the VMH.

3.1.7. Effect of Subcutaneous Compared to VMH Application of Morphine Sulphate

The three rats used in this study responded to VMH application of morphine (5.3 nmole) by eating more than 1.5 g of food pellets in three hours. They were injected with saline (into the VMH), saline (0.2 ml, given subcutaneously) or morphine sulphate (2 µg in 0.2 ml saline, given subcutaneously). Drugs were applied in a Latin Square design. The quantity of morphine sulphate given into the hypothalamus was equal to the amount given subcutaneously (2 µg).

3.1.8. Studies on Specificity of the VMH Opiate Receptors Involved in Feeding

3.1.8.1. Levorphanol Tartrate Monohydrate, Dextrorphan Tartrate Monohydrate and Codeine Phosphate

Seven rats were given VMH injections of the following

opiates according to a Latin Square design: levorphanol (1.8 and 5.3 nmole); dextrorphan (5.3 nmole); or codeine (5.3 nmole).

3.1.8.2. Morphine Sulphate, Ketocyclazocine Hydrochloride and Phencyclidine Hydrochloride

Seven rats were given VMH injections of saline or the following agents which represent various classes of opiate agonists, again according to a Latin Square design: morphine (5.3 nmole, μ agonist); ketocyclazocine (5.3 nmole, κ agonist); or phencyclidine (5.3 nmole, σ agonist).

3.1.9. Effects of Quaternary Opiates in the VMH on Feeding and Temperature

3.1.9.1. Effects on Guinea Pig Ileum and Rat Blood Pressure

Preliminary studies were done to ascertain the effects of morphine methiodide and codeine methiodide on the stimulated guinea pig ileum and on rat blood pressure. Details of methods and results are given in the Appendix.

3.1.9.2. Morphine Methiodide Compared to Morphine Sulphate

Morphine methiodide (10.6 and 21.2 nmole) or morphine

sulphate (5.3 nmole) were instilled into the VMH in a Latin Square design. A trial day involving injection of saline alone intervened between each drug trial day.

3.1.9.3. Morphine Methiodide Compared to Codeine Methiodide

Six rats received morphine methiodide (21.2 nmole) or codeine methiodide (21.2 nmole) instilled into the VMH in a crossover design.

3.1.10. Effects of Opioid Peptides in the VMH on Feeding and Temperature

3.1.10.1. D-Ala^2, D-Leu^5-enkephalin (DADLE)

D-Ala^2, D-Leu^5-enkephalin (DADLE) was given into the VMH of two groups of rats. The first group (16 rats) received initially morphine (5.3 nmole) and subsequently DADLE (1.4, 2.7 and 5.3 nmole), doses of peptide being given in a Latin Square design. Each drug trial alternated with a saline trial day when animals received saline alone. The second group (11 rats) received 5.3 nmole morphine, then DADLE (0.35 and 0.70 nmole), by Latin Square protocol. Again, saline trial days alternated with drug trials.

3.1.10.2. β-Endorphin

The two studies examining VMH actions of various doses of β-endorphin were arranged similarly to the above studies of DADLE (Section 3.1.10.1.). Each consisted of a morphine treatment (5.3 nmole) followed by β-endorphin treatments, given in a Latin Square design. Drug trials alternated with saline trials. In the first study, six rats received 1.4 or 2.7 nmole β-endorphin, while in the second, four rats received 0.35 or 0.70 nmole.

3.1.11. Effects of Naloxone Hydrochloride on the Alterations in Feeding and Core Temperature Induced by VMH Injection of Morphine Sulphate, Morphine Methiodide, DADLE and β-Endorphin

3.1.11.1. Morphine Sulphate

Eight rats received a VMH injection of naloxone (10.6 nmole) or saline followed, after a five-minute interval, by an injection of morphine (5.3 nmole) into the same site.. A crossover design was used.

In five rats, naloxone (10.6 nmole) was given five minutes before saline, both injections being made into the VMH.

In a companion study nine rats received morphine (5.3 nmole) followed one hour later by naloxone (10.6 nmole) or

saline, all drugs being given into the VMH. Again, a cross-
over design was employed. Food intake was monitored
following naloxone or saline injection.

Ten rats were given subcutaneous injections of saline
or naloxone (2 or 10 mg/kg). Ten minutes later they were
given an injection of morphine (5.3 nmole) into the VMH.
A crossover experimental design, as above, was used.

3.1.11.2. Morphine Methiodide

In five rats, naloxone (10.6 nmole) or saline was
instilled into the VMH five minutes prior to instillation
of morphine methiodide (21.2 nmole) into the VMH.

In seven rats, a subcutaneous injection of naloxone
(2 or 10 mg/kg) or saline preceded by 10 minutes the
hypothalamic administration of morphine methiodide.

3.1.11.3. DADLE

Seven rats received naloxone (10.6 nmole) or saline
administered into the VMH and followed after five minutes
by DADLE (5.3 nmole) given by the same route. A Latin
Square design was used.

3.1.11.4. β-Endorphin

Four rats received naloxone (10.6 nmole) or saline administered into the VMH and followed after five minutes by β-endorphin (1.4 nmole) given by the same route. As above, a Latin Square design was used.

3.1.12. Involvement of Noradrenergic, Dopaminergic, and Serotonergic Neurons in Opioid-Influenced Feeding and Temperature Changes

3.1.12.1. General

In the following studies, whenever a series of two injections was given on a trial day, food intake was always measured from the second injection. All injections were made into the VMH. Occasionally, drugs were injected into the VMH in a volume of 1 μl; the use of this volume being dictated by the low solubility of the drug in saline.

3.1.12.2. Effects of Phentolamine Hydrochloride on Noradrenaline Hydrochloride

An initial study was performed in seven rats to determine the dose of phentolamine sufficient to block the effect of a noradrenaline (NA) injection given five minutes later. All injections were made into the VMH. The experimental design was:

```
      Injection 1 ———— (5 min) ———————— Injection 2
```

Trial 1	–	saline
Trial 2	saline	NA (30 nmole)
Trial 3	phentolamine (30, 60 nmole)	NA (30 nmole)
Trial 4	saline	NA (30 nmole)

3.1.12.3. Effects of Phentolamine Hydro-chloride on Morphine Sulphate

To determine whether phentolamine would affect feeding due to morphine, the antagonist or saline was applied to the VMH either five minutes before (seven rats) or one hour after (six rats) an identically placed dose of morphine. The design was as follows:

```
      Injection 1 —— (5 min) ——— Injection 2
```

Trial 1	–	saline
Trial 2	saline	morphine (5.3 nmole)
Trial 3	phentolamine (60 nmole)	morphine (5.3 nmole)
Trial 4	saline	morphine (5.3 nmole)

```
      Injection 1 —— (1 hour) —— Injection 2
```

Trial 1	saline	–
Trial 2	phentolamine (60 nmole)	–
Trial 3	morphine (5.3 nmole)	saline
Trial 4	morphine (5.3 nmole)	phentolamine (60 nmole)
Trial 5	morphine (5.3 nmole)	saline

3.1.12.4. Effects of Morphine Sulphate Given with Noradrenaline Hydrochloride

In six rats, interaction of morphine and noradrenaline given together to the VMH was investigated. On the initial

trial day, the rats received a saline injection. On the second and third trial days, they received 5.3 nmole morphine or 30 nmole noradrenaline in a crossover pattern. On the final trial day, all rats received an injection of 5.3 nmole morphine followed after five minutes by a 30 nmole noradrenaline injection.

3.1.12.5. Effects of Phentolamine Hydro-chloride on DADLE.

In nine rats, phentolamine or saline was applied to the VMH five minutes before DADLE. The sequence of trials was:

	Injection 1 ——— (5 min) ———	Injection 2
Trial 1	–	saline
Trial 2	saline	DADLE (5.3 nmole)
Trial 3	phentolamine (60 nmole)	DADLE (5.3 nmole)
Trial 4	saline	DADLE (5.3 nmole)

3.1.12.6. Effects of Prazosin Hydrochloride on Morphine Sulphate and on Noradrenaline Hydrochloride

Injections of prazosin (1 µl) or saline into the VMH were given to rats one hour after VMH morphine in the following sequence:

```
            Injection 1 —— (1 hour) —— Injection 2

Trial 1    saline                              —
Trial 2    prazosin (0.26 pmole)               —
Trial 3    morphine (5.3 nmole)                saline
Trial 4    morphine (5.3 nmole)                prazosin (0.26 pmole)
Trial 5    morphine (5.3 nmole)                saline
```

Injections of noradrenaline into the VMH were given
15 minutes after injections of VMH prazosin (1 µl) or
saline in six rats as follows:

```
            Injection 1 —— (15 min) —————— Injection 2

Trial 1    —                                   saline
Trial 2    saline                              NA (30 nmole)
Trial 3    prazosin (0.26 pmole)               NA (30 nmole)
Trial 4    saline                              NA (30 nmole)
```

3.1.12.7. Effects of Yohimbine Hydrochloride on Morphine Sulphate

Injections of yohimbine (1 µl) or saline into the
VMH were given to six rats one hour after morphine in
the following sequence:

```
            Injection 1 — (1 hour) —— Injection 2

Trial 1    saline                              —
Trial 2    yohimbine (9.1 nmole)               —
Trial 3    morphine (5.3 nmole)                saline
Trial 4    morphine (5.3 nmole)                yohimbine (9.1 nmole)
Trial 5    morphine (5.3 nmole)                saline
```

3.1.12.8.   Effects of Propranolol Hydro-
            chloride, 5-HT, Methysergide
            Bimaleate, Dopamine Hydrochloride,
            Haloperidol and Apomorphine
            Hydrochloride on Morphine
            Sulphate

The following design was used to test various agonist

and antagonist drugs for effects on morphine-induced

feeding and temperature changes:

Injection 1 ——— (1 hour) ——— Injection 2

Trial 1   saline   –
Trial 2   drug   –
Trial 3   morphine (5.3 nmole)   saline
Trial 4   morphine (5.3 nmole)   drug
Trial 5   morphine (5.3 nmole)   saline

The drugs used were propranolol (60 nmole, five rats),

5-HT (30 nmole, five rats), methysergide (28.3 nmole,

five rats), dopamine (30 nmole, seven rats), haloperidol

(8 nmole given in 1 µl, seven rats), and apomorphine

(25 nmole given in 1 µl, seven rats). The haloperidol was

dissolved in a small amount of acetic acid and made up

to volume with saline and bicarbonate. The final pH was

5.5.

3.1.12.9.   Effects on Feeding of Morphine
            Sulphate Applied to Various Brain
            Sites

Feeding responses to morphine were tested at several

brain sites, including the ventromedial hypothalamus, posterior hypothalamus, paraventricular hypothalamus, internal capsule, lateral hypothalamus, fornix, amygdala, caudate-putamen and thalamus. For each site, a unilateral cannula was implanted in three to five rats, as described in Section 3.1.2. The stereotaxic co-ordinates for these sites were chosen from the atlas of Pellegrino et al. (1979), with bregma used as a reference point. On four trial days, which were not consecutive days, animals received an injection of morphine dissolved in sterile, pyrogen-free saline (0.2 µl, Abbott Laboratories, Montreal) or saline alone (0.2 µl). The order of injections was: saline, morphine (5.3 nmole), saline and, finally, morphine (5.3 nmole). Injections were made between 1215 and 1245 h and administered over 20 seconds through 30 gauge hypothermic tubing (Small Parts, Miami, Fla.) which extended 0.5 mm beyond the permanent cannula. The injection cannula was joined by PE 10 tubing (Intramedic) to a 10 µl syringe (Hamilton Co.).

Rats were allowed ad lib food pellets (Purina) and water. Food intake and core temperature was measured as in Section 3.1.3.

3.2. Histology

After completion of the early studies, rats were anaesthetized with Equithesin.and perfused through the left ventricle of the heart with 10% buffered formalin phosphate (Fisher Scientific Co.). In later studies, rats were killed with chloroform vapours. All brains were removed and stored in buffered formalin for at least ten days at room temperature.

Transverse sections (50 μ) in the approximate plane of the cannula tract were sliced from the brains on a freezing microtome (Leitz). Sections were placed on microscope slides coated with 1% gelatin solution. At least 24 hours later, sections were processed with thionin, a basic cell stain (see Appendix), and the stained slides were mounted with DePex mounting medium (British Drug Houses Chemicals, Ltd.). Appropriate cannula place-ments were verified from the stained sections.

3.3. Analysis of Results

3.3.1. Feeding and Temperature Responses after Injections

For studies in which two treatments were compared, Student's t-test for paired data was used. These included naloxone studies, where animals received VMH

injections of saline or naloxone with an opioid (one-tail); the methiodide study where effects of morphine methiodide were compared to codeine methiodide (two-tail); and studies of effects of morphine or saline at various brain sites (two-tail).

In all other VMH studies, where more than two groups were compared, results were assessed by a randomized block analysis of variance. Where the F-ratio showed significance among groups, results were further analyzed by the Studentized range test (Goldstein, 1964).

4. <u>Results</u>

4.1 Studies Involving Application of Drugs to Specific Brain Sites

4.1.1. Alterations in Feeding and Temperature Following VMH Application of Morphine Sulphate and Noradrenaline Hydrochloride

Fig. 2 illustrates a typical cannula placement in the VMH.

Although noradrenaline caused hyperphagia, in this study no dose-dependency was observed over the dosage range examined. During the first hour after injection, animals ate significantly more in response to all doses of noradrenaline than to saline (Fig. 3). However, three-hour food intake after 40 nmole noradrenaline was not significantly different from saline. At this dose, the effect of the drug varied greatly among animals; some rats ate large amounts (e.g. seven g) while others exhibited greatly decreased locomotion and ate nothing. Generally, feeding began within five minutes of noradrenaline injection following much movement and sniffing and continued for 15 to 40 minutes. Core temperature one hour after injection of 10 and 20 nmole noradrenaline was significantly higher than control temperature. At two and three hours post-injection only 40 nmole

FIGURE 2

Photograph of a coronal section through a rat
brain, illustrating a representative placement site
for a cannula aimed at the ventromedial hypothalamus.
The plane of the section follows the atlas of Pellegrino
et al. (1979), with co-ordinates: +0.4 mm anterior to
bregma, -0.5 mm lateral to the midline and a depth
of 8.3 mm from the surface of the brain.

FIGURE 3

Food intake and corresponding core temperature over 3 hours following administration of several doses of noradrenaline and saline into the ventromedial hypothalamus of rats (n = 6).

Histobars represent mean cumulative food intake after injection for:

☐ 1 hour

▨ 2 hours

◨ 3 hours
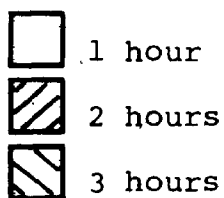
Graph represents core temperatures following injection of:
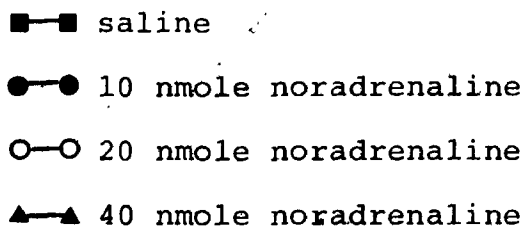
■—■ saline

●—● 10 nmole noradrenaline

○—○ 20 nmole noradrenaline

▲—▲ 40 nmole noradrenaline

Vertical lines indicate S.E.M. Significant differences from saline (p < 0.05) are indicated as * .

FOOD EATEN (g)

NORADRENALINE (nmol)

CORE TEMPERATURE (°C)

TIME FROM INJECTION (hours)
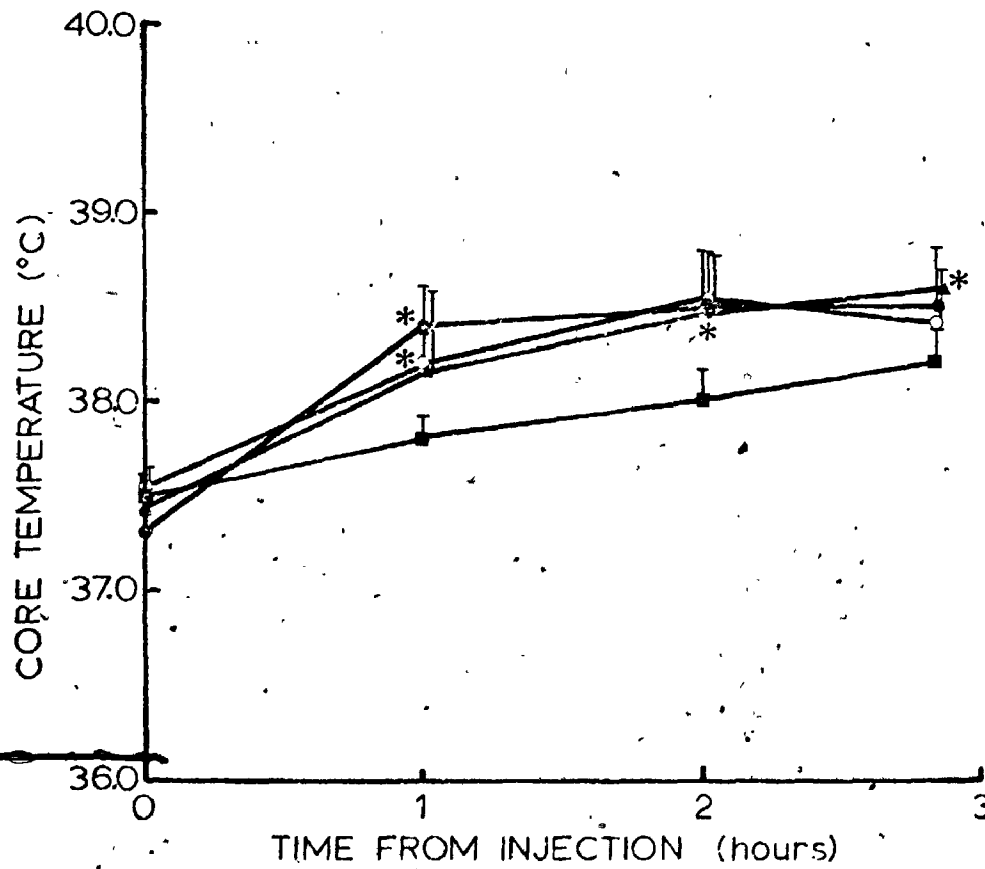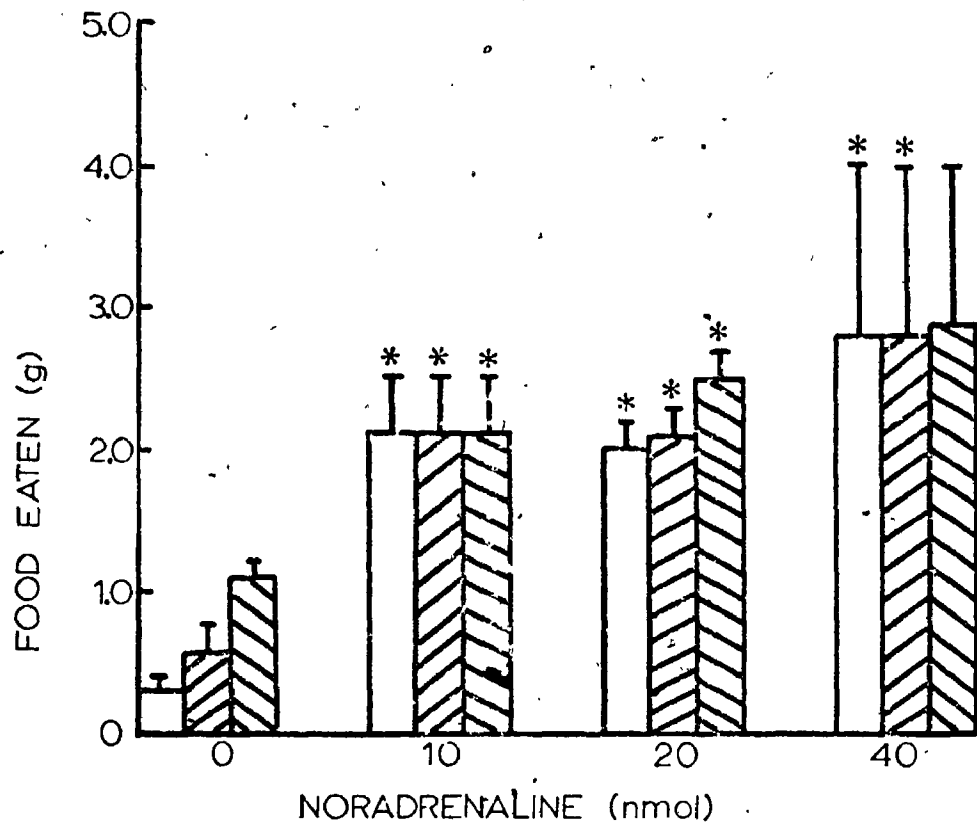
noradrenaline caused a significant rise in temperature over that after the saline control.

Fig. 4 illustrates the changes in feeding and core temperature following injections of morphine or saline into the VMH of rats, at the same site where noradrenaline increased food intake. Tolerance to either parameter was not apparent with this paradigm. All three doses of morphine produced a significant increase in the amount of food consumed over the three-hour period of measurement, and the higher doses (5.3 and 10.6 nmoles) of morphine led to significantly more feeding than the lowest (p < 0.05). The total quantity of food ingested after morphine was comparable to that following noradrenaline injection.

The pattern of feeding after morphine treatment, however, was very different from that after the catecholamine. There was a short latency before intense eating commenced after noradrenaline, but animals receiving morphine began eating only after a latent period that lasted from 30 to 120 min. As shown in Fig. 4, quantities eaten during the first hour following morphine injection were not significantly different from the saline control feeding. The duration of the latency generally related to the quantity of drug injected. It was characterized by a "trance-like" state, where animals would either sit

FIGURE 4

Food intake and corresponding core temperature over 3 hours following administration of several doses of morphine and saline into the ventromedial hypothalamus of rats (n = 6).

Histobars represent mean cumulative intake after injection for:

☐ 1 hour
▨ 2 hours
◩ 3 hours
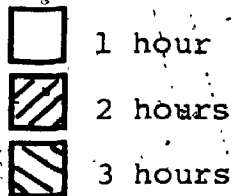
Graph represents temperature following injection of:

■—■ saline

●—● 2.7 nmole morphine

○—○ 5.3 nmole morphine

▲—▲ 10.6 nmole morphine
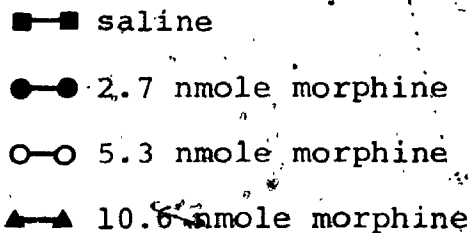
Vertical lines indicate S.E.M. Significant differences from saline ($p < 0.05$) are indicated as *
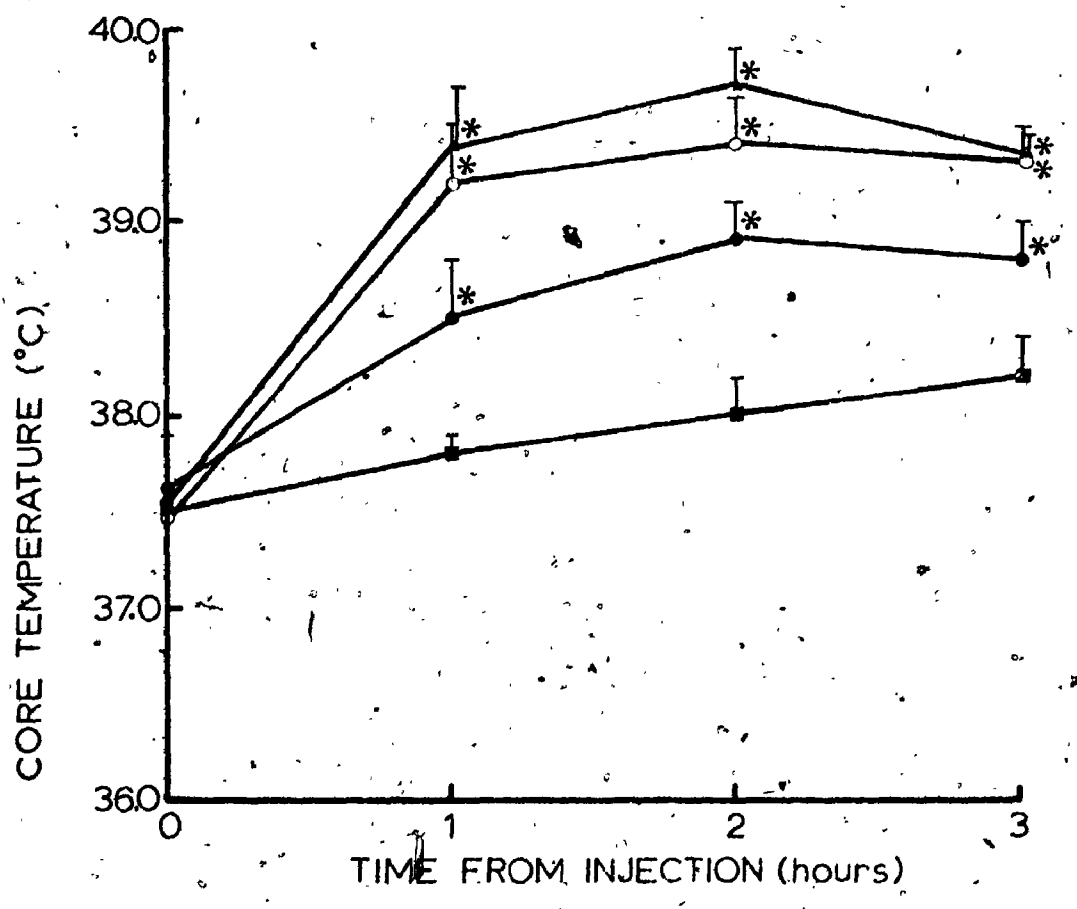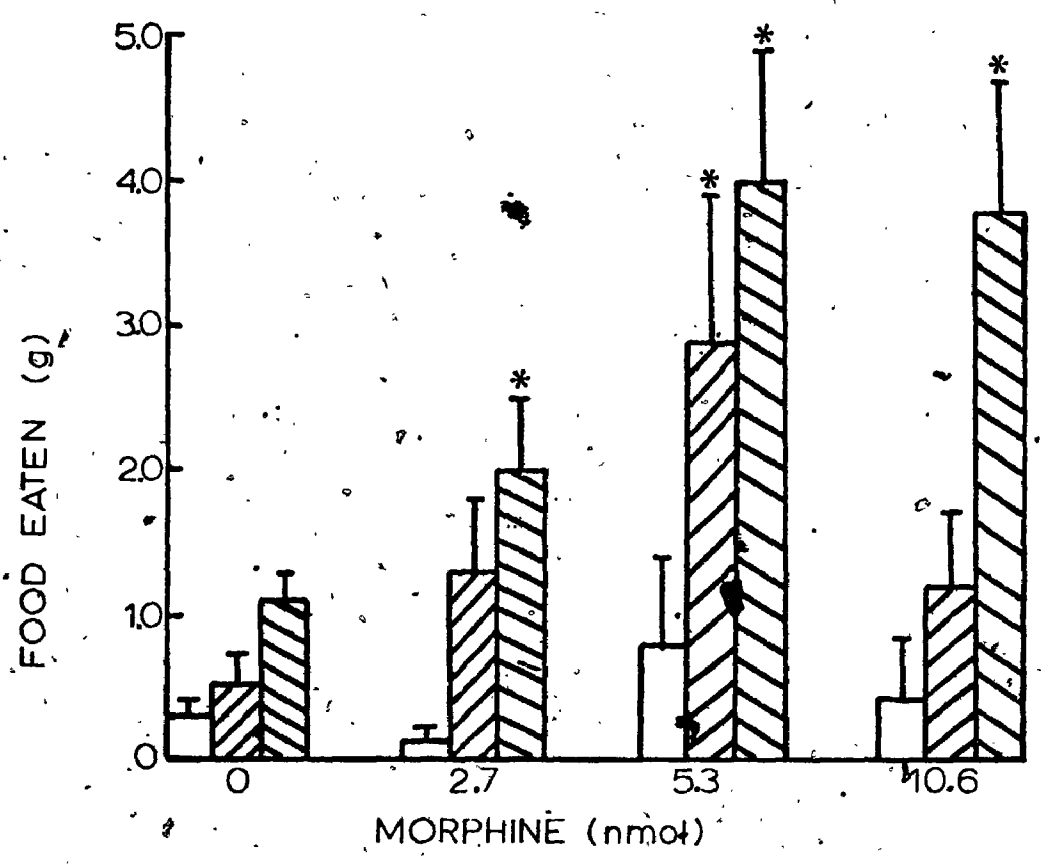
or stand at the back of the cage with eyes open. They were always capable of movement if gently provoked. Following this phase, animals generally began a prolonged feeding session. After the highest dose, animals were often found to be still eating at the end of the three-hour period, whereas feeding with noradrenaline had essentially ceased by the end of the first hour. After morphine was administered, the animals appeared apprehensive during feeding, and when approached closely would often stop or drop their food and stand with front paws up at the back of the cage. Noradrenaline did not appear to make animals as reactive to external stimuli.

At all doses, morphine evoked a persistent hyper-thermia; core temperature remained significantly elevated over that after saline for three hours. The elevation in temperature was dose-related; at two hours following the injection of morphine, 10.6 nmole elicited a significantly higher mean temperature than did 2.7 nmole. The effect on temperature of noradrenaline appeared to be smaller than that of morphine and not dose-dependent. Although levels of feeding and core temperature were both elevated over controls by the end of the three-hour morphine sessions, increases in temperature occurred before increases in feeding.

4.1.2. Effect of Procaine Hydrochloride Applied to the VMH on Feeding and Temperature

The effects of procaine on feeding and temperature did not differ appreciably from those after saline (Table 1). Total food intake following procaine was 0.7 ± 0.3 g and after saline was 1.0 ± 0.2 g; peak temperature following procaine was $38.3 \pm 0.4^{\circ}$ C and after saline was $38.1 \pm 0.2^{\circ}$ C.

4.1.3. Effect of Subcutaneous Compared to VMH Application of Morphine Sulphate

Morphine, given into the VMH, stimulated significantly more three-hour intake than did the same amount of morphine given subcutaneously, or saline administered by either route (Fig. 5). Similarly, only centrally-applied morphine effected a significant elevation in temperature at one, two and three hours after injection. Following sub-cutaneous morphine, food intake and temperatures were no different from saline.

4.1.4. Studies Investigating Specificity of the Opiate Receptor in the VMH Involved in Feeding

4.1.4.1. Levorphanol Tartrate Monohydrate, Dextrorphan Tartrate Monohydrate and Codeine Phosphate

Table 1

Food Eaten (g) Following VMH Injection of Procaine (110 nmole) or Saline

	Initial Hour	Two Hours	Three Hours
Procaine:	0.4 ± 0.3	0.4 ± 0.3	0.7 ± 0.3
Saline:	0.2 ± 0.1	0.7 ± 0.2	1.0 ± 0.2

Core Temperature (^{O}C), Following VMH Injection of Procaine (110 nmole) or Saline

	Initial Hour	Two Hours	Three Hours
Procaine:	37.3 ± 0.2	38.0 ± 0.4	38.3 ± 0.4
Saline:	37.4 ± 0.1	38.0 ± 0.1	38.1 ± 0.2

FIGURE 5

Mean cumulative food intake and core temperature
of rats (n = 3) over 3 hours following administration
of:

         O—O saline into the ventromedial
               hypothalamus

         ●—● morphine (5.3 nmole) into the
               ventromedial hypothalamus

         △—△ saline, subcutaneously

         ▲—▲ morphine (2 μg) subcutaneously

Vertical lines represent S.E.M. Significant
differences (p'< 0.01) from both saline given intra-
hypothalamically and from morphine and saline injected
subcutaneously are indicated as * .
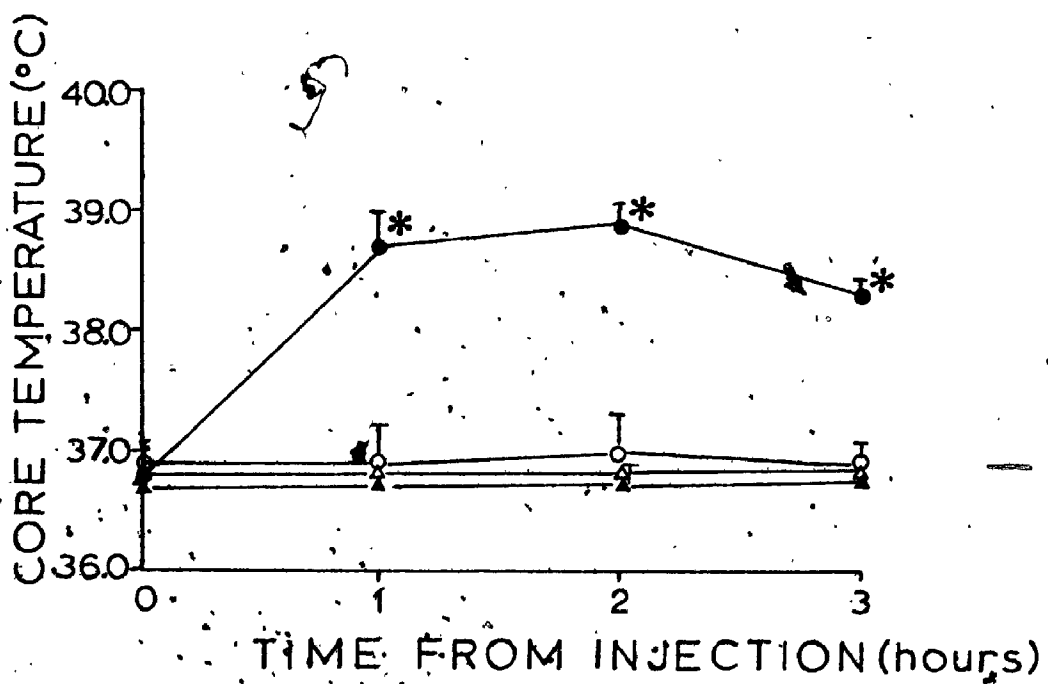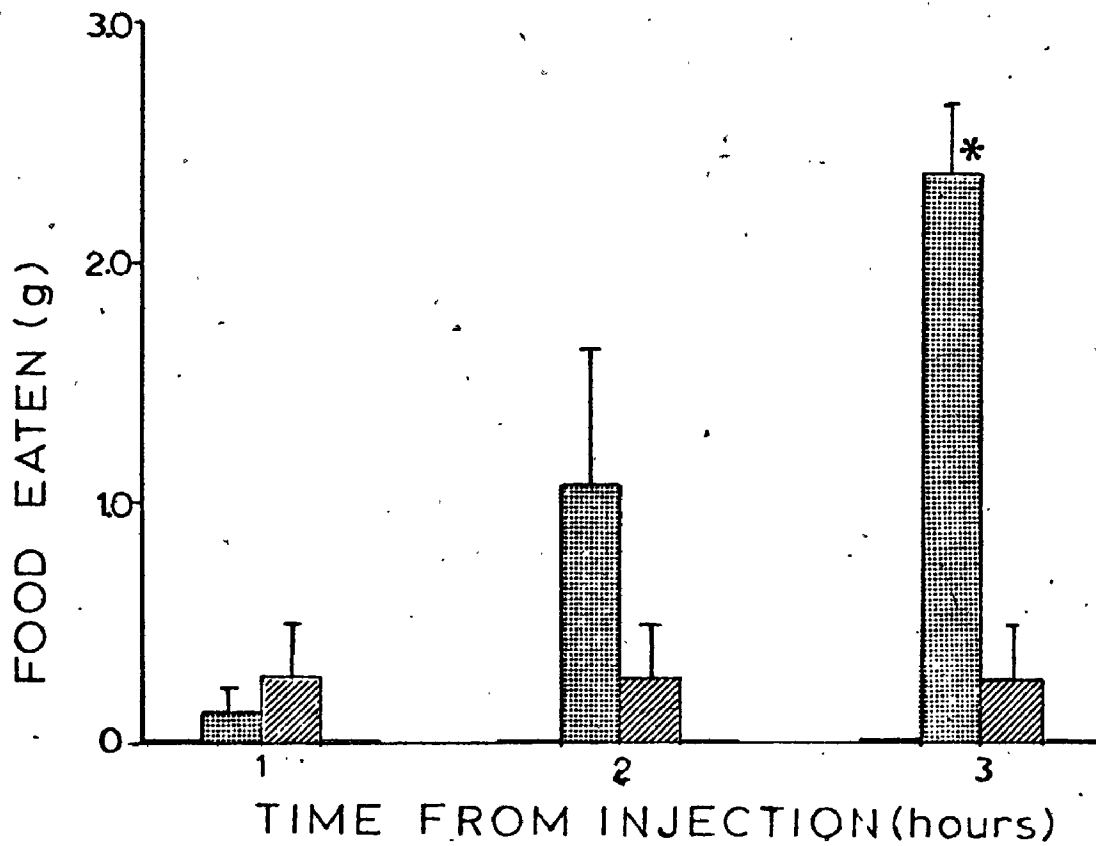
Injection of levorphanol (1.8 and 5.3 nmole, Fig. 6) was followed by a period of grooming and exploratory activity, and then dose-dependent feeding, which increased slowly during the three-hour period to reach maximum levels of 1.2 ± 0.6 g and 1.9 ± 0.4 g respectively. Cumulative food intakes both two and three hours after injection of levorphanol (5.3 nmole) were significantly greater than those occurring after treatment with an equimolar quantity of dextrorphan. Little was ingested after codeine and the animals, although awake, were not active. The weight of food consumed in the three hours following injection of codeine (5.3 nmole) was significantly less than following levorphanol (5.3 nmole), but not different from the dextrorphan. There were no significant differences in core temperature among the four treatment groups.

4.1.4.2. Morphine Sulphate, Ketocyclazocine Hydrochloride and Phencyclidine Hydrochloride

Only minimal ingestion followed injection of saline and ketocyclazocine (Fig. 7). Feeding after the latter was occasionally preceded by a short (less than 10 min) period of depressed activity followed by sporadic nibbling and sleeping. There was no food intake following phencyclidine. (Only one animal ate 0.1 g food during

FIGURE 6

Mean cumulative food intake and core temperature
of rats (n = 7) over 3 hours following administration
into the ventromedial hypothalamus of:

       ●—● levorphanol (1.8 nmole)
       ■—■ levorphanol (5.3 nmole)
       O—O dextrorphan (5.3 nmole)
       □—□ codeine (5.3 nmole).
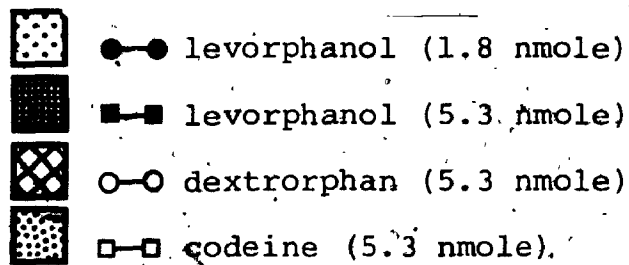
Vertical lines represent S.E.M. Significant
differences (p < 0.05) from dextrorphan are indicated
as * and from codeine as ▼ .

FOOD EATEN (g)

3.0

2.0

1.0

0

1   2   3

TIME FROM INJECTION (hours)

CORE TEMPERATURE (°C)

40.0

39.0

38.0

37.0

36.0

0   1   2   3

TIME FROM INJECTION (hours)
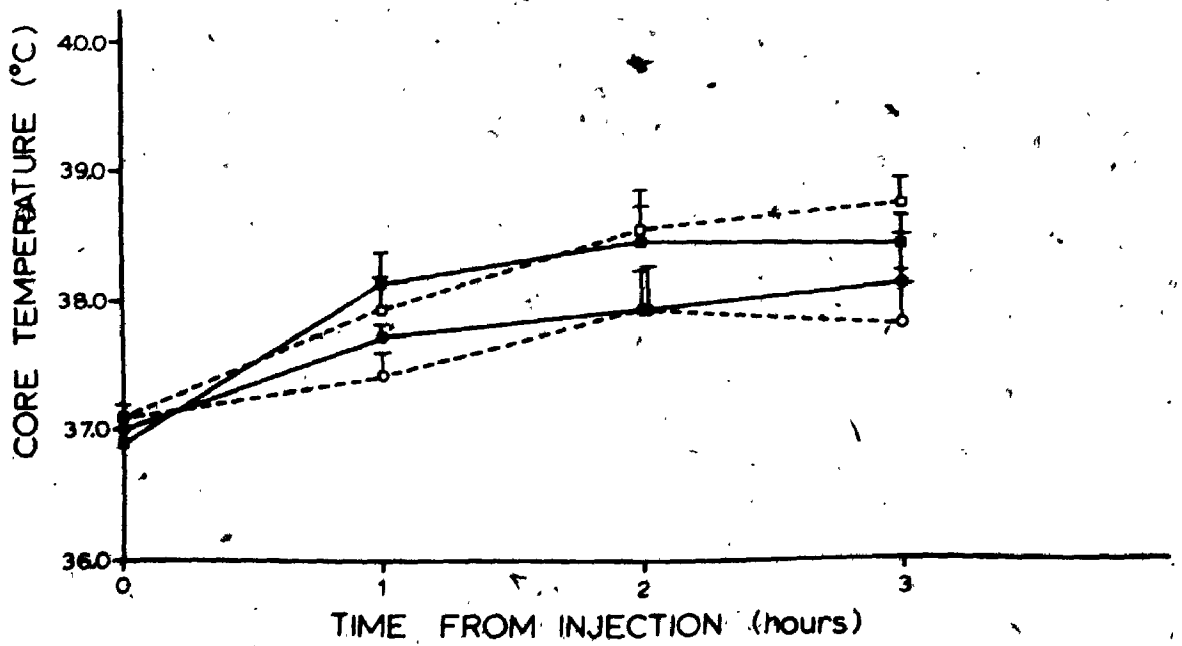
FIGURE 7

Mean cumulative food intake and core temperature
of rats (n = 7) over 3 hours following administration
into the ventromedial hypothalamus of:

☐ o—o saline

── ●—● phencyclidine (5.3 nmole)

▨ ■—■ ketocyclazocine (5.3 nmole)

▨ □—□ morphine (5.3 nmole)
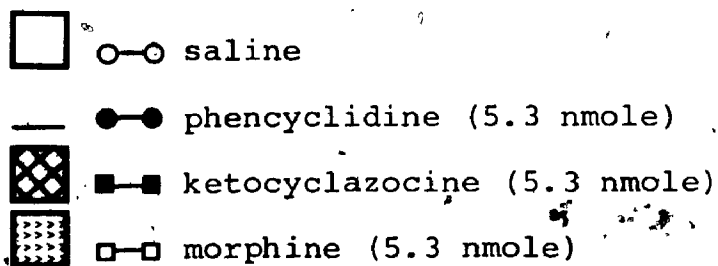
Vertical lines represent S.E.M. Significant
differences (p < 0.01) from saline, phencyclidine
and ketocyclazocine are indicated as * .
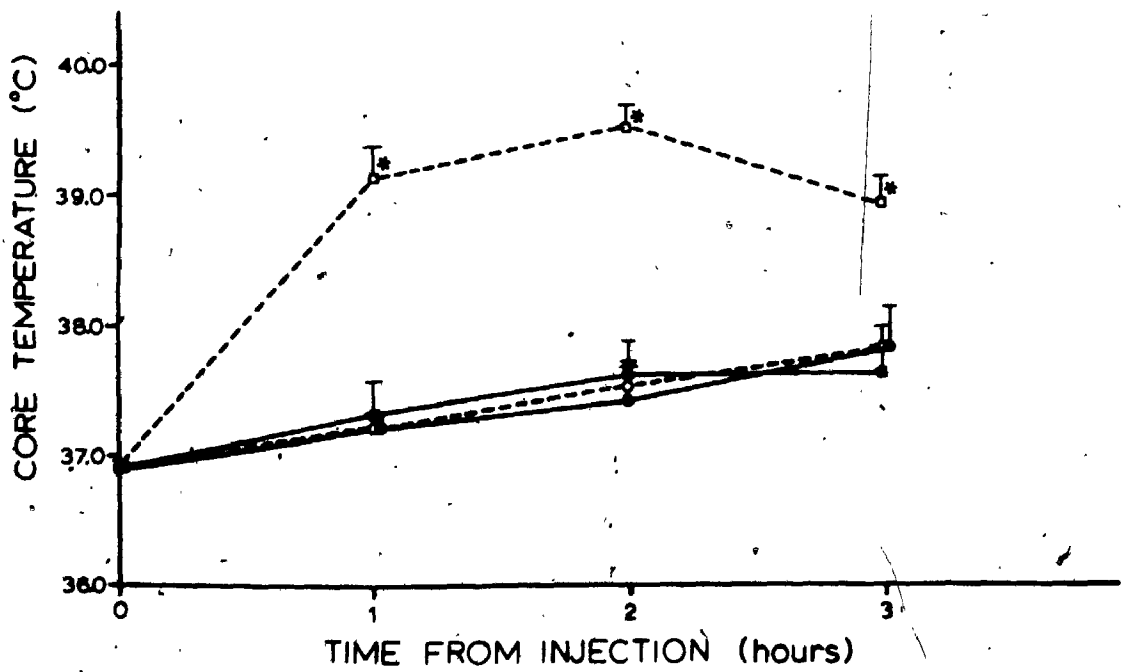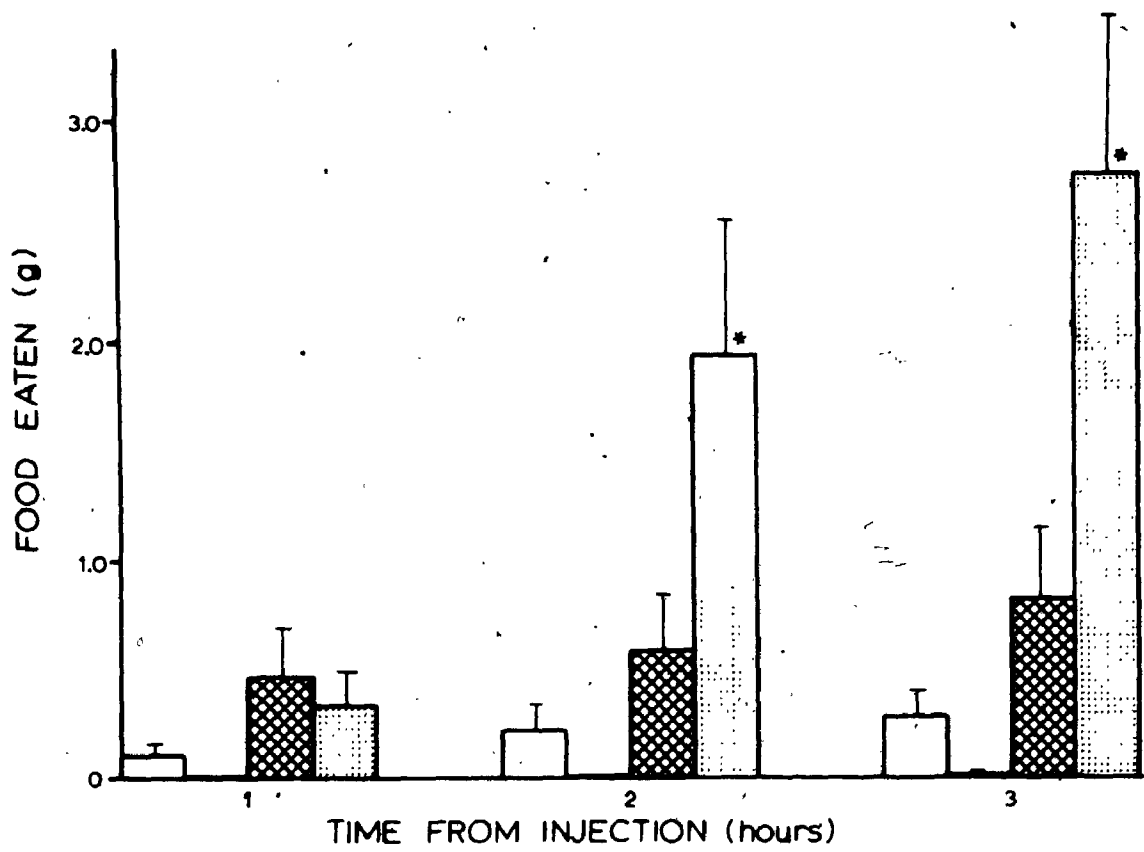
the third hour of the study). Although these animals assumed a natural sleep position immediately following injection, they could be aroused. Morphine, as previously noted (Section 4.1.1.), evoked an initial lengthy period of behavioural depression which lasted at least 30 minutes, followed by a prolonged period of food intake. Morphine stimulated food intake to a level (2.8 ± 0.7 g) that was significantly greater than that observed following injection of the other three agents. Moreover, only morphine elevated core temperature compared with the other substances.

4.1.5. Effects of Quaternary Opiates in the VMH on Feeding and Temperature

4.1.5.1. Morphine Methiodide Compared to Morphine Sulphate

Rats treated with morphine methiodide (21.2 nmole) or morphine (5.3 nmole) ate similar quantities of food at each of one, two and three hours after injection (Fig. 8). This level of ingestion was significantly greater than after saline administration. The amount of food eaten after the lower dose of morphine methiodide (10.6 nmole) was not different from saline or the other drug injections. The behaviour of morphine methiodide-treated rats was notably different from morphine sulphate-treated

FIGURE 8

Mean cumulative food intake and core temperature of rats (n = 6) over 3 hours following administration into the ventromedial hypothalamus of:

△—△ saline .

▲—▲ morphine methiodide (10.6 nmole)

●—● morphine methiodide (21.2 nmole)

○—○ morphine (5.3 nmole)
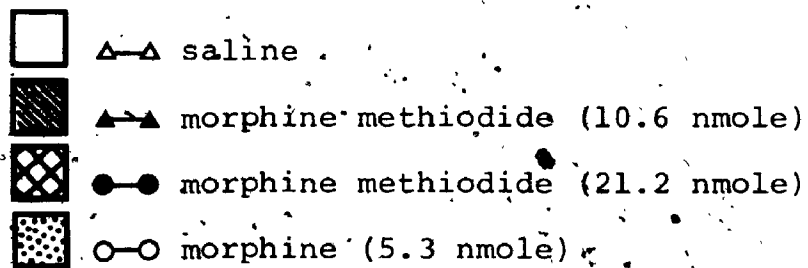
Vertical lines represent S.E.M. Significant differences (p < 0.05) from saline are indicated as * and from morphine methiodide (10.6 and 21.2 nmole) as ◆

FOOD EATEN (g)

TIME FROM INJECTION (hours)

CORE TEMPERATURE (°C)

TIME FROM INJECTION (hours).
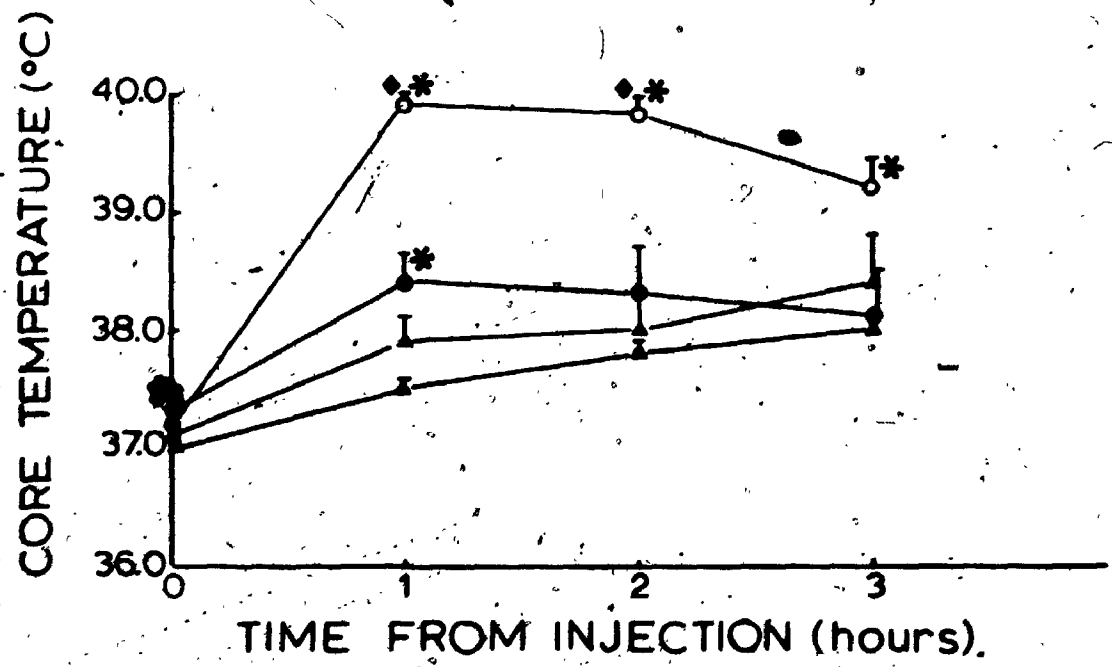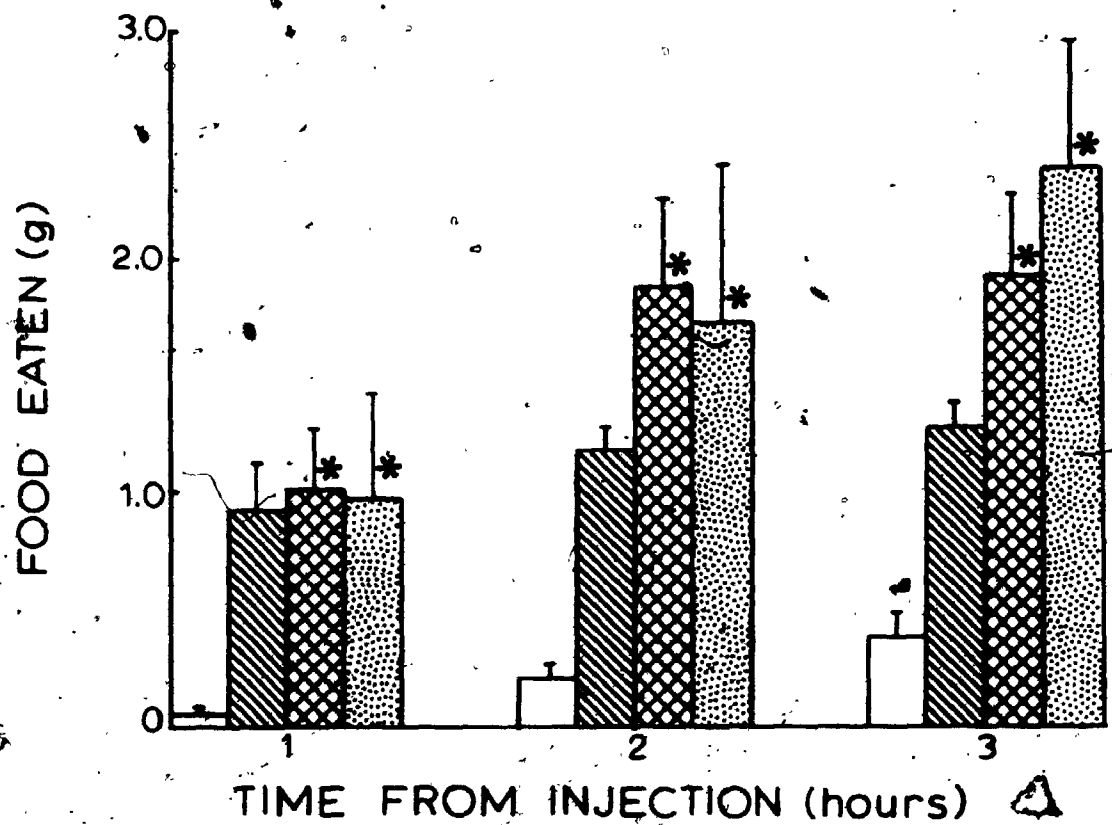
animals in that the quaternary agent did not appear to cause a prolonged depression in activity. After treatment with either dose of the methiodide the activity of the rats included sniffing, grooming and sleeping for short periods before beginning to feed.

Morphine elicited hyperthermia which was persistently higher than core temperature after saline, and higher than temperatures of morphine methiodide-treated animals at the first and second hour after injection. Temperatures of morphine methiodide-treated animals (21.2 nmole) were significantly elevated over temperatures following morphine methiodide (10.6 nmole) and saline at one hour, but not two or three hours, after administration.

4.1.5.2. Morphine Methiodide Compared to Codeine Methiodide

Morphine methiodide stimulated feeding which continued throughout the experimental period, while subsequent to codeine methiodide injection there was a small amount of feeding (0.3 ± 0.1 g) only during the first hour after injection (Fig. 9). The differences in food intake between treatments became significant at the second and third hours following injection. Codeine methiodide did not appear to reduce the activity of the rats; following injection they exhibited their normal

FIGURE 9

Mean cumulative food intake and core temperature
of rats (n = 6) over 3 hours following administration
into the ventromedial hypothalamus of:

■—■ morphine methiodide (21.2 nmole)
□—□ codeine methiodide (21.2 nmole)

. Vertical lines represent S.E.M. Significant
differences (p < 0.05) from codeine methiodide are
indicated as * .

FOOD EATEN (g)

TIME FROM INJECTION (hours)

CORE TEMPERATURE (°C)

TIME FROM INJECTION (hours)
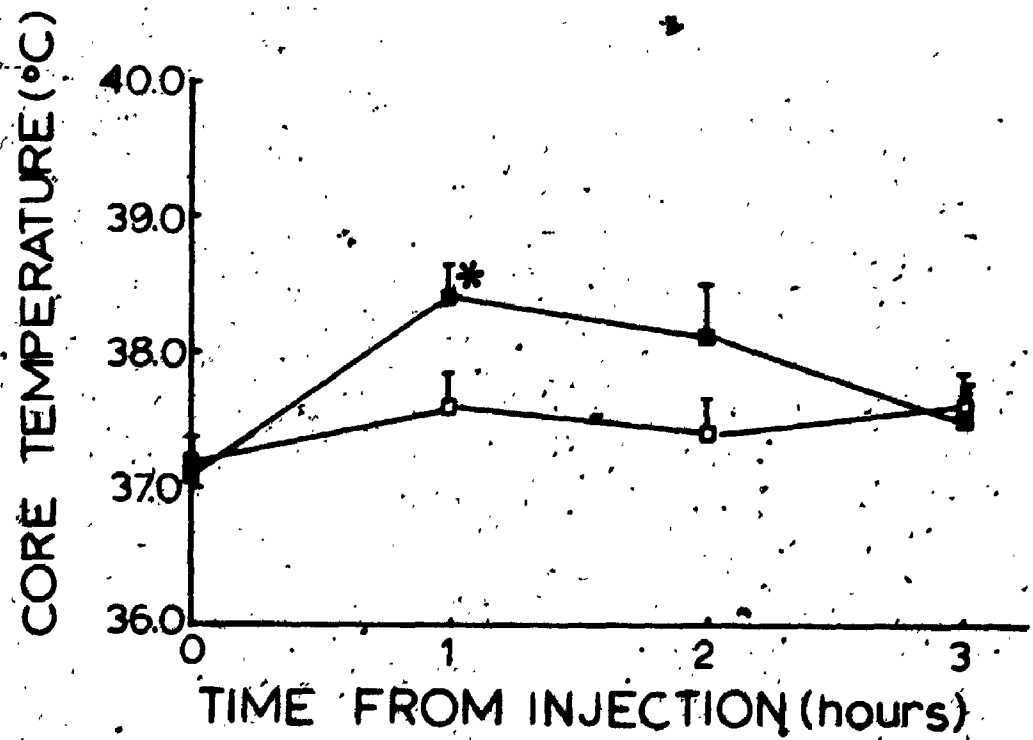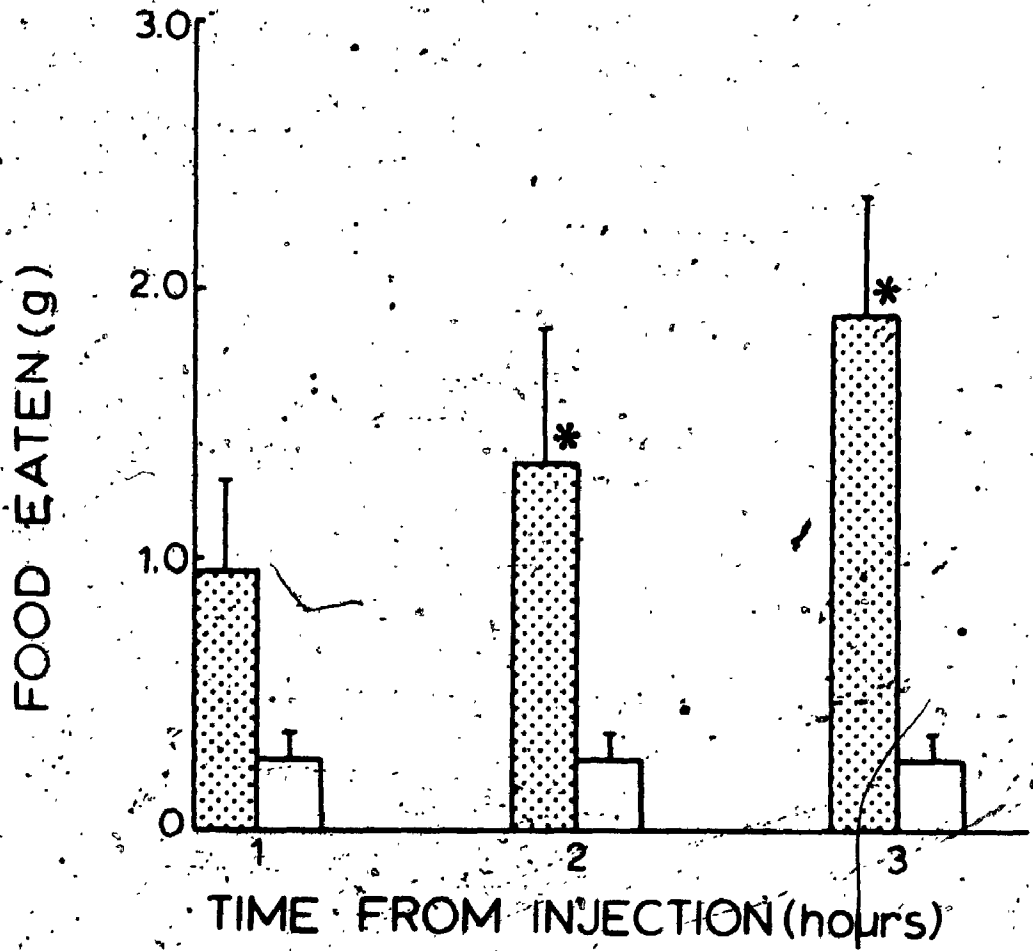
patterns of sniffing, exploratory activity and sleeping.

Morphine methiodide elevated core temperatures significantly compared to codeine methiodide one hour after injection. However, at two and three hours after morphine methiodide was given, although animals were still feeding, core temperatures were not significantly different from those after codeine methiodide.

4.1.6. Effects of Opioid Peptides in the VMH on Feeding and Temperature

4.1.6.1. $D-Ala^2$, $D-Leu^5$-Enkephalin (DADLE)

DADLE injection elicited a dose-dependent increase in ingestion over saline. After a delay of 10 to 25 minutes, eating began, and was mainly completed by one hour after drug treatment. During the initial pre-feeding period there was often a phase of increased activity, and occasionally a brief phase of decreased locomotion, which lasted about 10 minutes at the highest doses and was absent at the lowest. Again, morphine produced prolonged depression in activity and then persis-tent feeding.

The peak of the dose-response curve for feeding plateaued at 1.4 nmole DADLE, since higher doses (2.7 and
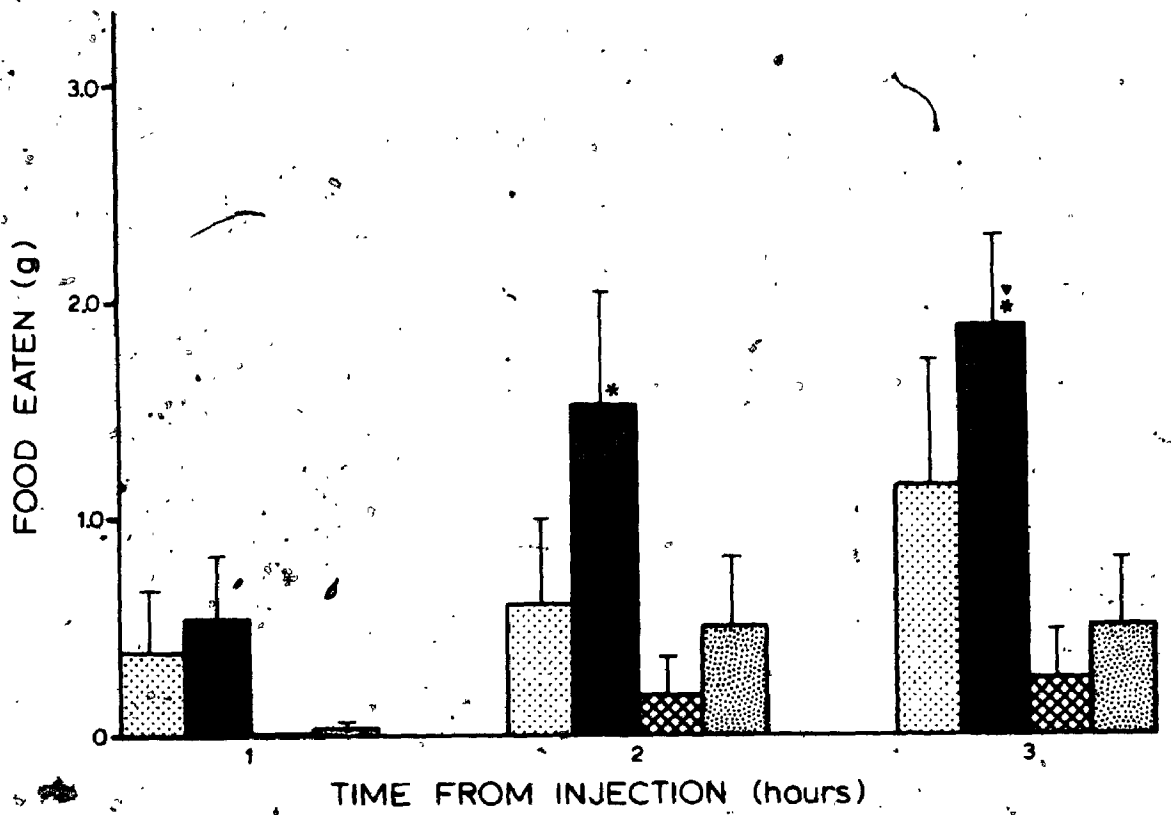
FIGURE 10

Mean cumulative food intake and core temperature of rats (n = 16) over 3 hours following administration into the ventromedial hypothalamus of saline, morphine or D-Ala^2, D-Leu^5-enkephalin (DADLE) as follows:

● ● DADLE (1.4 nmole)

■ ■ DADLE (2.7 nmole)

▲ ▲ DADLE (5.3 nmole)

□ □ morphine (5.3 nmole)

O O saline

Vertical lines represent S.E.M. Significant differences (p < 0.01) from saline are indicated as ✳ , from DADLE (1.4 and 2.7 nmole) as ◆ , and from DADLE (5.3 nmole) as ◇ .
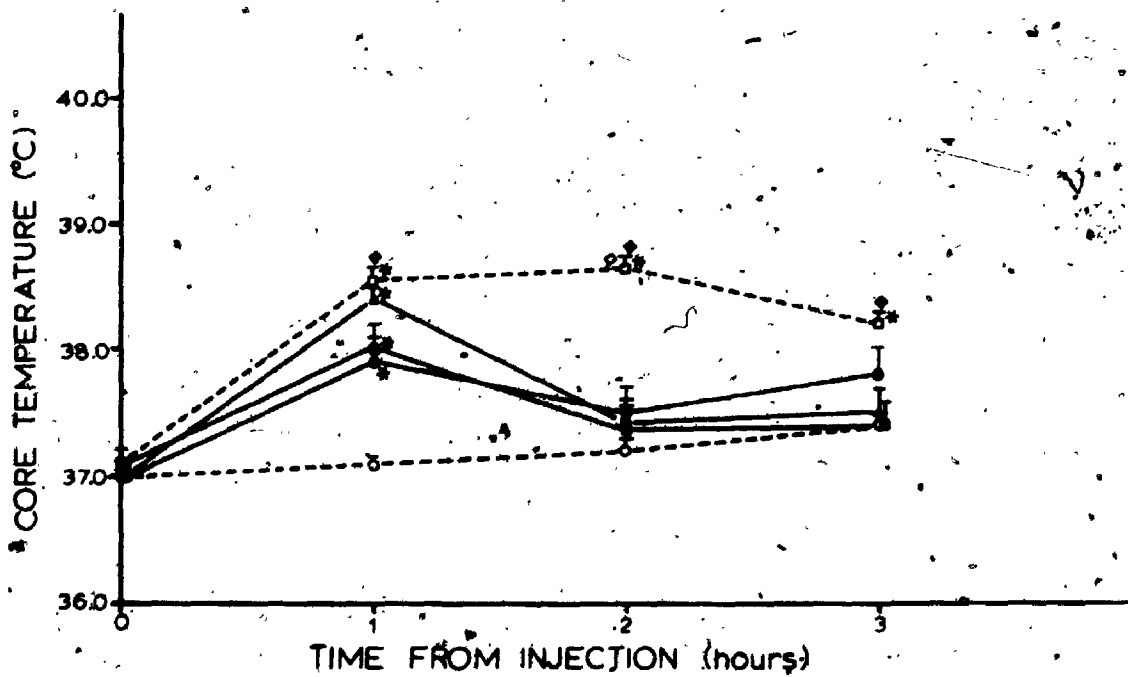
FIGURE 11

Mean cumulative food intake and core temperature of rats (n = 11) over 3 hours following administration into the ventromedial hypothalamus of saline, morphine, or D-Ala^2,D-Leu^5 enkephalin (DADLE) as follows:

▼—▼ DADLE (0.35 nmole)

■—■ DADLE (0.70 nmole)

□—□ morphine (5.3 nmole)
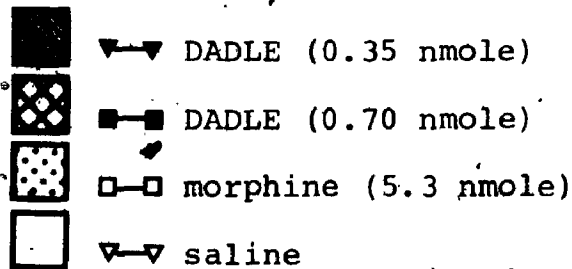
▽—▽ saline

Vertical lines represent S.E.M. Significant differences (p < 0.05) from saline are indicated as *, from DADLE (0.35 nmole) as ■ , and from DADLE (0.70 nmole as △ .
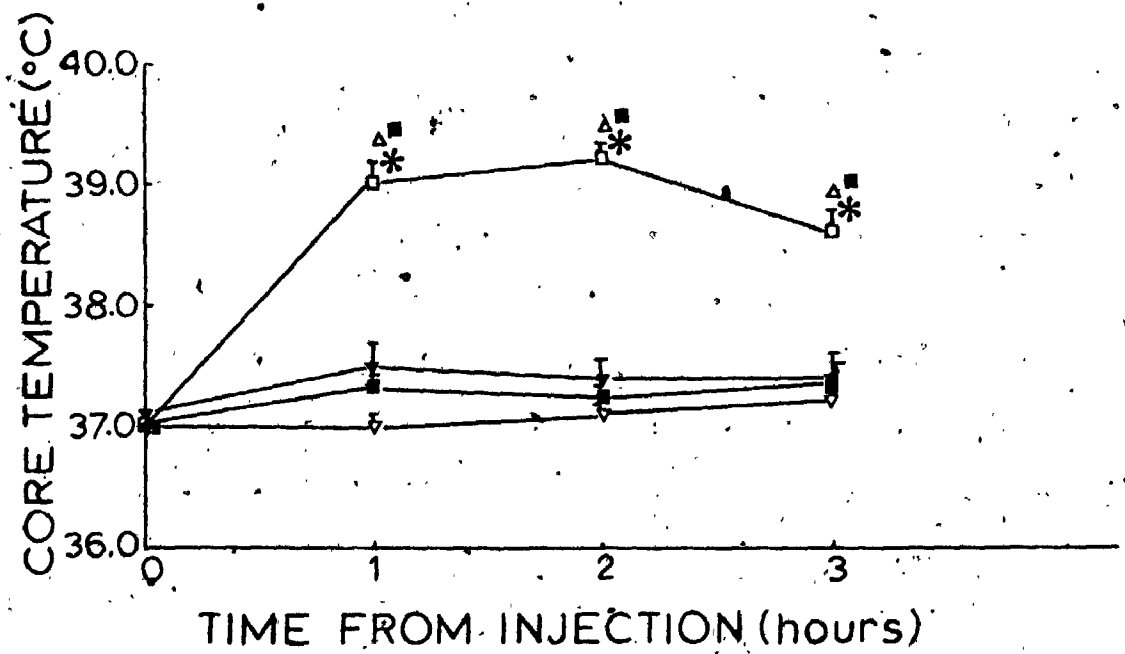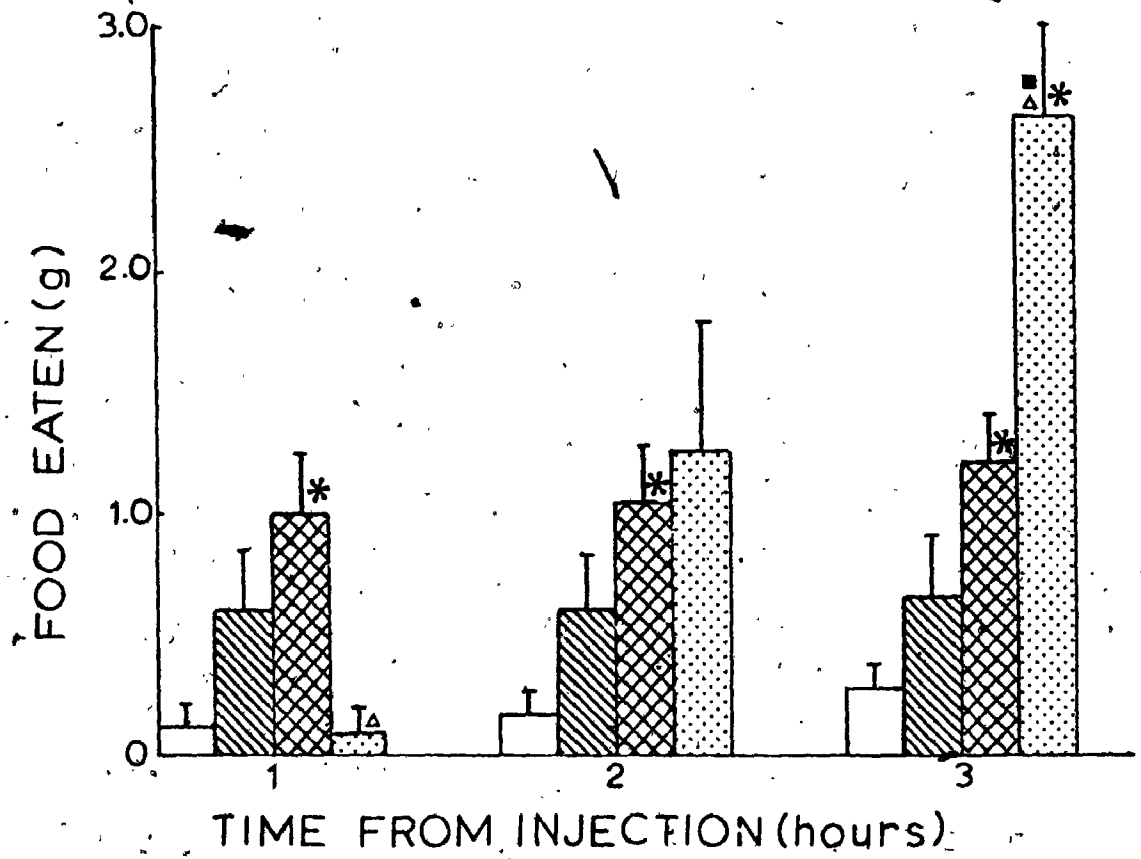
5.3 nmole) did not further enhance food intake (Fig. 10).
The level of feeding produced in one hour was comparable
to the total three-hour food intake following morphine.
In a separate group of rats, ingestion following DADLE
(0.7 nmole) was about one-half as great as with morphine,
while a lower dose (0.35 nmole) had no effect (Fig. 11).

The prolonged hyperthermia caused by morphine was
in contrast to the short-lived elevation in temperature
produced by the three highest doses of DADLE. The peptide-
induced hyperthermia was dose-related and appeared at the
first time of measurement; returning to saline control
levels by the end of the second hour after treatment.
Moreover, although the level of feeding with 1.4 and 2.7
nmole was equivalent to that induced by morphine, the
temperatures recorded after one hour were significantly
lower. The hyperthermia elicited at the first measurement
interval by the highest dose of DADLE (5.3 nmole) was
comparable to that after morphine injection. Neither of
the two lower doses (0.7 and 0.35 nmole) altered
temperatures from values recorded in saline-treated rats.

4.1.6.2. β-Endorphin

The pattern of behaviour following β-endorphin
resembled that after morphine; there was an initial
interval of decreased activity followed by persistent

FIGURE 12

Mean cumulative food intake and core temperature
of rats (n = 6) over 3 hours following administration
into the ventromedial, hypothalamus of:

□—□ β-endorphin (1.4 nmole)

■—■ β-endorphin (2.7 nmole)

●—● morphine (5.3 nmole)
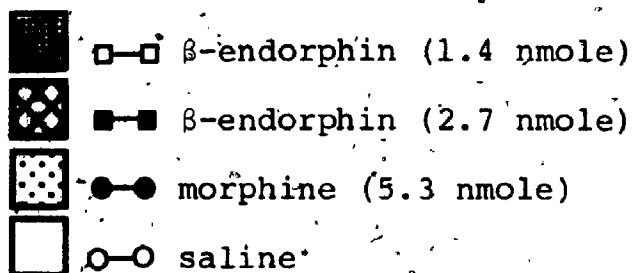
O—O saline

Vertical lines represent S.E.M. Significant
differences (p < 0.05) from saline are indicated as
* , from β-endorphin (1.4 nmole) as ▲ , and from
β-endorphin (2.7 nmole) as ● :
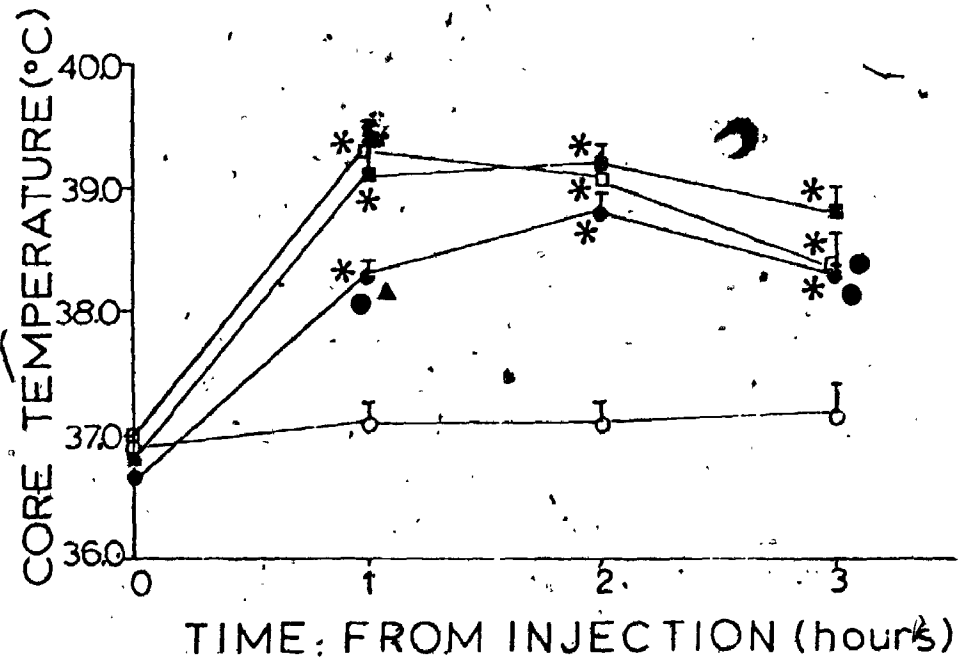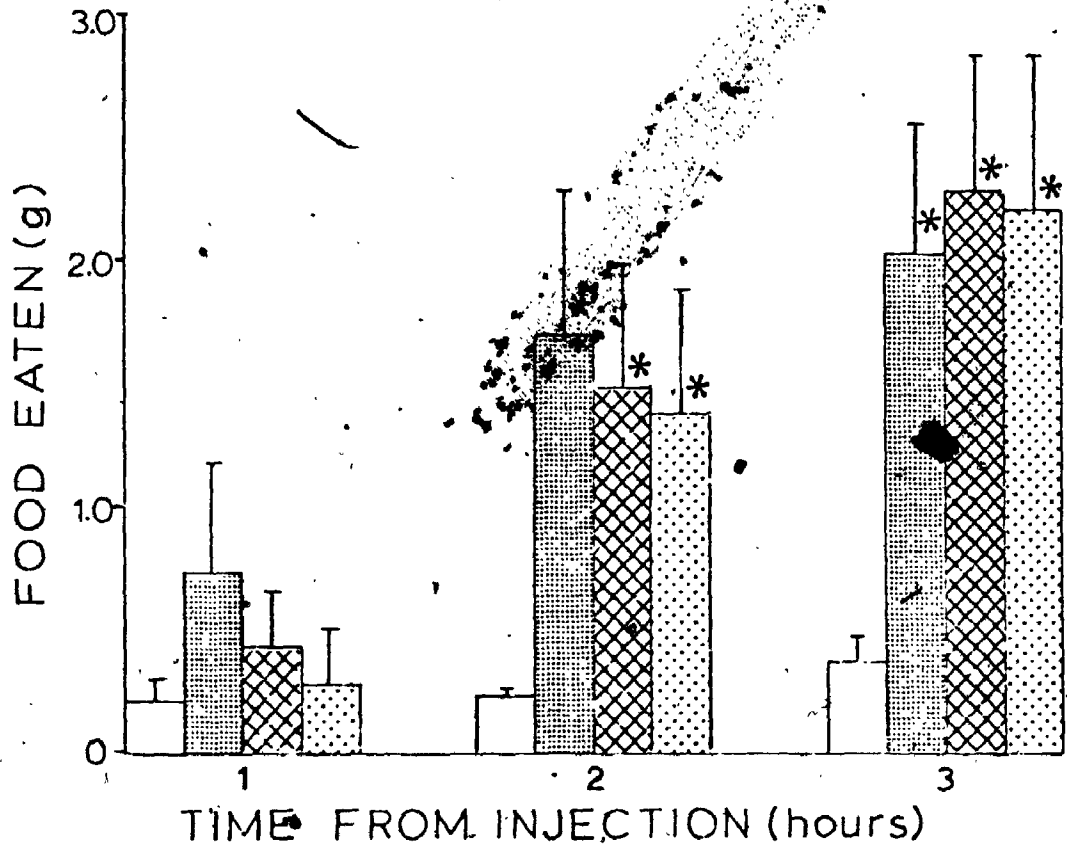
feeding. The one, two and three hour quantities of food
eaten after administration of the peptide (1.4 and 2.7
nmole) were equivalent to that after morphine (5.3 nmole,
Fig. 12). At one hour post-injection, food intake for
the peptide and narcotic were no different from saline,
but at two and three hours, intake was considerably
lower following saline than after the drug treatments.
The lower doses of β-endorphin tested (0.35 and 0.70
nmole) never evoked more feeding than did saline, and
caused significantly less total three-hour consumption than
did morphine (5.3 nmole, Fig. 13).

β-Endorphin (1.4 and 2.7 nmole) elevated rat core
temperatures over those in saline control animals, but the
peptide-evoked hyperthermia did not appear to be dose-
dependent. Morphine (5.3 nmole) also elevated temperatures
significantly over those recorded in saline-treated
animals, but one hour after injection the elevation in
temperature elicited by morphine was significantly less
than that after β-endorphin (1.4 and 2.7 nmole) and three
hours after injection, morphine and β-endorphin (1.4
nmole) produced significantly lower temperatures than the
higher dose of β-endorphin (2.7 nmole). Core temperatures
of rats following injection of the lower doses of β-
endorphin (0.35 and 0.70), as well as after morphine,
were significantly higher than those after saline one hour

FIGURE 13

Mean cumulative food intake and core temperature of rats (n = 4) over 3 hours following administration into the ventromedial hypothalamus of:

▨ □—□ β-endorphin (0.35 nmole)

▨ ■—■ β-endorphin (0.70 nmole)

▨ ●—● morphine (5.3 nmole)
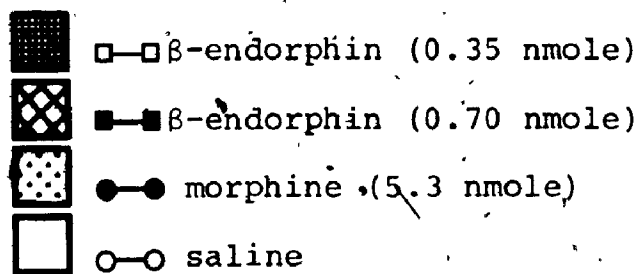
☐ ○—○ saline

Vertical lines represent S.E.M. Significant differences (p < 0.05) from saline are indicated as ✳ , from β-endorphin (0.35 nmole) as ● , from β-endorphin (0.70 nmole) as ▲ , and from morphine as ◆

FOOD EATEN (g)

TIME FROM INJECTION (hours)

CORE TEMPERATURE (°C)

TIME FROM INJECTION (hours)
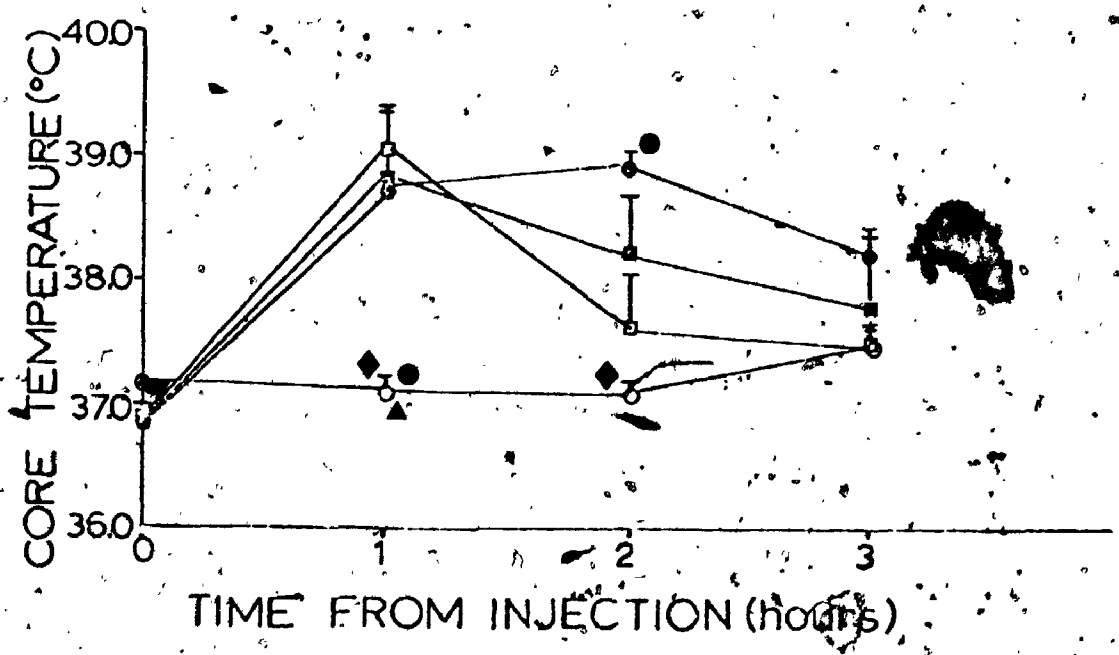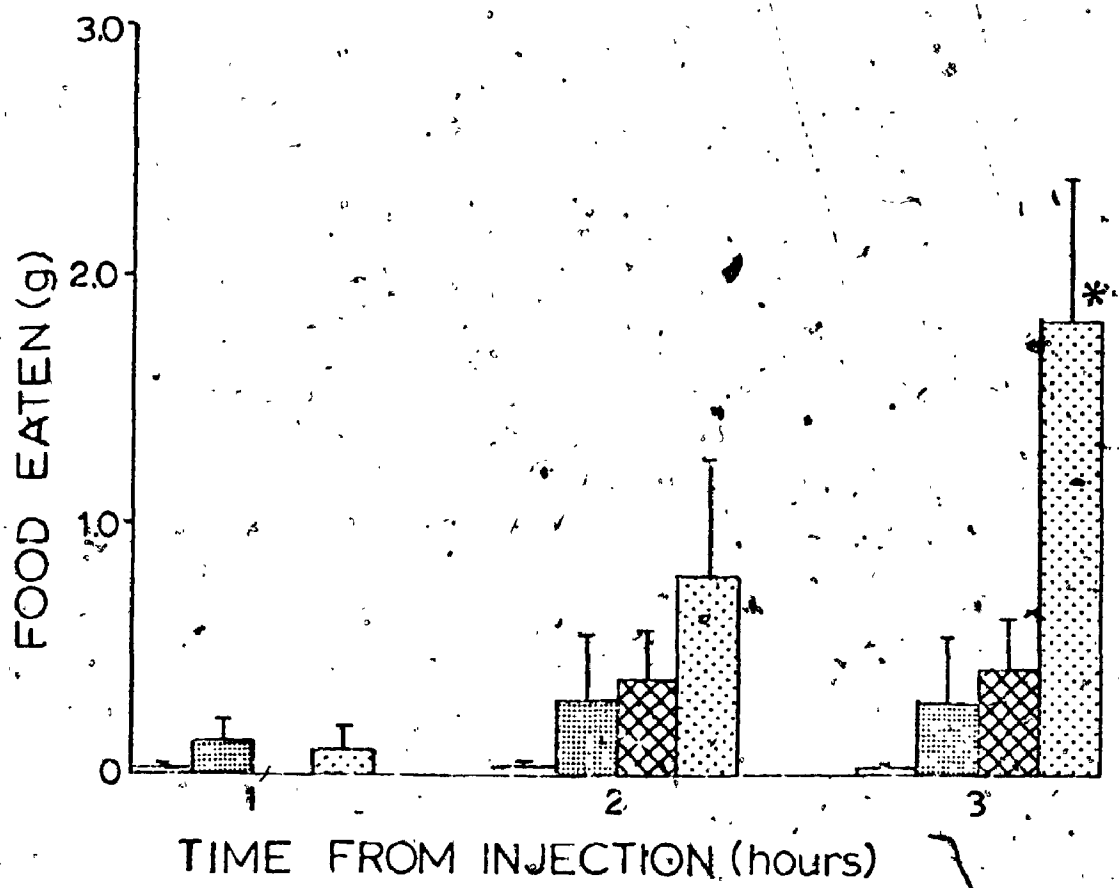
2
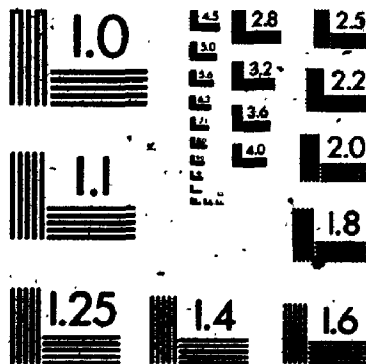
after injection (Fig. 13). Two hours after injection
the temperature after morphine was still elevated over
that after saline, but the β-endorphin-induced temper-
atures had fallen, so that the response to the 0.70
nmole dose was no different from that after morphine
or saline, and the effect of the 0.35 nmole dose was no
different from that after saline and was significantly
lower than morphine. Three hours after injection, there
were no significant differences in temperatures among the
different groups.

4.1.7.   Effects of Naloxone Hydrochloride on the Altera-
          tions in Feeding and Core Temperature Induced
          by VMH Injection of Morphine Sulphate, Morphine
          Methiodide, DADLE and β-Endorphin

    4.1.7.1.   Morphine Sulphate

    Pretreatment with naloxone, given into the VMH,
depressed the small amount of feeding and the hyperthermia
significantly for the initial hour following morphine
injection (Fig. 14). Both parameters then rose to levels
comparable to those occurring in rats after combined
saline and morphine injections. When naloxone was
administered before saline (three-hour intake — 0 ±
0.3 g; core temperature - initial: 37.0 ± 0.2° C, one hour:
37.1 ± 0.3° C, two hour: 37.6 ± 0.2° C, three hour:
37.6 ± 0.2°C), there were no significant changes in

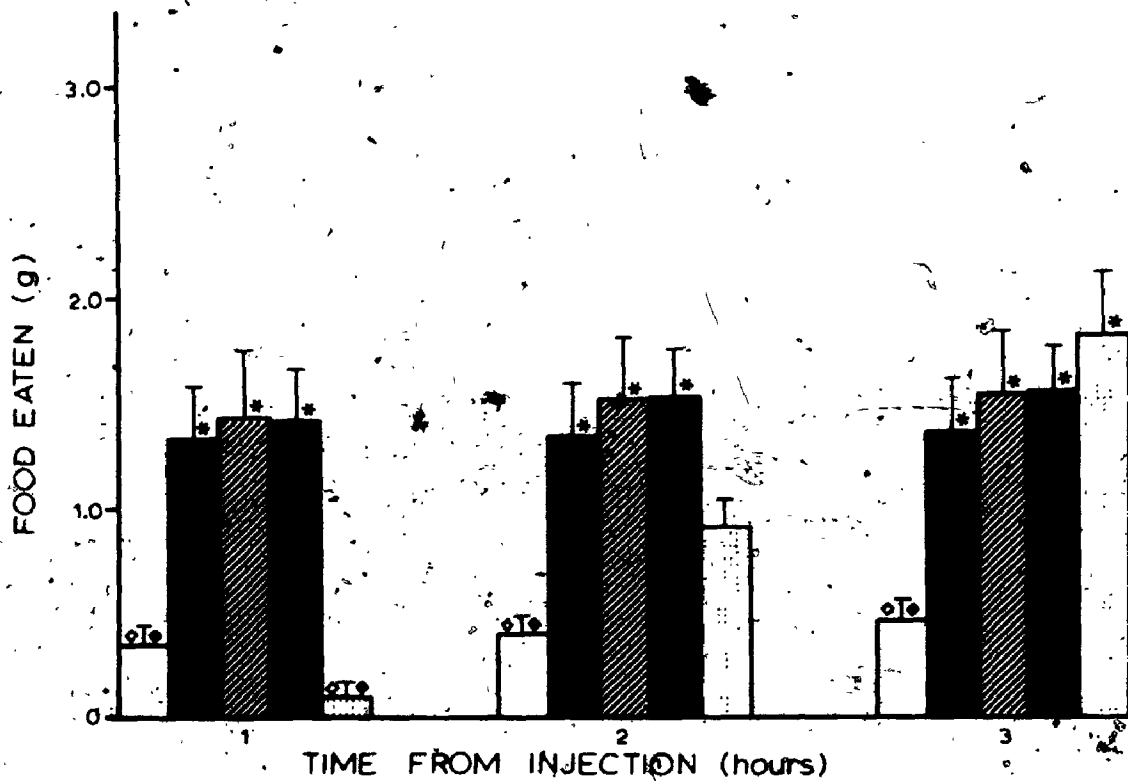FIGURE 14

Mean cumulative food intake and core temperature
of rats (n = 8) over 3 hours following administration
of morphine (5.3 nmole) which was preceded by:

□ ■—■ saline

or ◪ ○—○ naloxone (10.6 nmole)

All injections were made into the ventromedial
hypothalamus. Vertical lines represent S.E.M.
Significant differences from saline (p < 0.05) are
indicated as * .
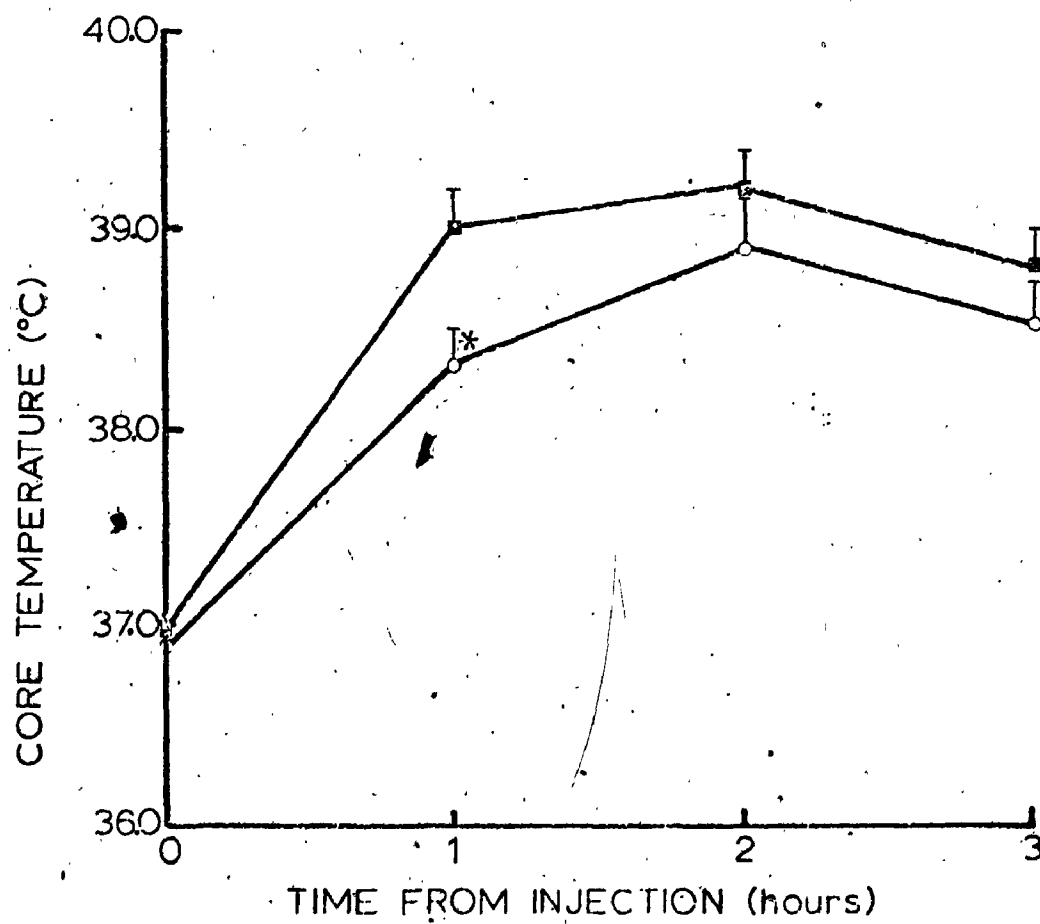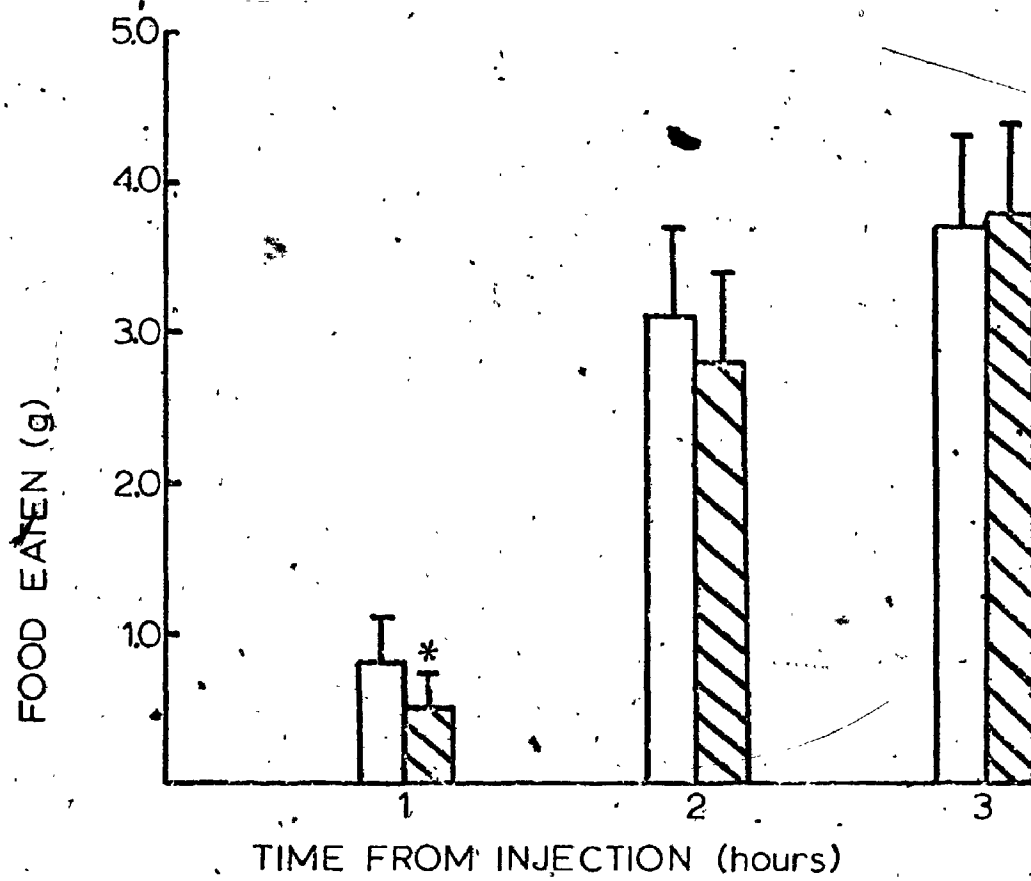
feeding and temperature from values recorded for saline alone (three-hour intake - 0.8 ± 0.4 g; core temperature - initial: $37.0 \pm 0.1^\circ$ C, one hour: $37.2 \pm 0.2^\circ$ C, two hour: $37.9 \pm 0.4^\circ$ C, three hour: $38.1 \pm 0.4^\circ$ C). However, two of the five rats ate small amounts during the hour after receiving saline, but none ate during this interval when they received naloxone.
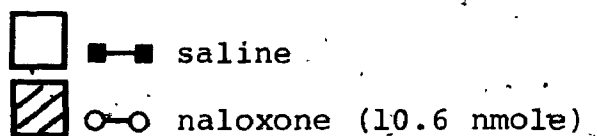
When naloxone was delayed until one hour after morphine (Fig. 15), its effects on feeding and temperature were no different from measurements in rats receiving saline. It should be noted that in Fig. 15, food intake was measured cumulatively after injection of naloxone or saline.

The first hour after treatment, naloxone hydrochloride (10 mg/kg), given subcutaneously, was significantly more effective in lowering food intake induced by morphine than was subcutaneous saline (Fig. 16). Cumulative feeding at two and three hours following morphine administration was significantly lower after both doses of naloxone (two and 10 mg/kg) than after the saline control treatment, and the reduction was dose related. For two hours, both doses of naloxone were equally effective in reducing the morphine-related hyperthermia in the rats, but this effect was maintained for the entire three hours only after the dose of 10 mg/kg.

FIGURE 15

Mean food intake and core temperature of rats
(n = 9) over 3 hours following morphine (5.3 nmole)
which was administered 1 hour prior to

☐ ■—■ saline

▨ o—o naloxone (10.6 nmole)

All injections were made into the ventromedial
hypothalamus. The arrow indicates injection of
saline or naloxone and the abscissa denotes time
from this injection. Food intake is cumulative from
the time of the saline and naloxone injections.
Vertical lines indicate S.E.M.

FOOD EATEN (g)

TIME FROM INJECTION (hours)

CORE TEMPERATURE (°C)

TIME FROM INJECTION (hours)
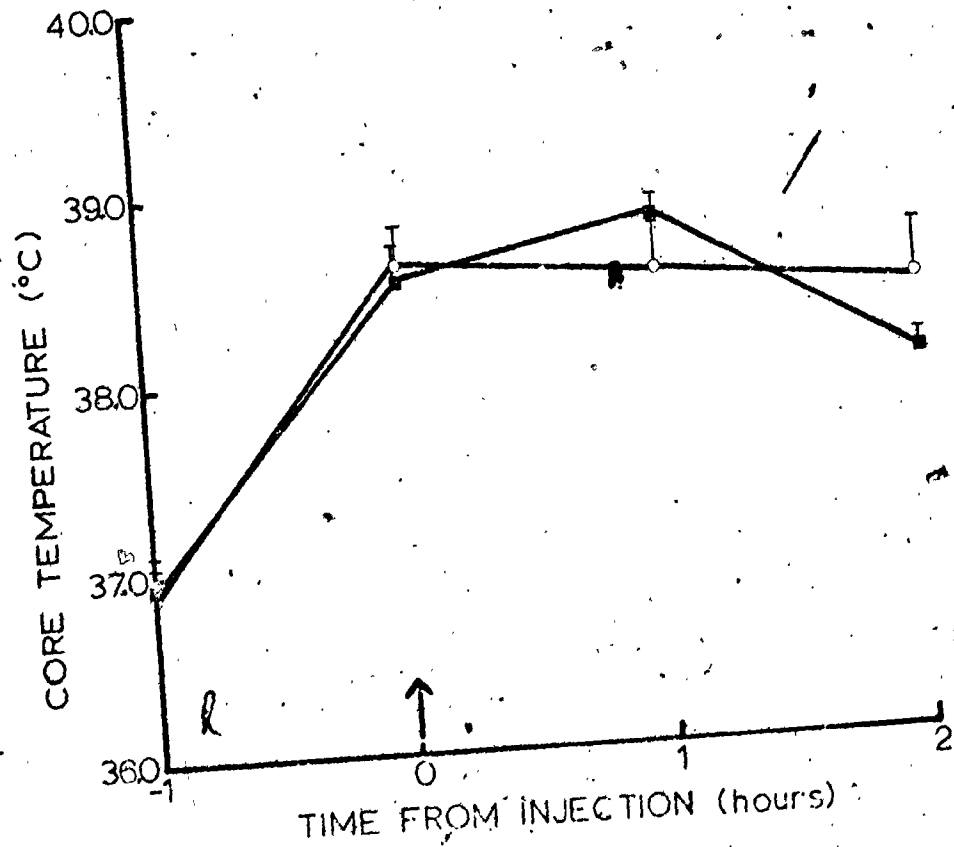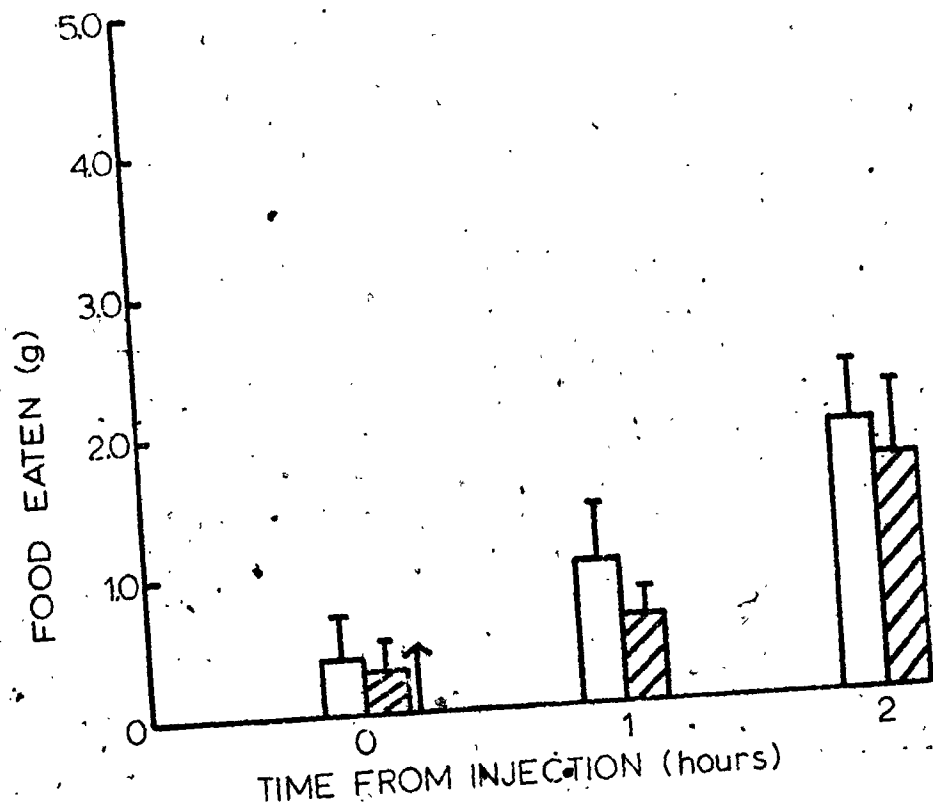
FIGURE 16

Mean cumulative food intake and core temperature
of rats (n = 10) over 3 hours after administration
into the ventromedial hypothalamus of morphine (5.3
nmole), which was preceded by subcutaneous injection
of

☐ ■—■ saline

▨ ●—● naloxone (2 mg/kg)

or ◨ ○—○ naloxone (10 mg/kg)

Vertical lines indicate S.E.M. Significant
differences from saline ($p < 0.05$) are indicated as
*
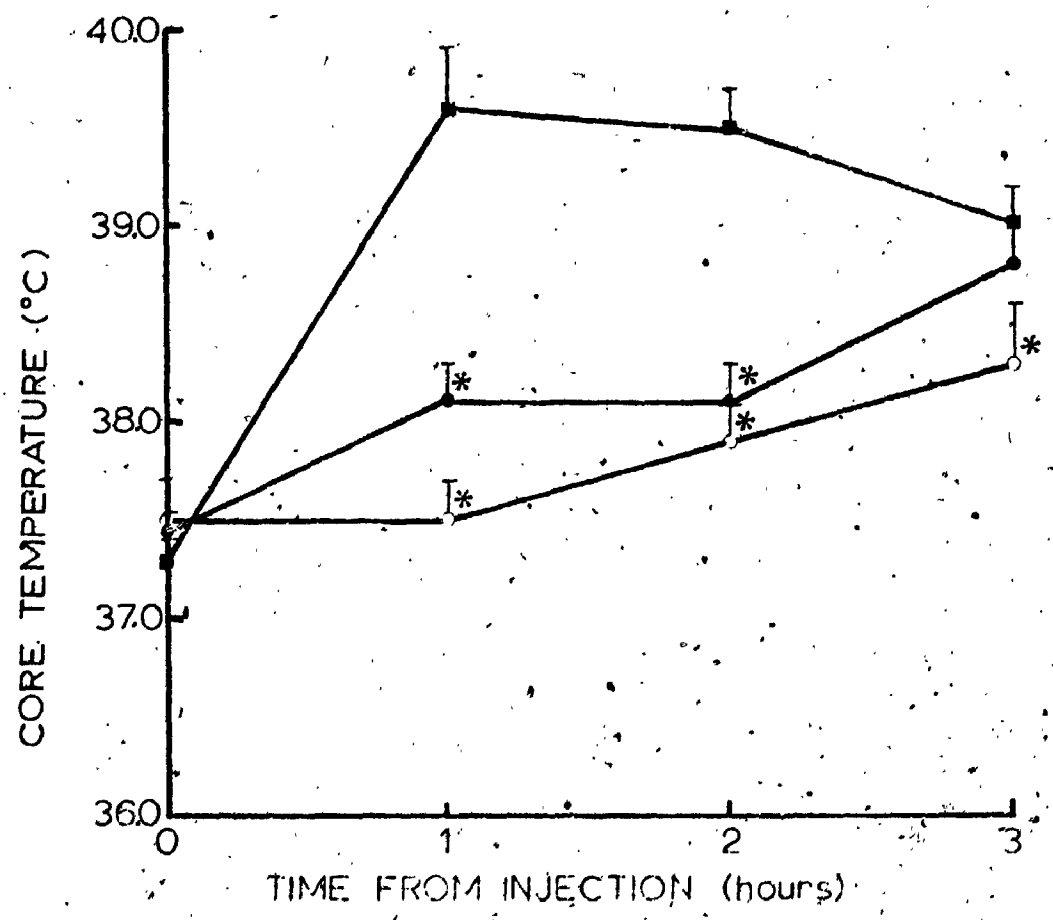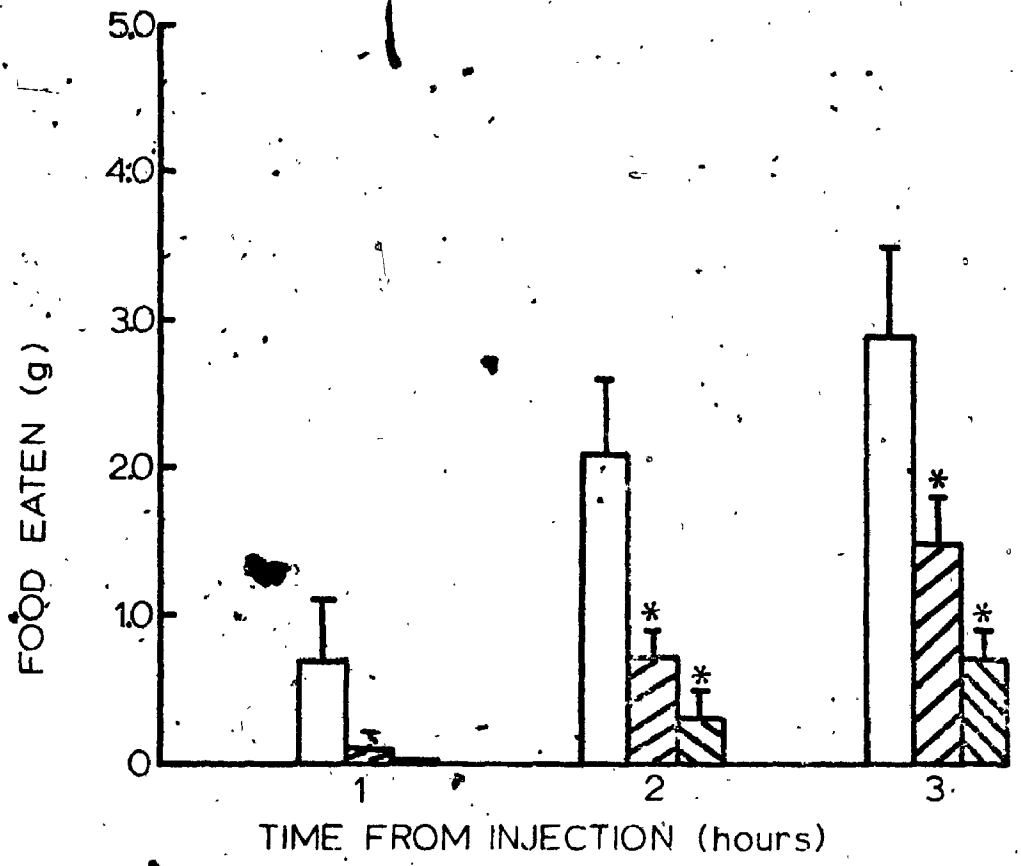
4.1.7.2. Morphine Methiodide
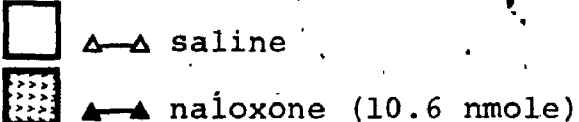
Intrahypothalamic naloxone did not alter food intake following morphine methiodide, but did result in a small, but significant, increase in temperature one hour after injections were given (Fig. 17).

When given subcutaneously, prior to morphine methiodide, naloxone (two and 10 mg/kg) significantly reduced the cumulative food intake for three hours (Fig. 18). During the third hour, the higher dose of naloxone maintained almost total suppression of food intake. However, a rebound feeding occurred during the third hour after the lower dose of naloxone was given with morphine methiodide, which was intense enough that the quantity of pellets eaten (1.1 ± 0.2 g) was not significantly different from the amount eaten after saline and morphine were given (0.8 ± 0.3 g). In terms of total, three-hour, food intake, naloxone (2 mg/kg) allowed significantly more ingestion than naloxone (10 mg/kg), but significantly less than saline.

One hour after parenteral injection, naloxone was able to reduce core temperature significantly.

FIGURE 17

Mean cumulative food intake and core temperature
of rats (n = 5) over 3 hours following administration
of morphine methiodide (21.2 nmole) which was preceded
by:

$\square$ △—△ saline

or ▨ ▲—▲ naloxone (10.6 nmole)

All injections were made into the ventromedial
hypothalamus. Vertical lines represent S.E.M.
Significant differences from saline (p < 0.05) are
indicated as * .

FOOD EATEN (g) vs TIME FROM INJECTION (hours)

CORE TEMPERATURE (°C) vs TIME FROM INJECTION (hours)
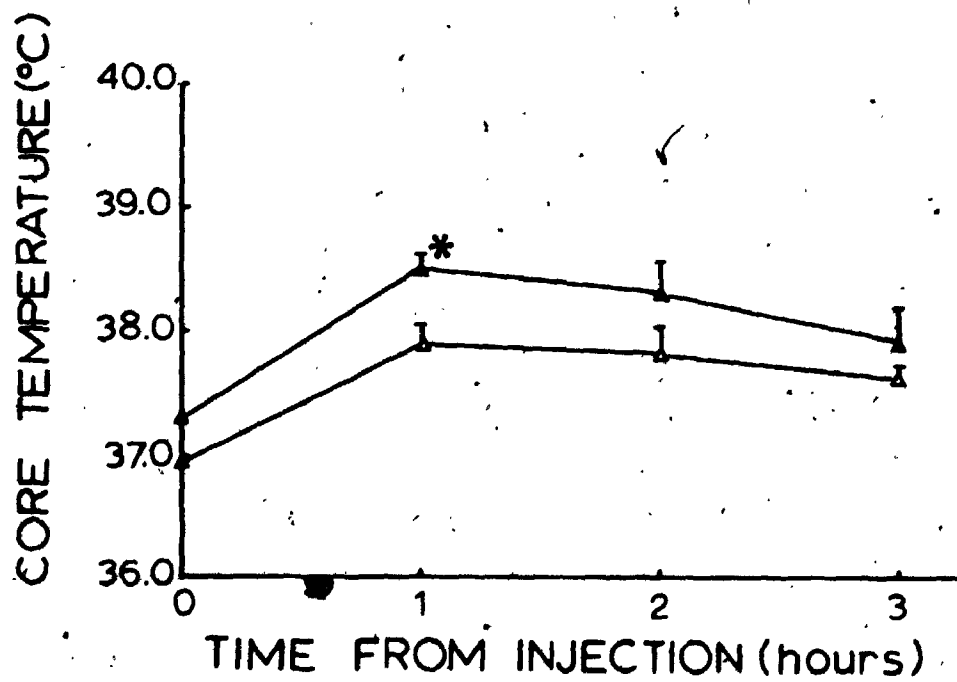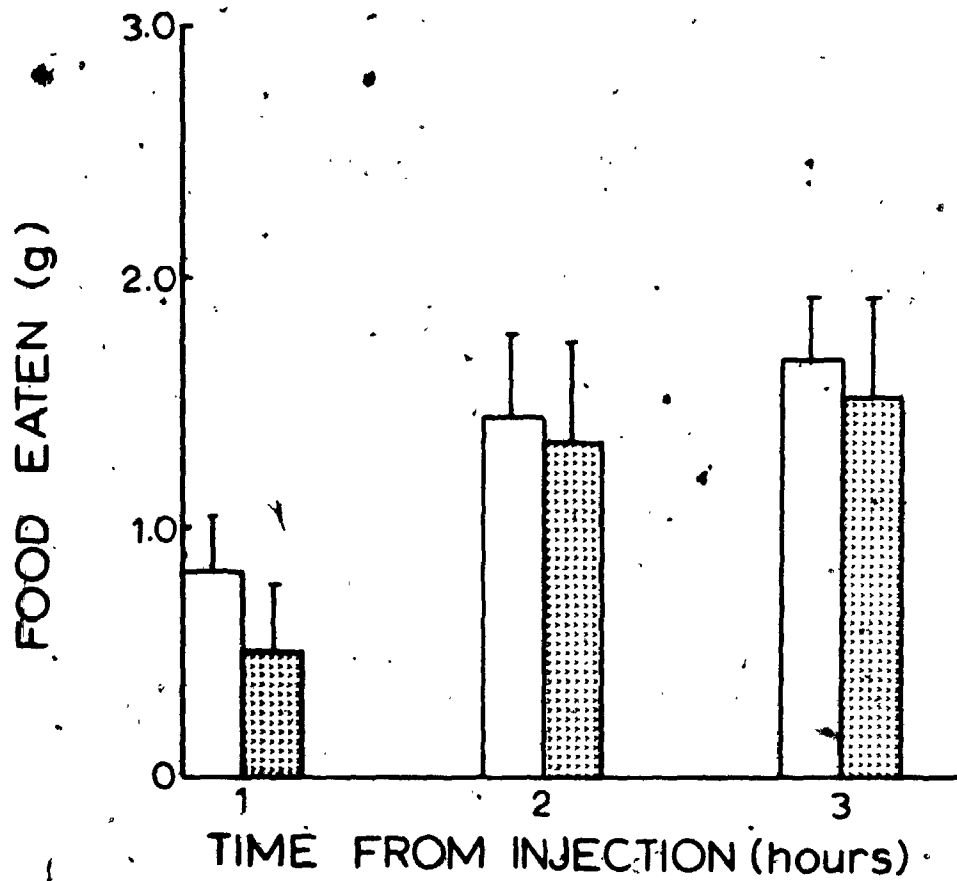
FIGURE 18

Mean cumulative food intake and core temperature of rats (n = 7) over 3 hours after administration into the ventromedial hypothalamus of morphine methiodide (5.3 nmole), which was preceded by subcutaneous injection of

△—△ saline

●—● naloxone (2 mg/kg)

or ■—■ naloxone (10 mg/kg)

Vertical lines indicate S.E.M. Significant differences (p < 0.01) from naloxone (2 mg/kg) are indicated as ✳ and from naloxone (10 mg/kg) as ◆.
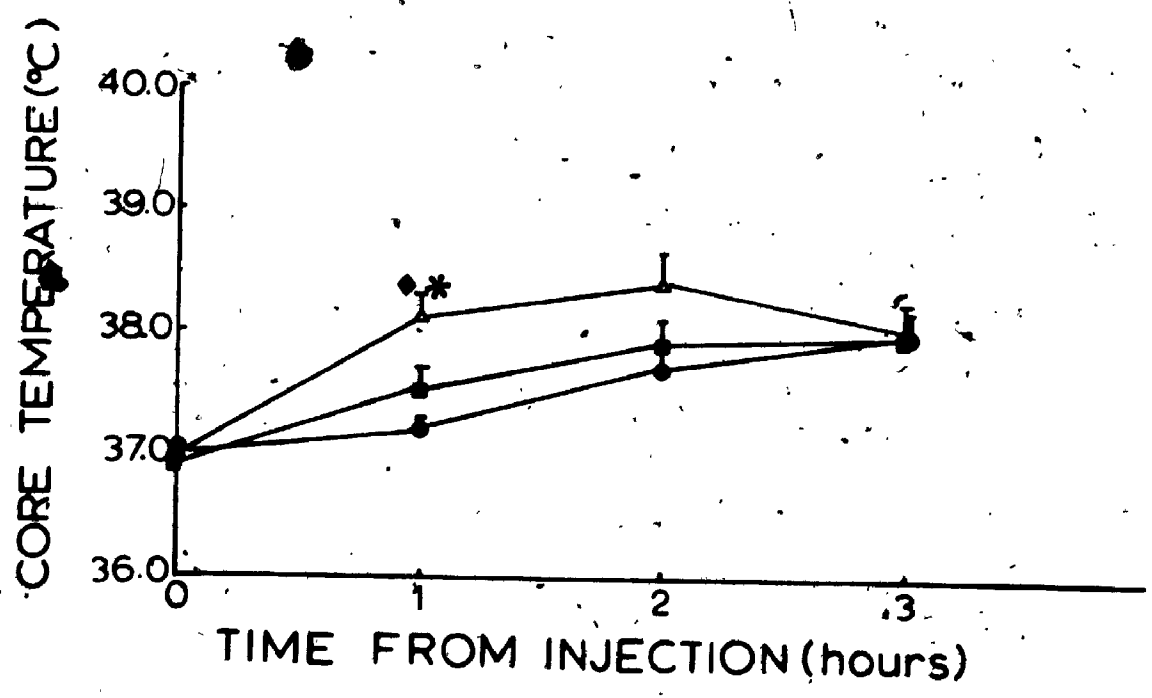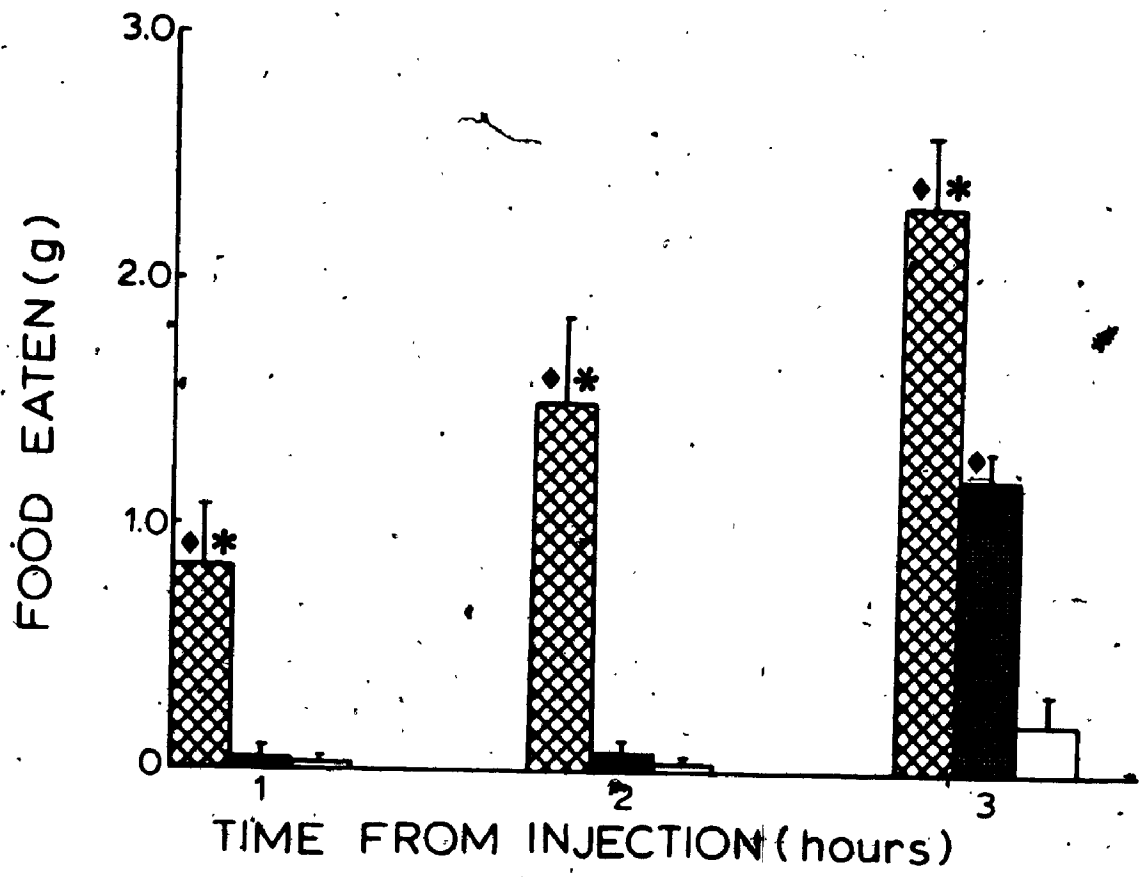
4.1.7.3. DADLE

Enkephalin-induced food consumption was not significantly altered when a ventromedial intrahypothalamic injection of naloxone (10.6 nmole) was given five minutes before the peptide (5.3 nmole) (Fig. 19). However, with naloxone treatment but not saline, three of the seven animals continued to eat into the second hour of the study. Naloxone had no effect on enkephalin-induced hyperthermia.

4.1.7.4. β-Endorphin

Neither the feeding nor the elevation in temperature following injection of β-endorphin (1.4 nmole) were reduced by naloxone (10.6 nmole) given five minutes before endorphin, just as naloxone did not reduce these parameters following DADLE injection (Fig. 20).
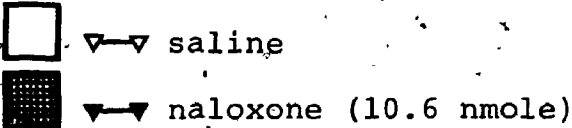
4.1.8. Involvement of Noradrenergic, Dopaminergic and Serotonergic Neurons in Opioid-Influenced Feeding and Temperature Changes

4.1.8.1. Effects of Phentolamine Hydrochloride on Noradrenaline Hydrochloride

Noradrenaline-induced feeding (30 nmole) was significantly reduced by the α-adrenergic receptor antagonist phentolamine (60 nmole) at both one and two hours after

FIGURE 19

Mean cumulative food intake and core temperature
of rats (n = 7) over 3 hours following administration
of D-Ala^2,D-Leu^5-enkephalin (5.3 nmole) which was
preceded by:

☐ ▽—▽ saline

or ▩ ▼—▼ naloxone (10.6 nmole)

All injections were made into the ventromedial
hypothalamus. Vertical lines represent S.E.M.

FOOD EATEN (g)

TIME FROM INJECTION (hours)

CORE TEMPERATURE(°C)

TIME FROM INJECTION (hours)
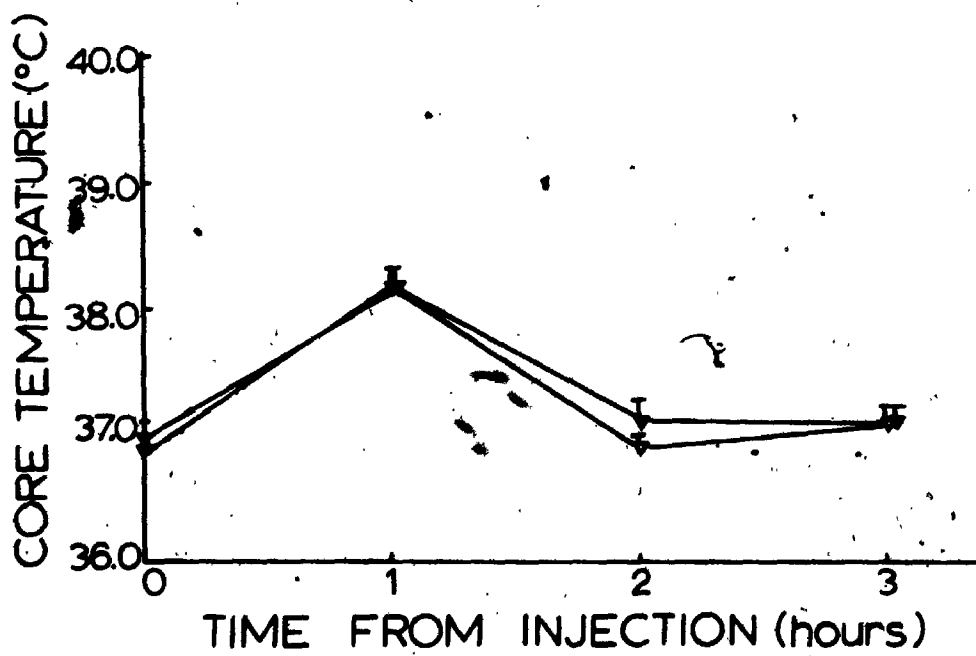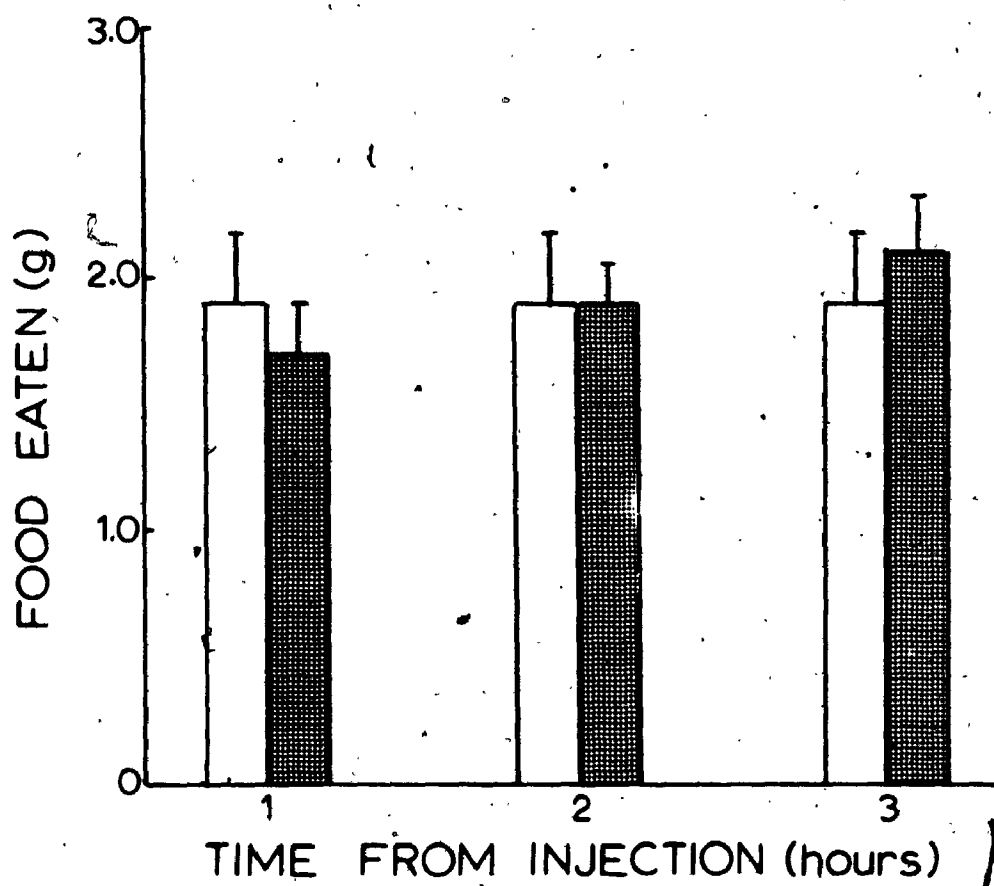
FIGURE 20

Mean cumulative food intake and core temperature
of rats (n = 4) over 3 hours following administration
of β-endorphin (1.4 nmole) which was preceded by:

☐ △—△ saline

or ▨ ▲—▲ naloxone (10.6 nmole)

All injections were made into the ventromedial
hypothalamus. Vertical lines represent S.E.M.
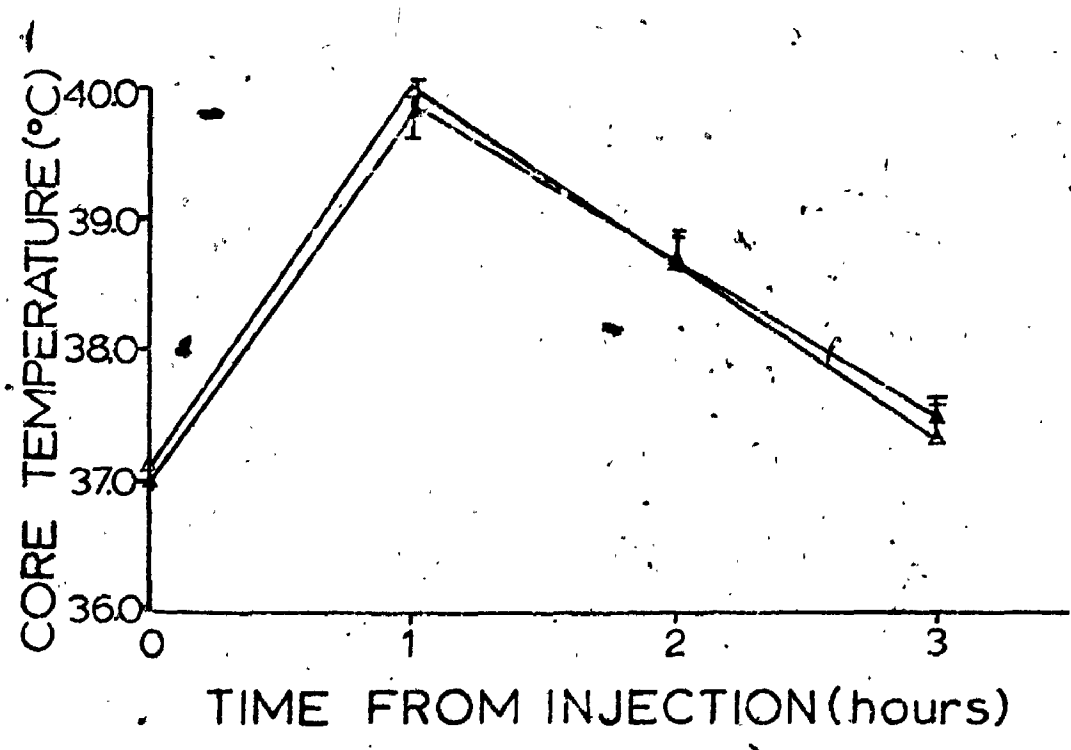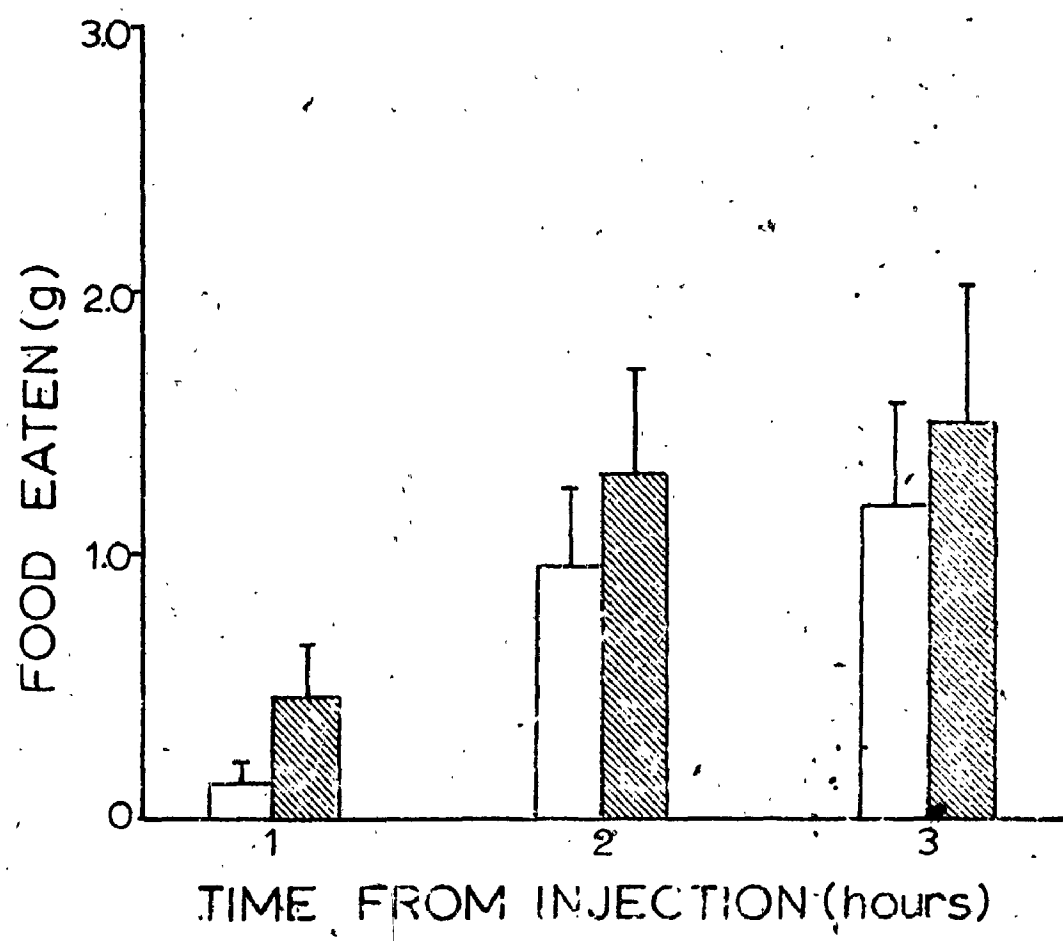
injection (Fig. 21). A smaller dose of phentolamine
(30 nmole) did not have a significant effect. The two-
hour intake after saline treatment was 0.4 ± 0.1 g.
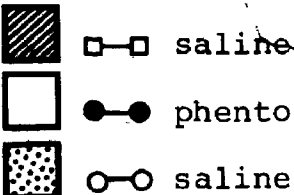
Core temperature varied little following the first
trial day in which saline and noradrenaline were injected.
On later trial days, however, the core temperatures two
hours after the sequential injections of phentolamine
plus noradrenaline and of saline plus noradrenaline
were significantly elevated over temperatures obtained
following the initial saline plus noradrenaline treatment.
When saline was injected independently, temperatures were:
initial 36.9 ± 0.1° C, one hour 37.1 ± 0.2° C, two hour
37.0 ± 0.2° C.

4.1.8.2. Effects of Phentolamine Hydrochloride on Morphine Sulphate

The dose of phentolamine (60 nmole) which decreased
noradrenaline-induced feeding (Section 4.1.7.1) was able
to lower morphine-induced feeding when injected one hour
after (Fig. 22), but not five minutes before, the injection
of morphine (Fig. 23). When given after morphine,
phentolamine tended to reduce feeding during the initial
hour (p < 0.10) and significantly reduced the cumulative
two-hour intake.

FIGURE 21

Mean cumulative food intake and core temperature
of rats (n = 7) over 2 hours following administration
of noradrenaline (30 nmole), preceded on consecutive
drug administration trial days by:

▨ □—□ saline

☐ ●—● phentolamine (60 nmole)

and ▦ ○—○ saline

All injections were made into the ventromedial
hypothalamus. Vertical lines represent S.E.M.
Significant differences (p < 0.05) from the initial
saline + noradrenaline trial are indicated as * and
from the later saline + noradrenaline trial as ◆ .

FOOD EATEN (g) vs TIME FROM INJECTION (hours)

CORE TEMPERATURE (°C) vs TIME FROM INJECTION (hours)
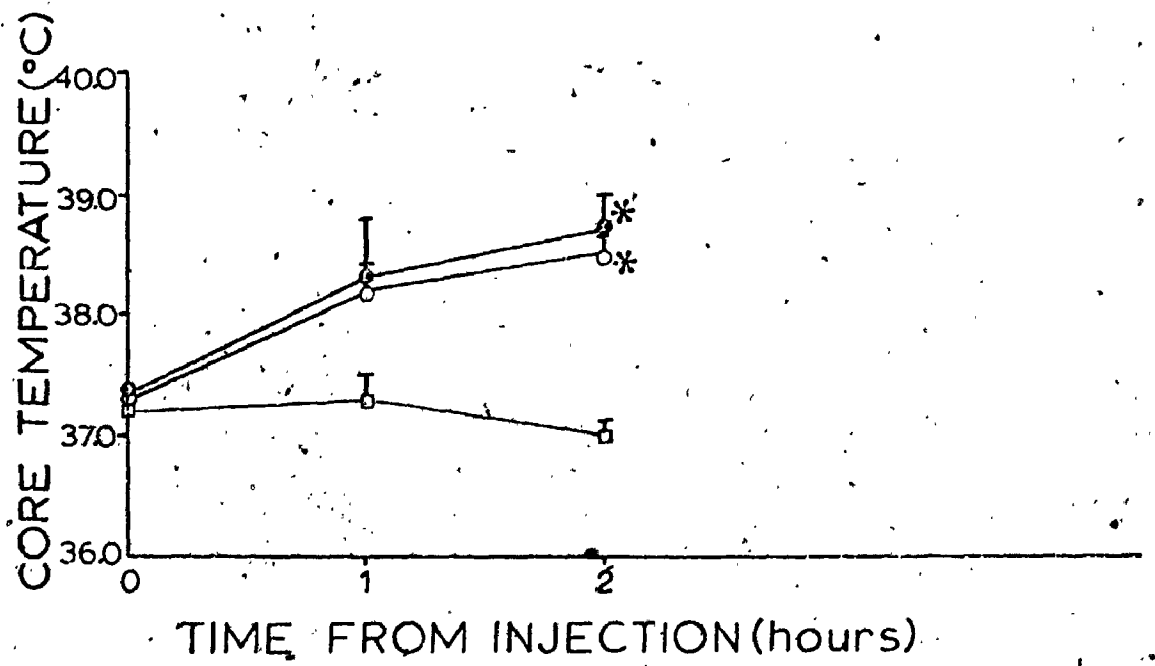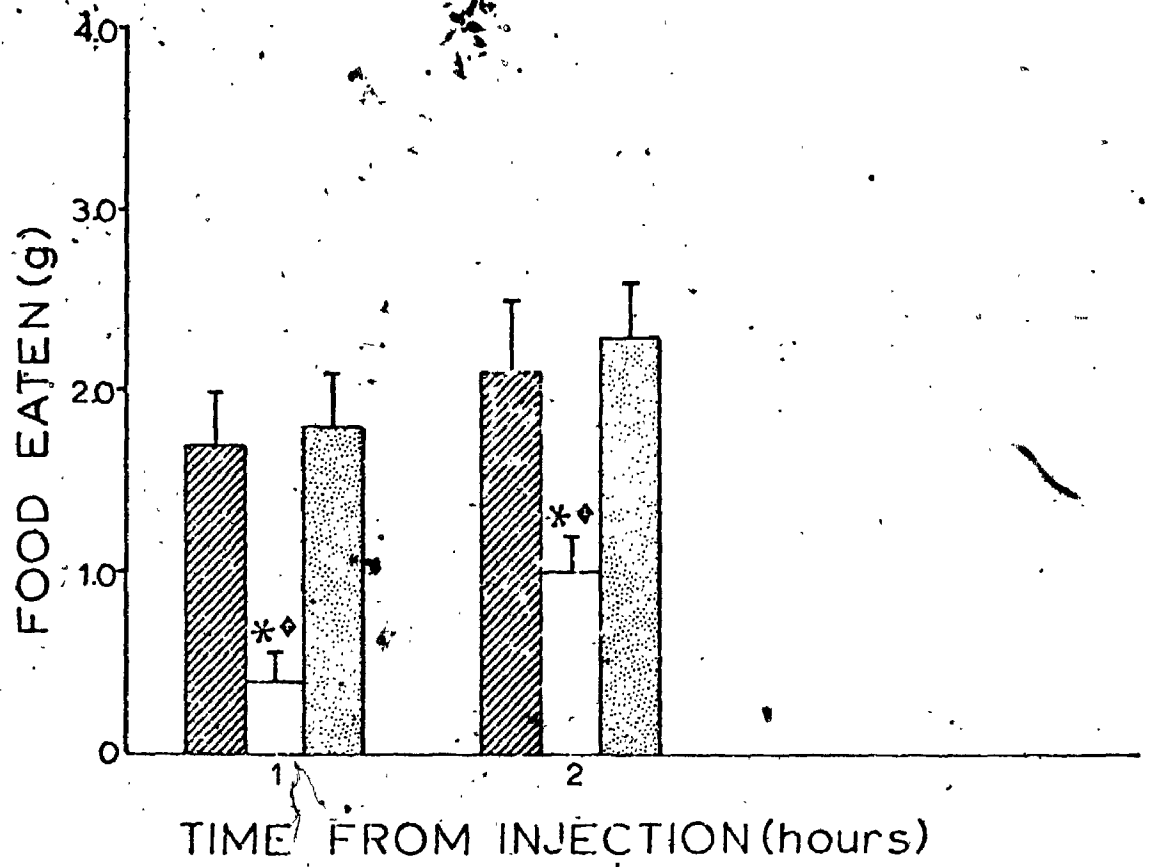
Although feeding was unaffected when phentolamine was injected prior to morphine, both one and two hours after administration of morphine preceded by either phentolamine or the final saline, core temperature was significantly elevated over the initial saline plus morphine regime. When morphine was injected an hour before the α-antagonist or saline, the temperature measured two hours after phentolamine administration was significantly elevated over that at two hours after the second saline treatment.

4.1.8.3. Morphine Sulphate and Noradrenaline Hydrochloride

Noradrenaline-induced feeding was, as described previously, rapid in onset and complete by one hour after injection (Fig. 24). Morphine-induced feeding was slow in onset, building gradually through the three-hour measurement period. Although the rats ingested less food the first two hours after injection of morphine than after noradrenaline, the total food intake following morphine injection was not significantly less than total intake following noradrenaline injection. When morphine and noradrenaline were injected sequentially into the same animal the usual period of inactivity observed after morphine treatment was eliminated; feeding began shortly

FIGURE 22

Mean cumulative food intake and core temperature of rats (n = 6). Administration of morphine was followed after 1 hour, on consecutive drug trial days by:

        ▨ □—□ saline

        ☐ ●—● phentolamine (60 nmole)

and   ⊠ O—O saline

Amount of food eaten was measured following the saline or phentolamine injection. Recording of temperature was begun just before morphine injection, with the arrow on the abscissa denoting administration of saline or phentolamine. Food intake and core temperature following phentolamine alone (60 nmole) are indicated by ■—■ and ▦ . All injections were made into the ventromedial hypothalamus. Vertical lines represent S.E.M. Significant differences (p < 0.01) from the initial morphine + saline trial are indicated as ◆ and from the later morphine + saline trial as ✻ .

FOOD EATEN (g)

TIME FROM INJECTION (hours)

CORE TEMPERATURE (°C)

TIME FROM INJECTION (hours)
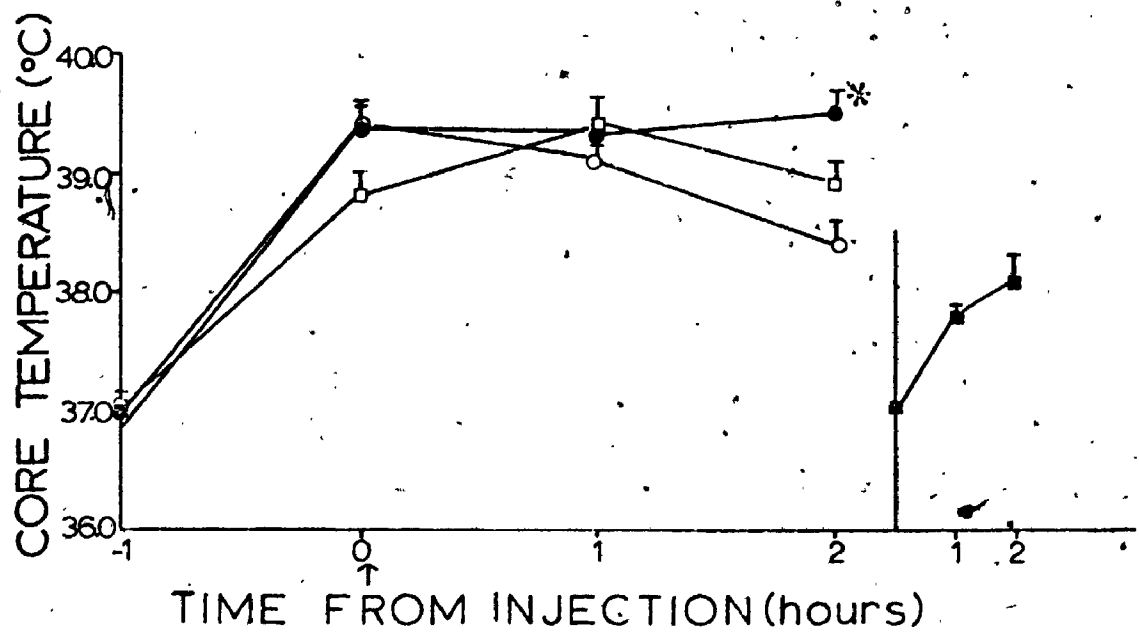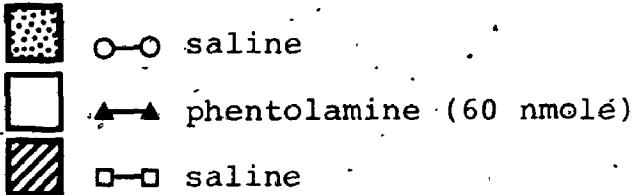
FIGURE 23

Mean cumulative food intake and core temperature of rats (n = 7) over 2 hours following administration of morphine (5.3 nmole) preceded on consecutive drug administration trial days by:

○—○ saline

▲—▲ phentolamine (60 nmole)

and   □—□ saline

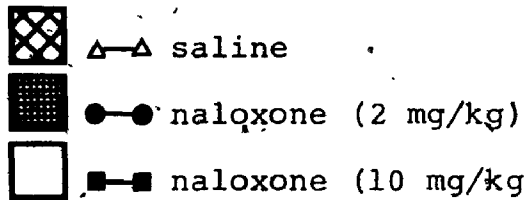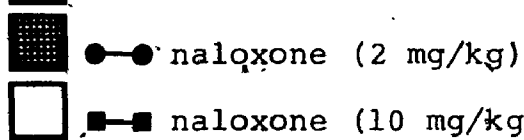All injections were made into the ventromedial hypothalamus. Vertical lines represent S.E.M. Significant differences (p < 0.01) from the initial saline + morphine trial are indicated as * .
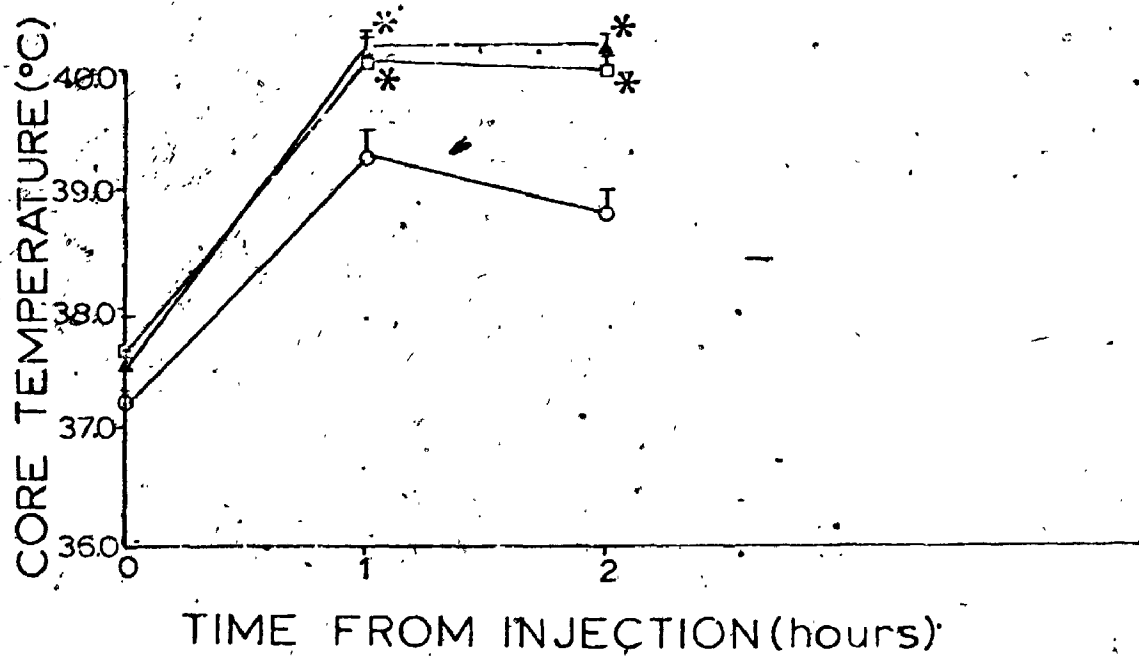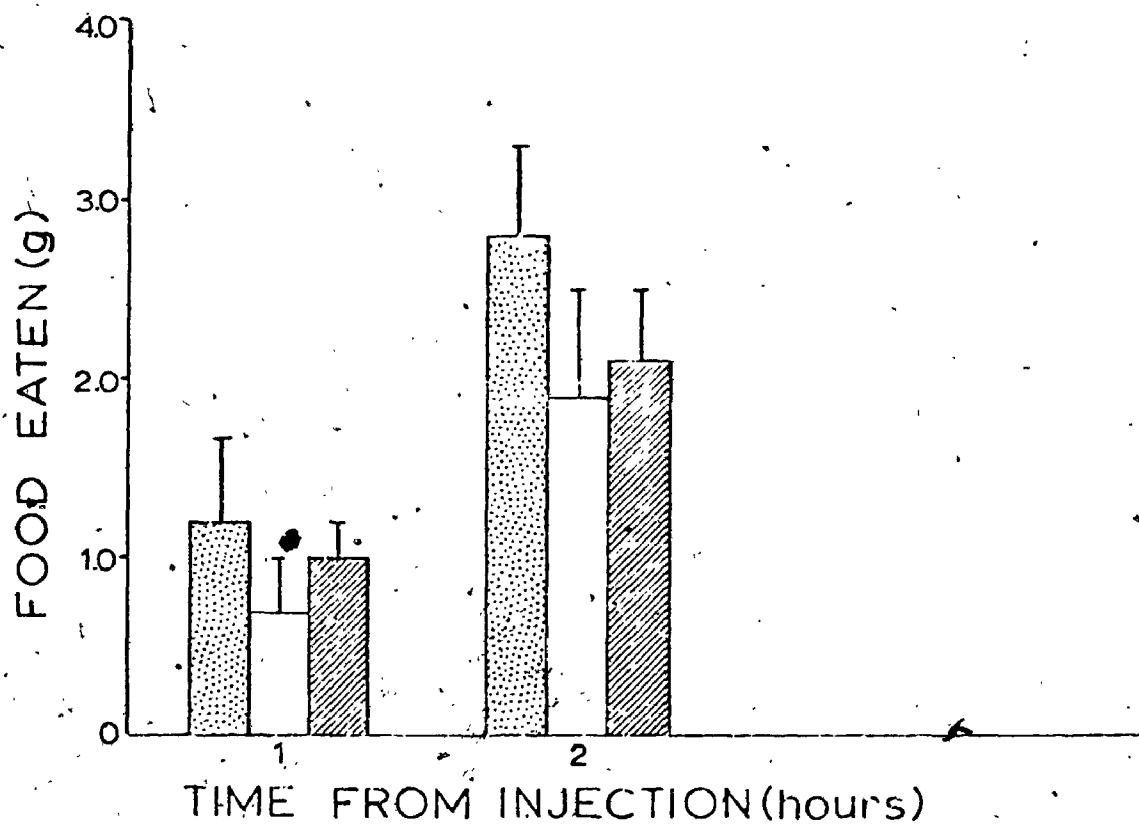
after injection and continued during the course of the experiment. One-hour food intake due to, the dual injection of the opiate and the catecholamine was equivalent to that induced by noradrenaline alone and greater than that induced by morphine alone; two-hour and total intake were both greater than that induced by either morphine or noradrenaline given alone. When the quantity of food eaten following the independent injections of morphine and noradrenaline were summed, the total proved to be equivalent at each hourly measurement to the consumption following the sequential injection of morphine and noradrenaline. Injection of saline alone elicited a three-hour total food intake of 0.3 ± 0.1 g.

Morphine, when injected alone or when given in combination with noradrenaline, produced the same degree of hyperthermia, which was significantly higher at one, two and three hours after injection than the temperature following noradrenaline injection. Following saline injection, temperatures remained stable (initial - 36.9 ± 0.1° C; one hour - 36.9 ± 0.1° C; two hour - 36.9 ± 0.1° C; three hour - 37.0 ± 0.1° C).

4.1.8.4. Effects of Phentolamine Hydrochloride on DADLE

Phentolamine significantly reduced enkephalin-induced

FIGURE 24

Mean cumulative food intake and core temperature of rats (n = 6) over 3 hours following administration into the ventromedial hypothalamus of:

■ ●—● noradrenaline (30 nmole)

■ ▲—▲ morphine (5.3 nmole)

▨ □—□ morphine (5.3 nmole) + noradrenaline (30 nmole)

▨ represents the sum of the food intake following the separate noradrenaline and morphine injections.

Vertical lines represent S.E.M. Significant differences ($p < 0.05$) from noradrenaline are indicated as ✱ , from morphine + noradrenaline as ● , and from the sum of noradrenaline + morphine as ◆ .
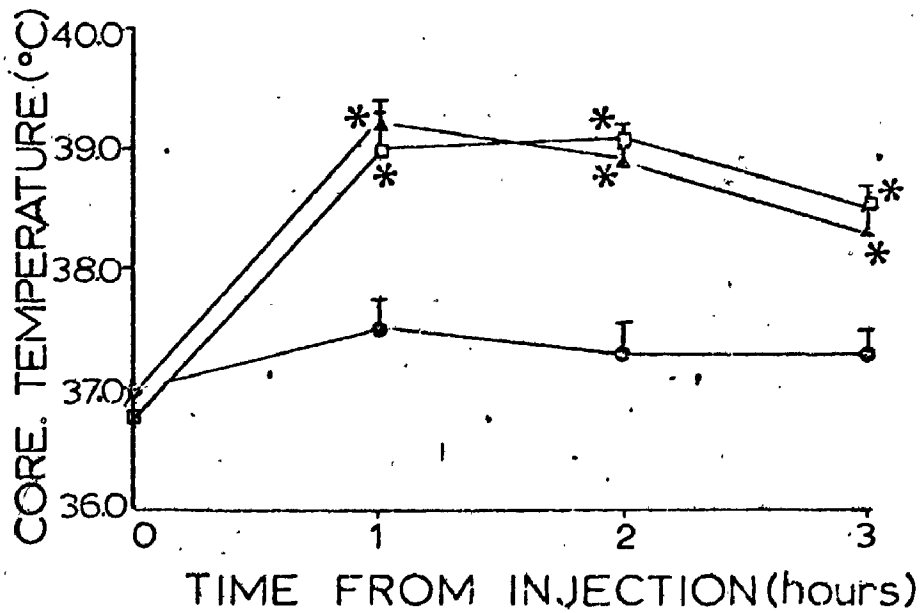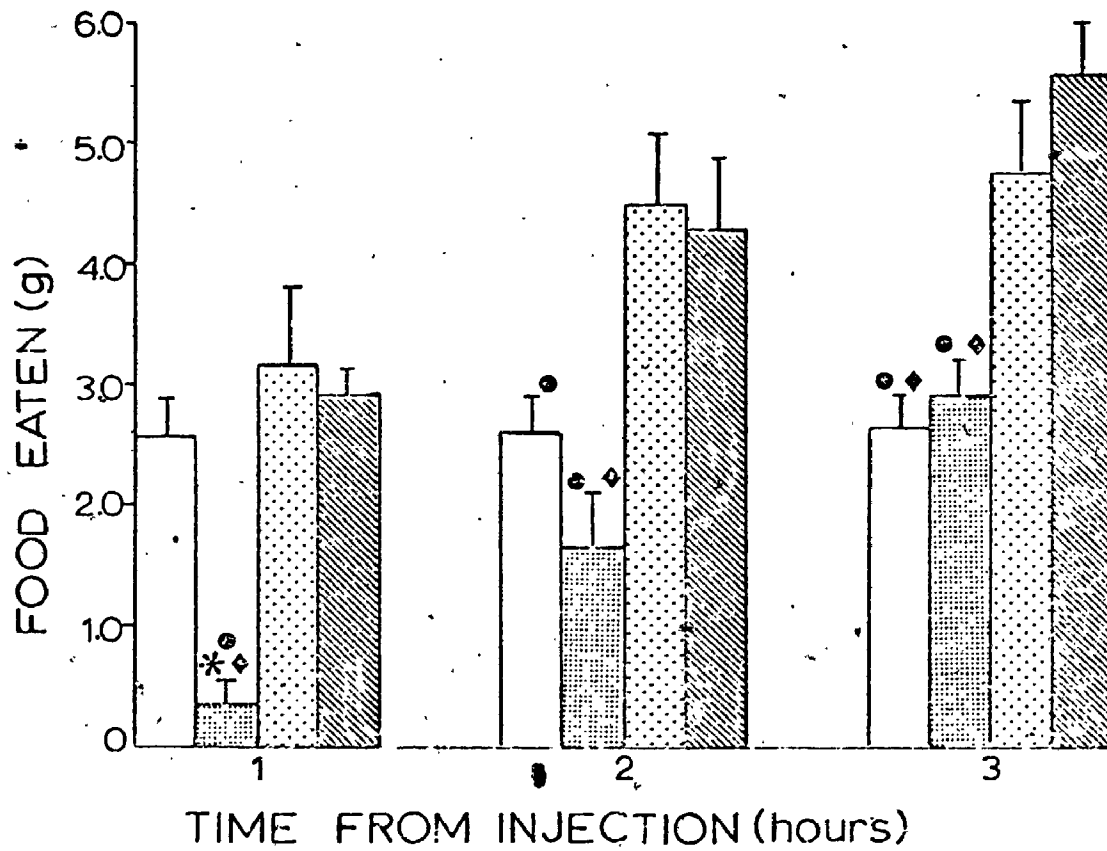
feeding from that of the saline plus enkephalin controls which preceded and followed it (Fig. 25). There was no significant difference in amount of food eaten between these controls, although on the second control treatment day, a few animals ate a small amount during the second test hour. When saline alone was injected, the three-hour intake was 0.4 ± 0.2 g.

Hyperthermia was observed one hour after the initial saline plus DADLE injection, but not at later times. When phentolamine accompanied DADLE, hyperthermia persisted throughout the duration of the experiment. Following the final saline plus DADLE injections, temperatures remained elevated after the one-hour measurement; the two- and three-hour temperatures recorded in the final phase of the experiment were significantly higher than those observed after the first saline plus DADLE injections. Following injection of saline alone, temperatures ranged from an initial $36.9 \pm 0.1^\circ$ C, to $37.1 \pm 0.1^\circ$ C, measured three hours later.

4.1.8.5. Effects of Prazosin Hydrochloride on Morphine Sulphate and Noradrenaline Hydrochloride

Prazosin (0.26 pmole) did not alter feeding or core temperature following morphine injection (Fig. 26). Two-

FIGURE 25

Mean cumulative food intake and core temperature of rats (n = 9) over 3 hours following administration of D-Ala^2,D-Leu^5-enkephalin (5.3 nmole), preceded on consecutive drug administration trial days by:

▽—▽ saline

■—■ phentolamine (60 nmole)

and   □—□ saline

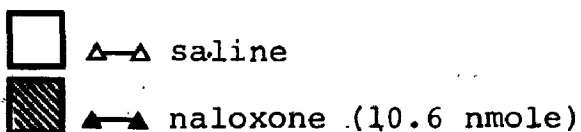All injections were made into the ventromedial hypothalamus. Vertical lines represent S.E.M. Significant differences (p < 0.05) from the initial saline + DADLE trial are indicated as * , and from the later saline + DADLE trial as ● .
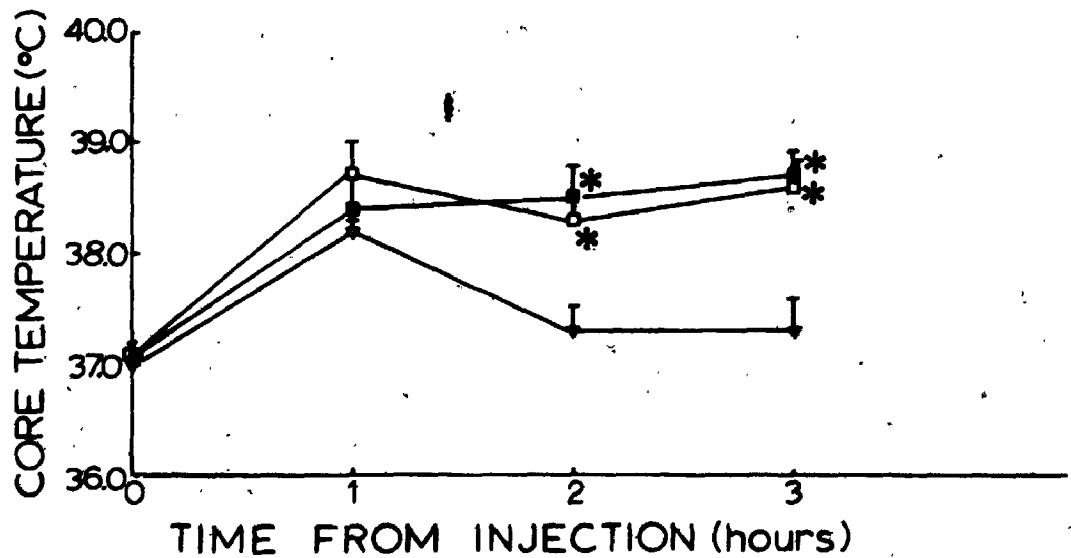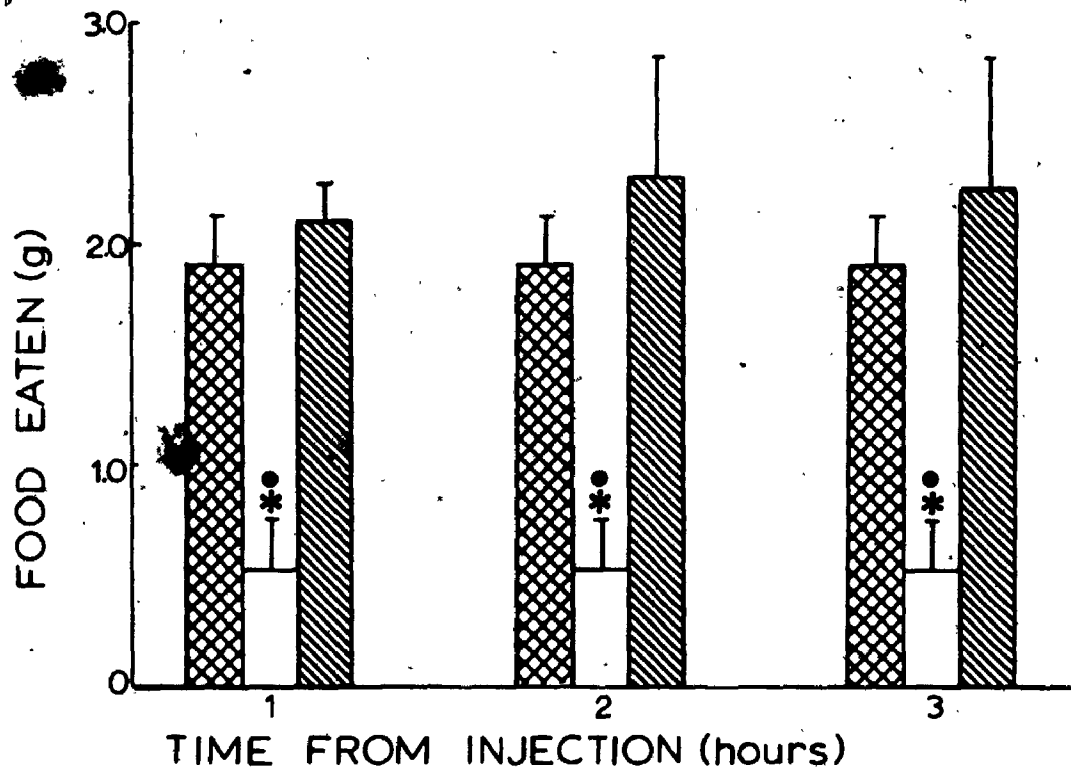
hour saline feeding was 0.6 ± 0.3 g, with temperature ranging from the initial 37.1 ± 0.1° C, to 37.1 ± 0.3° C. As shown, cumulative feeding following prazosin alone was small (0.7 ± 0.2 g) and the accompanying temperature was slightly elevated.

The ability of the same dose of prazosin to decrease noradrenaline (30 nmole) feeding was tested. Prazosin was unable to alter feeding following noradrenaline (Fig. 2-7). However, on the second occasion prazosin was given with saline and when prazosin plus noradrenaline were given the core temperature two hours after treatment was significantly higher than that measured two hours after the initial saline plus noradrenaline treatment. When saline was injected alone, feeding reached 0.3 ± 0.2 g in two hours, and temperatures remained quite stable (initial temperature - 37.3 ± 0.1° C, one hour - 37.1 ± 0.2° C, two hours - 36.9 ± 0.1° C).

4.1.8.6. Effect of Yohimbine Hydrochloride on Morphine Sulphate

Yohimbine (9.1 nmole) caused a significant increase in feeding when injected one hour after morphine (5.3 nmole). The effect was most evident during the first hour after injection of the antagonist, when food intake was more than double that after the two morphine plus saline controls

FIGURE 26

Mean cumulative food intake and core temperature
of rats (n = 8). Administration of morphine was
followed after 1 hour, on consecutive drug trial
days by:

       ▨ △—△ saline

       ☐ ●—● prazosin (0.26 pmole)
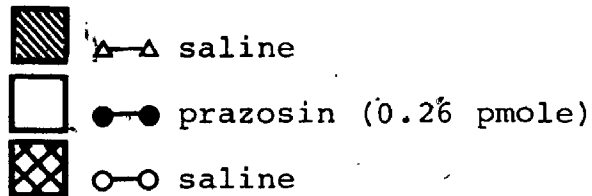
       ▧ ○—○ saline

Amount of food eaten was measured following the
saline or prazosin injection. Recording of temperature
was begun just before morphine injection, with the
arrow on the abscissa denoting administration of
saline or prazosin. Food intake and core temperature
following prazosin alone (0.26 pmole) are indicated
by ▦ and ■—■ . All injections were made into
the ventromedial hypothalamus. Vertical lines
represent S.E.M.
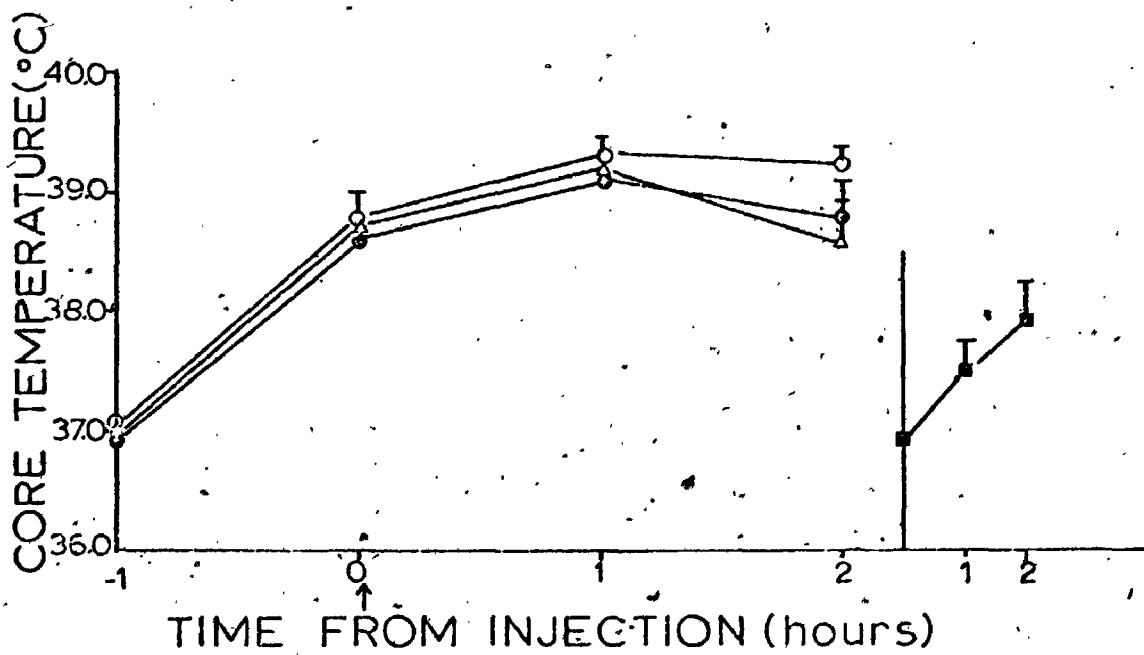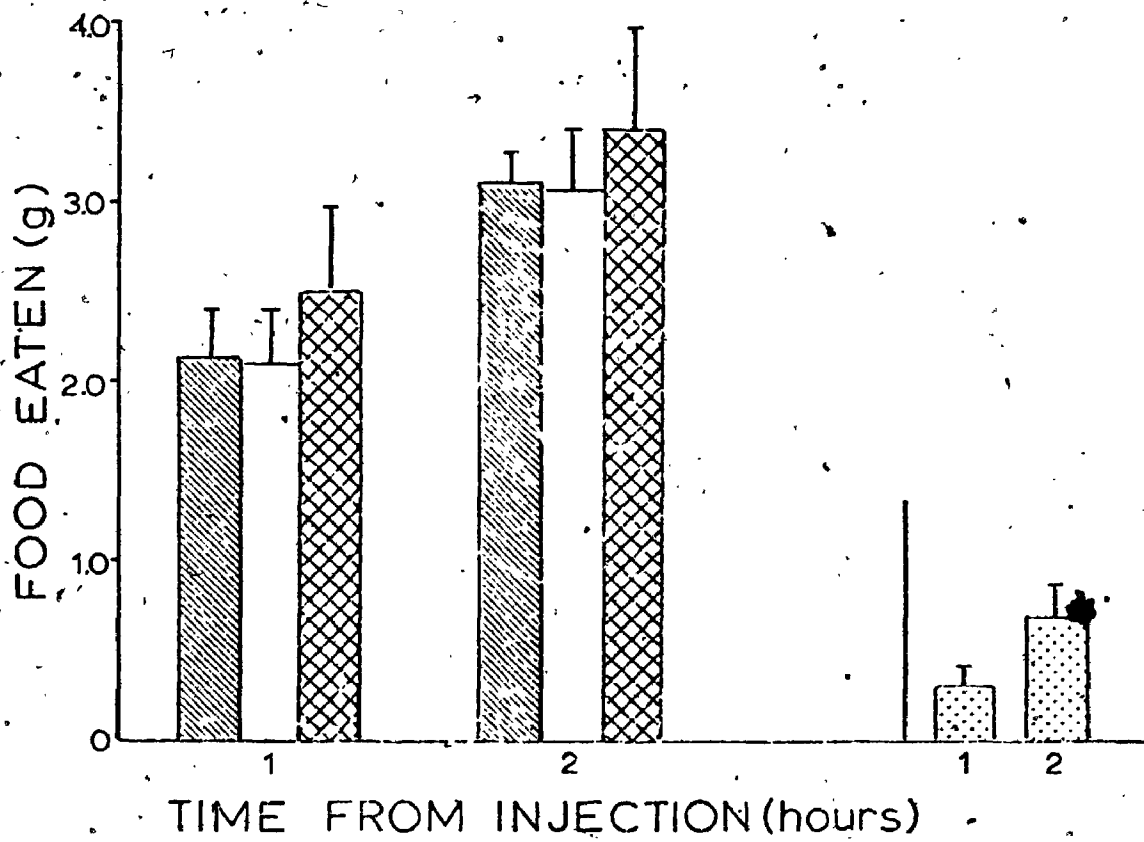
FIGURE 27

Mean cumulative food intake and core temperature of rats (n = 6) over 2 hours following administration of noradrenaline (30 nmole), preceded on consecutive drug administration trial days by:

△—△ saline

●—● prazosin (0.26 pmole)
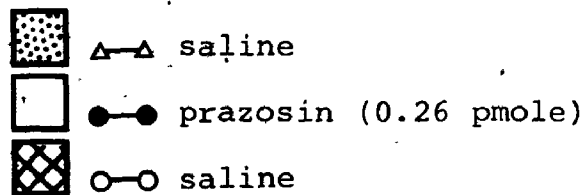
○—○ saline

Food intake and core temperature following prazosin alone (0.26 pmole) are indicated by [▨] and ■—■ . All injections were made into the ventromedial hypothalamus. Vertical lines represent S.E.M. Significant differences from the initial saline + prazosin trial are indicated as

FOOD EATEN(g)

TIME FROM INJECTION(hours)

CORE TEMPERATURE(°C)

TIME FROM INJECTION(hours)
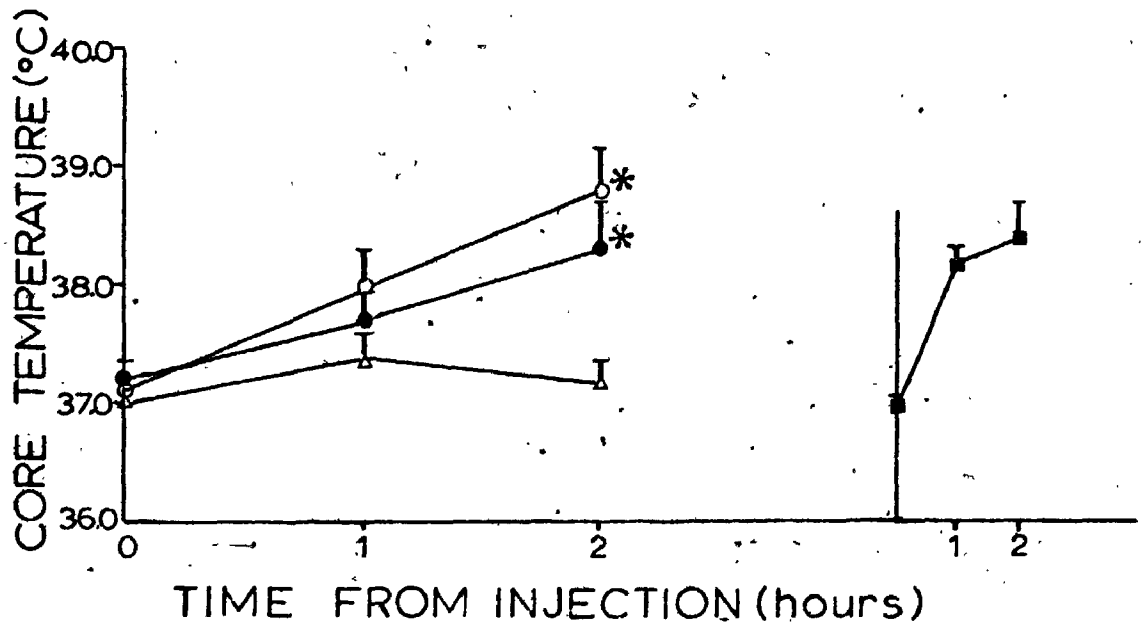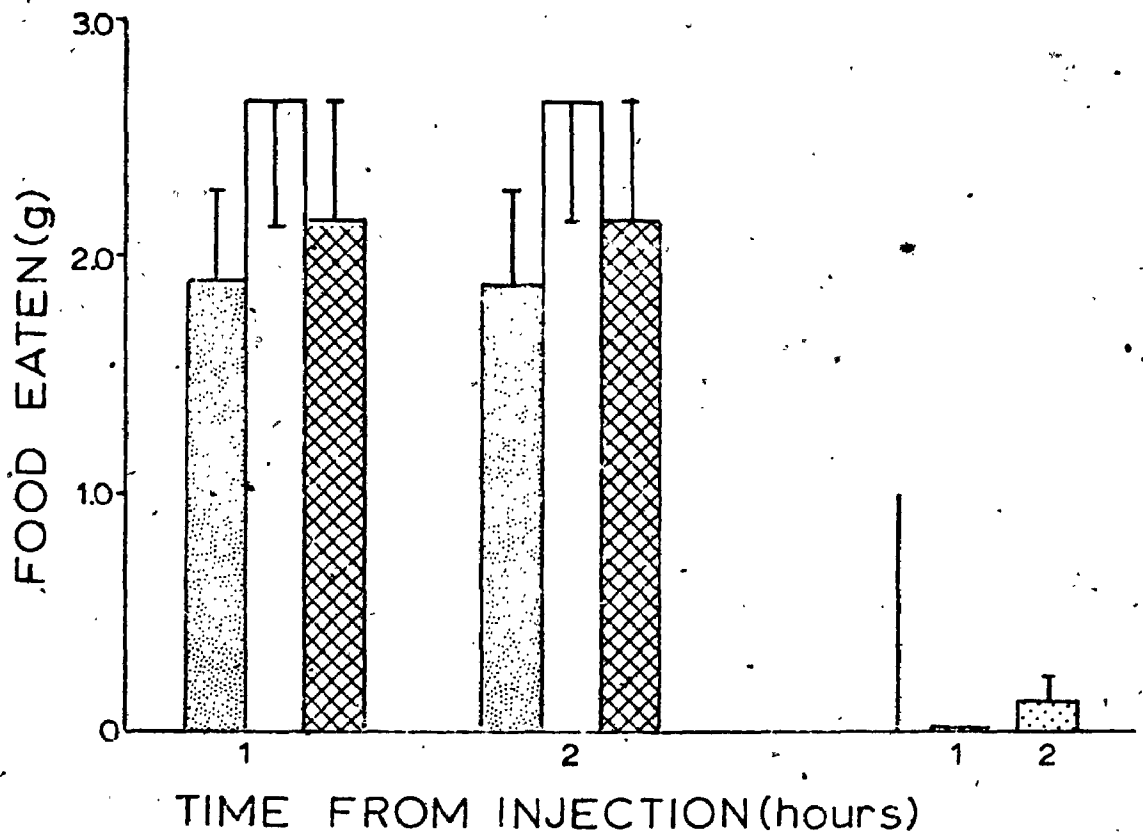
(Fig. 28). The animals' cumulative two-hour food intake
following administration of morphine plus yohimbine only
exceeded the food intake following the second morphine
plus saline control. Little feeding followed injection
of yohimbine alone (0.3 ± 0.3 g), and cumulative feeding
following saline administration in these animals was only
0.2 ± 0.2 g.
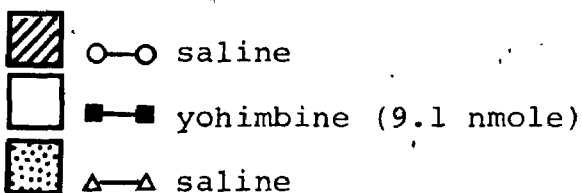
There were no differences in core temperatures among
the three groups. When yohimbine was injected on its own,
temperatures ranged from 37.1 ± 0.1° C, to 38.0 ± 0.4°C,
and following saline administration temperatures ranged
from 37.0 ± 0.1°C, initially to 36.9 ± 0.1° C.

4.1.8.7. Effects of Propranolol Hydrochloride, 5-HT, Methysergide Bimaleate, Dopamine Hydrochloride, Haloperidol and Apomorphine Hydrochloride

Most of the monoamine agonists and antagonists tested
had little effect on feeding activity. In contrast to the
hypophagic effect of the α-adrenergic antagonist phento-
lamine, the β-adrenergic antagonist propanolol was
ineffective (Fig. 29). Neither 5-HT nor its antagonist,
methysergide, significantly affected morphine-induced
feeding, nor induced feeding by themselves (Fig. 30 and 31).
Again, neither the dopamine-receptor agonist apomorphine
nor its antagonist haloperidol had any significant effect
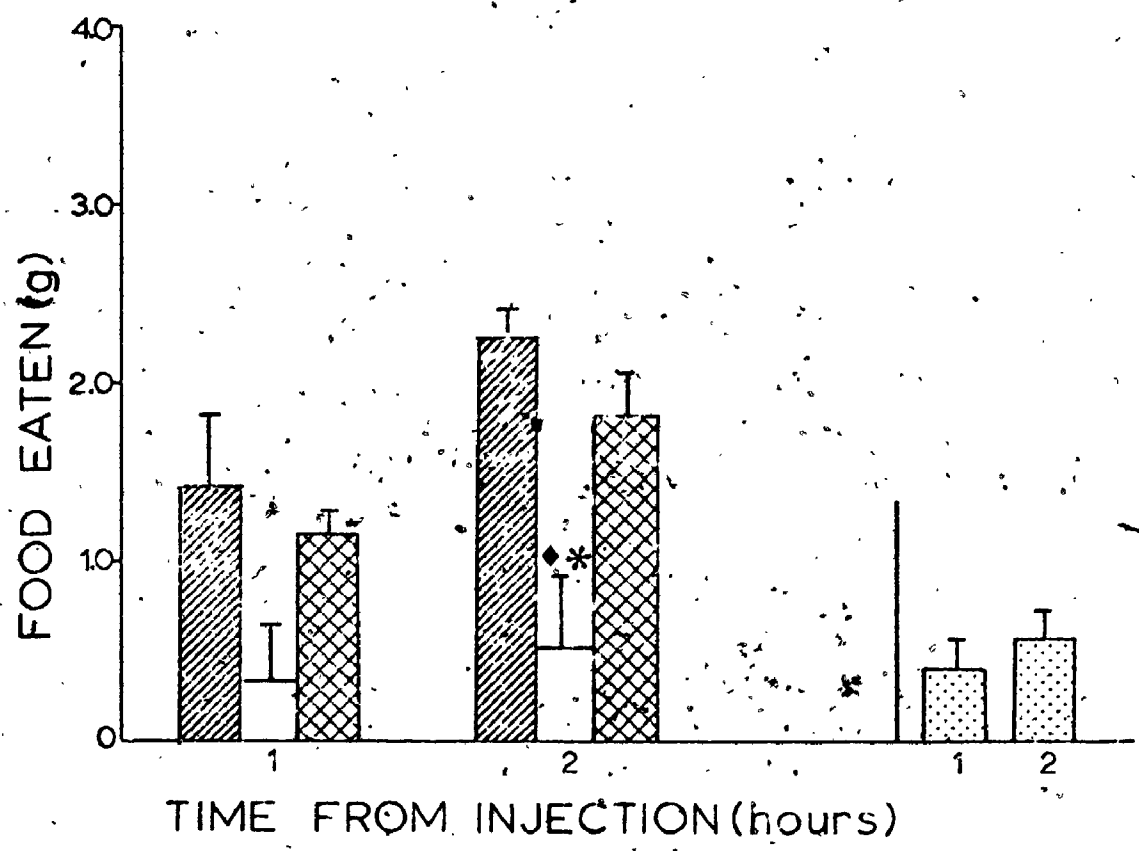
FIGURE 28

Mean cumulative food intake and core temperature
of rats (n = 6). Administration of morphine was
followed after 1 hour, on consecutive drug trial
days by:

⬜ (hatched) O—O saline

⬜ (blank) ■—■ yohimbine (9.1 nmole)

and ⬜ (dotted) △—△ saline

Amount of food eaten was measured following the
saline or yohimbine injection. Recording of temper-
ature was begun just before morphine injection, with
the arrow on the abscissa denoting administration
of saline or yohimbine. Food intake and core
temperature following yohimbine alone (9.1 nmole)
are indicated by ⬜ (dotted) and ▲—▲ . All injections
were made into the ventromedial hypothalamus. Vertical
lines represent S.E.M. Significant differences
($p < 0.05$) from the initial morphine + saline trial
are indicated as * and from the later morphine +
saline trial as ◆ .
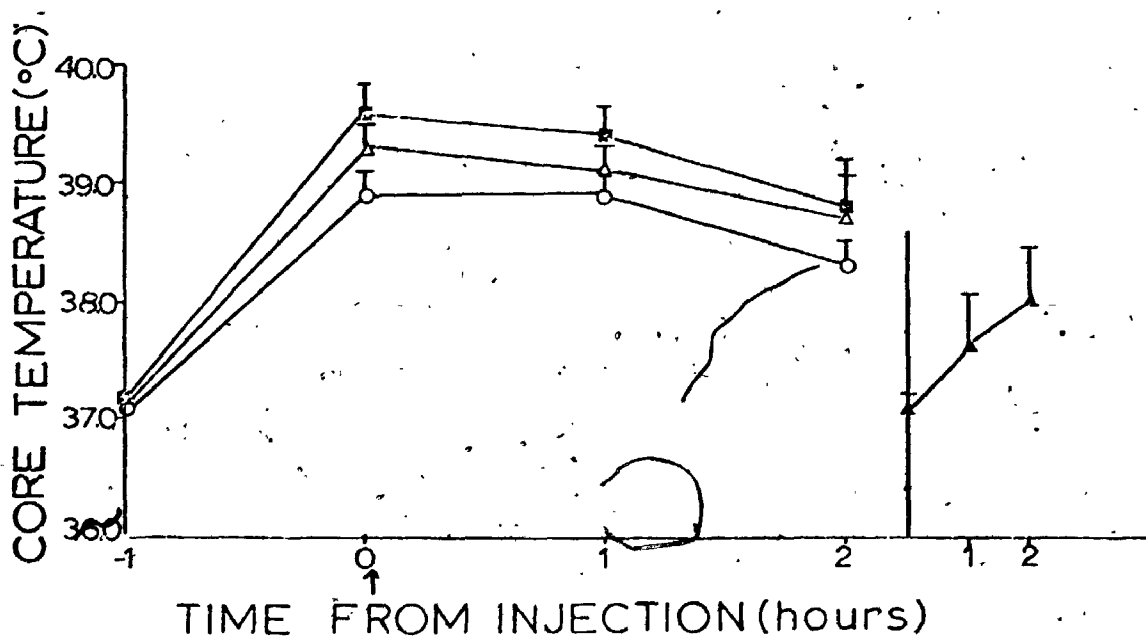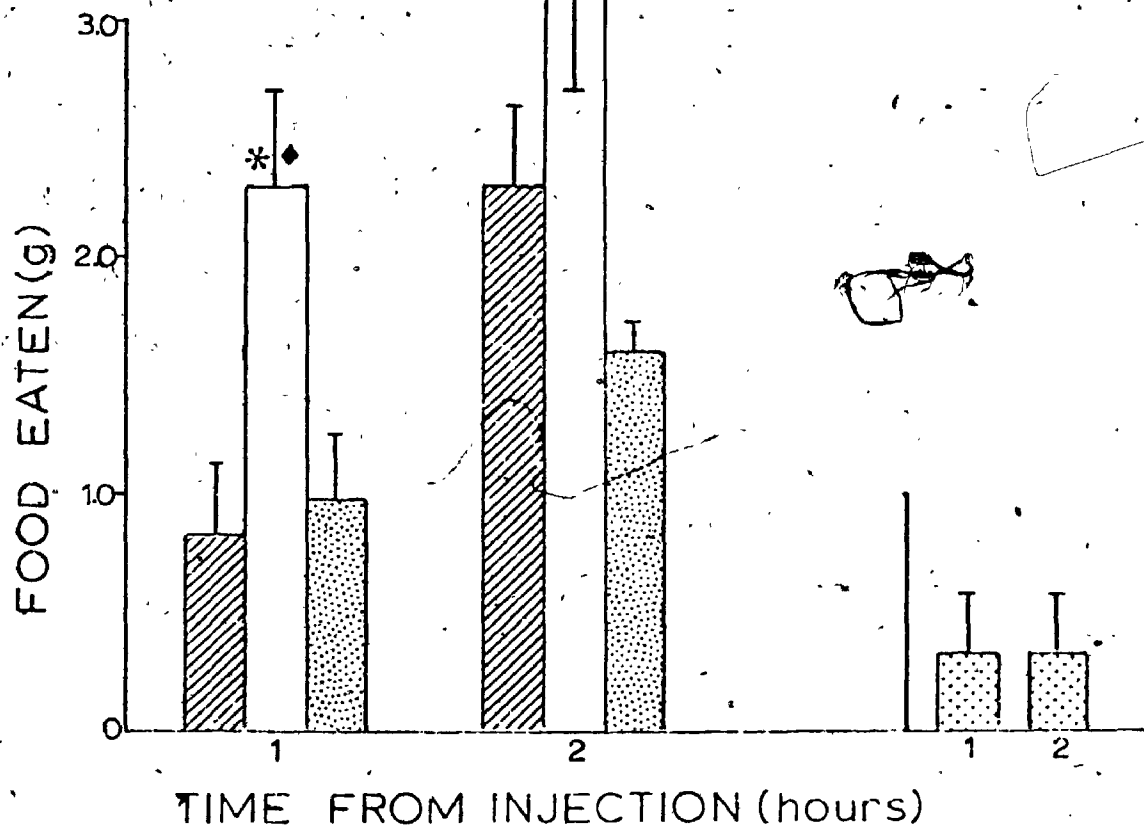
on spontaneous feeding or that induced by morphine (Fig.
32 and 33). However, dopamine itself tended to increase
feeding (p < 0.10) during the hour following injection,
and significantly elevated the two-hour food intake
($\blacklozenge$ < 0.05, Fig. 34). In each study, the initial saline
plus morphine feeding was no different from that after
saline plus morphine treatment following the drug trial.
As stated, the amount of food ingested in the two hours
following injection of the test drugs by themselves (Fig.
29 to 34) was small and resembled the low quantities
ingested following saline control injections.

Most of these monoamine agonists and antagonists
injected after morphine did not influence the morphine-
provoked hyperthermia (Fig. 29 to 34). However, one hour
after propranolol injection (Fig. 29) the core temperature
was significantly higher than at the same interval
following saline. Also, two hours after injection of
apomorphine, temperatures were significantly lower than
after the saline controls (Fig. 32).

4.1.9. Effects on Feeding and Temperature of Morphine
        Sulphate Applied to Various Brain Sites

The brain sites to which morphine or saline was
administered are depicted in Fig. 35.

Morphine was generally effective when injected into
the hypothalamic sites selected, especially the more

FIGURE 29

Mean cumulative food intake and core temperature
of rats (n = 5). Administration of morphine was
followed after 1 hour, on consecutive drug trial
days, by:

        ⊠ △—△ saline

        ☐ ●—● propranolol (60 nmole)

and   ▦ □—□ saline

Amount of food eaten was measured following the
saline or propranolol injection. Recording of tem-
perature was begun just before morphine injection,
with the arrow on the abscissa denoting administration
of saline or propranolol. Food intake and core
temperature following propranolol alone (60 nmole)
are indicated by ▨ and ■—■ . All injections
were made into the ventromedial hypothalamus. Vertical
lines represent S.E.M. Significant differences
($p < 0.05$) from morphine + propranolol are indicated
as * .
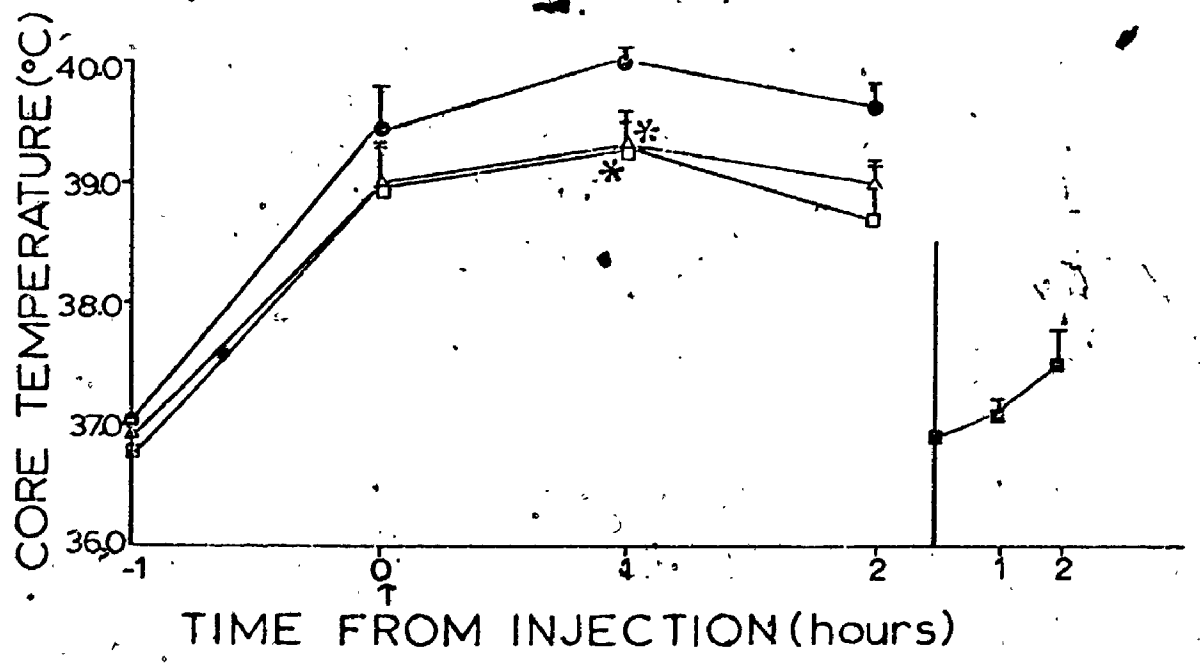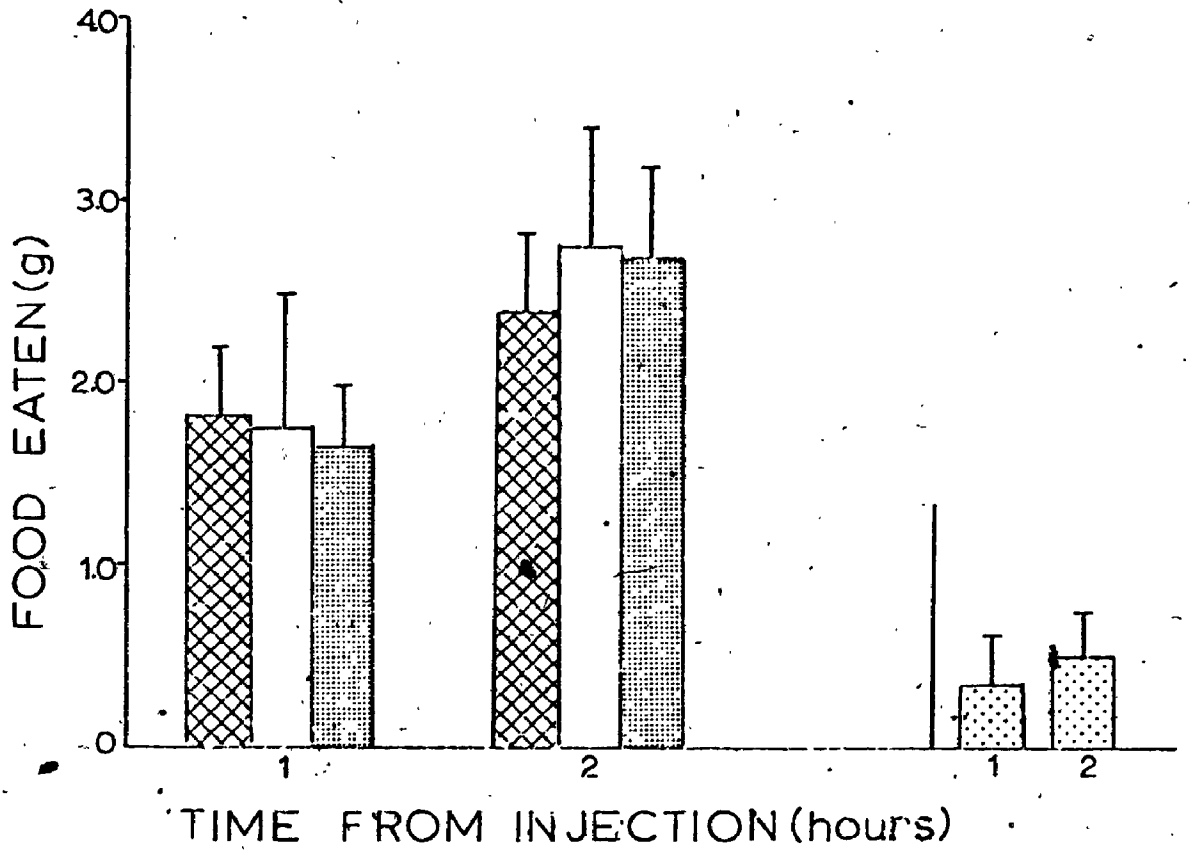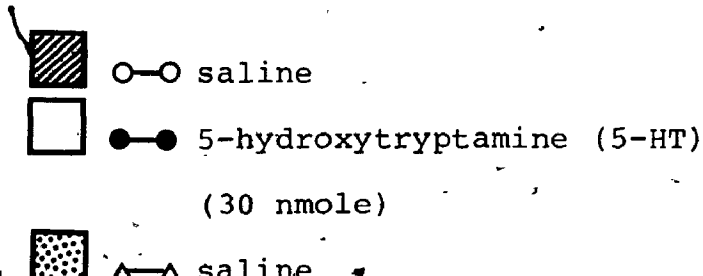
FIGURE 30

Mean cumulative food intake and core temperature
of rats (n = 5). Administration of morphine was
followed after 1 hour, on consecutive drug trial
days, by:

� O—O saline

□ ●—● 5-hydroxytryptamine (5-HT)

(30 nmole)

and ▦ △—△ saline

Amount of food eaten was measured following the
saline or 5-HT injection. Recording of temperature
was begun just before morphine injection, with the
arrow on the abscissa denoting administration of
saline or 5-HT. Food intake and core temperature
following 5-HT alone (30 nmole) are indicated by ▦
and ■—■. All injections were made into the ventro-
medial hypothalamus. Vertical lines represent S.E.M.
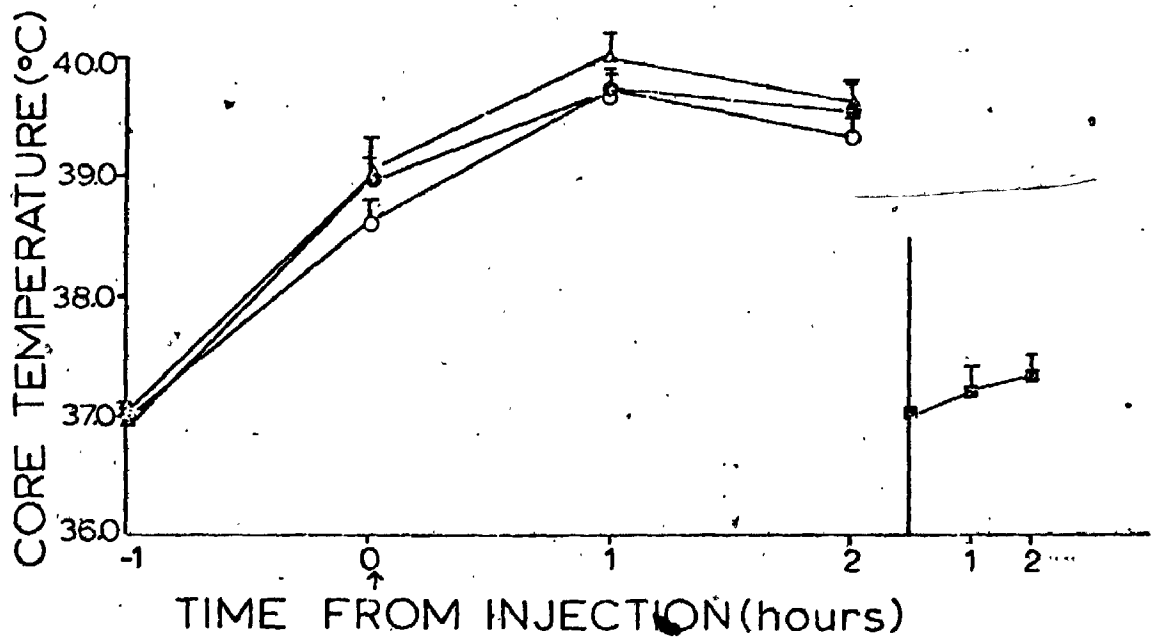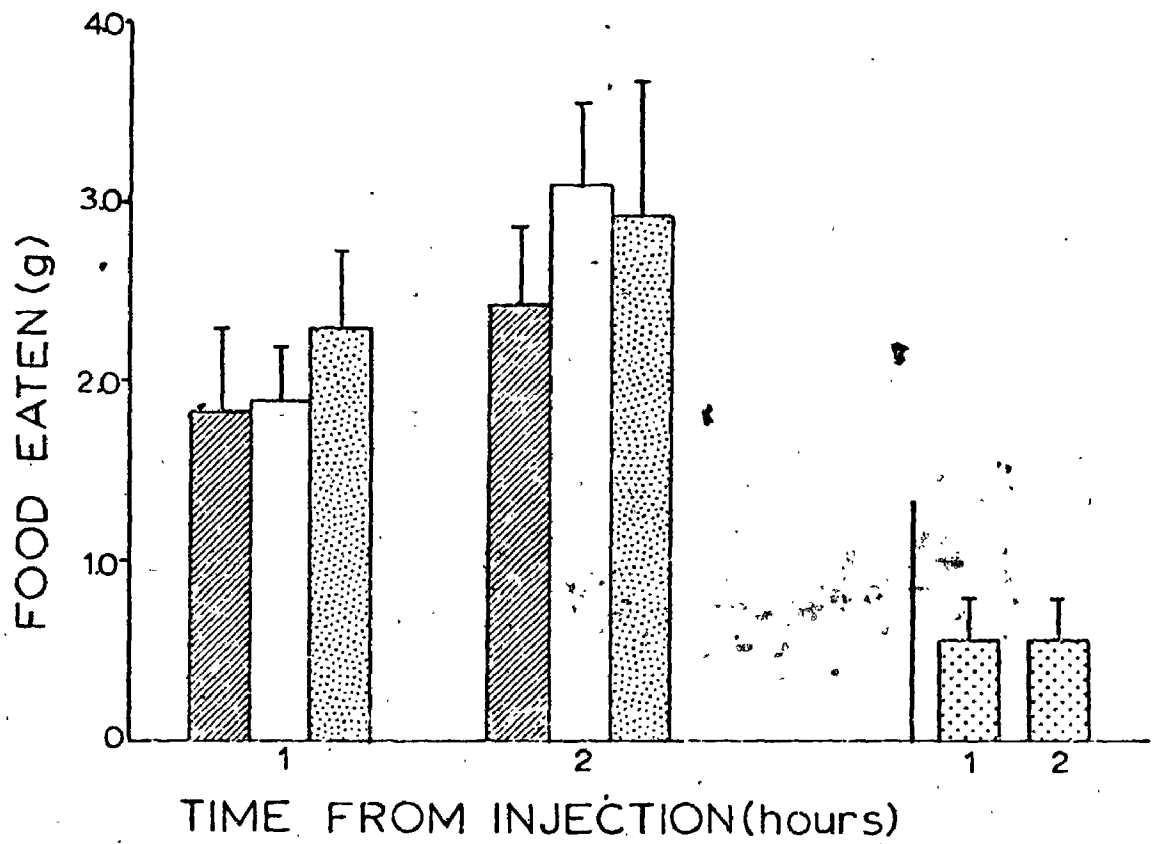
FIGURE 31

Mean cumulative food intake and core temperature
of rats (n = 5). Administration of morphine was
followed after 1 hour, on consecutive drug trial
days, by:

              ▨ O—O saline

            □ ▲—▲ methysergide (28.3 nmole)

  and ⊠ □—□ saline

Amount of food eaten was measured following
the saline or methysergide injection. Recording of
temperature.was begun just before morphine injection,
with the arrow on the abscissa denoting administration
of saline or methysergide. Food intake and core
temperature following methysergide alone (28.3 nmole)
are indicated by ▣ and ■—■ . All injections were
made into the ventromedial hypothalamus. Vertical
lines represent S.E.M.

TIME FROM INJECTION (hours)

TIME FROM INJECTION (hours)
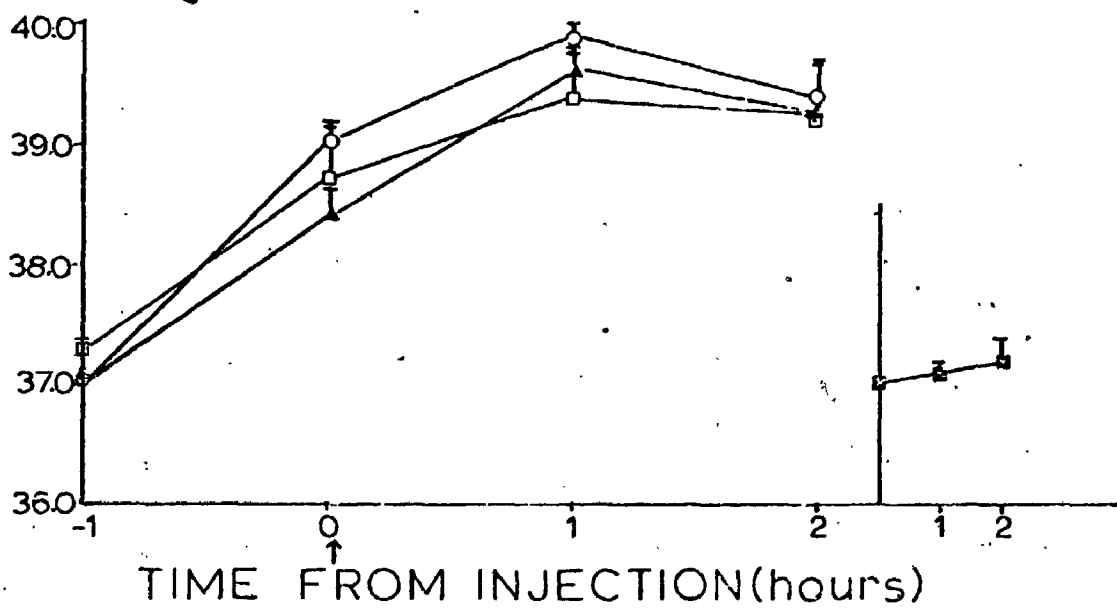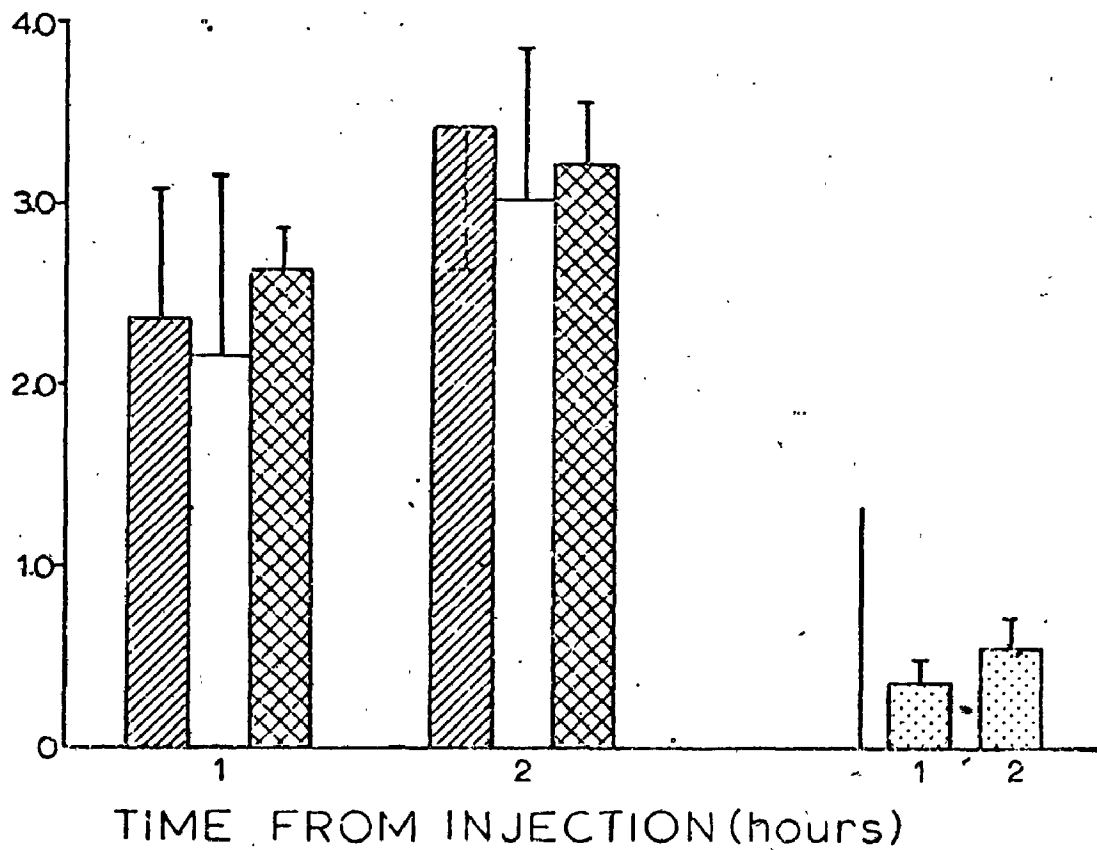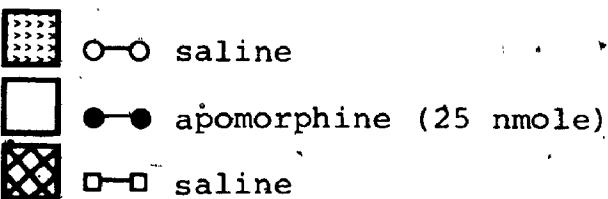
FIGURE 32

Mean cumulative food intake and core temperature
of rats (n = 7). Administration of morphine was
followed after 1 hour, on consecutive drug trial
days, by:

▦ O—O saline

▢ ●—● apomorphine (25 nmole)

and ▨ □—□ saline

Amount of food eaten was measured following the
saline or apomorphine injection. Recording of temper-
ature was begun just before morphine injection, with
the arrow on the abscissa denoting administration of
saline or apomorphine. Food intake and core temperature
following apomorphine alone (25 nmole) are indicated
by ▨ and ■—■ . All injections were made into the
ventromedial hypothalamus. Vertical lines represent
S.E.M. Significant differences ($p < 0.05$) from
morphine + saline are indicated as ◆ .
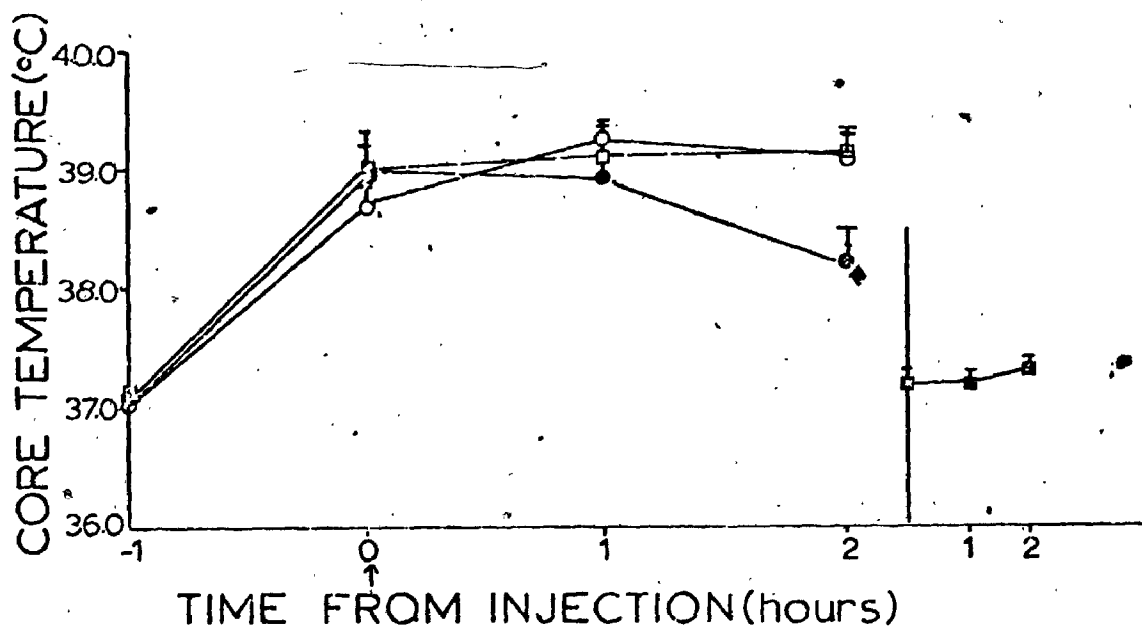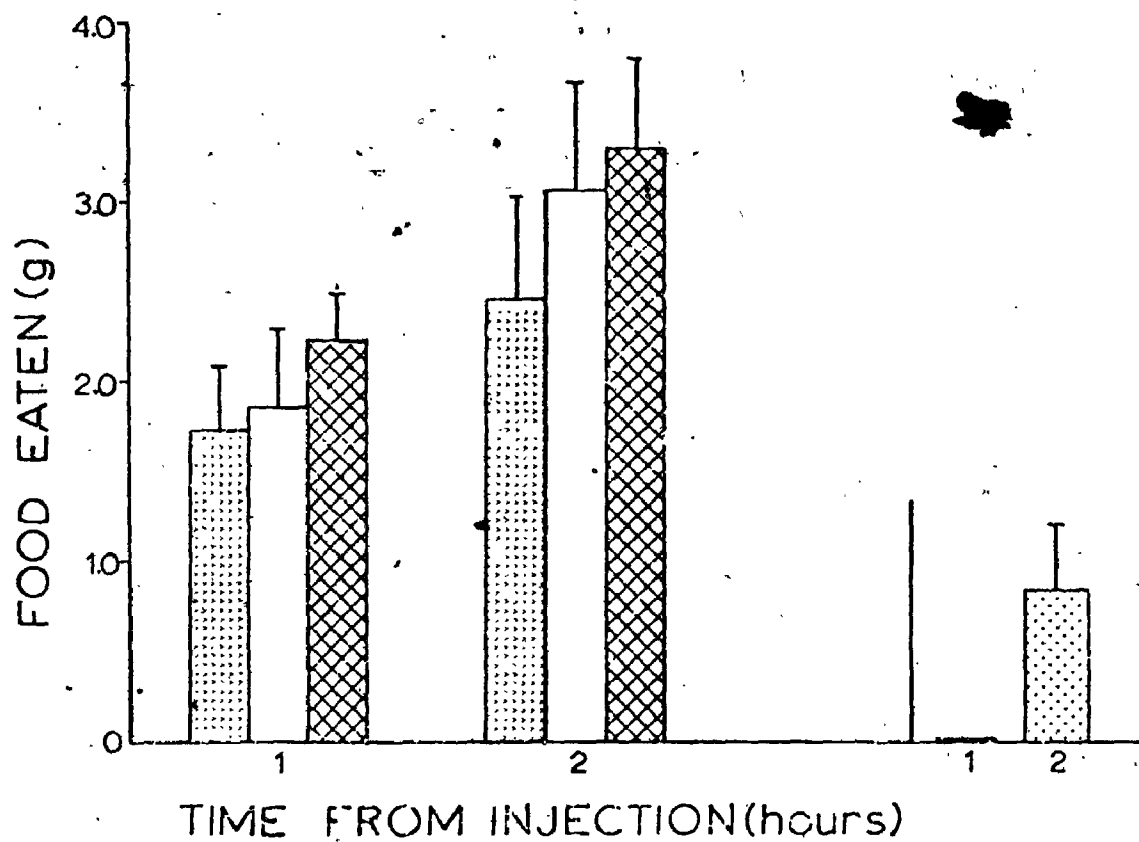
FIGURE 33

Mean cumulative food intake and core temperature
of rats (n = 7). Administration of morphine was
followed after 1 hour, on consecutive drug trial
days, by:

◻ o—o saline

◻ ●—● haloperidol (8 nmole)

and ◻ △—△ saline

Amount of food eaten was measured following
the saline or haloperidol injection. Recording of
temperature was begun just before morphine injection,
with the arrow on the abscissa denoting administration
of saline or haloperidol. Food intake and core tem-
perature following haloperidol alone (8 nmole) are
indicated by ◻ and ■—■ . All injections were made
into the ventromedial hypothalamus. Vertical lines
represent S.E.M.
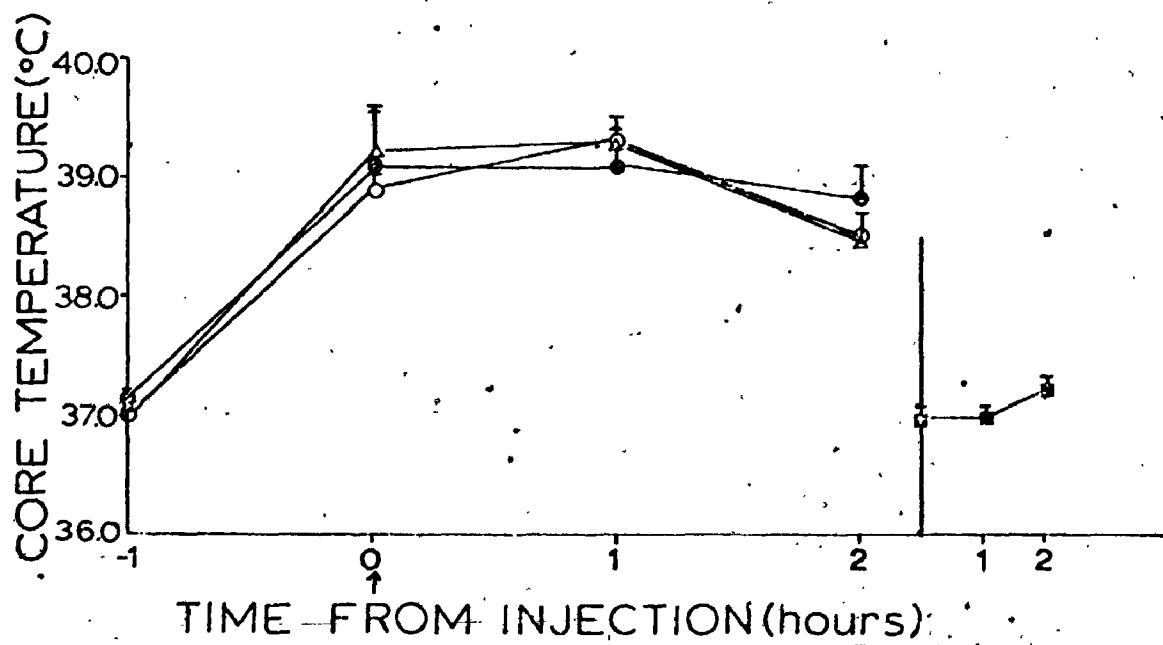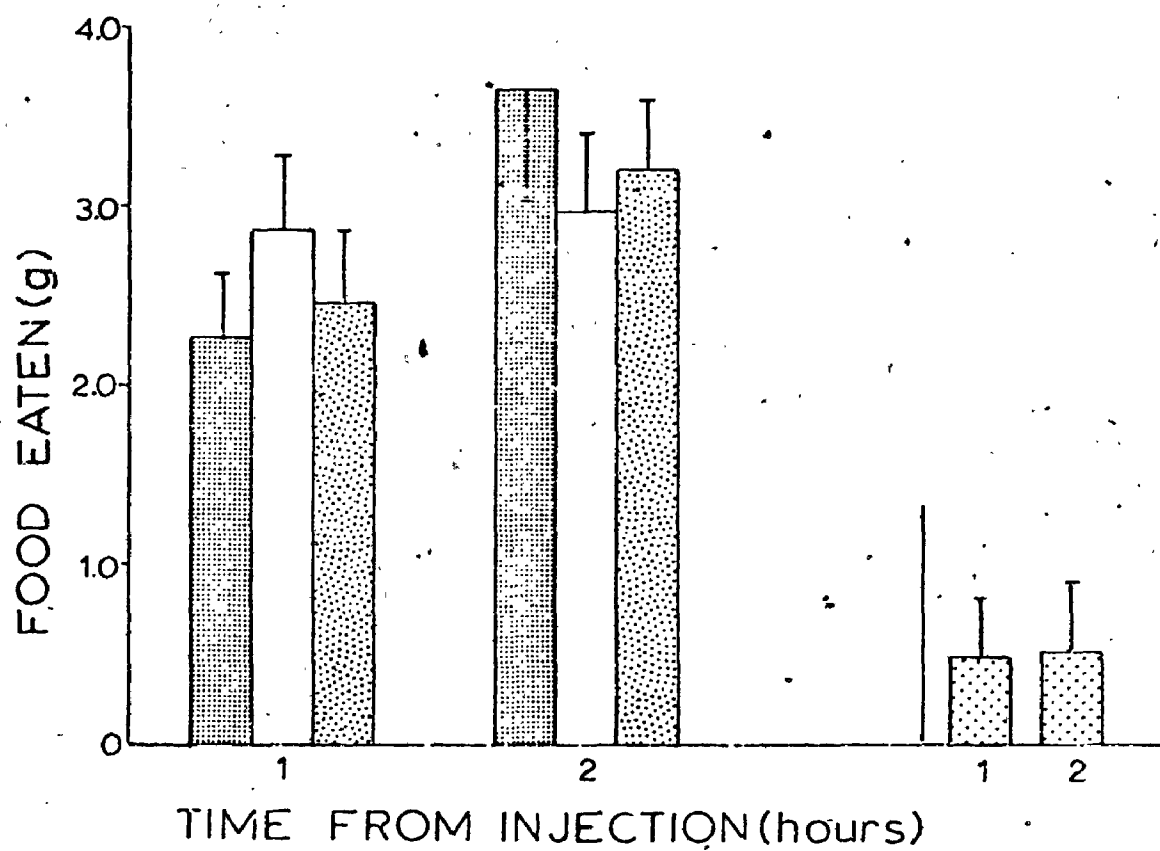
FIGURE 34

Mean cumulative food intake and core temperature of rats (n = 7). Administration of morphine was followed after 1 hour, on consecutive drug trial days, by:

O—O saline

●—● dopamine (30 nmole)

and △—△ saline

Amount of food eaten was measured following the saline or dopamine injection. Recording of temperature was begun just before morphine injection, with the arrow on the abscissa denoting administration of saline or dopamine. Food intake and core temperature following dopamine alone (30 nmole) are indicated by and ■—■ . All injections were made into the ventromedial hypothalamus. Vertical lines represent S.E.M. Significant differences ($p < 0.05$) from both morphine + saline injections are indicated as * .
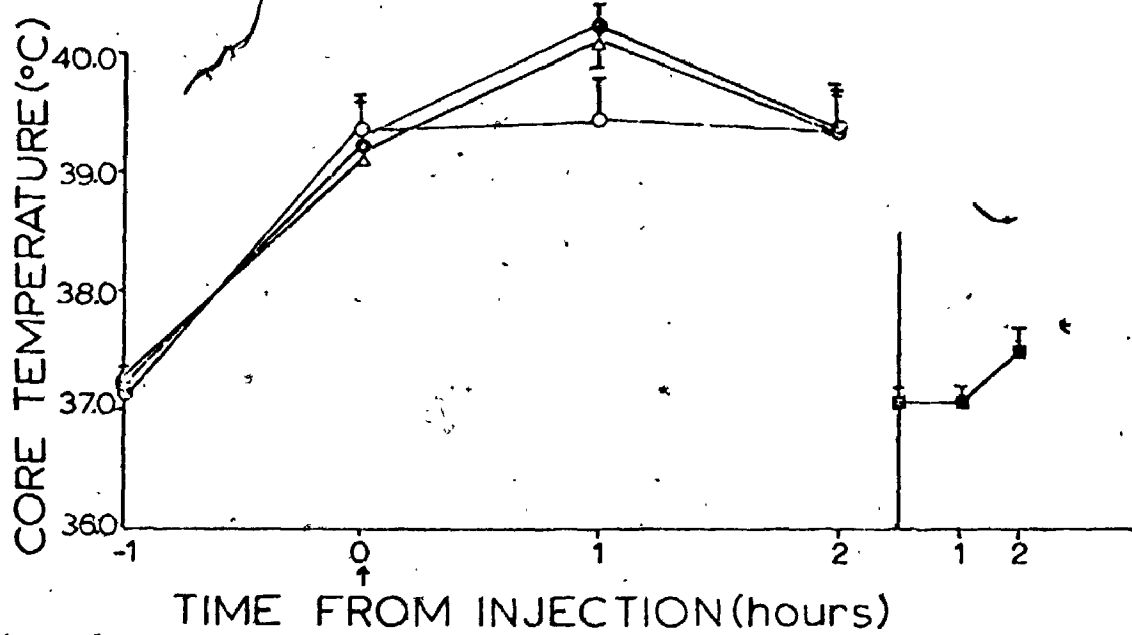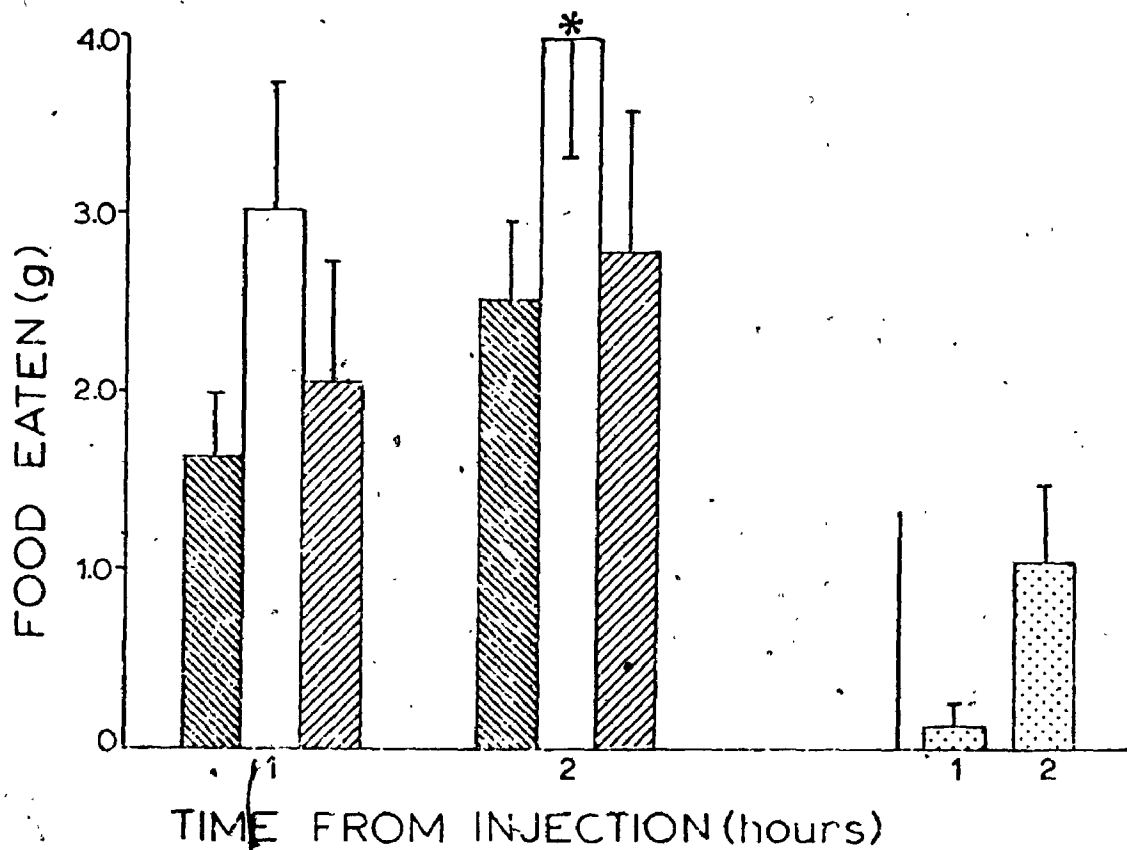
rostral loci (Fig. 36).· In the VMH, morphine produced, as previously, prolonged feeding and hyperthermia. In the paraventricular hypothalamus, a similar pattern was observed, although unlike the VMH, only total three-hour food intake following morphine injection was significantly greater than that after saline. At this site, the loco-motor depression appeared to have a longer duration (about 60 to 120 minutes rather than 30 to 70 minutes as at the VMH). Core temperature was significantly elevated one hour after injection at the VMH and paraventricular hypothala-mus, and it remained elevated for the duration of the study.

Morphine applied to the lateral hypothalamus elicited little locomotor depression. As in the paraventricular hypothalamus, only the total three-hour food intake was increased significantly over control levels. Opiate introduction into the sites within the lateral hypothala-mus was followed by rising temperatures which appeared to peak after two hours and returned to control levels by three hours. The posterior hypothalamus was the only hypothalamic site tested where morphine did not significant-ly influence food intake or core temperature (Fig. 36).

The extrahypothalamic sites to which morphine was administered afforded varying results (Fig. 36 and 37).

FIGURE 35

Diagrams of coronal sections of rat brain exhibiting
sites where morphine was injected. The plane of the
section follows the atlas of Pellegrino et al. (1979),
and the numbers give anterior-posterior positions in
relation to bregma. The letters indicate the following
sites:

    ♥   : ventromedial hypothalamus
    b,   : posterior hypothalamus
    p   : paraventricular hypothalamus
    a   : internal capsule
    u   : lateral hypothalamus 1
    h   : lateral hypothalamus 2
    x   : fornix
    y   : amygdala
    c   : caudate-putamen
    t   : thalamus

+1.2

−0.4

+1.0

−1.0

+0.8

−1.2

+0.4

−1.4

+0.2
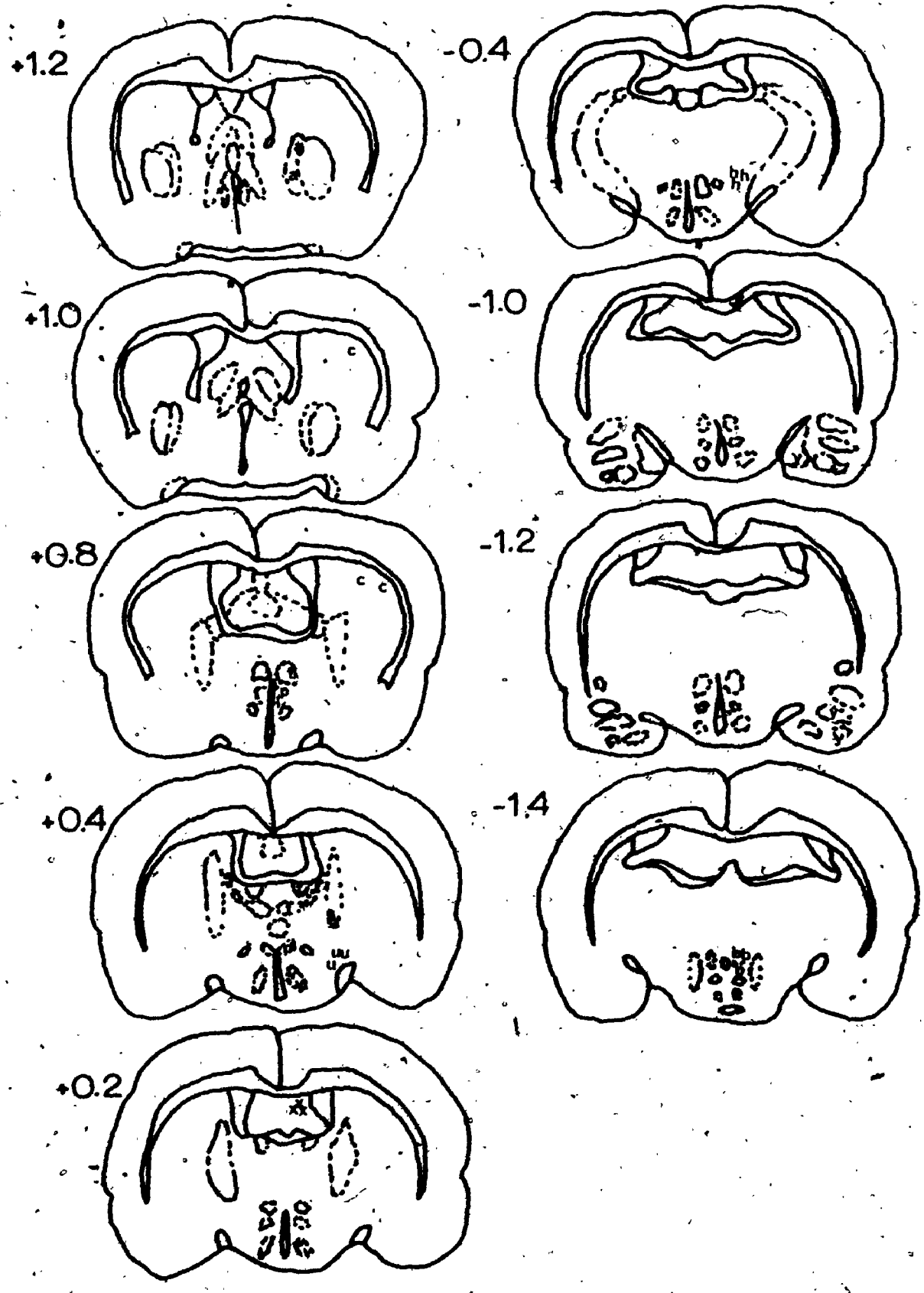
The thalamus was the only site at which an injection of morphine caused significantly more feeding over the three-hour period than a control injection of saline. This was preceded by a short interval of inactivity. Intrathalamic morphine elicited hyperthermia at one and two hours after administration, but by three hours, at the time when feeding was elevated, it was no different from the control temperature. Morphine did not enhance ingestion or produce activity levels different from saline treatment when administered to the caudate-putamen, fornix or amygdala. Administration to the internal capsule produced a trend towards increased food intake (p < 0.10 from two- and three-hour measurements), preceded by a depression in activity. Morphine elicited hyperthermia one and two hours after injection into the thalamus and at two hours after injection into the internal capsule, but had no thermogenic activity when applied to the caudate-putamen, fornix or amygdala.

FIGURE 36 and 37

Mean cumulative food intake and core temperature following injection of:

☐ o—o saline

or ●—● morphine (5.3 nmole)
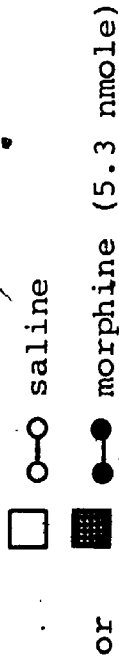
into various brain sites. Vertical lines represent S.E.M. Significant differences ($p < 0.05$) from saline are indicated as *****.

FIGURE 36

Brain sites include: ventromedial hypothalamus (VMH), paraventricular hypothalamus (PVH), lateral hypothalamus (LH1), lateral hypothalamus (LH2), posterior hypothalamus (PH), and thalamus (T).

FIGURE 37

Brain sites include: internal capsule (CI), caudate-putamen (CP), fornix (FX), and amygdala (A).
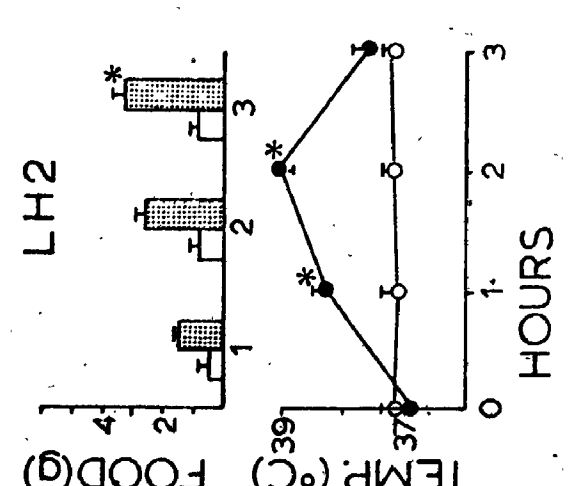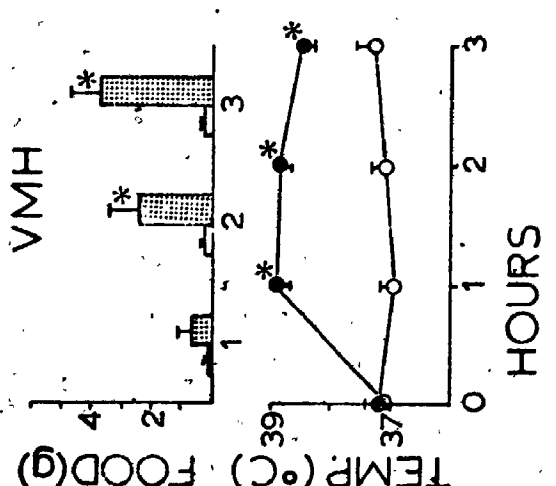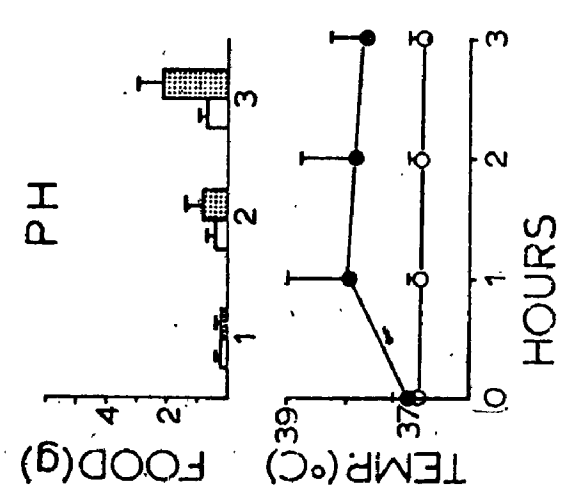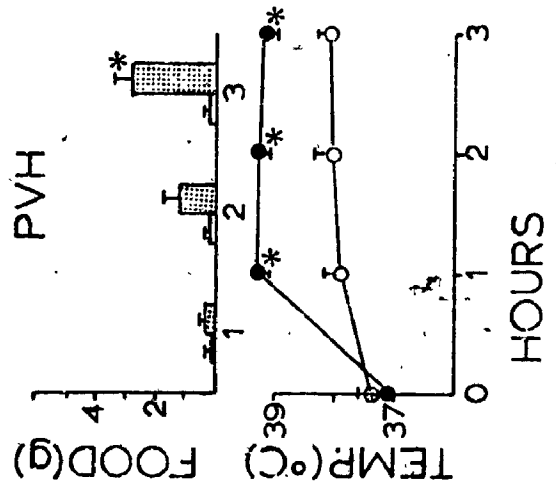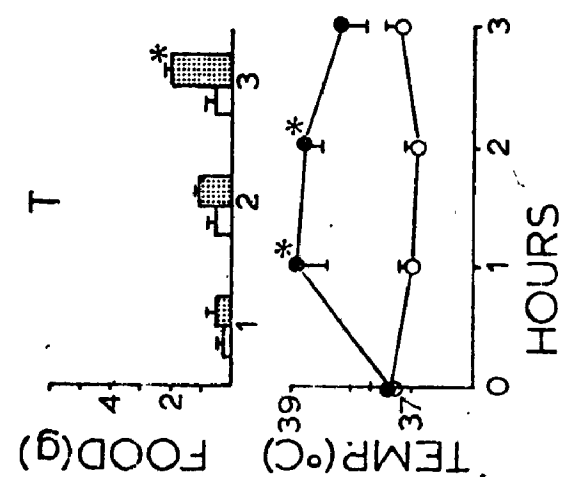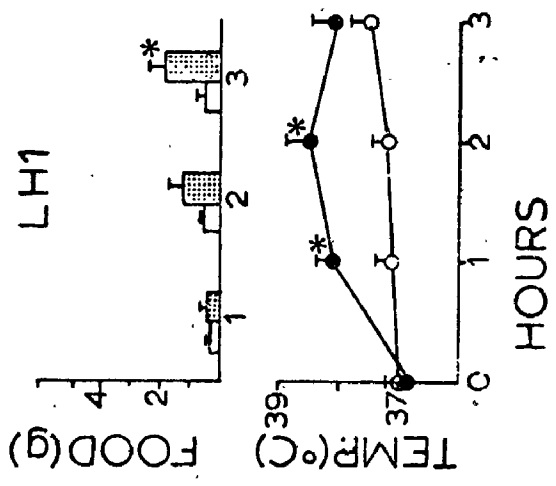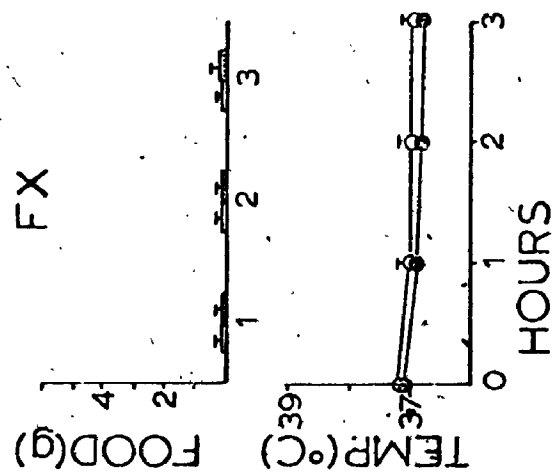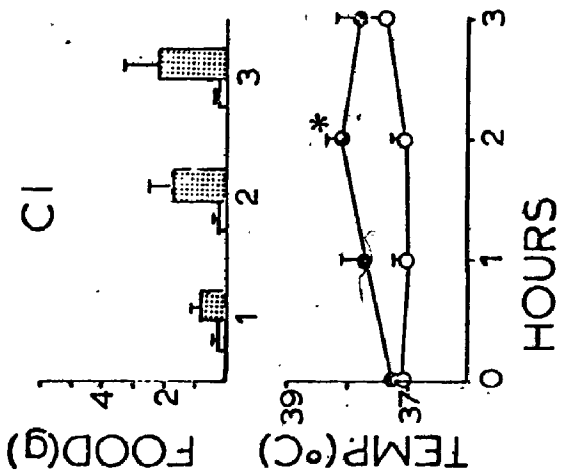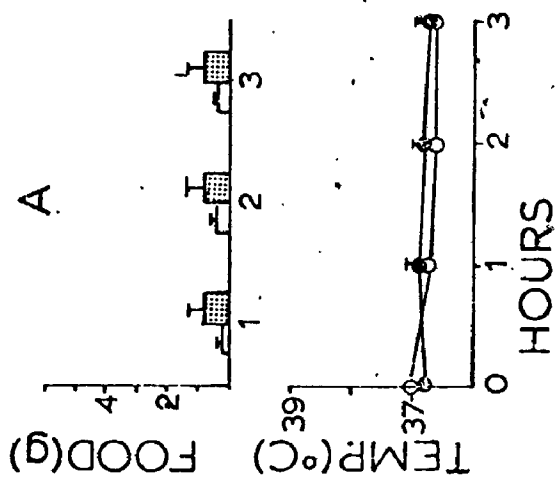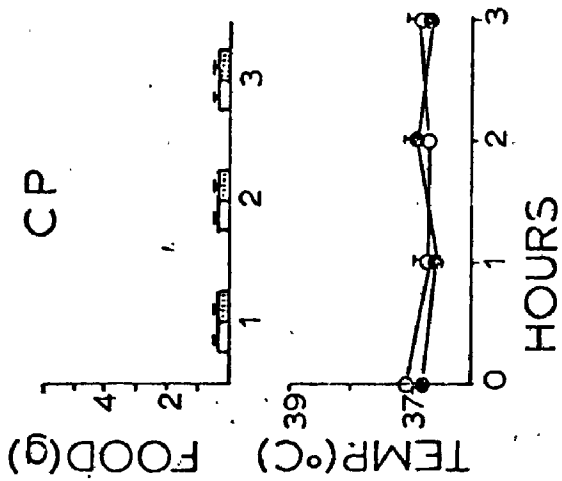
5. Discussion

5.1. Opiates, Opioid Peptides and Naloxone in the VMH: Feeding and Temperature Responses

When given subcutaneously to rats, morphine stimulates food intake. The diverse mechanisms that promote feeding activity in combination with the many probable sites of action of the drug, make it difficult to deduce how the drug-induced stimulus is transformed into the response. To further complicate the situation, the response shows some unusual characteristics. After subcutaneous injection the feeding behaviour occurred after a period of depressed motor activity. With continuing administration the rats became tolerant to the depressive actions of the morphine, the latency to feeding was reduced and the feeding activity was more pronounced (Thornhill et al., 1978a). It was hoped that injection of morphine into a specific hypothalamic site would provide some indication of the central component of this behaviour.

In the VMH, morphine produced reliable feeding following each injection. The feeding was, moreover, dose-dependent over the range tested. The pattern of this behaviour was reminiscent of effects after sub-cutaneous injection and quite consistent; there was a

long, dose-dependent interval of greatly decreased activity followed by a gradual increase in feeding. As the local anesthetic, procaine, did not duplicate the feeding or behavioural effects of morphine, it is probable that the action of the opiate did not arise through a temporary lesion at the site. Moreover, a dose of morphine that stimulated feeding when administered to the VMH was ineffective when given subcutaneously. These experiments indicate that the observed effects are of central origin. The sensitivity of the VMH to the drug-induced behaviour suggests further that this may be a central site involved in the feeding response to opiates.

As has been demonstrated previously (Leibowitz, 1970), vigorous feeding can be produced when noradrenaline is injected into the VMH: in fact, at the same sites where morphine was effective. Although noradrenaline produced reliable feeding, like morphine, a different pattern of ingestive behaviour was associated with the monoamine. Noradrenaline-induced feeding was rapid in onset and generally had ended by one hour after injection. This temporal dissimilarity in drug-induced behaviour suggested that differing mechanisms of action could be occurring, and this issue will be discussed in greater detail later.

It is common practice in experiments with narcotic analgesics as with other agonist drugs to attempt to reverse

their actions with appropriate antagonists. Accordingly, an attempt was made to suppress the feeding effect with the opiate antagonist, naloxone. Naloxone had no significant effects of its own when administered to the VMH. Peripheral injections of naloxone were able to decrease ingestion when stimulated by VMH application of β-endorphin (Grandison and Guidotti, 1977), intra-cerebroventricular administration of dynorphin (Morley and Levine, 1981) and subcutaneous injection of morphine (Thornhill et al., 1978a). In the present study, subcutaneous injections of naloxone reduced morphine-stimulated feeding in the second hour following ingestion, the time when food consumption was well-established, and reduced total food intake. However, results of studies involving peripheral administration of the antagonist must be interpreted cautiously. It is possible that subcutaneous naloxone may have caused nausea, since there is some suggestion that it may induce conditioned taste aversion at higher doses, although at lower doses this does not appear to be the case (Frenk and Rogers, 1979; Lowy, Maickel and Yim, 1980; Ostrowski, Foley, Lind and Reid, 1980; Pilcher and Stolerman, 1975). Moreover, as mentioned above, there is a loss of specificity of site of action. Thus, a peripheral injection of naloxone, which is highly lipid-soluble and would distribute widely, could interact with both central and peripheral

opiate receptors. This type of injection, therefore,
could antagonize VMH-induced feeding through an action at
opiate receptor sites other than those in the VMH. For
example, King and his colleagues (King, Castellanos,
Kastin, Berzas, Mauk, Olson and Olson, 1979) found that
intraperitoneal naloxone was able to reduce food intake
in rats with bilateral lesions of the VMH. Moreover,
naloxone may have some non-specific effects (Sawynok et
al., 1979).

The problem of site specificity was addressed
by placing naloxone directly into the VMH. This is not
without its own inherent difficulties. As naloxone is a
lipophilic substance, then it would be expected to distri-
bute to various central and peripheral sites just as would
happen after a subcutaneous injection, although the time
course of distribution would differ. If centrally-applied
doses were large enough, then effective concentrations
could be present at these sites after diffusion. Various
studies have tested the ability of central injections of
naloxone to antagonize consumption by rats. Doses of
100 to 2100 nmole naloxone have decreased food intake
which had been elevated by morphine, opioid peptides or
restricted feeding schedules when injected into the
paraventricular area or lateral ventricles (Leibowitz and
Hor, 1980; Thornhill, Taylor, Marshall and Parent, 1982;

Yim, Lowy, Holsapple and Nichols, 1980). Some of these
doses would suppress feeding if given peripherally,
for quantities as small as 0.3 to 0.5 mg/kg have been
reported to reduce opiate-induced feeding (Hynes,
Gallagher and Yacos, 1981; McCarthy, Dettmar, Lynn and
Sanger, 1981; Yim et al., 1980). Lower doses of naloxone
injected into the cerebral ventricles gave mixed results.
Jones and Richter (1981) were able to reduce feeding by
administering 80 nmole naloxone and Thornhill et al.
(1982) decreased food intake in a portion of the rats
tested with 67 nmole naloxone, but in other hands, doses
from 2.7 to 270 nmole were ineffective (Hynes et al.,
1981; Yim et al., 1980).

In the present study, an attempt was made to limit
the amount of naloxone injected into the VMH in order to
reduce the possible artifact arising from inhibiting
opiate receptors in other locations. At the same time,
it was important to give sufficient naloxone to antagonize
the opiate-induced effects at this site. Naloxone is
known to have a greater binding affinity than morphine
for opiate receptors from in vitro studies (Pert and
Snyder, 1974), and in in vivo studies, equimolar and even
lower quantities of naloxone given centrally to rats
were able to abolish morphines effects on drinking and
exploratory behaviour (Chance and Rosencrans, 1977; File

and Rogers, 1979). Moreover, the total hypothalamic content of naloxone found 15 minutes after subcutaneous injection of 10 mg/kg naloxone was only 7.0 ± 1.5 ng/mg (0.019 ± 0.004 nmole). This decreased over the next 120 minutes with a half-life of about 40 minutes (Tepperman, Smith and Hirst, unpublished results). Bearing these factors in mind, the dose of naloxone chosen for the present study was twice the dose of the applied morphine. This quantity of naloxone was able to reduce only the small amount of feeding that occurred during the first hour following morphine treatment, but was without effect on the peak feeding during the second hour. Since the lack of a potent effect by naloxone may have reflected a rapid dispersion of the antagonist from the site of injection, the activity of naloxone was also determined when injected one hour after morphine, when ingestion was well-established. It was found to be ineffective.

The ability of subcutaneous naloxone to reduce morphine-induced feeding suggested the involvement of opiate receptors. However, the more direct intrahypotha-lamic injection of the antagonist caused only a small reduction in ingestion, which contrasts with the inter-pretation of action on such receptors. Hence, it was essential to complement this data with additional information to support the view that an action on specific

opiate receptors was responsible for the observed increase in ingestion. A demonstration of stereospecificity of the opiate receptors involved in ingestion was considered to be an important step in this process.

The stereoisomeric pair, levorphanol and dextrorphan, have been utilized in various studies investigating the functions of the opiate receptor. The L(-)isomer, levorphanol, is a potent narcotic analgesic in man, while the D(+) stereoisomer, dextrorphan is inactive in this regard. When these agents were injected subcutaneously in rats, levorphanol caused much more food intake than dextrorphan (Lowy, Starkey and Yim, 1981; Thornhill et al., 1979). In a similar fashion, levorphanol injected into the VMH in the present study induced significantly more feeding than dextrorphan, demonstrating the requirement for appropriate stereochemistry.

Stereospecificity has been observed with narcotic antagonist pairs. Two benzomorphan compounds (Mr 1452 and Mr 2266), known to act as opiate antagonists, reduced quantities of food eaten by food-deprived rats, while their (+)-isomers, non-antagonists (Mr 1453 and Mr 2267) had no effect (Sanger, McCarthy and Metcalf, 1981).

The lack of effect of codeine on feeding behaviour is further support for the role of opiate receptors in the

VMH. Codeine is a weak ligand for opiate receptors (Pert and Snyder, 1973). It can enhance feeding in rats after peripheral injection, but these effects are probably the result of hepatic biotransformation of the codeine to morphine (Johannesson, Rogers, Fouts and Woods, 1965; Johannesson and Schou, 1963; Thornhill, Hirst and Gowdey, 1978b).

Levorphanol, as mentioned, is a more potent analgesic agent in man than is morphine. It is also more effective in suppressing electrically-evoked twitches in the guinea pig ileum. In both circumstances, levorphanol is about seven times more potent than morphine (Kosterlitz, Waterfield and Berthoud, 1974). In the present study, however, levorphanol induces a morphine-like pattern of food intake, with a slow onset and prolonged duration, but the amount of feeding elicited by equimolar amounts of the two narcotics was equivalent. This discrepancy may reflect the more lipophilic nature of levorphanol, and a more rapid diffusion than morphine from the administration site into the surrounding tissue. The consequence of this could be an apparent reduction in potency of levorphanol relative to morphine. If this was not so then a different subspecies of receptor might be considered to be involved in feeding from that in analgesia.

There were several features of the behaviour associated with morphine- and levorphanol-induced feeding which distinguished it from the behaviour following injection of another feeding stimulus, noradrenaline, into the same VMH site. One prominent feature was the lengthy interval between injection and onset of food intake, which was noted also following injection of β-endorphin into the VMH (Grandison and Guidotti, 1977; and this study). This has been noted subsequently following the injection of opiates into other regions of the brain: namely, morphine or D-Ala^2-Met^5-enkephalinamide into the paraventricular hypothalamus (McLean and Hoebel, 1980) and dynorphin into the lateral cerebral ventricles of rats (Morley and Levine, 1981). The extended time period may have been an indication of the redistribution of the opiates to other brain sites such as the lateral hypothalamus or amygdala, which have been implicated in feeding regulation. Alternatively, the time taken until ingestion began may have been associated solely with the sequence of events which followed the interaction of opiates at VMH opiate receptors. There is some evidence that morphine will remain in a brain region to which it is administered; Lomax (1966) found that in a period of more than 24 hours, morphine (1 µl) did not migrate in a radius of more than one mm from its injection site in the anterior hypothalamus, although its concentration had decreased considerably.

Since morphine in the present study was injected in a volume of 0.5 µl, the radius of migration may have been even smaller. However, in an attempt to further establish that the feeding observed following morphine and levorphanol injections was due, in fact, to binding with VMH opiate receptors, studies employing the quaternary opiate agonists morphine methiodide and codeine methiodide were undertaken. Their charged cationic structures would be expected to deter movement across lipid membranes. For this reason, they should be less likely to migrate from their site of administration in the VMH than the other narcotics tested. It was hoped that the codeine-based product would be a control for the methiodide of morphine, for, as mentioned, unlike morphine, codeine itself has little if any influence on feeding after VMH application.

Because these quaternary compounds are structurally distinct from morphine, preliminary in vitro and in vivo tests were done to establish their relative potencies, as well as their comparative potency to morphine. In vitro, morphine methiodide was not a highly active agonist. On the stimulated guinea pig ileum, morphine methiodide was about one thirty-fifth as effective as morphine in reducing twitch height. On a similar bioassay, Foster, Jenden and Lomax (1967) found morphine methiodide to be one-tenth as potent as the parent alkaloid. Codeine methiodide, however, was inactive at the ileal receptors suggesting

that these receptors interact with the opiate liganding portions of the methiodide.

The effects of morphine sulphate, morphine methiodide and codeine methiodide were also tested on rat blood pressure. Curiously, morphine caused a short-lived decrease in blood pressure only at the smallest dose studied. The decrease may have been due to an action of morphine on the vasomotor centre, but the reason that the higher doses were ineffective is unclear. The methiodides caused short-lived, dose-related declines in blood pressure. This was not unexpected because, although their quaternary nature would probably prevent them from crossing the blood-brain barrier and directly affecting blood pressure, their lipophilic foundation and quaternary cationic charge make them putative ganglionic blockers. The fall in blood pressure could be due, therefore, to reductions in sympathetic tone. The similar actions of morphine methiodide and codeine methiodide suggest that the methiodide portion of the molecule was active in this context. As noted above, a compound like codeine methiodide was a necessary control for the experiment with morphine methiodide for it could be that any observed effect could arise through a central intersynaptic blocking action. In studies, morphine methiodide, but not codeine methiodide, was able to elicit feeding when administered

into the VMH, suggesting that the effect was due to binding to the opiate receptors, not because of any interactions of the methiodide portion of the molecule. Morphine proved to be only about four times more efficient than the quaternary compound at provoking food intake. This may indicate a difference between opiate receptors in the guinea pig ileum and the rat hypothalamus, or alternative-ly, the methiodide was remaining at the site of injection, whereas the morphine was dispersing from this location during the experimental period.

Feeding following injection of morphine methiodide was dose-dependent, delayed in onset and prolonged after initiation. Since administration of the quaternary opiate did not reduce the lengthy interval before the onset of feeding from that observed following morphine injection, the delay may not indicate movement of the opiate to other feeding-sensitive locations.

Morphine initially depressed motor activity in treated rats prior to the beginning of feeding. This was not observed following injection of morphine methiodide or levorphanol; the rats actively groomed and sniffed, as well as rested. Dextrorphan and codeine methiodide, which did not alter feeding, produced no evident behavioural changes. Thus, while the behaviour associated with feeding due to the opiate compounds was generally similar, doses

of morphine methiodide or levorphanol, which caused comparable food intake to morphine, had less effect on locomotion. There may then be some differences in the hypothalamic influence on motor activity and feeding, but other studies would be required to develop this further.

The opiate antagonist, naloxone, was administered in the same doses that were used in conjunction with morphine-induced feeding in order to determine if the quaternary opiate had a comparable sensitivity to the effects of the antagonist. Subcutaneously-injected naloxone was found to decrease food intake significantly at each measurement interval, which was similar to its action against morphine. There was little feeding during the first two hours of measurement, but by the third hour the effect of the lower dose of naloxone on morphine methiodide-induced feeding appeared to be diminishing. This may have been because enough of the antagonist had been eliminated from the body that its reduced concentration was not as effective as at previous times. Even so, the same dose of naloxone which, when injected into the VMH, caused a small but significant reduction in morphine-induced feeding, did not influence morphine-methiodide-induced food intake. The reason for this is not known.

As alluded to in the Introduction, the opiate receptor is not a single entity, but consists of a variety

of subspecies. The concept of multiple opiate receptors originated with Martin and his colleagues (Gilbert and Martin, 1976; Martin et al., 1976) who, from studies on the chronic spinal dog, postulated the existence of three distinct receptor populations, all producing different pharmacological syndromes. These receptors were labelled "μ" for morphine and morphine-like drugs; "κ" for antinociceptive agents with similar properties to ketocyclazocine and ethylketocyclazocine; and "σ" for SKF 10,047 (N-allylnormetazocine). Lord et al. (1977) later postulated that the receptors to which opioid peptides bind are separate again from the above-named receptors. The new subclass of receptors was named "δ". Subsequently, the peptide β-endorphin has been shown to bind to an additional receptor sub-population named "ε" (Schultz et al., 1981).

This complex picture has been further confused by the finding that many of the commonly used opiates and opioid peptides bind with varying affinities to several of these receptor subtypes. This has been verified in physiological studies which test the ability of agonists to depress the electrically-evoked contractions of tissues with high content of a particular opiate receptor (guinea pig ileum for μ receptors, mouse vas deferens for δ receptors and rat vas deferens for ε receptors) and in binding assays

done on brains or brain parts of various species. By these means, morphine has been found to cross-react to a small extent with κ-sites (Kosterlitz, Paterson and Robson, 1981). κ-Opiate agonists, such as ethylketo-cyclazocine, were found to exhibit a high degree of cross-reactivity with μ-binding sites, and a smaller amount of affinity for δ-binding sites (Gillan and Kosterlitz, 1982), while the newly discovered opioid peptide, dynorphin, has been established as having selectivity for κ-receptors, but can cross-react also with μ- and δ-receptors. The selectivity of dynorphin (1-13) and dynorphin (1-17) for the κ-receptor may only be two to four times greater than for the μ and δ receptors (Chavkin et al., 1982; Corbett, Paterson, McKnight, Magnan and Kosterlitz, 1982; Quirion and Pert, 1981). The enkephalins, which bind to δ-receptors, can cross-react with μ-receptors. In fact, physiological and binding studies suggest that these naturally-occurring peptides may be anywhere from two times to 100 times selective for their respective binding sites (Chang et al., 1979; Chang and Cuatrecasas, 1979; Lord et al., 1977; Quirion and Pert, 1981; Schulz, Wuster, Krenss and Herz, 1980). Even D-Ala^2, D-Leu^5-enkephalin, (DADLE), a long acting opioid peptide which is more selective for δ-receptors than the natural peptides, does not bind exclusively to the δ-site, but interacts to a lesser degree with the μ site

(Gillan and Kosterlitz, 1982; Kosterlitz et al., 1981; Wuster et al., 1979),and very slightly with κ-receptors (Chavkin et al., 1982). β-Endorphin has high affinity for the δ-receptor, as well as the μ- and ε-receptors (Lord et al., 1977; Schultz et al., 1981; Wuster et al., 1979).

In the present work, the involvement of the receptor types in the feeding response of the VMH was investigated with the understanding that the agonists used could have overlapping activity at several opiate receptors. Phencyclidine was used as a σ-opiate receptor ligand. In binding studies, this compound was displaced quite potently by the prototype σ-opiate receptor ligand, SKF 10,047, but not by morphine, ketocyclazocine or opioid peptides (Quirion, Hammer, Herkenham and Pert, 1981). In discriminative tests in rats, phencyclidine produced effects indistinguishable from SKF 10,047 (Holtzman, 1980). However, in the present study, it was without effect on feeding.

Ketocyclazocine, the prototype κ-opiate agonist analgesic did not increase significantly the amount of food eaten when given directly into the VMH. The feeding that did occur may have been due to an action at μ-receptors. This contrasts with effects observed when

κ-opiate ligands were given peripherally; ketocyclazocine
and ethylketocyclazocine given subcutaneously to rats
were both of equal or greater potency to morphine in.
stimulating food intake (Morley, Levine, Grace and Kniep,
1982a; Sanger and McCarthy, 1981; Yim, Lowy, Holsapple
and Nichols, 1980). Dynorphin, the endogenous κ-ligand,
stimulated ingestion when injected into the lateral
cerebral ventricles of rats (Morley and Levine, 1981),
although some portion of this response might be due to
stimulation of μ-receptors. Nevertheless, these findings
suggest that feeding is a complex phenomenon involving
opiate receptors in other brain or peripheral sites. In
the VMH, the results indicate that μ-, but not σ- or κ-
receptors influence ingestion.

Feeding is also elicited upon administration of
opioid peptides. As mentioned previously, β-endorphin,
$D-Ala^2-Met^5$-enkephalinamide and dynorphin have all
stimulated feeding after being injected into the VMH,
paraventricular hypothalamus and lateral ventricles,
respectively (Grandison and Guidotti, 1977; McLean and
Hoebel, 1980; Morley and Levine, 1981). In another study,
a very small quantity of β-endorphin (0.06 nmole) was able
to stimulate ingestion of a liquid diet when injected into
the lateral cerebral ventricle of rats that were on a
restricted feeding schedule (McKay, Kenney, Edens, Williams

and Woods, 1981). Unlike rats in the present study and in the study by Grandison and Guidotti (1977), where there was a long delay until the beginning of ingestion, the animals began to eat during the first half-hour after injection of the β-endorphin. Whether this reflects the different site of administration, the use of a liquid rather than solid diet, or is related to the hungrier condition of the rats (rats in the other studies were not fasted) cannot be deduced. The peptides met-enkephalin and D-Ala^2-Met^5-met-enkephalinamide were, however, ineffective in this paradigm. In the present study, the initial decrease in activity, followed by persistent feeding which was observed after the higher doses of β-endorphin were tested, resembled the pattern of behaviour following morphine injection. Lower doses of endorphin were ineffective. As β-endorphin has affinity for the μ-receptor as well as the δ- and ε-opiate receptors (Wuster et al., 1979), the similarity of responses to those of morphine suggest that its μ-agonist properties may predominate for feeding activity.

DADLE is a long-acting structural analogue of Leu-enkephalin, being resistant to enzymatic degradation. As mentioned, its primary activity is at δ-opiate receptors, although it has some affinity for μ-receptors and binds very slightly to κ-receptors. This peptide signifi-

cantly elevated ingestion, but unlike morphine or β-endorphin, the enkephalin initially elicited a short period of enhanced locomotion, which was, on occasion at higher doses, preceded briefly by depressed motor activity. The absence of prolonged depression in activity was reminiscent of behaviour following levorphanol and morphine methiodide administration. Kosterlitz et al. (1980) have reported that levorphanol has some δ-binding activity. Thus, reduced locomotion may be related to µ-receptor binding; its presence after DADLE administration may then be due to cross-reactivity of the higher doses with µ-receptors.

The intense hyperphagia which followed enkephalin injection generally ended after one hour. The time course of events resembled that by Tseng (1981),who monitored analgesia caused by intrathecal administration of DADLE in rats. He found that inhibition of the tail flick response started five to 10 minutes after injection and lasted about 30 minutes. While the duration of action was much shorter than that of morphine or β-endorphin, DADLE (1.4 nmole) stimulated as much feeding in one hour as morphine (5.3 nmole) or β-endorphin (1.4 nmole) did in three hours. The dose of DADLE required to significantly increase feeding was low enough that this proved to be the most potent substance tested.

The present data demonstrates an overlap of activities of the μ- and δ-agonists. It is possible, then, that the response to DADLE was through μ-receptor binding activity in this in vivo system. Nonetheless, the animals were more sensitive to food intake provoked by the enkephalin and the time course of consumption was different, which could point to an independent influence on feeding. If the activity of DADLE were due solely to μ-receptor binding, one might expect the pattern to mimic that seen with morphine and β-endorphin.

The hypothalamic opioid peptides may contribute to the physiological regulation of feeding. Fasted rats have been found to have diminished hypothalamic, but not pituitary, levels of β-endorphin, although other opioids were not measured (Gambert, Garthwaite, Pontzer and Hagen, 1980). κ-Opiate receptors have been postulated to be important in feeding regulation (Morley et al., 1982a) and the amount of food eaten in one hour following intracerebroventricular injection of the κ-opioid peptide, dynorphin (Morley and Levine, 1981) was comparable to that following DADLE in the present study, yet, with DADLE, the onset of feeding following injection was faster. If dynorphin specificity is as low as some studies indicate (Quirion and Pert, 1981) some component of the ingestion may be due to δ or μ- receptor binding. Even if this is not the case, the

sensitivity and rapidity of the response to DADLE could be construed as an indication of the possible significance of the activation of the δ receptor in initiating feeding.

Feeding was not influenced when naloxone was given into the VMH prior to β-endorphin or DADLE. While the lack of action against other opiate ligands has been discussed, it is known that naloxone has a low affinity for δ receptors (Kosterlitz and Hughes, 1975; Lord et al., 1977; Waterfield et al., 1979).

Temperature-sensitive sites in the brain respond to morphine with either hypo- or hyperthermia. Hyperthermia exclusively has been identified following morphine admin- istration into the VMH (in the present study) and the anterior hypothalamus (Lin, 1982). Other locations in the brain elicited hypothermia and/or hyperthermia; namely the pre-optic anterior hypothalamic region and the lateral ventricles (Cox et al., 1976; Ferri et al., 1978; Trzcinka et al., 1977). Besides the different loci of injection, hypothermia is associated with doses of morphine that were considerably larger than those used here. In addition, in the other cited studies the animals were often restrained; restraint is a confounding issue for it tends to heighten hypothermia and attenuate hyperthermic effect of morphine (Blasig et al., 1979; Lotti et al., 1965; Martin

and Morrison, 1978).

The thermogenic effects of noradrenaline are variable. When given into the anterior hypothalamus, it has, in previous studies, been found to cause a rise, a fall, or a biphasic effect (Avery, 1971; Lomax et al., 1969; Veale and Wishaw, 1976). In the present work, noradrenaline in the VMH caused a small rise in temperature, which unlike that following morphine injection into the anterior hypothalamus (Lin, 1982) or VMH, was not dose-related. The temperature response to morphine methiodide was a small yet significant, short-lived rise in temperature at one hour after injection. The hyperthermic effect of this quaternary opiate was less than the temperature at the same time following morphine injection. Codeine methiodide was without effect. Consideration was given to the possibility that morphine had a greater effect because it was better able to diffuse to neighbouring, highly temperature-sensitive areas such as the POAH than the less lipophilic morphine methiodide. However, levorphanol, which is more lipophilic than morphine, did not alter temperature at all after injection into the VMH. Subcutaneous injection of levorphanol can produce an elevation in temperature (Thornhill et al., 1979), but this may be through an action at other sites.

The opioid peptides can also alter temperature. DADLE, in doses from 1.4 to 5.3 nmole caused a transient hyperthermia when injected into the VMH, but the lower doses tested did not. Intrathecal injection of small amounts of DADLE sufficient to cause analgesia failed to alter core temperature of rats (Tseng, 1981). When given intracerebroventricularly, large doses of DADLE were able to cause a slight hypothermia at 15 minutes after injection, which returned to normal 30 minutes later, but smaller doses had no effect on temperature (Tseng, 1981; Tseng, Ostwald, Lo and Li, 1979).

Low doses of β-endorphin have been observed to produce hyperthermia when given into the hypothalamus or intrathecally (Holaday et al., 1977; Lin, 1982; Tseng, 1981; Martin and Bacino, 1979). In the present study, the prolonged hyperthermia evoked by the higher doses of β-endorphin was similar to that seen with morphine, while the lower doses caused a short-lived hyperthermia.

The interrelationship between feeding and temperature following opiate administration was examined. It had been established previously that repeated daily subcutaneous injections of low doses of heroin or morphine (Thornhill et al., 1976, 1978a) caused increasing food intake and hyperthermia, both of which occurred progressively sooner

after administration. The times of post-injection peak hyperthermia and peak feeding activity were observed to coincide, suggesting a close relationship between the two events. When morphine was injected into the VMH, a similar temporal relationship was present, with hyper-thermia tending to appear before feeding began, and continuing during ingestion. There were, however, several indications that the feeding and temperature responses effected by opiates may not be integrally related. Doses of morphine and morphine methiodide which produced equivalent amounts of food intake did not produce uniform changes in temperature. Following morphine methiodide, the temperature increase was not only smaller than that following morphine, but it was transient and did not last through the duration of the ingestion period. More-over, a dose of levorphanol, which elicited food intake in a comparable manner and quantity to that produced by an equimolar dose of morphine, had no effect on temperature. Of the peptides, β-endorphin, at the higher doses tested, produced effects reminiscent of morphine; there was a prolonged hyperthermia which corresponded to the feeding period. The lower doses, however, caused a brief rise in temperature but no change in food intake. The higher doses of DADLE also caused increased food intake with a corresponding rise in temperature, but in this case, a

lower dose elicited food intake unaccompanied by a change in temperature. The differences between DADLE, with its predominantly δ-activity and β-endorphin, with μ-activity are apparent. Stimulation of δ receptors appears to have more influence on feeding while temperature seems to be altered more effectively by stimulation of μ receptors in the VMH. The notable differences in effects on these parameters produced by such diverse opioid agents as morphine methiodide, morphine, levorphanol, DADLE and β-endorphin leaves open the possibility that subclasses of opiate receptors are involved in generation of these responses.

Naloxone, injected peripherally, has been found to reliably reduce temperatures elevated by opioids such as subcutaneously or intracerebroventricularly administered morphine (Ferri et al., 1978; McGilliard, Tulunay and Takemori, 1976; Thornhill et al., 1978a); met-enkephalin, injected into the lateral cerebral ventricles (Ferri et al., 1978) and the enkephalin derivative, FK 33-824, administered subcutaneously (Blasig et al., 1979). Naloxone, given subcutaneously, proved to be similarly effective in reducing morphine-induced hyperthermia in the present study. The higher dose of naloxone antagonized the hyperthermic effects of morphine for three hours, while the effect of the lower dose had diminished by this

time. The temperature response to β-endorphin appears to be less influenced by naloxone; intraperitoneal injections of naloxone did not alter the rise in temperature following intrathecal β-endorphin (Tseng, 1981) and only partially attenuated the hyperthermia following injection of β-endorphin into the pre-optic anterior hypothalamus (Martin and Bacino, 1979). In contrast, the increase in temperature evoked by β-endorphin injected into the spinal subarachnoid space could be prevented by treatment with intraperitoneally-administered naloxone (Martin and Bacino, 1979).

Following intrahypothalamic application of naloxone, there was a reduction in morphine-induced hyperthermia at the first hour after injection, but no decrease in temperatures elevated by morphine methiodide, DADLE or β-endorphin. Lin (1982) was unable to alter the elevation in temperature produced by morphine or β-endorphin injected into the anterior hypothalamus with naloxone administered into the same hypothalamic location. Similarly, Tseng (1981) found that the increase in body temperature induced by β-endorphin given intrathecally was not altered by naloxone given at that site.

5.2. Noradrenergic Mediation of Opiate-Induced Feeding and
Changes in Temperature

Although small amounts of various opiates and opioid
substances were able to influence feeding, the delay in
the response was unusual and gave rise to speculations
on whether "feeding" receptors were directly stimulated
or a change in levels of an intermediary substance was
induced which in turn stimulated feeding. As mentioned
previously, there was an extended interval (usually 30 to
90 minutes) following injection of most μ-agonists until
feeding began. Even after injection of DADLE, where the
delay was slightest, feeding was generally later in onset
than after injection of noradrenaline, another potent
stimulator of feeding behaviour. Furthermore, although
pretreatment with an intrahypothalamic injection of
naloxone reduced food intake, naloxone administered by the
same route an hour after morphine (the beginning of the
feeding period) was ineffective. It has been shown that the
concentration of morphine is rapidly cleared by passing
into the bloodstream after injection into the brain, a
high dose of ^{14}C-morphine (50 μg) having fallen to minimal
levels by two hours after being placed into the anterior
hypothalamus (Lomax, 1966). In the present studies,
feeding often persisted three hours after injection. If
indeed the effect was occurring in the absence of the

initiating drug, there would be a mandatory requirement for
a secondary system to implement the effects.

There is evidence that opiates may interact with
other substances to elicit their effects. Thus, central
5-HT and noradrenaline have been implicated in the hypo-
thermia caused by morphine (Burks and Rosenfeld, 1979),
while β-endorphin-induced hyperthermia may involve pro-
staglandins and 5-HT (Martin and Bacino, 1979). Moreover,
the growth-hormone-releasing action of morphine appears
to require functional noradrenergic, but not serotonergic
or dopaminergic neurons (Koenig, Mayfield, Coppings, McCann
and Krulich, 1980).

Since the VMH is sensitive to feeding stimulated by
noradrenaline, this catecholamine was evaluated as a
possible mediator. All agents used in these studies were
given directly into the VMH to reduce the possibility of
non-specific actions which might affect ingestion. A
dose of phentolamine which reduced noradrenaline-induced
feeding was established initially. This dose of phento-
lamine did not, however, alter ingestion when given just
before morphine. Administration of phentolamine one hour
after morphine successfully attenuated ingestion. This was
the converse of the results obtained with the naloxone
and morphine injections. The phentolamine-induced effect

was pursued with DADLE as the stimulant for feeding. While not anticipated, phentolamine pre-treatment reduced DADLE-induced ingestion, which often began within minutes after injection of the peptide. These experiments demonstrated that the feeding effect of these differing opioids are suppressed by an α-sympathetic antagonist more effectively than by an opiate antagonist. It is conceivable that activation of both μ and δ opiate receptors trigger the release of noradrenaline, although there may be a dissimilarity in the timing of such release. The results suggest that the peptide causes a faster output of noradrenaline than may be likely after morphine.

There is substantial evidence that opioid substances interact with catecholamines in the brain. Opiates appear to activate dopaminergic pathways (Biggio, Casu, Corda, DiBello and Gessa, 1978). There is also evidence that the opioid peptide dynorphin may activate feeding through dopamine, since haloperidol, given peripherally, inhibited this effect (Morley et al., 1982b), although they did not examine the effects of dynorphin on the adrenergic system. Burks and Rosenfeld (1979) postulated that morphine-induced hypothermia may be due to activation of the noradrenergic system. Results from the present work suggest that opioids may cause a liberation of neuronal noradrenaline. If true, this would be unexpected

in light of the body of literature which indicates that
morphine and opioid peptides cause a decrease in release
of noradrenaline from nerve terminals. This has been shown
in the hypothalamus and frontal cortex of the rat, and
in electrically-stimulated brain slices. Opiates also
depress the firing rate of noradrenaline-containing
cell bodies in the locus coeruleus (Korf, Bunney and
Aghajanian, 1974; Montel, Starke and Weber, 1974;
Nakamura, Tepper, Young, Ling and Groves, 1982; Taube,
Starke and Borowski, 1977). Moreover, the increases in
hypothalamic noradrenaline levels, in rats, observed after
intracerebroventricular injections of β-endorphin or mor-
phine were attributed to a decrease in catecholamine
release (McIntosh, Vallans and Barfield, 1980). While it
may be argued that inconsistancies between the literature
and the present data may be associated with variations
in doses, routes of application and times of measurements,
the suggestion that opiates enhance noradrenergic function
should be explored, for this would have broader implications
than solely affecting feeding behaviour and thermoregula-
tion. The time course of noradrenaline liberation would
be the critical issue for food intake following opiate
administration may possibly be due to a rebound phenomenon.
Thus the initial binding of opiates could cause a
temporary inhibition of noradrenaline release (perhaps even

contributing to the state of depressed activity). A rebound increase in noradrenaline levels that stimulate feeding could then occur during an acute "withdrawal" phase. This hypothesis does have constraints for it presupposes a much faster rate of recovery or "withdrawal" after DADLE than after morphine, associated with the faster appearance of feeding activity. To determine satisfactorily whether a direct or rebound mechanism applies, studies of the time course of opioid influences on the dynamics of the VMH noradrenergic systems would be, as mentioned, well worth pursuing.

Phentolamine, given alone, elevated core temperature following administration to the VMH. Although in the present studies application of noradrenaline to this hypothalamic site did not cause hypothermia, noradrenaline has been implicated elsewhere as an endogenous agent predisposed to lowering body temperature (Avery, 1971; Lomax et al., 1969). Hypothermia elicited by high doses of morphine was inhibited by intracerebroventricular administration of phentolamine, leading to the postulation of involvement of noradrenaline in opiate-induced depression in temperature (Burks and Rosenfeld, 1979). It is worthy of note that these authors do not mention what occurred when phentolamine was given alone. As noted earlier, phentolamine reduced noradrenaline-elicited

feeding, but by itself caused an elevation in body
temperature. When this dose of phentolamine did not
reduce morphine-induced hyperthermia, and extended the
time course of the hyperthermia occurring after DADLE,
this was attributed to an interaction with the thermo-
genic action of the antagonist. Hyperthermia was still
prominent in the second control trial when noradrenaline,
morphine or DADLE was given with saline and no sympatho-
lytic was present. This induceable long-lasting hyper-
thermia resembled that achieved in the mid-test trial,
when phentolamine was given after the particular opioid
substance, or before noradrenaline. This phentolamine-
influenced effect cannot be explained on the basis of some
indiscriminate damage at the injection site, for DADLE
induced an equivalent amount of food intake both before
and after it had been given with the antagonist. It could
be that phentolamine may have altered the susceptibility
of temperature-sensitive neurons in the VMH, but if so,
the underlying mechanism for this effect is not clear.

The influence of noradrenaline on morphine-induced
feeding and temperature was examined directly. The dose
of noradrenaline chosen for this purpose (30 nmole) was
sufficient to elicit the same amount of feeding as
morphine (5.3 nmole) over the course of the three-hour
measurement. When morphine and noradrenaline were

injected sequentially into the VMH, their effect appeared to be additive, since the amount of food intake was equivalent to a summation of their separate effects. Feeding following the dual injection was rapid in onset, as occurred following noradrenaline injection. The feeding was prolonged, however, as normally seen with administration of morphine. There was no sign of tachyphylaxis that might have presented if the injected noradrenaline had caused a presynaptic inhibition of the noradrenaline release postulated to occur following morphine administration. It was noted also that the locomotor depression regularly observed after morphine was not present following the dual opiate-catecholamine injections. This observation enhances the earlier-presented consideration that a decrease in noradrenaline release might be involved in the behavioural depression. Noradrenaline appeared to have no effect on morphine-induced hyperthermia.

To further refine the study of the influence of noradrenaline on morphine-elicited feeding, the effects of more specific α-adrenergic antagonists, prazosin and yohimbine, were pursued.

Prazosin is reported to have predominantly post-synaptic; α_1, blocking activity (Cambridge, Davey and Massingham, 1977; Doxey, Smith and Walker, 1977) and hence

was of interest, but it did not prove useful. The amount used in the present study (0.1 µg) was the maximum that would dissolve in the largest volume of saline used in these studies (1 µl). When morphine-induced feeding remained unaltered, the antagonist activity of this dose of prazosin was assessed by measurement of its ability to inhibit noradrenaline-induced feeding. The prazosin proved to be ineffective again. Accordingly, it was assumed that the dose used was too low to affect food intake.

This small dose of prazosin, nevertheless, seemed to elevate core temperature when administered by itself. Further, when injected before noradrenaline, significant hyperthermia resulted. Moreover, core temperature was elevated following the injections of saline plus noradrenaline associated with the second control trial, just as it was following the second control injections of saline plus noradrenaline which followed the phentolamine plus noradrenaline trial. While this suggests that prazosin, as well as phentolamine, may have altered temperature-sensitive neurons in the vicinity of the VMH, this effect is inexplicable and there is no literature that shows that others have encountered this phenomenon.

Yohimbine preferentially antagonizes pre-synaptic (α_2)-adrenergic receptors (Doxey et al., 1977; Starke, Borowski and Endo, 1975), which normally allow noradrenaline to regulate its own release. Blocking α_2-receptors would cause much larger than normal quantities of noradrenaline to be released following a particular stimulus, and there would be increased stimulation, therefore, of postsynaptic α_1-receptors (Anden, Pauksens and Svenson, 1982; Doxey et al., 1977). Yohimbine, given parenterally, has been found to increase food consumption by rats (Gregson and Stribling, 1983). This might be due to an increase in the synaptic content of noradrenaline in the hypothalamus. The intrahypothalamic dose (9.1 nmole) used in the present study, however, did not alter food intake on its own. When this dose of yohimbine was injected an hour after morphine, feeding increased considerably. This may be taken as further evidence that morphine can cause release of noradrenaline. Without an intact feedback mechanism, the synaptic concentration, and hence the effect on feeding of noradrenaline, became greatly amplified. Singer and his colleagues (Singer et al., 1971) reported a small decrease in food intake in the rat following intrahypothalamic injection of yohimbine. This latter study was designed to measure reductions in food intake and the animals were only

allowed to feed for two hours each day. In addition,

doses of yohimbine were large and administered in such a

volume of saline that other brain regions were likely

affected (Myers, 1964). The marked locomotor depression

which was observed could be construed as influential,

rendering the animals unable to feed.

In the present study, yohimbine did not affect core

temperature when administered an hour after morphine. Lin

(1982), however, found that pretreatment in the anterior

hypothalamus with a small amount of yohimbine, which

did not influence temperature by itself, reduced the peak

of hyperthermia due to injection of morphine at that site.

Most other biogenic amines and their antagonists which

were tested in the VMH had little effect on feeding,

emphasizing that the hyperphagic actions of morphine

involve noradrenaline specifically. The work described

here supported the finding (Leibowitz,1970) that α- and not

β-adrenergic receptors are involved in the stimulation of

feeding in the VMH, for propranolol was ineffective against

morphine-induced feeding. Although 5-HT has been

previously implicated in ingestion (Goldman et al., 1971;

Hoebel, 1977), neither it, nor its antagonist, methysergide,

influenced feeding evoked by morphine given to this site.

Dopamine has a weak (and often-delayed) hyperphagic effect

in the present study and other studies (Booth, 1968;
Slangen and Miller, 1969). In the present study it
augmented food intake when given in conjunction with
morphine. Apomorphine and haloperidol, however, had no
effect. It is possible, then, that the dopamine might
simply have been acting as additional substrate for
conversion to noradrenaline in local noradrenergic
neurons.

Most of the amines tested did not change the elevation
in core temperature elicited by morphine. Neither 5-HT
nor methysergide were effective here, although 5-HT has
been proposed to be involved in both the hyperthermia due
to β-endorphin and the hypothermia provoked by morphine
(Burks and Rosenfeld, 1979; Martin and Bacino, 1979).
However, the β-antagonist, propranolol, injected after
morphine, significantly raised the already high core
temperature and also produced sensitivity to the temper-
ature effects of a later injection of morphine. Needless
to say, this finding does not assist any explanation of
similar effects shown by the α-antagonists, phentolamine
and prazosin. In a different site, the anterior hypo-
thalamus, pretreatment with an injection of propranolol
depressed the small elevation in temperature observed
following morphine injection (Lin, 1982). Haloperidol
and dopamine, given into the VMH in the present study did
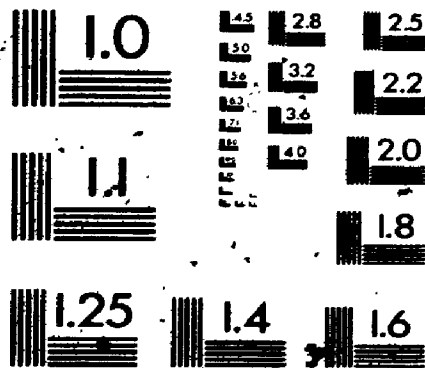
not alter the hyperthermia induced by morphine. Cox and colleagues (Cox et al., 1978) found that haloperidol injected into the preoptic anterior hypothalamus did not alter core temperature in rats, whereas dopamine in the same site caused a short-lived fall in temperature that appeared to return to control temperature one hour after injection. Apomorphine, given into the same hypothalamic site (Cox et al., 1978), caused a prolonged hypothermia, while in the present study, morphine-induced hyperthermia was significantly lowered at two hours after the administration of apomorphine.

5.3. Feeding and Temperature Responses in the Rat to Administration of Morphine into Various Brain Sites

Opiate-induced feeding has only been demonstrated centrally in two hypothalamic locations (the paraventricular hypothalamus and VMH), although several studies have used non-specific applications to the lateral cerebral ventricles. The extent of sites in the brain which can be stimulated by opiates to produce ingestion has not been particularly well-defined. Several sites in the hypothalamus were chosen for this study, as well as several scattered extrahypothalamic locations. The level of opioid innervation in these locations is known to vary.

The abundance of met- and leu-enkephalin and β-endorphin, as well as enkephalinergic and endorphinergic fibres and cell bodies found in the hypothalamus, suggests a neuronal role. The β-endorphin-containing cell bodies arise in the arcuate region and fibres are found through the hypothalamus; especially close to the ventricle. Enkephalinergic fibres have been seen in almost all hypothalamic nuclei, but are especially dense in the periventricular area and ventromedial nucleus, and are more diffuse in the lateral hypothalamus (Adler, 1980; Bloom, Rossier, Battenberg, Bayon, French, Henricksen, Siggins, Desal, Browne, Ling and Guillemin, 1978; Dupont, Lepine, Langelier, Merand, Rouleau, Vaudry, Gros and Barden, 1980; Rossier, French, Rivier, Ling, Guillemin and Bloom, 1977). Endorphinergic fibres distribute rostrally to the mid-line structures of the thalamus, while enkephalinergic fibres and cell bodies are less dense in the thalamus than in the VMH (Bloom et al., 1978; Cuello, 1983). β-Endorphin-containing fibres do not extend to the caudate-putamen, but this region has a high concentration of enkephalins and a dense population of receptors (Adler, 1980; Brann and Emson, 1980; Cuello, 1983; Dupont et al., 1980; Simantov, Kuhar, Pasternak and Snyder, 1977). The amygdala contains both enkephalinergic and endorphinergic systems (Adler, 1980; Dupont et al.,

1980; Uhl et al., 1978). In the more ventral aspects of the fornix there is a small concentration of enkephalins (Cuello, 1983).

Of the regions tested in the present study, the hypothalamus appeared to respond most sensitively to morphine. As stated above, this region contains enkephalins and endorphins, and is traversed by enkephalinergic and endorphinergic neurons. The VMH, furthermore, was the hypothalamic locus where injection of morphine caused the greatest increase in ingestion. There was not as rapid, or as great an increase in food intake following injection into the paraventricular hypothalamus. However, the more intense locomotor depression that morphine elicited at this site may have obscured some of the feeding response. Morphine at both of these hypothalamic sites caused a prolonged hyperthermic response, with temperatures following paraventricular injection being, perhaps, slightly higher than following VMH injection. A significant increase in three-hour feeding also occurred following administration of morphine into the lateral hypothalamus, with the more medial sites (LH2) being more effective than the lateral sites (LH1). Administration of morphine into the thalamus caused feeding which was comparable to that after injection into the lateral hypothalamus (LH1). There was some separation

of feeding and temperature effects due to morphine at the lateral hypothalamic and thalamic sites; the initial opiate-elicited hyperthermia fell so that it was no different from control temperatures by the third hour, while food intake became significantly greater than control levels during the third hour. The only part of the hypothalamus into which morphine was applied without significant effects on feeding and/or temperature was the posterior hypothalamus. As mentioned, this region is well-innervated with opioid neurons. Morphine-induced feeding appeared to centre around the VMH, and become weaker at the more lateral and ventral sites, while the posterior hypothalamic site tested was unresponsive. Injections into the more distal sites, including the internal capsule, caudate-putamen, amygdala and fornix did not cause increased feeding, even though the caudate-putamen and the amygdala contain moderate to high levels of opioid peptides.

The implied relationship between noradrenaline and morphine would be enhanced if there was overlap between brain sites that afforded feeding responses to these drugs. While this was not developed directly, the sensitivity of the various loci tested with morphine was compared to the data from the extensive study by Leibowitz (1978), who observed feeding following injections of

noradrenaline. She found extrahypothalamic injections of
the monoamine into the caudate-putamen and amygdala
to be ineffective, while sites in the periventricular
nucleus of the thalamus proved moderately responsive.
The posterior hypothalamus was unresponsive as well as
hypothalamic locations which were more than 1.3 mm lateral
to the midline. In the present study, although all
lateral hypothalamic sites to which morphine was
administered elicited feeding, those closer to the mid-
line appeared more effective. Food intake was greater
following injection of noradrenaline into the para-
ventricular hypothalamus than the VMH, whereas the
converse was true for morphine-elicited feeding. In
general, stimulation of particular brain regions by
morphine or noradrenaline produced similar effects on
ingestion, providing supportive evidence for the
interrelation of these agents in feeding processes.

6. <u>Summary</u>

1. Morphine, administered into the ventromedial hypo-
   thalamus (VMH), elicited feeding in a dose-related
   manner. Injection of the narcotic was always
   followed by a latent period, characterized by a
   decrease in activity, and then prolonged feeding.

2. Another potent stimulator of food intake, noradrena-
   line, given into the VMH, caused a different temporal
   pattern of ingestive behaviour. Feeding began after
   a very brief interval and was completed within an hour
   of injection.

3. An attempt was made to reverse the effects of morphine,
   given into the VMH, with the opiate antagonist,
   naloxone. Given subcutaneously, naloxone suppressed
   morphine-induced feeding for the full three hours of
   the study. Pretreatment with naloxone instilled into
   the VMH reduced only the feeding during the first
   hour after administration of morphine. When given
   centrally an hour after morphine, the antagonist had
   no effect.

4. The stereospecificity of the VMH opiate receptors was
   considered. The narcotic agonist, levorphanol,
   stimulated feeding, while its non-narcotic stereoisomer,

dextrorphan, did not. Feeding after levorphanol injection, as with morphine, began after a long latent period and had an extended duration.

The inability of codeine to stimulate ingestion further supported the role of opiate receptors in feeding, for codeine itself is a weak ligand at opiate receptors.

5. The quaternary opiates, morphine methiodide and codeine methiodide, were examined for their effects on feeding. In preliminary studies, their actions on guinea pig ileum and rat blood pressure were compared to morphine. When injected into the VMH, the inability of codeine methiodide to alter feeding resembled the lack of effect of the tertiary alkaloid, codeine. Although morphine methiodide was less potent than morphine, there was a parallel in its temporal feeding pattern. Because the methiodides would be less likely to migrate from the site of injection than the tertiary bases tested, the long interval following narcotic administration before ingestion may not indicate movement of the opiates to other central feeding-sensitive locations.

Morphine methiodide-induced feeding was signifi-cantly decreased by subcutaneously-administered

naloxone, but naloxone given into the VMH was unable to antagonize food intake.

.6. The involvement of various opiate receptor types in the feeding response of the VMH was investigated, with the understanding that the agonists used could have overlapping activities at several opiate receptor subspecies. Morphine (μ-receptor agonist), keto-cyclazocine (κ-receptor agonist) and phencyclidine (σ-receptor agonist) were tested, but only morphine influenced ingestion when given at this site.

7. Opioid peptides were found to elicit feeding when injected into the VMH. D-Ala^2, D-Leu^5-enkephalin (DADLE), a structural analogue of Leu-enkephalin, with largely δ-opiate receptor activity, and β-endorphin, a naturally-occurring peptide with μ-, δ- and ϵ-opiate receptor activity, were tested. Injection of DADLE elicited a short interval of enhanced locomotion, occasionally preceded by depressed motor activity. This was followed by intense hyperphagia which generally ended by one hour after injection. After application of β-endorphin, there was a long delay followed by prolonged feeding, similar to the behaviour observed following morphine.

Naloxone, given into the VMH, did not block the effects of either peptide.

8. Core temperatures were influenced by VMH injections of some opiates and opioid peptides. The changes did not always correspond temporally to fluctuations in ingestion, indicating that these effects may not be integrally related. Intrahypothalamic morphine induced dose-dependent, prolonged hyperthermia, which continued during the course of food intake. However, the rise in temperature elicited by morphine methiodide was brief enough that the majority of food was ingested when the rats were normothermic. Furthermore, core temperature was not altered by a dose of levorphanol that induced the same amount of food intake as did morphine.

    Of the peptides tested, β-endorphin produced a pattern of hyperthermia and feeding reminiscent of that caused by morphine, while at lower doses the elevation in temperature was not accompanied by feeding. DADLE induced a brief rise in temperature which coincided with food intake, but at lower doses food was consumed without a change in temperature.

    Naloxone, given subcutaneously, reversed the thermogenic actions of morphine and morphine methiodide.

When administered into the VMH, naloxone attenuated morphine-induced hyperthermia but had no effect on temperatures changed by morphine methiodide, β-endorphin or DADLE.

9. The question was addressed of whether opiate receptor activation was directly able to stimulate feeding, or if this changed activity of another system which in turn stimulated feeding. Noradrenaline, an agent which sensitively stimulated ingestion when injected into the VMH, was found to be a likely mediator of opiate influenced feeding in the VMH. The actions of noradrenaline and morphine on food intake proved to be additive. Moreover, the α-adrenergic antagonist, phentolamine, blocked noradrenaline- as well as morphine- and DADLE-induced feeding. The interaction of opiate and noradrenergic systems could not be extended to core temperature changes. Phentolamine exerted a hyperthermic effect which reappeared even when injections of noradrenaline, morphine or DADLE were no longer given with the antagonist.

To further refine this study, the effects of more specific α-adrenergic antagonists were pursued. Prazosin, an α_1-blocker, was ineffective because insufficient dosage could be given. Yohimbine, an

α_2-antagonist, significantly elevated food intake when given in conjunction with morphine over intake following a morphine plus saline co-treatment. This was probably due to blockage of the noradrenergic feedback mechanism, and hence an increase in synaptic concentration of the noradrenaline released by morphine. Yohimbine did not alter temperature, but prazosin exerted a hyperthermic action reminiscent of phentolamine.

10: Most other biogenic amines and their antagonists which were tested in the VMH had little effect on feeding, emphasizing that the hyperphagic actions of morphine involve noradrenaline specifically. The β-adrenergic antagonist, propranolol, or 5-HT or its antagonist, methysergide, did not change the amounts eaten. Dopamine augmented food intake when given with morphine. Apomorphine and haloperidol, the dopamine receptor agonist and antagonist, respectively, had no effect; this suggested that dopamine might have been acting as additional substrate for conversion to noradrenaline in local noradrenergic neurons.

The elevation in temperature due to morphine was not altered by most amines tested. Some changes were induced by propranolol and apomorphine.

11. Feeding and temperature responses of various brain
    sites to morphine were determined. The most vigorous
    feeding appeared to occur following injection of
    morphine into the VMH and became weaker at more
    lateral, ventral and caudal locations. Even some
    sites with high levels of opioid-containing neurons
    did not cause an increase in feeding after morphine
    injection. These feeding results were compared to
    findings from a study in the literature determining
    ingestion after noradrenaline injection into various
    brain sites.

Appendix I

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Appendix I

Preparation of Morphine Methiodide and Codeine Methiodide

Sodium hydroxide was added to morphine sulphate and codeine phosphate to raise the pH to 9. The precipitated morphine and codeine bases were filtered and dried in air. The bases were then dissolved in methanol and diethyl ether, and excess methyl iodide was added. After several days at room temperature, morphine methiodide and codeine methiodide were filtered and recrystallized from methanol/ diethyl ether. HPLC analysis of the morphine methiodide did not reveal any detectable morphine levels.

Appendix II

Appendix II

Potency of Morphine Methiodide, Codeine Methiodide and Morphine Sulphate on Guinea Pig Ileum

Method

Much of this study was conducted by Mr. S. Tse.

Male guinea pigs (about 300 g) were killed by a blow to the head. Segments of ileum (about 2 cm in length) were taken approximately ten cm from the ileocaecal junction and rinsed well with Tyrode's solution. These were placed in an organ bath containing Tyrode's solution at 37° which was gassed with a 5% carbon dioxide - 95% oxygen mixture. Both ends of the tissue were connected to a Grass Stimulator (Model S4KR); one end was impaled on a stainless steel hook and the other was attached by a stainless steel suture which was loose enough that it did not affect tissue contractions. The sutured end was also connected by a linen thread to a Harvard apparatus auxotonic smooth muscle transducer with applied tension of approximately 500 mg. Contractions were recorded on a Rikadenki recorder (Model B-24).

The tissue was allowed to equilibrate for 30 minutes and then stimulated with square wave pulses of 0.1 Herz

frequency and 0.5 mseconds duration. Voltage was adjusted
to obtain maximal contractions (approximately 60 V).
The stimulated tissue was allowed to equilibrate for an
additional 30 minutes. Drugs were added serially; when
the maximum contraction for a particular dose was reached
the next dose was added. After each drug, the ileal
segment was washed two to three times and allowed to
equilibrate for 30 minutes.

Results

The log dose-response curves obtained for morphine
methiodide and morphine sulphate are shown in Fig. 38.
Both agents were able to reduce the height of electrically-
evoked twitches of the ileal segment, although they were
not equally effective. Morphine sulphate reduced twitch
height to 44% of the control, while morphine methiodide
only decreased the contraction to 61% of the control.
The relation between the dose administered and the percent
of inhibition of the twitch was linear for both morphine
sulphate (r = 0.83) and morphine methiodide (r = 0.90).
The statistically-derived, best straight lines through
the points are shown in Fig. 38; the slopes of these lines
do not differ significantly. The quaternary morphine was
found to be approximately 35 times less potent than the

tertiary morphine. Codeine methiodide, however, was
ineffective in reducing twitch height.

FIGURE 38

The % inhibition of the twitch response of the
electrically-stimulated guinea pig ileum by various
bath concentrations of:

● morphine sulphate

O morphine methiodide
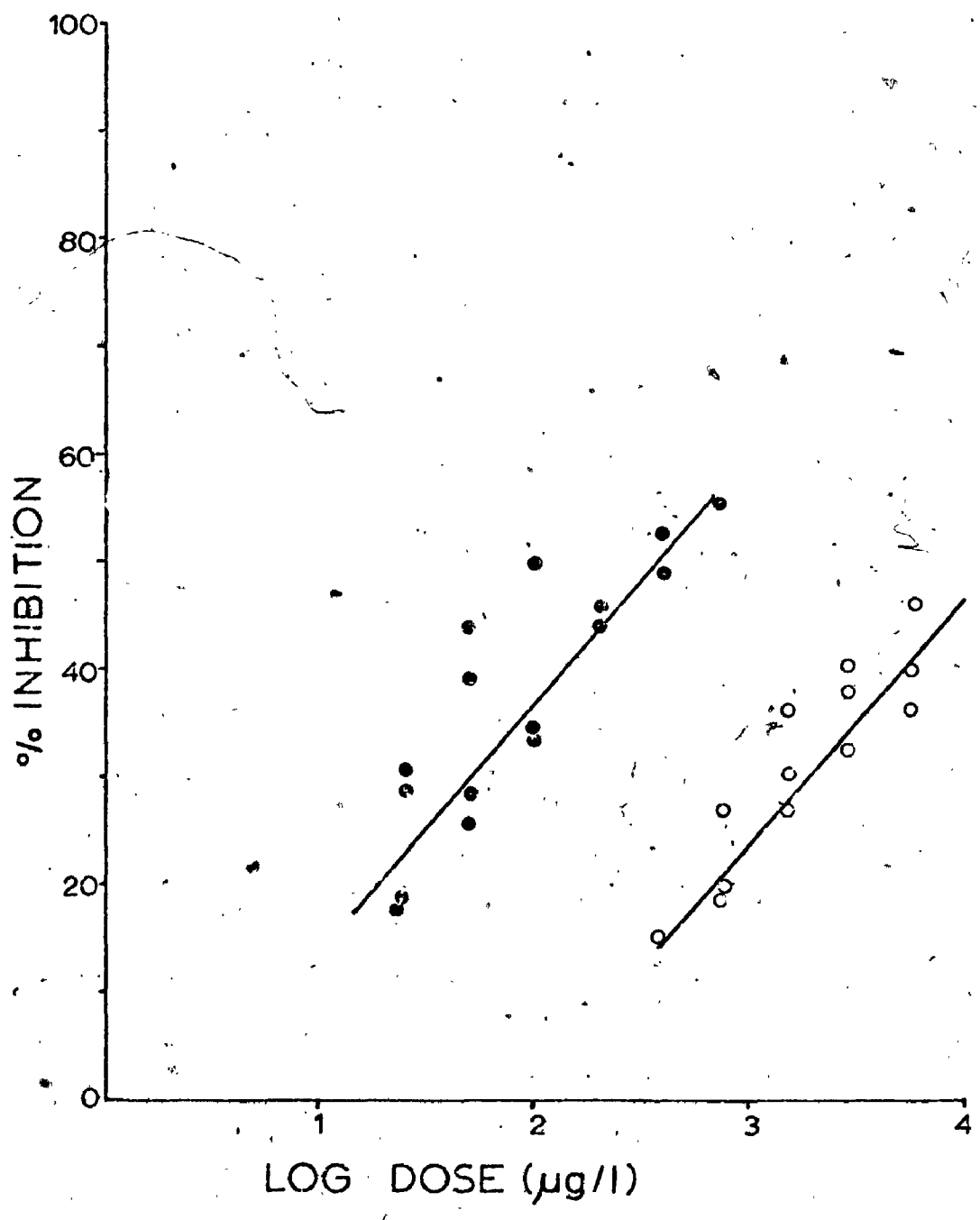
Appendix III

Appendix III

Potency of Morphine Methiodide, Codeine Methiodide and Morphine Sulphate on Rat Blood Pressure

Method

Four male, Sprague-Dawley rats weighing about 400 g were anaesthetized with pentobarbital. Their carotid artery was cannulated with polyethylene tubing (Intramedic PE50), which was connected to a pressure transducer (Gould Stratham Instruments, Inc. P23ID) so that blood pressure tracings could be recorded on a Grass Model 79D EEG and Polygraph Recording System. The jugular vein was also cannulated and used for drug administration; between infusions, the cannula was kept patent with heparinized saline (10 units/ml). The animals remained anaesthetized during the course of the study. An additional dose of a particular drug was not given until blood pressure was stable for at least five minutes, and between different drugs, animals were not infused for a minimum of 30 minutes. The doses of morphine methiodide administered ranged from 0.5 to 20 mg/kg, for codeine methiodide from 10 to 20 mg/kg and for morphine sulphate from 0.5 to 4.5 mg/kg.

Results

Table II details the changes observed in blood pressure.

A decrease in blood pressure was evident with all drugs tested; the higher doses of methiodides exerted the greatest effect. Both morphine methiodide and codeine methiodide caused a dose-related fall in blood pressure, the maximum drop observed being 45.0 ± 3.5 and 36.3 ± 5.5 mm Hg, respectively. The duration of the decrease was also dose-related, the mean interval being no more than 5.1 minutes. Morphine sulphate reduced blood pressure at the lowest dose tested, but the two higher doses had no apparent activity.

Table II Effects of Intravenous Administration of
Morphine Sulphate, Morphine Methiodide and
Codeine Methiodide on Rat Blood Pressure

Drug	Dose (mg/kg)	Molar Ratio Compared to Morphine Sulfate	Maximum Reduction in BP (mm Hg)	Duration of Reduction (min)
Morphine Sulphate	0.5	1	17.5 ± 8.5	1.3 ± 0.9
	1.5		0	0
	4.5		0	0
Morphine Methiodide	0.5	0.89	0	0
	1.5		1.3 ± 1.3	0.1 ± 0.1
	4.5		11.3 ± 1.3	2.3 ± 0.3
	10.0		26.3 ± 8.5	3.3 ± 1.2
	20.0		45.0 ± 3.5	3.9 ± 0.6
Codeine Methiodide	10.0	0.86	22.5 ± 6.3	2.8 ± 0.3
	20.0		36.3 ± 5.5	5.1 ± 2.2

Appendix IV

Appendix IV

Procedure for Thionin Staining

At least 24 hours after the brain sections were placed on slides, they were stained with thionin, according to the following procedure:

Immersion solution	Time Slides Immersed
1) absolute ethanol	two minutes
2) 95% ethanol	two minutes
3) 70% ethanol	two minutes
4) distilled water	rinse
5) thionin stain	15 minutes
6) distilled water	rinse
7) 70% ethanol	two minutes
8) 95% ethanol	two minutes
9) 95% ethanol	two minutes
10) isopropyl alcohol	rinse
11) xylene	three minutes

Thionin Stain

The thionin stain contained 0.6% acetic acid (180 ml), 0.8% sodium acetate anhydrous (20 ml) and 1.0% thionin (5 ml). These ingredients were combined and filtered.

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