Pharmacology Biochemistry & Behavior, Vol. 39, pp. 1003–1009. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

RAPID COMMUNICATION

Hypothermia and Feeding Induced Simultaneously in Rats by Perfusion of Neuropeptide Y in Preoptic Area

AMY K. ROSCOE AND R. D. MYERS[1]

Departments of Pharmacology and Psychiatric Medicine, School of Medicine
East Carolina University, Greenville, NC 27858

Received 6 May 1991

ROSCOE, A. K. AND R. D. MYERS. *Hypothermia and feeding induced simultaneously in rats by perfusion of neuropeptide Y in preoptic area.* PHARMACOL BIOCHEM BEHAV **39**(4) 1003–1009, 1991.—Changes in the body temperature (T_{bo}) of the unrestrained rat as well as the hyperphagic-like ingestion of food were simultaneously determined during the sustained elevation of neuropeptide Y_{1-36} (NPY) within the anterior hypothalamic preoptic area (AH/POA). A single guide tube was implanted stereotaxically in each rat for repeated perfusions by means of push-pull cannulae of either the CSF solvent vehicle or NPY. Following postoperative recovery, each site in the AH/POA was perfused for 6.0 min at a rate of 20 μl/min over four successive intervals at a concentration of 100 ng/1.0 μl or 250 ng/1.0 μl, with an interval of 6.0 min intervening between perfusions. During repeated perfusions of NPY in the fully sated and normothermic rat, concentration-dependent eating, or a hypothermia or both responses occurred simultaneously. Mean cumulative intakes of food over 3.0 h were 12.1 ± 1.4 and 21.5 ± 2.7 g following the 100 and 250 ng concentrations of NPY, respectively. The mean maximal declines in T_{bo} were -0.92 ± 0.21 and -1.1 ± 0.28 °C, respectively after the lower and higher concentrations of the peptide. Push-pull perfusions of artificial CSF control vehicle at homologous anatomical sites in the AH/POA were without effect on feeding or the T_{bo} of the rats. These results demonstrate that repeated and sustained elevation of NPY in the AH/POA can cause a perturbation of the neuronal mechanisms underlying the normal "set-point" for body temperature as well as satiety. Further, as long as the level of NPY in the hypothalamus is chronically elevated over time, a shift in T_{bo} or persistent eating occurs either simultaneously or independently depending upon the population of neurons affected. It is thus envisaged that NPY plays an integrative role metabolically within neurons of the AH/POA in the control of energy metabolism coupled with caloric intake.

Neuropeptide Y (NPY) Feeding Paraventricular nucleus PVN Body temperature Eating Food intake Peptides Push-pull perfusion Hunger Hypothalamus Thermoregulation Water intake Satiety mechanism Obesity

HISTORICALLY, the neurohumoral systems in the diencephalon underlying the processes of thermoregulation, hunger and satiety have been considered to operate concordantly (22). In early studies it was shown that the infusion of the catecholamine neurotransmitter, norepinephrine (NE), in the anterior hypothalamic, preoptic area (AH/POA) of the satiated monkey causes a decline in core temperature (T_{bo}) and spontaneous eating concomitantly over a one-hour period (37). This finding corresponded well to the observation that a protracted lowering of the body temperature (T_{bo}) of the rat induces a compensatory long-term increase in the ingestion of food (7). In both cases the two seminal concepts of Brobeck thus were supported: that an interrelationship exists between the energy requirement of the animal and its level of T_{bo}, and that feeding is one form of thermoregulatory behavior (3).

Noradrenergic neurons in the hypothalamus are thought now to be a principal class of cells underlying either feeding or a thermoregulatory change or both responses (22). Considerable physiological and pharmacological evidence has accumulated over the past decade in support of this viewpoint. For example, NE acts on medial hypothalamic structures to evoke feeding, but in the AH/POA to induce hypothermia in the rat, cat monkey and other species (16, 17, 25). Further, a hyperthermic episode, the act of feeding or a glucoprivic challenge evoke the release of NE from circumscribed sites in the anterior and medial hypothalamus (18, 25, 32).

Previously, it was shown that an intracerebroventricular (ICV) injection in the rat or mouse of neuropeptide Y (NPY) produces intense feeding behavior (4,30) and a fall in T_{bo} (5,11). These observations raise the question in parallel to that of NE in terms

[1]Requests for reprints should be addressed to R. D. Myers, Department of Pharmacology, ECU School of Medicine, Greenville, NC 27858-4354.

of the respective hypothalamic role of NPY in hunger and thermoregulatory mechanisms. The purpose of this study, therefore, was to characterize both the ingestive and temperature responses in the rat following the central administration of NPY. In contrast to procedures in which NPY was given by the anatomically nonspecific ICV route (22), NPY was perfused by means of a push-pull cannula system within a circumscribed area of tissue in the AH/POA. This region of the telencephalon was selected because of its well known involvement in the functional control of T_{bo} and feeding (24,33). By means of the recurrent and sustained elevation of NPY in the AH/POA, the temporal course of the responses of NPY as well as maximal effects could be ascertained during perfusion of nanogram concentrations of the peptide.

METHOD

Male Sprague-Dawley rats (N = 9), weighing between 562 and 746 g, were housed individually and kept on a 12-h light-dark cycle (0730–1930 h) at an ambient temperature of 22 ± 1.5°C. Water and Purina rat chow were provided ad lib to the animals, and their intakes as well as body weights were recorded daily.

Surgery and Test Procedures

Each rat was anesthetized with 35–45 mg/kg sodium pentobarbital administered intraperitoneally and placed in a Kopf stereotaxic instrument. A 20-ga thin-walled stainless steel guide tube, 12 mm in length, was implanted stereotaxically so that the tip was positioned 6.0 mm ventral to the dura mater. The coordinates according to Paxinos and Watson (31) were as follows: AP, 8.5; LAT, 0.8; and HOR, 6.0. A 23-ga stylet of the same length and tip bevel was inserted into the guide tube to prevent its occlusion. Postoperatively, each rat was given specially prepared chocolate biscuits consisting of 38% ground Purina rat chow, 30% sucrose, 14% w/v commercial evaporated milk, and 18% Nestle Quik chocolate powder (29). After 3 days, each rat was adapted to the experimental procedures. The T_{bo} of the animals was monitored daily over a 3–4-h interval by means of a YSI 401 temperature probe, which was inserted 4.0 cm into the colon and held by surgical tape wrapped gently around the base of the tail.

The anatomical position of the intended sites of microinjection was validated for each rat by a standard norepinephrine (NE) test (22,29). After the rat was placed in a standard open perfusion chamber, 1.5 μg/μl of NE HCl (Sigma), dissolved in a pyrogen-free artificial CSF vehicle (34), was microinjected over a 1.0-min interval into a site within the AH/POA. Changes in temperature and food intake were recorded over the next 1.0-h period. The criterion for a positive feeding response was a cumulative intake of ≥5.0 g food at 0.5 h after the injection of NE. Subsequently, a perfusion of NPY was undertaken only at the sites at which NE elicited feeding.

Synthesis of NPY

NPY_{1-36} was kindly synthesized in the laboratory of Dr. J. A. Nyce at the ECU School of Medicine by means of Fmoc-BOP chemistry. An automated BioSearch model 9600 peptide synthesizer was used to prepare the samples. The side-chain protected amino acids (Milligen BioSearch) were: Tyr (tBu [tert-butyl]), Thr (tBu), Ser (tBu), Glx (Tmob [2,4,6-trimethoxybenzyl]), Asx (Tmob), Arg (Mtr [4-methoxy-2,3,6-trimethylbenzenesulfonyl]), His (Trt [triphenylmethyl]), Asp (Ot Bu [t-butyl ester]) and Lys (Boc [t-butyloxycarbonil]). The nonprotected amino acids were Ile, Leu, Ala, Met, Pro and Gly. To deprotect the final product, TFA/thioanisole/ethanedithiol/anisole was used in molar excess (10 ml/g), which serves to separate the peptide from the resin (PAL Resin) and removes side chain protecting groups. After completion of the deprotection procedures, the compound was purified over a 1 × 25 cm VYDAC 5 micron C_{18} column using a Waters model 600-E HPLC with 0.1% TFA →60% acetonitrile in 0.1% TFA applying a linear gradient over 45 min. The main peak detected by UV absorbance at 215 nm was collected and repeatedly lyophilized. The purity of the end product was ≥95%.

Push-Pull Perfusion Procedures

For the push-pull perfusion of NPY or CSF control vehicle, a standard concentric cannula system was used, which consisted of a 28-ga stainless steel inner or push cannula and a 23-ga thin-walled outer or pull cannula (23, 26, 29). The inner and outer cannulae were connected by PE 20 and PE 50 tubing, respectively, to 1.0 ml Teflon tipped Hamilton gas-tight syringes mounted on the infusion-withdrawal decks of a Harvard model 934 pump. The syringes, tubing and cannula assembly were kept in 70% ethanol, but prior to an experiment the system was flushed repeatedly with pyrogen-free artificial CSF (16,20). The artificial CSF vehicle and vehicle containing the peptide were filtered through a 0.22 μm Millipore filter before each experiment.

The sequence of perfusions was initiated ordinarily at 900–1000 h and continued through 1300–1400 h. Immediately before a perfusion was begun, a 1.0 mm bubble of air was introduced into the pull tubing line so that the flow of perfusate could be visually monitored continuously (23). Then the cannula assembly was positioned in the guide tube so that the tip rested at the site at which NE evoked feeding (vide supra). The first perfusion consisted of control CSF delivered to the site over a 6.0-min interval and at a flow rate of 20 μl/min. Following a 6.0-min rest period a second CSF perfusion was undertaken identically. Then either 100 or 250 ng/1.0 μl of NPY were perfused at a flow rate of 20 μl/min. Three additional perfusions were carried out consecutively, following identical procedures, again with a 6.0-min interval elapsing between each. The actual concentration of NPY was estimated to be 10–15% of the perfused concentration because the recovery of solute from the cerebral parenchyma in this push-pull system is approximately 85–90% (26).

The set of perfusions with NPY was always followed by two or more postcontrol perfusions of CSF at a time which depended on the T_{bo} of the animal. A counter-balanced design was used for the order of administration of the two concentrations of NPY. Throughout each experiment, both commercial food pellets and chocolate biscuits were equally available to the animal ad lib. During the series of perfusions, cumulative intakes of food and measures of T_{bo} were recorded at 15 min, 30 min, 1.0 h and every h thereafter.

Histological and Statistical Analysis

Following the experiments, each rat was given an overdose of sodium pentobarbital and perfused transcardially with 10% buffered neutral formalin. The brain was removed from the cranium and sectioned on a cryostat through the plane of the perfusion site. Sections were then stained with cresyl violet according to standard histological procedures (32). From an analysis of the histological material under light microscopy, the position of the cannulae and respective sites of perfusion were subsequently verified and "mapped" anatomically.

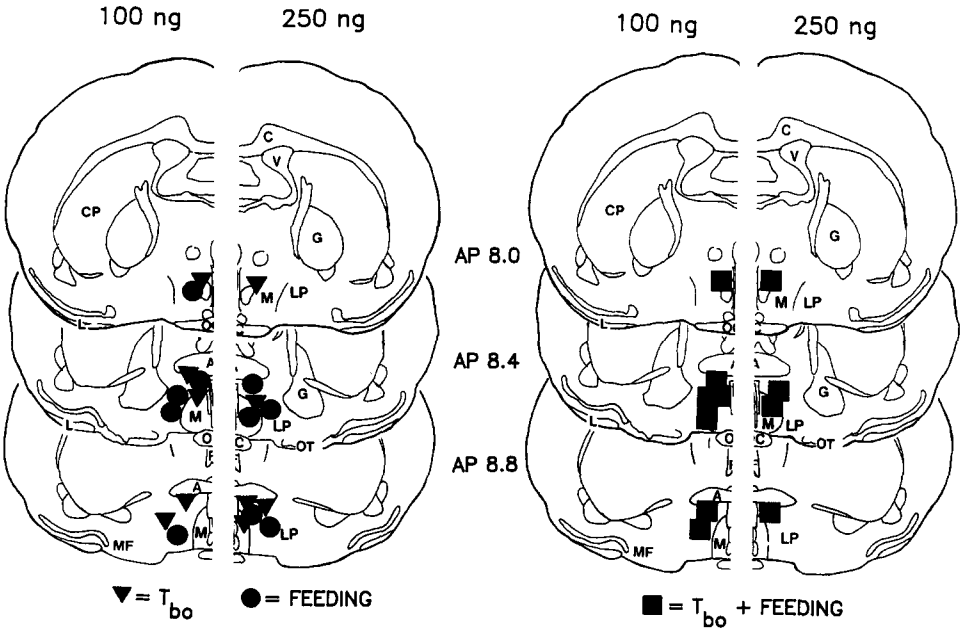

FIG. 1. Anatomical mapping of sites within coronal planes AP 8.0, 8.4 and 8.8 at which a concentration of 100 ng/μl NPY (left section), 250 ng/μl NPY (right section) or control CSF was perfused repeatedly at a rate of 20 μl/min by means of push-pull cannulae. Each site was reactive to NPY in terms of its mediation of: (left) hypothermia (▼) in the normothermic rat or spontaneous feeding (●) in the fully sated animal; and (right) both feeding and hypothermic responses (■) simultaneously. Anatomical abbreviations are: A, anterior commissure; C, corpus callosum; CP, caudate-putamen; F, fornix; G, globus pallidus; L, lateral olfactory tract; LP, lateral preoptic area; M, medial preoptic area; MF, medial forebrain bundle; OC, optic chiasm; OT, olfactory tubercle; S, septum pellucidum; V, lateral cerebral ventricle.

The data were analyzed by computer by means of the Stat-Mate software program. One-way analyses of variance were performed followed by Newman-Keuls tests when appropriate. For comparisons of the results of two sets of food intake or temperature measures, Student's *t*-tests also were performed. A *p*-value of <0.05 was considered to be statistically significant.

RESULTS

A composite anatomical mapping of sites of perfusion is presented in Fig. 1 for both the lower (left) and higher (right) concentrations of the perfused peptide. Sites in which NPY evoked a spontaneous feeding response concomitant with hypothermia, feeding alone or only a change in temperature are differentiated by separate symbols as designated. The two criteria for a positive feeding and/or temperature response after the onset of perfusions of NPY at the NE-reactive site (vide supra) were: (a) a cumulative intake of ≥ 5.0 g food at the 0.5 h interval; and (b) a change in T_{bo} of ≥ 0.3°C within 15 min after the cessation of perfusions.

Temperature Responses to NPY

Both concentrations of NPY evoked a significant hypothermia in response to NPY which commenced after a combined mean latency of 0.48 ± 0.2 h following the onset of a perfusion. As illustrated in Fig. 2, the significant decline below the controls in mean T_{bo} reached its nadir by 1.0 h following repeated perfusions of the 100 ng concentration of NPY, $F(1,82) = 148.8$, $p<0.01$, and by 1.5 h after 250 ng NPY, $F(1,82) = 263.9$, $p<0.01$. Whereas the T_{bo} of the rats returned to the mean baseline level after the lower concentration of NPY, 250 ng NPY caused a substantially more protracted delay in the recovery of T_{bo}. Parallel perfusions with the artificial CSF control vehicle were without significant effect on the T_{bo} of the rats (Fig. 2).

Feeding Responses to NPY

A significant increase in the intake of food in the rats, compared to that of the controls, was produced by NPY perfused in both the lower, $F(1,70) = 310.32$, $p<0.01$, and higher, $F(1,76) = 496.18$, $p<0.01$, concentrations. As portrayed in Fig. 3, the mean cumulative intake of food over the 3.0-h test interval of the rats perfused with the 250 ng concentration was significantly higher than that of rats given the 100 ng/1.0 μl concentration, $F(1,64) = 81.03$, $p<0.01$. The CSF solvent vehicle perfused at the same sites produced no significant effect on the consumption of food by the control rats (Fig. 3).

Simultaneous Feeding and Hypothermia

The relationship between the intakes of food and simultaneous decline in T_{bo} of the rats to the respective intervals of perfusion is presented in Fig. 4. An overall analysis of the amount of food consumed by the rats following the initial two perfusions of CSF control vehicle and the 100 and 250 ng concentrations of NPY revealed significant differences between the groups, $F(2,107) = 73.18$, $p<0.01$. Although the intakes between the two NPY-treated groups were similar initially, the higher concentration of the peptide sustained the ingestion of food significantly, $F(1,66) = 19.37$, $p<0.01$, well after the perfusions had ceased, as shown in Fig. 4.

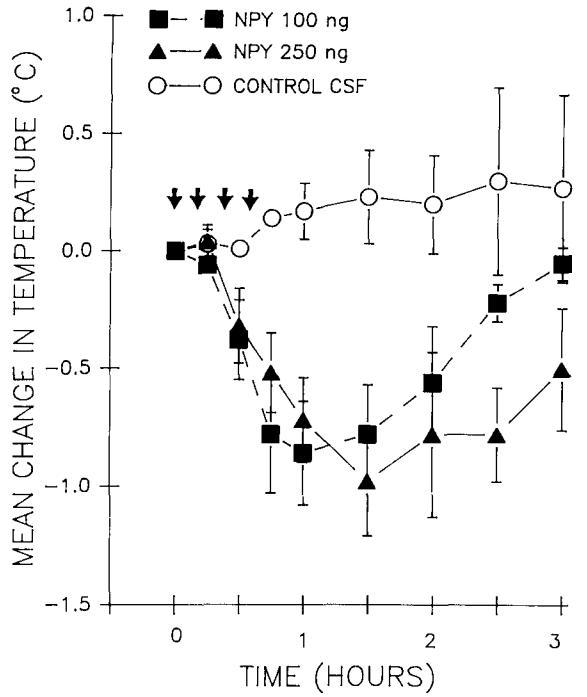

FIG. 2. Mean ± S.E. changes in T_{bo} in °C from baseline during repeated perfusions (arrows) of 100 ng/μl NPY (n = 5), 250 ng/μl NPY (n = 5) or control CSF (n = 7) at sites within the AH/POA which mediated only an effect on colonic temperature.

The feeding response subsequent to the first perfusion of either concentration of NPY essentially paralleled the significant decline, $F(2,107) = 23.88$, $p < 0.01$, in the respective slopes of the T_{bo} of the rats (Fig. 4) in comparison to the controls. The overall decrease in T_{bo} of the group perfused with 250 ng concentration of NPY was significantly greater than that produced by the lower concentration, $F(1,66) = 4.58$, $p < 0.05$.

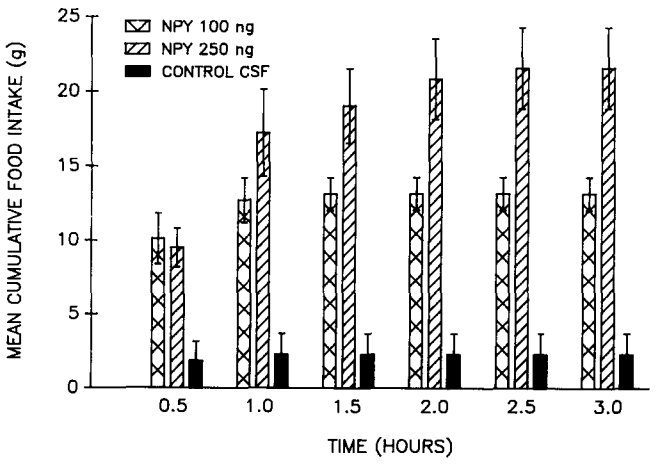

FIG. 3. Mean ± S.E. cumulative intakes of food of fully sated rats in which 100 ng/μl NPY (n = 5), 250 ng/μl NPY (n = 6) or control CSF (n = 7) were perfused at sites within the AH/POA which mediated only an effect on feeding. The time of onset of the 6.0-min intervals of the four successive perfusions was as follows: 0, 12, 24 and 36 min.

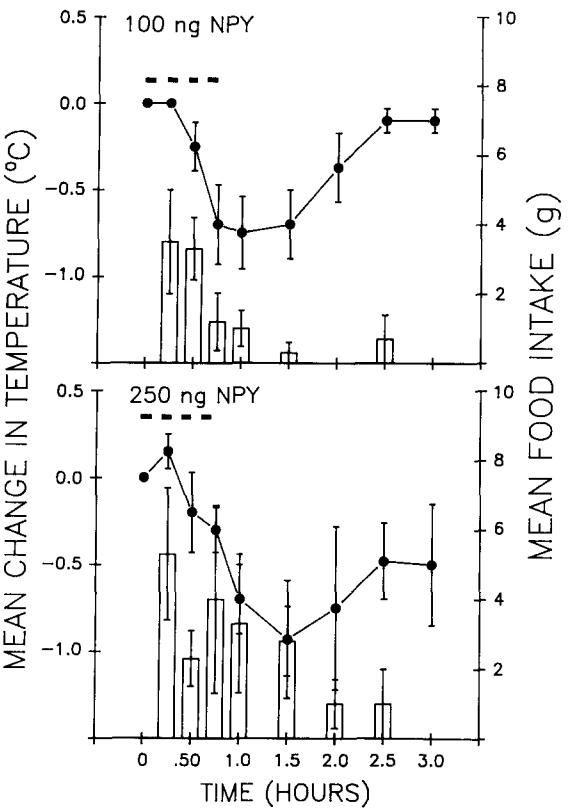

FIG. 4. Mean ± S.E. changes in T_{bo} in °C (lines) and intakes of food in g (bars) from baseline during repeated perfusions of 100 ng/μl NPY (top, n = 6) and 250 ng/μl NPY (bottom, n = 6) at sites in the AH/POA in which both responses were activated in normothermic and fully sated rats. The 6.0-min intervals of the successive perfusions are denoted by the horizontal bars.

Individual Responses to NPY

The specific characteristics of the feeding and temperature responses of the fully sated and normothermic rat during the perfusion of either concentration of NPY depended on the locus of their application within the AH/POA. To illustrate, Fig. 5 (top) shows that the T_{bo} of a representative rat began to fall during the initial perfusion of the 100 ng/μl concentration of NPY at a site adjacent to the third cerebral ventricle contiguous to the medial preoptic nucleus (Fig. 5, top, histological inset). Then, the T_{bo} continued to decline steadily during the next three perfusions. However, the rat did not begin to eat until the second perfusion of NPY and discontinued feeding after the third. Thereafter, only one more bout of feeding occurred at the 1.5-h test interval which corresponded precisely to the nadir in T_{bo} of the rats (Fig. 5, top).

In contrast, NPY delivered in the 250 ng/μl concentration at a more rostral preoptic site just ventral to the anterior commissure (Fig. 5, bottom, histological inset), did not affect the T_{bo} of the rat until the third perfusion commenced. However, the rat began to feed just after the first perfusion and during the second and third perfusions before any significant change occurred in the T_{bo} of the rat. Again, the rat fed also at the 1.5- and 2.0-h intervals (Fig. 5, bottom), thus paralleling the point of maximal decrease in the animal's T_{bo}.

DISCUSSION

The present results demonstrate that repeated perfusions of NPY to elevate its level in the AH/POA cause a protracted hy-

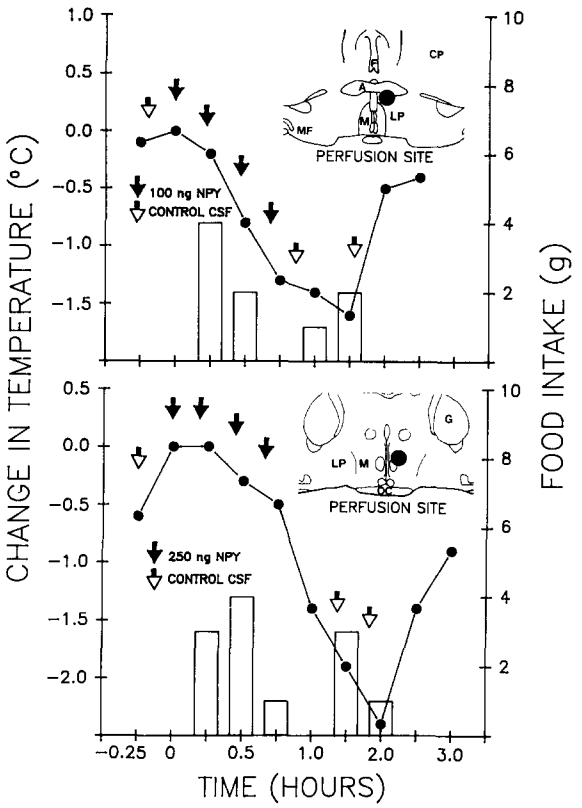

FIG. 5. Changes in T_{bo} (lines) and intake of food (bars) from baseline of two representative normothermic and fully sated rats in which a concentration of 100 ng/μl NPY (top) and 250 ng/μl NPY (bottom) was perfused repeatedly (arrows) in the AH/POA. The individual site of perfusion depicted by the filled circle in the respective histological inset was (top) contiguous to the PVN and (bottom) adjacent to midline.

pothermia, a "stimulus-bound" type of feeding or both responses simultaneously. The temporal characteristics as well as the magnitude of these functional changes depend not only on the concentration of the peptide perfused directly in the tissue but on the anatomical site of the perfusion within the AH/POA (19,29). That is, successive push-pull perfusions over time of NPY at either of the two concentrations sustain spontaneously evoked feeding, the decline in temperature or both responses which differed according to the level of NPY present within each respective anatomical site of its elevation. Since reflexive-like eating could occur without a change in T_{bo} and, conversely, T_{bo} of the rat declined without its ingesting any food, a separate system or population of neurons must exist in the AH/POA which are "coded" to subserve these functions independently. However, the anatomical maps of Fig. 1 strongly suggest that neurons reactive to NPY which stimulate eating or dissipation of body heat are contiguous to one another if not overlapping entirely. Anatomically, therefore, the cells functionally responsible for the activation of feeding (e.g., glucoreceptors) or loss of heat (thermodetectors) must be densely packed and interspersed together within the AH/POA (15,22).

Initially it was reported that NPY has minimal effects on the core temperature of the rat (6). However, in the dog, NPY given ICV in a relatively high dose evokes a mild hypothermia (21) and attenuates partially the hyperthermia induced by a prostaglandin (8). More recently, Esteban (5) showed that 2–5 μg NPY injected ICV produces a short-lived dose-dependent hypothermia in the mouse; since the animals had no food available, a functional interaction between feeding and temperature consequently was not ascertained. A high dose of NPY infused ICV in the rat also impairs motor coordination in the rat, lowers its T_{bo}, alters feeding and muscle tone, and even elicits catalepsy (11). Taken together, it is apparent from these data that a neuroanatomically specific approach is required to elucidate the intricate functional properties of NPY (14,36).

Anatomically, the sites reactive to NPY are in large part homologous to those within which an injection of NE evokes a hypothermia or a feeding response, namely the medial aspect of the POA. Thus the hypothermia in response to NPY may be due to a local modulatory action of the peptide on catecholaminergic neurons within the AH/POA (29). Both dopaminergic and noradrenergic cells which comprise in part the thermosensitive and pyrogen reactive region of the diencephalon (1,22) are thought to underlie the mechanism for heat dissipation in the rodent (24). Although dopaminergic neurons in the rostral hypothalamus have been implicated also in the thermoregulatory mechanisms for heat loss (34), the cells of origin of the heat loss pathway in the AH/POA are considered to be principally noradrenergic in nature (24,27). In this connection, NPY releases NE in the hypothalamus of the rat, possibly through an α_2 adrenoreceptor mechanism (38). Thus the peptide may evoke hypothermia and feeding by activation of this subtype of catecholamine receptor. However, peripheral treatment of the mouse with yohimbine, an α_2 adrenoreceptor antagonist which blocks clonidine hypothermia, does not alter the magnitude of hypothermia induced by NPY infused centrally (5). Hence, NPY may function partially through another subtype of NE receptor on neurons within the AH/POA. Alternatively, NPY could act on dopaminergic neurons perhaps through the release of DA from thermosensitive neurons in the AH/POA (28,34).

The physiological interrelationship between the control of T_{bo} in an animal and its intake of calories is well known (2,12). One would envisage, therefore, that the interdependence of these two vital processes is represented neuronally by a closely knit conglomerate of neurons in the POA or AH. These neurons conceivably serve in the functional integration of feeding behavior, contingent upon a specific state of hunger, and the defence of the "set-point" for T_{bo} against fluctuations in the environmental temperature to which the animal is perpetually challenged. From a functional standpoint, the magnitude of food intake covaries on a moment by moment or daily basis with the ambient temperature to which a rat is exposed, independently of the strain or age of the animal (9). For example, at a low ambient temperature, feeding behavior is enhanced as a reaction in defense against the physiological challenge of a cold environment in that frequency of feeding increases simultaneously with the onset of a low ambient temperature (12). In accord with this, our results show that NPY perfused at certain sites within the AH/POA stimulates feeding only after the thermal displacement in the T_{bo} of the rat. Thereafter, bouts of feeding continue well after the perfusion of NPY is concluded and while the hypothermic response is still in progress.

Although the temporal course of food intake and perturbation of body temperature was not reported, NPY given ICV to the rat evokes feeding and hypothermia (11). Apparently, the portion of the molecule responsible for the functional component underlying energy metabolism and/or heat loss ostensibly comprises the N-terminus tyrosine residue. That is, NPY_{2-36}, but not other fragments, acts to augment food intake in a dose-dependent manner similar to NPY but fails to significantly alter the temperature of the rat (10). Alternatively, the receptor recognition sites for NPY, already characterized in the hypothalamus of the rat (13), may simply not accept the NPY_{2-36} fragment, devoid of

tyrosine at the 1 position, on the respective thermosensitive neurons in AH/POA which possess an affinity for the native molecule (35).

Finally, our results strongly suggest that at the level of neurons of the AH/POA, NPY participates in the integrative mechanisms responsible for the control of energy metabolism, heat conservation and caloric intake. Physiologically, a neuronal signal for heat loss or a nutritional deficit generated centrally in the AH/POA by an elevation in the level of NPY could nullify both the peripheral sensors underlying the temperature set-point in the normothermic rat as well as those reflecting a condition of satiety in the fully fed animal.

ACKNOWLEDGEMENTS

This research was supported by National Science Foundation Grant BNS 84-10663 to R.D.M. We thank Miles F. Lankford and Sherry Leonard for their excellent technical assistance. We are particularly grateful to Dr. Jonathan W. Nyce of the Department of Pharmacology in the School of Medicine for the synthesis of NPY as well as for his constructive suggestions throughout.

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