Neuropeptide Y Perfused in the Preoptic Area of Rats Shifts Extracellular Efflux of Dopamine, Norepinephrine and Serotonin During Hypothermia and Feeding

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(Accepted February 2, 1996)

This study examined the localized action of neuropeptide Y (NPY) on monoamine transmitter activity in the hypothalamus of the unrestrained rat as this peptide induced hypothermia, spontaneous feeding or both responses simultaneously. A guide tube was implanted in the anterior hypothalamic pre-optic area (AH/POA) of Sprague-Dawley rats. Then either control CSF vehicle or NPY in a dose of either 100 ng/μl or 250 ng/μl was perfused by push-pull cannulae in this structure in the fully satiated, normothermic rat. Successive perfusions were carried out at a rate of 20 μl/min for 6.0 min with an interval of 6.0 min elapsing between each. Samples of perfusate were assayed by HPLC for their levels of dopamine (DA), norepinephrine (NE), serotonin (5-HT) and their respective metabolites. Whereas control CSF was without effect on body temperature (Tb) or feeding, repeated perfusions of NPY over 3.0 hr caused dose-dependent eating from 4 to 39 g of food, hypothermia of 0.9 to 2.3°C or both responses concurrently. As the rats consumed 11–39 g of food, the efflux of NE, MHPG, DOPAC and 5-HT increased significantly while the Tb of the rat declined simultaneously with eating behavior, the levels in perfusate of DOPAC and HVA increased significantly while MHPG declined. During perfusion of the AH/POA with NPY the turnover of NE declined while DA and 5-HT turnover increased during hypothermia alone or when accompanied by feeding. These results demonstrate that the sustained elevation in NPY within the AH/POA causes a selective alteration in the activity of the neurotransmitters implicated in thermoregulation, satiety and hunger. These findings suggest that both DA and NE comprise intermediary factors facilitating the action of NPY on neurons involved in thermoregulatory and ingestive processes. The local activity of NPY on hypothalamic neurons apparently shifts the functional balance of serotonergic and catecholaminergic neurons now thought to play a primary role in the control of energy metabolism and caloric intake.

KEY WORDS: Neuropeptide-Y; feeding; norepinephrine; dopamine; serotonin; food intake; NPY; anterior hypothalamus; 5-HT; push-pull perfusion; hunger; pre-optic area; body temperature; eating; peptides; thermoregulation; satiety mechanism.

INTRODUCTION

Although the steady state elevation of neuropeptide Y₁₋₃₆ (NPY) at circumscribed sites in the medial or anterior hypothalamus of the rat evokes intense feeding and/or hypothermia (8,33,35,41), these responses generally are characterized by a long latency. The nature of the prolonged delay is a perplexing phenomenon and is in marked contrast to the almost immediate hyperphagic and hypothermic action of other neuroactive factors injected centrally including norepinephrine (NE), calmo-

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dulin excess or Ca²⁺ ions (11,13,22,25,30). Previously it was proposed that NPY may not act directly on pre- or post-synaptic receptors on hypothalamic neurons (29) but rather modulates monoamine neurotransmitter activity in the hypothalamus to evoke a change in body temperature (Tb) or feeding (10,29,33).

Evidence for this idea was provided by the finding that NPY perfused in the medial hypothalamus of the rat evokes a selective shift in the efflux of monoamines and their metabolites during insatiable eating (29). That is, the extracellular efflux of both NE and dopamine (DA) was enhanced along with elevated levels of their respective metabolites in NPY containing perfusates (29). Also, the turnover of 5-HT as reflected by the level of 5-HIAA increased in the medial hypothalamus as the rat consumed food. Thus, the amine neurotransmitters could comprise an intermediary step in the functional role played by NPY in the hypothalamus in integrating the control of energy metabolism and caloric intake.

The present experiments were carried out to determine whether NPY alters the normal function of monoaminergic neurons in the AH/POA which are involved in thermoregulatory and consummatory processes (35). In these experiments, NPY was perfused at NE—sensitive sites in the pre-optic area of the rat where the peptide acts to alter thermogenesis and induce a consequent decline in Tb, robust feeding or both responses (35). Then the in vivo activity was determined for norepinephrine (NE), dopamine (DA) and serotonin (5-HT), and their respective metabolites was determined in samples of perfusate collected from the AH/POA. Assays were undertaken by HPLC-EC in order to monitor concurrently the fluctuations in the activity of these neurotransmitters within the AH/POA as a decline in Tb ensued and/or as the rats ingested food.

**EXPERIMENTAL PROCEDURE**

Male Sprague-Dawley rats (N = 14) weighing 646 g ± 33 g at the time of surgery were housed individually in a room kept at an ambient temperature of 22 ± 1.5°C and on 12 hr illumination with lights on at 0630 hr. Water and Purina rat chow were provided ad lib to each animal and intakes as well as body weights were recorded daily at 0930 hr. All experimental procedures used for these studies were approved by the internal animal research committee of the School of Medicine and were in strict compliance with the National Institutes of Health guidelines for the care and usage of laboratory animals.

**Surgical Procedures.** Each rat was anesthetized with 45-60 mg/kg sodium pentobarbital administered intraperitoneally and placed in a Kopf stereotaxic instrument. Following aseptic procedures described earlier (29), a 20 ga thin-walled stainless steel guide tube was implanted stereotaxically according to the following coordinates: (34):

- AP, 8.0-9.0; LAT, 0.2-1.2; and HOR, -6.0. A 23 ga stylet of the same length and bevel was inserted in the guide tube to prevent its occlusion. In a second procedure, a Mini-mitter Model VM-FM temperature—activity transmitter (Mini-mitter Co. Inc. Sunriver, OR) was surgically implanted within the intraparenchymal space of 6 of the rats. Signals from the transmitter were delivered through a computer-linked receiver to the Dataquest data acquisition system for the continuous recording of core Tb. In the remaining rats, the Tb was monitored by a VSI 401 temperature probe inserted 3.0 cm into the colonic and held by surgical tape wrapped gently around the base of the tail.

After 5-7 days elapsed post-operatively, a catecholamine reactive site was determined in each rat by a standard test (29) in which 0.8-1.0 µg of norepinephrine (NE) HCl, dissolved in a concentration of 1.0 mg/ml in a pyrogen-free CSF vehicle (23), was micro-injected into the AH/POA by a Sage pump over 1.0 min. The Tb and intakes of food then were recorded, and the site was considered responsive to NE if either one or both conditions were met within 30 min as follows: a decline in Tb of ≥0.5°C or the consumption of more than 5.0 g of food or both. Thereafter, only rats with reactive sites were used for the experiments.

**Synthesis of NPY.** Using Fmoc-BOP chemistry, NPY was synthesized as described earlier by Dr. J. W. Nyce here at the School of Medicine by means of an automated BioSearch model 9600 peptide synthesizer. The side chain protected amino acids (Milligen Biosearch) were: Tyr (Bu [tert-Butyl]), Thr (Bu), Ser (Bu), Glx (Tmob [2,4,6-Trimethoxybenzyl]), Asx (Tmob), Arg (Mtr [4-Methoxy-2,3,6-trimethylbenzenesulfonyl]), His (Tri [Triphenylmethyl]), Asp (Ot Bu [t-Butyl ester]), and Lys (Boc [t-Butyloxycarboxy]). The non-protected amino acids used were: Ile, Leu, Ala, Met, Pro, and Gly. The final product was deprotected with 10 ml/g molar excess of TFA/thioanisole/ethanedithiol/anisole which both separates the peptide from the PAL resin and removes side chain protecting groups. After the deprotection process was completed, the compound was purified over a 1.0 × 25 cm VYDAC 5 micron C18 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. Purity of the final product was estimated to be 95%.

**Procedures for Push-pull Perfusion.** A standard concentric push-pull perfusion 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 (23,35). 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 reciprocating decks of a Harvard infusion-withdrawal pump. The cannula assembly, tubing and syringes were stored in 70% ethanol, and prior to an experiment were flushed repeatedly with pyrogen-free artificial CSF (31). Before each experiment, the control CSF and solution containing NPY were filtered through a 0.22 mm non-protein binding Acrodisc syringe filter (Gelman).

Prior to a perfusion, a 2.0 mm bubble of air was introduced in the pull tubing so that the flow rate of perfusate was continuously monitored visually. Then the push-pull cannula assembly was lowered into the guide tube to the depth of the NE reactive locus. At the beginning of each experiment, the site was perfused with control CSF over a 6.0 min interval at a flow rate of 20 µl/min with a 4.6-6.0 min interval between each subsequent perfusion in the series. Each 120 µl sample of perfusate was collected in a mini-vial containing 10 µl of 0.1 M perchloric acid (PCA) plus 20 µl of the internal standard, 3,4-dihydroxy-benzylamine (DHBA) in a final concentration of 100 µg/ml. Each sample was transferred from the pull tubing line immediately to a 1.5 ml Eppendorf micro-centrifuge tube, containing 5.0 µl of 100