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Forebrain and Hindbrain Involvement of Neuropeptide Y in Ingestive Behaviors of Rats

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STEINMAN, J. L., M. W. GUNION AND J. E. MORLEY. *Forebrain and hindbrain involvement of neuropeptide Y in ingestive behaviors of rats.* PHARMACOL BIOCHEM BEHAV 47(2) 207-214, 1994.—Neuropeptide Y (NPY) is an extremely potent orexigenic agent. These studies demonstrate that the effect of NPY on food and water intake are seen after infusion into either the third (3V) or fourth (4V) ventricle and that this is a specific effect, as it was not seen with the deaminated form. There was a nonsignificant tendency for lateral midbrain knife cuts to increase food intake. Both 3V and 4V NPY infusions showed an attenuated increases in food intake at 1 but not 2 h following NPY infusion in the lateral knife cut rats compared to the sham controls. Medical knife cuts resulted in significantly greater food intake in the basal state and a lesser increase in food intake in response to NPY infused into the 3V. These midbrain data suggest a role for both medical and lateral fibers in mediating the effects of NPY on food intake. Lateral fibers appear to be more important, but their transection only delays the time of onset of the stimulating effect of NPY to the second hour. Lateral knife cuts virtually abolish the effect of 4V NPY on stimulating water intake. 3V NPY in the presence of NPY has a less clear effect at 1 h, but mildly attenuated the NPY effect on water intake at 2 h in lateral knife cut rats. Medial knife cuts slightly attenuate the effect of 3V NPY on water intake. However, medial knife cuts markedly increased basal water ingestion. These studies demonstrate the importance of neuronal communications between third and fourth ventricle associated structures in the modulation of ingestive behavior. Further, it appears that NPY, when infused either into the 3V or 4V, stimulates more than one pathway to modulate ingestive behavior.

Neuropeptide Y Eating Drinking Midbrain transection Forebrain Hindbrain

A NUMBER of neuropeptides have been suggested to play a physiological role in the modulation of food intake (11). Over the past several years, increasing evidence has accumulated indicating that endogenous neurochemicals belonging to the pancreatic polypeptide family act as potent stimulators of feeding behavior (3,10,13,17,18). Of these peptides, neuropeptide Y, a 36-amino-acid peptide which occurs in high concentrations in the hypothalamus, appears to be a particularly important orexigenic agent (7). Intracerebroventricular administration of antisera to NPY results in a decreased food intake in food-deprived rats (20). NPY secretion from the paraventricular nucleus of the hypothalamus is modified by hunger status of the rat (8), and NPY levels and NPY mRNA are altered in hyperphagic animals (1,16,24). In rats, NPY increases fluid as well as food ingestion (3,10,18). The neuronal substrates underlying the modulation of feeding and drinking by NPY have not been elucidated. In addition, it is unknown whether the neural pathways mediating the orexigenic actions of NPY in the rat can be dissociated from those involved in drinking.

In addition to the well-documented role of the hypothalamus in feeding, other neural regions also are thought to influence consummatory behaviors (19). Peripheral input from the gastric viscera to the brain is transmitted via the vagus nerve to brainstem nuclei (9). Accordingly, transection of fibers traversing through the midbrain diminishes the orexigenic efficacy of NE infusions to the PVN (23) as well as the inhibitory actions of peripherally administered cholecystokinin (CCK) (5). Since NPY-containing structures have been visualized within brainstem regions (2), it seemed possible that infusions of NPY directly to this region might modulate feeding and/or drinking in addition to its already known action in forebrain

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(e.g., hypothalamic) regions. The present experiments were designed to analyze both these issues. First, we established whether NPY would increase food and water intake following infusions to either the third ventricle (3V) of the forebrain or the fourth ventricle (4V) of the brainstem. After establishing dose-response curves for these two regions, we examined the effects of partial bilateral midbrain transections on the efficacy of NPY following 3V and 4V infusions. A final component of this study examined the effects of more medially located (midline) transections, previously shown to block the effects of NE on food intake (23), on modulating NPY-induced feeding following 3V infusions. These findings have been presented earlier in abstract form (21,22).

METHODS

Subjects

A total of 70 male adult Sprague-Dawley rats weighing 250–350 g at the start of the experiment were used. Each rat was housed individually in a wire-mesh cage in a light- and temperature-controlled room (12-h light–dark cycle, lights on at 0630). Water and Purina rodent chow (pellets) were available ad lib in food hoppers attached to the front of the cage, except during testing periods (see below).

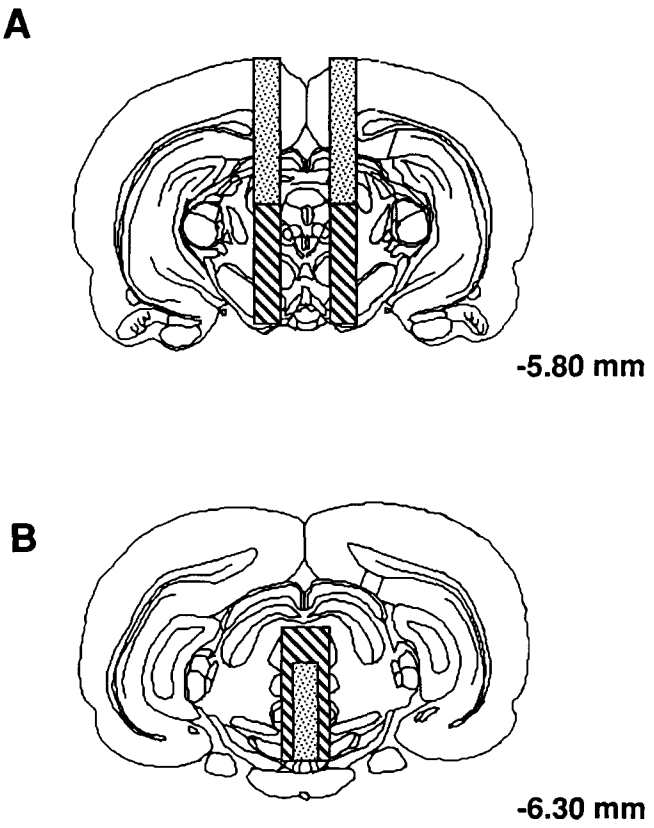

FIG. 1. (A) Localization of lateral knife cuts (LKCs) placed bilaterally at the level of the midbrain, indicating the minimal (hatched) and maximal (stippled) extent of tissue damage. (B) Localization of midline knife cuts (MKCs) placed bilaterally approximately 1 mm caudad to the position for LKCs. The distance (in mm) from bregma is indicated in the lower right corner of each figure. See Methods section for additional details.

Surgery

Stainless steel injection cannulae (Plastic Products, CA) were implanted stereotaxically into the third or fourth ventricles. Ten rats received double cannulae (both 3V and 4V), whereas the remaining 60 were matched for body weight and received either a 3V (N = 40) or a 4V (N = 20) cannula. Under anesthesia (80 mg/kg ketamine plus 10 mg/kg xylazine, IP), using flat skull coordinates, 3V cannulae were placed 3.0 mm caudal to bregma, directly on midline and 6.5 mm ventral to dura; 4V cannulae were placed 11.6 mm caudal to bregma, directly on midline and 6.1 mm ventral to dura. The cannula was attached to a 12″ piece of PE-50 tubing and both cannula and tubing were filled with saline. The tip of the tubing was clamped with hemostats. After a dura hole was made and the cannula penetrated the brain 0.5 mm, the hemostats were removed and the clamped end of the tubing was cut off. The cannula was then lowered to its approximate depth. When the cannula tip entered the ventricle, a drop in the saline was visualized at the end of the PE tubing. This method was used to ensure entry into both 3V and 4V, and histological verification revealed 95% success for ventricular impalement. After implantation, the cannulae were secured to the skull with acrylic cement and stainless steel screws that were anchored to the cranium.

The knife cuts were chosen because they had previously been demonstrated to modulate ingestive behavior (15,23). For animals receiving lateral knife cuts (LKCs: N = 40), a 1.5-mm-wide stainless steel knife fashioned from stock was lowered into the brain, 0.5 mm laterally from the midline, 6.5 mm caudal to bregma, and to a depth of 7.7 mm ventral to dura. Knife cuts were performed 7 to 10 days prior to cannula implants. For animals receiving midline knife cuts (MKCs: N = 20), an encephalotome (Kopf Instruments) with a 1.5-mm wire blade was used to create a midline transection. The blade guide was lowered 0.8 mm from the midline, extended medially, and then moved ventrally to create the transection. The coordinates used for MKC placements were 7.6 mm caudal to bregma, 0.8 mm laterally from the midline, and 7.0 mm ventral to dura. Both LKCs and MKCs were performed bilaterally. Figure 1 illustrates the extent of each transection.

General Testing Procedures

After a 10- to 14-day recovery period following cannula implantation, animals were acclimated to testing procedures. During this postoperative period rats were handled daily, weighed every other day, exposed to vehicle injections, and given food pellets on the floor of their cages in a mock testing procedure. On the morning of the experiments, hopper food and water were removed 20 min before testing. Each animal was given one pellet of food to reduce the effect of novel food in the cage at the time of testing. At the time of testing, the rat was removed from its cage and given a preweighed amount of food and water. Spillage was collected under the cage with paper. Food and water intake were measured 1 and 2 h after injection. The latencies to first eating and drinking also were recorded by an independent observer blind to the drug treatment of the animals. Animals were tested every other day in a counterbalanced design so that each rat received each dose once.

Drugs (human and porcine forms of neuropeptide Y and human form of the free acid) were purchased from Peninsula Laboratories (Belmont, CA) and Sigma Chemical Company (St. Louis). These compounds were administered in doses of

0, 2.5, 5.0, or 10.0 μg per 3 μl of saline/1% bovine serum albumin (vehicle).

Histological Analysis

At the end of experimental testing rats were exposed to Ethrane anesthesia until heavily sedated. Three microliters of dye was delivered to each ventricle. In the case of animals receiving double cannulae, green dye was injected into the 3V and blue dye into the 4V. Five to ten minutes later, while still maintained under anesthesia, the animal was decapitated, the cannula and brain were removed, and the brain stored in formalin until sectioning. At the time when the brain was removed from the cranium, the base of the brain was examined and the presence of dye within the ventral ventricle recorded. Fifty-micron sections were cut on a freezing stage and the sections were stained with cresyl violet and examined for cannula placement as well as knife cuts. The precise placement of cannulae and knife cuts were determined by comparing histological sections to brain maps in the atlas of Paxinos and Watson (14).

After elimination of animals based on histological analysis of lesions and cannulae implants, the total number of animals per group for the LKC experiment were 3V sham-treated = 5, 3V LKC = 9, 4V sham-treated = 9, and 4V LKC = 9. For the MKC experiments, the total number of rats per group were 3V sham-treated = 7 and 3V MKC = 8.

Statistical Analyses

For the initial studies evaluating the effects of NPY administered to the third or fourth ventricles ("double cannulae" rats), the effects of drug, time postinjection, and injection site were analyzed using a four-way analysis of variance (ANOVA) with repeated measures for injection site and experimental subjects. For the studies examining the effects of knife cuts on 3V and 4V administration of NPY, four-way ANOVAs also were used. Post hoc analyses were performed using Duncan's multiple range test for nearly equal *n*s. In addition to analyzing the amount of food or water consumed 1 and 2 h after injection, the total amount of food consumed over 2 h was assessed, as well as the change in food or water intake, calculated by subtracting the values during vehicle (e.g., 0 μg NPY) treatment from the values during drug (e.g., 5 μg NPY) treatment. Unless otherwise stated, all values are presented as mean ± SE.

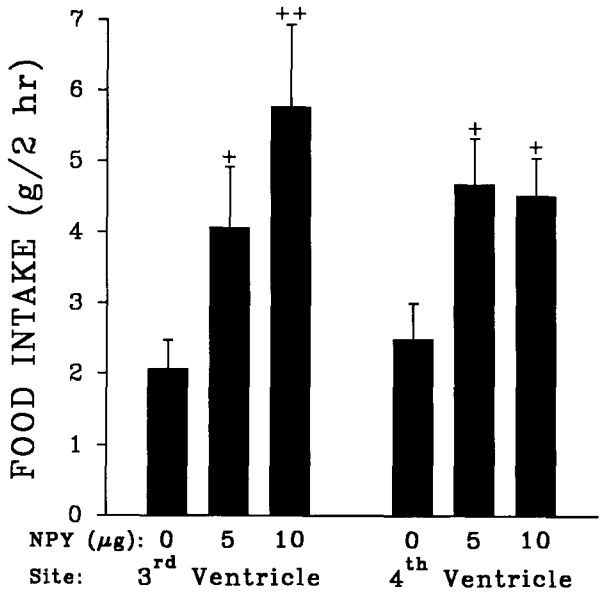

FIG. 2. The cumulative amount of food eaten during a 2-h period was significantly elevated in response to 3V or 4V infusions of neuropeptide Y (5 or 10 μg/3 μl vehicle) as compared to vehicle infusions alone. The *p* values are based on a significant effect for dose, $F(2, 36) = 11.91, p < 0.0003$. $^{+}p < 0.05$, $^{++}p < 0.02$, compared to corresponding 0 dose.

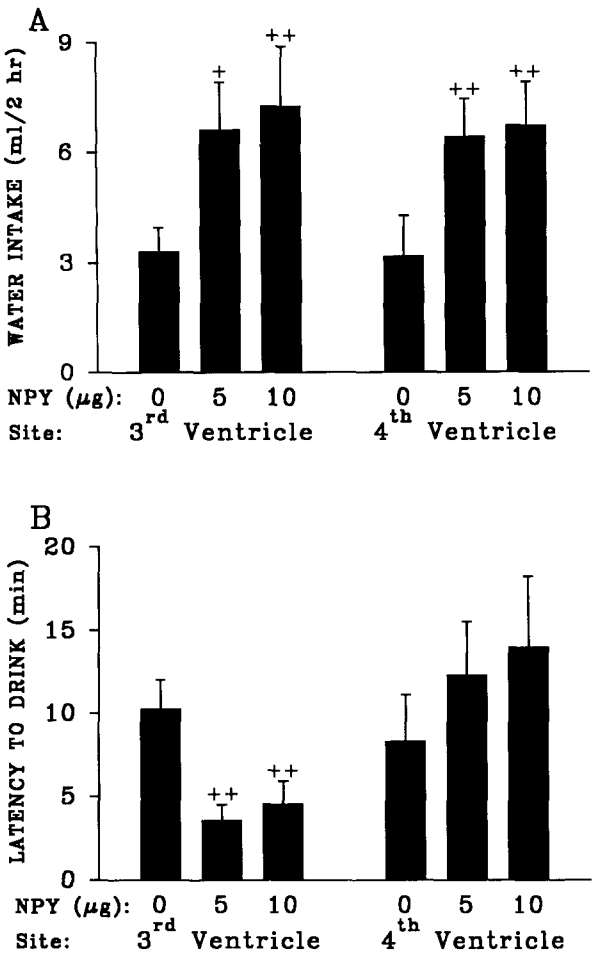

FIG. 3. (A) Neuropeptide Y significantly elevated water intake when infused into both 3V and 4V. Data presented here were collected when food was present in the cage, but the dipsogenic effects of 3V and 4V infusions of NPY were also observed in the absence of food (data not shown), $F(2, 36) = 11.39, p < 0.0004$. (B) The latency to drink was significantly shorter after 3V infusions of NPY, whereas the latency appeared longer after 4V infusions when compared to vehicle infusions, $F(1, 24) = 6.69, p = 0.016$; $^{+}p < 0.05$; $^{++}p < 0.02$, compared to corresponding 0 dose.

RESULTS

Third Versus Fourth Ventricle Infusions—"Double Cannulae" Group

The first series of experiments examined the effects of 0, 5, and 10 μg NPY on food and water intake when adminis-

tered to either the third or fourth ventricles in the same rats ("double cannulae"). As depicted in Fig. 2, animals ate 2.1 ± 0.4 and 2.5 ± 0.5 g when given vehicle (0 μg NPY) injections into the third and fourth ventricles, respectively. By comparison with these control values, the total amount of food consumed over the 2-h postinjection period differed significantly following the administration of 5 and 10 μg of porcine NPY for both ventricles ($ps < 0.05$).

In addition to the effects of NPY on food intake, both 3V and 4V infusions of NPY significantly elevated the amount of water ingested. When tested both in the presence (Fig. 3A) and absence of food in the cage, the amount of water consumed following infusion of 5 or 10 μg of porcine NPY differed significantly as compared to the values obtained following vehicle infusions ($ps < 0.05$). The latency to drink was significantly shorter after both doses of NPY when infused into the third ventricle ($ps < 0.02$), but tended to be longer than for vehicle infusion when given into the fourth ventricle (Fig. 3B).

To determine the biological potency of NPY, we compared the effects of porcine NPY to the human form as well as to its free acid (deaminated) form. The orexigenic (Fig. 4A) and dipsogenic (Fig. 4B) efficacy of NPY appears to depend upon the amination at the C terminal, for the free acid form of NPY (10 μg/3 μl) did not elevate food or water consumption significantly as compared to vehicle injections ($ps > 0.05$) when administered to either ventricle. However, 10 μg of the human form of NPY administered to both 3V and 4V significantly elevated the cumulative amount of food and water consumed over a 2-h period relative to the amounts consumed following vehicle or free acid infusions ($ps < 0.02$).

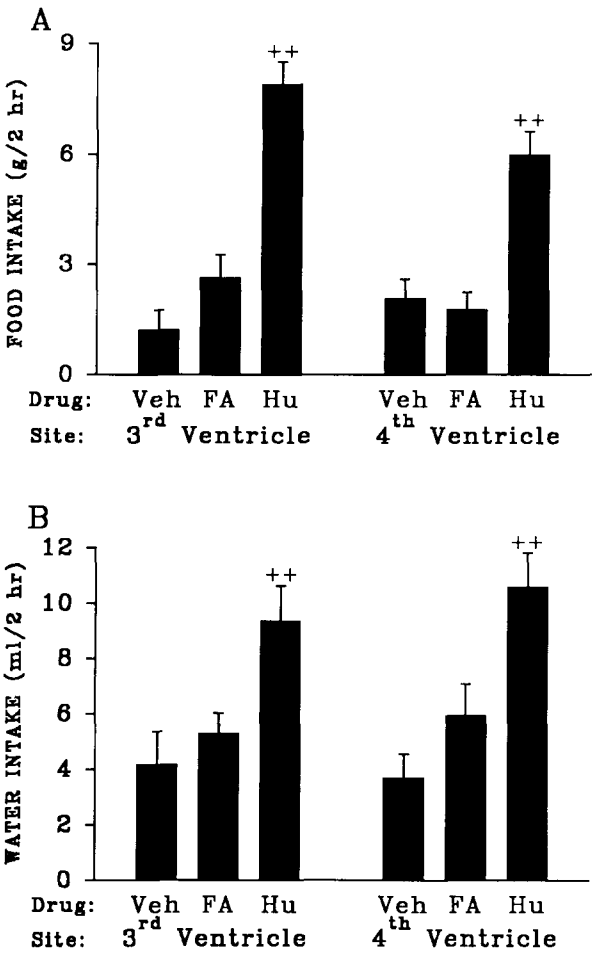

FIG. 4. Cumulative 2-h food intake was significantly elevated by 3V and 4V infusions of the human (HU) form of NPY, whereas the deaminated free acid (FA) form was ineffective in stimulating eating. Veh = vehicle B. Water intake also was significantly increased by 3V and 4V infusions of both porcine and human NPY, but not the free acid form. $^{++}p < 0.02$, compared to 0 dose.

Lateral Knife Cuts

The effects of lateral midbrain transections on food and water intake following infusion of 2.5, 5.0, and 10.0 μg of NPY were evaluated in additional groups of operated and sham-treated rats which had single injection cannula for either the third or the fourth ventricle. As observed in rats with double cannulae, the sham-treated animals with either 3V or 4V cannulae ate significantly more food following infusion of NPY than following vehicle solution (Fig. 5A). This increase in food intake induced by NPY infusions could be attributed to significant elevations occurring at both 1 and 2 h postinfusion.

Cumulative food intake—2 h (Fig. 5A). For the 3V LKC group, the total amount of food consumed over a 2-h period following vehicle infusions did not differ significantly as compared to the sham-treated group, although there was a tendency for the rats with knife cuts to eat more than their sham cohorts ($p > 0.05$). The 5- and 10-μg doses of NPY elevated food intake significantly during the total 2-h period as compared to the amount consumed following vehicle in the 3V LKC group ($ps < 0.05$).

The amount of food consumed by sham-treated rats receiving infusions of 0, 2.5, or 10 μg NPY to the fourth ventricle did not differ significantly as compared to sham-treated rats with 3V infusions. The 4V LKC group did not differ significantly from the 4V sham-treated group. All three doses of NPY elevated food intake significantly as compared to vehicle control values for the 4V LKC group ($ps < 0.05$).

First hour postinfusion (Fig. 5B). For the 1-h period after administration, NPY significantly elevated food intake over vehicle in the 3V (10.0 and 2.5 μg > 0 μg) and 4V sham-treated (2.5, 5.0, and 10.0 μg > 0 μg) groups. In the groups receiving LKCs, only 5 μg NPY significantly elevated food intake over vehicle infusions in the 3V group, whereas only the highest dose (10 μg) of NPY was effective in the 4V LKC group. Due to fluctuations in the mean absolute food intake values during vehicle infusions across the four groups, difference scores were calculated to compare the amount of food eaten by each rat following 2.5, 5.0, and 10.0 μg of NPY infusions to vehicle control infusions. Figure 5B illustrates that the relative amount of food consumed by the 3V LKC group following 10 μg NPY infusions was significantly lower than for the 3V sham-treated group, whereas the difference score for the 4V LKC group following infusions of 5 μg NPY was significantly lower as compared to the 4V sham group ($p < 0.05$).

Second hour postinfusion. By the second hour, food intake was still significantly elevated following 10 μg of NPY in the 3V sham-treated and operated groups as compared to vehicle ($ps < 0.05$); however, food intake in the 4V sham-treated and operated groups was no longer elevated. Food intake was not significantly greater in the second hour for any other doses of NPY as compared to vehicle. Between-group

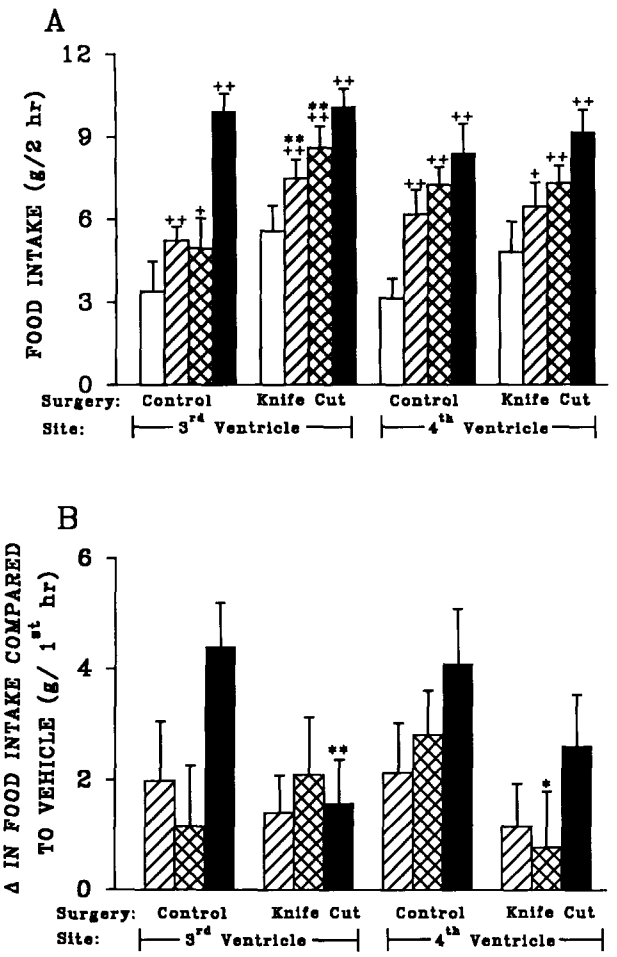

FIG. 5. (A) Cumulative food intake over a 2-h period was significantly elevated by 2.5, 5, and 10 μg NPY infused into the third or fourth ventricle in rats receiving lateral knife cuts (LKCs). For LKC-treated rats receiving infusions into the fourth ventricle, all three doses of NPY significantly elevated food intake over vehicle infusion values, $F(1, 28) = 5.63$, $p < 0.025$. $*p < 0.05$, $**p < 0.01$, compared to same dose given to corresponding sham-treated group. (B) Difference scores comparing the amount of food ingested during the first hour of NPY trials with that measured during vehicle trials indicates that the LKC-treated rats receiving 10 μg of NPY into the 3V or 5 μg of NPY into the 4V showed a significantly lower orexigenic response to NPY when compared to sham-treated rats, $F(2, 56) = 15.52$, $p < 0.0001$. + + is for comparisons vs. shams and + is for comparisons vs. vehicle.

comparisons were not significantly different at any dose at 2 h postinfusion.

Cumulative water intake—2 h (Fig. 6C). As had been observed in the double cannulae animals, NPY infusions of 5.0 and 10.0 μg significantly elevated the amount of water consumed over the 2-h postinfusion period in sham-operated groups with single 3V or 4V cannulae ($ps < 0.05$). The low dose of NPY (2.5 μg) was sufficient to elevate water intake significantly in the 4V ($p < 0.01$) but not the 3V ($p > 0.05$) sham-treated groups. In the 3V LKC group, 2.5 and 5 μg (but not 10 μg) of NPY significantly elevated water intake as compared to the 0-μg dose. However, the amount of water consumed by 3V LKC rats was significantly lower than that consumed by the 3V sham-treated control group at the 5 and 10 μg doses. At the 10-μg dose, the 4V LKC group drank significantly less than the 4V sham-treated group ($p < 0.05$).

First hour postinfusion (Figs. 6A and B). These differences in cumulative water intake over 2 h were attributable to knife-cut-related changes occurring primarily in the first hour after infusion (see Fig. 6A). One hour postinjection, both the 3V and 4V sham-treated groups showed significant elevations in water intake at all doses of NPY ($ps < 0.05$). By contrast, only the 2.5-μg dose of NPY significantly elevated water intake in the 3V LKC group ($p < 0.05$) and 10 μg of NPY actually resulted in a significantly lower amount of water ingested during this period relative to the vehicle ($p < 0.01$). During the first hour after drug infusion there were no signifi-

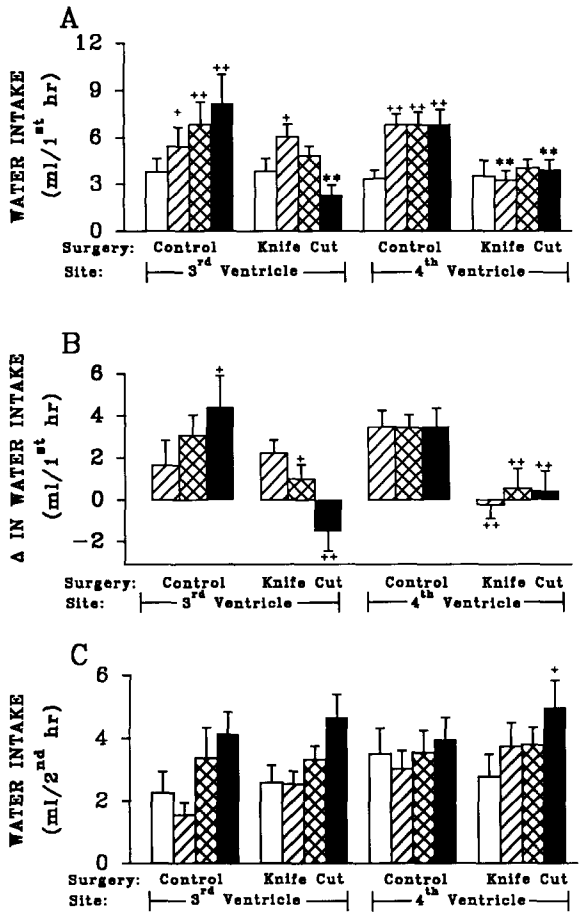

FIG. 6. (A) The amount of water consumed during the first hour postinfusion was not significantly elevated by 5 or 10 μg NPY when infused into the 3V in LKC-treated rats. Fourth ventricular infusion of NPY in LKC-treated rats did not significantly elevate drinking during this period at any of the doses tested, $F(3, 84) = 30.02$, $p < 0.0001$. (B) Difference scores, comparing the amount of water ingested after NPY infusion (first hour) with that consumed following vehicle infusion showing that both 3V and 4V LKC groups did not increase their drinking in response to 5 or 10 μg NPY. $*p < 0.05$, $**p < 0.01$, as compared to corresponding sham-treated group, based on $F(2, 56) = 15.52$, $p < 0.0001$. (C) During the second hour, 10 μg of NPY infused into the 4V significantly elevated drinking in the LKC rats, although 3V infusions did not. For significant differences among LKC and sham-treated groups, $F(3, 84) = 7.97$, $p < 0.0001$.

cant differences between any dose of NPY and vehicle in the 4V LKC group (*p*s > 0.05). The effects of LKCs on NPY-induced drinking during the first hour are clearest when the data are expressed as difference scores (Fig. 6B).

Second hour postinfusion. A different pattern was observed in the second hour postinfusion among groups (see Fig. 6C). NPY no longer significantly elevated water intake in either sham-treated group (*p*s > 0.05). In the 3V LKC group, water intake did not differ significantly across doses, whereas the 4V LKC group showed a significant elevation in water intake during the second hour following 10 μg of NPY ($p < 0.01$).

Medial Knife Cuts

Cumulative food and water intake (Fig. 7). Animals receiving MKCs showed significantly higher food intake values following vehicle infusions when compared to their 3V sham-treated controls (Fig. 7A). Both 5 and 10 μg NPY significantly increased food intake above vehicle values in both the MKC and control groups during the total 2-h period. However, the between-group comparisons revealed that the amount of food consumed by the 3V MKC group following 10 μg NPY was significantly lower than that for the sham-treated group.

In the sham-treated group, the cumulative amount of water consumed over the 2-h period was significantly elevated by both 5 and 10 μg of NPY (Fig. 7B). The overall amount of water consumed by rats receiving MKCs was not significantly elevated by either dose; however, this was partly due to the fact that the 3V MKC rats drank significantly more water during the first hour after vehicle (0 μg) infusions (7.95 ± 1.14 g) as compared to the sham-treated rats (4.01 ± 1.12 g).

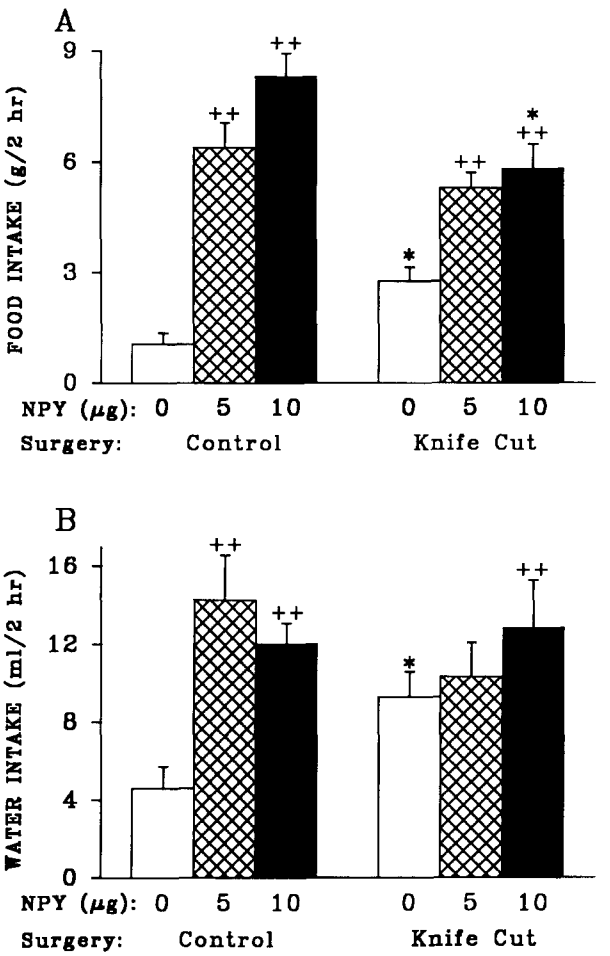

FIG. 7. (A) Animals in the MKC group, which received injections into the 3V, ate significantly more than controls after vehicle infusion. Both 5 and 10 μg of NPY significantly elevated food intake in both the sham-treated and MKC-treated groups when compared to their own vehicle infusion values; however, the total amount of food consumed over the 2-h period in response to both 5 and 10 μg of NPY was significantly lower in the MKC-treated rats as compared to the sham-treated rats, $F(2, 26) = 8.66$, $p < 0.016$. (B) The total amount of water consumed following vehicle infusions was significantly higher in the MKC-treated rats as compared to those receiving sham treatment. Although both 5 and 10 μg of NPY significantly elevated drinking in the sham-treated group, only the 10-μg dose was effective in the MKC-treated group when compared to vehicle infusions.

DISCUSSION

The present studies showed that infusion of neuropeptide Y into the third as well as the fourth ventricle significantly elevated food and water consumption in rats. This demonstrates that NPY acts at the brainstem level to stimulate both feeding and drinking. A similar observation has been reported by Corp et al. (4). Infusions of the deaminated, free acid form of NPY did not significantly alter food or water intake, indicating that the biological potency of NPY depends upon the presence of the amine at the C terminus. This has also been demonstrated to be the case in mice, but only for food and not water ingestion (12). These studies also indicate that the transection of fibers passing through the lateral portions of the midbrain partially reduced the ability of third and fourth ventricular infusions of NPY to elevate food intake. However, the elevation in water intake following NPY infusions to both the third and fourth ventricles was clearly and dramatically reduced by lateral transections of the midbrain. Hence, these findings suggest that the modulation of food and water intake by NPY can be dissociated, since each consummatory response was differentially modulated by the knife cuts. Further support of this hypothesis stems from the findings in rats with more medially placed transections, which also showed differential responses to NPY on food as compared to water intake.

The present findings contribute to and extend the growing body of knowledge pertaining to the role of the pancreatic polypeptide family in feeding behavior. Originally, it was found that infusions of NPY, as well as peptide YY, into the third ventricle produced a robust increase in food intake in female rats (3). Based upon immunohistochemical staining of NPY-containing cell bodies and fibers throughout the nervous system, subsequent experiments revealed that NPY acts within discrete regions of the hypothalamus—notably the paraventricular nucleus (PVN), ventromedial nucleus (VMN), and anterior regions—to modulate feeding in male rats (6,19). The 3V implants used in the present study were aimed at the ventricular region adjacent to the PVN, but infusions probably reached other hypothalamic structures as well. Hence, we were able to demonstrate not only that NPY produced a reliable increase in food and water intake within 1 h after infusion near hypothalamic structures, but also that the free acid form of this peptide is not biologically active in this region. Similarly, infusion of NPY, but not the free acid form, through fourth ventricular implants aimed at brainstem sites known to

be rich in NPY-containing neuronal elements was effective in elevating food and water intake.

A long range efferent NPY projection involves medullary A1 and C1 to C3 catecholaminergic cell groups that also contain NPY. These projections innervate both the paraventricular and supraoptic nuclei (2). All these NPY projections are not necessarily also catecholaminergic projections (6). It is likely that fibers traversing from brainstem regions through the midbrain are involved in the mediation of feeding induced by 4V infusions of NPY. Accordingly, LKCs which transected ascending fibers diminished the effectiveness of NPY on both feeding and drinking when injections were made into the 4V, although the latter was more markedly reduced. By contrast, laterally placed knife cuts reduced the effects of 3V infusions of only the high NPY dose on food intake, leaving the efficacy of lower doses intact. This suggests that the hypothalamic regions in which NPY acts may be less dependent on traversing fibers in their modulation of feeding. However, the elevating effect of NPY on drinking following 3V infusions was markedly attenuated. This finding indicates that fibers descending from the third ventricular region are involved in the forebrain activation of water intake by NPY.

Sahu et al. (15) reported that a low dose of NPY infused into the 3V significantly elevated food intake over a 1-h period in rats receiving a bilateral mesencephalic knife cut when compared to sham-treated cohorts. When a higher dose of NPY was given, the animals receiving transections did not show the proportional increase in food intake that was observed in the controls. The results of the present study are in agreement with these findings in that the low and high doses of NPY were equally efficacious in stimulating food intake in LKC rats within 1 h after infusion. However, the present findings extend this observation, since the 3V LKC rats did show a dose-response sensitivity when the cumulative food intake over 2 h was measured. Hence, this study demonstrates that there may be an initial delay in the stimulatory effect of NPY when infused into the third ventricle of LKC rats, but eventually food intake is nevertheless increased significantly. Whether this delayed effect is due to diffusion of NPY to areas that are not influenced by the LKCs is unknown.

Since 3V infusions of all doses of NPY produced equivalent elevations in food intake in the LKC rats, it is possible, as proposed by Sahu et al. (15), that these rats are supersensitive to NPY and, accordingly, eat more in response to a lower dose. This interpretation corresponds with the fact that laterally placed knife cuts decrease NPY and NE levels in the hypothalamus (15). However, our results raise the alternative possibility that the NPY receptors available after lateral midbrain transections are insufficient to permit the higher doses of NPY to stimulate food intake. When examined as the change in food intake relative to vehicle, the present findings showed that the 3V LKC group did not differ from the sham-operated animals, indicating that the dose-response effect may be masked partially by a slight, but relevant, elevation in basal food intake.

MKCs increased basal food intake and attenuated the ability of NPY to increase food intake after 3V injections. This finding is similar to that of Weiss and Leibowitz (23), that MKCs significantly elevated daily food intake and decreased the food intake produced by norepinephrine (NE) infused into the PVN of the hypothalamus. Although our MKCs were more ventrally located than those described by Weiss and Leibowitz, the extent to which the periventricular gray was damaged is similar in the two studies. This type of knife cut reduced NE-induced food intake by more than 60% (23), whereas we did not see such a substantial reduction in NPY-induced feeding following 3V infusions (approximately 30%). Lateral coronal knife cuts through the midbrain had no effect on NE-induced feeding. Interestingly, in the present study lateral midbrain transections did not alter basal drinking, whereas the more medially placed transections did.

In summary, these studies have demonstrated that structures associated with both the third and fourth ventricle can be stimulated to produce increases in ingestive behavior by NPY. LKCs markedly decreased the ability of 4V infusions of NPY to enhance food and water intake. There was a tendency for NPY to show increased sensitivity as far as food intake was concerned in rats with LKCs. MKCs decreased food intake after 3V NPY infusion and significantly elevated basal water intake, masking any effects of NPY on drinking. These studies confirm the importance of communication between the third and fourth ventricles in the modulation of ingestive behavior.

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