Hypothalamic Localization of the Feeding Effect of Agouti-Related Peptide and \(\alpha\)-Melanocyte-Stimulating Hormone


The melanocortin-4 receptor (MC4R) in the hypothalamus is thought to be important in physiological regulation of food intake. We investigated which hypothalamic areas known to express MC4R are involved in the regulation of feeding by using \(\alpha\)-melanocyte-stimulating hormone (\(\alpha\)-MSH), an endogenous MC4R agonist, and agouti-related peptide (Agrp), an endogenous MC4R antagonist. Cannulae were inserted into the rat hypothalamic paraventricular (PVN), arcuate (Arc), dorsomedial (DMN), and ventromedial (VMN) nuclei; the medial preoptic (MPO), anterior hypothalamic (AHA), and lateral hypothalamic (LHA) areas; and the extrahypothalamic central nucleus of the amygdala (CeA). Agrp (83–132) (0.1 nmol) and [\(\text{Nle}^6, \text{D-Phe}^7\)]\(\alpha\)-MSH (NDP-MSH) (0.1 nmol), a stable \(\alpha\)-MSH analog, were administered to fed and fasted rats, respectively. The PVN, DMN, and MPO were the areas with the greatest response to Agrp and NDP-MSH. At 8 h postinjection, Agrp increased feeding in the PVN by 218 ± 23% (\(P < 0.005\)), in the DMN by 268 ± 42% (\(P < 0.0005\)), and in the MPO by 236 ± 31% (\(P < 0.01\)) compared with a saline control group for each nucleus. NDP-MSH decreased food intake in the PVN by 52 ± 6% (\(P < 0.005\)), in the DMN by 44 ± 6% (\(P < 0.0001\)), and in the MPO by 55 ± 6% (\(P < 0.0001\)) at 1 h postinjection. Injection into the AHA and CeA resulted in smaller alterations in food intake. No changes in feeding were seen after the administration of Agrp into the Arc, LHA, or VMN, but NDP-MSH suppressed food intake in the Arc and LHA. This study indicates that the hypothalamic nuclei expressing MC4R vary in their sensitivity to Agrp and \(\alpha\)-MSH with regard to their effect on feeding. Diabetes 49:177–182, 2000
administration of the COOH-terminal fragment of Agrp (83–132) induced an increase in food intake over a 24-h period (19). The anorectic effect of ICV α-MSH was blocked by both prior and simultaneous injection of Agrp (83–132). The injection of Agrp (83–132) and NDP-MSH into discrete hypothalamic nuclei is now being used to identify possible areas that regulate feeding through the melanocortin-Agrp system.

RESEARCH DESIGN AND METHODS

Peptides. Agrp (83–132) was purchased from Phoenix Pharmaceuticals (Mountain View, CA) (23). [Nle6, D-Phe1,Tyr6]-MSH (NDP-MSH) was purchased from Peninsular (St. Helens, Merseyside, U.K.).

Animals. Male Wistar rats (Interfauna, Huntingdon, U.K.) weighing 250–300 g were maintained in individual cages under controlled temperature (21–23°C) and light (12:12-h light, dark cycle, lights on 7:00 a.m.) with ad libitum access to food (RM1 diet; SDS, Witham, U.K.) and water. Animal procedures undertaken were all approved by the British Home Office Animals Scientific Procedures Act of 1986 (project license no. PIL 90/1077).

Intraneural cannulation and injections. Animals were anesthetized with an intraperitoneal injection of xylazine (20 mg/kg) (Rompun; Bayer, Suffolk, U.K.) and ketamine (100 mg/kg) (Ketalar; Parke-Davis, Pontypool, Gwent, U.K.). Permanent 26-gauge stainless steel cannulae (Plastics One, Roanoke, VA) were stereotaxically placed into eight sites (MPO, PVN, AHA, CeA, DMN, VMN, LHA, and Arc) with 15 animals for each site according to coordinates obtained from the Paxinos and Watson rat brain atlas (21). The cannulae tips were positioned 1 mm above the target areas. Guide cannulae were held in place by dental cement glued to three stainless steel screws driven into the skull. After surgery, a thin wire stylet was inserted into each cannula to prevent blockage. After a 7-day recovery period, animals were handled daily for a 1-week period to minimize stress. All compounds were dissolved in 0.9% saline, and 0.5 µl of peptide or saline was injected in each study. Substances were administered by a 31-gauge stainless-steel injector placed in and projecting 1 mm below the tip of the guide cannula. The injector was connected by polyethylene tubing to a Hamilton syringe (Reno, NV) in a pump set to dispense 0.5 µl of solution each minute. After injection, animals were returned to their home cages that contained preweighed food and were observed. All studies were of a random cross-over design, in which half of the animals in each group received peptide while the remainder received saline. After a 4-day washout period, animals that had previously received peptide received saline and vice versa.

Agrp (83–132) 0.1 nmol (0.6 µg) or saline was injected into fed animals, and NDP-MSH 0.1 nmol (0.2 µg) or saline was injected into 24-h fasted animals during the early light phase. At 1, 2, 4, 8, and 24 h after each injection, food remaining in the cage dispenser was weighed to the nearest 0.1 g. The amounts of peptides chosen were those determined to be the lowest fully effective from pilot studies performed in the PVN (data not shown).

After all studies were completed, animals were killed by decapitation after an injection of 1 µl black ink. Brains were removed and immediately frozen in liquid nitrogen and were stored at –70°C until they were sliced on a cryostat (Bright, Huntingdon, U.K.) into 15-µm coronal sections and stained with cresyl violet for histological confirmation of correct cannula placement. Only those animals with correct placement of cannulae were included in the analysis of data.

Food intake is expressed as mean ± SE of %control. Statistical analysis of food intake for a given peptide at each nucleus and time point was measured by paired Student’s t test. Statistical analysis of intraneural comparison of food intake for a given peptide at each time point was determined by analysis of variance (ANOVA) followed by a post hoc least significant difference (LSD) pairwise comparison. Values of P < 0.05 were considered significant.

RESULTS

PVN. Agrp (83–132) increased food intake significantly from 2 h postinjection (238 ± 32% control, P < 0.005), and this effect was persistent for 24 h (24-h food intake 211 ± 23% control, P < 0.005) (Fig. 2). NDP-MSH decreased food intake from 1 h postinjection (52 ± 6% control, P < 0.005) and was persistent during the 24-h period although with a reduced effect (24-h food intake 86 ± 4% control, P < 0.005) (Fig. 2).

DMN. Animals cannulated into the DMN showed a significant increase in food intake in response to the Agrp (83–132) from 4 h postinjection, and a further increase in food intake was observed at 8 h (268 ± 42% control, P < 0.005) (Fig. 2). This effect persisted for 24 h but was reduced at 24 h (150 ± 5% control, P < 0.01). NDP-MSH significantly decreased 1-h food intake (44 ± 6% control, P < 0.0001). The effect of NDP-MSH remained significant up to 8 h postinjection (72 ± 7% control, P < 0.01).

MPO. In the MPO cannulated animals, Agrp (83–132) caused an increase in food intake from 4 h postinjection (220 ± 25% control, P < 0.005) with the greatest increase seen at 8 h (236 ± 31% control, P < 0.01, Fig. 2). This effect lasted for 24 h but...
FIG. 2. Effect of Agrp (83–132) (●) and NDP-MSH (▲) on food intake over a 24-h period in the eight different sites cannulated. *P < 0.05, **P < 0.01, ***P < 0.001 Agrp (83–132) vs. control group; ●P < 0.05, ••P < 0.01, •••P < 0.001 NDP-MSH vs. control group.
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with reduced magnitude (162 ± 11% control, P < 0.005). NDP-MSH significantly reduced 1-h food intake (55 ± 6% control, P < 0.0001). The effect was seen up to 4 h postinjection (63 ± 10% control, P < 0.005).

AHA. Agrp (83-132) administration into the AHA caused a smaller increase in food intake that was significant at 2 and 8 h postinjection (8-h food intake 175 ± 19% control, P < 0.01) (Fig. 2) but not at 24 h postinjection. The anorectic effect of NDP-MSH was observed from 1 h postinjection (71 ± 11% control, P < 0.005) and remained significant up to 8 h (68 ± 7% control, P < 0.005).

CeA. Agrp (83-132) administered into the CeA induced a weak and delayed increase in food intake significant at only 8 and 24 h postinjection (8-h food intake 171 ± 20% control, P < 0.001) (Fig. 2). NDP-MSH caused no alteration in food intake when injected into the CeA.

LHA. In the LHA, Agrp (83-132) did not induce a significant increase in food intake. Interestingly, Agrp showed a tendency to suppress 2-h food intake, although it did not reach significance (53 ± 16% control, P = 0.07) (Fig. 2). Injection of NDP-MSH showed a delayed decrease in food intake significant at only 8 h postinjection (80 ± 7% control, P < 0.05).

**FIG 3. Comparison among different nuclei of Agrp (83-132)-stimulated food intake at 2 h (A) and 8 h (B) postinjection. Horizontal line indicates 100% control food intake, which is identical to the average food intake of saline control group. **P < 0.05, **P < 0.01, ***P < 0.001 vs. LHA; P < 0.05, ***P < 0.001 vs. VMN; P < 0.01, ***P < 0.001 vs. Arc; P < 0.05, **P < 0.01 vs. CeA; P < 0.01 vs. AHA.

Agrp (83-132) caused nonsignificant changes in food intake when injected in the Arc (Fig. 2). However, injection of NDP-MSH caused a decrease in food intake from 1 h postinjection that persisted to 4 h (1-h food intake 63 ± 9% control, P < 0.05; 4-h food intake 58 ± 8% control, P < 0.05).

VMN. Neither Agrp (83-132) nor NDP-MSH induced significant changes in food intake in the VMN (Fig. 2).

**DISCUSSION**

Within the hypothalamus, both Agrp (83-132) and NDP-MSH are most effective at altering food intake when injected into the PVN, MPO, and DMN. A similar time course is seen at all of these nuclei with Agrp (83-132) increasing food intake by 4 h and persisting throughout 24 h and with the NDP-MSH inhibiting by 1 h and persisting for at least 4 h. Agrp (83-132) also increases feeding at the AHA and CeA, although the effect is smaller, whereas NDP-MSH causes a reduction in food intake only at the AHA but not the CeA. The LHA and
The basis for concluding this is that the feeding response
food intake at 1 h (A). VMN is responsive to neither peptide. This finding suggests
Arc are only weakly responsive to NDP-MSH, whereas the VMN is responsive to neither peptide. This finding suggests
that the PVN, MPO, and DMN may be the major sites of action for Agrp and the melanocortins to regulate feeding
within the hypothalamus.

Although only a very small dose of each peptide was administered in this study, the possibility of diffusion of peptide
from one site to the other must always be considered when interpreting data from studies such as this one. However, our data suggest that for the doses chosen in this study the diffusion effect at the DMN and MPO to the PVN is small. The basis for concluding this is that the feeding response elicited from injection into the AHA, which is much closer to the PVN than the DMN or MPO, is much less than that of the latter two nuclei (Table 1 and Fig. 1). In addition, the time-course effects at the three most responsive nuclei are very similar. If the effect was due to diffusion, a delayed response might be expected. In comparison, the small but lasting feeding effect seen after NDP-MSH injection in the Arc and AHA may be because of diffusion.

Lesioning studies classically implicated the LHA and VMN in the regulation of food intake (16,17). The LHA but not the VMN is reported to be innervated by Agrp and α-MSH neurons, although fibers are seen to surround the VMN in the rat. Therefore, it is surprising that injection into these two nuclei results in little or no change in food intake. In contrast, administration of Agrp and α-MSH into the PVN, DMN, and MPO, which also show presence of immunoreactive fibers containing these two peptides, results in large and sustained changes in food intake.

The area with the greatest response to Agrp and NDP-MSH on food intake is the PVN. Injection of Agrp (83–132) into this nucleus produces a rapid and sizable increase in food intake. Agrp more than doubles the 24-h food intake when administered in the PVN, a dramatic effect likely to result in gross obesity if such an effect of high endogenous release were to be maintained for days or weeks. The involvement of the PVN in the control of appetite is well documented. NPY, the most potent orexigenic peptide yet discovered, has its maximal effect when administered into the perifornical area just latero-caudal to the PVN (22). Several other peptides have also been shown to alter feeding when directly injected into the PVN, including galanin, melanin-concentrating hormone, and orexin-A (23–25). Recently, Giraudo et al. (26) demonstrated the effects of SHU9119 and MT II, a melanocortin 3/4 receptor antagonist and agonist, respectively, on feeding when injected into the PVN. They found an increase in food intake from 2 h after injection of 0.05 nmol of SHU9119 that persisted for 24 h with a resultant increase to ~150% of the control group. MT II at the same dose caused a reduction of ~50% in feeding at 2 h, which again persisted for 24 h but with reduced effect. No other nuclei were examined in this study.

The DMN may also be involved in the regulation of feeding because disruption of the DMN, although not resulting in obesity, does result in altered feeding patterns (27). NPY, galanin, and the opioids increase feeding when directly injected into the DMN (23,28,29). Interestingly, though wild-type mice are normally devoid of NPY mRNA expression, MC4R knockout mice show a high level of expression of NPY in the DMN once obesity is established (30). The strong response elicited by MPO injection with both Agrp (83–132) and NDP-MSH is surprising, because it is an area that is thought to be mainly associated with reproductive function rather than feeding regulation. Ingestion of a palatable meal has, however, been shown to increase fos-like immunoreactivity and NPY content in the MPO of the rats (31,32).

In the CeA, Agrp-induced feeding is less than that seen at the PVN, MPO, and DMN, although Agrp nerve fibers and MC4R are also present in the CeA. In addition, NDP-MSH, despite having high affinity for MC4R, has no effect on feeding when injected into the CeA. The response of the AHA to both Agrp (83–132) and NDP-MSH was also unexpected, because it is an area that does not appear to be associated with feeding control.

Although the Arc and LHA have a similar array of Agrp and melanocortin projections, only NDP-MSH in this study causes an alteration of feeding at these sites. The discrepancy between agonist (NDP-MSH) and antagonist (Agrp) was unexpected, because these peptides have been suggested to act through the same receptor (MC4R). It is, however, possible that in the Arc and LHA there is no tonic agonism of the MC4R; therefore, antagonism by Agrp does not elicit an increase in feeding.

In conclusion, we have shown that the main nuclei in the intricate hypothalamic melanocortin-Agrp system that control
feeding appear to be the PVN, DMN, and MPO. This study indicates that the hypothalamic nuclei expressing MC4R vary in their sensitivity to Agrp and α-MSH with regard to their effects on feeding.

REFERENCES