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Intracerebroventricular Administration of Melanin-Concentrating Hormone Suppresses Pulsatile Luteinizing Hormone Release in the Female Rat

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Abstract

Melanin-concentrating hormone (MCH) has been reported to be involved in the regulation of feeding behaviour in rats and mice. Because many neuropeptides that influence ingestive behaviour also regulate reproductive function, the present study was designed to determine if central administration of MCH changes pulsatile secretion of luteinizing hormone (LH) in the rats. Wistar-Imamichi strain female rats were ovariectomized and implanted with oestradiol to produce a moderate inhibitory feedback effect on LH release. The effects of i.c.v. injections of MCH on LH release were examined in freely moving animals. Blood samples were collected every 6 min for 3 h through an indwelling cannula. After 1 h of sampling, MCH (0.1, 1 or 10 μg/animal) or vehicle (saline) was injected into the third cerebroventricle. Because MCH is also reported to affect the hypothalamo-pituitary-adrenal (HPA) axis, which in turn, can influence reproductive function, plasma corticosterone concentrations were determined in the same animals at 30-min intervals during the first and last hours and every 12 min during the second hour of the 3-h sampling period. When expressed as per cent changes, mean plasma LH concentrations after MCH administration were significantly lower in the animals injected with all doses of the peptide compared with vehicle-treated animals; LH pulse frequency was significantly lowered by 1 μg of MCH. Per cent changes in mean plasma corticosterone levels were not significantly affected by MCH administration. These results in oestradiol-treated ovariectomized rats indicate that central MCH is capable of inhibiting pulsatile LH secretion. We have previously shown that 48-h fasting suppresses pulsatile LH release in the presence of oestrogen. Take together, these results raise the possibility that MCH could play a role in mediating the suppression of LH secretion during periods of reduced nutrition.

Melanin-concentrating hormone (MCH) was first isolated from the salmon pituitary and identified as a substance regulating body colour in the fish (1). This cyclic 19-amino acid peptide has been identified in the rat central nervous system (CNS) with neuronal cell bodies predominately located in the lateral hypothalamus and zona incerta (2, 3), the former of which is considered to be a feeding centre. MCH has been found to stimulate feeding in the mouse (4, 5), and MCH mRNA levels in the brain increase by fasting or glucoprivation (4, 6). In the rat, central administration of MCH induces feeding behaviour during both periods of dark and light (7). These findings clearly suggest that MCH has an orexigenic action within the brain (8). Further, MCH alters the hypothalamo-pituitary-adrenal (HPA) axis (9, 10). MCH functionally antagonizes activation of MC3/4 receptors, the principal brain melanocortin receptor subtypes (8, 11), and MCH antagonizes α-melanocyte-stimulating hormone (α-MSH) actions on feeding and glucocorticoid secretion (11). Recently, MCH receptors were cloned (12, 13), and are distributed in the several brain areas, including ventromedial (VMH) and dorsomedial (DMH) hypothalamic nuclei, brain regions that are considered to play a role in feeding behaviour.

Regulation of food intake and reproduction may share a number of common neuroendocrine pathways. Fasting

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suppresses gonadal activity (14), and glucoprivation suppresses luteinizing hormone (LH) secretion, but stimulates feeding behaviour (15). Noradrenergic neurones projecting to the paraventricular nucleus stimulate feeding behaviour and suppress LH secretion (16, 17). Peptides involved in the regulation of feeding behaviour are also closely associated with the activity of reproductive axis. Leptin, a peptide that suppresses feeding, reverses suppression of LH secretion in fasted rats and *ob*/*ob* mice (18–20). We have recently demonstrated that motilin, a gastrointestinal peptide, stimulates feeding and suppresses pulsatile LH release (21). Neuropeptide Y and galanin have been reported to stimulate feeding behaviour and to be involved in the regulation of LH secretion in rats (22). Based upon the feeding functions associated with MCH, it is reasonable to hypothesize that MCH participates in suppressing the activity of the hypothalamo-pituitary-gonadal axis. Gonzalez *et al*. have reported the effect of central administration of MCH on LH secretion in anaesthetized ovariectomized (OVX) adrenalectomized rats injected with 5 μg oestradiol (23). In that study, MCH stimulated LH secretion when administered into the preoptic area or median eminence. However, the effect of central MCH is still unknown in conscious adrenal-intact animals.

To determine if central MCH could play an inhibitory role in the regulation of reproductive neuroendocrine function, the present study investigated the acute effect of i.c.v. injection of MCH on pulsatile LH secretion in freely moving female rats. OVX rats chronically implanted with oestradiol were used, since this oestrogen treatment enhances inhibitory effect of fasting and glucoprivation on LH release (15, 24). We monitored changes in plasma corticosterone levels after central administration of MCH to determine if this peptide affects the HPA axis, which in turn, may influence pulsatile LH secretion. It is known that corticotrophin-releasing hormone (CRH) mediates the suppression of pulsatile LH secretion induced by fasting and glucoprivation (25, 26), and it is possible that MCH could mediate this reduction in gonadotropin-releasing hormone (GnRH) secretion through activation of CRH.

Materials and methods

Animals and surgery

Wistar-Imamichi strain female rats (180–230 g BW) were maintained under controlled conditions (14 L:10D, lights on at 05.00 h, temperature at $22 \pm 2\,^{\circ}C$) with free access to food (Labo-MR-stock, Nihon Nosan Kogyo Co., Yokohama, Japan) and water. Animals having at least two consecutive 4-day oestrous cycles were ovariectomized and implanted s.c. with Silastic tubing (i.d., 1.5 mm; o.d., 3.0 mm; length, 25 mm; Dow-Corning, Midland, MI, USA) containing 20 μg/ml oestradiol-17β (E_2, Sigma, St Louis, MO, USA) dissolved in peanut oil for 7 days. We have previously shown that animals subjected to this E_2 treatment had plasma E_2 levels of 35.8 ± 1.2 pg/ml (24) and this plasma E_2 concentration was within the range found at dioestrus in rats (27), resulting in a moderate negative feedback effect on pulsatile LH release (17, 24). Our previous studies have also demonstrated that this E_2 treatment enhanced the inhibitory effects of 48-h fasting and glucoprivation on pulsatile LH secretion in OVX rats (15, 24). The animals were then immediately implanted with a stainless-steel guide cannula (23 gauge, Plastic Products Co., Roanoke, VA, USA) with its tip located in the third cerebroventricle (0.8 mm posterior and 7.5 mm ventral to the bregma at the midline). The females were allowed to recover from brain surgery for 1 week before blood sampling.

Experimental protocols

One week after OVX, E_2 implantation and brain surgery, blood samples (110 μl) were collected from the freely moving conscious animals at 6-min intervals for 3 h beginning at 13.00 h through Silastic tubing that was inserted into the right atrium on the previous day. An equivalent volume of rat red blood cells taken from donor animals was suspended in heparinized saline and replaced through the cannula after the removal of each blood sample. Plasma was separated immediately, aliquotted for LH (50 μl) and corticosterone (0.5 μl) assay, and stored at $-30\,^{\circ}C$ until assayed.

MCH (Sigma) was dissolved in saline at a concentration of 0.1, 1 or 10 μg/5 μl. After the first hour of blood sampling, 5 μl of MCH solution or equal volume of the vehicle was slowly (1 μl/min) infused into the third ventricle with a microinjection pump (EP-60, EICOM, Kyoto, Japan) through an inner cannula inserted into the outer cannula. The doses of MCH were chosen because previous studies in rats determined that 0.1 μg of MCH increased LH secretion and that 1.5–15 μg of this peptide significantly increased food intake (6, 23). Plasma corticosterone levels were determined in plasma samples collected every 30 min for the first and last hours and every 12 min for the second hour of the 3-h sampling period. The plasma samples for corticosterone assay were taken from the same samples for LH assay.

Hormone assay

Plasma LH concentrations were determined by a double-antibody radioimmunoassay (RIA) with a rat LH RIA kit provided by the National Hormone and Pituitary Program (Baltimore, MD, USA), and are expressed in terms of NIDDK rat LH RP-3. The least detectable level of LH was 0.156 ng/ml for 50 μl of plasma sample. The intra- and interassay coefficients of variation were 4.2% for 3.3 ng/ml and 11.4% for 1.5 ng/ml, respectively.

Plasma corticosterone levels were determined by RIA, with synthetic corticosterone (Sigma) used as a reference standard. Rabbit anticorticosterone serum and tritiated corticosterone were purchased from Teikoku-Zoki (Tokyo, Japan) and Amersham (Tokyo, Japan), respectively. The least detectable level of corticosterone was 2.5 ng/ml for 5 μl of plasma sample. The intra- and interassay coefficients of variation were 5.6% for 51.5 ng/ml and 8.8% for 28.7 ng/ml, respectively.

Statistical analysis

LH pulses were identified by the PULSAR computer program (28). The criteria for LH pulse detection have been described elsewhere (29). Per cent changes in mean plasma LH and corticosterone levels and the frequency and amplitude of LH pulses before and after MCH or vehicle injection in individuals were determined as follows: (A-B)/B*100 (A, the mean levels of each values at postinjection period; B, the mean levels of each values at preinjection period). Statistical differences in the per cent change in each LH pulse parameter and mean corticosterone concentrations between groups were detected by the one-way anova followed by Fisher's least significant difference test.

Results

Figure 1A shows profiles of plasma LH levels in the animals with injections of 0.1, 1, or 10 μg MCH into the third ventricle. The lowest dose (0.1 μg) of MCH had little effect on pulsatile LH secretion, though in 2/4 animals pulsatile LH secretion was transiently depressed. Central administration of the intermediate dose (1 μg) of MCH immediately suppressed pulsatile LH release, and this inhibition lasted more than 2 h in most animals. Interestingly, the high dose (10 μg) of MCH only transiently inhibited LH secretion. Mean LH concentrations, expressed as per cent changes, were significantly ($P < 0.05$, Fisher's least significant difference test) suppressed by all doses of MCH compared with vehicle-treated group (Fig. 1B). The middle dose (1 μg) of MCH significantly ($P < 0.05$) suppressed LH pulse frequency. LH pulse amplitude was not significantly affected by any dose of MCH.

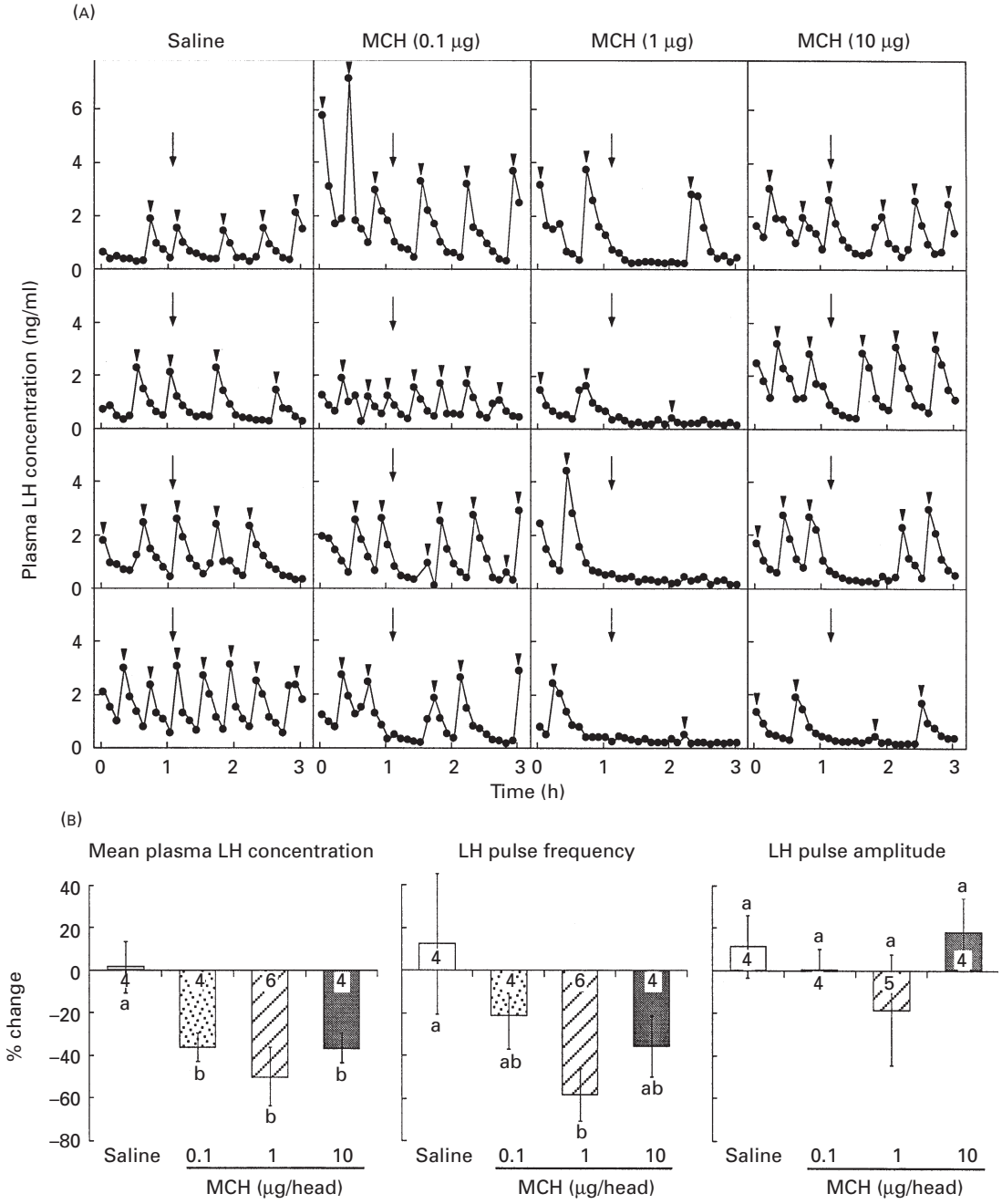


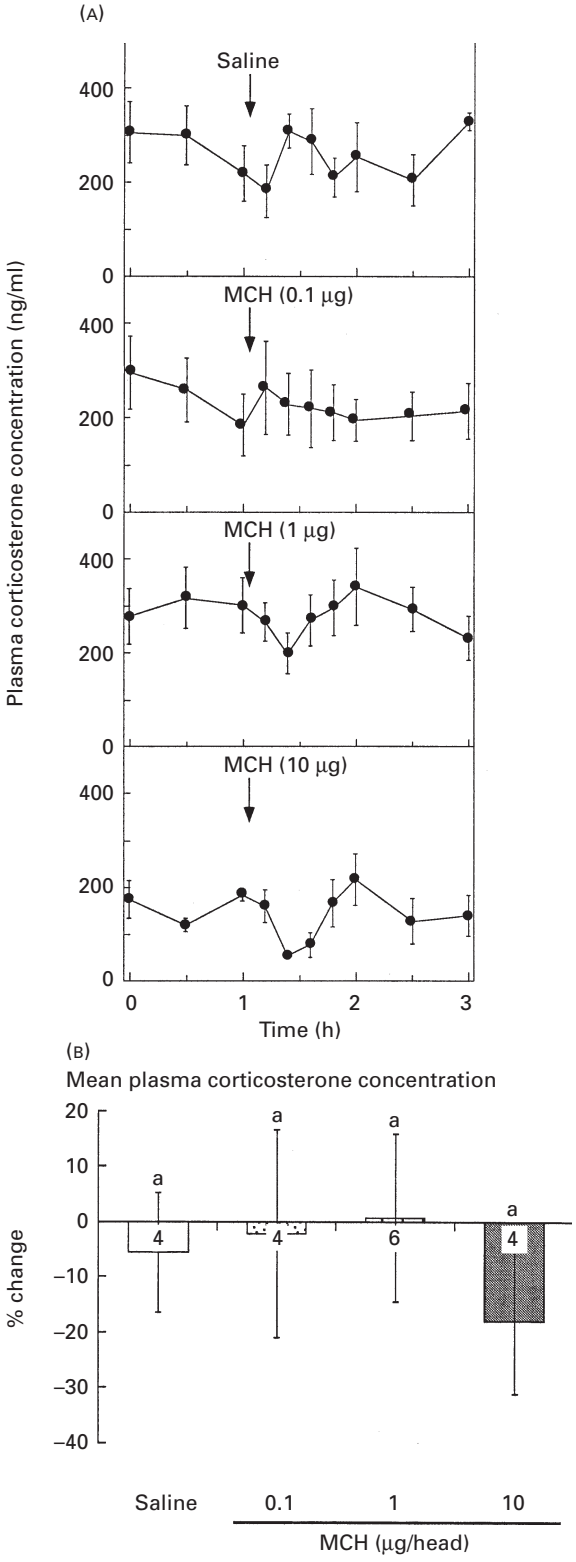
FIG. 1. Profiles of plasma LH concentrations in four individuals (A) and per cent changes (means ± SEM) in plasma LH concentrations and frequency and amplitude of LH pulses (B) in oestradiol-primed ovariectomized rats before and after i.c.v. injection of MCH at 0.1, 1 or 10 μg in 5 μl of saline. Control animals were injected with the same volume of saline. Blood samples were collected every 6 min for 3 h through an indwelling atrial cannula. MCH was injected into the third ventricle after the first hour of the blood sampling. Arrows indicate the timing of the i.c.v. injections. Arrowheads represent LH pulses identified with the PULSAR computer program. Numbers in/under the column indicate the numbers of the animals used. Values with different letters are significantly different from each other ($P < 0.05$, anova followed by Fisher's least significant difference test).

Figure 2A presents mean concentrations of plasma corticosterone in the groups receiving the three different doses of MCH. Plasma corticosterone levels fluctuated unpredictably in the saline-and MCH-injected groups. When expressed as percent changes, mean corticosterone levels were not significantly altered by central administration of any doses of MCH (Fig. 2B).

Discussion

In the present study, central MCH at all doses significantly decreased mean LH concentrations in freely moving oestrogen-treated OVX rats. None of the doses used in our study stimulated LH secretion. Because the intermediate dose (1 μg) of MCH significantly decreased LH pulse frequency, it raises

the possibility that MCH may participate in suppressing GnRH release during fasting. The increase in MCH gene expression detected after food deprivation or glucoprivation (4, 6) appears to be consistent with this notion. MCH may mediate the action of leptin on reproductive activity during fasting (plasma leptin levels decrease) because MCH mRNA levels decrease with central leptin administration (30), and leptin receptors have been found in the MCH-containing neurones in the lateral hypothalamus (31). In support of this hypothesis, peripheral administration of leptin reverses fasting-induced suppression of LH secretion (18).



In the present study, central MCH did not produce a dose-dependent decrease in LH secretion: The middle dose (1 μg) of MCH clearly suppressed pulsatile LH release, while the highest dose (10 μg) of the peptide had only a moderate action on LH release. This could be explained by the hypothesis proposed by De Meyts *et al.* (32) for why some hormones, such as growth hormone (GH), produce a bell-shaped dose–response curves. This hypothesis arose from their studies of the effects of GH on lipogenesis in rat adipocytes. According to their model, high doses of ligands prevent receptor dimerization events critical to specific physiological or cellular responses. When this model is applied to our results for the effects of MCH on LH secretion, high doses would produce responses that are blunted or even diminished. However, at physiologic ligand concentrations of MCH, the receptors would dimerize to generate specific physiological responses to regulate LH secretion.

In the present study, saline-injected controls did not show a significant increase in plasma corticosterone levels, because the blood samples were collected prior to the onset of daily afternoon corticosterone surge occurring around the lights-off (19.00 h in our light schedule). The administration of any doses of MCH into the third ventricle did not significantly affect plasma corticosterone levels. This result may imply that the inhibition of LH release by MCH is not mediated by increase in CRH expression or activation of the HPA axis. It has been shown that environmental factors, such as physical stress or glucoprivation, activate the HPA axis and suppress GnRH/LH secretion through CRH (26, 33, 34). Under the above-mentioned circumstances, CRH is released into the portal circulation as well as within the hypothalamic regions to suppress GnRH release. It is most likely that MCH inhibits GnRH/LH release via other mechanism than CRH release in the hypothalamus. The effects of MCH on the HPA axis are still controversial. The inhibitory action of MCH on HPA axis is reported in a previous study, in which i.c.v. injection of MCH to the lateral ventricle did not affect basal ACTH secretion but even suppressed stress-induced ACTH release in male rats (9). On the other hand, Jezova *et al.* suggested that the administration of MCH into the lateral ventricle stimulated ACTH secretion and this response was blocked

FIG. 2. Mean plasma corticosterone concentrations (A) and per cent changes in mean plasma corticosterone concentrations (B) in oestradiol-primed, ovariectomized rats before and after the i.c.v. injection of MCH at 0.1, 1 or 10 μg in 5 μl of saline. Control animals were injected with the same volume of saline. Blood samples were collected every 30 min for the first and last hours and every 12 min for the second hour of the 3-h sampling period. Values are means ± SEM. Numbers in/under each column indicate the numbers of the animals used. Per cent change values were not significantly different from each other (anova followed by Fisher's least significant difference test).

by CRH antiserum in the male rat (10). The effect of central MCH on the HPA axis should be clarified in future studies.

The finding of an inhibitory action of MCH on LH release in the present study does not agree in concept with the previous report in which local injection of MCH into the preoptic area or median eminence increased plasma LH concentrations (23). This inconsistency may be due to several differences. The first is that different sites were used for the administration of MCH. In the study by Gonzalez *et al.* (23), MCH was injected locally into the preoptic area or median eminence whereas we administered the peptide into the third ventricle. Because MCH immunoreactive neurones have been found to project to the various brain regions in rats (2), MCH injected in the ventricular system may exert diverse actions on brain sites different from the previous study. In this regard, the present study found that the highest dose of MCH exerted only a moderate inhibitory action on LH release. Perhaps, a more broad action by the high dose of MCH may overcome the inhibitory effect of this peptide on LH secretion. It is possible that degraded peptide in the cerebroventricle has a different action from intact peptide. The second potential difference for the opposite effect (inhibitory and stimulatory) of MCH on LH release might be due to the difference in oestrogen treatment. Gonzalez *et al.* (23) used OVX animals bearing single injection of oestrogen (5 μg/animal), which could induce LH surge when it followed by progesterone administration; our oestrogen treatment only exerts an inhibitory feedback effect on tonic LH secretion (17, 24). Because synaptic remodelling in the hypothalamus, which is highly correlate with induction of LH surge, is induced by oestrogen (35), MCH may exert inhibitory or stimulatory effect on LH release depending on the oestrogenic milieu. The third explanation for the conflicting results of MCH on LH release could be due to the difference in the sampling conditions. In the study by Gonzalez *et al.* (23), blood samples were taken from the tail vein in the animals anaesthetized with Saffan, which acts via γ-aminobutyric acid (GABA) receptor (36). Because the anaesthetic agent (Saffan), as well as pentobarbital, which also activates GABA receptors, has been suggested to affect LH secretion (37, 38), the anaesthetized animal model may not be optimal for study of brain mechanisms regulating gonadotropin secretion. We have determined that our blood sampling system is free of stress and is suitable for evaluating pulsatile LH secretion under various conditions (17, 25, 26, 29). The fourth difference between the two studies may be related to the presence (our study) or absence (Gonzalez *et al.* (23) study) of the adrenal gland. Adrenal glucocorticoids are reported to stimulate MCH expression in the rat hypothalamus (39), raising the possibility that some changes in the MCH expression can occur in the hypothalamus of adrenalectomized animals (23).

Recently, MCH receptor has been cloned (12, 13), and *in situ* hybridization and immunohistochemical data have now demonstrated that MCH receptors are widely distributed in the brain. Within the hypothalamus, MCH receptors were located mainly in the VMH and DMH, suggesting that central MCH may regulate feeding and reproduction by acting on those brain areas. Furthermore, MCH-immunoreactive fibres have been found in various brain areas, including hindbrain (2). Possible brain action sites of MCH to suppress LH secretion are the nucleus of the solitary tract (NTS) in the medulla oblongata and/or paraventricular nucleus. These nuclei include projections of both MCH and α-MSH-containing neurones (2, 40, 41) and express MC4R mRNA (42). Both the paraventricular nucleus and NTS have been proposed to play a key role in inducing feeding behaviour and suppressing GnRH/LH secretion through a noradrenergic pathway in fasted or glucoprived rats (14, 16, 17, 25, 43). Therefore, the increased MCH release during fasting could act at the NTS and/or paraventricular nucleus to mediate the fasting-induced suppression of GnRH/LH secretion. The arcuate nucleus, which is involved in ingestive behaviour and reproductive functions, could also be a candidate for the action site of MCH to regulate food intake and LH secretion. The arcuate receives axonal projections from both MCH- and α-MSH-containing neurones and this hypothalamic nucleus also expresses MC3R mRNA (2, 40, 44). Further studies will be required to clarify the sites of action for MCH to suppress LH release.

In conclusion, i.c.v. administration of MCH suppresses LH secretion in the oestradiol-primed OVX rat, raising the possibility that central MCH plays an inhibitory role in the regulation of reproductive axis. Considering the role of MCH in regulating feeding behaviour, MCH may partly mediate the suppression of LH secretion during fasting or undernutrition.

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