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Corticotropin-releasing factor receptor type 1, but not type 2, in the ventromedial hypothalamus modulates dopamine release in female rats

Tomohiro Muramatsu *, Koki Inoue, Shinichi Iwasaki, Tsuneo Yamauchi, Teruaki Hayashi, Nobuo Kiriike

Department of Neuropsychiatry, Osaka City University Medical School, 1-4-3 Asahi-machi, Abeno-ku, Osaka City, Osaka 545–8585, Japan

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Abstract

Corticotropin-releasing factor (CRF) plays an important role in stress responses and is mediated through two subtypes of receptors, CRF receptor type 1 (CRFR1) and CRF receptor type 2 (CRFR2). Each CRF receptor might have a different function through several neurotransmitter systems; however, the mechanism remains unclear. To clarify the role of each receptor in dopamine (DA) metabolism, we measured the change of extracellular concentrations of DA and the metabolites in the ventromedial hypothalamus (VMH) that played important roles in the stress response of freely moving female rats in response to the direct administration of comparative CRFR1 selective agonist, CRF, or CRFR2 selective agonist, Urocortin II (Ucn II), into the brain region. Administration of 10 μg CRF increased extracellular concentrations of DA compared with 2 μg CRF immediately after injection, and this effect was not observed after 60 min of 10 μg CRF injection. On the other hand, this change did not always occur after Ucn II administration. These results suggest that the activation of CRFR1, but not CRFR2, modulates the release of DA in VMH.

Keywords: CRF; Urocortin II; Dopamine; Ventromedial hypothalamus; Microdialysis; Microinjection; Sex; Rat; Stress

1. Introduction

Corticotropin-releasing factor (CRF) is known to be a key mediator of the stress response (Vale et al., 1981). CRF administration in the central nervous system changed behavior, increasing locomotor activity and anxiety-like behavior, or inhibiting feeding and sexual behavior (Dunn and Berridge, 1990). Urocortin I (Ucn I) (Vaughan et al., 1995), Urocortin II (Ucn II) (Hsu and Hsueh, 2001; Reyes et al., 2001), and Urocortin III (Ucn III) (Hsu and Hsueh, 2001; Lewis et al., 2001), known as the CRF family, were recently identified. CRF and the CRF family have different affinity for two kinds of CRF receptors, CRF receptor type 1 (CRFR1) (Chang et al., 1993; Chen et al., 1993; Vita et al., 1993), and CRF receptor type 2 (CRFR2) (Lovenberg et al., 1995; Perrin et al., 1995). CRF had comparably high affinity for CRFR1, Ucn I had high affinity for both CRFR1 and CRFR2, and Ucn II and Ucn III bound CRFR2 selectively (Vaughan et al., 1995; Hsu and Hsueh, 2001; Lewis et al., 2001; Reyes et al., 2001). Two types of CRF receptors were distributed differently in the brain. CRFR1 was widely distributed, whereas CRFR2 was distributed in a more limited brain area, being highly distributed in the hypothalamic ventromedial nucleus (VMH), lateral septal nucleus, and choroid plexus (Chalmers et al., 1995).

Both CRFR1 and CRFR2 were involved in behavioral regulation, such as the control of food intake and anxiety, but two types of CRF receptors were revealed to play different roles (Reyes et al., 2001; Valdez et al., 2002; Zorrilla et al., 2004). In feeding, CRFR1 activation inhibited food intake immediately, and CRFR2 activation showed a delayed inhibitory effect (Reyes et al., 2001; Inoue et al., 2003). On the other hand, CRFR1 activation had an anxiogenic effect (Lundkvist et al., 1996; Okuyama et al., 1999; Weninger et al., 1999), and CRFR2 was associated with both anxiolytic (Valdez et al., 2002, 2003; Venihaki et al., 2004) and anxiogenic effects (Pelleymounter et al., 2002, 2004). The difference in the exact mechanism between two CRF receptor subtypes in the control of such behavior remains unclear.

* Corresponding author. Tel.: +81 6 6645 3821; fax: +81 6 6636 0439.
E-mail address: muramatsut@med.osaka-cu.ac.jp (T. Muramatsu).

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The association of CRF administration with DA metabolism has been reported in various brain areas (Dunn and Berridge, 1990). VMH has neural connections with many other areas of the brain that have been implicated in feeding behavior, and the brain region has many receptors that respond to such as DA and serotonin to affect feeding behavior (King, 2006). Both CRFR1 and CRFR2 were distributed in rat VMH (Chalmers et al., 1995), and the exact interaction between two types of CRF receptors and the dopaminergic system in the area has not been demonstrated. Thus, CRF and CRF-related peptides might be involved in the dopaminergic system through VMH. Female rats react to physical and psychological stressors with an endocrine response which is quantitatively greater than the response of male rats (Handa et al., 1994). To elucidate the mechanism of stress-related mental disorders we used female rats. In this study, CRF or Ucn II were microinjected into the VMH of female rats, and changes of extracellular concentrations of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in the brain region were continuously measured by microdialysis technique to characterize the interaction and metabolism of DA.

2. Methods

2.1. Subjects

Female Wistar rats aged eleven weeks (Keari Co., Osaka, Japan) were individually housed in plastic cages (30 × 30 × 35 cm) under controlled temperature (22 °C) and reversed dark–light cycle (lights on 20:00–8:00 h). Rats had free access to laboratory chow (CE-2, CLEA Japan, Inc., Tokyo, Japan) and water. They were cared for in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985) and the Guidelines for Animal Experimentation of Osaka City University.

2.2. Drugs

Synthetic rat/human CRF was obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA), and human Ucn II was obtained from Phoenix Pharmaceuticals, Inc. (Belmont, CA, USA). CRF and Ucn II were dissolved in artificial cerebrospinal fluid (aCSF) (128 mM NaCl, 1.3 mM $CaCl_2$, 0.9 mM $MgCl_2$, and 2.6 mM KCl, pH 7.4). The dissolved neuropeptide was divided into several Eppendorf tubes and stored at −80 °C for later use. The stored solution was thawed at room temperature just before the experiment, and the defrosted neuropeptide was used only once.

2.3. Surgery

All rats were allowed at least 1 week to acclimate to the colony room before surgery. Rats were anesthetized with sodium pentobarbital (400 mg/kg, IP) and positioned in a stereotaxic instrument (SR-5N, Narishige Scientific Instrument Lab., Tokyo, Japan). A 12-gauge guide cannula (MI-AG-12, Eicom, Kyoto, Japan) was implanted above the left VMH (2.7 mm posterior, 0.5 mm lateral to the bregma, and 7.5 mm ventral to the brain surface). The coordinate followed the atlas of Paxinos and Watson (1998). The guide cannula was fixed in the skull with screws and dental cement, and a wire stylet was placed in the guide cannula to keep it free of debris. After surgery, the rats were housed individually and allowed 1 week to recover.

2.4. Microinjection and microdialysis procedures

On the day before the experiment a rat was placed into the microdialysis apparatus with a counterbalance arm attached to a spring and a liquid swivel to allow free movement. A 2 mm microdialysis probe with a microinjector (MI-A-I-12–02, Eicom, Japan), whose tip was just intermediate of the probe, detached beside the probe, was inserted into the guide cannula. The aCSF was perfused at a rate of 1.0 μl/min using a micro syringe pump (EP-60, Eicom, Japan) for about 4 h to stabilize the dialysate samples. Samples were collected at 20 min intervals with an autosampler (9:00–16:00 h) and injected automatically (AS-10, Eicom, Japan) into a high-pressure liquid chromatograph (HPLC) (EP-10, Eicom, Japan) equipped with a reverse-phase 2.1 × 150 mm ODS 5 μm microbore column (SC-5ODS, Eicom, Japan). The HPLC mobile phase (0.02 mM EDTA, 0.65 mM sodium 1-octan sulfonate, 47.5 mM sodium acetate trihydrate, 33.5 mM citric acid monohydrate, and 19% methylalchol) was pumped through the column at a flow rate of 230 μl/min.

Either aCSF (2 μl), CRF (2 or 10 μg in 2 μl aCSF), or Ucn II (1 or 5 μg in 2 μl aCSF) was injected into the VMH through the microinjector over 2 min. The dose of the neuropeptide in this experiment referred to past studies (Butler et al., 1990; Inoue et al., 2003; Zorrilla et al., 2004; de Groote et al., 2005). The injection was performed carefully to protect against tissue damage from infusion pressure, and we confirmed whether VMH was not damaged by verifying brain slices as described in the Histology section. Before microinjection, three baseline samples were collected. Samples were collected at 20 min intervals for 3 h after microinjection. Extracellular concentrations of DA, DOPAC, and HVA were identified on chromatographs by comparing the retention times with referential standards. The amount of DA, DOPAC, and HVA in each dialysate sample was quantified from the respective peak area using linear regression analysis of the peak area obtained from reference standards each time after the experiment.

2.5. Data analysis

Baseline levels of DA, DOPAC, and HVA were determined for each rat from the mean of dialysate samples collected before microinjection. The content of DA, DOPAC, and HVA in the dialysate samples was individually represented as a percentage value of the average of three baseline samples. All results were expressed as the mean ± SEM. The means of the baseline sample were compared respectively by repeated-measures ANOVA between groups. The effects of microinjection on DA, DOPAC, and HVA levels were assessed by repeated-measures ANOVA during 60 min time slots (0–60, 60–120, and 120–180 min). Additionally, one-way ANOVA following Dunnett's test was used to evaluate the effects of treatment at individual time points

within the time slot showing a significant dose effect. Statistical significance was accepted at $p<0.05$. All statistical analyses were carried out using StatView 5.0J (SAS Institute Inc., Cary, NC, USA).

2.6. Histology

After the experiment, rats were killed by an overdose of diethyl ether. Brains were excised and refrigerated with ice. Chilled brains were then cut in coronal sections, and the slices verified whether the tract of the microdialysis probe with an microinjector was in the VMH. Only data from a correctly positioned microinjector and microdialysis probe were included in the analyses.

3. Results

The mean±SEM baseline levels of DA, DOPAC, and HVA in 20 µl of analyzed dialysates from the VMH across all treatment groups ($n=39$) were 8.5±1.5, 62.8±9.1, and 41.0±6.1 pg, respectively. There was no significant difference in all baseline dialysate concentrations between treatment groups (Table 1).

A higher dose of CRF (10 µg) elevated dialysate concentrations of DA in VMH immediately after microinjection, and the extracellular concentrations of DA peaked at 0–20 min after injection (Fig. 1(a)). Repeated-measures ANOVA indicated a significant effect of treatment [$F(2,22)=3.644$, $p<0.05$] but no significant effect of time [$F(2,22)=0.476$, NS] and no significant treatment×time interaction [$F(4,44)=0.576$, NS] during 0–60 min after microinjection of aCSF or CRF. Additionally, one-way ANOVA indicated a significant difference [$F(2,22)=4.951$, $p<0.05$] between groups at 0–20 min after microinjection. Dunnett's post-hoc comparisons indicated that the 10 µg CRF group differed from the aCSF group at that time ($p<0.05$). No significant treatment effect was observed during the other time slots (60–120, 120–180 min). DOPAC levels after CRF injection into VMH seemed to show biphasic change. DOPAC concentrations after the administration of 2 µg CRF increased from the concentrations after aCSF injection, and the concentrations after the microinjection of 10 µg CRF fell (Fig. 1(b)); however, DOPAC levels were not significantly altered. HVA levels after aCSF or CRF injection showed similar changes during the time course (Fig. 1(c)). CRF administration did not produce statistically significant changes in HVA levels.

Extracellular concentrations of DA after the injection of both 1 and 5 µg Ucn II in VMH seemed to decrease gradually compared with the concentrations of aCSF (Fig. 2(a)); however,

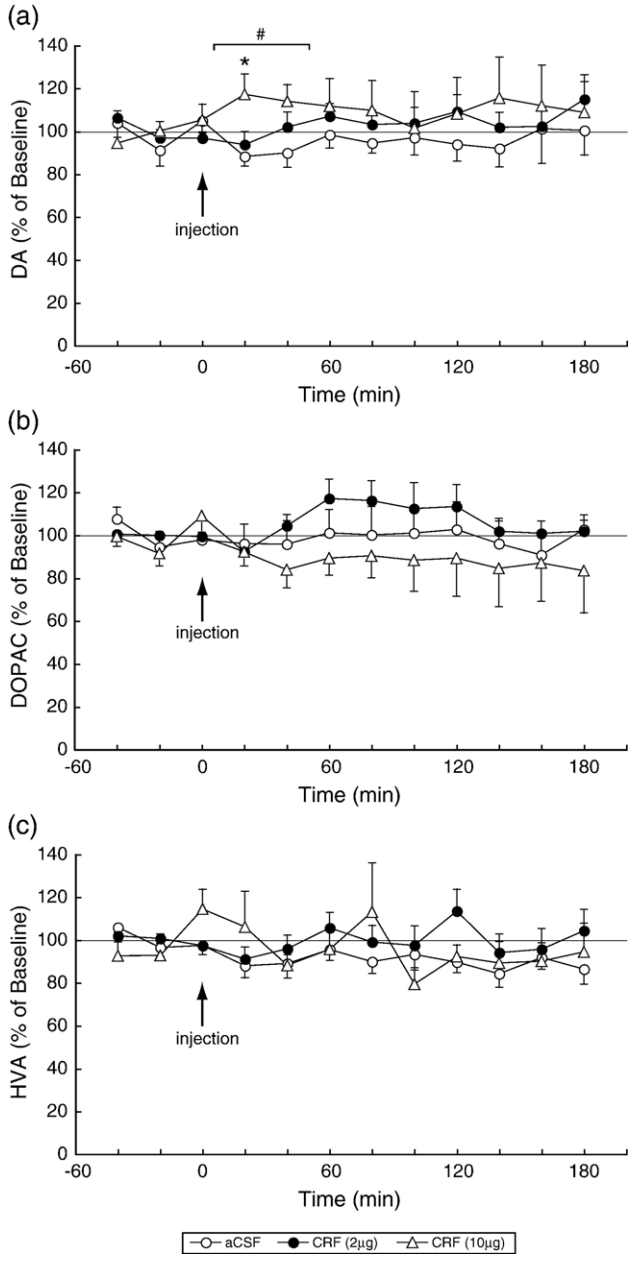

Fig. 1. Effects of microinjection of CRF or aCSF on extracellular concentrations of DA (a), DOPAC (b) HVA (c) in the VMH. The *x*-axis indicates the time before and after microinjection of CRF or aCSF, and the *y*-axis indicates extracellular levels of DA, DOPAC, or HVA, whose values represent the mean percentage of the baseline (±SEM). The arrow indicates the time of microinjection of CRF or aCSF into the VMH. $^{\#}p<0.05$ between groups during 0–60 min after microinjection; $^{*}p<0.05$ 10 µg CRF versus aCSF.

Table 1
Basal levels of DA, DOPAC, and HVA in dialysate samples from the VMH

	aCSF	CRF (2 µg)	CRF (10 µg)	Ucn II (1 µg)	Ucn II (5 µg)
DA	8.6±3.0	11.8±4.7	4.0±0.9	9.5±4.4	8.8±2.4
DOPAC	76.2±22.2	68.5±20.8	29.6±11.0	89.6±27.1	50.2±13.8
HVA	52.5±16.0	64.5±17.4	18.8±2.6	43.3±11.5	22.7±7.6
	$n=9$	$n=8$	$n=8$	$n=7$	$n=7$

Data represent the means±SEM in pg/20 µl sample.

the change after the injection of neither 1 nor 5 µg Ucn II reached statistical significance. 1 µg Ucn II seemed to elevate dialysate concentrations of DOPAC in VMH from within 60 min after injection, whereas 5 µg Ucn II decreased the concentrations from about 60 min after injection (Fig. 2(b)). However, repeated-measures ANOVA indicated no significant effect of treatment on DOPAC levels. Administration of 1 µg Ucn II gradually elevated dialysate concentrations of HVA in VMH, whereas aCSF and 5 µg Ucn II similarly changed during

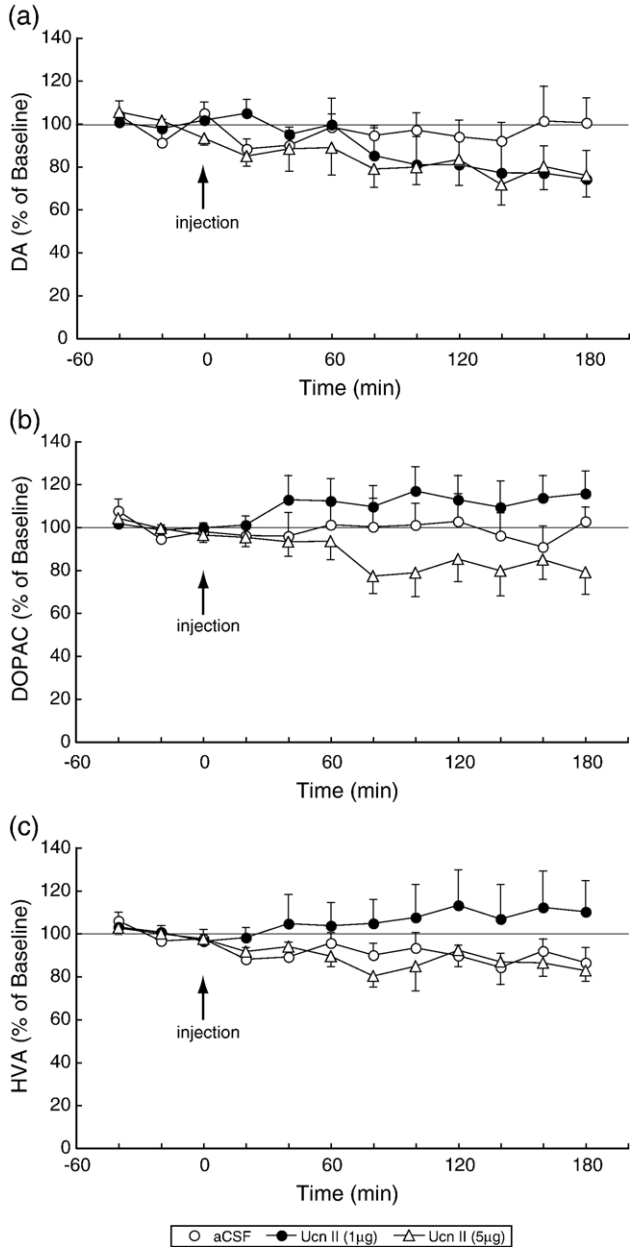

Fig. 2. Effects of microinjection of Ucn II or aCSF on extracellular concentrations of DA (a), DOPAC (b) HVA (c) into the VMH. The *x*-axis indicates the time before and after microinjection of Ucn II or aCSF, and the *y*-axis indicates extracellular levels of DA, DOPAC, or HVA, whose values represent the mean percentage of the baseline (±SEM). The arrow indicates the time of microinjection of Ucn II or aCSF into the VMH.

the time course (Fig. 2(c)). No significant difference in HVA levels was found.

4. Discussion

In this study, direct administration of CRF (10 μg) in the VMH of female rats increased extracellular concentrations of DA in the same brain region, but Ucn II induced no such change. These results suggest that DA release in VMH is modulated by CRFR1, but not by CRFR2.

These data were partly consistent with previous studies that intracerebroventricular (ICV) injection of 0.1, 1, or 2 μg CRF increased dialysate concentrations of DA, DOPAC, and HVA during 2–3 h after administration in the medial prefrontal cortex and the medial hypothalamus (Lavicky and Dunn, 1993). Lowry et al. (2001) also found that ICV 25 or 50 ng CRF increased DA levels 30 min after treatment in the dorsomedial hypothalamus, but there was no significant treatment effect on DOPAC levels or DOPAC:DA ratio in the same brain region. This was consistent with our study showing that DA levels were elevated after CRF administration, but DOPAC:DA ratios were not significantly changed by injection of CRF or Ucn II into VMH (data not shown). However, the elevation of DA levels in VMH returned to the control level within the 60 min slot after injection of 10 μg CRF, and the extracellular concentration of DA did not change after injection of 2 μg CRF. Moreover, neither 2 nor 10 μg CRF changed DOPAC and HVA levels. The reactivity of DA metabolism for CRF in VMH might be weaker than reactivity in the medial prefrontal cortex, the medial hypothalamus, or the dorsomedial hypothalamus as previously reported (Lavicky and Dunn, 1993; Lowry et al., 2001). In contrast, Butler et al. (1990) reported that CRF microinjection into the locus coeruleus did not alter DA or DOPAC concentrations in the amygdala, posterior hypothalamus, and bed nucleus of the stria terminalis. DOPAC:DA ratios also showed that the ratios were significantly increased in the hypothalamus by ICV administration of 1 μg CRF (Dunn and Berridge, 1987; Dunn, 2000). Matsuzaki et al. (1989) also reported that ICV administration of 1 and 10 μg CRF increased DOPAC:DA ratios in the frontal cortex, striatum, hippocampus, and amygdala. Almost all studies selected ICV administration to study the DA system, but we injected drugs directly into the VMH to exclude interaction with any other brain region. The dose of CRF in our experiment was the same as the dose which elevated DA levels in the preceding reports. The effect of CRF on the DA system in VMH may be less than the effect in other brain regions as CRFR1 mRNA expression in VMH is lower than CRFR2 mRNA expression (Chalmers et al., 1995).

Few studies have examined the interaction between CRFR2 and the DA system. Using an in vitro superfusion study following electrical stimulation, Bagosi et al. (2006) observed that CRF administration increased DA release in the striatum and that Ucn II did not significantly change DA release. They indicated that CRF mediated DA release and that Ucn II was not involved in this process, although CRFR2 activity in the basal ganglia was very low. In this study, microinjection was performed in VMH where CRFR2 is highly distributed (Chalmers et al., 1995), and we used an in vivo microdialysis technique under spontaneous conditions whose technique was different from that used by Bagosi et al. Even in this condition, the injection of Ucn II into VMH did not change the extracellular concentrations of DA, DOPAC and HVA. Our findings support that the activation of CRFR1, but not CRFR2, is involved in DA release. It is important to examine the DA system in the projection of VMH to evaluate the direct effect of activating CRF receptors. VMH has a rather widespread output, such as to the bed nucleus of the stria terminalis, which receives dopaminergic inputs that make direct synaptic contact

with CRF neurons (Phelix et al., 1994). In our results, whether the mechanism of CRF activates the DA system directly or indirectly remains unclear.

CRFR2 activation in VMH is important, because CRFR2 is abundant in VMH. However, Ucn II infusion did not alter extracellular concentrations of DA, DOPAC, or HVA during 3 h after microinjection of 1 or 5 μg Ucn II in our study. Ucn II may influence stress-related behavior mainly through another neurotransmitter system like the serotonergic system. Microdialysis studies indicated that ICV injection of Ucn II increased extracellular concentrations of serotonin in the hippocampus (de Groote et al., 2005), and that microinjection of Ucn II directly into the dorsal raphe nucleus increased extracellular concentrations of serotonin in the basolateral amygdala (Amat et al., 2004). Both studies reported that maximal concentrations of serotonin were reached within 60 min after the administration of Ucn II. Previous studies reported that the central administration of Ucn II induced a delayed anorectic effect (Reyes et al., 2001; Inoue et al., 2003) or delayed anxiolytic effect (Valdez et al., 2002). Our results suggested that CRFR2 activation was irrelevant to changes of DA or DA metabolites, because DA levels were not changed by the injection of Ucn II into the VMH as immediately as serotonin levels. DA levels might be changed by Ucn II-induced behavior, however, we did not observe any change of DA metabolism in VMH, and we did not evaluate rat behavior after Ucn II administration.

When using female rats, DA and DA metabolites in the brain are different during the estrous cycle (Doge, 1993). Variation of the mean baseline DA and DA metabolites levels was considerable in our results, partly because we did not consider the estrous cycle in the experiment. Hence, we compared the percentage change from baseline samples before intra-VMH injection of aCSF or CRF agonists. It may be necessary to control the responsive difference of the DA system due to the estrous cycle. It is important to note, however, that in spite of taking no account of the estrous cycle, direct administration of CRF into the VMH of female rats effected DA release. As to Ucn II, the injection did not change extracellular concentrations of DA or DA metabolites, partly because the experiment was carried out without considering the estrous cycle. Role of CRFR2, which is abundant in VMH, might be associated with the estrous cycle. Further study should be conducted in future.

Orosco and Nicolaidis (1992) reported that DA increased in the rostromedian hypothalamus following food intake by using the in vivo microdialysis technique. Meguid et al. (1997) indicated that DA levels in VMH decreased during eating by microdialysis. Taken together, eating behavior alters DA levels in the VMH. Hence, it is necessary to consider the change of DA levels induced by food intake to evaluate the interaction between CRF receptors and the dopaminergic system. Moreover, central administration of CRF or Ucn II reduced food intake. In this study, we did not need to consider the change, because the rats were deprived food during our experiment to exclude the influence that activation of CRF receptors inhibited feeding behavior.

In summary, direct administration of CRF into VMH increased the extracellular concentration of DA, whereas Ucn II showed no significant effect on the concentration of DA or DA metabolites. These results suggest that CRFR1, but not CRFR2, is involved in the DA system in the VMH. These differences between the two receptors might play important roles in the control of stress-related behavior.

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