Centrally administered adrenomedullin 2 activates hypothalamic oxytocin-secreting neurons, causing elevated plasma oxytocin level in rats

Hashimoto, Hirofumi, Susumu Hyodo, Makoto Kawasaki, Takashi Mera, Lei Chen, Atsushi Soya, Takeshi Saito, Hiroaki Fujihara, Takashi Higuchi, Yoshio Takei, and Yoichi Ueta. Centrally administered adrenomedullin 2 activates hypothalamic oxytocin-secreting neurons, causing elevated plasma oxytocin level in rats. Am J Physiol Endocrinol Metab 289: E753–E761, 2005.—We examined the effects of intracerebroventricular (ICV) administration of adrenomedullin 2 (AM2) on plasma oxytocin (OXT) and arginine vasopressin (AVP) levels in conscious rats. Plasma OXT levels were markedly increased 5 min after ICV administration of AM2 (1 nmol/rat) compared with vehicle and remained elevated in samples taken at 10, 15, 30, and 60 min. By contrast, plasma AVP levels were not significantly elevated in samples taken between 5 and 180 min after ICV administration of AM2 except at the 30-min time point. Fos-like immunoreactivity (Fos-LI) was observed in various brain areas, including the paraventricular (PVN) and the supraoptic nuclei (SON) after ICV administration of AM2 (2 nmol/rat) in conscious rats. (measured at 90 min post-AM2 infusion). Dual immunostaining for OXT/Fos and AVP/Fos showed that OXT-LI neurons predominantly exhibited nuclear Fos-LI compared with AVP-LI neurons in the PVN and the SON. In situ hybridization histochemistry showed that ICV administration of AM2 (0.2, 1, and 2 nmol/rat) caused marked induction of the expression of the c-fos gene in the PVN and the SON. This induction was significantly reduced by pretreatment with both the calcitonin gene-related peptide (CGRP) antagonist CGRP-(8–37) (3 nmol/rat) and the AM receptor antagonist AM-(22–52) (27 nmol/rat). These results suggest that centrally administered AM2 mainly activates OXT-secreting neurons in the PVN and the SON, at least in part through the CGRP and/or AM receptors with marked elevation of plasma OXT levels in conscious rats.

c-fos; fos; paraventricular nucleus; supraoptic nucleus; vasopressin; CGRP (calcitonin gene-related peptide); AM (adrenomedullin); SON (supraoptic nucleus); PVN (paraventricular nucleus)

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onist), the effects of ICV administration of AM2 on the expression of the c-fos gene in the PVN and the SON were examined by in situ hybridization histochemistry.

MATERIALS AND METHODS

Animals. Adult male Wistar rats, weighing 200–250 g, were housed individually in plastic cages in an air-conditioned room (24 ± 1°C) under a 12:12-h light (0700–1900)-dark (1900–0700) cycle.

Surgical procedures. Animals to be administered ICV AM2 or 0.9% saline vehicle were implanted with stainless steel cannula aimed at the lateral ventricle. For implantation surgery, animals were anesthetized with pentobarbital sodium (50 mg/kg body wt ip injection) and then placed in a stereotaxic frame. A stainless steel guide cannula (550 μm outer diameter, 10 mm length) was implanted stereotaxically at the following coordinates: 0.8 mm posterior to the bregma, 1.4 mm lateral to midline, and 2.0 mm below the surface of the left cortex such that a tip of the cannula was 1.0 mm above the left cerebral ventricle (18). Two stainless steel anchoring screws were fixed to the skull, and the cannula was secured in place by acrylic dental cement. The animals were then returned to their cages and allowed to recover for at least 7 days. They were then handled every day and housed in cages before the start of the experiments.

Central administration of AM2 and vehicle. For ICV administration of AM2 or vehicle, a stainless steel injector (300 μm outer diameter) was introduced through the cannula at a depth of 1.0 mm beyond the end of the guide. The total volume of injected solution of AM2 and saline into the lateral ventricle was 10 μl. Rat AM2, human CGRP-(8–37), and AM-(22–52) were purchased from the Peptide Institute (Minoh, Japan). AM2, CGRP-(8–37), and AM-(22–52) were dissolved in pyrogen-free sterile 0.9% saline solution (Otsuka Pharmaceutical).

Experimental procedures. For studies of circulating levels of OXT and AVP, animals were decapitated 5, 10, 15, 30, 60, or 180 min after ICV administration of AM2 (1 nmol/rat) or vehicle (n = 6 in each group) in conscious rats for dose-response studies and 30 min after ICV administration of AM2 (at doses of 0.2, 1, and 2 nmol/rat) or vehicle (n = 6–8 in each group) in conscious rats for time course studies. Trunk blood was collected for measuring plasma concentrations of OXT and AVP, using RIA.

Studies of the colocalization of c-fos activity with OXT or AVP were performed on animals that were infused with AM2 (2 nmol/rat) or vehicle (n = 3 or 5 in each group). After ICV administration of the solution (90 min), the animals were anesthetized deeply (pentobarbital sodium, 75 mg/kg body wt ip), perfused with fixative, and processed for immunohistochemistry of Fos, OXT, and AVP (see below).

Animals used for in situ hybridization histochemistry for c-fos mRNA were decapitated 5, 10, 15, 30, 60, or 180 min after ICV administration of AM2 (1 nmol/rat) or vehicle (n = 6 in each group) in conscious rats for time course studies or at 30 min after ICV administration of AM2 (0.2, 1, and 2 nmol/rat) or vehicle (n = 6–8 in each group) in conscious rats for dose-response studies. Brains were rapidly removed and placed on powdered dry ice for in situ hybridization histochemistry for c-fos mRNA (see below).

For studies of CGRP or AM inhibitor action, animals received 5 μl saline (vehicle), CGRP-(8–37) (3 nmol/rat) in 5 μl saline, or AM-(22–52) (27 nmol/rat) in 5 μl saline ICV (n = 6 in each group). After infusion of inhibitor or vehicle (10 min), rats were given a second ICV injection containing 5 μl saline or AM2 (1 nmol/rat) in 5 μl saline and decapitated 30 min after the second infusion. Brains were removed and placed on powdered dry ice for in situ hybridization histochemistry for c-fos mRNA.

All procedures in the present study were done in accordance with guidelines on the use and care of laboratory animals as set out by the Physiological Society of Japan and under the control of the Ethics Committee of Animal Care and Experimentation, University of Occupational and Environmental Health (Kitakyushu, Japan).

RIA for OXT and AVP. Plasma concentrations of OXT and AVP were determined by RIA with specific anti-OXT and anti-AVP antisera, as described previously (6, 7). Coefficients of inter- and intra-assay variations were 9.7 and 4.7% for OXT and 7.3 and 3.2% for AVP, respectively. The minimum detection limit was 0.76 fmol/ml for OXT and 0.68 fmol/ml for AVP.
Colocalization of Fos-like immunoreactivity with OXT-like immunoreactivity or AVP-like immunoreactivity. Deeply anesthetized animals were perfused transcardially with 0.1 M phosphate buffer (PB, pH 7.4) containing heparin (1,000 U/l saline) followed by 4% paraformaldehyde and 0.2% picric acid in 0.1 M PB. The brains were then removed, coronally cut, and divided into three blocks (forebrain, hypothalamus, and brain stem). Blocks were postfixed with 4% paraformaldehyde and 0.2% picric acid in 0.1 M PB for 48 h at 4°C and then cryoprotected in 20% sucrose in 0.1 M PB for another 48 h at 4°C. Serial sections of either 30 µm for staining for Fos and OXT/AVP or 40 µm for immunostaining for Fos were cut using a microtome. Sections were rinsed twice with 0.1 M PBS containing 0.3% Triton X-100, incubated in 0.1 M PBS containing 0.3% Triton X-100 with 0.3% hydrogen peroxidase for 60 min, and then rinsed twice with 0.1 M PBS containing 0.3% Triton X-100. Floating sections were incubated with a primary Fos antibody (sc-52; Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:500 in 0.1 M PBS containing 0.3% Triton X-100 for 4°C for 4 days. After being washed for 20 min in 0.1 M PBS containing 0.3% Triton X-100, the sections were incubated for 120 min with a biotinylated secondary antibody solution (1:250) and finally with an avidin-biotin peroxidase complex (Vectastain ABC kit; Vector Laboratories, Burlingame, CA) for 120 min. The peroxidase in the sections was visualized with 0.02% diaminobenzidine in a Tris buffer containing 0.05% hydrogen peroxidase for 3 min. In the dual staining for OXT or AVP, sections were subsequently incubated in OXT antibody (Chemicon International, Temecula, CA; diluted 1:5,000) or AVP antibody (Incstar, Stillwater, MN; diluted 1:10,000) for 3 days at 4°C. The avidin-biotin peroxidase complex was made visible by using nickel sulfate. Sections were mounted on gelatin-coated slides, air-dried, dehydrated in 100% ethanol, cleared with xylene, and then finally covered with a coverslip. The presence of the dark brown label that appeared in round structures was judged as indicative of Fos-like immunoreactivity (LI)-positive nuclei (36), and that of violet label that appeared in spindle-shaped structures was judged as indicative of OXT- or AVP-LI. Details of the immunohistochemistry have been published elsewhere (8, 16). To count the double-labeled cells, three serial sections that included the PVN and the SON per animal were chosen and counted under a light microscope by two independent investigators.

In situ hybridization histochemistry for c-fos mRNA. In situ hybridization histochemistry was performed on frozen 12-µm-thick coronal sections.
brain sections cut on a cryostat at -20°C, thawed, and mounted on gelatin/chrome alum-coated slides. Brain tissue was stored at -80°C before cutting. The locations of the PVN, the SON and the nucleus tractus solitarius (NTS) were determined according to coordinates given by the atlas of Paxinos and Watson (18). The sections including the PVN were chosen from plate 18 in the atlas. The sections including the SON were chosen from plate 17 in the atlas. The sections including the NTS were chosen from plate 42 in the atlas. Ten sets of two sections containing the PVN and the SON or four sections containing the NTS were used from each rat to measure the density of autoradiography. Slides were warmed to room temperature, allowed to dry for 10 min, and then fixed in 4% formaldehyde in PBS for 5 min. They were then washed twice in PBS and incubated in 0.9% NaCl containing 0.25% acetic anhydride (vol/vol) and 0.1 M triethanolamine at room temperature for 10 min. The sections were dehydrated using a series of 70% (1 min), 80% (1 min), 95% (2 min), and 100% (1 min) ethanol solutions consecutively and delipidated in 100% chloroform for 5 min. The slides were then partially rehydrated first in 100% (1 min) and then 95% (1 min) ethanol and allowed to air-dry briefly.

Hybridization was performed at 37°C overnight in a 45-µl buffer solution consisting of 50% formamide and 4% saline sodium citrate (SSC; 1× SSC = 150 mM NaCl and 15 mM sodium citrate), which contained 500 µg/ml sheared salmon sperm DNA (Sigma, St. Louis, MO), 250 µg/ml baker’s yeast total RNA (Roche Molecular Biochemicals, Mannheim, Germany), 1× Denhardt’s solution, and 10% dextran sulfate (500,000 molecular weight; Sigma). The hybridization was performed under a Nesofilm (Bando Chemical IMD, Osaka, Japan) coverslip. A 32P-3′-end-labeled deoxyoligonucleotide that was complementary to transcripts coding for c-fos (bases 138–185 of rat c-fos nucleotides) was used. The specificity of the probes has been described previously (5, 10). A total of 1 × 106 cpm/slide for c-fos transcripts. After hybridization, the sections were washed for 1 h in four separate 1× SSC rinses at 55°C and for another hour in two changes of 1× SSC at room temperature. All independent experimental sections were treated simultaneously to minimize the variable effects of hybridization and wash stringency. Hybridized sections containing the PVN, the SON, and the NTS were apposed to autoradiography film (Hyperfilm; Amersham, Buckinghamshire, UK) for 2–3 wk for c-fos transcripts. The autoradiographic images were quantified using a MCID imaging analyzer (Imaging Research, St. Catherines, Ontario, Canada). The images were captured by a charge-coupled device camera (DAGE-MTI, Michigan City, IN) with ×40 magnification. The mean optical density (OD) of autoradiographs was measured by comparing it with simultaneously exposed 14C microscale samples (Amersham). 14C was used as the standard for quantification of the OD of autoradiographs for in situ hybridization histochemistry. The standard curve was fitted by OD of the 14C microscale on the same film. Slides hybridized with the c-fos probe were dipped in a nuclear emulsion (K-5; Ilford, Cheshire, UK) and exposed for a further 6 wk.

Statistical analysis. A mean deviation from control (percentage) ± SE was calculated from data obtained from measurement of plasma OXT and AVP levels, immunohistochemistry for Fox, and in situ hybridization for c-fos mRNA. Each group within an experiment was compared with the control group. The data were analyzed using a one-way fractional ANOVA followed by a Bonferroni correction for multiple comparisons. The statistical significance was set at P < 0.05.

RESULTS

Effects of ICV administration of AM2 on plasma concentrations of OXT and AVP. The concentrations of plasma OXT and AVP were measured 5, 10, 15, 30, 60, and 180 min after ICV administration of AM2 (1 nmol/rat) or vehicle. The concentrations of plasma OXT were profoundly increased 5–60 min after ICV administration of AM2 (1 nmol/rat) compared with vehicle (Fig. 1A). The concentrations of plasma AVP were not increased at any of the sample times after ICV administration of AM2 (1 nmol/rat) compared with vehicle except at the 30-min sample time (Fig. 1B). The plasma concentrations of OXT and AVP measured at 30 min after ICV administration of AM2 (0.2, 1, and 2 nmol/rat) or vehicle showed large, significant increases in OXT levels at all AM2 doses (Fig. 1C) but smaller or nonsignificant changes in AVP levels (Fig. 1D). Only the 1 nmol/rat dose was associated with a small but significant increase in plasma AVP.

Colocalization of Fos-LI and OXT-LI or AVP-LI in the PNG and SON. Fos-LI cells in the magnocellular parts of the PNG after ICV administration of AM2 (2 nmol/rat) colocalized to OXT-LI rather than AVP-LI cells (Figs. 2–4). The numerous Fos-LI in the parvocellular parts of the PNG were reactive for neither AVP nor OXT (Fig. 2, A–D). In the SON, OXT-LI cells predominated in the nuclear Fos-LI region compared with AVP-LI cells (Fig. 2, E–H). Taking the total OXT-LI-positive cells counted in each nucleus as 100%, the percentage of...
Fos-LI-positive cells (n = 5) was 85.3 ± 2.5% in the PVN (Fig. 3, A and B) and 89.5 ± 1.7% in the SON (Fig. 4, A and B) after ICV administration of AM2 (2 nmol/rat). Similarly, taking the total AVP-LI-positive cells counted in each nucleus as 100%, the percentage of Fos-LI-positive cells (n = 5) was 10.0 ± 1.2% in the PVN (Fig. 3, C and D) and 13.6 ± 1.1% in the SON (Fig. 4, C and D) after ICV administration of AM2 (2 nmol/rat).

Functional mapping by Fos expression. Many intense Fos-LI were found in various regions of the brain after ICV administration of AM2 (2 nmol/rat; Fig. 6, right side of each brain section). On the other hand, only a small number of Fos-LI was observed in the CNS after ICV administration of saline (Fig. 6, left side of each brain section). In the forebrain, many Fos-LI were localized to the piriform cortex and the central amygdaloid nucleus (CeA; Fig. 7F). In the hypothalamus, many Fos-LI were observed in the medial preoptic nucleus, the SON (Fig. 7B), the PVN (Fig. 7D), the arcuate hypothalamic nucleus, the periventricular regions of the third ventricle, and the ventromedial hypothalamic nucleus (VMH; Fig. 7H). In the midbrain and the brain stem, there were many Fos-LI in the locus coeruleus (LC; Fig. 7J), the area postrema (AP), the nucleus of the NTS, and the dorsal motor nucleus vagus (Fig. 7L). On the other hand, only a few Fos-LI were observed in corresponding areas in controls injected ICV with a vehicle (Fig. 7, A, C, E, G, I, and K).

Effects of ICV pretreatment with CGRP and AM receptor antagonists on AM2-induced c-fos gene expression in the PVN and SON. In situ hybridization histochemistry revealed that the c-fos gene expression in the PVN and the SON was significantly increased after ICV administration of AM2 (1 nmol/rat) with pretreatment of saline, CGRP-(8–37) (3 nmol/rat; CGRP receptor antagonist), or AM-(22–52) (27 nmol/rat; AM receptor antagonist; Fig. 8). However, the induction of c-fos expression in the PVN and the SON as caused by ICV administration

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Fig. 5. Effects of ICV administration of saline (vehicle) or AM2 (0.2, 1, and 2 nmol/rat) on c-fos transcript prevalence in the SON (A, D, G, and J), the PVN (B, E, H, and K) and the NTS (C, F, I, and L). A-C: effects of ICV administration of vehicle or AM2 (0.2, 1, and 2 nmol/rat) on c-fos transcript prevalence. D-F: effects of ICV administration of vehicle or AM2 (1 nmol/rat) on c-fos transcript prevalence. G-L: representative images of emulsion-dipped sections hybridized to a 35S-labeled oligodeoxynucleotide probe for c-fos mRNA in the SON (G and J), the PVN (H and K), and the NTS (I and L) 30 min after ICV administration of vehicle (saline; G-I) or AM2 (1 nmol/rat; J-L). Values represent means ± SE (n = 6). **P < 0.01 compared with vehicle-administered rats. Bars indicate 100 μm.
and demonstrate activation of cells using combined Fos and OXT immunohistochemistry. In situ hybridization histochemistry was used to generate dose-response functions for AM2 activation of the PVN and the SON neurons and to demonstrate the efficacy of an AM inhibitor. In contrast, AVP-containing neurons in the PVN or the SON showed little or no activation, and circulating AVP levels did not change. We conclude that centrally administered AM2 mainly activates OXT-producing magnocellular neurosecretory cells in the SON and the PVN, at least in part via CGRP and/or the AM receptors in rats.

**DISCUSSION**

The present study provides the first evidence that centrally administered AM2 causes a marked increase in plasma OXT levels and activates OXT-secreting neurons in the PVN and the SON in conscious rats. We measured circulating levels of OXT of AM2 was significantly reduced by pretreatment with CGRP-(8–37) and AM-(22–52). The expression of the c-fos gene in the PVN and the SON after ICV administration of the vehicle did not change.

**Fig. 6.** Distribution of Fos-LI in the central nervous system 90 min after ICV administration of AM2 (2 nmol/rat; red dots on right side) or saline (vehicle; black dots on left) in a conscious rat. AP, area postrema; Arc, arcuate hypothalamic nucleus; CeA, central amygdaloid nucleus; DLPAG, dorsolateral periaqueductal gray; DM, dorsomedial hypothalamic nucleus; DMPAG, dorsomedial periaqueductal gray; DMV, dorsal motor nucleus vagus; DR, dorsal raphe nucleus; ECIC, external cortex of the inferior colliculus; LC, locus coeruleus; LV, lateral ventricle; MD, mediodorsal thalamic nucleus; ME, median eminence; Mve, medial vestibular nucleus; opt, optic tract; ox, optic chiasma; Pir, piriform cortex; Pn, pontine nuclei; POA, preoptic area; PV, paraventricular thalamic nucleus; SuM, supramammillary nucleus; VMH, ventromedial hypothalamic nucleus; VTA, ventral tegmental area. Bar indicates 100 μm.

**Fig. 7.** Photomicrographs showing changes in Fos-LI in the SON (A and B), the PVN (C and D), the CeA (E and F), the VMH (G and H), the LC (I and J), and the AP and NTS (K and L). Bars indicate 100 μm.
Brain regions activated by AM2. The present study demonstrates that centrally administered AM2 in conscious rats activates various areas in the forebrain, hypothalamus, and brain stem, using immunohistochemistry for Fos. We focus here on Fos-LI cells that were found in the PVN, the SON, the NTS, and the AP. Among these structures, Fos-LI was predominantly localized in OXT-LI cells in the SON and the PVN, although a few AVP-LI cells exhibited nuclear Fos-LI after ICV administration of AM2. These results are in agreement with the large increases in plasma OXT levels and the contrastingly small changes in plasma AVP levels after ICV administration of AM2.

Taylor et al. (34) showed that AM2 contents in the hypothalamus are very high compared with those in brain stem and cerebellum in rat (34). They also showed that posterior pituitary also contains some amount of AM2 (34). Although there are no available data to confirm the existence of AM2 in the hypothalamo-neurohypophyseal system, these findings suggested the possibility that AM2 may be produced by magnocellular neurosecretory cells in the hypothalamus and regulate the neuronal activity of OXT-secreting neurons and release of OXT in the posterior pituitary. Verbalis et al. (37) demonstrated that central OXT is involved in the inhibition of salt appetite and feeding behavior. In earlier work, we have shown that AM levels are increased in the cerebrospinal fluid after chronic salt loading or fasting (3). Centrally administered AM inhibits salt appetite in rats (23), and AM2 inhibits feeding and water drinking in rats (34). AM2 and AM may both be involved in the central regulation of salt appetite, feeding, and water balance.

The presence of Fos-LI was also observed in the parvocellular parts of the PVN. These parts of the PVN comprise cells containing hypophysiotropic factors, such as corticotropin-releasing hormone- and thyrotropin-releasing hormone-producing cells. It is well known that AM may play an important role in the regulation of the hypothalamo-pituitary-adrenal axis (13, 24, 27, 28).

Fig. 8. Effects of ICV administration of saline (vehicle) or AM2 (1 nmol/rat) on c-fos transcript prevalence in the PVN (A and B) and the SON (C and D) after pretreatment with calcitonin gene-related peptide (CGRP) antagonist [CGRP-(8-37), 3 nmol/rat; A and C] and AM antagonist [AM-(22-52), 27 nmol/rat; B and D]. Representative images of emulsion-dipped sections hybridized to a 35S-labeled oligodeoxynucleotide probe for c-fos mRNA in the PVN (E and H) and the SON (I and L) 30 min after ICV administration of AM2 (1 nmol/rat) and pretreatment with CGRP antagonist [CGRP-(8-37), 3 nmol/rat] and AM antagonist [AM-(22-52), 27 nmol/rat]. Values represent means ± SE (n = 6). P < 0.01 compared with vehicle-administered rats (***) and compared with antagonist-administered rats (###). Bars indicate 100 μm.
istration of AM2, although it is unknown whether centrally administered AM2 activated LC neurons, including the noradrenergic neurons, directly or indirectly.

Centrally administered AM2 induced the expression of the c-fos gene and Fos in the NTS and the AP. Allen and Ferguson (1) reported in an in vitro study that AM excites AP neurons directly and modulates the activity of the NTS neurons indirectly. Other studies have demonstrated that AM acts on the AP directly and produces a significant cardiovascular response (2, 26, 39), indicating that the AP is one of the most potent sites of action for centrally administered AM. Therefore, we expected and found that central administration of AM2 induced the expression of the c-fos gene and Fos in both the AP and the NTS. Taylor et al. (34) reported that intracerebroventricularly administered AM2 elevated mean arterial pressure, and these effects were abrogated by the α-adrenergic receptor antagonist phentolamine and the CGRP receptor antagonist CGRP-(8–37). Although the site of action of AM2 in the regulation of cardiovascular function in the CNS is unclear, our immunohistochemistry studies for Fos suggest that the PVN, the LC, and the brain stem (the NTS and the AP) are activated by ICV administration of AM2 and may be involved in the central regulation of the cardiovascular system.

How are OXT cells activated by AM2? Physiological stimuli such as hyperosmolality and hypovolemia stimulate the release of both AVP and OXT (29). We found that AM2 was largely selective for the activation of OXT neurons in hypothalamic areas, and this activity correlated with the increased circulating levels of OXT. Systemic cholecystokinin (CCK) is also known to stimulate the release of OXT, but not AVP, in rats (38). It has been demonstrated in previous studies that peripheral administration of CCK activates OXT-secreting cells in the SON and the PVN via noradrenergic neurons in the caudal NTS in the region of the brain stem receiving visceral vagal afferents (11, 15). Verbalis et al. (37) reported that central OXT may inhibit salt appetite and feeding behavior. They suggested that release of OXT in the systemic circulation may correlate with the activation of the central oxytocinergic pathway.

Previous studies showed that ICV administration of AM had no significant effect on the plasma concentrations of AVP in rats (40) or sheep (17), and, furthermore, AM was reported to inhibit the release of AVP that is normally evoked by hypertonic and hypovolemic stimulation in rats (40). We found a few AVP-LI cells in the SON and the PVN that exhibited novel Fos-LI after ICV administration of AM2 and a small increase in the plasma concentration of AVP at 30 min after ICV administration of AM2. The activation of AVP neurons by centrally administered AM2 is different from the actions of centrally administered AM. There is a possibility that AM2 may interact with additional receptors.

Contrasting AM with AM2. Our previous study demonstrated that centrally administered AM increases plasma OXT level, activates OXT-secreting neurons in the PVN and the SON in conscious rats (25), and excites the OXT-secreting cells in the PVN in urethane-anesthetized rats (35). The excitatory effects of centrally administered AM2 on OXT-secreting cells in the rat hypothalamus seem to be similar with those of AM.

Fujisawa et al. (4) showed that peripheral administration of AM and AM2 changed renal function and the effects of AM lasted longer than that of AM2. In the present study, central administration of AM2 caused long-lasting elevation of OXT in plasma. Although the peripheral and central effects of AM and AM2 on OXT secretion and autonomic responses were not compared in the present study, a comparison of our findings with those of a previous study (25) shows that the effects of centrally administered AM2 on OXT secretion are stronger than those of centrally administered AM. Previous studies have reported that AM2 activates a cAMP-dependent pathway via complexes of calcitonin receptor-like receptor (CRLR) and receptor-activity-modifying proteins (RAMPs) with CGRP and AM (20, 32). The rank of potency for the stimulation of CRLR/RAMP1, CRLR/RAMP2, and CRLR/RAMP3 was shown in these studies to be CGRP > AM2 = AM, AM > AM2 > CGRP and AM > AM2 > CGRP, respectively. The relative potencies are consistent with the effects of peripherally administered AM2 on renal function but are not consistent with the effects of centrally administered AM2 on OXT secretion. The different effects of peripherally administered AM2 on renal function compared with centrally administered AM2 on OXT secretion suggest that the distribution of AM and AM2 receptors in the CNS may be different from that in peripheral organs.

The present study showed that centrally administered AM2 induced the expression of the c-fos gene in the PVN and the SON, and this induction was significantly reduced by pretreatment with both the CGRP and AM receptor antagonists. Therefore, we presume that central AM2 activates OXT-secreting neurons in the PVN and the SON, at least in part, through CGRP and/or AM receptor. The possibility that unknown specific receptors for AM2 may exist in the CNS cannot be excluded and requires further study.

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