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Review

Roles of the Calcitonin Gene-Related Peptide Family in the Central Nervous System

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SUMMARY

The calcitonin gene-related peptide (CGRP) family is composed of CGRP, adrenomedullin (AM), AM2 (identical to intermedin), AM5 and amylin. These peptides are widely distributed in peripheral tissues and the central nervous system (CNS). They activate a cAMP-dependent pathway via complexes of calcitonin receptor or calcitonin receptor-like receptor and receptor activity-modifying proteins. They are involved in several physiological and pathological functions such as water intake, feeding, and modulation of cardiovascular and neuroendocrine systems. Previous studies have shown that a central administration of these neuropeptides activated various areas in the CNS and induced physiological effects. Our previous studies showed that centrally administered AM family (AM, AM2 and AM5) induces the expressions of the c-*fos* gene in the oxytocin-secreting neurons in the supraoptic and the paraventricular nuclei of the hypothalamus and elicits a marked increase in plasma oxytocin levels in conscious rats. Here, I review the roles of the CGRP family in the CNS.

Key Words : adrenomedullin, amylin, hypothalamus, intermedin, oxytocin

INTRODUCTION

The calcitonin gene-related peptide (CGRP) family is composed of CGRP, adrenomedullin (AM), AM2, AM5 and amylin[1~6]. Their structures are similar, each having an internal disulfide bridge with a ring and amidated carboxyl terminal (Fig. 1). Both these structural traits are essential for receptor binding and subsequent signaling[7,8]. CGRP family is widely distributed in the peripheral tissues and the central nervous system (CNS) and have various physiological and pathophysiological effects. The activation of CGRP family was inhibited or attenuated by $CGRP_{8-37}$ and/or AM_{22-52}, which is used as CGRP and AM antagonist, respectively[9]. Afterward, it has been clarified that they are regulated by a combination of calcitonin receptor (CR) or calcitonin receptor-like receptor (CRLR), and the receptor activity-modifying proteins (RAMPs). CR and CRLR (CR/CRLR) is a G protein-coupled receptor, and these receptors have unique system. CR/CRLR needs the receptor activity-modified protein for their activation. Three receptor activity-modified proteins, RAMP1-3, were identified in rats and mice[10]. CR/CRLR formed a complex with each RAMP and was translocated from endoplasmic reticulum to cell membrane[10]. CR/CRLR or RAMPs alone were not activated as receptor. Namely, CR/CRLR cannot translocate to cell membrane and cannot be activated without RAMPs[11]. $CGRP_{8-37}$ blocked the

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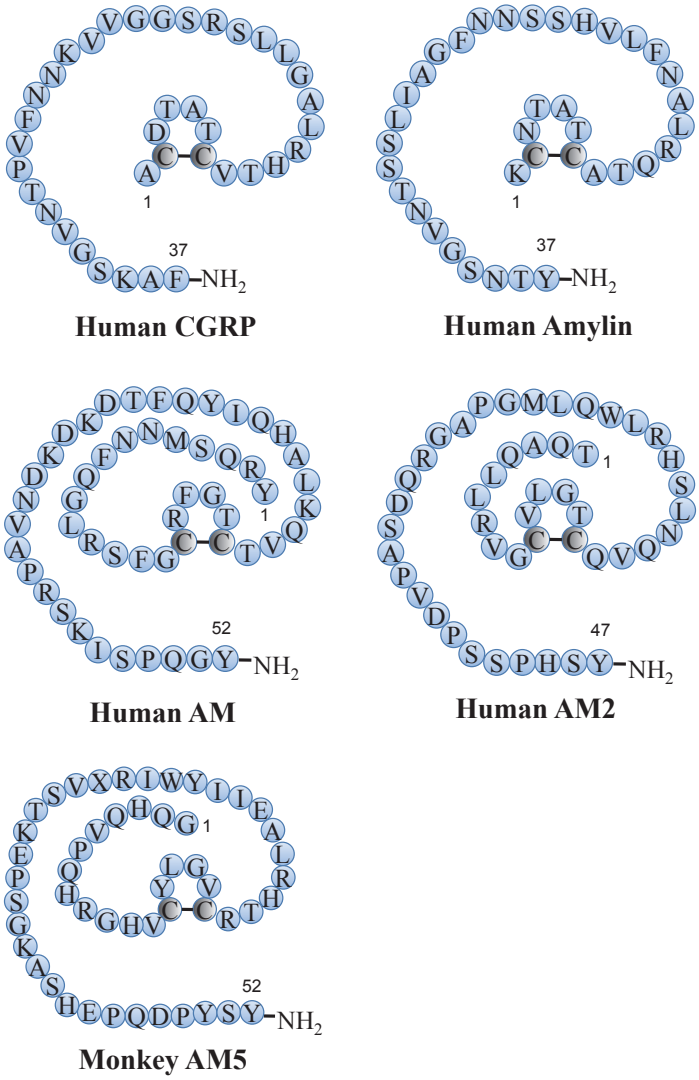

Fig. 1 Structure of CGRP family

activation of the CRLR/RAMP1 and AM_{22-52} blocked the activation of the CRLR/RAMP2 and CRLR/RAMP3[12].

Many previous studies have shown that centrally administered neuropeptides activated various areas in brain and affected physiological and pathophysiological functions. Our previous studies showed that central administration of AM family (AM, AM2 and AM5) induced the expression of the c-*fos* gene in the supraoptic (SON) and the paraventricular nuclei (PVN) of the hypothalamus and elicited the marked increase of plasma oxytocin levels in conscious rats[13~15]. In this review, I focus on (1) the distribution, (2) receptor, and (3) physiological roles of CGRP family in the CNS.

Calcitonin gene-related peptide (CGRP)

Distribution and synthesis of CGRP

CGRP is a 37-amino acid peptide (Fig. 1) and is produced by alternative splicing of RNA from the calcitonin gene[16,17]. CGRP is principally located in unmyelinated sensory C-fibers and myelinated Aδ-fibers of peripheral nerves[18~20]. CGRP is co-located with substance P in C-fibers[20]. In the periphery, CGRP is also localized in the adrenal gland, pancreas and nerves at the adventitial-medial border of coronary blood vessels[17,21,22] (Table 1). In the CNS, the CGRP mRNAs were also distributed in the peripeduncular nucleus (PP), nucleus of oculomotor nerve, parabrachial nuclei (PBN), nucleus of trigeminal nerve, superior olivary nucleus, motor nucleus of facial nerve, nucleus ambig-

Table 1 Distribution of CGRP family gene in tissues

	CGRP	AM	AM2	AM5	Amylin
brain		+		+	
pituitary		+	+	+	
heart		+			
lung		+	+		
adrenal gland	+	+		+	
kidney		+	+		
liver		+			
bone		+			
pancreas	+	+	+		+
esophagus			+		
stomach		+	+		+
small intestine		+	+		+
large intestine		+	+		+
rectum			+		
mesentery			+		
spleen		+	+	+	
thymus		+	+	+	
submaxirally		+	+		
testis		+			
ovary		+	+		
uterus		+			
References	17	37	4, 5	6	92, 93, 116, 117

uus, nucleus of hypoglossal nerve[23] (Table 2). In immunohistochemistry studies, CGRP-like immunoreactivity (LI) was localized in the olfactory bulb (OB), central nucleus of the amygdala (CeA), PVN, lateral hypothalamic area (LHA), dorsomedial hypothalamic nucleus (DMH), ventromedial nucleus of hypothalamus (VMH), arcuate nucleus (Arc), superior olivary nucleus, parabigeminal nucleus, superior colliculus, vestibular nuclei, posterior agranular insular area (INS), PP, PBN, area postrema (AP), nuclei of solitary tract (NTS), nucleus of hypoglossal nerve[17,24]. As these areas are involved in nociception, feeding, stress, emotion and autonomic function (Table 2), CGRP may involve in these physiological functions.

Distribution of CGRP receptor

Some previous studies showed that mRNAs encoding the CR and CRLR gene products are expressed in many tissues such as heart, spleen, spinal cord, and predominantly in the lungs[25,26]. In the CNS, the CRLR mRNAs are expressed in the caudate putamen and amygdala[27,28]. As well as CRLR, mRNAs encoding RAMPs are expressed in many tissues, including heart, skeletal muscle, gastrointestinal tract, spinal cord, pituitary, adrenal, and thyroid glands[10]. CGRP activates the combination of CRLR with RAMP1. Oliver *et al.* showed the distribution of RAMP1 mRNAs in the rat CNS in detail[28]. RAMP1 mRNAs was widespread in the CNS, especially abundantly expressed in the olfactory tubercles, piriform cortex, nucleus accumbens, caudate putamen, cortex, and amygdala[28].

Table 2 Distribution of CGRP family in the CNS

	physiological role	CGRP mRNA	CGRP -LI	AM -LI	AM2 -LI	amylin -LI
Telencephalon						
bed nucleus of stria terminalis	fear, feeding, social behavior, pain		+	+		+
central amygdaloid nucleus	emotion, memory, stress		+	+		+
globus pallidus	motivation, voluntary movement		+	+		
hippocampus	memory, learning		+	+		
innominate substance	memory			+		
medial septal nucleus	memory		+	+		+
olfactory bulb (OB)	odor system		+	+		+
posterior agranular insular area (INS)	emotion, addiction, taste		+			
striatum	motor, learning, memory			+		
Diencephalon						
arcuate nucleus (Arc)	feeding		+	+	+	
circularis nucleus	osomosencer			+		
dorsomedial nucleus (DMH)	feeding, drinking, body-weight regulation, circadian activity		+	+		+
habenular nucleus	addiction, emotion			+		+
lateral hypothalamic area (LHA)	feeding		+	+		
mammillary body	memory			+		+
median preoptic nucleus (MnPO)	drinking, osomosenser				+	
paraventricular nucleus (PVN)	feeding, stress, pain, social behavior, autonomic system		+	+	+	+
preoptic nucleus	sexual and parental behavior, sleep-awake cycle, thermoregulation		+			+
suprachiasmatic nucleus	circadian rhythm					+
supraoptic nucleus (SON)	neuroendocrine system			+	+	+
vascular organ of the lamina terminalis (OVLT)	drinking, osomosenser				+	
ventromedial nucleus of hypothalamus (VMH)	feeding				+	+
Mesencephalon						
cochlear nuclei	auditory system		+			+
cuneate nucleus	pain, somatosensory system		+	+		+
interfascicular nucleus	reward system, motivation, cognition, addiction			+		
nucleus of oculomotor nerve	origin of oculomotor nerve	+	+	+		+
parabigeminal nucleus	vision system					+
peripeduncular nucleus (PP)	sexual behavior, milk ejection	+	+			
superior colliculus	somatosensory sytem, eye movements, auditory system		+	+		+
Pons						
area postrema (AP)	vomiting centre, cardiovascular system, feeding, metabolism		+	+	+	
nucleus of facial nerve	origin of facial nerve	+	+			+
nucleus of trigeminal nerve	origin of trigeminal nerve	+	+	+		+
parabrachial nuclei (PBN)	nociception, feeding, taste	+	+			
pontine nuclei	motor system			+		+
red nucleus	motor system			+		+
substantia nigra	reward system, motor system		+	+		+
superior olivary nucleus	auditory system	+	+	+		+
trapezoid body	auditory system			+		+
Cerebellum						
cerebellar nuclei	motor system					+
Myencephalon						
inferior olivary nucleus	motor system, sensory system					+
locus coeruleus nucleus (LC)	sleep-wake cycle, memory, attetion, emotion, stress			+	+	
nuclei of solitary tract (NTS)	pain, taste, intestinal motility, cardiovascular system, stress		+		+	+
nucleus ambiguus	speech	+	+		+	+
nucleus of hypoglossal nerve	origin of hypoglossal nerve	+	+			+
vestibular nuclei	vestibular system		+	+		+
References		23	17, 24	39, 40	75	92

Physiological role of CGRP in the CNS

Previous studies have shown effects of centrally administered CGRP on food intake and intestinal motility. Centrally administered CGRP suppresses food intake in food-deprived rats[29,30]. Although peripherally administered CGRP decreases food intake in rat and mice[29,31], the effects of peripheral administered CGRP were less effective than those of centrally administered CGRP in rats[29]. Krahn *et al.* also showed that centrally administered CGRP decreased saccharin intake by one-bottle conditioned aversion test, and suggested that the centrally administered CGRP-induced food suppression involves an aversive mechanism[32]. While, centrally administered CGRP increases food intake and rumination, and decreases the frequency of forestomach motility in the sheep[33]. In goldfish, centrally administered CGRP significantly decreases the food intake[34]. The effects of centrally administered CGRP may be different among the species.

Centrally administered CGRP significantly increases the mean arterial pressure (MAP) and the heart rate (HR) in rats[35]. The effects of CGRP on MAP and HR are attenuated by pretreatment with CGRP receptor antagonist[35]. The effects of CGRP on increasing MAP are inhibited by pretreatment with alpha adrenoreceptor-blocker. The effects of CGRP on increasing HR are suppressed by pretreatment with alpha- and beta-adrenoreceptor-blocker. Moreover, the effects of CGRP on increasing MAP and HR are suppressed by the chemical sympathectomy. Centrally administered CGRP also increased plasma adrenaline and noradrenaline[35]. Central administered CGRP may stimulate sympathetic nervous system and increase MAP and HR.

As described above, some previous studies showed the central effects of CGRP on feeding and cardiovascular function. In immunohistochemistry study for CGRP, CGRP-LI cells were found in the OB, Arc, bed nucleus of stria terminalis, DMH, LHA, medial septal nucleus, PVN, preoptic nucleus, cuneate nucleus, INS, PBN, AP and NTS (Table 2). These areas are involved in feeding, neuroendocrine system, sleep-awake cycle, thermoregulation, cardiovascular function, pain, emotion, and memory. Beside feeding and cardiovascular function, the further study may clarify the physiological role of CGRP in the nociception, neuroendocrine system, sleep-awake cycle, thermoregulation, emotion and memory.

Adrenomedullin (AM)

Distribution and synthesis of AM

AM is a 52-amino acid neuropeptide (Fig. 1) that was originally isolated from tissue extracts of human pheochromocytoma[36]. AM was widely distributed in peripheral organs and the CNS[37~42] (Table 1). In peripheral organs, AM mRNAs were expressed in adrenal glands, lung, kidney, heart, spleen, duodenum, and submandibular glands[37] (Table 1). A large amount of AM is synthesized and secreted from the endothelial cells and vascular smooth muscle cells[38,43~45]. Some previous studies showed that the plasma AM levels increased in the cardiovascular disease, including hypertension, chronic renal failure, congestive heart failure, stroke, and septic shock[46~50]. In the CNS, at first, AM-LI cells were found in the SON, PVN, and circularis nucleus in rats[40,41]. Serrano *et al.* investigated the distribution of AM-LI cells in rats in detail[39]. AM-LI cells were found in multipolar neurons and pyramidal cells in the layers IV-VI of the cerebral cortex, mitral cells and granule cells of the olfactory bulb, caudate putamen, globus pallidus, amygdala, bed nucleus of the stria terminalis, LHA, DMH, PVN, Arc, medial and lateral habenular nuclei, cuneate nucleus, interfascicular nucleus, reticulate and lateralis of the substantia nigra, nucleus of oculomotor nerve, superior colliculus, lateral and medial superior olivary nucleus, vestibular nuclei, locus coeruleus nucleus (LC), and AP in rats[39]. AM-LI cells were co-localized with oxytocin (OXT) and arginine vasopressin (AVP) in the SON and PVN in rats[41]. In human, AM-LI cells were found in the SON and PVN[42]. These areas are involved with cardiovascular system, feeding, social recognition, emotion, reward system, learning, water balance, and autonomic function (Table 2).

Distribution of AM receptor

AM activates the combination of CRLR with RAMP2 and with RAMP3. Oliver *et al.* showed the distribution of RAMP2 and PAMP3 mRNAs in the rat CNS in detail[28]. RAMP2 mRNAs are expressed in the olfactory tubercles, the cortex, hippocampus, SON,

PVN, Arc, VMH, DMH, amygdala, nucleus of hypoglossal nerve, paragigantocellular reticular nucleus, and ventrolateral medulla[28,51]. RAMP3 mRNAs are expressed in the olfactory tubercles, piriform cortex, nucleus accumbens, hippocampus, habenula nucleus, PVN, Arc, VMH, DMH, and granule cell layer of the cerebellum[28].

Physiological role of AM in the CNS

The expression of the c-*fos* protein has been widely used to detect the neuronal activity in the CNS[52]. Some previous studies investigated the central effects of CGRP family using c-*fos* protein activity. After intracerebroventricular (icv) administration of AM, Fos-LI cells were found in various areas of brain in rats, including the SON, PVN, VMH, CeA, lateral habenular nucleus, PBN, LC, AP, NTS, and rostal ventrolateral medulla (RVLM) in rats[53,54]. Moreover, AM-induced Fos expression was attenuated by pretreatment with a CGRP receptor antagonist ($CGRP_{8-37}$) in rats[54]. Centrally administered AM activated OXT-secreting neurons in the SON and PVN, and also increased plasma OXT level in rats[55,56]. Fos-LI cells were co-localized with neuronal nitric oxide synthase (nNOS)-LI neurons in the SON, PVN, NTS, and nucleus paragigantocelluaris lateralis after icv administration of AM in rats[54]. AM-induced Fos expressions in nNOS-LI cells were attenuated by pretreatment with CGRP receptor antagonist[54].

I focused on neurohypophysial hormone, AVP and OXT. AVP and OXT are nine amino acid neuropeptides, and the structure of AVP differs from that of OXT by only 2 amino acids. Both peptides are synthesized in the SON and PVN that terminate their axons in the posterior pituitary[57]. AVP plays a role in water homeostasis and cardiovascular regulation, and also controls osmolality and blood pressure. OXT is well known for its roles in reproduction, especially during and after childbirth. OXT is also involved in several physiological functions, such as antinociception, anxiety, feeding, social recognition and stress responses[58~62]. Central administration of AM also activates OXT-secreting neurons[14,55] and increases plasma OXT levels in rats[55]. While, icv administration of AM had no significant effect on the plasma concentrations of AVP in rats[63] and sheep[64]. Furthermore, AM was reported to inhibit the release of AVP that is normally evoked by hypertonic and hypovolemic stimulation in rats[63].

As well as CGRP, centrally administered AM inhibits its feeding in rats[65]. Pretreatment with a CGRP receptor antagonist ($CGRP_{8-37}$) attenuates this AM-induced anorexia[65]. After icv administration of AM, Fos-LI cells were found in the PVN, VMH, and PBN[53,54]. As these areas are related to the central regulation of feeding behavior, centrally administered AM may activate neurons in these areas and inhibit food intake.

Water intake is controlled by body fluid and osmolality. In general, when we investigate the effects of neuropeptides on water intake, we used water deprived model, angiotensin II (AII) induced water-intake model, and polyethylene glycol induced isotonic hypovolemia model[66~68]. In water deprived rats, centrally administered AM significantly decreases the water intake[69,70]. Centrally administered AM significantly decreases AII-induced water intake[70]. Centrally administered AM also significantly decreased the water intake in hyperosmotic stimuli in rats[70]. Moreover, centrally administered AM inhibits salt appetite in rats[71]. These results suggested that AM may involve in the regulation of body fluid in the CNS.

As AM is produced in the vascular smooth muscle, AM is considered a potent vasodilator Peripheral administration of AM increased heart rate, decreases arterial blood pressure, and inhibits urine flow[72~75]. Centrally administered AM as well as peripherally administered, has a cardiovascular action. Centrally administered AM increases the MAP and the HR in rats[35,74~76] and in rabbit[77]. These effects of AM on MAP and HR are significantly suppressed by pretreatment with an AM receptor antagonist (AM_{22-52}) or a CGRP receptor antagonist ($CGRP_{8-37}$) in rat[76]. In sheep, although peripherally administered AM decreases MAP and increases HR, centrally administered AM does not change either MAP or HR[64].

As described above, some previous studies showed the central effects of AM on feeding, drinking, neuroendocrine system, and cardiovascular function. In immunohistochemistry study for AM, AM-LI cells were found in the OB, Arc, bed nucleus of stria terminalis, DMH, LHA, medial septal nucleus, PVN, SON, innominate substance, cuneate nucleus, habenular

nucleus, mammillary body, AP and LC (Table 2). These areas are involved in feeding, neuroendocrine system, cardiovascular function, pain, addiction, emotion, and memory. Besides feeding, neuroendocrine system, and cardiovascular function, the further study may clarify the physiological role of AM in pain, addiction, emotion, and memory.

Adrenomedullin 2 (AM2)

Distribution and synthesis of AM2

A 47-amino acid neuropeptide (Fig. 1), AM2 was first isolated from pufferfish[3,5] and later from mammals[4,5] by the bioinformatics technique. AM2 is identical to intermedin, which was discovered by Roh *et al.*[5]. AM2 is also widely distributed in peripheral organs and the CNS (Table 1). AM2 mRNAs are expressed in the submaxillary gland, kidney, stomach and mesentery, pituitary, lung, pancreas, intestines, spleen, thymus and ovary in mice[4] (Table 1). In human, AM2 mRNAs are expressed in the esophagus, stomach, jejunum, ileum, ileocecum, ascending colon, transverse colon, descending colon, and rectum[5] (Table 1). AM2 was also detected in the lung, heart, stomach kidney cerebellum, pituitary, brain stem, and hypothalamus in rats[69]. We showed that AM2-LI were observed in the intermediate lobe of pituitary, vascular organ of lamina terminalis (OVLT), median preoptic nucleus (MnPO), VMH, SON, PVN, Arc, LC, AP, NTS, and nucleus ambiguus in rats[78]. These areas are involved with cardiovascular system, feeding, sleep-awake cycle, water balance, and autonomic function (Table 2). Moreover, we showed that after salt loading, AM2-LI cells were significantly visualized stronger than those of control rats in the SON and the magnocellular parts of the PVN[78]. Colchicine treatment is known to block axonal transport, resulting in neuropeptide accumulation in the cell body[78~80]. After colchicine treatment, AM2-LI cells were significantly visualized stronger than those of control rats in the SON, the magnocellular parts of the PVN and LC[78]. As neurons in the SON and PVN terminate their axons to the posterior pituitary (PP), produced AM2 in the SON and PVN may axon-transport to PP and secrete from PP to circulation in rats. Accordingly, these findings in salt loading and colchicine study indicated that AM2 was produced in the SON and PVN, and mainly secreted from posterior pituitary by the axonal transports.

Distribution of AM2 receptor

The information about receptor of AM2 has not been known in detail. As the induction of c-*fos* gene after icv administration of AM2 were attenuated by pretreatment with CGRP and/or AM receptor antagonists[13,14], AM2 activates the combination of CRLR with RAMPs. We showed that the induction of c-*fos* gene after icv administration of AM were attenuated by pretreatment with either the CGRP or AM receptor antagonists and completely blocked by pretreatment with both the CGRP and AM receptor antagonists[14]. We also showed that the induction of c-*fos* gene after icv administration of AM2 was attenuated by pretreatment with either the CGRP or AM receptor antagonists, as well as AM. Interestingly, unlike AM, the induction of c-*fos* gene after icv administration of AM2 was attenuated, not completely blocked, by pretreatment with both the CGRP and AM receptor antagonists[14]. Therefore, AM2 may activate neurons via other pathway directly or indirectly, including unknown receptor.

Physiological role of AM2 in the CNS

As well as AM, icv administration of AM2, Fos-LI cells were found in the SON, PVN, CeA, VMH, LC, AP, and NTS (Fig. 2)[13]. Centrally administered AM2 also induced the expression of c-*fos* gene in the SON and PVN, and this induction was significantly reduced by pretreatment with both the CGRP ($CGRP_{8-37}$ 3 nmol) and AM (AM_{22-52} 27 nmol) receptor antagonists (Fig. 3)[13,14]. Icv administration of AM2 also activated OXT-secreting neurons and elevated circulating OXT levels in rats[13,14]. I also found a few AVP-LI cells in the SON and the PVN that exhibited nuclear 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, unlike AM[13,15]. The activation of AVP neurons by centrally administered AM2 is different from the actions of centrally administered AM. Although the effects of centrally administered AM2 were similar to those of AM[13,14,55,65,70,71,74,75] and were mediated by both AM and CGRP receptors[81], I also showed that centrally administered

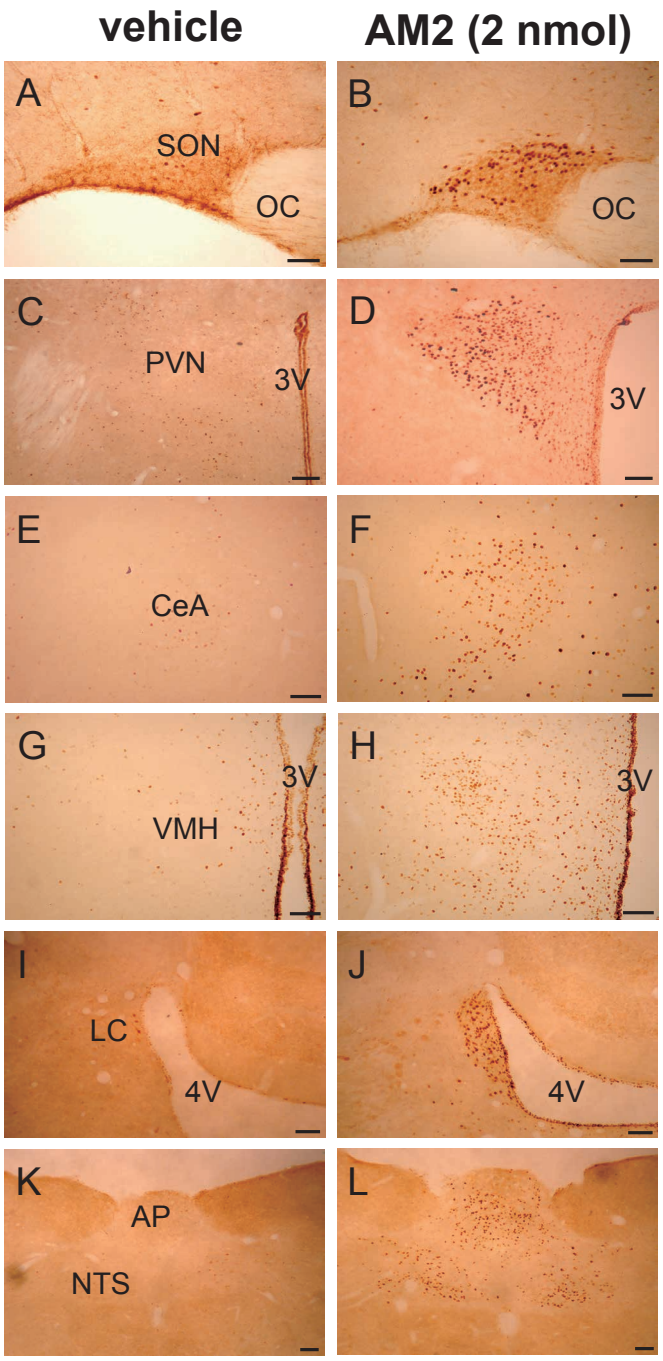

Fig. 2
Photomicrographs showing changes in Fos- like immunoreactivity in the supraoptic nucleus (SON) (A and B), the paraventricular nucleus (PVN) (C and D), the central nucleus of the amygdala (CeA) (E and F), the ventromedial nucleus of the hypothalamus (VMH) (G and H), the locus coeruleus (LC) (I and J), and the area postrema (AP) and the nucleus of the solitary tract (NTS) (K and L). OC, optic chiasma ; 3V, third ventricle ; 4V, fourth ventricle. Bars indicate 100 m. Modified with permission from Fig. 7 in ref. [13].

effects of AM2 (1 nmol) were stronger than those of AM (1 nmol) in the expression of the c-*fos* gene in the SON and PVN (Fig. 3 A and B), plasma OXT level, and blood pressure (Fig. 3 C) in rats[14]. The combination of AM and CGRP receptor blockade completely inhibited c-*fos* gene expression after centrally administered AM, while there was an incomplete inhibition after centrally administration of AM2[14].

Centrally administered AM2 inhibits food intake in rats in the same manner as it does when given peripherally[69]. We showed that Fos-LI cells were found in the PVN and VMH after centrally administration of AM2 in rats[13]. The VMH and PVN are related to the central regulation of feeding behavior. Especially, the VMH is called a satiety center, and I presume that the activation of the VMH may inhibit the feeding behavior after central administration of AM2. In the effect for water drinking, as well as AM,

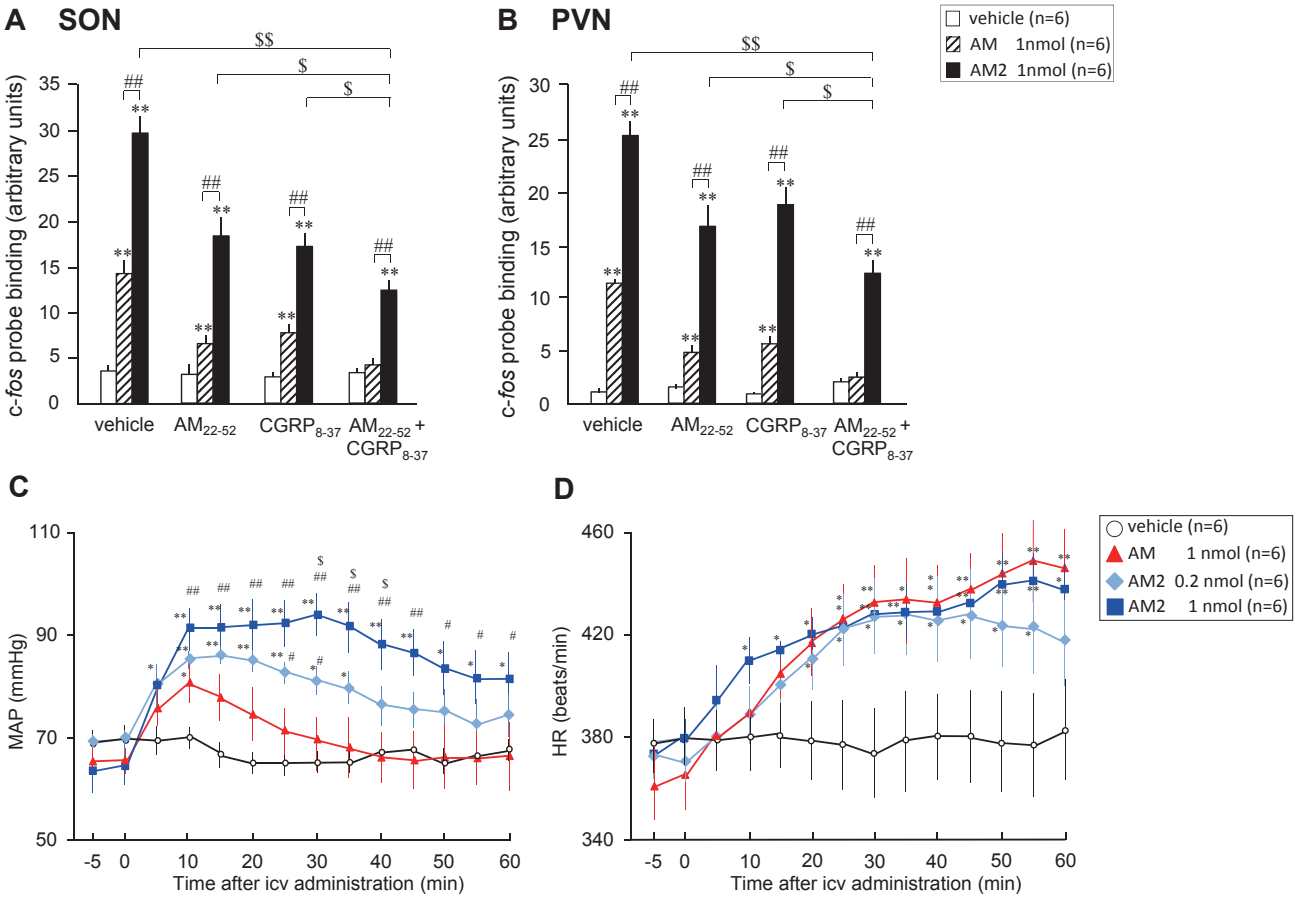

Fig. 3

Effects of intracerebroventricular (icv) injection of saline (vehicle), AM (1 nmol/rat), or adrenomedullin 2 (AM2) (1 nmol/rat) on c-*fos* transcript prevalence in the supraoptic nucleus (SON) (A) and the paraventricular nucleus (PVN) (B) 10 min after pretreatment with AM antagonist (AM_{22-52} 27 nmol/rat) or/and CGRP antagonist ($CGRP_{8-37}$ 3 nmol/rat). Rats were decapitated 30 min after icv injection of AM (1 nmol/rat), AM2 (1 nmol/rat) or vehicle. Values represent the mean ± SEM (n = 6). $^{**}P<0.01$, compared with vehicle-administered rats ; $^{\#\#}P<0.01$, compared with AM-administered rats. $^{\$\$}P<0.01$ and $^{\$}P<0.05$, compared with AM_{22-52} + $CGRP_{8-37}$ + AM2-administered rats. Time courses of changes in mean arterial pressure (MAP) (C) and heart rate (HR) (D) after icv injection of vehicle, AM (1 nmol/rat) or AM 2 (0.2 and 1 nmol/rat) in urethane-anesthetized rats. Values represent the mean ± S.E.M. (n = 6). $^{*}P<0.05$ and $^{**}P<0.01$, compared with vehicle-administered rats. $^{\#}P<0.05$ and $^{\#\#}P<0.01$, compared with AM-administered rats. $^{\$}P<0.05$, compared with AM2 0.2 nmol-administered rats. Modified with permission from Fig. 1 and Fig. 5 in ref. [14].

centrally administered AM2 significantly decreases water intake in water-deprived rats[69,70].

Peripherally administered AM2 decreases MAP, inhibits urine flow, increases renal sympathetic nerve activity as well as AM[5,72,73]. By all accounts, the effects of the AM and AM2 are qualitatively and quantitatively similar[4,5,72,73]. However, the actions of AM or AM2 given centrally is quite different from their actions when given peripherally. Unlike peripheral administration, centrally administered AM2 increased MAP and HR[69]. I showed that centrally administered AM and AM2 increased BP and HR in rats (Fig. 3 C and D)[14]. The effects of AM2 on MAP were stronger and longer than those of AM[14]. I showed that icv administration of AM2 activated neurons in the NTS in rats (Fig. 2)[13]. Recently, Li *et al.* showed that microinjection of AM2 into the NTS increased the MAP and the sympathetic outflow via cAMP/PKA signaling pathway in rats[82]. Centrally administered AM2 may be involved in the central regulation of the cardiovascular system via the NTS.

In immunohistochemistry study for AM2, AM2-LI

cells were found in the Arc, MnPO, OVLT, PVN, SON, VMH, AP, LC, and NTS (Table 2). These areas are involved in feeding, drinking, neuroendocrine system, cardiovascular function, memory, emotion, stress, sleep-awake cycle. As described above, some previous studies showed the central effects of AM2 on feeding, drinking, neuroendocrine system, and cardiovascular function. The further study may clarify the physiological role of AM2 in stress, sleep-awake cycle, emotion, and memory.

Adrenomedullin 5 (AM)

Distribution and synthesis of AM5

AM5 is a 52-amino acid neuropeptide (Fig. 1). Takei and colleagues identified five AMs (AM1-5) in teleost fish, and they form an independent subfamily[3,5]. They searched the orthologs of the AMs in the genome and established sequence tag databases and showed that AM, AM2, and AM5 in mammals are respectively identical to AM1, AM2, and AM5 in teleost fish[6,83]. In the pig, AM5 mRNAs are expressed in the spleen and thymus[84]. Although AM5 was identified from pig, ox, sheep, horse, dog, cat, AM5 could not be identified in rodents and AM5-like sequence exists in human[84]. However, we showed that central administered porcine AM5 activated OXT-secreting neurons in the SON and PVN in rats. These results indicated that porcine AM5 activate OXT secreting neuron in the hypothalamus partly via AM/CGRP receptor or unknown receptor system for AM5 in rats.

Distribution of AM5 receptor

The information about receptor of AM5 has not been known in detail. As the induction of c-*fos* gene after icv administration of AM5 were attenuated by pretreatment with CGRP and/or AM receptor antagonists[13,14], AM5 activates the combination of CRLR with RAMPs. We showed that the induction of c-*fos* gene after icv administration of AM were attenuated by pretreatment with either the CGRP or AM receptor antagonists and completely blocked by pretreatment with both the CGRP and AM receptor antagonists[14]. We also showed that the induction of c-*fos* gene after icv administration of AM5 was attenuated by pretreatment with either the CGRP or AM receptor antagonists, as well as AM. Interestingly, unlike AM, the induction of c-*fos* gene after icv administration of AM5 was attenuated, not completely blocked, by pretreatment with both the CGRP and AM receptor antagonists[14]. Therefore, AM5 may activate neurons via other pathway directly or indirectly, including unknown receptor, as well as AM2.

Physiological role of AM5 in the CNS

After icv administration of AM5, Fos-LI cells were found in the CeA, MnPO, SON, PVN, VMH, Arc, LC, AP, and NTS[15]. As these nuclei are related with emotion, memory, drinking, feeding, stress, sleep-awake cycle, vomiting, pain, and cardiovascular system, AM5 may have a role in these physiological functions. As well as AM and AM2, central administration of AM5 activates OXT-secreting neurons and increases plasma OXT levels in rats[15]. Centrally administered AM5 induces the expression of c-*fos* gene in the SON and the PVN, and this induction was significantly reduced by pretreatment with both the CGRP ($CGRP_{8-37}$) and AM (AM_{22-52}) receptor antagonists[15]. Therefore, I presume that central administered AM5 may activate OXT-secreting neurons in the SON and PVN partly through the CGRP and/or AM receptor, as well as AM2. The further study is required to explore the possibility that unknown specific receptors for AM5 and/or AM2 may exist in the CNS.

Centrally administered AM5, as well as AM and AM2, increases BP and HR in rats[6], but the effects of centrally administered AM5 were larger and shorter-lasting than those of centrally administered AM[6]. The effects of centrally administered AM2 were larger and longer than those of centrally administered AM (Fig. 3 C and D). Therefore, the central effects of AM2 and AM5 were different from those of AM on MAP and HR in potency and duration in rats[6,14]. Centrally administered AM2 and AM5 may increase MAP and HR partially via unknown specific receptors for AM2 and AM5 in the CNS.

Amylin

Distribution and synthesis of amylin

Amylin, also called islet amyloid polypeptide (IAPP), is a 37-amino acid peptide that was originally

isolated from pancreatic amyloid deposit in diabetes (Fig. 1)[85,86]. It is produced by pancreatic β-cells[87,88], and secreted with insulin by glucose stimulation[89~91]. Amylin mRNAs are expressed in the pancreas, stomach, duodenum, jejunum, ileum, colon, dorsal root ganglion, and trigeminal ganglion[92~94]. Unlike other members of the CGRP family, at first amylin mRNAs had not been distributed in the CNS, probably due to technical problem[94]. However, Amylin-LI cells were found in the dorsal root ganglion, jugular nodose ganglion, and trigeminal ganglion, olfactory bulb (OB), preoptic nucleus, the bed nucleus of the stria terminalis, suprachiasmatic nucleus, PVN, SON, DMH, VMH, mammillary body of the hypothalamus, habenular nucleus, the substantia nigra, red nucleus, pontine nuclei, inferior olivary nucleus, NTS, and cuneate nucleus, and vestibular nuclei[95,96]. These areas are involved with feeding, circadian rhythm, addiction, emotion, water balance, pain, reproductive behavior, stress, learning, motor system and autonomic function (Table 2).

Distribution of amylin receptor

Amylin has three receptors, amylin receptor 1-3 (AMY_{1-3}), which is combination of CR with RAMP 1-3[9,97]. As described above, RAMP1-3 is fairly widespread in the CNS. CR mRNA is distributed in the nucleus accumbens, preoptic area, suprachiasmatic nucleus, PVN, DMH, VMH, Arc, amygdala, LC, and raphe nucleus in rats[98].

Physiological role of amylin in the CNS

Peripherally administered amylin induced Fos expression in the AP, NTS, anterior hypothalamic area, and dorsomedial hypothalamic nucleus in mice[99]. To the best of our knowledge, no previous study has examined Fos-LI expression after centrally administered amylin. After icv administration of CGRP, Fos-LI cells were found in the preoptic area and PVN in rats[99]. To elucidate the central mechanism of CGRP family, I should investigate where centrally administered CGRP and amylin induce Fos-LI expressions in the CNS.

Amylin is known to inhibit basal and insulin-stimulated periphery glucose uptake and glycogen synthesis[100,101]. Some previous studies reported that peripherally administered amylin decreases food intake in rats[102~105]. This amylin-induced anorexia was significantly abolished by pretreatment with CGRP receptor antagonist ($CGRP_{8-37}$) in rats[106]. Icv administration of amylin decreases food intake in rats[107~109] and mice[96,110]. Icv administration of amylin also inhibits gastric acid secretion in rats[111]. Centrally administered amylin decreases gastric emptying and intestinal transit in rats[112]. Intrahypothalamic administration of amylin also decreased food intake in rats[103]. After intrahypothalamic administration of amylin, concentrations of dopamine, tryptophan, and their metabolites were significantly increased in the hypothalamus, corpus striatum and nucleus accumbens in rats[103]. These results indicated that intrahypothalamic administration of amylin inhibited feeding by acting directly on hypothalamic neurons to alter metabolism.

Peripherally administered amylin decreases MAP and increases HR in rats[113,114]. Icv administration of amylin increases HR, and O_2 consumption rate in anesthetized rats[115]. These effects of centrally administered amylin were eliminated by the electrolytic ablation of the AP[115]. AP is known as the vomiting center and involve in autonomic nervous system. I presume that centrally administered amylin may increase MAP via AP/NTS and be involved in the central regulation of the cardiovascular system.

In immunohistochemistry study for amylin, amylin-LI cells were found in the OB, bed nucleus of stria terminalis, amygdala, PVN, preoptic nucleus, suprachiasmatic nucleus, SON, VMH, and NTS (Table 2). These areas are involved in feeding, neuroendocrine system, cardiovascular function, circadian rhythm, memory, emotion, stress, and pain. As above described, some previous studies showed the central effects of amylin on feeding and cardiovascular function. The further study may clarify the physiological role of amylin in stress, neuroendocrine system, circadian rhythm, memory, emotion, stress, and pain.

CONCLUSION

Many neuropeptides have been discovered. Many studies showed physiological and pathophysiological role of these neuropeptides. Some neuropeptides, such as human atrial natriuretic peptide (hANP), brain natriuretic peptide (BNP), and orexin receptor antag-

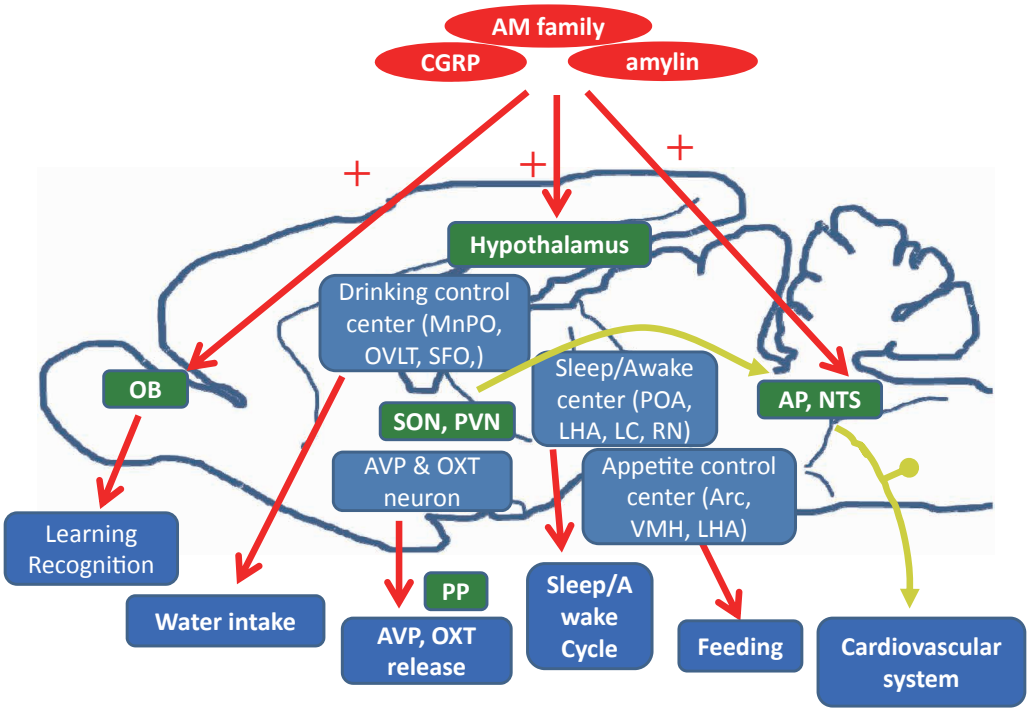

Fig. 4

Schema of central effects of CGRP family in sagittal section of diencephalon and brain stem. CGRP family activates neurons in the central nervous system. They activated neurons in the hypothalamus and medulla, which is related with appetite, water balance, sleep-awake cycle, learning, and cardiovascular system. They may involve in the regulation of feeding, drinking, cardiovascular system, and neuroendocrine system. Arc, arcuate nucleus ; AP, area postrema ; LC, locus coeruleus nucleus ; LHA, lateral hypothalamic area ; MnPO, median preoptic nucleus ; NTS, nuclei of solitary tract ; OVLT, vascular organ of lamina terminalis ; OB, olfactory bulb ; POA, preoptic area ; PP, posterior pituitary ; PVN, paraventricular nucleus ; RN, raphe nucleus ; SFO, subfornical organ ; SON, supraoptic nucleus ; VMH, ventromedial nucleus of the hypothalamus.

onist, are used as medical drugs or diagnosis. I have investigated the central effects of neuropeptides and reported my results obtained in some previous studies. Lots of studies about the peripherally effects of CGRP family were reported and some clinical trials for CGRP, AM and amylin are ongoing. However, the mechanism for central effects of CGRP family is unknown. Although CGRP family is considered that they cannot pass through the blood brain barrier, they and their receptor exist in the CNS and have some effects when it is administered centrally. In this review, I summarized that central administered CGRP family have some effects on autonomic function (Fig. 4). There is a possibility that unknown specific receptors for AM2 and/or AM5 may exist in the CNS. I anticipate that further studies can clarify the mechanism of the central effects of CGRP family, including exploration of unknown receptors in the CNS.

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Conflict of interest statement

Author has no conflict of interest.

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