Bidirectional roles of the brain 2-arachidonoyl-*sn*-glycerol in
the centrally administered vasopressin-induced adrenomedullary
outflow in rats

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Abstract

Previously, we reported that intracerebroventricularly (i.c.v.) administered arginine-vasopressin evokes the secretion of noradrenaline and adrenaline from adrenal medulla through the brain phospholipase C- and diacylglycerol-mediated and cyclooxygenase-mediated mechanisms in rats. Diacylglycerol can be hydrolyzed by diacylglycerol lipase to 2-arachidonoyl-*sn*-glycerol, which may be further degraded by monoacylglycerol lipase to free arachidonic acid, a representative substrate of cyclooxygenase. Recently, 2-arachidonoyl-*sn*-glycerol has been recognized as a major endocannabinoid, which can modulate synaptic transmission in the brain. In the present experiment, therefore, we examined (1) a role of the brain 2-arachidonoyl-*sn*-glycerol as a precursor of arachidonic acid in the centrally administered vasopressin-induced elevation of plasma noradrenaline and adrenaline, and (2) a regulatory role of the brain 2-arachidonoyl-*sn*-glycerol as an endocannabinoid on the vasopressin-induced response, using urethane-anesthetized rats. The vasopressin (0.2 nmol/animal, i.c.v.)-induced elevation of plasma catecholamines was reduced by RHC-80267 (diacylglycerol lipase inhibitor) (1.3 and 2.6 μ mol/animal, i.c.v.) and also reduced by MAFP (monoacylglycerol lipase inhibitor) (0.7 and 1.4 μ mol/animal, i.c.v.). MAFP (1.4

μmol/animal, i.c.v.) also attenuated the 2-arachidonoyl-*sn*-glycerol (0.5 μmol/animal, i.c.v.)-induced elevation of plasma catecholamines. AM 251 (cannabinoid CB₁ receptor antagonist) (90 and 180 nmol/animal, i.c.v.) potentiated the vasopressin (0.2 nmol/animal, i.c.v.)-induced response, while AM 630 (cannabinoid CB₂ receptor antagonist) (198 and 793 nmol/animal, i.c.v.) was largely ineffective. In addition, WIN 55212-2 (cannabinoid CB receptor agonist) (188 and 470 nmol/animal, i.c.v.) dose-dependently reduced the vasopressin-induced response. These results suggest that the brain 2-arachidonoyl-*sn*-glycerol generated from diacylglycerol plays a role as a precursor of arachidonic acid in the centrally administered vasopressin-induced activation of the adrenomedullary outflow, and also negatively regulates the peptide-induced central response through the brain cannabinoid CB₁ receptors in rats.

Keywords: Vasopressin; Adrenal medulla; Brain; 2-Arachidonoyl-*sn*-glycerol; Cannabinoid CB₁ receptor

1. Introduction

Vasopressin has been recognized as an important neuropeptide involved in water conservation (Acher, 1993) and pituitary adrenocorticotrophic hormone secretion (Antoni, 1984; Rivier et al., 1984). The peptide is also recognized as a neurotransmitter or neuromodulator to modulate diverse brain functions such as memory and behavior (De Wied et al., 1991; Drago et al., 1997; Bielsky et al., 2005), fever (Wilkinson and Kasting, 1987) and central cardiovascular regulation (Milutinovic et al., 2006). Previously, we reported that the centrally administered vasopressin elevated plasma noradrenaline and adrenaline and this elevation was attenuated by centrally administered indomethacin, an inhibitor of cyclooxygenase, and also abolished by bilateral adrenalectomy (Okada et al., 2003). These results suggest that the brain vasopressin activates the central adrenomedullary outflow by the brain cyclooxygenase-mediated mechanisms in rats.

Recently, we reported that the vasopressin-induced elevation of plasma catecholamines is mediated by the brain phospholipase C- and diacylglycerol-mediated mechanisms in rats (Shimizu et al., 2004). Phospholipase C cleaves the phosphodiester bond of membrane phospholipids, resulting the formation of diacylglycerol (Rebecchi and Pentyala, 2000). Diacylglycerol can be hydrolyzed by diacylglycerol lipase, which is more selective for the *sn*-1 over the *sn*-2 position of diacylglycerol (Bisogno et al., 2003), to produce 2-arachidonoyl-*sn*-glycerol (Konrad et al., 1994; Stella et al., 1997; Bisogno et al., 2003; Gauthier et al., 2005; Tang et al.

2006). 2-Arachidonoyl-*sn*-glycerol can be further metabolized by monoacylglycerol lipase to free arachidonic acid, a representative substrate of cyclooxygenase (Konrad et al., 1994; Karlsson et al., 1997; Dinh et al., 2002; Tang et al. 2006).

2-Arachidonoyl-*sn*-glycerol has been recognized as a major endocannabinoid for the brain cannabinoid CB receptors (Sugiura et al., 1995; Sugiura and Waku, 2000; De Petrocellis et al., 2004). Endocannabinoids such as 2-arachidonoyl-*sn*-glycerol and anandamide have been shown to have regulatory effects on the sympathetic nervous system (Ishac et al., 1996; Seagard et al., 2004; Brozoski et al., 2005), in addition to a wide range of central effects including pain processing (Hohmann et al., 2005), neuroprotection (Melis et al., 2006; Kreitzer and Malenka, 2007), depression (Gobbi et al., 2005) and neuroendocrine secretion (Patel et al., 2004).

In the present study, therefore, we pharmacologically examined (1) a role of the brain 2-arachidonoyl-*sn*-glycerol as a precursor of arachidonic acid in the centrally administered vasopressin-induced elevation of plasma catecholamines. Furthermore, we examined (2) a regulatory role of 2-arachidonoyl-*sn*-glycerol as an endocannabinoid on the vasopressin-induced response in rats.

2. Materials and methods

2.1. Experimental procedures

Male Wistar rats weighing about 350 g were maintained in an air-conditioned room at 22-24°C under a constant day-night rhythm for more than 2 weeks and given food (laboratory chow, CE-2; Clea Japan, Hamamatsu, Japan) and water ad libitum. Under urethane anesthesia (1.2 g/kg, i.p.), the femoral vein was cannulated for infusion of saline (1.2 ml/h), and the femoral artery was cannulated for collecting blood samples. After these procedures, the animal was placed in a stereotaxic apparatus, as shown in our previous papers (Yokotani et al., 1995; Shimizu et al., 2004). The skull was drilled for intracerebroventricular administration of test substances using stainless-steel cannula (0.3 mm outer diameter). The stereotaxic coordinates of the tip of cannula were as follows (in mm): AP -0.8, L 1.5, V 4.0 (AP, anterior from the bregma; L, lateral from the midline; V, below the surface of the brain), according to the rat brain atlas (Paxinos and Watson, 1986). Three hours were allowed to elapse before the start of each experiment.

RHC-80267 [1,6-bis(cyclohexyloximinocarbonylamino)hexane], MAFP (methyl arachidonyl fluorophosphonate), AM 251 [*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide], AM 630 [(6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl)(4-methoxyphenyl)methanone], WIN 55212-2 [(*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*de*]-1,4-

benzoxazin-6-yl]-1-naphthalenylmethanone], and 2-arachidonoyl-*sn*-glycerol dissolved in 2.5 μ l of 100% *N,N*-dimethylformamide (DMF)/animal were slowly administered into the right lateral ventricle using a 10- μ l Hamilton syringe. Vasopressin dissolved in sterile saline in a volume of 10 μ l/animal was intracerebroventricularly (i.c.v.) administered using a 25- μ l Hamilton syringe 30 min after application of RHC-80267, MAFP, AM 251 and AM 630 and 60 min after application of WIN 55212-2, due to their slightly elevating effects on the basal plasma levels of catecholamines. 2-Arachidonoyl-*sn*-glycerol was i.c.v. administered 30 min after application of MAFP. Only one dose of these reagents was applied to each animal.

All experiments were conducted in compliance with the guiding principles for the care and use of laboratory animals approved by the Kochi University.

2.2. Measurement of plasma catecholamines

Blood samples (250 μ l) were collected through an arterial catheter and were preserved on ice during experiments. Plasma was prepared immediately after the final sampling. Catecholamines in the plasma were extracted by the method of Anton and Sayre (1962) with a slight modification and were assayed electrochemically with high performance liquid chromatography (HPLC) (Shimizu et al., 2004). Briefly, after centrifugation, the plasma (100 μ l) was transferred to a centrifuge tube containing 30 mg of activated alumina, 2 ml of double deionized water, 1 ml of 1.5 M Tris Buffer (pH 8.6) containing 0.1 M disodium EDTA and 1 ng of 3,4-

dihydroxybenzylamine as an internal standard. The tube was shaken for 10 min and the alumina was washed three times with 4 ml of ice-cold double deionized water. Then, catecholamines adsorbed onto the alumina were eluted with 300 μ l of 4% acetic acid containing 0.1 mM disodium EDTA. A pump (EP-300: Eicom, Kyoto, Japan), a sample injector (Model-231XL; Gilson, Villiers-le-Bel, France) and an electrochemical detector (ECD-300: Eicom) equipped with a graphite electrode were used with HPLC. Analytical conditions were as follows: detector, +450 mV potential against a Ag/AgCl reference electrode; column, Eicompact CA-50DS, 2.1 x 150 mm (Eicom); mobile phase, 0.1 M NaH_2PO_4 - Na_2HPO_4 buffer (pH 6.0) containing 50 mg/l disodium EDTA, 0.75 g/l sodium 1-octanesulfonate and 15% methanol at a flow of 0.18 ml/min; injection volume, 40 μ l. The amount of catecholamines in each sample was calculated using the peak height ratio relative to that of 3,4-dihydroxybenzylamine. By this assay, coefficients of variation for intra- and inter-assay were 3.0% and 3.7% respectively, and 0.5 pg of noradrenaline and adrenaline was accurately determined.

2.3. Treatment of data and statistics

All values are expressed as the means $\pm$ S.E.M. The data were analyzed by repeated-measure analysis of variance (ANOVA), followed by post-hoc analysis with the Bonferroni method (Figs. 1, 3 and 4). When only two means were compared, an unpaired Student's *t*-test was used (Fig. 2). *P* values less than 0.05 were taken to indicate statistical significance.

2.4. Compounds

The following drugs were used: synthetic arginine-vasopressin (vasopressin) (Peptide Institute, Osaka, Japan); RHC-80267 and 2-arachidonoyl-*sn*-glycerol (Biomol Research Lab., Plymouth Meeting, PA, USA); MAFP (Cayman Chemical, Ann Arbor, MI, USA); AM 251 and WIN 55212-2 mesylate (Tocris Cookson Inc., Ellisville, Missouri, USA); AM 630 (Alexis Biochemicals, San Diego, CA, USA). All other reagents were the highest grade available (Nacalai Tesque, Kyoto, Japan).

3. Results

3.1. Effects of RHC-80267 (an inhibitor of diacylglycerol lipase) and MAFP (an inhibitor of monoacylglycerol lipase) on the centrally administered vasopressin-induced elevation of plasma catecholamines

Treatments with vehicle-1 (2.5 μ l DMF/animal, i.c.v.) and vehicle-2 (10 μ l saline/animal, i.c.v.) had no effect on the basal plasma levels of noradrenaline and adrenaline (Fig. 1, A and B). Pretreatment with RHC-80267 [2.6 μ mol (1000 μ g)/animal, i.c.v.] or MAFP [1.4 μ mol (500 μ g)/animal, i.c.v.] also had no effect on the basal plasma levels of both catecholamines (Fig. 1, A and B).

Since we previously reported that vasopressin (0.1, 0.2 and 0.5 nmol/animal, i.c.v.) dose-dependently elevated plasma levels of both catecholamines (Okada et al., 2002), we used a dose of 0.2 nmol/animal in the present experiment. I.c.v. administered vasopressin (0.2 nmol/animal) rapidly increased plasma levels of both catecholamines. These responses reached a maximum 5 min after administration of vasopressin and then declined toward their basal levels (Fig. 1, A and B). Intravenous administration of this dose of vasopressin had no effect on the basal plasma levels of catecholamines (data not shown).

The vasopressin (0.2 nmol/animal, i.c.v.)-induced elevation of both catecholamines was reduced by pretreatment with RHC-80267 in a dose-dependent manner [1.3 and 2.6 μ mol (500 and 1000 μ g)/animal, i.c.v.] (Fig. 1A). The peptide-

induced response was also attenuated by pretreatment with MAFP [0.7 and 1.4 μmol (250 and 500 μg)/animal, i.c.v.] (Fig. 1B).

3.2. Effect of MAFP (an inhibitor of monoacylglycerol lipase) on the centrally administered 2-arachidonoyl-sn-glycerol-induced elevation of plasma catecholamines

Treatments with vehicle-1 (2.5 μl DMF/animal, i.c.v.) and vehicle-2 (2.5 μl DMF/animal, i.c.v.) had no effect on the basal plasma levels of noradrenaline and adrenaline (Fig. 2). Pretreatment with MAFP [1.4 μmol (500 μg)/animal, i.c.v.] also had no effect on the basal plasma levels of both catecholamines (Fig. 2).

I.c.v. administered 2-arachidonoyl-sn-glycerol [0.1, 0.5 and 1.0 μmol (38, 189 and 379 μg)/animal] elevated plasma levels of both catecholamines and a maximal response was obtained at 0.5 μmol /animal (data not shown). 2-Arachidonoyl-sn-glycerol [0.5 μmol /animal, i.c.v.] rapidly increased plasma levels of both catecholamines (adrenaline > noradrenaline) and these responses reached a maximum 5-10 min after administration of this reagent (Fig. 2). The reagent-induced response was effectively attenuated by pretreatment with MAFP (1.4 μmol /animal, i.c.v.) (Fig. 2).

3.3. Effects of AM 251 (a selective antagonist of cannabinoid CB₁ receptors) and AM 630 (a selective antagonist of cannabinoid CB₂ receptors) on the centrally administered vasopressin-induced elevation of plasma catecholamines

Pretreatment with AM 251 [180 nmol (100 µg)/animal, i.c.v.] or AM 630 [793 nmol (400 µg)/animal, i.c.v.] had no effect on the basal plasma levels of noradrenaline and adrenaline (Fig. 3, A and B).

Pretreatment with AM 251 [90 and 180 nmol (50 and 100 µg)/animal, i.c.v.] dose-dependently potentiated the vasopressin (0.2 nmol/animal, i.c.v.)-induced elevation of plasma levels of both catecholamines (Fig. 3A). On the other hand, the vasopressin-induced response was largely unaffected by pretreatment with AM 630 [198 and 793 nmol (100 and 400 µg)/animal, i.c.v.] (Fig. 3B).

3.4. Effect of WIN 55212-2 (an agonist of cannabinoid CB receptors) on the centrally administered vasopressin-induced elevation of plasma catecholamines

Treatments with vehicle-1 (2.5 µl DMF/animal, i.c.v.) and vehicle-2 (10 µl saline/animal, i.c.v.) had no effect on the basal plasma levels of noradrenaline and adrenaline (Fig. 4). Pretreatment with WIN 55212-2 [470 nmol (250 µg)/animal, i.c.v.] also had no effect on the basal plasma levels of both catecholamines (Fig. 4).

The vasopressin (0.2 nmol/animal, i.c.v.)-induced elevation of both catecholamines was effectively reduced by pretreatment with WIN 55212-2 in a dose-dependent manner [188 and 470 nmol (100 and 250 µg)/animal, i.c.v.] (Fig. 4). Intravenous pretreatment of WIN 55212-2 [470 nmol (250 µg)/animal] had no effect on the vasopressin-induced elevation of both catecholamines (data not shown).

4. Discussion

2-Arachidonoyl-*sn*-glycerol is formed from arachidonic acid-enriched membrane phospholipids through phospholipase C- and diacylglycerol lipase-mediated mechanisms (Stella et al., 1997; Gauthier et al., 2005; Tang et al. 2006). Diacylglycerol lipase overexpression in mouse neuroblastoma increased basal 2-arachidonoyl-*sn*-glycerol levels and this effect was accompanied by enhanced utilization of 1-stearoyl,2-arachidonoyl-*sn*-glycerol (a precursor of 2-arachidonoyl-*sn*-glycerol) (Jung et al., 2007). RHC-80267 has been shown to selectively inhibit diacylglycerol lipase activity in human adrenal glomerulosa cells and rat thyroid lobes (Levasseur et al., 1984; Natarajan et al., 1988), and also to inhibit 2-arachidonoyl-*sn*-glycerol release in rat brain neurons and bovine coronary endothelial cells (Stella et al., 1997; Gauthier et al., 2005). In the first experiment, we examined the effect of RHC-80267 on the centrally administered vasopressin-induced elevation of plasma noradrenaline and adrenaline. The reagent effectively reduced the vasopressin-induced response, suggesting that 2-arachidonoyl-*sn*-glycerol generated from diacylglycerol by diacylglycerol lipase is involved in the vasopressin-induced activation of the central adrenomedullary outflow in rats.

2-Arachidonoyl-*sn*-glycerol can be oxygenated directly by cyclooxygenase to generate glycerol esters of prostaglandins and thromboxane A₂ (Kozak et al., 2000, 2002), which may serve as a precursor of each prostanoid (Rouzer and Marnett, 2005). We previously reported that the sequential actions of the

brain cyclooxygenase and thromboxane A₂ synthase are involved in the centrally administered vasopressin-induced elevation of plasma catecholamines in rats (Okada et al., 2003). However, thromboxane A₂ synthase is far lesser efficient to catalyze 2-arachidonoyl-*sn*-glycerol-derived glycerol ester of prostaglandin H₂ than prostaglandin H₂ to the corresponding thromboxanes by the sequential action of cyclooxygenase (Kozak et al., 2002). The evidence suggests that the brain thromboxane A₂ is synthesized from the other pathway than glycerol ester of thromboxane A₂ in the vasopressin-induced activation of adrenomedullary outflow in rats.

2-Arachidonoyl-*sn*-glycerol can be further hydrolyzed by monoacylglycerol lipase to generate free arachidonic acid (Konrad et al., 1994; Dinh et al., 2002; Tang et al. 2006), which is further metabolized to prostanoids (prostaglandins and thromboxane A₂) by the sequential actions of cyclooxygenase and each prostanoid synthase. In the next experiment, we examined the effect of MAFP on the centrally administered vasopressin-induced elevation of plasma catecholamines. MAFP has been shown to be an inhibitor of phospholipase A₂ or fatty acid amide hydrolase, but it is currently recognized as a very potent (IC₅₀ value 2-3 nM) inhibitor of 2-arachidonoyl-*sn*-glycerol hydrolyzing enzymatic activity (Goparaju et al., 1999; Saario et al., 2004). MAFP has been shown to accumulate endogenous 2-arachidonoyl-*sn*-glycerol in rat brain sections by functional autoradiography (Palomaki et al., 2007). In the present experiment, MAFP effectively attenuated the vasopressin-induced elevation of plasma catecholamines. Since the vasopressin-induced response

was not influenced by central pretreatment with mepacrine, an inhibitor of phospholipase A₂ (Shimizu et al., 2004), the attenuating effect of MAFP on the vasopressin-induced response seems to be due to the inhibition of 2-arachidonoyl-*sn*-glycerol hydrolyzing enzymatic activity, thereby reducing arachidonic acid production from 2-arachidonoyl-*sn*-glycerol.

We further examined the effect of MAFP on the centrally administered 2-arachidonoyl-*sn*-glycerol-induced elevation of plasma noradrenaline and adrenaline. The reagent also effectively attenuated the reagent-induced elevation of plasma catecholamines. Since centrally administered arachidonic acid itself elevated plasma catecholamines in rats (Yokotani et al., 2000), the attenuating effect of MAFP on the 2-arachidonoyl-*sn*-glycerol-induced response is due to the inhibition of arachidonic acid production from 2-arachidonoyl-*sn*-glycerol. These lines of evidence further support a possibility that the brain arachidonic acid generated from 2-arachidonoyl-*sn*-glycerol by monoacylglycerol lipase is involved in the vasopressin-induced activation of adrenomedullary outflow in rats.

Recently, 2-arachidonoyl-*sn*-glycerol has been shown to act as an endocannabinoid on cannabinoid CB receptors in the central nervous system (Sugiura et al., 1995; Sugiura and Waku, 2000; De Petrocellis et al., 2004). So far two types of cannabinoid receptors (CB₁ and CB₂) have been identified (Matsuda et al., 1990; Munro et al., 1993). Cannabinoid CB₁ receptors are expressed predominantly in the central nervous system, whereas cannabinoid CB₂ receptors are present mainly in the immune system (Howlett et al., 2002). Recent studies

have also revealed the functional presence and expression of cannabinoid CB₂ receptors in the central nervous system (Van Sickle et al., 2005; Onaivi, 2007).

AM 251 and AM 630 have been shown to be a selective antagonist of cannabinoid CB₁ and CB₂ receptors, respectively (AM 251 is 306-fold selective over cannabinoid CB₂ receptors and AM 630 is 165-fold over CB₁ receptors) (Lan et al., 1999; Ross et al., 1999). AM 251 displays a K_i value of 7.49 nM at cannabinoid CB₁ receptors (Lan et al., 1999) and AM 630 has a K_i value of 31.2 nM at cannabinoid CB₂ receptors (Ross et al., 1999). AM 251 reverses the cannabinoid CB receptor agonist WIN 55212-2-induced synaptic suppression at hippocampal excitatory synapses on murine pyramidal neurons (Kawamura et al., 2006) and the intra-CA1 injection of WIN 55212-2-induced anxiogenic-like effect in rats (Roohbakhsh et al., 2007). AM 630 prevents the intrathecally administered CP55940 (a synthetic cannabinoid)-induced analgesia in rat pain models (Romero-Sandoval and Eisenach, 2007).

In the next experiment, we examined a regulatory role of the brain 2-arachidonoyl-*sn*-glycerol generated from diacylglycerol as an endocannabinoid on the vasopressin-induced elevation of plasma catecholamines in regard to the cannabinoid receptor subtypes. Central pretreatment with a selective antagonist of cannabinoid CB₁ receptors AM 251 (180 nmol/animal) significantly potentiated the vasopressin-induced responses. On the other hand, central pretreatment with a selective antagonist of cannabinoid CB₂ receptors AM 630 even at a larger dose (793 nmol/animal) had a little effect on the vasopressin-induced responses. These results suggest that the

brain 2-arachidonoyl-*sn*-glycerol generated from diacylglycerol also has a negatively regulating effect on the vasopressin-induced activation of adrenomedullary outflow through the brain cannabinoid CB₁ receptor-mediated mechanisms in rats.

WIN 55212-2 has been shown to be a non-selective agonist of cannabinoid CB receptors (Felder et al., 1995). This reagent increases tail-flick reflexes, exerts antihyperalgesic effects and induces hypothermia in rats by central cannabinoid CB₁ receptors (Martin et al., 1998; Fox et al., 2001; Rawls et al., 2002). In the present experiment, central pretreatment with WIN 55212-2 effectively reduced the vasopressin-induced elevation of plasma catecholamines, but peripheral pretreatment with this reagent had no effect. These results further support a possibility that the brain 2-arachidonoyl-*sn*-glycerol generated from diacylglycerol also acts as an endocannabinoid, thereby negatively regulating the vasopressin-induced activation of adrenomedullary outflow in rats.

Cannabinoid has marked effects on peripheral autonomic neurons. Cannabinoid presynaptically inhibits the release of noradrenaline from many postganglionic sympathetic neurons (Malinowska et al., 1997; Niederhoffer and Szabo, 1999). On the other hand, only a few studies have been carried out to characterize the central effects of cannabinoid on the sympathetic nervous system. Intracisternally administered WIN 55212-2 increased blood pressure and plasma noradrenaline concentration in rabbits and rats, respectively (Niederhoffer and Szabo, 2000; Pfitzer et al., 2004). Similarly, unilateral microinjection of WIN 55212-2 into the rostral ventrolateral

medulla oblongata, a crucial outflow pathway for centrally mediated sympathomodulatory effects, induced sympathoexcitation and hypertension in rats (Padley et al., 2003). These lines of evidence suggest a possibility that centrally administered cannabinoid elicits sympathetic activation by acting on cardiovascular centers in the medulla oblongata. On the other hand, it is also reported that endocannabinoid released in the nucleus tractus solitarius prolongs baroreflex inhibition of renal sympathetic nerve activity (Seagard et al., 2004; Brozoski et al., 2005), suggesting the presence of endocannabinoid-induced sympathoinhibitory system in the brain. However, a central effect of cannabinoid on sympathetic nervous system in supramedullary cardiovascular centers such as hypothalamus has been scarcely studied. Further studies are necessary to clarify the mechanisms by which the brain 2-arachidonoyl-*sn*-glycerol modulates the vasopressin-induced activation of adrenomedullary outflow in rats.

In summary, we demonstrated here a possibility that the brain 2-arachidonoyl-*sn*-glycerol has bidirectional roles in the centrally administered vasopressin-induced activation of adrenomedullary outflow in rats: (1) a source of free arachidonic acid for the brain cyclooxygenase, thereby activating adrenomedullary outflow; (2) a role of an endocannabinoid, thereby negatively regulating the vasopressin-induced response by brain cannabinoid CB₁ receptors-mediated mechanisms.

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Legends to figures

Fig. 1. Effects of RHC-80267 (an inhibitor of diacylglycerol lipase) and MAFP (an inhibitor of monoacylglycerol lipase) on the vasopressin-induced elevation of plasma catecholamines. (A) RHC-80267 (1.3 and 2.6 $\mu\text{mol}/\text{animal}$) or vehicle-1 (2.5 μl DMF/ animal) was intracerebroventricularly (i.c.v.) administered 30 min before the administration of vasopressin (0.2 nmol/ animal , i.c.v.) or vehicle-2 (10 μl saline/ animal , i.c.v.). (B) MAFP (0.7 and 1.4 $\mu\text{mol}/\text{animal}$) or vehicle-1 (2.5 μl DMF/ animal) was i.c.v. administered 30 min before the administration of vasopressin (0.2 nmol/ animal , i.c.v.) or vehicle-2 (10 μl saline/ animal , i.c.v.). Δ Noradrenaline and Δ Adrenaline: increments of noradrenaline and adrenaline above the basal. Arrows indicate the intracerebroventricular administrations of RHC-80267/MAFP/vehicle-1 and vasopressin/vehicle-2. Each point represents the mean $\pm$ S.E.M. * $p < 0.05$, significantly different from vehicle-1- and vasopressin-treated group. Vehicle-1-treated groups in Fig. 1B were cited from Fig. 1A. The actual values for noradrenaline and adrenaline at 0 min were 309 ± 37 and 516 ± 115 pg/ml in the group pretreated with vehicle-1 ($n=14$); 346 ± 31 and 518 ± 38 pg/ml in the group pretreated with RHC-80267 (1.3 $\mu\text{mol}/\text{animal}$) ($n=6$); 348 ± 38 and 436 ± 144 pg/ml in the group pretreated with RHC-80267 (2.6 $\mu\text{mol}/\text{animal}$) ($n=11$); 395 ± 13 and 503 ± 101 pg/ml in the group pretreated with MAFP (0.7 $\mu\text{mol}/\text{animal}$) ($n=7$); 343 ± 23 and 506 ± 58 pg/ml in the group pretreated with MAFP (1.4 $\mu\text{mol}/\text{animal}$) ($n=11$), respectively.

Fig. 2. Effect of MAFP (an inhibitor of monoacylglycerol lipase) on the 2-arachidonoyl-*sn*-glycerol (2-AG)-induced elevation of plasma catecholamines. MAFP (1.4 μ mol/animal) or vehicle-1 (2.5 μ l DMF/animal) was i.c.v. administered 30 min before the administration of 2-AG (0.5 μ mol/animal, i.c.v.) or vehicle-2 (2.5 μ l DMF/animal). * p <0.05, significantly different from vehicle-1- and 2-AG-treated group. Other conditions were the same as those of Fig. 1. The actual values for noradrenaline and adrenaline at 0 min were 121 $\pm$ 23 and 92 $\pm$ 18 pg/ml in the group pretreated with vehicle-1 (n=7); 145 $\pm$ 17 and 109 $\pm$ 13 pg/ml in the group pretreated with MAFP (1.4 μ mol/animal) (n=8), respectively.

Fig. 3. Effects of AM 251 (a selective antagonist of cannabinoid CB₁ receptors) and AM 630 (a selective antagonist of cannabinoid CB₂ receptors) on the vasopressin-induced elevation of plasma catecholamines. (A) AM 251 (90 and 180 nmol/animal) or vehicle-1 (2.5 μ l DMF/animal) was i.c.v. administered 30 min before the administration of vasopressin (0.2 nmol/animal, i.c.v.) or vehicle-2 (10 μ l saline/animal, i.c.v.). (B) AM 630 (198 and 793 nmol/animal) or vehicle-1 (2.5 μ l DMF/animal) was i.c.v. administered 30 min before the administration of vasopressin (0.2 nmol/animal, i.c.v.) or vehicle-2 (10 μ l saline/animal, i.c.v.). Vehicle-1-treated groups were cited from Fig. 1A. * p <0.05, significantly different from vehicle-1- and vasopressin-treated group. Other conditions were the same as those of Figs. 1 and 2. The actual values for noradrenaline and adrenaline at 0 min were 402 $\pm$ 65 and 520 $\pm$ 126 pg/ml in the group pretreated with AM 251 (90

nmol/animal) (n=5); 358±58 and 584±156 pg/ml in the group pretreated with AM 251 (180 nmol/animal) (n=12); 387±23 and 587±89 pg/ml in the group pretreated with AM 630 (198 nmol/animal) (n=6); 343±16 and 481±80 pg/ml in the group pretreated with AM 630 (793 nmol/animal) (n=9), respectively.

Fig. 4. Effect of WIN 55212-2 (an agonist of cannabinoid CB receptors) on the vasopressin-induced elevation of plasma catecholamines. WIN 55212-2 (188 and 470 nmol/animal) or vehicle-1 (2.5 µl DMF/animal) was i.c.v. administered 60 min before the administration of vasopressin (0.2 nmol/animal, i.c.v.) or vehicle-2 (10 µl saline/animal, i.c.v.). * $p < 0.05$, significantly different from vehicle-1- and vasopressin-treated group. Other conditions were the same as those of Figs. 1-3. The actual values for noradrenaline and adrenaline at 0 min were 286±36 and 385±129 pg/ml in the group pretreated with vehicle-1 (n=13); 214±24 and 331±41 pg/ml in the group pretreated with WIN 55212-2 (188 nmol/animal) (n=6); 247±35 and 369±119 pg/ml in the group pretreated with WIN 55212-2 (470 nmol/animal) (n=10), respectively.

Figure-1

Figure-2

Figure-3

Figure-4