Possible inhibitory roles of endogenous 2-arachidonoylglycerol during corticotropin-releasing factor-induced activation of central sympatho-adrenomedullary outflow in anesthetized rats

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Abstract

We previously reported that intracerebroventricularly (i.c.v.) administered corticotropin-releasing factor (CRF) (0.5-3.0 nmol/animal) dose-dependently elevates plasma noradrenaline and adrenaline through brain phospholipase C-, diacylglycerol lipase- and prostanoids-mediated mechanisms in rats. Diacylglycerol produced by phospholipase C from phospholipids can be hydrolyzed by diacylglycerol lipase into 2-arachidonoylglycerol, which may be further hydrolyzed by monoacylglycerol lipase into arachidonic acid, a precursor of prostanoids. Recently, 2-arachidonoylglycerol has been recognized as a major brain endocannabinoid, which can modulate synaptic transmission through presynaptic cannabinoid CB₁ receptors. Released 2-arachidonoylglycerol is rapidly deactivated by uptake into cells and enzymatic hydrolysis. In the present study, therefore, we examined (1) the involvement of brain 2-arachidonoylglycerol, (2) the regulatory role of 2-arachidonoylglycerol as a brain endocannabinoid, and (3) the effect of exogenous cannabinoid receptor agonist, on the CRF-induced elevation of plasma noradrenaline and adrenaline using anesthetized rats. The elevation of both catecholamines induced by a submaximal dose of CRF (1.5 nmol/animal, i.c.v.) was reduced by i.c.v. administered MAFP (monoacylglycerol lipase inhibitor) (0.7 and 1.4 μ mol/animal), AM 404 (endocannabinoid uptake-inhibitor) (80 and 250 nmol/animal) and ACEA (cannabinoid CB₁ receptor agonist) (0.7 and 1.4 μ mol/animal, i.c.v.), while AM 251 (cannabinoid CB₁ receptor antagonist) (90 and 180 nmol/animal, i.c.v.) potentiated the response induced by a small dose of CRF (0.5 nmol/animal, i.c.v.). These results suggest a possibility that 2-arachidonoylglycerol is endogenously generated in the brain during CRF-induced

activation of central sympatho-adrenomedullary outflow, thereby inhibiting the peptide-induced response by activation of brain cannabinoid CB₁ receptors in anesthetized rats.

Keywords: Corticotropin-releasing factor; Sympatho-adrenomedullary outflow; Brain; 2-Arachidonoylglycerol; Monoacylglycerol lipase; Cannabinoid CB₁ receptor

1. Introduction

The endogenous cannabinoids that have received the most experimental attention are the arachidonic acid-containing lipids known as anandamide and 2-arachidonoylglycerol. 2-Arachidonoylglycerol has been recognized as a major brain endocannabinoid because it acts as a full agonist at cannabinoid CB receptors while anandamide acts as a partial agonist (Sugiura and Waku, 2000) and 2-arachidonoylglycerol is several 100-fold more abundant than anandamide in the brain (Stella et al., 1997; Sugiura and Waku, 2000). 2-Arachidonoylglycerol produced on demand from membrane phospholipid of postsynaptic neurons acts on presynaptic cannabinoid CB₁ receptors, thereby inhibiting the release of neurotransmitters (Stella et al., 1997; Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2001; Freund et al., 2003). The released 2-arachidonoylglycerol into the synaptic cleft is considered to be rapidly inactivated by uptake into neurons (Di Marzo, 2006; Centonze et al., 2007; Bisogno, 2008). The 2-arachidonoylglycerol-induced retrograde signaling process seems to be involved in the widespread central effects including synaptic plasticity (Heifets and Castillo, 2009), neuroprotection (Centonze et al., 2007), epilepsy (Ludányi et al., 2008), depression (Vinod and Hungund, 2006), food intake (Di Marzo et al., 2009), and also in the baroreflex-evoked central sympathetic modulation (Brozoski et al., 2005).

2-Arachidonoylglycerol is generated from diacylglycerol, which is produced by hydrolysis of arachidonic acid-enriched membrane phospholipids through phospholipase C (Stella et al., 1997; Di Marzo, 2006; Tang et al., 2006; Bisogno, 2008). Diacylglycerol containing arachidonic acid in position 2 is hydrolyzed to 2-

arachidonoylglycerol by *sn*-1 selective diacylglycerol lipase (Stella et al., 1997; Bisogno et al., 2003; Di Marzo, 2006; Tang et al., 2006; Bisogno, 2008). Actually, overexpression of the lipase in mouse neuroblastoma increases basal 2-arachidonoylglycerol levels (Jung et al., 2007). 2-Arachidonoylglycerol can be further hydrolyzed by monoacylglycerol lipase to generate arachidonic acid and glycerol (Dinh et al., 2002; Di Marzo, 2006; Tang et al., 2006; Bisogno, 2008). Recently, we reported the involvement of brain arachidonic acid generated by brain phospholipase C-, diacylglycerol lipase- and monoacylglycerol lipase-mediated mechanisms in centrally administered arginine-vasopressin-induced elevation of plasma noradrenaline and adrenaline in rats (Shimizu et al., 2004; Shimizu and Yokotani, 2008). Brain arachidonic acid has already been shown to be involved as a precursor of prostanoids in this peptide-induced elevation of plasma catecholamines (Yokotani et al., 1995, 2000; Murakami et al., 2002; Okada et al., 2003a). Furthermore, it is interesting to note that 2-arachidonoylglycerol, a precursor of arachidonic acid, plays a modulating role as an endocannabinoid to inhibit the arginine-vasopressin-induced response (Shimizu and Yokotani, 2008).

Previously, we also reported that brain phospholipase C, diacylglycerol lipase and prostanoids are involved in the centrally administered corticotropin-releasing factor (CRF)-induced elevation of plasma noradrenaline and adrenaline in rats (Yokotani et al., 2001; Okada et al., 2003b). Therefore we propose, on the basis of recent studies in our laboratory, to examine (1) the involvement of brain 2-arachidonoylglycerol in the CRF-induced response. Furthermore, we examined (2) the regulatory role of 2-arachidonoylglycerol as a brain endocannabinoid, and (3) the effect

of centrally administered cannabinoid receptor agonist on the CRF-induced response, in anesthetized rats.

2. Materials and methods

2.1. Experimental procedures

All experiments were conducted in compliance with the guiding principles for the care and use of laboratory animals approved by Kochi University, which are in accordance with the “Guidelines for proper conduct of animal experiments” from the Science Council of Japan. 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*. In the morning (09:00~10:00), the femoral vein was cannulated for infusion of saline (1.2 ml/h) and the femoral artery was cannulated for collecting blood samples, under urethane anesthesia (1.2 g/kg, i.p.). After these procedures, the animal was placed in a stereotaxic apparatus until the end of each experiment, 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 a stainless-steel cannula (0.3 mm outer diameter). The stereotaxic coordinates of the tip of the 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, 2005). Three hours were allowed to elapse before the application of reagents.

MAFP (a monoacylglycerol lipase inhibitor), AM 251 (a cannabinoid CB₁ receptor antagonist), AM 404 (an uptake-inhibitor of endocannabinoid) and ACEA (a cannabinoid CB₁ receptor agonist) dissolved in 3 µl of 100% *N,N*-dimethylformamide

(DMF)/animal were intracerebroventricularly (i.c.v.) administered using the cannula connected to a 10- μ l Hamilton syringe, which was retained for 15 min to avoid the leakage of these reagents and then removed from the ventricle. CRF dissolved in sterile saline in a volume of 10 μ l/animal was then slowly administered into the ventricle, 30 min after application of MAFP, AM 251 and ACEA and 60 min after application of AM 404, due to their slightly elevating effects on the basal plasma levels of catecholamines, using the same cannula connected to a 25- μ l Hamilton syringe. The cannula was retained until the end of the experiment.

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 (1,500 g for 10 min, at 4°C), the plasma (100 μ l) was transferred to a centrifuge tube containing 30 mg of activated alumina, 2 ml of twice 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 twice 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 an Ag/AgCl reference electrode; column, Eicompac 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 rate of 0.18 ml/min; injection volume, 40 μl . 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 the 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*

Increments of plasma catecholamines above the basal level at each time are expressed as pg/ml. The area under the curve (AUC) is also expressed as pg/2 h. The number of animals in each group is shown in all figures. All values are expressed as the mean $\pm$ S.E.M. Statistical differences are determined using repeated-measure (treatment $\times$ time) or one-way analysis of variance (ANOVA), followed by *post-hoc* analysis with the Bonferroni method. *P* values less than 0.05 were taken to indicate statistical significance.

2.4. *Compounds*

The following drugs were used: synthetic CRF (rat/human) (Peptide Institute, Osaka, Japan); MAFP (methyl arachidonoyl fluorophosphonate), AM 251 [*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide], AM 404 [*N*-(4-hydroxyphenyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetrenamide] and ACEA (arachidonyl 2'-chloroethylamide) (Cayman Chemical, Ann Arbor, MI, U.S.A.). All other reagents were of the highest grade available (Nacalai Tesque, Kyoto, Japan).

3. Results

3.1. Effect of MAFP on the centrally administered CRF (1.5 nmol/animal)-induced elevation of plasma catecholamines

Repeated-measure ANOVA showed for plasma levels of catecholamines (noradrenaline and adrenaline) significant effects of treatment [noradrenaline, $F(4,18)=26.42$, $P<0.05$; adrenaline, $F(4,18)=53.34$, $P<0.05$], time [noradrenaline, $F(4,69)=28.97$, $P<0.05$; adrenaline, $F(4,68)=48.07$, $P<0.05$] and the interaction between these two factors [noradrenaline, $F(16,69)=11.31$, $P<0.05$; adrenaline, $F(16,68)=13.21$, $P<0.05$] (Fig. 1A). One-way ANOVA also revealed significant effects of treatment on plasma catecholamines [noradrenaline, $F(4,17)=26.39$, $P<0.05$; adrenaline, $F(4,18)=74.24$, $P<0.05$] (Fig. 1B).

Treatments with vehicle-1 (3 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 MAFP (an inhibitor of monoacylglycerol lipase) [1.4 μ mol (500 μ g)/animal, i.c.v.] had a slight, but significant inhibitory effect on the basal plasma noradrenaline level, but had no significant effect on the basal plasma adrenaline level (Fig. 1, A and B).

Since we previously reported that CRF (0.5, 1.5 and 3.0 nmol/animal, i.c.v.) dose-dependently elevated plasma levels of noradrenaline and adrenaline (Yokotani et al., 2001), we used a sub-maximum dose of 1.5 nmol/animal in the present study. Administration of CRF (1.5 nmol/animal, i.c.v.) gradually elevated the plasma levels of noradrenaline and adrenaline and the responses reached a maximum 90 min after the

administration of this peptide (Fig. 1A). The CRF-induced elevation of both catecholamines was reduced by MAFP in a dose-dependent manner [0.7 and 1.4 μmol (250 and 500 μg)/animal, i.c.v.] (Fig. 1, A and B).

3.2. Effect of AM 251 on the centrally administered CRF (0.5 nmol/animal)-induced elevation of plasma catecholamines

Repeated-measure ANOVA showed for plasma levels of catecholamines (noradrenaline and adrenaline) significant effects of treatment [noradrenaline, $F(4,20)=21.85$, $P<0.05$; adrenaline, $F(4,20)=30.04$, $P<0.05$], time [noradrenaline, $F(4,79)=14.89$, $P<0.05$; adrenaline, $F(4,74)=11.81$, $P<0.05$] and the interaction between these two factors [noradrenaline, $F(16,79)=7.37$, $P<0.05$; adrenaline, $F(16,74)=4.73$, $P<0.05$] (Fig. 2A). One-way ANOVA also revealed significant effects of treatment on plasma catecholamines [noradrenaline, $F(4,20)=21.09$, $P<0.05$; adrenaline, $F(4,19)=27.78$, $P<0.05$] (Fig. 2B).

Pretreatment with AM 251 (a selective antagonist of cannabinoid CB₁ receptors) [180 nmol (100 μg)/animal, i.c.v.] had no significant effect on the basal plasma levels of both catecholamines (Fig. 2, A and B).

In this experiment, we used a small dose of CRF (0.5 nmol/animal, i.c.v.) to clarify the potentiating effect of cannabinoid CB₁ receptor antagonist on plasma catecholamines induced by this peptide. Administration of CRF (0.5 nmol/animal, i.c.v.) slightly, but significantly elevated plasma levels of noradrenaline and adrenaline during 120 min after the administration of this peptide (Fig. 2A). The CRF-induced

elevation of both catecholamines was potentiated by AM 251 in a dose-dependent manner [90 and 180 nmol (50 and 100 µg)/animal, i.c.v.] (Fig. 2, A and B).

3.3. Effect of AM 404 on the centrally administered CRF (1.5 nmol/animal)-induced elevation of plasma catecholamines

Repeated-measure ANOVA showed for plasma levels of catecholamines (noradrenaline and adrenaline) significant effects of treatment [noradrenaline, $F(4,21)=27.24$, $P<0.05$; adrenaline, $F(4,21)=24.18$, $P<0.05$], time [noradrenaline, $F(4,82)=75.33$, $P<0.05$; adrenaline, $F(4,81)=38.22$, $P<0.05$] and the interaction between these two factors [noradrenaline, $F(16,82)=12.65$, $P<0.05$; adrenaline, $F(16,81)=7.81$, $P<0.05$] (Fig. 3A). One-way ANOVA also revealed significant effects of treatment on plasma catecholamines [noradrenaline, $F(4,21)=25.86$, $P<0.05$; adrenaline, $F(4,21)=23.18$, $P<0.05$] (Fig. 3B).

Treatments with vehicle-1 (3 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. 3, A and B). Pretreatment with AM 404 (an uptake-inhibitor of endocannabinoid) [250 nmol (100 µg)/animal, i.c.v.] had no significant effect on the basal plasma levels of both catecholamines (Fig. 3, A and B).

The CRF (1.5 nmol/animal, i.c.v.)-induced elevation of plasma noradrenaline and adrenaline was reduced by AM 404 in a dose-dependent manner [80 and 250 nmol (30 and 100 µg)/animal, i.c.v.] (Fig. 3, A and B).

3.4. *Effect of ACEA on the centrally administered CRF (1.5 nmol/animal)-induced elevation of plasma catecholamines*

Repeated-measure ANOVA showed for plasma levels of catecholamines (noradrenaline and adrenaline) significant effects of treatment [noradrenaline, $F(4,17)=33.66$, $P<0.05$; adrenaline, $F(4,17)=18.45$, $P<0.05$], time [noradrenaline, $F(4,67)=42.99$, $P<0.05$; adrenaline, $F(4,64)=23.94$, $P<0.05$] and the interaction between these two factors [noradrenaline, $F(16,67)=9.84$, $P<0.05$; adrenaline, $F(16,64)=6.58$, $P<0.05$] (Fig. 4A). One-way ANOVA also revealed significant effects of treatment on plasma catecholamines [noradrenaline, $F(4,17)=31.29$, $P<0.05$; adrenaline, $F(4,17)=23.40$, $P<0.05$] (Fig. 4B).

Pretreatment with ACEA (a selective agonist of cannabinoid CB₁ receptors) [1.4 μ mol (500 μ g)/animal, i.c.v.] had no significant effect on the basal plasma levels of both catecholamines (Fig. 4, A and B).

The CRF (1.5 nmol/animal, i.c.v.)-induced elevation of noradrenaline and adrenaline was reduced by ACEA in a dose-dependent manner [0.7 and 1.4 μ mol (250 and 500 μ g)/animal, i.c.v.] (Fig. 4, A and B). On the other hand, intravenous administration of ACEA (1.4 μ mol/animal) had no effect on the CRF-induced response (data not shown).

4. Discussion

2-Arachidonoylglycerol generated by phospholipase C and *sn*-1 selective diacylglycerol lipase (Stella et al., 1997; Bisogno et al., 2003; Di Marzo, 2006; Tang et al., 2006; Bisogno, 2008) can be hydrolyzed by monoacylglycerol lipase to arachidonic acid (Dinh et al., 2002; Di Marzo, 2006; Quistad et al., 2006; Tang et al., 2006; Blankman et al., 2007; Bisogno, 2008). In addition, arachidonic acid can be also produced from diacylglycerol containing the fatty acid in position 2 by *sn*-2 selective diacylglycerol lipase (Bell et al., 1979). Brain phospholipase C, diacylglycerol lipase and prostanoids, active metabolites of arachidonic acid, have been shown to be involved in the centrally administered CRF-induced elevation of plasma noradrenaline and adrenaline (Yokotani et al., 2001; Okada et al., 2003b). Therefore, we examined whether 2-arachidonoylglycerol is involved as a precursor of arachidonic acid in the CRF-induced response using MAFP. This reagent is currently recognized as a very potent inhibitor of 2-arachidonoylglycerol hydrolyzing enzymatic activity with an IC₅₀ value of 2-3 nM (Goparaju et al., 1999; Saario et al., 2004) and actually accumulates endogenous 2-arachidonoylglycerol in rat brain sections (Palomäki et al., 2007). Previously we also reported that centrally administered MAFP also reduced the elevation of plasma catecholamines induced by centrally administered 2-arachidonoylglycerol (Shimizu and Yokotani, 2008). In the present experiment, centrally administered MAFP effectively reduced the CRF-induced elevation of plasma catecholamines, suggesting the involvement of brain 2-arachidonoylglycerol generated by *sn*-1 selective diacylglycerol lipase in the CRF-induced activation of central sympatho-adrenomedullary outflow in rats.

Recently, 2-arachidonoylglycerol has been recognized as a major endogenous ligand for two cannabinoid receptors, CB₁ and CB₂ (Matsuda et al., 1990; Munro et al., 1993). Cannabinoid CB₁ receptors are predominantly expressed in the central nervous system, whereas cannabinoid CB₂ receptors are present mainly in the immune system (Howlett et al., 2002; Mackie, 2008). Brain 2-arachidonoylglycerol has been shown to inhibit neurotransmission through presynaptic cannabinoid CB₁ receptors (Ohno-Shosaku et al., 2001; Freund et al., 2003) and we recently reported that brain cannabinoid CB₁ receptors, but not cannabinoid CB₂ receptors, play an inhibitory role in the centrally administered arginine-vasopressin-induced elevation of plasma catecholamines (Shimizu and Yokotani, 2008). Therefore we examined whether the endogenously generated 2-arachidonoylglycerol has an inhibitory role as an endocannabinoid in the centrally administered CRF-induced elevation of plasma catecholamines with regard to cannabinoid CB₁ receptors. In the present experiment, we used a selective cannabinoid CB₁ receptor antagonist AM 251 (Lan et al., 1999), which actually reverses the orexigenic effect induced by 2-arachidonoylglycerol administered into pontine parabrachial nucleus in rats (DiPatrizio and Simansky, 2008) and the protective effect against brain ischemia caused by electroacupuncture-induced elevation of brain 2-arachidonoylglycerol content in rats (Wang et al., 2009). Centrally pretreated AM 251 effectively potentiated the elevation of plasma catecholamines induced by a small dose of CRF, suggesting a possibility that endogenously generated 2-arachidonoylglycerol has an inhibitory effect on the CRF-induced activation of central sympatho-adrenomedullary outflow through cannabinoid CB₁ receptors in rats.

The released 2-arachidonoylglycerol into the synaptic cleft in the brain seems to be rapidly uptaken into neurons and degraded for termination of the endocannabinoid

signaling (Di Marzo, 2006; Centonze et al., 2007; Bisogno, 2008). The uptake is considered to occur through a plasma membrane transporter that has not been isolated or cloned yet (Bisogno, 2008). Several reagents, including AM 404, have been shown to inhibit the cellular uptake of 2-arachidonoylglycerol (Centonze et al., 2007). AM 404 was originally reported as an inhibitor of anandamide transport (Beltramo et al., 1997), and this reagent actually induces anandamide accumulation in the rat brain (Giuffrida et al., 2001) and increases an activity-dependent anandamide level in rat hypothalamic slices (Di et al., 2005). Interestingly, AM 404 also elicits an activity-dependent increase of 2-arachidonoylglycerol in rat hypothalamic slices (Di et al., 2005) and inhibits the uptake of 2-arachidonoylglycerol in C6 glioma cells (Bisogno et al., 2001). In the present experiment, central pretreatment with AM 404 effectively reduced the centrally administered CRF-induced elevation of plasma catecholamines, suggesting a possibility that AM 404-induced synaptic accumulation of endogenously generated brain 2-arachidonoylglycerol negatively modulates the CRF-induced activation of central sympatho-adrenomedullary outflow in rats.

In the next experiment, we examined the effect of exogenously administered ACEA, a selective agonist of cannabinoid CB₁ receptors (Hillard et al., 1999), on the centrally administered CRF-induced elevation of plasma catecholamines. This agonist administered into the dorsolateral periaqueductal gray induces an anxiolytic effect in rats and centrally administered this reagent decreases the frequency of epileptiform activity in epilepsy model rats (Moreira et al., 2007; Kozan et al., 2009). In the present experiment, centrally administered ACEA effectively reduced the CRF-induced elevation of plasma catecholamines, but peripherally administered ACEA had no effect. The result indicates that exogenously administered cannabinoid CB₁ receptor agonist

into the brain further inhibits the CRF-induced activation of central sympatho-adrenomedullary outflow in addition to the inhibition induced by CRF-induced endogenously generated brain 2-arachidonoylglycerol.

Centrally administered cannabinoid CB₁ receptor agonists can inhibit the activation of central sympatho-adrenomedullary outflow. However, one of the major drawbacks of the use of agonists is that cannabinoid CB₁ receptors have a widespread distribution in the brain (Howlett et al., 2002; Mackie, 2008) and global activation of these receptors is associated with adverse side effects such as sedation, dependence, cognitive impairment and psychosis (Kalant, 2004; Pacher et al., 2006). On the other hand, endocannabinoid uptake-inhibitors can elevate the synaptic content of endocannabinoid at sites of high endocannabinoid turnover. From our studies, brain 2-arachidonoylglycerol can be endogenously generated during the CRF-induced activation of central sympatho-adrenomedullary outflow and the activation was effectively inhibited by centrally administered AM 404 (an uptake-inhibitor of endocannabinoid). Furthermore, Brozoski et al. reported that AM 404 administered in the nucleus tractus solitarius after an increase in blood pressure prolongs baroreflex inhibition of renal sympathetic nerve activity in rats (Brozoski et al., 2005). These lines of evidence suggest a possibility that endocannabinoid uptake-inhibitors can locally activate cannabinoid CB₁ receptors at sites of high endocannabinoid turnover and inhibit the activation of central sympatho-adrenomedullary outflow. Taken together, it is suggested that endocannabinoid uptake-inhibitors rather than agonists of cannabinoid CB₁ receptors are beneficial targets for therapeutic drugs to inhibit central sympatho-adrenomedullary outflow without adverse side effects on other brain functions.

In summary, it seems likely that brain 2-arachidonoylglycerol is endogenously generated during the centrally administered CRF-induced activation of sympatho-adrenomedullary outflow, thereby inhibiting the peptide-induced response by brain cannabinoid CB₁ receptor-mediated mechanisms in anesthetized rats.

Acknowledgments

The authors thank K. Nakamura for technical assistance (measurement of plasma noradrenaline and adrenaline). This work was supported in part by a Grant-in-Aid for Young Scientists (B) (No. 21790627 to T.S.) from The Ministry of Education, Culture, Sports, Science and Technology, a Grant-in-Aid for Scientific Research (C) (No. 20590702 to K.Y.) from the Japan Society for the Promotion of Science, a Grant from The Smoking Research Foundation in Japan, a Discretionary Grant of the Head of the Kochi Medical School Hospital, and specific Research Grants from Kochi University.

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Legends to figures

Fig. 1. Effect of MAFP on the centrally administered CRF (1.5 nmol/animal)-induced elevation of plasma catecholamines. MAFP (an inhibitor of monoacylglycerol lipase) (0.7 or 1.4 μ mol/animal) or vehicle-1 (3 μ l DMF/animal) was i.c.v. administered 30 min before the administration of CRF (1.5 nmol/animal, i.c.v.) or vehicle-2 (10 μ l saline/animal, i.c.v.). (A) Increments of plasma catecholamines above the basal level at each time point are expressed as pg/ml. Arrows indicate the administration of MAFP/vehicle-1 and CRF/vehicle-2. Δ Noradrenaline and Δ adrenaline: increments of noradrenaline and adrenaline above the basal level. (B) The area under the curve (AUC) of the CRF-induced elevation of plasma catecholamines above the basal level is expressed as pg/2 h. Each point represents the mean $\pm$ S.E.M. # $P<0.05$ versus vehicle-1- and vehicle-2-treated group (Bonferroni method), * $P<0.05$ versus vehicle-1- and CRF-treated group (Bonferroni method).

Fig. 2. Effect of AM 251 on the centrally administered CRF (0.5 nmol/animal)-induced elevation of plasma catecholamines. AM 251 (a selective antagonist of cannabinoid CB₁ receptors) (90 or 180 nmol/animal) or vehicle-1 (3 μ l DMF/animal) was i.c.v. administered 30 min before the administration of CRF (0.5 nmol/animal, i.c.v.) or vehicle-2 (10 μ l saline/animal, i.c.v.). (A) Increments of plasma catecholamines above the basal level. Arrows indicate the administration of AM 251/vehicle-1 and CRF/vehicle-2. (B) AUC of the CRF-induced elevation of plasma catecholamines above the basal level. The vehicle-1- and vehicle-2-treated group is the same as that in Fig. 1. # $P<0.05$ versus vehicle-1- and vehicle-2-treated group (Bonferroni method), * $P<0.05$

versus vehicle-1- and CRF-treated group (Bonferroni method). Other conditions were the same as those of Fig. 1.

Fig. 3. Effect of AM 404 on the centrally administered CRF (1.5 nmol/animal)-induced elevation of plasma catecholamines. AM 404 (an uptake-inhibitor of endocannabinoid) (80 or 250 nmol/animal) or vehicle-1 (3 μ l DMF/animal) was i.c.v. administered 60 min before the administration of CRF (1.5 nmol/animal, i.c.v.) or vehicle-2 (10 μ l saline/animal, i.c.v.). (A) Increments of plasma catecholamines above the basal level. Arrows indicate the administration of AM 404/vehicle-1 and CRF/vehicle-2. (B) AUC of the CRF-induced elevation of plasma catecholamines above the basal level. # $P<0.05$ versus vehicle-1- and vehicle-2-treated group (Bonferroni method), * $P<0.05$ versus vehicle-1- and CRF-treated group (Bonferroni method). Other conditions were the same as those of Figs. 1 and 2.

Fig. 4. Effect of ACEA on the centrally administered CRF (1.5 nmol/animal)-induced elevation of plasma catecholamines. ACEA (a selective agonist of cannabinoid CB₁ receptors) (0.7 or 1.4 μ mol/animal) or vehicle-1 (3 μ l DMF/animal) was i.c.v. administered 30 min before the administration of CRF (1.5 nmol/animal, i.c.v.) or vehicle-2 (10 μ l saline/animal, i.c.v.). (A) Increments of plasma catecholamines above the basal level. Arrows indicate the administration of ACEA/vehicle-1 and CRF/vehicle-2. (B) AUC of the CRF-induced elevation of plasma catecholamines above the basal level. # $P<0.05$ versus vehicle-1- and vehicle-2-treated group (Bonferroni method), * $P<0.05$ versus vehicle-1- and CRF-treated group (Bonferroni method). Other conditions were the same as those of Figs. 1-3.

Figure-1

A

B

Figure-2

A

B

Figure-3

A

B

Figure-4

A

- Vehicle-1 + Vehicle-2 (n=5)
- ACEA (1.4 μ mol) + Vehicle-2 (n=4)
- Vehicle-1 + CRF (1.5 nmol) (n=5)
- ▼ ACEA (0.7 μ mol) + CRF (1.5 nmol) (n=4)
- ACEA (1.4 μ mol) + CRF (1.5 nmol) (n=4)

B

- Vehicle-1 + Vehicle-2 (n=5)
- ▨ ACEA (1.4 μ mol) + Vehicle-2 (n=4)
- ▧ Vehicle-1 + CRF (1.5 nmol) (n=5)
- ▩ ACEA (0.7 μ mol) + CRF (1.5 nmol) (n=4)
- ACEA (1.4 μ mol) + CRF (1.5 nmol) (n=4)