2-Arachidonoylglycerol and Anandamide Oppositely Modulate Norepinephrine Release from the Rat Heart Sympathetic Nerves

Junichi Kurihara[1],*, Michiru Nishigaki[1], Shigeto Suzuki[1], Yoko Okubo[1], Yoshinobu Takata[1], Shinji Nakane[2], Takayuki Sugiura[2], Keizo Waku[2] and Hitoshi Kato[1]

[1]*Department of Pharmacology and* [2]*Department of Hygienic Chemistry and Nutrition, Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-0195, Japan*

ABSTRACT—Anandamide (10^{-7} and 10^{-6} M) as well as a synthetic cannabinoid HU210 (10^{-8} to 10^{-6} M) suppressed the norepinephrine release evoked by perivascular nerve stimulation (PNS) of the rat heart Langendorff's preparation. The effects of HU210 and the lower dose of anandamide were completely blocked by the cannabinoid CB_1-receptor antagonist AM251, while that of anandamide at 10^{-6} M was partly mediated by arachidonate-derived metabolites. 2-Arachidonoylglycerol (2-AG), at 10^{-6} M in the presence of DFP and indomethacin, increased PNS-evoked norepinephrine release, which was completely blocked by AM251. The present results suggest that the two endocannabinoids may oppositely participate in the CB_1-receptor-mediated modulation of sympathetic norepinephrine release.

Keywords: 2-Arachidonoylglycerol, Anandamide, Norepinephrine release

Effects of cannabinoids, i.e., Δ^9-tetrahydrocannabinol (THC) and its related compounds, have been extensively studied, focusing on their psychotropic effects through central CB_1 cannabinoid receptors (1). Recent studies further reveal that cannabinoid receptors are also involved in the regulation of the peripheral nervous system and cardiovascular system (2 – 5). At present, two lipid compounds, *N*-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG) are putatively considered as endogenous ligands of cannabinoid receptors (6 – 8). However, their pharmacological potentials as well as physiological roles at the autonomic nerve endings are not well characterized. The present study was conducted to investigate the effects of these endocannabinoids, in comparison with the THC-like synthetic cannabinoid HU210, on the norepinephrine release from the rat heart sympathetic nerves.

Male Wistar rats weighing 300 to 400 g were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and injected with heparin sodium (1000 U/kg, i.v.). The hearts were removed and perfused with 37°C Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.03 mM KH_2PO_4, 1.2 mM $MgSO_4$, 11.1 mM D-glucose, 0.067 mM EDTA · 2Na, 0.14 mM ascorbic acid,

0.001 mM atropine, 0.01 mM cocaine and 0.001 mM hydrocortisone) at the rate of 5 ml/min according to Langendorff's technique. The perfusion medium was gassed with a mixture of 95% O_2 and 5% CO_2. Perivascular nerve stimulation (PNS) was applied for 10 s at 30-min intervals to the proximal portion of the ascending aorta through bipolar platinum plate electrodes; pulse duration, amplitude and frequency were set at 2 ms, 80 V and 1 Hz, respectively. A preliminary experiment confirmed that the effect of PNS was completely blocked by tetrodotoxin (10^{-6} M). Norepinephrine in the effluents was extracted by activated alumina and measured by HPLC (GINA50; Gynkotek, Germering, Germany) and electrochemical detector (5200A; Esa, Chelmsford, MA, USA). PNS-evoked norepinephrine release was calculated by subtracting the amount in 1-min effluent just prior to PNS (basal release: 0.054 ± 0.004 ng/min, n = 80) from the amount in the effluent collected for 1 min from the start of PNS. PNS was applied totally 9 times to each preparation and the average of the second and third responses (1.15 ± 0.05 ng, n = 80) was regarded as the control value. Cannabinoids were dissolved in dimethyl sulfoxide (DMSO; Wako Pure Chemical Industries, Ltd., Osaka), mixed in the perfusion medium and continuously perfused from 20 min prior to the fourth PNS until the end of the experiment. The final concentration of DMSO in the perfusate was 0.001%, which had no effect on the norepinephrine release. 2-AG

*Corresponding author.   FAX: +81-426-85-3726
 E-mail: jun-kuri@pharm.teikyo-u.ac.jp

was synthesized according to the method described previously (9). Other drugs including HU210 ((6a*R*)-*trans*-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[*b*,*d*]pyran-9-methanol), AM251 (*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) and anandamide were obtained from Wako Pure Chemical Industries, Ltd. The data were analyzed by two-way ANOVA with repeated measure. When there is no interaction between the factors, the difference from the value of the DMSO-treated group was analyzed by Dunnett's post hoc procedure. When the interaction existed, the data were re-analyzed by one-way ANOVA followed by Bonferroni/Dunn's procedure for multiple comparisons.

As shown in Fig. 1, HU210 suppressed the PNS-evoked norepinephrine release in a dose-related manner. The effect developed gradually, reaching a statistically significant range in 80 and 110 min of perfusion at 10^{-6} and 10^{-7} M, respectively. The effect of 10^{-8} M was not statistically significant. Basal release of norepinephrine was not affected by any dose of HU210 (data not shown). Co-administration of AM251 (10^{-6} M), a selective CB_1-receptor antagonist, completely blocked the effect of HU210 (Fig. 1). The results indicate the existence of inhibitory CB_1 receptors on the rat heart sympathetic nerve endings, which is in line with the previous results from isolated rat vas deferens and atria (10) as well as human atrial appendages (4). In addition, AM251 alone affected neither the basal release

nor the PNS-evoked norepinephrine release (data not shown), suggesting that CB_1 receptors are not tonically stimulated by endogenous ligands under the conditions used in the present study.

Anandamide (10^{-7} and 10^{-6} M) suppressed the PNS-evoked norepinephrine release, as shown in Fig. 2; the effects developed earlier than HU210. AM251 completely blocked the effect of a lower dose of anandamide (Fig. 2A). However, the effect of higher dose was somewhat resistant to AM251; only the early effect observed at 50 min of perfusion was blocked (Fig. 2B). Since anandamide is likely converted to arachidonate by free fatty acid amidohydrolase, the possible involvement of arachidonate-derived prostanoids in the effect of anandamide was further investigated. Thus, in the presence of indomethacin (10^{-5} M), the effect of the higher dose, but not the lower dose, of anandamide was partially reduced (Fig. 2). In addition, arachidonate (10^{-6} M) caused about 50% suppression of PNS-evoked norepinephrine release, which was completely blocked by indomethacin (data not shown). The results suggest that anandamide may suppress the PNS-induced norepinephrine release acting on CB_1 receptors and that arachidonate-derived metabolites may be also involved in the effect of anandamide during persistent administration of a higher dose.

As shown in Fig. 3A, 2-AG (10^{-6} M) caused maximally 36% suppression of the PNS-evoked norepinephrine release. The effect of 2-AG was completely blocked by
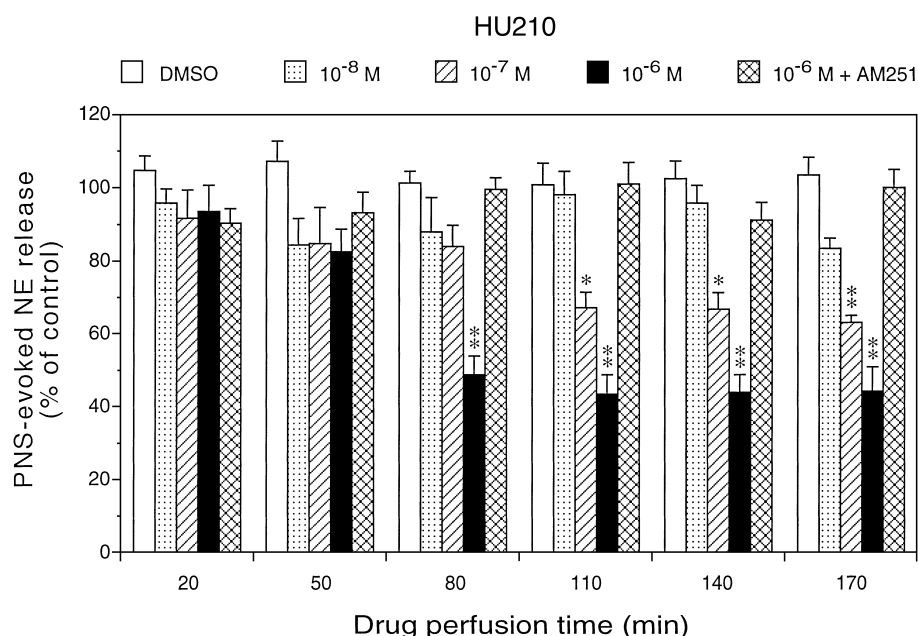

Fig. 1. Effects of HU210 on the perivascular nerve stimulation (PNS)-evoked norepinephrine (NE) release. PNS was applied totally 9 times at 30-min intervals, and the average of the second and third responses was regarded as the control. AM251 (10^{-6} M) was co-administered with HU210. Data are means ± S.E.M. of 5 experiments. *$P<0.05$, **$P<0.01$: significantly different from the DMSO-treated group at the same time point.
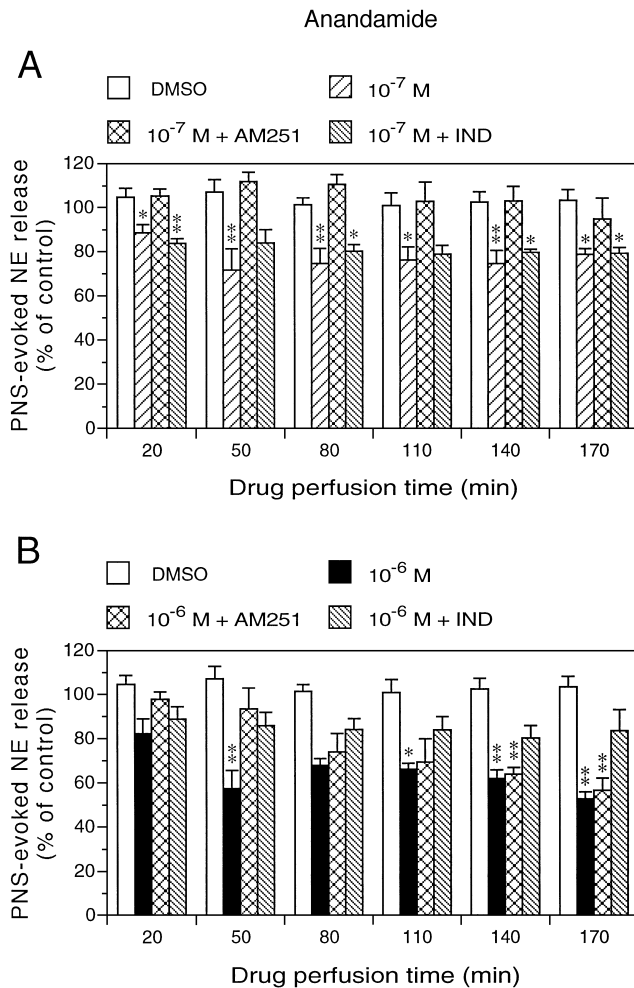
Anandamide

2-Arachidonoylglycerol

Fig. 2. Effects of anandamide at 10^{-7} M (A) and 10^{-6} M (B) on the perivascular nerve stimulation (PNS)-evoked norepinephrine (NE) release. The control is as noted in Fig. 1. IND: indomethacin (10^{-5} M). AM251 (10^{-6} M) was co-administered with anandamide. Data are means $\pm$ S.E.M. of 5 experiments. *$P<0.05$, **$P<0.01$: significantly different from the DMSO-treated group at the same time point.
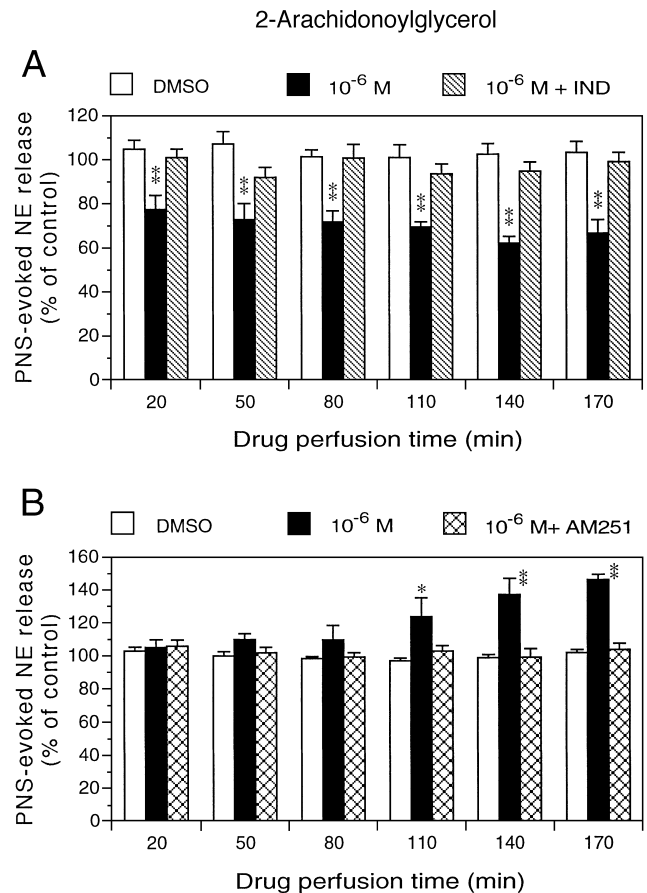
Fig. 3. Effects of 2-arachidonoylglycerol (2-AG) on the perivascular nerve stimulation (PNS)-evoked norepinephrine (NE) release. The control is as noted in Fig. 1. A: in the absence of DFP. IND: indomethacin (10^{-5} M). B: in the presence of DFP (10^{-4} M) and IND (10^{-5} M). AM251 (10^{-6} M) was co-administered with 2-AG. Data are means $\pm$ S.E.M. of 5 experiments. *$P<0.05$, **$P<0.01$: significantly different from the DMSO-treated group at the same time point.

indomethacin. The result is in agreement with the fact that 2-AG is easily metabolized by esterase to produce arachidonate and glycerol. Then, as shown in Fig. 3B, the effect of 2-AG was re-evaluated in the presence of DFP (10^{-4} M), an esterase inhibitor, and indomethacin (10^{-5} M) to unmask a potential activity of 2-AG in the non-degraded form. Under this condition, 2-AG (10^{-6} M) gradually enhanced the PNS-evoked norepinephrine release, in contrast with anandamide and HU210. About 45% increase was observed at 170 min of perfusion. Basal norepinephrine release was not significantly affected (data not shown). The effect of 2-AG was completely blocked by AM251, suggesting that this facilitatory effect is also mediated by CB$_1$ receptors. The results provide the first evidence that 2-AG and anandamide may modulate sympathetic neuro-

transmission to an opposite direction sharing AM251-sensitive CB$_1$ receptors at the sympathetic nerve endings.

CB$_1$ receptor is a member of the G$_{i/o}$ protein-coupled receptors with seven hydrophobic transmembrane domains. It has been shown that signal transduction pathways of CB$_1$ receptors include adenylate cyclase, ion channels and MAP kinase (1). Thus, activation of CB$_1$ receptors may cause inhibition of adenylate cyclase, inhibition of N- and Q/P-type voltage-dependent calcium channels, stimulation of inwardly rectifying potassium channels and reduction of cAMP-dependent inhibition of A-type potassium channels, which likely reduce neurotransmitter release. The inhibitory effects of HU210 and anandamide in the present study can be explained by these signal transduction pathways.

There are only few studies on the neuronal effect of 2-AG. Mechoulam et al. (7) showed that 2-AG suppressed the contraction of isolated mouse vas deferens induced by field electrical stimulation, although no direct measurement

of norepinephrine release was carried out. Stella et al. (11) showed that 2-AG inhibited cAMP production in the rat cortical cell culture and formation of long term potentiation in the rat hippocampal slices, which were blocked by the CB_1 antagonist SR141716A. These results are in line with the above-mentioned signal transduction pathways postulated for CB_1 receptors. On the other hand, the present result on 2-AG is unique in that 2-AG unexpectedly facilitated the PNS-evoked norepinephrine release from the rat heart sympathetic nerves in the presence of DFP and indomethacin. At present, the mechanism of facilitation is not clear except the involvement of AM251-sensitive CB_1 receptors. If 2-AG, HU210 and anandamide might share the same AM251-sensitive CB_1 receptors to cause the effects observed in the present study, binding of 2-AG might activate intracellular signaling pathways different from those activated by HU210 and anandamide. Recently, accumulating evidences suggest that CB_1 receptors are coupled with both $G_{i/o}$ and G_s proteins. Thus, pertussis toxin unmasks a stimulatory effect of cannabinoids on cAMP production in rat striatal culture (12) and Chinese hamster ovary cells (12, 13). Interestingly, anandamide is markedly less efficacious in stimulating cAMP production than in inhibiting it (13). Therefore, one of the possible mechanisms of opposing effects of 2-AG and anandamide is that 2-AG and anandamide might preferentially activate G_s protein-coupled and $G_{i/o}$ protein-coupled pathways, respectively. Furthermore, the possible existence of unknown subtypes of AM251-sensitive CB_1 receptor, which is different from the receptors in the central nervous system, cannot be excluded.

In conclusion, the present study suggests that two endocannabinoids, 2-AG and anandamide, may oppositely participate in the CB_1-receptor-mediated modulation of sympathetic norepinephrine release in the rat heart.

REFERENCES

1   Ameri A: The effects of cannabinoids on the brain. Prog Neurobiol **58**, 315 – 348 (1999)
2   Malinowska B, Godlewski G, Bucher B and Schlicker E: Cannabinoid CB_1 receptor-mediated inhibition of the neurogenic vasopressor response in the pithed rat. Naunyn Schmiedebergs Arch Pharmacol **356**, 197 – 202 (1997)
3   Wagner JA, Varga K and Kunos G: Cardiovascular actions of cannabinoids and their generation during shock. J Mol Med **76**, 824 – 836 (1998)
4   Molderings GJ, Likungu J and Göthert M: Presynaptic cannabinoid and imidazoline receptors in the human heart and their potential relationship. Naunyn Schmiedebergs Arch Pharmacol **360**, 157 – 164 (1999)
5   Niederhoffer N and Szabo B: Cannabinoids cause central sympathoexcitation and bradycardia in rabbits. J Pharmacol Exp Ther **294**, 707 – 713 (2000)
6   Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A and Mechoulam R: Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science **258**, 1946 – 1949 (1992)
7   Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, Pertwee RG, Griffin G, Bayewitch M, Barg J and Vogel Z: Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol **50**, 83 – 90 (1995)
8   Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A and Waku K: 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochim Biophys Res Commun **215**, 89 – 97 (1995)
9   Sugiura T, Kodaka T, Nakane S, Miyashita T, Kondo S, Suhara Y, Takayama H, Waku K, Seki C, Baba N and Ishima Y: Evidence that the cannabinoid CB1 receptor is a 2-arachidonoylglycerol receptor. Structure-activity relationship of 2-arachidonoylglycerol, ether-linked analogues, and related compounds. J Biol Chem **274**, 2794 – 2801 (1999)
10 Ishac EJN, Jiang L, Lake KD, Varga K, Abood ME and Kunos G: Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB_1 receptors on peripheral sympathetic nerves. Br J Pharmacol **118**, 2023 – 2028 (1996)
11 Stella N, Schweitzer P and Piomelli D: A second endogenous cannabinoid that modulates long-term potentiation. Nature **388**, 773 – 778 (1997)
12 Glass M and Felder CC: Concurrent stimulation of cannabinoid CB_1 and dopamine D_2 receptor augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB_1 receptor. J Neurosci **17**, 5327 – 5333 (1997)
13 Bonhaus DW, Chang LK, Kwan J and Martin GR: Dual activation and inhibition of adenylyl cyclase by cannabinoid receptor agonists: evidence for agonist-specific trafficking of intracellular responses. J Pharmacol Exp Ther **287**, 884 – 888 (1998)