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

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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\textsubscript{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\textsubscript{1}-receptor-mediated modulation of sympathetic norepinephrine release.

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was synthesized according to the method described previously (9). Other drugs including HU210 ((6aR)-\textit{trans}-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,f]pyran-9-methanol), AM251 (N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-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, arachidionate ($10^{-6}$ 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
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 CB₁ receptors. The results provide the first evidence that 2-AG and anandamide may modulate sympathetic neurotransmission to an opposite direction sharing AM251-sensitive CB₁ receptors at the sympathetic nerve endings.

CB₁ receptor is a member of the Gₛ/o protein-coupled receptors with seven hydrophobic transmembrane domains. It has been shown that signal transduction pathways of CB₁ receptors include adenylyl cyclase, ion channels and MAP kinase (1). Thus, activation of CB₁ receptors may cause inhibition of adenylyl 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 CB₁ antagonist SR141716A. These results are in line with the above-mentioned signal transduction pathways postulated for CB₁ 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 CB₁ receptors. If 2-AG, HU210 and anandamide might share the same AM251-sensitive CB₁ 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 CB₂ receptors are coupled with both Gᵢ/ₒ and Gᵢ 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 Gᵢ protein-coupled and Gᵢ/ₒ protein-coupled pathways, respectively. Furthermore, the possible existence of unknown subtypes of AM251-sensitive CB₁ 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 CB₁-receptor-mediated modulation of sympathetic norepinephrine release in the rat heart.

REFERENCES

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