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2-Arachidonoylglycerol, an endogenous cannabinoid receptor agonist: identification as one of the major species of monoacylglycerols in various rat tissues, and evidence for its generation through Ca^{2+}-dependent and -independent mechanisms

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Abstract The molecular species compositions of monoacylglycerols obtained from various rat tissues were examined by reverse-phase high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) analyses. We confirmed that 2-arachidonoylglycerol, an endogenous cannabinoid receptor agonist, is one of the most abundant molecular species of monoacylglycerols in the brain. Substantial amounts of 2-arachidonoylglycerol were also found in the liver, spleen, lung and kidney, but the levels were considerably lower than that in the brain. We found that a small amount of 2-arachidonoylglycerol was generated in a brain homogenate during incubation in the absence of Ca^{2+}. Importantly, the generation of 2-arachidonoylglycerol was markedly augmented in the presence of Ca^{2+}, suggesting that Ca^{2+} plays a key role in regulation of the generation of 2-arachidonoylglycerol in this tissue.

Key words: 2-Arachidonoylglycerol; Monoacylglycerol; Cannabinoid; CB1 receptor

1. Introduction

Δ^9-Tetrahydrocannabinol is a psychoactive constituent of marijuana, and is known to exert a variety of biological effects in experimental animals and man, although its mechanisms of action remained unclear until recently. A decade ago, Devane et al. [1] demonstrated the presence of a specific binding site for cannabinoids in rat brain synaptosomes. Soon after, Matsuda et al. [2] cloned the cDNA for the cannabinoid CB1 receptor from a rat brain cDNA library. These findings constitute evidence that the actions of cannabinoids are mediated largely through specific receptor(s), and suggested the occurrence of an endogenous cannabinoid receptor ligand(s). Evans et al. [3] demonstrated that (a) material(s) having the ability to bind to the cannabinoid receptor is released from A23187-stimulated rat brain slices, although its chemical structure has not yet been elucidated. In 1992, an endogenous cannabinoid receptor ligand, named anandamide, was isolated from pig brain by Devane et al. [4]. This compound has been shown to exhibit various cannabimimetic activities in vitro and in vivo [5]. However, the levels of anandamide in various living tissues were found to be very low [6–10]. Furthermore, the biosynthetic pathways for anandamide (*N*-acylphosphatidylethanolamine pathway [7,8,11,12] and the condensation pathway [8,13–15]) do not appear to be able to provide a large amount of anandamide, at least under normal conditions. These observations raised the possibility that another type of endogenous cannabinoid receptor ligand may exist.

In 1995, we [16], and Mechoulam et al. [17] proposed that 2-arachidonoylglycerol is another type of endogenous ligand for the cannabinoid receptor(s). We obtained evidence that 2-arachidonoylglycerol exhibits binding activity toward the cannabinoid receptor in rat brain synaptosomes [16], and that 2-arachidonoylglycerol induces rapid elevation of the intracellular free Ca^{2+} concentration through the cannabinoid CB1 receptor on NG108-15 cells [18,19], and suggested that the cannabinoid CB1 receptor is originally a 2-arachidonoylglycerol receptor [18–20]. Mechoulam et al. [17] demonstrated that 2-arachidonoylglycerol binds to either CB1 or CB2 receptors, and inhibits adenylate cyclase in spleen cells and the twitch response in the mouse vas deferens. Furthermore, recently Stella et al. [21] reported that 2-arachidonoylglycerol inhibits long-term potentiation (LTP) in the rat hippocampus via a cannabinoid CB1 receptor-dependent mechanism. Nevertheless, not much is known concerning the tissue levels of 2-arachidonoylglycerol. Mechoulam et al. [17] detected 2-arachidonoylglycerol in canine gut, yet the exact tissue level is not known. We detected 3.3 nmol of arachidonoylglycerol [16], and Stella et al. [21] detected 4.0 nmol/g tissue of 2-arachidonoylglycerol in rat brain. However, little information is so far available concerning the tissue levels of monoacylglycerols, especially those containing arachidonic acid, except in the case of brain. Furthermore, it has not yet been established whether or not mammalian tissues actually contain both enzyme activities and the substrates for the generation of substantial amounts of 2-arachidonoylglycerol.

In this study, we developed a new method for the quantification of monoacylglycerols. We found that various tissues other than blood plasma contain significant amounts of 2-arachidonoylglycerol besides other species of monoacylglycerols, and that a large amount of 2-arachidonoylglycerol is produced in a rat brain homogenate upon incubation.

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Abbreviations: LTP, long-term potentiation; BHT, butylated hydroxytoluene; DFP, diisopropylfluorophosphate; TMS, trimethylsilyl; GABA, γ-aminobutyric acid; Fatty acids are designated in terms of number of carbon atoms/number of double bonds, e.g. 20:4 for arachidonic acid

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2. Materials and methods

2.1. Chemicals

1(3)-Myristoylglycerol, 1(3)-palmitoylglycerol, 2-palmitoylglycerol, 1(3)-stearoylglycerol, 1(3)-oleoylglycerol, 1(3)-eicosaenoylglycerol and various types of fatty acids were obtained from Sigma (St. Louis, MO, USA). 1(3)-Linoleoylglycerol was purchased from Doosan Serdary Res. Lab. (Englewood Cliffs, NJ, USA). 1-Anthroyl cyanide, diisopropylfluorophosphate (DFP), *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), and butylated hydroxytoluene (BHT) were obtained from Wako Pure Chem. Ind. (Osaka, Japan). Quinuclidine was from Molecular Probes, Inc. (Eugene, OR, USA). Various types of 2-monoacylglycerol were prepared as described previously [18]. 1(3)-Palmitoylglycerol, 1(3)-*cis*-vaccenoylglycerol, 1(3)-arachidonoylglycerol, and 1(3)-docosahexaenoylglycerol were synthesized from appropriate fatty acid anhydrides and glycerol, and then purified by borate-impregnated thin-layer chromatography (TLC).

2.2. HPLC analysis of monoacylglycerols

Various species of standard monoacylglycerols were converted to dianthroyl derivatives as described previously [19]. Briefly, monoacylglycerols were treated with 1-anthroyl cyanide (0.2 mg) in 200 μl of dehydrated acetone containing 0.08% quinuclidine at 45°C for 60 min. The reaction was stopped by the addition of 50 μl of methanol. The resultant dianthroyl derivatives of monoacylglycerol were purified by TLC and developed with petroleum ether/diethyl ether/acetic acid (65:35:1, v/v). The purified dianthroyl derivatives of monoacylglycerols were analyzed with a HPLC system equipped with a reverse phase column (Shiseido; CAPCELL PAK C18 SG, 4.6×250 mm), and a fluorescence detector (excitation at 370 nm; emission at 470 nm) as described previously [19].

2.3. Extraction and purification of monoacylglycerols from various rat tissues

Various organs were taken from male Wistar rats (200–280 g body weight). Blood was taken by means of cardiac puncture using heparin as an anticoagulant, and platelet-poor plasma was separated by centrifugation. Total lipids were extracted by the method of Bligh and Dyer [22]. BHT (final, 0.05%) was added to avoid lipid peroxidation. Monoacylglycerol was purified and analyzed as described previously [19]. Briefly, a portion of the total lipids (equivalent to 1 g or 0.5 g wet tissue), with 1 nmol of 2-heptadecanoylglycerol added as an internal standard, was fractionated by TLC and developed with petroleum ether/diethyl ether/acetic acid (20:80:1, v/v) in a tank sealed with N_2 gas. The area corresponding to standard monoacylglycerol was scraped off the TLC plate, followed by extraction from the silica gel by the method of Bligh and Dyer. The extraction was conducted in the presence of BHT (0.001%) in an N_2 gas-sealed tube. Monoacylglycerols were further purified by TLC and developed with petroleum ether/diethyl ether/acetic acid (20:80:1, v/v). The purified monoacylglycerols were converted to their 1-anthroyl derivatives and then analyzed as described above.

2.4. GC-MS analysis of trimethylsilyl (TMS) derivatives of monoacylglycerols obtained from rat brain

Monoacylglycerols obtained from rat brain (total 23 g) were purified by silicic acid column chromatography, and then by TLC and developed with petroleum ether/diethyl ether/acetic acid (20:80:1, v/v). Monoacylglycerols were further purified by TLC and developed with the same solvent system twice. The purified monoacylglycerols were converted to TMS derivatives by treatment with *N,O*-bis-trifluorotrimethylsilylacetamide (Wako Pure Chem. Ind., Osaka, Japan) at 30°C for 30 min. The resultant TMS derivatives of monoacylglycerols were then subjected to GC-MS analysis. Electron impact (70 eV) mass spectra of TMS derivatives of the monoacylglycerols were obtained using a JEOL JMS-SX102A mass spectrometer (accelerating voltage, 10 kV; ionizing current, 300 μA) coupled with a gas chromatograph equipped with a fused silica column (J&W Scientific, DB-1701, 30 m×0.25 mm I.D., 0.25 μm thickness). The column temperature was increased from 200°C to 280°C at the rate of 10°C/min. The temperature for the injection port, interface and ion source was 250°C, respectively.

2.5. Generation of 2-arachidonoylglycerol in a rat brain homogenate

Brains were taken from Wistar male rats immediately after decapitation. The brains were homogenized in 10 volumes of 0.32 M sucrose/5 mM TES buffer (pH 7.4) containing 0.5 mM EDTA and 1 mM DFP using a Potter-Elvehjem glass-Teflon homogenizer. The protein content was determined by the method of Lowry et al. [23]. Each brain homogenate was diluted with 12 volumes of 20 mM TES buffer (pH 7.4) containing 122 mM NaCl, 3.1 mM KCl, 0.4 mM KH_2PO_4, 1 mM $NaHCO_3$, 1.2 mM $MgSO_4$, 1.3 mM $CaCl_2$, 10 mM glucose and 1 mM DFP, which was added to block monoacylglycerol lipase activity, to a final concentration of 5 mg protein/5 ml, followed by incubation at 37°C for 5 min. In some cases, 2.5 mM EGTA was added instead of 1.3 mM $CaCl_2$. After the incubation, the reaction was stopped by the addition of chloroform and methanol. Total lipids were extracted by the method of Bligh and Dyer, and monoacylglycerols were purified and analyzed as dianthroyl derivatives using 2-heptadecanoylglycerol as an internal standard as described above.

3. Results

We developed a new method for the separation of various species of dianthroyl derivatives of monoacylglycerols on reverse-phase HPLC. Various species of 1(3)-monoacylglycerols and 2-monoacylglycerols were separated well from each other with this HPLC method, although the separation of myristic acid-containing and palmitoleic acid-containing species, and that of palmitic acid-containing and oleic acid- or *cis*-vaccenic acid-containing species were not satisfactory, at least under the present experimental conditions (data not shown). We confirmed that the fluorescence intensity increased almost linearly, at least from 10 pmol to 3 nmol of 1(3)- or 2-monoacylglycerols (data not shown). We also confirmed that the fluorescence intensities of the same amounts of dianthroyl derivatives of 2-arachidonoylglycerol, 1(3)-arachidonoylglycerol, 2-palmitoylglycerol and 1(3)-palmitoylglycerol were not so different, at least in the range described above (data not shown). Employing this novel analytical method, we determined the levels of monoacylglycerols in various rat tissues.

Table 1 summarizes the results of such analyses. We found that various rat tissues contain substantial amounts of monoacylglycerols. The highest value was noted in brain. Noticeably, brain contains a high amount of 2-arachidonoylglycerol besides a lower amount of 1(3)-arachidonoylglycerol, the level of total arachidonoylglycerol being 4.75 nmol/g tissue and

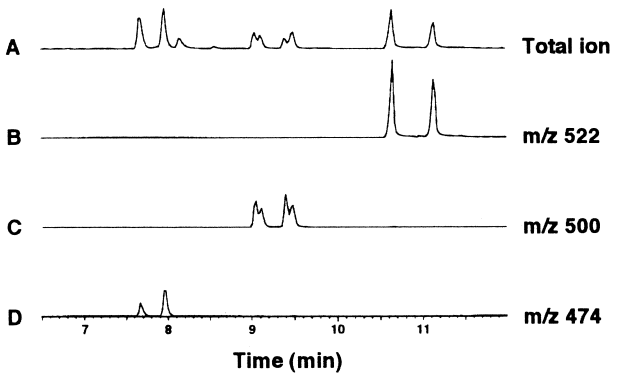

Fig. 1. Mass chromatograms ($[M]^+$) of TMS derivatives of monoacylglycerols obtained from rat brain by GC-MS. A: Total ion. B: *m/z* 522 (2-arachidonoylglycerol (retention time, 10.59 min) and 1(3)-arachidonoylglycerol (retention time, 11.11 min)). C: *m/z* 500 (2-oleoylglycerol (retention time, 9.03 min), 2-*cis*-vaccenoylglycerol (retention time, 9.14 min), 1(3)-oleoylglycerol (retention time, 9.38 min), and 1(3)-*cis*-vaccenoylglycerol (retention time, 9.46 min)). D: *m/z* 474 (2-palmitoylglycerol (retention time, 7.65 min) and 1(3)-palmitoylglycerol (retention time, 7.91 min)).

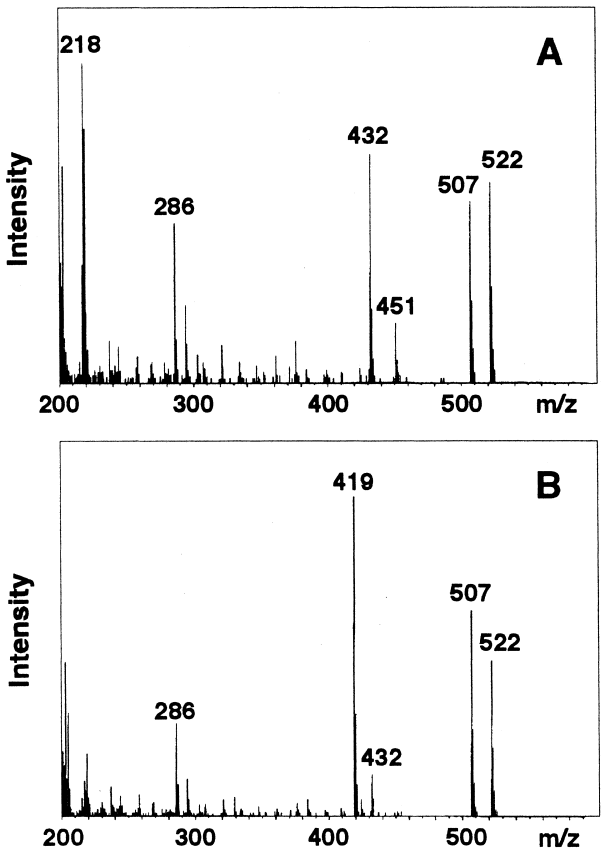

Fig. 2. Electron-impact mass spectra of TMS derivatives of arachidonoylglycerols obtained from rat brain. A: 2-Arachidonoylglycerol. B: 1(3)-Arachidonoylglycerol.

accounting for 44.1% of the total monoacylglycerols. Other tissues, such as liver, spleen, lung and kidney, also contain substantial amounts of arachidonoylglycerols. However, the amounts of arachidonoylglycerols (nmol/g tissue) in these tissues were markedly lower than those in brain, being about 29–38% of those in brain. The relative proportions of arachidonoyl species in the total monoacylglycerols in these tissues (17–27% of total monoacylglycerols) were also considerably lower than those in brain. We also found that substantial amounts of monoacylglycerols are present in blood plasma. The levels of arachidonoylglycerols in blood plasma were, however, very low, which is quite different from the levels in other tissues.

The structures of monoacylglycerols in brain were further examined by GC-MS analyses, because the separation of palmitic acid-containing, oleic acid-containing, and *cis*-vaccenic acid-containing species was not successful on HPLC analyses (Table 1). Fig. 1 illustrates mass chromatograms ($[M]^+$) of TMS derivatives of monoacylglycerols obtained from rat brain. We obtained evidence that rat brain actually contains 2-palmitoylglycerol, 1(3)-palmitoylglycerol, 2-oleoylglycerol, 1(3)-oleoylglycerol, 2-*cis*-vaccenoylglycerol and 1(3)-*cis*-vaccenoylglycerol besides 2-arachidonoylglycerol and 1(3)-arachidonoylglycerol. The electron impact mass spectra of TMS-derivative of arachidonoylglycerols in brain are shown in Fig. 2. Fig. 2A: 2-arachidonoylglycerol (*m/z* 522 for $[M]^+$, *m/z* 507 for $[M\text{-}CH_3]^+$, *m/z* 451 for $[M\text{-}71]^+$, *m/z* 432 for $[M\text{-}TMSOH]^+$, *m/z* 218 for $[M\text{-}RCOOH]^+$); Fig. 2B: 1(3)-arachidonoylglycerol (*m/z* 522 for $[M]^+$, *m/z* 507 for $[M\text{-}CH_3]^+$, *m/z* 432 for $[M\text{-}TMSOH]^+$, *m/z* 419 for $[M\text{-}TMSOCH_2]^+$).

Finally, we examined whether or not 2-arachidonoylglycerol is generated in a rat brain homogenate. As shown in Fig. 3A, we found that a large amount of 2-arachidonoylglycerol was generated in a rat brain homogenate when it was incubated in the presence of Ca^{2+} at 37°C for 5 min (6.4-fold and 98.5 pmol/mg protein increases over the level at time 0). We also found that substantial amounts of 2-palmitoylglycerol plus 2-oleoylglycerol plus 2-*cis*-vaccenoylglycerol (Fig. 3C), and 1(3)-palmitoylglycerol plus 1(3)-oleoylglycerol plus 1(3)-*cis*-vaccenoylglycerol (Fig. 3D) were generated during the incubation in the presence of Ca^{2+} (3.5- and 2.5-fold, and 58.6 and 26.2 pmol/mg protein increases over the levels at time 0, respectively), while the increases were less pronounced compared with that of 2-arachidonoylglycerol. We also confirmed that the amounts of several other minor species, such as 2-docosahexaenoylglycerol and 1(3)-docosahexaenoylglycerol, increased to some extent during the incubation in the presence of Ca^{2+} (Fig. 3E,F). Importantly, the addition of EGTA instead of $CaCl_2$ reduced the generation of 2-arachidonoyl-

Table 1
Molecular species compositions of monoacylglycerols obtained from various rat tissues

Monoacylglycerol species	Brain		Liver		Spleen		Lung		Kidney		Plasma	
	nmol/g tissue	(%)	nmol/g tissue	(%)	nmol/g tissue	(%)	nmol/g tissue	(%)	nmol/g tissue	(%)	nmol/ml	(%)
1(3)-14:0+1(3)-16:1(n-7)	0.00 ± 0.00	(0.0)	0.03 ± 0.06	(0.4)	0.00 ± 0.00	(0.0)	0.04 ± 0.08	(0.5)	0.01 ± 0.02	(0.2)	0.012 ± 0.008	(0.7)
2-14:0+2-16:1(n-7)+ 1(3)-18:2(n-6)	0.01 ± 0.01	(0.1)	0.69 ± 1.08	(8.6)	0.32 ± 0.16	(4.7)	0.34 ± 0.32	(4.3)	0.12 ± 0.11	(2.3)	0.258 ± 0.147	(15.7)
1(3)-16:0+1(3)-18:1 (n-7,n-9)	1.64 ± 0.21	(15.3)	2.08 ± 1.38	(26.0)	1.25 ± 0.15	(18.4)	3.90 ± 1.45	(49.4)	1.86 ± 0.74	(34.9)	0.288 ± 0.118	(17.5)
2-16:0+2-18:1(n-7,n-9)	3.66 ± 0.34	(34.0)	1.66 ± 0.92	(20.8)	2.50 ± 1.13	(36.8)	1.53 ± 0.40	(19.4)	1.44 ± 0.18	(27.0)	0.540 ± 0.212	(32.8)
1(3)-18:0	0.18 ± 0.23	(1.7)	0.14 ± 0.25	(1.8)	0.27 ± 0.05	(4.0)	0.14 ± 0.31	(1.8)	0.08 ± 0.11	(1.5)	0.034 ± 0.076	(2.1)
2-18:2(n-6)	0.05 ± 0.04	(0.5)	1.27 ± 1.24	(15.9)	0.60 ± 0.18	(8.8)	0.46 ± 0.37	(5.8)	0.26 ± 0.10	(4.9)	0.500 ± 0.250	(30.3)
1(3)-20:4(n-6)	1.39 ± 0.49	(12.9)	0.55 ± 0.56	(6.9)	0.63 ± 0.49	(9.3)	0.59 ± 0.30	(7.5)	0.48 ± 0.20	(9.0)	0.004 ± 0.005	(0.2)
2-20:4(n-6)	3.36 ± 1.34	(31.2)	1.15 ± 0.45	(14.4)	1.17 ± 0.55	(17.2)	0.78 ± 0.23	(9.9)	0.98 ± 0.46	(18.4)	0.012 ± 0.004	(0.7)
1(3)-22:6(n-3)	0.21 ± 0.16	(2.0)	0.17 ± 0.20	(2.1)	0.03 ± 0.03	(0.4)	0.07 ± 0.06	(0.9)	0.04 ± 0.03	(0.7)	0.000 ± 0.000	(0.0)
2-22:6(n-3)	0.25 ± 0.17	(2.3)	0.25 ± 0.12	(3.1)	0.03 ± 0.02	(0.4)	0.04 ± 0.03	(0.5)	0.06 ± 0.03	(1.1)	0.000 ± 0.000	(0.0)
Total	10.75 ± 1.87	(100.0)	7.99 ± 5.37	(100.0)	6.80 ± 1.05	(100.0)	7.89 ± 2.24	(100.0)	5.33 ± 1.29	(100.0)	1.648 ± 0.701	(100.0)

Monoacylglycerols were analyzed as dianthroyl derivatives as described in Section 2. The data are the means ± S.D. of five determinations.

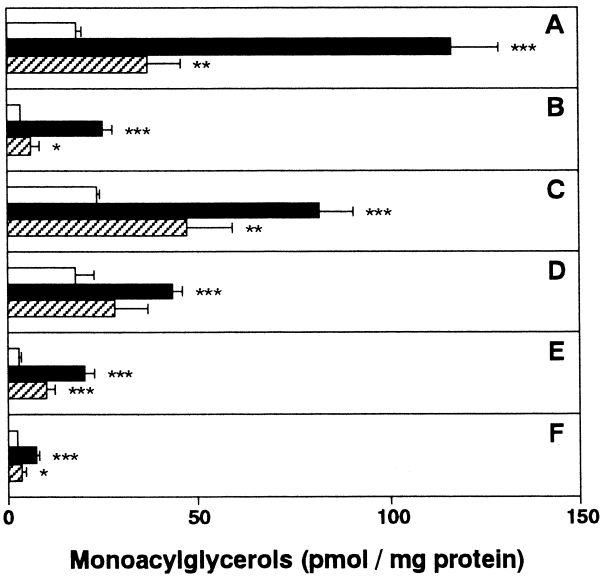

Fig. 3. Effects of Ca^{2+} on the generation of 2-arachidonoylglycerol and other molecular species of monoacylglycerols in a rat brain homogenate. A: 2-Arachidonoylglycerol. B: 1(3)-Arachidonoylglycerol. C: 2-Palmitoylglycerol plus 2-oleoylglycerol plus 2-*cis*-vaccenoylglycerol. D: 1(3)-Palmitoylglycerol plus 1(3)-oleoylglycerol plus 1(3)-*cis*-vaccenoylglycerol. E: 2-Docosahexaenoylglycerol. F: 1(3)-Docosahexaenoylglycerol. Open bars, before incubation; closed bars, after 5 min incubation in the presence of 1.3 mM $CaCl_2$; hatched bars, after 5 min incubation in the presence of 2.5 mM EGTA. The data are the means ± S.D. of four determinations. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (compared to the control (before incubation)) (Student's *t*-test).

glycerol markedly (Fig. 3A). However, noticeably, the generation of 2-arachidonoylglycerol was not totally abolished even in the presence of EGTA, suggesting that a mechanism for Ca^{2+}-independent generation of 2-arachidonoylglycerol exists besides that for Ca^{2+}-dependent generation in this tissue.

4. Discussion

This is the first detailed comparative study on the molecular species of monoacylglycerols in various mammalian tissues. The results obtained here clearly indicate that 2-arachidonoylglycerol is one of the major species of monoacylglycerols in several rat tissues, especially the brain. In a previous study, we found that rat brain contains a high level of arachidonoylglycerol [16], yet no information has been presented so far concerning other tissues. Stella et al. [21] also demonstrated that 2-arachidonoylglycerol is present in rat brain, while they did not describe either other molecular species in brain or monoacylglycerols in other tissues. Therefore, the present data concerning the molecular species compositions of monoacylglycerols in various rat tissues are of importance, highlighting the possible physiological significance of 2-arachidonoylglycerol in mammalian tissues.

Among the data shown here, the abundance of 2-arachidonoylglycerol in the monoacylglycerol fraction in brain (Table 1) is particularly noteworthy in view of the fact that various species of monoacylglycerols other than 2-arachidonoylglycerol do not exhibit appreciable cannabimimetic activity toward neuronal cells [18], and in view of the fact that the cannabinoid receptor is highly abundant in the brain [24]. Indeed, the level of the cannabinoid receptor(s) in the brain is known to be almost the same as that of glutamic acid receptors or γ-aminobutyric acid (GABA) receptors [24], suggesting that the endogenous cannabinoid receptor ligand(s) is a rather common molecule and is present abundantly in this tissue. Apparently, 2-arachidonoylglycerol, but not anandamide, fulfills this requirement. Possibly, 2-arachidonoylglycerol is a physiologically important molecule, like glutamic acid or GABA, in the brain. It should also be noted that 2-arachidonoylglycerol is present not only in brain but also in spleen, kidney and lung (Table 1). Cannabinoid receptors have been reported to exist in these tissues besides in brain [24–26]; 2-arachidonoylglycerol may play some important physiological roles in these tissues as well.

Obviously, GC-MS analysis is essential and indispensable for verifying the structures of monoacylglycerols. Several investigators have developed methods for GC-MS analyses of monoacylglycerols [27–30]. However, not much attention has been directed toward monoacylglycerols actually present in mammalian tissues. In a recent report, Stella et al. [21] presented the GC-MS spectrum of the TMS derivative of 2-arachidonoylglycerol obtained from rat brain, although the mass spectra for other species of monoacylglycerols were not given. They attributed $[M\text{-}71]^+$ to the loss of the pentyl radical. However, we detected $[M\text{-}71]^+$ in other species of 2-monoacylglycerols, such as 2-palmitoylglycerol, similar to 2-arachidonoylglycerol (data not shown). It is possible, therefore, that the formation of $[M\text{-}71]^+$ is not due to cleavage at the double bond. We assume that the structure of $[M\text{-}71]^+$ is $[RCOH{+}O\text{-}Si(CH_3)_2{+}O\text{-}Si(CH_3)_3]^+$ based on the results of elementary analyses of $[M\text{-}71]^+$ involving high resolution mass spectrometry (Tokumura, A. and Sugiura, T., unpublished results).

One of the most important findings shown here is the explosive generation of 2-arachidonoylglycerol in a rat brain homogenate during incubation, especially in the presence of Ca^{2+} (Fig. 3). This provides clear evidence that rat brain contains both the substrates and enzyme activities responsible for the generation of 2-arachidonoylglycerol. We assume that most of 1(3)-arachidonoylglycerol was formed from 2-arachidonoylglycerol by isomerization. It should be noted that the amount of 2-arachidonoylglycerol generated upon incubation in the presence of Ca^{2+} exceeded those of other species of monoacylglycerols (Fig. 3), indicating that Ca^{2+} stimulated the generation of 2-arachidonoylglycerol preferentially. Calcium ionophore-dependent generation of 2-arachidonoylglycerol in cultured neuronal cells was recently reported by several investigators [21,31]. Importantly, the generation of 2-arachidonoylglycerol proceeded to some extent even in the presence of EGTA (Fig. 3). Possible reasons for this may be as follows: (i) There are at least two separate pathways (Ca^{2+}-dependent and -independent ones) responsible for the generation of 2-arachidonoylglycerol. (ii) The enzyme activities responsible for the generation of 2-arachidonoylglycerol do not require the presence of Ca^{2+} but are stimulated by Ca^{2+}. Previously, we pointed out that 2-arachidonoylglycerol can be formed through several metabolic pathways, such as the hydrolysis of arachidonic acid-containing diacylglycerol, and the hydrolysis of arachidonic acid-containing 2-acyl lysophospholipids, derived from membrane phospholipids, by specific phospholipase C [16]. It is noteworthy that some of the enzyme activities involved in these pathways are known to be regulated by

Ca^{2+}. In any case, the mechanisms underlying the generation of 2-arachidonoylglycerol appear to be rather complicated, and further detailed studies are necessary to elucidate the mechanisms underlying the Ca^{2+}-dependent and -independent generation of 2-arachidonoylglycerol in this tissue.

Ca^{2+}-dependent or -stimulated generation of 2-arachidonoylglycerol is particularly noticeable in view of the fact that various types of neurotransmitters such as glutamic acid induce elevation of the intracellular free Ca^{2+} concentration in postsynapses. We previously demonstrated that 2-arachidonoylglycerol reduces depolarization-induced elevation of the intracellular free Ca^{2+} concentration in NG108-15 cells [32]. Stella et al. [21] also demonstrated that 2-arachidonoylglycerol reduces LTP in rat hippocampal slices, in which elevation of the postsynaptic free Ca^{2+} concentration is crucially important. Thus, 2-arachidonoylglycerol generated by activated neuronal cells may play an important role in stabilizing these cells following stimulation, leading to negative feedback control of neurotransmission. Such a mechanism should be of great physiological significance, because sustained elevation of Ca^{2+} is deleterious and toxic to neuronal cells. So far, not much attention has been paid to the physiological significance of monoacylglycerols. The physiological roles, dynamics and metabolism of 2-arachidonoylglycerol, a cannabimimetic monoacylglycerol, in the nervous system as well as in other systems will be clarified in the near future.

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