An acid amidase hydrolyzing anandamide as an endogenous ligand for cannabinoid receptors

Natsuo Ueda, Kenji Yamanaka, Yuka Terasawa, Shozo Yamamoto*

Department of Biochemistry, Tokushima University, School of Medicine, Kuramoto-cho, Tokushima 770-8503, Japan

Received 24 May 1999; received in revised form 9 June 1999

Abstract Anandamide loses its cannabimimetic activities upon hydrolysis to arachidonic acid and ethanolamine. So far the anandamide hydrolyzing activity widely distributed in mammalian organs has been attributed exclusively to an enzyme referred to as anandamide amidohydrolase with an optimum pH around 9. We found another enzyme hydrolyzing anandamide in a human megakaryoblastic cell line (CMK). The enzyme present in the 12 000 × *g* pellet of the cell homogenate was solubilized by freeze-thaw. The solubilized enzyme showed an optimal pH around 5, and was almost inactive at alkaline pH. The enzyme activity was increased by the addition of dithiothreitol. In contrast, anandamide amidohydrolase of RBL-1 cells was mostly insoluble even after freeze-thaw, showed an optimal pH at 9, and was not affected by dithiothreitol. Furthermore, the enzyme of CMK cells was much less sensitive to phenylmethylsulfonyl fluoride and methyl arachidonoyl fluorophosphonate potently inhibiting anandamide amidohydrolase, and effectively hydrolyzed palmitoylethanolamide, which was a poor substrate for anandamide amidohydrolase. Thus, the enzyme of CMK cells is distinguishable from anandamide amidohydrolase.

Key words: Cannabinoid; Anandamide; Palmitoylethanolamide; Fatty acid amide hydrolase; Megakaryoblastic cell

1. Introduction

Anandamide (arachidonoylethanolamide) was discovered as an endogenous ligand for cannabinoid receptors [1], and reported to show a variety of cannabimimetic activities [2–4]. The activities are lost when anandamide is hydrolyzed to arachidonic acid and ethanolamine [5]. The enzyme hydrolyzing anandamide has been referred to as *N*-acylethanolamine amidohydrolase [6], anandamide amidase [5], anandamide amidohydrolase [7,8], or fatty acid amide hydrolase (FAAH) [9]. However, these enzymes are now thought to be identical [4]. In this article, we will refer to the enzyme as anandamide amidohydrolase. Recently cDNAs for this enzyme were cloned from the liver of rat, mouse and human [9,10].

The anandamide hydrolyzing activities found in various mammalian organs and cell lines have been attributed exclusively to anandamide amidohydrolase [11,12]. Therefore, specific inhibitors for the enzyme are expected to be useful for the study on physiological significance of anandamide [13]. Although some differences in the catalytic properties were described between the enzymes of $N_{18}TG_2$ neuronal cells and rat basophilic leukemia (RBL) cells [14], the presence of isozymes is still unclarified.

CMK cell is a human megakaryoblastic cell line established from the peripheral blood of an acute megakaryoblastic leukemia patient with Down syndrome [15,16]. In this study, we report an anandamide hydrolyzing enzyme in CMK cells which is clearly distinct from anandamide amidohydrolase in terms of catalytic properties.

2. Materials and methods

2.1. Materials

[1-^{14}C]arachidonic acid was purchased from Amersham International (Amersham, UK), [1-^{14}C]palmitic acid from Du Pont NEN (Boston, MA, USA), arachidonic and palmitic acids from Nu-Chek-Prep (Elysian, MN, USA), anandamide, palmitoylethanolamide and methyl arachidonoyl fluorophosphonate (MAFP) from Cayman Chemical Company (Ann Arbor, MI, USA), phenylmethylsulfonyl fluoride (PMSF) from Sigma (St. Louis, MO, USA), dithiothreitol (DTT) from Wako Pure Chemical Company (Osaka, Japan), protein assay dye reagent concentrate from Bio-Rad (Hercules, CA, USA), and precoated silica gel 60 F254 glass plates for thin-layer chromatography (TLC) (20 cm × 20 cm, 0.25 mm thickness) from Merck (Darmstadt, Germany). [Arachidonoyl-1-^{14}C]anandamide and [palmitoyl-1-^{14}C]palmitoylethanolamide were chemically prepared from ethanolamine and [1-^{14}C]arachidonic acid or [1-^{14}C]palmitic acid [17]. [1-^{14}C]oleamide [18] and 2-[1-^{14}C]arachidonoyl-*sn*-glycerol [19] were prepared as described previously. Fetal calf serum was purchased from PAA (Linz, Austria) and RPMI 1640 and Dulbecco's modified Eagle's medium from Gibco BRL (Gaithersburg, MD, USA). CMK cells were kindly provided by Dr. Eiji Shimizu of the Third Department of Internal Medicine of our school with a permission of Dr. Takeyuki Sato of Chiba University, and RBL-1 cells by Dr. Shuh Narumiya of Kyoto University.

2.2. Cell culture and enzyme preparation

CMK cells or RBL-1 cells (3×10^6 cells/dish) were plated in a 150 mm plastic dish with 30 ml of medium. The medium used for CMK cells was RPMI 1640 containing 10% fetal calf serum, 2 g/l of sodium bicarbonate and 50 mg/l of streptomycin sulfate, and that for RBL-1 cells was Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 3.7 g/l of sodium bicarbonate, 100 mg/l of streptomycin sulfate and 10^5 Units/l of penicillin G. The dish was placed in a humidified 7% CO_2/93% air incubator at 37°C. The cells were subcultured every 3 days.

CMK or RBL-1 cells were harvested and suspended in phosphate-buffered saline at pH 7.4 (PBS) at a density of 1×10^7 cells/ml. The following procedures were carried out at 4°C. The cells were subjected to sonic disruption three times for 15 s. The homogenate of CMK cells was centrifuged at 800 × *g* for 15 min and at 12 000 × *g* for 30 min, successively. The 12 000 × *g* pellet was suspended in PBS and subjected to freeze-thaw twice. The sample was centrifuged again at 12 000 × *g* for 30 min. The resultant supernatant was designated as the CMK enzyme and used in the assays of Figs. 1–4. In the case of RBL-1 cells, the homogenate was freeze-thawed twice, and centrifuged at 105 000 × *g* for 40 min. The resultant pellet was suspended in PBS, and used as the RBL-1 enzyme in the assays of Figs. 1–4. All the samples thus prepared were stored at −80°C until use. Protein con-

*Corresponding author. Fax: +81 (88) 633-6409.

PII: S0014-5793(99)00820-0

centration was determined by the method of Bradford [20] with bovine serum albumin as a standard.

2.3. Enzyme assay

The enzyme was incubated with 100 μM [^{14}C]anandamide (10000 cpm in 5 μl of dimethyl sulfoxide) at 37°C for 30 min in 100 μl of 50 mM citrate-sodium phosphate (pH 5.0) containing 3 mM DTT. Termination of the reaction, separation of the product by TLC, and quantification of the radioactivity were carried out as described previously [8]. Assays were performed in triplicate.

For the enzyme assays using whole cells, the cells (1×10^6) were suspended in 0.5 ml of serum-free RPMI 1640 medium, and were incubated with 10 μM [^{14}C]anandamide (10000 cpm in 5 μl of ethanol) for different time periods in a humidified 7% CO_2/93% air incubator at 37°C. The medium was then acidified, and lipids were extracted from the medium containing the cells with the combination of 1 ml of chloroform and 0.5 ml of methanol. The extract was evaporated, dissolved in diethyl ether and subjected to TLC.

3. Results

Human megakaryoblastic cells (CMK) were subjected to sonic disruption, and various subcellular fractions were prepared by sequential centrifugation. Each fraction was incubated with ^{14}C-labelled anandamide, and the produced [^{14}C]arachidonic acid was determined. When the incubation was performed at pH 5, approximately 43%, 12% and 3% of the total activity of the whole homogenate were recovered in the $12000\times g$ pellet (mitochondrial-lysosomal fraction), the $800\times g$ pellet (nuclear fraction) and the $105000\times g$ pellet (microsomal fraction), respectively. As shown in Table 1, the specific activity of the anandamide hydrolysis was the highest in the $12000\times g$ pellet followed by the $800\times g$ pellet and the $105000\times g$ pellet. The $105000\times g$ supernatant (cytosol) did not hydrolyze anandamide. The enzyme activity of the $12000\times g$ pellet was solubilized in a yield of 92% by freeze-thaw even in the absence of detergent. The soluble enzyme showed a specific activity of 0.84 nmol/min/mg protein which was about 8-fold higher than that of the homogenate. Interestingly, the enzyme activity of each fraction was hardly detectable at pH 9 (Table 1).

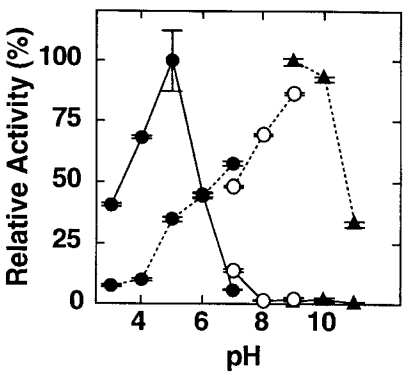

Fig. 1. pH-dependence of the anandamide hydrolysis by the CMK enzyme and the RBL-1 enzyme. The CMK enzyme (solid line, 40 μg protein) and the RBL-1 enzyme (broken line, 80 μg protein) were allowed to react with 100 μM [^{14}C]anandamide at the indicated pH. pH was adjusted with the following buffers (50 mM): citrate-Na_2HPO_4 (closed circles), Tris-HCl (open circles), Na_2CO_3-$NaHCO_3$ (closed triangles). The highest activity was expressed as 100%; the CMK enzyme (pH 5.0), 0.80 nmol/min/mg protein; RBL-1 enzyme (pH 9.0), 0.84 nmol/min/mg protein. Mean values ± S.D. are shown ($n=3$).

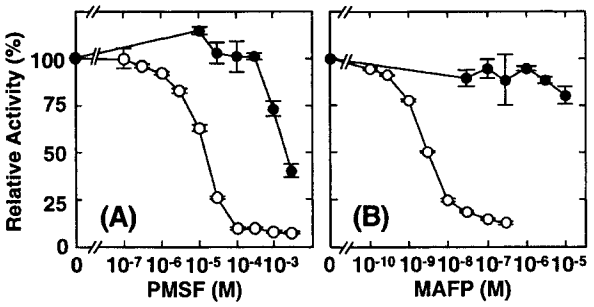

Fig. 2. The inhibitory effect of PMSF and MAFP on the anandamide hydrolysis. The CMK enzyme (closed circles, 40 μg protein) and the RBL-1 enzyme (open circles, 140 μg protein) were allowed to react with 100 μM [^{14}C]anandamide at pH 5.0 in the presence of the indicated concentrations of PMSF (A) or MAFP (B) dissolved in 5 μl of dimethyl sulfoxide. The enzyme was preincubated with the inhibitors at 37°C for 5 min before anandamide was added. DTT at 3 mM was included in the reaction mixture. The activity in the absence of the inhibitors was expressed as 100%; the CMK enzyme, 0.89 (A) or 0.94 (B) nmol/min/mg protein; the RBL-1 enzyme, 0.24 (A) or 0.23 (B) nmol/min/mg protein. Mean values ± S.D. are shown ($n=3$).

The same procedure was applied to rat basophilic leukemia (RBL-1) cells which were previously demonstrated to express anandamide amidohydrolase [12,14]. The specific anandamide hydrolyzing activity was the highest in the $12000\times g$ pellet, and the reaction at pH 9 proceeded about 3-fold faster than that at pH 5 (Table 1). The enzyme was solubilized in a low yield from the $12000\times g$ pellet of RBL-1 cells by freeze-thaw.

When the anandamide hydrolase activity of the solubilized CMK enzyme was examined between pH 3 and 11, the enzyme was active at acidic pH, and the optimal pH was around 5 (Fig. 1). The activity was hardly detected at alkaline pH. In contrast, the RBL-1 enzyme showed the highest activity at pH 9 under the same assay conditions.

PMSF [5,8] and MAFP [21,22] were reported to inhibit anandamide amidohydrolase. As shown in Fig. 2A, PMSF inhibited the RBL-1 enzyme dose-dependently with an IC_{50} of about 20 μM, while the CMK enzyme was inhibited with an IC_{50} of as high as about 3 mM. Similarly, MAFP, which

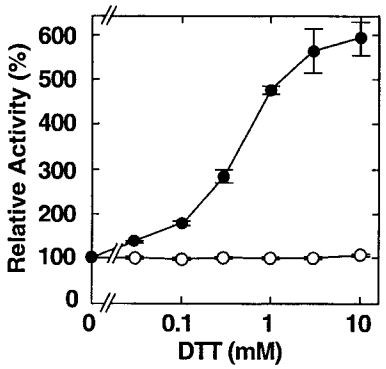

Fig. 3. The stimulatory effect of DTT on the anandamide hydrolysis. The CMK enzyme (closed circles, 40 μg protein) and the RBL-1 enzyme (open circles, 140 μg protein) were allowed to react with 100 μM [^{14}C]anandamide at pH 5.0 in the presence of the indicated concentrations of DTT. The activity in the absence of DTT was expressed as 100%; the CMK enzyme, 0.17 nmol/min/mg protein; the RBL-1 enzyme, 0.17 nmol/min/mg protein. Mean values ± S.D. are shown ($n=3$).

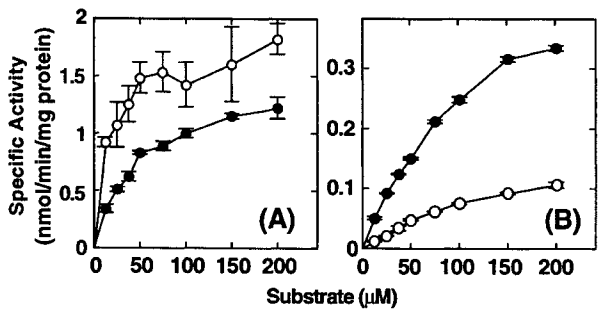

Fig. 4. Hydrolysis of palmitoylethanolamide by the CMK enzyme and the RBL-1 enzyme. The CMK enzyme (A, 15–30 μg protein) and the RBL-1 enzyme (B, 150 μg protein) were incubated at pH 5.0 with the indicated concentrations of [^{14}C]anandamide (closed circles) or [^{14}C]palmitoylethanolamide (open circles). Mean values ± S.D. are shown ($n = 3$).

potently inhibited the RBL-1 enzyme (IC_{50}, 3 nM), hardly inhibited the CMK enzyme up to 10 μM (Fig. 2B). Furthermore, the added DTT 6-fold increased the anandamide hydrolyzing activity of the CMK enzyme as the concentration of the added DTT was raised up to 10 mM, whereas the RBL-1 enzyme was not affected by DTT at as high as 10 mM (Fig. 3).

We also examined the substrate specificity of the two enzyme preparations. At pH 5 the RBL-1 enzyme hydrolyzed palmitoylethanolamide at only one third the rate of the anandamide hydrolysis at 200 μM (Fig. 4B). In contrast, palmitoylethanolamide was 1.5-fold more active than anandamide with the CMK enzyme (Fig. 4A). Apparent K_m values of the CMK enzyme for anandamide and palmitoylethanolamide were approximately 40 and 10 μM, respectively. Furthermore, the CMK enzyme hydrolyzed oleamide (an endogenous sleep inducer [18]) and 2-arachidonoylglycerol (another endogenous cannabinoid receptor ligand [23,24]), which were reported to be substrates for anandamide amidohydrolase [9,19], at 12% and 740% of the rate of the anandamide hydrolysis as compared at 100 μM concentration.

In order to examine whether or not the anandamide hydrolyzing enzyme actually worked in CMK cells, we incubated the whole cells with [^{14}C]anandamide. Lipids were then extracted and separated by TLC. Anandamide decreased time-dependently while the radioactivity corresponding to non-polar lipids and polar lipids increased. After 4 h 85% of anandamide disappeared. About 3% of the total radioactivity was detected as free arachidonic acid between 0.5 and 4 h. When [^{14}C]anandamide was incubated in a cell-free medium for 4 h, 98% of the added anandamide remained unchanged. These results suggested uptake and hydrolysis of anandamide in CMK cells, followed by incorporation of the generated free arachidonic acid to neutral lipids and phospholipids.

4. Discussion

Anandamide amidohydrolase has been isolated from brain, liver and other mammalian tissues, and its catalytic properties were investigated extensively [5–8,11,12,14,25,26]. cDNAs for the enzyme were cloned [9,10], and the recombinant enzymes were also characterized [9,10,19,27,28]. Thus, the following properties were confirmed with native and recombinant enzymes: (1) The enzyme is membrane-bound, and detergents are required for its solubilization from the membrane [6,8]. (2) The optimal pH is around 9 [6,8,12,14,19,25,26]. Recently, the enzyme isolated from human brain was also reported to have an optimal pH at 9.0 [29]. (3) The enzyme is inhibited by serine protease inhibitors such as PMSF [5,8,19]. (4) MAFP is an extremely potent inhibitor for the enzyme [19,21,22]. (5) Palmitoylethanolamide, which does not bind to cannabinoid receptors [30,31] but shows anti-inflammatory effects [32], is a less active substrate than anandamide [7,8,14].

We found now an anandamide hydrolyzing activity in human megakaryoblastic cells (CMK). The enzyme showed several unique properties different from those of the known anandamide amidohydrolase: (1) The enzyme was solubilized from the 12 000 × *g* pellet by freeze-thaw in the absence of detergent. (2) The optimal pH was around 5. (3) The enzyme was much less sensitive to PMSF and MAFP than anandamide amidohydrolase. (4) The enzyme activity was several-fold increased by DTT, while the enzyme of RBL-1 cells was not affected by DTT. Earlier it was reported that the anandamide hydrolyzing activity of neuroblastoma cells was 1.3-fold increased by 1 mM DTT [26]. (5) The enzyme of CMK cells hydrolyzed palmitoylethanolamide faster than anandamide. However, we can not rule out the possibility that the two substrates are separately hydrolyzed by different enzymes with different specificities contained in our enzyme preparation.

In the present work the enzyme of RBL-1 cells was also assayed under the same conditions, and showed properties

Table 1
Subcellular distribution of the anandamide hydrolase activity in CMK cells and RBL-1 cells

	Specific anandamide hydrolase activity (nmol/min/mg protein)			
	Cell line CMK		Cell line RBL-1	
	pH 5.0	pH 9.0	pH 5.0	pH 9.0
Homogenate	0.11 ± 0.00	0.01 ± 0.00	0.13 ± 0.00	0.29 ± 0.00
800 × *g* pellet	0.12 ± 0.00	0.01 ± 0.00	0.16 ± 0.00	0.39 ± 0.02
12 000 × *g* pellet	0.28 ± 0.01	n.d.[b]	0.40 ± 0.00	1.10 ± 0.06
105 000 × *g* pellet	0.02 ± 0.00	n.d.	0.19 ± 0.00	0.52 ± 0.01
105 000 × *g* supernatant	n.d.	n.d.	0.03 ± 0.00	0.04 ± 0.01
Soluble fraction after freeze-thaw[a]	0.84 ± 0.06	0.02 ± 0.00	0.24 ± 0.02	0.19 ± 0.02
Insoluble fraction after freeze-thaw[a]	0.19 ± 0.01	0.01 ± 0.00	0.69 ± 0.00	2.03 ± 0.04

Each enzyme preparation (15–150 μg protein) was allowed to react with 100 μM [^{14}C]anandamide in 50 mM citrate-Na_2HPO_4 (pH 5.0) or 50 mM Tris-HCl (pH 9.0). The values shown are means ± S.D. ($n = 3$).
[a]The 12 000 × *g* pellet was suspended in PBS, freeze-thawed twice, and centrifuged again at 12 000 × *g* for 30 min.
[b]n.d., below detection limit.

typical of anandamide amidohydrolase as described before [14]. Thus, our results strongly suggest that the enzyme of CMK cells is an enzyme distinct from the known anandamide amidohydrolase. Especially we should note that the enzyme is less sensitive to PMSF since the compound has been used to protect anandamide from the enzymatic inactivation in pharmacological and behavioral experiments [33–36]. We used only CMK cells as a source of the new enzyme, but further studies on the tissue distribution of the enzyme may lead to elucidation of its physiological role. Previously it was suggested that the particulate fraction of rat brain contained a non-specific short chain amidohydrolase, which hydrolyzed *N*-palmitoylethanolamide [7]. This brain enzyme may be related to our enzyme.

The localization of the CMK enzyme in the $12\,000 \times g$ pellet (mitochondrial-lysosomal fraction) and its optimal pH around 5 suggest that the enzyme is one of lysosomal hydrolases. Lysosomes contain a large number of hydrolases which are active around pH 5, and some of them are lipid hydrolyzing enzymes such as acid lipase, phospholipases A_1 and A_2, sphingomyelinase and ceramidase [37]. Further investigation is now being undertaken to find out whether or not the anandamide hydrolase activity of CMK cells is attributed to an already known lysosomal hydrolase.

Acknowledgements: This work was supported by grants-in-aid for scientific research from the Ministry of Education, Science, Sports and Culture of Japan, Human Frontier Science Program, the Japanese Foundation of Metabolism and Disease, Uehara Memorial Foundation, the Japan Foundation for Applied Enzymology, Ono Pharmaceutical Company, Kissei Pharmaceutical Company, Sankyo Company, Japan Tobacco Incorporation, and Takeda Pharmaceutical Industry.

References

[1] Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A. and Mechoulam, R. (1992) Science 258, 1946–1949.
[2] Mechoulam, R., Hanus, L. and Martin, B.R. (1994) Biochem. Pharmacol. 48, 1537–1544.
[3] Pertwee, R.G. (1997) Pharmacol. Ther. 74, 129–180.
[4] Di Marzo, V. (1998) Biochim. Biophys. Acta 1392, 153–175.
[5] Deutsch, D.G. and Chin, S.A. (1993) Biochem. Pharmacol. 46, 791–796.
[6] Schmid, P.C., Zuzarte-Augustin, M.L. and Schmid, H.H.O. (1985) J. Biol. Chem. 260, 14145–14149.
[7] Desarnaud, F., Cadas, H. and Piomelli, D. (1995) J. Biol. Chem. 270, 6030–6035.
[8] Ueda, N., Kurahashi, Y., Yamamoto, S. and Tokunaga, T. (1995) J. Biol. Chem. 270, 23823–23827.
[9] Cravatt, B.F., Giang, D.K., Mayfield, S.P., Boger, D.L., Lerner, R.A. and Gilula, N.B. (1996) Nature 384, 83–87.
[10] Giang, D.K. and Cravatt, B.F. (1997) Proc. Natl. Acad. Sci. USA 94, 2238–2242.
[11] Katayama, K., Ueda, N., Kurahashi, Y., Suzuki, H., Yamamoto, S. and Kato, I. (1997) Biochim. Biophys. Acta 1347, 212–218.
[12] Bisogno, T., Katayama, K., Melck, D., Ueda, N., De Petrocellis, L., Yamamoto, S. and Di Marzo, V. (1998) Eur. J. Biochem. 254, 634–642.
[13] Bisogno, T., Melck, D., De Petrocellis, L., Bobrov, M.Yu., Gretskaya, N.M., Bezuglov, V.V., Sitachitta, N., Gerwick, W.H. and Di Marzo, V. (1998) Biochem. Biophys. Res. Commun. 248, 515–522.
[14] Bisogno, T., Maurelli, S., Melck, D., De Petrocellis, L. and Di Marzo, V. (1997) J. Biol. Chem. 272, 3315–3323.
[15] Sato, T., Fuse, A., Eguchi, M., Hayashi, Y., Ryo, R., Adachi, M., Kishimoto, Y., Teramura, M., Mizoguchi, H., Shima, Y., Komori, I., Sunami, S., Okimoto, Y. and Nakajima, H. (1989) Br. J. Haematol. 72, 184–190.
[16] Komatsu, N., Suda, T., Moroi, M., Tokuyama, N., Sakata, Y., Okada, M., Nishida, T., Hirai, Y., Sato, T., Fuse, A. and Miura, Y. (1989) Blood 74, 42–48.
[17] Ueda, N., Yamamoto, K., Yamamoto, S., Tokunaga, T., Shirakawa, E., Shinkai, H., Ogawa, M., Sato, T., Kudo, I., Inoue, K., Takizawa, H., Nagano, T., Hirobe, M., Matsuki, N. and Saito, H. (1995) Biochim. Biophys. Acta 1254, 127–134.
[18] Cravatt, B.F., Prospero-Garcia, O., Siuzdak, G., Gilula, N.B., Henriksen, S.J., Boger, D.L. and Lerner, R.A. (1995) Science 268, 1506–1509.
[19] Goparaju, S.K., Ueda, N., Yamaguchi, H. and Yamamoto, S. (1998) FEBS Lett. 422, 69–73.
[20] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[21] Deutsch, D.G., Omeir, R., Arreaza, G., Salehani, D., Prestwich, G.D., Huang, Z. and Howlett, A. (1997) Biochem. Pharmacol. 53, 255–260.
[22] De Petrocellis, L., Melck, D., Ueda, N., Maurelli, S., Kurahashi, Y., Yamamoto, S., Marino, G. and Di Marzo, V. (1997) Biochem. Biophys. Res. Commun. 231, 82–88.
[23] Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N.E., Schatz, A.R., Gopher, A., Almog, S., Martin, B.R., Compton, D.R., Pertwee, R.G., Griffin, G., Bayewitch, M., Barg, J. and Vogel, Z. (1995) Biochem. Pharmacol. 50, 83–90.
[24] Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A. and Waku, K. (1995) Biochem. Biophys. Res. Commun. 215, 89–97.
[25] Hillard, C.J., Wilkison, D.M., Edgemond, W.S. and Campbell, W.B. (1995) Biochim. Biophys. Acta 1257, 249–256.
[26] Maurelli, S., Bisogno, T., De Petrocellis, L., Di Luccia, A., Marino, G. and Di Marzo, V. (1995) FEBS Lett. 377, 82–86.
[27] Kurahashi, Y., Ueda, N., Suzuki, H., Suzuki, M. and Yamamoto, S. (1997) Biochem. Biophys. Res. Commun. 237, 512–515.
[28] Arreaza, G., Devane, W.A., Omeir, R.L., Sajnani, G., Kunz, J., Cravatt, B.F. and Deutsch, D.G. (1997) Neurosci. Lett. 234, 59–62.
[29] Maccarrone, M., van der Stelt, M., Rossi, A., Veldink, G.A., Vliegenthart, J.F.G. and Agro, A.F. (1998) J. Biol. Chem. 273, 32332–32339.
[30] Showalter, V.M., Compton, D.R., Martin, B.R. and Abood, M.E. (1996) J. Pharmacol. Exp. Ther. 278, 989–999.
[31] Sheskin, T., Hanus, L., Slager, J., Vogel, Z. and Mechoulam, R. (1997) J. Med. Chem. 40, 659–667.
[32] Schmid, H.H.O., Schmid, P.C. and Natarajan, V. (1990) Prog. Lipid Res. 29, 1–43.
[33] Childers, S.R., Sexton, T. and Roy, M.B. (1994) Biochem. Pharmacol. 47, 711–715.
[34] Pertwee, R.G., Fernando, S.R., Griffin, G., Abadji, V. and Makriyannis, A. (1995) Eur. J. Pharmacol. 272, 73–78.
[35] Adams, I.B., Ryan, W., Singer, M., Thomas, B.F., Compton, D.R., Razdan, R.K. and Martin, B.R. (1995) J. Pharmacol. Exp. Ther. 273, 1172–1181.
[36] Compton, D.R. and Martin, B.R. (1997) J. Pharmacol. Exp. Ther. 283, 1138–1143.
[37] Holtzman, E. (1976) Cell Biol. Monogr. 3, 1–27.