Pharmacological Effects in Mice of Anandamide and Its Related Fatty Acid Ethanolamides, and Enhancement of Cataleptogenic Effect of Anandamide by Phenylmethylsulfonyl Fluoride

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Anandamide (*N*-arachidonylethanolamine) and six fatty acid ethanolamides were synthesized and their pharmacological effects in mice were assessed using catalepsy, hypothermia and pentobarbital-induced sleep prolongation as indices. The effects of phenylmethylsulfonyl fluoride (PMSF) pretreatment on anandamide effects were also evaluated and discussed in relation to inhibition of anandamide amidohydrolase in mouse brain and liver. The cataleptogenic effect of anandamide ($ED_{50}=6.0$ mg/kg, i.v.) was 4 to 6 times more active than those of *N*-oleoyl- ($ED_{50}=26.5$ mg/kg, i.v.) and *N*-linoleylethanolamine ($ED_{50}=37.5$ mg/kg, i.v.), although the peak time in the effect was observed within 1 min after i.v. administration. None of the saturated fatty acid ethanolamides (*N*-myristoyl-, *N*-palmitoyl-, *N*-stearoyl- and *N*-arachidylethanolamine) showed a positive response in the cataleptogenic effect even at a dose up to 40 mg/kg i.v. Anandamide, *N*-linoleoyl-, *N*-oleoyl- and *N*-myristylethanolamine (10 mg/kg, i.v.) produced a significant hypothermia (0.19 to 0.59 °C) at 5 to 15 min after administration. The duration of the effects of these ethanolamides was also relatively short. Anandamide, *N*-linoleoyl-, *N*-oleoyl- and *N*-palmitylethanolamine (5 or 10 mg/kg, i.v.) significantly prolonged pentobarbital-induced sleeping time by 148–207% of control sleeping time. The cataleptogenic effect of anandamide was markedly potentiated by pretreatment of mice with PMSF (100 mg/kg, i.p.). The ED_{50} (mg/kg, i.v.) of anandamide was 0.48 (0.24–0.96) in PMSF-pretreated mice. The pretreatment of mice with PMSF significantly decreased the metabolic clearance rate of anandamide in microsomal fractions of liver and brain. Thus, the V_{max}/K_m values of brain and hepatic microsomes were 26 and 10%, respectively, as compared with those of control mice. The present study demonstrated that anandamide and *N*-acylethanolamines of unsaturated fatty acids exhibited cannabinoid-like effects in mice, and that anandamide amidohydrolase has an important role in the pharmacological effects of anandamide *in vivo*.

Key words anandamide; catalepsy; hypothermia; sleep prolongation; phenylmethylsulfonyl fluoride; anandamide amidohydrolase

Anandamide (*N*-arachidonylethanolamine) was isolated from porcine brain and identified as an endogenous ligand for the cannabinoid receptor.¹⁾ The amide was also isolated from calf brain and identified as a regulator of L-type calcium channels.²⁾ Fride and Mechoulam³⁾ demonstrated that anandamide produced the same pharmacological effects as those of tetrahydrocannabinol (THC), an active constituent of marijuana. Several studies also demonstrated that anandamide parallels Δ^9 -THC in competing with the binding of agonists to cannabinoid receptors^{4,5)} and inhibits adenylate cyclase.^{6,7)} Anandamide was also reported to be a potent inhibitor of gap junction conductance⁸⁾ and a vasorelaxant in the endothelium suggesting that the amide has a biologically important role in the cardiovascular system.⁹⁾

Hanus *et al.*¹⁰⁾ identified other fatty acid ethanolamides, homo- γ -linoleylethanolamine and docosatetraenylethanolamine, in porcine brain that bind the cannabinoid receptor and inhibit the electrically evoked twitch response of the mouse isolated vas deferens similarly to anandamide and cannabinoids.¹¹⁾ In *in vitro* structure-activities studies, Felder *et al.*⁷⁾ suggested that the end pentyl chain may play some role similar to the pentyl side chain of THC. Adams *et al.*¹²⁾ demonstrated that the level of saturation of the arachidonic acid moiety of anandamide was critical to receptor affinity. Pinto *et al.*⁴⁾ suggested that the bulk and length of the arachi-

donic acid moiety are important for affinity of the anandamide analogs to the central cannabinoid receptor (CB1). Wise *et al.*¹³⁾ reported that a conjugated triene derivative of anandamide bound to the cannabinoid receptor. Palmitylethanolamine has been reported to be an agonist for the peripheral cannabinoid receptor (CB2) on mast cells and may produce an antiinflammatory effect.¹⁴⁾ These data suggest that anandamide and other fatty acid ethanolamides may have some cannabimimetic effects, although more extensive pharmacological studies *in vivo* of fatty acid ethanolamides are required to give conclusive evaluation of the structural importance of anandamide.

Studies from several laboratories have shown that anandamide is mainly degraded by hydrolysis with membrane-bound enzyme, anandamide amidohydrolase.^{15–17)} Anandamide has been thought to be rapidly hydrolyzed by the *in vivo* system, since the pharmacological effects of anandamide are produced with rapid onset and a rather short duration of action.^{18,19)} (*R*)-Methanandamide, a metabolically more stable derivative of anandamide, exhibited greater pharmacological effects such as hypothermia, hypokinesia, antinociception, ring immobility in mice than those produced by anandamide.²⁰⁾ Phenylmethylsulfonyl fluoride (PMSF) was found to be a potent inhibitor of the hydrolase.^{15,17,21)} The addition of PMSF significantly decreased the K_i value

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for anandamide in competing [^3H]CP55940 binding to the cannabinoid receptor in rat brain P2 membrane preparations.¹²⁾ These data suggest that PMSF may potentiate the biological effects of anandamide *in vivo*. Recently, Compton and Martin²²⁾ reported that PMSF potentiated antinociception and immobility produced by anandamide, although PMSF itself showed some pharmacological effects at rather high doses. However, the limited data available at present with respect to *in vivo* hydrolysis of anandamide and its pharmacological effects hinders the assessment of the importance of peripheral and central anandamide amidohydrolase in mediating the biological effects of anandamide.

The present paper describes the pharmacological effects of anandamide and six fatty acid ethanolamides, and the effects of pretreatment with PMSF on the cataleptogenic effect of anandamide in relation to inhibition of anandamide amidohydrolase in mouse brain and liver.

MATERIALS AND METHODS

Pharmacological Experiments Male ddY mice weighing 20 to 30 g were used for the experiments. The animals received food and water *ad libitum*. The pharmacological experiments were carried out at ambient temperature of 22 to 24 °C.

Catalepsy The cataleptogenic effect was assessed by the simple bar test as described previously²³⁾ after i.v. injection of anandamide and other six fatty acid ethanolamides at various time intervals. Mouse front paws were placed on a horizontal bar (0.5 cm in diameter), 5–6 cm height, and the mouse was forced to stand on its hind legs. When the mouse kept the unusual position for more than 30 s, the cataleptogenic effect was regarded to be positive. ED₅₀ values of test compounds were evaluated by the method of Litchfield-Wilcoxon²⁴⁾ by using at least 3 different doses (5 to 40 mg/kg i.v.). Five groups consisting of 8 mice were used for the time course experiments at each time point (1, 5, 10, 30 and 60 min) for anandamide.

Hypothermia Rectal temperature of mice was measured with a thermistor thermometer (Natsume Seisakusho Ltd., Tokyo), recording for 30 min after i.v. injection of test compounds as described previously.²⁵⁾ The control mice received 1% Tween 80-saline only.

Pentobarbital-Induced Sleep Sodium pentobarbital (40 mg/kg, i.p.) was injected 1 min after i.v. injection of anandamide and other six fatty acid ethanolamides (5 or 10 mg/kg i.v.). The sleeping time was measured as loss of the righting reflex.²⁵⁾ Each group consisted of 8 to 25 mice.

Cataleptogenic Effect in PMSF-Treated Mice Mice were pretreated with PMSF at a dose of 100 mg/kg i.p. Mice pretreated with the dose of PMSF did not show any positive cataleptogenic effect, although Compton and Martin²²⁾ reported that PMSF reduced spontaneous activity of mice at doses greater than 100 mg/kg. Anandamide (10 mg/kg i.v.) was injected into 6 groups consisting of 8 mice each. In the time course experiments, the cataleptogenic effect was assessed at 1, 5, 10, 30, 60 and 90 min after the anandamide injection. ED₅₀ values for anandamide in PMSF-treated mice was determined by the same procedure described as above, but the dose range was 1 to 10 mg/kg i.v.

Drugs Anandamide and its related fatty acid ethanol-

amides (*N*-myristoyl-, *N*-palmitoyl-, *N*-stearoyl-, *N*-oleoyl-, *N*-linoleoyl-, *N*-arachidoyl-) were synthesized by the method described.²⁶⁾ Fatty acid chlorides were prepared by reacting free fatty acids with oxalyl chloride and then reacted with ethanolamine. The *N*-acylethanolamines synthesized were purified by column chromatography using a solvent system of methylene chloride/methanol (50:1). Sodium pentobarbital and PMSF were purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan) and Sigma Chem. Co., respectively. Sodium pentobarbital (40 mg/kg) and PMSF (100 mg/kg) were dissolved in physiological saline and olive oil, respectively, and injected i.p.

Assay of Anandamide Amidohydrolase Activity Livers and brains of mice were removed at 60 min after i.p. injection of olive oil (control) or PMSF (100 mg/kg i.p.). Hepatic and brain microsomes were prepared from control and PMSF-pretreated ddY male mice. Anandamide amidohydrolase activity was assayed as described previously.²⁷⁾ A typical incubation mixture consisted of microsomes (0.02 g liver or 0.05 g brain equivalent) and anandamide (10–230 μM) in sodium potassium phosphate buffer (pH 7.4) to make a final volume of 1 ml. The incubations were carried out at 37 °C for 20 min. The arachidonic acid formed was extracted with *n*-hexane (4 ml) after addition of 0.5 ml of 1 M KH_2PO_4 and an internal standard (methyl linolate, 10 μg). The *n*-hexane extract was methylated with diazomethane in ethyl ether, and analyzed by GC.^{21,27)}

Analysis of Data The data are expressed as means and limits of errors as standard errors for the hypothermic effect and pentobarbital-induced sleep prolongation. The ED₅₀ values and their 95% confidence limits in the cataleptogenic effects of anandamide and fatty acid ethanolamides were calculated by the method of Litchfield-Wilcoxon.²⁴⁾ The effects of anandamide and other fatty acid ethanolamides on the body temperature of mice were calculated by subtracting rectal temperature measured just before drug administration and thereafter for 30 min. The significance of difference between means was evaluated by ANOVA, and individual data were compared by F-test for the hypothermic effect and pentobarbital-induced sleep prolongation. The significance of differences in the time course experiment of cataleptogenic effect between control and PMSF-treated groups was calculated by χ^2 -test.

RESULTS

The time course in the cataleptogenic effect of anandamide is shown in Fig. 1. At the dose of 10 mg/kg i.v., anandamide exhibited a positive response in 75% of the mice at 1 min after the injection. The effect rapidly attenuated and none of the mice showed a positive response at 30 min after the injection of anandamide. Thus, the peak time in the cataleptogenic effect of anandamide was observed at 1 min after the injection. Therefore, the cataleptogenic effects of anandamide and other fatty acid ethanolamides were assessed at 1 min after their injections. The ED₅₀ (with their 95% confidence limits) of anandamide, *N*-oleoyl- and *N*-linoleylethanolamine were 6.0 (4.1–8.7), 37.5 (25.9–54.4) and 26.5 (20.7–33.9) mg/kg, respectively (Table 1). However, saturated fatty acid ethanolamides (*N*-myristoyl-, *N*-palmitoyl-, *N*-stearoyl- and *N*-arachidoylethanolamine) did not show any

Fig. 1. Time Course for the Cataleptogenic Effect of Anandamide

Five groups consisting of 8 mice each were injected with anandamide at a dose of 10 mg/kg, i.v. The cataleptogenic effect was assessed at 1, 5, 10, 30 or 60 min after the injection by a simple bar test.²³⁾ The bar test was carried out by placing the front paws of mice on a horizontal bar (0.5 cm in diameter), 5–6 cm in height, and forcing mice to stand on their hind legs. When mice maintained the unusual position for more than 30 s, the cataleptogenic effect was regarded to be positive.

Table 1. Cataleptogenic Effects for Anandamide and Six Fatty Acid Ethanolamides in Mice

Ethanolamides	Animals/group	ED ₅₀ (mg/kg, i.v.)
Anandamide	8	6.0 (4.1–8.7) ^{a)}
<i>N</i> -Myristoylethanolamine	8	No effect at 40 mg/kg
<i>N</i> -Palmitoylethanolamine	8	No effect at 40 mg/kg
<i>N</i> -Stearoylethanolamine	8	No effect at 40 mg/kg
<i>N</i> -Oleoylethanolamine	8	37.5 (25.9–54.4)
<i>N</i> -Linoleoylethanolamine	8	26.5 (20.7–33.9)
<i>N</i> -Arachidoylethanolamine	8	No effect at 40 mg/kg

The cataleptogenic effects for anandamide and other fatty acid ethanolamides were assessed by the method described previously (Yoshimura *et al.*, 1978). Dose-response curves were estimated from at least three doses of the ethanolamides. ^{a)} 95% Confidence limits.

positive cataleptogenic effect in mice even at a dose up to 40 mg/kg i.v.

The effects of anandamide and its related fatty acid ethanolamides (10 mg/kg, i.v.) on the rectal temperature of mice are illustrated in Fig. 2. Anandamide exhibited the highest potency in this parameter, while *N*-myristoyl-, *N*-oleoyl- and *N*-linoleoylethanolamine caused a significant hypothermic effect at 5 to 15 min after their injections. The maximal hypothermia produced were 0.59 ± 0.08 (anandamide, 10 min), 0.39 ± 0.07 (*N*-oleoylethanolamine, 5 min), 0.32 ± 0.07 (*N*-linoleoylethanolamine, 5 min) and 0.19 ± 0.10 °C (*N*-myristoylethanolamine, 5 min). However, their hypothermic effects lasted only for 15 min and a significant hypothermia was not observed at 30 min after the administration. *N*-Palmitoyl- and *N*-arachidoylethanolamine did not show a significant hypothermic effect at 10 mg/kg, i.v.

The effects of anandamide and other fatty acid ethanolamides on pentobarbital-induced sleeping time are shown in Table 2. Five mg/kg i.v. of anandamide and *N*-oleoylethanolamine significantly prolonged pentobarbital-induced sleeping time, although other fatty acid ethanolamides examined in the present study did not prolong significantly the sleeping time at the same dose. At 10 mg/kg, i.v., anandamide, *N*-palmitoyl-, *N*-oleoyl- and *N*-linoleoylethanolamine significantly prolonged the sleeping time by 207, 145,

Fig. 2. Time Course for the Effects of Anandamide and Fatty acid Ethanolamides on Rectal Temperature of Mice

Anandamide and other fatty acid ethanolamides were injected to each group of 10 mice at a dose of 10 mg/kg, i.v. Rectal temperature of mice was recorded just before injection and at each time point thereafter for 30 min. ○, control; ●, anandamide; □, *N*-myristoylethanolamine; ■, *N*-palmitoylethanolamine; △, *N*-oleoylethanolamine; ▲, *N*-linoleoylethanolamine; ◇, *N*-arachidoylethanolamine. Each symbol represents the mean value and standard errors. * and ** indicate the values that are significantly different from their corresponding control groups at $p < 0.05$ and $p < 0.01$, respectively, by ANOVA with F-test.

Table 2. Effects of Anandamide and Six Fatty Acid Ethanolamides on Pentobarbital-Induced Sleeping Time in Mice

Treatments	Dose (mg/kg i.v.)	Animals (n)	Sleeping time (min)
Control	—	25	17.0 ± 1.7
Anandamide	5	10	29.0 ± 4.2*
	10	10	35.2 ± 3.5**
<i>N</i> -Myristoylethanolamine	5	8	15.4 ± 2.2
	10	8	15.6 ± 2.1
<i>N</i> -Palmitoylethanolamine	5	8	23.3 ± 3.6
	10	8	24.6 ± 2.8*
<i>N</i> -Stearoylethanolamine	5	8	20.8 ± 3.5
	10	9	20.7 ± 3.5
<i>N</i> -Oleoylethanolamine	5	10	26.6 ± 3.4*
	10	10	25.2 ± 3.0*
<i>N</i> -Linoleoylethanolamine	5	10	19.7 ± 2.7
	10	10	29.2 ± 4.2**
<i>N</i> -Arachidoylethanolamine	5	8	20.7 ± 3.0
	10	9	17.9 ± 2.4

Control animals received the vehicle (1% Tween 80 in physiological saline). Anandamide and other fatty acid ethanolamides (5 or 10 mg/kg i.v.) suspended in physiological saline containing 1% Tween 80 were injected through tail vein of mice before pentobarbital injection. Sleeping times were measured following 40 mg/kg i.p. injection of pentobarbital. The data are expressed as the mean value ± standard errors. * and ** indicate the values that are significantly different from the control group at $p < 0.05$ and $p < 0.01$, respectively.

148 and 172%, respectively, as compared with the control group (17.0 min).

The time course for the cataleptogenic effect of anandamide in PMSF-treated mice is shown in Fig. 3. PMSF-pre-treatment significantly potentiated the anandamide effect at 5, 10 and 30 min after administration and prolonged the effect. The positive cataleptogenic effect in PMSF-treated group (10 mg/kg, i.v.) lasted for 60 min. The ED₅₀ value of anandamide in PMSF-treated mice was 0.48 (0.24–0.96) mg/kg i.v., indicating 13 times potentiation of the cataleptogenic effect as compared with that in control mice (Table 1). This ED₅₀ value is 6 to 7 times lower than those of THC₁ and comparable to those of 11-hydroxy-THCs, active metabolites of THC₁.²⁸⁾

Fig. 3. Time Course for the Cataleptogenic Effect of Anandamide in PMSF-Pretreated Mice

Six groups consisting of 10 mice each were injected with PMSF (100 mg/kg, i.p.). Anandamide (10 mg/kg, i.v.) was injected 60 min after the injection of PMSF. The cataleptogenic effect was assessed at 1, 5, 10, 30, 60 and 90 min after the injection of anandamide by the bar test as in Fig. 1. The data for the control group is from Fig. 1. ○, control; ●, PMSF-treated. * and ** indicate the values that are significantly different from their corresponding control groups at $p < 0.05$ and $p < 0.01$, respectively, by χ^2 -test.

Table 3. Effects of PMSF on Kinetic Parameters of Microsomal Anandamide Amidohydrolase in Mouse Brain and Liver

Tissues	Treatments	K_m (μM)	V_{max} ^{a)}	V_{max}/K_m (%)
Brain	Control	20.9 ± 1.4	3.03 ± 0.05	0.145 (100)
	PMSF	$8.9 \pm 1.0^{**}$	$0.34 \pm 0.06^{**}$	0.038 (26)
Liver	Control	164 ± 37	11.7 ± 2.0	0.072 (100)
	PMSF	96 ± 36	$0.67 \pm 0.07^{**}$	0.007 (10)

Mice were sacrificed at 60 min after i.p. administration of PMSF (100 mg/kg) or the vehicle (olive oil). Each group consisted of 8 mice. Brain and hepatic microsomes were prepared, and anandamide amidohydrolase activity was assayed according to the method described in the MATERIALS AND METHODS. ^{a)} nmol/min/mg protein. ** indicates the values that are significantly different from the corresponding control groups at $p < 0.01$.

The potentiation of the anandamide effect may be due to the inhibition of anandamide hydrolysis by PMSF-treatment. In order to examine this possibility, the change in kinetic parameters for anandamide hydrolysis in hepatic and brain microsomes from PMSF-treated mice was determined (Table 3). In this experiment, mice were treated with PMSF (100 mg/kg, i.p.). One hour after the administration of PMSF, microsomal fractions were prepared and anandamide amidohydrolase activities were determined. Table 3 shows that PMSF-treatment caused a significant decrease in K_m and V_{max} values for anandamide amidohydrolase activities in hepatic and brain microsomes of mice. K_m values for anandamide in both PMSF-treated microsomes were about half as compared with those of control. V_{max} values in brain and hepatic microsomes of PMSF-treated mice were 11.2 and 5.7%, respectively, compared with those of control mice. Thus, clearance rates as V_{max}/K_m were 26 and 10%, respectively, in brain and hepatic microsomes from PMSF-treated mice as compared with those from the control mice.

DISCUSSION

The present study demonstrated that anandamide and two unsaturated fatty acid ethanolamides exhibited pharmacological effects in mice such as catalepsy, hypothermia and barbiturate-induced sleep prolongation, although saturated fatty

acid ethanolamides (*N*-myristoyl-, *N*-palmitoyl-, *N*-stearoyl- and *N*-arachidoyl-) produced little effects. Among fatty acid ethanolamides tested, anandamide showed the highest activity in all pharmacological indices. Fride and Mechoulam³⁾ have reported the hypothermic effect of anandamide in mice. At a dose of 10 mg/kg i.p., maximal hypothermia produced by anandamide was reported to be 1.62 °C. However, the maximal hypothermia by anandamide was 0.59 °C in the present study. The discrepancy between the present result and the previous one may reflect some differences in the experimental conditions used. *N*-Oleoyl- and *N*-linoleoyl ethanolamine exhibited the pharmacological effects some extent, whereas ethanolamides of saturated fatty acids were far more less active. Felder *et al.*⁷⁾ suggested the end pentyl chain of anandamide might play an important role in the receptor binding. Adams *et al.*¹²⁾ also reported that the levels of saturation of anandamide in the fatty acid moiety were critical to receptor affinity and *in vivo* pharmacological effects. A looped conformation of anandamide analogs has been suggested to be energetically favourable, and there is some structural similarity of anandamide to Δ^9 -THC.²⁹⁾ These findings and the present results conclusively indicate that saturation of the fatty acid moiety is one of most important factors in exhibiting cannabimimetic effects for the anandamide analogs. The pharmacological experiments indicate that the effects of anandamide, *N*-oleoyl- and *N*-linoleoyl ethanolamine are qualitatively similar to those of THC, although the potency of these *N*-acyl ethanolamines is less than those of THCs reported previously from our laboratory under similar conditions.^{25,28)} *N*-Palmitoylethanolamine exhibited a significant effect only on the prolongation of pentobarbital-induced sleep indicating that the ethanolamide may have some different effects from the other *N*-acyl ethanolamines examined in the present study.

The onset of catalepsy by anandamide was very rapid, and the ED₅₀ value for anandamide was about half as active as those of Δ^8 - and Δ^9 -THC.^{25,30)} Mechoulam *et al.*³¹⁾ and Smith *et al.*¹⁸⁾ reported that the pharmacological effects in mice of anandamide were less potent than those of Δ^9 -THC. The rapid onset in the cataleptogenic effect indicates that anandamide rapidly crosses the blood-brain barrier. Thus, the peak time in the effect of anandamide was around 1 min after i.v. administration and faster than those of THC and its metabolites reported previously.^{23,25)} Smith *et al.*¹⁸⁾ reported that maximal antinociception was observed immediately after administration of anandamide in mice. The peak time (5 to 15 min) of anandamide in hypothermic effect is also rapid onset as compared with that of THC reported previously.^{25,32)} The effect of anandamide rapidly disappeared after the injection, suggesting the rapid degradation or excretion of anandamide in mice *in vivo*. Crawley *et al.*³³⁾ reported that the sedative effect of anandamide was shorter in duration of action than that of THC in rats. Abadji *et al.*²⁰⁾ reported that (*R*)-methanandamide, possessing remarkable stability to hydrolysis, produced a relatively higher activity to inhibit electrically evoked contractions of the mouse vas deferens. These results suggest that anandamide is rapidly hydrolyzed *in vivo* to arachidonic acid and ethanolamine. Willoughby *et al.*¹⁹⁾ reported that anandamide was quickly distributed, and biotransformed to arachidonic acid and polar metabolites in brain of mice. They suggested that rapid metabolism of anan-

damide in brain may play a key role in the time course of its pharmacological effects. Anandamide amidohydrolase, which is the main enzyme to hydrolyze anandamide, is known to be presented in liver and brain of mice.²¹⁾ The presence of the enzyme in brain suggests that anandamide penetrating into brain from peripheral tissues by i.v. administration may also be rapidly hydrolyzed for inactivation.

It is well known that *in vitro* affinity of anandamide for the cannabinoid receptor is increased by PMSF, a potent inhibitor of anandamide amidohydrolase.⁵⁾ At a concentration of 0.5 mM, PMSF decreased the K_d of anandamide for rat brain membrane from 1.3 μ M to 143 nM. To examine the importance of anandamide amidohydrolase on the *in vivo* effects of anandamide, the cataleptogenic effect of anandamide was assessed in mice pretreated with PMSF. The cataleptogenic effect of anandamide was markedly enhanced by PMSF-pretreatment together with prolonging the duration of the effect. Recently, Compton and Martin²²⁾ reported that PMSF (30 mg/kg i.p.) highly potentiated the effects of anandamide on antinociception, spontaneous activity and mobility in mice. Kinetic parameters (V_{max}/K_m) for anandamide amidohydrolase in brain and liver of mice pretreated with PMSF were significantly decreased indicating a remarkable decrease in clearance of anandamide causing the high potentiation of the anandamide effect by PMSF-treatment.

The present study demonstrated that the short duration of action of anandamide is due to its rapid degradation by anandamide amidohydrolase, which has an important role for the regulation of the biological effect of anandamide in mice. Other possibilities that PMSF acts on other neuromodulatory molecules which may contribute to the enhancement of the anandamide effects by PMSF must also be evaluated.

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