ELSEVIER

European Journal of Pharmacology 272 (1995) 73–78

ejp

Effect of phenylmethylsulphonyl fluoride on the potency of anandamide as an inhibitor of electrically evoked contractions in two isolated tissue preparations

Roger G. Pertwee [a,*], Susanthi R. Fernando [a], Graeme Griffin [a], Vasiliki Abadji [b], Alexandros Makriyannis [b]

[a] *Department of Biomedical Sciences, Marischal College, University of Aberdeen, Aberdeen AB9 1AS, Scotland, UK*
[b] *School of Pharmacy, University of Connecticut, Storrs, CT 06269, USA*

Received 21 July 1994; revised MS received 26 September 1994; accepted 7 October 1994

Abstract

The endogenous cannabinoid receptor ligand, anandamide, produced a concentration related inhibition of electrically evoked contractions of the guinea-pig myenteric plexus preparation. Its potency was markedly enhanced by phenylmethylsulphonyl fluoride (2.0–200 μM) which presumably acts by inhibiting the hydrolysis of anandamide in this preparation. The degree of this potentiation increased with the concentration of phenylmethylsulphonyl fluoride used. The methyl analogue of anandamide, *R*-(+)-arachidonyl-1'-hydroxy-2'-propylamide, also inhibited contractions of the guinea-pig myenteric plexus preparation. The potency of this compound was much less affected by phenylmethylsulphonyl fluoride than was the potency of anandamide, confirming its greater resistance to hydrolysis. Phenylmethylsulphonyl fluoride did not alter the inhibitory potency of the cannabinoid, CP 55,940 ((−)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-[3-hydroxypropyl]cyclohexan-1-ol), which is not an amidase substrate. Nor did phenylmethylsulphonyl fluoride affect the ability of anandamide to inhibit electrically evoked contractions of the mouse vas deferens, suggesting that anandamide does not undergo hydrolysis in this tissue.

Keywords: Cannabinoid; Phenylmethylsulphonyl fluoride; Anandamide; *R*-(+)-Arachidonyl-1'-hydroxy-2'-propylamide; Vas deferens, mouse; Myenteric plexus preparation, guinea-pig

1. Introduction

The endogenous cannabinoid receptor ligand, anandamide, is highly susceptible to enzymatic hydrolysis (Deutsch and Chin, 1993). As a result, its ability to bind to cannabinoid receptors is only detectable in some preparations if its metabolic degradation is blocked by the amidase inhibitor, phenylmethylsulphonyl fluoride (Abadji et al., 1994; Childers et al., 1994). The present experiments were directed at searching for preparations in which phenylmethylsulphonyl fluoride influences the ability of anandamide to elicit pharmacological responses. The preparations used were the mouse isolated vas deferens and the myenteric plexus preparation of guinea-pig small intestine. These are both preparations in which psychotropic cannabinoids are known to be highly potent inhibitors of electrically evoked contractions and to exhibit marked stereoselectivity (Pertwee et al., 1992). Whilst it was already known that anandamide can inhibit evoked contractions of the mouse vas deferens (Devane et al., 1992; Pertwee et al., 1993; 1994), its ability to inhibit contractions of the myenteric plexus preparation had not been investigated before.

The effect of phenylmethylsulphonyl fluoride on the pharmacological potencies of two other cannabinoids were studied. These were *R*-(+)-arachidonyl-1'-hydroxy-2'-propylamide (*R*-(+)-methanandamide), which

* Corresponding author. Tel. +44-224-273040, fax +44-224-273019, eMail pha040@aberdeen.ac.uk.

SSDI 0014-2999(94)00618-0

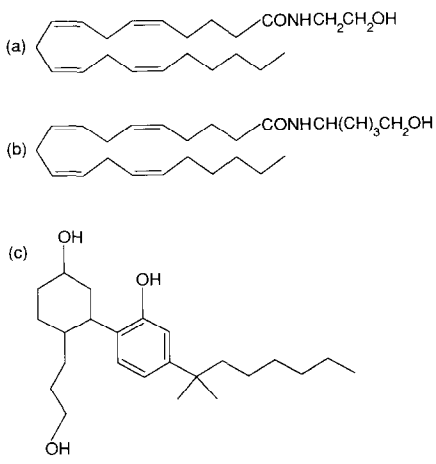

Fig. 1. Structures of (a) anandamide, (b) *R*-(+)-arachidonyl-1'-hydroxy-2'-propylamide (*R*-(+)-methanandamide) and (c) CP 55,940.

is a synthetic analogue of anandamide that possesses a fair degree of metabolic stability (Abadji et al., 1994), and CP 55,940 ((−)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-[3-hydroxypropyl]cyclohexan-1-ol) which is not susceptible to hydrolytic cleavage as it lacks an amide linkage. The structures of these two compounds and of anandamide are shown in Fig. 1.

2. Materials and methods

2.1. Drugs

Anandamide, *R*-(+)-methanandamide, CP 55,940 and phenylmethylsulphonyl fluoride were each mixed with two parts of Tween 80 by weight and dispersed in water (phenylmethylsulphonyl fluoride) or in a 0.9% aqueous solution of NaCl (saline) as described previously for Δ^9-tetrahydrocannabinol (Pertwee et al., 1992). CP 55,940 was supplied by Dr. L.S. Melvin (Pfizer) and phenylmethylsulphonyl fluoride by Sigma. Anandamide and *R*-(+)-methanandamide were synthesized as described by Abadji et al. (1994). Our first experiments with phenylmethylsulphonyl fluoride were performed using a concentration of 50 μM as this is known to increase the binding potency of anandamide (Abadji et al., 1994; Childers et al., 1994).

2.2. Tissue experiments

Tissues were mounted in 4 ml organ baths at an initial tension of 0.5 g using the method described by Pertwee et al. (1993). The baths contained Krebs solution which was kept at 37°C and bubbled with 95% O_2 and 5% CO_2. The composition of the Krebs solution was (mM): NaCl 118.2, KCl 4.75, KH_2PO_4 1.19, $NaHCO_3$ 25.0, glucose 11.0 and $CaCl_2 \cdot 6H_2O$ 2.54. In experiments with the myenteric plexus preparation, 1.29 mM $MgSO_4 \cdot 7H_2O$ was also present. Isometric contractions were elicited by electrical field stimulation through platinum electrodes attached to the upper and lower ends of each bath. Stimuli were generated by a Grass S48 stimulator, then amplified (Med-Lab channel attenuator) and divided to yield separate outputs to four organ baths (Med-Lab StimuSplitter). Contractions were monitored by computer (Apple Macintosh LC) using a data recording and analysis system (MacLab) that was linked via preamplifiers (Macbridge) to Dynamometer UF1 transducers (Pioden Controls). Drug additions were made in a volume of 10 μl.

Strips of myenteric plexus-longitudinal muscle were dissected from the small intestine of male albino Dunkin-Hartley guinea-pigs (320–630 g) and set up for field stimulation by the method of Paton and Zar (1968) in an inverted 'V' configuration. They were stimulated with single pulses of 110% maximal voltage, 0.5 ms duration and 0.1 Hz frequency (Pertwee et al., 1992). Phenylmethylsulphonyl fluoride was added after the tissues had equilibrated. Unless stated otherwise, twitch inhibitors were added 15 min later and then at 30 min intervals. Once phenylmethylsulphonyl fluoride had been added, tissues were incubated for up to 225 min without replacing the fluid in the bath.

Vasa deferentia were obtained from albino MF1 mice weighing 32–61 g. The tissues were stimulated with 0.5 s trains of three pulses of 110% maximal voltage (train frequency 0.1 Hz; pulse duration 0.5 ms). Each tissue was subjected to several periods of stimulation, the first of these beginning after the tissue had equilibrated but before drug administration and continuing for 11 min. Subsequent stimulation periods lasted 5 min after which baths were washed out by overflow and the tissues subjected to a stimulation-free period of 10 min.

Phenylmethylsulphonyl fluoride was added after the first stimulation period (time zero) and also after each bath wash. Anandamide was added immediately after the second addition of phenylmethylsulphonyl fluoride and after all subsequent additions of this compound.

2.3. Analysis of data

Values are expressed as means and limits of error as standard errors. Inhibition of the electrically evoked twitch response is expressed in percentage terms and has been calculated by comparing the amplitude of the twitch response 15–30 min after each addition of a twitch inhibitor with its amplitude immediately before the first addition of the inhibitor. Potency ratios, their 95% confidence limits and drug concentrations producing a 50% reduction in twitch amplitude (IC_{50} values) have all been determined by symmetrical (2 + 2) dose parallel line assays (Colquhoun, 1971) as described

previously (Pertwee et al., 1993). In none of these assays did pairs of log concentration-response curves deviate significantly from parallelism ($P > 0.05$). The significance of differences between means ($P < 0.05$) have been evaluated by Scheffé's test using Super ANOVA (Abacus Concepts, Berkeley) or by Student's t-test (two-tail).

3. Results

3.1. Effect of 50 μM phenylmethylsulphonyl fluoride on the ability of anandamide to inhibit electrically evoked contractions of the mouse vas deferens

Administration both of phenylmethylsulphonyl fluoride and of its vehicle, Tween 80, led to significant reductions in the amplitude of evoked contractions of vasa deferentia. Mean decreases in amplitude of 10.4 ± 2.0% and 11.5 ± 2.7% respectively were observed within 15 min of the administration of phenylmethylsulphonyl fluoride and Tween 80 ($P < 0.001$; $n = 7$). The inhibitory effect of anandamide on evoked contractions of the vas deferens is shown in Fig. 2. Its ability to inhibit the twitch response was not affected by phenylmethylsulphonyl fluoride.

3.2. Effect of phenylmethylsulphonyl fluoride on the ability of anandamide, R-(+)-methanandamide and CP 55,940 to inhibit electrically evoked contractions of the myenteric plexus preparation

Anandamide produced a concentration-related inhibition of evoked contractions of the myenteric plexus preparation (Fig. 2). Its inhibitory potency was increased by 50 μM phenylmethylsulphonyl fluoride which produced a 22-fold leftward parallel shift in the log concentration-inhibitory response curve of anandamide (Fig. 2). The degree of potentiation of anandamide was related to the concentration of phenylmethylsulphonyl fluoride used, the threshold concentration lying between 0.2 μM and 2.0 μM (Fig. 3). The effect of phenylmethylsulphonyl fluoride on the potency of anandamide was long lasting (Table 1). Both R-(+)-methanandamide and CP 55,940 shared the ability of anandamide to produce a concentration-related inhibition of evoked contractions of the myenteric plexus preparation (Fig. 4). Whilst phenylmethylsulphonyl fluoride potentiated R-(+)-methanandamide, it did not affect the inhibitory potency of CP 55,940 (Fig. 4). None of the cannabinoids investigated were potentiated by Tween 80 (results not shown).

Control experiments performed in the absence of cannabinoids showed that administration of phenylmethylsulphonyl fluoride was followed by falls in twitch amplitude. For example, decreases in amplitude were 1.6 ± 1.1%, 15 min after administration of 200 μM

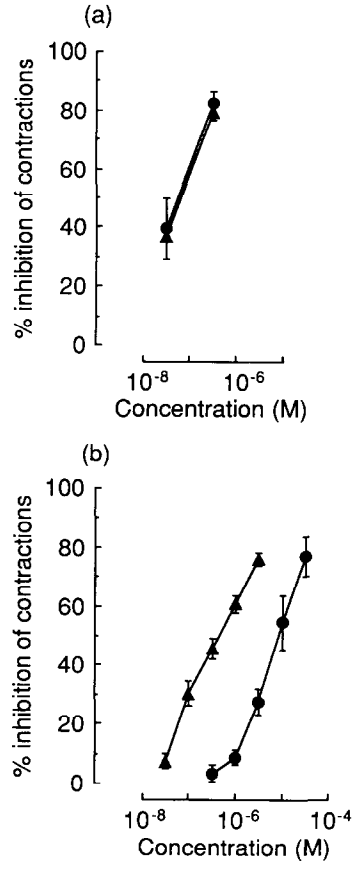

Fig. 2. Mean concentration-response curves for anandamide (a) in the mouse isolated vas deferens ($n = 7$) and (b) in the myenteric plexus preparation of guinea-pig small intestine ($n = 6$) constructed in the presence of 50 μM phenylmethylsulphonyl fluoride (triangles) or in its absence (circles). Each symbol represents the mean value ± S.E. of inhibition of electrically evoked contractions expressed as a percentage of the amplitude of the twitch response measured immediately before addition of anandamide to the organ bath. The relative potency of anandamide in phenylmethylsulphonyl fluoride-treated versus control tissue is 0.9 (0.4 and 1.7) in the vas deferens and 21.5 (11.3 and 38.5) in the myenteric plexus preparation (mean and 95% confidence limits).

phenylmethylsulphonyl fluoride, and 0.7 ± 3.8% at the end of a 135 min exposure to this concentration ($n = 6$). Although small, these reductions in amplitude are statistically significant ($P < 0.01$; Student's t-test for paired data). Significant reductions in twitch amplitude also occurred after the addition of Tween 80 alone (e.g. 5.27 ± 2.21%, 15 min after administration; $n = 6$). Concentrations of phenylmethylsulphonyl fluoride of up to 200 μM did not affect the resting tension of the myenteric plexus preparation or its rate of relaxation subsequent to electrical stimulation.

3.3. The potencies of anandamide, R-(+)-methanandamide and CP 55,940 in the vas deferens relative to their potencies in the myenteric plexus preparation

Table 2 shows the ratio of inhibitory potency in the vas deferens to that in the myenteric plexus prepara-

Table 1
Inhibitory effect of 3162 nM anandamide, added either 15 min or 135 min after 200 μM phenylmethylsulphonyl fluoride (PMSF), on the twitch response of the myenteric plexus preparation

Time interval between PMSF and anandamide (min)	Inhibition of twitch response (% ± S.E.)	n
15 [a]	62.1 ± 4.9	6
135 [a]	57.3 ± 6.8	6
135 [b]	73.7 ± 5.5	6

[a] The only addition of anandamide to be made. [b] Added after four other doses of anandamide, during the construction of a concentration-response curve. Anandamide produced the same degree of inhibition in all three of these experiments (Scheffé's test).

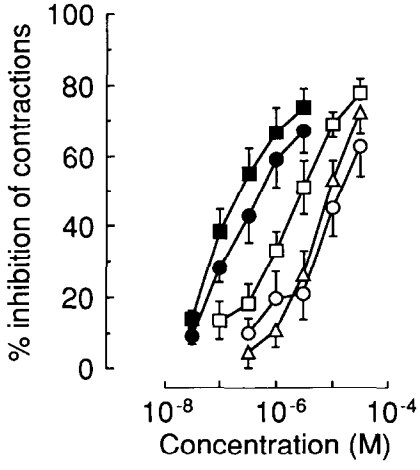

Fig. 3. Mean concentration-response curves for anandamide in the myenteric plexus preparation of guinea-pig small intestine constructed in the presence of Tween 80 (open circles) or phenylmethylsulphonyl fluoride at concentrations of 0.2 μM (open triangles), 2 μM (open squares), 20 μM (filled circles) and 200 μM (filled squares). Each symbol represents the mean value ± S.E. of inhibition of electrically evoked contractions expressed as a percentage of the amplitude of the twitch response measured immediately before addition of anandamide to the organ bath ($n = 6$–8 different myenteric plexus preparations). Significant leftward shifts in the concentration-inhibitory response curve of anandamide were produced by phenylmethylsulphonyl fluoride at concentrations of 2 μM and above.

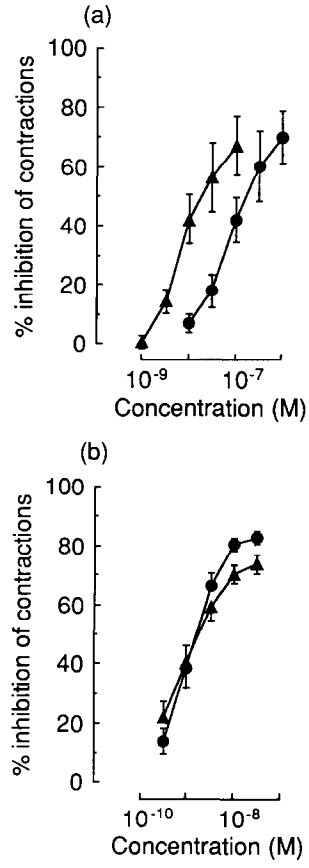

Fig. 4. Mean concentration-response curves for (a) *R*-(+)-methanandamide and (b) CP 55,940 in the myenteric plexus preparation of guinea-pig small intestine constructed in the presence of 200 μM phenylmethylsulphonyl fluoride (triangles) or in its absence (circles). Each symbol represents the mean value ± S.E. of inhibition of electrically evoked contractions expressed as a percentage of the amplitude of the twitch response measured immediately before addition of anandamide to the organ bath ($n = 5$–8 myenteric plexus preparations). The relative potency of *R*-(+)-methanandamide in phenylmethylsulphonyl fluoride-treated versus control tissue is 8.8 (2.0 and 35.0) (mean and 95% confidence limits).

Table 2
Relative inhibitory potencies of anandamide, *R*-(+)-arachidonyl-1'-hydroxy-2'-propylamide (*R*-(+)-methanandamide) and CP 55,940 on evoked contractions of the guinea-pig myenteric plexus preparation and mouse vas deferens

Twitch inhibitor	Concentrations used in 2 + 2 assay (nM)	Tissue	IC_{50} (nM)	Potency ratio	95% confidence limits	n
Anandamide	3162, 31620	Myenteric plexus	8823	155.8	64.4 and 322.1	6
	10, 100	Vas deferens	56.6			6
R-(+)-Methanandamide	31.62, 316.2	Myenteric plexus	155	14.6	9.2 and 23.7	6
	3.162, 31.62	Vas deferens	10.6			6
Anandamide	31.62, 316.2	Myenteric plexus [a]	289	4.7	2.1 and 11.7	6
	10, 100	Vas deferens	61			6
R-(+)-Methanandamide	3.162, 31.62	Myenteric plexus [a]	21.3	1.7	0.9 and 3.7	5
	3.162, 31.62	Vas deferens	12.4			5
CP 55,940	0.3162, 3.162	Myenteric plexus	2.62	7.04	3.5 and 18.9	6
	0.3162, 3.162	Vas deferens	0.37			6

[a] In the presence of 200 μM PMSF.

tion for anandamide, *R*-(+)-methanandamide and CP 55,940. One set of potency ratios in Table 2 has been calculated from data obtained in the absence of phenylmethylsulphonyl fluoride. Examination of this set shows that the potency ratio for anandamide is significantly greater than that for CP 55,940 whilst the potency ratios for *R*-(+)-methanandamide and CP 55,940 do not differ significantly from each other. A second set of potency ratios in Table 2 has been calculated using data from experiments in which the inhibitory effects of anandamide and *R*-(+)-methanandamide on the myenteric plexus preparation were measured in the presence of 200 μM phenylmethylsulphonyl fluoride. In this set, potency ratios for anandamide, *R*-(+)-methanandamide and CP 55,940 do not differ significantly from each other.

4. Discussion

Our results show that as in the mouse vas deferens (Abadji et al., 1994; Pertwee et al., 1993) so too in the myenteric plexus preparation of guinea-pig small intestine, anandamide, *R*-(+)-methanandamide and CP 55,940 share the ability of other cannabinoids to inhibit electrically evoked contractions (Pertwee et al., 1992). They also demonstrate that phenylmethylsulphonyl fluoride can markedly increase the inhibitory potency of anandamide in this preparation. This observation is in line with earlier reports that phenylmethylsulphonyl fluoride increases the binding potency of anandamide in brain preparations that contain cannabinoid receptors (Abadji et al., 1994; Childers et al., 1994). The concentration of phenylmethylsulphonyl fluoride used in these binding experiments (50 μM) is within the range of concentrations found in the present investigation to increase the pharmacological potency of anandamide in the myenteric plexus preparation. In contrast to its effect on anandamide potency in this preparation, phenylmethylsulphonyl fluoride (50 μM) did not increase the inhibitory potency of anandamide in the mouse vas deferens.

The metabolic degradation of anandamide is catalysed by an amidase that can be inhibited by phenylmethylsulphonyl fluoride (Deutsch and Chin, 1993). It is probable, therefore, that phenylmethylsulphonyl fluoride can potentiate anandamide by increasing the concentration of this amide at its site of action through inhibition of its metabolism. If this is true, our finding that phenylmethylsulphonyl fluoride does not potentiate anandamide in mouse vas deferens would indicate that this is a tissue in which anandamide is not metabolized, at least not through the action of any enzyme susceptible to phenylmethylsulphonyl fluoride. Such a difference in ability to metabolize anandamide could explain the greater potency that is shown by this compound in the mouse vas deferens than in the myenteric plexus preparation (Table 2).

A second way in which phenylmethylsulphonyl fluoride might increase the potency of anandamide is by somehow augmenting tissue sensitivity to anandamide. However, there are two reasons for rejecting this hypothesis. Firstly, phenylmethylsulphonyl fluoride fails to potentiate the cannabinoids, CP 55,940 and WIN 55,212-2 ((+)-(*R*)-4,5-dihydro-2-methyl-4-(4-morpholinylmethyl)-1-(1-naphthalenylcarbonyl)-6*H*-pyrrolo[3,2,-1-*ij*]quinolin-6-one), that are not amidase substrates but that presumably do have the same mode of action as anandamide. More specifically, it was found in the present experiments that phenylmethylsulphonyl fluoride does not increase the inhibitory potency of CP 55,940 in the myenteric plexus preparation and Childers et al. (1994) have found that although phenylmethylsulphonyl fluoride increases the binding of anandamide to cannabinoid receptors it does not affect the binding potency of WIN 55,212-2. Secondly, our results demonstrate that phenylmethylsulphonyl fluoride potentiates anandamide in only one of two cannabinoid bioassays indicating that its ability to increase the pharmacological potency of anandamide does not extend to all cannabinoid-responsive preparations.

Another observation made in the present investigation was that phenylmethylsulphonyl fluoride can enhance the inhibitory potency in the myenteric plexus preparation of *R*-(+)-methanandamide, an analogue of anandamide. The potency of this compound was much less affected by phenylmethylsulphonyl fluoride than that of anandamide. This supports the notion that *R*-(+)-methanandamide is significantly more resistant to enzymatic hydrolysis than anandamide (Abadji et al., 1994).

Results from previous experiments have shown the psychotropic cannabinoids, Δ^9-tetrahydrocannabinol and the dimethylheptyl homologue of 11-hydroxy-Δ^8-tetrahydrocannabinol, to be about 10 times more potent as inhibitors of the twitch response in the mouse vas deferens than in the myenteric plexus preparation (Pertwee et al., 1992). The present experiments indicate that this rule extends to CP 55,940. It also applies to anandamide, but only if the inhibitory potency of this compound in the myenteric plexus preparation is measured in the presence of phenylmethylsulphonyl fluoride (Table 2). When the ratio of the inhibitory potency of anandamide in the vas deferens to that in the myenteric plexus preparation is calculated from data gathered in the absence of phenylmethylsulphonyl fluoride, the value of this ratio is much higher (156). Presumably this is because anandamide is more stable in the vas deferens than the myenteric plexus preparation. For *R*-(+)-methanandamide, the ratio of inhibitory potency in the vas deferens to that in the myenteric plexus preparation, fails to deviate signifi-

cantly from 10 only if it is calculated from data obtained in experiments performed in the absence of phenylmethylsulphonyl fluoride. The ability of this cannabimimetic analogue to inhibit contractions of the myenteric plexus preparation in the presence of phenylmethylsulphonyl fluoride was found to be no different from its ability to inhibit contractions of the vas deferens in the absence of phenylmethylsulphonyl fluoride. This may indicate that at least one type of prejunctional receptor, possibly a second type of cannabinoid receptor, responds more readily to *R*-(+)-methanandamide than to other cannabinoids such as Δ^9-tetrahydrocannabinol or CP 55,940. Further experiments are required to establish whether anandamide really does differ from *R*-(+)-methanandamide in being less potent as an inhibitor of evoked contractions in the myenteric plexus preparation than in the vas deferens. This is because it remains possible that even the highest concentration of phenylmethylsulphonyl fluoride used in this investigation (200 μM) may have inhibited the enzymatic hydrolysis of anandamide less completely than that of *R*-(+)-methanandamide, the more stable compound (Abadji et al., 1994).

As well as being an amidase inhibitor, phenylmethylsulphonyl fluoride is an inhibitor of several other enzymes, including acetylcholinesterase (Zollner, 1989). However, it is unlikely that any of the concentrations of phenylmethylsulphonyl fluoride used in the present investigation produced significant inhibition of acetylcholinesterase, at least in the myenteric plexus preparation. Inhibition of this enzyme in this preparation is known to provoke an increase in resting tension and to decrease rate of relaxation after electrical stimulation (Cowie et al., 1978). Phenylmethylsulphonyl fluoride produced neither of these effects.

The present observation, that phenylmethylsulphonyl fluoride can enhance the pharmacological potency of anandamide in one tissue but not in another, is consistent with a previous report that anandamide is not metabolized by all tissues (Deutsch and Chin, 1993). Further experiments are now required to confirm whether or not anandamide is indeed metabolized by amide hydrolysis in the myenteric plexus preparation and if it is, to establish the degree of amidase inhibition that is produced in this preparation by concentrations of phenylmethylsulphonyl fluoride that potentiate anandamide. It may also be of advantage to develop inhibitors of anandamide metabolism with greater selectivity than phenylmethylsulphonyl fluoride as these could well serve as important pharmacological tools for investigating the physiological roles of anandamide in the whole organism and might also come to be exploited therapeutically. If, as our results suggest, the guinea-pig myenteric plexus preparation does metabolize anandamide by amide hydrolysis, it could be an excellent bioassay with which to develop such drugs. On the other hand, the apparent inability of the mouse vas deferens to metabolize anandamide, lends further support to the suitability of this preparation as a bioassay for characterizing the structure-activity relationships of metabolically unstable anandamide analogues.

Acknowledgements

This work was supported by grants 034924 and 039538 (to R.G.P.) from the Wellcome Trust and by grants DA-3801 and DA-7215 (to A.M.) from the National Institute on Drug Abuse. We thank Dr. L.S. Melvin (Pfizer) for providing us with CP 55,940.

References

Abadji, V., S. Lin, G. Taha, G. Griffin, L.A. Stevenson, R.G. Pertwee and A. Makriyannis, 1994, *R*-Methanandamide: a chiral novel anandamide possessing higher potency and metabolic stability, J. Med. Chem. 37, 1889.

Childers, S.R., T. Sexton and M.B. Roy, 1994, Effects of anandamide on cannabinoid receptors in rat brain membranes, Biochem. Pharmacol. 47, 711.

Colquhoun, D., 1971, Lectures on Biostatistics (Oxford University Press, Oxford).

Cowie, A.L., H.W. Kosterlitz and A.A. Waterfield, 1978, Factors influencing the release of acetylcholine from the myenteric plexus of the ileum of the guinea-pig and rabbit, Br. J. Pharmacol. 64, 565.

Deutsch, D.G. and S.A. Chin, 1993, Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist, Biochem. Pharmacol. 46, 791.

Devane, W.A., L. Hanuš, A. Breuer, R.G. Pertwee, L.A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger and R. Mechoulam, 1992, Isolation and structure of a brain constituent that binds to the cannabinoid receptor, Science 258, 1946.

Paton, W.D.M. and M.A. Zar, 1968, The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strips, J. Physiol. 194, 13.

Pertwee, R.G., L.A. Stevenson, D.B. Elrick, R. Mechoulam and A.D. Corbett, 1992, Inhibitory effects of certain enantiomeric cannabinoids in the mouse vas deferens and the myenteric plexus preparation of guinea-pig small intestine, Br. J. Pharmacol. 105, 980.

Pertwee, R.G., L.A. Stevenson and G. Griffin, 1993, Cross-tolerance between delta-9-tetrahydrocannabinol and the cannabimimetic agents, CP 55,940, WIN 55,212-2 and anandamide, Br. J. Pharmacol. 110, 1483.

Pertwee, R.G., G. Griffin, L. Hanuš, and R. Mechoulam, 1994, Effects of two endogenous fatty acid ethanolamides on mouse vasa deferentia, Eur. J. Pharmacol. 259, 115.

Zollner, H., 1989, Handbook of Enzyme Inhibitors (VCH publishers, Weinheim).