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Short communication

Further evidence for the presence of cannabinoid CB_1 receptors in mouse vas deferens

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Abstract

Our results provide further evidence for the hypothesis that the mouse vas deferens contains cannabinoid CB_1 receptors. Thus we found that in the presence of forskolin, the cannabinoid receptor agonist, CP 55,940 ((−)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol) produced a concentration related inhibition of cyclic AMP production by the vas deferens (EC_{50} = 6.0 nM). At 100 nM, SR141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride) attenuated this effect of CP 55,940, producing a parallel rightward shift in its log concentration-response curve (K_d = 4.3 nM). We also found that cyclic AMP production was inhibited by (−)-11-hydroxy-1′,1′-dimethylheptyl-Δ^8-tetrahydrocannabinol but not by the (+) enantiomer.

Keywords: Cannabinoid receptor; Cannabinoid receptor antagonist; SR141716A; Vas deferens, mouse; cAMP; Adenylate cyclase

1. Introduction

Because of the high potency and remarkable stereoselectivity of certain cannabinoids as inhibitors of electrically evoked contractions of the mouse vas deferens and because the cannabinoid CB_1 receptor antagonist, SR141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride), can antagonize this effect it is likely that the vas deferens contains cannabinoid receptors (Pacheco et al., 1991; Pertwee et al., 1992, 1995; Rinaldi-Carmona et al., 1994). The present experiments have explored this hypothesis further by investigating the ability of the cannabinoid receptor agonist, CP 55,940 ((−)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol) to inhibit cyclic AMP production by the vas deferens. This strategy was adopted as CB_1 receptors are coupled to G_i and are able to inhibit adenylate cyclase (see Pertwee, 1995). As an inhibitory effect by CP 55,940 was detected, further experiments were carried out to measure the ability of SR141716A to attenuate the degree of inhibition produced. The question of whether cannabinoids show stereoselectivity as inhibitors of cyclic AMP production by the vas deferens was also addressed using (+)- and (−)-11-hydroxy-1′,1′-dimethylheptyl-Δ^8-tetrahydrocannabinol, the stereochemical purity of which is particularly high (see Mechoulam and Fride, 1995).

2. Materials and methods

2.1. Drugs, chemicals and analytical reagents

The 11-hydroxy-1′,1′-dimethylheptyl analogues of (+)- and (−)-Δ^8-tetrahydrocannabinol were obtained from Professor Mechoulam, Hebrew University of Jerusalem, CP 55,940 from Pfizer and SR141716A from Sanofi. Each compound was mixed with 2 parts of Tween 80 by weight and dispersed in Tyrode's solution of the following composition (mM): NaCl 137.1, KCl 2.7, $NaHCO_3$ 12.0, $MgCl_2 \cdot 6H_2O$ 14.6, glucose 5.5 and $CaCl_2 \cdot 2H_2O$ 1.4. In control experiments, Tween 80 (Sigma) was added instead of SR141716A or a cannabi-

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noid. Forskolin and 3-isobutyl-1-methylxanthine were dissolved in dimethyl sulfoxide. These compounds were obtained from Sigma as was bovine serum albumin. Folin-Ciocalteu's phenol reagent was supplied by Fison's Scientific Equipment and the liquid scintillation cocktail, Ultima Gold XR, by Canberra Packard. Other chemicals were obtained from BDH Chemicals. All drugs were added in a volume of 10 μl.

2.2. Cyclic AMP assay

Vasa deferentia were obtained from albino MF1 mice weighing 36–60 g. Each tissue was preincubated for 30 min in the presence or absence of SR141716A in 500 μl of Tyrode's solution. It was then transferred to a second 500 μl sample of this solution, now also containing 500 μM 3-isobutyl-1-methylxanthine and usually also a cannabinoid or Tween 80. After a 30 min incubation, each tissue was transferred to another 500 μl sample of this solution containing 100 nM forskolin. After 15 min, 500 μl 0.1 M HCl was added. Each solution was allowed to stand for 15 min at 37°C and was then centrifuged for 2 min at 15 600 × *g*. The supernatant was removed and then brought to a pH of 8–9 by the addition of 40–60 μl of 1 M NaOH. The concentration of cyclic AMP present in the supernatant was determined by radioimmunoassay (Amersham kit, UK). All incubations were carried out at 37°C.

2.3. Protein assay

After the centrifugation step just described, each vas deferens was homogenized in 1 ml of a 2% (w/v) aqueous solution of the solubilizing agent sodium dodecylsulphate containing 0.1 M NaOH. After 60 min at 37°C, a 50-fold dilution of the homogenate was made with 0.5 M NaOH and its protein content measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

2.4. Analysis of data

Values are given as means and limits of error as standard errors. The dissociation constant (K_d) of SR141716A was calculated from a single dose ratio

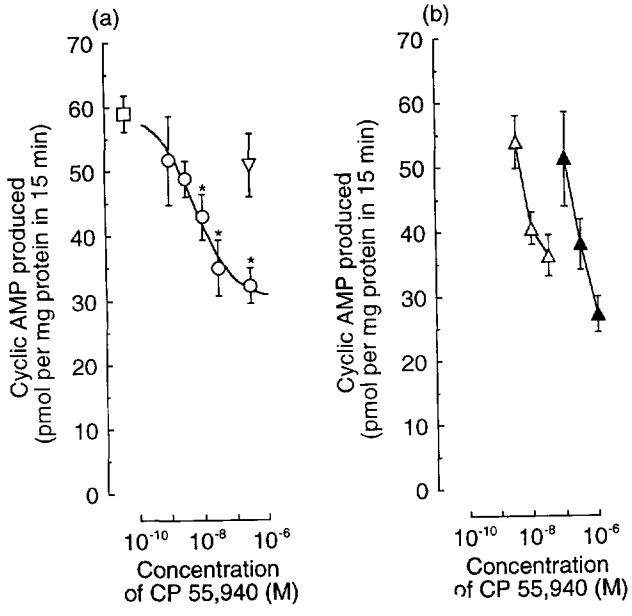

Fig. 1. Mean concentration-response curves for CP 55,940 constructed (a) in the absence of other agents (open circles) or (b) in the presence of 100 nM SR141716A (filled triangles) or of Tween 80 (open triangles). The square symbol and inverted triangle shown in (a) denote respective responses to 100 nM forskolin and to a dose of Tween 80 identical to the dose added in combination with 316.2 nM CP 55,940. Each symbol represents the mean value ± S.E. of cyclic AMP produced by vasa deferentia in the presence of 100 nM forskolin (n = 6–12 different vasa deferentia). The upper plateau of the sigmoid curve shown in (a) has been constrained to pass through the mean value of cyclic AMP production stimulated by 100 nM forskolin in the absence of CP 55,940 or Tween 80 (see square symbol). The mean value of forskolin-stimulated cyclic AMP production through which the lower plateau of the curve passes is 30.0 pmol/mg of protein/15 min (95% confidence limits = 18.3 and 42.3 pmol/mg of protein/15 min). The significance of differences between the effect of forskolin by itself and the effect of forskolin in the presence of CP 55,940 or Tween 80 shown in (a) was evaluated by analysis of variance followed by Dunnett's 1-tailed test (*$P < 0.05$).

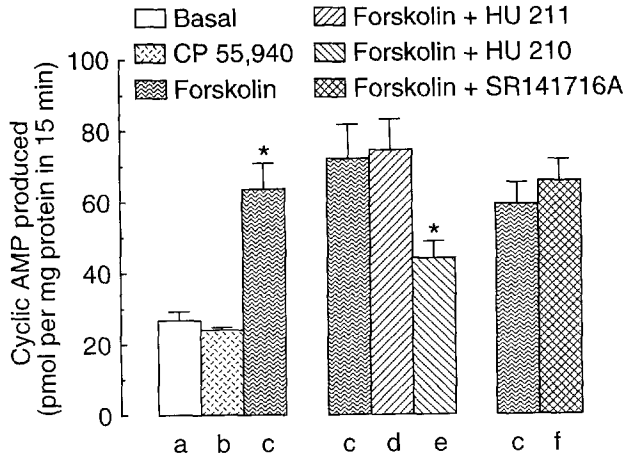

Fig. 2. Each column represents the mean value ± S.E. of cyclic AMP produced by vasa deferentia (a) in the absence of forskolin, (b) in the presence of 316.2 nM CP 55,940 and absence of forskolin, (c) in the presence of 100 nM forskolin, (d) in the presence of 100 nM forskolin and 1 μM (+)-11-hydroxy-1′,1′-dimethylheptyl-Δ^8-tetrahydrocannabinol (HU 211), (e) in the presence of 100 nM forskolin and 25 nM (−)-11-hydroxy-1′,1′-dimethylheptyl-Δ^8-tetrahydrocannabinol (HU 210) and (f) in the presence of 100 nM forskolin and 100 nM SR141716A (n = 8–12 different vasa deferentia). The asterisk denotes a significant difference ($P < 0.05$) between Basal and Forskolin (Dunnett's 2-tailed test) or between Forskolin and Forskolin + HU 210 (Dunnett's 1-tailed test). There is no significant difference between Basal and CP 55,940 or Forskolin and Forskolin + HU 211 (Dunnett's test) or between Forskolin and Forskolin + SR141716A (Student's t-test for unpaired data).

value by Schild analysis (Pertwee et al., 1995). A symmetrical (2 + 2) dose parallel line assay was used to calculate the dose ratio value and the 95% confidence limits of this value and also to establish whether any shift in the log concentration-response curve of CP 55,940 produced by SR141716A deviated significantly from parallelism (Pertwee et al., 1995). The concentration of CP 55,940 at the mid point of its log concentration-response curve (EC_{50}) and its 95% confidence limits have been calculated by non-linear regression analysis (GraphPAD InPlot). Mean values have been compared by Student's t-test for unpaired data or by analysis of variance followed by Dunnett's test (Super Anova). A P value < 0.05 was considered to be significant.

3. Results

Cyclic AMP production was inhibited by CP 55,940 in a concentration-related manner (Fig. 1a). The log concentration-response curve fitted a sigmoid curve ($r^2 = 0.978$), the shape of which suggests that maximal concentrations of CP 55,940 are not completely inhibitory. The mean EC_{50} of CP 55,940 with its 95% confidence limits shown in brackets is 6.0 nM (1.1 and 31.1 nM). SR141716A (100 nM) behaved as a competitive surmountable antagonist, producing a parallel rightward shift in the log concentration-response curve of CP 55,940 (Fig. 1b). The mean K_d value of SR141716A with its 95% confidence limits shown in brackets was calculated to be 4.3 nM (1.8 and 424.4 nM). By itself, SR141716A did not detectably affect cyclic AMP production (Fig. 2).

Cyclic AMP production by the vas deferens occurred in the absence of forskolin but was greater in its presence (Fig. 2). A concentration of CP 55,940 (316.2 nM) that was significantly inhibitory in the presence of forskolin (Fig. 1) had no detectable effect in its absence (Fig. 2). Forskolin-stimulated cyclic AMP production was significantly inhibited by 25 nM (−)-11-hydroxy-1′,1′-dimethylheptyl-Δ^8-tetrahydrocannabinol but not by 1 μM (+)-11-hydroxy-1′,1′-dimethylheptyl-Δ^8-tetrahydrocannabinol (Fig. 2).

4. Discussion

Our experiments show that CP 55,940 has a dose-related inhibitory effect on cyclic AMP production by the vas deferens and that this effect can be attenuated by SR141716A. The K_d value of SR141716A calculated from our data is similar to values obtained previously (Pertwee et al., 1995). These results suggest that it is cannabinoid CB_1 receptors through which CP 55,940 acts to inhibit cyclic AMP production by the vas deferens and consequently also provide further evidence for the presence of such receptors in this tissue. Support

for these conclusions comes from our experiments with (+)- and (−)-11-hydroxy-1′,1′-dimethylheptyl-Δ^8-tetrahydrocannabinol. Only the (−) enantiomer is a potent cannabinoid receptor agonist (Pertwee et al., 1992; Mechoulam and Fride, 1995) and it was only this enantiomer that significantly attenuated cyclic AMP production.

The highest concentration of CP 55,940 that we used to inhibit cyclic AMP production in the presence of forskolin (316.2 nM) had no detectable effect on basal production. A similar observation has been made previously with certain other preparations, for example rat brain slices and mouse synaptosomes (Bidaut Russell et al., 1990; Little and Martin, 1991). Another of our observations, that maximal concentrations of CP 55,940 do not completely inhibit cyclic AMP production by the vas deferens, is also in line with previous reports (Howlett et al., 1988; Pacheco et al., 1993). One possible explanation for this finding is that not all the cyclic AMP production that occurs in tissues containing cannabinoid receptors is regulated by these receptors.

In conclusion, our results seem to provide another example of a tissue in which cannabinoid receptors are negatively coupled to adenylate cyclase. It remains to be established whether it is this transduction mechanism that mediates cannabinoid-induced inhibition of the twitch response of the mouse vas deferens as cannabinoid CB_1 receptors are also negatively coupled to N-type Ca^{2+} channels (see Deadwyler et al., 1995). Cannabinoids probably reduce the twitch response by acting prejunctionally to decrease release of contractile transmitters (Pertwee and Griffin, 1995) and such an effect could well result from cannabinoid-induced inhibition of Ca^{2+} influx. Our finding that CP 55,940 is markedly less potent as an inhibitor of cyclic AMP production by the mouse vas deferens (EC_{50} = 6 nM) than as an inhibitor of electrically evoked contractions of this tissue (EC_{50} = 0.48 nM) (Pertwee et al., 1995) is consistent with this hypothesis. However, because the immediate effects of cytosolic cyclic AMP are presumably amplified by subsequent steps of the signal transduction cascade, there is another possible explanation for this potency difference. Thus it may be that some concentrations of CP 55,940 decrease cyclic AMP concentrations by amounts that are too small to detect by radioimmunoassay but that are sufficient when amplified by the signal transduction process, to bring about measurable reductions in electrically evoked contractions.

Acknowledgements

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