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A preliminary investigation of the mechanisms underlying cannabinoid tolerance in the mouse vas deferens

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Abstract

Vasa deferentia taken from mice treated with Δ^9-tetrahydrocannabinol (20 mg/kg i.p., once daily for 2 days) showed tolerance to the inhibitory effect of the cannabinoid, *R*-(+)-arachidonyl-1'-hydroxy-2'-propylamide, on electrically evoked twitches. This treatment did not induce tolerance to the inhibitory effects on the twitch response of morphine or clonidine or of selective μ-, δ- or κ-opioid receptor agonists. Nor did it affect the contractile potencies of noradrenaline or β,γ-methylene-L-ATP. We suggest that cannabinoid tolerance in the vas deferens is attributable neither to downregulation of opioid receptors or α_2-adrenoceptors nor to an increased sensitivity of this tissue to its main contractile transmitters noradrenaline and ATP. A concentration of Δ^9-tetrahydrocannabinol that inhibits electrically evoked twitches of the vas deferens (100 nM) did not alter the ability of noradrenaline or β,γ-methylene-L-ATP to induce contractions suggesting that Δ^9-tetrahydrocannabinol inhibits the twitch response by acting prejunctionally.

Keywords: Cannabinoid; Opioid; Noradrenaline; β,γ-Methylene-L-ATP; Vas deferens, mouse; Tolerance

1. Introduction

Previous experiments have shown that vasa deferentia obtained from mice pretreated with Δ^9-tetrahydrocannabinol show significant reductions in their sensitivity to the inhibitory effect of cannabinoids on the electrically evoked twitch response (Pertwee et al., 1993, 1994). As a first step in establishing the mechanism underlying this phenomenon, we have now investigated whether pretreatment with Δ^9-tetrahydrocannabinol induces tolerance to certain non-cannabinoid inhibitors of the twitch response. In particular, we have examined whether Δ^9-tetrahydrocannabinol desensitizes the mouse isolated vas deferens to the inhibitory effect of morphine on the twitch response. This we have done because there is evidence that Δ^9-tetrahydrocannabinol can induce tolerance to at least one effect of morphine in mice, its hypothermic effect (Bloom and Dewey, 1978). In addition, we have investigated the effect of in vivo treatment with Δ^9-tetrahydrocannabinol on the sensitivity of mouse isolated vasa deferentia to the selective μ-opioid receptor agonist, [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin, the selective δ-opioid receptor agonist, [D-Ala^2,D-Leu^5]enkephalin, and the selective κ-opioid receptor agonist, (5α,7α,8β)-(+)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxa-spiro-[4.5]dec-8-yl]-benzeneacetamide (U 69,593) (Corbett et al., 1993). These experiments were carried out because it has been reported that Δ^9-tetrahydrocannabinol can induce tolerance to the antinociceptive effect of κ-opioid receptor agonists in mice without attenuating the antinociceptive effects of μ- or δ-opioid receptor agonists (Smith et al., 1994). We have also examined the effect of Δ^9-tetrahydrocannabinol on the sensitivity of the vas deferens to the α_2-adrenoceptor agonist, clonidine. This experiment was prompted by a report that the ability of clonidine to inhibit the twitch response of the mouse vas deferens can be attenuated by a pretreatment with morphine that also renders tissues tolerant to morphine-induced inhibition of the twitch response (McCulloch and Pollock, 1985).

Finally, we have addressed the question of whether cannabinoid tolerance in the mouse vas deferens can be attributed to an increase in sensitivity of the smooth

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muscle of this tissue to its main junctional neurotransmitters, noradrenaline and ATP (Stjärne and Åstrand, 1985; Von Kügelgen et al., 1989b). This was accomplished by determining whether or not the contractile potencies of noradrenaline or of the purinergic receptor agonist, β,γ-methylene-L-ATP, are greater in cannabinoid tolerant vasa deferentia than in non-tolerant tissues. The second of these compounds was selected because it is more stable than ATP and because it shows selectivity towards the type of postjunctional purinergic receptor, P_{2X}, that is thought to be contractile in the mouse vas deferens (Boland et al., 1992; Kennedy, 1990; Von Kügelgen et al., 1989b). These additional experiments were considered necessary not least because of a report by McCulloch and Pollock (1985) that mouse isolated vasa deferentia exhibiting partial tolerance to the inhibitory effect of morphine on electrically evoked contractions show supersensitivity to the contractile effect of noradrenaline.

2. Materials and methods

2.1. Drugs

Drugs used in the present investigation were morphine HCl, [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin, [D-Ala^2,D-Leu^5]enkephalin, U 69,593 and clonidine HCl. Experiments were also carried out with Δ^9-tetrahydrocannabinol, with the novel synthetic cannabinoid receptor agonist, *R*-(+)-arachidonyl-1′-hydroxy-2′-propylamide (Abadji et al., 1994), with noradrenaline bitartrate and with the tetrasodium salt of β,γ-methylene-L-ATP. Δ^9-Tetrahydrocannabinol and *R*-(+)-arachidonyl-1′-hydroxy-2′-propylamide were each mixed with 2 parts of Tween 80 by weight and dispersed in a 0.9% aqueous solution of NaCl (saline) as described previously (Pertwee et al., 1992). Δ^9-Tetrahydrocannabinol was supplied by the American National Institute on Drug Abuse and *R*-(+)-arachidonyl-1′-hydroxy-2′-propylamide by Professor Alexandros Makriyannis (University of Connecticut). Other drugs were dissolved in saline and were supplied by Koch Light Laboratories (noradrenaline bitartrate), MacFarlan Smith (morphine HCl), Peninsula ([D-Ala^2,D-Leu^5]enkephalin), Sigma (clonidine HCl), Upjohn (U 69,593) or Research Biochemicals International.

2.2. Vasa deferentia

These were obtained from albino MF1 mice weighing 34–65 g. 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 Mg^{2+}-free Krebs' solution kept at 37°C and bubbled with 95% O_2 and 5% CO_2. 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.

2.3. Drug-induced contractions

Dose-contractile response curves for noradrenaline and β,γ-methylene-L-ATP were constructed using dose cycles of 5 min and 15 min respectively. Some of these experiments were carried out in the presence of Δ^9-tetrahydrocannabinol, which was added 15 min before each addition of noradrenaline or β,γ-methylene-L-ATP to produce and maintain a bath concentration of 100 nM Δ^9-tetrahydrocannabinol.

2.4 Electrically evoked contractions

Isometric contractions were elicited by electrical field stimulation through a platinum electrode attached to the upper end of each bath and a stainless steel electrode attached to the lower end. 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). 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 at the end of which the bath contents were washed out by overflow. Twitch inhibitors were first added immediately after the first stimulation period and then after each bath wash, in progressively increasing doses. The time interval between each addition of a twitch inhibitor and onset of the subsequent stimulation period was always 25 min for Δ^9-tetrahydrocannabinol and 5 min for the other inhibitors.

2.5. Production of cannabinoid tolerance

Mice were injected with Δ^9-tetrahydrocannabinol (20 mg/kg i.p.) once daily for 2 days, a pretreatment that is known to induce tolerance to the inhibitory effects of cannabinoids on the twitch response of the isolated vas deferens (Pertwee et al., 1993, 1994). Control animals received Tween 80 (40 mg/kg i.p.). Mice were killed 24 h after the second injection and their vasa deferentia removed.

2.6. Analysis of data

Values are expressed as means and limits of error as standard errors. The amplitudes of drug-induced con-

tractions have been expressed in grams. Inhibition of the electrically evoked twitch response is expressed in percentage terms and has been calculated by comparing the amplitude of the twitch response immediately before the injection of a twitch inhibitor with its amplitude during each of the postinjection periods of stimulation. Degrees of tolerance to twitch inhibitors were assessed by comparing the inhibitory potency of each agent in vasa deferentia obtained from Δ^9-tetrahydrocannabinol treated mice with its potency in tissues obtained from Tween treated animals. Potency ratios and their 95% confidence limits were determined by symmetrical (2 + 2) dose parallel line assays (Colquhoun, 1971), using responses to pairs of concentrations located on the steepest part of each log concentration-response curve. In none of these assays did pairs of log concentration-response curves show significant deviation from parallelism ($P > 0.05$). The significance of differences between means was evaluated by Student's t-test for unpaired data ($P < 0.05$).

3. Results

3.1. Effects of in vivo treatment with Δ^9-tetrahydrocannabinol on the sensitivity of the vas deferens to twitch inhibitors

Vasa deferentia obtained from mice that had been injected with Δ^9-tetrahydrocannabinol did not show any detectable reduction in sensitivity to the inhibitory effects of morphine, [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin, [D-Ala^2,D-Leu^5]enkephalin, U 69,593 or clonidine on electrically evoked contractions (Table 1 and Fig. 1). This is a treatment that has previously been shown to reduce the sensitivity of isolated vasa deferentia to the inhibitory effects on evoked contractions of several cannabinoids, including Δ^9-tetrahydrocannabinol, CP 55,940, WIN 55,212-2 and anandamide (Pertwee et al., 1993, 1994; and Table 1). The same treatment with Δ^9-tetrahydrocannabinol has now also been shown to induce tolerance to the cannabinoid

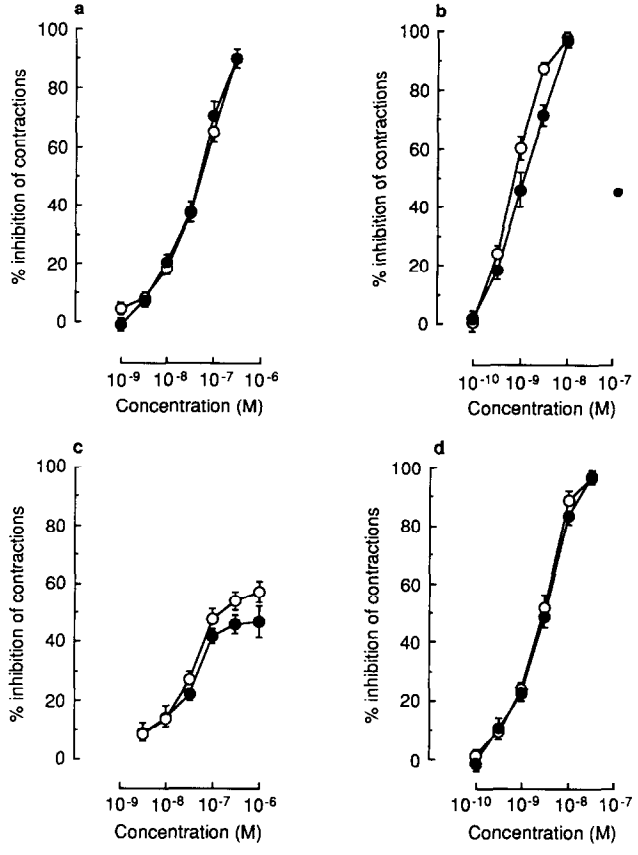

Fig. 1. Mean concentration-response curves for (a) [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin, (b) [D-Ala^2,D-Leu^5]enkephalin, (c) U 69,593 and (d) clonidine in vasa deferentia obtained from mice pretreated once daily for 2 days with Δ^9-tetrahydrocannabinol at a dose of 20 mg/kg i.p. (filled circles) or with Tween 80 (open circles) at a dose of 40 mg/kg i.p. 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 [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin, [D-Ala^2,D-Leu^5]enkephalin, U 69,593 or clonidine to the organ bath (n = 7 or 8 different vasa deferentia). The relative potencies of these compounds in Tween pretreated versus Δ^9-tetrahydrocannabinol pretreated tissues are respectively 0.8 (0.6 and 1.2), 1.2 (1.0 and 1.4), 1.4 (1.0 and 1.8) and 1.1 (0.9 and 1.4) (mean and 95% confidence limits). The degree of inhibition produced by 316 nM or 1000 nM U 69,593 was not significantly less in tissues obtained from Δ^9-tetrahydrocannabinol pretreated mice than in tissues obtained from control animals (Student's t-test).

Table 1
Effect of in vivo treatment with Δ^9-tetrahydrocannabinol (Δ^9-THC) on inhibition of electrically evoked contractions of mouse isolated vasa deferentia by morphine and Δ^9-THC added in vitro

In vivo treatment drug	In vivo treatment dose (mg/kg i.p.)	In vitro agonist	In vitro concentration (nM)	Potency ratio	95 % confidence limits	n
Δ^9-THC + Tween	20 + 40	Δ^9-THC	31.6, 100	30.0 [a]	17.5 and 83.3 [a]	12
Tween	40	Δ^9-THC	3.16, 10			
Δ^9-THC + Tween	20 + 40	Morphine	100, 1000	1.6	1.0 and 2.6	8
Tween	40	Morphine	100, 1000			

The potency ratios listed above indicate the extents by which in vivo treatment with Δ^9-THC reduced the potencies of Δ^9-THC and morphine as inhibitors of electrically evoked contractions of isolated vasa deferentia.
[a] From Pertwee et al. (1993).

receptor agonist, *R*-(+)-arachidonyl-1′-hydroxy-2′-propylamide (Fig. 2), confirming that this treatment does indeed reduce the sensitivity of vasa deferentia to cannabinoids.

3.2. Effects of Δ^9-tetrahydrocannabinol on the sensitivity of the vas deferens to the contractile effects of noradrenaline and β,γ-methylene-L-ATP

Vasa deferentia taken from Δ^9-tetrahydrocannabinol treated mice did not show any detectable change in sensitivity to the contractile effects of noradrenaline or β,γ-methylene-L-ATP (Fig. 3). Nor was the ability of noradrenaline or β,γ-methylene-L-ATP to induce contractions of mouse isolated vasa deferentia significantly affected by Δ^9-tetrahydrocannabinol when this was added in vitro at a concentration of 100 nM (Fig. 4). This is a concentration of Δ^9-tetrahydrocannabinol that produces a marked inhibition of electrically evoked contractions (Pertwee et al., 1992, 1993).

4. Discussion

Our results indicate that the contractile potencies of noradrenaline and β,γ-methylene-L-ATP are the same in vasa deferentia taken from Δ^9-tetrahydrocannabinol pretreated mice as in tissues obtained from control animals. Our experiments also indicate that pretreatment with Δ^9-tetrahydrocannabinol does not induce tolerance to the non-cannabinoid twitch inhibitors

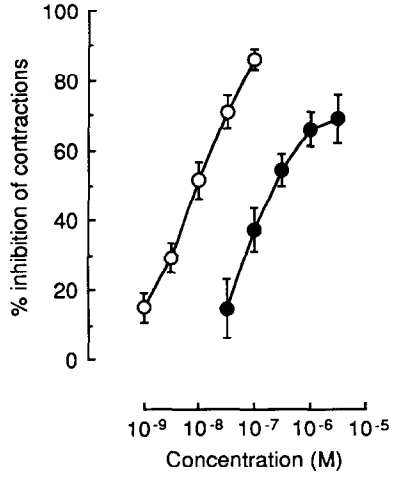

Fig. 2. Mean concentration-response curves for *R*-(+)-arachidonyl-1′-hydroxy-2′-propylamide in vasa deferentia obtained from mice pretreated once daily for 2 days with Δ^9-tetrahydrocannabinol at a dose of 20 mg/kg i.p. (filled circles) or with Tween 80 (open circles) at a dose of 40 mg/kg i.p. Each symbol represents mean tension ± S.E. ($n = 14$ different vasa deferentia). The relative potency of *R*-(+)-arachidonyl-1′-hydroxy-2′-propylamide in Tween pretreated versus Δ^9-tetrahydrocannabinol pretreated tissues is 24.3 (14.4 and 45.3) (mean and 95% confidence limits).

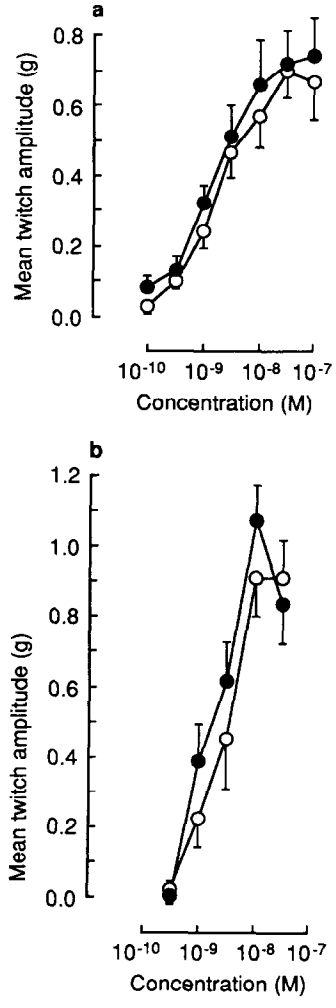

Fig. 3. Mean concentration-response curves for (a) noradrenaline and (b) β,γ-methylene-L-ATP in vasa deferentia obtained from mice pretreated once daily for 2 days with Δ^9-tetrahydrocannabinol at a dose of 20 mg/kg i.p. (filled circles) or with Tween 80 (open circles) at a dose of 40 mg/kg i.p. Each symbol represents mean tension ± S.E. ($n = 6$–8 different vasa deferentia). The relative potencies of noradrenaline and β,γ-methylene-L-ATP in Tween pretreated versus Δ^9-tetrahydrocannabinol pretreated tissues are respectively 0.6 (0.2 and 1.3) and 0.6 (0.3 and 1.2) (mean and 95% confidence limits).

morphine, [D-Ala^2,*N*-Mc-Phc^4,Gly-ol^5]enkcphalin, [D-Ala^2,D-Leu^5]enkephalin, U 69,593 or clonidine. Previous experiments have shown that the Δ^9-tetrahydrocannabinol treatment used in the present study does produce cannabinoid tolerance in the mouse vas deferens (Pertwee et al., 1993, 1994). This was confirmed in the present investigation by the demonstration that this treatment renders isolated vasa deferentia tolerant to the inhibitory effect of the synthetic cannabinoid receptor agonist, *R*-(+)-arachidonyl-1′-hydroxy-2′-propylamide. We conclude from these results that the production of cannabinoid tolerance in the vas deferens does not depend on the development of supersensitivity of this tissue to noradrenaline or ATP, its main contractile neurotransmitters (Stjärne and Åstrand,

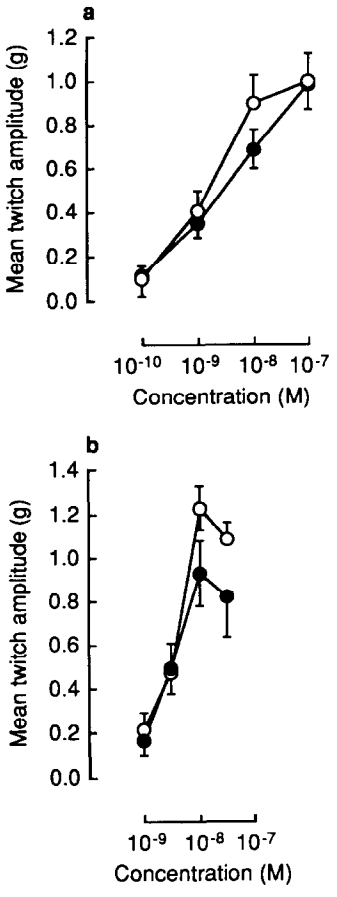

Fig. 4. Mean concentration-response curves for (a) noradrenaline and (b) β,γ-methylene-L-ATP in mouse isolated vasa deferentia constructed in the presence of 100 nM Δ^9-tetrahydrocannabinol (filled circles) or in its absence (open circles). Each symbol represents mean tension ± S.E. ($n = 6$ different vasa deferentia). The contractile effect of 10 nM or 31.6 nM β,γ-methylene-L-ATP or of 10 nM noradrenaline was not significantly less in the presence of Δ^9-tetrahydrocannabinol than in its absence (Student's t-test).

1985; Von Kügelgen et al., 1989b). We also conclude that cannabinoid tolerance cannot be attributed to downregulation of prejunctional μ-, δ-, κ-opioid receptors or α_2-adrenoceptors, at least in the vas deferens. Whilst these results support the hypothesis that the development of cannabinoid tolerance in the mouse vas deferens takes place at cannabinoid-specific sites such as cannabinoid receptor recognition sites, the evidence for this is not conclusive. This is because, in addition to opioid receptors and α_2-adrenoceptors, the vas deferens contains several other non-cannabinoid receptors that are capable of mediating inhibition of the twitch response, for example purinergic receptors and receptors for γ-aminobutyric acid, 5-hydroxytryptamine, dopamine and neuropeptide Y (Seong et al., 1990; Smith and Rowland, 1989; Stjärne et al., 1986; Stone, 1981; Von Kügelgen et al., 1989b).

If it transpires that Δ^9-tetrahydrocannabinol can specifically desensitize the vas deferens to cannabinoids, it will become possible to distinguish a cannabinoid from a non-cannabinoid twitch inhibitor by establishing whether or not the inhibitor is less potent in cannabinoid-tolerant vasa deferentia than in a non-tolerant tissue. There is still a need for such a bioassay as, apart from binding assays or assays that rely on the measurement of responses mediated by cannabinoid receptors that have been transfected into cells (Felder et al., 1992; Howlett et al., 1992), all existing cannabinoid bioassays that are capable of providing quantitative data with reasonable speed, lack specificity. In line with the hypothesis that tolerance can develop specifically to cannabinoids, is evidence from previous investigations that onset of cannabinoid tolerance is accompanied by a decrease in cannabinoid receptor density as measured by the maximum binding capacity of cannabinoid receptors (B_{max}) (Oviedo et al., 1993; De Fonseca et al., 1994). However, there is also evidence firstly, that it is possible for cannabinoid tolerance to develop without any concomitant reduction in B_{max} (Abood et al., 1993) and secondly, that Δ^9-tetrahydrocannabinol can induce tolerance to non-cannabinoids (see Introduction).

Finally, our experiments have shown that a concentration of Δ^9-tetrahydrocannabinol that markedly inhibits electrically induced contractions of the mouse vas deferens (100 nM) does not decrease the ability of noradrenaline or β,γ-methylene-L-ATP to induce contractions of this tissue. Consequently, it is unlikely that the inhibitory effect of Δ^9-tetrahydrocannabinol on electrically evoked contractions of the vas deferens stems from any ability of this drug to reduce the sensitivity of the smooth muscle of this tissue to the excitatory actions of noradrenaline or ATP, both of which are presumably released from prejunctional nerve terminals during electrical stimulation (Stjärne and Åstrand, 1985; Von Kügelgen et al., 1989a,b). It follows, therefore, that Δ^9-tetrahydrocannabinol probably exerts its inhibitory effect on the electrically evoked twitch response of the vas deferens by acting prejunctionally to decrease the release of excitatory neurotransmitters into the neuromuscular junction rather than by acting directly on the smooth muscle of this tissue.

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