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Inhibitory effects of certain enantiomeric cannabinoids in the mouse vas deferens and the myenteric plexus preparation of guinea-pig small intestine

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1 The psychoactive cannabinoids (−)-Δ^9-tetrahydrocannabinol ((−)-Δ^9-THC) and the 1,1-dimethylheptyl homologue of (−)-11-hydroxy-Δ^8-tetrahydrocannabinol ((−)-DMH) both inhibited electrically-evoked contractions of the mouse isolated vas deferens and the myenteric plexus-longitudinal muscle preparation of the guinea-pig small intestine.

2 Concentrations of (−)-Δ^9-THC and (−)-DMH that decreased twitch heights by 50% were 6.3 and 0.15 nM respectively in the mouse vas deferens and 60 nM and 1.4 nM respectively in the myenteric plexus preparation. (−)-DMH was about 40 times more potent than (−)-Δ^9-THC in both preparations, supporting the notion that their mode of action in each tissue is the same.

3 The psychically inactive cannabinoid, (+)-DMH, had no inhibitory effect in the mouse vas deferens at a concentration of 30 nM, showing it to be at least 1000 times less potent than (−)-DMH. In the myenteric plexus preparation, (+)-DMH was about 500 times less potent than its (−)-enantiomer.

4 The inhibitory effects of sub-maximal concentrations of (−)-Δ^9-THC were not attenuated by 300 nM naloxone.

5 The findings that (−)-Δ^9-THC and (−)-DMH are highly potent as inhibitors of the twitch response of the mouse vas deferens and guinea-pig myenteric plexus preparation and that DMH shows considerable stereoselectivity suggest that the inhibitory effects of cannabinoids in these preparations are mediated by cannabinoid receptors.

Keywords: Δ^9-Tetrahydrocannabinol; cannabinoids; mouse vas deferens; myenteric plexus; guinea-pig small intestine; stereoselectivity

Introduction

Cannabinoids owe many of their pharmacological properties to an ability to produce functional changes in neuronal membranes and there is good evidence that this ability depends on the molecular shape of cannabinoids rather than on their marked lipophilicity (Thomas *et al.*, 1990; Pertwee, 1990). Some structure-dependent effects of cannabinoids may be produced by non-receptor-mediated processes involving, for example, the induction of conformational changes in membrane phospholipids (Hillard *et al.*, 1990; Pertwee, 1990). Other effects, however, are now thought to result from the activation of cannabinoid receptors, there being evidence that cannabinoids are highly potent, that they show marked chemical and stereochemical selectivity (Pertwee, 1990), that certain areas of the brain in which the cannabinoids are thought to initiate some of their central effects contain specific, high affinity cannabinoid binding sites (Bidaut-Russell *et al.*, 1990; Devane *et al.*, 1988; Herkenham *et al.*, 1990; 1991) and that functional cannabinoid receptors can be cloned (Matsuda *et al.*, 1990).

This investigation was directed at identifying isolated tissue preparations that would serve as models with which to elucidate further the modes of action of psychotropic cannabinoids. Our strategy was to identify tissues in which cannabinoids show a significant degree of stereoselectivity and in which (−)-Δ^9-tetrahydrocannabinol [(−)-Δ^9-THC], the main psychoactive constituent of cannabis, produces effects when present in the medium at concentrations yielding tissue levels no higher than those achieved following *in vivo* administration of (−)-Δ^9-THC at submaximal psychotropic doses (below 1 μM; Pertwee, 1990). The present experiments compared the potency of certain enantiomeric cannabinoids as inhibitors of electrically-evoked contractions of the myenteric plexus-longitudinal muscle preparation of guinea-pig small intestine which was selected for study because its response to electrical stimulation was already known to be readily inhibited by psychotropic cannabinoids (Pertwee, 1990) and because cannabinoids can inhibit intestinal motility *in vivo* (Anderson *et al.*, 1975). The effects of cannabinoids on evoked contractions of a second nerve-smooth muscle preparation, the mouse isolated vas deferens, were also investigated. The cannabinoids studied were (−)-Δ^9-THC (see above) and the 1,1-dimethylheptyl homologues of (+)- and (−)-11-hydroxy-Δ^8-tetrahydrocannabinol [abbreviated to (+)- and (−)-DMH]. (+)- and (−)-DMH were chosen because only the (−)-enantiomer is psychoactive (Little *et al.*, 1989; Pertwee & Wickens, 1991) and because the stereochemical purity of the two isomers is particularly high (Mechoulam *et al.*, 1990). The chemical structures of (−)-Δ^9-THC and DMH are shown in Figure 1.

Some of the results described in this paper have been presented to the British Pharmacological Society (Pertwee *et al.*, 1991).

Methods

Tissue preparations

All tissues were mounted in 3 ml organ baths under an initial tension of 0.5 g. Isometric contractions were evoked by elect-

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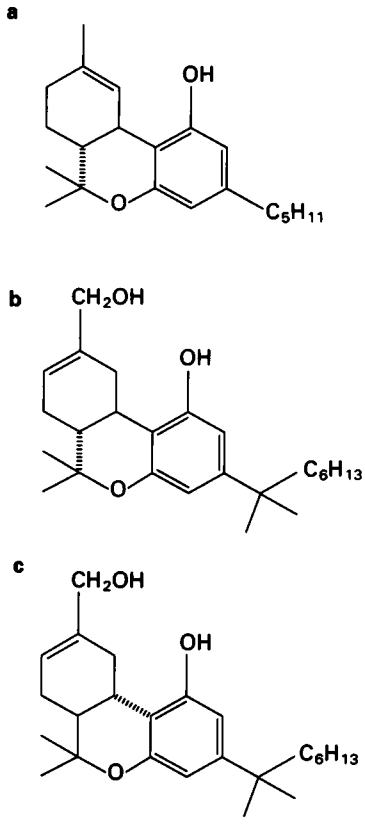

Figure 1 The chemical structures of (a) (−)-Δ^9-tetrahydrocannabinol [(−)-Δ^9-THC] and of the 1,1-dimethylheptyl homologues of (b) (−)- and (c) (+)-11-hydroxy-Δ^8-tetrahydrocannabinol [(−)- and (+)-DMH].

rical field stimulation through platinum electrodes attached to the upper and lower ends of each bath and were registered on a polygraph recorder (Grass model 7D) using Pye Ether UF1 transducers. The baths contained Krebs-Henseleit solution which was kept at 37°C and bubbled with 5% CO_2 in 95% O_2. They were siliconized at the beginning of each day (Sigmacote) and rinsed between experiments with ethanol followed by distilled water. Drug additions were made in volumes of 10 or 30 μl after the tissues had equilibrated. Only one dose of one drug was added to each tissue, pilot experiments having shown that the rates of onset of action of the cannabinoids used in this investigation are slow and that it is impossible to reverse their effects by perfusing the organ baths with drug-free Krebs-Henseleit solution. Once a drug had been added, tissues were incubated for up to 210 min without replacing the fluid in the bath.

Strips of myenteric plexus-longitudinal muscle were dissected from the small intestine of male albino Dunkin-Hartley guinea-pigs (180–400 g) by the method of Paton & Zar (1968) and set up for field stimulation in an inverted 'V' configuration. Each tissue was bathed in Krebs-Henseleit solution of the following composition (mM): NaCl 118.2, KCl 4.75, $MgSO_4.7H_2O$ 1.29, KH_2PO_4 1.19, $NaHCO_3$ 25.0, glucose 11.0 and $CaCl_2.6H_2O$ 2.54. The solution also contained hexamethonium bromide (70 μM), mepyramine maleate (0.125 μM) and choline chloride (20 μM) (Kosterlitz *et al.*, 1970) and was stimulated with single bipolar rectangular pulses of 110% maximal voltage, 0.5 ms duration and 0.1 Hz frequency.

Vasa deferentia from TO mice (33–49 g) were bathed with Mg^{2+}-free Krebs-Henseleit solution (Hughes *et al.*, 1975; Corbett *et al.*, 1984). The tissue was stimulated with trains of 3 pulses of 110% maximal voltage and 0.5 ms duration at intervals of 250 ms. The trains were repeated at a frequency of 0.1 Hz. Pilot experiments showed that the amplitude of the twitch response of untreated vasa deferentia usually decreased quite markedly with time when trains of stimuli were applied continuously but remained constant when the tissue was subjected to 10 min periods of stimulation separated by 60 min periods in which there was no stimulation. Consequently, this pattern of intermittent stimulation was adopted for all the vas deferens experiments, the drug additions being made at the end of the first 11 min period of stimulation.

Drugs

(+)- and (−)-DMH (code numbers HU-211 and HU-210 respectively) were synthesized at the Hebrew University, Jerusalem, Israel (Mechoulam *et al.*, 1990) and (−)-Δ^9-THC was donated by the National Institute on Drug Abuse, U.S.A. Naloxone hydrochloride was obtained from Sigma and was dissolved in 0.9% w/v NaCl solution (saline). The cannabinoids were stored as ethanolic solutions which were kept in the dark at −20°C. Each cannabinoid was prepared for administration by mixing it with 2 parts of Tween 80 by weight, removing the ethanol by evaporation and then adding saline to form a 1 ml dispersion containing 30 nmol of the drug. To ensure that the dispersion would be homogeneous, the saline was added in a series of aliquots of increasing volume (50 μl × 2, 100 μl × 2, 200 μl and 500 μl), the mixture being shaken between additions with a vortex mixer. Dilutions were made serially, each dilution step involving the mixture of 1 volume of dispersion with up to 9 volumes of saline. Fresh dispersions were made up each day and were protected from light. Control experiments were performed with Tween 80.

Analysis of data

Values have been expressed as means and limits of error as standard errors. The degree of inhibition of the twitch response is expressed in percentage terms and has been calculated by comparing the amplitude of the electrically-evoked twitch response immediately before drug administration with the amplitude of the twitch response at various times after drug administration. The concentration of a cannabinoid that causes 50% inhibition of the twitch response (IC_{50}) was used to characterize its potency. Non-linear regression analysis of the data obtained in our experiments with (−)-Δ^9-THC and (−)-DMH (GraphPAD InPlot, GraphPAD Software, San Diego) showed the log concentration-response curves of these drugs in the vas deferens and myenteric plexus-longitudinal muscle preparation to be sigmoidal (correlation coefficients = 0.998 to 1.0). The log concentration at the midpoint of each of the sigmoid curves generated from our data by GraphPAD InPlot was estimated ($pD_2 \pm$ s.e.).

Results

(−)-Δ^9-THC and (−)-DMH each produced concentration-dependent decreases in the amplitude of evoked contractions in both the myenteric plexus preparation and the mouse vas deferens (Figures 2 to 4). Figure 2 shows the time courses for inhibition of the evoked twitch response of the myenteric plexus preparation by various concentrations of these drugs and indicates that they were both rather slow in their onset of action. The rate of onset of inhibition was concentration-dependent, the degree of inhibition reaching a plateau progressively more quickly as the concentration of either drug was increased. At concentrations that were approximately equi-effective, the onset of action of (−)-DMH was even slower than that of (−)-Δ^9-THC (Figure 2). In the mouse vas deferens, (−)-Δ^9-THC produced its maximal inhibitory effect within 70 min and (−)-DMH within 140 min (Figure 5). Log concentration-response curves (Figures 3 and 4) were constructed from measurements made after the inhibitory effects of these drugs had reached a maximum. For (−)-Δ^9-THC,

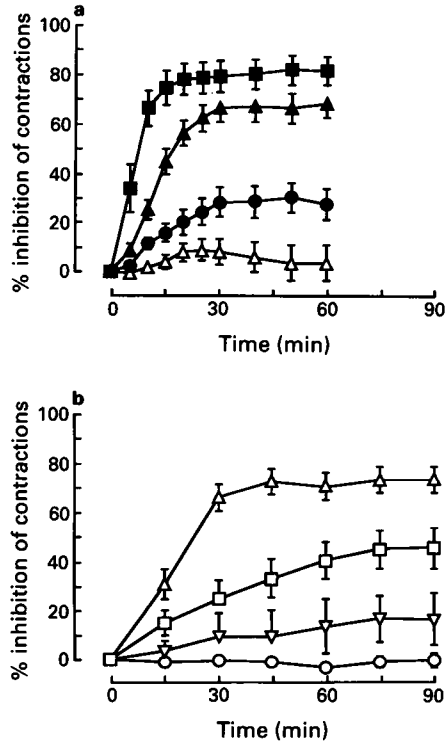

Figure 2 Time courses of the inhibitory effects of (−)-Δ^9-tetrahydrocannabinol ((−)-Δ^9-THC, a) and the 1,1-dimethylheptyl homologue of (−)-11-hydroxy-Δ^8-tetrahydrocannabinol ((−)-DMH, b) on the electrically-evoked contractions of the myenteric plexus-longitudinal muscle preparation of guinea-pig small intestine. The symbols represent mean % inhibition of the twitch response by cannabinoid concentrations of 10^{-6} M (■), 10^{-7} M (▲), 3×10^{-8} M (●), 10^{-8} M (△), 10^{-9} M (□) or 3×10^{-10} M (▽). The effect of Tween 80 added in the amount required to produce a cannabinoid bath concentration of 10^{-6} M is denoted by (○). The vertical bars show standard errors ($n = 6$).

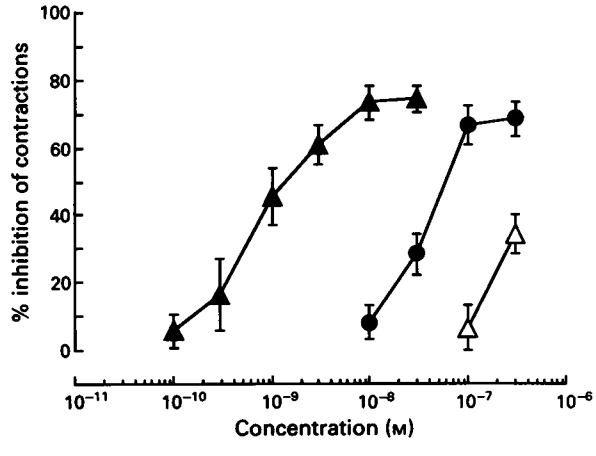

Figure 3 Effect of (−)-Δ^9-tetrahydrocannabinol ((−)-Δ^9-THC, ●), the 1,1-dimethylheptyl homologue of (−)-11-hydroxy-Δ^8-tetrahydrocannabinol ((−)-DMH, ▲) and (+)-DMH (△) on the size of electrically-evoked contractions of the myenteric plexus-longitudinal muscle preparation of guinea-pig small intestine measured 30 min ((−)-Δ^9-THC) or 90 min after drug administration. The symbols represent mean % inhibition of the twitch response and the vertical bars show standard errors ($n = 6$).

the measurements used were those made 30 min after its addition to the myenteric plexus preparation and 60 to 70 min after its addition to the vas deferens. For (+)- and (−)-DMH, the measurements used were those made 90 min after their addition to the first of these preparations and 130 to 140 min after their addition to the second.

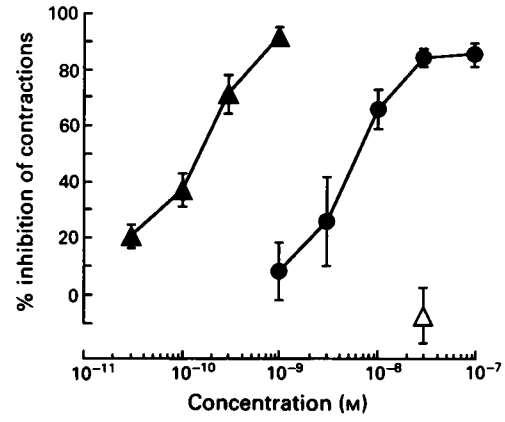

Figure 4 Effect of (−)-Δ^9-tetrahydrocannabinol ((−)-Δ^9-THC, ●), the 1,1-dimethylheptyl homologue of (−)-11-hydroxy-Δ^8-tetrahydrocannabinol ((−)-DMH, ▲) and (+)-DMH (△) on the size of electrically-evoked contractions of the mouse vas deferens measured 60 min ((−)-Δ^9-THC) or 130 min after drug administration. The symbols represent mean % inhibition of the twitch response and the vertical bars show standard errors ($n = 6$).

By itself, the vehicle Tween 80 did not inhibit the twitch response of either preparation when it was added at a dose 3.3 times above the upper limit of its dose-range in the cannabinoid experiments. Indeed, additions of this dose of Tween 80 were followed by slight increases in twitch amplitude. More specifically, when Tween 80 was added to the mouse vas deferens in the amount (1.9 μg) that would have been required to expose the tissue to a (−)-Δ^9-THC concentration of 1 μM, the mean twitch response increased by 12.2 ± 7.3%, over the first 70 min, and by 4.8 ± 4.1%, over 140 min ($n = 6$). In the myenteric plexus preparation, addition of 1.9 μg Tween 80 was followed by an increase in mean twitch amplitude of 0.7 ± 1.9%, over the first 30 min, and of 0.6 ± 2.9%, over 90 min ($n = 6$). In contrast, a dose of 5.7 μg of Tween 80, which is 10 times above the upper limit of the dose range of vehicle used in the (−)-Δ^9-THC experiments, did inhibit the twitch response of the myenteric plexus preparation, the degree of inhibition after 30 and 90 min being respectively 16.8 ± 4.7% and 42.7 ± 9.5% ($n = 6$). Each of these mean changes in twitch response are significantly differ-

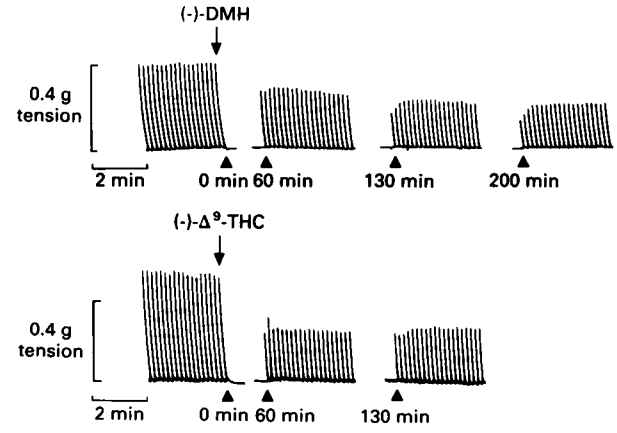

Figure 5 The effects of the 1,1-dimethylheptyl homologue of (−)-11-hydroxy-Δ^8-tetrahydrocannabinol ((−)-DMH) and of (−)-Δ^9-tetrahydrocannabinol ((−)-Δ^9-THC) on electrically-evoked twitch contractions of the mouse isolated vas deferens. The drugs were added at time zero. The preparations were stimulated for 11 min before drug addition and then intermittently for 10 min periods commencing 60, 130 or 200 min after drug addition. They were stimulated with trains of 3 pulses of 110% maximal voltage and 0.5 ms duration at intervals of 250 ms. The trains were repeated at a frequency of 0.1 Hz.

ent ($P<0.01$; Student's t test for unpaired data) from those observed to occur at the corresponding time after the addition of 1.9 μg of Tween 80 (see above).

As shown in Figures 3 and 4, concentrations of (−)-Δ^9-THC that decreased twitch heights by 50% were 60 nM in the myenteric plexus preparation ($pD_2 = 1.668 \pm 0.037$) and 6.3 nM in the vas deferens ($pD_2 = 0.73 \pm 0.03$). For (−)-DMH, the corresponding IC_{50} values were respectively 1.4 nM ($pD_2 = -0.124 \pm 0.085$) and 0.15 nM ($pD_2 = -0.716 \pm 0.006$), indicating that in both preparations it is about 40 times more potent than (−)-Δ^9-THC as an inhibitor of the twitch response. Although (+)-DMH also inhibited the twitch response of the myenteric plexus preparation, it was about 500 times less potent than its (−)-isomer (Figure 3). At a concentration of 30 nM, (+)-DMH had no detectable effect on the evoked twitch response of the mouse vas deferens, the results obtained demonstrating that, in this preparation, (−)-DMH is at least 1000 times more potent than (+)-DMH (Figure 4).

The ability of submaximal concentrations of (−)-Δ^9-THC to inhibit the twitch response of the myenteric plexus preparation (75 nM) or the mouse vas deferens (10 nM) was not attenuated by naloxone, 300 nM, when this was added 10 min before the cannabinoid. Nor were the effects of these concentrations of (−)-Δ^9-THC on either preparation reversed by naloxone (300 nM), added 60 or 70 min after the cannabinoid (data not shown).

Discussion

The results obtained in the present study demonstrate that cannabinoids can produce a concentration-related inhibition of the electrically-evoked twitch response of the mouse isolated vas deferens and of the myenteric plexus preparation of the guinea-pig small intestine. More importantly, they demonstrate the 1,1-dimethylheptyl homologue of 11-hydroxy-Δ^8-tetrahydrocannabinol (DMH) to be highly potent as an inhibitor of the twitch response of both preparations and to exhibit a remarkable degree of stereoselectivity, (−)-DMH being considerably more potent than (+)-DMH. The results from the experiments with (−)-Δ^9-THC showed that this cannabinoid can also inhibit the electrically-evoked twitch response of the mouse isolated vas deferens and, in addition, confirmed its ability to inhibit the twitch response of the myenteric plexus preparation (Pertwee, 1990). Our findings are in agreement with a report, published during the preparation of this paper, that (−)-Δ^9-THC is a potent inhibitor of the twitch response of the vas deferens of Swiss Webster mice (Pacheco *et al.*, 1991). Indeed, Pacheco *et al.* (1991) found the IC_{50} of (−)-Δ^9-THC to be 4 nM which is very close to the IC_{50} of this drug determined in the present investigation with TO mice (6.3 nM). Our findings are also consistent with an earlier observation that (−)-Δ^9-THC is more potent than its (+)-isomer in inhibiting the twitch response of the guinea-pig myenteric plexus preparation (Roth, 1978). However, the potency difference between (−)- and (+)-DMH observed in the present experiments with the myenteric plexus preparation (about × 500) was much greater than the potency difference between (−)- and (+)-Δ^9-THC (× 25) observed by Roth (1978), an indication perhaps that the samples of (−)- and (+)-DMH used in this investigation were of a higher stereochemical purity than the samples of (−)- and (+)-Δ^9-THC used in the previous study (Mechoulam *et al.*, 1990).

The electrically-evoked twitch response of the myenteric plexus preparation of guinea-pig small intestine and of the mouse vas deferens can be readily inhibited by opioid receptor agonists (Lesley, 1987). It is unlikely, however, that cannabinoids inhibit the twitch response of these preparations by acting through opioid receptors, as it was found in the present study that the inhibitory effect of (−)-Δ^9-THC on the twitch response of the myenteric plexus preparation and mouse vas deferens was not attenuated by naloxone when this was applied at a concentration known to antagonize μ, δ, and κ opioid receptors (300 nM; Kosterlitz & Paterson, 1990).

An important practical difficulty associated with the study of the pharmacology of cannabinoids, is the relatively low aqueous solubility of this group of drugs (Roth & Williams, 1979). This property together with their high lipophilicity (Roth & Williams, 1979; Thomas *et al.*, 1990) may well have accounted for the rather slow onset of action of (−)-Δ^9-THC and DMH that was observed in this investigation. More specifically, it is likely that the lipophilic nature of these cannabinoids will cause them to be sequestered by the solubilizing agent, Tween 80 (Roth & Williams, 1979) and will delay equilibration between cannabinoid molecules in free solution and those present in the tissue. The low solubility of these drugs in water would be expected to delay their onset of action by limiting their rate of diffusion across the aqueous phase from solubilizing agent to tissue. The finding that the rate of onset of action of (−)-DMH was less than that of (−)-Δ^9-THC is consistent with these ideas as (−)-DMH is known to be somewhat more lipophilic than (−)-Δ^9-THC (Thomas *et al.*, 1990). Interestingly, (−)-DMH has also been reported to have a slower onset of action than (−)-Δ^9-THC *in vivo* (Järbe *et al.*, 1989).

(−)-DMH and (−)-Δ^9-THC were each about 10 times more potent as inhibitors of the twitch response in the mouse vas deferens than in the myenteric plexus preparation, (−)-DMH being the more potent of the two cannabinoids in both preparations. (+)-DMH was less potent than (−)-Δ^9-THC, the order of potency of the three cannabinoids studied correlating with their psychotropic activity (Little *et al.*, 1989). The relative potency of these cannabinoids was approximately the same in the myenteric plexus preparation as in the mouse isolated vas deferens, supporting the notion that their mode of action in each tissue is the same. A fuller comparison of the structural requirements for inhibition of the twitch response with those for psychotropic activity is now required, as such experiments would help to establish whether the myenteric plexus preparation and the mouse vas deferens could be used as models for studying the central pharmacology of cannabinoids or whether they are more suitable as models for investigating the peripheral pharmacology of these drugs. The observations made in this investigation, that (−)-Δ^9-THC and (−)-DMH are highly potent as inhibitors of the twitch response of the guinea-pig myenteric plexus preparation and the mouse vas deferens and that DMH shows considerable stereoselectivity, suggest that inhibition of the twitch response by cannabinoids in both tissues are mediated by cannabinoid receptors. If such receptors are indeed present, it should be possible to demonstrate that, like certain areas of the brain (Herkenham *et al.*, 1990; 1991), the guinea-pig small intestine and the mouse vas deferens contain specific high affinity cannabinoid binding sites.

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