Δ^9-Tetrahydrocannabinol: Effects on Mammalian Nonmyelinated Nerve Fibers

Abstract. *Δ^9-Tetrahydrocannabinol can be applied to tissue in vitro by dissolving it in Pluronic F68 and ethanol. It causes a decrease in size of the compound action potential of the nonmyelinated fibers of the vagus nerve of the rabbit. This effect appears to be dose-related and chloride-dependent. Effects on other measurable parameters of nerve function seem to be minimal. Although the amounts required seem to be higher than those required to produce hallucinogenic effects in man, this effect is consistent with other work on Δ^9-tetrahydrocannabinol and may ultimately account for a significant portion of the pharmacological activity of this drug.*

Despite a great interest in the pharmacological properties of marihuana constituents there is almost no information about the effects of these substances on elemental nervous tissue. This lack of information is largely the result of the difficulty in dissolving the tetrahydrocannabinols in nontoxic aqueous media. We report here first, a method of dissolving Δ^9-tetrahydrocannabinol (Δ^9THC) in an innocuous medium, and second, a well-defined effect of Δ^9THC on nervous conduction.

Δ^9-Tetrahydrocannabinol is not readily soluble in water, which presents a major difficulty in studies of this compound on isolated tissues. It can be carried into solution by Tween 80 (*1*), but the high concentrations of the detergent required (up to 2 percent) are themselves toxic to many preparations. In the experiments reported here, which examine the effect of Δ^9THC on mammalian nonmyelinated nerve fibers, the difficulty has been overcome through the use of Pluronic F68 (*2*). This is a high molecular weight surfactant compound that has general applications in bringing into solution compounds which are otherwise insoluble in water; for example, the present experiments suggested its use (successfully) in studies of axon fluorescence with a relatively water-insoluble merocyanine dye (*3*). A small amount of Pluronic (100 mg) was dissolved (by gentle heating to less than 60°C) in 0.15 or 0.2 ml of ethanol containing 15 mg of Δ^9THC (*4*). The resulting solution could be readily dissolved in 100 ml of Locke solution (*5*) to produce a 500 μM solution of Δ^9THC. This solution, which was initially clear, became increasingly cloudy over the next few hours, as if some colloidal suspension of Δ^9THC were being formed. No analytical determinations of the amounts of Δ^9THC in solution were made. All experiments to test solutions of Δ^9THC were started within half an hour of preparation.

A desheathed cervical vagus nerve from a rabbit, killed by injection of air into an ear vein, was mounted either for monophasic electrical recording in the sucrose gap apparatus (*6*), or for diphasic recording in a glass capillary chamber (1 mm in diameter) in which five annular platinum electrodes for stimulation and recording were embedded (*7*). The compound action potential of the nonmyelinated fibers was elicited every minute; the conduction distance was 10 to 25 mm. The temperature was about 20°C. Changing the perfusion fluid from normal Locke solution to one containing Pluronic F68 (100 mg/100 ml) and ethanol (0.2 ml/100 ml) produced little or no effect on the size of the compound action potential (or on the size of the spike in the giant fibers of lobster and squid used in later experiments). However, as Fig. 1 shows, changing to a solution that also contained 500 μM Δ^9THC produced a fall in the height of the compound action potential of 15.2 ± 1.6 percent (mean ± standard error, 12 experiments) by the end of 30 minutes. About half of the experiments were done with monophasic recording, and the other half with diphasic recording. No significant difference was seen in the two groups, so the results of all experiments were treated as a single population. In four experiments a similar exposure to 100 μM Δ^9THC produced an average fall of 12.9 ± 3.7 percent, and in four experiments an exposure to 30 μM Δ^9THC produced an average fall of 8.7 ± 2.8 percent. In three experiments 10 μM Δ^9THC produced a fall of 7.3 ± 1.7 percent. In two experiments 3 μM Δ^9THC produced just visible responses of 0 and 2.8 percent.

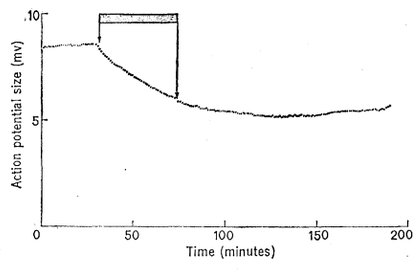

Fig. 1. Effect of 500 μM Δ^9THC, applied to the nerve during the interval between the arrows, on the size of the monophasic compound action potential of the nonmyelinated fibers of the desheathed rabbit cervical vagus nerve at 21.1°C.

The effect (Fig. 1) continued to develop during the time of exposure to the drug (up to 1 hour). In seven experiments with 500 μM Δ^9THC the average size of the compound action potential fell by 7.5, 13.3, and 18.6 percent of its initial value after exposures to the drug of 15, 30, and 45 minutes, respectively. In experiments in which the exposure to Δ^9THC was further continued, the fall after 1 hour (four experiments) was about twice, and after 4 hours (one experiment) four times, that obtained after a 30-minute exposure. When the Δ^9THC was removed and the nerve was returned to Locke solution containing just Pluronic F68 and ethanol, the height of the compound action potential always stopped declining. It usually remained constant as long as it was subsequently observed (30 to 60 minutes), although on the rare occasions when it was followed for 1 to 2 hours (as in Fig. 1), some late recovery toward the pre-Δ^9THC values sometimes did occur. A similar delayed recovery from the tetrahydrocannabinols, still incomplete after many hours, had already been observed in experiments in which the guinea pig ileum was stimulated transmurally (*8*).

The reduction in size of the compound action potential seemed to be accompanied by a slowing in conduction velocity. This effect was, however, small. In five experiments a 30-minute exposure to 500 μM Δ^9THC caused the conduction velocity to fall by 2.7 ± 0.8 percent. However, since a 30-minute exposure to normal Locke solution was itself accompanied by a slight decrease in conduction velocity the true effect of Δ^9THC was smaller, being 1.8 ± 0.9 percent. There was no marked or consistent effect on the electrical threshold, nor was there any evidence that the sodium pump was affected, 500 μM Δ^9THC producing no great change in the time constant of decay of the post-tetanic hyperpolarization recorded with the nerves in a Locke solution in which

75 to 100 percent of the chloride had been replaced by isethionate (*9*).

Complete replacement of the chloride of the Locke solution by the presumably impermeant anion isethionate led to a marked reduction in the effect of Δ^9THC. In six experiments a 30-minute exposure to 500 μM Δ^9THC produced a decrease of 17.2 ± 2.5 percent in the height of the compound action potential when applied in normal Locke solution containing chloride; in similar experiments on paired nerves from the same rabbits carried out in chloride-free Locke solution the fall was only 5.8 ± 1.7 percent.

These experiments demonstrated that Δ^9THC directly affects nerve fibers, an action that is particularly interesting in the light of the reported absence of any action on ganglia (*10*). It is important to establish which of the fundamental properties of the nerve membrane is altered by the Δ^9THC to produce the effects seen. All fibers might be affected, the sodium conductance, for example, being turned off earlier, or the potassium conductance being turned on earlier; either of these effects, by shortening the duration of the elemental spikes in each elemental single fiber, would reduce the size of the compound action potential. Alternatively, some particular Δ^9THC-sensitive fibers may be blocked completely. Unfortunately, experiments done to examine these possibilities with internal electrodes—on giant axons of lobsters, in which the maximum rate of rise of the spike was measured, and of squid, in which the sodium and potassium currents in voltage-clamp experiments were measured—proved negative. But because of the preliminary nature of these experiments we cannot say whether the apparent lack of effect of Δ^9THC on marine invertebrate axons reflects a true absence of action on these axons.

Quantitatively similar results to those just described were also obtained with a water-soluble THC analog, ADL 1137 (*11*). In concentrations of 100 to 500 μM this drug produced a fall in the size of the compound action potential of the nonmyelinated fibers of the rabbit vagus nerve of about the same size as that produced by Δ^9THC. Furthermore, the water-soluble analog shared with Δ^9THC the ability to reduce the size of the compound action potential of the myelinated fibers of this nerve. The myelinated fibers may prove more suitable for a voltage-clamp examination of the action of Δ^9THC, in which the effects on the sodium and potassium currents of the nerve membrane can be examined, and such studies are now under way.

We have not yet determined the minimum concentration of Δ^9THC required to produce the kind of effect described here when applied for 1 to 2 hours or more. Threshold effects were obtained with half-hour exposures to 3 μM Δ^9THC, which is equivalent to 1 mg/liter. At first sight, this seems a good deal higher than the psychoactively effective doses in humans, which are 50 to 250 μg/kg (*12*). However, the experimental conditions are quite different, so the two values are not really comparable.

A more relevant comparison might be based on the amount of Δ^9THC actually taken up by the brain when given intravenously in animals. Furthermore, the opportunity for biotransformation or metabolism of the Δ^9THC is probably more limited in the nerve in vitro than in vivo. If the effects of Δ^9THC depend to some extent on the production of active metabolic products, as has been suggested (*13*), it might not be appropriate to compare the concentrations used in the present experiments with psychoactive doses in man. It should be noted that the proposed active metabolite 11-OH-Δ^9THC showed, in a single experiment, qualitatively similar effects on the vagal nonmyelinated fibers to those just described. Furthermore, the psychologically inactive compound, cannabinol, was similarly found to be inactive on nerve.

An effect of THC on the conduction system was postulated by Lapa *et al.* (*14*) on the basis of experiments in which they recorded potentials from the sensory nucleus of the trigeminal nerve. The present experiments provide direct confirmation of this suggestion. And an action of the sort described here on the fine nonmyelinated terminals of neurons and dendrites might explain some of the many effects of this drug on nervous tissue.

The question must certainly arise: Are these results on isolated nerve at this dosage pertinent to the known pharmacological effects of marihuana in man? Unfortunately, dosage per se may not be relevant, since the rabbit vagus nerve may resemble the human nervous system only insofar as both share the same basic physiological mechanisms of conduction. The sensitivity of different parts of nervous systems to drugs may vary considerably, and it may well be that some parts of rabbit or human nervous systems are far more sensitive than our selected test object. Even the minimum concentrations used in these experiments are at least an order of magnitude greater than those reported in human plasma during peak psychedelic action (*15*). This means that the significance of these results for understanding the human response is at the moment unclear. However, this is the first demonstrated pharmacological effect of marihuana constituents on a simple nerve preparation, and it would seem to provide at least a starting point in working out a mode of action.

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4. The Δ^9THC was kindly supplied by the Center for Studies of Narcotic and Drug Abuse, National Institute of Mental Health, Bethesda, Maryland.
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