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Role of the central autonomic nervous system in the hypotension and bradycardia induced by (−)-Δ^9-*trans*-tetrahydrocannabinol

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(−)-Δ^9-*trans*-Tetrahydrocannabinol (Δ^9-THC), when given intravenously (2 mg kg^{-1}) to cats, produced marked decreases in blood pressure and heart rate which developed gradually and were of prolonged duration. Cervical spinal transection (C_1–C_2) abolished these effects whereas surgical removal of neurogenic tone to the myocardium selectively eliminated the bradycardia. Bilateral vagotomy alone did not modify the action of Δ^9-THC upon heart rate or blood pressure. Recordings of spontaneous sympathetic outflow in the inferior cardiac nerve indicated a rapid reduction in neural discharge rate after Δ^9-THC administration. These observations support the hypothesis that Δ^9-THC produces a cardiodecellerator and hypotensive effect by acting at some level within the sympathetic nervous system. Experiments conducted to investigate transmission in the superior cervical and stellate ganglia demonstrated that Δ^9-THC did not alter ganglionic function. Also, responses to intravenous isoprenaline and noradrenaline were unchanged which suggested that Δ^9-THC did not interact with α- or β- adrenoceptors. The possible action of Δ^9-THC on central sympathetic structures was investigated by perfusion of Δ^9-THC into the lateral cerebral ventricle. Δ^9-THC so administered produced a significant reduction in heart rate without a substantial lowering of blood pressure. Tritiated or ^{14}C-Δ^9-THC perfused into the lateral ventricle demonstrated that the amount of radioactive compound passing into the peripheral circulation was insignificant and could not account for the decrease in heart rate. The current data are in agreement with the proposal that Δ^9-THC produces cardiovascular alterations by an action on the central nervous system which results in a decrease in sympathetic tone.

(−)-Δ^9-*trans*-Tetrahydrocannabinol (Δ^9-THC), a psychoactive constituent of marihuana, and several other tetrahydrocannabinols produce cardiovascular alterations in man (Hollister, 1971; Isbell, Gorodetsky & others, 1967) as well as animals. Marked decreases in blood pressure and/or heart rate have been demonstrated in dogs (Hardman, Domino & Seevers, 1957; Dewey, Harris & others, 1970; Dewey, Yonce & others, 1970; Cavero, Solomon & others, 1973a), cats (Dagirmanjian & Boyd, 1962; Hosko & Hardman, 1971) and rabbits (Lipparini, Scotti DeCarolis & Longo, 1969).

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The mechanism by which these effects occur is not completely resolved, however there is evidence for an action of Δ⁹-THC on central nervous system sites. In support of this hypothesis, decreases in heart rate (Cavero & others, 1973a) and blood pressure (Cavero, Solomon & others, 1973b) have been demonstrated following the administration of Δ⁹-THC into the cerebral circulation of the vascularly isolated, neurally intact head of recipient dogs in cross circulation experiments. Also, Hosko & Hardman (1971) have reported that Δ⁹-THC causes a reduction in the pressor responses to electrical stimulation of central vasomotor centres. Similarly, Dagirmanjian & Boyd (1962) suggested that the dimethyl heptyl pyran derivative of tetrahydrocannabinol (DMPH) may produce its depressor effect by acting on the medullary reticular formation.

We have attempted to clarify the relative importance of peripheral and central nervous system sites of action in the hypotension and bradycardia produced by Δ⁹-THC.

METHODS

Cats of either sex, 2·0–3·5 kg, were anaesthetized with α-chloralose (60 mg kg^{-1}, i.v.) and mechanically ventilated (Harvard respirator, Model 606). Aortic blood pressure was recorded from a catheter inserted into the left femoral artery. Heart rate was obtained using a tachograph (Grass 7P4D) triggered by the pulse pressure.

(−)-Δ⁹-*trans*-Tetrahydrocannabinol (Δ⁹-THC) was suspended in a saline solution of 10% polyvinylpyrrolidone as described by Fenimore & Loy (1971) at a concentration of 2·5 mg ml^{-1}. In preliminary experiments the effects of the solvent on the cardiovascular system were found to be negligible. However, the solvent was administered in each experiment 30 to 60 min before Δ⁹-THC. A dose of 2 mg kg^{-1} (i.v.) was chosen since in preliminary studies this amount of Δ⁹-THC gave consistently reproducible cardiovascular effects.

Nine cats received no surgical or pharmacological pretreatment before the administration of Δ⁹-THC. Additional groups of cats were subjected to (a) cervical transection at C_1-C_2 (N = 5); (b) total cardiac denervation (N = 5) or (c) bilateral vagotomy (N = 4) before the intravenous administration of Δ⁹-THC.

The effect of Δ⁹-THC on stellate ganglionic transmission was investigated by stimulation of pre-ganglionic fibres emerging from the right stellate ganglion. Maximal increases in heart rate were recorded during a 30 s stimulation at supramaximal voltage (3–6 V; frequencies of 0·25–4·0 Hz; duration of stimuli 1 ms). During these experiments the dose-response effects to isoprenaline (0·025–0·5 μg kg^{-1} i.v.) induced cardiac acceleration were also obtained. Transmission across the superior cervical ganglion was investigated in vagotomized spinal sectioned (C_1–C_2) cats by recording responses of the nictitating membrane to electrical stimulation of preganglionic sympathetic fibres. Frequency-response curves for developed tension (5 g initial tension) were obtained at supramaximal voltage (3–6 V; frequencies of 0·25–16·0 Hz; duration of stimuli 2 ms).

The interaction of Δ⁹-THC with the pressor responses to intravenous noradrenaline (0·125–0·5 μg kg) and the response to 30 s occlusion of both carotid arteries was conducted in vagotomized cats (Heymans & Neil, 1958) before Δ⁹-THC and at 30, 60 and 90 min after Δ⁹-THC administration. Since the magnitude of the bilateral carotid occlusion pressor response is directly related to the mean arterial pressure (Prochnik, Maison & Stutzman, 1950), the hypotensive effect of Δ⁹-THC alone should result in a

lesser response. Therefore, to investigate an action of Δ^9-THC directly upon the neural pathways of this reflex system, blood pressure was restored to pre-Δ^9-THC levels by continuous infusion of (—)-noradrenaline (0·1–0·25 μg kg min^{-1}) and bilateral carotid occlusion was repeated.

The central mechanism of Δ^9-THC was investigated by administering the compound into the lateral cerebral ventricle as described by Bhattacharya & Feldberg (1958) (N = 6). Δ^9-THC, 2·0 mg kg^{-1}, was perfused for 10 min at a rate of 0·1 ml min^{-1}. In some animals tritiated or [^{14}C]Δ^9-THC (2 mg kg^{-1}) was used to estimate the amount entering the brain tissue. In this preparation the perfusate was recovered via a cannula placed into the cisterna magna through the allanto-occipital membrane. In addition, venous plasma was sampled at 10 min intervals up to 90 min after termination of the perfusion of radioactive Δ^9-THC to determine whether the compound was leaking into the peripheral circulation.

The effects of Δ^9-THC on sympathetic efferent fibres was investigated by recording spontaneous neural activity of the inferior cardiac accelerator nerve distal to the right stellate ganglion. The nerve was placed on a bipolar platinum electrode. Sympathetic discharges were amplified by using a wide band AC eeg preamplifier (Grass 7P5B) and the total electrical activity for 10 s intervals was integrated using a Grass 7P108 integrator.

All values are represented as mean ± standard error of the mean (s.e.). Significant differences between means within a group were determined using a paired *t*-test and significance between the means of two groups were calculated using Student's *t*-test. A *P* value of less than 0·05 was considered to be significant.

RESULTS

Effects of Δ^9-THC on blood pressure and heart rate

Intravenous administration of (—)-Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC) (2 mg kg^{-1}, i.v.) to cats with normal intact neurogenic tone to the vasculature and myocardium (control group) resulted in marked hypotension and bradycardia (Tables 1 and 2). These cardiovascular modifications were gradual in onset, prolonged in duration and maximal between 15–30 min after the injection of Δ^9-THC (Tables 1 and 2). Perfusion of the compound through the lateral cerebral ventricle (2 mg kg^{-1} over 10 min) also resulted in a significant attentuation of heart rate. However, in comparison with control animals, the bradycardia developed more slowly with a maximal decrease

Table 1. *Effects of Δ^9-THC (2 mg kg^{-1}) upon heart rate in cats anaesthetized with α-chloralose.*

Procedure before Δ^9-THC administration	Route	n	Heart rate (beats min^{-1}) ± s.e. Pre-Δ^9-THC	5	15	Min after Δ^9-THC 30	60	90
None (control group)	i.v.	9	188 ± 8	160 ± 6†	143 ± 6†	131 ± 4†	133 ± 5†	137 ± 5†
Cervical spinal section§	i.v.	5	164 ± 13	160 ± 13	163 ± 14	164 ± 12*	168 ± 15*	—
Total cardiac denervation	i.v.	5	123 ± 8*	119 ± 7*	122 ± 9	121 10	122 ± 10	120 ± 9
Vagotomy	i.v.	4	195 ± 15	143 ± 9†	133 ± 6†	130 ± 7†	124 ± 11†	134 ± 11†
Lateral cerebral ventricle cannulation	IVT	6	169 ± 12	167 ± 12	159 ± 12	146 ± 11†	130 ± 11†	123 ± 22†

* Significantly different (P <0·05: unpaired *t*-test) from the group receiving no pretreatment.
† Significant response (P <0·05: paired *t*-test) within the group.
§ Blood pressure maintained at pre-spinal section levels by intravenous infusion of noradrenaline (0·1–0·25 μg kg^{-1} min^{-1}).

IVT—intraventricular administration.

occurring at 30–60 min. Also, the hypotensive effect was significantly less than that observed when the same dose was given intravenously (Table 2). Perfusion of tritiated or [^{14}C] Δ⁹-THC indicated less than 2% of the total drug infused passed into the brain tissue, the remainder was recovered in the perfusate. No significant amount of radioactivity could be detected in samples of venous plasma.

Intravenous administration of Δ⁹-THC to spinal cats with vasomotor and cardiac tone re-established by continuous infusion of noradrenaline caused no measurable changes in heart rate and blood pressure (Tables 1 and 2). In contrast, Δ⁹-THC induced hypotension but not bradycardia in cats in which the heart was surgically denervated (Tables 1 and 2).

The absence of a significant contribution of efferent and afferent vagal activity to the hypotensive and/or bradycardic effects of Δ⁹-THC was demonstrated in vagotomized animals (Tables 1 and 2). Furthermore, no reduction in the bradycardia was observed when atropinization (0·5 mg kg^{-1}, i.v.) or bilateral vagotomy were performed 90 min after intravenous or intracerebroventricular administration of Δ⁹-THC to intact animals.

Table 2. *Effect of Δ⁹-THC (2 mg kg^{-1}) upon mean blood pressure of cats anaesthetized with α-chloralose.*

Procedure before Δ⁹-THC administration	Route	n	Mean blood pressure (mm Hg) ± s.e. Pre-Δ⁹THC	5	15	30	60	90
				Min after Δ⁹-THC				
None (control group)	i.v.	9	113 ± 6	87 ± 9†	70 ± 7†	70 ± 6†	72 ± 5†	75 ± 6†
Cervical spinal section§	i.v.	5	94 ± 10	101 ± 9	98 ± 10*	98 ± 12*	93 ± 13	—
Total cardiac denervation	i.v.	5	107 ± 3	71 ± 10†	61 ± 5†	62 ± 6†	68 ± 7†	76 ± 4†
Vagotomy	i.v.	4	117 ± 11	53 ± 9†	63 ± 12†	65 ± 11†	69 ± 9†	74 ± 12†
Lateral cerebral venricle cannulation	IVT	6	124 ± 15	125 ± 16*	119 ± 17*	111 ± 18*	107 ± 19*	112 ± 20*

* Significantly different ($P < 0·05$: unpaired t-test) from the group receiving no pretreatment.
† Significant response ($P < 0·05$: paired t-test) within the group.
§ Blood pressure maintained at pre-spinal section levels by intravenous infusion of noradrenaline (0·1–0·25 μg kg^{-1} min^{-1}.
IVT—intraventricular administration.

Studies on ganglionic transmission and spontaneous neural activity

Δ⁹-THC (2 mg kg^{-1}, i.v.) did not affect ganglionic transmission across the superior cervical and stellate ganglia. The contractile tension developed by the nictitating membrane following electrical stimulation of preganglionic fibres to the superior cervical ganglion was unaltered by Δ⁹-THC, which confirms the previous observation of Dewey & others (1970). Similarly, Δ⁹-THC did not modify the positive chronotropic responses elicited by stimulating pre-ganglionic sympathetic fibres to the myocardium. Cats with total cardiac denervation were used for this procedure since the responses to nerve stimulation would not be influenced by a change in baseline heart rate following Δ⁹-THC (Table 1).

The role of sympathetic efferent outflow was also evaluated by recording spontaneous neural discharges in the inferior cardiac accelerator nerve distal to the right stellate ganglion. Electrical activity was markedly decreased within 2 min of Δ⁹-THC administration. This reduction is clearly evident in tracings of neural activity integrated over 10 s intervals (Fig. 1).

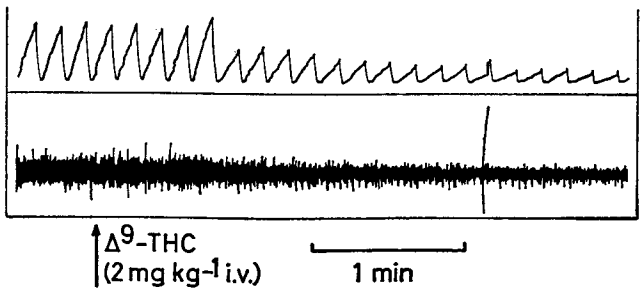

FIG. 1. The effect of Δ^9-THC (2·0 mg kg^{-1}, i.v. as indicated by ↑) upon spontaneous sympathetic discharges in the inferior cardiac nerve in an α-chloralose anaesthetized cat. Lower tracings: total electrical activity. Upper tracing: the integration of total electrical activity over 10 s intervals.

Effects of Δ^9-THC on bilateral carotid occlusion and responses to vasoactive drugs

Δ^9-THC markedly attenuated the large vasopressor response elicited by occlusion of the common carotid arteries in bilaterally vagotomized cats. This inhibitory action of Δ^9-THC was not related to the drug induced hypotension since the pressor response to bilateral carotid occlusion could not be elicited even when aortic blood pressure was re-established to pre-$^9\Delta$-THC levels by infusing noradrenaline (Table 3). The pressor responses to noradrenaline (0·125, 0·25 and 0·5 μg kg^{-1}, i.v.) were greater after Δ^9-THC but this potentiation only occurred at the lower blood pressure levels (Table 3). In fact, no significant change in the peak blood pressure to noradrenaline was observed after Δ^9-THC. Also, there was no difference in the blood pressure changes when noradrenaline was administered to animals in which the hypotensive effect of Δ^9-THC had been abolished by a continuous infusion of noradrenaline.

The positive chronotropic responses to intravenous isoprenaline were studied in cats in which neurogenic tone to the myocardium was removed by total cardiac denervation. Δ^9-THC failed to significantly alter the positive chronotropic effects of isoprenaline. Before Δ^9-THC, isoprenaline (0·05, 0·1 and 0·2 μg kg^{-1}) gave increases ($\bar{X} \pm$ s.e.) in heart rate (beats min^{-1}) of 21 ± 2; 35 ± 3 and 50 ± 6, respectively; following Δ^9-THC these responses were 24 ± 3, 43 ± 4 and 57 ± 4, respectively.

Table 3. *Effect of Δ^9-THC (2 mg kg^{-1}, i.v.) upon pressor responses ($X \pm$ s.e.) to noradrenaline and occlusion of both carotid arteries in α-chloralose anaesthetized cats ($n = 4$).*

	Mean blood pressure (mm Hg)								
	Pre-Δ^9-THC			30 min after Δ^9-THC			90 min after Δ^9-THC		
Procedure	Control	Peak	Δ	Control	Peak*	Δ	Control§	Peak	Δ
Carotid occlusion in vagotomized cats	114 ± 10	199 ± 15	85 ± 7	85 ± 15	99 ± 17	+ 14 ± 2	121 ± 13	133 ± 10	12 ± 2
Noradrenaline (μg kg^{-1}, i.v.)									
0·125	111 ± 12	138 ± 9	+27 ± 3	67 ± 11	127 ± 12	+ 60 ± 6†			
0·25	111 ± 10	148 ± 10	+37 ± 4	69 ± 10	151 ± 8	+ 82 ± 3†			
0·50	106 ± 10	164 ± 14	+58 ± 7	68 ± 9	170 ± 10	−102 ± 14†			

* All peak responses to noradrenaline following Δ^9-THC are not significantly different from pre-drug peaks.
† Significant response ($P < 0.05$).
§ The hypotensive effect of Δ^9-THC has been abolished by infusing noradrenaline.

DISCUSSION

(−)-Δ⁹-*trans*-Tetrahydrocannabinol (2 mg kg^{-1}, i.v.) caused a marked decrease in blood pressure and heart rate in cats under α-chloralose anaesthesia. These changes are consistent with previous reports on Δ⁹-THC and several other tetrahydrocannabinols (Dagirmanjian & Boyd, 1962; Hosko & Hardman, 1971).

The present data clearly indicate that the depressor and bradycardic effects of Δ⁹-THC require an operative sympathetic autonomic innervation to the heart and vasculature. This conclusion is supported by the fact that removal of the normal neurogenic control (transection of the cervical spinal cord and vagi) prevented both actions of Δ⁹-THC even though blood pressure and heart rate had been re-established to pre-spinal section levels with noradrenaline. Furthermore, the hypotension and bradycardia were not measurably dependent upon the integrity of the vagus nerve. This observation contrasted with experiments using dogs in which a mechanism involving afferent vagal fibres also contributed to the reduction in heart rate (Cavero & others, 1973). Therefore it seems that Δ⁹-THC does not produce its cardiovascular effects by acting upon sympathetic ganglionic or neural transmission since the measured responses to electrical stimulation of pre- and/or post-ganglionic fibres of the stellate and superior cervical ganglia were not significantly altered which confirms previous work of Dewey & others (1970). The dose-response relations to noradrenaline and isoprenaline after Δ⁹-THC were essentially equal to control values, thus an interaction of Δ⁹-THC with α- or β-adrenoceptors appears unlikely.

The failure to demonstrate an interaction of Δ⁹-THC at peripheral autonomic sites supports the hypothesis that this compound inhibits neurogenic tone via an action upon the central nervous system. Recordings of spontaneous sympathetic outflow from the inferior cardiac accelerator nerve strengthened this conclusion by demonstrating that adrenergic neuronal activity was rapidly diminished following Δ⁹-THC administration. This finding may explain the markedly decreased pressor response to bilateral carotid occlusion following Δ⁹-THC, even though an action on the afferent limb of this reflex mechanism cannot be ruled out. To eliminate the possibility that the attenuation of the bilateral carotid occlusion response was due only to the decrease in the mean arterial pressure (Prochnik & others, 1950), blood pressure was re-established at pre-Δ⁹-THC levels by infusion of noradrenaline, but even then the decreased pressor response to carotid occlusion was still evident.

The pronounced bradycardia which gradually developed after the perfusion of Δ⁹-THC into the lateral cerebral ventricle supports the likelihood that a central site of action is involved in this effect of the compound. It is unlikely that leakage of the compound to the periphery contributed to this central action since an insignificant amount of radioactive Δ⁹-THC was detected in venous plasma following ventricular perfusion of labelled Δ⁹-THC (tritiated or ^{14}C). A significant reduction in blood pressure did not accompany the decrease in heart rate which may be because the central structures responsible for the cardiac decelleration were more accessible to Δ⁹-THC in the cerebrospinal fluid than areas involved in the depressor action.

The current data also indicate that the contribution of the decrease in heart rate to the hypotensive action of Δ⁹-THC is negligible. This conclusion was drawn from experiments in which the hypotension induced by Δ⁹-THC, following the removal of neurogenic tone to the myocardium, was not significantly different from the depressor effect observed in intact cats. Δ⁹-THC did not alter the intrinsic atrial heart rate in

these animals or the heart rate of spinal cats receiving noradrenaline infusions. Thus a direct negative chronotropic action of Δ^9-THC may be excluded.

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