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EFFECTS OF Δ¹-TRANS-TETRAHYDROCANNABINOL ON MECHANICAL PERFORMANCE OF ISOLATED HEART MUSCLE PREPARATIONS

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1 The effects of four concentrations of Δ¹-trans-tetrahydrocannabinol (Δ¹-THC) (0.1, 1, 5 and 20 μg/ml) were compared to that of the vehicle (ethanol, 0.5 μl/ml) on the mechanical performance of isolated cardiac muscle of the cat and rat.

2 In rat isolated papillary muscles, Δ¹-THC (20 μg/ml) caused a decline in mechanical performance (−8% in developed tension and −11% in V_{max}) in contrast to the apparent lack of effect of ethanol.

3 All other parameters of mechanical performance studied in both rat and cat papillary muscles were unaffected by Δ¹-THC when compared with ethanol.

4 It was concluded that Δ¹-THC in concentrations up to 20 μg/ml had negligible effect on contractile performance, the time course of contraction or muscle elasticity in rat and cat isolated papillary muscle preparations under the conditions studied.

Introduction

Δ¹-Trans-tetrahydrocannabinol (Δ¹-THC) is the principal psychoactive material found in *Cannabis sativa*. The cardiovascular effects of the cannabinoids have been studied in man and in a number of different animal preparations. An increase in heart rate has generally been found in man (Weiss, Watanabe, Lemberger, Tamarkin & Cardon, 1972; Kanakis, Pouget & Rosen, 1976) but not in animals (Cavero, Buckley & Jandhyala, 1973). Haemodynamic measurements in animals suggest that Δ¹-THC probably exerts no direct inotropic effect (Cavero *et al.*, 1973) but clinical studies have described increased cardiac output (Malit, Johnstone, Bourke, Kulp, Klein & Smith, 1975) and changes in systolic time intervals which were interpreted as indicating a positive inotropic effect (Kanakis *et al.*, 1976). However, assessment of contractile state in the intact heart is notoriously difficult and these studies must leave doubt about the possible inotropic effects of Δ¹-THC. Indeed, it is difficult to evaluate possible inotropic interventions without studying their effects in isolated heart muscle preparations (Henderson, Van Ocken & Brutsaert, 1973a; Linden & Harry, 1976). The present study was carried out in order to examine whether Δ¹-THC does have any direct inotropic action on mammalian ventricular heart muscle.

Methods

Isolated papillary muscle preparations of cat and rat hearts were mounted and their mechanical performance studied as previously described (Henderson, Brutsaert, Parmley & Sonnenblick, 1969; Henderson, Claes & Brutsaert, 1973b). Rats (male Wistar, 400–450 g) were killed by stunning, and cats were anaesthetized with sodium pentobarbitone (40 mg/kg intraperitoneal injection). Hearts were quickly excised and papillary muscles dissected out in oxygenated buffer solution, the posterior left ventricular papillary muscles from rats and right ventricular papillary muscles from cats being used. Muscles were mounted vertically by clipping the lower end to an underwater force transducer and tying the upper tendinous end by a short silk thread to the electro-magnetic lever system, as previously described (Henderson *et al.*, 1973a). The bathing medium contained (mM): NaCl 118, KCl 4.7, $Mg_2SO_4.7H_2O$ 1.2, KH_2PO_4 1.1, $NaHCO_3$ 24, $CaCl_2.6H_2O$ 2.4 and glucose 10. It was equilibrated with 95% O_2 and 5% CO_2 in a temperature-controlled water bath (50 ml capacity) at 29°C. Muscles were mass-stimulated through platinum plate electrodes placed parallel to the muscle, using 5 ms rectangular pulses of just suprathreshold voltage, at

a frequency of 6/min (0.1 Hz, rat) or 12/min (0.2 Hz, cat) unless otherwise stated. Data were recorded at slow paper speed (1 mm/s) on a 4-channel Devices pen recorder (MX4) and, for individual contractions, on a storage oscilloscope (Tektronix 567 with Polaroid camera C12).

Muscles were equilibrated until mechanical performance stabilized (approx. 3 h), and resting length was adjusted to L_{max} (representing the peak of the length-active tension relationship). The characteristics of isometric contractions and maximum unloaded shortening velocity (V_{max}) were measured, each under stable loading conditions (Henderson *et al.*, 1973a). V_{max} was measured directly by abruptly reducing load to zero at the beginning of contraction as previously described (Brutsaert, Claes & Sonnenblick, 1971; Henderson *et al.*, 1973b). The steady state isometric force-frequency relationship (over the range 0.3 to 24/min) and maximum post extrasystolic potentiation (using the shortest effective coupling interval) were measured in cat muscles. Total elasticity (of muscle and equipment) was characterized by recording directly the force-length curve obtained when force was abruptly (<5 ms) reduced to zero at the moment of peak isometric force. Possible changes in elasticity were examined by comparing (at common force) the curves obtained before and after intervention.

In each muscle, measurements were made before addition of Δ^1-THC, and were repeated 15 min after the addition of 0, 5 μg, 50 μg, 250 μg and 1 mg Δ^1-THC, each in 25 μl vehicle (ethanol), with intervening washes with pre-equilibrated (for temperature and oxygenation) buffer, to give Δ^1-THC final bath concentrations of 0, 0.1 μg/ml, 1 μg/ml, 5 μg/ml and 20 μg/ml.

In additional experiments with rat papillary muscles the effects of 20 μg/ml Δ^1-THC on hypoxic contracture ($n = 4$) and on potassium contracture ($n = 4$) were studied. Progressive hypoxic depression was induced by changing the gas mixture to 95% N_2 and 5% CO_2, as previously described (Henderson, Most, Parmley, Gorlin & Sonnenblick, 1970) and the Δ^1-THC was added after 15 min of the 30-min hypoxic period. Potassium contracture was induced by addition of 74 mM KCl and then 2.5 mM $CaCl_2$ to calcium-free buffer, as previously described (Henderson & Cattell, 1976).

Muscle length (at L_{max}) and lightly-blotted wet weight were measured at the end of each experiment, and mean cross-sectional area calculated assuming cylindrical shape and specific gravity of 1. Results are expressed as mean $\pm$ standard error, compared by paired t test, and considered significantly different where $P < 0.05$. Rat muscles ($n = 9$) were of length 6.5 $\pm$ 0.4 mm, weight 5.65 $\pm$ 0.76 mg, mean cross-sectional area 0.86 $\pm$ 0.1 mm^2, with total peak force (P_0) under control conditions 55.67 $\pm$ 11.38 mN/mm^2, resting force (RF) 12.15 $\pm$ 2.18 mN/mm^2 and V_{max} 3.2 $\pm$ 0.32 muscle lengths/second. Cat muscles ($n = 4$) were of length 8.12 $\pm$ 0.72 mm, weight 6.0 $\pm$ 1.16 mg, mean cross-sectional area 0.76 $\pm$ 0.16 mm^2, with P_0 55.35 $\pm$ 4.41 mN/mm^2, RF 9.86 $\pm$ 1.19 mN/mm^2 and V_{max} 1.41 $\pm$ 0.1 muscle lengths/second.

Results

Figure 1a shows that in rat muscles Δ^1-THC induced no significant change in most parameters measured, although a general decline in performance throughout the sequential dose-response experiments reached significance with respect to V_{max} at the highest concentration of Δ^1-THC (−7% in DT, −9% in V_{max}). There was no change in the relative time course of contraction. To test the possibility that this minimal decline in performance might be due to a non-specific drift during the 5 h duration of these experiments, the highest concentration of Δ^1-THC was added directly to 2 further muscles (Figure 1b), and a similar very slight decline in performance was observed (−8% in DT, −11% in V_{max}), in contrast to the apparent lack of effect on addition of vehicle alone.

In the light of these results, only the highest concentration (20 μg/ml) of Δ^1-THC was studied in cat muscles. This caused again a very slight decline in mechanical performance (−3% in DT, −2% in V_{max}) but in this case no greater than that caused by the addition of the vehicle alone (−4% in DT, −2% in V_{max}). The force-frequency relationship and post extrasystolic potentiation, studied in cat muscles ($n = 4$), were not altered by 20 μg/ml of Δ^1-THC. Elasticity was not altered by 20 μg/ml of Δ^1-THC in rat ($n = 4$) or cat ($n = 2$) muscles. Hypoxic and potassium contracture in rat muscles were unaffected by the addition of 20 μg/ml of Δ^1-THC.

Discussion

The data show that Δ^1-THC in concentrations up to 20 μg/ml had negligible effect on contractile performance, the time course of contraction, or muscle elasticity in isolated papillary muscle preparations of the rat and cat under the conditions studied. A possible very slightly depressant effect of Δ^1-THC was observed at the highest concentration studied (20 μg/ml), but this appeared to be due, at least in cat muscles, to the low concentration of alcohol present in the vehicle. A comprehensive study of mechanical characteristics was carried out. V_{max} and P_0 provide the two intercepts of the time-independent major portion of the force-velocity-length interrelationship that characterizes the shortening capabilities (contractile state) of the muscle (Brutsaert & Henderson 1973;

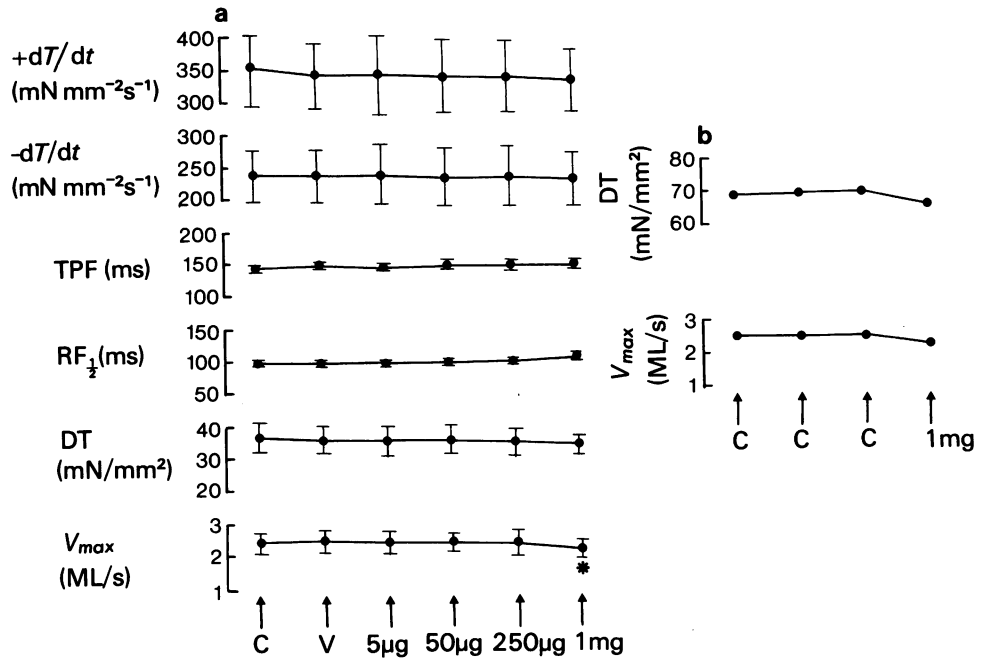

Figure 1 Mechanical performance of rat papillary muscle preparations in response to Δ¹-trans-tetrahydrocannabinol (Δ¹-THC). (a) Δ¹-THC was added in increasing concentrations, with intervening 20 min washes, and its effects compared with the control state (C) and with addition of vehicle (25 μl ethanol) alone (V). Mean values ($n = 7$) are shown of peak rate of tension development (+dT/dt), peak rate of tension decline (−dT/dt), time to peak force (TPF), half isometric relaxation time ($RF_{\frac{1}{2}}$), developed tension (DT), maximum unloaded shortening velocity (V_{max}), expressed as mN (milliNewtons) and ML (muscle lengths) where appropriate. Vertical lines show s.e. mean. *Indicates significant difference from control ($P < 0.01$). (b) Shows effect of direct addition of maximum dose of Δ¹-THC (1 mg = final concentration 20 μg/ml) after series of control readings at 20 min intervals ($n = 2$).

Henderson *et al.*, 1973a). V_{max}, peak dT/dt, and P_0 also represent parameters of contractile performance which are measured at progressively later moments during the contraction, thus providing a measure of the relative time course of contraction, while the time to peak force (TPF) and half isometric relaxation time ($RF_{\frac{1}{2}}$) provide measures of the total time course of contraction and relaxation. Changes in elasticity might influence force development but not V_{max}. Consideration of all these measurements together provides mutually supportive evidence for the generally negative findings. The mechanical behaviour of rat heart muscle is in many ways peculiar (Henderson *et al.*, 1969; Henderson, Brutsaert, Forman & Sonnenblick, 1974), so that the effects of Δ¹-THC were studied also in cat papillary muscle preparations, with similarly negative findings. Force-frequency relationships and post extrasystolic potentiation may reflect more subtle changes in excitation-contraction coupling than are manifest in mechanical performance at any one frequency, but again Δ¹-THC caused no change in these phenomena. Reversible contractures (increased resting force at constant length) can be induced by severe hypoxia or chemical depolarization, possibly by different mechanisms, but Δ¹-THC was equally without effect on these.

The findings of this study provide no support for the view that Δ¹-THC directly augments contractile performance in mammalian cardiac tissue. This contrasts with the conclusion, drawn indirectly and with uncertain validity from haemodynamic studies in man, that Δ¹-THC had a positive inotropic effect (Malit *et al.*, 1975; Kanakis *et al.*, 1976). It remains possible that the reported clinical effects might have been mediated in part by a hypothetical, inotropically-active metabolite of Δ¹-THC, as there suggested (Kanakis *et al.*, 1976) and not here tested.

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