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Effects and Interactions of Natural Cannabinoids on the Isolated Heart (42181)

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Abstract. A Langendorff perfused rat heart preparation was designed to process dose–response effects of cardioactive drugs on rate, coronary flow, and supraaortic differential pressure (ΔP; an index of cardiac performance). In this preparation, Δ^{9-}-tetrahydrocannabinol (THC) 2×10^{-6} *M* to 10^{-5} *M* induces in the isolated perfused rat heart a biphasic increase in rate (maximal at 8 $\times 10^{-6}$ *M*). Tachycardia is associated with decreases in (ΔP) and no change or decreased coronary flow. Cardiac toxicity is observed with 3×10^{-5} *M*. Cannabidiol (CBD) at concentrations of 9 $\times 10^{-6}$ *M* to 10^{-4} *M* has limited effect on rate while increasing ΔP and coronary flow. Cannabinol (CBN) 8×10^{-6} *M* to 3×10^{-4} *M* depresses rate and ΔP while coronary flow remains constant. Simultaneous equimolar administration of THC with CBD antagonizes or mitigates the cardiac effects of THC on rate, ΔP, and coronary flow.

The most consistent biological marker after intoxication with Δ^9-tetrahydrocannabinol (THC), the psychoactive substance of cannabis, or THC-containing preparations, is a dose-related cardiac chronotropic effect: tachycardia in man (1, 2) and bradycardia in most other animal species (3–7). Cannabis preparations also contain nonpsychoactive cannabinoids, mainly cannabidiol (CBD), a natural substance contained in the flowering top of the plant, and cannabinol (CBN) which results mostly from the chemical degradation of THC in stored preparations (8). Furthermore, after its absorption, THC is biotransformed into psychoactive metabolites like 11-hydroxy-Δ^9-tetrahydrocannabinol which has THC-like properties, and nonpsychoactive metabolites like 11-hydroxycannabinol which has CBN-like properties. These are the three principal cannabinoids which so far have been investigated for their biological effects. Therefore, body fluid and tissues of cannabis users contain a mixture of various cannabinoids which have THC-like, CBD-like, or CBN-like properties. Cardiac properties of cannabis preparation have been attributed mainly to THC. While CBD and CBN do not have any measurable effect on heart rate (9), they possess biological activity (10, 11) and interactions between CBD and THC have been reported (12). Such interactions may best be studied on the isolated perfused heart. Some authors (13–16) have reported that THC reduces myocardial contractility with little or no effect on rate in the perfused rat heart of the guinea pig heart–lung preparation. Others (17) have observed that THC and CBN decrease contractile force and increase rate, while CBD produces bradycardia, arrhythmias, and asystole. However, the experimental design used by these investigators resulted in an accumulation of cannabinoids in the heart and prevented the study of dose–response effects and of the cardiac interaction of these different cannabinoids.

The aim of the present study was to investigate the effect of THC, CBD, and CBN administered separately or in combination to a heart preparation specially designed to avoid cumulative effects of the drug and to obtain dose–response effects.

Methods. Wistar rats weighing 200 to 300 g and maintained on a standard laboratory diet were anesthetized with ether. The heart is excised and placed in a saline buffered heparinized solution at 4°C. The aorta is cannulated and attached to the base of the retrograde perfusion column of a modified Langendorff preparation which has been described in a separate publication (18). This system allows for continuous storage, analysis, and display of coronary flow, heart rate, and differential pressure (ΔP) between minimal diastolic and peak systolic supraaortic pressures. This latter measurement may be considered an index of cardiac performance.

The cannabinoids supplied by NIDA (National Institute of Drug Abuse) with chro-

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matographic analysis indicating 99.5% purity are stored in the dark at 4°C. Before usage, these samples were reanalyzed by mass spectrometry liquid–gas chromatography and similar results were obtained. Δ^9-THC was supplied diluted in ethanol, while CBD and CBN supplied in crystalline form were resuspended in ethanol. All drugs were diluted in a solution containing 10% Tween 80 in buffered saline and 2.5% ethanol, a solution in which the fat soluble cannabinoids are well solubilized (19). The test substance is administered during 1 min by means of an electrically powered syringe containing 100 μl as described (18), to obtain dose–response effects. Dose–response effects of each cannabinoid were studied on four to seven hearts.

The pharmacokinetic profile of Δ^9-THC in this system was determined by administering in a single bolus the tritiated compound and recovering coronary output every 30 sec for the next 10 min. Retention and elimination time of the drug was then calculated so as to perform subsequent dose–response curves of the drug at proper intervals and avoid its accumulation in the heart. It was observed that after administration of a single bolus of tritiated THC in the system, 98% of the radioactivity had been eliminated after 10 min. Cannabinoids were therefore administered at intervals of 10 min or more, after all variables had been restored to their initial control values, and the measurement performed during the period just preceding drug administration was considered to be a control measurement.

Analysis of data. The analog to digital conversion of the physiological markers recorded by this system will give a greater precision than that of conventional recording methods. Under "controlled," stable conditions, the reproducibility of coronary outflow, pressure, and rate measurements are better than ±1%. Therefore a variation in these measurements performed on the same preparation during its 1-hr period of stability and exceeding ±3% is significant ($P < 0.01$). Effects produced by the different compounds administered are processed by the computer and expressed as a fraction of the preceding control measurement which has been previously recorded and stored. A change greater than 2% (0.02) from control is significant ($P \leq 0.05$), and for a change greater than 3% (0.03), $P \leq 0.01$.

Results. Administration of the vehicle (10% Tween 80% isotonic saline, 2.5% ethanol) did not alter any of these recorded markers.

THC administered to five preparations (Fig. 1) produced a biphasic effect on cardiac frequency, first an increase with THC concentrations of 2.05 to 7.75×10^{-6} *M*. Maximum recorded increase was 24% above control with 7.75×10^{-6} *M*. With higher dosage there was a progressive decrease in frequency toward and below control (Fig. 1). With similar doses of THC, ΔP and coronary flow decreased progressively. ΔP was 50% below control when THC concentration was 3×10^{-5} *M*. All cardiac activity stopped with THC 3.4×10^{-5} *M*.

CBD administered to seven preparations (Fig. 1) produced small increments in frequency limited to 8% above control, and a dose-related increase of pulse pressure (4 to 42%) and coronary flow (7 to 55%). Cardiac arrest occurred with 2.66 to 3.4×10^{-4} *M*, a dose nine times higher than that occurring with THC (Fig. 1).

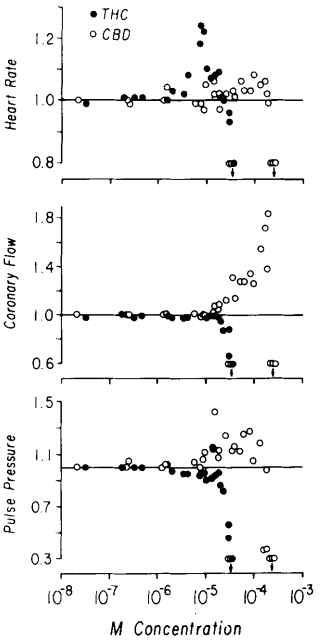

FIG. 1. Dose–response effects of Δ^9-THC (THC) and cannabidiol (CBD) on isolated rat hearts (five for THC, seven for CBD). Each result is expressed as a fraction of the preceding control measurement. A change greater than 2% (0.02) from control is significant ($P < 0.05$) and a change greater than 3% (0.03) $P < 0.01$. The black arrows near the ordinates are indicative of asystole.

When THC and CBD (Fig. 2) are administered simultaneously to five preparations in equimolar concentrations, 10^{-7} to 10^{-4} *M*, the effects of CBD predominate. There is a limited change in rate (±4 to 7%), while contractile force and coronary flow increase with THC concentrations of 1.23×10^{-6} *M* to 5.6×10^{-5} *M*.

Cannabinol (CBN) 10^{-6} *M* to 10^{-4} *M* tends to decrease rate and pulse pressure in the presence of a stable coronary flow (Fig. 3).

Discussion. The cardiac chronotropic effects of THC *in vitro* are observed within a very narrow range of concentrations: 4.2×10^{-6} *M* to 2.0×10^{-5} *M*. Higher concentrations are toxic: 3.3×10^{-5} *M* THC produces asystole. Other *in vitro* biological effects of THC on lymphocytes (10) or nerve cells are observed within a similar narrow range of concentrations: 10^{-6} *M* to 10^{-5} *M*. The concentrations of THC used in this study were calculated from amount administered and coronary flow; they are 10 to 50 times higher than plasma concentrations associated with cardiac effects (1, 2). However, THC and cannabinoids tend to adhere to glass and other surfaces, and tissue concentrations of these drugs, following their *in vitro* administration, are consequently reduced by these amounts and may be lower by a factor of 10 than the concentrations administered (19).

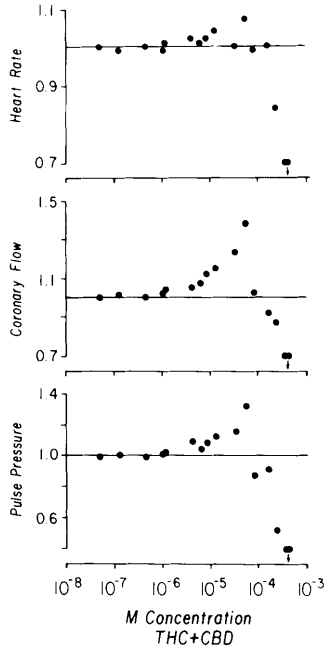

FIG. 2. Dose-response effects of THC and CBD administered in equimolar concentration on four isolated rat hearts.

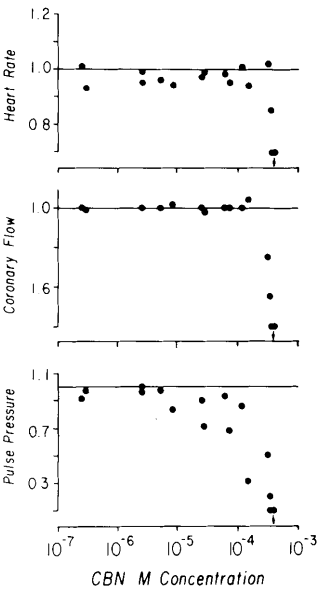

FIG. 3. Dose response effects of cannabinol (CBN) on five isolated rat hearts.

CBD which will induce asystole with concentrations of 2.3×10^{-4} *M*, nine times the concentration of THC which stops cardiac activity. CBN appears to have a cardiac activity different from that of THC and CBD: decreasing rate and pulse pressure in the presence of a constant coronary flow.

The cellular mechanisms which mediate the cardiac effects of these cannabinoids are not known. The tachycardia induced by THC in the isolated heart could be due in part to its action on catecholaminergic receptors which the drug has been reported to potentiate (20). The brief duration of the tachycardia induced by THC and its "biphasic" effect might be caused by the release and depletion by THC of catecholamine stores from the isolated rat heart. These stores are limited; THC might in addition interfere with uptake mechanisms. The mechanisms of the depressing effect of THC on contractile force and coronary flow cannot be presently ascertained. There is some limited evidence in man of a weakening of cardiac contractility by THC (21): in a study

of marihuana and placebo cigarette smokers with coronary heart disease, THC per se contributed to the observed reduction of stroke index, cardiac index, and ejection fraction.

The mechanisms of the cardiac effect of CBD are unclear. CBD 10^{-6} *M* decreases by 50% calcium ATPase activity (22), a property which might account in part for its effects on coronary flow and ΔP.

The antagonistic effects between THC and CBD have also been reported in animal preparations since the first observation of Carlini *et al.* (12): CBD blocks THC-induced catatonia in mice, corneal areflexia in rabbits, aggressiveness in REM sleep-deprived rats, and minimizes in unanesthetized rabbits the depressant effect of THC on heart rate, respiration, and rectal temperature (23, 24).

CBD antagonizes the effect of THC on operant behavior of rodents and Rhesus monkeys (25, 26). It has also been reported that CBD inhibits the epileptic seizures induced by THC in the genetically epileptic rabbit. In man, according to the same authors (28, 29), CBD blocks the increase in heart rate of THC when simultaneously administered with this drug; others (30, 31) failed to observe such antagonistic effects of CBD. Therefore, it is not clear to what extent the direct cardiac effects of THC could be antagonized in man by CBD.

Furthermore, CBD, which is not psychoactive, has low somatic toxicity and is well tolerated in man (28, 31), has been tested with some success in the treatment of secondary generalized epilepsy (32). Its cardioactive properties might be worth investigating.

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