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Effects of Chronic Delta-9-THC Treatment on Cardiac Beta-Adrenoceptors in Rats

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EVANS, E. B., E. SEIFEN, R. H. KENNEDY, R. KAFILUDDI, M. G. PAULE, A. C. SCALLET, S. F. ALI AND W. SLIKKER, JR. *Effects of chronic delta-9-THC treatment on cardiac beta-adrenoceptors in rats.* PHARMACOL BIOCHEM BEHAV **28**(2) 171–174, 1987.—This study was designed to determine if chronic treatment with delta-9-tetrahydrocannabinol (THC) alters cardiac beta-adrenoceptors in the rat. Following daily oral administration of 10 or 20 mg/kg THC or an equivalent volume of control solvent for 90 days, rats were sacrificed, and sarcolemmal membranes were prepared from ventricular myocardium. Beta-adrenoceptor density and binding affinity estimated with (−)[^{3}H]dihydroalprenolol, a beta-adrenergic antagonist, were not significantly affected by treatment with THC when compared to vehicle controls. These results suggest that the tolerance to cardiovascular effects of THC which develops during chronic exposure in the rat is not associated with alterations in cardiac beta-adrenoceptors as monitored by radiolabeled antagonist binding.

Rat heart Sarcolemmal membranes Beta-adrenoceptors B_{max} K_d Chronic delta-9-THC

DESPITE the widespread use of marijuana and a substantial body of information concerning effects of marijuana and its major active ingredient, delta-9-tetrahydrocannabinol (THC), little is known about its mode of action on the cardiovascular system. There are marked differences between cardiovascular effects of marijuana in animals and those in man. Typically in humans, marijuana produces tachycardia with no change or a slight increase in blood pressure [6, 12, 13, 16, 17, 23]. The major cardiovascular effects of marijuana in most animal species are bradycardia and hypotension [1, 9, 11, 27]. In rodents, chronic administration of THC has been reported to cause tolerance to the hypotensive and bradycardiac effects [1, 7, 9, 14, 21]. Information concerning the mechanism(s) underlying the development of this tolerance is currently not available. Since cardiovascular effects of THC are mediated to some extent by the autonomic nervous system or, more specifically, the sympathetic nervous system [3, 4, 10, 13, 27], tolerance may result from changes in the affinity and/or number of adrenergic receptors. Therefore, the present study examined effects of chronic administration of THC on cardiac beta-adrenoceptors in the rat.

METHOD

Chronic THC Treatment of Rats

Male, Sprague-Dawley rats (initial average weight: 245 g) were housed individually in suspended wire cages and maintained on a 12:12 light:dark cycle with lights on at 0600 hr (National Center for Toxicological Research, Jefferson, AR). Pseudomonas-free water was available ad lib. Animals were pair-fed during the treatment to prevent differences in body weight. On Mondays through Fridays animals were dosed orally (1 ml/kg) with either vehicle (1% Triton X-100, 10% ethanol, 89% normal saline) (group C), 10 mg/kg THC (group B) or 20 mg/kg THC (group A). Dosing continued for 90 days. This prolonged administration of THC was chosen since the animals were used to replicate studies on behavioral deficits established by others [25,26] with this treatment scheme. Forty-eight hr after the last treatment, animals (mean weight: 380 g) were sacrificed by decapitation. Hearts were immediately removed, and the ventricles were dissected free from atria and connective tissue, rinsed in normal saline, blotted dry, frozen in liquid nitrogen and stored at −70°C.

Beta-Adrenoceptor Binding Experiments

A ventricular plasma membrane preparation similar to that described by Glaubiger and Lefkowitz [8] was used in these experiments. The frozen ventricles (average weight: 0.8 g) were removed from cold storage and minced in 10 ml of ice-cold homogenizing buffer (0.25 M sucrose, 1 mM $MgCl_2$, 5 mM Tris-HCl, pH 7.4). Further procedures were

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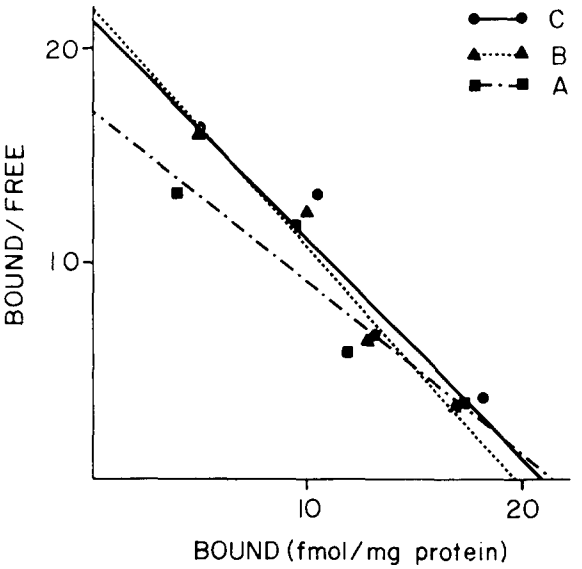

FIG. 1. Scatchard plots for specific [^{3}H]dihydroalprenolol binding to rat ventricular sarcolemmal membranes. Each plot represents one of three individual experiments performed with preparations from rats treated daily for 90 days with 20 mg/kg THC (plot A), 10 mg/kg THC (plot B), or solvent used as vehicle (plot C).

performed at 4°C. The minced tissue was rinsed once with 15 ml of homogenizing buffer and homogenized by two 20 sec bursts with a Polytron homogenizer at a setting of 7. The homogenate was centrifuged for 10 min at 480 × g, and the resulting supernatant was centrifuged at 30,000 × g for 10 min. The pellet was resuspended in 20 ml of buffer (10 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.5) using 6 strokes of a motor driven Teflon/glass homogenizer. The resulting homogenate was centrifuged at 30,000 × g for 10 min. This washing procedure was repeated once, and the final pellet was suspended in 4 ml of a buffer containing 25 mM $MgCl_2$ and 75 mM Tris-HCl, pH 7.65.

The beta-adrenergic receptor binding assay was carried out using a method similar to those described earlier [2,8]. The binding reaction was started by adding 100 μl of the ventricular membrane preparation to 50 μl of preincubated (4 min, at 37°C) solutions containing 25 mM $MgCl_2$, 75 mM Tris-HCl (pH 7.65), and (−)[^{3}H]dihydroalprenolol ([^{3}H]DHA) (final concentrations from 0.3 to 5 nM) with and without 10^{-7} M nadolol. Total reaction volume was 150 μl. The mixture was incubated for 15 min at 37°C in a shaking water bath after which time the reaction was stopped by the addition of 3 ml of ice-cold buffer (25 mM $MgCl_2$, 75 mM Tris-HCl, pH 7.65). Bound and free [^{3}H]DHA were separated via filtration through glass fiber filters (Gelman type A-E, Gelman Sciences Inc., Ann Arbor, MI). Filters were washed twice with 3 ml of the ice-cold buffer and placed in scintillation vials containing 10 ml of scintillation fluid (667 ml toluene, 333 ml Triton X-100, 5.5 g PPO, 0.1 gPOPOP). After sitting over night, the vials were shaken for 2 hr, and radioactivity was determined by liquid scintillation spectrometry. Counting efficiency, approximately 30%, was monitored by external standard. Specific binding of DHA, expressed as fmol/mg protein, was calculated by subtracting the amount bound in the presence of 10^{-7} M nadolol (nonspecific binding) from that bound in the absence of nadolol (total binding). Protein determinations were done following the method of Lowry *et al.* [20].

Binding data were subjected to Scatchard analysis [24]. Slopes ($-1/K_d$) and x-axis intercepts (B_{max}) were estimated by linear regression analysis. Data were evaluated by Student's *t*-test, and *p* values smaller than 0.05 were used as criteria for significance.

Nadolol was a generous gift from E. R. Squibb & Sons, Inc., Princeton, NJ. (−)[^{3}H]dihydroalprenolol (90.3 Ci/mmol) was purchased from New England Nuclear, Boston, MA. Delta-9-tetrahydrocannabinol was supplied courtesy of the National Institute on Drug Abuse. All other chemicals were of reagent grade.

RESULTS

The average body weight of rats from the two groups receiving THC did not differ significantly from the control group (vehicle only) after 90 days treatment (375–390 g). Cardiovascular effects of THC were not assessed during the THC administration. It is, however, safe to assume that the tolerance which is already well established after 28 days of treatment with similar THC doses [1, 9, 14] was maintained, at least to some extent, with continued administration of THC.

Initial experiments in rat ventricular sarcolemmal preparations of the control group (group C; treated with vehicle only) demonstrated that specific [^{3}H]DHA binding was saturable and equilibrium was achieved within 15 min at 37°C (data not shown). Scatchard analysis of the binding data (Fig. 1) suggested that there was a single population of binding sites for DHA with an apparent dissociation constant (K_d) of 1.31±0.51 nM. The density of specific DHA binding sites (B_{max}) was estimated to be 21.4±4.0 fmol/mg protein in these vehicle controls. A single receptor population has also been described by other investigators in rat sarcolemma with DHA concentrations similar to those used in this study [5, 8, 15].

Chronic treatment for 90 days with 10 or 20 mg/kg THC PO did not significantly alter beta-adrenoceptor density or affinity in rat myocardium. In preparations obtained from rats of group B (10 mg/kg THC), K_d for DHA was 1.17±0.37 nM, and B_{max} amounted to 21.6±2.7 fmol/mg protein. Corresponding values obtained in preparations from group A (20 mg/kg THC) were 1.41±0.30 nM (K_d) and 22.8±1.6 fmol/mg protein (B_{max}), respectively. Representative Scatchard plots (Fig. 1), one for each experimental group, show similar slopes and x-axis intercepts.

DISCUSSION

This project was designed to determine if chronic administration of THC alters beta-adrenoceptors in rat ventricular myocardium. Specific binding of [^{3}H]DHA, a radiolabeled beta-adrenergic antagonist, was monitored in sarcolemmal membranes prepared from rat ventricular muscle isolated after 90 days treatment with 20 mg/kg THC (group A), 10 mg/kg THC (group B) or the solvent used as vehicle (group C). In membrane preparations obtained from vehicle controls, B_{max} averaged 21.4±4.0 fmol/mg protein, and K_d was estimated to be 1.31±0.51 nM DHA. These values are in good agreement with those described by others in nontreated animals [5,15]. Chronic administration of THC did not affect the affinity or the number of specific binding sites. There were no marked differences in the slopes or x-axis intercepts of the Scatchard plots (Fig. 1), and values for B_{max} and K_d of all three groups were not significantly different. Because of the limited amount of tissue available, it was not

possible to determine if chronic THC treatment affected beta-adrenergic agonist binding or its modulation by guanine nucleotides in these sarcolemmal membranes.

Effects of THC on cardiac beta-adrenoceptors have not been reported previously; however, other investigators [10] have reported that THC alters beta-adrenergic antagonist, but not agonist, binding to cerebral cortical membranes when present in the binding assay medium. This suggests that THC will not affect the responsiveness to beta-adrenoceptor agonists, at least not via alterations in the agonist-receptor interaction, and studies in isolated rat heart have demonstrated that THC does not alter the chronotropic action of isoproterenol [18]. The earlier work examining beta-receptor binding in cortical membranes in the presence of THC [10] is not comparable with the present data. In current experiments, sarcolemmal membranes were prepared from hearts isolated from rats after chronic treatment with THC, and these membrane preparations were subsequently assayed for [^{3}H]DHA binding in the absence of THC.

The cardiovascular effects of marijuana and its major active ingredient, THC, are well known. In man, they typically consist of tachycardia associated with a slight increase or no effect in blood pressure [6, 12, 13, 16, 17, 23]; whereas, in most animal species including the rat, bradycardia and hypotension are observed [1, 9, 11, 27]. It is not well established whether these responses in animals are elicited by direct or indirect actions, i.e., whether they are mediated by effects on the heart and vasculature or by reflex or central nervous system actions. It has been reported that THC elicits a direct negative chronotropic response in isolated rat heart which is not affected by atropine or propranolol [18] and that THC reduces adenylate cyclase activity [19] in rat ventricular tissue, an effect which may alter basal cardiac function. On the other hand, the observation that cardiovascular effects of THC in cats can be attenuated by cervical spinal transection but not by bilateral vagotomy [27] suggests that the effects are central in origin and mediated primarily by the sympathetic nervous system. Therefore, it seemed possible that chronic THC administration would alter adrenoceptor responsiveness and/or number [8,15] due to changes in efferent sympathetic activity. It is well known that chronic administration induces tolerance to the cardiovascular effects of THC in rodents [1, 9, 14] as well as other species [7, 21, 22]. The current data, however, indicate that chronic exposure to THC did not alter the number of specific binding sites nor the affinity for DHA in sarcolemmal membranes isolated from rat cardiac muscle. This finding suggests that tolerance develops via non-receptor mechanisms or that the alteration in cardiac beta-adrenoceptors is specific for agonist binding.

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