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Short communication

Δ^9-Tetrahydrocannabinol enhances presynaptic dopamine efflux in medial prefrontal cortex

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Acute administration of 1.0-2.0 mg/kg Δ^9-tetrahydrocannabinol (Δ^9-THC) increased presynaptic dopamine (DA) efflux in the medial prefrontal cortex of rats, as measured by intracerebral microdialysis in awake, behaving rats. These data are congruent with suggestions that (1) marijuana's euphorigenic effects and abuse potential may be related to augmentation of presynaptic DA mechanisms, and (2) the medial prefrontal cortex may be an important site of action for drugs of abuse in general and for Δ^9-THC in particular.

Marijuana; Δ^9-Tetrahydrocannabinol; Dopamine; Cortex (medial prefrontal); Microdialysis; Drugs of abuse

1. Introduction

The neuropharmacological basis for marijuana's abuse liability has been obscure. That habit-forming drugs derive significant abuse liability from direct enhancement of brain reward, and that this enhancement involves a portion of mesolimbic/mesocortical dopamine (DA) projections to the forebrain, has become a seminal conception. Congruent with this conception, we have shown: (1) that Δ^9-tetrahydrocannabinol (Δ^9-THC), the psychoactive ingredient in marijuana, augments brain stimulation reward (electrical intracranial self-stimulation) in the rat medial forebrain bundle at low doses believed pharmacologically relevant to moderate human use of marijuana (Gardner et al. 1988); and (2) that low doses of Δ^9-THC augment potassium-stimulated presynaptic DA efflux in rat neostriatum as measured by both in vivo voltammetric electrochemistry and in vivo brain microdialysis (Ng Cheong Ton et al., 1988). We also have recently found that Δ^9-THC enhances mesolimbic presynaptic DA efflux in the nucleus accumbens (Chen et al., 1989). We now report that Δ^9-THC also enhances presynaptic DA efflux in the medial prefrontal cortex, a forebrain convergence of reward-relevant mesocortical DA circuitry (Smith and Dworkin, 1986).

2. Materials and methods

Brain microdialysis was performed in freely-moving 250-300 g male Lewis rats (Charles River). They were housed individually on ad libitum food and water in an animal room kept at 72° F with a 12 h light-dark cycle. Before experiments started, all rats were implanted under pentobarbital anesthesia with microdialysis probes in the medial prefrontal cortex on the left side of the brain by standard surgical and stereotaxic technique. Loop-shaped dialysis probes (Ng Cheong Ton et

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al., 1988) were implanted longitudinally in medial prefrontal cortex (stereotaxic coordinates A + 2.7 (from bregma), L 0.4 (from midline), V −5.5 (from dura)) 20-24 h before experiments began. Each probe loop consisted of a 5 mm cellulose tubular dialysis membrane (Spectrum, MCO 6000). As determined by in vitro tests, relative recovery rates for the probes used in these experiments were approximately 6% for DA, 8% for 3,4-dihydroxyphenylacetic acid (DOPAC) and 9% for homovanillic acid (HVA). The perfusate was a modified Ringer solution (189 mM NaCl, 3.9 mM KCl, 3.37 mM $CaCl_2$, pH 6.0) perfused through the dialysis probes at 2 μl/min. Dialysis samples (40 μl) were collected every 20 min and immediately assayed for DA and for the DA metabolites DOPAC and HVA by high performance liquid chromatography with electrochemical detection. The compounds were separated by reverse phase chromatography with a Brownlee Velosep RP-18 (3 μm particles) 100 × 3.2 mm column and a mobile phase (pH 3.0), containing 0.1 M chloroacetic acid, 0.1 mM EDTA, 0.2 mM sodium octyl sulfate and 4.5% methanol. The mobile phase flow rate was 1.0 ml/min. Electrochemical measurements of the eluting species were made with a glassy carbon electrode (Bioanalytical Systems model TL-5A) coupled to a LC-4B potentiostat (Bioanalytical Systems) set at +0.7 V. The minimum detection limit was approximately 6-16 fmol for all chemicals measured.

Δ^9-THC (National Institute on Drug Abuse, U.S. Public Health Service) was prepared for in vivo administration by an adaptation of methods we have previously described (Ng Cheong Ton et al., 1988). Briefly, the Δ^9-THC was dissolved in a 20% solution of polyvinylpyrrolidone (PVP) (Aldrich Chemical Company) prepared in ethanol. This mixed solution was then evaporated under nitrogen and the dried, concentration Δ^9-THC-PVP solution was suspended in saline at a concentration of either 1.0 or 2.0 mg/ml. A 20% PVP solution was used for control injections. All administrations of drug or vehicle were given by i.p. injection.

Three to four consecutive stable samples were collected before drug or vehicle was administered. Mean levels of DA, DOPAC and HVA of the pre-injection samples were used as baselines and analysis was performed on percent changes after drug or vehicle injections. Data were analyzed by two-way ANOVA with repeated measures, followed by post-hoc Tukey-Kramer tests of individual comparisons. In view of the fact that the animals were fully conscious and freely-moving, behavioral observations were made of all animals throughout all dialysis experiments. Standard histology was used to verify dialysis probe locations after the completion of experiments.

3. Results

Basal DA and metabolite levels preceding drug or vehicle injection were 48.6 ± 6.4 fmol/40 μl per 20 min DA (N = 12), 2.44 ± 0.57 pmol/40 μl per 20 min DOPAC (N = 12), and 3.54 ± 0.49 pmol/40 μl per 20 min HVA (N = 12). As shown in fig. 1, 1.0 mg/kg and 2.0 mg/kg Δ^9-THC significantly enhanced DA efflux in medial prefrontal cortex (Δ^9-THC effect: $F(2,9) = 10.7$; $P <$

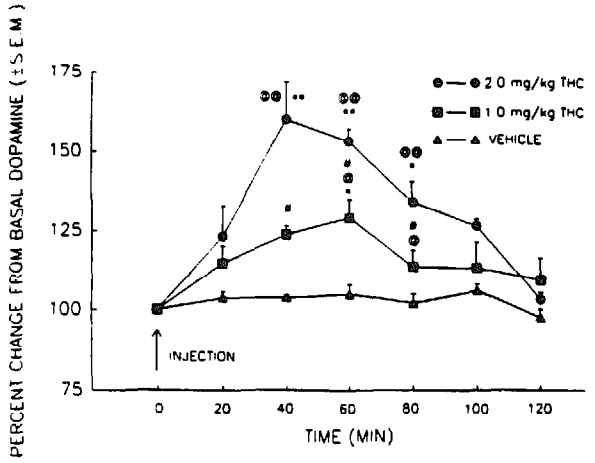

Fig. 1. Effect of acute Δ^9-THC on presynaptic dopamine (DA) efflux in medial prefrontal cortex of conscious, freely moving rats (N = 4 for each 1.0, 2.0 mg/kg Δ^9-THC and control group). Values are expressed as means ± S.E.M. percentages of pre-injection basal DA levels. Comparison of individual sample with pre-injection basal DA level (* $P < 0.05$, ** $P < 0.01$); comparison of individual sample at each corresponding time between 1.0 mg/kg and 2.0 mg/kg Δ^9-THC (# $P < 0.05$) comparison of individual sample at each corresponding time between Δ^9-THC-treated rats and vehicle-treated rats (@ $P < 0.05$, @@ $P < 0.01$).

0.005), with 2.0 mg/kg Δ^9-THC having earlier peak effect (time × Δ^9-THC interaction effect: $F(10,45) = 4.3$; $P < 0.0005$). Δ^9-THC did not significantly alter DOPAC or HVA. Vehicle injections did not alter DA, DOPAC or HVA.

The rats were behaviorally unaffected by i.p. injections of the PVP vehicle. They showed no evidence of stereotypy or psychomotor stimulation following i.p. injections of Δ^9-THC; to the contrary, all animals seemed slightly quiescent following Δ^9-THC, but still retained full motoric capability.

4. Discussion

The present study with Δ^9-THC shows that this psychoactive ingredient in marijuana augments basal extracellular DA efflux in medial prefrontal cortex. These findings are in agreement with previous reports that Δ^9-THC augments DA synthesis and release in synaptosomes (Poddar and Dewey, 1980) and inhibits DA uptake into synaptosomes (Banerjee et al., 1975). Also, the present findings are in close agreement with our own previous in vivo findings that: (a) Δ^9-THC augments brain stimulation reward (electrical intracranial self-stimulation) in the rat medial forebrain bundle (Gardner et al., 1988); and (b) Δ^9-THC augments potassium-evoked DA efflux in rat caudate-putamen and basal DA efflux in nucleus accumbens, perhaps by inhibiting presynaptic DA reuptake mechanisms (Ng Cheong Ton et al., 1988; Chen et al., 1989).

Importantly, the present findings together with our previous findings in caudate putamen and nucleus accumbens (Ng Cheong Ton et al., 1988; Chen et al., 1989) constitute the first studies of Δ^9-THC's effect on brain DA biochemistry to use doses of Δ^9-THC that approximate those found in human use of marijuana. Rosenkrantz et al. (1975) translated doses of Δ^9-THC in rats to inhalation doses in humans; taking their assumptions and calculations, the dose range of 1.0-2.0 mg/kg Δ^9-THC used in the present experiments translates into two to three marijuana cigarettes of moderate Δ^9-THC content. Thus, the presently observed robust effects of Δ^9-THC on DA brain systems implicated in mediating brain reward would appear to be pharmacologically and physiologically relevant to human marijuana use.

The medial prefrontal cortex, a principal terminal of the mesocortical DA projection, has been hypothesized to be an essential anatomical location in the pharmacological action of drugs of abuse (Goeders and Smith, 1983; Smith and Dworkin, 1986). Direct self-administration of cocaine into the medial prefrontal cortex has been reported (Goeders and Smith, 1983). Furthermore, lesions of the ventral tegmental area (containing the DA neurons for the mesolimbic/mesocortical dopaminergic pathways) is known to modify cocaine self-administration, but the degree of attenuation does not correlate with the decrease in DA content in the nucleus accumbens (Roberts and Koob, 1982), indicating that dopaminergic innervation of other structures such as medial prefrontal cortex may participate in the neuronal processes mediating reinforcement. Also, activation of DA innervation in the prefrontal cortex after administration of phencyclidine (PCP, angel dust) has been reported (Deutch et al., 1987).

Smith and Dworkin (1986) have pointed out that drug self-administration is a complex behavioral phenomenon involving many pharmacological actions of the abused drug in addition to the reinforcing effect per se, such as sensory, perceptual, motivational and motoric actions, as well as stimulus- and/or response-modulating actions. In addition, Smith and his colleagues have pointed out that brain sites of initiation of reinforcing neuronal activity may be different from brain sites involved in general reinforcement processes. Moreover, these workers have delineated two anatomically distinct neuronal systems hypothesized to be involved in drug self-administration. One of these systems, a medial prefrontal cortex-striatum-medial prefrontal cortex circuit, is proposed to partially mediate the reinforcement engendered by administration of abused drugs (Smith and Dworkin, 1986). This medial prefrontal cortex-striatum-medial prefrontal cortex circuit may act as a reinforcement initiation system (because the medial prefrontal cortex will support direct intracranial drug self-administration) or as part of a generalized reinforcement system (be-

cause the medial prefrontal cortex is one of the brain sites activated by electrical brain-stimulation reward in the ventral tegmental area, as determined by quantitative radioactive 2-deoxyglucose autoradiographic estimation of local cerebral glucose utilization), or possible as both. On the basis of intracranial drug self-administration studies coupled with neurochemically-specific denervation and pharmacological manipulations, these same workers have suggested that both pre- and postsynaptic DA mechanisms in medial prefrontal cortex play an important role in mediating the reinforcement processes in medial prefrontal cortex involved in drug-engendered reward (Smith and Dworkin, 1986).

While not speaking directly to the accuracy of these provocative and important hypotheses, the present data are definitely congruent with them insofar as they suggest that DA mechanisms in the medial prefrontal cortex constitute as important site of action of the cannabinoid class of abused drugs. In addition, the present findings, taken with our previous demonstrations (Gardner et al., 1988; Ng Cheong Ton et al., 1988; Chen et al., 1989), suggest that marijuana is not as anomalous a drug of abuse a formerly thought, and may in fact resemble other drugs of abuse (ethanol, opiates and cocaine) in producing, as a final common neuropharmacological action, acute facilitation of the mesocorticolimbic DA reward-relevant circuitry.

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