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Blockade of dorsal hippocampal dopamine receptors inhibits state-dependent learning induced by cannabinoid receptor agonist in mice

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ABSTRACT

To clarify the interaction between cannabinnoid CB1 receptors and the dopaminergic system in memory processes, the effects of dopamine receptor agents on the state-dependent learning induced by the non-selective CB1/CB2 receptor agonist, WIN55,212-2 have been investigated in mice. Animals implanted with unilateral cannula at the CA1 region of the dorsal hippocampus and microinjected with WIN55,212-2 and/or dopaminergic agents, were tested using a single-trial step-down passive avoidance task. Intra-CA1 microinjections of WIN55,212-2 (0.1–1 μg/mouse) immediately after training, decreased the step-down latency, indicating an amnesic effect of the drug. The amnesia was reversed by pre-test administration of the drug, suggesting state-dependent learning by the cannabinoid. Pre-test microinjection of apomorphine, a D1/D2 dopamine receptor agonist (0.1–0.3 μg/mouse) into the CA1 region reversed the amnesia induced by post-training WIN55,212-2 (1 μg/mouse). Moreover, pre-test co-administration of apomorphine with an ineffective dose of WIN55,212-2 (0.01 μg/mouse), showed a reversion of the impairment on retention performance. Pre-test administration of the same doses of apomorphine did not show any response by itself. Pre-test intra-CA1 administration of a D1 dopamine receptor antagonist, SCH23390 (0.05–0.3 μg/mouse) or D2 dopamine receptor antagonist, sulpiride (0.125–0.5 μg/mouse) inhibited the expression of WIN55,212-2-induced state-dependent learning. Pre-test microinjection of the same doses of SCH23390 or sulpiride had no effect on WIN55,212-2-induced amnesia. Moreover, single injection of SCH23390 (0.2 and 0.3 μg/mouse) or sulpiride (0.125 μg/mouse) decreased memory retrieval. The results suggest that the dorsal hippocampal dopaminergic system participates in the modulation of WIN55,212-2-induced state-dependent learning.

1. Introduction

Cannabinoids, as psychoactive drugs cause different effects in a large number of species. The agents exert their effects through two different CB1 (Matsuda et al., 1990) and CB2 (Munro et al., 1993) cannabinoid receptor subtypes. The CB1 receptors are mainly found in the central nervous system, but they are also found in peripheral tissues (for review see Svízenská et al., 2008). However, there is a report that CB2 receptors existed only in the periphery (Chaperon and Thiébot, 1999), but CB2 receptors have been found in the brain areas such as the hippocampus (Mackie and Stella, 2006; Brusco et al., 2008). Although, the existence of a non-CB1/CB2 (so-called CB3) receptor in mouse hippocampus has been suggested (Hajos et al., 2001; Hajos and Freund, 2002), but has been denied by other investigators (Kawamura et al., 2006; Takahashi and Castillo, 2006). Cannabinoid receptors are widely distributed in the hippocampus, cortex, basal ganglia and the cerebellum (Davies et al., 2002; Wilson and Nicoll, 2002). There are high levels of expression of CB1 receptors in the hippocampal formation (Ameri, 1999; Hampson and Deadwyler, 1999; Herkenham et al., 1990; Pettit et al., 1998) especially in the dorsal hippocampus which is a structure essential both for memory (Izquierdo and Medina, 1995; Nguyen et al., 1994) and long-term potentiation (LTP) (Bliss and Collingridge, 1993). Therefore, it is

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assumed that the role of endocannabinoid systems in hippocampal memory processing is controlled by CB1 receptors (Clarke et al., 2008; Riedel and Davies, 2005). Several lines of evidence reveal that administration of cannabinoid receptor agonists block LTP in hippocampal slices (Collins et al., 1995; Paton et al., 1998; Stella and Schweitzer, 1997; Terranova et al., 1995). Hippocampal LTP is enhanced in CB1 knock-out mice (Bohme et al., 2000). Furthermore, the activation of CB1 receptors in the CA1 regions of dorsal hippocampus by post-training administration of WIN-55,212-2 inhibits long-term memory formation in the object recognition task which is due to disruption of consolidation (Clarke et al., 2008).

Cannabinoids may interact with several neurotransmitter systems, including the dopaminergic system (Rodriguez de Fonseca et al., 1991). They modulate monoamine synthesis (Moranta et al., 2004) and release dopamine (Kathmann et al., 1999; Wu et al., 2008) through the activation of CB1 receptors. The activation of the mesolimbic dopaminergic system has also been suggested to be involved in the rewarding effect of cannabinoids (French, 1997; French et al., 1997; Tanda et al., 1997). On the other hand, the hippocampus receives a dopaminergic input from the ventral tegmental area which forms a functional loop designed to detect novelty (Lisman and Grace, 2005) and has different types of DA receptors (Meador-Woodruff et al., 1994). Previous studies have also indicated that hippocampal dopamine receptors have an important role in synaptic plasticity which is the basis of learning and memory (Lemon and Manahan-Vaughan, 2006; Morris et al., 2003; Navakkode et al., 2007; Tran et al., 2008). Considering the involvement of CB1 receptors and dopamine receptor system of dorsal hippocampus in memory processes, the effects of dopaminergic receptor mechanism(s) on cannabiniod-induced state-dependent learning, using a step-down passive avoidance task have been investigated in the present study.

2. Materials and methods

2.1. Animals

Male albino NMRI mice (22–25 g at the time of surgery) were used. The animals were maintained under a 12/12-h light–dark cycle, with light beginning at 7:00 a.m. and in a controlled temperature ($22 \pm 2\ ^{\circ}C$), with *ad libitum* access to food and water. The animals were housed ten per cage. Ten animals were used in each experimental group. Each animal was used once. All procedures were carried out in accordance with institutional guidelines for animal care and use.

2.2. Surgical and infusion procedures

Mice were anesthetized with intraperitoneal injection of ketamine hydrochloride (50 mg/kg) plus xylazine (5 mg/kg) in a volume of 10 ml/kg and placed in a stereotaxic apparatus. The skin was incised and the skull was cleaned. One 22-gauge guide cannula was placed 1 mm above the intended site of injection according to the atlas of Paxinos and Franklin (2001). Stereotaxic coordinates for the CA1 region of the dorsal hippocampus were AP: −2 mm from bregma, L: −1.6 from the sagittal suture and V: −1.5 mm from the skull surface. The cannula was secured to anchor jewelers' screws with dental acrylic. A stainless steel stylet (27-gauge) was inserted into the guide cannula to keep it free of debris. All animals were allowed 1 week to recover from surgery and to clear anesthetic.

For drug injection, the animals were gently restrained by hand; the stylet was removed from the guide cannula and replaced by 27-gauge injection needle (1 mm below the tip of the guide cannula). The injection solutions were administered in a total volume of 0.5 μl/mouse over a 60 s period. The injection needle was left in place for an additional 60 s to facilitate the diffusion of the drugs.

2.3. Apparatus

The inhibitory avoidance apparatus was a wooden box (30 cm × 30 cm × 40 cm). The floor of the apparatus consisted of parallel stainless steel bars (0.3 cm in diameter spaced 1 cm apart). A wooden platform (4 cm × 4 cm × 4 cm) was placed on the center of the grid floor.

2.4. Training

A single-trial step-down passive avoidance task was used. In the training session, animals were placed on the platform and their latencies to step down on the grid floor with all four paws were recorded. Immediately after stepping down on the grid floor each animal received an electric shock (1 Hz, 0.5 s, 45 V DC) continuously for 15 s, which was delivered to the grid floor by an isolated stimulator. Retention test session was carried out 24 h after the training and was procedurally identical to the training, except that no shock was given. Step-down latency was used as a measure of inhibitory avoidance memory. An upper cut-off time of 300 s was set. The training and testing sessions were carried out between 8:00 a.m. and 2:00 p.m.

2.5. Drugs

The drugs used in the present study were WIN55,212-2mesylate (Tocris Cookson, Bristol, UK), apomorphine, SCH 23390 (R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-3,4,5-tetrahydro-1H-3-benzazepine hydrochloride) and sulpiride (Sigma Chemical Co., St Louis, CA, USA). WIN55,212-2 was dissolved in dimethylsulphoxide (DMSO; up to 10%, v/v) and sterile 0.9% saline and a drop of Tween 80, which was also used as vehicle. Apomorphine and SCH 23390 were dissolved in sterile 0.9% saline and sulpiride was dissolved in vehicle (the vehicle was one drop of glacial acetic acid with a Hamilton microsyringe and made up to a volume of 5 ml with sterile 0.9% saline and then diluted to the required volume) just before the experiment. Control animals received either saline or appropriate vehicle. All the drugs were injected intra-CA1. The doses used and time intervals are based on our previous experiments (Zarrindast et al., 2006a) and a pilot study.

2.6. Verification of cannula placements

After completion of the experimental sessions, each animal was killed with an overdose of ether. Animals received bilateral intra-CA1 injection of ink (a 0.5 μl/mouse; 1% aquatic methylene blue solution). The brains were then removed and fixed in a 10% formalin solution for 10 days before sectioning. Sections were examined to determine the location of the cannula aimed for the CA1 region. The cannula placements were verified using the atlas of Paxinos and Franklin (2001).

2.7. Statistical analysis

The step-down latencies are expressed as the median and interquartile range. All data were analysed by Kruskal–Wallis non-parametric one-way analysis of variance (ANOVA), followed by the Mann–Whitney *U*-test. The latter test was also used where two groups were compared with each other. The criterion for statistical significance was $P < 0.05$. Calculations were performed using the SPSS statistical package.