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Effects of intrahippocampal cannabinoid receptor agonist and antagonist on radial maze and T-maze delayed alternation performance in rats

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ABSTRACT

Brain cannabinoid receptors are abundantly distributed in the hippocampus, however their detailed role in learning and memory remains unclear. This study investigated the role of hippocampal cannabinoid receptors for performing two kinds of working memory tasks. In experiment 1, intrahippocampal infusion of cannabinoid receptor agonist WIN 55,212-2 (1-2 μg/side) dose-dependently disturbed radial maze performance in rats. In experiment 2, WIN 55,212-2 (2 μg/side) disturbed the performance of delayed alternation in a T-maze by increasing the errors and successive errors, and on the other hand, a cannabinoid receptor antagonist AM 281 (1 μg/side) did not have any significant effects. Disruptive effect of WIN 55,212-2 on the number of errors in delayed alternation was blocked by the pretreatment with intraperitoneal AM 281 (2 mg/kg). Results suggest that hippocampal cannabinoid receptors are involved in the performance of working memory tasks. A possible role of endogenous cannabinoid system in the hippocampus was discussed in terms of an inhibitory adjustment of behavior based on the outcome of animals' previous response.

1. Introduction

Cannabinoids are known to affect sensory, motor and cognitive functions including learning and memory both in humans and rodents (Iversen, 2003). There are at least two types of G protein-coupled cannabinoid receptors identified presently. CB1 receptors are mainly expressed in the central nervous system (Matsuda et al., 1990), and their expression is abundant in the basal ganglia, cerebellum and hippocampus (Herkenham et al., 1990; Moldrich and Wegner, 2000; Tsou et al., 1998). The second cannabinoid receptors, CB2, are expressed in tissues of the immune system (Munro et al., 1993). In addition it is suggested recently that the third, putative cannabinoid receptors (CB3) exist in the central nervous system (Wilson and Nicoll, 2002). According to their localization, the effects of cannabinoids on cognitive functions are thought to arise through CB1 or CB3 receptor mechanisms. It has been suggested that activation of cannabinoid receptors inhibits long-term potentiation (LTP) in rat hippocampal slices (Collins et al., 1995; Misner and Sullican, 1999; Stella et al., 1997; Terranova et al., 1995), which is recognized as a neural base of learning and memory. On the other hand, hippocampal slices from mice lacking CB1 receptors exhibit larger LTP than those from wild-type animals (Bohme et al., 2000). Therefore, hippocampal CB1 receptors presumably are involved in learning and memory process.

A number of behavioral studies have shown that cannabinoid receptors play a role in the performance of various memory tasks which are closely related to the hippocampal functions. For example, systemic or intrahippocampal administration of several cannabinoid receptor agonists impaired the performance of radial maze task (Egashira et al., 2002; Iwasaki et al., 1992; Lichtman et al., 1995; Molina-Holgado et al., 1995) and of delayed alternation task in rats (Nava et al., 2000, 2001). On the other hand, systemic administration of a CB1 antagonist improved the performance in delay-interposed radial maze task (Lichtman, 2000; Wolff and Leander, 2003). These data suggest the possibility that the cannabinoid receptor blockade enhances the maintenance of memory information, while the activation deteriorates it. Furthermore, when CB1 receptor knockout mice were tested in several memory tasks, they showed better object recognition memory (Reibaud et al., 1999), and they continued to swim to the original platform position in the reversal task of Morris water maze compared to wild-type mice (Varvel and Lichtman, 2002). These observations also give support to the hypothesis that cannabinoid receptors are involved in learning and memory in an inhibitory manner.

However there is only a little evidence of hippocampal cannabinoid receptor involvement in learning and memory based on behavioral studies using direct administration of the drugs into the hippocampus. Furthermore, in previous studies, effects of cannabinoid receptor agonists and antagonists have not been examined in an identical learning task, so the effects of cannabinoid receptor activation and blockade on the memory task performance could not be

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compared directly. Therefore, we investigated the effects of intrahippocampal administration of cannabinoid receptor agonist and antagonist on two kinds of working memory tasks in rats. If the hippocampal cannabinoid receptors are involved in inhibition of working memory, a cannabinoid receptor agonist would disturb working memory performance, while an antagonist would improve it. First, we examined the effects of cannabinoid receptor agonist WIN 55,212-2 (R-(+)-(2,3-Dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo (1,2,3-de)-1,4-benzoxazinyl)-(1-naphthalenyl) methanone; WIN) on the radial maze performance (experiment 1). Next, we tested the effects of WIN and a cannabinoid receptor antagonist AM 281 (1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-4-morpholinyl-1*H*-pyrazole-3- carboxamide; AM) on the delayed alternation performance in a T-maze (experiment 2). The effect of pretreatment with intraperitoneal AM prior to hippocampal WIN treatment was also tested in this experiment. We used the task since we could easily operate the difficulty of the task by changing the length of intertrial interval (ITI), and thus we could examine the effects of both activation and blockade of these receptors in the same task.

2. Materials and methods

2.1. Subjects

Forty male Wistar–Imamichi rats (8–12 weeks old) were used as subjects, and their mean body weight at the beginning of behavioral tests was 310 g. They were housed in individual cages on a 12:12 h light–dark cycle, and maintained at 80–90% of their expected free feeding weight. Water was freely available. Seven rats were used in the radial maze task (experiment 1). Thirty-three rats were used in the delayed alternation task (experiment 2), and they were assigned to one of the three groups of drug treatment, WIN ($n=13$), AM ($n=11$) or AM+WIN ($n=9$). Animal experiments were approved by the University of Tsukuba Committee on Animal Research.

2.2. Surgery

Rats pretreated with atropine sulfate (0.05 mg, i.p.) were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and ketamine (10 mg, i.m.), and placed on a stereotaxic instrument. Guide cannulae were implanted bilaterally into the dorsal hippocampus with the stereotaxic coordinates (mm) AP: −3.8 from bregma, ML: ±2.7, DV: −3.0 from skull surface (Paxinos and Watson, 1998), and they were fixed on the skull with dental cement and small screws.

2.3. Drugs

Cannabinoid receptor agonist WIN (Sigma, MO) was dissolved in 45% 2-hydroxypropyl-β-cyclodextrin (HBC, Sigma) solution. Cannabinoid antagonist AM (Tocris, MO) was dissolved in dimethyl sulfoxide (DMSO; Wako, Osaka).

In intracerebral administration, drugs were bilaterally injected into the dorsal hippocampus 10 min prior to each trial (radial maze task) or each session (delayed alternation) via injection canulae, which were inserted into the guide cannulae and advanced 1.0 mm below the tips of them. The flow rate was kept 0.5 μl/min with a microsyringe pump (ESP-32, Eicom, Kyoto). After the drug injection, the injection cannulae were held to the site for additional 1 min to diffuse the drug from the tips. In AM+WIN group of experiment 2, AM or DMSO was administered intraperitoneally 15 min prior to hippocampal WIN injection.

2.4. Histology

After the behavioral tests, rats were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and perfused intracardially with 0.02 M-phosphate buffered saline followed by 10% formalin solution (Wako). The brains were further fixed in 10% formalin solution, then immersed in 20% sucrose solution. They were frozen by carbon dioxide, and sectioned in the coronal plane (40 μm) using a cryostat (CM3000, Leica, Heidelberg). Sections were Nissl-stained with cresyl violet to assess the location of tips of injection cannulae.

2.5. Behavioral procedures

2.5.1. Radial maze task (experiment 1)

2.5.1.1. Apparatus. An elevated eight-arm radial maze made of black polyvinyl chloride was used. The maze consisted of an octagonal center platform (32 cm in diameter) and 8 arms (60 cm×12 cm) radiated from the platform. A food well (1 cm in diameter, 0.5 cm deep) was carved out at each end of the arms. Plexiglas guillotine doors (15 cm high) divided the arms from the center platform, and each of them was operated automatically. The sidewalls of the arms were 4 cm high, except 12 cm from guillotine doors (12 cm high). The maze was elevated 70 cm above the floor. There were extra-maze visual cues (e.g. curtain, desk, colored drawing paper and door) around the maze in the experiment room. Control and analysis of the behavioral experiment were carried out using Image RM (O'Hara Co. Ltd, Tokyo), modified software based on the public domain NIH Image program (developed at the U.S. National Institutes of Health).

2.5.1.2. Training. Rats were given 5 min handling for three days and then three daily sessions of habituation to the apparatus. In the habituation session, all the guillotine doors were opened, and 20 mg food pellets (Research Diets, Inc., NJ) were placed on the platform and arms. In the first two sessions, 5 rats were placed in the maze together for 30 min, and in the last session, each rat was put in the maze individually for 15 min.

Rats were trained the radial maze task one trial a day. At the beginning of each trial, a 20 mg food pellet was placed in each food well. The rat was placed on the center platform and all the doors were opened. A choice was counted when the rat completely entered an arm, then all the doors except the chosen arm were closed. When the rat returned to the center platform, the door was closed and the rat was confined there for 5 s. After that, all the doors were reopened, and the rat was allowed the next choice. This procedure was repeated until the animal had consumed all the pellets, it had made 16 choices, or 10 min had elapsed since the start of the trial. A correct choice was defined as the rat entered an arm which had not been chosen in the trial and consumed the pellet, and the other choices were counted as errors. The learning criterion was defined as the 5 consecutive trials in which 7 or more correct choices in the first 8 choices were attained. Rats' choice responses and time spent to complete a trial were recorded. After the rats attained the criterion, they received the surgery of guide cannulae implantation.

2.5.1.3. Drug tests. After a week of recovery period from surgery, rats were retrained in the radial maze task. The procedures and the criterion were the same as in the acquisition training. Rats which reattained the criterion were given the drug tests. In the drug test, HBC (1 μl/side) and WIN (1.0–2.0 μg/1 μl/side) were tested in a random order. After drug injection trials, rats were given drug-free trials that continued until the criterion of 7 or more correct choices in the first 8 choices for 2 consecutive trials was attained.

2.5.2. Delayed alternation task (experiment 2)

2.5.2.1. Apparatus. A T-maze made of gray polyvinyl chloride was used. The maze consisted of a start box (20×12 cm, 30 cm high), an adjoining stem (40×12 cm, 30 cm high), and two arms (60×12 cm, 30 cm high). A food well (3 cm in diameter, 1 cm deep) was carved out at each end of the arms. Gray guillotine doors made of polyvinyl