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Effect of scopolamine on the hippocampal theta rhythm during an eight-arm radial maze task in rats

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Abstract

The changes in the hippocampal theta rhythm during an impairment of reference and working memory of radial maze task induced by scopolamine administration were studied. Intraperitoneal injection of scopolamine at doses of 0.5 and 1.0 mg/kg caused a significant increase in the number of total, reference memory and working memory errors. On the other hand, scopolamine significantly increased the hippocampal theta power (5–12 Hz) at doses (0.5 and 1.0 mg/kg) that caused an impairment of reference and working memory. A significant increase in the peak frequency of the hippocampal theta rhythm was also observed with scopolamine, even at a dose of 0.2 mg/kg. At doses of 0.2, 0.5 and 1.0 mg/kg, scopolamine caused a decrease in the locomotor activity during the radial maze task. From these results, it may be concluded that an increase in amplitude of the hippocampal theta rhythm induced by scopolamine is closely associated with memory/learning function of the eight-arm radial maze.

Keywords: Scopolamine; Radial maze; Hippocampus; Theta power; Peak frequency

1. Introduction

It is generally recognized that the hippocampal theta rhythm is closely correlated with memory and attention. For instance, Olvera-Cortés et al. (2004) reported that an increase of the hippocampal theta activity in the Morris water maze reflects learning rather than motor activity. We also demonstrated that the hippocampal theta wave is intimately associated with the memory acquisition of the eight-arm radial maze in rats (Masuoka et al., 2003). On the other hand, it has been reported that scopolamine, which is one of the most famous amnesic drugs, caused changes in the theta power in rats (Bennett et al., 1971; Bland, 1986; Markowska et al., 1995). However, the hippocampal theta rhythm differed greatly according to the conditions of electroencephalographic measurement. For example, scopolamine increased the hippocampal theta power in freely moving rats (Dimpfel, 2005), whereas the drug decreased the hippocampal theta power under urethane anesthesia in rats (Kinney et al., 1999). However, little work has been performed in the study of the hippocampal electroencephalogram after scopolamine treatment during the radial maze task. In the present study, therefore, the changes in the hippocampal theta rhythm during an impairment of reference and working memory of the radial maze task induced by scopolamine administration were investigated.

2. Materials and methods

2.1. Animals

Male Wistar rats, 7 weeks old (body weight, 200–220 g), were purchased from Japan SLC, Shizuoka, Japan. Animals were maintained in an air-conditioned room with controlled temperature (24±2 °C) and humidity (55±15%). They were housed in aluminum cages with sawdust and kept under a light–dark cycle (lights on from 07:00 to 19:00). The animals were allowed free access to food and water except during the experiments. All procedures involving animals were carried out in accordance with the Guidelines of the Animal

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2.2. Surgery

The rats were anesthetized with pentobarbital sodium (Nembutal®, 35 mg/kg, i.p., Abbott Laboratories, North Chicago, IL, USA) then fixed to a stereotaxic apparatus (SR-5, Narishige, Tokyo, Japan). For hippocampal electroencephalogram recording, stainless steel wire electrodes (200 μm) were chronically implanted into the right and left dorsal hippocampus (A: 3.0, L: ±2.5, H: 2.5) according to the atlas of de Groot (1959). Electrodes were connected to a miniature receptacle and the whole assembly was fixed to the skull with dental cement. One week was allowed for recovery from the surgery.

2.3. Eight-arm radial maze

The apparatus used was described in our previous paper (Chen et al., 1999). The procedure was as follows (Taga et al., 2001; Nishiga and Kamei, 2003). To familiarize them with the radial maze, rats received one daily habituation session for 3 days prior to training. On the first day, food pellets (45 mg, each, Bio-Serv, A Holton Industries, Frenchtown, N.J., USA) were scattered over the entire maze surface, and 3 or 4 rats were simultaneously placed on the radial maze and allowed to take pellets freely. Over the next 2 days, a pellet was placed in the food cup in each of the 8 arms, and the rat was allowed to explore freely until it had taken all the pellets. After adaptation, all rats were trained with 1 trial per day. In each trial, only 4 arms were baited, and the sequence was not changed throughout the experiment. The rat was placed on the center platform, which was closed off by a door. After 20 s, the door was opened and the rat was allowed to make an arm choice to obtain food pellets until all 4 pellets had been eaten or 10 min had elapsed. Rats were trained until reaching the criterion of at most 1 error per trial for 5 successive trials. The number of entries into the unbaited arms was scored as the total error. The first entry into never-baited arms was scored as a reference memory error, while re-entry into arms where the pellet had already been taken was scored as a working memory error. Scopolamine was injected the next day after reaching the criterion. Test trials were carried out 30 min after scopolamine treatment.

2.4. Electroencephalogram measurement and analysis

The electroencephalogram of the rat was recorded with a polygraph system (RM-6000, Nihon Kohoden, Tokyo, Japan) with a telemetric technique during the eight-arm radial maze task. Twenty seconds before each task, the telemetry transmitter (ZB-701J) was connected to a miniature receptacle on the head. The electroencephalogram signals were transmitted to the telemetry receiver (ZR-701J) and recorded with a thermal recorder (WT-645G). The analogue signals were converted into digital values using a multi-channel A–D converter (GENIUS, Medical Research Equipment, Tokyo, Japan) and fast Fourier transformer (FFT); spectral powers were calculated in real time using a personal computer (PC-9801 BX-2, NEC, Tokyo, Japan). In this system, data sampling was carried out at a rate of 50 Hz for 2.56 s. The power spectrum densities and peak frequencies of hippocampal theta rhythm were integrated and averaged for a whole task. In the present study, the hippocampal theta rhythm was defined as a 5–12 Hz band. The power spectrum of 5–12 Hz in the group administrated saline was defined as 100% in each rat. Hippocampal theta activity during a radial maze performance was recorded using the rats reaching the learning criterion.

2.5. Drugs

The following drugs were used: (-)scopolamine hydrobromide (Sigma, St. Louis, Mo., USA). Scopolamine was dissolved in saline and injected intraperitoneally at 30 min before each task.

2.6. Histology

The rats were anesthetized with pentobarbital sodium (Nembutal®, 35 mg/kg, i.p., Abbott Laboratories) and perfused transcardially with 10% formaldehyde neutral buffered solution (Wako, Tokyo, Japan) containing 5% potassium ferrocyanide (II) trihydrate (Wako). The brains were removed and placed in 10% formaldehyde neutral buffer solution for 3 days, and the area including the blue stain coronally removed. The sections were embedded in paraffin and sectioned (10 μm) coronally. The sections were then stained with hematoxylin and eosin. The lesion positions were checked under the microscope and reconstructed according to the atlas of Paxinos and Watson (1997). We only used the hippocampal electroencephalogram measured in the dorsal hippocampus CA1 area in the present study.

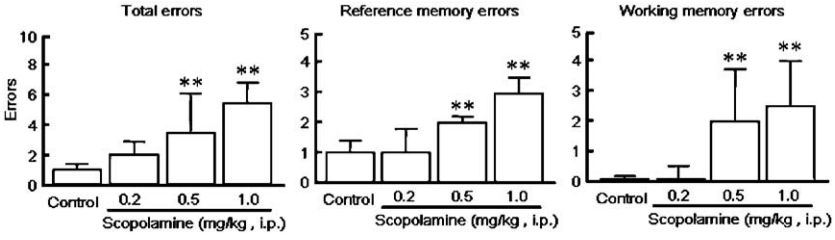

Fig. 1. Effect of scopolamine on radial maze performance in rats. Trials were performed 30 min after intraperitoneal injection of scopolamine. Columns and vertical bars represent median±median standard error ($n=16$). **: Significantly different from the control group at $P<0.01$.

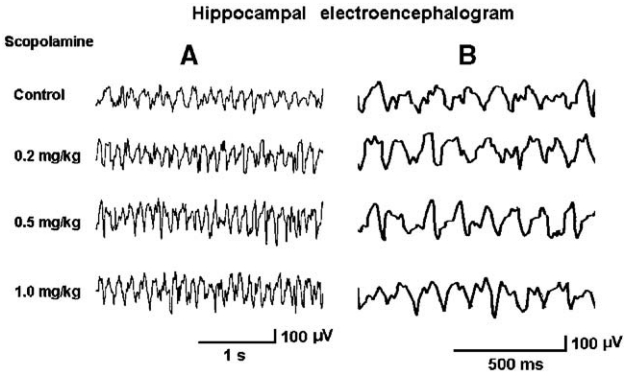

Fig. 2. Representative hippocampal electroencephalogram recording during the radial maze task A: Amplitude, B: Frequency.

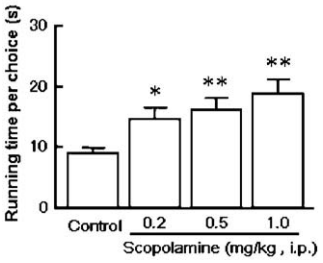

Fig. 4. Effect of scopolamine on running time per choice during the radial maze task in rats Columns and vertical bars represent means±S.E.M. (n=13–16). *, **: Significantly different from the control group at $P<0.05$ and $P<0.01$, respectively.

2.7. Statistical analysis

One-way analysis of variance with the Kruskall–Wallis test or Mann–Whitney U-test was used for the statistical analysis of total, reference memory and working memory errors in the eight-arm radial maze performance. One-way analysis of variance (ANOVA) with Dunnett's test was used for statistical analysis of theta power, theta peak frequency and running time per choice.

3. Results

Fig. 1 shows the effect of scopolamine on the radial maze performance in rats. Scopolamine, at a dose of 0.2 mg/kg, showed no significant effect on the number of total, reference memory and working memory errors. On the other hand, at doses of 0.5 and 1.0 mg/kg, scopolamine caused a significant increase in the number of total, reference memory and working memory errors. Fig. 2 shows a representative hippocampal electroencephalogram during the eight-arm radial maze task. Scopolamine caused a dose-dependent increase of amplitude and frequency of the hippocampal theta wave. Quantitative results in the amplitude and frequency of the hippocampal theta wave are shown in Fig. 3. Scopolamine increased the hippocampal theta power dose-dependently, and a significant increase was observed at doses of 0.5 and 1.0 mg/kg. Furthermore, when the frequency bands were divided into three categories (5–7, 7–9 and 9–12 Hz), scopolamine caused a significant increase in 7–9 Hz band at a dose of 0.5 mg/kg, and at a dose of 1.0 mg/kg, it also caused significant increase in 9–12 Hz bands. In addition, a significant increase was observed in the peak frequency of the hippocampal theta wave at all doses used. Fig. 4 shows the running time per choice, measured as an index of the locomotor activity. Scopolamine significantly increased the running time per choice at doses of 0.2, 0.5 and 1.0 mg/kg, indicating that scopolamine showed a decrease of locomotor activity. Fig. 5 shows the histological location of the hippocampal electrode. The electrodes were inserted in the

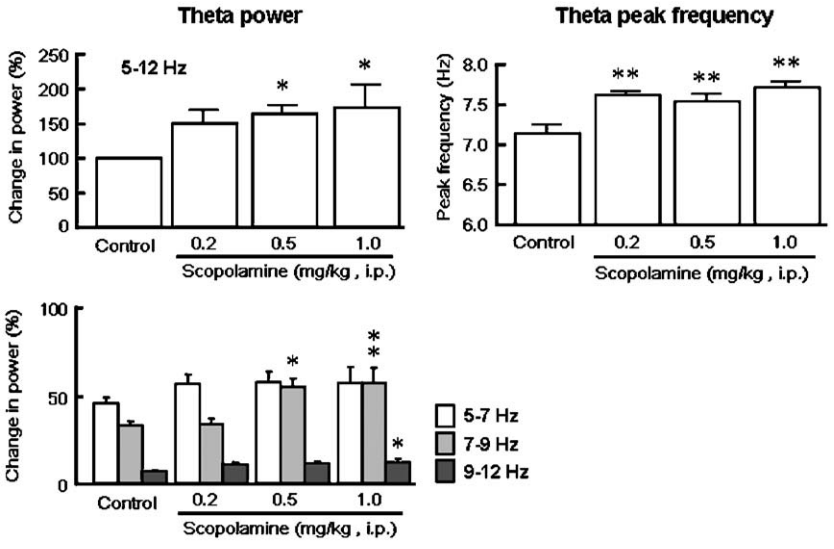

Fig. 3. Effect of scopolamine on hippocampal theta rhythm during the radial maze task in rats Columns and vertical bars represent means±S.E.M. (n=16). *, **: Significantly different from the control group at $P<0.05$ and $P<0.01$, respectively.

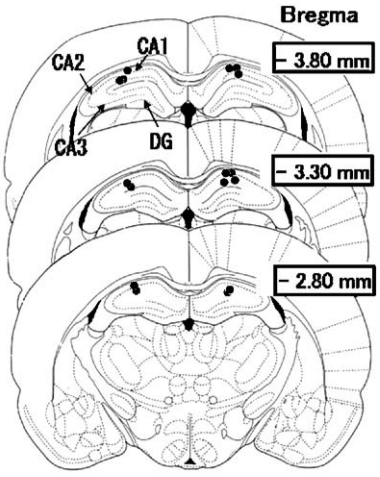

Fig. 5. Histological location of the hippocampal electrode. Anatomical diagram adapted from Paxinos and Watson (1997).

dorsal hippocampus CA1 area when a clear hippocampal theta wave was observed.

4. Discussion

In the present study, it was confirmed that scopolamine at doses of 0.5 and 1.0 mg/kg caused an impairment of the reference and working memory. Almost the same findings were reported by Okaichi et al. (1989), that scopolamine at doses of 0.4 and 0.8 mg/kg increased both reference and working memory errors using the same eight-arm radial maze task with four-arms baited. However, some reports showed that scopolamine impaired only the working memory of the radial maze task at doses of 0.1, 0.4 and 0.8 mg/kg (Wirsching et al., 1984), and at a dose of 0.5 mg/kg (Beatty and Bierley, 1985). Lydon and Nakajima (1992) reported that scopolamine at a dose of 0.5 mg/kg impaired both reference and working memory when rats were trained to a higher criterion of learning; a maximum of 1 error in 3 consecutive trials. On the other hand, scopolamine at the same dose impaired only working memory when rats were trained to a lower criterion; at least 3 correct responses on the first 4 choices for 3 consecutive trials. In our present results, the rats showed a higher criterion of learning; a maximum of 1 error in 5 consecutive trials. Therefore, it is reasonable to understand that scopolamine caused an impairment of both reference and working memory.

It was also found that scopolamine showed a significant increase in the hippocampal theta power (5–12 Hz) at doses of 0.5 and 1.0 mg/kg, which caused an impairment of reference and working memory. At doses of 0.2, 0.5 and 1.0 mg/kg, scopolamine caused a decrease in the locomotor activity during the radial maze task. Dimpfel (2005) reported that scopolamine at a dose of 0.5 mg/kg increased the hippocampal theta power (4.75–6.75 Hz) and alpha 1 power (7.00–9.50 Hz) in freely moving rats. This finding is essentially the same as our present results, though the conditions under which the electroencephalogram was recorded were quite different. As for locomotor activity, Okaichi et al. (1989) also reported that with scopolamine, at doses of 0.4 and 0.8 mg/kg, the running time per choice in the eight-arm radial maze performance was significantly increased, indicating that the locomotor activity was decreased. It is generally accepted that an increase in locomotor activity resulted in an induction of the hippocampal theta activity (Whishaw and Vanderwolf, 1973; McFarland et al., 1975; Bland, 1986). However, it was shown in the present data that scopolamine caused a decrease in the locomotor activity during the radial maze task. From these findings, it is reasonable to presume that an increase in the hippocampal theta power induced by scopolamine during the radial maze task does not depend on an increase in the locomotor activity.

We have reported that an increase in the hippocampal theta power was observed in the early stage of acquisition and decreased after the rats aquired spatial memory in the eight-arm radial maze task (Masuoka et al., 2003). Futhermore, there is a close relationship between decrease in theta powers of 7–9 Hz band and acquisition of spatial reference memory. These findings indicate that activation of the hippocampal function was recognized during the acquiring radial maze task. In addition, in the present study, an increase in the hippocampal theta power of 7–9 Hz band was observed in parallel with the memory deficit caused by scopolamine administration. That is to say, activation of hippocampal theta rhythm (7–9 Hz) was observed not only during the acquisition, but also during the impairment of spatial memory. These findings cleary indicated that the hippocampal theta rhythm is closely related to the memory/learning function.

It was also found that a significant increase in the peak frequency of the hippocampal theta rhythm was observed at doses of 0.2, 0.5 and 1.0 mg/kg. Givens and Olton (1995) reported that scopolamine at doses of 0.2 and 0.4 mg/kg impaired the working memory in a T-maze spatial alternation task, and at the same doses of scopolamine shifted the hippocampal theta activity to a higher peak frequency. However, in our present study, scopolamine caused no significant impairment in spatial memory at the dose of 0.2 mg/kg, whereas a significant increase in the peak frequency of the hippocampal theta rhythm was observed. These findings suggest that the changes in hippocampal theta rhythm are more sensitive to the changes in memory deficits in the radial maze.

From these results, it may be concluded that an increase in amplitude of the hippocampal theta rhythm induced by scopolamine is closely associated with the memory/learning function of the eight-arm radial maze.

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