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Apamin improves spatial navigation in medial septal-lesioned mice

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Abstract

We investigated the effects of acute i.p. injections of the Ca^{2+}-dependent K^+ channel blocker, apamin, on water maze spatial navigation, Y-maze and passive avoidance behavior in intact and medial septal-lesioned mice. Apamin 0.02, 0.06 or 0.2 mg/kg (i.p.) administered 30 min before or immediately after the training did not affect the performance of intact mice. Apamin 0.02 or 0.06 mg/kg (i.p.) administered immediately after the daily training did not affect the performance of medial septal-lesioned mice. Apamin 0.02 and 0.06 mg/kg (i.p.) administered 30 min before daily training reversed the navigation failure present in medial septal-lesioned mice during the initial and reversal learning stages of the water maze task. Apamin had no effect on the cognitive performance in Y-maze or passive avoidance tests. The results indicate that blockade of Ca^{2+}-dependent K^+ channels may facilitate acquisition of spatial navigation performance, but has no effect on consolidation, inhibitory avoidance and spontaneous alternation behavior in mice. © 1998 Elsevier Science B.V.

Keywords: Septal lesion; Medial; Apamin; K^+ channel, Ca^{2+}-dependent; Spatial navigation; Reversal learning; Memory; (Mouse)

1. Introduction

Apamin is a neurotoxin extracted from bee venom, which specifically inhibits a particular class of Ca^{2+}-dependent K^+ channels. These channels are characterised by their relatively high sensitivity to intracellular Ca^{2+} concentration, lack of voltage dependence and small conductance (Dreyer, 1990; Habermann, 1984; Lazdunski et al., 1988; Strong, 1990), and they are involved in the generation of slow afterhyperpolarization that occurs subsequently to the action potential in many excitable cells (Kawai and Watanabe, 1986). In mice and rats, apamin at high doses produces signs of poisoning such as tremors, ataxia and lethal respiratory insufficiency (Lallement et al., 1995). There are many apamin binding sites in some of the brain areas implicated in learning and memory processing such as the septum, the hippocampal formation, cingulate cortex and the anteroventral thalamic nuclei (Mourre et al., 1986; Gehlert and Gackenheimer, 1993). Apamin has been shown to block the slow afterhyperpolarization and increase the firing of cholinergic neurons in a slice preparation of the medial septum-diagonal band region (Matthews and Lee, 1991), suggesting that drugs acting via Ca^{2+}-dependent K^+ channels may modulate cholinergic function.

Recent pharmacological studies have revealed that blockade of Ca^{2+}-dependent K^+ channels may stimulate some forms of memory and learning in rodents. Apamin administered before or after the training has been shown to facilitate memory processes in appetitively-motivated bar-pressing response in mice (Messier et al., 1991). Another study (Deschaux et al., 1997) showed that apamin can improve learning in an object recognition task in rats. Injections of apamin before the training were shown to be effective, but injections after the training were ineffective. Moreover, apamin increases the expression of immediate early genes c-*fos* and c-*jun* in the hippocampal formation induced by learning (Heurteaux et al., 1993). These immediate early genes are thought to be involved in the activation of neurons during the memory process, and some studies have reported the induction of c-*fos* gene in dentate gyrus following the induction of long term potentiation (Dragunow et al., 1989). This finding suggests that a blocker of Ca^{2+}-dependent K^+ channels could modulate memory via these immediate early genes.

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Medial septal-lesioning causes a cholinergic deficit, which removes most of the cholinergic input to the hippocampus. This defect can be used as a model for the degeneration of the cholinergic cells (Hagan and Morris, 1988; Riekkinen et al., 1997), thought to be associated with the development of cognitive disorders in Alzheimer's disease (Dunnett et al., 1991; Fibiger, 1991; Bowen et al., 1992; Dunnett and Fibiger, 1993). The most common approach to treat this cognitive defect has been to use cholinesterase inhibitors, such as tacrine and metrifonate. Tacrine is already being used for treatment for human patients with Alzheimer's disease, and metrifonate has been shown to be effective in reversing the cognitive defect present in medial septal-lesioned rats (Riekkinen et al., 1996) and mice (our unpublished results).

In order to study further the role of Ca^{2+}-dependent K^+ channels in memory processing, we investigated the effects of apamin on the performance of young mice in the Morris water maze, Y-maze and passive avoidance tasks. We also wanted to study the possible therapeutic role of potassium channel inhibitors in cognitive disorders associated with a cholinergic deficit, and therefore in addition to intact mice, we also studied the effects of apamin on memory failure induced by medial septal-lesion in mice. Medial septal-lesioning impairs spatial learning, which disrupts the performance in the Morris water maze (Hagan and Morris, 1988; Riekkinen et al., 1997). In order to characterise the different cognitive processes modulated by apamin, we studied the action of this drug on acquisition of spatial reference and reversal memory in the water maze, on spontaneous alternation behavior in a Y-maze, and on passive avoidance performance. Spontaneous alternation behavior is supposed to reflect a primitive form of spatial working memory.

2. Materials and methods

2.1. Animals

Young (3–4-month-old; $n = 151$) female C57BL/6J//Kuo mice were used in the present study. The mice were housed five per cage, except the sham- and medial septal-lesioned mice, which were housed one per cage. The environment conditions were controlled and constant (21 ± 1°C, humidity at 50 ± 10%, light period 0700–1900). Food and water were available ad libitum. The study plan was approved by the municipal government of Kuopio county.

2.2. Drugs

Apamin (Sigma) was dissolved in NaCl 0.9% and injected intraperitoneally (i.p.) at 0.02, 0.06 and 0.2 mg/kg (10 ml/kg). Controls received vehicle injections of equal volume. The groups received two injections: 30 min before and immediately after the daily water maze, Y-maze or passive avoidance training. No drug or vehicle injections were given during the passive avoidance testing day. Details of the treatment groups are presented in Table 1.

2.3. Surgery

Medial septal (A: 0.9 mm, M: 0.0 mm, D: −4.7 mm; relative to the bregma) lesions were made by passage of an anodal DC current (1 mA, 15 s) via tungsten electrodes (diameter 0.0625 mm, 0.5 mm tip uninsulated). Sham-lesioned mice were treated identically, but no current was applied. Mice were deeply anaesthetised with a 1:1 mixture of Dormicum (Roche) and Hypnorm (Janssen Pharmaceutica) (s.c.) during the operations and for analgesia the mice were given a 0.1 mg/kg injection of buprenorfin (Temgesic; Reckitt and Colman) (s.c.) after the surgery. The mice were allowed to recover from the surgery for 2 weeks before starting the first experiments.

2.4. Water maze

We used a black plastic circular pool, diameter 120 cm, and a black painted stainless steel square platform; 14×14 cm, 1.0 cm below the water line. The pool was divided into three annuli of equal surface area, and the submerged escape platform was always in the middle annulus. The starting locations, which were labelled North, South, East and West, were located arbitrarily on the pool rim (Riekkinen et al., 1990). The timing of the latency to find the

Table 1
The number of mice in each treatment group

		Vehicle	Apamin (mg/kg)		
			0.02	0.06	0.2
Before the daily training	intact (3.1.)	9	10	10	10
	sham (3.2.)	10	–	–	–
	ms-lesioned (3.2.)	9	–	8	8
	ms-lesioned (3.3.)	7	6	–	–
After the daily training	intact (3.1.)	10	10	10	10
	ms-lesioned (3.4.)	7	8	9	–

The mice that received the apamin or vehicle injections 30 min before the daily training received also a vehicle injection immediately after the daily training, and the mice that received the apamin or vehicle injections immediately after the daily training, received also a vehicle injection 30 min before the daily training.

submerged platform was started and ended by the experimenter. A computer connected to an image analyser (HVS Image, Hampton, UK) monitored the swim pattern. Mice were placed in the water with their nose pointing towards the wall at one of the starting points in a random manner. If the mouse failed to find the platform in the maximum time, it was placed there by the experimenter. Mice were allowed to stay on the platform for 5 s. A 30-s recovery period was allowed between the training trials. The temperature of the water was kept constant throughout the experiment (20.5 ± 0.5°C).

The training schedule consisted of 8 consecutive days of testing. Four platform trials of 60 s were assessed per day during the first 5 training days. The platform location was kept constant (the Southwest quadrant) during this period of training. On the sixth day the platform was removed from the pool and the mice were allowed to swim for 50 s. Immediately after this spatial bias test, the platform was placed in the Northeast quadrant and five 50 s platform trials were assessed. The schedule on the seventh day consisted also of five 50 s platform trials (platform in the Northeast quadrant). The water maze experiment was finished on the eighth day with a spatial bias test (a 50 s trial without the platform). During the platform training trial, the following parameters were measured: escape length, percentage of animals that found the platform and swimming speed. During the spatial bias test the number of counter crossings was measured. Counter crossing was defined as crossing a circular area, in which the platform had previously been located, and which was three times larger than the platform (radius = 12.1 cm).

2.5. Y-maze

The Y-maze experiment was started 24 h after the water maze testing sessions. The Y-maze used in this experiment had black plastic walls that were 10 cm high. Its arms consisted of three compartments (10 cm × 10 cm) connected with 4 cm × 5 cm passages. The mouse was placed in one of the arm compartments and was allowed to move freely for 6 min without reinforcers. An arm entry was defined as the body of a mouse except for its tail completely entering into an arm compartment. The sequence of arm entries was manually recorded. An alternation was defined as the entry into all three arms on consecutive choices. The number of maximum spontaneous alternations was then the total number of arms entered minus 2, and the percent alternation was calculated as (actual alternations/maximum alternations) × 100. The test was run on two consecutive days (days 9–10).

2.6. Passive avoidance

Passive avoidance training trial was performed immediately after the Y-maze trial on the tenth day. The passive avoidance box consisted of a lit and a dark compartment. During the training trial the mice were placed in the lit compartment and 30 s later the sliding guillotine door was opened. After the mice entered the dark compartment (the latency was measured), the door was closed and a foot shock of 0.1 mA (0.5 s) was given. Then the mice were returned to their home cage and 24 h later they were again placed in the lit compartment during the testing trial and the latency to enter the dark compartment was measured (900 s maximum latency). The final latency was defined as a difference between the latencies on the first and the second day.

2.7. Histology

After the passive avoidance testing trial, the lesioned mice were decapitated. The brains of the mice were removed and immersed for 1–2 days in 4% formaldehyde solution. Fifty-micrometer sections were cut with a vibratome and the sections were stained. The medial septum area was stained with cresyl fast violet to see the position of the lesion. The hippocampal sections were stained with acetylcholinesterase staining (Hedreen et al., 1985; butylcholinesterase inhibitor was included in the assay mixture)

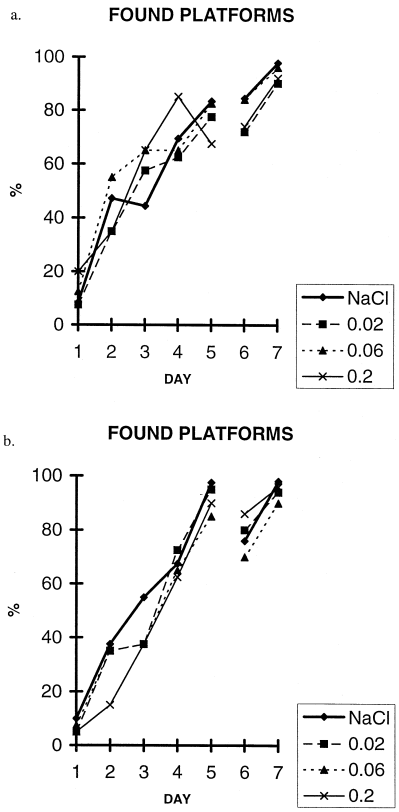

Fig. 1. Administration of apamin 0.02, 0.06 and 0.2 mg/kg 30 min before (a) or immediately after. (b) the daily training had no effect on reference memory or reversal learning, as there were no group differences in the number of found platforms. In both panels, the *X*-axis indicates the reference memory training days 1–5 (fixed platform location) and reversal learning days 6–7 (reversal of the platform location). The values are daily group means.

in order to confirm the decrease of acetylcholine-containing fibers in hippocampus.

2.8. Statistics

We evaluated the effect of the drugs on water maze escape distance and speed using analysis of variance for repeated measurements. We evaluated the effect of the drugs on the ability of the mice to find the water maze platform and the latencies in passive avoidance using Mann–Whitney test for two independent samples. The Bonferroni correction was used with analysis of variance and Mann–Whitney U-test. For analysis of the activity in Y-maze we used a one-way analysis of variance followed by Scheffe's post-hoc multiple group comparison.

3. Results

3.1. Intact mice; apamin administered before and after the daily training

3.1.1. Water maze

During the first 5 training days, apamin treatment administered before (0.02, 0.06 and 0.2 mg/kg) or after (0.02, 0.06 and 0.2 mg/kg) the daily training had no effect on the number of found platforms ($P > 0.05$) (Fig. 1), escape distance (Data not shown) or swimming speed (group: $F(3,35)/(3,36) < 1.09$, $P > 0.05$, for all comparisons) (speed, apamin administered before the daily training: vehicle: 16.8 ± 1.6; 0.02 mg/kg: 17.2 ± 4.0; 0.06 mg/kg: 17.6 ± 3.0; 0.2 mg/kg: 18.1 ± 2.5; speed, apamin administered after the daily training: vehicle: 18.3 ± 3.9; 0.02 mg/kg: 18.1 ± 3.4; 0.06 mg/kg: 16.8 ± 2.0; 0.2 mg/kg: 18.9 ± 2.9; group mean $\pm$ S.D.). There were no group differences in counter crossings during the first bias assessment ($F(3,35)/(3,36) < 3.018$, $P > 0.042$, Scheffe's test: $P > 0.05$) (counter crossings, apamin administered before the daily training: vehicle: 2.78 ± 1.39; 0.02 mg/kg: 2.1 ± 1.85; 0.06 mg/kg: 1.9 ± 1.6; 0.2 mg/kg: 1.6 ± 1.43; counter crossings, apamin administered after the daily training: vehicle: 2.30 ± 1.42; 0.02 mg/kg: 3.0 ± 2.11; 0.06 mg/kg: 1.40 ± 1.17; 0.2 mg/kg: 1.00 ± 1.70; group mean $\pm$ S.D.)

During the platform reversal stage on days 6 and 7, apamin treatment before or after the daily training had no effect on the number of mice that found the platform ($P > 0.05$) (Fig. 1), escape distance (Data not shown) or swimming speed (group: $F(3,35)/(3,36) < 1.73$, $P > 0.05$, for all comparisons) (speed, apamin administered before the daily training: vehicle: 16.0 ± 3.3; 0.02 mg/kg: 17.0 ± 4.1; 0.06 mg/kg: 18.8 ± 2.8; 0.2 mg/kg: 17.3 ± 3.2; speed, apamin administered after the daily training: vehicle: 13.7 ± 4.5; 0.02 mg/kg: 15.5 ± 4.9; 0.06 mg/kg: 12.9 ± 3.2; 0.2 mg/kg: 16.1 ± 3.7; group mean $\pm$ S.D. cm/s). During the second bias assessment measured on the eighth testing day, there were no differences in counter crossings ($F(3,35)/(3,36) < 1.175$, $P > 0.05$, for all com-

Table 2
The effects of apamin on Y-maze and passive avoidance tests of intact animals (apamin 0.02, 0.06 and 0.2 mg/kg administered before and after the daily training) and medial septal-lesioned animals (apamin 0.06 and 0.2 mg/kg administered before the daily training)

	Y-maze				PA		
	Day 1		Day 2		day 1 (s)	day 2 (s)	diff. (s)
	tot	%	tot	%			
Intact pre							
vehicle	16.4 ± 6.2	63.6 ± 13.8	9.5 ± 3.5	70.0 ± 16.2	26.7 ± 37.2	480.0 ± 243.1	422.0 ± 232.1
0.02	16.1 ± 6.8	66.5 ± 14.1	10.2 ± 6.1	52.9 ± 24.7	13.0 ± 5.9	752.5 ± 197.3	739.5 ± 194.7[b]
0.06	16.5 ± 5.3	55.4 ± 8.2	10.7 ± 4.0	54.1 ± 20.8	9.5 ± 2.8	470.5 ± 324.3	461.0 ± 325.9
0.2	18.3 ± 5.7	72.6 ± 15.3	12.5 ± 4.4	60.2 ± 15.0	55.5 ± 90.8	623.5 ± 245.6	568.0 ± 228.7
Intact post							
vehicle	19.5 ± 5.3	63.8 ± 7.7	10.0 ± 2.1	61.7 ± 15.3	18.0 ± 11.1	267.5 ± 218.0	249.5 ± 220.3
0.02	18.6 ± 5.9	64.2 ± 13.9	11.5 ± 4.7	60.1 ± 18.7	19.0 ± 17.3	419.0 ± 240.2	400.0 ± 233.1
0.06	16.6 ± 4.3	67.9 ± 14.4	11.2 ± 4.3	62.4 ± 21.4	36.0 ± 53.0	513.5 ± 309.3	477.5 ± 291.4
0.2	18.7 ± 4.2	64.8 ± 11.2	11.9 ± 5.1	70.4 ± 12.0	10.0 ± 2.3	153.5 ± 138.4	143.5 ± 137.4
MS-lesion pre							
sham	16.9 ± 9.5	45.0 ± 26.3	10.1 ± 6.6	42.5 ± 32.6	40.5 ± 32.7	587.5 ± 329.0	547.0 ± 323.8
vehicle	2.1 ± 1.1[a]	22.2 ± 44.1	5.3 ± 9.6	13.9 ± 27.7	300.0 ± 0.0[c]	708.9 ± 293.5	408.9 ± 293.5
0.06	7.6 ± 7.4[a]	47.8 ± 41.7	10.6 ± 8.2	50.6 ± 38.1	158.1 ± 123.1[d]	604.4 ± 366.0	446.3 ± 286.8
0.2	4.6 ± 3.9[a]	30.8 ± 38.3	11.3 ± 14.0	23.9 ± 33.2	285.0 ± 42.4[c]	540.0 ± 337.2	255.0 ± 316.3

These tests were not performed on other groups. Intact pre = Intact mice, apamin administered before the training; Intact post = Intact mice, apamin administered after the training; MS-lesion pre = medial septal-lesioned mice, apamin administered before the training, I; Y-maze: day 1 = first day of training; day 2 = second day of training; tot = number of total alternations; % = percent alternations. Passive avoidance: day 1 = entry latency of the training day; Day 2 = entry latency of the testing day; Diff. = the difference between the latencies of the testing day and the training day.
[a] $P = 0.000$ vs. sham-lesioned group, [b] $P = 0.017$ vs. vehicle group, [c] $P < 0.002$ vs. sham-lesioned group, [d] $P = 0.021$ vs. medial septal-lesioned-vehicle group. The values are expressed as mean ± S.D.

parisons) (counter crossings, apamin administered before the daily training: vehicle: 3.89 ± 2.76; 0.02 mg/kg: 4.00 ± 2.49; 0.06 mg/kg: 4.1 ± 2.85; 0.2 mg/kg: 3.6 ± 2.72; counter crossings, apamin administered after the daily training: vehicle: 4.50 ± 2.80; 0.02 mg/kg: 4.20 ± 2.20; 0.06 mg/kg: 2.80 ± 1.99; 0.2 mg/kg: 4.50 ± 2.42; group mean ± S.D.).

3.1.2. Y-maze and passive avoidance

Apamin administered before or after the daily training had no effect on total moves or percent alternation during either of the testing days in Y-maze ($F(3,35)/(3,36) < 2.642$, $P > 0.05$, for all comparisons) (Table 2). In passive avoidance there were no group differences in the latency to enter the dark compartment during the training day ($P > 0.05$) (Table 2). Apamin 0.02 mg/kg administered before the training day increased the difference between the entry latency during the testing and training day ($P = 0.017$). In contrast, apamin administered after the daily training had no effect on passive avoidance ($P > 0.05$) (Table 2).

3.2. Medial septal-lesioned groups; apamin administered before the training; I

3.2.1. Water maze

A comparison between the sham-lesioned group and the medial septal-lesioned-vehicle group showed a clear impairment in platform finding ($P = 0.000$) (Fig. 2a) and escape length ($F(1,17) = 23.77$, $P = 0.000$) (Fig. 2b) during the first 5 days. The 0.06 mg/kg dose of apamin decreased the escape length significantly compared to the medial septal-lesioned-vehicle group ($F(1,15) = 14.44$, $P = 0.006$). Apamin 0.06 mg/kg also increased the number of found platforms ($P = 0.018$ vs. medial septal-lesioned-vehicle group). However, apamin 0.06 mg/kg did not reverse the navigation failure present in medial septal-lesioned mice completely, as the group's performance was also impaired when compared with the sham-lesioned group (number of found platforms: $P = 0.001$)). Escape length, however, was not impaired ($F(1,16) = 1.00$, $P > 0.05$). The apamin 0.2 mg/kg had no effect on escape length ($F(1,15) = 0.31$, $P > 0.05$) or the ability to find the platform ($P > 0.05$) compared to the medial septal-lesioned-vehicle group. There were no group differences in swimming speed during the first 5 days (group: $F(3,31) = 2.19$, $P > 0.05$) (Sham: 21.5 ± 3.5; vehicle: 19.2 ± 3.0; 0.06 mg/kg: 17.8 ± 4.0; 0.2 mg/kg: 18.6 ± 2.0; group mean ± S.D. cm/s) between the groups. On the sixth day, during the spatial bias test, no group differences were observed in counter crossings ($F(3,31) = 0.507$, $P > 0.05$) (Sham: 6.10 ± 2.73; vehicle: 5.00 ± 1.32; 0.06 mg/kg: 5.25 ± 2.87; 0.2 mg/kg: 5.00 ± 1.69; group mean ± S.D.).

During the platform reversal, the vehicle- and apamin 0.2 mg/kg treated medial septal-lesioned mice were not as effective in escaping from the pool compared to the sham-lesioned group: there were differences in the number of found platforms (vehicle or apamin 0.2 mg/kg treated medial septal-lesioned vs. sham-lesioned group: $P = 0.000$) (Fig. 2a) and in the escape length ((group: $F(3,31) = 11.65$, $P = 0.000$); vehicle treated medial septal-lesioned vs. sham-lesioned group: ($F(1,17) = 19.73$, $P = 0.001$); apamin 0.2 mg/kg treated medial septal-lesioned vs. sham-lesioned group: ($F(1,16) = 24.38$, $P = 0.001$)) (Fig. 2b). However, apamin 0.06 mg/kg treatment decreased the escape length ($F(1,15) = 23.80$, $P = 0.000$) and increased the probability of platform finding ($P = 0.000$) in medial septal-lesioned mice. Indeed, there was no significant difference between the 0.06 mg/kg treated medial septal-lesioned group and the sham-lesioned group in escape length ($F(1,16) = 0.27$, $P > 0.05$) or in the number of found platforms ($P > 0.05$). There were no group differences in swimming speed during the platform reversal trials (group: $F(3,31) = 0.71$, $P > 0.05$) (Sham: 21.0 ± 4.6; vehicle: 20.0 ± 2.8; 0.06 mg/kg: 18.3 ± 6.3; 0.2 mg/kg: 21.0 ± 2.4; group mean ± S.D. cm/s) between any of the groups. On the eighth day, during the spatial bias test, no group differences were observed in counter crossings

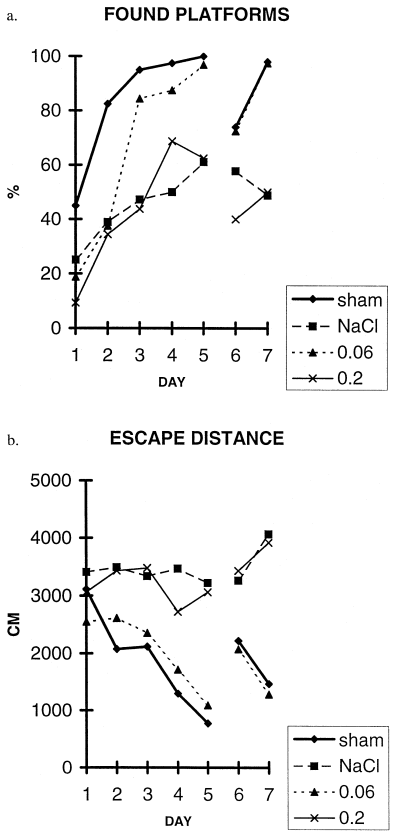

Fig. 2. Administration of apamin 0.06 mg/kg 30 min before the daily training alleviated the navigation failure present in medial septal-lesioned mice during both reference memory and reversal learning days by increasing the percentage of found platforms (a) and decreasing the escape length (cm) (b). In both panels, the *X*-axis indicates the reference memory training days 1–5 (fixed platform location) and reversal learning days 6–7 (reversal of the platform location). The values are daily group means.

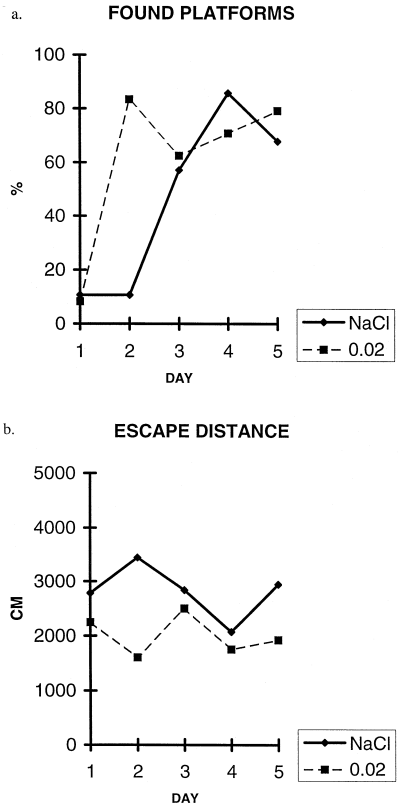

Fig. 3. Administration of apamin 0.02 mg/kg 30 min before the daily training alleviated the navigation failure present in medial septal-lesioned mice during reference memory training days by increasing the percentage of found platforms (a) and decreasing the escape length (cm) (b). In both panels, the *X*-axis indicates the reference memory training days 1–5 (fixed platform location). The values are daily group means.

($F(3,31) = 3.01$, $P = 0.045$, Scheffe's test: $P > 0.05$) (Sham: 6.60 ± 1.90; vehicle: 4.00 ± 1.80; 0.06 mg/kg: 5.13 ± 3.23; 0.2 mg/kg: 4.13 ± 1.13; group mean $\pm$ S.D.).

3.2.2. Y-maze and passive avoidance

During the first day in Y-maze all the medial septal-lesioned groups made significantly less total moves than the sham-lesioned group ($F(3,31) = 9.432$, $P = 0.000$). However, the percentage alternation was not affected ($F(3,31) = 0.899$, $P > 0.05$) (Table 2). During the second day in Y-maze there were no longer any group differences in total moves or percent alternation ($F(3,31) < 2.252$, $P > 0.05$) (Table 2).

The major proportion of medial septal-lesioned mice refused to enter the dark compartment already on the training day of passive avoidance test. The latency was greatest in medial septal-lesioned-vehicle group ($P = 0.001$ vs. sham-lesioned) and medial septal-lesioned-apamin 0.2 mg/kg group ($P = 0.002$ vs. sham-lesioned). The apamin 0.06 mg/kg treatment decreased the latencies on the training day compared to the medial septal-lesioned-vehicle group ($P = 0.021$) (Table 2). After the testing day, it was observed that there were no group differences in the difference between the latencies to enter the dark compartment on the testing day and the training day ($P > 0.05$) (Table 2).

3.3. Medial septal-lesioned mice; apamin administered before the training; II

3.3.1. Water maze

Administration of apamin 0.02 mg/kg increased the number of mice that found the platform ($P = 0.021$) (Fig. 3a) and it also decreased the escape length ($F(1,11) = 5.98$, $P = 0.033$) (Fig. 3b). There were no group differences in swimming speed ($F(1,11) = 0.41$, $P > 0.05$) (vehicle: 17.0 ± 3.1; 0.02 mg/kg: 15.6 ± 4.8; group mean $\pm$ S.D. cm/s). There were no group differences in counter crossings ($F(1,11) = 0.406$, $P > 0.05$) (vehicle: 5.00 ± 2.24; 0.02 mg/kg: 4.17 ± 2.48; group mean $\pm$ S.D.) during the bias assessment measured on the sixth day of the water maze training.

3.4. Medial septal-lesioned mice; apamin administered after the training

3.4.1. Water maze

Administration of apamin had no effect on the number of found platforms ($P > 0.05$) (Fig. 4a). There was an overall group difference in escape length (group: $F(2,21)$

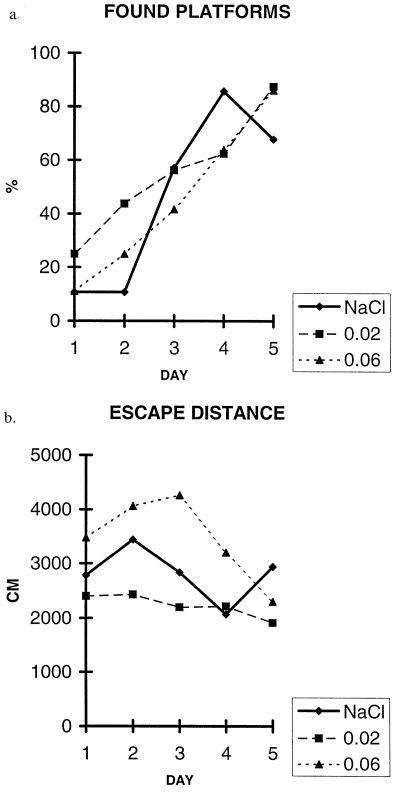

Fig. 4. Administration of apamin 0.02 and 0.06 mg/kg immediately after the daily training did not affect the number of found platforms (a) or escape length (cm) (b). In both panels, the *X*-axis indicates the reference memory training days 1–5 (fixed platform location). The values are daily group means.

$= 4.38$, $P = 0.026$) (Fig. 4b), but neither of the apamin-treated groups differed significantly from the vehicle group. In addition, the apamin 0.06 mg/kg group swam faster than the medial septal-lesioned-vehicle group ($F(1,14) = 12.31$, $P = 0.003$) (vehicle: 17.0 ± 3.1; 0.02 mg/kg: 16.9 ± 5.0; 0.06 mg/kg: 21.5 ± 2.0; group mean ± S.D. cm/s). There were no group differences in counter crossings ($F(2,21) = 1.225$, $P > 0.05$) (vehicle: 5.00 ± 2.24; 0.02 mg/kg: 3.50 ± 2.73; 0.06 mg/kg: 4.89 ± 1.27; group mean ± S.D.) during the bias assessment measured on the sixth day of the water maze training.

3.5. Histology

The locations of the lesions in medial septal-lesioned mice were confirmed by studying the cresyl fast violet -stained sections (Fig. 5a). The decrease of acetylcholine-

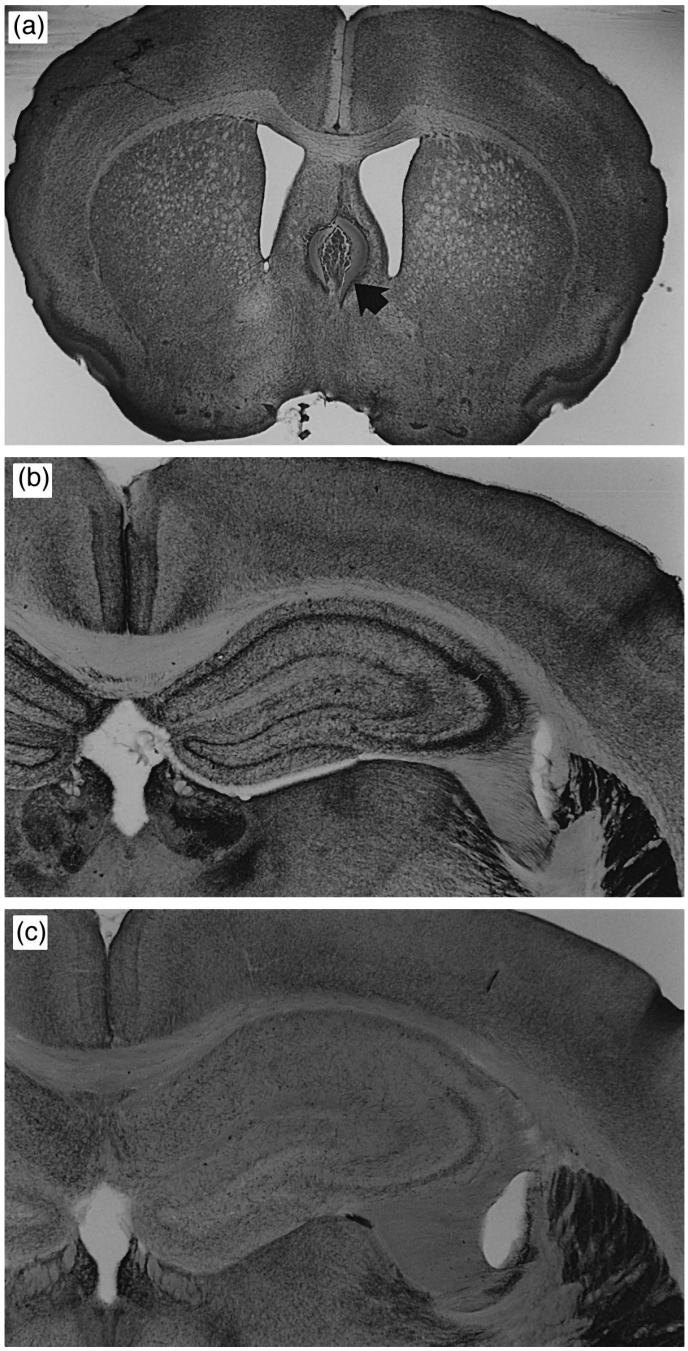

Fig. 5. (a) Cresyl violet staining of a coronal section containing medial septal-lesion site. An arrow indicates the location of the lesion. (b) Acetylcholinesterase staining of a coronal hippocampal section of an intact mouse. (c) Acetylcholinesterase staining of a coronal hippocampal section of an medial septal-lesioned mouse.

containing fibers in hippocampus was confirmed by acetylcholinesterase staining (Fig. 5, panels b and c).

4. Discussion

We described that in intact mice administration of apamin (0.02–0.2 mg/kg) before or after the training had no effect on water maze or Y-maze performance, but apamin 0.02 mg/kg at one dose administered before the training increased passive avoidance entry latency during the testing trial.

Previously, it has been shown that apamin could facilitate memory processing in a appetitively-motivated bar-pressing response task in intact mice. In the report of Messier et al., administration of apamin before the training at a dose of 0.2 mg/kg accelerated the acquisition of a bar-pressing response, but also increased the bar-pressing rates. Doses of 0.1 and 0.4 mg/kg were not effective. Administration of apamin at a dose of 0.2 mg/kg after the training also facilitated the memory process (Messier et al., 1991). In another article by Deschaux et al. (1997), apamin improved learning in an object recognition task in intact rats at a dose of 0.4 mg/kg. Doses of 0.1 mg/kg and 0.2 mg/kg were not effective, and apamin was also ineffective when administered after the training (Deschaux et al., 1997). We observed that apamin 0.02 mg/kg administered before the training trial increased passive avoidance entry latency. However, several non-mnemonic factors, such as altered sensitivity to foot shock, may affect passive avoidance performance. Indeed, the effects of apamin on pain threshold has not been studied and it is possible that the increased testing trial entry latency may have resulted from non-cognitive factors. One explanation for the failure of apamin to alter water maze and Y-maze performance might be, that these tests were too easy for intact mice, i.e., there may have been a ceiling effect. On the other hand, the tests used in this and previous studies probably impose different mnemonic demands, which could also explain the lack of effect of apamin in intact mice in our study. Indeed, water maze measures spatial reference memory and Y-maze contains a spatial working memory component. In contrast, the previous studies by Messier et al. (1991) and Deschaux et al. (1997) employed tests that do not require spatial memory.

We report here, that pretraining injections of apamin can markedly stimulate water maze behaviour of medial septal-lesioned mice. The effective doses were 0.06 mg/kg and 0.02 mg/kg. The 0.2 mg/kg dose, however, was ineffective, which may be a reflection of the possible toxic side-effects (Lallement et al., 1995). The lack of any effect of injections of apamin after the daily training trials on performance in Morris water maze raises the possibility that apamin does not enhance formation of spatial memory, but influences some other factor which modulates escape performance, such as anxiety, arousal, attention or motor behaviour. Indeed, the effects of apamin on these functions are largely unexplored. However, some of the present results tentatively suggest that apamin can stimulate cognitive processes in medial septal-lesioned mice.

First, the effects of apamin on locomotor activity and accuracy of the learning performance did not occur in the same behavioural tests used in this study. Apamin had no effect on swimming speed in water maze, suggesting that changes in motor activity do not account for the beneficial effect of apamin on spatial navigation. In contrast, apamin actually decreased the hypoactivity of medial septal-lesioned mice in Y-maze and passive avoidance tests, but produced no obvious cognitive improvement in these tests.

Second, we observed that the learning curves of apamin and vehicle treated mice started from the same level, but the drug treated group had a steeper learning curve. Indeed, an increase in the slope of the learning curve is classically interpreted as an improvement of memory formation. In addition, apamin treatment alleviated the defect observed during platform reversal in medial septal-lesioned mice, showing that the effect is not limited to one learning event in the spatial memory test. However, retrieval of previously learned spatial information was not stimulated by apamin treatment in medial septal-lesioned mice. Furthermore, apamin had no effect on spontaneous alternation behavior, a primitive measure of working memory, in Y-maze or passive avoidance functions, suggesting that the mechanisms underlying acquisition of spatial reference memory engrams may be especially amenable to the modulation of the function of Ca^{2+}-dependent K^+ channels.

The bias assessments on days 6 and 8 showed no significant differences between any of the sham-lesioned or medial septal-lesioned groups, even though there was a clear difference in platform finding and escape length. This may be due to a different search strategy of mice compared to rats who generally show a close correlation between reduced escape length and the bias performance (Morris, 1984; Riekkinen et al., 1997). Indeed, we have found that in rats a medial septal-lesion increases escape distance and reduces bias, and metrifonate, a cholinesterase inhibitor, can alleviate both changes (Riekkinen et al., 1997). In contrast, in mice, a medial septal lesion increased only escape distance, but had no effect on bias (Ikonen et al., unpublished). Furthermore, metrifonate reduced escape distance, but had no effect on spatial bias in mice (Ikonen et al., unpublished results). Therefore, it is possible that mice are not as sensitive as rats in developing bias in the water maze.

In conclusion, apamin alleviated the acquisition defect during reference memory testing in medial septal-lesioned mice and it had no effect on inhibitory avoidance or spontaneous alternation behavior in the Y-maze. The present and previous results (Deschaux et al., 1997; Messier et al., 1991) indicate that blockade of Ca^{2+}-dependent K^+ channels may facilitate reference memory function.

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