Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Letters 386 (2005) 5–8
Neuroscience Letters
www.elsevier.com/locate/neulet

Apamin produces selective improvements of learning in rats

O. Deschaux [a,*], J.-C. Bizot [b]

[a] *Centre d'Etudes du Bouchet, DGA, Vert le Petit, France*
[b] *Key-Obs SA, 16 rue Léonard de Vinci, 45074 Orléans Cedex 2, France*

Received 9 February 2005; received in revised form 4 May 2005; accepted 20 May 2005

Abstract

The effect of apamin on learning was examined using two behavioral tasks where the animals were subjected to two trials separated by a 24 h interval. In the Y maze task, apamin administered before the acquisition session did not enhance performance on both the acquisition session and the restitution session. In the second behavioral task, animals were trained to press a lever to obtain a food pellet (fixed ratio 1). Then, to study the effect of apamin on extinction, animals were submitted to two sessions where a press on the lever was not reinforced. Apamin administered before the acquisition session reduced the number of lever presses during the first 3-min period of the restitution session. These results suggest that the blockade of SK channels could improve the acquisition but not when the task requires the processing of spatial information.

Keywords: Apamin; Learning; Memory; Rat

It has been reported that apamin, which is an active principle of honey-bee venom and a selective blocker of slow conductance Ca^{2+}-activated K^+ channels (SK channels) [8] may enhance cognitive function.

In support of these findings, apamin binding sites are numerous in areas of the rat brain associated with learning and memory such as the hippocampus [6,11,12], and there is some evidence that apamin penetrates the blood–brain barrier [8]. Moreover, the expression of the immediate early genes c-fos and c-jun, which are thought to be involved in the initial activation of neurons during memory processes, is increased by apamin [9]. Additionally, apamin has been shown to increase the firing rate of cholinergic neurons of the medial septum diagonal band region, an area controlling both cholinergic and glutamatergic innervation of the hippocampal formation [10,13].

However, there are several inconsistencies in the literature concerning the effects of apamin on cognitive function in rodents. It has been shown that apamin improves acquisition in an object recognition task [4] and facilitates habituation in rats [3]. Fournier et al. [5] have demonstrated in rats that blockade of SK channels can facilitate consolidation of a new-odor-rewarded association in a non-spatial context memory task. In contrast to these findings, apamin was without effect in spatial memory tasks in rats such in Morris water maze [14], radial maze and passive avoidance tasks [3,14].

With respect to these studies, the type of memory improved by apamin still remains unclear. It appears that apamin improves learning in behavioral tasks which are not associated with a spatial strategy or a stressful situation in rats. In light of the inconsistencies, the present study was designed to clarify the effects of apamin in distinct memory processes in rats. We used two different cognitive tasks to study the effects of apamin on learning: a spatial memory task assessed by the Y maze and a non-spatial memory task as extinction of operant behavior. The Y maze task was chosen to study spatial memory in rats because it avoids deprivation and overt stress. It is likely used to show either disruption or improvement of memory processes by lesions or drugs [2,11]. This task has not been used to test the ability of apamin to improve learning in Wistar rats. The second task was performed to generate new data on the effect of apamin in a learned extinction operant behavior protocol. A previous study using rats has shown that apamin did not improve retention of the incompletely acquired lever-press response

* Corresponding author.
E-mail address: deschaux@unice.fr (O. Deschaux).

0304-3940/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2005.05.050

[14]. In this second task, rats first learned that a press on a lever was reinforced and then during extinction learning that a lever press was not reinforced. We hypothesized that blockade of SK channels in this protocol should improve the learning.

All studies were conducted using male Wistar rats (Janvier France) weighing 180–200 g. Rats were housed five per cage in a regulated environment with a 12 h light/dark cycle. They had free access to food and water except for rats used in operant behavior which had a strictly controlled diet from Monday to Thursday (12 g/rat/day at 17:00 h from Monday to Thursday) although food was freely available from Friday 17.00 h to Sunday 10.00 h. The animals were used for experimentation after habituation to laboratory conditions for at least 5 days. The experiments were performed in accordance with the European Community Guidelines on the care and use of laboratory animals (86/609/EEC).

The Y maze apparatus consisted of three white Plexiglas arms radiating at 120° (each 45-cm long, 16-cm wide, 32-cm high). Each arm was different from each other by the painting on its wall and all arms could be independently closed using a sliding door. In order to rules out the possibility of scent traces left in the arm and therefore the dependency of the recognition capacity of rats on the olfactory cue, the walls and the ground of the arm were washed with alcohol before and after each session. On the test day, at the first session (T1), the rat was allowed to explore 2 arms for 2 min. In the second session (T2), the 3 arms were opened and the rats given 1-min exploration time. In the first experiment conducted in order to demonstrate that rats could recognize the arms visited in T1, a short interval of 1 h separated T1 and T2. In the following experiment, since a promnesic effect of apamin is expected, a long interval of 24 h separated T1 and T2. The basic measure was the time (in second) taken by the rat in exploring each arms. Time was recorded by an image analysis system (Imetronic) connected to a PC computer.

The operant behavior experiment took place in four operant chambers (Campden Instruments Ltd., Leicester, UK) enclosed in sound-attenuating chambers. The food tray was located between two retractable levers. The chambers were connected to an IBM PC XT microcomputer via a computer interface (Paul Fray Ltd., UK). The experiments were controlled, and data recorded, using a program written using Spider Basic language (Paul Fray).

Initially, rats received pretraining sessions in which a press on either the left or the right lever was reinforced with a food pellet (45 mg, Campden) according to a fixed ratio 1 (FR1: one press for one pellet) schedule (15 sessions of 15 minutes) to reach stable performances. Then, depending on the rat, only a press on the right or the left lever was always reinforced. The criterion was arbitrarily defined as at least 60 presses on the correct lever. Finally, in the extinction task, two sessions separated by a 24 h interval took place in which any lever press was not reinforced.

Apamin (Latoxan, France) was dissolved in physiological saline solution (vehicle) and administered intraperitoneally.

Table 1
Experiment 1, mean ± S.E.M. with a 1 h interval

Treatment (mg/kg)	*n*	% of time spent in the new arm		% of time spent in the old arms/2	
		Mean	S.E.M.	Mean	S.E.M.
Saline	41	44.9	2.2	27.9	1.1
Apamin 0.1	12	46.0	5.2	27.0	2.6
Apamin 0.2	18	41.5	2.8	29.2	1.4
Apamin 0.4	12	47.6	6.4	26.1	3.2

All administrations were given in a volume of 1 ml/kg body weight. Control rats received an equal volume of vehicle. In the two experiments, drug or saline was administered 30-min before the acquisition session. Apamin was administered at 0.2 and 0.4 mg/kg in both experiments and 0.1 mg/kg in the extinction protocol (to check if this task was more sensitive than others). These doses have been shown to be behaviorally effective and not associated with motor or convulsive side effects [3,5].

Statistical analysis was carried out using Statview 4.02. In the Y maze protocol, the time spent in the novel arm and the previously visited arms was compared by two-way repeated-measures analysis of variance (ANOVA) using drug as between-group variable and arm (new versus old) as a repeated measure. Then, the time in exploration was compared between the new arm versus the old arms independently for each group by the paired Student's *t*-test.

In the extinction protocol, the number of lever presses was analyzed using blocks of 3-min and was compared using a two-way repeated measures analysis of variance (ANOVA) with drug as between-group variable and time as a repeated measure. Then, an ANOVA and subsequent PLSD's Fisher test post hoc analysis were performed to determine the treated-group significantly different from the saline.

In the first experiment (1 h inter-trial interval, see Table 1), apamin did not significantly modify the time spent in the arms instead of a repeated effect is showed ($F(2,154) = 19.3$; $p < 0.001$). The Student's *t*-test showed that all groups spent proportionally more time in the novel arm than the arms previously visited (saline treated group ($t(40) = 5.1$; $p < 0.01$), apamin 0.1 mg/kg ($t(11) = 2.4$; $p < 0.05$), apamin 0.2 mg/kg ($t(117) = 2.8$; $p < 0.01$) and apamin 0.4 mg/kg ($t(11) = 2.2$; $p < 0.05$)).

In the second experiment (24 h inter-trial interval, see Table 2), apamin had no effect on the time spent in the arms

Table 2
Experiment 2, mean ± S.E.M. with a 24 h interval

Treatment	*n*	% of time spent in the new arm		% of time spent in the old arms/2	
		Mean	S.E.M.	Mean	S.E.M.
Saline	53	34.5	1.9	32.7	0.9
Apamin 0.1	16	33.3	3.4	33.3	1.6
Apamin 0.2	22	41.2	4.1	29.3	2.0
Apamin 0.4	17	36.9	3.9	31.5	1.9