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The effects of some K^+ channel blockers on scopolamine- or electroconvulsive shock-induced amnesia in mice

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Abstract

The effects of three K^+ channel blockers, 4-aminopyridine, 3,4-diaminopyridine and apamin, on scopolamine- or electroconvulsive shock-induced amnesia were investigated in mice by using a one-trial step-down passive avoidance system. Scopolamine and electroconvulsive shock reduced the retention latency of passive avoidance, which indicated the amnestic effect of these treatments. 4-Aminopyridine, 3,4-diaminopyridine and apamin injected immediately after the acquisition trial, reversed the amnestic effect of scopolamine or electroconvulsive shock in a dose-dependent manner. None of the drugs or electroconvulsive shock treatment affected the rotarod or activity cage performance of the mice. These results indicate that K^+ channel blockers may improve cognitive deficits when memory is impaired by a drug or any other manipulation.

Keywords: 4-Aminopyridine; 3,4-Diaminopyridine; Apamin; Passive avoidance; (Mouse)

1. Introduction

After Hodgkin and Huxley's (1952) observation indicated that K^+ currents are responsible for the repolarisation phase of the action potential, a great deal of work was carried out on this area. These studies, most of which were of electrophysiological, kinetic, genetic and pharmacological nature, showed that K^+ channels exist in both excitable and non-excitable cells, and are of critical importance in a variety of cell functions (Rudy, 1988). In excitable cells, opening of K^+ channels stabilizes the membrane potential, sets the resting potential, repolarises action potentials and terminates periods of action potential firing, while they have a role in transmembrane transport, volume regulation, signal transduction and maintenance of cell resting potential in the non-excitable cells (Grissmer, 1997). The diversity and distribution of K^+ channels are much greater than any other ion channels observed; they vary widely as to kinetics, voltage-dependence, pharmacology, and single-channel behaviour (Rudy, 1988; Aronson, 1992).

As a result of these observations, there has been a growing interest in the role of K^+ channels in some diseases and in drugs which affect these channels. Results from these studies have shown the involvement of K^+ channels in learning and memory processes to be of interest. Many authors have demonstrated a critical role of K^+ channels in the mechanisms of memory and learning by applying different K^+ channel modulators in various experimental models (Barnes et al., 1989; Peterson and Gibson, 1982; Ghelardini et al., 1998; Deschaux and Bizot, 1997; Messier et al., 1991; Heurteaux et al., 1993). Most of these studies with few exceptions (Ikonen et al., 1998; Ikonen and Riekkinen, 1999) were done with intact animals showing no deficits in memory. In the present study with mice, we aimed to examine the effects of three K^+ channel blockers, 4-aminopyridine, 3,4-diaminopyridine and apamin, on the memory impaired by scopolamine or electroconvulsive shock.

2. Materials and methods

2.1. Animals

Male albino mice weighing 21–28 g obtained from Çukurova University Medical Sciences Research Center

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(TIBDAM) were used in the experiments. The animals were housed five per cage and kept in a regulated environment (24 ± 1°C, light–dark cycle, with the light on between 0600 and 1800 h) and provided with commercially available food and water ad libitum. All animals used for the experiments were naive to the passive avoidance apparatus. The experiments were conducted between 0900 and 1100 h in a semi-soundproof and semi-dark laboratory. All groups included 10 animals each.

2.2. Drugs

Scopolamine HBr, 4-aminopyridine, 3,4-diaminopyridine and apamin were purchased from Sigma. All drugs were dissolved in 0.9% NaCl solution and injected intraperitoneally (i.p.) in a volume of 0.1 ml/10 g body weight.

2.3. Passive avoidance

A step-down passive avoidance apparatus was used for evaluating memory retention (Sahgal, 1993). The apparatus consisted of two compartments, a small chamber ($20 \times 25 \times 20$ cm) and a larger darkened one ($20 \times 35 \times 30$ cm) divided by a guillotine door (8×8 cm). The small chamber was illuminated by a 60-W bulb and set 1 cm higher than the larger one. The large chamber was made up of an electrifiable grid floor and the shock was delivered to the animal's feet via a shock generator. On the first day (acquisition trial), each mouse was initially placed in the small illuminated chamber of the passive avoidance apparatus, the guillotine door was opened 10 s later and a timer was started simultaneously. When the mouse crossed with all four paws into the dark chamber, the guillotine door was closed, the timer was stopped, latency to enter the chamber was recorded, and footshock (50 Hz, 1 mA, 3 s) was delivered to the animal's feet immediately. After shock application finished, the animal was removed from the dark chamber and returned to its home cage. Any animal failing to cross from the illuminated to the dark chamber within 120 s was discarded from the experiment. Mice housed in a separate cage under the same experimental conditions were used instead of the eliminated ones in order to have 10 animals for each group. All drugs, except scopolamine, were administered intraperitoneally (i.p.) immediately after the acquisition trial. Scopolamine (1 mg/kg, i.p.) was injected 30 min before the acquisition trial. Retention latency was recorded again 24 h later, in the same manner as described above, but without applying shock.

2.4. Electroconvulsive shock

An electric current (60 Hz, 2 s and 20 mA) was delivered to the restrained mouse by holding its neck skin between the forefinger and the thumb, and pressing it gently against the table, while applying the corneal electrodes of the electroconvulsive treatment apparatus (Ugo Basile, 7801, Italy) with the other hand (Isaac et al., 1985). The shock was delivered immediately after the acquisition trial in control groups and just before drug injection in drug groups. This protocol was determined by preliminary studies performed in our laboratory.

2.5. Rotarod and activity cage tests

These tests were applied to examine the probability that K^+ channel blockers may elicit their modulatory effects on memory by changing either motor coordination or spontaneous locomotor activity in separate groups of mice. For rotarod testing, the mice receiving the highest effective doses of drugs, electroconvulsive shock treatment, or saline were placed on the rotating bar (20 rpm) of the rotarod apparatus (Rotarod treadmills, Ugo Basile, 7600, Italy) for 5 min and dropping time was determined. To examine spontaneous locomotor activity, the mice receiving the drugs, saline or electroconvulsive shock treatment were placed in the activity cage (Ugo Basile, 7400, Italy) and their activity was evaluated for 15 min.

2.6. Statistics

Results were expressed as the means ± S.E.M. One-way analysis of variance (ANOVA) followed by a post-hoc Tukey's honestly significant difference (HSD) test was used for the comparison of groups. Significance was set at $P < 0.05$.

3. Results

3.1. Effects of 3,4-diaminopyridine and 4-aminopyridine on the scopolamine-induced amnesia

Scopolamine (1 mg/kg, i.p.) induced amnesia as shown by the reduction of retention latency during passive avoidance test. Retention latencies of the mice that received saline (control) or scopolamine were 119 ± 0.6 and 22.4 ± 4.6, respectively ($P < 0.05$). 3,4-Diaminopyridine and 4-aminopyridine reduced the amnestic effect of scopolamine in a dose-dependent manner. Retention latencies were 40.2 ± 13.8, 57.2 ± 15, 108.7 ± 4.5 for 0.5, 1 and 5 μg/kg 3,4-diaminopyridine doses and 47.8 ± 5.7, 70.8 ± 5.9 and 112 ± 3.3 for 12.5, 25 and 50 μg/kg 4-aminopyridine doses, respectively (Fig. 1). The differences were significant when compared to the scopolamine group, with 3,4-diaminopyridine doses of 1 and 5 μg/kg ($P < 0.05$); and 4-aminopyridine doses of 25 and 50 μg/kg ($P < 0.05$).

3.2. Effects of 3,4-diaminopyridine and 4-aminopyridine on the electroconvulsive shock-induced amnesia

The amnestic effect of electroconvulsive treatment was also determined by the reduction of retention latency.

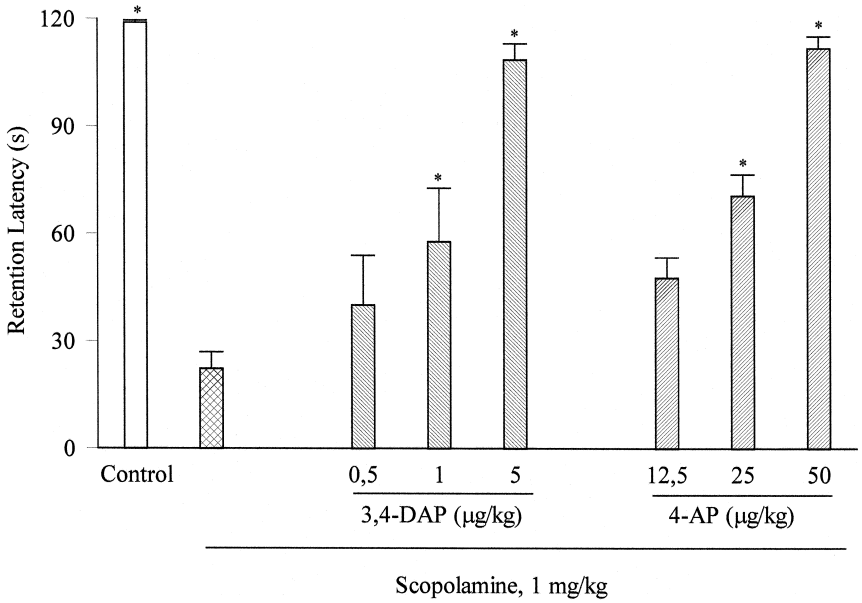

Fig. 1. Retention latencies of mice that received saline (control), scopolamine, 3,4-diaminopyridine + scopolamine and 4-aminopyridine + scopolamine, and the effects of 3,4-diaminopyridine and 4-aminopyridine on the amnesia induced by scopolamine. * $P < 0.05$; significantly different when compared to the scopolamine (hatched column) group, using one-way ANOVA followed by Tukey's HSD. 3,4-DAP: 3,4-diaminopyridine; 4-AP: 4-aminopyridine.

Retention latencies of the mice that received saline + sham operation (control) and saline + electroconvulsive shock were 114.5 ± 3.5 and 17.7 ± 1.8, respectively (significantly different from each other at $P < 0.05$ level). 3,4-Diaminopyridine and 4-aminopyridine dose dependently reversed this effect of electroconvulsive shock. Retention latencies were 39.6 ± 5.5, 88 ± 8.3 and 120 ± 0 for 0.5, 1 and 5 μg/kg 3,4-diaminopyridine doses and 39.9 ± 9.2, 56.7 ± 12.6 and 105.6 ± 4.6 for 12.5, 25 and 50 μg/kg 4-aminopyridine doses, respectively (Fig. 2). Significant differences between the electroconvulsive shock group and 3,4-diaminopyridine groups were observed at the 1 and 5

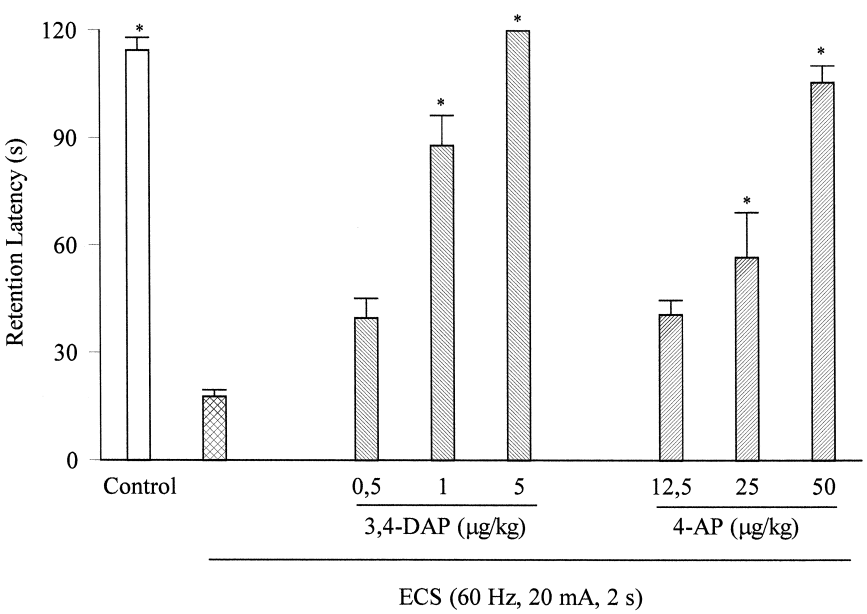

Fig. 2. Retention latencies in the saline + sham operation (control), saline + electroconvulsive shock, 3,4-diaminopyridine + electroconvulsive shock and 4-aminopyridine + electroconvulsive shock groups and the effects of 3,4-diaminopyridine and 4-aminopyridine on the amnesia induced by electroconvulsive shock. * $P < 0.05$; significantly different when compared to the ECS (hatched column) group, using one-way ANOVA followed by Tukey's HSD. ECS: electroconvulsive shock; 3,4-DAP: 3,4-diaminopyridine; 4-AP: 4-aminopyridine.

μg/kg doses of 3,4-diaminopyridine ($P < 0.05$). 4-Aminopyridine exerted significant effects with the doses of 25 and 50 μg/kg ($P < 0.05$).

3.3. Effects of apamin on the scopolamine- or electroconvulsive shock-induced amnesia

Effects of apamin on scopolamine- and electroconvulsive shock-induced amnesia are shown in Figs. 3 and 4. Apamin dose dependently reduced the amnestic effects of scopolamine or electroconvulsive shock by enhancing the retention latency of passive avoidance. Retention latencies were 42.1 ± 4.6, 86.1 ± 6.1 and 115.7 ± 3.4 for scopolamine + 0.05, 0.1 and 0.2 mg/kg apamin doses, and 47.3 ± 8.2, 87.4 ± 4.7 and 116.1 ± 2.0 for electroconvulsive shock + 0.05, 0.1 and 0.2 mg/kg apamin doses, respectively. Significant differences between scopolamine and apamin groups were observed at apamin doses of 0.1 and 0.2 mg/kg (Fig. 3, $P < 0.05$). Significant differences between electroconvulsive shock and apamin groups were observed at all doses of apamin (Fig. 4, $P < 0.05$).

3.4. Effects of K^+ channel blockers on the rotarod and activity cage tests

Neither drugs at their highest doses nor electroconvulsive shock treatment impaired physical strength or motor coordination as revealed by the rotarod test, or modified spontaneous locomotor activity as revealed by the activity cage test.

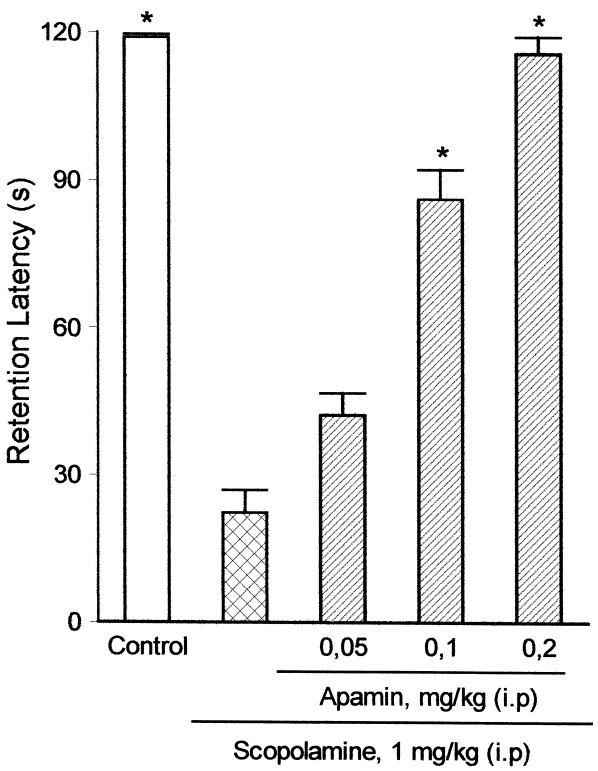

Fig. 3. The effects of apamin on the amnesia induced by scopolamine. * $P < 0.05$; significantly different when compared to the scopolamine (hatched column) group, using one-way ANOVA followed by Tukey's HSD.

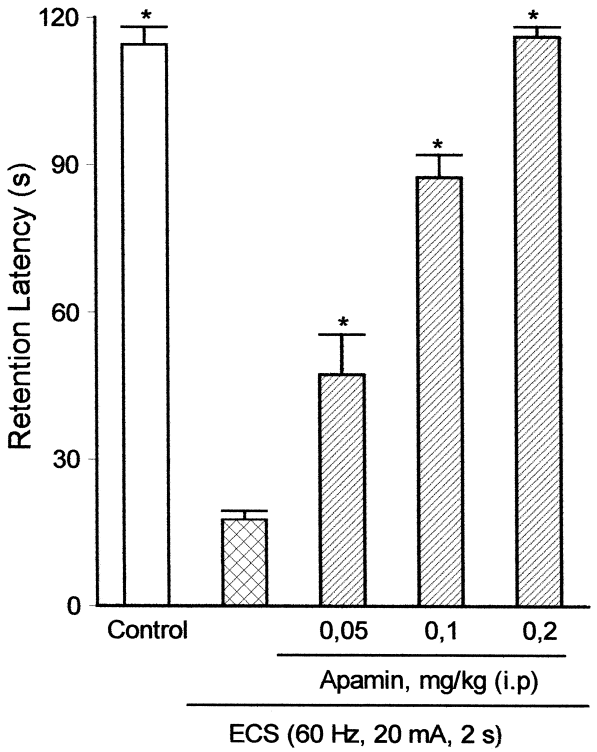

Fig. 4. The effects of apamin on the amnesia induced by electroconvulsive shock. * $P < 0.05$; significantly different from the electroconvulsive shock (hatched column) group, using one-way ANOVA followed by Tukey's HSD. ECS: electroconvulsive shock.

4. Discussion

In this study, aminopyridines, which predominantly inhibit voltage-activated K^+ channels, and apamin, which inhibits Ca^{2+}-activated K^+ channels, both attenuated the memory impairing effects of scopolamine or electroconvulsive shock in a step-down passive avoidance task. Passive avoidance procedures are widely used to measure the cognitive alterations following drug administration, lesions or behavioural manipulations (Sahgal, 1993). These procedures have some advantages as being simple to carry out and require little equipment and time; however, they also have disadvantages. The most important question is, what processes are being measured precisely when passive avoidance is used. Memory is certainly involved but motivational and other factors may influence the results. These procedures must therefore be considered as simple preliminary tests for evaluating the effects of drugs or lesions on memory. So, it may be important to investigate if a drug or any other manipulation affects passive avoidance performance, before using the more sophisticated techniques.

Several studies, with different techniques, have shown that K^+ channel modulators are effective to modulate the memory performance of rodents. In an early study, 3,4-diaminopyridine was shown to selectively improve the memory performance of aged rats and that, within this age group, it only improved performance in the short-term memory task which was assessed by the radial arm maze (Barnes et al., 1989). In accordance with this latter finding, Beninger et al. (1995) found that 3,4-diaminopyridine did not improve radial maze performance in the memory-impaired young rats. On the other hand, 4-aminopyridine, the other aminopyridine derivative, enhanced the retention of passive avoidance (Haroutunian et al., 1985). This finding is consistent with that from our present study and it may therefore be speculated that these drugs affect long-term memory (retrieval) in young animals, but not the short-term working memory.

Apamin has been shown to facilitate memory processes in the appetitively motivated bar-pressing response in mice (Messier et al., 1991), improve learning in an object recognition task in rats (Deschaux and Bizot, 1997), reverse the spatial navigation defect induced by a medial septal lesion (Ikonen et al., 1998), and increase the expression of immediate early genes c-*fos* and c-*jun* in the hippocampus (Heurteaux et al., 1993). On the other hand, apamin did not alter passive avoidance in intact mice and rats (Ikonen and Riekkinen, 1999; Ghelardini et al., 1998) but it prevented the K^+ channel opener-induced amnesia in the same test (Ghelardini et al., 1998). In our present study, apamin prevented the scopolamine- and electroconvulsive shock-induced amnesia in the passive avoidance task. These results suggest that apamin improves the retention of passive avoidance when memory is impaired by a drug or by any other manipulation, such as electroconvulsive shock. This possibility was supported by Ghelardini et al. (1998), who suggested that K^+ channel blockers did not enhance cognitive abilities when given alone.

The K^+ channel blockers used in the present study did not alter spontaneous locomotor activity or motor coordination of the mice; therefore, a non-specific effect of these drugs cannot have been responsible for the memory improving action. The ameliorating effects of K^+ channel blockers on scopolamine-induced amnesia may be due to the interaction of these agents with the cholinergic system. It is known that functional changes in central cholinergic activity affect learning and memory processes (Pepeu and Spignoli, 1989). Numerous studies have shown that cholinergic agents can enhance performance in learning and memory tasks, while acetylcholine receptor antagonists such as scopolamine impair performance in the same tasks (Flood et al., 1981; Haroutunian et al., 1985; Moye and Vanderryn, 1988; Savage et al., 1996; Spangler et al., 1986). 4-Aminopyridine and 3,4-diaminopyridine increase the release of acetylcholine from synaptic terminals, stimulate Ca^{2+} uptake into synaptosomes and ameliorate the hypoxia-induced deficit in the Ca^{2+}-dependent release of acetylcholine (Peterson and Gibson, 1982; Tapia and Stiges, 1981; Gibson and Peterson, 1981). 3,4-Diaminopyridine partially attenuates the decrease in release and synthesis of acetylcholine if given following hypoxic episodes in mice (Peterson and Gibson, 1982). Systemic administration of 4-aminopyridine in subconvulsive doses increases the cortical output of acetylcholine in anaesthetized rats (Casamenti et al., 1982). 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). Based on these results, it may be speculated that K^+ channel blockers improve the scopolamine-induced amnesia by releasing acetylcholine in the brain.

Our electroconvulsive shock data seem to be original and interesting, because there appear to be no studies available concerning the effects of K^+ channel blockers on electroconvulsive shock-induced amnesia. Electroconvulsive shock-induced amnesia could result from the activation of more than one mechanism in the brain. This treatment has been shown to influence some central nervous system functions by decreasing the acetylcholine level (Spignoli and Pepeu, 1986), increasing acetylcholine esterase activity (Appleyard et al., 1987), and releasing endorphins and enkephalins (Netto et al., 1986) in the brain. Electroconvulsive shock treatment also produces a significant enhancement of baclofen-induced inhibition of 5-HT release, possibly by interaction with the function of $GABA_B$ receptors (Gray and Green, 1987).

In conclusion, the present results strengthen the hypothesis that blockade of the voltage- and Ca^{2+}-activated K^+ channels improves the acquisition of passive avoidance performance when memory was impaired by scopolamine or electroconvulsive shock.

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References

Appleyard, M.E., Green, A.R., Greenfield, S.A., 1987. Acetylcholinesterase activity rises in rat cerebrospinal fluid post-ictally; effect of a substantia nigra lesion on this rise and on seizure threshold. Br. J. Pharmacol. 91, 149–154.

Aronson, J.K., 1992. Potassium channels in nervous tissue. Biochem. Pharmacol. 43 (81), 11–14.

Barnes, C.A., Eppich, C., Rao, G., 1989. Selective improvement of aged rat short-term spatial memory by 3,4-diaminopyridine. Neurobiol. Aging 10, 337–341.

Beninger, R.J., Wirsching, B.A., Mallet, P.E., Jhamandas, K., Boegman, R.J., 1995. Physostigmine, but not 3,4-diaminopyridine improves radial maze performance in memory-impaired rats. Pharmacol. Biochem. Behav. 51 (4), 739–746.

Casamenti, F., Corradetti, R., Löffelholz, K., Mantovani, P., Pepeu, G., 1982. Effects of 4-aminopyridine on acetylcholine output from the cerebral cortex of the rat in vivo. Br. J. Pharmacol. 76, 439–445.

Deschaux, O., Bizot, J.C., 1997. Effect of apamin, a selective blocker of Ca^{2+}-activated K^+-channel, on habituation and passive avoidance responses in rats. Neurosci. Lett. 227 (1), 57–60.

Flood, J.F., Landry, D.W., Jarvik, M.E., 1981. Cholinergic receptor interactions and their effects on long-term memory processing. Brain Res. 215, 177–185.

Ghelardini, C., Galeotti, N., Bartolini, A., 1998. Influence of potassium channel modulators on cognitive processes in mice. Br. J. Pharmacol. 123, 1079–1084.

Gibson, G.E., Peterson, C., 1981. Low oxygen decreases acetylcholine release in vitro. Fed. Proc. 40, 199.

Gray, J.A., Green, A.R., 1987. Increased $GABA_B$ receptor function in mouse frontal cortex after repeated administration of antidepressant drugs or electroconvulsive shocks. Br. J. Pharmacol. 92, 357–362.

Grissmer, S., 1997. Potassium channels still hot. Trends Pharmacol. Sci. 18, 347–349.

Haroutunian, V., Barnes, E., Davis, K.L., 1985. Cholinergic modulation of memory in rats. Psychopharmacology 87, 266–271.

Heurteaux, C., Meisser, C., Destrade, C., Lazdunski, M., 1993. Memory processing and apamin induce immediate early gene expressions in mouse brain. Mol. Brain Res. 3, 17–22.

Hodgkin, A.L., Huxley, A.F., 1952. A quantitative description of membrane currents and its applications to conduction and excitation in nerve. J. Physiol. 117, 500–544.

Ikonen, S., Riekkinen, P. Jr., 1999. Effects of apamin on memory processing of hippocampal-lesioned mice. Eur. J. Pharmacol. 382, 151–156.

Ikonen, S., Schmidt, B., Riekkinen, P. Jr., 1998. Apamin improves spatial navigation in medial septal-lesioned mice. Eur. J. Pharmacol. 347, 13–21.

Isaac, L., Advokat, C., Nelson, D.K., Browning, R.A., 1985. Motor paralysis in rats after repeated electroconvulsive shock comparison between aural and corneal stimulation. Life Sci. 37, 1257–1264.

Matthews, R.T., Lee, W.L., 1991. A comparison of extracellular and intracellular recordings from medial septum/diagonal band neurons in vitro. Neuroscience 42, 451–462.

Messier, C., Mourre, C., Bontempi, B., Sif, J., Lazdunski, M., Destrade, C., 1991. Effect of apamin, a toxin that inhibits Ca^{2+}-dependent K^+ channels, on learning and memory processes. Brain Res. 551, 322–326.

Moye, T.B., Vanderryn, J., 1988. Physostigmine accelerates the development of associative memory processes in the infant rat. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 95, 401–406.

Netto, C.A., Dias, R.D., Izquierdo, I., 1986. Differential effect of post-training naloxone, β-endorphine, Leu-enkephalin and electroconvulsive shock administration upon memory of an open-field habituation and of a water-finding task. Psychoneuroendocrinology 11 (4), 437–446.

Pepeu, G., Spignoli, G., 1989. Nootropic drugs and brain cholinergic mechanisms. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 13, 77–88.

Peterson, C., Gibson, G.E., 1982. 3,4-Diaminopyridine alters acetylcholine metabolism and behavior during hypoxia. J. Pharmacol. Exp. Ther. 222, 576–582.

Rudy, B., 1988. Diversity and ubiquity of K channels. Neuroscience 25 (3), 729–749.

Sahgal, A., 1993. Passive avoidance procedures. In: Sahgal, A. (Ed.), Behavioural Neuroscience: A Practical Approach vol. I Oxford Univ. Press, Oxford, pp. 49–56.

Savage, U.C., Faust, W.B., Lambert, P., Moerschbaecher, J.M., 1996. Effects of scopolamine on learning and memory in monkeys. Psychopharmacology 123, 9–14.

Spangler, E.L., Rigby, P., Ingram, D.K., 1986. Scopolamine impairs learning performance of rats in a 14-unit T-maze. Pharmacol. Biochem. Behav. 25, 673–679.

Spignoli, G., Pepeu, G., 1986. Oxiracetam prevents electroshock-induced decrease in brain acetylcholine and amnesia. Eur. J. Pharmacol. 126, 253–257.

Tapia, R., Stiges, M., 1981. Calcium dependent stimulation of transmitter release by 4-aminopyridine. Trans. Am. Soc. Neurochem. 12, 250.