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Influence of potassium channel modulators on cognitive processes in mice

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1 The effect of i.c.v. administration of different potassium channel openers (minoxidil, pinacidil, cromakalim) and potassium channel blockers (tetraethylammonium, apamin, charybdotoxin, glibenclamide) on memory processes was evaluated in the mouse passive avoidance test.

2 The administration of minoxidil (10 µg per mouse i.c.v.), pinacidil (5–25 µg per mouse i.c.v.) and cromakalim (10–25 µg per mouse i.c.v.) immediately after the training session produced an amnesic effect.

3 Tetraethylammonium (TEA; 1–5 µg per mouse i.c.v.), apamin (10 ng per mouse i.c.v.), charybdotoxin (1 µg per mouse i.c.v.), glibenclamide (3 µg per mouse i.c.v.) and glibenclamide (1 µg per mouse i.c.v.), administered 20 min before the training session, prevented the potassium channel opener-induced amnesia.

4 At the highest effective doses, none of the drugs impaired motor coordination, as revealed by the rota rod test, or modified spontaneous motility and inspection activity, as revealed by the hole board test.

5 These results suggest that the modulation of potassium channels plays an important role in the regulation of memory processes. On this basis, the potassium channel blockers could be useful in the treatment of cognitive deficits.

Keywords: Potassium channel openers; potassium channel antagonists; learning and memory; minoxidil; pinacidil; cromakalim; sulphonylureas; apamin; charybdotoxin; tetraethylammonium

Introduction

Several kinds of potassium channels with different electrophysiological characteristics and pharmacological sensitivities have been described in neurones (Halliwell, 1990; Aronson, 1992). In general, open potassium channels inhibit excitable cells by drawing the membrane potential toward the potassium equilibrium potential and away from action potential threshold. Potassium channels that are tonically open set the resting membrane potential. Stimuli that increase the activity of these potassium channels hyperpolarize cells and lower the effectiveness of excitatory inputs (Christie, 1995).

Hermisenda associative conditioning (Alkon *et al.*, 1982) and rabbit nictitating membrane conditioning (correlated with enhanced postsynaptic responses due to persistent reduction of voltage-dependent potassium current in hippocampal cells) (Sanchez-Andres & Alkon, 1991) have all strongly suggested a role for specific potassium channels in learning and memory. Recently, it has been shown that a reversible antisense inhibition of *Shaker*-like Kv1.1 potassium channel expression impairs associative memory in mouse and rat (Meiri *et al.*, 1997). Etcheberrigaray *et al.* (1993) found that a 113 pS tetraethylammonium (TEA)-sensitive potassium channel was consistently absent from Alzheimer's disease fibroblasts while it was present in young and aged control fibroblasts. It was observed that TEA depolarized and caused intracellular Ca²⁺ elevation in young and aged control fibroblasts but not in Alzheimer's disease fibroblasts. In patients with neurological and psychiatric disorders such as Parkinson's disease, senile dementia and Huntington's disease the signal induced by TEA was present. Furthermore, Etcheberrigaray *et al.* (1994) observed that treatment of fibroblasts with β -amyloid (10 nM)

induced the same potassium channel dysfunction previously shown to occur specifically in fibroblasts from patients with Alzheimer's disease, namely the absence of a 113 pS potassium channel.

Boakye *et al.* (1994) have observed a marked difference to the TEA response of the 115 pS potassium channels in olfactory neuroblasts from Alzheimer's patients in comparison with normal donors. In control neuroblasts 115 pS potassium channels were blocked by TEA 10 mM, whereas in Alzheimer's disease patients the same channel is not blocked by a dose of TEA 10 times higher.

TEA blocks different types of potassium channels in neurones including calcium-activated and voltage-dependent potassium channels, although TEA is not selective for any of them in particular (Cook & Quast, 1990; Halliwell, 1990). Charybdotoxin, as well as apamin, was originally shown to block specifically currents through the calcium-activated potassium channel (Miller *et al.*, 1985; Cook, 1988). In recent years, it has become clear that charybdotoxin can also block voltage-gated potassium channels (Stühmer *et al.*, 1989; Strong, 1990). Sulphonylureas, such as glibenclamide and glibenclamide, block K_{ATP} potassium channels in neurones, whereas minoxidil, pinacidil and cromakalim open the same type (K_{ATP}) of potassium channel (Longman & Hamilton, 1992; Edwards & Weston, 1993). Since the potassium channel functionality appears to be importantly involved in memory processes, we thought it worthwhile to employ potassium channel modulators to elucidate better the role of potassium channels in cognitive processes. To this purpose we have evaluated the effects produced by minoxidil, pinacidil and cromakalim as potassium channel openers and TEA, apamin, charybdotoxin, glibenclamide and glibenclamide as potassium channel blockers in the mouse passive avoidance test.

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Methods

Animals

Male Swiss albino mice (23–30 g) from the Morini (San Polo d'Enza, Italy) breeding farm were used. Fifteen mice were housed per cage. The cages were placed in the experimental room 24 h before the test of acclimatization. The animals were fed a standard laboratory diet and tap water *ad libitum* and kept at $23 \pm 1^\circ\text{C}$ with a 12 h light/dark cycle, lights on at 7 h 00 min. All experiments were carried out according to the guidelines of the European Community Council for experimental animal care.

Intracerebroventricular injection technique

Intracerebroventricular (i.c.v.) administration was performed under ether anaesthesia, according to the method described by Haley and McCormick (1957). Briefly, during anaesthesia, mice were grasped firmly by the loose skin behind the head. A 0.4 mm external diameter, hypodermic needle attached to a 10 μl syringe was inserted perpendicularly through the skull and no more than 2 mm into the brain of the mouse, where 5 μl were then administered. The injection site was 1 mm to the right or left from the midpoint on a line drawn through to the anterior base of the ears. Injections were performed into the right or left ventricle randomly. To ascertain that the drugs were administered exactly into the cerebral ventricle, some mice (20%) were injected with 5 μl of diluted 1:10 India ink and their brains examined macroscopically after sectioning. The accuracy of the injection technique was evaluated and the percentage of correct injections was 95.

Passive-avoidance test

The test was performed according to the step-through method described by Jarvik and Kopp (1967) with modifications. The apparatus consisted of a two-compartment acrylic box with a lighted compartment connected to a darkened one by a guillotine door. In the original method mice received a punishing electrical shock as soon as they entered the dark compartment, while in our modified method, mice, after their entry into the dark compartment, received a punishment consisting of a fall into a cold water bath (10°C). For this purpose the dark chamber was constructed with a pitfall floor. The latency times for entering the dark compartment were measured in the training test and after 24 h in the retention test. Tetraethylammonium, apamin, charybdotoxin, glibenclamide and gliquidone were injected intracerebroventricularly (i.c.v.) 20 min before the training session while minoxidil, pinacidil and cromakalim were injected i.c.v. immediately after termination of the training session. The drug administration schedule was chosen on the basis of preliminary experiments in which the time-course for each compound was determined. From these data it became apparent that potassium channel openers exerted their maximum amnesic effect when injected immediately after the training session, whereas the potassium channel blockers exerted their maximum anti-amnesic effect when administered 20 min before the training session. The maximum entry latency allowed in the retention session was 120 s. The degree of memory recall of received punishment (fall into cold water) was expressed as the difference (Δ s) between retention and training latencies.

Hole-board test

The hole-board test consisted of a 40 cm square plane with 16 flush mounted cylindrical holes (diameter 3 cm) distributed 4 by 4 in an equidistant, grid-like manner. Mice were placed on the centre of the board one by one and allowed to move about freely for a period of 10 min each. Two electric eyes, crossing the plane from mid-point to mid-point of opposite sides, thus dividing the plane into 4 equal quadrants, automatically signalled the movement of the animals (counts in 10 min) on the surface of the plane (locomotor activity). Miniature photoelectric cells, in each of the 16 holes, recorded (counts in 10 min) the exploration of the holes (exploratory activity) by the mice.

Rota-rod test

The apparatus consisted of a base platform and a rotating rod of 3 cm diameter with a non-slippery surface. The rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into 5 equal sections by 6 disks. Thus, up to 5 mice were tested simultaneously on the apparatus, with a rod-rotating speed of 16 r.p.m. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 30 s according to Vaught *et al.* (1985). Those mice scoring less than 3 and more than 6 falls in the pretest were rejected (20%). The performance time was measured before and 15, 30 and 45 min after treatment.

Drugs

The following drugs were used: minoxidil, pinacidil, tetraethylammonium chloride, apamin, charybdotoxin, glibenclamide (RBI); D-amphetamine hydrochloride (De Angeli); gliquidone (Boehringer Ingelheim); scopolamine hydrobromide, cromakalim (Sigma).

Drugs were dissolved in isotonic (NaCl 0.9%) saline solution, with the exception of pinacidil, glibenclamide and gliquidone that were dissolved in a water and dimethylsulphoxide (DMSO) (3:1) vehicle, immediately before use. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 5 μl per mouse by intracerebroventricular (i.c.v.) injection and 10 ml kg^{-1} by subcutaneous (s.c.) or intraperitoneal (i.p.) injection.

The potassium channel modulators were administered i.c.v. (minoxidil 0.01–10 μg per mouse; pinacidil 2.5–25 μg per mouse; tetraethylammonium chloride 0.5–5 μg per mouse; glibenclamide 0.1–1 μg per mouse; gliquidone 1–3 μg per mouse; cromakalim 1–25 μg per mouse) to avoid peripheral effects. Moreover, since the compounds have limited ability at crossing the blood brain barrier and apamin (1–10 ng per mouse) and charybdotoxin (0.1–1 μg per mouse) are peptides and therefore easily degradable by systemic administration, the i.c.v. administration route appeared to be the most appropriate.

Statistical analysis

All experimental results are given as the mean \pm s.e.mean. Analysis of variance (ANOVA), followed by Fisher's protected least significant difference (PLSD) procedure for *post-hoc* comparison, was used to verify significance between two means. Data were analysed with the StatView software for the Macintosh (1992). *P* values of less than 0.05 were considered significant.

Results

A comparison between two different aversive stimuli (electric shock and fall into cold water) applied to the mouse passive avoidance test is presented in Figure 1a. No difference between the entrance latency of retention and training sessions was observed.

The amnesic effect of scopolamine ($0.5\text{--}3\text{ mg kg}^{-1}$, i.p.) was of the same intensity in both experimental conditions (electric and cold water shock; Figure 1b).

Amnesic effect of potassium channel openers

The dose-response curves for minoxidil ($0.01\text{--}10\text{ }\mu\text{g}$ per mouse, i.c.v.), pinacidil ($2.5\text{--}25\text{ }\mu\text{g}$ per mouse, i.c.v.) and cromakalim ($1.0\text{--}25\text{ }\mu\text{g}$ per mouse, i.c.v.) in the mouse passive avoidance test are shown in Figure 2. All compounds, injected immediately after the training session, were endowed with amnesic properties. The maximum amnesic effect of minoxidil, cromakalim and pinacidil was reached at the dose of $10\text{ }\mu\text{g}$ per mouse i.c.v. Higher doses of the three potassium channel openers, because of their poor solubility in water, needed to be dissolved in a vehicle comprising water and DMSO 3:1. However, a stronger amnesic effect was not obtained. A dose of cromakalim ($25\text{ }\mu\text{g}$ per mouse i.c.v.) and pinacidil ($25\text{ }\mu\text{g}$ per

mouse i.c.v.), higher than the maximal dose soluble in water, produced an amnesia comparable to that obtained with the doses soluble in water (Figure 2). The vehicle employed did not modify the entrance latency in comparison with saline control when given alone (Figure 2).

Prevention of amnesia by potassium channel blockers

The amnesia induced by minoxidil ($10\text{ }\mu\text{g}$ per mouse i.c.v.), pinacidil ($10\text{ }\mu\text{g}$ per mouse i.c.v.) and cromakalim ($25\text{ }\mu\text{g}$ per mouse i.c.v.) was prevented, in the mouse passive avoidance test, by pretreatment with the potassium channel blocker TEA injected 20 min before the training session (Figure 3). TEA ($0.5\text{--}5\text{ }\mu\text{g}$ per mouse, i.c.v.) produced a dose-dependent antagonism of the minoxidil-induced amnesia. A dose of $0.5\text{ }\mu\text{g}$ per mouse i.c.v. was completely ineffective. TEA, at $1\text{ }\mu\text{g}$ per mouse i.c.v., partially prevented minoxidil amnesic effect, even if the statistical significance was not reached, while the dose of $5\text{ }\mu\text{g}$ per mouse i.c.v. enhanced the entrance latency (Δ between retention and training) up to a value comparable to that produced by control animals (Figure 3). A dose of TEA of $5\text{ }\mu\text{g}$ per mouse, i.c.v., was also able to prevent completely

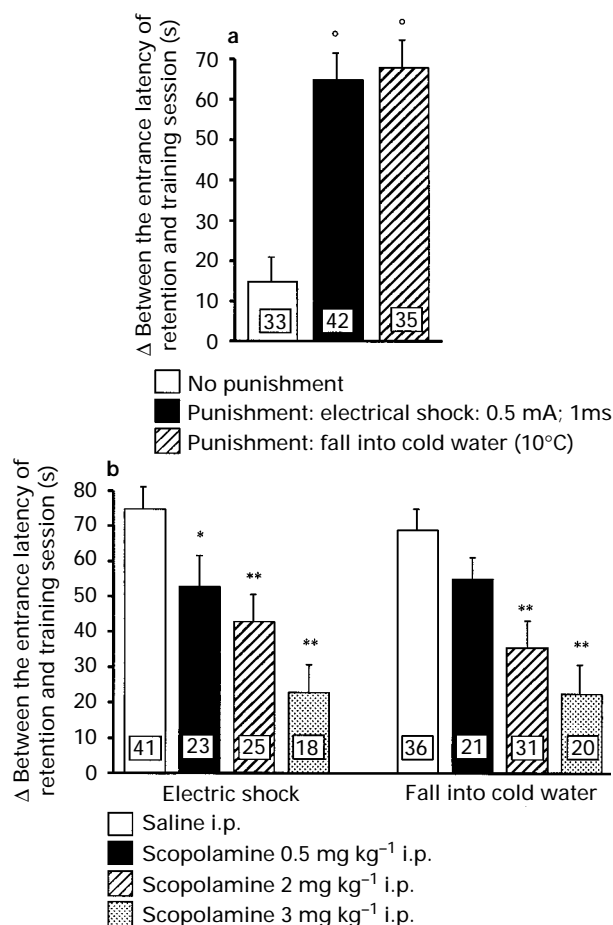


Figure 1 Comparison between two different aversive stimuli in the mouse passive-avoidance test (a). Dose-response curve for effect of scopolamine ($0.5\text{--}3\text{ mg kg}^{-1}$, i.p.) on mouse passive avoidance test in the presence of an aversive stimulus constituted by electric shock and by fall into cold water (b). The number of mice is indicated inside the columns. Scopolamine was injected immediately after the training session. * $P < 0.05$; ** $P < 0.01$ in comparison with saline controls; ° $P < 0.01$ in comparison with unpunished mice.

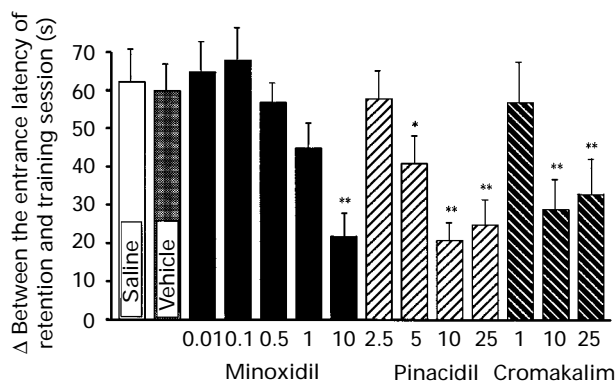


Figure 2 Dose-response curves for effects of minoxidil ($0.01\text{--}10\text{ }\mu\text{g}$, i.c.v.), pinacidil ($2.5\text{--}25\text{ }\mu\text{g}$, i.c.v.) and cromakalim ($1\text{--}25\text{ }\mu\text{g}$, i.c.v.) in the mouse passive avoidance test. Minoxidil, pinacidil and cromakalim were injected immediately after the training session. Each column represents the mean \pm s.e. mean of at least 12 mice. * $P < 0.05$; ** $P < 0.01$ in comparison with saline controls.

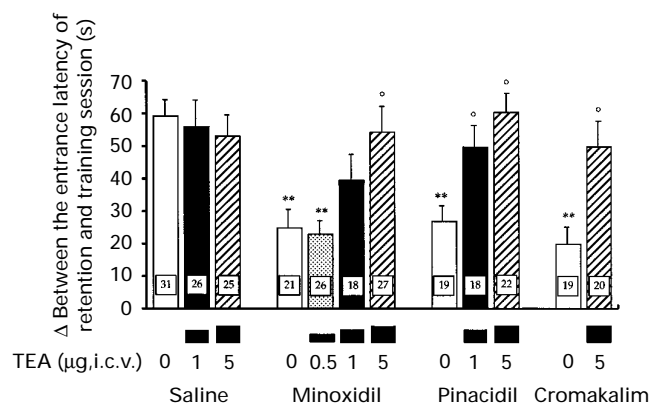


Figure 3 Effect of TEA ($0.5\text{--}5\text{ }\mu\text{g}$, i.c.v.) on amnesia induced by minoxidil ($10\text{ }\mu\text{g}$, i.c.v.), pinacidil ($10\text{ }\mu\text{g}$, i.c.v.) and cromakalim ($25\text{ }\mu\text{g}$, i.c.v.) in the mouse passive avoidance test. TEA was administered 20 min before the training session; minoxidil, pinacidil and cromakalim were injected immediately after the training session. The number of mice is indicated inside the columns. ** $P < 0.01$ in comparison with saline controls; ° $P < 0.01$ in comparison with minoxidil-, pinacidil- or cromakalim-treated mice.

pinacidil and cromakalim amnesia (Figure 3). Contrary to minoxidil, pinacidil amnesia was antagonized by TEA, 1 μ g per mouse i.c.v. (Figure 3). TEA did not produce any effect on mouse passive avoidance test in comparison with saline-treated mice when given alone (Figure 3).

Apamin (1 ng per mouse i.c.v.) and charybdotoxin (1 μ g per mouse i.c.v.), like TEA, were able to prevent both minoxidil and pinacidil amnesia. The two potassium channel blockers, at doses 10 times lower than those effective, were completely devoid of any anti-amnesic effect (Figure 4). At the active doses, apamin and charybdotoxin did not enhance the entrance latency in unamnesic mice in comparison with the control group (Figure 4).

The administration of glibenclamide (1 μ g per mouse i.c.v.) and gliclidone (3 μ g per mouse i.c.v.) antagonized the memory disruption produced by minoxidil and pinacidil without showing any memory facilitation activity when given alone (Figure 5). Glibenclamide and gliclidone were ineffective at preventing minoxidil-induced amnesia at lower doses (Figure 5).

No differences between the entrance latencies of each group in the training session of the passive-avoidance test were observed (data not shown).

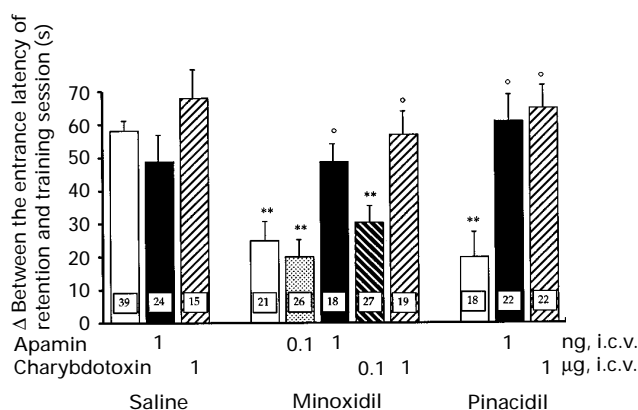


Figure 4 Effect of apamin (0.1–1 ng, i.c.v.) and charybdotoxin (0.1–1 μ g, i.c.v.) on amnesia induced by minoxidil (10 μ g, i.c.v.) and pinacidil (10 μ g, i.c.v.) in the mouse passive avoidance test. Apamin and charybdotoxin were administered 20 min before the training session; minoxidil and pinacidil were injected immediately after the training session. The number of mice is indicated inside the columns. ** P < 0.01 in comparison with saline controls; ° P < 0.01 in comparison with minoxidil- or pinacidil-treated mice.

Effect of potassium channel modulators on mouse rota rod and hole-board tests

It should be noted that the potassium channel openers (minoxidil, pinacidil, cromakalim) and potassium channel blockers (TEA, apamin, charybdotoxin, glibenclamide, gliclidone) under investigation elicited their modulatory effect on cognitive processes without changing either gross behaviour or motor coordination, as revealed by the rota-rod test (Table 1). None of the drugs, administered at the highest active doses, increased the number of falls from the rotating rod in comparison with saline and vehicle-treated mice (Table 1). The number of falls in the rota-rod test progressively decreased, since mice learned how to balance on the rotating rod.

The spontaneous motility and inspection activity of mice was not modified by administration of the above-mentioned potassium channel modulators, as revealed by the hole-board test in comparison with saline and vehicle-treated mice (Figure 6). In the same experimental conditions D-amphetamine (2 mg kg^{-1} , s.c.), used as a reference drug, increased both parameters evaluated.

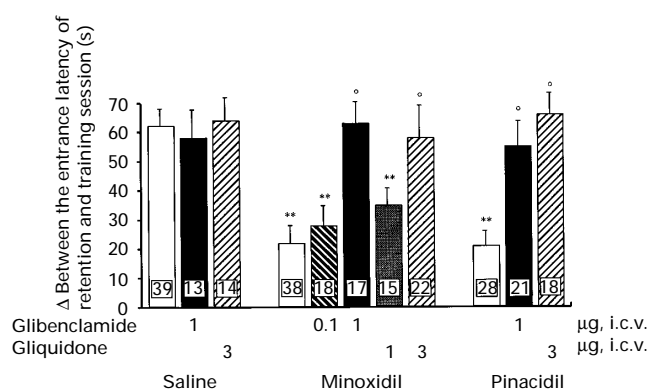


Figure 5 Effect of glibenclamide (0.1–1 μ g, i.c.v.) and gliclidone (1–3 μ g, i.c.v.) on amnesia induced by minoxidil (10 μ g, i.c.v.) and pinacidil (10 μ g, i.c.v.) in the mouse passive-avoidance test. Glibenclamide and gliclidone were administered 20 min before the training session; minoxidil and pinacidil were injected immediately after the training session. The number of mice is indicated inside the columns. ** P < 0.01 in comparison with saline controls; ° P < 0.01 in comparison with minoxidil- or pinacidil-treated mice.

Table 1 Effect of K^+ channel modulators in the mouse rota-rod test

Treatment	Dose i.c.v. per mouse	Before treatment	Number of falls in 30 s		
			15 min	After treatment 30 min	45 min
Saline	5 μ l	3.8 ± 0.4	2.6 ± 0.5	1.9 ± 0.4*	1.6 ± 0.3**
Vehicle	5 μ l	3.5 ± 0.5	2.7 ± 0.4	2.3 ± 0.5	1.5 ± 0.4**
Minoxidil	10 μ g	4.6 ± 0.4	2.9 ± 0.4*	1.5 ± 0.3**	1.2 ± 0.2**
Pinacidil	10 μ g	4.5 ± 0.3	3.3 ± 0.4	2.1 ± 0.6*	1.1 ± 0.3**
Cromakalim	25 μ g	3.7 ± 0.4	3.2 ± 0.4	1.9 ± 0.5*	0.9 ± 0.3**
TEA	5 μ g	4.6 ± 0.5	3.1 ± 0.4	2.1 ± 0.4*	1.5 ± 0.2**
Apamin	1 ng	4.8 ± 0.4	3.1 ± 0.5	2.4 ± 0.5*	1.2 ± 0.3**
Charybdotoxin	1 μ g	5.1 ± 0.4	3.6 ± 0.4	2.4 ± 0.4*	2.2 ± 0.3*
Glibenclamide	1 μ g	3.7 ± 0.6	3.3 ± 0.7	1.7 ± 0.5**	1.4 ± 0.5**
Gliclidone	3 μ g	4.4 ± 0.4	2.8 ± 0.3	1.4 ± 0.4**	1.3 ± 0.2**

Each value represents the mean ± s.e. mean of 8–10 mice.

Vehicle: H_2O + DMSO 3:1

* P < 0.05, ** P < 0.01 in comparison with the respective pre-test value.

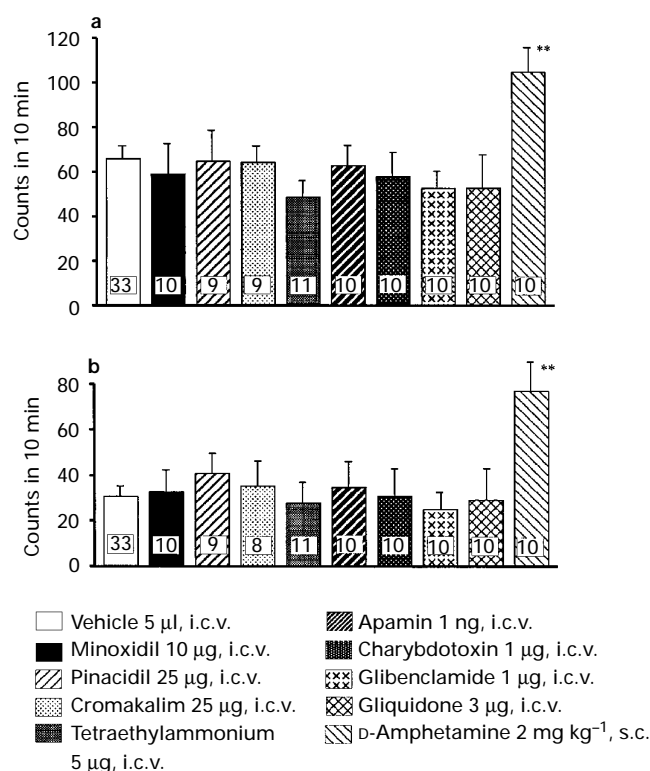


Figure 6 Lack of effect of minoxidil, pinacidil, cromakalim, TEA, apamin, charybdotoxin, glibenclamide and gliquidone on the hole-board test in comparison with D-amphetamine; (a) locomotor activity and (b) exploratory activity. The responses were recorded 15–25 min after drug administration. The number of mice is indicated inside the columns. ** $P < 0.01$ in comparison with saline controls.

Discussion

Potassium channel modulators appear to be involved in the regulation of cognitive processes in mice. Our results demonstrated that the administration of potassium channel openers (minoxidil, pinacidil, cromakalim) provokes amnesia in the mouse passive avoidance test of an intensity comparable to that induced by the amnesic drugs scopolamine and dicyclomine (Gualtieri *et al.*, 1994). The potassium channel blockers (TEA, apamin, charybdotoxin, glibenclamide, gliquidone) were able to prevent the minoxidil- and pinacidil-induced amnesia.

In our experimental conditions, the electric shock, described as the punishing stimulus in the original passive avoidance method (Jarvik & Kopp, 1967), was substituted by a stimulus consisting of a fall into cold water. Both foot shock and cold water appeared to be aversive to the animals, since no difference between the memory recall of the two punishments received was observed.

The importance of potassium channel functionality in the regulation of memory processes was evident in T lymphocytes from Alzheimer's disease (SDAT) patients, in which inactivity of the voltage-gated potassium channels was observed (Fudeberg *et al.*, 1984; Roberts, 1986). Later, a 113 pS TEA-sensitive potassium channel was found to be consistently absent from Alzheimer's disease fibroblasts, whilst it was present in young and aged control fibroblasts (Etcheberrigaray

et al., 1993). Boakye *et al.* (1994) observed a difference in the TEA response of the 115 pS potassium channels in olfactory neuroblasts from Alzheimer's patients in comparison with normal donors.

The amnesia in mice was produced by opening the K_{ATP} potassium channels and it was reversed by blocking K_{ATP} potassium channels. These data are in agreement with the observation that the mnemonic deficit observed in diabetic patients may be due not only to the cerebrovascular complications produced by the disease, but also by a functional modification of K_{ATP} potassium channels (Meneilly *et al.*, 1993). Furthermore, the treatment of diabetic patients with sulphonylureas provokes, simultaneously a decrease in the glycaemic levels, and a statistically significant improvement of neurological function (Meneilly *et al.*, 1993). However, prevention of the K_{ATP} potassium channel openers-induced amnesia in the mouse passive-avoidance test is also obtained by antagonizing other types of potassium channels, such as voltage-gated and calcium activated channels. Therefore, in the regulation of memory processes more than one type of potassium channel appears to be involved.

In agreement with the findings of Deschaux & Bizot (1997), the potassium channel blockers investigated did not enhance cognitive abilities when given alone. However these findings appear to be in contrast with other data in which a memory enhancing effect of apamin was observed in an object recognition task (Deschaux *et al.*, 1997). The discrepancy could be due to the fact that the blockade of calcium-activated potassium channels produced by apamin affects learning rather than memory and, therefore, is apparent only in an object recognition task. It should be taken into account that the effect of apamin was observed after an interval of 24 h, at a time when control animals did not remember the exploration of the objects presented in the first session.

The potassium channel modulators, at the highest doses used, did not modify animals gross behaviour. Moreover, these compounds did not impair motor coordination, as revealed by the rota rod test, nor modify locomotor and inspection activity, as indicated by the hole-board test. We can, thus, suppose that the effects produced by potassium channel modulators were not due to compromised behavioural paradigms. Higher doses of potassium channel openers were not investigated since they were not soluble in water. However, at the doses used, the maximum amnesic effect had already been reached. The use of a vehicle constituted by water and DMSO 3:1 to dissolve a dose of pinacidil and cromakalim higher than 10 µg per mouse i.c.v., did not increase the intensity of the amnesia. With regard to the potassium channel blockers TEA, apamin and charybdotoxin, at doses double those able to prevent completely potassium channel opener-induced amnesia, induced convulsions in mice.

In conclusion, these results suggest an important role played by potassium channels in the regulation of memory processes. The potassium channel blockers, therefore, could represent a promising pharmacological approach for the treatment of cognitive deficits.

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