Memory facilitation with atropine: a paradoxical effect

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Memory Facilitation with Atropine: A Paradoxical Effect

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The potential antiamnesic properties of atropine were investigated in the mouse passive avoidance test. Amnesia was induced by scopolamine (2 mg/kg i.p.) and by exposure to a hypoxic environment (5% O_2 in water-saturated nitrogen) for 8 min. Atropine (1–10 μg/kg s.c.) completely prevented disrupted acquisition without improving learning in mice with memory deficiency. At the same doses atropine did not produce any behavioural side effects in mice. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

The central cholinergic system has long been known to be involved in the modulation of learning and memory processes in animals and man. Drugs that affect the central cholinergic system have been found either to enhance or to hinder performance in learning and memory tests. Direct muscarinic agonists (oxotremorine, arecoline, AF-102B, RS 86 etc.) acetylcholine esterase inhibitors (physostigmine, DFP, eptastigmine, tacrine etc.) and acetylcholine releasers (AFDX 116, DuP 996, SM-21 etc.) potentiate test performance retention in rodents (Bartolini *et al*., 1994; Coyle, 1995). On the contrary, disruption of the cholinergic system produces impairment of cognitive processes. The administration of muscarinic antagonists (scopolamine, atropine, pirenzepine and dicyclomine) or inhibitors of choline uptake (hemicolinium-3), or lesions of nucleus basalis magnocellularis (NBM) or injection of the cholinotoxic agent AF64A, all induce amnesia (Coyle, 1995). Ghelardini *et al*. (1990) reported that the antimuscarinic drug atropine, in doses ranging from 1 to 10 μg/kg s.c., induced cholinergic antinociception as evidenced by its prevention by pretreatment with hemicolinium-3, with the M1 antagonists pirenzepine and dicyclomine, and by NBM lesions. We thought it worthwhile to investigate whether atropine, in the range of doses in which it produces antinociception through cholinergic system activation, was also endowed with antiamnesic properties.

METHODS

Animals. Male albino mice (20–25 g) from Morini (San Polo d'Enza - Italy) were used. All experiments were carried out according to the guidelines of the European Community Council on animal care.

Passive-avoidance test. The test was conducted in a two-compartment passive avoidance box performed according to the step-through method described by Jarvik and Kopp (1967), and modified for testing drugs endowed with analgesic properties. The apparatus comprises 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 receive a non-painful punishment consisting of a fall into a cold water bath (10°C) after their entry into the dark compartment. 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. For memory disruption, mice were: (1) injected with scopolamine (2 mg/kg i.p.) immediately after termination of the training session; (2) exposed to a hypoxic environment for 8 min up to 30 before passive avoidance training. The oxygen concentration used was 5.0% in water-saturated nitrogen. Atropine was injected subcutaneously (s.c.) 20 min before the training session. The maximum entry latency allowed in the retention session was 120 s. The degree of memory of the received punishment (fall into cold water) was expressed as the difference (Δ s) between retention and training latency.

Irwing test. The test was performed according to the method described by Irwing (1966).

Drugs. The following drugs were used: atropine sulphate, scopolamine hydrobromide, piracetam and physostigmine sulphate (Sigma). Drugs were dissolved, immediately before use, in isotonic (NaCl 0.9%) saline solution and prepared in such a way that they could be

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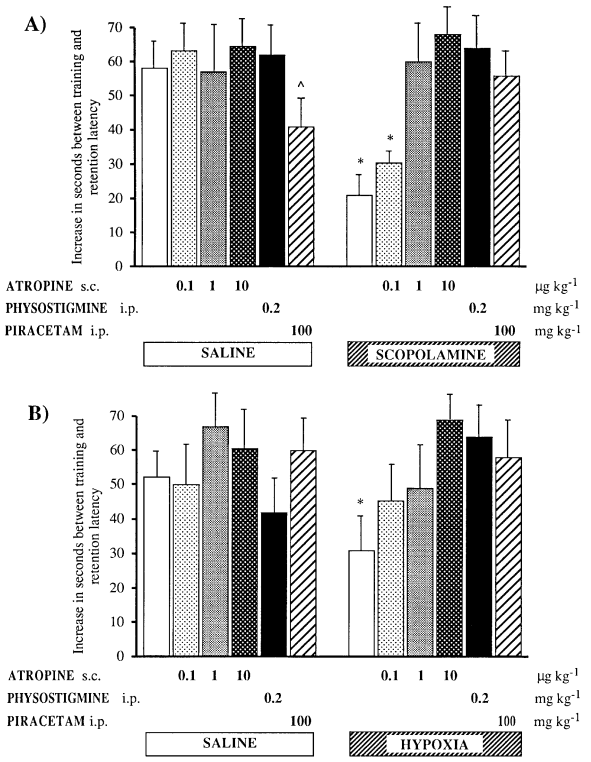

Figure 1. Effect of atropine, physostigmine and piracetam on amnesia induced by scopolamine (2 mg/kg i.p.) (*A*) and hypoxia (*B*) in the mouse passive avoidance test. $^p < 0.05$. $*p < 0.01$ in comparison with saline controls. Each column represents the mean of 10–25 mice.

administered in a volume of 10 mL/kg subcutaneously (s.c.).

Statistical analysis. Results are given as the mean ± SEM; analysis of variance, followed by Scheffe's *F* procedure for *post hoc* comparison, was used to verify the significance between two means. *p* values of less than 0.05 were considered significant. Data were analysed with the StatView for Macintosh (1992) computer program.

RESULTS

As shown in Fig. 1A the muscarinic antagonist scopolamine injected i.p. at doses of 2 mg/kg was able to disrupt the acquisition of a passive-avoidance conditioned response in mice, as demonstrated by the shortening of the entry latency into the dark compartment during the retention session. A similar cognition deficit was obtained by hypoxia exposure (Fig. 1B). Atropine (1–10 μg/kg s.c.) administered 20 min before the training session, completely prevented the acquisition disruption caused by both scopolamine and hypoxia. Atropine injected s.c. at the same doses was not able to improve learning in mice with any memory deficiency (Fig. 1). Atropine (10 μg/kg s.c.) did not modify mouse spontaneous locomotor activity (Table 1) as proved by the Animex test. Figure 1A, B also shows that under the same experimental conditions the two well-known nootropic drugs: piracetam (100 mg/kg i.p.) and physostigmine (0.2 mg/kg i.p.) were able, as expected, to prevent scopolamine and hypoxia amnesia. However, while atropine and physostigmine did not modify learning in mice free of memory impairment, piracetam reduced it.

DISCUSSION

The present study illustrates the paradoxical antiamnesic activity of the antimuscarinic drug atropine. In agreement with our results, other cholinomimetic effects of atropine were previously observed by Ferguson-Anderson (1952) and Brown (1990), who reported that in humans low doses of atropine increased gastric contraction frequency and amplitude and decreased heart rate. Bülbring (1946) and Ghelardini *et al*. (1990) described, respectively, the ability of atropine to potentiate cholinergic transmission *in vitro* at the isolated neuromuscular junction of rat phrenic hemidiaphragm and in the longitudinal muscle strip of guinea-pig.

The antiamnesic effect of atropine was obtained without any cholinergic symptoms such as tremors, scialorrhoea, diarrhoea, rhinorrhoea, lacrimation, abdomen flaccidity and also without any modification of spontaneous activity or motor coordination, as demonstrated by the Irwing test. On the other hand, physostigmine, used at a dose equiantiamnesic with atropine, provoked very clear cholinergic symptoms. The lack of cholinergic side effects suggests that atropine acts as an antiamnesic through a presynaptic mechanism. The hypothesis of a presynaptic mechanism is supported by the increase in endogenous ACh release induced by atropine (Bartolini *et al*., 1994) at doses at which it prevents scopolamine and hypoxia induced amnesia. Furthermore, in the same range of doses atropine-induced

Table 1. Comparison of atropine and physostigmine in the Irwing test

	Tremors	Salivation	Lacrimation	Diarrhoea	Abdominal tone	Spontaneous motility
Saline s.c.	0	0	0	0	4	4
Atropine 10 μg/mL s.c.	0	0	0	0	4	4
Physostigmine 200 μg/mL s.c.	2	6	+	+	2	0

Tremors	absent = 0		maximum score = 8
Salivation	absent = 0		maximum score = 8
Lacrimation	absent = 0		present +
Diarrhoea	absent = 0		present +
Abdominal tone	flaccid abdomen = 0	normal = 4	abdomen board-like = 8
Spontaneous motility	absent = 0	normal = 4	maximum score = 8

Each value represents the mean of mice. Spontaneous motility was evaluated by Animex test.

antinociception was antagonized by pretreatment with the choline uptake depletor hemicholinium-3 (Ghelardini *et al*., 1990). It is interesting to note that atropine elicits its indirect cholinomimetic effects at doses 1000 times lower than those able to provoke antimuscarinic effects, such as amnesia, through the blockade of postsynaptic receptors. The amplification of cholinergic responses induced by atropine appears to be much more useful than direct stimulation with cholinomimetic drugs such as physostigmine. Direct stimulation with anticholinesterases exerts a strong and continuous activation of postsynaptic receptors responsible for a large series of untoward effects at the same time as cognition facilitation.

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