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Short communication

The adenosine A_1 receptor antagonist BIIP 20 counteracts scopolamine-induced behavioral deficits in the passive avoidance task in the rat

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Abstract

The present study investigated the effects of the putative adenosine A_1 receptor antagonist BIIP 20 ((*S*)-(−)-8-(3-oxocyclopentyl)-1,3-dipropyl-7*H*-purine-2,6-dione) on counteracting scopolamine-induced behavioral deficits in the rat in the passive avoidance paradigm. A single oral application of BIIP 20 (1 and 3 mg/kg) 90 min before the rats received the noxious stimulus significantly attenuated the scopolamine-induced deficits observed during the retention trial of this task.

Keywords: Adenosine A_1 receptor antagonist; BIIP 20; Passive avoidance; Memory

1. Introduction

Adenosine is considered as a neuromodulator in the central nervous system (Williams, 1989) and acts by depressing cholinergic, noradrenergic and GABA (γ-aminobutyric acid)ergic transmission via adenosine A_1 receptors (Phillis and Kostopoulos, 1975; Hollins and Stone, 1980). That the cholinergic system is involved in learning and memory processes is well documented (Bartus et al., 1982). Thus, blocking of adenosine A_1 receptors may indirectly stimulate cholinergic neurotransmission and thereby enhance cognition.

The purpose of the present study was to investigate whether the novel adenosine A_1 receptor antagonist BIIP 20, which is the active enantiomer of KFM-19 (8-(3-oxocyclopentyl)-1,3-dipropyl-7*H*-purine-2,6-dione) (Schignitz et al., 1991) was able to antagonize scopolamine-induced deficits in the rat in a memory task. The step-through passive avoidance procedure was chosen for this purpose (King and Glasser, 1970).

In order to verify whether or not BIIP 20 had or had not modified the animal's response to pain during the acquisition of the task, the thresholds for various responses to electrical stimuli were also evaluated.

2. Material and methods

Procedures involving animals and their care were conducted in conformity with the institutional guidelines, in compliance with national and international laws and policies (EEC Council Directive 86/609, = J L 358, 1, Dec. 12, 1987; NIH Guide for Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985).

2.1. Animals

Different populations of male 2-month-old Sprague-Dawley rats (Charles River, Calco, Italy) weighing 200–220 g were used for the two studies (memory and nociception). The animals were housed in Makrolon cages (35 × 40 × 25 cm), five animals per cage, in a regulated environment (21 ± 1°C, 50–55% relative humidity, a 12 h light-dark cycle, lights on: 07.00 a.m.) with free access to food and water.

For both studies the rats were randomly divided into six experimental groups ($n = 10$ rats) as follows: vehicle + vehicle, vehicle + 1 mg/kg BIIP 20, vehicle + 3 mg/kg BIIP 20, vehicle + scopolamine, scopolamine + 1 mg/kg BIIP 20, scopolamine + 3 mg/kg BIIP 20.

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2.2. Step-through passive avoidance

2.2.1. Apparatus

For full details, see King and Glasser (1970). The apparatus was composed of a dark compartment with a grid floor connected to an illuminated platform. The electric shock was delivered to the grid floor by an isolated stimulator.

2.2.2. Procedure

During the first day (training trial) each rat was gently placed on the illuminated platform and 10 s later the guillotine door was opened. As soon as the rat had moved into the dark chamber and the door had been shut, a 1.6 mA footshock was applied for 1 s. Thereafter, the rat was immediately removed from the apparatus and returned to the home cage.

During the second day, the retention trial was performed. Each rat was placed in the lighted compartment again and the step-through latency was recorded. This is the time the rat remained on the illuminated platform. The test was stopped as soon as the rat entered the dark compartment, or remained on the illuminated platform for 180 s.

2.3. Nociceptive threshold

2.3.1. Apparatus

An operant chamber with a grid floor made of stainless-steel rods connected to a scrambled AC shock generator was used to determine the nociceptive threshold.

2.3.2. Procedure

The procedure described by Carli et al. (1992) was used. The rats were allowed 15 min to adapt to the chamber before a series of unavoidable shocks was delivered to the grid floor. Each series consisted of different stimulations with intensities of 0.1–1.5 mA in steps of 0.1 mA. The shock duration was 1 s and the shocks were delivered at 30 s intervals. The series of shocks was presented twice, once in an ascending and then in a descending order, at an interval of 15 min. The shock intensities were increased until a 'jump' response (removal of four paws from the grid floor) was observed. The shock intensities at which the rats displayed the 'flinch' or forepaws-off-the-grid-floor and vocalization were also recorded.

When the descending series of shock intensities was run, the last shock intensity at which a jump, flinch or vocalization response was observed was recorded. Thresholds for flinch, vocalization and jump response were estimated by averaging the two determinations.

2.4. Drugs

BIIP 20 ((*S*)-(−)-8-(3-oxocyclopentyl)-1,3-dipropyl-7*H*-purine-2,6-dione) (Boehringer-Ingelheim, Ingelheim, Germany) was given at a dose of 1 or 3 mg/kg, p.o., by gavage in suspension (distilled water + Tween-80) 90 min before the rats received the electric footshock in the passive avoidance study, or before nociception evaluation was undertaken. Scopolamine HBr (Sigma, St. Louis, MO, USA) was dissolved in saline and delivered at a dose of 0.75 mg/kg, i.p., 30 min before the rats received the noxious stimulus in the passive avoidance paradigm or before starting the pain reactivity study. Control animals received the vehicle (distilled water + Tween 80) p.o. The doses of compounds are expressed as the free bases. No drugs were administered during the retention trial.

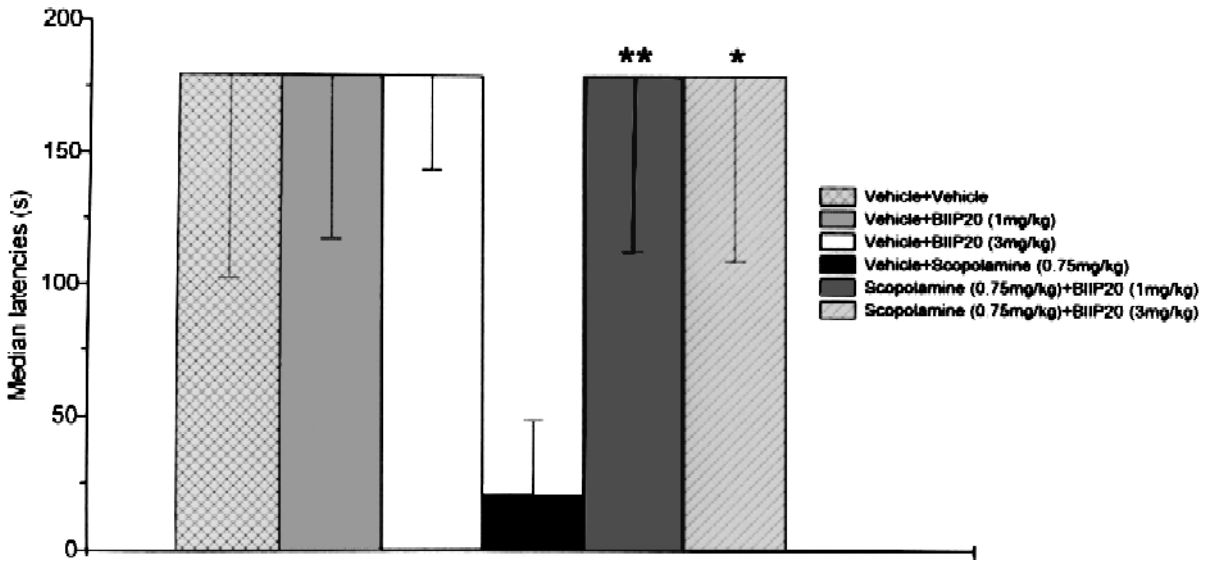

Fig. 1. Retention latencies (s) to enter the dark compartment in the passive avoidance task. Each value represents the median and interquartile ranges for 10 rats. * $P < 0.05$, ** $P < 0.01$ vs. the scopolamine + vehicle-treated group.

Table 1
Thresholds (mA) for responses to electric footshock

Group	*n*	Flinch Median	25 perc.	75 perc.
Vehicle + vehicle	10	0.250	0.20	0.30
Vehicle + BIIP 20 (1 mg/kg)	10	0.300	0.25	0.30
Vehicle + BIIP 20 (3 mg/kg)	10	0.275	0.25	0.30
Vehicle + scopolamine (0.75 mg/kg)	10	0.150	0.15	0.20
Scopolamine (0.75 mg/kg) + BIIP 20 (1 mg/kg)	10	0.150	0.15	0.20.
Scopolamine (0.75 mg/kg) + BIIP 20 (3 mg/kg)	10	0.175	0.15	0.20
Group	*n*	Jump Median	25 perc.	75 perc.
Vehicle + vehicle	10	0.550	0.45	0.60
Vehicle + BIIP 20 (1 mg/kg)	10	0.475	0.40	0.65
Vehicle + BIIP 20 (3 mg/kg)	10	0.525	0.45	0.60
Vehicle + scopolamine (0.75 mg/kg)	10	0.300	0.25	0.30
Scopolamine (0.75 mg/kg) + BIIP 20 (1 mg/kg)	10	0.300	0.30	0.30
Scopolamine (0.75 mg/kg) + BIIP 20 (3 mg/kg)	10	0.300	0.25	0.30
Group	*n*	Vocalization Median	25 perc.	75 perc.
Vehicle + vehicle	10	0.450	0.40	0.50
Vehicle + BIIP 20 (1 mg/kg)	10	0.475	0.35	0.60
Vehicle + BIIP 20 (3 mg/kg)	10	0.450	0.40	0.45
Vehicle + scopolamine (0.75 mg/kg)	10	0.350	0.25	0.40
Scopolamine (0.75 mg/kg) + BIIP 20 (1 mg/kg)	10	0.350	0.25	0.40
Scopolamine (0.75 mg/kg) + BIIP 20 (3 mg/kg)	10	0.375	0.30	0.40

n: number of rats per group.

2.5. Statistical analysis

The model of Weibull (Cox and Oakes, 1984) for censored data (considering as 'censored' each value not lower than 180 s) was applied on the complete experimental model: the main factors were the presence/absence of scopolamine, the dose of BIIP 20 (0, 1, 3 mg/kg) and their interactions; the significance level was at 0.05 and a two-tailed statistical test was adopted.

Nociceptive threshold data were analyzed by the Kruskal-Wallis non-parametric test.

3. Results

3.1. Step-through passive avoidance

Passive avoidance data are illustrated in Fig. 1. Treatment with scopolamine strongly impaired the rats' retention abilities with respect to the control populations ($P < 0.01$). A single administration of 1 or 3 mg/kg BIIP 20 resulted in a statistically significant enhancement of performance of the scopolamine-treated animals ($P < 0.01$ and $P < 0.05$, respectively, for the dose of 1 mg/kg and of 3 mg/kg BIIP 20). No effect on animal performance was observed when BIIP 20 was given alone. Interestingly, no differences were observed in the pre-shock latencies among the various experimental groups (data not shown).

3.2. Nociceptive threshold

The data are shown in Table 1. Rats treated with scopolamine displayed lower thresholds with respect to their control cohorts ($P < 0.01$) in the three parameters of pain reactivity recorded. Treatment with BIIP 20 did not modify the animal's response to pain and did not counteract the effects due to scopolamine treatment.

4. Discussion

Our results showed that acute administration of BIIP 20 significantly antagonized the scopolamine-induced deficits in a behavioral paradigm testing the retention abilities of rats. Acute treatment with BIIP 20, in line with a previous report (Normile and Barraco, 1991) concerning other adenosine A_1 receptor antagonists, did not have any effect on the vehicle-treated rats' performance. The lack of difference in training latencies between the animal groups may reflect that treatment with BIIP 20 does not influence motility.

An attempt to clarify whether changes in pain perception were involved in the effects of pre-training treatment demonstrated that various responses to electrical stimuli measured in the rats were not influenced by this adenosine A_1 receptor antagonist. This suggests that the response to the same stimulus (electric footshock) as used in the passive avoidance procedure was not affected by the treatment with BIIP 20.

The implication of the adenosine A_1 receptor in learning and memory processes is well known (Normile and Barraco, 1991; Whinsky and Harvey, 1987; Von Lubitz et

al., 1993; Zarrindast and Shafaghi, 1994). However, the results reported are controversial since both activation (Von Lubitz et al., 1993) and inhibition of the adenosine A_1 receptor (Normile and Barraco, 1991; Zarrindast and Shafaghi, 1994) seem to facilitate cognition in animals, depending on the treatment regimen of the adenosine A_1 receptor-related compounds. Acute treatment with adenosine A_1 receptor antagonists enhances cognition (Normile and Barraco, 1991) whereas subchronic treatment inhibits it (Von Lubitz et al., 1993). Repeated administration of adenosine A_1 receptor agonists improves memory (Von Lubitz et al., 1993) while acute treatment with these compounds attenuates retention abilities (Whinsky and Harvey, 1987; Zarrindast and Shafaghi, 1994). These findings suggest that adaptive changes in adenosine A_1 receptor responses occur upon repeated treatment with agonist and antagonist compounds.

In summary, BIIP 20, an adenosine A_1 receptor antagonist compound, counteracted scopolamine-induced performance deficits in the rat in the passive avoidance task. This compound needs further characterization in different memory tasks in order to be able to assess whether it will be suitable for clinically testing cognition-related problems in the elderly.

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