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Short communication

Itasetron (DAU 6215) prevents age-related memory deficits in the rat in a multiple choice avoidance task

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Abstract

The effects of itasetron (endo-*N*-8-methyl-8-azabicyclo-[3.2.1.]-octo-3-yl)-2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carboxamide hydrochloride), a 5-HT_3 receptor antagonist, on discrete memory abilities of the aged rat were assessed by using the multiple choice avoidance behavioral task. Chronic treatment with itasetron (i.p., 10 μg/kg, b.i.d., for three consecutive weeks), but not with vehicle, significantly improved retention abilities of the aged rats in this memory test. These results further support the important role of this 5-HT_3 receptor antagonist in counteracting age-related memory degeneration in rodents.

Keywords: Ageing; 5-HT_3 receptor; Itasetron (DAU 6215); Avoidance, multiple choice; Memory

1. Introduction

The important role of 5-HT_3 receptor antagonists in modulating learning and memory processes has recently been acknowledged (Barnes et al., 1990). It was demonstrated that the novel selective 5-HT_3 receptor antagonist, itasetron (DAU 6215), counteracted scopolamine-induced amnesia in the step-through passive avoidance task (Brambilla et al., 1993) and in the Morris water maze task in the young rat (Pitsikas et al., 1994). In addition, itasetron improved spatial learning and memory abilities in the aged rat, in the Morris water maze task (Pitsikas et al., 1993).

There is evidence that the aged rodent usually is not able to retain recently processed information and that its discrete memory abilities are seriously compromised (Bartus et al., 1980; Martinez and Rigter, 1983; Pitsikas et al., 1991; Zornetzer et al., 1982). The aim of the present study was to provide additional information on the mnemonic properties of itasetron. Thus, the ability of this compound to attenuate cognitive impairments in the aged rat was evaluated in the multiple choice avoidance task (Van Haaren and Van de Poll, 1984).

Compared to the usual step-through passive avoidance task, the design we now used offers the animal a second choice during the post-shock trial (the existence of a second dark chamber in which no shock is presented). Thus, if the aged rats suffer from exposure to light and their mnemonic capacities are not impaired, they are more likely to escape to this second dark compartment; conversely, if they escape to darkness in general during the post-shock trial, this implies that their memory is impaired. This design gives the aged rat an opportunity to avoid both the noxious stimulus in the dark compartment and the light, and to escape to another dark chamber where it has never been shocked.

In order to ascertain whether itasetron had or had not modified the animals' response to pain during the acquisition of the task, the thresholds for various responses to electrical stimuli were also evaluated.

2. Materials 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).

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2.1. Animals

Different populations of male 24-month-old and 2-month-old Sprague-Dawley rats (Charles River, Calco, Italy) were used for the two studies (cognition and nociception). These animals arrived at our animal house 1 month before the start of experimentation. The rats were housed in Makrolon cages (35 × 45 × 20 cm), two per cage, and maintained in a controlled 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 the multiple choice avoidance test, the rats were randomly divided into four experimental groups as follows: young-vehicle ($n = 20$), young-itasetron ($n = 20$), aged-vehicle ($n = 20$) and aged-itasetron ($n = 20$). For the pain reactivity study the same experimental design also was applied to a different population of animals ($n = 13–14$ rats per group).

The health status of the rats was continuously checked. Rats with evident malignancies, motor deficits, or foot problems were a priori excluded from behavioral testing. None of the animals that participated in either of the experiments presented evident signs of these health problems.

2.2. Drugs

Itasetron solution was prepared freshly each day. Itasetron (endo-*N*-(8-methyl-8-azabicyclo-[3.2.1.]-octo-3-yl)-2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carboxamide hydrochloride) (Boehringer Ingelheim Italia S.p.A., Milan, Italy) was dissolved in saline and administered at the dose of 10 μg/kg and in a volume of 1 ml/kg. This dose of itasetron, expressed as the base, was selected on the basis of previous studies in which it was found to be the most effective dose for antagonizing cognitive impairments (Brambilla et al., 1993; Pitsikas et al., 1994). Young and aged rats received either vehicle (saline) or the drug i.p. twice a day (09.00 and 14.00 h) for three consecutive weeks. The memory test was carried out during the 3rd, 4th and 5th day of the 3rd week of treatment. Nociception evaluation was performed during the 5th day of the 3rd week of treatment. Observations were made between 09.00 and 13.00 h starting 45 min after the morning injection.

2.3. Multiple choice avoidance

2.3.1. Apparatus

The apparatus (80 × 40 × 40 cm) consisted of two dark compartments (A and B) connected to a platform illuminated by a 25 W lamp, and each chamber was accessible from the platform through a guillotine door. To obtain discrimination between the two dark compartments, one (A) was fitted with an inside box consisting of a wire mesh floor and vertically stripped black and white inner walls, while the other (B) had a grid floor and flat grey inner walls. Scrambled footshocks could be delivered through the grids of the compartment B. Chambers A and B could be interchanged during the training period.

2.3.2. Procedure

On day 1, the animals were allowed to adapt to the apparatus. For half the animals, compartment A was on the left and compartment B on the right, and vice versa for the other half. The rats were first adapted to chamber A for 2 min during which the guillotine door was closed. Thereafter they were placed on the platform and the guillotine door of chamber A was raised and that of compartment B remained closed. The latency to enter compartment A was recorded. The same sequence was followed for adapting the animals to chamber B. Rats that did not enter the dark chamber within 300 s were eliminated from the test.

On day 2, the rats were placed on the platform as on day 1. The rats could visit only one chamber and the latency to enter this chamber was recorded. The animals started with compartment A and continued with compartment B. This procedure was repeated six times at intervals of 2 min. If the rats did not enter the dark chamber within 300 s they were eliminated from the test. When the animal entered chamber B at the sixth (last trial), the guillotine door was closed and a 1 mA footshock lasting 2 s was delivered.

On day 3, the rats' retention abilities were assessed. The animals were replaced on the platform and both guillotine doors were raised. The post-shock trial ended after an animal had entered one of the two compartments, or if the animal remained on the platform for a maximum of 300 s. The latencies of the animals to enter one of the two dark chambers were measured. Correct choices were recorded if the rat remained on the platform or entered the dark chamber in which it had not previously obtained a shock. Entrance within 300 s into the dark compartment in which the footshock was delivered was considered an index of impaired retention.

2.4. Nociceptive threshold

2.4.1. 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.

2.4.2. 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 responses were estimated by averaging the two determinations.

2.5. Statistical analysis

Post-shock behavior and preference for darkness in the multiple choice avoidance task were analyzed by using the exact probability test for 2×2 tables (Fisher's exact test). Nociceptive threshold data were analyzed with a factorial analysis of variance (ANOVA 2×2).

3. Results

3.1. Multiple choice avoidance

Three 2-month-old, seven 24-month-old rats treated with the vehicle and five 24-month-old rats treated with itasetron did not enter one of the two dark chambers within 300 s during the pre-shock training and thus were eliminated. Two 24-month-old rats treated with itasetron died during the treatment period.

The animals' retention abilities are illustrated in Table 1. The aged rats treated with the vehicle demonstrated poor retention capacities as compared to their younger counterparts (seven out of 13 aged rats displayed incorrect choices); $P = 0.042$. On the contrary, the old rats treated with itasetron displayed memory abilities (13 out of 13 correct choices) similar to those of their younger cohorts and significantly better than those of the aged rats treated with saline; $P = 0.0150$. Post-shock trial latencies indicate that those rats which took refuge in the dark chambers did so between 10–20 s, independently of age or of drug treatment.

Table 1
Retention abilities of young and aged rats treated either with itasetron or with vehicle in the multiple choice avoidance task

Age	Treatment	n	24 h post-shock retention			Total
			ES	ENS	NE	ENS + NE
Young	Vehicle	17	1	8	8	16
Young	Itasetron	20	2	6	12	18
Aged	Vehicle	13	6	6	1	7
Aged	Itasetron	13	0	9	4	13

n: number of rats per group; ES: number of rats entering the shock compartment; ENS: number of rats entering no-shock compartment; NE: number of rats not entering either compartment; ENS + NE: total of correct choices. Itasetron (10 μg/kg) or vehicle (saline) was given i.p., for three consecutive weeks twice a day. Aged-vehicle rats vs. young-vehicle rats, $P = 0.0093$ (Fisher's exact test). Aged-vehicle rats vs. aged-itasetron rats, $P = 0.0150$ (Fisher's exact test).

Table 2
Thresholds (mA) for responses to electric footshock of young and aged rats treated either with itasetron or with vehicle

Age	Treatment	n	Flinch		
			Median	25 perc.	75 perc.
Young	Vehicle	14	0.275	0.25	0.35
Young	Itasetron	13	0.250	0.20	0.30
Aged	Vehicle	14	0.325	0.25	0.35
Aged	Itasetron	13	0.300	0.25	0.35
Age	Treatment	n	Jump		
			Median	25 perc.	75 perc.
Young	Vehicle	14	0.525	0.40	0.60
Young	Itasetron	13	0.450	0.40	0.55
Aged	Vehicle	14	0.525	0.50	0.60
Aged	Itasetron	13	0.500	0.40	0.60
Age	Treatment	n	Vocalization		
			Median	25 perc.	75 perc.
Young	Vehicle	14	0.500	0.45	0.50
Young	Itasetron	13	0.500	0.35	0.50
Aged	Vehicle	14	0.625	0.55	0.75
Aged	Itasetron	13	0.600	0.50	0.70

n: number of rats per group. Itasetron (10 μg/kg) or vehicle (saline) was given i.p., twice a day for three consecutive weeks.

When the preference of aged animals for light (ENS, Table 1) or for darkness (ES + ENS, Table 1) was checked, Fisher's exact test indicated that the 24-month-old rats treated with the vehicle, when compared to the young controls, preferred to escape to the dark chambers rather than remain exposed to the light stimulus; $P = 0.0420$. The 24-month-old rats treated with itasetron did not show this difference from their younger cohorts; $P = 0.157$.

However, while the aged vehicle-treated animals entered either of the two dark chambers (correct or incorrect) during the post-shock trial, the 24-month-old rats treated with itasetron showed a statistically significant preference for escape to the 'correct' dark chamber (nine out of nine correct choices) when compared to their vehicle-treated counterparts; $P = 0.019$.

3.2. Nociceptive threshold

The data are shown in Table 2. Aged rats, independently of the pharmacological treatment, displayed raised thresholds for flinch ($F(3,50) = 4.33$, $P < 0.05$) and for vocalization ($F(3,50) = 25.54$, $P < 0.01$) but not for jumping, when compared to their younger cohorts. Interestingly, no effect of drug was observed for the three parameters of pain reactivity recorded.

4. Discussion

Accordingly to the literature, aged rats exhibit poor retention abilities as compared to those of their younger cohorts (Bartus et al., 1980; Martinez and Rigter, 1983; Pitsikas et al., 1991; Zornetzer et al., 1982). Chronic treatment with itasetron, however, had a beneficial effect on aged rats' memory since these animals performed as well as their young counterparts in this multiple choice avoidance task. It seems very difficult to assume that itasetron exerts its effects exclusively on the retention process. Since this drug was also given during the training period of the task, we cannot rule out possible beneficial effects on other memory processes such as attention, acquisition, consolidation or retrieval. Thus, we cannot attribute the positive effect of this 5-HT_3 receptor antagonist in this behavioral test to a specific memory mechanism.

The retention abilities of the young animals apparently were not affected by chronic treatment with itasetron. We cannot exclude, however, the possibility that a beneficial effect of this compound on cognitive abilities of the young animal would be observed in other behavioral tests.

An attempt to clarify whether changes in pain perception were involved in the effects of pre-training treatment demonstrated that various responses to electric footshock measured in young and aged animals were not influenced by this 5-HT_3 receptor antagonist. This suggests that the response to the same stimulus (electric shock) as used in the multiple choice avoidance procedure was not affected by the treatment with itasetron.

Post-shock behavior of the aged control animals confirmed earlier findings that old rats suffer from exposure to the light stimulus and prefer to escape to darkness (12 out of 13) (Goodrick, 1971). This seemed less pronounced in the population of aged rats treated with itasetron. Although these animals preferred to escape to darkness (9 out of 13) instead of remaining exposed to the light stimulus during the retention trial, this tendency did not reach statistical significance. Since itasetron has been reported to exert an effect on the behavior of animals in the light/dark exploratory test (Borsini et al., 1993) it seems unlikely that the anxiolytic activity of this 5-HT_3 receptor antagonist influenced the aged rats' post-shock behavior. In fact, when the 24-month-old rats treated with itasetron escaped to darkness during the retention trial, all chose the 'correct' chamber in which they did not receive a shock, and this suggests that their memory was not impaired.

These results extend and are in line with our previous findings that scopolamine-induced amnesia in the young rat (Brambilla et al., 1993; Pitsikas et al., 1994) and spatial learning deficits in the aged rat (Pitsikas et al., 1993) are antagonized by 10 μg/kg itasetron.

The mechanism of action of this 5-HT_3 receptor antagonist compound is still under investigation. An electrophysiological study showed that itasetron antagonized the 5-HT inhibitory effect on long-term potentiation induction in the rat hippocampus (Passani et al., 1994). Microdialysis experiments showed that, like other 5-HT_3 receptor antagonist compounds (Barnes et al., 1989; Bianchi et al., 1990), itasetron counteracts the inhibitory effect of the selective 5-HT_3 receptor agonist 2-CH_3-5-HT on acetylcholine release in the rat cerebral frontal cortex (Ladinsky et al., 1993).

In conclusion, in agreement with our prior results, treatment with itasetron protected memory abilities in the aged rat in a multiple choice avoidance task. It would be valuable to test this compound in the senescent human affected by memory problems.

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