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Short communication

Beneficial effect of the σ_1 receptor agonist PRE-084 against the spatial learning deficits in aged rats

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Abstract

Sigma$_1$ (σ_1) receptor agonists showed anti-amnesic properties in pharmacological amnesia models. We now investigated whether the selective σ_1 receptor agonist, 2-(4-morpholinoethyl)-1-phenylcyclohexane-1-carboxylate hydrochloride (PRE-084), could ameliorate spatial learning in aged animals, using a water-maze procedure. Wistar rats, 3 or 24 months old, were trained to locate a visible platform and then an invisible platform. Finally, a transfer test was performed during saline or PRE-084 treatment. Aged, but not adult, animals showed learning deficits unrelated to visual impairments. The PRE-084 treatment allowed aged animals to learn the new platform location, in terms of decreased latencies to the platform during training and increased presence in the quadrant during retention. The results of these experiments suggest a potential of selective σ_1 receptor agonists as cognitive enhancers during ageing.

Keywords: σ_1 Receptor; PRE-084; Water maze test; Learning; Memory; Aged, rat

1. Introduction

The sigma$_1$ (σ_1) receptor was recently cloned in several animal species and in the human (Hanner et al., 1996; Kekuda et al., 1996; Seth et al., 1997, 1998; Pan et al., 1998). The protein obtained shows a 223-amino acid sequence and shares no homology with any known protein, in particular with classical ionotropic or metabotropic neurotransmitter receptors. However, the protein mediates a very efficient neuromodulatory action, affecting several neurotransmitters systems, including the acetylcholine and *N*-methyl-D-aspartate (NMDA)-type of glutamatergic receptor (for review, see Maurice et al., 1999). In addition, it was recently reported that selective σ_1 receptor agonists potentiate intracellular calcium mobilisation through both the intracellular IP_3 receptor and the voltage-dependent calcium channels located on the plasma membrane (Hayashi et al., 2000). This neuromodulatory action is likely to be responsible for the numerous behavioural effects mediated by the σ_1 receptor ligands, including anti-amnesic effects, attenuation of the responses to stress and depression (Maurice et al., 1999), or attenuation of cocaine-induced reward or toxicity (McCracken et al., 1999, Romieu et al., 2000). The anti-amnesic effects induced by selective σ_1 receptor agonists have been extensively characterised, using several models of amnesia induced following administration of pharmacological agents. Indeed, learning impairments observed after blockade of the NMDA receptor with dizocilpine, or after blockade of muscarinic acetylcholine receptor using scopolamine, could be reversed by several σ_1 receptor agonists (Maurice et al., 1999).

Since these neurotransmission systems are markedly affected during ageing, it is expected that selective σ_1 receptor agonists may exert beneficial effects against the age-related learning deficits. A preliminary study showed that igmesine and PRE-084 improved the learning capacities in 12 months old senescence-accelerated SAMP/8 mice to levels similar to those observed in age-matching senescence-resistant SAMR/1 mice (Maurice et al., 1996). Memory capacities were evaluated using the spontaneous alternation behaviour and a passive and an active avoidance test. In the present study, we examined the efficiency of a selective σ_1 receptor agonist, PRE-084, to improve spatial memory capacities of aged rats. Animals with ages 3 or 24 months old were trained, using the water-maze procedure, to locate first, a visible platform and second, an invisible platform. Then aged animals were put through a

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transfer test during either saline or PRE-084 treatment. The results demonstrated that PRE-084 allowed a significant improvement of spatial learning ability in aged Wistar rats.

2. Methods

2.1. Animals

Male Wistar rats (Charles River, St-Aubin-lès-elbeuf, France) were used at 3 months of age, average weight: 425 ± 6 g; and at 24 months, average weight: 673 ± 21 g. The animals were housed in groups in plastic cages. They had free access to laboratory chow and water, except during behavioural experiments, and were kept in a regulated environment (23 ± 1 °C, 40–60% humidity) under a 12-h light/dark cycle (light on at 7:00 a.m.). Experiments were carried out between 1:00 and 6:00 p.m., in a soundproof and air-regulated experimental room, to which the animals were habituated for at least 30 min before each experiment. All animal procedures were conducted in strict adherence to the European Community Council Directive of 24 November 1986 (86-609/EEC).

2.2. Behavioural observations

The ability of animals to perform a spatial learning task was examined using the water-maze test. The maze was a circular pool, 150 cm in diameter, 40 cm in height, arbitrarily divided into four quadrants. The water temperature (24 ± 2 °C), light intensity, external cues in the room, and water opacity, obtained by suspension of lime carbonate, were rigorously reproduced. A transparent Plexiglas platform, 10 cm in diameter, could be immersed 2 cm under the water surface at the centre of each quadrant during training sessions. This quadrant was termed the training (T) quadrant and the others opposite (O), adjacent right (AR), and adjacent left (AL) quadrants, during the subsequent retention session. Swimming was recorded using a CCD camera connected to a computer, trajectories being analysed in terms of latencies and distances using the Videotrack® II (version 2.65) software (Viewpoint, Champagne-au-Mont-d'Or, France).

2.3. Experimental procedure

Training consisted of four swims per day from days 1–5, with an inter-trial time interval of 10 min. Start positions, set at each limit between quadrants, were randomly selected for each animal. Each animal was allowed a 90-s swim to find the platform and was left for a further 30 s on the platform. Animals failing to find the platform were placed on it manually. During the retention test, the platform was removed, and each animal was allowed a free 60-s swim. The percentage of time spent in each quadrant was determined. The experimental procedure was as follows: animals were first put through a training series with the platform located in the north–east quadrant and rendered visible with a white plastic flag. Training was carried out during days 1–5 and a retention test was performed on day 6. After 3 weeks, the animals were trained again with the invisible platform located in the south–west quadrant. Training was carried out during days 1–5 and a retention test was performed on day 6. Aged animals were subjected to a transfer test from days 7 to 9. The platform was moved to the south–east quadrant and training sessions were repeated. The animals were injected with either saline solution or PRE-084 (0.5 mg/kg) 20 min before the first swim on days 7–9. A retention test was again carried out on day 10.

2.4. Drug

2-(4-morpholinoethyl)-1-phenylcyclohexane-1-carboxylate hydrochloride (PRE-084) was provided by Dr T.P. Su (Cellular Pathobiology Unit, I.R.P., N.I.D.A./N.I.H., Baltimore, MD, USA) and dissolved in saline solution. It was administered subcutaneously (s.c.) in a volume of 100 μl per 100 g of body weight. The dose was selected according to results of previous studies (Maurice et al., 1994, 1995, 1996, 1998).

2.5. Statistical analysis

The median swim latency was calculated for each training session. The results were then expressed as means ± S.E.M. Swimming speeds, determined during the retention tests, were analysed using a Student's *t*-test. Latencies were analysed over trials using the non-parametric Friedman repeated measures test (Fr values), comparisons between groups being made using Dunn's test. Data from the retention sessions were analysed using Dunn's test after a Kruskal–Wallis analysis of variance (KW values). The statistical levels for significance were $P < 0.05$ and $P < 0.01$.

3. Results

3.1. Learning abilities of adult and aged rats in the water maze

The rats were first tested in the water-maze with the visible platform procedure to check their physical ability (Fig. 1A and B). For the young adult group, the latencies to finding the platform decreased over the course of acquisition training ($Fr(4,49) = 27.8$, $P < 0.01$; Fig. 1A). Between trials, there was a significant decline in latencies from trial 1 to trials 3 ($P < 0.05$), 4 ($P < 0.01$) and 5 ($P < 0.01$). For the aged animals group, the latencies also decreased with training ($Fr(4,84) = 38.3$, $P < 0.01$; Fig. 1A). Between trials, there was a significant decline in

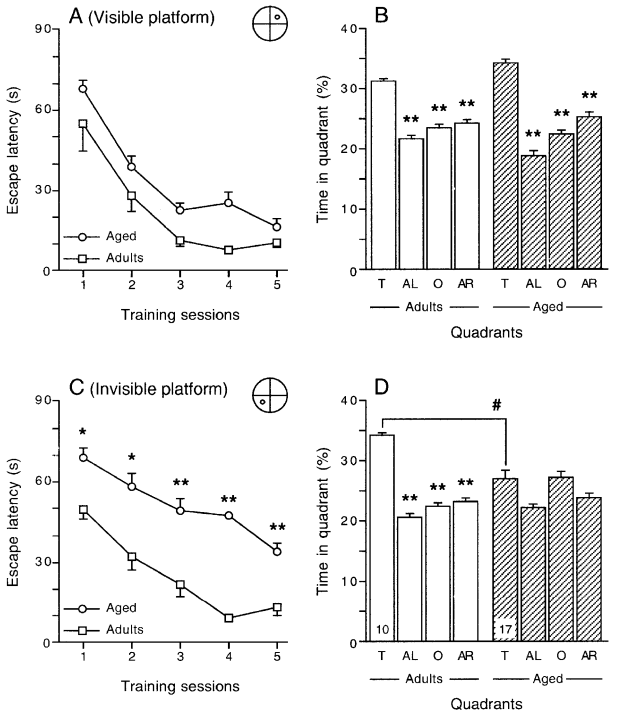

Fig. 1. Performance of adult and aged Wistar rats in the water-maze test: (A) training sessions and (B) retention test during the visible platform test; (C) training sessions and (B) retention test during the invisible platform test performed 3 weeks after. (A, C) The animals were put through four 90-s swim each day. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. performance of the young during the same training session. (B, D) Percentages of time spent in each quadrant during the 60-s swim. $^{**}P < 0.01$ vs. time spent in the T quadrant, $^{\#}P < 0.05$ vs. performance of the young (Mann–Whitney's test). Quadrants: T, training; AL, adjacent left; O, opposite; AR, adjacent right. The number of animals per group is indicated within the columns in (D).

latencies from trial 1 to trials 3 ($P < 0.01$), 4 ($P < 0.05$) and 5 ($P < 0.01$). The latencies shown by aged animals appeared slightly but non-significantly higher than the ones observed for young animals, a difference related to lower swimming speed of the aged animals (12.9 ± 0.6 cm/s, $n = 17$) as compared to that of the young (20.6 ± 0.9 cm/s, $n = 10$, $P < 0.001$, Student's t-test). During the retention test, performed 24 h after the last training session, both adult and aged animals swam preferentially in the T quadrant during the 60-s session (KW = 23.3, $P < 0.01$ for young; KW = 34.2 for aged; Fig. 1B). The time spent in this quadrant appeared significantly greater than the time spent in the other three, indicating that each group learned the visible platform location correctly.

The rats were then tested in the water-maze, using the invisible platform procedure (Fig. 1C and D). For young animals, the latencies to finding the platform decreased over the course of acquisition training ($Fr(4,49) = 22.2$, $P < 0.01$; Fig. 1C). Between trials, there was a significant decline in latencies between trials 1 and 5 ($P < 0.01$). For the aged group, the latencies also decreased over training, although less significantly than with the visible platform procedure ($Fr(4,84) = 11.0$, $P < 0.05$; Fig. 1C). Between trials, there was a significant diminution in latencies between trials 1 and 5 ($P < 0.05$). However, the latencies determined for aged animals were all significantly higher than those for young animals (Fig. 1C). During the retention test on day 6, young animals swam preferentially in the T quadrant (KW = 25.0, $P < 0.01$; Fig. 1C) and the time spent in this quadrant appeared significantly greater than the time spent in the other three. On the contrary, aged animals failed to swim differentially in the four quadrants (KW = 4.1, $P > 0.05$; Fig. 1D), indicating that they failed to locate the platform position. Furthermore, a statistically significant difference was measured for the time spent in the T quadrant by the young and the aged animals.

3.2. Effect of PRE-084 on the learning abilities of aged rats in the water maze

Aged rats, tested with the invisible platform procedure, were arbitrarily divided into two groups. Each group be-

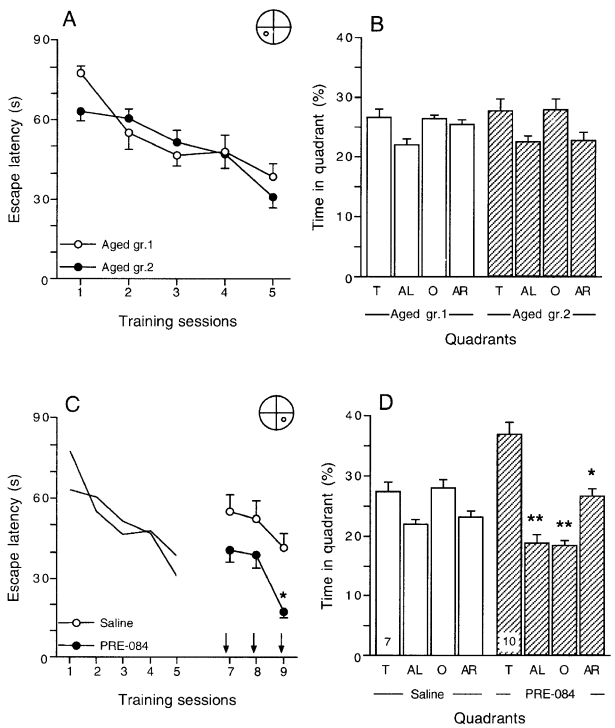

Fig. 2. Effect of PRE-084 on the performance of aged Wistar rats in the water-maze test. The aged animals were divided into two experimental groups that showed similar patterns of training from days 1 to 5 (A), and retention tested on day 6 (B). The animals were then given saline or PRE-084 (0.5 mg/kg, s.c.) and submitted to a transfer test from days 7 to 9 (C), and a retention test on day 10 (D). In (C), the animals were injected once a day, 20 min before the first training swim (indicated by the arrow). $^{*}P < 0.05$ vs, the saline-treated group during the same training session or in the T quadrant. In (D), $^{*}P < 0.05$, $^{**}P < 0.01$ vs. time spent in the T quadrant (Mann–Whitney's test). Quadrants: T, training; AL, adjacent left; O, opposite; AR, adjacent right. The number of animals per group is indicated within the columns in (D).

haved similarly in the test. They showed a limited and non-significant decrease in latencies during training acquisition ($Fr(4,34) = 6.3$, $P > 0.05$ for the aged group 1; $Fr(4,49) = 6.8$, $P > 0.05$ for the aged group 2; Fig. 2A) and no differences in the time spent within each quadrant during the retention test ($KW = 3.0$, $P > 0.05$ for the aged group 1; $KW = 2.0$, $P > 0.05$ for the aged group 2; Fig. 2B). Between days 7 and 9, each group was put through a transfer test, group 1 being injected s.c. with saline solution and group 2 with PRE-084 (0.5 mg/kg), 20 min before the first swim on each day. The saline-treated group failed to show any decrease in latencies over the training sessions ($Fr(2,20) = 1.5$, $P > 0.05$, Fig. 2C). The PRE-084-treated group showed some significant decrease in latencies during training ($Fr(2,29) = 3.7$, $P < 0.05$, Fig. 2C). Furthermore, the latency measured on day 9 was significantly lower for the PRE-084-treated group than for the saline-treated group (Fig. 2C). During the retention test performed on day 10, the saline-treated animals failed to a significant differences in time spent within each quadrant ($KW = 4.9$, $P > 0.05$; Fig. 2D), whereas the PRE-084-treated showed a significantly increased time in the T quadrant ($KW = 12.4$, $P < 0.01$; Fig. 2D). It must be noted that treatments did not affect the swimming speed measured during the retention test (12.7 ± 1.0 cm/s, $n = 7$, for the saline-treated group, and 13.1 ± 0.8 cm/s, $n = 10$, for PRE-084-treated group). These values were similar to the speeds measured during the first retention test ($P > 0.05$ each, paired t-test).

4. Discussion

The present study showed that aged Wistar rats developed spatial learning impairments in the water-maze procedure that could not be related to motor or visual deficits. Indeed, although 24 months old animals learned at the same rate as did young adults the location of a visible platform, they failed to learn the location of a hidden platform during the same learning procedure. The present study showed then that a repetitive treatment with the selective σ_1 receptor agonist PRE-084 allowed aged animals to learn significantly a new platform location during a transfer test.

These observations indicated that the anti-amnesic effect mediated by the σ_1 receptor, previously observed in different pharmacological models of amnesia, may be of interest for the enhancement of residual cognitive abilities during ageing. The σ_1 receptor agonists exerted a beneficial effect against learning deficits induced by blockade of the NMDA-type of glutamatergic neurotransmission (Maurice et al., 1994; Ohno and Watanabe, 1995), the muscarinic type of cholinergic neurotransmission (Matsuno et al., 1993), and voltage-dependent calcium channels (Maurice et al., 1995). These different systems are markedly affected during ageing, which can be regarded as the result of progressive deficits in the effectiveness of neurotransmission systems, partly due to an imbalance in several neuromodulatory controls. The wide-range neuromodulation exerted by the σ_1 receptor thus appeared to be a promising therapeutic strategy to alleviate the deficits resulting from ageing. The regional and subcellular distributions of the σ_1 receptor have recently been achieved by immunocytochemical labelling in the adult Wistar rat (Alonso et al., 2000). The most highly labelled structures appeared to be the olfactory bulb, hippocampus, superficial layer of the cortex, hypothalamic nuclei, amygdala, septum, motor nuclei of the hindbrain and dorsal horn of the spinal cord. Most of these structures are related to memory processes. However, examination of the expression of σ_1 receptor distribution during ageing has not yet been reported upon. It is expected from the present data showing the efficiency of a low dosage of PRE-084 that σ_1 receptor density may be well-preserved during ageing, at least in brain structures involved in cognitive functions.

The endogenous effector system related to the σ_1 receptor is still unidentified. However, a major endogenous hormonal system, namely the neuroactive steroids, including progesterone, pregnenolone or dehydroepiandrosterone, has been reported to interact directly and potently with the σ_1 receptor (Su et al., 1988; Monnet et al., 1995; Maurice et al., 1999). Neuroactive steroids, synthesised in the periphery or centrally, mainly through glial cells, act on nervous cells and mediate a potent modulation of several neuronal responses to neurotransmitters, including glutamate, γ aminobutyric acid (GABA) and acetylcholine. Part of their effects involves an interaction with the σ_1 receptor. In particular, the antiamnesic effect of dehydroepiandrosterone could be blocked using a selective σ_1 receptor antagonist (Maurice et al., 1997). Progesterone behaves as a potent endogenous σ_1 receptor antagonist (Monnet et al., 1995; Bergeron et al., 1996; Maurice and Privat, 1997). We recently demonstrated that endocrine manipulations of progesterone levels by surrenalectomy/castration and administration of inhibitors of the steroid biosynthesis enzymes (Phan et al., 1999), directly and potently affects the extent of the beneficial anti-amnesic effects mediated by PRE-084. Steroid levels decrease during ageing (Orentreich et al., 1984). The substantial age-related decrease of plasma levels of these steroids that occurs concurrently with involution of the zona reticularis indicates that these hormones might play a role in the incidence of age-related cognitive declines. Pregnenolone sulphate treatment improved memory in aged rodents (Vallée et al., 1997) and, in humans, dehydroepiandrosterone administration improved well-being in the elderly (Morales et al., 1994). These observations suggest that decreases in steroid levels observed during ageing are deleterious for cognitive functions and that cognitive declines may be due partly to a deficit in neuromodulatory control exerted by neuroactive steroids on several neurotransmission systems in the brain (Vallée et al., 1997;

Maurice et al., 1999). It is thus likely that selective σ_1 receptor agonists exert their efficient beneficial memory enhancing effect during ageing by compensating for the deficits in neuroactive steroids. It remains to be determined whether σ_1 receptor agonists alleviate the decreases in dehydroepiandrosterone or pregnenolone level, or whether the decrease in progesterone, i.e., in endogenous σ_1 receptor antagonist, led to facilitation of the σ_1 receptor agonist effect.

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