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RAPID COMMUNICATION

Atipamezole, an α_2 Antagonist, Stabilizes Age-Related High-Voltage Spindle and Passive Avoidance Defects

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RIEKKINEN, P. JR., J. SIRVIÖ, M. RIEKKINEN, R. LAMMINTAUSTA AND P. RIEKKINEN. *Atipamezole, an α_2 antagonist, stabilizes age-related high-voltage spindle and passive avoidance defects.* PHARMACOL BIOCHEM BEHAV 41(3) 611-614, 1992.—The present study investigates the effects of an α_2 antagonist, atipamezole (Ati), on the high-voltage spindle (HVS; Ati at 0.1, 1.0, and 3.0 mg/kg) activity, passive avoidance retention (PA; Ati at 3 mg/kg; injected before retention trial), and water maze (WM; Ati at 3 mg/kg; injected after daily training trials) acquisition of young and aged rats. PA retention trial performance defect of aged rats was partially alleviated by Ati at a 3-mg/kg dose. Ati at 3 mg/kg had no effect on the PA performance of young rats. Retention trial performance of nonshocked young or aged rats was not altered by a 3-mg/kg Ati dose. WM acquisition was not affected by posttraining Ati injections. Age-related increase of HVS was stabilized by Ati at 1 or 3 mg/kg. Ati at 1 and 3 mg/kg completely suppressed HVS of young rats. Ati at 0.1 mg/kg had no effect on HVS of young or aged rats. The results suggest that $\alpha_2$antagonist-administration-induced increase in noradrenergic activity may stabilize age-related HVS activity increase and PA performance defect.

Aging Atipamezole HVS, PA, and WM performance

THE noradrenergic system of the locus coeruleus (LC) has been shown to be importantly involved in the mechanisms underlying cognitive functions (4,8,12,13,17). First, pharmacological studies have shown that modulation of the noradrenergic system affects performance in some tasks used to assess learning and memory (8,12,13,17). For example, passive avoidance (PA) retention is impaired by preretention test injectioning of propranolol, a β-receptor blocker (8). On the other hand, drugs that stimulate noradrenergic activity in the brain improve retention of previously learned tasks (17). Second, neurophysiological studies demonstrated that noradrenaline may modulate thalamocortical activation (2,6,9). Neocortical high-voltage spindle (HVS) oscillations, which are closely dependent upon a thalamic pacemaker, nucleus reticularis thalamus, are decreased by α_2 antagonists and increased by α_2 agonists (2,9).

It is important to note that the noradrenergic system is adversely affected by aging (4,7,17). For example, previous studies have shown that the number and functioning of LC neurons may be affected during aging (4,17). Therefore, it is reasonable to believe that the age-related noradrenergic deficit may contribute to the impaired PA retention and HVS activity of aged rats. A pharmacological strategy for restoring the age-related decrease in noradrenergic activity is to disinhibit the LC neurons by the use of α_2 antagonists (5).

The present study investigates if the α_2 antagonist atipamezole (Ati) could alleviate age-related behavioral and neurophysiological defects. Previously, Ati has been shown to increase the turnover of noradrenaline (0.03-3 mg/kg) (5) and suppress HVS activity (0.1-10 mg/kg) (9) dose dependently in young rats. In the present study, we first investigated the dose-response curve for the HVS suppressing efficacy of Ati in aged rats. Next, the effects of an Ati dose effective in suppressing HVS activity was investigated on the PA retention and water maze (WM) acquisition performance of aged rats.

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METHOD

Animals

Young adult (3 months) and aged (26 months) female rats were used in the present experiment. The groups are shown in Table 1.

Drugs

Saline and Ati (Orion, Farmos Group, Finland) were injected SC (0.5 ml/kg) 20 min before PA retention test and EEG recordings. We have shown previously that Ati dose dependently induced catalepsy and floating in WM test (at a 3-mg/kg Ati dose, rats were completely unable to swim). Therefore, Ati was injected after WM training in the present experiment. In the neurophysiological study, the following recordings were made: baseline (saline), Ati 0.1 mg/kg, Ati 1 mg/kg, Ati 3 mg/kg, baseline.

Neurophysiology

Rats were anesthetized with chloral hydrate (350 mg/kg, IP) and placed in a stereotaxic frame with the incisor bar set at −3.3 mm. The active recording electrodes (stainless steel screws 0.5 mm in diameter) were located on the skull at the following coordinates: ML = 3.0 mm, AP = 2.0 mm. The reference and ground electrodes were located in the midline above the cerebellum. The electrodes and connected female pins were embedded in dental acrylic. A recovery period of 10 days was allowed before any recordings were made. The HVS activity (incidence × mean duration) was recorded between 1000–1400 h during a 20-min period of cumulative waking immobility (head held up, eyes open; waking immobility periods shorter than 10 s were excluded from the analysis).

Passive Avoidance

The rectangular plexiglas PA box was divided into lighted and dark (metal grid floor) compartments by a sliding guillotine door. Rats were placed in the lighted side and 60 s later the guillotine door opened. Five s after the entry into the dark side, a 1.0-mA shock was delivered to the rat's feet. In the control experiment, half of the aged and young Ati-treated rats received no shock treatment and were removed from the dark compartment by an experimenter. The shock remained on until the rat returned to the lighted side and an avoidance criterion of 1 min was attained. The latency to enter the dark compartment and number of reentries were measured during the training trial. Testing occurred 6 days later. The rat was put in the lighted side and the door opened 60 s later. The latency to enter was measured (max 600 s) during the retention trial.

TABLE 1

PASSIVE AVOIDANCE TRAINING TRIAL DATA

Group	Reentries	Entry Latency
First experiment		
Young c (n = 10)	1.3 ± .6	23 ± 12
Young Ati (n = 10)	1.3 ± .5	33 ± 15
Aged c (n = 10)	1.1 ± .3	25 ± 10
Aged Ati (n = 10)	1.4 ± .4	25 ± 8
Second experiment		
Replication		
Young c (n = 8)	1.5 ± .6	27 ± 8
Young Ati (n = 8)	1.2 ± .5	23 ± 13
Aged c (n = 8)	1.4 ± .3	30 ± 19
Aged Ati (n = 8)	1.3 ± .4	25 ± 12
Not shocked		
Young Ati (n = 8)	–	26 ± 18
Aged Ati (n = 8)	–	23 ± 13

Values are expressed as mean ± SD. No drug injections were made before acquistion trial. Ati, atipamezole 3 mg/kg; c, controls; n, number of rats per group.

–, not measured.

Water Maze

The circular WM swimming pool system has been described in detail previously (10). A computer calculates swim speed [in arbitrary computer units (pixels)], total swimming distance, and distance swum in all annuli (n = 3) and quadrants (n = 4) separately. Starting locations were labeled north, south, east, and west. Timing of the latency was started and ended by an experimenter. Testing consisted of 5 days of consecutive training (three 70-s trials in a day).

Statistics

One-way analysis of variance (ANOVA) followed by Duncan's posthoc multiple-group comparison was used to assess group differences in PA performance and HVS activity. Drug-induced changes in HVS activity were analyzed with Wilcoxon signed ranks test. ANOVA was used to analyze WM acquisition data.

RESULTS

Neurophysiology

Aged rats had higher baseline HVS activity than young rats, $F(1,9) = 32.7, p < 0.05$ (Fig. 1).

Ati at 0.1 mg/kg had no effect on HVS activity ($p > 0.05$) of young or aged rats. Ati at 1 and 3 mg/kg completely suppressed HVS activity of young rats and decreased HVS activity of aged ($p < 0.05$) rats.

Passive Avoidance

Experiment 1. Analysis of the PA acquisition trial data revealed no significant group differences [latency to enter: $F(3,37) = 0.2, p > 0.05$; reentries: $F(3,37) = 1.0, p > 0.05$] (Table 1).

A significant group effect was observed in the analysis of PA retention trial data, $F(3,37) = 6.9, p < 0.05$. Aged saline-treated rats were impaired ($p < 0.05$), but Ati-3 mg/kg-treated rats were not impaired ($p > 0.05$) compared with young rats (Fig. 2).

Experiment 2. In the PA control experiment, no significant group differences were found in the training trial data [latency to enter: $F(5,41) = 0.2$, $p > 0.05$; reentries: $F(3,27) = 0.4$, $p > 0.05$] (Table 1).

A significant group difference was found in the analysis of PA retention trial data, $F(5,41) = 5.9, p > 0.05$. Again, Ati-treated, shock-trained aged rats were not impaired ($p > 0.05$) but saline-treated, shock-trained aged rats were impaired ($p < 0.05$) (Table 2). Aged and young Ati-treated rats not shocked during the training trial entered the dark chamber as fast as during the training trial ($p > 0.05$ in both comparisons).

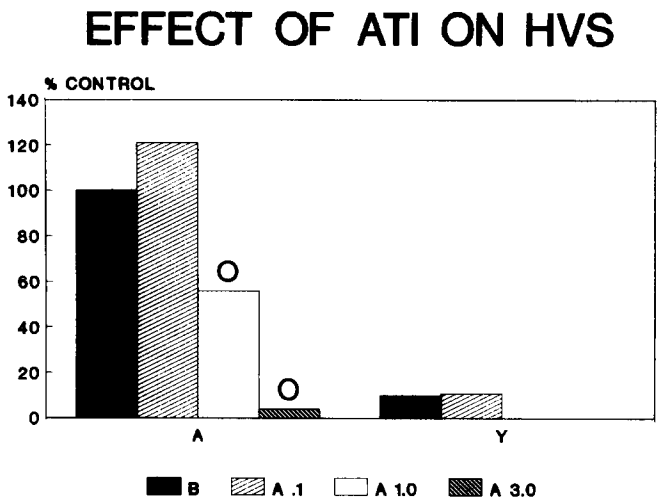

FIG. 1. Atipamezole (0.1, 1, and 3 mg/kg)-induced decrease in HVS activity (measured during a 20-min period of cumulative waking immobility: head held up, eyes open) of young and aged rats. Values are expressed as mean ± SD. Injections were made SC 20 min before recordings were started. A .1, A 1.0, and A 3.0 = atipamezole 0.1, 1.0, and 3.0 mg/kg; Y = young; A = aged. No HVS activity was recorded from young rats after injections of atipamezole at 1.0 and 3.0 mg/kg. ○, $p < 0.05$ vs. baseline HVS values.

Water Maze

A significant group effect was found in the analysis of the WM escape distance values, $F(3,195) = 13.3, p < 0.05$. Aged saline- and Ati-treated rats were impaired in WM acquisition [$F(1,97) > 8.0, p < 0.05$, in both comparisons] (Fig. 3). Ati-treated aged or young rats did not differ from aged or young controls, respectively [$F(1,97) < 0.5, p > 0.05$, for all data].

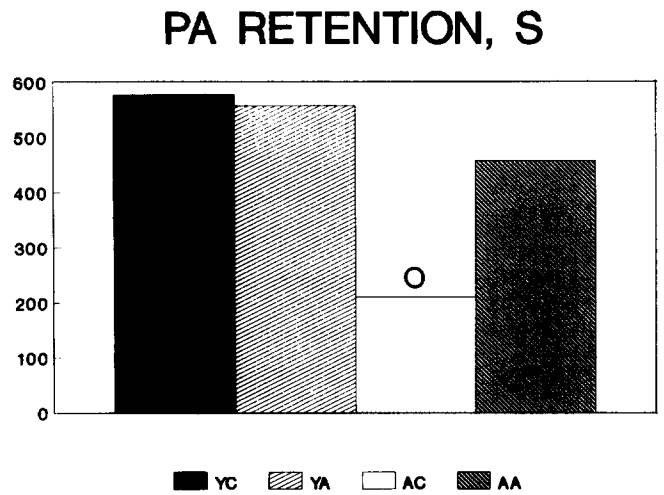

FIG. 2. Effects of atipamezole on passive avoidance retention performance (seconds) in young and aged rats. Values expressed are mean ± SD. Injections were made SC 20 min before testing. YC, young controls; YA, young atipamezole 3 mg/kg; AC, aged controls; AA, aged atipamezole 3 mg/kg. ○ $p < 0.05$ vs. young controls, Duncan's posthoc multiple-group comparison.

TABLE 2

EFFECTS OF ATIPAMEZOLE ON PASSIVE AVOIDANCE RETENTION PERFORMANCE (SECONDS) OF YOUNG AND AGED RATS

Group	Retention
Young	
Controls	523 ± 23
Atipamezole 3.0 mg/kg	545 ± 33
Not shocked, atipamezole 3.0 mg/kg	33 ± 12
Aged	
Controls	134 ± 97*
Atipamezole 3.0 mg/kg	356 ± 121
Not shocked, atipamezole 3.0 mg/kg	35 ± 15

*$p < 0.05$ vs. young control, Duncan's posthoc multiple-group comparison.

DISCUSSION

The present results demonstrate that in aged rats acute α_2 antagonist treatment may stabilize HVS increase dose dependently and that an Ati dose effective in suppressing HVS activity alleviates PA performance deficits. Interestingly, we could show that Ati had no effect on the PA retention test entry latency values of rats that received no shock treatment during the training trial. Therefore, it is reasonable to believe that the Ati-induced increase in the entry latency values of aged rats may not reflect nonspecific neurological changes affecting motor activity. Our results agree with previous studies suggesting that the noradrenergic system may be importantly involved in the mechanisms underlying cognitive functions and that the degeneration of LC may contribute to age-related functional deficits (4,8,11-14,17).

The present results demonstrating that Ati decreases HVS oscillations in aged rats is supported by previous studies investigating the role of the noradrenergic system in the regulation of thalamocortical activity (2,6,9). First, noradrenergic drugs may suppress oscillatory activity of neurotransmitters and thalamocortical relay neurons with in vitro preparations

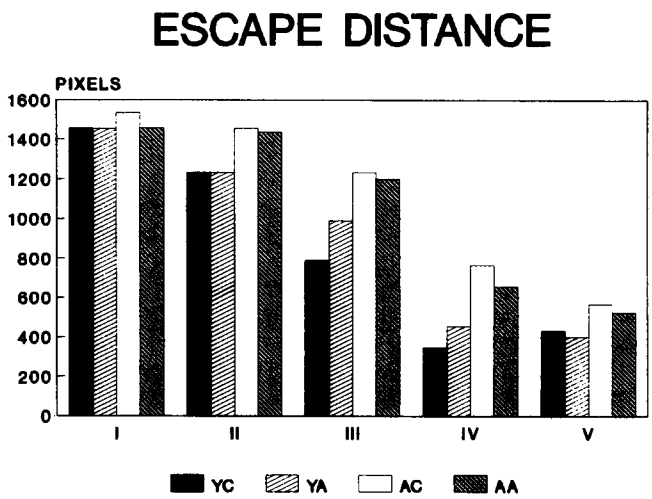

FIG. 3. Posttraining atipamezole (3 mg/kg) treatment and water maze spatial navigation performance. Note the lack of an effect of atipamezole on spatial navigation. Abbreviations: see Fig. 1. I, II, III, IV, and V indicate training days 1, 2, 3, 4, and 5, respectively.

(6). Second, in studies conducted using freely moving rats α_2 antagonist and α_1 agonist may suppress HVS oscillations by acting on thalamic adrenoreceptors (2). Therefore, the present HVS results may further suggest that noradrenergic deficit is involved in the age-related HVS release and that α_2 antagonist may be effective in restoring noradrenergic control of thalamocortical HVS oscillations.

Our PA results may be interpreted as supporting previous studies demonstrating that noradrenergic pathology is involved in PA deficit of aged rats and that increased activity of the LC system improves PA performance of aged rats (17). First, in young rats an α_2 agonist that decreased noradrenaline metabolism impaired PA retention of young rats (12). Second, activation of the LC system improved PA performance: Electrical stimulation of LC, intraventricular administration of noradrenaline, or peripheral injections of α_2 antagonist improved PA retention performance of aged rats (17). Furthermore, the age-related decrease in LC neuron number correlated with PA retention performance (4).

Our failure to observe a recovery of age-induced WM spatial learning defect by Ati could be explained several ways. First, only a single dose was used in the present study. Therefore, it could be proposed that the 3-mg/kg dose may have been too small to increase noradrenaline activity and improve WM consolidation. However, previously it has been shown that Ati produced a marked increase in noradrenaline metabolism at the dose used in the present study (5). Second, it could be proposed that the noradrenergic system may be more importantly involved in the acquisition than consolidation of the WM task. Importantly, Sirviö et al. (13) previously showed that pretraining injections of Ati at doses that increase noradrenaline metabolism have no improving effect on the WM defect of aged rats. Indeed, it is interesting to note that either lesioning or pharmacologically induced blockade of the activity of the LC neurons had no effect on WM performance (11–13,16). Therefore, it is reasonable to suggest that the noradrenergic system may not have an important role in age-related degeneration of WM spatial navigation performance.

The present results may also have some relevance to clinical disorders like Alzheimer's disease that are associated with a loss of noradrenergic activity and impaired cognitive and EEG activity (1,7,15). Therefore, effects of α_2 antagonist-induced restoration of noradrenergic deficit on the aging- and Alzheimer's disease-induced cognitive and EEG deficits should be further investigated.

In conclusion, the present results demonstrate that atipamezole dose dependently suppressed age-related HVS oscillations and that a high dose of Ati effective in decreasing HVS activity improved PA performance of aged rats.

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