

ALPHA₂-ADRENOCEPTOR ANTAGONISTS: NEW WAYS TO INFLUENCE ATTENTION AND MEMORY

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SUMMARY

In Alzheimer's disease the loss of cholinergic neurons is often thought to be essential but cholinesterase inhibitors have been helpful only for a part of patients. Experimental and clinical evidence supports the importance of noradrenergic neurons in arousal but also in attention and memory. Some of these neurones seem to end in contact with cholinergic neurons. In animal studies, both α_2 -adrenoceptor agonists and antagonists have been reported to improve some cognitive functions. The reported clinical tests with clonidine have been negative like our experiments with a less sedative α_2 -agonist, guanfacine, in rats.

An injection of atipamezole (ATI), α_2 -adrenoceptor antagonist (Farnos Group) decreased total motor activity, rearing and grooming in rats in

the open-field test. In the Morris water maze, after high doses (≥ 3 mg/kg) of ATI the rats did not swim, after low doses they made less errors. In the appetitive spatial memory test, after low doses of ATI, the rats showed slightly less forgetting than the controls. In the passive avoidance test, acute treatment with ATI improved the performance of rats. ATI enhanced the EEG of rats decreasing slow wave spikes like cholinergic drugs do. Autoradiographic localization of ATI binding sites in rat brain slices indicated that those sites are distributed throughout the whole brain and have a similar location to other imidazole containing α_2 -adrenoceptor antagonists. Several neurotransmitters may be involved in the actions of ATI since it seemed to release noradrenaline and serotonin in brain.

In preliminary clinical trial, ATI (0.05 and 0.1 mg/kg, i.v.) enhanced the EEG of healthy volunteers and patients with age-associated memory impairment (AAMI). In neurophysiological tests, ATI slightly increased the mean score of digit span, while in word recognition test and in Moss spatial recognition task the drug tended to impair the performance. The drugs like ATI seem to increase the attentional tone, which might be low in AAMI patients. Thus these drugs could improve performance in tasks which require concentration, but may impair complex divided attention, probably due to noradrenergic overdrive.

INTRODUCTION

In Alzheimer's disease usually a loss of both cholinergic and other neurons are found. Cholinergic drugs, like the cholinesterase inhibitor THA (tetrahydroaminoacridine), give some positive response only in a part of Alzheimer's disease patients, which seem to be possible to forecast with a pharmaco-EEG test² Drugs which could activate other defective neuronal systems, might be more useful to the other patients of Alzheimer's disease and age-associated memory impairment.

Studies on the biological basis of memory have largely focused on the cholinergic system, but in the recent years more and more findings about the role of the noradrenergic system in learning and memory processes have been reported. The coeruleo-cortical noradrenergic projection has been proposed to be involved in attention, learning and memory by many investigators who have studied the postsynaptic

affects of noradrenaline in target brain structures. Relatively few studies have been done with pharmacological manipulation of noradrenaline system before the retention test, to indicate the direct role of noradrenaline in memory retrieval processes. Blockade of beta receptors by propranolol before the retention test has been shown to produce amnesia for a passive avoidance task. α_2 -Adrenergic agonists, clonidine and guanfacine, have been reported to improve cognitive functions in aged monkeys on the delayed response task³. In the spatial navigation task, guanfacine, however, impaired the performance of rats⁹ and worsened the age-related changes in EEG⁷. α_2 -antagonists, yohimbine and idazoxan, have been reported to facilitate retrieval in rats when injected before the retention test in the maze forgetting paradigm⁸. We have used a more specific α_2 -antagonist, atipamezole^{10, 12} in biochemical, neurophysiological and behavioural tests in rats and man.

METHODS

Animals and drugs

Male Wistar young rats (3-4 months) were tested in the open field, elevated plus-maze tests, in the passive avoidance and in an appetitive spatial discrimination maze (multichoice maze) tasks. In some tests, septal lesioned or old rats were used. The group of rats with septal lesions were lesioned by current (DC 2mA, 7sec), their controls were sham-operated without current.

Atipamezole, a potent, specific α_2 -adrenoceptor antagonist¹², (Farnos Group, Finland), was dissolved in saline, and tested in doses 0.6, 1.0 and 3.0 mg/kg, s.c. (30-45 min, before the tests). Animals in the control group were injected with saline. In the passive avoidance task, atipamezole was injected 30-45 min. before the test trial.

Behavioural tests in rats

In the open-field (diameter 80 cm) test, the behaviour of rats was observed with a video camera and analyzed semi-manually on a micro-computer. Plus-maze test (elevated bridge consisted of two open arms

and two arms with walls) was used to evaluate anxiety of rats.

In Morris water maze, the rats had to learn and remember the place of a hidden platform. The swimming time and the path to find the platform were registered during the training and test trials. The platform was removed and the time spent in the expected quadrant was registered.

In the appetitive spatial memory task, the rats had to remember the right and left turns before reaching the goal with food¹. Again both errors and the time spent were registered. After the training in which the rats learned to find the correct path in five daily trials, they were left undisturbed for four weeks in the home cages. Reliable forgetting of a relatively complex maze is seen in rats when the training-to-test interval is four weeks⁸. After this time pharmacologic treatment with atipamezole was administered 45-50 min before the retention test in doses (0.6 and 3.0 mg/kg) found to be effective in blocking both peripheral and central α_2 -adrenoceptors in rats on the basis of measurements of noradrenaline metabolites. Animals in the control group were injected with saline.

In the passive avoidance task, the rats had to learn to avoid the dark part of the chamber, where they received a light electric shock. ATI (0.01 to 3 mg/kg) was given after the training trial. A long retention time was considered to be due to remembering the previous shock.

Neurophysiology

In the rats, quantitative EEG (QEEG) was registered in several locations of electrodes on the head after doses of 0.3-10 mg/kg s.c.

Preliminary clinical trial

In the clinical trial, four young healthy male volunteers and four AAMI (age-associated memory impairment) patients were included in the study. Atipamezole (0.05 and 0.1 mg/kg) was slowly infused i.v. in the young ones and 0.1 mg/kg in AAMI patients. The baseline QEEG was monitored and the first neuropsychological tests were done 50-20 min before the infusions of ATI. The second QEEG was started 20 min later.

The neuropsychological tests - (1) Digit span - series of numbers forward and backward; (2) Word recognition task - new words among the earlier mentioned; and (3) Moss spatial recognition task - the localisation of a large number of things, were performed again after one hour and the third QEEG was performed three hours after the infusion.

RESULTS

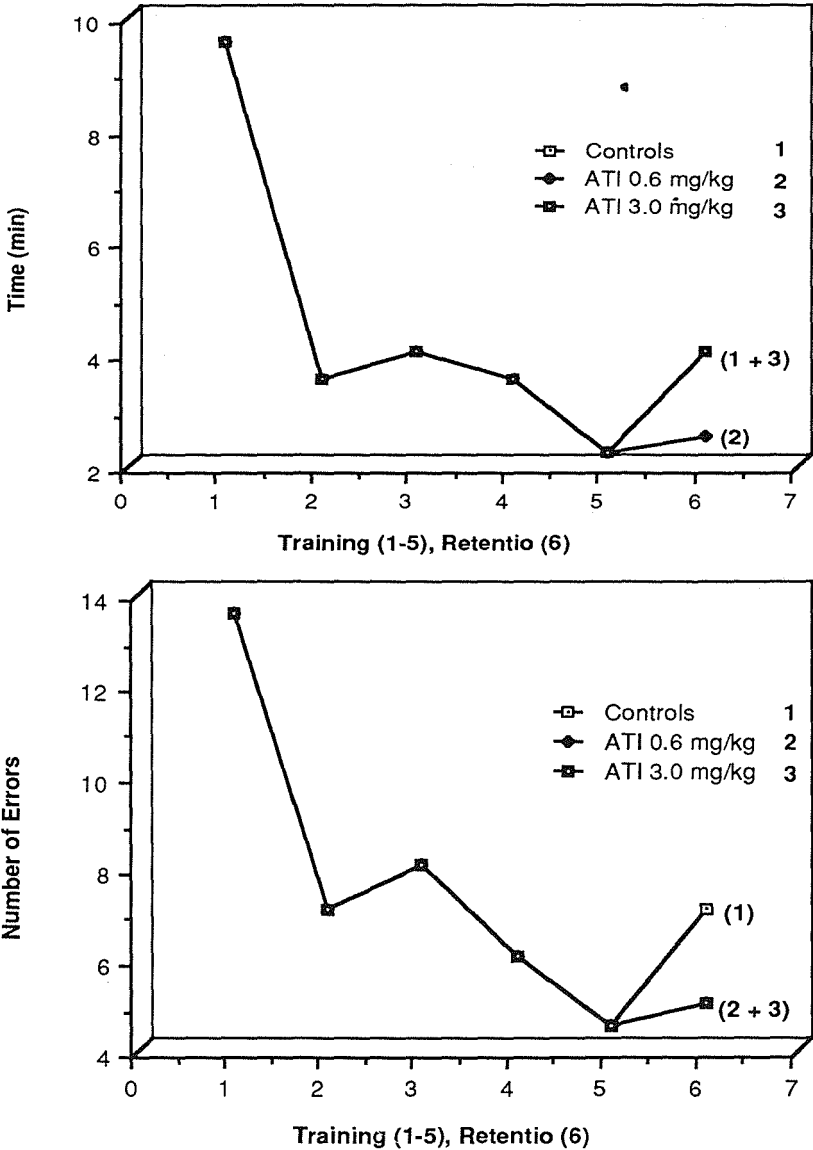
Behavioural tests in rats

In the open field and in the plus-maze tests, the animals in the atipamezole groups were less active than those of the control group. In the open field, the distance travelled by atipamezole administered rats was shorter than that of the controls. At the time those rats tended to decrease dose-dependently rearing and grooming behaviour. In the plus-maze test, atipamezole did not have any effect after acute injections, while the chronic treatment of rats tended to increase the proportion of time spent in the open arms and the proportion of entries made onto them.

The rats in each group showed forgetting, but it was less marked in animals receiving atipamezole 0.6 mg/kg. Forgetting was indicated by an increase in the number of errors and the longer time to run in the maze (Figure 1).

Already smaller doses increased the retention time in the passive avoidance task. In the lesioned rats only negligible difference was found between the treated and control animals.

Fig. 1. The effects of atipamezole on the time and number of errors in young Wistar rats ($n = 18$) in the serial T-maze. Atipamezole was injected 30 min before the retrieval test, which was performed four weeks after the training trials



Neurophysiological test in rats

High-voltage spindels, which are increased in aged rats, dose-dependently decreased with ATI, and in QEEG the higher frequency alpha and theta power increased, most significantly in prefrontal and centroparietal region.

Preliminary clinical trial

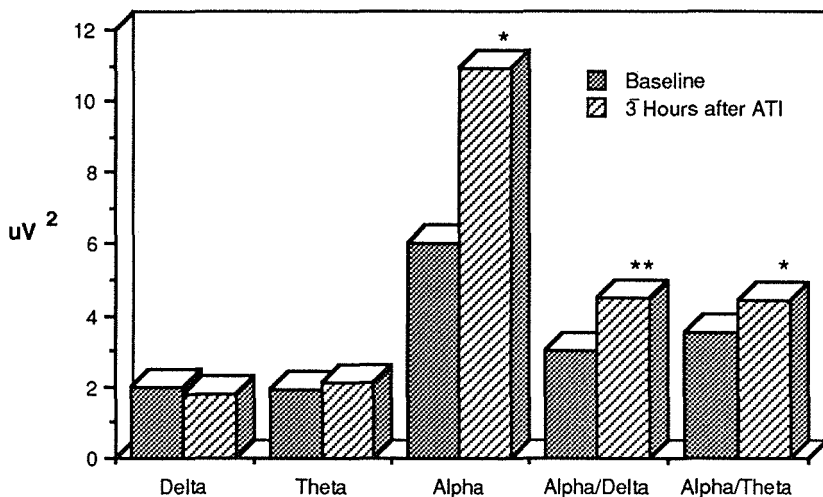
Atipamezole was very well tolerated. The dose of 0.1 mg/kg ATI produced some improvement in Digit span (DS) both in young persons and AAMI. With the dose of 0.05 mg/kg no change was observed in DS. Word recognition task was impaired, with 0.05 mg/kg the impairment was minor. The score of Moss tended to be impaired with 0.1 mg/kg of ATI, but the smaller dose tended to induce a slight improvement. The results of the tests with 0.1 mg/kg ATI are presented in Table 1, young persons and AAMI patients together since the effects were similar.

Table 1
The effects of atipamezole (0.1 mg/kg) on the neuropsychological tests one hour after the infusion

	Baseline	ATI 0.1 mg/kg
Digit Span (forward + backward)	10.9±0.6	12.1±1.0
Word Recognition Task Target Words	37.7±1.6	34.6±1.6
Word Recognition Task False Words	8.0±2.5	9.9±2.1
Moss Spatial Span	8.7±0.6	7.4±1.0

Analysis of QEEG revealed most salient improvement in centroparietal region (derivation C3-P3, Figure 2). The mean absolute alpha power and the mean total power increased significantly ($P < 0.02$; Student's *t*-test). Also the alpha-delta ratio ($P < 0.01$) and the alpha-theta ratio ($P < 0.05$) were found to increase. The changes in the prefrontal QEEG activity had the same trend as in the centroparietal region.

Fig. 2. The effects of atipamezole (0.1 mg/kg) on quantitative EEG in centroparietal region (derivation C3-P3) three hours after the infusion. Significant changes* $P < 0.05$, ** $P < 0.01$ (Student's t-test) compared to baseline



DISCUSSION AND CONCLUSIONS

Inactivating effect of high doses of atipamezole observed in the water maze and open-field tests might reflect an increased stage of fear in rats after acute injections, since after chronic administration the rats did not differ from their controls. Noradrenergic lesions have not induced changes in motor activity¹¹. The results of the elevated plus-maze test support this suggestion. Ethanol-induced increase in activity in the plus-maze test was potentiated by 1 mg/kg atipamezole, but no anxiogenic effect of the drug was measured (1 and 3 mg/kg)⁴. Alleviation of forgetting by treatment with atipamezole, a specific α_2 -antagonist, indicates that noradrenergic compounds can be used in modulating memory retrieval. They may only not have blocking effect directly on to cortical α_2 -receptors, but they also increase firing of locus coeruleus cells. The mechanisms of modulation of the complex behavioural processes like memory by noradrenergic agents are still largely unknown, but it may activate the cholinergic neurons as suggested by similar changes in EEG as after a cholinesterase inhibitor^{6,7}. The effects

may also be partly mediated via serotonergic, dopaminergic or other systems. In rat brain, atipamezole induced dose-dependent, rapid and relatively long-lasting increases in the central turnover of NA, reflected by increased levels of major NA metabolites¹⁰. The concentration of 5-HIAA, and in lesser degree of HVA, the metabolites of serotonin and dopamine, also increased.

Atipamezole induced significant changes in QEEG and also some trends in the neuropsychological tests. ATI is considered to increase noradrenergic activity in central nervous system. The most dense noradrenergic innervation has been shown in the somatosensory cortex of primates⁵. Also the prefrontal cortex has a pronounced quantity of noradrenergic nerve terminals. The changes in QEEG were noticed to occur especially in those regions.

The relationship between the optimal range of attention and reticular formation activity may have a shape of an inverted-U. Thus, both too high and too low activity of reticular midline nuclei may impair attention and optimal performance. One may assume that ATI produced slight noradrenergic overdrive in subjects being tested. Impairment in the tests requiring more complex divided attention, as WR and Möss may be a consequence of the overactivity. On the other hand, performance in tasks requiring very narrowly directed attention (DS) may need higher attentional tone. A compound like ATI may increase the level of attentional tone to an optimal level in patients with AAMI or Alzheimer's disease and perhaps in children with attention deficiency disorders (MBD), thereby improving the cognitive performance in the every day life of these patients.

Our preliminary clinical tests measured mainly attention after an acute ATI administration. Our animal tests also showed some promising effects on long term memory, but the effects of α_2 -antagonists on it in AAMI and Alzheimer's disease patients remain to be studied.

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