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The effects of a specific α_2-adrenoceptor antagonist, atipamezole, on cognitive performance and brain neurochemistry in aged Fisher 344 rats

Antti Haapalinna [a,*], Jouni Sirviö [a], Ewen MacDonald [b], Raimo Virtanen [a], Esa Heinonen [a]

[a] *Orion Pharma, Preclinical R&D, Orion, PO Box 425, FIN-20101 Turku, Finland*
[b] *Department of Pharmacology and Toxicology, University of Kuopio, Kuopio, Finland*

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Abstract

The present experiments investigated the effects of a specific and potent α_2-adrenoceptor antagonist, atipamezole, on cognitive performance and neurochemistry in aged rats. Aged control Fisher 344 rats, which had lower activities of choline acetyltransferase in the frontal cortex, were impaired in the acquisition of the linear arm maze task both in terms of repetition errors and their behavioural activity (the speed of arm visits), and they needed longer time to complete this task as compared to adult control rats. Atipamezole treatment (0.3 mg/kg) facilitated the acquisition of this task in the aged rats as they committed fewer errors and completed the task more quickly than saline-treated aged control rats. A separate experiment indicated that atipamezole enhanced the turnover of noradrenaline both in the adult and aged rats, but this effect was more pronounced in the aged rats. Furthermore, atipamezole enhanced significantly the turnover of serotonin and dopamine only in the aged rats when analysed in the whole brain samples. As α_2-adrenoceptor antagonists are known to alleviate akinesia in the experimental models of Parkinson's disease, the present results could be especially relevant for the development of palliative treatment for demented Parkinsonian patients.

Keywords: α_2-Adrenoceptor; Atipamezole; Ageing; Memory; Cholinergic system; Noradrenergic system

1. Introduction

Comprehensive data from electrophysiological, behavioural and anatomical investigations suggest an important role for the central noradrenergic system in the modulation of attention as well as learning and memory processes (Harley, 1987; Aston-Jones et al., 1991; Berridge et al., 1993; Sara et al., 1994). The activity of the noradrenergic neurones is regulated by α_2-adrenergic autoreceptors, and the blockade of these receptors increases the firing rate of the neurones and the release of noradrenaline in the target areas (Cedarbaum and Aghajanian, 1977; Simson and Weiss, 1987).

It has been postulated that an appropriate level of stimulation of the central noradrenergic system could improve cognitive functions. Accordingly, it has been reported that idazoxan, an α_2-adrenoceptor antagonist, improves selective attention, learning and memory retention in adult rats (Sara and Devauges, 1989; Devauges and Sara, 1990; Bunsey and Strupp, 1995). Atipamezole is a potent α_2-adrenoceptor antagonist which has a high α_2-/α_1-adrenoceptor selectivity ratio and does not display differential affinity for α_2-adrenoceptor subtypes (Virtanen et al., 1989; Haapalinna et al., 1997). Unlike various other α_2-adrenoceptor antagonists, it has negligible affinity for other receptors such as 5-HT_{1A} receptors (Winter and Rabin, 1992; Meana et al., 1996; Newman-Tancredi et al., 1998) and for non-adrenoceptor [H^3]idazoxan binding sites (imidazoline I_2 sites) (Savontaus et al., 1997). Thus it has been considered as an ideal tool to assess the involvement of α_2-adrenoceptors in the modulation of higher cerebral functions. Previous studies with adult animals have shown that atipamezole facilitates the excitability of granular cells in rat hippocampus in vivo and improves retention in a radial arm maze task (Ylinen et al., 1996), and it also improves the performance of rats in an attentional five-choice serial reaction time task under certain conditions (Sirviö et al., 1993). Furthermore, atipamezole enhanced the acquisition of a linear-arm maze test, improved choice accuracy in a three choice-maze test and facilitated memory consolidation when injected immediately after teaching in a one-trial appetite-maze test (Haapalinna et al., 1998).

* Corresponding author. Tel.: +358-2-2727670; fax: +358-2-2727766.
E-mail address: antti.haapalinna@orion.fi (A. Haapalinna)

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Even though the effects of atipamezole and idazoxan on cognitive functions in adult animals are in accordance with the theory on the noradrenergic modulation of learning and memory, it should be noted that α_2-adrenoceptors are not restricted to the noradrenergic nerves. Those receptors participate in the regulation of the release of many neurotransmitters (Gulat-Marney et al., 1989; Raiteri et al., 1990; Trendelenburg et al., 1994). Furthermore, it has been suggested that the population of α_2-adrenoceptors found on noradrenergic nerves is only a small fraction of the total amount of α_2-adrenoceptors in the brain (Dausse et al., 1982; Dooley et al., 1983; Ordway, 1995). Thus, the effects of α_2-adrenoceptor antagonists seen in vivo may not be due exclusively to an increase in the release of noradrenaline. Accordingly, it has been reported that α_2-adrenoceptor antagonists enhance the release of dopamine (Matsumoto et al., 1998) and acetylcholine (Tellez et al., 1997) in the prefrontal cortex of conscious freely moving rats.

There is some evidence that certain age or disease associated cognitive disturbances can be accompanied with the degeneration of central noradrenergic neurones (Zornetzer, 1985; McEntee and Crook, 1990). However, it is clear that other transmitter systems as well are deteriorated during ageing. Especially, the dysfunction of the central cholinergic system may play an important role in cognitive deterioration associated with ageing and Alzheimer's disease (Decker and McGaugh, 1991). Therefore, the enhanced release of various neurotransmitters by α_2-adrenoceptor antagonists predicts therapeutic potential for this type of drugs in the treatment of cognitive problems in neurological diseases associated with multiple neural deficits.

However, it has been reported that the α_2-adrenergic system per se is compromised in aged brain as the number and function of brain α_2-adrenoceptors may be altered during ageing (Kalaria and Andorn, 1991; Zsilla et al., 1997). It should be noted that many previous reports on the positive effects of α_2-antagonists on higher cerebral functions provide information about their effects in normally functioning brain only (Sara and Devauges, 1989; Devauges and Sara, 1990; Haapalinna et al., 1998). Interestingly, it has also been reported that although idazoxan improved performance of adult rats in a radial arm maze, its effect was less pronounced in aged rats, even impairing the performance of the rats (Dickinson et al., 1989). Furthermore, administration on an α_2-adrenoceptor *agonist* has been found to improve working memory in aged monkeys but not in adult monkeys, and yohimbine, an nonspecific α_2-adrenoceptor antagonist, had only a marginal effect in this model (Arnsten and Cai, 1993).

The aim of present experiments was to clarify whether atipamezole can improve learning and memory process also in aged subjects. Therefore, we studied the effects of atipamezole on the performance of aged rats in the linear arm maze task, since this agent improved the performance of adult rats in this task. To verify the existence of age associated dysfunction in the central cholinergic system in this model, choline acetyltransferase (ChAt) activities were measured in the post-mortem brain samples taken from the frontal cortex and hippocampus of the rats after the maze test had been completed. In order to assess possible age related alterations in the effects of this α_2-adrenoceptor antagonist on brain neurochemistry, we studied the effects of atipamezole on the levels of brain monoamines (noradrenaline, dopamine, 5-hydroxytryptamine (5-HT)) and their metabolites ex vivo.

2. Materials and methods

2.1. Animals

Male rats of the Fisher 344 (F-344) strain (Harlan, Netherlands) were used in all of the tests. In the linear-arm maze test, 20 (approximately 22 months old rats, weighing 373–524 g at the beginning of the study) and 10 (approximately 5 months old rats, weighing 296–360 g at the beginning of the study) were used. In the neurochemistry test, eight approximately 24 months old rats, weighing 405–501 g, and 10 approximately 3 months old rats, weighing 209–276 g, were used. The animals were housed in the groups of 4–5 in the same cage, under standard conditions (21 $\pm$ 1°C, light–dark cycle with lights between 6:00 and 18:00). Softwood granulated aspen was used as bedding. The animals had a free access to water and food until behavioural testing began. All experimentation was approved by the local laboratory animal care committee.

2.2. Test substances

Atipamezole hydrochloride (Orion, Orion Pharma, Finland), is a specific α_2-adrenoceptor antagonist (Virtanen et al., 1989; Haapalinna et al., 1997), which penetrates rapidly into the brain after its subcutaneous (s.c.) injection (Biegon et al., 1992). A dose of 300 μg/kg s.c. was used in the present experiments, since the dose is known to block central α_2-adrenoceptors, to increase central noradrenaline release and it was found to be effective in previous maze performance study (Haapalinna et al., 1997, 1998). Atipamezole was dissolved in distilled water, which was also used as control treatment. The route of administration was s.c. and the injection volume was 1 ml/kg in all the experiments. Commercial 45 mg pellets (Bio Serve, USA) were used as reward food in the maze tasks.

2.3. Behavioural test

2.3.1. The linear-arm maze

The linear-arm maze has been described (including its illustration) earlier by Liljequist et al. (1997). The maze is

a wooden platform in the shape of two crosses joined by a bridge another. The stem (starting arm) was 90 cm long and 20 cm wide. The five other arms (goal arms) were 50 cm long and 12 cm wide. Four goal arms were situated perpendicularly to the stem and to the fifth arm, which was located opposite to the stem. Edges, 2.0 cm high, surrounded all sides of the stem and the arms. At the end of each goal arm, was a hole 1 cm deep and 3 cm in diameter, which served as a food container. The starting platform (20×20 cm) was separated from the stem by a guillotine door, (12 cm high and 7 cm wide). The frame was 20 cm high and 20 cm wide. The holes at the end of the goal arms were baited with three pellets of reward food. The maze was elevated 31 cm above the floor in a test room that contained other objects, as well as the test apparatus (e.g., table, shelves and door) providing extra maze cues for spatial navigation.

2.3.2. Experimental protocol

In the linear-arm maze test, the habituation of the rats to handling (twice a day), administration of water, test room and reward food was started seven days before training. Two days before training, the animals were placed on a food deprivation schedule that reduced their body weights to 90–95% of their initial weights. One day before training, they were also habituated to the unbaited maze, five animals from the same cage at the same time for 10 min. On the next day, the goal arms were baited, and the teaching trial, with one rat at a time, was carried out. The rat received atipamezole (0.3 mg/kg) or distilled water and 60 min later it was placed on the starting platform. After 10 s, the door was opened and the rat was allowed to explore the maze until all the baits were found. The time to find all baits (time), re-entries made into already visited arms (errors) and correct choices made before the first error (corrects) were recorded. To evaluate behavioural activation, the total arm entries made per time (speed) were later calculated. At this first time (teaching), every rat was allowed to stay in the maze for at least 5 min. On the next day, the proper memory and learning testing began and these continued for 5 days. The rats were given a total of 10 trials, two trials per day. The inter-trial interval was 50 min. Atipamezole or distilled water was administered 30 min before the first trial of the day. Otherwise, testing trials were identical to the teaching trial. There were 10 rats in all three groups (adult/control, aged/control and aged/atipamezole). The test solutions were kept in coded bottles, so the investigator was blind to the treatment. The sequence of the adult and aged (and treatments) was randomised in the teaching trial and maintained thereafter constant for each individual rat during the experiment.

2.4. Rat brain neurochemistry

2.4.1. ChAt activity

One day after the last trial in the linear arm maze test, the rats were injected once more with their appropriate treatment, and the rats were sacrificed by decapitation three hours later. Then, the brains were removed, frozen and stored at -70°C. One month later the brains were thawed. The frontal cortex and hippocampus were dissected on ice, and the tissue samples were homogenised in distilled water. A sample of homogenate was suspended in 5 mM EDTA–NaOH solution, pH 7.4, containing 0.5% Triton-X 100 buffer. The ChAt activity was measured in duplicate according to the method of Fonnum (1975). Total protein was measured using the method of Lowry et al. (1951). Bovine serum albumin was used as the standard.

2.4.2. Effect on monoamines

Five adult rats and four aged rats were injected with distilled water and five adult and four aged were injected with atipamezole (0.3 mg/kg s.c.). The rats were sacrificed by decapitation three hours after the injection. The brains (cerebrum and cerebellum) were removed, frozen and stored at -70°C for one week before analysis. Biogenic amines and their metabolites were measured from the homogenate of brain tissue. Noradrenaline, 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), dopamine and homovanillic acid (HVA) were determined by electrochemical detection after separation by HPLC on a reverse phase C_{18} column. The metabolite of noradrenaline, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG–SO_4), was determined fluorometrically as described earlier (MacDonald et al., 1988; Haapalinna et al., 1997). Turnover rate was calculated as the main metabolite/corresponding transmitter ratio.

2.5. Statistical analysis

All the results are expressed as means ± standard error of mean (S.E.M.). In the linear-arm maze test, the normality assumption was not reached, and therefore the rank transformation of the data was applied (Conover and Iman, 1981). In the case of ties among the observations, midranks were applied. After rank transformation, the two-factor analysis of variance for repeated measurements (RM ANOVA) was used to analyse the ranked data. The comparisons were made between adult and aged control groups (= the effect of ageing) and between aged control and aged atipamezole treated animals (= the effect of treatment). As in the previous study with adult animals (Haapalinna et al., 1998), there were two separate analyses: (1) difference between teaching and the first trial (group (age or treatment) as factor and teaching time and trial 1 as repetition), (2) the effect of ageing or treatment in repeated trials (trials 1–10; group as factor and trial as repetition). Both initial overall analyses were followed by appropriate post-hoc analysis. The analyses were performed with SAS statistical software (SAS Institute, USA). The neurochemistry data were analysed by one way analysis of variance (ANOVA) followed by a Fisher's PLSD

(protected least significant difference) test (StatView 4.12 software). The criterion for a statistical significance was $P < 0.05$.

3. Results

3.1. Effect of atipamezole on linear arm-maze performance in aged rats

The performance of rats in the linear-arm maze is summarised in Fig. 1(A–D). In the analysis of the number of errors in the teaching trial and trial 1, there was a significant overall group effect (Fig. 1A), but there was no clear repetition effect or any interaction between group and repetition [group: $F(2,27) = 4.5$, $P = 0.02$; trial: $F(1,27) = 0.7$, $P = 0.41$; interaction: $F(2,27) = 1.6$, $P > 0.2$]. There was a significant overall difference between aged and adult controls ($P = 0.006$). The progression from a teaching trial to trial 1 was not statistically significant in the aged control group ($P > 0.8$) or in the adult control group ($P > 0.5$), but it approached significance in the atipamezole-treated aged group ($P = 0.07$). The RM ANOVA analysis revealed that there was a significant difference between the groups in the number of errors during the repeated trials (1–10), and the number of errors decreased clearly trial by trial in all groups [group: $F(2,27) = 27.25$, $P = 0.0001$; trial: $F(9.243) = 8.29$, $P = 0.0001$; interaction: $F(18.243) = 1.05$, $P > 0.4$]. The group difference was significant between aged and adult controls ($P = 0.0001$), but also between aged control and atipamezole treated groups ($P = 0.0014$).

There was also a clear increase in the number of correct choices from a teaching trial to trial 1 in all the groups with no apparent difference between the groups (Fig. 1B) [group: $F(2,27) = 2.4$, $P > 0.1$; trial: $F(1,27) = 45.26$, $P = 0.0001$; interaction: $F(2,27) = 1.03$, $P > 0.3$]. In the repeated trials, there was a significant difference between

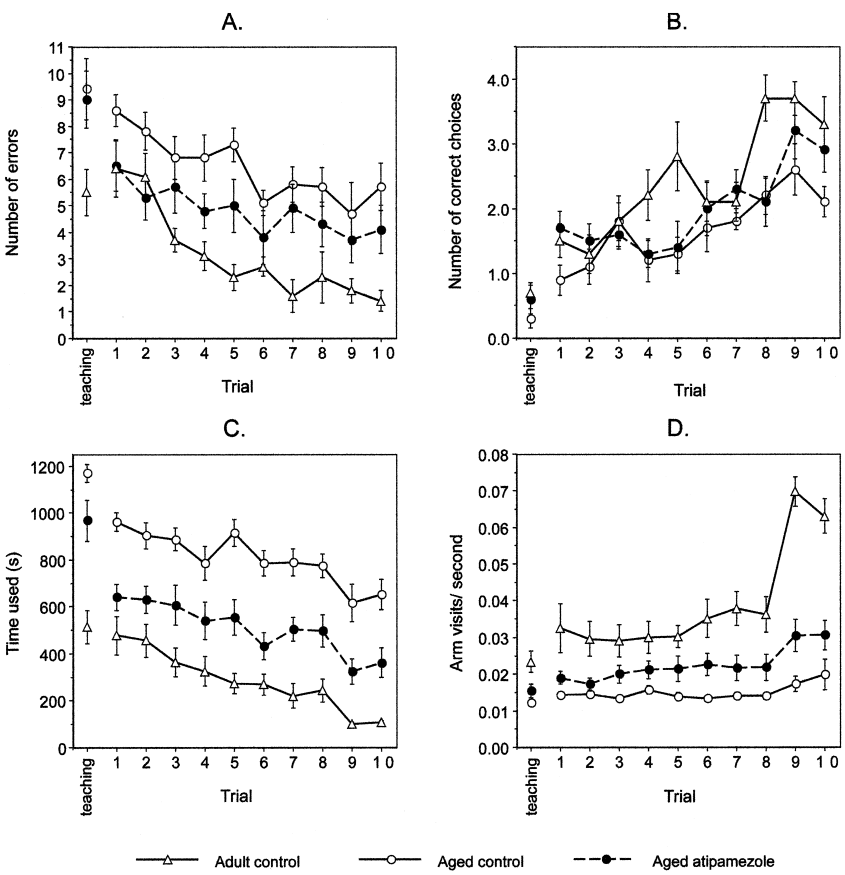

Fig. 1. The performance of adult (△), aged control (○) and atipamezole (0.3 mg/kg s.c.) treated aged (●) rats in the linear-arm maze. The results are expressed as mean ± S.E.M. (A) The number of errors/trial (Errors). (B) The number of correct choices/trial (Corrects). (C) The mean time used to complete the task/trial in seconds (Time). (D) The total number of arms visited/s in trial (Speed). In the teaching trial and trial 1, the RM ANOVA revealed a significant difference between the adult and the aged control groups in their performance measured by Errors, Time and Speed, and between the aged control and atipamezole-treated rats in Time and Speed. The RM ANOVA revealed a significant interaction between treatment and repetition in Time ($P < 0.002$) from the teaching time to the trial 1 and further analyses revealed significantly a more extensive change in aged atipamezole-treated rats than in aged controls ($P < 0.001$). In the repeated trials (1–10), RM ANOVA revealed a significant group and trial effects without any interaction between those effects in the performance (all the variables). There was a highly significant difference between adult and aged controls in all the variables as well as between aged control and aged atipamezole treated in Errors ($P < 0.002$), Time ($P < 0.0001$) and Speed ($P < 0.002$). All groups consisted of 10 animals.

Table 1
The cholineacetyltransferase (ChAt) activity in adult and aged control and atipamezole treated rat brain tested in the linear arm maze test
ANOVA was followed by the Fisher's PLSD test.
Values are mean ± S.E.M., $n = 10$ per group.

Age/treatment	ChAt activity (nmol/protein/min) in	
	Frontal cortex	Hippocampus
Adult/control	1.01 ± 0.11	0.81 ± 0.08
Aged/control	0.79 ± 0.04[a]	0.99 ± 0.06
Aged/atipamezole	0.80 ± 0.06	0.95 ± 0.09
ANOVA		
$F =$	2.92	1.52
$P =$	0.07	0.24

[a] $P < 0.05$, when compared to the adult control group.

the groups in the number of correct choices, and those were also increased trial by trial in all the groups with no interaction between group and trial effects [group: $F(2,27) = 6.14$, $P = 0.006$; trial: $F(9,243) = 10.85$, $P = 0.0001$; interaction: $F(18,243) = 1.13$, $P > 0.3$]. The difference was significant between the adult and aged controls ($P = 0.002$), but not between the aged control and atipamezole groups ($P = 0.15$).

The time needed to complete the task (Fig. 1C) diminished from the teaching trial to trial 1 slightly in all the groups, and there was a significant interaction between the group and repetition effects [group: $F(2,27) = 27.19$, $P = 0.0001$; trial: $F(2.27) = 31.98$, $P = 0.0001$; interaction: $F(2,27) = 7.82$, $P = 0.002$]. The progression from a teaching trial to trial 1 was significant in the aged control group ($P = 0.001$) and in the atipamezole group ($P = 0.0001$), but not in the adult control group ($P = 0.78$). There was a significant overall difference between adult and aged control groups ($P = 0.0001$) as well as between aged control and aged atipamezole treated groups ($P = 0.001$). During the repeated trials (1–10), the aged control animals needed more time per trial to complete the task than the atipamezole treated aged animals ($P = 0.0001$) or the adult control animals ($P = 0.0001$). The time also decreased from trial to trial in all the groups, but there was not a statistical interaction between the group and trial effects [group: $F(2,27) = 38.65$, $P = 0.0001$; trial: $F(9,243) = 22.72$, $P = 0.0001$; interaction: $F(18,243) = 0.76$, $P > 0.7$].

The speed of arm visits (Fig. 1D), i.e., the behavioural activity of rats, increased slightly from the teaching trial to trial 1 in all the groups showing no interaction between the group and repetition effects [group: $F(2,27) = 15.81$, $P = 0.0001$; trial: $F(1.27) = 5.79$, $P = 0.02$; interaction: $F(2,27) = 1.26$, $P > 0.3$]. The progression in speed from a teaching trial to trial 1 was significant between the adult and aged controls ($P = 0.0001$), as well as between the aged control and atipamezole groups ($P = 0.01$). The analysis of speed in the repeated trials by RM ANOVA revealed significant overall group and repetition effects and no interaction between them [group: $F(2,27) = 25.66$, $P = 0.0001$; trial: $F(9,243) = 7.35$, $P = 0.0001$; interaction: $F(18,243) = 1.31$, $P = 0.18$]. The difference in speed was significant between the aged control and aged atipamezole treated groups ($P = 0.002$) as well as between the aged and adult control groups ($P = 0.0001$).

3.2. Rat brain neurochemistry

The ChAt activities in the frontal cortex and hippocampus of the rats tested in the linear arm maze are presented in Table 1. The frontocortical ChAt activity was somewhat lower in the aged rats as compared to the adult rats. The difference was significant in the control groups, and it

Table 2
The effect of atipamezole (0.3 mg/kg s.c.) on the levels, including the main metabolites, (nmol/g) and the turnover rate of noradrenaline (NA), 5-hydroxytryptamine (5-HT) and dopamine (DA) in the brains of adult and aged rats 3 h after treatment
The results are expressed as mean ± S.E.M., $n = 5$ in the groups of adult rats and 4 in the groups of aged rats. ANOVA was followed by the Fisher's PLSD test.

Age/treatment	NA	MHPG–SO_4	MHPG–SO_4/NA	5-HT	5-HIAA	5-HIAA/5-HT	DA	HVA	HVA/DA
Adult/control	3.48 ± 0.16	0.42 ± 0.02	0.12 ± 0.01	2.59 ± 0.05	1.54 ± 0.02	0.60 ± 0.02	5.69 ± 0.03	0.42 ± 0.02	0.074 ± 0.003
Adult/atipamezole	3.20 ± 0.07	0.53 ± 0.02[a]	0.16 ± 0.01[a]	2.45 ± 0.07	1.63 ± 0.02	0.67 ± 0.02	5.92 ± 0.06[a]	0.46 ± 0.02	0.077 ± 0.005
Aged/control	3.53 ± 0.08	0.44 ± 0.01	0.12 ± 0.01	2.93 ± 0.01[b]	1.77 ± 0.05[b]	0.60 ± 0.02	5.55 ± 0.08	0.32 ± 0.01[b]	0.058 ± 0.002[b]
Aged/atipamezole	3.45 ± 0.03	0.72 ± 0.03[a,c]	0.21 ± 0.01[a,c]	2.78 ± 0.05[c]	2.18 ± 0.16[a]	0.79 ± 0.06[a,c]	5.66 ± 0.1[c]	0.40 ± 0.03[a]	0.072 ± 0.005[a]
ANOVA									
$F =$	2.13	37.64	38.81	15.4	14.17	7.39	5.13	6.93	3.89
$P <$	0.2	0.0001	0.0001	0.0001	0.001	0.01	0.05	0.01	0.05

[a] Refers to significant difference ($P < 0.05$) atipamezole group from the corresponding age control group.
[b] Refers to significant difference between adult and aged control groups ($P < 0.05$).
[c] Refers to significant difference between adult and aged atipamezole treated groups ($P < 0.05$).

approached significance between the adult control and the aged atipamezole treated groups ($P < 0.06$). In the hippocampus, there was no difference in ChAt activities between the group of rats.

The effects of atipamezole after an acute (0.3 mg/kg) injection on central monoamine metabolism in adult and aged rats are shown in Table 2. There were no apparent age associated differences in the metabolism of noradrenaline between the control groups. The administration of atipamezole increased the amount of MHPG–SO_4 in the brain of adult (26%, $P < 0.01$) and aged (63%, $P < 0.001$) rats. This effect was significantly higher in the aged rats than in the adult rats ($P < 0.001$). The 5-HT and 5-HIAA levels were significantly higher in the aged control rats as compared to the adult control group, but there was no difference in the basal turnover rate (5-HIAA/5-HT ratio) between the control groups. The injection of atipamezole did not have any effects in the adult group, but caused a marked elevation ($P < 0.01$) in the 5-HIAA/5-HT ratio in the aged group. The dopamine level was slightly reduced, and HVA ($P < 0.01$) and HVA/dopamine ratio ($P < 0.05$) were significantly lower in the aged control group as compared to the adult control group. Atipamezole had a modest effect on the central dopamine metabolism in the adult rats, whereas it caused a significant increase in the levels of dopamine and HVA as well as in HVA/dopamine ratio ($P < 0.05$ for all) in the aged rats.

4. Discussion

The primary objective of the present study was to investigate the effects of the specific α_2-adrenoceptor antagonist, atipamezole, on the cognitive performance of aged rats. Secondly, its influence on the central monoamine metabolism was also assessed in order to evaluate possible age related alterations in the effects of atipamezole on brain neurochemistry.

The performance of the adult control F-344 rats in the linear-arm maze test in the present study is in general agreement with the performance of adult Sprague–Dawley control rats assessed previously (Haapalinna et al., 1998), i.e., there was a slight or no improvement from the teaching trial to trial 1, but there was a consistent improvement in their performance during the repeated trials. In the present study, aged F-344 rats showed a clear decline in their performance in the linear-arm maze task, and this inferiority to the adult rats was seen already in the teaching trial, especially in the number of repetition entries and in the time needed to complete the task. In the repeated trials, there was a highly significant difference between the adult and aged rats in all the variables measured. This is in accordance with other studies, where the cognitive performance of F-344 rats at ages of 4–5 months and 22–25 months have been compared in tests such as the radial-arm maze (Luine et al., 1990), Morris water maze (Yavich et al., 1996) or inhibitory avoidance task (Collier et al., 1987).

In the previous study with adult Sprague–Dawley rats, where atipamezole improved their performance in a linear-arm maze test, the most clear-cut effect of atipamezole was obtained between the teaching trial and the first proper test trial. In the repeated trials, the improvement by atipamezole was less profound, because of the good performance level of the adult control rats (ceiling effect) (Haapalinna et al., 1998). In the present study, the facilitation of performance induced by atipamezole treatment in the aged rats was less pronounced during the transition from the teaching trial to trial 1. However, the reduction in the number of repetition entries from the teaching trial to trial 1 approached significance only in the atipamezole group. Importantly enough, atipamezole treatment shortened significantly the time needed to complete the task in aged animals, as compared to the aged control rats, from a teaching trial to trial 1. In the repeated trials, although the aged control animals improved their performance, atipamezole-treated aged animals maintained at better performance level both in the terms of the number of errors committed and the time needed to complete this task. Their behavioural activity as expressed in the speed of arm visits was higher than in the aged control group, even though it was still lower than that of the adult control group. In the repeated trials, the number of correct choices was increased in all the groups, but there was no significant difference between the atipamezole treated and the control aged rats. The effect of age on this variable, though it was significant, was not, however, as clear as on the other variables of maze performance measured.

Interestingly, these results are different from those obtained with idazoxan (1 mg/kg s.c.) in aged rats performing a radial arm maze task (Dickinson et al., 1989). Those authors reported that idazoxan improved the performance of young rats (decreasing the number of errors and time used), but it failed to show a reduction in the number of errors committed by aged rats. It was also reported that the improvement in time needed to complete the task over repeated trials was due to a significant impairment caused by idazoxan in the first training trial. It is difficult to make comparisons between results obtained in different tests with different rat strains, but one major difference is that we did not observe any impairment in the performance of atipamezole treated aged rats in the teaching trial. Differences in the habituation protocols could be an important factor between the studies.

Studies on the age related changes in the cholinergic markers of a rodent brain have resulted in contradictory results, partly due to differences in brain sampling as well as the strain, sex and age of cohorts (Decker, 1987). In the present study, the aged male F-344 rats studied in the linear-arm maze test had a lower level of ChAt activity in the frontal cortex, but not in the hippocampus, as compared to the adult rats. Even though ChAt is not the

rate-limiting step in the synthesis of acetylcholine, the decreased activity indicates that the basalo-cortical cholinergic system is affected in this model. This is in accordance with previous studies reporting both neurochemical and histological evidence for age-related cholinergic dysfunction in the F-344 rats (see Schwartz et al., 1990). The causality between the decreased frontocortical ChAt activity and the performance deficits in the linear-maze task is, of course, unclear. However, the recent pertinent studies in rats have revealed that a selective lesion of the nucleus basalis, which decreased neocortical ChAt activity, failed to affect performance in various tests of learning and memory, but it did cause deficits in a 5-choice serial reaction time task, that measures sustained, selective and divided attention (Robbins et al., 1997). One intriguing interpretation is that the age associated performance deficits seen in the linear-arm maze task were partially due to the dysfunction of attention mechanisms such as response selection, rather than spatial memory deficit per se. Interestingly, the effect of ageing on the number of correct choices was not as clear as on the other variables of the maze performance and the effect of atipamezole on it was not statistically significant. Thus, it is possible that the number of correct choices is an indicator for spatial learning that is dependent on normally functioning hippocampus. Atipamezole and other α_2-adrenoceptor antagonists have been found to enhance acetylcholine release in the prefrontal cortex of adult rats (Tellez et al., 1997). Thus, these drugs may be effective to diminish performance deficits caused by a cortical cholinergic dysfunction. However, age-related changes in various other neurotransmitter systems as well have been reported to occur in F-344 rat brain (Buzsáki et al., 1990; Luine et al., 1990; Friedemann and Gerhardt, 1992).

Interestingly enough, the number and function of brain α_2-adrenoceptors may be altered during ageing both in the central nervous system of rodents and primates (Qi and Nomura, 1988; Gelbman and Müller, 1990; Kalaria and Andorn, 1991; Pascual et al., 1991; Zsilla et al., 1997). In a study with isolated hearts from F-344 rats at various ages, it was found that the activity of extraneuronal uptake mechanism for noradrenaline increased and α_2-adrenoceptor-mediated autoregulation of noradrenaline decreased with age (Daly et al., 1989). On the other hand, age-related changes in peripheral noradrenergic system can be different from those in the central noradrenergic system. For example, a decrease in noradrenaline content was found in the heart of aged rats, and an increase in the levels of hippocampal noradrenaline in those subjects (Sirviö et al., 1994). However, an α_2-adrenoceptor antagonist was found to stimulate noradrenaline release from hippocampal slices taken from rats of ages four and 12 months, but not from the slices taken from rats of 24 months age (Zsilla et al., 1997). Here, we studied the possible effects of ageing on the ability of atipamezole to stimulate central noradrenaline release in vivo. The effect of an acute atipamezole injection on brain neurochemistry in the adult F-344 rats is in a general agreement with the previous results obtained with adult Sprague–Dawley (Haapalinna et al., 1997) and Wistar rats (Scheinin et al., 1988), since there was a significant increase in MHPG–SO_4 level and noradrenaline turnover rate as well as a trend for increased 5-HT and dopamine turnover rates. There was no difference in the whole brain levels of noradrenaline and MHPG–SO_4 between adult and aged control rats. Interestingly, atipamezole increased noradrenaline turnover significantly more in the aged than in adult rats. Although the activity and number of noradrenergic neurones in the locus coeruleus have been reported to decline during ageing in rodents, there are reports that in the terminal fields, like in the frontal cortex and hippocampus, there is no change or even an increase in noradrenergic activity (see Sirviö et al., 1994). There are also contradictory findings with aged F-344 rats (MacIntosh and Westfall, 1987; Luine et al., 1990). Although, the measurement of whole brain transmitter changes is not valid for studying the effect of ageing on the regional transmitter status or on the role of α_2-adrenoceptors for local transmitter release, the present study indicated that atipamezole clearly stimulated the release of central noradrenaline also in the aged rats in vivo.

Bearing in mind the limitations in measuring whole brain transmitter levels, it is still interesting to note the increased 5-HT and 5-HIAA levels in aged control rats, which were not, however, reflected as a change in 5-HT turnover. This is in line with the previous studies on the 5-HT and 5-HIAA levels in various brain regions of aged rodents (Luine et al., 1990; McEntee and Crook, 1991). As atipamezole accentuated the age related increase in the levels of serotonin metabolite, the improvement in cognitive performance by atipamezole may not be due to its effect of 5-HT system since the enhanced serotonergic activity may be detrimental to cognitive functions during ageing (McEntee and Crook, 1991).

With respect to the dopaminergic system, a decrease in the whole brain dopamine and HVA levels as well as in dopamine turnover was observed in aged rats. This supports the findings of previous reports in various species showing that the central dopaminergic system is affected by ageing (see Decker and McGaugh, 1991), including F-344 rats (Buzsáki et al., 1990; Luine et al., 1990; Friedemann and Gerhardt, 1992). Atipamezole enhanced dopamine transmission in aged rats to the level obtained in adult rats. The same dose increased behavioural activation, most clearly seen as slightly increased speed (arm visits/s) in the linear arm maze test. This is in accordance with the role of dopamine in the modulation of decision making and locomotor functions.

The present studies do not allow us to specify the cause for a more potent effect of atipamezole on monoamine metabolism in aged rats. It is possible that the metabolism and/or distribution volume of atipamezole is altered due

to ageing, resulting in higher concentrations of atipamezole in the brains of aged than adult rats. As the effect of atipamezole on central noradrenaline turnover has been found to be dose dependent (Scheinin et al., 1988; Haapalinna et al., 1997), age -elated alterations in the pharmacokinetics of atipamezole may account for the more profound stimulation of central noradrenaline turnover in aged rats. However, it is noteworthy that atipamezole caused a significant increase in the central 5-HT and dopamine turnover rate only in the aged rats. In previous studies, atipamezole did not significantly increase 5-HT and dopamine turnover even at a high dose (10 mg/kg s.c.) (Haapalinna et al., 1997) or after a continuous infusion (0.1 mg/kg h) for 24 hours or 10 days in adult rats (Haapalinna et al., 1999). Therefore, there may also be age related alterations in the pharmacodynamic effects of atipamezole. The interactions between neurotransmitter systems should not be underestimated. For example, it has been reported that the effect of noradrenaline on acetylcholine release in the frontal cortex of rat is a net result from multiple interactions between various neurotransmitters and their receptors (Beani et al., 1986). Moreover, a lesion of the serotonergic system is reported to prevent the stimulatory effect of idazoxan on dopamine release in the frontal cortex (Matsumoto et al., 1998). Thus, the age-related changes in the net effect of atipamezole on the turnover of the monoamines found in the present study is not necessarily explained by alterations at the level of α_2-adrenoceptors per se.

The present data may have some potential clinical relevance. A recent study in patients with the dementia of frontal lobe type, showed that idazoxan improved their performance in the tests of planning, sustained attention, verbal fluency and episodic memory, but impaired performance in the test of spatial working memory. Thus, it was suggested that α_2-adrenergic drugs may be effective in some cognitive deficits that are based on frontal lobe dysfunction (Coull et al., 1996). Furthermore, the basalocortical cholinergic system is affected in demented patients with Parkinson's disease (Sirviö et al., 1989; Jellinger, 1999). As α_2-adrenoceptor antagonists, including atipamezole, have also anti-parkinsonian effects in the experimental models of Parkinson's disease (Leino et al., 1997; Chopin et al., 1999), this type of drugs could be beneficial in the palliative treatment of demented Parkinson's disease patients. It is also plausible that an α_2-adrenoceptor antagonist could improve attentional functions in patients with Alzheimer's disease.

Taken as a whole, the present results showed that atipamezole enhanced central noradrenergic activity both in adult and aged rats, and it effectively improved performance deficits in a relational learning and memory test among the aged rats which had a cholinergic dysfunction in the frontal cortex. Moreover, atipamezole enhanced behavioural activity and dopamine turnover that were decreased in aged rats. These results strongly indicate that some of the behavioural and neurochemical effects of α_2-adrenoceptor antagonists can be preserved in the ageing brain. They also provides an impetus to test the hypothesis that α_2-adrenoceptor antagonists could be used in the treatment of the cognitive deficits seen in Parkinson's disease (Coull, 1996).

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References

Arnsten, A.F.T., Cai, J.X., 1993. Postsynaptic α_2-receptor stimulation improves memory in aged monkeys: indirect effects of yohimbine versus direct effects of clonidine. Neurobiol. Ageing 14, 597–603.

Aston-Jones, G., Chiang, C., Alexinsky, T., 1991. Discharge of noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance. In: Barnes, C.D., Pompeiano, O. (Eds.), Neurobiology of the Locus Coeruleus 88 Elsevier, Amsterdam, pp. 501–520.

Beani, L., Tanganelli, S., Antonelli, T., Bianchi, C., 1986. Noradrenergic modulation of cortical acetycholine release is both direct and γ-aminobutyric acid-mediated. J. Pharmacol. Exp. Ther. 236, 230–236.

Berridge, C.W., Arnsten, A.F.T., Foote, S.L., 1993. Noradrenergic modulation of cognitive function — clinical implications of anatomical, electrophysiological and behavioural studies in animal models. Psychol. Med. 23 (3), 557–564.

Biegon, A., Mathis, C.A., Budinger, T.F., 1992. Quantitative in vitro and ex vivo autoradiography of the α_2-adrenoceptor antagonist [^{3}H]atipamezole. Eur. J. Pharmacol. 224, 27–38.

Bunsey, M.D., Strupp, B.J., 1995. Specific effects of idazoxan in a distraction task: evidence that endogenous norepinephrine plays a role in selective attention in rats. Behav. Neurosci. 109, 903–911.

Buzsáki, G., Laszlovszky, I., Lajtha, A., Vadász, C., 1990. Spike-and-wave neocortical patterns in rats: genetic and aminergic control. Neuroscience 38, 323–333.

Cedarbaum, J.M., Aghajanian, G.K., 1977. Catecholamine receptors on locus coeruleus neurons: pharmacological characterization. Eur. J. Pharmacol. 44, 375–385.

Chopin, P., Colpaert, F.C., Marien, M., 1999. Effects of α_2-adrenoceptor agonists and antagonists on circling behavior in rats with unilateral 6-hydroxydopamine lesion of the nirgostriatal pathway. J. Pharmacol. Exp. Ther. 288, 789–804.

Collier, T.J., Gash, D.M., Sladek, J.R., 1987. Transplantation of norepinephrine neurons into aged rats improved performance of a learned task. Brain Res. 448, 77–87.

Conover, W., Iman, R., 1981. Rank transformation as a bridge between parametric and nonparametric statistics. Am. Stat. 35, 124–128.

Coull, J.T., 1996. α_2-Adrenoceptors in the treatment of dementia: an attentional mechanism. J. Psychopharm. 10, 43–48.

Coull, J.T., Sahakian, B.J., Hodges, J.R., 1996. The α_2 antagonists idazoxan remediates certain attentional and executive dysfunction in patients with dementia of frontal type. Psychopharmacology 123, 239–249.

Daly, R.N., Goldberg, P.B., Roberts, J., 1989. The effect of age on presynaptic α_2 adrenoceptor autoregulation of norepinephrine release. J. Gerontol. 44, B59–B66.

Dausse, J.P., Le Quan-Bui, K.H., Meyer, P., 1982. α_1- and α_2-adrenoceptors in rat cerebral cortex: effects of neonatal treatment with 6-hydroxydopamine. Eur. J. Pharmacol. 78, 15–20.

Decker, M.W., 1987. The effect of ageing on hippocampal and cortical projections of the forebrain cholinergic system. Brain Res. Rev. 12, 423–438.

Decker, M.W., McGaugh, J.L., 1991. The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory. Synapse 7, 151–168.

Devauges, V., Sara, S.J., 1990. Activation of the noradrenergic system facilitates an attentional shift in the rat. Behav. Brain Res. 39, 19–28.

Dickinson, S.L., Gadie, B., Woodmansey, N.R., 1989. Effect of repeated idazoxan treatment on radial arm maze performance in adult and in aged rats. Br. J. Pharmacol. 97, P384.

Dooley, D.J., Bittiger, H., Hauser, K.L., Bischoff, S.F., Waldmeier, P.C., 1983. Alteration of central α_2- and β-adrenergic receptors in the rat after DSP-4, a selective noradrenergic neurotoxin. Neuroscience 9, 889–898.

Fonnum, F., 1975. A rapid radiochemical method of the determination of choline acetyltransferase. J. Neurochem. 24, 407–409.

Friedemann, M.N., Gerhardt, G.A., 1992. Regional effects of ageing on dopaminergic function in the Fischer-334 rat. Neurobiol. Ageing 13, 325–332.

Gelbman, G.M., Müller, W.E., 1990. Specific decrease of high-affinity agonist states of α_2-adrenoceptors in the ageing mouse brain. J. Neural Transm. 79, 131–136.

Gulat-Marney, C., Lafitte, A., Arrang, J.-M., Schwartz, J.-C., 1989. Modulation of histamine release and synthesis in the brain mediated by α_2-adrenoceptors. J. Neurochem. 53, 519.

Haapalinna, A., MacDonald, E., Viitamaa, T., Salonen, J.S., Sirviö, J., Virtanen, R., 1999. Comparison of the effects of acute and subchronic administration of atipamezole on reaction to novelty and active avoidance learning in rats. Naunyn-Schmiedeberg's Arch. Pharmacol. 359, 194–203.

Haapalinna, A., Sirviö, J., Lammintausta, R., 1998. Facilitation of cognitive functions by a specific α_2-adrenoceptor antagonist, atipamezole. Eur. J. Pharmacol. 347 (1), 29–40.

Haapalinna, A., Viitamaa, T., MacDonald, E., Savola, J.-M., Tuomisto, L., Virtanen, R., Heinonen, E., 1997. Evaluation of the effects of a specific α_2-adrenoceptor antagonist, atipamezole, on α_1- and α_2-adrenoceptor subtype binding, brain neurochemistry and behaviour in comparison with yohimbine. Naunyn-Schmiedeberg's Arch. Pharmacol. 356, 570–582.

Harley, C.W., 1987. A role for norepinephrine in arousal, emotion and learning? Limbic modulation by norepinephrine and the Kety hypothesis. Prog. Neuropsychopharmacol. Biol. Psychiatry 11, 419–458.

Jellinger, K.A., 1999. Post mortem studies in Parkinson's disease — is it possible to detect brain areas for specific symptoms? J. Neural Transm. 56, 1–29.

Kalaria, R.N., Andorn, A.C., 1991. Adrenergic receptors in ageing and Alzheimer's disease: decreases α_2 receptors demonstrated by [^{3}H]*p*-aminoclonidine binding in prefrontal cortex. Neurobiol. Ageing 12, 131–136.

Leino, T., Haapalinna, A., Heinonen, E., 1997. α_2-adrenoceptor antagonists potentiate anti-parkinsonian effects of levodopa and apomorphine and reduce dopaminergic adverse effects. Soc. Neurosci. Abstr. 23, 414.

Liljequist, R., Haapalinna, A., Åhlander, M., Ying, H.L., Männistö, P.T., 1997. Catechol *O*-methyltransferase inhibitor tolcapone has minor influence on performance in experimental memory models in rats. Behav. Brain Res. 82, 195–202.

Lowry, O., Rosebrough, N., Farr, A., Randall, R., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Luine, V., Bowling, D., Hearns, M., 1990. Spatial memory deficits in aged rats: contributions of monoaminergic systems. Brain Res. 537, 271–278.

MacDonald, E., Scheinin, H., Scheinin, M., 1988. Behavioural and neurochemical effects of medetomidine, a novel veterinary sedative. Eur. J. Pharmacol. 158, 119–127.

MacIntosh, H.H., Westfall, T.C., 1987. Influence of ageing on catecholamine levels, accumulation and release in F-344 rats. Neurobiol. Ageing 8, 233–239.

Matsumoto, M., Yoshioka, M., Togashi, H., Mori, K., Ueno, K., Saito, H., 1998. Effects of idazoxan on dopamine release in the prefrontal cortex of freely moving rat. Eur. J. Pharmacol. 343, 165–170.

McEntee, W.J., Crook, T.H., 1990. Age-associated memory impairment: a role for catecholamines. Neurology 40, 526–530.

McEntee, W.J., Crook, T.H., 1991. Serotonin, memory, and the ageing brain. Psychopharmacology 103, 143–149.

Meana, J.J., Callado, L.F., Pazos, A., Grijalba, B., Garciá-Sevilla, J.A., 1996. The subtype-selective α_2-adrenoceptor antagonists BRL 44408 and ARC 239 also recognize 5-HT$_{1A}$ receptors in the rat brain. Eur. J. Pharmacol. 312, 358–388.

Newman-Tancredi, A., Tancredi, J.-P., Audinot, V., Gavaudan, S., Verrièle, L., Touzard, M., Chaput, C., Richard, N., Millan, M., 1998. Actions of α_2-adrenoceptor ligands at alpha-2A and 5-HT-1A receptors: the antagonist, atipamezole, and the agonist, dexmedetomidine, are highly selective for alpha-2A adrenoceptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 358, 197–206.

Ordway, G.A., 1995. Effect of noradrenergic lesions on subtypes of α_2-adrenoceptors in rat brain. J. Neurochem. 64, 1118–1126.

Pascual, J., del Arco, C., Gonzáles, A.M., Diaz, A., del Olmo, E., Pazos, A., 1991. Regionally specific age-dependent decline in α_2-adrenoceptors: an autoradiographic study in human brain. Neurosci. Lett. 133, 279–283.

Qi, S.-B., Nomura, Y., 1988. Age related changes in the inhibitory effect of clonidine on high K-evoked noradrenaline release from brain slices of rats. J. Pharmacobio-Dyn. 11, 483–485.

Raiteri, M., Maura, G., Folghera, S., Cavazzani, P., Andrioli, G.C., Schlicker, E., Schalnus, R., Göthert, M., 1990. Modulation of 5-hydroxytryptamine release by presynaptic inhibitory α_2-adrenoceptors in the human cerebral cortex. Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 508–512.

Robbins, T.W., McAlonan, G., Muir, J.L., Everitt, B.J., 1997. Cognitive enhancers in theory and practice: studies of the cholinergic hypothesis of cognitive deficits in Alzheimer's disease. Behav. Brain Res. 83, 15–23.

Sara, S.J., Devauges, V., 1989. Idazoxan, an α-2 antagonist, facilitates memory retrieval in the rat. Behav. Neural Biol. 51, 401–411.

Sara, S.J., Vankov, A., Hervé, A., 1994. Locus coeruleus-evoked responses in behaving rats: a clue to the role of noradrenaline in memory. Brain Res. Bull. 35, 457–465.

Savontaus, S., Raasmaja, A., Rouru, J., Koulu, M., Pesonen, U., Virtanen, R., Savola, J.-M., Huupponen, R., 1997. Anti-obesity effect of MPV-1743 A III, a novel imidazoline derivative, in genetic obesity. Eur. J. Pharmacol. 328, 207–215.

Scheinin, H., MacDonald, E., Scheinin, M., 1988. Behavioural and neurochemical effects of atipamezole, a novel α_2-adrenoceptor antagonist. Eur. J. Pharmacol. 157, 243–244.

Schwartz, P., Bernabel, A.A., Spencer, C.J., 1990. Loss of muscarinic M1 receptors with ageing in the cerebral cortex of Fisher 344 rats. Pharmacol. Biochem. Behav. 35, 589–593.

Simson, P.E., Weiss, J.M., 1987. α_2-receptor blockade increases responsiveness of locus coeruleus neurons to excitatory stimulation. J. Neurosci. 7, 1732–1740.

Sirviö, J., Jäkälä, P., Mazurkiewicz, M., Haapalinna, A., Riekkinen, P. Jr., Riekkinen, P.J., 1993. Dose- and parameter-dependent effects of atipamezole, an α_2-antagonist, on the performance of rats in a five-choice serial reaction time task. Pharmacol. Biochem. Behav. 45, 123–129.

Sirviö, J., Lahtinen, H., Riekkinen, P.J., Riekkinen, P., 1994. Spatial learning and noradrenaline content in the brain and periphery of young and aged rats. Exp. Neurol. 125, 312–315.

Sirviö, J., Rinne, J.O., Valjakka, A., Rinne, U.K., Riekkinen, P.J.,

Paljärvi, L., 1989. Different forms of brain acetylcholinesterase and muscarinic binding in Parkinson's disease. J. Neurol. Sci. 90, 23–32.

Tellez, S., Colpaert, F., Marien, M., 1997. Acetylcholine release in the rat prefrontal cortex in vivo: modulation by α_2-adrenoceptor agonists and antagonists. J. Neurochem. 68, 778–785.

Trendelenburg, A.U., Starke, K., Limberger, N., 1994. Presynaptic alpha-2A-adrenoceptors inhibit the release of endogenous dopamine in rabbit caudate nucleus slices. Naunyn-Schmiedeberg's Arch. Pharmacol. 350, 473–481.

Virtanen, R., Savola, J.-M., Saano, V., 1989. Highly selective and specific antagonism of central and peripheral α_2-adrenoceptors by atipamezole. Arch. Int. Pharmacodyn. Ther. 297, 190–204.

Winter, J.C., Rabin, R.A., 1992. Yohimbine as a serotonergic agent: evidence from receptor binding and drug discrimination. J. Pharmacol. Exp. Ther. 263, 682–689.

Yavich, L., Sirviö, J., Haapalinna, A., Puumala, T., Koivisto, E., Heinonen, E., Riekkinen, P.J., 1996. The systemic administration of tacrine or selegiline facilitate spatial learning in aged Fisher 344 rats. J. Neural Transm. 103, 619–626.

Ylinen, A., Pitkänen, M., Pussinen, R., Haapalinna, A., Koivisto, E., Lappalainen, R., Sirviö, J., Riekkinen, P.S., 1996. A selective antagonists of α_2-adrenoceptors, facilitates the excitability of the dentate gyrus and improves the retention of the radial arm maze. Soc. Neurosci. Abstr. 22, 680.

Zornetzer, S.F., 1985. Catecholamine system involvement in age-related memory dysfunction. Ann. N.Y. Acad. Sci. 444, 242–254.

Zsilla, G., Zelles, T., Mike, A., Kékes-Szabó, A., Milusheva, E., Vizi, E.S., 1997. Differential changes in presynaptic modulation of transmitter release during ageing. Int. J. Dev. Neurosci. 12, 107–115.