Pharmacology Biochemistry and Behavior, Vol. 42, pp. 903-907, 1992
Printed in the U.S.A. All rights reserved.

0091-3057/92 $5.00 + .00
Copyright © 1992 Pergamon Press Ltd.

BRIEF COMMUNICATION

Effects of Atipamezole, An α_2-Adrenoceptor Antagonist, on the Performance of Rats in a Five-Choice Serial Reaction Time Task

PEKKA JÄKÄLÄ,*[1] JOUNI SIRVIÖ,* PAAVO RIEKKINEN, JR.,*
ANTTI HAAPALINNA† AND PAAVO RIEKKINEN*

**Department of Neurology, University of Kuopio, P.O. Box 1627, SF-70211 Kuopio, Finland*
†Orion Corporation Farmos, R&D Pharmaceuticals, Turku, Finland

Received 29 July 1991

JÄKÄLÄ, P., J. SIRVIÖ, P. RIEKKINEN, JR., A. HAAPALINNA AND P. RIEKKINEN. *Effects of atipamezole, an α_2-adrenoceptor antagonist, on the performance of rats in a five-choice serial reaction time task.* PHARMACOL BIOCHEM BEHAV 42(4) 903-907, 1992. – The present study investigates whether pharmacological activation of the noradrenergic system improves attention. The effects of atipamezole, a potent α_2-adrenoceptor antagonist, on the performance of adult male rats in the five-choice serial reaction time task were studied. Before drug testings, food-deprived rats were trained to detect and respond to brief flashes of light presented randomly by the computer in one of five spatially diverse locations until a stable level of performance had been reached (about 3 months). Single-dose administration of atipamezole (0.03-3.0 mg/kg) slightly increased the number of premature and perseverative responses during the intertrial interval and slightly decreased the reaction times to incorrect responses, indicating increased behavioral activation. Atipamezole did not affect discriminative accuracy. However, in a subpopulation of rats with the poorest discriminative accuracy according to pretest performance seven of eight rats improved their discriminative accuracy when treated with 0.3 mg/kg atipamezole as compared to controls. At the other doses tested, no improvement was found. The present results suggest that acute administration of atipamezole, an α_2-adrenoceptor antagonist, slightly increases behavioral activation, although the effects on baseline performance in the task measuring selective attention are modest.

Atipamezole Five-choice serial reaction time task Rats Selective attention

EXTENSIVE electrophysiological, anatomical, and behavioral data demonstrate that the noradrenergic system may have an important role in the regulation of neocortical arousal and vigilance (2,4,6,8-11). Recent psychopharmacological studies suggest a role for the dorsal ascending noradrenergic projection in the processes of attention (4,6,12).

The locus coeruleus (LC) is the major source of the noradrenergic innervation of the forebrain (3). The firing rate of LC noradrenergic neurons is regulated by α_2-adrenergic autoreceptors (1,2,5). Blockade of these receptors increases the firing rate of LC and concomitantly increases the turnover of noradrenaline in brain (1,2,5).

We are currently investigating whether pharmacological stimulation of the noradrenergic system by α_2-adrenergic antagonists would improve cognitive functions. Atipamezole, a potent and selective α_2-adrenoceptor antagonist (13,14), increases the turnover of noradrenaline in rat brains in a dose-dependent manner (0.03-3.0 mg/kg) (13). Our previous results suggest that atipamezole may suppress thalamic oscillatory activity and thus may facilitate the transfer of information through the thalamus to the neocortex (10,11). The present experiment investigates whether atipamezole could improve attention in rats. Therefore, the effects of single-dose administration of atipamezole on the performance of adult male rats in the five-choice serial reaction time task were studied. This task, which requires the subject to detect and respond to brief flashes of light in spatially diverse locations, has been adapted for rats from Leonard's five-choice serial reaction time task (15) and is proposed to assess selective attention (4, 7,12).

[1] To whom requests for reprints should be addressed.

METHOD

Animals

Twenty male Kuo:Wistar rats were used in the experiment. Rats were 4 and 7 months old at the beginning of behavioral training and drug testing, respectively. Rats were singly housed in Makrolon (University of Kuopio) cages in a controlled environment (temperature 20°C, humidity 50–60%, lights on 0700–2100 h). During training and drug testing, rats were deprived of food for 22–23 h before training/testing sessions. After daily behavioral training/testing, rats received 10–12 g food pellets (Astra-Ewos, Sweden) so that they were maintained at approximately 85% of free-feeding weight. Water was available ad lib except in the test apparatus.

Behavioral Training and Testing

Apparatus. The test apparatus (4,7), which was made in the Technical Center (University of Kuopio, Finland), consisted of a 25 × 25 cm aluminium chamber with a curved rear wall. Set in the curved wall were nine 2.5-cm square holes 4 cm deep and 2.5 cm above floor level. Each hole had an infrared photocell beam crossing the entrance vertically and illuminating a photoelectric cell. A standard 3-W bulb at the rear of the hole provided illumination for that hole. The entrances to holes 2, 4, 6, and 8 were blocked with a metal cap. Food pellets (45 mg, dustless, Bioserv. Inc., Holton Instruments Co., Frenchtown, NJ) could be dispensed automatically into a magazine at the front of the chamber. Access was gained to the magazine through a Perspex door. The distances from the panel to the illuminated holes at the rear of the box were all 25 cm. The chamber was illuminated by a 3-W houselight mounted in the roof. Animals were introduced to the chamber through a Perspex door in the top half of the front wall. The apparatus was housed in a dark, soundproof compartment. On-line control of the apparatus and data collection was performed using microprocessors that were programmed using Spider (Paul Fray Ltd., Cambridge, UK).

Training. Rats were trained in the following manner to discriminate spatially a brief visual stimulus presented randomly by the computer in one of the five holes (from left, holes 1, 3, 5, 7, and 9). On the first 3 days of behavioral training, all rats were magazine trained by being placed in the chambers for 20 min with the houselight on and the food tray containing 30–40 food pellets. On the next 2 days, rats were placed in the chambers for 20 min and a food pellet was delivered every 15 s into the magazine. The houselight was on during this phase. In the third phase, one of the holes was illuminated all the time during the 20-min training period and every time the rat made a response (nose-poke) on the illuminated hole it was reinforced by a food pellet on the panel. After learning this, rats entered the next phase, which started by a free delivery of a single food pellet. The first trial started when the panel was opened to collect the food pellet. After a fixed delay [intertrial interval (ITI)], the light at the rear of one of the holes was illuminated for a short period (stimulus duration). The light stimulus was presented in each of the holes for an equal number of times during each complete session, and the order of presentations was randomized by the computer. Responses by the rat in the illuminated hole and responses in that particular hole for a short period after illumination (the limited hold) were rewarded with the delivery of a food pellet and a correct response was recorded. A response in any other hole (incorrect response) or a failure to respond at all during the visual stimulus or the limited hold period (omission) resulted in a punishment period of darkness (time-out). Any response made during the time-out period restarted the time-out. Responses made in the holes during the ITI period were recorded as premature responses and responses made in the panel during the ITI period were recorded as perseverative responses and both resulted in a period of time-out. The next trial was initiated by pushing the panel either to collect the reinforcing food pellet after a correct response or following completion of a time-out period. The latency between the onset of the stimulus and response, whether correct or incorrect, was measured, as well as the latency to collect the earned food pellet following completion of a correct response. Each daily training session (five sessions a week) consisted of 15 min of training. During the first training session, the stimulus duration and limited hold periods were set at 5.0 and 1.0 s, respectively. These durations were then progressively reduced and increased to 0.5 and 3.5 s, respectively, during the training. The ITI and time-out were both set at 2 s for the first session of training and then increased to 5 and 4 s, respectively, during the training. Rats were trained on this schedule depending upon individual performance until a stable performance had been reached. It took about 40–50 training sessions to reach a stable level when no improvement in performance could be observed. The level of percent correct responses reached ranged from 65–85% in rats used in the present experiment. According to their attained level of percent correct responses, rats were divided into two groups (group p = the poorest 2/5 of rats with less than 75% correct responses; group r = the rest of the rats with at least 75% correct responses) to further analyze the effects of atipamezole on discriminative accuracy.

Drug testing. Following the completion of the training period, the effects of atipamezole HCl (Orion Corp. Farmos, Finland) (0.03–3.0 mg/kg) on this task were tested. Drug was dissolved in isotonic saline and 0.5 ml/kg was administrated (SC) 25 min before testing. Saline was used as a control treatment. During testing, the ITI was set to 5.0 s, stimulus duration to 0.5 s, limited hold to 3.5 s, and time-out to 4.0 s. Rats were injected once with saline, 0.03, 0.1, 0.3, and 1.0 mg/kg atipamezole in a counterbalanced order every second day. Two days after the last treatment, all rats were treated with 3.0 mg/kg atipamezole. Previously, it has been shown in rats that high doses of atipamezole ($\geq$3.0 mg/kg) increase the rate of breathing and at higher doses this may be accompanied by piloerection, anxiousness, and increased vocalization (13).

Behavioral variables. The parameters selected for analysis of variance (MANOVA) were: a) *trials* = the total number of trials (correct + incorrect + omissions) made during a 15-min testing period; b) *ITI responses* = the number of premature responses in the holes or perseverative responses in the panel during the intertrial interval; c) *omissions* = the number of errors of omissions made; d) *response latencies* = the latency to respond (the time between the onset of the stimulus and a nose-poke) was recorded separately for correct and incorrect responses; e) *magazine latency* = the latency to collect earned food pellets from the magazine after a correct response had been made; f) *discriminative accuracy* = the proportion of correct responses [correct responses/(correct + incorrect responses)], expressed as a percentage.

Statistics. The MANOVA (multivariance test for repeated measurements) and the posthoc Wilcoxon signed-rank test were used to analyze the treatment effects on the variables

TABLE 1

EFFECTS OF ATIPAMEZOLE (0.03-3.0 mg/kg, SC 25 min BEFORE TESTING) ON THE NUMBER OF TRIALS, ITI RESPONSES, AND OMISSIONS DURING A 15-min TESTING PERIOD

	Trials	ITI Hole Responses	ITI Panel Pushes	Omissions
Saline	53.8 ± 3.7	22.1 ± 2.4	58.5 ± 10.3	3.6 ± 0.8
Ati 0.03	53.4 ± 3.8	25.3 ± 3.2	58.7 ± 9.2	3.3 ± 0.6
Ati 0.1	51.7 ± 3.3	25.6 ± 2.9	58.1 ± 7.8	3.4 ± 0.6
Ati 0.3	49.7 ± 3.0	27.3 ± 2.4	55.1 ± 8.2	2.8 ± 0.5
Ati 1.0	53.8 ± 3.0	23.1 ± 2.4	57.9 ± 9.5	3.6 ± 0.6
Ati 3.0	56.2 ± 3.2	24.6 ± 3.2	69.5 ± 9.0*	3.4 ± 0.6
MANOVA: $F(5, 95)$	1.18, $p > 0.1$	1.08, $p > 0.1$	3.33, $p < 0.01$	0.39, $p > 0.1$

Results are expressed as mean ± SEM. Ati, atipamezole; ITI, intertrial interval.
*Two-tailed $p < 0.01$ vs. saline treatment (Wilcoxon).

mentioned above. Before MANOVA analysis, the percent correct data were transformed using arcsine transformation.

RESULTS

In the analysis of the total number of trials made by rats during a 15-min testing period (Table 1), no significant drug treatment effects were observed.

For the number of omissions (Table 1), the overall drug treatment effects were also nonsignificant.

At 0.3 mg/kg, atipamezole slightly increased the number of premature hole responses ($p = 0.07$) (Table 1). The number of perseverative panel pushes during the ITI was significantly increased by atipamezole at 3.0 mg/kg (Table 1).

There were no significant differences in the latency to collect earned food pellets from the magazine after a correct response following atipamezole treatment (Table 2). Atipamezole decreased the latencies to incorrect responses, at the dose of 0.03 mg/kg slightly ($p = 0.09$) and at the doses of 0.3 and 3.0 mg/kg significantly, but it did not affect the latencies to correct responses (Table 2).

The analysis of percent correct responses (Fig. 1) did not reveal an overall drug treatment effect. However, atipamezole at 0.3 mg/kg slightly increased percent correct responses in the subpopulation of rats with the poorest discriminative accuracy according to pretest performance (seven of eight rats improved their discriminative accuracy).

DISCUSSION

The present experiment investigated whether pharmacological activation of the noradrenergic system could improve attention. Therefore, the effects of atipamezole, a potent and selective α_2-adrenoceptor antagonist (13,14) that increases the turnover of noradrenaline in a dose-dependent manner in rat brains after systemic administration (13), on the performance of adult rats were studied in the five-choice serial reaction time task, which is analogous to the Leonard's five-choice serial reaction time task used in the analysis of different forms of arousal (15). The task has been adapted for rats and is considered to assess selective attention (4,7,12).

The highest atipamezole dose (3.0 mg/kg) used significantly increased the number of perseverative panel pushes and atipamezole tended also to increase the number of premature hole responses made by rats during the ITI. These effects suggest a behaviorally activating effect of atipamezole. Previously, it has been shown that amphetamine also increases the number of premature hole and perseverative panel re-

TABLE 2

EFFECTS OF ATIPAMEZOLE (0.03-3.0 mg/kg, SC, 25 min BEFORE TESTING) ON THE LATENCIES (SECONDS) TO CORRECT AND INCORRECT RESPONSES AND THE LATENCY TO COLLECT EARNED FOOD PELLETS FROM THE MAGAZINE AFTER A CORRECT RESPONSE DURING A 15-min TESTING PERIOD

	Correct Response	Incorrect Response	Panel
Saline	0.74 ± 0.02	1.35 ± 0.10	3.04 ± 1.00
Ati 0.03	0.74 ± 0.02	1.18 ± 0.11	2.24 ± 0.41
Ati 0.1	0.76 ± 0.02	1.22 ± 0.06	2.43 ± 0.45
Ati 0.3	0.77 ± 0.02	1.08 ± 0.07*	2.48 ± 0.36
Ati 1.0	0.78 ± 0.03	1.17 ± 0.07	2.35 ± 0.34
Ati 3.0	0.73 ± 0.02	1.11 ± 0.09*	2.66 ± 0.28
MANOVA: $F(5, 95)$	1.32, $p > 0.1$	1.73, $p > 0.1$	0.38, $p > 0.1$

Results are expressed as mean ± SEM. Ati, atipamezole.
*Two-tailed $p < 0.05$ vs. saline treatment (Wilcoxon).

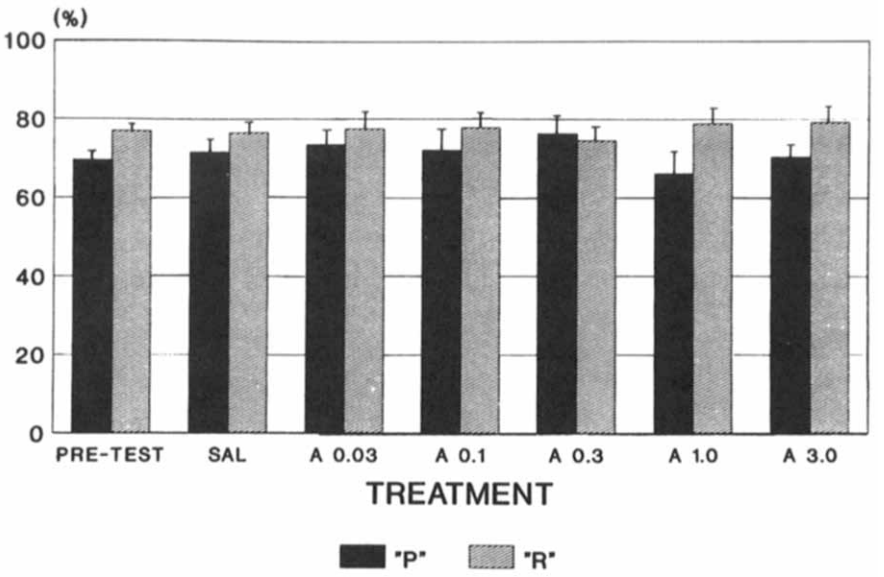

FIG. 1. Effects of atipamezole (0.03–3.0 mg/kg, SC, 25 min before testing) on the discriminative accuracy (percent correct responses) of adult rats in the five-choice serial reaction time task during a 15-min testing period. Results are expressed as mean ± SEM. In the whole population, the percent correct responses did not differ between saline (74.5 ± 1.4)-, atipamezole 0.03 mg/kg (75.9 ± 1.9)-, 0.1 mg/kg (75.6 ± 2.1)-, 0.3 mg/kg (75.3 ± 1.8)-, 1.0 mg/kg (73.9 ± 2.8)-, or 3.0 mg/kg (75.7 ± 2.1)-treated rats. However, atipamezole 0.3 mg/kg slightly increased the percent correct responses in the subpopulation of rats with the poorest discriminative accuracy according to pretest performance. PRE-TEST, the mean of the percent correct responses during the last five training sessions before the drug tests; SAL, saline; A, atipamezole; P, the subpopulation of rats with the poorest pretest discriminative accuracy (n = 8); R, the rest of the rats (n = 12).

sponses during the ITI in rats performing the five-choice serial reaction time task, possibly due to dopaminergic activation (7). Atipamezole slightly increases the turnover of dopamine at the dose of 3.0 mg/kg (13). Therefore, there is a possibility that the increase in perseverative panel pushes induced by atipamezole at this dose could be mediated by dopaminergic mechanisms. In addition, at the 3.0-mg/kg dose a direct α_1-adrenoceptor agonistic effect cannot be excluded either (13,14).

On the other hand, acute administration of moderate to high doses of atipamezole (≥1.0 mg/kg) may induce freezing behavior in a novel situation (personal observations). Furthermore, acute administration of lower doses (0.03–0.3 mg/kg) may reduce exploratory behavior in a novel situation (personal observations). However, in the present experiment rats were already familiar with the testing situation, that is, they had been pretrained for about 3 months before drug testings.

Atipamezole did not increase the latency to collect delivered food pellets from the magazine after a correct response, nor did it increase the number of omissions. In addition, atipamezole tended to decrease the latencies to incorrect responses. Therefore, it is likely that atipamezole did not decrease motivation in the present task.

The effects of atipamezole treatment on the discriminative accuracy (percent correct responses) were modest. At 0.3 mg/kg, atipamezole slightly improved discriminative accuracy only in the subpopulation of rats with the poorest discriminative accuracy according to pretest performance. Lower doses were not effective in adult rats. At 0.3 mg/kg, atipamezole may slightly decrease thalamic oscillation (10,11). On the other hand, the doses (1.0 and 3.0 mg/kg) that have been shown to decrease thalamic oscillation markedly at single-dose administration (10,11) did not improve attention in the present task. The dose–response curves of atipamezole could be explained by the well-known inverted U-shaped relationship between performance vs. arousal (12). Therefore, low doses (0.03–0.1 mg/kg) may not sufficiently increase the level of arousal for optimal performance, whereas high doses (1.0–3.0 mg/kg) may increase the level of arousal too much for optimal performance in the present task.

It is important to note that previously extensive depletion of noradrenaline in the forebrain induced by lesion of the dorsal noradrenergic bundle did not impair the baseline performance of adult rats in the five-choice serial reaction time task that was also used in the present experiment (4). On the other hand, some specific parametric manipulations of the task, such as shortening the rate of presentation of visual stimuli, were found to impair performance of rats, especially dorsal-noradrenergic-bundle-lesioned rats (4). However, lesion models may not be suitable for testing the effectiveness of α_2-adrenergic antagonists because partial lesions may not be effective in young rats and, furthermore, after complete/near complete lesions presynaptic terminals are lost and concomitantly the proposed mechanism of action of α_2-adrenergic antagonists in increasing LC firing and release of noradrena-

line from presynaptic terminals (1,2,5,13) would no longer be effective.

In conclusion, a single-dose administration of atipamezole slightly increased the level of behavioral activation and had a modest effect on selective attention in adult rats. Manipulation of the present task to make it more demanding for adult rats or its use in aged rats are needed in the future experiments to conclude whether pharmacological stimulation of the noradrenergic system using α_2-adrenoceptor antagonists improves attentional functions.

ACKNOWLEDGEMENTS

This study was financially supported by The Finnish Academy of Sciences and The Northern Savo Foundation of The Finnish Cultural Foundation.

REFERENCES

1. Aghajanian, G. K.; VanderMaelen, C. P. α_2-Adrenoceptor-mediated hyperpolarization of locus coeruleus neurons: Intracellular studies in vivo. Science 215:1394–1396; 1982.
2. Aston-Jones, G.; Shipley, M. T.; Ennis, M.; Williams, J. T.; Pieribone, J. A. Restricted afferent control of locus coeruleus neurones revealed by anatomical, physiological and pharmacological studies. In: Heal, D. J.; Marsden, C. A., eds. The Pharmacology of noradrenaline in the central nervous system. New York: Oxford University Press; 1990:187–247.
3. Björklund, A.; Lindvall, O. Catecholaminergic brain stem regulatory systems. In: Plum, F.; Mountcastle, V., eds. Handbook of physiology. The nervous system, vol. IV. Bethesda, MD: American Physiological Society; 1987:155–235.
4. Carli, M.; Robbins, T. W.; Evenden, J. L.; Everitt, B. J. Effects of lesions to ascending noradrenergic neurones on performance of a 5-choice serial reaction task in rats: Implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. Behav. Brain Res. 9:361–380; 1983.
5. Cederbaum, J. M.; Aghajanian, G. K. Noradrenergic neurons of the locus coeruleus: Inhibition by epinephrine and activation by the α-antagonist piperoxane. Brain Res. 112:413–419; 1976.
6. Clark, C. R.; Geffen, G. M.; Geffen, L. B. Catecholamines and attention I: Animal and clinical studies. Neurosci. Behav. Rev. 11:341–352; 1987.
7. Cole, B. J.; Robbins, T. W. Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi on performance of a 5-choice serial reaction time task in rats: Implications for theories of selective attention and arousal. Behav. Brain Res. 33:165–179; 1989.
8. Foote, S. L.; Morrison, J. H. Extrathalamic modulation of cortical function. Annu. Rev. Neurosci. 10:67–95; 1987.
9. McCormick, D. A. Cholinergic and noradrenergic modulation of thalamocortical processing. Trends Neurosci. 12:215–221; 1989.
10. Riekkinen, P. J.; Riekkinen, P., Jr.; Aaltonen, M.; Sirviö, J.; Airaksinen, M.; Lammintausta, R. Relationship between electrocortical arousal and memory deficits in aged rats—effects of atipamezole, α2-antagonist. Psychopharmacology (Berl.) 101(suppl. 1):S47; 1990.
11. Riekkinen, P., Jr.; Sirviö, J.; Jäkälä, P.; Lammintausta, R.; Riekkinen, P. Interaction between the α2-noradrenergic and muscarinic systems in the regulation of neocortical high voltage spindles. Brain Res. Bull. 25:147–149; 1990.
12. Robbins, T. W.; Everitt, B. J. Psychopharmacological studies of arousal and attention. In: Stahl, S. M.; Iversen, S. D.; Goodman, E. C.; eds. Cognitive neurochemistry. New York: Oxford University Press; 1987:135–170.
13. Scheinin, H.; MacDonald, E.; Scheinin, M. Behavioral and neurochemical effects of atipamezole, a novel α2-adrenoceptor antagonist. Eur. J. Pharmacol. 151:35–42; 1988.
14. Virtanen, R.; Savola, J.-M.; Saano, V. Highly selective and specific antagonism of central and peripheral α2-adrenoceptors by atipamezole. Arch. Int. Pharmacodyn. Ther. 297:190–204; 1989.
15. Wilkinson, R. T. Interaction of noise with knowledge of results and sleep deprivation. J. Exp. Psychol. 66:332–337; 1963.