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Short communication

Methoctramine moderately improves memory but pirenzepine disrupts performance in delayed non-matching to position test

Juhana Aura [a,*], Jouni Sirviö [b], Paavo Riekkinen Jr. [a]

[a] *Department of Neuroscience and Neurology, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland*
[b] *A.I. Virtanen Institute, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland*

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Abstract

The present study was designed to investigate the effect of i.c.v. administration of various muscarinic receptor antagonists in rats on memory performance in delayed non-matching to position test. The drugs chosen were the non-selective antagonist scopolamine (3 and 10 μg), the muscarinic M_1 receptor-selective antagonist pirenzepine (10 and 30 μg) and the muscarinic M_2 receptor-selective antagonist methoctramine (2, 5 and 20 μg). Scopolamine delay-independently decreased % correct choices and reduced motor activity. Pirenzepine also delay-independently decreased % correct choices. In contrast, methoctramine 2 μg, but not at 5 or 20 μg, improved slightly, but significantly, % correct performance delay-dependently. The present data suggests that the decrease in activation of inhibitory muscarinic M_2 autoreceptors induced by methoctramine produces a specific improvement of short-term memory at long forgetting delays. © 1997 Elsevier Science B.V.

Keywords: Muscarinic M_1 receptor; Muscarinic M_2 receptor; Scopolamine; Pirenzepine; Methoctramine; (Delay-dependent and -independent); (% correct choice); Alzheimer's disease

1. Introduction

Pharmacological studies have provided evidence for two major subtypes of muscarinic acetylcholine receptors in the brain and peripheral organs; muscarinic M_1 and M_2 receptors that respectively possess high and low affinity for pirenzepine (Hammer et al., 1980; Watson et al., 1985). The anatomical distribution of muscarinic M_1 receptors has been analysed and this receptor subtype is largely restricted to the forebrain areas, such as cortex, hippocampus, nucleus accumbens and striatum in rats (Wamsley et al., 1984; Cortes and Palacios, 1986) and humans (Cortes et al., 1986). Furthermore, muscarinic M_1 receptors are largely post-synaptic. Muscarinic M_2 receptors predominate in the thalamic nuclei and brainstem (Tonnaer et al., 1988). However, inhibitory presynaptic muscarinic M_2 receptors are also found in limbic and cortical regions, and they may regulate cholinergic activity in the forebrain structures (Cortes et al., 1987; Miyoshi et al., 1989; Levey et al., 1991).

Dunnett (1985) has developed an operant delayed non-matching to position task which can measure short-term spatial memory in rats. The advantage of this test is that specific impairments in short-term memory are reflected by a delay-dependent disruption of % correct choice performance, whereas non-specific defects are manifested as % correct choice deficits across all delays (i.e., delay-independent deficits).

Previous studies have indicated that peripheral injection of scopolamine, a subtype non-selective muscarinic antagonist, can cause a delay-independent impairment in % correct choice performance and also disturbs motor activity in the test (Dunnett, 1985; Pitkänen et al., 1995). Interestingly, hippocampal muscarinic receptors may be important for learning and memory, since intrahippocampal administration of scopolamine caused a delay-dependent impairment of short-term memory in rats in the delayed non-matching to position task (Dunnett et al., 1990). On the contrary, administration of scopolamine into the medial prefrontal cortex produced a non-mnemonic and delay-independent performance defect in the delayed non-matching to position test (Dunnett et al., 1990; Herremans et al., 1996), suggesting that cholinergic activation of

* Corresponding author. Tel.: (358-17) 162-696; Fax: (358-17) 162-048; e-mail: aura@jalus.uku.fi

the prefrontal cortex may be a prerequisite for effective performance in this memory test.

Pharmacological studies have also evaluated the effect of peripheral administration of M_1 (Vincent and Sepinwall, 1992; Brandeis et al., 1995) and M_2 (Pike and Hamm, 1995) subtype specific drugs on performance in tests used to assess learning and memory. These studies suggest that stimulation of post-synaptic muscarinic M_1 and inhibition of presynaptic muscarinic M_2 receptors can both enhance acquisition of spatial reference memory in the water maze test. However, interpreting the overall effects of drugs administered before daily water maze training simply in terms of altered memory is problematic, since non-specific effects such as altered arousal or motivation may modify the animals spatial escape performance.

Therefore, we designed the present study to investigate the hypothesis that muscarinic M_1 receptor activation is necessary for accurate performance in memory tests and that inhibition of muscarinic M_2 receptors may stimulate short-term memory performance. To test this hypothesis, we compared the effect of three drugs, scopolamine, which does not differentiate between muscarinic receptor subtypes, pirenzepine, a selective muscarinic M_1 receptor antagonist (Hammer et al., 1980) and methoctramine, a selective muscarinic M_2 receptor antagonist (Melchiorre et al., 1989), on delayed non-matching to position short-term memory performance in rats. We administered all the drugs into the lateral cerebral ventricle to circumvent any possible peripheral side-effects that may be detrimental to performance in this test.

2. Materials and methods

2.1. Animals

Male Han: Wistar rats were used in the present experiments. The rats were housed singly in a controlled environment (temperature: 20 ± 2°C; humidity: 50–60%; light period: 07.00–19.00 h). During training and testing the rats had free access to water but were food restricted to 85% of their free feeding weights. At the beginning of the test the rats were 10 months old.

2.2. Apparatus

The experiments were conducted in four Campden operant chambers with two retractable levers and a food pellet dispenser with 45 mg dustless pellets (Campden Instruments). The operant chambers were under the on-line control of microprocessors (Paul Fray, Cambridge, UK) programmed using Spider (Sirviö et al., 1991).

2.3. Delayed non-matching to position

The rats were trained to the task during 20 min daily sessions using a training schedule, which has been described in detail previously (Sirviö et al., 1991). The final training to the delayed non-matching to position was done in 30 min sessions. The rats were trained to asymptotic level, i.e., when the rats performed at a stable level and did not improve the percent correct total responses (69–75%) during last ten training trials. Each trial of the task consists of three phases: the sample, delay and choice phases, the interval between the trials being 5 s. In the sample phase, a randomly selected right or left lever is presented. If the rat does not respond within 20 s, the sample lever is retracted, and the lights are dimmed for 5 s, after which a new trial is started. Pressing the sample lever starts a delay period (0, 1, 2, 4, 8 or 16 s) during which the magazine of the food pellet dispenser is illuminated. After the delay period, the first nose poke into the magazine starts the choice phase, where the magazine light is turned off and both levers are presented. If the rat pushes the lever opposite to the sample lever, both levers are retracted and a reward pellet is provided. If it makes the wrong choice and pushes the sample lever, both levers are retracted, the lights are turned off for 5 s and no reward is provided. The % correct responses at different delays and the total % correct were utilized as an index of performance accuracy. The percentage of omissions in the sample phase and the latency to press the sample lever (s) were also measured to analyse the effect of treatment on motor function.

2.4. Surgery

After the rats had been trained to an asymptotic level, they were returned for free feeding 7 days prior to surgery. The rats were anaesthetised with chloral hydrate (350 mg/kg i.p.) and positioned in a Kopf stereotaxic frame with incisor bar 3.3 mm below the interaural line. A 22-gauge guide cannula with an internal 28-gauge infusion cannula was unilaterally implanted to the right (50% of the rats) or left (50% of the rats) lateral ventricle at the following co-ordinates: AP = −0.8 mm posterior, L = 1.5 mm lateral to bregma and V = −3.5 mm relative to bregma. The guide cannulae and the supporting screws were covered in dental acrylic cement.

2.5. Drugs

Scopolamine (Sigma, St. Louis, MO, USA) 3 and 10 μg, pirenzepine 10 and 30 μg, and methoctramine 2, 5 and 20 μg (both from Research Biochemicals International, Natick, MA, USA) were dissolved in 0.1 M phosphate buffer (pH 7.4) used as a control vehicle. The doses of the drugs were chosen according to earlier studies (Ohno et al., 1994) and our preliminary studies with the delayed non-matching to position task (data not shown). The drugs were administered 10 min before the testing session during 2.5 min using a minipump. After the infusion (2.5 μl) the drugs were allowed to diffuse for 1 min before replacing the infusion cannulae with stylets. During

the infusions the rats were gently restrained with a blue terry towel. The effects of the drugs were tested every third day in a counter balanced order, i.e., half of the rats received the drugs in an ascending order from the vehicle to the highest dose, and the other half in a descending order from the highest dose to the vehicle.

2.6. Histology

After the last tests the rats were decapitated. The brain area containing the infusion site was placed into 4% formalin in 0.1 M phosphate-buffered saline for 24 h. The tissue was then immersed in 30% sucrose in 0.1 M phosphate buffered saline for 24 h. Serial coronal sections (60 μm) were cut and stained with cresyl violet to determine whether the cannulae had been correctly placed into the lateral ventricle.

2.7. Statistics

The overall effect of treatment on performance was analysed with multivariate analysis of variance. Paired samples *t*-test was used to analyse the effects of different drug doses on the number of omissions, the latency of sample press, total % correct and % correct at different delays. Levene test for variance was used to examine the possible 'bottom' effect in the case of methoctramine 2 μg. Note that the group sizes vary, since the animals having zero responses on any of the delays were left out of the study (for groups scopolamine: $n = 7$, pirenzepine: $n = 12$ and methoctramine: $N = 11$).

3. Results

An overall analysis of % correct values measured after treatment with scopolamine 3 and 10 μg revealed a delay-independent decrease in % correct ($F(2,6) = 4.0$, $P < 0.05$) (Fig. 1, top left). Scopolamine 3 μg decreased total % correct ($F(1,7) = 3.9$, $P < 0.05$), but the higher 10 μg dose did not significantly lower total % correct ($F(1,7) = 1.1$, $P > 0.05$). Scopolamine treatment increased omissions and latency to sample press ($F(2,14) > 3.30$, $P < 0.05$, for both comparisons). Scopolamine 10 μg decreased the number of trials completed and increased the percentage of omissions during the sample phase ($F(1,7) = 9.6$, $P < 0.05$), but scopolamine 3 μg had no effect on the number of trials or omissions ($F(1,7) = 1.3$, $P > 0.05$) (Table 1).

An overall analysis of % correct values measured after treatment with pirenzepine 10 and 30 μg revealed a delay-independent decrease in % correct ($F(2,22) = 3.53$, $P < 0.05$) (Fig. 1, top right). Pirenzepine 30 μg decreased total % correct ($F(1,11) = 6.4$, $P < 0.05$), but the lower 10 μg dose did not significantly diminish total % correct ($F(1,11) = 2.2$, $P > 0.05$). Pirenzepine did not significantly increase omissions ($F(2,22) = 1.83$, $P > 0.05$) but increased the latency to sample press ($F(2,22) = 4.32$, $P < 0.05$) (Table 1).

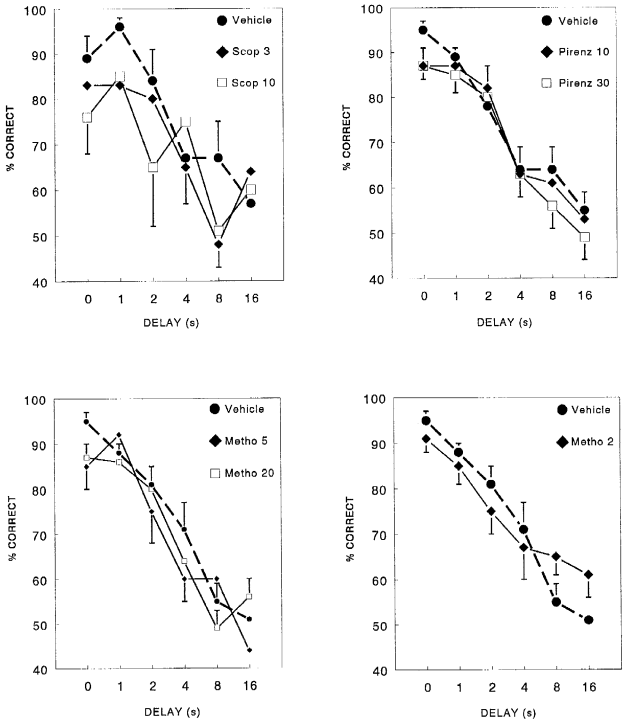

Fig. 1. The percent correct responses of scopolamine (top left) pirenzepine (top right) and methoctramine (bottom left and right) treated rats. Note that scopolamine and pirenzepine induced a delay-independent defect of % correct, and methoctramine 2 μg induced an improvement of % correct at long delays. The results are expressed as the group means at different delays, with bars expressing the standard error of mean. Treatment abbreviations: Scop = scopolamine; Pirenz = pirenzepine; Metho = methoctramine. The drug doses are expressed as μg/rat. The drugs were infused into the lateral ventricle in 2.5 μl vehicle.

An overall analysis of % correct values measured after treatment with methoctramine 2, 5 and 20 μg revealed no significant treatment effect ($F(3, 30) = 1.42$, $P > 0.05$)

Table 1
The percentage of omissions in the sample phase and the latency of sample presses (mean ± standard error of mean)

Drugs	% Omissions	Sample press
Vehicle	25 ± 8	5.2 ± 0.5
Scop 3	31 ± 6	6.2 ± 0.5
Scop 10	49 ± 7 [a]	6.6 ± 0.6
Vehicle	11 ± 3	3.8 ± 0.3
Pirenz 10	15 ± 4	4.4 ± 0.4 [a]
Pirenz 30	17 ± 5	4.8 ± 0.5 [a]
Vehicle	19 ± 6	4.8 ± 0.6
Metho 2	16 ± 6	4.8 ± 0.5
Metho 5	21 ± 7	5.0 ± 0.6
Metho 20	25 ± 6	5.0 ± 0.3

Scop, scopolamine; Pirenz, pirenzepine; Metho, methoctramine. The drug doses are expressed as μg/rat. The drugs were infused into the lateral ventricle

[a] $P < 0.05$.

(Fig. 1, bottom left and right) but the effect of delay * treatment on % correct was almost significant ($F(15,150) = 1.69$, $P = 0.058$). A comparison of the effects of methoctramine 2, 5 and 20 μg on % correct accuracy at different delays revealed that the lowest dose improved accuracy at the longest 8 and 16 s delays ($F(1,10) = 7.5$, $P < 0.05$), significantly at the 16 s delay ($F(1,10) = 4.1$, $P < 0.05$), and non-significantly at the 8 s delay ($F(1,10) = 4.1$, $P = 0.07$). With the lowest dose 2 μg, the variances did not differ significantly between the delays ($F(5,60) = 1.7$, $P > 0.1$), suggesting that there is no significant bottom effect. Methoctramine 5 and 20 μg failed to improve % correct accuracy at the longest delays ($F(1,10) < 1.1$, $P > 0.05$), for both comparisons). Methoctramine 2, 5 and 20 μg did not significantly increase omissions or latency to sample press ($F(3,30) < 0.51$, $P > 0.05$, for both comparisons) (Table 1).

3.1. Histology

All the cannulae of the rats included in the analysis were correctly placed into the left or right ventricle.

4. Discussion

The present data demonstrates that both centrally administered scopolamine 3–10 μg and pirenzepine 30 μg delay-independently impaired performance accuracy, indicating that the drugs do not impair short-term memory per se. Pirenzepine (10 and 30 μg) and scopolamine 10 μg increased sample press latency reflecting a decrease in motor activity during memory testing. Furthermore, scopolamine 10 μg decreased the motivation of the rats, as indicated by the increase in errors of omissions. Our results imply that subtype non-selective and muscarinic M_1 receptor selective antagonists disrupt delayed non-matching to sample performance by affecting non-mnemonic performance factors and that the drugs act via centrally located receptors. Indeed, there is previous evidence to suggest that scopolamine impairs attention and arousal (Jäkälä et al., 1992; Moore et al., 1992) and induces response bias (Pontecorvo et al., 1991; Steckler et al., 1995) which may cause non-specific performance failure. Interestingly, our present data suggests that the effect of scopolamine on the % correct accuracy seems to be due to the antagonism of muscarinic M_1 receptors, since pirenzepine (30 μg) delay-independently disrupted the accuracy of memory performance. Therefore, it is possible that the previously reported deleterious effect on acquisition performance induced by pirenzepine administered prior to daily training in water maze (Hagan et al., 1987; Hunter and Roberts, 1988) was a consequence of non-specific defects in attention or arousal. Similarly, the ability of muscarinic M_1 receptor agonists, such as AF102B (*cis*-2-methylspiro-(1,3-oxathiolane-5,3′)-quinuclidine), to improve escape performance in water maze navigation (Brandeis et al., 1990) may result from improved attention capacity that can aid in the acquisition of spatial navigation strategies.

Our data, showing that methoctramine 2 μg modestly stimulated % correct choice performance selectively at long delays, agrees with previous evidence indicating that treatment with muscarinic M_2 receptor antagonists may enhance performance in tests used to assess learning and memory performance. For example, administration of BIBN 99 (5,11-dihydro-8-chloro-11-[[4-[3-[(2,2-dimethyl-1-oxopentyl)ethylamino]propyl]-1-piperidinyl]acetyl]-6H-pyrido[2,3-*b*][1,4]benzodiazepin-6-one), another muscarinic M_2 receptor antagonist, enhanced acquisition of reference memory in a water maze spatial navigation test in young rats subjected to head trauma (Pike and Hamm, 1995) and in aged, cognitively impaired rats (Quirion et al., 1995). Furthermore, BIBN 99 increased acetylcholine release from the cortex in the aged rats, suggesting that the effect of the drug on water-maze navigation is mediated via blockade of presynaptic muscarinic M_2 autoreceptors (Quirion et al., 1995). However, as explained above, it is difficult to interpret the beneficial effects on performance of drugs administered prior to daily water maze testing simply in terms of improved learning and memory. Therefore, the present results are important, since they reveal a genuine delay-dependent, although modest, improvement, indicating a moderate improvement of short-term memory per se. Interestingly, Packard et al. (1990) showed that the administration of AF-DX 116 ([*R*,*S*]-11-[[2-[diethylamino)-methyl]-1-piperidinyl]acetyl]-5,11-dihydro-[2,3-*b*][1,4]-benzodiazepin-6-one), a muscarinic M_2 receptor antagonist, stimulated memory consolidation in two different radial arm maze tasks. Therefore, the present and previous (Packard et al., 1990) data suggests that M_2 antagonist may improve maintenance of short-term memory and consolidation of reference memory. The failure of higher doses of methoctramine to improve memory function may be attributed to the loss of M_2 vs. muscarinic M_1 receptor selectivity. Indeed, the elegant recent study of Ohno et al. (1995) described how only a dose of methoctramine high enough to block muscarinic M_1 receptors could impair three-panel runaway performance and that AF102B, a muscarinic M_1 receptor agonist, attenuated this performance failure. A second possibility is that higher doses of methoctramine produce too robust an increase in synaptic acetylcholine levels and this disrupts the physiological, phasic variation in the cholinergic signal transmission and introduces ‘noise’ to the system processing spatial short-term memory.

It is difficult to pinpoint the exact location where centrally administered scopolamine and pirenzepine act to disrupt performance in the delayed non-matching to position test, but it is possible that prefrontal muscarinic M_1 receptors mediate, at least to some extent, the impairing effect of these drugs on performance in this test (Dunnett

et al., 1990). On the contrary, several independent studies indicate that the effect of methoctramine may be mediated via enhanced activity of the septo-hippocampal cholinergic projection (Dunnett, 1985; Steckler et al., 1995). First, aspirative lesions of the fimbria-fornix which destroyed cholinergic and non-cholinergic fibers connecting different subcortical nuclei and hippocampus delay-dependently impaired % correct choice performance (Dunnett, 1985). Second, the importance of the cholinergic fibre loss for the memory failure induced by the non-selective fimbria-fornix lesion is supported by a recent study showing that a selective lesion of hippocampal cholinergic fibers induced by an immunotoxin, saporin, rendered the lesioned rats more sensitive to the action of scopolamine to cause a delay-dependent impairment of % correct choice accuracy (Steckler et al., 1995). Third, local microinfusion of scopolamine into the hippocampus delay-dependently impaired % correct choice (Dunnett et al., 1990). Therefore, the present and previous evidence indicate that muscarinic M_2 receptor antagonists may be effective in enhancing behavioural functions that depend on the activity of the septohippocampal cholinergic system.

In conclusion, the present results demonstrate that central administration of a subtype non-selective muscarinic and a selective muscarinic M_1 receptor antagonist both produced a delay-independent non-mnemonic performance defect. In contrast, a selective muscarinic M_2 receptor antagonist facilitated memory performance at long delays. These results provide support for the hypothesis that a muscarinic M_1 receptor agonist or an anticholinesterase when combined with a muscarinic M_2 receptor antagonist could form the basis for effective treatment to activate the function of damaged basal forebrain projection cells.

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