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Progress in Neuro-Psychopharmacology & Biological Psychiatry 30 (2006) 104 – 111
Progress In Neuro-Psychopharmacology & Biological Psychiatry
www.elsevier.com/locate/pnpbp

Influence of chronic treatment with olanzapine, clozapine and scopolamine on performance of a learned 8-arm radial maze task in rats

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Accepted 26 August 2005
Available online 13 October 2005

Abstract

Cognitive deficit is a significant symptom in schizophrenic patients. Use of atypical antipsychotics has been demonstrated to improve some cognitive functions in schizophrenics, as well as in patients with dementia. However, side effects like sedation and muscarinic antagonism induced by these drugs have detracted from this improvement. We are interested in determining the behavioural effect of acute and chronic treatments with olanzapine and clozapine, two atypical antipsychotics, in a paradigm of working memory, and the influence on behavioural response of possible motor effects during test performance. Unspecific muscarinic antagonist scopolamine has been used for comparison. Male Wistar rats were trained on the 8-arm radial maze up to an accuracy level in choice of 80%. Distance travelled in the maze was also measured during test performance. Acute olanzapine, clozapine and scopolamine caused significant impairment of correct performance. Rats treated with olanzapine and clozapine presented a decrease in motor activity level at the same time. After the test at acute dosage, rats were chronically treated for 14 days with olanzapine, clozapine or scopolamine and 24 h after the last dose were again tested in the 8-arm radial maze. Under this procedure, chronic treatment with olanzapine, clozapine and scopolamine did not impair correct task performance and did not modify distance travelled. We concluded that the sedative effect masked a possible effect on working memory after acute administration of olanzapine and clozapine, whereas chronic treatment with olanzapine, clozapine and scopolamine did not adversely affect working memory performance. In the case of scopolamine, it suggests that chronic muscarinic antagonism does not induce memory impairment and for atypical antipsychotics, it suggests that chronic treatment induced a tolerance to acute motor effects of these drugs.

Keywords: Clozapine; Memory; Motor activity; Olanzapine; Radial arm maze; Scopolamine

1. Introduction

In recent years, the characterization of the cognitive deficit profile in schizophrenia has been the focus of many research studies. Data from studies with typical and atypical antipsychotics are still the subject of discussion (Flashman and Green, 2004; Mishara and Goldberg, 2004; Sharma and Antonova, 2003). However, substantial and clinically significant improvements in several domains of cognitive function have been reported with several atypical antipsychotics (Flashman and Green, 2004; Kasper and Resinger, 2003). In these studies, different memory functions have been evaluated using different tests, drug treatments and protocols, and evidence has been demonstrated that clozapine, olanzapine, quetiapine, risperidone and melperone each improve some cognitive functions in schizophrenic patients. (Bilder et al., 2002; Good et al., 2002; Kim and Kang, 2004; Meltzer and McGurk, 1999; Sumiyoshi et al., 2003; Velligan et al., 2002).

Working memory and attention are characteristically impaired in patients with schizophrenia (Elvevag and Goldberg, 2000). Working memory is the ability to maintain or hold temporary, active representations of information for further processing or recall (Ellis and Nathan, 2001). Various different neurotransmitter system have been studied in relation to working memory, dopamine and acetylcholine being among the most studied. In human subjects, increases in dopamine levels facilitate working memory performance (Luciana and Collins, 1997; Muller et al., 1998). Since dopamine agonists may facilitate working memory, it would follow that dopamine receptor antagonists may impair working memory performance. There have been studies using D2 receptor antagonists

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doi:10.1016/j.pnpbp.2005.08.020

sulpiride (Mehta et al., 1999) and haloperidol (Luciana and Collins, 1997) showing an impairment of spatial working memory. Typical antipsychotics like haloperidol, which have D2 antagonistic properties, have been shown to impair working memory in schizophrenic patients, whereas atypical antipsychotics with less D2 antagonistic properties have been shown to improve working memory in schizophrenia (Bilder et al., 2002; Honey et al., 1999; Sharma and Mockler, 1998).

The cholinergic neurotransmitter system also has a well-established relationship with human memory (Ellis and Nathan, 2001). Manipulation of both muscarinic and nicotinic cholinergic receptors may modulate working memory processes leading to an improvement or impairment in performance when an increase or decrease in cholinergic function is induced. Scopolamine, an unspecific muscarinic receptor antagonist, has been used as the "gold standard" in memory impairment in both animal and human studies of working memory (Blokland, 1995).

However, there is experimental evidence that scopolamine induces impairment of new paired-associate learning but not impairment in cued recall of previously learned associates (Atri et al., 2004). In this context, the amnesic effect induced by scopolamine could be related to its influence on sensory/attentional processes (Blokland, 1995). Moreover, acute administration of scopolamine induces hypermotility in activity tests in rats (Braida et al., 1998; Pitsikas et al., 2001). However, scopolamine-induced hypermotility assessed by a specific motor activity task only represents a behavioural effect similar to that caused by many anticholinergic drugs that do not affect cognitive performance (Bushnell, 1987). Generally, animal motor activity is not assessed during cognitive test performance and, therefore, there are no experimental data about the influence of motor alterations on memory impairment induced by scopolamine.

Atypical antipsychotics, such as clozapine and olanzapine, act as dopamine and muscarinic antagonists and, theoretically, these mechanisms of action could account for memory impairment in working memory tasks. In addition, these drugs induce a sedative effect that is one of their adverse effects (Casey, 1996). Hence, the coexistence of different possible mechanisms does not inform us about the effect of atypical antipsychotics on learned working memory tasks. Therefore the authors were interested to determine the effect of acute and chronic administration of atypical antipsychotics, olanzapine and clozapine, on the level of correct performance of a learned task in the 8-arm radial maze in rats and the possible influence of treatment-induced motor activity variations on behavioural task performance. Scopolamine treatment was used for determining unspecific antimuscarinic effect.

2. Methods

2.1. Animals

Albino male Wistar rats (200–250 g) were used. All animals were provided by the "Servicio de Experimentación y Producción Animal" (SEPA) at the University of Cadiz. Rats were housed three per cage during an adaptation period of one week. They were maintained under standard conditions: 12 h light/dark schedule (lights on at 8:00 h) with ad libitum water and at constant temperature (21±1 °C). The animals were on ad libitum feeding for one week and then kept at approximately 70% of ad lib levels. The rats were fed daily after testing. At the start of the experiments, animals were housed individually.

Our study was conducted according to guidelines for use of laboratory animals established by the Federation of European Laboratory Animal Science Associations (FELASA) (Rehbinder et al., 1996). The experimental protocol was approved by the Local Committee for Animal Experimentation of the Faculty of Medicine at the University of Cadiz.

2.2. Drugs

The following drugs were used: olanzapine (2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-*b*][1,5]benzodiazepine) (Lilly, USA), clozapine (8-Chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[*b*,*e*][1,4]-diazepine) (Sigma, St Louis, MO, USA), and (−)-scopolamine hydrochloride (Sigma, St Louis, MO, USA).

Drugs were ip administered: in acute treatment, olanzapine at doses of 1.25, 2.5, 5 and 10 mg/kg; clozapine at doses of 5, 10, 20 and 40 mg/kg; and scopolamine 0.3, 0.6, 1.2 and 2.4 mg/kg. Chronic treatment lasted for 14 days and doses used were: 2.5, 5 and 10 mg/kg for olanzapine and clozapine; 0.3 and 0.6 mg/kg for scopolamine. Saline solution was used as control treatment. Olanzapine and clozapine were dissolved in distilled water by adding 20 μl of acetic acid. Scopolamine was dissolved in normal saline solution.

2.3. Apparatus

The experiments were conducted in an eight-arm radial maze. The maze was made of black painted wood. Each arm of the maze was 10 cm wide and 65 cm in length, with walls extending 20 cm in height. The arms extended from an octagonal centre compartment that was 30 cm in diameter and the same height as the arms. The maze was placed on the floor in a room with fixed extra-maze visual cues. Each arm was baited with a piece of sweetened cereal that was located at the end of the arm. No arm was rebaited after testing began.

After the rat had been placed in the central area of the maze, timing was begun and the rat was free to explore. Arm choices were recorded after the rat entered completely into the arm. If the rat re-entered an arm, it was counted as an error. Thus this procedure tested working memory for cues encountered during a specific session of a task (Olton, 1987). The trial was judged complete when the rat had chosen all 8 baited arms or had spent 10 min. In addition, time required to complete the task and distance travelled in the maze were recorded.

Percentage of correct performance of the task has been used as experimental parameter for evaluating the effects of acute or chronic drug treatments on task execution. This parameter was calculated by the following formula: correct performance

%=number of correct choices/(maximum number of correct choices+number of errors)*100.

2.4. Motor activity measurement

In order to determine possible influences of drug treatment on motor activity we have measured the total distance travelled in the maze for each rat during the test. We have used an automated computer-based system (SMART, Letica) to quantify the trace of rats in the eight-arm radial maze during the test. This system allowed us to monitor each rat in the maze with a CCD camera equipped with a personal computer.

2.5. Experimental procedure

Rats were trained for 10 to 25 sessions until reaching a choice accuracy level of 80%. This parameter was calculated by the following formula: choice accuracy %=number of correct choices/(number of correct choices+number of errors)*100. The day before testing (both acute and chronic), the rats were completely deprived of food.

We conducted two experiments in order to test acute and chronic effect of drug treatment. For the acute experiment, each rat was placed into the maze for testing 30 min after intraperitoneal drug injection. For the chronic experiment, conducted after the acute test, rats returned to restricted food schedule for 14 days. During this period, chronic drug treatment was administered, once a day, and the test was performed one day after the last dose.

2.6. Statistical analysis

The results are expressed as mean±S.E.M. of each experimental parameter. Statistical analysis was performed using a one-way ANOVA followed by the Dunnett test for comparisons with control group. A value of $p<0.05$ was considered to be significant.

3. Results

3.1. Correct performance of the task

At the range of dose used, acute administration of olanzapine and clozapine induced a dose-related sedative effect in treated animals in contrast with animals treated with scopolamine or saline. This adverse effect was reflected on the results obtained with these animals.

Thirty minutes after administration, olanzapine induced a dose-related reduction in percentage of correct performance of the task ($F_{(4,42)}=22.796$; $p<0.001$) (Fig. 1). This reduction was statistically significant at doses of 2.5, 5 and 10 mg/kg ($p<0.05$). Acute administration of clozapine also induced a dose-related reduction in percentage of correct performance of the task ($F_{(4,34)}=24.864$; $p<0.001$) (Fig. 1). This reduction was statistically significant at doses of 5, 10, 20 and 40 mg/kg ($p<0.05$). Similarly, acute administration of scopolamine also induced a significant reduction in percentage of correct performance of the task ($F_{(4,42)}=6.106$; $p<0.005$) (Fig. 1). However, this scopolamine effect was not dose-dependent but

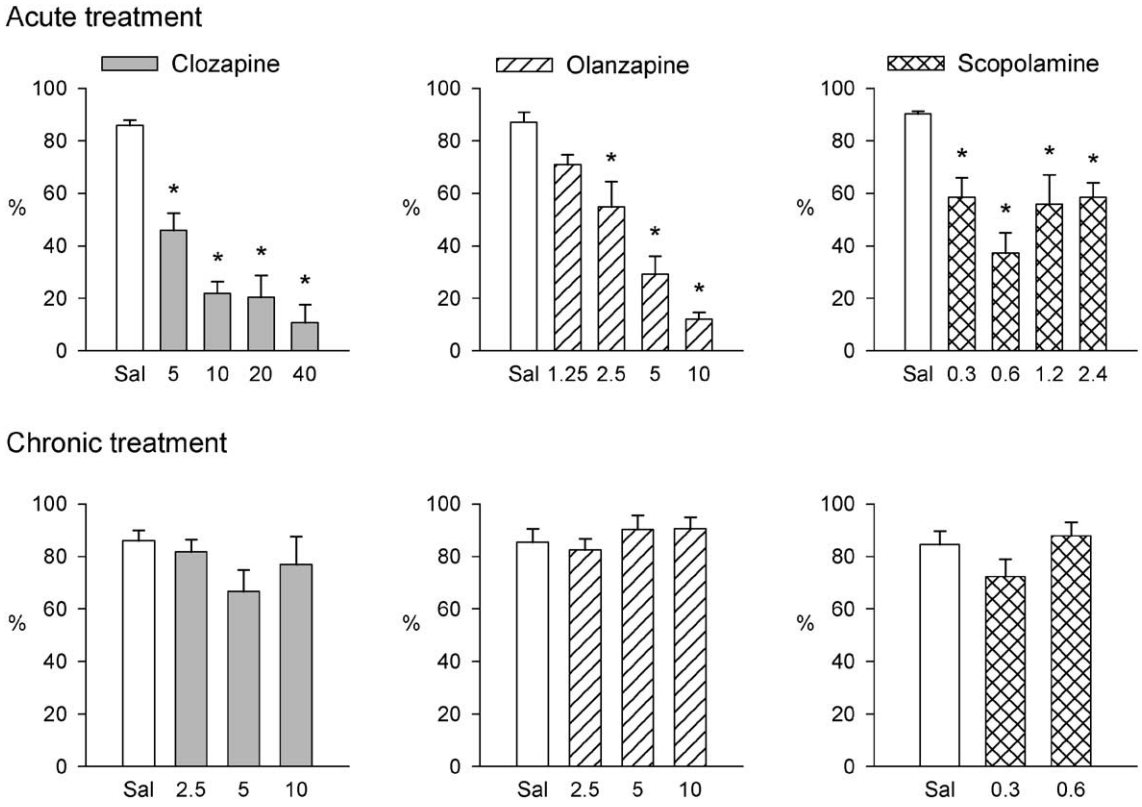

Fig. 1. Effect of acute and chronic administration of clozapine, olanzapine and scopolamine on percentage of correct performance of the task (doses in mg/kg, $n=7$–10; *$p<0.05$ vs. saline).

Table 1
Effect of acute and chronic administration of olanzapine, clozapine and scopolamine on time taken to complete the task

Drug	*n*	Time (s)	Drug	*n*	Time (s)	Drug	*n*	Time (s)
Acute treatment								
Saline	9	238.33±57.88	Saline	8	162.50±29.30	Saline	9	128.00±10.69
OLZ 1.25	9	370.00±73.11	CLO 5	8	557.63±30.69[a]	SCO 0.3	10	484.60±46.59[a]
OLZ 2.5	10	509.30±60.73[a]	CLO 10	8	600.00±0.00[a]	SCO 0.6	10	556.40±40.37[a]
OLZ 5	10	600.00±0.00[a]	CLO 20	8	600.00±0.00[a]	SCO 1.2	10	450.70±58.33[a]
OLZ 10	9	600.00±0.00[a]	CLO 40	7	600.00±0.00[a]	SCO 2.4	8	578.38±21.62[a]
Chronic treatment								
Saline	8	278.00±54.10	Saline	7	203.71±23.56	Saline	8	255.00±47.91
OLZ 2.5	7	272.71±54.23	CLO 2.5	7	271.86±60.06	SCO 0.3	8	301.00±54.56
OLZ 5	8	316.00±66.69	CLO 5	8	423.63±68.67	SCO 0.6	8	206.25±19.34
OLZ 10	8	260.75±64.56	CLO 10	7	312.83±32.51			

Results expressed as mean±S.E.M.
[a] $p<0.05$ vs. saline.

was similar for all doses used. This reduction was statistically significant at doses of 0.3, 0.6, 1.2 and 2.4 mg/kg ($p<0.05$) when compared to saline control.

After chronic treatment, rats were tested 24 h after the last dose of the drug, and any sign of sedation was observed during performance. Chronic treatment with olanzapine, clozapine and scopolamine did not induce statistically significant modifications in percentage of correct performance of the task (Fig. 1).

3.2. Time taken to complete the task

The sedative effect observed in animals treated acutely with olanzapine and clozapine was reflected in the time taken to complete the task. Olanzapine ($F_{(4,42)}=9.964$; $p<0.001$) and clozapine ($F_{(4,34)}=98.435$; $p<0.001$) induced a significant increase of time taken to complete the task (Table 1). Thus, the increase was significant for olanzapine at doses of 2.5, 5 and 10 mg/kg ($p<0.05$), reaching maximum time allowed (600 s) at 5 and 10 mg/kg. Clozapine induced significant increases at all doses used ($p<0.05$) reaching maximum time allowed (Table 1). However, although scopolamine considerably increased the time taken to complete the task ($F_{(4,42)}=18.236$; $p<0.001$) (Table 1), these increases were significant at all doses ($p<0.05$) but no experimental group mean reached the maximum time allowed (600 s).

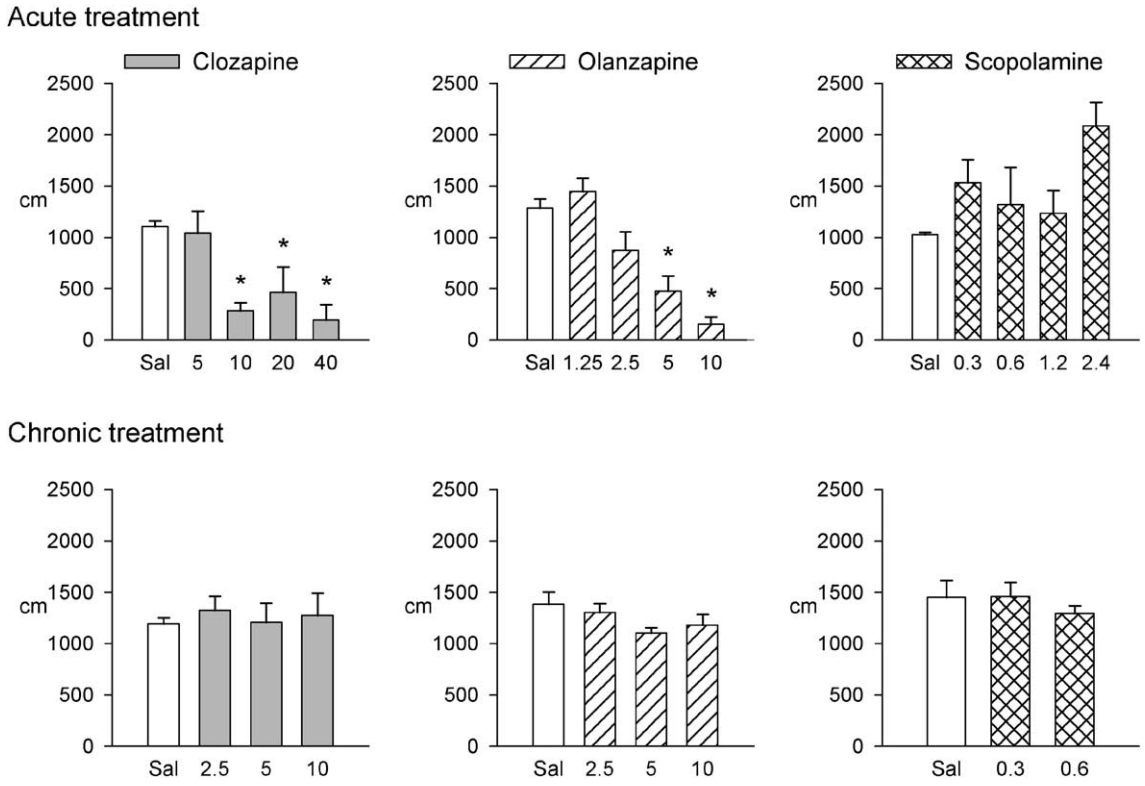

Fig. 2. Effect of acute and chronic administration of clozapine, olanzapine and scopolamine in total distance travelled in the maze (doses in mg/kg, $n=7–10$; *$p<0.05$ vs. saline).

Chronic treatment with olanzapine, clozapine or scopolamine did not induce statistically significant modifications (Table 1) in time taken to complete the task.

3.3. Total distance travelled in the maze

Acute drug administration of olanzapine, clozapine and scopolamine induced variations in total distance travelled during test performance. Thirty minutes after olanzapine administration, there was a marked dose-dependent decrease in this parameter ($F_{(4,42)}=15.892$; $p<0.001$). Decreases in distance travelled were statistically significant at 5 and 10 mg/kg ($p<0.05$) (Fig. 2). Clozapine administration induced a more marked decrease than olanzapine ($F_{(4,34)}=6.335$; $p<0.005$). Decreases in distance travelled were statistically significant at 10, 20 and 40 mg/kg ($p<0.05$) (Fig. 2).

Scopolamine-treated rats did not show any sign of sedation during performance of the test. In addition, there was a non-statistically significant increase in distance travelled during the test for all doses used ($F_{(4,42)}=1.837$; $p=0.140$) (Fig. 2).

Chronic treatment with olanzapine, clozapine and scopolamine did not produce significant variations in distance travelled in the maze (Fig. 2).

4. Discussion

4.1. Effects induced by acute administration of olanzapine, clozapine and scopolamine on radial arm performance

Our results have shown an impairment of radial arm performance in rats acutely treated with olanzapine, clozapine and scopolamine. On the one hand, in animals treated with olanzapine and clozapine, this effect could be closely related to its sedative effect as indicated by the significant dose-dependent increase in time taken to complete the task and the significant decrease of distance travelled in the maze. On the other hand, in animals treated with scopolamine, memory impairment could not be related to a sedative effect since animals did not show any sign of sedation. In addition, scopolamine increased significantly the time taken to complete the task and tended to increase the total distance travelled. Therefore, the negative effect could be explained as an impairment of working memory.

From our acute experiment protocol, we cannot determine the real effect of olanzapine and clozapine administration on 8-arm radial maze performance. This effect is masked by its dose-related sedative effect (Casey, 1996), reflected by clinical signs and results observed in distance travelled. At the range of dose used we cannot establish an unequivocal effect of olanzapine and clozapine in this working memory test in rats. On this point, there have been few studies of the influence of antipsychotics (typical or atypical) on working memory in animals. Haloperidol was or was not able to induce retention-impairment in a spatial working memory task in 8-arm radial maze, depending on the administration schedule used (Beatty and Rush, 1983). In addition, haloperidol had a greater negative effect on reference memory than on working memory in a spatial cone field task (Blokland et al., 1998). In contrast, different results have been obtained in the 8-arm radial maze using low doses of haloperidol, clozapine and risperidone. Low doses of clozapine induced a significant decrease of choice accuracy levels, whereas very low doses of haloperidol and risperidone alone did not affect memory performance (Addy and Levin, 2002). The effect of drug treatment on the learning process has also been evaluated. Thus, acute treatment with sertindole and quetiapine did not affect spatial performance in the Morris water maze; clozapine impaired performance in the first 2 days but showed no effect in the last 2 days; ziprasidone, olanzapine, risperidone and haloperidol markedly impaired spatial performance (Skarsfeldt, 1996).

In another working memory test, a delayed response task in rats, low doses of haloperidol, clozapine and risperidone induced a delay-independent impairment, but sertindole treatment did not show an effect (Didriksen, 1995). In another delayed non-match to sample task, olanzapine and risperidone acute oral administration after the information phase reduced the number of errors during the retention phase, whereas clozapine, ziprasidone and haloperidol failed to affect this parameter (Wolff and Leander, 2003). Moreover, clozapine impaired delayed response performance in monkeys in a spatial delayed response test (Murphy et al., 1997). However, low doses of iloperidone improved choice accuracy of rats in a delayed not-matching-to-position paradigm, whereas low doses of clozapine and haloperidol had no effect (Gemperle et al., 2003). Rosengarten and Quartermain (2002b) have reported that prenatal administration of haloperidol, risperidone and quetiapine disrupted spatial learning in adult rats whereas olanzapine did not. In these animals, short-term retention was only affected by haloperidol and risperidone. Therefore, heterogeneity of data and differences in protocols and schedules used do not give us a clear idea of the possible effect of olanzapine and clozapine on radial arm performance.

In contrast to olanzapine and clozapine, scopolamine tended to increase total distance travelled in the maze. This finding does not correlate with performance deficit, thus suggesting a clear scopolamine-induced working memory impairment. In this respect, scopolamine has been widely used as standard in memory impairment studies and it has been argued that cognitive deficit observed after its use is related directly to a decrease in central cholinergic function (Blokland, 1995). In addition, the increase in distance travelled observed in these rats could reflect scopolamine-induced hyperlocomotion (Mueller and Peel, 1990).

4.2. Effects induced by chronic administration of olanzapine, clozapine and scopolamine on radial arm performance

Chronic treatment with none of the drugs used induced any significant variation in either radial arm performance, time taken or distance travelled. This finding suggests that chronic administration induces a tolerance to motor side effects of acutely administered drugs.

Regarding this lack of effect of chronic olanzapine and clozapine treatment on this working memory task, Rosengarten

and Quartermain (2002a) have reported the effect of oral chronic treatment, administered in drinking water, with low doses of typical (haloperidol), and atypical (clozapine, risperidone and olanzapine) antipsychotics on acquisition and retention on a working memory task in young and old rats. Haloperidol and risperidone disrupted learning in both young and old rats, whereas clozapine impaired acquisition only in old rats; retention was impaired by haloperidol, risperidone and clozapine in both groups of rats and olanzapine had no effect on either parameter. In that study only haloperidol and risperidone induced motor behaviour impairment in old rats. Another study using a prior chronic treatment with olanzapine and haloperidol for 90 days (not for 45 days) reported impaired learning performance in water maze (Terry et al., 2002). However, the negative effect with haloperidol was greater than that induced by olanzapine. Recently, Schroder et al. (2005) reported that prior haloperidol and clozapine chronic treatments both impaired short-term recognition memory in rats. These authors suggest a relationship between memory impairment and modifications of hippocampal oxidative stress induced by drug treatment (Schroder et al., 2005). This negative effect of clozapine and haloperidol on hippocampal oxidative stress has not been reported for olanzapine (Reinke et al., 2004). Nevertheless, in the present study we have used different doses, administration route and experimental schedule. We have tested the effect of chronic treatment with olanzapine and clozapine at higher doses, injected intraperitoneally, once a day, after acquisition period. Therefore, apart from acute motor effects, it is reasonable to think that these drug treatments had no effect on performance of a previously learned task.

4.3. Effect induced by chronic antimuscarinic effect on radial arm performance

In relation to olanzapine and clozapine antimuscarinic effect and its possible negative influence on working memory performance, results obtained with scopolamine chronic treatment suggests that chronic muscarinic antagonism is not related to memory impairment of learned tasks. On the one hand, there is tolerance to the motor stimulatory effect of scopolamine (Rosic et al., 1980) as a consequence of a significant upregulation of muscarinic receptors in the cortex, hippocampus and striatum (Russell et al., 1986). On the other hand, scopolamine chronic treatment before the acquisition period of the Morris water maze improved the rate of learning (Abdulla et al., 1993). This paradoxical effect could be associated with both muscarinic receptor upregulation and a scopolamine-induced increase in acetylcholine release in the ventral hippocampus after acute administration (Mishima et al., 2000). These results could explain the lack of negative effect of chronic scopolamine treatment on working memory performance.

In this regard, systemic administration of olanzapine and clozapine has been reported to increase acetylcholine release in the rat hippocampus by up to 1500% and 500%, respectively (Shirazi-Southall et al., 2002). However, typical antipsychotics (haloperidol, thioridazine and chlorpromazine) and other atypical ones (risperidone and ziprasidone) produced only a modest increase of about 50–100% above basal release. It must be taken into account that these increases were obtained by microdialysis techniques using inhibition of acetylcholine degradation with neostigmine. Moreover, these authors used selective antagonists of 5-HT2A, 5-HT2C, 5-HT6, D2 receptors, α1- and α2-adrenoceptors, and a selective agonist of 5-HT1A, to test their ability to increase acetylcholine efflux, but none of them had an effect similar to that observed with olanzapine and clozapine. These types of receptor cover a large part of the known receptor profile for olanzapine and clozapine, although these authors did not test selective antagonists of muscarinic or histaminergic receptors that might account for the large increase in acetylcholine release. An action via histaminergic receptors is improbable since antagonism of hippocampal H1 receptors was reported to induce a working memory impairment that was reversed by increasing acetylcholine levels (Nakazato et al., 2000). There is evidence pointing to a muscarinic receptor involvement, mainly through the M2 subtype. Although olanzapine has shown potent non-specific antimuscarinic properties in vitro, muscarinic occupancy by olanzapine has been studied in vivo in schizophrenic patients using SPECT. That study revealed a potent and subtype-selective muscarinic antagonism with an M2 anatomical distribution pattern (Raedler et al., 2000). In addition, a novel selective M2 antagonist produced a dose-related increase in acetylcholine release in rat hippocampus, cortex and striatum and improved performance in working memory in monkeys (Carey et al., 2001). In addition, Weiner et al. (2004) have reported that *N*-desmethylclozapine, the principal metabolite of clozapine, but not clozapine itself, is a potent and efficacious muscarinic receptor agonist. These findings suggest a theoretical beneficial effect on the memory process.

5. Conclusion

We conclude that the sedative effect masked a possible effect of acute administration of olanzapine and clozapine on a learned working memory task. However, chronic treatment with olanzapine, clozapine and scopolamine did not negatively affect working memory performance. In the case of scopolamine, it suggests that chronic muscarinic antagonism does not induce memory impairment; and for atypical antipsychotics, it suggests that chronic treatment induced a tolerance to acute motor effects of these drugs. However, many animal and long term treatment studies are necessary to determine how atypical antipsychotics can affect working memory performance and other aspects of cognitive processes.

Acknowledgments

We thank Blanca Lorena Cobo Realpe, Pedro Romero Gómez and Enrique Fierro Carballo for their technical assistance. This study was partially funded by Eli Lilly (Spain). Thanks also to Rodrigo Escobar for Project Management.

References

Abdulla, F.A., Calaminici, M.R., Stephenson, J.D., Sinden, J.D., 1993. Chronic treatments with cholinoceptor drugs influence spatial learning in rats. Psychopharmacology (Berl) 111, 508–511.

Addy, N., Levin, E., 2002. Nicotine interactions with haloperidol, clozapine and risperidone and working memory function in rats. Neuropsychopharmacology 27, 534–541.

Atri, A., Sherman, S., Norman, K.A., Kirchhoff, B.A., Nicolas, M.M., Greicius, M.D., Cramer, S.C., Breiter, H.C., Hasselmo, M.E., Stern, C.E., 2004. Blockade of central cholinergic receptors impairs new learning and increases proactive interference in a word paired-associate memory task. Behav. Neurosci. 118, 223–236.

Beatty, W.W., Rush, J.R., 1983. Spatial working memory in rats: effects of monoaminergic antagonists. Pharmacol. Biochem. Behav. 18, 7–12.

Bilder, R.M., Goldman, R.S., Volavka, J., Czobor, P., Hoptman, M., Sheitman, B., Lindenmayer, J.P., Citrome, L., McEvoy, J., Kunz, M., Chakos, M., Cooper, T.B., Horowitz, T.L., Lieberman, J.A., 2002. Neurocognitive effects of clozapine, olanzapine, risperidone, and haloperidol in patients with chronic schizophrenia or schizoaffective disorder. Am. J. Psychiatry 159, 1018–1028.

Blokland, A., 1995. Acetylcholine: a neurotransmitter for learning and memory? Brain. Res. Rev. 21, 285–300.

Blokland, A., Honig, W., Prickaerts, J., 1998. Effects of haloperidol and D-amphetamine on working and reference memory performance in a spatial cone field task. Behav. Pharmacol. 9, 429–436.

Braida, D., Paladini, E., Griffini, P., Lamperti, M., Colibretti, L., Sala, M., 1998. Long-lasting antiamnesic effect of a novel anticholinesterase inhibitor (MF268). Pharmacol. Biochem. Behav. 59, 897–901.

Bushnell, P.J., 1987. Effects of scopolamine on locomotor activity and metabolic rate in mice. Pharmacol. Biochem. Behav. 26, 195–198.

Carey, G.J., Billard, W., Binch III, H., Cohen-Williams, M., Crosby, G., Grzelak, M., Guzik, H., Kozlowski, J.A., Lowe, D.B., Pond, A.J., Tedesco, R.P., Watkins, R.W., Coffin, V.L., 2001. SCH 57790, a selective muscarinic M(2) receptor antagonist, releases acetylcholine and produces cognitive enhancement in laboratory animals. Eur. J. Pharmacol. 431, 189–200.

Casey, D.E., 1996. Side effect profiles of new antipsychotic agents. J. Clin. Psychiatry 57 (Suppl 11), 40–45.

Didriksen, M., 1995. Effects of antipsychotics on cognitive behaviour in rats using the delayed non-match to position paradigm. Eur. J. Pharmacol. 281, 241–250.

Ellis, K.A., Nathan, P.J., 2001. The pharmacology of human working memory. Int. J. Neuropsychopharmacol. 4, 299–313.

Elvevag, B., Goldberg, T.E., 2000. Cognitive impairment in schizophrenia is the core of the disorder. Crit. Rev. Neurobiol. 14, 1–21.

Flashman, L.A., Green, M.F., 2004. Review of cognition and brain structure in schizophrenia: profiles, longitudinal course, and effects of treatment. Psychiatr. Clin. North Am. 27, 1–18.

Gemperle, A.Y., McAllister, K.H., Olpe, H.R., 2003. Differential effects of iloperidone, clozapine, and haloperidol on working memory of rats in the delayed non-matching-to-position paradigm. Psychopharmacology (Berl) 169, 354–364.

Good, K.P., Kiss, I., Buiteman, C., Woodley, H., Rui, Q., Whitehorn, D., Kopala, L., 2002. Improvement in cognitive functioning in patients with first-episode psychosis during treatment with quetiapine: an interim analysis. Br. J. Psychiatry Suppl. 43, s45–s49.

Honey, G.D., Bullmore, E.T., Soni, W., Varatheesan, M., Williams, S.C., Sharma, T., 1999. Differences in frontal cortical activation by a working memory task after substitution of risperidone for typical antipsychotic drugs in patients with schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 96, 13432–13437.

Kasper, S., Resinger, E., 2003. Cognitive effects and antipsychotic treatment. Psychoneuroendocrinology 28 (Suppl. 1), 27–38.

Kim, Y.T., Kang, B.J., 2004. A preliminary study of the comparative effects of olanzapine and risperidone on cognition in schizophrenia. Hum. Psychopharmacol. 19, 265–269.

Luciana, M., Collins, P.F., 1997. Dopamanergic modulation of working memory for spatial but not object cues in normal humans. J. Cogn. Neurosci. 9, 330–347.

Mehta, M.A., Sahakian, B.J., McKenna, P.J., Robbins, T.W., 1999. Systemic sulpiride in young adult volunteers simulates the profile of cognitive deficits in Parkinson's disease. Psychopharmacology (Berl) 146, 162–174.

Meltzer, H.Y., McGurk, S.R., 1999. The effects of clozapine, risperidone, and olanzapine on cognitive function in schizophrenia. Schizophr. Bull. 25, 233–255.

Mishara, A.L., Goldberg, T.E., 2004. A meta-analysis and critical review of the effects of conventional neuroleptic treatment on cognition in schizophrenia: opening a closed book. Biol. Psychiat. 55, 1013–1022.

Mishima, K., Iwasaki, K., Tsukikawa, H., Matsumoto, Y., Egashira, N., Abe, K., Egawa, T., Fujiwara, M., 2000. The scopolamine-induced impairment of spatial cognition parallels the acetylcholine release in the ventral hippocampus in rats. Jpn. J. Pharmacol. 84, 163–173.

Mueller, K., Peel, J.L., 1990. Scopolamine produces locomotor stereotypy in an open field but apomorphine does not. Pharmacol. Biochem. Behav. 36, 613–617.

Muller, U., von Cramon, D.Y., Pollmann, S., 1998. D1- versus D2-receptor modulation of visuospatial working memory in humans. J. Neurosci. 18, 2720–2728.

Murphy, B.L., Roth, R.H., Arnsten, A.F., 1997. Clozapine reverses the spatial working memory deficits induced by FG7142 in monkeys. Neuropsychopharmacology 16, 433–437.

Nakazato, E., Yamamoto, T., Ohno, M., Watanabe, S., 2000. Cholinergic and glutamatergic activation reverses working memory failure by hippocampal histamine H1 receptor blockade in rats. Life Sci. 67, 1139–1147.

Olton, D.S., 1987. The radial arm maze as a tool in behavioral pharmacology. Physiol. Behav. 40, 793–797.

Pitsikas, N., Rigamonti, A.E., Cella, S.G., Locatelli, V., Sala, M., Muller, E.E., 2001. Effects of molsidomine on scopolamine-induced amnesia and hypermotility in the rat. Eur. J. Pharmacol. 426, 193–200.

Raedler, T.J., Knable, M.B., Jones, D.W., Lafargue, T., Urbina, R.A., Egan, M.F., Pickar, D., Weinberger, D.R., 2000. In vivo olanzapine occupancy of muscarinic acetylcholine receptors in patients with schizophrenia. Neuropsychopharmacology 23, 56–68.

Rehbinder, C., Baneux, P., Forbes, D., van Herck, H., Nicklas, W., Rugaya, Z., Winkler, G., 1996. FELASA recommendations for the health monitoring of mouse, rat, hamster, gerbil, guinea pig and rabbit experimental units. Report of the Federation of European Laboratory Animal Science Associations (FELASA) Working Group on Animal Health accepted by the FELASA Board of Management, November 1995. Lab. Anim. 30, 193–208.

Reinke, A., Martins, M.R., Lima, M.S., Moreira, J.C., Dal-Pizzol, F., Quevedo, J., 2004. Haloperidol and clozapine, but not olanzapine, induces oxidative stress in rat brain. Neurosci. Lett. 372, 157–160.

Rosengarten, H., Quartermain, D., 2002a. The effect of chronic treatment with typical and atypical antipsychotics on working memory and jaw movements in three- and eighteen-month-old rats. Prog. Neuro-Psychopharmacol. Biol. Psychiat. 26, 1047–1054.

Rosengarten, H., Quartermain, D., 2002b. Effect of prenatal administration of haloperidol, risperidone, quetiapine and olanzapine on spatial learning and retention in adult rats. Pharmacol. Biochem. Behav. 72, 575–579.

Rosic, N., Bokonjic, D., Overstreet, D.H., 1980. Task-dependent development of tolerance to scopolamine. Pharmacol. Biochem. Behav. 13, 183–186.

Russell, R.W., Ehlert, F.J., Hwa, J.J., 1986. Relation between behaviorally augmented tolerance and upregulation of muscarinic receptors in the CNS: effects of chronic administration of chronic administration of scopolamine. Psychopharmacology (Berl) 88, 33–39.

Schroder, N., de Lima, M.N., Quevedo, J., Dal Pizzol, F., Roesler, R., 2005. Impairing effects of chronic haloperidol and clozapine treatment on recognition memory: possible relation to oxidative stress. Schizophr. Res. 73, 377–378.

Sharma, T., Antonova, L., 2003. Cognitive function in schizophrenia. Deficits, functional consequences, and future treatment. Psychiatr. Clin. North Am. 26, 25–40.

Sharma, T., Mockler, D., 1998. The cognitive efficacy of atypical antipsychotics in schizophrenia. J. Clin. Psychopharmacol. 18, 12S–19S.

Shirazi-Southall, S., Rodriguez, D.E., Nomikos, G.G., 2002. Effects of typical and atypical antipsychotics and receptor selective compounds on acetyl-

choline efflux in the hippocampus of the rat. Neuropsychopharmacology 26, 583–594.

Skarsfeldt, T., 1996. Differential effect of antipsychotics on place navigation of rats in the Morris water maze. A comparative study between novel and reference antipsychotics. Psychopharmacology (Berl) 124, 126–133.

Sumiyoshi, T., Jayathilake, K., Meltzer, H.Y., 2003. The effect of melperone, an atypical antipsychotic drug, on cognitive function in schizophrenia. Schizophr. Res. 59, 7–16.

Terry, A.V. Jr., Hill, W.D., Parikh, V., Evans, D.R., Waller, J.L., Mahadik, S.P., 2002. Differential effects of chronic haloperidol and olanzapine exposure on brain cholinergic markers and spatial learning in rats. Psychopharmacology (Berl) 164, 360–368.

Velligan, D.I., Newcomer, J., Pultz, J., Csernansky, J., Hoff, A.L., Mahurin, R., Miller, A.L., 2002. Does cognitive function improve with quetiapine in comparison to haloperidol? Schizophr. Res. 53, 239–248.

Weiner, D.M., Meltzer, H.Y., Veinbergs, I., Donohue, E.M., Spalding, T.A., Smith, T.T., Mohell, N., Harvey, S.C., Lameh, J., Nash, N., Vanover, K.E., Olsson, R., Jayathilake, K., Lee, M., Levey, A.I., Hacksell, U., Burstein, E.S., Davis, R.E., Brann, M.R., 2004. The role of M1 muscarinic receptor agonism of *N*-desmethylclozapine in the unique clinical effects of clozapine. Psychopharmacology (Berl) 177, 207–216.

Wolff, M.C., Leander, J.D., 2003. Comparison of the effects of antipsychotics on a delayed radial maze task in the rat. Psychopharmacology (Berl) 168, 410–416.