SEPARATELY ADMINISTERED SELECTIVE DOPAMINE D₁ AND D₂ ANTAGONISTS DO NOT AFFECT PERFORMANCE ACCURACY IN A CONDITIONAL RESPONSE TASK IN RATS

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Abstract

The acute effects of separately administered selective dopamine D₁ and D₂ receptor antagonists - SCH 23390 and raclopride, respectively - on the performance of a conditional response task were tested in a preliminary (small group, n = 6) study in rats. The effects of four doses of each drug (SCH 23390: 0-0.1 mg/kg; raclopride: 0-0.4 mg/kg) were tested consecutively in the same rats, using blinded Latin square designs. The conditional response task was performed in T-maze using tactile discrimination stimuli and spatial responses. The number of errors was not affected by the dopamine antagonists. However, the average response latency was significantly increased after administration of the highest doses of SCH 23390 and raclopride compared to saline. It seems that antagonism at either D₁ or D₂ receptors alone is not sufficient to produce impairments in performance accuracy using the present conditional response task.

Key words: Dopamine antagonist; D1, D2, Conditional response, Tactile discrimination

INTRODUCTION

In several neuropsychiatric disorders, e.g. schizophrenia and obsessive compulsive disorder, the neuropsychological symptoms include impaired performance on tasks requiring behavioral shifting (Pantelis et al. 1999; Chamberlain et al. 2005), pointing to deficits in cognitive or behavioral flexibility. In order to develop drugs that can stimulate behavioral flexibility in such brain disorders, more knowledge about the neurochemical basis of cognitive and behavioral flexibility in the healthy brain is called for.

The present study was a component of a series of studies scrutinising the neurochemical substrate of behavioral flexibility in a rat model. In the present study the acute effects of selective dopamine D₁ and D₂ antagonists on performance of a conditional response task were

investigated. Conditional response tasks - or conditional discrimination tasks - are tasks in which different stimuli, presented one at a time, indirectly signal which of a number of different response possibilities are correct. Performance of conditional response tasks makes high demands on the ability to flexibly shift behavioral responses guided by an external signal.

It has been demonstrated in experimental lesion studies, that performance of various conditional response tasks is mediated by the prefrontal cortex (Goldman & Rosvold 1970; Stamm 1973; Podbros et al. 1980; Stamm 1973) as well as caudate-putamen (Robbins et al. 1990; Adams et al. 2001) in rats and monkeys. The prefrontal cortex and caudate-putamen have extensive neural connections and are both dependent upon dopaminergic

neurotransmission via D₁ and D₂ receptors for normal functioning (Robbins 2005). Non-selective dopamine D₁ and D₂ antagonists such as the neuroleptic -flupenthixol (Robbins et al. 1990) and dopamine releasers such as *d*-amphetamine and phencyclidine (Moerschbaeche & Thompson 1980; Dunn et al. 2005; Dunn & Killcross 2006a) have been found to impair the performance of various conditional response tasks. However, information about the relative importance of the different individual dopamine receptors for performance of conditional tasks is sparse (however, see Dunn & Killcross 2006b), and the acute effect of selective dopamine antagonists administered alone has not been tested in conditional tasks. Consequently, the aim of the present study was to scrutinize the effect of two selective dopamine receptor antagonists - the D₁ receptor antagonist SCH 23390 and the D₂ receptor antagonist raclopride - on the performance of a conditional response task in rats. The conditional response task of our own design was performed in T-maze using tactile discrimination stimuli and spatial responses. The hypothesis was that the D₁ and/or the D₂ antagonist would impair performance accuracy on the task through disturbance of prefrontal and/or striatal functioning, resulting in an increase in the number of errors. Based on earlier findings (e.g. Liao et al. 2001; Choi et al. 2005), it was also expected that high doses of both drugs would increase the response latency due to locomotor suppression. The drug doses were chosen because comparable doses have been demonstrated to exert significant effects on the performance of a delayed response task in the rat, a type of task considered mediated by the prefrontal cortex like conditional response tasks (Didriksen 1995).

MATERIALS AND METHODS

Subjects

Six male Wistar rats (Charles River Labs., Wilmington, MA), weighing approximately 250 g at the start of the experiment, were used in the experiments. Female rats were not included in the study to avoid confounding variables associated with oestrus cycle variation. The rats were housed singly in Macrolone Type III cages (Scanbur, Køge, Denmark) with aspen bedding (Tapvei Estonia, Harjumaa, Estonia) in a temperature (21 °C +/- 1 °C) and humidity (45-65%)-controlled environment and maintained at a 12 h/12 h light/dark cycle. Testing was conducted during the light phase. Daily food intake of Altromin 1314 (Altromin GmbH, Lage, Germany) was restricted, and the rats were maintained at 90 % of their free-feeding weight, adjusted for growth throughout the

studies (7 g per week). Acidified (citric acid) tap water was available *ad libitum*. The present study was approved by the relevant Danish authorities and was in compliance with the European Communities Council directive 86/609/EEC and the recently revised Appendix A to the Council of Europe Convention ETS 123.

Apparatus

The apparatus was a beige-coloured Plexiglas T-maze (width 10 cm; height 20.5 cm; length of start alley 38 cm; length of response arms 30.5 cm) without start box. The maze had no bottom, allowing differently textured pads to be placed underneath the maze as tactile discrimination stimuli. Three cm into each of the response arms a vertical flap was placed covering the width of the arm. The rats had to run through the flap in order to gain access to food. Each flap was made of plastic coated white paper and suspended from a horizontal string. The maze was placed in a dimly lit room without ceiling lights. Light sources were placed symmetrically around the maze.

Habituation

Figure 1 shows an overview of the experiments. Habituation started on the second day of the food deprivation period. All rats were subjected to one 20 min habituation session in the T-maze on each of three consecutive days. During habituation, the rats were allowed to explore the maze freely with mashed food (a mixture of 1/3 rat chow, 2/3 water was used during the entire study) present in a bowl at the end of both maze arms. The experimenter was located at the end of the start alley. In all phases of the experiment, the rats were weighed before and after the sessions to determine their food intake in the maze. After each habituation session all rats were subjected to a 5 min handling session.

Shaping

During shaping trials, the rats were released in the start alley of the maze and allowed to run into one of the response arms of their own choice and eat of the food reward. Mashed food was present in both maze arms. Each rat received 20 shaping trials in each session, and one session was performed per day. Shaping continued until a performance criterion demanding that 90 % of the trials of the session should be completed in less than 10 s for two sessions in a row (6-9 sessions). Upon meeting the criterion, the individual rats continued with training on the conditional response task.

Conditional response task

The task was a conditional response task with tactile discrimination stimuli and spatial responses, performed in a T-maze. Under the maze - in the choice point area - was, in each trial, placed one out of two different black pads; a grooved cardboard pad or a smooth rubber pad. For half of the rats, the grooved cardboard pad signalled that a response to the right was correct, and the smooth rubber pad signalled that a response to the left was correct. For the other half of the rats, the opposite stimulus-response pairs were used. The food bowl in the incorrect maze arm was covered by a plastic mesh. The mesh made the food inaccessible to the rats, but because of the flaps situated in the response arms, the rats could not see the mesh until they had run through the flap and a clear choice of arm therefore had been made. No rat withdrew its response before it had run all the way through the flap. The rats were allowed to eat the food if they entered the correct arm, but were picked up if they entered the incorrect maze arm after access to food was denied by the mesh. All instances of rearing in the choice point were noted, since the rats could possibly see the mesh placed in one of the food bowls if rearing. However, rearing happened so rarely that it had no significant effect on the results. The type of pad placed under the choice point in each trial was determined by a pseudo-randomisation procedure, in which each of the pads was presented for the same number of trials in each session, but maximally used for three trials in a row. The rats were trained for 20 trials in each session and were subjected to one session per day. Training continued until a performance criterion of 90 % correct for each of two consecutive sessions was observed (11-15 sessions). Thereafter, the individual rats received a training pause until all rats had reached the criterion. Subsequently, all rats received one common training session, and since all rats performed at criterion level on this session, drug testing was started in the following week.

Drug testing

The acute effects of the selective dopamine D₁ receptor antagonist SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1 *H*-3-benzazepine hydrochloride) (Sigma-Aldrich, Germany) and the selective D₂ receptor antagonist raclopride (*S*(-)-raclopride L-tartrate) (Sigma-Aldrich, Germany) on performance of the described conditional response task were examined. In the main experiments, the effects on task performance of three doses of each drug and saline were tested consecutively in the same rats. In these experiments, the experimenter was always kept ignorant regarding the

treatment given to individual rats on the drug-testing day. The rats in the first experiment received 0 mg/kg, 0.05 mg/kg, 0.1 mg/kg or 0.2 mg/kg raclopride dissolved in isotonic saline. The drug doses given to the individual rats on each of the four drug-testing days were randomised according to a Latin square design. After the raclopride experiment had been finished, the same rats in a second experiment received 0 mg/kg, 0.025 mg/kg, 0.05 mg/kg or 0.1 mg/kg SCH 23390 dissolved in isotonic saline, on one weekly drug-testing day for each of four consecutive weeks, also following a Latin square design. Both drugs were injected i.p. 30 min prior to testing on the task, and injection volumes were 10 ml/kg body weight. In the post-injection pause the rats were placed in their home cages in a rodent container in the testing room. Response latencies were measured for each trial, and if a rat failed to respond for 5 min, the testing of that rat was stopped. In all drug-testing weeks, the rats also received a non-blinded saline session on the day before the drug testing session. Apart from not being blinded, these saline sessions were performed in the same way as the drug sessions, and served as an extra control obtained under more similar circumstances than the blinded control (the same stimulus randomisation sequence and the same training level as the drug testing session). When considered necessary for keeping the rats to perform at criterion level, all the rats received a training session without injections on the day before the saline session. Generally, a novel randomisation of the discrimination stimuli was used in each session. However, an identical randomisation was used in the saline session and the drug-testing session performed in the same week. In between the two main experiments, the effect of 0.4 mg/kg raclopride on task performance was tested blindly. Half of the rats received this dose and half received saline in a randomised design. In all experiments there were at least four days between the drug testing sessions and the next saline or drug testing session.

Data and data analysis

For each rat data consisted of the number of errors and average response latency on the task after receiving saline or different doses of SCH 23390 or raclopride. In cases where a rat failed to respond for the maximum allowed time (5 min), the average response latency of that rat was set to 16.9 s ((5 min + 19 · 2 s) / 20 trials), in order to obtain a minimum response latency for the statistical analysis. Naturally, error scores for rats that failed to respond were not included in the data set. Data for the four doses blindly tested in the main experiments (including 0 mg/kg) were compared by one-way ANOVA ($p=0.05$ significance level) for each of the drugs. In

addition, data for each of the blindly tested drug doses were compared by t-test to data for the non-blinded saline session performed by the same rats in the same week, and following the same stimulus randomisation ($p=.05$ significance level, one-tailed analysis, testing the hypothesis that the drugs increased the number of errors and the response latency). Comparisons with the non-blinded saline controls were performed because ANOVA did not reveal any significant effects of the drugs comparing the blinded doses and blinded control. Before concluding that the drugs had no effects on task performance, we wanted to test if any significant effects could be found using these comparisons. If a significant difference was revealed by one-tailed t-test, it was noted if the difference also reached significance in two-tailed analysis.

Table 1. Experimental overview.

1. Habituation

3 sessions

2. Shaping

Response latency criterion

6-9 sessions

3. Acquisition of conditional task

Performance criterion

11-15 sessions

4. Raclopride testing I

Four doses (0-0.2 mg/kg)

Latin square, blinded

Non-blinded saline sessions

Four weeks

5. Raclopride testing II

Two doses (0 and 0.4 mg/kg)

Randomised, blinded

One day

6. SCH 23390 testing

Four doses (0-0.1 mg/kg)

Latin square, blinded

Non-blinded saline sessions

Four weeks

RESULTS

Neither for SCH 23390, nor for raclopride, where any significant differences revealed by ANOVA between the

number of errors obtained for the three blindly tested drug doses and the blinded control, and there were no statistical trends in that direction (Fig. 2A, Fig. 3A). For the average response latency there was a strong trend towards an effect of SCH 23390 ($p=.056$, ANOVA) only (Fig. 2B, Fig. 3B).

Figure 2A. Number of errors.

When comparing data obtained for the blindly tested drug doses to data obtained in the associated non-blinded saline sessions (see section 2.7), there was no significant effect of raclopride (Fig. 2A, Fig. 2B), but the average response latency was significantly increased for 0.05 mg/kg SCH 23390 ($p<.05$, one-tailed) and 0.1 mg/kg SCH 23390 ($p<.05$, one-tailed and two-tailed) compared to saline (Fig. 3B).

Figure 2B. Response latency.

Also in the additional blinded experiment, comparing the effect of 0.4 mg/kg raclopride to the effect of saline on task performance, the average response latency was

significantly increased for 0.4 mg/kg raclopride compared to saline ($p < .001$, one-tailed and two-tailed) (not shown). This was due to all raclopride-treated rats failing to respond on the task for 5 min. There were rats that failed to respond on the task after 0.05 mg/kg SCH 23390 (one rat out of six) and 0.1 mg/kg SCH 23390 (two rats out of six) as well as after 0.4 mg/kg raclopride (three rats out of three). All rats that failed to respond did so from the first trial of the drug session.

Figure 3A. Number of errors.

DISCUSSION

None of the tested doses of the selective dopamine receptor antagonists - the D_1 antagonist SCH 23390 and the D_2 antagonist raclopride - affected the performance accuracy in the conditional response task (Fig. 2A, Fig. 3A). Thus, the hypothesis that rats would be impaired in performance of the task after acute administration of the drugs was not confirmed. Comparable doses have been demonstrated to exert significant effects on the performance of a delayed response task in the rat, a type of task considered mediated by the prefrontal cortex like conditional response tasks (Didriksen 1995). The investigation included six rats, which is a rather small group size. Since there were no statistical trends towards impaired performance accuracy after administration of the drugs - neither comparing the four drug doses nor comparing the drug doses individually to associated saline sessions - it is unlikely that an increased group size would lead to another result of the experiment. Therefore the experiment was not extended with a second group of rats.

In the experiment testing the effect of SCH 23390, there was a strong trend towards an effect of the drug on response latency, but the effect did not reach statistical significance. However, the highest doses of SCH 23390 significantly increased the average response time compared

to associated non-blinded saline sessions (Fig. 3B), partly due to rats that failed to respond on the task after drug administration. For the highest dose of SCH 23390 (0.1 mg/kg) 1/3 of the rats failed to respond on the task for five minutes. Also all rats receiving 0.4 mg/kg raclopride - in a separate experiment - failed to respond on the task.

Figure 3B. Response latency.

For that reason higher drug doses were not tested. Drug-induced increases in response latency were not unexpected, as both drugs in comparable doses have previously been found to have a locomotor suppressing effect (Liao et al. 2001; Choi et al. 2005). For neither drug did rats with clearly increased average response time after drug administration seem to make more errors than saline treated rats; e.g. for rats capable of completing the testing, the two rats with the highest average response times for each of the drugs had error scores at the typical saline level (0-10% errors). However, it cannot be excluded that the rats that failed responding on the task after administration of the antagonists were also impaired in cognitive functions necessary for performing the task. Likewise, it cannot be excluded that the antagonists could potentially have an improving effect on task performance, since improvement in performance accuracy would be difficult to show in the present study due to ceiling effect.

It is noteworthy, that the selective D_1 and D_2 antagonists did not impair performance accuracy of the present task while the non-selective D_1 and D_2 antagonist -flupenthixol have been demonstrated to produce impairments in performance accuracy in a visual as well as an auditory version of a conditional response task (Robbins et al. 1990). The task used by Robbins et al. (1990) was a conditional task involving temporal frequency discriminations. Performance of the task was also impaired after dorsal striatum lesions. There are several possible explanations for why impairments were

found in the study of Robbins and co-workers, while none were found in the present study.

Firstly, the kind of conditional task - e.g. the discriminations and/or responses involved - could be of importance for determining if the task performance is dependent upon activation of D₁ and D₂ receptors. While the task used by Robbins and colleagues included visual or acoustic temporal frequency discriminations and (spatial) panel pressing responses, the present task included tactile discriminations and (spatial) maze running responses. However, the type of discrimination stimuli and responses involved in conditional response tasks are generally considered to be relatively unimportant regarding the involvement of the dopaminergic system, the prefrontal cortex and the striatum in performance of these tasks. It is believed that it is the conditional response principle that makes these tasks susceptible to prefrontal-striatal brain lesions and dopaminergic manipulations (Adams et al. 2001; Robbins et al. 1990).

Secondly, a perhaps more likely explanation is that action by -flupenthixol at other receptors than D₁ and D₂ receptors (-flupenthixol is also a D₃, D₄, 5-HT_{2A}, 5-HT_{2C} and 1-adrenergic receptor antagonist (Glaser et al. 1998)), or the combination of D₁ and D₂ antagonism exerted by the drug, may have been responsible for the performance impairing effect of -flupenthixol seen in the study of Robbins and his colleagues. Performance of conditional tasks has also been demonstrated to be disrupted by the dopamine releasers *d*-amphetamine and phencyclidine (Moerschbaecher & Thompson 1980; Dunn et al. 2005; Dunn & Killcross 2006a). Acute selective D₁ antagonists and chronic - but not acute - selective D₂ antagonists can attenuate disruption of conditional task performance induced by *d*-amphetamine (Dunn & Killcross 2006b). This shows that D₁ and D₂ receptors do have a role in at least some conditional tasks. Dunn and Killcross (2006b) have argued that there possibly is an interactive relationship between D₁ and D₂ antagonists in this attenuation of task disruption. Thus, it also seems likely, that only acute combined dopamine D₁ and D₂ receptor antagonists - and not selective D₁ or D₂ antagonists - impair conditional tasks performance when administered alone.

It will be relevant in future studies to test the acute effect of a combination of the selective D₁ and D₂ receptor antagonists SCH 23390 and raclopride, as well as the non-selective D₁/D₂ receptor antagonist -flupenthixol on performance in the present conditional response task, using doses that impaired performance accuracy in other conditional tasks. In addition, the acute effect of selective

D₁ and D₂ antagonists as used in the present study should be tested on performance in other conditional tasks.

In conclusion, the selective dopamine D₁ and D₂ antagonists did not impair the performance accuracy on the present conditional response task in the doses tested. The doses were not increased, as the highest of the presently tested doses caused rats to fail to respond on the task, probably due to the locomotor suppressing effect of the drugs. It seems likely that antagonism at only D₁ receptors or at only D₂ receptors - following acute administration of antagonists - may not be sufficient to impair performance accuracy in conditional tasks. However, it can not be ruled out that it is not all types of conditional response tasks where performance is dependent upon D₁ and/or D₂ receptors.

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