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Pharmacology, Biochemistry and Behavior 76 (2003) 1–8
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Haloperidol and clozapine affect social behaviour in rats postnatally lesioned in the ventral hippocampus

Axel Becker*, Gisela Grecksch

Faculty of Medicine, Institute of Pharmacology and Toxicology, Otto-von-Guericke University, Leipziger Str. 44, 39120 Magdeburg, Germany

Received 14 January 2003; received in revised form 25 April 2003; accepted 8 May 2003

Abstract

Neonatal ibotenic acid lesion of the ventral hippocampus results in altered patterns of social behaviour. After puberty, lesioned animals spent less time in social interaction and the nonaggressive/aggressive behaviour ratio shifted towards increased aggressiveness. In this study, the effects on social behaviour of the neuroleptic drugs haloperidol (HAL) and clozapine (CLO) after acute and subchronic treatment were studied. Seven-day-old rats were lesioned and social behaviour was tested at the age of 13 weeks. Drug effects were tested after acute (HAL 0.025 mg/kg, CLO 1.0 mg/kg) and subchronic (10 injections, HAL 0.075 mg/kg, CLO 5.0 mg/kg) administration. For comparison, diazepam (DZP, 0.5 mg/kg) was used in the acute experiment. After acute administration, DZP had no effect on social behaviour in sham-lesioned rats, but nonaggressive behaviour increased significantly in lesioned animals. CLO and HAL did reduce the time sham-lesioned rats spent in social contact, and CLO also increased % nonaggressive behaviour in lesioned rats. Here, HAL had no effect. Subchronic administration did not alter social behaviour in sham-lesioned animals. However, CLO increased the time lesioned animals spent in social interaction, whereas HAL had an effect on nonaggressive behaviour. The results of this study indicate that the lesion model is sensitive to differentiated effects of classical neuroleptic drugs such as HAL and atypical neuroleptic drugs like CLO. It might be a useful tool in the search for potential neuroleptic drugs.

Keywords: Schizophrenia; Rat; Ventral hippocampus; Social behaviour; Haloperidol; Clozapine; Diazepam

1. Introduction

Schizophrenia is characterised by a highly variable phenomenology, treatment response and illness course (Andreasen and Carpenter, 1993). Its aetiology was discussed regarding multiple factors including genetic predisposition, psychosocial factors, changes in neurotransmitter systems, viral vectors and neuroanatomical alterations (Ashton, 1992; Duncan et al., 1999). These authors proposed that schizophrenia unfolds in three distinct phases. An initial insult to the CNS (Stage 1) is followed by facilitated sensitisation in the dopaminergic system (Stage 2). Such endogenous sensitisation could progress to a self-limiting degenerative phase (Stage 3) manifested by persistent morbidity, resistance to treatment and clinical deterioration. Growing evidence suggests the neurodevelopmental abnormalities in the processes of neurogenesis, neuronal migration, differentiation, synaptogenesis and myelination basically contribute to schizophrenia (Bogerts, 1993; Heyman and Murray, 1992; Weinberger, 1995; Harrison, 1997; Arnold, 1999; Pilowsky et al., 1993; Lieberman, 1999; Bogerts, 1997). It was proposed that the heterogeneity of schizophrenia might be the product of complex interactions of different factors acting at different stages in ontogenesis.

The aetiological and clinical heterogeneity of schizophrenia is difficult to explain by a unitary animal model. Thus, a set of valid models focusing on specific aspects of this disease is needed. Recently, it was concluded (Cannon and Murray, 2000) that the study of the neonatal and childhood precursors of schizophrenia allow the possibility of prevention and early intervention in at least some cases of the disorder. Lipska and Weinberger (1993) introduced a schizophrenia model in rats based on neonatal ventral hippocampus lesion, which induced a delayed emergence of alterations in the processes of neurotransmission and behaviour. Such a lesion might mirror an initial insult (Stage 1, see above) to the CNS. This model addresses an

* Corresponding author. Tel.: +49-391-6715351; fax: +49-391-67190149.
E-mail address: axel.becker@medizin.uni-magdeburg.de (A. Becker).

0091-3057/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0091-3057(03)00139-4

important schizophrenic aspect of postpubertal onset (Lipska et al., 1995). Recently, we reported on social behaviour in rats lesioned with ibotenic acid in the ventral hippocampus at Day 7 of life (Becker et al., 1999). At the age of 13 weeks, neonatally lesioned rats showed a significantly decreased time spent in social interaction. The ratio nonaggressive/aggressive behaviour was shifted towards increased aggressiveness. It was concluded that social interaction might be a relevant tool to study potential neuroleptic drugs. To prove this assumption, we tested the effects of haloperidol (HAL) and clozapine (CLO) after acute and subchronic administration. For comparison, the effects of diazepam (DZP) were studied after acute injection.

2. Materials and methods

The experiments were conducted in accordance with the National Act on the Use of Experimental Animals (Germany).

2.1. Animals

Pairs of Sprague–Dawley rats (Shoe:SPRD) were housed in Macrolone (Type IV) cages under controlled climatic conditions (temperature 20 ± 2 °C, relative air humidity 55–60%, light/dark cycle of 12:12, lights on at 6:00 a.m.). The rats were fed with commercial pellets (Altromin 1316) and tap water ad libitum. After birth, the number of pups per litter was balanced to 8 to 10. Male pups were weaned 21 days after birth and housed litterwise in the same room as where the parents were sheltered. Following Week 4 of life, rats were fed with Altromin 1324 pellets.

Naive male Sprague–Dawley rats, which were used for the investigation of drug effects on locomotor activity, were housed in groups of five under identical conditions to those specified above.

2.2. Surgery

Bilateral ventral hippocampal lesions were produced in pups on their Postnatal Day 7 according to the procedure described by Becker et al. (1999). Male pups of each litter were randomised to either lesion group or control group. Anaesthetised pups (hypothermia on wet ice for 15 min) were placed in a modified stereotactic apparatus (David Kopf Instruments). An incision was made in the skin overlying the skull and 0.3 μl ibotenic acid (Tocris, UK) (15 μg/μl), dissolved in sterile physiological saline, was infused bilaterally into the ventral hippocampus (AP = − 2.8 mm, ML = 3.5 mm, VD = 5.0 mm, coordinates relative to bregma) at a rate of 0.15 μl/min. For control, the solvent was infused (sham-lesioned). The injector was withdrawn 3 min after completion of the infusion and the incision was closed with surgical tissue adhesive (Histoacryl, Braun Surgical, Melsungen, Germany). The pups were placed on an electric warming pad and then returned to their parents after body temperature had recovered.

2.3. Behavioural testing

Animals were tested in the social interaction test 12 weeks after surgery when the animals were 13 weeks old.

2.4. Social interaction test

The general design of the model was adapted from File (1993). The test arena ($100 \times 100 \times 40$ cm) was made of smooth black polyvinyl chloride. The animals' behaviour was scored and recorded in an adjacent room via a video camera mounted on the ceiling of the experimental room. Lighting in the test room was 30 lx and was diffused to prevent shadow in the test arena.

Ten days prior to testing, rats were housed singly (Makrolon type III cages with food and water ad libitum). The cages were located together in racks so that auditory and olfactory contact was maintained.

Two days prior to testing, the rats were familiarised with the test arena. Each animal was given two 7-min trials to explore the apparatus. Between each test, the apparatus was cleaned and wiped. To standardise the olfactory background, 0.1% disinfectant Teta Extra, Fresenius Germany, containing 2.5% polyhexanide and 8% didecyldimethylammonium, was used for cleaning before the first and after each test.

The day prior to testing, rats were allocated to test partners on the basis of surgery (i.e. lesioned with ibotenic acid or infusion of sterile saline solution), pretreatment (DZP, HAL and CLO, respectively, or saline) and body weight. The difference between the two partners was within 20 g.

Social behaviour was tested for a 7-min period. The time spent in social interaction was scored and separated into nonaggressive (sniffing, following and grooming the partner, social play) and aggressive behaviour (kicking, boxing, wrestling, aggressive grooming, biting). Each pair was used once only. Per test 6–16 pairs were used.

The drug dosages and pretreatment times were selected based on previous published reports (Sams-Dodd, 1999; Compton et al., 2001) and sets of pilot studies. First, the acute effects of DZP (Faustan, Arzneimittelwerk Dresden, Germany, 0.75 mg/kg), HAL (Haldol, Janssen-Cilag, Germany, 0.075 mg/kg) and CLO (Leponex, Wander Pharma, Germany, 1 mg/kg) on social behaviour were studied 30 min (DZP and HAL) or 1 h (CLO) after injection. In a set of pretests, it was confirmed that these are the maximum doses without effect on motor/locomotor behaviour as measured for 20 min after single injection (pretreatment times, see above) in the Moti-Test apparatus (Table 1) (TSE, Bad Homburg, Germany). Illumination in the testing room was 30 lx.

Table 1
Activity time (time spent in horizontal + time spent in vertical activity) measured over 20 min (MOTI test)

Treatment	n	Activity time (s)	F	P
Acute				
Saline	10	694.7 ± 23.5	0.54	.47
DZP (0.75 mg/kg)	10	669.0 ± 25.9		
Saline	10	678.7 ± 25.4	1.86	.19
HAL (0.075 mg/kg)	10	635.0 ± 19.5		
Saline	10	638.5 ± 14.6	0.37	.55
CLO (1.0 mg/kg)	10	652.1 ± 16.7		
Subchronic				
Saline	12	523.8 ± 15.1	0.11	.74
HAL (0.075 mg/kg)	13	531.17 ± 17.0		
Saline	12	565.2 ± 22.5	1.1	.3
CLO (5.0 mg/kg)	13	601.2 ± 27.0		

Acute effects were measured 30 min after DZP and HAL injection, and pretreatment time for CLO was 1 h. Subchronic treatment was over a period of 10 days with one injection per day; effects on activity time were measured 24 h after the final injection, 30 lx, mean ± S.E.M.

Second, effects of HAL (0.025 and 0.075 mg/kg) and CLO (1.0 and 5.0 mg/kg) were studied after subchronic administration. When the rats were housed singly, they received one injection daily (8:00–10:00 a.m.) over a period of 10 days. Twenty-four hours after the final injection, social behaviour was scored. At this time, the substances were expected to have been cleared.

The substances were dissolved in isotonic saline. To dissolve CLO, three drops of Tween 80 were added. Control animals received the solvent in an identical manner. Injections were given intraperitoneally in a volume of 1 ml/100 g body weight.

2.5. Histological verification

After completion of the experiments, the rats were deeply anaesthetised with an overdose of chloral hydrate. After decapitation, the brains were quickly removed from the cranium and fixed in 8% formalin. After soaking in 10% sucrose solution, frozen coronal sections (6 μm) were cut and subjected to morphological inspection under a light microscope. The atlas of Paxinos and Watson (1997) served as a reference. Animals with incorrect or unilateral lesion were excluded from the study.

2.6. Statistics

The statistical analysis was based on analysis of variance and post hoc Bonferroni test (SPSS+ software). The significance threshold was set at .05.

3. Results

In our experiments, social behaviour was measured in terms of time spent in social interaction, percentage of nonaggressive behaviour and percentage of aggressive behaviour. The latter components are complementary and therefore, we focus on the percentage of nonaggressive behaviour.

3.1. Effects of DZP, CLO and HAL after acute administration

We did not find any significant difference between saline-injected sham-lesioned animals from the three experimental groups and between saline-injected lesioned animals, respectively. Therefore, the controls were pooled.

As published earlier (Becker et al., 1999), neonatal lesion in the ventral hippocampus resulted in decreased time spent in social contact [sham/saline vs. lesioned/saline; $F(1,23) = 8.876$, $P = .007$, Fig. 1A–C]. The percentage of nonaggressive behaviour was significantly reduced in IBS (ibotenic acid) lesioned animals [$F(1,23) = 111.75$, $P < .001$].

3.1.1. Effect of acute DZP

We did analyse quantitative aspects of social behaviour (i.e. time spent in social interaction) and qualitative parameters (i.e. the ratio nonaggressive/aggressive behaviour). It was found a significant difference between the four experimental groups in time spent in social interaction [$F(3,37) = 6.05$, $P = .002$] and in % nonaggressive behaviour [$F(3,37) = 60.15$, $P < .001$].

After single injection of 0.75 mg/kg DZP, there was no change in the time spent in social interaction either in the sham-lesioned (saline vs. DZP, $P = .9$) or in the lesioned group (saline vs. DZP, $P = .33$). Percentage of nonaggressive behaviour in lesioned animals increased significantly (saline vs. DZP, $P = .006$). There was no difference between saline-injected, sham-lesioned rats and DZP-treated lesioned animals ($P = .9$, Fig. 1A).

3.1.2. Effects of acute CLO

Time spent in social interaction: $F(3,42) = 5.17$, $P = .004$

% Nonaggressive behaviour: $F(3,42) = 36.19$, $P < .001$

Acute administration of 1.0 mg/kg CLO exerted similar effects. Time spent in social interaction was reduced in treated sham-lesioned rats compared with saline-injected controls ($P = .017$) and percentage of nonaggressive behaviour increased considerably (lesioned saline vs. CLO, $P = .01$) in lesioned rats after CLO injection resulting in insignificant differences between saline-injected, sham-lesioned animals and CLO-treated lesioned rats ($P = .9$, Fig. 1B). This means that the substance normalised this aspect of social behaviour.

3.1.3. Effects of acute HAL

Time spent in social interaction: $F(3,39) = 4.93$, $P = .005$

% Nonaggressive behaviour: $F(3,39) = 26.6$, $P < .001$

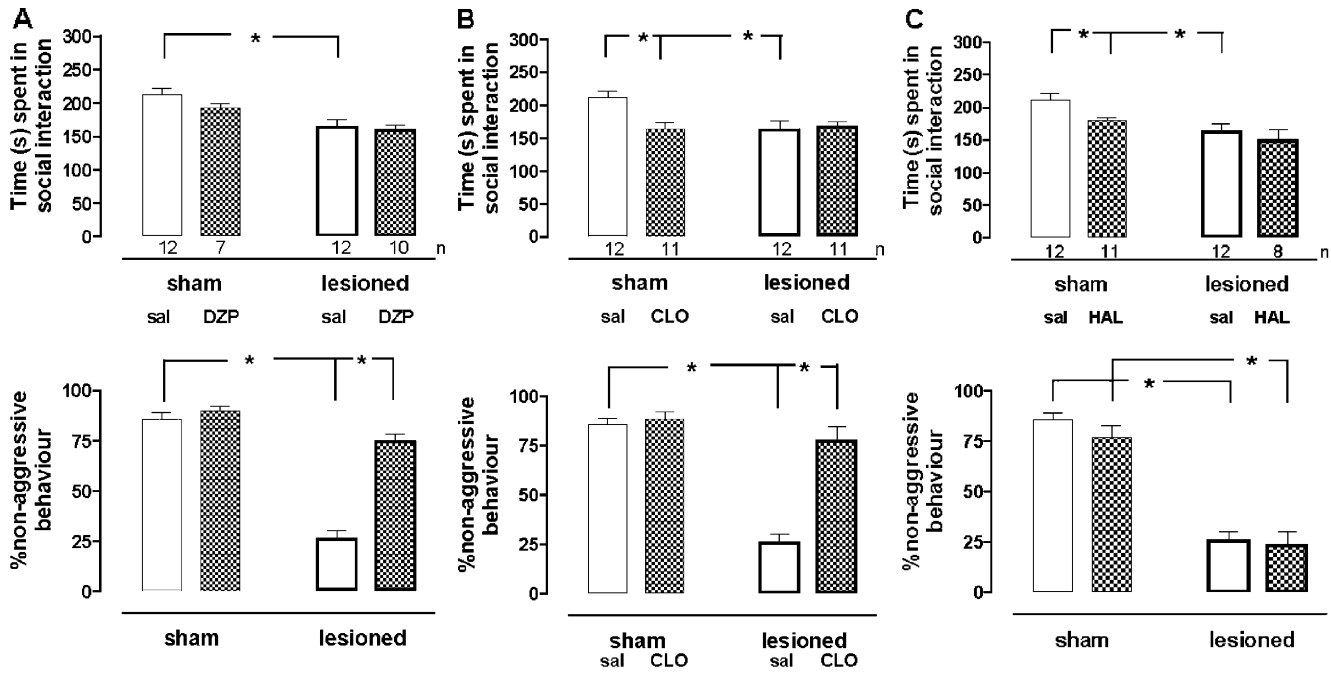

Fig. 1. Social behaviour in 13-week-old rats postnatally lesioned with ibotenic acid in the ventral hippocampus. Time (s) spent in social interaction (above) and percentage of nonaggressive behaviour (below), sal = saline. (A) Social behaviour after single injection of 0.5 mg/kg DZP. (B) Social behaviour after single injection of 1.0 mg/kg CLO. (C) Social behaviour after single injection of 0.075 mg/kg HAL. Mean ± S.E.M., $*P<.05$, n = number of pairs tested.

A single injection of 0.075 mg/kg HAL reduced the time spent in social interaction of sham-lesioned animals (sham-lesioned saline vs. HAL, P=.05), but there was no effect on the percentage of nonaggressive behaviour in lesioned animals (saline/sham-lesioned vs. HAL-lesioned, $P<.001$). There is no difference between HAL-treated sham-lesioned and HAL-treated lesioned rats in this parameter (P=.99, Fig. 1C).

3.2. Effects of CLO and HAL after subchronic administration

In the pilot experiment, two doses of each substance were tested. However, there were no clear dose–response relationships. Therefore, we completed only those experiments where the dose showed effects on social behaviour (i.e. 5.0 mg/kg CLO and 0.075 mg/kg HAL, Fig. 2).

3.2.1. Chronic CLO

Time spent in social interaction: $F(3,48) = 3.16$, $P = .033$

% Nonaggressive behaviour: $F(3,48) = 10.55$, $P<.001$

Chronic administration of CLO did not affect social behaviour in sham-lesioned animals (time spent in social interaction: sham-lesioned saline vs. CLO, $P=0.9$; percentage of nonaggressive behaviour, P=.153). However, as a consequence of treatment, the time spent in social interaction was normalised in the lesioned rats (lesioned saline vs. CLO, P=.027). There is no difference between saline-injected, sham-lesioned rats and lesioned rats repetitively injected with the substance (P=.91). There was no effect on the percentage of nonaggressive behaviour, and in all subchronically treated groups, the percentage was significantly lower compared with saline-injected, sham-lesioned rats (sham-lesioned saline vs. lesioned saline, P=.004; sham-lesioned saline vs. lesioned CLO, P=.021, Fig. 2A).

3.2.2. Chronic HAL

Time spent in social interaction: $F(3,41) = 3.3$, $P = .03$

% Nonaggressive behaviour: $F(3,41) = 5.62$, $P<.03$

Similar to CLO, subchronic treatment with HAL did not interfere with social behaviour in sham-lesioned animals (time spent in social interaction: sham-lesioned saline vs. HAL, $P=0.92$; percentage of nonaggressive behaviour, P=.9). Interestingly enough, HAL had the opposite effect in lesioned rats compared with CLO. The time spent in social interaction was not changed (sham vs. lesioned saline, P=.027; sham-lesioned saline vs. lesioned HAL, P=.93), but 0.075 mg/kg significantly increased the percentage of nonaggressive behaviour (sham-lesioned saline vs. lesioned saline, P=.004; lesioned saline vs. lesioned HAL, P=.021). Thus, there was no difference between

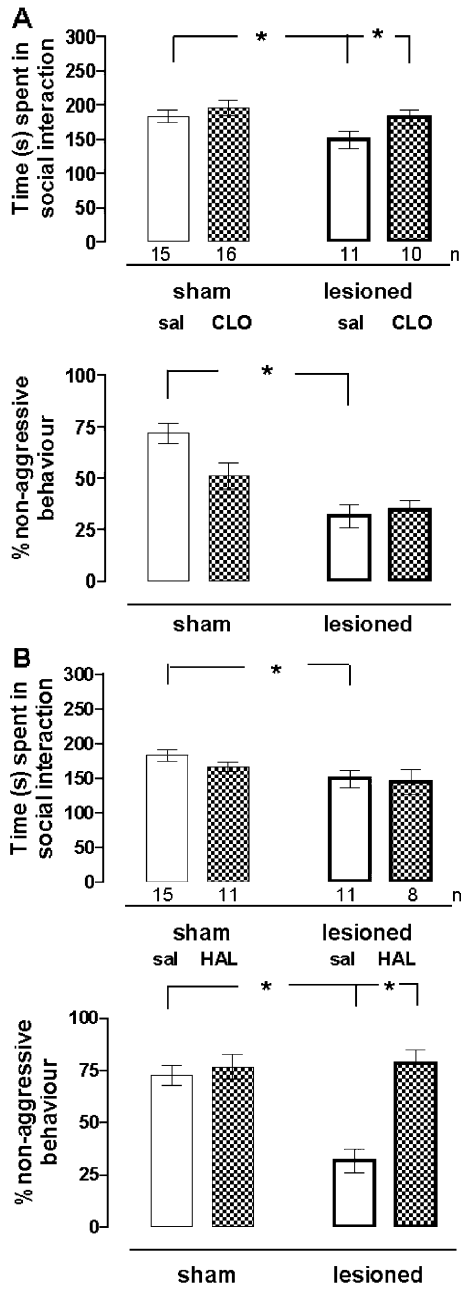

Fig. 2. Social behaviour in 13-week-old rats postnatally lesioned with ibotenic acid in the ventral hippocampus. Time (s) spent in social interaction (above) and percentage of nonaggressive behaviour (below) after subchronic (10 ×) treatment, sal = saline. (A) Social after subchronic treatment with 5.0 mg/kg CLO. (B) Social behaviour after subchronic treatment with 0.075 mg/kg HAL. Mean ± S.E.M., * $P < .05$, n = number of pairs tested.

saline-treated, sham-lesioned rats and lesioned rats repetitively injected with 0.075 mg/kg HAL (P=.558, Fig. 2B).

4. Discussion

Previously, we reported on alterations in social behaviour in rats neonatally lesioned in the ventral hippocampus (Becker et al., 1999). These rats showed reduced time spent in social contact and the ratio nonaggressive/aggressive behaviour was shifted towards an increased level of aggressiveness. It was speculated that this model might be a useful tool to test potential neuroleptic drugs. To test this assumption, the effects of HAL and CLO after acute and subchronic application were studied. For comparison, the anxiolytic drug DZP was used in the acute experiment.

The experiments have shown that:

1. After single injections at doses that did not affect locomotor activity: DZP had no effect on time spent in social contact either in sham-lesioned or lesioned rats, but the percentage of nonaggressive behaviour was elevated in lesioned animals (Fig. 1A). CLO decreased time spent in social interaction in shams only and increased the percentage of nonaggressive behaviour (Fig. 1B). HAL decreased time spent in social interaction in shams (Fig. 1C).
2. 24 h after discontinuation of subchronic administration of CLO and HAL, we did not find significant alterations in social behaviour in sham-lesioned rats. Interestingly, in lesioned rats, CLO increased the time spent in social interaction but there was no effect on the qualitative component, i.e. percentage of nonaggressive behaviour: aggressive behaviour (Fig. 2A). HAL exerted the opposite effects. There was no change in the time spent in social interaction in lesioned rats, but the percentage of nonaggressive behaviour was significantly increased (Fig. 2B).

In the clinical setting, DZP is used to control increased aggressiveness and anxiety. The substance exerts no antipsychotic effectiveness in the classical sense (Lingjaerde, 1991), but it has been used in the treatment of early signs of exacerbation in schizophrenia (Carpenter et al., 1999). In our study, the substance did not affect social behaviour in sham-lesioned rats but the ratio nonaggressive/aggressive behaviour was normalised in lesioned animals. This might be the result of the anxiolytic and anti-aggressive action of DZP (Corbett et al., 1993; Wongwitdecha and Marsden, 1996; Fernandes and File, 1999). Although the social interaction test is supposed to be sensitive to anxiolytic drugs at a dose range free from motor effects, the substance had no effect on sham-lesioned rats. This aspect requires further consideration. Rats tested after an acute dose of 2 mg/kg DZP showed an anxiolytic effect, measured by an increase in the time spent in social interaction (Fernandes and File, 1999). However, locomotor activity in rats from the Sprague–Dawley strain as used in our experiments showed motor impairments after treatment with doses higher than 0.75 mg/kg. Considering the ratio in sham-lesioned rats, a further increase in nonaggressive behaviour seems to be less likely.

After administration of the psychotomimetic drug phencyclidine (PCP), rats developed alterations in their social

behaviour. Thus, (Sams-Dodd, 1997, 1999) proposed that PCP-induced social abnormalities might be a possible animal model of the positive and negative symptoms of schizophrenia. In a subsequent study, Sams-Dodd (1998) tested DZP in this paradigm and found that drugs without antipsychotic activity did not inhibit the behavioural effects of PCP. This clearly demonstrates the differences between both models. We also found no effect on time spent in social contact but a detailed analysis of the components of social behaviour indicated an increase in the percentage of non-aggressive behaviour after DZP administration reflecting anti-aggressive effectiveness. Obviously, a more detailed analysis of behaviour (i.e. quantitative aspects and qualitative aspects) results in a more comprehensive detection of drug effects. In the studies by Sams-Dodd (1998, 1999), rats were tested in the social interaction test on the day of the last injection. Thus, drug interference might contribute to the difference found. The role of the benzodiazepinergic system in schizophrenia is controversial. It was suggested that the DZP-binding inhibitor may have a symptom modulatory role (Van Kammen et al., 1993). Abi-Dargham et al. (1999) failed to identify alterations of BZD receptor densities in schizophrenia. The authors concluded that, if this illness is associated with deficits in GABA transmission, these deficits do not substantially involve BZD receptor expression or regulation.

In our study, the antipsychotic drugs HAL and CLO did diminish social interaction in control rats, but there was no effect on the qualitative components after acute administration. This is not due to unspecific effects on locomotor behaviour because the doses were found to be ineffective as shown with the MOTI test (Table 1). It is the more surprising because there was no effect on time spent in social contact in lesioned rats (Fig. 1B and C). As published earlier (Grecksch et al., 1999), no difference was found in the locomotor activity between neonatal ibotenic acid-lesioned rats and control animals. Thus, this difference remains open for explanation. In the social interaction test, there were no differences in locomotor activity between sham-lesioned and lesioned animals. This is consistent with results published by Grecksch et al. (1999). However, Sams-Dodd et al. (1991) found hyperlocomotion in ibotenic acid-lesioned animals. These data are not conflicting. Our experiments were performed under an illumination level of 30 lx, whereas Sams-Dodd (1997) measured locomotor activity in a brightly lit room. The effects of stress have been implicated in the exacerbation of symptoms in schizophrenia (Lieberman et al., 1997).

As shown in Fig. 1B, CLO balanced the ratio of nonaggressive/aggressive behaviour in lesioned rats. It is speculated that the anxiolytic potential of CLO (Canon, 1979; Canon and Lippa, 1977; Bruhwyler et al., 1990; Rex et al., 1998) led to this shift towards normalisation. It was found that DZP increased social behaviour in familiar and unfamiliar rats, HAL decreased social behaviour in both paradigms and CLO increased social behaviour between pairs of unfamiliar but not familiar rats (Corbett et al., 1993). This is not in contradiction to our results because the authors used nonlesioned animals and different doses (e.g. CLO 10.0 mg/kg, DZP 1.25–5.0 mg/kg). In Sprague–Dawley rats as used in our experiments, these doses clearly depressed locomotor activity obscuring any conclusion about effects on social behaviour. As outlined in the Materials and methods section, social behaviour was scored 24 h after the final injection. At this time, HAL and CLO were expected to have been cleared because their half-life in the rat is 1.5 h (Cheng and Paalzow, 1992; Baldessarini et al., 1993). This is in line with a behavioural study by Öhman et al. (1977), demonstrating that shuttlebox performance was normal 24 h after an injection of 0.25 mg/kg HAL. However, these authors also reported a four-phase elimination of the drug. The fourth phase of elimination is the slowest, with a half-life of 4 days. It is debatable whether elimination phase 4 is of importance for the behavioural effects found in our experiments.

According to clinical experience, neuroleptic drugs generally do not exert their antipsychotic effects until they have been administered over longer periods of time lasting from days to weeks. A number of studies have shown that repeated administration has qualitatively distinct effects on dopamine systems and changes a number of dopamine parameters (see O'Donnell and Grace, 1996). Therefore, we investigated the effects of CLO and HAL after subchronic administration. Subchronic administration of CLO and HAL did not affect social behaviour in sham-lesioned rats but we found significant effects in lesioned animals (Fig. 2A and B): CLO did normalise the quantitative aspect of social behaviour, i.e. time spent in social contact was significantly increased (Fig. 2A). This is consistent with results by Qiao et al. (2001) demonstrating that reduced social behaviour in PCP-treated mice was attenuated by subchronic treatment with CLO as measured 24 h after the final injection. HAL had opposite effects. Subchronic treatment shifted the qualitative aspect, i.e. the ratio of nonaggressive/aggressive behaviour was normalised. Differences in receptor binding profile might explain these specific effects on social behaviour. HAL shows subnanomolar affinity for D_2 receptors and it also binds to D_3 and D_4 receptors with affinities in the nanomolar range (Hartman et al., 1996). Changes in D_4 receptor affinity seem to be region-specific (Schoots et al., 1995; Kusumi et al., 1995). It was speculated that cerebrocortical D_2-like and striatolimbic D_4-like receptors contribute to antipsychotic actions of both typical and atypical drugs (Tarazi et al., 1997). CLO is characterised by high-affinity binding in the nanomolar range to both D_4 and D_2 receptors, somewhat lower affinity to the D_1 receptor and submicromolar affinities for the D_3 and D_5 receptors (Hartman et al., 1996). In more detail, CLO was reported to exhibit relatively potent 5-HT_{2A} antagonism compared to their dopamine D_2 antagonistic effects and overexcitatory 5-HT_{2A} receptors were suspected to be involved in the expression of negative symptoms (e.g.

social withdrawal) of schizophrenia (Schmidt et al., 1995; Meltzer, 1989, 1991, 1999; Nakazato and Okuyama, 2000; Worrel et al., 2000; McDonald et al., 2002). Beside 5-HT_2 antagonism (Gobbi and Janiri, 1999) reported on CLO mediated 5-HT_3 antagonism. They concluded that CLO at the medial prefrontal cortex level exerts a complex modulatory activity on dopamine receptors, that is directly at the dopaminergic D_1 and D_2 receptors and through 5-HT receptors on the same neurones. Such interaction with serotonergic parameters of neurotransmission fits in with anxiolytic action (Graeff et al., 1998; Menard and Treit, 1999) of CLO. This anxiolytic effect was found 1 h after acute administration but not 24 h after the final injection in the course of subchronic treatment. This suggests that the anxiolytic effect of CLO is transient. Subsequent experiments are needed to investigate social behaviour after subchronic CLO treatment and different periods between the final injection and testing.

A number of clinical studies have shown that CLO was at least superior in the treatment of core negative symptoms of schizophrenia (Breier et al., 1994; Miller et al., 1994; Brar et al., 1997). Social withdrawal was classified as one of the core negative symptoms and this might explain different effects of the substance compared with HAL after subchronic treatment (Fig. 2A and B). In lesioned rats pretreated with CLO, there was no significant difference in the time spent in social interaction compared with sham-lesioned controls. This might be interpreted in terms of particular effectiveness against negative symptoms.

The results of our study demonstrate that the social interaction test in rats neonatally lesioned in the ventral hippocampus may have predictive and face validity to measure symptoms of schizophrenia. Moreover, the test seems to be sensitive enough to distinguish effects by classical and atypical neuroleptics as exemplified using HAL and CLO.

Acknowledgements

We thank D. Apel, M. Bohn, P. Dehmel, B. Reuter and G. Schulze for excellent technical assistance. Furthermore, we gratefully acknowledge the linguistic assistance by Mr. Andrew K. Toms (UK).

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