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PRELIMINARY COMMUNICATION

OPPOSITE EFFECTS OF OLANZAPINE AND HALOPERIDOL IN RAT ULTRASONIC VOCALIZATION TEST

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Opposite effects of olanzapine and haloperidol in rat ultrasonic vocalization test. M. SIEMIĄTKOWSKI, P. MACIEJAK, H. SIENKIEWICZ-JAROSZ, A.I. CZŁONKOWSKA, J. SZYNDLER, A. GRYCZYŃSKA, A. PŁAŹNIK. Pol. J. Pharmacol., 2001, 53, 669–673.

The opposite effects of the classical antipsychotic, haloperidol, and atypical neuroleptic, olanzapine, in the rat ultrasonic vocalization test of anxiety were observed. The present data are discussed in relation to growing body of evidence of specific brain biochemical changes after pretreatment with different antipsychotics.

Key words: *antipsychotics, ultrasonic vocalization, anxiety, rat*

INTRODUCTION

The involvement of dopamine and dopamine D_2 receptor in anxiety has been widely documented, however, little is known about physiological mechanisms that underlie the observed phenomena. Our previous behavioral studies focused on the changes in rat emotional behavior following pretreatment with classical antipsychotics reported their anxiogenic-like action in the open field test of neophobia and Vogel's conflict procedure, as opposed to the dopamine D_2 receptor agonist, quinpirole [10]. The aim of the present study was to examine the anxiety-related effects of the classical antipsychotic, haloperidol, and atypical neuroleptic, olanzapine, in the rat anxiety model. In the light

correspondence

of the commonly known facts that dopaminergic drugs may influence both motor performance of rats as well as affect their motivational states underlying approach and avoidance drives such a thirst, the use of aforementioned tests may evoke some complications. Accordingly, an alternative procedure seemed to be essential allowing not to attribute potential behavioral changes after dopaminergic drugs to the inhibition of locomotor activity. In this context, shock-induced ultrasonic vocalization procedure represents an interesting anxiety model for testing dopamine receptor ligands [1, 5].

MATERIALS and METODS

Animals

Male Wistar rats (200 ± 20 g), bought from a licensed breeder, were housed in standard laboratory conditions under a 12-h light/dark cycle (lights on at 6 a.m.), at a constant temperature (21 ± 2°C) and 70% humidity. All experimental procedures using animal subjects were approved by the Committee for Animal Care and Use at the Institute of Psychiatry and Neurology.

Drugs

Quinpirole (Eli Lilly, USA), haloperidol (Polfa, Warszawa, Poland), buspirone (Astra, Sweden) were dissolved in saline for *ip* administrations (2 ml/kg). Olanzapine (generously supplied by Eli Lilly, Poland) was dissolved in 50 µl of 0.2 M HCl and the volume was made up with the saline. Animals were tested 30 min after injection of a single dose of the drugs with the exception of olanzapine (60 min).

Ultrasonic vocalization test

A polycarbonate cages (17 cm each side) were located on a metal grid floor connected to a shock--generator, and the whole cage was placed into a sound-protected test chamber (W 45 cm, L 45 cm, H 50 cm). Ultrasonic vocalizations were recorded by a microphone (Mini-3 Bat Detector, Noldus Information Technology) attached to the ceiling of the chamber and processed by an interface (Ultravox, Noldus Information Technology) to select 22 ± 4 kHz signals and to digitize them in a IBM compatible PC. In an adaptation phase, each rat was placed in the test cage for a 2-min time period. In the priming phase, the rats received one stimulation session daily on 3 consecutive days. A stimulus session consisted of an adaptation period of 3 min in the test cage, followed by 5 electric footshocks (500 ms, 1.0 mA) delivered *via* the grid floor and scrambled during 3 min time period, followed by 3 min shock-free period. The testing phase was initiated with 5-min shock-free period, followed by one electric footshock (500 ms, 1.0 mA) and terminated with another 5-min shock-free period. In both 5-min periods, the frequency, mean and total duration of ultrasonic vocalizations were automatically recorded.

Open field test

The test was performed in a soundproof chamber under dim light and continuous white noise (65 dB). The open field apparatuses consisted of two round arenas (80 cm diameter) with walls 30 cm high. Locomotor activity of naive rats, the number of central entries and the time spent in the central sector of the open field (50 cm diameter) during one 15-min session were recorded and analyzed with the PC-based Videomot System (TSE, Bad Homburg, Germany). The anti-thigmotactic effect was calculated as a ratio of the number of entries into the central part of the open field to the rat locomotor activity, and multiplied by 1000. This parameter was calculated for each rat separately and then the mean value for each experimental group was computed.

Statistical analysis

The obtained data were submitted to one-way analysis of variance (ANOVA), followed by post-hoc LSD test. The confidence limit of $p < 0.05$ was considered statistically significant.

RESULTS

Haloperidol at the dose of 0.2 mg/kg increased the number and tended to enhance the duration of post-shock vocalization episodes. The drug also decreased the mean duration and tended to increase the number and total duration of pre-shock ultrasonic vocalization episodes on the testing day (Tab. 1). Olanzapine at the dose of 1.0 mg/kg *ip*, which was shown to be inactive in locomotor activity test (Tab. 2), reduced the number and total duration of contextual pre-shock ultrasonic calls. Both buspirone and quinpirole lowered the contextual and post-shock parameters of rat ultrasonic vocalizations (Tab. 1).

Table 1. The effects of pretreatment with buspirone, haloperidol, olanzapine and quinpirole on the ultrasonic vocalizations (USV) in the first part (pre-shock, 0–5 min) and second part (post-shock, 5–10 min) of the experiment

	n	USV episodes (0–5 min)	USV total duration (0–5 min) (s)	USV mean duration of the episodes (0–5 min) (s)	USV episodes (5–10 min)	USV total duration (5–10 min) (s)	USV mean duration of the episodes (5–10 min) (s)
control	12	71.7 ± 20.1	78.8 ± 23.8	1.11 ± 0.15	75.9 ± 22.3	85.8 ± 24.9	1.15 ± 0.11
buspirone 2.4 mg	13	5.2 ± 2.8**	5.7 ± 3.1**	1.11 ± 0.06	8.2 ± 5.6**	5.6 ± 3.9**	0.58 ± 0.12**
control	13	13.6 ± 7.9	21.1 ± 12.6	1.54 ± 0.09	15.0 ± 8.1	21.2 ± 13.0	0.88 ± 0.24
haloperidol 10 µg	14	18.9 ± 10.0	21.6 ± 11.6	0.99 ± 0.15*	49.1 ± 19.4	52.8 ± 21.3	0.85 ± 0.16
haloperidol 200 µg	13	37.4 ± 13.2	40.7 ± 16.2	1.01 ± 0.12*	66.8 ± 22.2*	56.4 ± 17.9	0.85 ± 0.15
control	12	34.8 ± 12.3	42.2 ± 16.0	1.15 ± 0.24	72.4 ± 19.3	79.7 ± 21.2	1.01 ± 0.21
olanzapine 10 µg	13	15.5 ± 9.1	20.8 ± 12.8	1.15 ± 0.23	54.5 ± 16.4	55.6 ± 17.9	0.80 ± 0.15
olanzapine 100 µg	13	34.0 ± 14.6	36.2 ± 16.5	1.00 ± 0.21	54.0 ± 15.9	61.6 ± 20.3	1.02 ± 0.21
olanzapine 1000 µg	11	6.7 ± 6.9*	9.6 ± 10.7*	1.08 ± 0.21	41.8 ± 19.0	54.3 ± 25.7	0.97 ± 0.28
control	13	36.9 ± 16.7	35.8 ± 15.8	0.77 ± 0.19	55.7 ± 19.9	53.7 ± 20.9	0.80 ± 0.16
quinpirole 1.0 mg	12	1.3 ± 0.7*	0.6 ± 0.3*	0.45 ± 0.02	4.9 ± 2.5**	2.4 ± 1.3**	0.40 ± 0.03**
quinpirole 2.0 mg	12	1.3 ± 1.1*	0.5 ± 0.4*	0.45 ± 0.03	8.3 ± 5.6*	3.8 ± 2.6**	0.44 ± 0.02**

n – number of rats in each experimental group. The data are shown in seconds (where applicable) as the means ± SD. * $p < 0.05$, ** $p < 0.01$ vs the control group

Table 2. Motor activity, the number of central entries, the anti-thigmotactic effect (calculated as a ratio of the number of entries into the central part of the open field to the rat locomotor activity, and multiplied by 1000), and the time spent in the central sector of the open field during one 15-min session

	Motor activity (m)	Entries into central sector	Anti-thigmotactic effect	Time spent in central sector (s)
control	23.5 ± 2.6	1.3 ± 0.3	61.5 ± 13.6	3.6 ± 1.3
olanzapine 100 µg	26.4 ± 1.9	3.4 ± 0.8*	131.2 ± 31.6	9.1 ± 1.9*
olanzapine 1000 µg	17.9 ± 2.2	3.0 ± 0.7	156.4 ± 39.3*	16.3 ± 5.1*

The anti-thigmotactic ratio was calculated for each rat separately and then the mean value for each experimental group was computed. The number of rats in each experimental group varied from 8 to 10. The data are shown as means ± SD. * $p < 0.05$ vs respective control group

DISCUSSION

The main finding of this study is the demonstration of the opposite effects of the classical antipsychotic, haloperidol, and atypical neuroleptic, olanzapine, in the ultrasonic vocalization test. Haloperidol (0.2 mg/kg) increased the number and duration of post-shock vocalization episodes suggesting its anxiogenic-like potential (Tab. 1). On the other hand, acute treatment with olanzapine (1.0 mg/kg) reduced the pre-shock contextual vocalizations and tended to diminish the post-shock ultrasonic vocalizations. The effects of olanzapine may suggest its anxiolytic-like potential as they correspond to the action of buspirone used as a pharmacological validation of the model (Tab. 1). In this test, buspirone at the dose of 2.4 mg/kg, which was previously proved to be inactive in locomotor activity test [9], markedly lowered both the number and total duration of contextual vocalization episodes as well as the number, mean and total duration of post-shock rat ultrasonic vocalizations.

As the results with olanzapine could be theoretically confounded by its nonspecific enhancement

or impairment of motor performance, we also examined the drug effects in the open field test. Olanzapine at the dose of 1.0 mg/kg increased exploratory parameters in the open field test, the number of entries into, and the time spent in the central sector of the open field (Tab. 2). The drug also revealed its anxiolytic-like action lowering the tigmotaxis. In the previous report, haloperidol at the presently examined doses of 0.01 and 0.2 mg/kg and quinpirole at the dose of 1.0 mg/kg were shown not to influence locomotor activity of rats [10].

In line with the results with olanzapine and haloperidol in the ultrasonic vocalization test, also the present effects of olanzapine in the open field test were directly opposed to the haloperidol-induced changes observed in the aforementioned study. The dissimilarities may correspond to some different biochemical effects after administration of typical and atypical antipsychotics. In a microdialysis study, olanzapine dose-dependently increased the extracellular dopamine and norepinephrine levels in the rat prefrontal cortex and striatum [11]. In contrast, haloperidol at the doses of 0.5 and 2 mg/kg did not alter dopamine or norepinephrine levels in the prefrontal cortex (PFC). Consistently, olanzapine increased c-fos expression in the PFC [7], whereas this effect was not observed following acute haloperidol injection [8]. Furthermore, microinfusions of the dopamine receptor antagonist, *cis*-flupentixol, into the medial PFC of rats exerted anxiogenic-like effect in two conflict procedures, on the other hand, the increased dopamine turnover after dopamine receptor agonist, apomorphine, resulted in anxiolytic-like changes in behavior [3]. Also in the present study, quinpirole, the dopamine D_2 receptor agonist, revealed its anxiolytic-like properties lowering both pre-shock and post-shock parameters of ultrasonic vocalizations (Tab. 1). Interestingly, olanzapine altered ultrasonic vocalizations in the manner similar to that of quinpirole. It has been previously suggested that dopamine neurotransmission in the PFC may directly regulate rat anxiety behavior [2] independently of the induction method, namely, stressful stimuli or administration of anxiogenic drugs. The other difference in profile of action between haloperidol and olanzapine is that haloperidol preferentially affected these parameters in the ultrasonic vocalization test which are directly shock-induced, i.e. the number of post-shock vocalization episodes (significantly) and their total duration after the electric shocks (a tendency), whereas the drug did not change the contextual vocalizations except for their mean duration (Tab. 1). On the contrary, olanzapine influenced only pre-shock contextual parameters. The observed phenomena should also be analyzed in the light of the cited above differences in targeting of different brain structures. Olanzapine changing dopamine metabolism in the frontal cortex may affect rather emotional (contextual) memory, whereas haloperidol perhaps interacts with another structure involved in the development of emotional reaction, and thus differs in effects on ultrasonic vocalizations.

On the other hand, the hypothesis that anxiolytic-like properties of olanzapine may be underlied by its influence on neurosteroids level cannot be excluded. For example, Marx et al. [6] have recently reported that olanzapine dose-dependently increased up to fourfold cerebral cortical allopregnanolone level, the neurosteroid potentiating $GABA_A$ receptor chloride channel function and demonstrating anxiolytic activity. Accordingly, allopregnanolone administered intracerebroventricularly selectively blocked picrotoxin- and bicuculline-induced seizures [4].

In conclusion, the present findings show directly opposed effects of representatives of classical and atypical antipsychotics, independent of their influence on motor behavior. The possible explanation may refer both to different alterations in dopaminergic neurotransmission in the PFC after typical and atypical neuroleptics as well as mediating the effects by other modulators, e.g. neurosteroids.

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