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RAPID COMMUNICATION

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Similarity of clozapine's and olanzapine's acute effects on rats' lapping behavior

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Abstract As a way of further comparing the behavioral effects of clozapine and olanzapine, dose ranges of these drugs were studied in a task emphasizing fine motor detail of rats' tongue movements during lapping behavior. Rats lapped drops of tap water from a force-sensing disk. From this behavior four variables were derived: peak-force of tongue strikes, duration of tongue contact, number of separate tongue contacts in 2 min, and the rhythm of the lapping behavior as quantified by Fourier analysis. Both clozapine (0.5–4.0 mg/kg, IP, 45 min) and olanzapine (0.25–2.0 mg/kg, IP, 45 min) dose dependently reduced all four measures of behavior. With respect to lick rhythm, a behavioral marker which clearly distinguishes haloperidol from clozapine in this behavioral paradigm, olanzapine was about twice as potent as clozapine, with the two drugs having parallel dose-effect functions. Within-session decrements in behavior previously reported for haloperidol in the lick task were not produced by clozapine nor by olanzapine. Taken together, these data strengthen the idea that the behavioral effects of clozapine and olanzapine are strikingly similar, and thereby emphasize the potential of olanzapine as an atypical antipsychotic agent.

Key words Clozapine · Olanzapine · Rat · Lapping behavior

Introduction

Clozapine is an atypical neuroleptic used to treat the positive and the negative symptoms of otherwise treatment resistant schizophrenic patients (Helmchen 1989; Kane et al. 1989; Meltzer 1992). Clozapine is considered to be atypical because it produces few if any motor side effects (Meltzer 1992). Although clozapine's widespread use has been limited by its relatively high incidence of agranulocytosis (Claas 1989; Fitton and Heel 1990), its therapeutic success has stimulated the search for clozapine-like drugs without leukocytopenic side effects. Behavioral methods have been prominent in this search (e.g., Costall and Naylor 1975; Ljungberg and Ungerstedt 1979; Faustman et al. 1981; Rebec et al. 1982; Sanger 1985; Bruhwyler et al. 1993; Moore et al. 1993; Wiley et al. 1993). Olanzapine (Lilly), a candidate clozapine-like drug is a thienobenzodiazepine derivative with high affinity for $5HT_2$, D_1, D_2, α_1, and muscarinic receptors, a profile which is very similar to the dibenzodiazepine derivative clozapine (Moore et al. 1993). Moreover, several behavioral effects of olanzapine are similar to clozapine's in mice and rats (Moore et al. 1993; Stanford and Fowler 1994). A recent report (Das and Fowler, 1995a) showed that acute clozapine reduced rats' lick emission and lick rhythm; with the same methods, however, haloperidol was reported not to appreciably affect the lick rhythm (Fowler and Das 1994), even though this typical neuroleptic greatly reduced number of licks emitted.

The purpose of the current study was to compare olanzapine and clozapine in the lingual motor task for rats (Fowler and Mortell 1992; Fowler and Das 1994). Accordingly, rats were administered acute doses of clozapine or olanzapine, and the drugs' effects on rat's lapping behavior were characterized by quantitative measurements of lick force, lick duration, rhythm of tongue movements (with Fourier methods), and overall number of licks emitted in a brief test session. These dose effect profiles were then compared to one another and to published data for haloperidol.

An additional purpose of the study was to compare clozapine and olanzapine with respect to within-session decrements (Sanger 1986; Sanger and Perrault 1995) in behavior. Several typical neuroleptics (e.g., haloperidol) produce within-session decrements, whereas clozapine

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and several atypical candidates do not produce within-session decrements (Sanger 1986; Das and Fowler 1995; Sanger and Perrault 1995b). Thus, within-session analyses were seen as an additional way to compare the preclinical behavioral effects of clozapine and olanzapine.

Materials and methods

Subjects

Thirty male, Sprague-Dawley rats (Harlan Co., Indianapolis, Ind.) with an average weight of 360 g were the subjects. Animals were maintained on a restricted watering regime with 5–6 min of water-access time 30 min after the 2-min experimental session (in which water was also consumed). The restricted water-access allowed slow weight gain (about 5 g/week) while providing for high motivation to perform the task. Measurements of licking performance occurred between 0830 and 1130 hours daily during the light portion of the light-dark cycle (on at 0800, off at 2000 hours) in the vivarium. The age of the animals at the beginning of the current experiment was 4 months. The rats had previously received subchronic treatment with clozapine (1.5, 3.0, and 4.5 mg/kg, IP, 45 min) 7 days at each dose. Twenty-eight days had elapsed since the last drug experience and the first drug treatment of this study.

Apparatus

The single lick-force recording chamber has been described in detail (Fowler and Mortell 1992). It consisted of a modified Gerbrands rodent operant chamber fitted with a front panel containing a 6-cm square hole at the floor level to which was affixed a 3-cm long transparent plastic enclosure. The 18 mm diameter lick disk was positioned 5 mm below the inside surface of the plastic enclosure. The lick-disk was rigidly attached to the shaft of a force transducer. The natural frequency of the transducer assembly was 160 Hz, but the support circuitry limited the high frequency band pass of the transducer to 100 Hz for force-waveform data collection. The transducer gain was calibrated to resolve force to 0.2 g equivalent weights. Through a Labmaster interface, the computer program measured the number of licks, peak force, and lick duration (the time the lick force remained above the detection threshold for a single lick), and recorded each 2-min session of force-time data digitized at 100 samples/s.

Procedure

The rats were trained to lick water through the circular hole in the rectangular recession in the front panel leading to the force-sensing disk. Training was as previously described (Fowler and Das 1994). By the end of training rats licked continuously for 2 min on an FR12 schedule with 4 g or more of tongue peak force leading to programmed consequences (advancement of the FR count and delivery of 0.055 ml water to the lick surface). At the beginning of the experiment the rats had already experienced 56 daily 2-min experimental sessions. Acute drug testing was carried out every third day with 2 vehicle days separating each drug day. Doses of clozapine (0.5, 1.0, 2.0, and 4.0 mg/kg, IP, 45 min) and olanzapine (0.25, 0.5, 1.0, and 2.0 mg/kg, IP, 45 min) were administered in a counterbalanced order such that all the rats received all the doses of clozapine and olanzapine once over the drug testing period of 24 days. In the first half of the testing period half of the rats received the four doses of clozapine while the other half of the rats received the four doses of olanzapine. This order was then reversed in the second half of the testing period to complete the experimental design.

Drugs

Clozapine (a gift from Sandoz) and olanzapine (a gift from Lilly) were dissolved in a vehicle prepared by a drop of lactic acid per 10 ml saline according to the concentration desired.

Quantitative analysis

Drug and dose effects were assessed in terms of four dependent variables: 1) number of licks, 2) lick duration, 3) the peak force of each lick event, and 4) the dominant rhythm of licking measured in Hertz by spectral analysis (Fourier) techniques. The mean of the lick forces emitted in a session served as the dependent variable for statistical analysis. Likewise, the mean duration was the mean of the individual lick durations for each subject's session. The lick rhythm for each rat was determined as previously described (Fowler and Mortell 1992). Briefly, the dominant rhythm of the lick oscillations was taken as the frequency associated with the spectral peak in the 3.5- to 6.5-Hz region of the spectrum computed by Fourier methods for each rat each session. With these methods the dominant rhythm is a measure of the periodic tendency of the licking behavior; therefore, the rhythm of the oscillatory process of licking can be largely independent of the number of licks. Prior to dose-effect one-way analyses of variance, data were expressed as a proportion of the vehicle control for each rat. The vehicle performance for each subject was the average of 8 vehicle days immediately preceding the drug-treatment days.

Data analyses for the within-session effects were carried out as described by Das and Fowler (1995b). Data for each session were divided into first and second 1-min halves. Then a change score was obtained by subtracting the first half from the second half. Resulting negative change scores represented within-session decrements and positive change scores represented within-session increments in behavior. Within-session rhythm-data analyses were not undertaken because of the difficulty of using 1-min epochs to estimate the lick rhythm when the drugs reduced the availability of an adequate number of sufficiently long bursts of continuous lapping. One-way repeated measures ANOVAs were applied to the change scores to assess dose effects. In the repeated measures ANOVAs, the conservative Wilk's Lambda multivariate F-test was used to decide on statistical significance with a probability criterion no greater than 0.01. This same probability criterion was used as the decision rule for the one-degree-of-freedom trend tests because of the relatively large number of tests conducted. Occasionally, the degrees of freedom in statistical tests were less than would be expected for 30 rats because some variables had missing values (such as peak force) when a rat emitted no licks at all.

Results

Group mean performance data for the 8 consecutive vehicle treatment days are arrayed in Table 1. Repeated measures ANOVAs for each of the four measures did not detect any changes across time. Consistency of means and standard errors suggests stability of responding across the vehicle sessions.

Clozapine dose-dependently reduced lick emission, $F(3,27)=77.168$, $P<0.001$ (Fig. 1, bottom left), and olanzapine displayed a similar pattern of dose-dependent reduction in the number of tongue contacts emitted in 2 min, $F(3,27)=31.853$, $P<0.001$. The peak-force-diminishing effects (Fig. 1, top left) of clozapine, $F(3,16)=34.148$, $P<0.001$, were similar to those of olanzapine, $F(3,26)=22.910$, $P<0.001$. Significant reductions in lick duration (Fig. 1, top right) were observed after the administration of both clozapine, $F(3,16)=11.869$,

Table 1 Groups means for four measures of the lapping behavior of 30 rats on each of the 8 vehicle injection days that immediately preceded the day when rats were treated with an injection of clozapine or olanzapine. *SEM* standard error of the mean

Measure	Statistic	Consecutive/Vehicle/Days							
		1	2	3	4	5	6	7	8
Peak force (g)	mean	30.5	30.7	32.2	30.2	30.5	30.2	31.1	31.3
	SEM	1.0	1.1	1.0	1.0	0.7	0.9	1.0	1.1
Duration (s)	mean	0.063	0.062	0.063	0.062	0.062	0.062	0.064	0.063
	SEM	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
No. of licks	mean	551	551	541	538	552	547	545	546
	SEM	9.9	13.5	13.3	19.3	13.3	14.9	11.8	11.4
Rhythm (Hz)	mean	5.20	5.22	5.20	5.19	5.21	5.18	5.16	5.12
	SEM	0.05	0.04	0.04	0.05	0.05	0.05	0.05	0.06

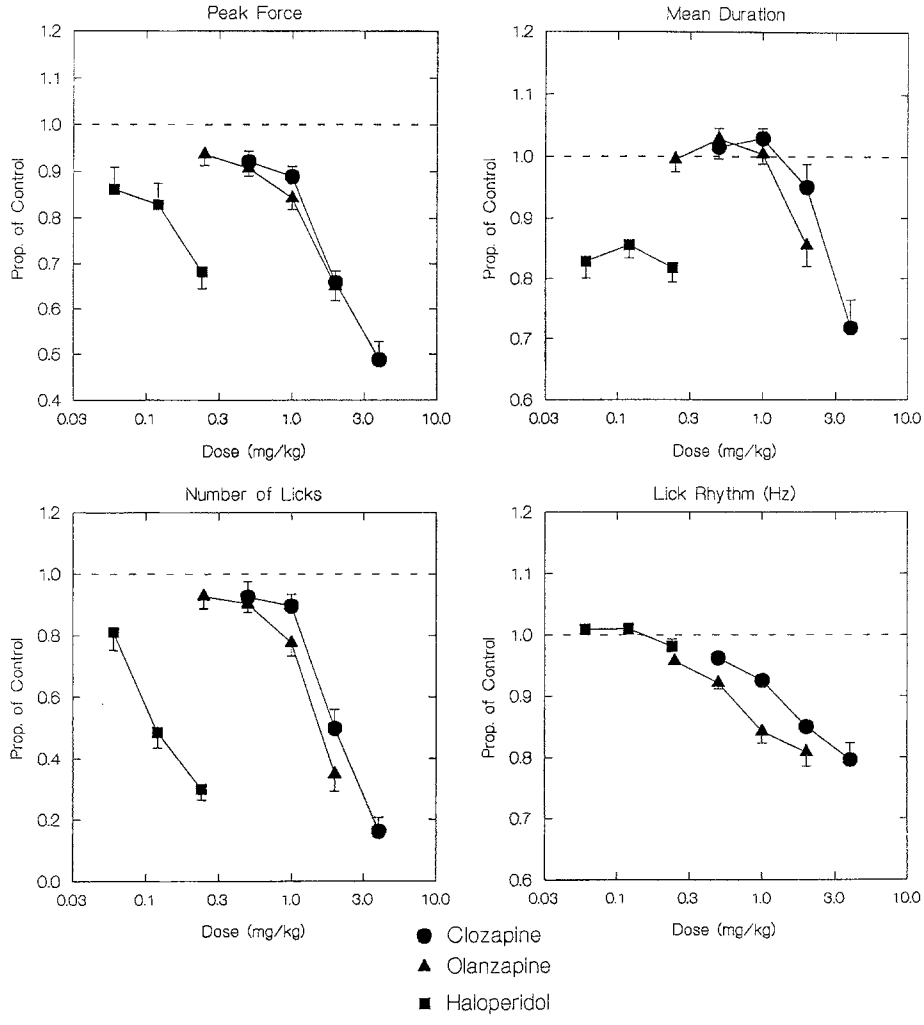

Fig. 1 Effects of clozapine (*filled circles*) and olanzapine (*filled triangles*) on the indicated measures of tongue dynamics as rats licked drops of water from a force-sensing disk. The results shown for haloperidol (*filled squares*: 0.06, 0.12, 0.24 mg/kg) were published previously (Fowler and Das 1994). Data are expressed as proportion of vehicle control. *Brackets* indicate 1 SEM. Symbols without an error bar reflect the fact that the SEM was smaller than the size of the symbol used

$P<0.001$, and olanzapine, $F(3,26)=7.041$, $P<0.005$. Both clozapine and olanzapine exhibited a graphic tendency to elevate response duration before decreasing it.

Lapping rhythm (Fig. 1, lower right) was significantly slowed both by clozapine, $F(3,11)=17.463$, $P<0.001$, and by olanzapine, $F(3,23)=38.35$, $P<0.001$. The parallel dose-effect functions for rhythm provided the opportunity to calculate, with regression methods, a potency comparison of clozapine and olanzapine for the dose that reduced rhythm to 0.90 of control [approximately a half-maximal effect; once rhythm drops to about 4.0 Hz, lapping generally ceases (unpublished observations)]. These doses were for clozapine 1.16 mg/kg (0.95 confidence interval: 1.00–1.32 mg/kg) and for olanzapine 0.55 mg/kg (0.95 confidence interval: 0.43–0.67 mg/kg).

In order to compare the shapes of the dose effect functions for the two compounds, polynomial trend tests were conducted. Significant linear log-dose effects were obtained for both drugs for all four measures of lapping behavior. Significant quadratic trends were the same for

both drugs, with one exception. Olanzapine produced a significant quadratic log-dose effect on peak force of tongue movement, but clozapine did not.

Clozapine produced a within-session increment (not a decrement) in number of licks, $F(3,27)=5.102$, $P=0.006$, whereas olanzapine produced no (neither increment nor decrement) within-session effect on number of licks, $F(3,27)=0.360$, $P>0.01$. Neither clozapine, $F(3,18)=1.538$, $P>0.01$, nor olanzapine, $F(2,26)=0.863$, $P>0.01$, produced a within-session change in peak force of tongue contact. A similar negative result was obtained for lick duration; clozapine: $F(3,18)=2.019$, $P>0.01$; olanzapine: $F(3,26) = 0.863$, $P>0.01$.

Discussion

The expectation of similar behavioral effects for clozapine and olanzapine (e.g., Moore et al. 1993; Stanford et al. 1994) was generally confirmed in the present study. Both drugs dose-relatedly decreased lick emission, lick peak force, lick duration, and lick rhythm. The behavior-modulatory effects of olanzapine were reported to be more potent than clozapine's in the rat-lick-while-press-task (Stanford and Fowler 1994) and in the conditioned avoidance task (Moore et al. 1993). The current results showed that for each of the four dependent measures the log dose-effect functions for olanzapine were located to the left of the clozapine functions (Fig. 1), indicating a greater potency of olanzapine relative to clozapine. Whether the small differences in the shapes of the peak force dose-effect functions (i.e., quadratic trend for olanzapine but not for clozapine) are of any import beyond differences in the dose scales selected for study, must await further work.

With respect to rhythm slowing, the dependent variable that most clearly distinguished clozapine from haloperidol in this paradigm [compare current data with haloperidol data taken from Fowler and Das (1994) and replotted in Fig. 1], olanzapine was about twice as potent as clozapine. In addition, the parallel log dose-effect functions on this measure suggest similarities of pharmacological mechanism for clozapine and olanzapine. It is interesting to note that olanzapine's "half maximal" dose in reducing lick rhythm observed here was quite close to the reported ED_{50} (0.57 mg/kg, IP) of olanzapine's antagonism of quipazine-induced elevation of corticosterone (Moore et al. 1993). The antagonism of quipazine's effects is taken as a measure of activity against serotonin receptors.

With regard to the issue of neuroleptic-induced within-session decrements in behavior, the results indicated that neither clozapine nor olanzapine produced within-session decrements – outcomes different from those reported for haloperidol in the same task (Das and Fowler 1995b). Previous work has shown that several typical neuroleptics produce within-session decrements (Sanger 1986; Fowler et al. 1990; Das and Fowler 1995b), whereas atypical neuroleptics such as clozapine do not (Sanger 1986; Sanger and Perrault 1995). There was a qualitative difference between clozapine and olanzapine on the within-session measure: clozapine produced a within-session increment in number of licks, but olanzapine produced no within-session effect on this variable. In this same task scopolamine produced a within-session increase in number of licks (Das and Fowler 1995b). This latter result raises the possibility that the observed difference in within-session trends for clozapine and olanzapine may be related to different muscarinic profiles, but the published data do not support this idea (Moore et al. 1993).

Because the rats used in this study were exposed to clozapine (7-day treatment periods at each of three doses, 1.5, 3.0 and 4.5 mg/kg) before the dose-effect determinations for the present study, it is appropriate to consider the likelihood that carry-over effects may have played a role in producing similarities between clozapine and olanzapine. Rats with a prior history of acute exposure to several neuroleptics, including haloperidol, displayed clozapine dose effects on lick rhythm that were quantitatively quite close to clozapine's effects on rats with no prior drug history (Das and Fowler 1995a). Thus, clozapine's ability to slow lick rhythm was not altered by prior haloperidol treatment. An additional finding of this work was that drug-naive rats exhibited no evidence for tolerance or sensitization to the rhythm-slowing effects of clozapine across 7 days of subchronic treatment at 1.5, 3.0, and 4.5 mg/kg (i.e., no evidence for neuroadaptive changes across 7 days of treatment on three separate occasions). On the other hand, another measure of the behavior, number of licks per session, did exhibit tolerance effects across 7 days of subchronic dosing. This study also suggested that tolerance effects for number of licks substantially (but not completely) subsided with a wash-out period of only 4 days off the drug. The wash-out period between initial subchronic dosing and dose-effect determinations in the present study was 28 days. Evidence in hand suggests no neuroadaptive change for the lick rhythm measure after 7 days of clozapine treatment, but rapid development of, and rapid loss of, tolerance for the number of licks measure.

Results derived from a different behavioral task are also relevant to the consideration of carry-over effects. With a single forelimb rats pressed a force transducer and simultaneously licked water from a dipper (Fowler et al. 1990, 1994; Stanford and Fowler 1994). In this task the prominent rhythmic oscillation detected in the rat's forelimb force as it presses the force transducer is produced by the licking behavior. Both clozapine (Fowler et al. 1994) and olanzapine (Stanford and Fowler 1994) dose dependently slowed the lick rhythm measured in the forelimb-lick paradigm. The rats treated with olanzapine in the forelimb-lick task (Stanford and Fowler 1994) had a complex prior drug history including acute and subchronic dosing with haloperidol (doses up to 0.12 mg/kg). Thus, the olanzapine dose-related rhythm slowing was observed regardless of the presence (Stanford and Fowler 1994) or absence (current study) of haloperidol in the rats' prior history. Available data, there-

fore, are consistent with the conclusion that, especially for the lick rhythm measure, modest prior drug exposure did not significantly influence the current results.

The data reported here suggest a close similarity between clozapine's and olanzapine's behavioral effects, and with respect to the slowing of lapping rhythm and a lack of within-session decrements both drugs were clearly different from the reported effects of haloperidol in the same task on the same measures of behavior (Fowler and Das 1994; Das and Fowler 1995b).

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