Effects of JL13, a Pyridobenzoxazepine with Potential Atypical Antipsychotic Activity, in Animal Models for Schizophrenia

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ABSTRACT

JL13 [5-(4-methylpiperazin-1-yl)-8-chloro-pyrido[2,3-b][1,5]benzoxazepine fumarate] is a substance with a close structural resemblance to clozapine. However, it is less sensitive to oxidation and may therefore have less hematological side effects. In the present study, JL13 was compared with clozapine and haloperidol in several animal models for schizophrenia. The paw test represents a screening model for antipsychotic drugs that can discriminate between drugs with extrapyramidal side effects and drugs without. Haloperidol increased both forelimb retraction time and hindlimb retraction time (HRT), whereas both clozapine and JL13 increased only HRT. In the prepulse inhibition paradigm, all three drugs reversed the apomorphine- and theamphetamine-induced disruption of prepulse inhibition. However, whereas haloperidol was equally effective against both dopaminergic drugs, JL13 and clozapine were more effective against amphetamine. Finally, only JL13 was able to increase prepulse inhibition in normal rats, whereas only clozapine reduced basal startle amplitude. Taken together, these data suggest that JL13 may be an effective antipsychotic drug, with a profile similar to clozapine.

Antipsychotic drugs have been the first choice in the treatment of schizophrenia ever since the introduction of chlorpromazine and haloperidol. Despite the tremendous advantages of these drugs in the therapy of schizophrenic patients, they have unmistakable limitations. The most important ones being the induction of extrapyramidal and haloperidol effects. In the present study, JL13 was compared with clozapine and haloperidol in several animal models for schizophrenia. The paw test represents a screening model for antipsychotic drugs that can discriminate between drugs with extrapyramidal side effects and drugs without. Haloperidol increased both forelimb retraction time and hindlimb retraction time (HRT), whereas both clozapine and JL13 increased only HRT. In the prepulse inhibition paradigm, all three drugs reversed the apomorphine- and theamphetamine-induced disruption of prepulse inhibition. However, whereas haloperidol was equally effective against both dopaminergic drugs, JL13 and clozapine were more effective against amphetamine. Finally, only JL13 was able to increase prepulse inhibition in normal rats, whereas only clozapine reduced basal startle amplitude. Taken together, these data suggest that JL13 may be an effective antipsychotic drug, with a profile similar to clozapine.

JL13 [5-(4-methylpiperazin-1-yl)-8-chloro-pyrido[2,3-b][1,5]benzoxazepine fumarate] was also developed in the search for a clozapine-like substance (Bruhwyluer et al., 1992; Liégeois et al., 1994). Although it is structurally related to clozapine, differing in only two positions in the tricyclic structure, JL13 possesses different physicochemical properties. Indeed, JL13, unlike clozapine, was found to be less sensitive to oxidation (Liégeois et al., 1997, 2000). Therefore, according to other results (Liégeois et al., 1999) showing a correlation between the oxidation profile of drugs and their potential to induce hematological side effects, JL13 should be less prone to induce hematological side effects than clozapine. Biochemically, JL13 was found to bind predominantly to the 5-HT2 and the D1 receptor, with much less potency for either the D2 or the muscarinic receptor (Bruhwyluer et al., 1992; Liégeois et al., 1994). In behavioral experiments, JL13 was shown to inhibit the amphetamine-induced locomotor activity and the amphetamine-induced climbing in mice. However, the drug did not induce catalepsy nor did it influence the apomorphine- or theamphetamine-induced stereotypy (Bruhwyluer et al., 1997). Using a microdialysis procedure, JL13 was found to increase selectively extracellular dopamine concentration in the prefrontal cortex (Invernizzi et al., 2000). This profile of action of JL13 resembles that of clozapine and is reminiscent of other atypical antipsychotics (Arnt, 1998).

The aim of the present study was to investigate the effects of JL13 on the expression of extrapyramidal side effects in animal models for schizophrenia.

ABBREVIATIONS: EPS, extrapyramidal side effects; JL13, 5-(4-methylpiperazin-1-yl)-8-chloro-pyrido[2,3-b][1,5]benzoxazepine fumarate; 5-HT2, serotonin; FRT, forelimb retraction time; HRT, hindlimb retraction time.
of JL13 in two other models for schizophrenia and directly compare it with haloperidol and clozapine. The models we choose include a screening test and a simulation model (Ellenbroek and Cools, 2000). The paw test was used as a screening model. This test was selectively developed for differentiating classical from atypical antipsychotic drugs and has so far proved to be reliable (Ellenbroek et al., 1987; Ellenbroek and Cools, 1988; Cools et al., 1990, 1995). The disruption of prepulse inhibition was used as a simulation model. As discussed elsewhere, this test seems to represent one of the best animal simulation models for schizophrenia to date (Swerdlow et al., 1994; Ellenbroek et al., 2000). Since preliminary experiments had shown that the disruption induced by apomorphine and amphetamine may be pharmacologically different, we decided to test the antipsychotic drugs against both dopamine agonists.

### Materials and Methods

#### Rats and Housing.
For these experiments, male Wistar rats (Harlan Laboratories, Horst, The Netherlands) were used weighing between 220 and 280 g. The rats were housed in groups of two or three males in standard Macrolon cages (26 × 42 × 15 cm), in temperature controlled rooms (23 ± 1°C). The rats were on a standard 12-h light/dark cycle with light on from 7 AM to 7 PM, with water and food available ad libitum except during the experiments. One day prior to the experiments, the rats were individually housed in the standard Macrolon cage. All experiments were done in accordance with the Helsinki Declaration and with national and institutional guidelines. Rats were only used once.

#### The Paw Test.
The paw test was performed 30 min after an intraperitoneal injection of either haloperidol, clozapine, or JL13. In this test, a rat was placed on a Perspex platform (30 × 30 cm with a height of 20 cm) containing two holes for the forelimbs (40 mm), two for the hindlimbs (50 mm), and a slit for the tail (Ellenbroek et al., 1987). The distance between the right and the left forelimb and hindlimb holes was 15 mm, and the distance between forelimb and hindlimb holes was 55 mm. The rat was held behind the forelimbs, and the hindlimbs were gently placed in the holes. The rat was then lowered and the forelimbs positioned in the holes. The forelimb retraction time (FRT) and the hindlimb retraction time (HRT) were defined as the time the animal needed to withdraw one forelimb and one hindlimb from the hole, respectively. The minimum time was set at 1 s, since it was difficult to determine the exact starting time. When the rat did not withdraw its fore- or hindlimb within 30 s, the animal was taken out and the FRT or HRT was set at 30 s. The paw test was repeated at 40 and 50 min after injection. No statistically significant increases or decreases were found with repeated testing (data not shown). The average FRT and HRT (the mean of the three measurements) were then calculated for each rat.

#### The Prepulse Inhibition.
On the day of the experiment, the animals were transported to a room adjacent to the startle chamber room and left undisturbed for at least 30 min. In the prepulse inhibition paradigm, four standard startle chambers of San Diego Instruments (San Diego, CA) were used. The startle chamber consisted of a Plexiglas tube (diameter 8.2 cm, length 25 cm), placed in a sound-attenuated chamber, in which the rats were individually placed. The tube was mounted on a plastic frame, under which a piezoelectric accelerometer was mounted, which recorded and transduced the motion of the tube. After the rats were placed into the chamber, they were allowed to habituate for a period of 5 min, during which a 70 dB[a] background white noise was present. After this period, the rats received 10 startle trials, 10 no-stimulus trials, and 30 prepulse inhibition trials. The intertrial interval was between 10 and 20 s, and the total session lasted about 17 min. The startle trial consisted of a single 120 dB[a] white noise burst lasting 20 ms. The prepulse inhibition trials consisted of a prepulse (20 ms burst of white noise with intensities of 73, 75, or 80 dB[a]) followed, 100 ms later, by a startle stimulus (120 dB[a], 20 ms white noise). Each of the three prepulse trials (73, 75, and 80 dB[a]) were presented 10 times. During the no-stimulus trial, no stimulus is presented, but the movement of the rat is scored. This represents a control trial for detecting differences in overall activity. The 50 different trials were presented pseudorandomly, ensuring that each trial was presented 10 times and that no two consecutive trials were identical. The resulting movement of the rat in the startle chamber was measured during 100 ms after startle stimulus onset (sampling frequency 1 kHz), rectified, amplified, and fed into a computer that calculated the maximal response over the 100-ms period. Basal startle amplitude was calculated as the mean amplitude of the 10 startle trials. Prepulse inhibition was calculated according to the formula 100 – 100% × (PPx/P120), in which PPx is the mean of the 10 prepulse inhibition trials (PP73, PP75, or PP80), and P120 is the basal startle amplitude.

#### Drugs.
In both the paw test and the prepulse inhibition test, JL13 (administered as fumarate salt), clozapine (Sigma/RBI, Zwijndrecht, The Netherlands), or haloperidol (Janssen, Tilburg, The Netherlands) were given intraperitoneally 30 min prior to the test. JL13 was dissolved in propylene glycol and diluted to the right concentration with saline. Given the limited solubility of JL13 (maximally 10 mg/ml) the two highest doses had to be administered as 2 ml/kg (making 20 mg/kg), respectively, and 4 ml/kg (making 40 mg/kg). Clozapine was dissolved in a drop of N HCl and diluted with saline. The pH was adjusted to about 4.5 to 5 using NaHCO3. Haloperidol was dissolved in lactic acid and diluted with saline. Immediately before the prepulse inhibition session, rats were injected with either saline, apomorphine (1 mg/kg), or amphetamine (10 mg/kg) administered subcutaneously.

#### Statistics.
Given the nonparametric nature of the paw test scores, the differences in FRT and HRT were analyzed with the Mann-Whitney U test.

Differences in basal startle amplitude were analyzed by an analysis of variance. The overall effect on prepulse inhibition was determined by an analysis of variance with repeated measures for the different prepulse intensities and drug as the between-subject factor. In case of a significant effect, post hoc Duncan tests were performed to evaluate the statistical differences between the groups for each prepulse intensity.

### Results

#### The Paw Test.
The results of the paw test are depicted in Fig. 1. The figure shows that haloperidol led to a strong, dose-dependent increase in both FRT and HRT. On the other hand, JL13 and clozapine increased only HRT, but appeared to be without effect on the FRT. This was confirmed by statistical analysis. The Mann-Whitney U test showed that, at doses of 0.5 mg/kg i.p. and higher, haloperidol induced a significant increase in both FRT and HRT. Clozapine and JL13 induced a significant increase in HRT at doses of 5 mg/kg and higher. Table 1 shows the minimal effective dose for increasing FRT and HRT for the three drugs tested, as well as the ratio.

#### The Prepulse Inhibition Paradigm.
The effects of the three antipsychotic drugs on the apomorphine-induced changes in startle and prepulse inhibition are shown in Fig. 2. Apomorphine did not affect basal startle amplitude ($F_{(1,22)} = 0.59; p = 0.45$), but significantly reduced prepulse inhibition ($F_{(1,22)} = 21.0; p < 0.001$).

When added to apomorphine, haloperidol did not affect
basal startle amplitude \( F(1,26) = 2.8; p = 0.1 \). JL13 and clozapine on the other hand did reduce basal startle amplitude when added to apomorphine (JL13: \( F(1,34) = 21.1; p < 0.001 \); clozapine: \( F(1,34) = 15.5; p < 0.001 \)). Post hoc analyses showed that the highest dose of JL13 and clozapine were significantly different from apomorphine alone.

As Fig. 2 clearly shows, all drugs reversed the apomorphine-induced disruption of prepulse inhibition (haloperidol: \( F(1,26) = 8.6; p < 0.01 \); JL13: \( F(1,34) = 7.5; p < 0.01 \); clozapine:

\[ F(1,26) = 4.4; p < 0.04 \]). Post hoc analyses showed that all doses of haloperidol were effective, whereas only the highest dose of JL13 and clozapine significantly reversed the effects of apomorphine on prepulse inhibition.

Figure 3 shows the effects of amphetamine. Like apomorphine, amphetamine did not affect basal startle amplitude \( F(1,22) = 1.2; p = 0.3 \). Amphetamine did, however, disrupt prepulse inhibition \( F(1,22) = 12.5; p < 0.002 \).

When added to amphetamine, JL13 \( F(1,32) = 1.8; p = 0.12 \) did not alter basal startle amplitude. When either haloperidol \( F(1,26) = 12.9; p < 0.001 \) or clozapine \( F(1,25) = 5.8; p < 0.025 \) was added, a significantly lower basal startle amplitude, compared with amphetamine alone, was observed. Post hoc analyses showed that both doses of haloperidol were significant, whereas no single dose of clozapine was significant.

All three drugs reversed the effects of amphetamine on prepulse inhibition (haloperidol: \( F(1,26) = 24.9; p < 0.001 \); JL13: \( F(1,32) = 6.2; p < 0.02 \); clozapine: \( F(1,25) = 6.0; p < 0.025 \)). Post hoc analyses showed that both doses of haloperidol and clozapine were significant, whereas only the highest two doses of JL13 were significant.

Figure 4 shows the effects of haloperidol, JL13, and clozapine when given alone. The results show that neither haloperidol \( F(1,26) = 1.0; p > 0.7 \) nor JL13 \( F(1,26) = 0.7; p > 0.4 \) affected baseline startle amplitude. Clozapine, on the other
hand, significantly decreased baseline startle amplitude \( (F(1,26) = 58.3; p < 0.001) \). Post hoc analysis showed that both doses of clozapine strongly reduced baseline startle amplitude. With respect to prepulse inhibition, neither haloperidol \( (F(1,26) = 0.2; p > 0.7) \), nor clozapine \( (F(1,26) = 0.1; p > 0.7) \) significantly altered prepulse inhibition. JL13 showed an increase in prepulse inhibition, which just reached significance \( (F(1,26) = 4.1; p < 0.05) \).

Discussion

The results of the present paper show that all three drugs reversed the effects of apomorphine and amphetamine in the prepulse inhibition paradigm, and increased the HRT in the paw test. In addition, haloperidol, but not JL13 and clozapine, also increased the FRT in the paw test.

The paw test was designed many years ago to distinguish classical antipsychotics from atypical antipsychotics on the basis of positive criteria (Ellenbroek et al., 1987). It was shown that all classical antipsychotics had an equal potency for increasing FRT and HRT, whereas atypical antipsychotics were more potent on HRT (Meert and Awouters, 1991; Prinssen et al., 1999). So far, clozapine was the only drug found not to increase FRT even at the highest dose (up to 100 mg/kg) tested. This lack of effect was confirmed in the present paper. However, JL13 also did not increase FRT at the highest dose tested (40 mg/kg, see Fig. 1), making it very similar to clozapine. Haloperidol, on the other hand, increased both FRT and HRT, with similar potency, confirming its classical profile. It has been shown that the best predictive parameter is the ratio between the minimal effective dose for increasing FRT and HRT (Ellenbroek, 1993). A ratio of 1.0 is indicative of a classical antipsychotic, whereas a ratio of more than 1 is indicative of an atypical antipsychotic drug. Table 1 shows that the ratio accurately predicts the clinical profile of both haloperidol and clozapine. It also predicts that JL13 will have an atypical profile similar to clozapine. We showed several years ago that the effects of haloperidol in the paw test could be reversed by the D2/3 agonist quinpirole, whereas the effects of clozapine could be reversed by the D1 agonist SKF38393 (Ellenbroek et al., 1991). Moreover, the effect of clozapine on the HRT was reversed by the 5-HT2 agonist \( (\sim) \)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (Ellenbroek et al., 1994). Since JL13 has a relatively strong affinity for the 5-HT2 receptor and also binds stronger to the D1 than the D2 receptor (Wilkerson and Levin, 1999), it is tempting to speculate that the effects of JL13 on the HRT in the paw test is also due to a combined 5-HT2/D1 antagonism. However, more pharmacological studies need to be done. Irrespective of the underlying mechanism, JL13 shows a profile similar to other atypical antipsychotics like clozapine, olanzapine, ris-
peridine, and quetiapine (Ellenbroek et al., 1987, 1996; Cools et al., 1995), suggesting that it may have a limited capacity for inducing extrapyramidal side effects in humans as well. Moreover, in nonhuman primates sensitized to haloperidol either in acute or chronic experiments, JL13 showed a good tolerance with moderate and dose-related increased sedation and decreased locomotor activity. In acute experiment, a mild dystonia and a parkinsonian symptom of slow movement developed at the highest dose tested (50 mg/kg p.o.) in only 50% animals (Casey et al., 2001).

Although screening tests like the paw test have been useful in identifying potential new antipsychotic drugs, simulation models might be more promising to evaluate the possible clinical effects of these new drugs. One of the models that has gained a tremendous amount of interest in this respect is the prepulse inhibition paradigm (Swerdlow et al., 1994; Geyer and Markou, 1995; De Hert and Ellenbroek, 2000; Ellenbroek et al. 2000). This interest is based on the fact that schizophrenic patients have a deficient prepulse inhibition (Braff et al., 1978) and that prepulse inhibition can be measured in rats with virtually identical methods. Prepulse inhibition has been referred to as sensory gating, reflecting the brain's capacity to filter incoming sensory information. It is important, however, to realize that prepulse inhibition in itself is not a simulation model for schizophrenia, it is the disruption thereof that is of particular interest. However, prepulse inhibition can be disrupted by many manipulations (both pharmacological and nonpharmacological). For instance, dopamine agonists (like amphetamine and apomorphine), N-methyl-d-aspartate antagonists (such as phencyclidine and ketamine), and serotonin agonists [such as 8-hydroxy-2-(+)-5,7-dimethoxy-4-iodoamphetamine hydrochloride] disrupt prepulse inhibition (Mansbach et al., 1988; Mansbach and Geyer, 1989; Sipes and Geyer, 1994, 1995). Likewise, isolation rearing (Geyer et al., 1993) and maternal deprivation (Ellenbroek et al., 1998) disrupt prepulse inhibition. As discussed elsewhere, it is so far unclear which of these different ways of disrupting prepulse inhibition most closely resembles the deficit seen in schizophrenic patients (Ellenbroek and Cools, 2000). Swerdlow and his colleagues (1994) suggested that the apomorphine-induced disruption of prepulse inhibition showed the strongest predictive validity, as all currently known antipsychotic drugs reverse the effects of apomorphine. In agreement with this, we found in the present study that all three drugs investigated reversed the effects of apomorphine on prepulse inhibition. Haloperidol was by far the most potent, with JL13 and clozapine being only effective at the highest doses tested. So far, very few studies have investigated the pharmacology of the amphetamine-induced disruption of prepulse inhibition. Preliminary experiments in our laboratory (data not shown) had indicated that there might be differences in the pharmacology of the disruption of prepulse inhibition by these two dopamine agonists. The present study seems to confirm this, as it showed that JL13 and clozapine reversed the effects of amphetamine at a dose of 5 resp. 10 mg/kg, whereas higher doses (10 resp. 20 mg/kg) were necessary to reverse the effects of apomorphine. Haloperidol, on the other hand, was equally effective against both drugs. At present, it is not clear why JL13 and clozapine were effective at lower doses against amphetamine than against apomorphine. Amphetamine, in addition to releasing dopamine, also releases serotonin, and as mentioned earlier, serotonin agonists also reduce prepulse inhibition. Thus, the strong serotonin blocking effect of both clozapine and JL13 might be partially responsible for the different effects of these drugs against amphetamine than against apomorphine.

Finally, JL13 was found to induce a small, yet significant increase in prepulse inhibition in normal rats. However, the effect was only marginal and seen at only one dose and one prepulse intensity, but it was not seen for clozapine or haloperidol. Depoortere and his colleagues (1997) found an increase in prepulse inhibition with some antipsychotics but not with others. Interestingly, the two most effective antipsychotics in this respect were haloperidol and clozapine, whereas remoxipride and risperidone were without effect. Likewise, Schwarzkopf and his colleagues (1993) also found an increase in prepulse inhibition after haloperidol. Although it is not entirely clear why these two studies differ from the present one, strain differences may well have played a role (Swerdlow et al., 1998; Kinney et al., 1999). In any case, it seems that potentiation of prepulse inhibition does not have much predictive validity for antipsychotic drugs (Depoortere et al., 1997).

Overall, data show that the behavioral effects of JL13 closely resemble those of clozapine (Table 2), although there are also some differences. This seems to be in agreement with other previous reports. Thus, both clozapine and JL13 reverse amphetamine-induced locomotion and amphetamine-induced climbing in mice, without affecting apomorphine- and amphetamine-induced stereotypy (Bruhwyl et al., 1997). Moreover, both clozapine and JL13 do not induce catalepsy (Bruhwyl et al., 1997), and increase immobility in the forced swim test (Bruhwyl et al., 1995). Both drugs also reduce rearing and defecation in the open field (Bruhwyl et al., 1995). Finally, JL13 showed a 70% generalization to clozapine in clozapine-trained rats (Goudie and Taylor, 1998). Our data add two further similarities, namely a selective enhancement of HRT in the paw test and a reversal of both apomorphine- and amphetamine-induced disruption of prepulse inhibition. These data strongly suggest that JL13 has a clinical profile similar to clozapine, although studies in patients will ultimately be needed.

References

| Table 2: Summary of the results reported in the present paper |

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<tr>
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<th>Apomorphine</th>
<th>Amphetamine</th>
<th>Control</th>
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<tr>
<td>Haloperidol</td>
<td>HRT = FRT</td>
<td>↓↓↓</td>
<td>↓↓↓</td>
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<tr>
<td>JL13</td>
<td>HRT &gt; FRT</td>
<td>↓↓↓</td>
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<tr>
<td>Clozapine</td>
<td>HRT &gt; FRT</td>
<td>↓↓↓</td>
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BSA, basal startle amplitude; PPI, prepulse inhibition; ↓↓↓, strong reduction; ↑, increase; –, no effect.