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Activity of "Seroquel" (ICI 204,636) in Animal Models for Atypical Properties of Antipsychotics: A Comparison with Clozapine

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The pharmacologic treatment of schizophrenia still suffers from two major problems: (1) most antipsychotic drugs still induce severe neurologic (extrapyramidal) side effects; (2) few antipsychotic drugs are effective in treating the negative symptoms of schizophrenia. In the present study, we have evaluated the effects of ICI 204,636 in the rat paw test and the amphetamine-induced social isolation in monkeys and compared them with the effects of clozapine. The paw test has been shown to be a valid model for differentiating classic and atypical neuroleptic drugs. The monkey social isolation model seems to represent one of the few animal models with validity for the negative symptoms of schizophrenia. The results show that both ICI 204,636 and clozapine had the profile of an atypical antipsychotic in the paw test, suggesting a reduced propensity to induce extrapyramidal side effects in humans. Likewise, ICI 204,636 and clozapine were found to prevent the amphetamine-induced social isolation in monkeys, suggesting a good therapeutic effect mitigating the negative symptoms in schizophrenia. Overall, the data suggest that ICI 204,636 may represent a new and interesting antipsychotic drug, closely resembling clozapine. ***[Neuropsychopharmacology 15:406–416, 1996]***

KEY WORDS: *Schizophrenia; Clozapine; Seroquel; ICI 204,636; Paw test; Monkey social isolation*

Despite more than 40 years of experience with antipsychotic drugs, the pharmacologic treatment of schizophrenia is still suffering from two major problems: (1) the relative high incidence of extrapyramidal side effects and (2) the relative lack of therapeutic efficacy with respect to the negative symptoms (for a recent review, see Kane 1995). These problems stem, at least to a certain degree, from the lack of adequate animal models with predictive and face validity (Ellenbroek 1993).

Animal models with predictive validity are usually based on a comparison of a new drug with a well-known antipsychotic (usually haloperidol). If the new drug produces the same effect, it is suggested to be an antipsychotic agent. These models have been very effective in detecting antipsychotics, but they seem less able to distinguish between classic and atypical antipsychotics, viz antipsychotics that induce much fewer extrapyramidal side effects. Indeed, atypical antipsychotics like clozapine or risperidone are usually either not active in these models or produce an effect similar to that of haloperidol. Several years ago we developed a rodent model able to differentiate classic and atypical antipsychotics on the basis of positive criteria (Ellenbroek et al. 1987). In the paw test, classic antipsychotics (like haloperidol, chlorpromazine, or fluphenazine) affect both the forelimb retraction time as well as the hindlimb re-

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traction time to a similar extent. Atypical antipsychotics (like clozapine, thioridazine, or risperidone) are much more effective in increasing hindlimb retraction time. This model has been extensively validated, using a set of criteria obtained from clinical practice (Ellenbroek and Cools 1988; Ellenbroek 1993) and has been very useful in detecting and/or confirming the atypical profile of antipsychotics (Meert and Awouters 1991; Cools et al. 1995) and in studying the underlying neuronal mechanisms (Prinssen et al. 1994ab 1995).

Animal models with face validity are designed to model one or more of the symptoms of a disease. In the case of schizophrenia, most of the symptoms are of a cognitive or emotional nature, only apparent upon introspection and thus unsuitable for animal modeling, although the recent observation that certain types of hallucinations can be linked to activation of specific brain regions might provide new opportunities (Silbersweig et al. 1995). However, one aspect of the negative symptom cluster (i.e., social withdrawal) can be measured, particularly in monkeys. Indeed, it has been suggested that the amphetamine-induced social isolation might be a promising animal model with face validity for the negative symptoms of schizophrenia (Miczek and Yoshimura 1982; Ellenbroek 1991), especially in light of the findings that classic antipsychotics do not affect this social isolation (Schiorring 1979; Miczek and Yoshimura 1982).

ICI 204,636 is a novel dibenzothiazepine with affinity for multiple brain receptors including serotonergic type 2 (5-HT_2) and dopaminergic type 2 (D_2) receptors (IC_{50} = 148 and 329 nM, respectively) (Saller and Salama 1993). Differences in receptor affinity also distinguish ICI 204,636 from risperidone. Although both have greater affinity for 5-HT_2 receptors than for D_2 receptors, absolute affinities at D_2 receptors differ: ICI 204,636 has weak D_2 receptor affinity, like clozapine, whereas risperidone has a very high affinity, like haloperidol (Leysen et al. 1988). ICI 204,636 exhibits antipsychotic-like activity in the conditioned avoidance response paradigm in both rats and monkeys (Migler et al. 1993) and blocks amphetamine-induced locomotor activity. In electrophysiologic experiments, it exhibits the profile of an atypical antipsychotic, as illustrated by its selective action on mesolimbic A_{10} neurons (Goldstein et al. 1993). However, the validity of this model has recently been questioned by Mereu et al. (1995), who suggested that the depolarization inactivation of A_9 and A_{10} neurons is an anesthesia artifact. Nevertheless, the preliminary clinical studies suggest that it has a low liability for inducing extrapyramidal side effects (Fabre 1993; Fabre et al. 1995; Wetzel et al. 1995; Borison et al. 1996). Since the clinical studies also indicate that ICI 204,636 may have therapeutic efficacy with respect to the negative symptoms, we decided to study the effects of this putative atypical antipsychotic in the paw test and the amphetamine-induced monkey social isolation paradigm and compare them with the effects of clozapine.

MATERIALS AND METHODS

All animal experiments were performed in accordance with the Helsinki Declaration and with institutional guidelines.

Paw Test

The paw test has been extensively described elsewhere (Ellenbroek et al. 1987, 1994). Male Wistar rats, obtained from the Central Animal Laboratory (Nijmegen, the Netherlands) weighing 240 to 300 g were used. The animals were individually housed at least 24 hours before the experiments with water and food freely available (except during experiment). All experiments were performed between 10:00 A.M. and 4:00 P.M. On the day of the experiment, the rats were placed into the test room and allowed at least 30 minutes for habituation. After this habituation, each rat was injected IP with either ICI 204,636 (10 to 100 mg/kg), clozapine (1 to 100 mg/kg) or solvent (saline, or diluted HCl) in a volume of 1 ml/kg. The paw test was performed 30 minutes after the intraperitoneal injection. 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. 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 needs to withdraw one forelimb and one hindlimb from the hole, respectively. The minimum time was set at 1 second, because it was difficult to determine the exact starting time. When the rat did not withdraw its forelimb or hindlimb within 30 seconds, the animal was taken out and the FRT or HRT was set at 30 seconds. The paw test was repeated at 40 and 50 minutes 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. Each dose of ICI 204,636 and of clozapine (as well as the saline control) was tested in a group of eight drug-naive rats. The median FRT and HRT per group was determined and displayed in Figure 1. The Mann Whitney U test for independent groups was used to evaluate statistical differences between different doses. The minimal effective dose (MED) was de-

fined as the lowest dose that induced a significant increase compared with saline.

Monkey Social Isolation Paradigm

The experiments were conducted in a group of eight Java monkeys (*Macaca fascicularis*), consisting of three adult males (age 7 to 9 years), two adult females (age 8 years), and three juvenile monkeys (age 0.5 to 2 years). The animals were housed in an experimental room (measuring 3 × 2 × 2 meters) with a standard light–dark cycle of 12 hours (light on 6:00 A.M. to 6:00 P.M.). The animals were fed twice daily (in the morning and afternoon), and water was freely available. A remote controlled videocamera was installed to allow recording of the monkey's behavior in a separate room. All animals were drug naive before this series of experiments.

Four monkeys were selected for the drug treatments: the two females and the two lower ranking males. *d*-Amphetamine sulphate was obtained from RBI (Natick, USA), and ICI 204,636 and clozapine were kindly provided by ZENECA (Wilmington, USA). Each monkey received every drug treatment in a pseudorandom order, so as not to treat one monkey more than once a week. Apart from saline and *d*-amphetamine sulphate (0.5 mg/kg) controls, monkeys were treated with a combination of *d*-amphetamine sulphate (0.5 mg/kg) and ICI 204,636 (1, 3.3, and 10 mg/kg) or a combination of *d*-amphetamine sulphate (0.5 mg/kg) and clozapine (0.33, 1, and 3.3 mg/kg). All experiments were repeated three (in case of the combination treatments) or four times (in case of the saline and amphetamine) and were performed on Mondays, Wednesdays, and Fridays, between 9:30 and 11:30 A.M. The experiments with ICI 204,636 were performed first, the experiments with clozapine later. Control and amphetamine treatments were given intermingled with the ICI 204,636 and the clozapine experiments. The monkey to be treated was caught in a small cage attached to the monkey's experimental cage, where it was intramuscularly injected. Immediately after this injection procedure (which typically lasted between 2 and 5 minutes), the monkey was allowed to join the other animals, and the videorecording was started. From earlier studies, it was known that this procedure induces some stress, which disappears in about 15 minutes (Ellenbroek et al. 1989). For that reason, we only analyzed the data from 30 to 60 minutes after the injection. The videotapes were analyzed by a rater who was blind to the treatment. Because our prime interest was to study the effects of ICI 204,636 and clozapine on amphetamine-induced social isolation, a limited ethogram was used. Basically, five different behavioral categories were scored (Table 1) for every monkey treated. Three behaviors (proximity, grooming, and submissive behavior) were further subdivided in an active and a passive form. These behaviors were scored in terms of duration with one exception: submissive behaviors. Because the latter behavior is usually short-lasting, frequency data are much more informative than duration data (see Ellenbroek et al. 1989).

The data were statistically evaluated per category of behavior (see Table 1). The effects of amphetamine were analyzed in a one-way ANOVA, comparing saline control and amphetamine. The effects of ICI 204,636 and clozapine on amphetamine were analyzed by comparing all doses of ICI 204,636 (or clozapine) with amphetamine in a one-way ANOVA. If the latter ANOVA was significant, a post hoc Duncan test was performed to analyze the individual doses of ICI 204,636 (or clozapine) separately. A p value of .05 or less was considered to indicate a significant difference.

RESULTS

Paw Test

Control injections of saline induced only a very small FRT (mean 1.0 ± 0.0 seconds) and a small HRT (3.1 ± 0.9 seconds). ICI 204,636 produced a dose-dependent increase in HRT (see Figure 1A), with an MED of 25 mg/kg. ICI 204,636 was much less effective in increasing the FRT. A significant increase was only at the highest dose tested (100 mg/kg). The ratio of MEDs (FRT to HRT) was therefore 4 (100/25). Clozapine also pro-

Table 1. Ethogram Used in the Monkey Social Isolation Experiment

Behavior	Subtype	Definition
Alone		All nonsocial activities
Social		All social activities
Grooming	Active	Actively picking scraping or licking the fur of another monkey or other monkeys
	Passive	Being groomed by another monkey or monkeys
Proximity	Active	Actively moving toward another monkey and coming within arm's reach of another monkey
	Passive	Being approached by another monkey who comes within arm's reach of the reference monkey
Submissive	Active	Actively showing submissive gestures like grinning and lipsmacking toward another monkey
	Passive	Receiving submissive gestures like grinning and lipsmacking from other monkeys

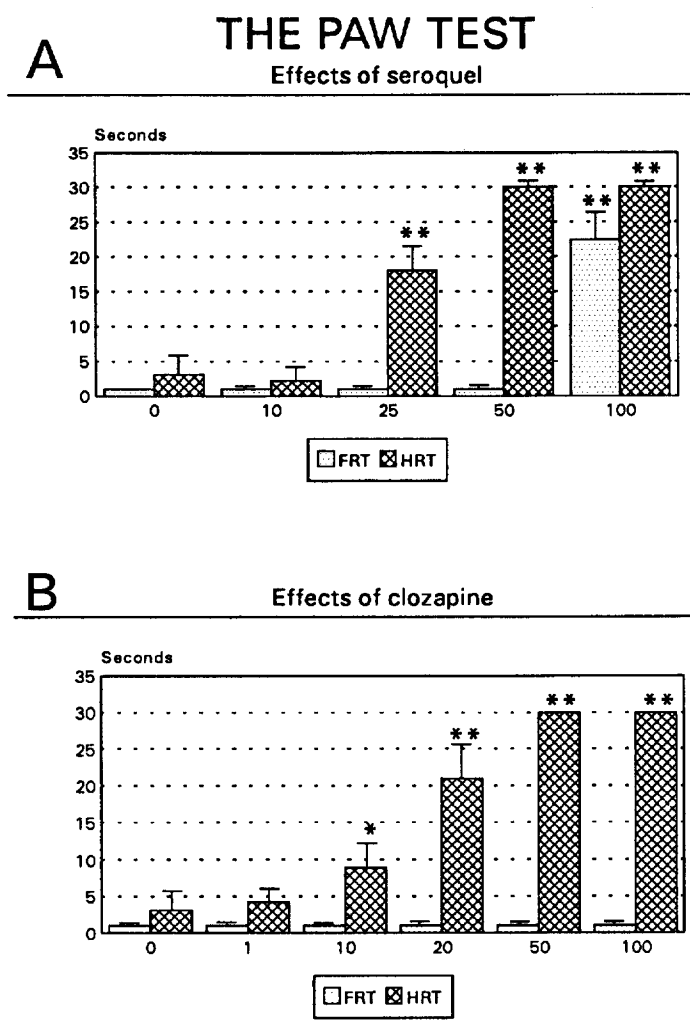


Figure 1. Effects of increasing doses of ICI 204,636 **(A)** or clozapine **(B)** on FRT and HRT. Represented are the median values ± SEM of groups of eight rats per dose. $p < .01$ Mann Whitney U test vs. control.

duced a dose-dependent increase in HRT, with an MED of 10 mg/kg. However, it did not significantly increase FRT in the dose range tested. Importantly, at the highest dose tested (100 mg/kg), some rats showed clear signs of convulsion and could not be tested in the paw test.

Monkey Social Isolation Paradigm

The effects of the different drugs on the social behavior of Java monkeys are depicted in Figures 2 to 9. We will first discuss the effects of ICI 204.636 and after that the effects of clozapine. Because both active grooming and passive submissive behavior rarely occurred, these data will not be discussed.

Effects of ICI 204,636. The overall duration of time spent alone under control conditions was rather high, as can be seen in Figure 2. Under control conditions, the animals spent approximately 75% of their time alone. Despite this relatively high baseline level, the overall time spent was further increased by 0.5 mg/kg amphetamine to 91%. Statistical analysis showed that this increase was significant ($F_{(1,22)} = 8.80$, $p < .007$). ICI

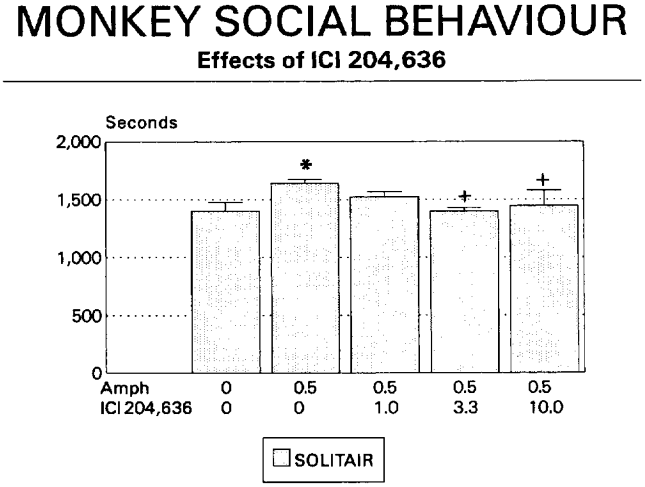


Figure 2. Effects of amphetamine and different doses of ICI 204,636 on time spent alone in Java monkeys. * $p < .05$ vs. control (ANOVA); $^{+}$ $p < .05$ vs. amphetamine 0.5 mg/kg (post hoc Duncan).

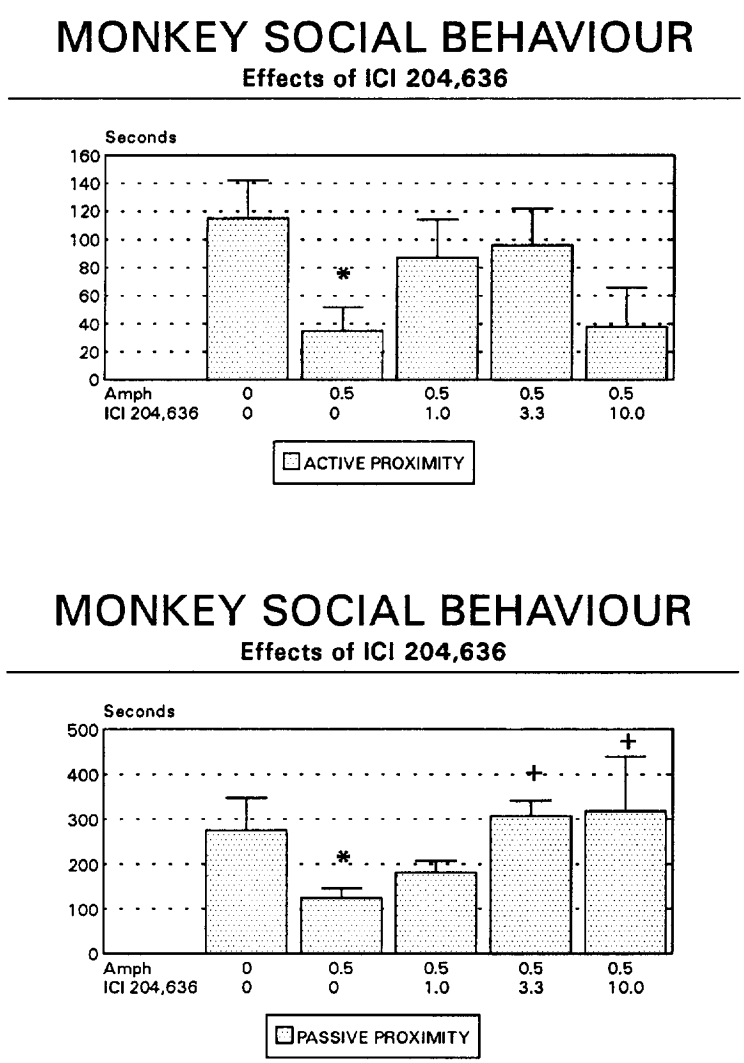


Figure 3. Effects of amphetamine and different doses of ICI 204,636 on the duration of active (*top*) and passive (*bottom*) proximity in Java monkeys. $^{*}p < .05$ vs. control (ANOVA); $^{+}p < .05$ vs. amphetamine 0.5 mg/kg (post hoc Duncan).

204,636 antagonized this amphetamine-induced increase in time spent alone. This was confirmed by the statistical evaluation (ANOVA $F_{(3,45)} = 5.70$; $p < .002$). Post hoc Duncan analysis showed that the doses of 3.3 and 10 mg/kg ICI 204,636 significantly antagonized the effects of amphetamine.

The overall increase in time spent alone seen after amphetamine treatment was due to a reduction in both active and passive proximity (Figure 3). These observations were confirmed by statistical analysis (active proximity: $F_{(1,22)} = 6.27$, $p < .02$; Passive proximity: $F_{(1,22)} = 4.04$, $p < .05$). ICI 204,636 dose dependently antagonized the effects of amphetamine on passive grooming ($F_{(3,45)} = 6.42$; $p < .001$). Post hoc Duncan analysis revealed that the combinations of amphetamine + ICI 204,636 3.3 and amphetamine + ICI 204,636 10 were significantly larger than amphetamine alone. With respect to active proximity, a slightly different effect was seen. Although a clear tendency toward reversal of the effects of amphetamine was seen at the lower doses of ICI 204,636 (1.0 and 3.3), no such effect was observed at the highest dose. Visual analysis of the video tapes showed that at this high dose of ICI 204,636 the monkeys were very sedated. This was especially true for the males, who were lying on the floor or on one of the platforms for prolonged periods of time. This sedative effect was less apparent in the treated females, although they also showed an overall slowness of movement.

Amphetamine also induced a reduction in passive grooming (Figure 4). However, due to the large individual variability in the control group, this effect did not reach statistical significance ($F_{(1,22)} = 3.62$, $p = .06$). ICI 204,636 induced a nonsignificant reversal of the effects of amphetamine (Figure 4).

Figure 5 shows that there was an increase in the frequency of submissive behaviors, which just reached statistical significance ($F_{(1,22)} = 4.00$, $p < .05$). ICI 204,636 significantly reduced this increase in the frequency of submissive behaviors (Figure 4: $F_{(3,45)} = 2.75$, $p < .05$). However, no dose dependency was observed, as all doses of ICI 204,636 were significantly lower than amphetamine alone.

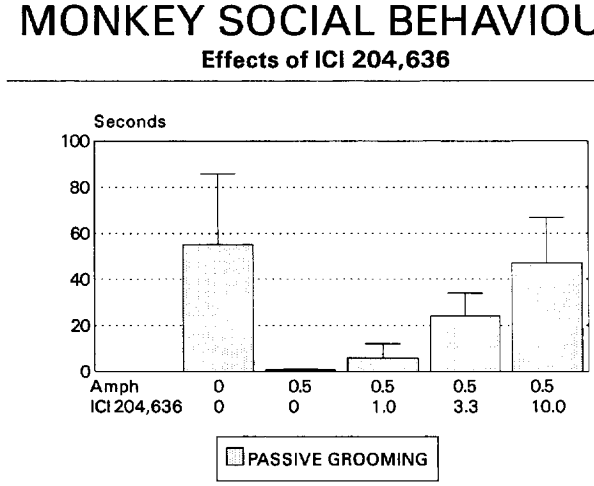


Figure 4. Effects of amphetamine and different doses of ICI 204,636 on the duration of passive grooming behavior in Java monkeys.

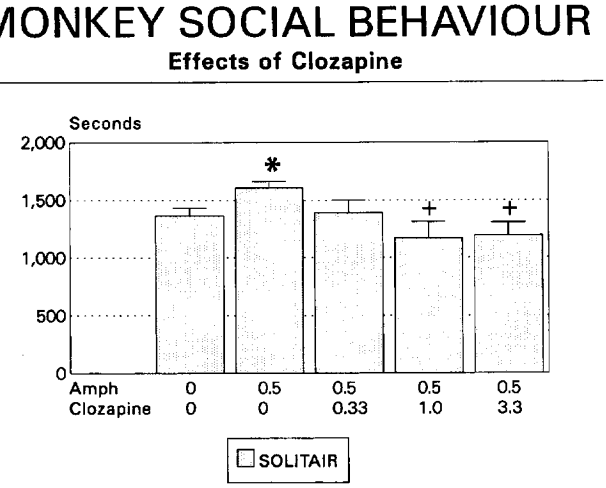


Figure 6. Effects of amphetamine and different doses of clozapine on time spent alone in Java monkeys. * $p < .05$ vs. control (ANOVA); + $p < .05$ vs. amphetamine 0.5 mg/kg (post hoc Duncan).

Effects of Clozapine. The effects of clozapine on the amphetamine-induced social isolation are depicted in Figures 6 to 9. Amphetamine induced very similar effects as in the experiments with ICI 204,636: it increased time spent alone (Figure 6, $F_{(1,30)} = 7.56$, $p < .01$); active proximity (Figure 7A, $F_{(1,30)} = 9.08$, $p < .005$), passive proximity (Figure 7B, $F_{(1,30)} = 4.10$, $p < .05$), and passive grooming (Figure 8, $F_{(1,30)} = 5.12$, $p < .05$). Amphetamine also enhanced the frequency of submissive behaviors (Figure 9, $F_{(1,30)} = 3.98$, $p = .05$).

Clozapine significantly reduced the amphetamine-induced increase in time spent alone ($F_{(3,48)} = 4.17$, $p < .01$). Post hoc Duncan tests showed that the effects of 1 and 3.3 mg/kg clozapine were significantly different from amphetamine alone (Figure 6). This reversal of amphetamine-induced social isolation was due to an increase in both active and passive proximity (Figure 7). ANOVA analysis showed a significant effect for both parameters (active proximity: $F_{(3,48)} = 9.1$, $p < .005$; passive proximity $F_{(3,48)} = 4.02$, $p < .01$). As with seroquel, the active proximity showed a bell-shaped dose response curve (Figure 7, top). However, post hoc Duncan tests showed that both 1.0 and 3.3 mg/kg clozapine were significantly different from amphetamine. The passive proximity showed a clear dose-dependent reversal of the amphetamine-induced reduction. Post hoc Duncan tests showed that 1.0 and 3.3 mg/kg were significantly different from amphetamine alone. Clozapine also reversed the amphetamine-induced decrease in passive grooming ($F_{(3,48)} = 4.01$, $p < .01$). Figure 8 shows that all doses of clozapine were significantly different from amphetamine alone (post hoc Duncan). The effects of clozapine on the amphetamine-induced increase in the frequency of submissive behaviors is displayed in Figure 9. Clozapine reversed the effects of amphetamine ($F_{(3,48)} = 5.14$, $p < .004$). As with seroquel, no dose dependency was observed. Post hoc Duncan tests showed a significant decrease for all doses of clozapine tested.

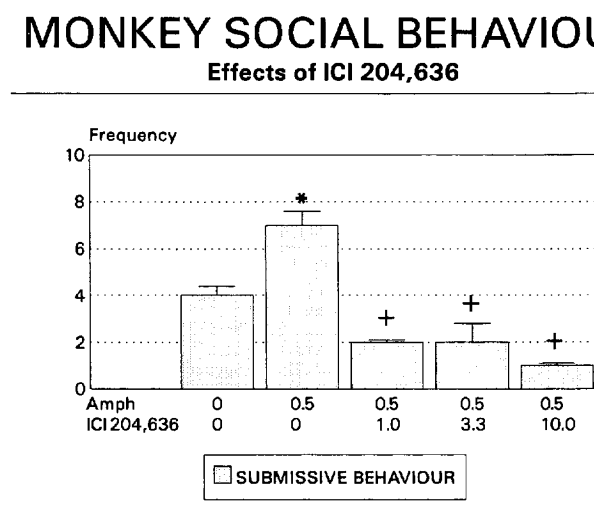
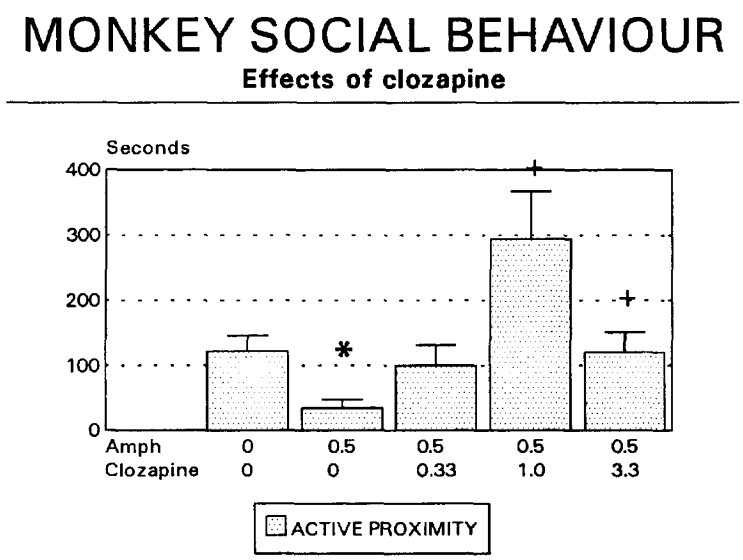


Figure 5. Effects of amphetamine and different doses of ICI 204,636 on the frequency of active submissive behavior in Java monkeys. * $p < .05$ vs. control (ANOVA); + $p < .05$ vs. amphetamine 0.5 mg/kg (post hoc Duncan).

DISCUSSION

The results of the present study show that in the paw test, ICI 204,636 displayed the profile of an atypical antipsychotic drug, closely resembling clozapine, as



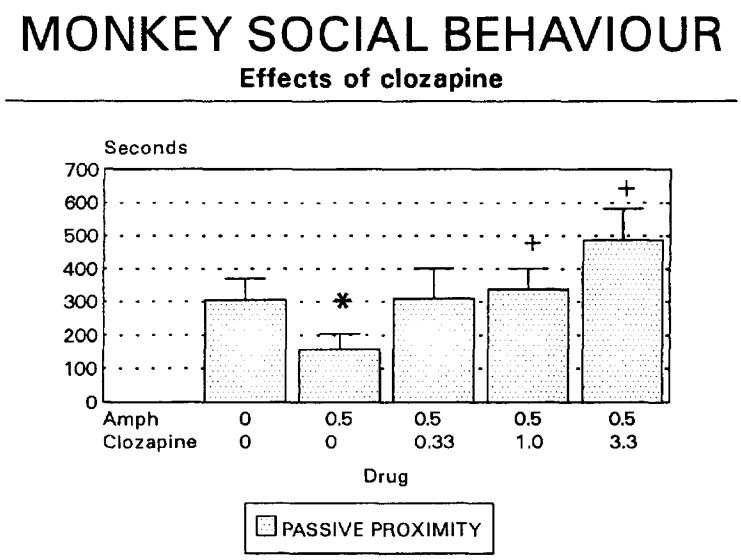


Figure 7. Effects of amphetamine and different doses of clozapine on the duration of active (*top*) and passive (*bottom*) proximity in Java monkeys. $^{*}p < .05$ vs. control (ANOVA); $^{+}p < .05$ vs. amphetamine 0.5 mg/kg (post hoc Duncan).

shown by a stronger effect on HRT than FRT. Like all known atypical antipsychotics tested so far (clozapine, thioridazine, risperidone), ICI 204,636 had a MED ratio larger than 1 (Ellenbroek et al. 1987, 1994). Classic antipsychotics (haloperidol, chlorpromazine, fluphenazine, flupenthixol) have been found to have an equal effect on both FRT and HRT, expressed in an MED ratio of 1 (Ellenbroek et al. 1987). As discussed in the introduction, ICI 204,636 appears to have a binding profile more similar to clozapine than to risperidone, with a strong affinity for 5-HT_2 receptors and a somewhat weaker affinity for D_2 receptors (Saller and Salama 1993; Leysen et al. 1988). We have recently shown (Ellenbroek et al. 1994) that the 5-HT_2 blocking potency of risperidone is responsible for its lack of effect on the FRT. It is tempting to speculate that the same holds true for ICI 204,636. We must realize, however, that the role of 5-HT_2 receptors in the effects of antipsychotics in the paw test is highly complex (Ellenbroek et al. 1994), and differs significantly between different antipsychotics. Furthermore, ICI 204,636 is a potent α_1 antagonist (Saller and Salama 1993), and we have recently shown that α_1-receptors can also play a role in the effects of antipsychotics in the paw test (Prinssen et al. 1994a,b). In other words, more detailed pharmacologic experiments are necessary to determine the role of these individual receptors in the effects of ICI 204,636 in the paw test. Nevertheless, ICI 204,636 resembles clozapine, risperidone, and other atypical antipsychotics in the paw test, which is in good agreement with the first clinical studies (Fabre 1993; Fabre et al. 1995; Hirsch 1994; Wetzel et al. 1995; Borison et al. 1996), where ICI 204,636 was shown to have a low propensity for inducing extrapyramidal side effects.

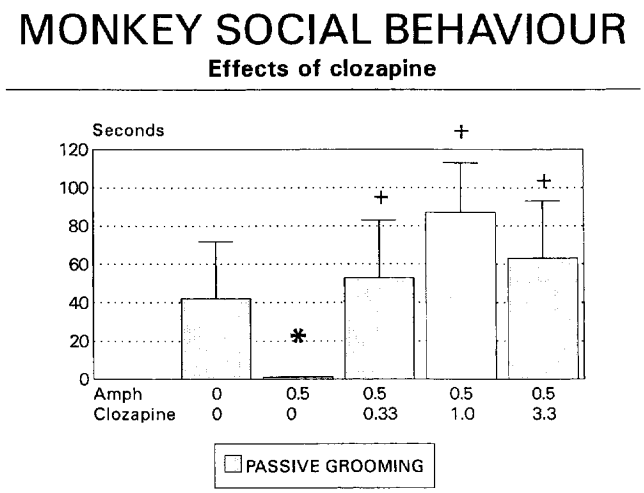


Figure 8. Effects of amphetamine and different doses of clozapine on the duration of passive grooming behavior in Java monkeys. * $p < .05$ vs. control (ANOVA); $^{+}$ $p < .05$ vs. amphetamine 0.5 mg/kg (post hoc Duncan).

An interesting observation was that clozapine, in a dose of 100 mg/kg IP induced convulsion-like behavior. Although we did not study this in any detail, it is a well-known side effect of clozapine in schizophrenic patients (Haller and Binder 1990).

Apart from an atypical profile in the paw test, both ICI 204,636 and clozapine were found to prevent the occurrence of social isolation in monkeys induced by amphetamine. Although the overall social behavior of the group was rather low (about 25%) as compared with earlier studies (Ellenbroek et al. 1989), amphetamine still reduced this social behavior to less than 10% (Figures 2 and 6). This effect was seen in all monkeys treated. Moreover, amphetamine significantly reduced

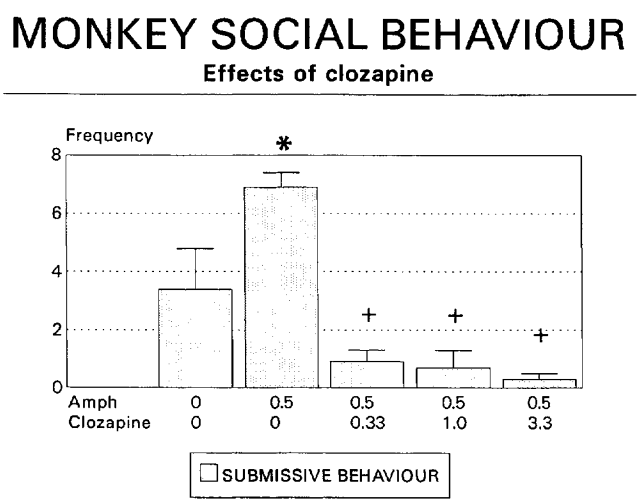


Figure 9. Effects of amphetamine and different doses of clozapine on the frequency of active submissive behavior in Java monkeys. * $p < .05$ vs. control (ANOVA); $^{+}$ $p < .05$ vs. amphetamine 0.5 mg/kg (post hoc Duncan).

active and passive proximity, whereas it showed a strong tendency to reduce passive grooming as well (which was significant in the experiments with clozapine). Apart from the general decrease in social activities, amphetamine is also known to enhance certain social behaviors, most prominently submissive gestures (Haber et al. 1977; Schlemmer and Davis 1981). Given the fact that this increase in submissive behaviors is not accompanied by an increase in aggressive behaviors toward the treated monkey (data not shown), this has been interpreted as a disturbed perception of reality: viz. perceiving the environment as more hostile than it is (Knobbout et al. 1996). The increase in submissive behavior has been suggested to represent an animal model with face validity for paranoid delusions (see Haber et al. 1977; Schlemmer and Davis 1981; Ellenbroek 1991). This is strengthened by the finding that, in contrast to the other effects of amphetamine, the increase in submissive behavior is sensitive to classic antipsychotic drugs (Schlemmer and Davis 1981). The effects on submissive behaviors are shown in Figures 5 and 8. Amphetamine significantly increased the frequency of submissive gestures, although this enhancement was small. This was primarily due to the fact that only the males showed a clear increase in submissive gestures. The two females hardly ever showed submissive behavior under any treatment. In summary, amphetamine reduced the time spent in social contact and thus induced social isolation in all monkeys treated. Moreover, amphetamine induced an increase in submissive gestures, especially in the males.

In the past the amphetamine-induced social isolation in monkeys has been regarded as an animal model for the negative symptoms of schizophrenia (Miczek and Yoshimura 1982; Ellenbroek 1991). This is based partly on a phenomenologic similarity between the social withdrawal in schizophrenic patients and the amphetamine-treated monkeys and partly on pharmacologic similarities. Thus, the amphetamine-induced social isolation in monkeys is unaffected by classic antipsychotics (Schiorring 1977; Scraggs and Ridley 1979; Miczek and Yoshimura 1982), by α_1 or β antagonists (Scraggs and Ridley 1979), by benzodiazepines (Scraggs and Ridley 1979), and by opiate antagonists (Winslow and Miczek 1988). There is ample evidence that the same drugs are also ineffective in reducing negative symptoms in schizophrenia (antipsychotics: Johnstone et al. 1978; Angrist et al. 1980; Kucharski et al. 1984; Scottish Schizophrenia Research Group 1987; α_1 antagonist: Albus et al. 1986; β antagonists: Myers et al. 1981; benzodiazepines: Csernansky et al. 1988; opiate antagonists: Githin et al. 1981). Unfortunately, there has been very little positive pharmacologic evidence for linking amphetamine-induced social isolation to the negative symptoms of schizophrenia. In the past, we have shown that the selective D_1 antagonist SCH 23390 is able to re-

verse the amphetamine-induced social isolation (Ellenbroek et al. 1989). Unfortunately, this drug has a very short duration of action and is therefore clinically not useful. However, recently DenBoer and his colleagues showed that another selective D_1 antagonist (SCH 39166) was able to attenuate the negative symptoms of schizophrenia, although the effect was small (DenBoer et al. 1995). The only effective treatment of the negative symptoms currently available seems to be clozapine (Kane et al. 1988). The present finding that clozapine reversed all aspects of the amphetamine-induced social isolation therefore constitutes an important improvement of the validity of the model. One important aspect that needs attention is that in the present study both ICI 204,636 and clozapine were found to be effective upon acute administration, whereas clinically there appears to be a therapeutic lag. One should realize, however, that we induced an acute stage of social isolation (by amphetamine), which is clearly different from the chronic schizophrenic illness. Moreover, there also have been several reports indicating a significant effect of antipsychotics after as little as 1 or 3 days of therapy (for a review, see Ellenbroek 1993). Nevertheless, it might be worthwhile to study the effects of antipsychotics after chronic amphetamine administration, which is also known to lead to social isolation (Ridley et al. 1979).

ICI 204,636 in different doses induced effects very similar to clozapine in the monkey social isolation paradigm. In fact there were only two clear differences: (1) clozapine significantly reversed the amphetamine-induced active proximity and passive grooming, whereas ICI 204,636 showed a strong, but nonsignificant tendency. (2) clozapine appeared to be more potent than ICI 204,636. Especially the latter finding is somewhat surprising, given the fact that the two drugs appeared to be equipotent in earlier studies in monkeys (Migler et al. 1993). It is at present unclear whether these differences are due to different routes of administration (IM in the present study, PO in Migler et al. 1993), to differences in the monkeys used (*M. fascicularis* in the present study; *Saimiri sciureus* and *Cebus apella* in Migler et al. 1993), or to other reasons.

Despite these differences, the similarities between the effects of clozapine and ICI 204,636 are obvious. Both significantly reverse the decrease in total social isolation, the decrease in active proximity, and the increase in submissive gestures. Moreover, both drugs had an inverted U-shaped dose–response curve with respect to active proximity and showed no clear dose dependency with respect to submissive behaviors. In the case of ICI 204,636, the inverted U-shaped dose response relationship was most likely due to the fact that the highest dose of ICI 204,636 tested (10 mg/kg IM) led to severe sedation and akinesia in the monkeys. This was especially apparent in the males, who were lying on the floor or on one of the sitting places for several minutes. Females appeared less sedated by this dose of ICI 204,636, although their overall locomotor activity was less and speed of locomotion was clearly reduced (data not shown). This may also provide an explanation for the large variation seen at this dose (Figures 2 and 3). However, no obvious sedation was seen in the animals treated with clozapine. The lack of dose dependency in the effects of clozapine and ICI 204,636 on submissive behaviors is due to the fact that already the lowest dose of both drugs almost completely abolished the display of submissive behavior.

The present data appear to be in line with the clinical effects of ICI 204,636 published so far. Thus, Wetzel and his colleagues, in an open label study, recently showed that ICI 204,636 produced a significant reduction in total Scale for the Assessment of Negative Symptoms scores within 3 weeks, a finding also reported by Arvanitis et al. (1995) in two recent double-blind trials.

The results of the present preclinical study suggest that ICI 204,636 is an atypical antipsychotic drug with a low propensity to induce extrapyramidal side effects. Moreover, it appears to have an improved therapeutic profile with respect to the negative symptoms of schizophrenia. Although this is supported by preliminary clinical studies, double-blind placebo or antipsychotic controlled trials are necessary to further substantiate this claim.

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