Psychopharmacological Profile of Amisulpride: An Antipsychotic Drug with Presynaptic D₂/D₃ Dopamine Receptor Antagonist Activity and Limbic Selectivity

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Accepted for publication August 19, 1996

ABSTRACT
Amisulpride, a benzamide derivative, is an antipsychotic drug with a pharmacological profile distinct from that of classical neuroleptics such as haloperidol and from that of another benzamide, remoxipride. In mice, amisulpride antagonized hypothermia induced by apomorphine, quinpirole or (±) 7-hydroxy-2-(di-n-propylamino)-tetralin, an effect involving D₂/D₃ receptors, at similar doses (ED₅₀ ~ 2 mg/kg i.p.), which were much lower than doses that blocked apomorphine-induced climbing, an effect involving postsynaptic D₂ and D₁ receptor activation (ED₅₀ = 21 mg/kg i.p.). Much higher doses (ED₅₀ = 54 mg/kg i.p.) of amisulpride were needed to block grooming behavior observed after a short period in water, a D₁ receptor-mediated behavior. In rats, amisulpride preferentially inhibited effects produced by low doses of apomorphine (hypomotility and yawning), related to stimulation of presynaptic D₂/D₃ dopamine autoreceptors (ED₅₀ = 0.3 and 0.19 mg/kg i.p.). By contrast, amisulpride antagonized apomorphine-induced hypermotility, a postsynaptic dopamine receptor-mediated effect, at a much higher dose (ED₅₀ = 30 mg/kg i.p.). Amisulpride (100 mg/kg i.p.) only partially inhibited apomorphine-induced stereotypies (gnawing) and had no effect on stereotypies induced by d-amphetamine. However, d-amphetamine-induced hyperactivity was antagonized by doses of amisulpride as low as 3 mg/kg i.p., which may indicate selectivity of this drug for limbic dopaminergic mechanisms. In addition, in contrast to haloperidol or remoxipride, which produced catalepsy at doses 2 or 3 times higher than those that antagonized stereotypies induced by apomorphine, amisulpride did not induce catalepsy up to a dose of 100 mg/kg i.p., which occupies 80% of striatal D₂ receptors. This pharmacological profile of amisulpride, characterized by a preferential blockade of effects involving presynaptic mechanisms and limbic structures, may explain the clinical efficacy of this drug against both negative and positive symptoms of schizophrenia and its low propensity to produce extrapyramidal side effects.

Although the therapeutic efficacy of drugs currently used in the treatment of positive symptoms of schizophrenia is well established, about 30% of patients remain unimproved, and the efficacy of most drugs in treating negative symptoms is low. Neurological side effects, such as extrapyramidal syndromes, and other adverse effects, such as sedation, hypotension and endocrine effects, also limit the utility of most marketed drugs and justify the development of new drugs with greater efficacy and fewer adverse effects.

The strategy for discovering new antipsychotic drugs remains based on the dopamine hypothesis (Carlsson, 1978) and on the belief that relative hyperactivity of dopaminergic neurotransmission is involved in positive symptoms of schizophrenia, whereas hypofunction of these neurons may be involved in negative symptoms. This hypothesis rests on the observation that clinically effective drugs share D₂ dopamine receptor antagonist properties (Seeman, 1992). In addition, it has been shown that occupancy of about 50%–60% of central D₂ dopamine receptors is needed to produce antipsychotic activity, whereas higher receptor occupancy (70%–80%) is associated with extrapyramidal effects (Farde et al., 1992). On the basis of the hypothesis that antipsychotic effects are related to activity at limbic dopamine receptors, whereas antagonism of dopamine receptors in the striatum is responsible for extrapyramidal side effects, a compound possessing selectivity for limbic structures might be an antipsychotic with less propensity to produce motor disturbances. This hypothesis has been validated by the clinical efficacy of sulpiride and clozapine, drugs that preferentially block limbic dopamine receptors (Zivkovic et al., 1975; Scatton et al., 1977; Köhler et al., 1979; Csernansky et al., 1993).

Recent developments in molecular biology have shown the diversity of dopamine receptors and their differential regional cerebral localization (Sokoloff et al., 1990; Sibley and

ABBREVIATIONS: ANOVA, analysis of variance; 7-OH-DPAT, 7-hydroxy-2-(di-n-propylamino)-tetralin; MED, minimal effective dose; ND, not determined.
Monsma, 1992; Van Tol et al., 1991) and have provided new approaches in the search for more selective antidopaminergic drugs. Research on the D2 receptor subfamily, for which all clinically effective neuroleptics have affinity, has shown the preferential limbic localization of the D3 and D4 receptor subtypes in comparison with the more widespread distribution of the D2 receptor subtype. On the basis of these findings, the hypothesis has been advanced that agents with selective antagonist effects at D3 or D4 receptors may be effective antipsychotic agents with reduced side effects. Clozapine is the prototype of compounds selective for D4 receptors (Van Tol et al., 1991), whereas benzamide derivatives have high affinity for D3 dopamine receptors (Sokoloff et al., 1990; 1992a).

Amisulpride [(±)-amino-4-N-(1-ethyl-2-pyrrolidinyl)methyl sulphonyl-5-methoxy-2-benzamide] is an antipsychotic agent that shows clinical efficacy against both positive and negative symptoms of schizophrenia at high or low dosage, respectively, with a low incidence of extrapyramidal side effects (Delcker et al., 1990; Boyer et al., 1995). Recent studies (see Schoemaker et al., 1997; Sokoloff et al., 1990; 1992a) have shown that amisulpride is a specific dopamine receptor antagonist with high and similar affinities for the dopamine D3 and D4 receptor subtypes. An original property of this drug is its selectivity, at low doses, for presynaptic dopamine autoreceptors that control dopaminergic transmission. Moreover, amisulpride preferentially interacts with limbic dopamine D2-like receptors (Schoemaker et al., 1997). The present studies were carried out to define precisely the psychopharmacological profile of amisulpride in mice and rats and to assess the relative activity of the drug in behavioral tests involving activation of presynaptic and postsynaptic D3/D4 dopamine receptors. Amisulpride was compared with haloperidol and also with remoxipride, another benzamide that has been described as an atypical neuroleptic (Ogren et al., 1984; 1990).

Materials and Methods

Male CD1 mice (18–24 g) and male Sprague-Dawley rats (180–220 g) were supplied by Charles River (St Aubin les Elbeuf, France). Mice and rats were housed in groups of 25 and 5, respectively, and maintained on a 12-hr light-dark cycle (lights on at 7:00 A.M.) with free access to food and water. Housing rooms were temperature- and humidity-controlled. All procedures were performed in accordance with current French legislation on animal experimentation.

Dopamine agonist-induced hypothermia in mice. Hypothermia induced by apomorphine (1 mg/kg) injected by the s.c. route was recorded by a rectal probe (ARM6, Ellab Instruments, Copenhagen) in mice placed individually in small boxes (21 × 9 × 9 cm high) 45 min before drug treatment. Temperature was measured simultaneously with antagonist drug treatment, immediately before apomorphine and 30 min after injection of apomorphine, as previously described (Costentin, et al., 1975; Puech et al., 1981). Differences between the two first measures provided an assessment of the effect of the antagonist drug alone on body temperature. The same procedure was used to measure hypothermia induced either by quinpirole (1 mg/kg i.p.) or by 7-OH-DPAT (3 mg/kg i.p.).

Apomorphine-induced climbing in mice. Climbing behavior was measured according to the method described by Protais et al. (1976) and Costall et al. (1978). Immediately after injection of apomorphine (1 mg/kg s.c.), mice were placed in individual Plexiglas cylindrical cages with walls made of wire mesh (diameter 14 cm, height 15 cm). Fifteen minutes later, the time spent climbing (animal gripped onto the wire mesh with at least two paws) was noted for each mouse during 1 min.

Grooming behavior in mice. Grooming behavior was induced by a short period of swimming as described by Cheshire and Jackson (1981). After drug treatment, mice were placed individually in swimming chambers (8 × 8 × 18 cm high) filled with water (32°C) for 1 min. Immediately after removal of a mouse from the water, the presence (score = 1) or absence (score = 0) of grooming was observed every 2 min for 20 min in an observation cage. The global score for each mouse was the total of the 10 observations.

Locomotor activity in rats. Locomotor activity was measured in individual photocell activity cages (38 × 38 × 25 cm high). Each cage was fitted with two perpendicular photobeams 2 cm above the floor. Beam breaks were recorded automatically. Four experimental procedures were used:

Apomorphine-induced hyperactivity was recorded for 15 min, 15 min after administration of apomorphine (0.25 mg/kg s.c.) to rats previously placed in the activity cages for a 30-min habituation period. These experimental conditions yield low base-line activity suitable for assessing increases in locomotion produced by apomorphine.

d-Amphetamine-induced hyperactivity was recorded for 20 min, 30 min after injection of d-amphetamine (2 mg/kg i.p.) immediately after rats were placed in the activity cages without habituation.

Apomorphine-induced hypomotility was measured for 20 min in rats placed in activity cages immediately after injection of apomorphine (0.05 mg/kg s.c.). These experimental conditions yield high base-line activity suitable for assessing the depressant effect of low doses of apomorphine.

Spontaneous locomotor activity was measured for 20 min immediately after rats were placed in the activity cages. These conditions produce high base-line levels of activity suitable for assessing the general depressant effects of drugs.

Apomorphine- or d-amphetamine-induced stereotypies in rats. Stereotypies induced by either apomorphine (0.5 mg/kg s.c.) or d-amphetamine (3 mg/kg i.p.) were observed every 10 min for 30 min immediately after apomorphine or 30 min after d-amphetamine in rats placed in individual Plexiglas cages (25 × 20 × 14 cm high). For scoring stereotypies, two scales adapted from Costall and Naylor (1973) were used. In the case of apomorphine, the scale was as follows: 0: asleep; 1: awake, quiet; 2: locomotion, head bobbing; 3: sniffing; 4: licking; 5: chewing/gnawing. In the case of d-amphetamine, the following scale was used: 0: asleep; 1: quiet, weak sniffing; 2: sniffing, head bobbing, locomotion; 3: sniffing, discontinuous head bobbing, locomotion, rearing; 4: sniffing, frequent rearing, continuous head bobbing; 5: climbing on the wall, continuous head bobbing and sniffing, occurrence of licking and gnawing. For each rat, a global score was calculated by averaging the three stereotypy scores obtained at 10-min intervals.

Apomorphine-induced yawning. As described by Mogilnicka and Klimek (1977), low doses of apomorphine elicit yawning. The number of yawns was counted for 20 min, starting 10 min after injection of apomorphine (0.075 mg/kg s.c.) in rats placed in individual Plexiglas cages (25 × 20 × 14 cm high).

Catalepsy. The occurrence of catalepsy in rats was assessed using the four-cork test (according to the method described by Worms and Lloyd, 1979). This measurement was performed by placing each paw of the rat on a cork 2.5 cm high (diameter 1.2 cm). The distance between contralateral corks was 8 cm, and that between ipsilateral corks was 13 cm. Catalepsy time was measured for a maximum of 2 min at 2 hr and 4 hr after i.p. drug treatment. Catalepsy was also measured 6 hr after amisulpride injection.

Statistical analyses. Hypothermia and time spent climbing induced by apomorphine in mice were analyzed by one-way ANOVA followed by post-hoc Dunnett’s test. Similar analyses were used for yawning and locomotor activity in rats. The nonparametric Kruskall-Wallis test was used to analyze grooming behavior in mice and stereotypies produced in rats by apomorphine or d-amphetamine. All
experimental procedures were performed in separated groups of animals. Observers were blind to the drug injected in the case of the assessment of grooming, catalepsy, stereotypies, and yawning behaviors.

ED_{50} values were calculated using log-probit analysis from values expressed as a percentage of control values. For catalepsy, the ED_{50} value was determined as the dose that produced catalepsy (all four paws on the corks for more than 10 sec) in 50% of tested rats. MED was determined as the first dose that produced a statistically significant effect as compared with control values.

Drugs. Amisulpride and remoxipride HCl were synthesized by the chemistry department of Synthelabo Recherche. Apomorphine HCl and haloperidol were purchased from Sigma Chemical Co, St. Louis, MO, d-amphetamine sulfate from Boyer, Paris, France, and (±) 7-OH-DPAT HB from RBI, Natick, MA. Amisulpride and haloperidol were dissolved in sterile water to which a few drops of HCl (amisulpride) or 10% w/w ascorbic acid (haloperidol) were added (final pH for both solutions: 3–4). Remoxipride, 7-OH-DPAT and quinpirole were dissolved in sterile saline with a few drops of Tween 80; apomorphine was dissolved in sterile saline and protected from the light. Haloperidol and remoxipride were administered 30 min (2 and 4 hr for catalepsy) before tests, and amisulpride 60 min (2, 4 and 6 hr for catalepsy) before tests. Refer to individual methodological descriptions of tests for agonist injection times. Drug weights refer to the base, except for quinpirole (weight of the salt). Injection volumes were 20 ml/kg i.p. and 10 ml/kg s.c. for mice and 5 ml/kg i.p. and s.c. for rats.

Results

Antagonism of apomorphine-induced effects (hypothermia and climbing) and grooming behavior in mice. Before drug treatment, core temperature was similar in different groups of mice in each experiment, as indicated by ANOVA: \( F(5,54) = 0.55 \), \( P > 0.05 \) (amisulpride); \( F(6,60) = 0.79, P > 0.05 \) (remoxipride) and \( F(6,49) = 0.56, P > 0.05 \) (haloperidol). In addition, none of the compounds tested produced changes in core temperature over the dose range studied \( F(5,54) = 1.47, P > 0.05 \) (amisulpride); \( F(6,60) = 1.08, P > 0.05 \) (remoxipride); \( F(6,49) = 2.24, P > 0.05 \) (haloperidol). Administration of apomorphine (1 mg/kg s.c.) produced similar decreases in core temperature in each experiment (\( \Delta \theta = 5.3^{\circ}C, 5.5^{\circ}C \) and 5.3\(^{\circ}C \) for amisulpride, remoxipride and haloperidol, respectively). As shown in figure 1, amisulpride, haloperidol and remoxipride antagonized hypothermia and climbing induced by apomorphine and also grooming behavior produced by a short period of immersion in water. However, as indicated by the figure and the ED_{50} values presented in table 1, the order of potency to inhibit these effects differed among the three drugs. In contrast to haloperidol, which antagonized all these effects at similar doses, remoxipride inhibited climbing and hypothermia induced by apomorphine at doses lower than those that antagonized grooming behavior. Unlike both compounds, amisulpride inhibited hypothermia at much lower doses than those that antagonized climbing or grooming behavior.

Antagonism of selective D_3 agonist-induced hypothermia in mice (quinpirole and 7-OH-DPAT). As shown in figure 2, quinpirole (1 mg/kg i.p.) produced similar decreases in core temperature in each group (\( \Delta \theta \) control values = 4.8\(^{\circ}C, 5.0^{\circ}C \) and 4.9\(^{\circ}C \) for amisulpride, remoxipride, and haloperidol, respectively). All three compounds antagonized this hypothermia in a dose-dependent manner. ED_{50} values, in mg/kg i.p., were as follows: amisulpride: 1.6 (0.7–3.9), remoxipride: 0.9 (0.4–1.8) and haloperidol: 0.06 (0.01–0.26).

In experiments using 7-OH-DPAT (3 mg/kg i.p.; fig. 2), values of hypothermia were similar to those observed with quinpirole (\( \Delta \theta = 4.1^{\circ}C, 4.8^{\circ}C \) and 4.8\(^{\circ}C \) for amisulpride, remoxipride and haloperidol groups, respectively). These compounds reversed the decreases in core temperature produced by 7-OH-DPAT at doses close to those that inhibited quinpirole-induced hypothermia as indicated by the following ED_{50} values, in mg/kg i.p.: 1.9 (1.2–3.3) for amisulpride, 1.5 (0.09–3.4) for remoxipride and 0.06 (ND) for haloperidol.

Antagonism of apomorphine-induced hypermotility in rats. In rats previously habituated to the activity cages, apomorphine (0.25 mg/kg s.c.) produced large increases (by a factor of 6 to 8) in spontaneous locomotor activity (fig. 3). This hyperactivity was antagonized by amisulpride \( F(6,76) = \)
9.27, P < .001], remoxipride [F(4,49) = 4.03, P < .001] and haloperidol [F(4,52) = 5.18, P < .001] with MED values of 30, 3 and 0.15 mg/kg i.p., respectively (table 2).

Antagonism of d-amphetamine-induced hypermotility in rats. In contrast to apomorphine, d-amphetamine produced hyperactivity in rats without habituation to the test chambers (fig. 4). This effect was antagonized by amisulpride [F(5,64) = 8.47, P < .001] at doses lower (MED = 3 mg/kg i.p.) than those needed to affect hypermotility induced by apomorphine, as illustrated in figure 3 and table 2. Remoxipride [F(5,64) = 6.16, P < .001] and haloperidol inhibited d-amphetamine-induced hyperlocomotion [F(4,49) = 5.99, P < .001] at doses similar to those that antagonized apomorphine-induced hyperactivity. In addition, in the case of haloperidol, this antagonism occurred at doses that affected spontaneous locomotor activity (fig. 8).

Antagonism of apomorphine- and d-amphetamine-induced stereotypies in rats. Apomorphine (0.5 mg/kg s.c.) produced stereotypies dominated by chewing and gnawing as indicated by control values very close to the maximal score.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Apomorphine-Induced Hypothermia ED50 (mg/kg i.p.)</th>
<th>Grooming After Swimming Hypothermia Climbing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amisulpride</td>
<td>1.9 (1.6–2.3)</td>
<td>21 (17.2–25.1)</td>
</tr>
<tr>
<td>Remoxipride</td>
<td>1.8 (0.9–4.3)</td>
<td>1.6 (1.4–2.0)</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>0.078 (0.029–0.49)</td>
<td>0.11 (ND)</td>
</tr>
</tbody>
</table>

**ED50 values with 95% confidence limits (in parentheses) were calculated using log probit analysis determined from dose-response curves shown in figure 1. ND: The dose-response curve did not fit the regression analysis with 95% confidence limits.**
In contrast to haloperidol and remoxipride, which inhibited stereotypies in a dose-dependent manner \((H = 21.0, P < .001)\) and \((H = 52.22, P < .001)\), respectively, high doses of amisulpride were needed to decrease stereotypy score partially \((H = 51.2, P < .001; \text{fig. 5 and table 2})\).

When d-amphetamine was used to induce stereotypies, haloperidol \((H = 24.8, P < .001)\) and remoxipride \((H = 26.7, P < .001)\) antagonized stereotypies at doses similar to those that inhibited stereotypies induced by apomorphine. Unlike haloperidol and remoxipride, amisulpride at doses up to 100 mg/kg did not block stereotypies induced by d-amphetamine \((H = 5.14, P > .05; \text{fig. 5 and table 2})\).

**Antagonism of yawning induced by apomorphine in rats.** A low dose of apomorphine \((0.075 \text{ mg/kg s.c.})\) induced yawning behavior, which was antagonized by the three compounds \((\text{fig. 6})\). Amisulpride \([F(4,87) = 9.04, P < .001]\) and remoxipride \([F(4,66) = 5.82, P < .001]\) displayed very similar potencies \((\text{table 2})\). Haloperidol also antagonized yawning \([F(3,57) = 6.66, P < .001]\), but only at the highest dose of 0.1 mg/kg \((P < .01)\).

**Antagonism of hypomotility induced by apomorphine in rats.** As shown in figure 7, haloperidol, up to 0.3 mg/kg, did not affect hypomotility induced by apomorphine \([F(4,43) = 1.97, P > .05]\). In contrast, amisulpride potency antagonized apomorphine-induced hypomotility \([F(5,60) = 7.25, P < .001]\), statistically significant effects being observed at all doses from 0.3 mg/kg to 30 mg/kg \((P < .01, \text{Dunnnett’s test})\). Remoxipride inhibited hypomotility induced by apomorphine \([F(4,69) = 9.1, P < .001]\) from 0.3 mg/kg to 3 mg/kg. Higher doses of haloperidol and remoxipride were not tested, because such doses produced decreases in spontaneous locomotor activity \((\text{fig. 8})\).

**Effects on spontaneous locomotor activity.** Remoxipride decreased spontaneous locomotion \([F(4,64) = 47.29, P < .001]\) at doses of 10 mg/kg and 30 mg/kg \((P < .01)\). Haloperidol also produced a marked decrease in spontaneous locomotion \([F(4,75) = 26.51, P < .001]\) at 0.3 mg/kg and 0.6 mg/kg \((P < .01)\). Amisulpride decreased locomotion to a similar extent \([F(4,31) = 3.57, P < .05]\), a statistically significant effect being observed only at the highest dose \((100 \text{ mg/kg, } P < .01; \text{fig. 8})\).

**Induction of catalepsy.** Figure 9 and table 3 show that, in contrast to haloperidol and remoxipride, amisulpride did not produce catalepsy measured 2 hr and 4 hr after administration. Six hours after administration of the highest dose \((100 \text{ mg/kg i.p.})\) of amisulpride, catalepsy occurred in only 36% of tested rats, and the duration of catalepsy was very short \((11.8 \pm 5.1 \text{ sec})\) as compared with those measured with the highest dose of haloperidol \((105.0 \pm 7.7 \text{ sec})\) and remoxipride \((83.5 \pm 8.8 \text{ sec})\) 4 hr after administration. Similar levels of catalepsy were produced by haloperidol 2 hr and 4 hr after administration, whereas the catalepsy produced by remoxipride was slightly greater at 4 hr than at 2 hr.

**Discussion**

These results clearly show that amisulpride displays a pharmacological profile different from that of the typical neuroleptic, haloperidol, and that of the benzamide remoxipride, which is considered an atypical neuroleptic \((\text{Ögren et al., 1984})\).

In mice, in contrast to haloperidol, which antagonized effects induced by apomorphine (hypothermia, climbing) and grooming behavior observed after a short period of swimming at similar doses, amisulpride inhibited hypothermia at doses 10 times lower than those that antagonized climbing behavior and 30 times lower than those that blocked grooming behavior.

There is increasing evidence that grooming behavior induced either by immersion in water \((\text{Chesher and Jackson, 1981})\) or by i.c.v. injection of various neuropeptides \((\text{Van Wimersma Greidanus et al., 1989})\) involves mainly D1 dopamine receptor stimulation. In mice, grooming can be elicited by the D1 dopamine agonist SKF 38393 \((\text{Molloy and Bradford, 1984})\) and can be selectively blocked by D1 antagonists \((\text{Van Wimersma Greidanus et al., 1989})\) or by i.c.v. administration of an oligodeoxynucleotide antisense to the D1 dopamine receptor \((\text{Zhang et al., 1994})\). The present results, showing that amisulpride inhibited grooming only at very high doses, are consistent with the lack of affinity of this compound for D1 receptors \((\text{Schoemaker et al., 1997})\). The effect of amisulpride on grooming may be related to a nonspecific general depressant activity, as is observed with remoxipride, which is also a selective D2 antagonist \((\text{Ögren et al., 1984})\). Less separation was observed between doses antagonizing climbing and grooming behavior. This difference may be explained by the marked central depressant effects of this compound, as compared with amisulpride, as shown in rats \((\text{fig. 8})\). Although haloperidol shows some, but low, affinity for D1 receptor \((\text{Schoemaker et al., 1997})\), its effect on grooming could be related to a nonspecific central depressant activity rather than to its D1 antagonist properties.

Unlike haloperidol and remoxipride, amisulpride antagonized hypothermia induced by apomorphine at doses much lower than those that inhibited climbing behavior. This difference may indicate that apomorphine-induced hypother-
mia and climbing are not mediated by the same dopamine receptor subtype. Since the initial report on the specific involvement of dopamine receptor activation in the hypothermia produced in mice by apomorphine (Fuxe and Sjöqvist, 1972), hypothermia has been related to D2 dopamine receptor stimulation (Colboc and Costentin, 1980; Meller et al., 1989), whereas climbing has been shown to require both D1 and D2 receptor stimulation (Vasse et al., 1988; Moore and Axton, 1990). More recently, very similar decreases in body temperature were observed with the preferential D3 dopamine receptor agonists quinelorane, quinpirole, and 7-OH-DPAT (Sanchez and Arnt, 1992; Millan et al., 1994), and these decreases were correlated with their affinities for D3 rather than D2 dopamine receptors (Millan et al., 1995). This suggests that the D3 dopamine receptor subtype may play a major part in the apomorphine-induced hypothermia. Supporting evidence for this view is also derived from the correlation observed between the potency of neuroleptics to antagonize apomorphine-induced hypothermia and their affinity for the D3 receptor (Millan et al., 1994). It has been reported that, in contrast to conventional neuroleptics (including haloperidol), which show higher affinity for the D2 than for the D3 receptor, amisulpride displays similar affinity for D3 and D2 dopamine receptors (Sokoloff et al., 1990, 1992a; Schoemaker et al., 1997). It is therefore possible that the preferential antagonism by amisulpride of hypothermia, as compared with climbing, induced by apomorphine is linked to its high affinity for the D3 receptor involved in apomorphine-induced hypothermia. Results showing that amisulpride antagonized hypothermia produced by quinpirole or by 7-OH-DPAT at the same doses as those that inhibited hypothermia induced by apomorphine support this hypothesis. Although hypothermia is produced by much lower doses of dopamine agonists than those needed to produce stereotypies (Menon et al., 1979), the observation that pretreatment with α-methyl-
p-tyrosine does not affect hypothermia induced by dopamine agonists (or, in the case of quinpirole, increases it), suggests that postsynaptic rather than presynaptic D₂/D₃ dopamine receptors may be involved in this effect (Sanchez and Arnt, 1992).

In rats, amisulpride displayed a pharmacological profile different from those observed with haloperidol and remoxipride. Haloperidol inhibited different apomorphine-induced effects at very similar doses, whereas amisulpride showed a selective antagonism of dopamine agonist-induced effects involving presynaptic dopamine autoreceptor stimulation (hypomotility, yawning) as compared with those involving postsynaptic dopamine receptor stimulation (hypermotility, gnawing; table 2). Remoxipride displayed an intermediate profile; unlike haloperidol, it partially reversed hypomotility induced by apomorphine at doses lower than or close to those that inhibited postsynaptic effects. These latter results are consistent with previous reports showing that neither haloperidol (Stähle and Ungerstedt, 1986) nor remoxipride (Stähle et al., 1987) fully reversed apomorphine-induced hypomotility.

Although some controversy exists (Stähle, 1992), there is evidence that apomorphine-induced yawning and hypolocomotion involve presynaptic dopamine receptor stimulation (DiChiara et al., 1976; Yamada and Furukawa, 1980; Mogilnicka et al., 1984; Dourish and Hutson, 1985). In addition, dopamine receptor agonists, including quinpirole, quinelorane and 7-OH-DPAT, which at low doses produce similar hypolocomotion (Feenstra et al., 1983; Eilam and Szechtman, 1989; Depoortere et al., 1996) and yawning (Damsma et al., 1993; Kurashima et al., 1995) have been shown to have higher affinity for D₃ than for D₂ dopamine receptors (Sokoloff et al., 1990; Sautel et al., 1995). These data suggest that D₃ dopamine receptors might play an important role in the low-dose apomorphine-induced effects. The wide separation between doses of amisulpride that antagonize the different apomorphine-induced effects may be related to a preferential blockade of presynaptic D₂/D₃ receptors involved in apomorphine-induced hypomotility and yawning and also to the preferential affinity of amisulpride for D₃ (vs. D₂) dopamine receptors as compared with other neuroleptics (Sokoloff et al., 1992b; Schoemaker et al., 1997). This hypothesis is supported by neurochemical studies showing that, in vivo, amisulpride increased the release of dopamine in the olfactory tubercle evoked by electrical stimulation of the ascending dopaminergic pathways (an index of nerve terminal D₂/D₃ autoreceptor blockade) in the rat at doses very close to those that antagonized hypomotility induced by apomorphine (Schoemaker et al., 1997). An interaction of amisulpride at low doses with presynaptic dopamine autoreceptors was also demonstrated by the increase in extracellular dopamine levels in striatum and nucleus accumbens of the rat (Schoemaker et al., 1997). Much higher doses were needed to decrease striatal ACh levels (an index of postsynaptic D₂ receptor blockade; Scatton, 1982; Schoemaker et al., 1997) as well as to reverse apomorphine-induced hypermotility or stereotypies. A preferential blockade by amisulpride of D₂/D₃ presynaptic receptors is also consistent with other behavioral studies showing a reversal by
Amisulpride produced only a weak antagonism of stereoty- 
pies induced by apomorphine and did not affect stereoty- 
pies produced by d-amphetamine, whereas lower doses of 
this drug markedly antagonized hypermotility induced both 
by apomorphine and by d-amphetamine. This lack of activity 
against stereotypies even at doses that produced a striatal D2 
receptor occupancy of 80% may be related to the preferential 
selectivity of amisulpride for the limbic as compared with the 
striatal D2/D3 receptors demonstrated in in vivo binding 
Studies (Schoemaker et al., 1997). Although a similar limbic 
selectivity has been claimed for remoxipride (Ogren et al., 
1984), this compound inhibited dopamine agonist-induced 
hypermotility and stereotypies at similar doses (table 2). It 
is also interesting to note that in a recent study, remoxipride 
produced a pattern of disruption of operant responding sim- 
ilar to that observed with haloperidol, whereas amisulpride 
was more similar to the atypical neuroleptics clozapine and 
risperidone (Sanger and Perrault, 1995). These results con- 
firm that remoxipride does not share all pharmacological 
properties with amisulpride; in particular, as we have noted, 
it lacks selectivity for presynaptic D2/D3 dopamine receptors. 
On the basis of the hypothesis that extrapyramidal side 
effects of neuroleptics are linked to the blockade of nigrostri-

tal dopaminergic transmission, whereas dopamine receptor 
blockade in limbic structures may be responsible for antipsy-
chotic activity (Zivkovic et al., 1975; Scatton et al., 1977), it 
is possible to speculate that the limbic selectivity of amisul-
pride, together with its high affinity for D3 receptors, 
explains why this drug exhibits antipsychotic effects while 
having low propensity to produce motor disturbances (Delcker 
et al., 1990).

The low potential of amisulpride to induce catalepsy (re-
lated to D3 striatal dopamine receptor blockade; Costall 
and Olley, 1971) provides a further experimental index of the 
atypical clinical profile of this compound. Haloperidol and 
remoxipride produced catalepsy at doses 2 to 3-fold higher 
than those that inhibited apomorphine-induced gnawing, but 
amisulpride, even at doses that produced a striatal D3 recep-
tor occupancy of 70% to 80% (Schoemaker et al., 1997), 
induced almost no catalepsy. Previous studies have shown that 
similar levels of occupation of striatal D3 receptors by most 
antipsychotic drugs, including the atypical neuroleptic ris-
peridone, give rise to catalepsy (Nielsen and Andersen, 1992; 
Schotte et al., 1993).

The lack of cataleptogenic potential of amisulpride may be
explained by three properties that characterize this molecule as shown by biochemical studies (Schoemaker et al., 1997): 1) its high specificity for D2/D3 dopamine receptor subtypes, 2) its selectivity for limbic areas and 3) its preferential blockade of presynaptic dopamine autoreceptors. In the striatum, preferential blockade by amisulpride of presynaptic D2 receptors (there are very few D2 receptors in this structure: Bouthenot et al., 1991; Sokoloff et al., 1992b; Landwehrmeyer et al., 1993) may produce an increase in dopamine release that can stimulate postsynaptic D1 receptors for which amisulpride does not have affinity. Such a stimulation might partly counteract the blockade of postsynaptic D2 receptors produced by amisulpride and prevent the drug from inducing catalepsy. A similar compensatory mechanism may not occur in limbic structures, which contain significant numbers of D2 receptors, for which amisulpride has high affinity. The preferential affinity of amisulpride for D2 receptors (localized in limbic areas) and for limbic D2 receptors may also explain the lack of catalepsy induced by this drug.

In conclusion, these results demonstrate striking differences between the psychopharmacological profile of amisulpride and the profiles of haloperidol, a nonselective antagonist of D2 receptor family subtypes (D2, D3, D4), and remoxipride, another benzamidine that selectively binds to D2 receptors without having affinity for D1 receptors (Sokoloff et al., 1992a,b; Van Tol et al., 1991). Amisulpride preferentially blocked behavioral responses linked to dopamine receptor activation in the limbic system and, at low doses, selectively antagonized effects of dopamine receptor agonists mediated by presynaptic dopamine receptors. These properties may explain the lack of motor side effects of this drug in the clinic. They may also account for the efficacy of low doses against negative symptoms and of high doses against positive symptoms of schizophrenia (Delcker et al., 1990). In addition to its importance in schizophrenia, there is evidence that dopamine plays an important role in antidepressant drug action. Chronic treatment with antidepressants increased dopaminergic neurotransmission, probably by desensitizing dopamine D1 receptors to the effects of apomorphine: A hypothermic effect of apomorphine reduces the cataleptic state of rats. Am J Psychiatry 145: 164–173, 1978.


