Effects of Acute and Repeated Administration of Amisulpride, a Dopamine D₂/D₃ Receptor Antagonist, on the Electrical Activity of Midbrain Dopaminergic Neurons

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ABSTRACT

Electrophysiological techniques were used to study the effects of amisulpride, a D₂/D₃ dopamine receptor blocker, on the activity of dopaminergic neurons in the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA). Administration of single bolus doses of amisulpride (8–32 mg/kg i.v.) induced a dose-dependent increase in the basal activity of dopaminergic neurons, in both the SNc and the VTA. The effect of amisulpride was more evident in the VTA, where it elicited a maximal excitation of 38.5 ± 12%, whereas in the SNc it caused a peak excitation of only 22.1 ± 9.8%. Amisulpride also increased the bursting activity of dopaminergic neurons in the VTA but not in the SNc. Microiontophoretic application of amisulpride (10–40 nA) into the SNc and the VTA caused an increase in the basal firing rate of the majority of dopaminergic neurons sampled. The excitation induced by 40 nA amisulpride was more marked in the VTA (36.1 ± 21%) than in the SNc (25.0 ± 18%). Moreover, microiontophoretic amisulpride (40 nA) increased the bursting activity of dopaminergic neurons in the VTA only. Repeated administration of amisulpride (20 and 50 mg/kg i.p.) for 21 consecutive days produced a significant decrease in the number of spontaneously active dopaminergic neurons in the VTA but not in the SNc. Repeated administration of haloperidol (0.5 mg/kg i.p.) decreased the number of dopaminergic cells both in the SNc and the VTA. The effect of repeated administration of amisulpride on the activity of VTA dopaminergic neurons was reversed by apomorphine, suggesting that these neurons were probably under a state of depolarization block. Taken together, these data confirm previous findings indicating that low doses of amisulpride preferentially increase dopaminergic transmission in the mesolimbic system. Moreover, results obtained from long-term experiments are consistent with clinical data indicating that amisulpride given at high doses is an effective antipsychotic agent, associated with a low incidence of extrapyramidal side effects.

Amisulpride [(±)amino-4-N-(1-ethyl-2 pyrrolidinyl)methylsulphonyl-5-methoxy-2-benzamide] is a substituted benzamide that binds with high and similar affinity to D₂ and D₃ dopamine receptor subtypes (Sokoloff et al., 1990, 1992; Schoemaker et al., 1997). Recent biochemical studies have shown that low doses (≤10 mg/kg) of amisulpride in rodents block preferentially presynaptic D₂/D₃ dopamine receptors, thus enhancing dopamine release and synthesis, particularly in the mesolimbic system (Schoemaker et al., 1997). However, at higher doses (40–80 mg/kg), amisulpride also blocks postsynaptic D₂ receptors (Schoemaker et al., 1997) and antagonizes D₃-mediated behavior in rodents, without causing catalepsy (Perrault et al., 1997). Consistent with these preclinical data, several clinical studies have shown that amisulpride given at doses of 50 to 300 mg/day is effective in the treatment of dysthymia and negative symptoms of schizophrenia, whereas at higher doses (400–1200 mg) amisulpride is active against the positive symptoms of schizophrenia (Boyer and Lecrubier, 1996; de Sousa, 1996; Smeraldi et al., 1996; Delcker et al., 1990; Boyer et al., 1995). Interestingly, repeated administration of antipsychotic doses of amisulpride in schizophrenic patients is associated with a low incidence of extrapyramidal side effects (Delcker et al., 1990; Boyer et al., 1995). On the basis of these clinical data, amisulpride can be considered an antipsychotic drug with an atypical pharmacological profile (Seeman, 1990), inasmuch as typical antipsychotics (e.g., chlorpromazine, haloperidol, trifluoperazine) are known to induce, after repeated administration, various extrapyramidal side effects including Parkinson-like syndrome (Klawans, 1976; Klein et al., 1980).

As already mentioned, the available data indicating that amisulpride is capable of increasing dopaminergic transmission are derived from biochemical studies, whereas electrophysiological data are still lacking. Electrophysiological techniques that allow researchers to record from neurochemically

ABBREVIATIONS: SNc, substantia nigra pars compacta; VTA, ventral tegmental area; ANOVA, analysis of variance; DOPAC, 3,4-dihydroxyphenylacetic acid.
identified dopaminergic neurons in the midbrain have proved to be particularly useful for the study of drugs acting on dopaminergic systems (Bunney et al., 1973, 1987; Chiodo and Bunney, 1984). Thus, both direct and indirect dopaminergic agonists such as apomorphine, quinpirole and d-amphetamine potently inhibit dopaminergic neurons (Skirboll et al., 1979; Kelland et al., 1989; Bunney and Aghajanian, 1976; Walters et al., 1975). There is evidence that the effects of systemically administered dopaminergic agonists are mediated preferentially by D2 dopaminergic autoreceptors (White and Wang, 1984) that are located in the somatodendritic area of dopaminergic neurons (Beckstead, 1988; Morelli et al., 1988). More recently it was also found that somatodendritic D3 autoreceptors (Bouthenet et al., 1991; Levant, 1997) can regulate the firing activity of dopaminergic neurons in the VTA (Lejeune and Millan, 1995). Consistent with the prominent role played by D2 dopaminergic autoreceptors in the tonic control of dopaminergic cell activity (Lacey et al., 1987; White, 1996), it has been found that (−)-sulpiride, a selective D2 receptor antagonist, increases the basal firing rate of dopaminergic neurons in the SNc (Mereu et al., 1985; Puca and Grace, 1994) and the VTA (White and Wang, 1984).

Therefore, it is conceivable that acute administration of amisulpride, which blocks both D2 and D3 dopaminergic receptors, would increase the basal activity of midbrain dopaminergic neurons. Moreover, repeated administration of amisulpride is expected to cause a selective reduction in the number of spontaneously active dopaminergic neurons in the VTA. Thus, several studies have shown that repeated treatment with typical antipsychotic drugs causes a marked decrease in the number of spontaneously active dopaminergic neurons, both in the SNc and the VTA (Bunney and Grace, 1978; Chiodo and Bunney, 1983; White and Wang, 1983; Grace et al., 1997). On the other hand, repeated administration of atypical antipsychotic drugs induces a decrease in the spontaneous activity of dopaminergic neurons only in the VTA (Chiodo and Bunney, 1983; White and Wang, 1983; Grace et al., 1997).

Based on the hypothesis that psychotomimetic disorders could be caused by hyperfunctioning of the mesolimbic and mesocortical dopaminergic systems originating in the VTA (Stevens, 1973; Matthysse, 1973; Hökfelt et al., 1974), it has been suggested that the reduced function of VTA dopaminergic neurons may be partly responsible for the therapeutic efficacy of antipsychotic drugs, whereas the decreased activity of the nigrostriatal dopaminergic system may contribute to the motor disturbances produced by these drugs (Chiodo and Bunney, 1983). Considering that in humans, many of the therapeutic and side effects of antipsychotic drugs develop after days or weeks of treatment (Crane, 1973; Crow et al., 1980; Beckman et al., 1979), this experimental model may be particularly useful for assessing the potential antipsychotic activity of new drugs and to predict their liability for inducing extrapyramidal side effects.

In this study the effect of acute administration of amisulpride on the activity of dopaminergic neurons in the SNc and the VTA were investigated by using electrophysiological techniques. In another series of experiments, the effect of repeated (21 days) treatment with amisulpride on the spontaneous activity of dopaminergic neurons in the SNc and the VTA was determined.

Surgical and recording procedures. Male Sprague Dawley rats (Charles River, Italy) weighing 250 to 350 g were anesthetized with chloral hydrate (400 mg/kg i.p.) and mounted on a stereotaxic apparatus. Supplemental doses of anesthetic were administered via a lateral tail vein cannula. Throughout the experiment the animal’s body temperature was maintained at 36° to 37°C by a thermostatically regulated heating pad. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., suppl. 40, 18 February, 1992) and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, Dec. 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication no. 85–23, 1985 and Guidelines for the Use of Animals in Biomedical Research, Thromb Haemost 58:1078–1084, 1987). After reflecting the scalp, the skull overlying both the SNc and VTA was removed. The coordinates, relative to the interaural line, for placement of the recording electrode were for the SNc: anterior, 2.7 to 3.4 mm; lateral, 1.8 to 2.2 mm; 6.5 to 7.5 mm ventral to the level of exposed tissue, and for the VTA: anterior, 2.7 to 3.4 mm; lateral, 0.1 to 0.5 mm; ventral, 7 to 8 mm (Paxinos and Watson, 1986). Extracellular recordings were performed by using either single- or five-barrel glass micropipettes. The single micropipettes, measuring 1 μm at the tip, were filled with 2% pontamine sky blue dye in 2 M NaCl (in vitro resistance 4–7 MΩ). Five-barrel micropipettes were pulled to an optimal wide tip angle and mechanically bevelled under microscopic control to a final tip diameter of 4 to 5 μm. The protruding center barrel, filled with 2% pontamine sky blue in 2 M NaCl, was used for recording (in vitro resistance, 4–8 MΩ) while one of the side barrels, filled with 2 M NaCl, was used for continuous automatic current balancing. The remaining barrels contained one of the after solutions: amisulpride (30 mM, pH 4), dopamine (100 mM, pH 4) and γ-hydroxybutyric acid (1 mM, pH 4). These solutions were retained with a –10 nA current between ejection periods. Dopaminergic neurons were identified by their location, waveform, firing rate and pattern (Bunney et al., 1973; Grace and Bunney, 1980). Electrical signals of spike activity were passed through a high input impedance amplifier whose output was led into an analog oscilloscope, audio monitor and window discriminator. Unit activity was then converted to an integrated histogram by a rate-averaging computer and displayed as spikes per 10-sec intervals on a chart recorder. Only cells whose electrophysiological characteristics matched those previously established for midbrain dopaminergic neurons were sampled (Bunney et al., 1973).

After each experiment, the sites of recording were marked by the ejection of pontamine sky blue dye from the electrode using a –20 μA current for 10 min. Brains were then removed and placed in 10% buffered formalin solution for 2 days before histological examination. Frozen sections were cut at 40-μm intervals and stained with formalthionin solution. Microscopic examination of the sections were carried out to verify that the location of the electrode tip was within the SNc or the VTA.

Drug administration protocols. Amisulpride (8–32 mg/kg) (dissolved in 200 μl of 10% acetic acid, made up to almost the required volume with distilled water and brought to pH 6) was administered i.v. (via a lateral tail vein) in single bolus injections in a volume of 150 μl. A group of control rats was given 150 μl of vehicle i.v. Only one cell per animal was studied. For the long-term studies, two doses of amisulpride (20 and 50 mg/kg) were injected i.p. for 21 consecutive days. The effect of amisulpride was compared with that of the typical antipsychotic drug haloperidol (0.5 mg/kg i.p.; dissolved in 200 l of 10% acetic acid, made up to almost the required volume with distilled water and brought to pH 6) was administered i.v. (via a lateral tail vein) in single bolus injections in a volume of 150 μl. A group of control rats was given 150 μl of vehicle i.v. Only one cell per animal was studied. For the long-term studies, two doses of amisulpride (20 and 50 mg/kg) were injected i.p. for 21 consecutive days. The effect of amisulpride was compared with that of the typical antipsychotic drug haloperidol (0.5 mg/kg i.p.; dissolved in the same way as amisulpride), given for the same period of time. A control group of animals was injected repeatedly i.p. with the vehicle for 21 consecutive days. One group of rats treated chronically with amisulpride was given apomorphine (0.5 mg/kg, i.p.) 30 min before the beginning of the experiment. At the end of the repeated treatment period (i.e. on day 21) spontaneously firing dopaminergic cells within both the SNc and the VTA regions were counted by
lowering the electrode through a block of tissue (240.00 μm²), which could be reproducibly located from animal to animal (Chiodo and Bunney, 1983). Twelve electrode tracks (separated from each other by 200 μm), whose sequence was kept constant from animal to animal, were made in each region. Only cells whose electrophysiological characteristics matched those previously established for midbrain dopaminergic neurons were sampled (Bunney et al., 1973).

Data and statistical analyses. Data acquisition and analysis were accomplished using an S3286-based PC and an integrated software package for electrophysiology (RISI, Symbolic Logic, Dallas, TX). In experiments involving the administration of bolus doses of amisulpride, the data are expressed as the mean differences (±S.E.M.) between the firing rate calculated at the peak of the drug effect (averaged over 500 spikes) and the basal firing rate (calculated as the mean of the 500 spikes occurring immediately before the injection of the drug). The modifications in firing rate induced by microiontophoretic application of amisulpride were calculated as percentages of drug-induced changes relative to the base line.

Burst analysis of dopaminergic neurons was performed by using the RISI program running on a PC computer. A total of 500 consecutive spikes were recorded for each neuron before and at the peak of the drug effect. Burst-firing, when present, was detected using an algorithm similar to that previously described by Grace and Bunney (1984). In the experiments involving bolus injections of amisulpride, the absolute change in the percentage of spikes occurring in bursts [i.e., the difference (Δ) between the percentage of spikes fired within bursts during the baseline period from the percentage of spikes fired within bursts after drug administration] was used as a measure of drug-induced changes in bursting. The modifications in burst firing induced by microiontophoretic application of amisulpride and after repeated administration of amisulpride and haloperidol were calculated as percentages of drug-induced changes relative to the baseline line. All the data obtained were subjected to one-way ANOVA. When significant effects were found, post-hoc comparisons were made with Tukey’s test. The effect of amisulpride and haloperidol on the number of cells per track were analyzed by one-way ANOVA, followed by Tukey’s test. To test the hypothesis that the VTA neurons are more sensitive to the effects of amisulpride, a two-way ANOVA with midbrain region (SNc or VTA) and dose of amisulpride as factors was performed. Post-hoc comparisons were made with Tukey-Kramer’s test.

Drugs. Amisulpride was kindly provided by Dr. B. Scatton (Synthélabo Recherche, Bagneux, France); haloperidol, apomorphine HCl, dopamine HCl and γ-hydroxybutyric acid were purchased from Sigma Chemical (St. Louis, MO).

Results

Effect of acute bolus administration of amisulpride on the basal activity of dopaminergic neurons in the SNc and the VTA. Administration of the vehicle in a group of control rats (n = 5) did not cause relevant changes in the basal firing rate of SNc dopaminergic neurons. However, there was an overall tendency toward a slight, nonsignificant reduction in the basal activity of these neurons in response to vehicle injection (fig. 1A). Amisulpride, at the dose of 8 mg/kg (n = 6) increased the basal firing rate of dopaminergic neurons in the SNc by 7.4 ± 3.2%, although this effect did not reach statistical significance (fig. 1A). A similar, nonsignificant effect was observed after 16 mg/kg amisulpride (n = 7), which increased the basal activity of dopaminergic neurons by 4.3 ± 3.3% (fig. 1). However, the dose of 32 mg/kg of this drug (n = 6) caused a statistically significant enhancement (+21.1 ± 9.8%) of the firing activity of SNc dopaminergic neurons (fig. 1A). On figure 1B is a representative rate histogram showing the typical effect of 32 mg/kg amisulpride on a SNc dopaminergic neuron. Neither dose of the drug caused significant changes of the bursting activity of dopaminergic neurons in the SNc (not shown). Also in the VTA, control injection of the vehicle (n = 5) caused a small, nonsignificant reduction in the basal activity of dopaminergic neurons (fig. 2A). The effect of amisulpride in the VTA was much more evident compared with that observed in the SNc. Thus, the dose of 8 mg/kg (n = 6) increased the basal activity of VTA dopaminergic neurons by 22 ± 15.4% (fig. 2A). The maximal excitatory effect of amisulpride was observed at the dose of 16 mg/kg (n = 6), which caused a 38.5 ± 12.0% increase over the base-line rate (fig. 2A). Figure 2B reports a representative

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**Fig. 1.** Effect of single bolus administration of amisulpride on SNc. Histograms show mean percentage of change (±S.E.M.) in firing rate of dopaminergic neurons after i.v. amisulpride (n = 5–7 rats per group). A, Representative rate histogram showing the typical excitatory effect of i.v. amisulpride (32 mg/kg). F(1.37) = 3.22; *P < .05 compared with the vehicle group (one-way ANOVA, followed by Tukey’s test). B, Representative rate histogram showing the typical excitatory effect of i.v. amisulpride (32 mg/kg). F(1.32) = 3.39; **P < .01 compared with the vehicle group (one-way ANOVA, followed by Tukey’s test).

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**Fig. 2.** Effect of single bolus administration of amisulpride on VTA. A, Histograms showing mean percentage of change (±S.E.M.) in firing rate of dopaminergic neurons after i.v. amisulpride (n = 5–6 rats per group). B, Representative rate histogram showing the typical excitatory effect of i.v. amisulpride (16 mg/kg). F(1.22) = 3.36; **P < .01 compared with the vehicle group (one-way ANOVA, followed by Tukey’s test).
rate histogram showing the typical effect of 16 mg/kg amisulpride on a VTA dopaminergic neuron. However, the response to this dose of amisulpride was variable, in that it ranged from 7.2% to 65.2%. This differential response did not depend on the basal firing rate of the neuron sampled. As can be seen in figure 2A, the effect of 32 mg/kg amisulpride was less evident compared with the dose of 16 mg/kg, in that it increased the firing rate of dopaminergic neurons in the VTA by only 32.6 ± 6.8%. In addition, amisulpride was also capable of enhancing the bursting activity of VTA dopaminergic neurons. Thus, 16 and 32 mg/kg amisulpride significantly increased the number of events in bursts (fig. 3). To test the hypothesis that the VTA neurons are more sensitive to the effects of amisulpride, a two-way ANOVA with midbrain region (SNc or VTA) and dose of amisulpride as factors was performed. This statistical analysis revealed that there was a significant difference between the effect of amisulpride in the VTA and the SNc [F(7,46) = 4.44; P < .01].

Effect of microiontophoretic application of amisulpride on the basal activity of dopaminergic neurons in the SNc and the VTA. Amisulpride applied locally by microiontophoresis caused a clear-cut excitation in the majority of dopaminergic cells tested both in the SNc (4 of 7) and the VTA (5 of 8). As can be seen in figure 4, the excitatory effect of amisulpride was related to the amount of current applied, which ranged from 10 to 40 nA. The current-response curves, showing the effect of amisulpride in the SNc and the VTA from neurons that responded, are represented in figure 5. The excitation induced by 40 nA amisulpride was more marked in the VTA (36.1 ± 21%) than in the SNc (25.0 ± 18%). Moreover, microiontophoretic amisulpride (40 nA) increased the percentage of spikes occurring in bursts of dopaminergic neurons in the VTA (vehicle = 15.5 ± 10.3; amisulpride = 34.8 ± 9.1; mean ± S.E.M.; F(3,19) = 3.40; P < .01; by one-way ANOVA). Administration of amisulpride at lower currents did not cause any change in bursting activity of dopaminergic neurons either in the VTA or the SNc. Microiontophoretic coadministration of amisulpride and dopamine prevented the inhibitory effect of dopamine on the basal activity of dopaminergic neurons, both in the SNc (n = 4) and the VTA (n = 4) (not shown).

Effect of repeated administration of amisulpride on the number of spontaneously active dopaminergic neurons in the SNc and the VTA. Repeated i.p. treatment with amisulpride (20 and 50 mg/kg) for 21 consecutive days caused a significant reduction in the number of spontaneously active dopaminergic neurons in the VTA (n = 9), but not in the SNc (n = 6) (fig. 6). The effect of amisulpride in the VTA tended to be dose dependent, in that 20 mg/kg reduced by 33 ± 12% the number of cells per track, whereas 50 mg/kg induced a 45 ± 7% decrease in the number of spontaneously active dopaminergic neurons. Repeated i.p. administration of haloperidol (0.5 mg/kg) for 21 consecutive days decreased the number of dopaminergic cells both in the SNc (46 ± 6%) (n = 6) and the VTA (50 ± 8%) (n = 9) (fig. 6). A statistical analysis of bursting in remaining activated dopaminergic neurons was performed by one-way ANOVA. The analysis was carried out on at least 200 spikes for each neuron. Because the recording length varied among the various neurons sampled, not all the neurons recorded were included in the analysis. Nevertheless, the numerosity was homogeneous among the different experimental groups and ranged from 34 to 39 for the VTA, and from 42 to 51 for the SNc. There was a significant increase in the percentage of spikes occurring in bursts in the VTA after repeated administration of 50 mg/kg amisulpride [vehicle = 49.2 ± 9.7%; amisulpride = 72.5 ± 4.8%; mean ± S.E.M; F(3,145) = 4.87; P < .01]. There were no statistically significant differences in bursting activity among the other experimental groups either in the VTA or the SNc. The effect of repeated administration of amisulpride (50 mg/kg; n = 6) on the activity of VTA dopaminergic neurons was completely reversed by apomorphine (0.5 mg/kg...
the vehicle group (one-way ANOVA, followed by Tukey’s test).

Discussion

This study shows that amisulpride administered intravenously in single bolus doses increased the basal firing rate of dopaminergic neurons in the SNc or the VTA. One important finding of this study is that the excitatory effect of amisulpride on dopaminergic neurons was more marked in the VTA compared with the SNc. Thus, amisulpride increased the basal activity of dopaminergic neurons in the SNc by only 22%, whereas the maximal excitatory effect of amisulpride in the VTA reached 38.5%. Moreover, amisulpride enhanced the bursting activity of dopaminergic neurons in the VTA, but not in the SNc. This finding is of particular importance because it is known that bursting activity is a strong stimulus in inducing dopamine release in the terminal fields of the dopaminergic system (Gonon, 1988). The “limbic selectivity” of amisulpride has been confirmed by statistical analysis showing that the effect of this drug on VTA dopaminergic cell firing was significantly higher than that on SNc dopaminergic neurons. The present data are consistent with previous studies showing that the mesolimbic dopaminergic system is more sensitive than the mesostriatal system to the stimulating effects of amisulpride (Schoemaker et al., 1997). Thus, microdialysis studies have shown that although 10 mg/kg amisulpride caused an essentially similar increase in dopamine release in the striatum and the nucleus accumbens, dialysate DOPAC levels were increased to a greater extent in the nucleus accumbens (Schoemaker et al., 1997). Because extracellular DOPAC levels in the projection fields are considered to reflect changes in the impulse flow of dopaminergic neurons (Waters et al., 1994), it was predicted that amisulpride would increase dopaminergic neuronal activity to a greater extent in the mesolimbic than in the mesostriatal system (Schoemaker et al., 1997). That amisulpride may act directly in the somatodendritic region of midbrain dopaminergic neurons was confirmed by the experiments showing that microiontophoretic application of amisulpride enhanced the basal firing rate of dopaminergic cells both in the SNc and the VTA. Interestingly, the effect of microiontophoretic amisulpride was more marked in the VTA than in the SNc. Thus, the maximal excitation elicited by 40 nA amisulpride was 36.1 ± 21% in the VTA vs. 25 ± 18% in the SNc. In addition, application of 40 nA amisulpride increased the bursting activity of dopaminergic neurons in the VTA only. Therefore, it is possible to argue that the effects of systemically administered amisulpride on dopaminergic activity are mediated, at least in part, by blockade of D2/D3 dopamine receptors which are present in the SNc and the VTA (Beckstead, 1988; Morelli et al., 1988; Bouthonet et al., 1991; Levent, 1997). Thus, amisulpride shows high similar affinity for both D1 and D2 receptor subtypes (Schoemaker et al., 1997), which play a relevant role in the autoregulation of midbrain dopaminergic neurons (Lacey et al., 1987; White, 1996; Lejeune and Millan, 1995). That D2 dopamine receptors exert a tonic inhibitory influence on the basal activity of dopaminergic neurons is confirmed by the finding that (−)-sulpiride, a selective D2 receptor antagonist, enhanced the basal activity of VTA dopaminergic neurons when applied locally by microiontophoresis (White and Wang, 1984). Moreover, the rate-enhancing effect of systemically administered sulpiride on SNc dopaminergic neurons was not abolished by hemisection of the striatongiral projection, thus indicating that the effect was mediated by local action on the somatodendritic D2 autoreceptors (Pucak and Grace, 1994). However, it is difficult to establish the relative contribution of D3 receptor subtypes in the action of amisulpride, as much as it has been shown that administration of a selective D3 receptor antagonist did not alter the basal firing rate of VTA dopaminergic neurons (Lejeune and Millan, 1995). Therefore, it appears that D3 dopamine receptors are not involved in the tonic control of dopaminergic activity, although administration of D3 agonists inhibits their function (Lejeune and Millan, 1995).

The disinhibitory effect of amisulpride on the activity of mesolimbic dopaminergic neurons might be relevant for its...
clinical effect in the treatment of dysthymia and the negative symptoms of schizophrenia (Boyer and Lerchbuerer, 1996; de Sousa, 1996; Smeraldi et al., 1996). Considering the similarity between the symptoms of dysthymia and the behavioral effects of high doses of haloperidol in humans (Belmaker and Wald, 1977), it is possible to argue that dysthymia could be caused by a reduced functioning of the dopaminergic system. Moreover, it has been suggested that a reduced function of the mesocorticolimbic dopaminergic system might be responsible for the negative symptoms of schizophrenia (Deutch et al., 1991). Therefore, the use of drugs enhancing dopamine release such as amisulpride could be considered a good strategy in the treatment of these psychiatric disorders. However, it is presently impossible to know if long-term treatment with low doses (50–300 mg/day) of amisulpride in humans would attenuate its capability to increase mesocorticolimbic dopaminergic function.

Another interesting finding of our study is that amisulpride, given repeatedly, produced a selective decrease in the number of spontaneously active dopaminergic neurons in the VTA. This phenomenon was probably due to induction of depolarization block, because the effect of repeated administration of amisulpride on VTA dopaminergic neurons was reversed by apomorphine (White and Wang, 1983); apomorphine, however reduced the number of spontaneously active dopaminergic neurons in the SNc, their activity not being affected by repeated administration of amisulpride. Repeated administration of the typical antipsychotic drug haloperidol produced, as expected, a reduction in the number of spontaneously active dopaminergic neurons both in the SNc and the VTA. The fact that repeated administration of amisulpride selectively induces depolarization block in the VTA might be explained by its ability to increase preferentially the burst firing of dopaminergic neurons in this area, after acute injection. Thus, burst firing is considered a precursor of depolarization block (Grace and Bunney, 1986). In this respect, it is interesting to note that repeated administration of 50 mg/kg amisulpride selectively increased the percentage of spikes occurring in bursts of the remaining activated dopaminergic cells in the VTA. Therefore, it is possible to argue that repeated treatment with amisulpride would lower the threshold of mesolimbic dopaminergic neurons toward burst firing, thus rendering these neurons more vulnerable to depolarization block. Considering that burst firing produced by systemic administration of D₃ antagonists may be mediated by the forebrain inputs to the midbrain (Pucak and Grace, 1994), in the case of VTA by inputs from the frontal cortex (Murase et al., 1993; Overton et al., 1996; Tong et al., 1996), it is conceivable that repeated administration of amisulpride could cause some functional changes in these circuits. A number of electrophysiological studies have shown that long-term treatment with typical antipsychotic drugs can reduce the spontaneous activity of midbrain dopaminergic neurons (Bunney and Grace, 1978; Chiody and Bunney, 1983; White and Wang, 1983; Grace et al., 1997), probably resulting from the induction of a state of depolarization block (Grace et al., 1997). One particular feature of the atypical antipsychotic drug, clozapine, assayed in this model is that its repeated administration reduced the number of spontaneously active dopaminergic neurons in the VTA but not in the SNc (Chiody and Bunney, 1983; White and Wang, 1983; Grace et al., 1997), an effect similar to that induced by amisulpride.


Lacey MG, Mercuri NB and North RA (1987) Dopamine acts on D3 receptors to increase potassium conductance in neurons of the rat substantia nigra zona compacta. *J Physiol (Lond)* **392**:397–416.


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