d-Sulpiride Inhibits Oral Behaviour Elicited From the Nucleus Accumbens of Freely Moving Rats

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ABSTRACT: The present study analyzed the effect of intra-accumbens administration of the stereoisomers of sulpiride upon (3,4-dihydroxyphenylimino)-2-imidazoline (DPI)-induced changes in oral behaviours and electromyographic patterns of jaw muscles. In line with earlier findings, DPI (5 μg) administered into the nucleus accumbens increased chewing and tremor. l-Sulpiride (2–50 ng) had no effect on DPI-induced oro-facial behaviours. d-Sulpiride (10–50 ng) significantly antagonized the DPI-induced increase in chewing and had a biphasic effect on tremor with potentiation (10 ng) followed by attenuation (50 ng). When administered alone, l- or d-sulpiride did not affect oro-facial behaviours. The electromyographic signals, which were analyzed according to a previously described method, were described with the help of three classes: A (the seconds marked by frequency 3 Hz), B (the seconds marked by the frequencies 4–6 Hz); C (the seconds marked by the frequencies 7–15 Hz). DPI enhanced Class B and C of the masseter muscle but did not significantly affect any frequency class of the digastric muscle. l-Sulpiride (2–50 ng) had no effect on DPI-induced (5 μg) changes in electromyographic signals. d-Sulpiride (50 ng) antagonized the effects of DPI on Class B of the masseter muscle. Furthermore, d-sulpiride had a biphasic effect on Class C with potentiation (10 ng) followed by attenuation (50 ng). When administered alone, l- or d-sulpiride did not affect the frequency classes of the jaw muscles. It is concluded that d-sulpiride inhibits DPI-induced changes in oral behaviour and electromyographic patterns. It is suggested that d-sulpiride may be effective in the pharmacotherapy of oro-facial dyskinesias in man.

KEY WORDS: Oro-facial dyskinesia, Electromyography, Jaw muscle, DPI, Stereoisomers, Neuroleptic.

INTRODUCTION

The nucleus accumbens modulates oro-facial behaviour in rats [3,5,8,17,19,22]. More specifically, intra-accumbens administration of (3,4-dihydroxyphenylimino)-2-imidazoline (DPI), which is considered to be a dopamine DA, receptor agonist [6,7], increases several oro-facial behaviours in rats [22]. This effect is subregion-specific, because DPI affects oral behaviour when administered into the shell, but not the core or the rostral pole of the nucleus accumbens; moreover, DPI-injections into sites adjacent to this nucleus remain ineffective [8,22, unpublished observations]. The shell, being the region in which DPI induces oral behaviours, is especially important, because the dopaminergic activity in the shell, but not the core, of the nucleus accumbens in rats is sensitive to stressors [10]. Sensitivity to stressors is also a characteristic of oro-facial dyskinesias in man [16]. Moreover, intra-accumbens administration of high doses of DPI induces dyskinetic-like oral behaviour (large amplitude chewing [22]), in contrast with administration of dopamine D1 and D2 agonists [17]. Therefore, DPI-induced oro-facial behaviour in rats has been suggested to be an interesting animal model of oro-facial dyskinesias in man [22].

Because the pharmacotherapeutic treatment of oro-facial dyskinesias in man is a major problem [14,15], it became of interest to find drugs that attenuate the DPI-induced oral behaviours. A possible candidate for such a drug is sulpiride. It has been found that sulpiride shares its high affinity for the so-called mesolimbic l-sulpiride binding site (7 nM) in the nucleus accumbens with that of DPI [2–5 nM] [9; Csernansky, personal communication]. Interestingly, in contrast to its affinity for dopamine D1 receptors, the affinity of sulpiride to the l-sulpiride binding site is not stereospecific [l-sulpiride: 7 nM; d-sulpiride: 9 nM] [9]. Therefore, the effects of intra-accumbens administration of different doses of l- and d-sulpiride on spontaneous and DPI-induced oro-facial behaviour were analyzed in the present study. Given the possibility that the sulpiride stereoisomers might increase or decrease the effects of DPI, a relatively low dose of DPI was chosen [5 μg] [22]. Because the effects of DPI-injections into the border region of the core and shell (the so-called “shore” [22]), which is easy to hit correctly, are largely comparable to DPI-injections into the shell, which is difficult to hit [22], it was decided to use “shore” injections in the present study. Finally, together with the behavioural assessment, electromyographical (EMG) measurements of jaw muscles were made. The analysis of the signals according to a previously described method [21] provided another, and, importantly, a more objective analysis of changes in oral behavior.

MATERIALS AND METHODS

Subjects

All experiments were performed in accordance with the Helsinki declaration and institutional respectively national guide-
lines. Naive, male Wistar rats (Central Animal Laboratory, Nijmegen) were used. Before operation they weighed between 180 and 220 g. Rats were individually housed and kept on a 12-h day/night cycle with lights on at 7:00 am. Food and water were available ad lib. Animals were used only once.

**Surgery**

Animals were anaesthetized with sodium pentobarbital (Narcoret, 60 mg/kg IP). Guide cannulae (0.65 mm outer diameter, 0.3 mm inner diameter) were stereotactically implanted into the nucleus accumbens, viz. the border region of the core and shell (ant. 9.8 mm; vert. 2.7 mm; lat. 1.2 mm). Cannulae (5 mm length) were angled 10 degrees from the midsagittal plane to avoid the ventricular system and to leave space for an electromyographic (EMG) connector. The cannulae and a five-pinched plug connected with five biomed wires (Cooner Wire, AS 632-4SS) were attached to the skull with dental acrylic cement (Durelon, ESP). Four of the five biomed electrodes were subcutaneously led to the jaw muscles (for details see [21]). Two electrodes were unilateraly placed in the right anterior superficial part of the maseter muscle; two other electrodes were unilaterally placed in the right anterior part of the digastric muscle. The fifth wire was placed subcutaneously in the neck and served as a grounding electrode.

**Apparatus**

During the experiments rats were placed in a cage of Plexiglas (25 cm × 25 cm × 35 cm). A mirror (angled 45 degrees) was mounted beneath the cage allowing the precise recording of behaviour, especially of oro-facial movements. Behaviour was registered using a protocol panel with 16 channels. The behavioural protocols and the EMG signals (transmitted by electric wires coupled to a swivel) were directly read into a computer and analyzed by a computer program that calculated the frequency and duration of every scored behaviour. The analysis of the EMG signals is described below.

**Procedure**

The procedure is described elsewhere in detail [22]. After surgery the rats were housed individually in the original stockroom and allowed recovery from operation for at least 1 week. After the recovery period, the rats were handled on three subsequent days and tested on the fourth day (between 9.00 and 17.00 h). The handling and test procedures were preceded by a habituation period, and the needle was left in situ for another 5 min. Finally, the animals received their last intracerebral injection with /-sulpiride, d-sulpiride or distilled water at / = 60 min and lasted 30 min (ex­­perimental session). The animals were given a second injection with DPI or its control (distilled water) at / = 55 min. The drug effects started at / = 60 min and lasted 30 min (ex­­perimental session). The injections were given by means of a 5 μl Hamilton syringe with a 0.25 mm needle that extended 2 mm into the brain tissue below the tip of the permanent embedded cannula. The volume was 0.5 μl per side injected over a 10 s period, and the needle was left in situ for another 10 s to minimize diffusion along the needle tract.

After the experiment the rats were sacrificed, and the brains were dissected. Brain sections were stained with cresyl violet and microscopically analyzed. Only data from subjects with injections made into the desired sites (about 90% of total) were further analyzed. All groups consisted of six to nine animals after excluding rats with misplaced injections (N = 11).

**Behavioural Observations**

The ethogram of behavioural elements was similar to that described earlier [22]: (1) chew (movement of the lower jaw vertical and/or lateral in a single or repetitive fashion without an object between the teeth); (2) abnormal chewing, being large amplitude chewing (wide opening and closing of the lower jaw in a brisk repetitive fashion); (3) gnaw (movement of the lower jaw vertical and/or lateral in a single or repetitive fashion with an object (pieces of straw, fences, or the box wall) between the teeth); (4) tremor (rapid oscillations of check and/or lower jaw); (5) tongue protrusion (not aimed at an object); (6) lick (tongue protrusion aimed at an object); (7) yawn (wide opening of the lower jaw with bare teeth). Continuous succession of identical elements of behaviour (bouts) were scored as a single event. Duration (except for tongue protrusion and yawn) and frequency of all behaviours were analyzed.

**Analysis of EMG Signals**

The analysis of the EMG signals was based upon an earlier described method [21]. However, some significant modifications were made, for example, signals were directly read in the computer and were on-line analyzed. Furthermore, the settings for minimum values concerning the assignment of a frequency to a second (see below) were slightly modified. This modified method will be shortly summarized. First, EMG signals were high-pass filtered (80 Hz) before they entered the computer. The signals of the masticator and the digastic muscle were analyzed independently, but in the same manner. The signals were divided in parts of 1 s, rectified and smoothed. Subsequently, the data were subjected to a fast-fourier transformation (FFT) per block of 1 s. This analysis offered two parameters: (a) the power per second being the area under the curve of the EMG signal per second; and (b) the frequency distribution curve per second. The power was used as a criterion for the assignment of a frequency to a second (see below). The dominant frequency per second was determined in the following manner. The proportional contribution of the area belonging to a frequency (e.g., the area from 5.5 to 6.5 for frequency 6) under the frequency distribution curve as compared to the total area under the frequency distribution curve (from 3 to 20 Hz) was calculated per second. The proportionally greatest area under the curve was labelled as the dominant frequency. To assign the dominant frequency to a second, two conditions had to be fulfilled. First, the dominant frequency had to be a salient feature of the second under study. For that reason, the proportional contribution of the dominant frequency per second had to surmount a minimum percentage [25%, present study vs. 14% in our previous study (21)] of the total area under the frequency distribution curve (3 to 20 Hz) of that second. Second, the EMG activity per se had to be a salient feature of the second under study. For that reason, the power per second had to surmount a minimum, which was 500 and 50 for the masticator and digastic muscle, respectively. Only when both restrictions were fulfilled was the dominant frequency accepted as a marker of the second under study; otherwise the second was not taken into account. The total number of seconds marked by a distinct dominant frequency was the dependent variable.

One more restriction was made before the final EMG results were further analyzed. A preliminary, unpublished study in which
the display of behaviour was directly correlated with the presence of the dominant frequency per second had shown that the display of both chewing and grooming correlates with the presence of the frequencies 4–6; moreover, it had been found that the display of tremor correlates with the presence of the frequencies 7–15. To prevent that the presence of long-lasting grooming bouts would mask the detection of chewing in the EMG-analysis, EMG data that were collected during the display of grooming were therefore discarded in the present EMG analysis.

**Drugs**

(3,4-Dihydroxyphenylimino)-2-imidazoline (DPI, Boehringer Ingelheim, FRG) was dissolved in water. The stereoisomers of sulpiride (RBI, Natick, USA) were dissolved in distilled water and a drop of acetic acid after which the pH was adjusted to 6–7. Drugs were injected bilaterally.

**Statistical Analysis**

Like in our previous study [22], the scores collected per rat during the pretreatment session were subtracted from the scores collected per rat during the experimental session to control for the individual variation in oro-facial behaviours. Only the resulting data were statistically analyzed. The effects of DPI on different behaviours or on the number of seconds characterized by a frequency were analyzed with a one-way analysis of variance (ANOVA) with two levels (water and DPI). The effects of different doses of l- or d-sulpiride upon the DPI-induced effects were analyzed with one-way analysis of variance (ANOVA) with four levels (DPI and 2, 10, and 50 ng of l-sulpiride; DPI and 10, 25, and 50 ng of d-sulpiride), followed by the post hoc Student–Newman–Keuls test when appropriate. Possible significant effects of l-sulpiride or d-sulpiride alone (10 and 50 ng) were determined with one-way ANOVAs.

**RESULTS**

**Behavioural Analysis: Effects of DPI**

Because the scores in the pretreatment session were subtracted from those in the experimental session (see Materials and Methods section), the resulting scores could be either negative or positive (Figs. 1 and 2; cf. [22]). In line with earlier studies, DPI (5 μg) increased oro-facial behaviours: the frequency of chewing [F(1, 17) = 10.4; p < 0.01; Fig. 1], large amplitude chewing [F(1, 17) = 5.7; p = 0.03], gnawing [F(1, 17) = 8.65; p < 0.01], tremor ([F(1, 17) = 4.3; p = 0.05]; Fig. 1), and tongue protrusions [F(1, 17) = 6.73; p = 0.02], but not of licking [F(1, 17) = 1.85; p = 0.19] and yawning [F(1, 17) = 1.31; p = 0.27], was significantly increased. DPI also significantly increased the duration of chewing [F(1, 17) = 11.76; p < 0.01; Fig. 1], large amplitude chewing [F(1, 17) = 5.19; p = 0.04], and gnawing [F(1, 17) = 10.53; p < 0.01], but not of tremor ([F(1, 17) = 2.76; p = 0.11]; Fig. 1) and licking [F(1, 17) = 0.21; p = 0.65].

**Behavioural Analysis: Effects of Sulpiride Stereoisomers on DPI**

L-Sulpiride did not affect any oral behaviour induced by DPI. d-Sulpiride significantly affected the frequency [F(3, 20) = 5.02; p < 0.01] and duration of chewing ([F(3, 20) = 5.87; p < 0.01]. With respect to both chewing frequency and duration, there was an apparent dose-dependent decrease (Fig. 1). d-Sulpiride significantly affected the frequency [F(3, 20) = 6.02; p < 0.01] and the duration of tremor [F(3, 30) = 7.56; p < 0.01; Fig. 1]. Tremor duration was enhanced by the lowest dose of d-sulpiride and attenuated by the highest dose of d-sulpiride. Tremor frequency showed similar, but nonsignificant, effects (Fig. 1). None of the other DPI-induced behaviours (i.e., large amplitude chewing, gnawing, and tongue protrusions) were significantly affected by d-sulpiride according to the ANOVA. However, inspection of the data suggested that this was due to the fact that DPI had small
effects (cf. [22]) rather than to a lack of effect of d-sulpiride, which nonsignificantly attenuated all these oral behaviors (data not shown).

Behavioural Analysis: Effects of Sulpiride Stereoisomers Alone

The effects of the sulpiride stereoisomers alone were studied in a similar way as were the effects of the stereoisomers on DPI (using baseline-controlled values, see Materials and Methods section). However, the experimental session values of the H_2O/H_2O control group for the frequency of gnawing (mean ± SEM: 0.1 ± 0.1), tongue protrusions (5.1 ± 2.0), yawning (0.8 ± 0.5) and large amplitude chewing (0.1 ± 0.1) were very low, and therefore a possible attenuating effect of the sulpiride stereoisomers could not be determined. Although a possible decrease in these behaviors could not be determined, neither l-sulpiride nor d-sulpiride induced an increase in any of these behaviors (data not shown). The experimental session value of the H_2O/H_2O control group for the tremor frequency was also reasonably low (mean ± SEM: 11.8 ± 3.5). Therefore, apart from analyzing the tremor frequency in the normal way (using baseline-controlled values, see above), it was also analyzed by comparing the experimental session values of the sulpiride stereoisomer groups with those of the H_2O/H_2O control group (data not shown). L-Sulpiride and d-sulpiride did not influence chewing: neither baseline-controlled values nor the experimental session values (Fig. 2) were significantly different from those of the controls. The experimental session value of the H_2O/H_2O control group for the chewing frequency was very high (means ± SEM: 77.0 ± 6.0); therefore, this parameter could be analyzed in a normal way. None of the doses tested of l-sulpiride or d-sulpiride affected chewing (Fig. 2). With respect to the duration of the different behaviors, l- and d-sulpiride showed the same lack of effect as for frequency.

Electromyographical Analysis: Effects of Sulpiride Stereoisomers on DPI

As for the behavioral items, the EMG data were baseline-controlled, and the resulting scores could be either negative or positive (Fig. 3). In line with an earlier study [22], the drug-induced effects on distinct frequencies could easily be described with the help of three main classes of frequencies, Class A (frequency 3 Hz), Class B (frequency 4–6 Hz), and Class C (frequency 7–15 Hz). DPI had no effect on Class A. On the other hand, DPI significantly enhanced Class B [F(1, 17) = 8.29; p = 0.01] and Class C [F(1, 17) = 4.84; p = 0.04] of the masseter muscle. DPI did not affect any frequency class of the digastric muscle.

Electromyographical Analysis: Effects of Sulpiride Stereoisomers on DPI

l-Sulpiride did not significantly affect the DPI-induced EMG effects (Fig. 3). d-Sulpiride significantly affected Classes B [F(3, 28) = 3.84; p < 0.05] and C [F(3, 28) = 10.9; p < 0.01], but not Class A, of the masseter muscle. Class B was significantly attenuated by 50 ng d-sulpiride (Fig. 3), whereas Class C was enhanced by a low dose (10 ng; p < 0.05) and attenuated by a high dose (50 ng; p < 0.05; Fig. 3). Although the EMG results of the digastric muscle were not significantly affected by DPI, d-sulpiride showed an overall effect on Class C [F(3, 28) = 5.95; p < 0.01]. Again, an enhancement (10 ng) of DPI was followed by a decrease (50 ng; data not shown).
Electromyographical Analysis: Effects of Sulpiride Stereoisomers Alone

As for the behavioral analysis, both the baseline-controlled values and the experimental values of the Classes A–C were analyzed. No significant effect was found for one of the classes of either the masseter or the digastric muscle.

DISCUSSION

The effects of intra-accumbens administration of l- and d-sulpiride on 1,4-dihydroxyphenylimino)-2-imidazoline (DPI)-induced oral behaviours were studied in freely moving rats. DPI is known to affect oral behaviour when injected into the shell, but not into more rostral, ventral, dorsal, or caudal regions (see Introduction section). Moreover, sulpiride is known to be very hydrophilic and to diffuse little after intracerebral administration [2]. This, together with the fact that the injections were placed in the central nucleus accumbens (the border region of the core and shell, see Introduction section), allows the conclusion that all the observed drug-induced effects were due to pharmacological actions within the nucleus accumbens.

In line with an earlier study [23], DPI significantly increased the oral behaviours chewing and tremor. These effects were not affected by l-sulpiride. On the other hand, d-sulpiride significantly attenuated the effects of DPI on chewing and tremor. This was not due to a nonspecific effect of d-sulpiride, because it did not inhibit these behaviours when administered alone. Although the observer was not blind to the treatment, the validity of the acquired data is strongly supported by the results of the electromyographic (EMG) analysis. First, the EMG effects of DPI on the masseter muscle were antagonized by d-sulpiride, but not l-sulpiride. Second, d-sulpiride given alone showed no EMG effect. And, finally, the behavioural changes in chewing co-occurred with the frequencies 4–6 Hz, whereas those in tremor co-occurred with the frequencies 7–15 Hz; these observations fully fit in with the finding in our previously unpublished study showing that the display of chewing and that of tremor nicely correlate with the occurrence of the frequencies 4–6 Hz and that of the frequencies 7–15 Hz, respectively (see Materials and Methods). Taken together, these EMG data strongly support the behavioural results.

Comparing both methods, it is clear that both the behavioural analysis and the EMG analysis are able to detect drug-induced changes in oral behavior. However, it is important to note that EMG analysis alone is insufficient for detecting which oral behaviour is affected: apart from the noted correlations (tremor and frequencies 7–15 Hz; chewing and frequencies 4–6 Hz), no other correlations were found. Nevertheless, the value of this method is evident: it is an objective method that can be used to (in)validate the assessment of the behavioural method.

The finding that d-sulpiride attenuates the effects of DPI on oro-facial behaviours suggests that dopamine D1,4 receptors were not involved, because d-sulpiride has a relatively small affinity for these receptors [1,13,26,27,29,30]. Moreover, actions on dopamine D2 or D3 receptors causing the attenuation of DPI-induced oral behaviours can be excluded because l-sulpiride, which is a stronger antagonist of both receptors than d-sulpiride (see below), is without effect on DPI-induced oral behaviours. This is underscored by the finding that a low dose of l-sulpiride (2 ng) with a dopamine D2 and D3 receptor blockade comparable to the effective dose of d-sulpiride (50 ng; [1,11,26,27] did not affect...
DPI-induced behaviours. The stereoselective action of \(d\)-sulpiride also suggests that the so-called mesolimbic \(l\)-sulpiride binding site is not involved, because \(d\)-sulpiride and \(l\)-sulpiride have identical affinities for this site \([9]\). Still, it cannot be excluded that the ineffectiveness of \(l\)-sulpiride was due to its potency to interact with both the mesolimbic \(l\)-sulpiride binding site and dopamine \(D_2\) or \(D_3\) receptors; for, combined binding may limit the relative occupancy and, thus, the degree of antagonism of \(l\)-sulpiride at each of the binding sites separately. Further research is required to characterize the actual target site of \(d\)-sulpiride.

Irrespective of the precise mechanism involved, \(d\)-sulpiride strongly attenuated DPI-induced oral behaviour. The present data imply that \(d\)-sulpiride may ameliorate oro-facial dyskinesias in man (see Introduction). Because \(d\)-sulpiride has a very low affinity for dopamine \(D_2\) receptors, it may have therapeutic effects on oro-facial dyskinesias in schizophrenic patients as well as in Parkinson's patients without aggravating the extrapyramidal side-effects of neuroleptics or without attenuating the therapeutic effects of \(l\)-DOPA, respectively. As far as we know, no clinical studies with \(d\)-sulpiride have been performed. However, the racemic mixture of sulpiride has been studied in schizophrenic patients: it ameliorates oro-facial dyskinesias when given alone \([4,12,23]\) and when given in combination with other neuroleptics \([4,25]\). These findings seem to support the clinical implication postulated above, although it cannot be excluded that the anti-dyskinetic effect of \(dl\)-sulpiride seen in the clinic was (partly) due to \(D_2\) receptor blockade caused by \(l\)-sulpiride. For, \(D_2\) antagonists like classical neuroleptics are known to ameliorate oro-facial dyskinesias \([14,15]\). Nevertheless, the present findings suggest that \(d\)-sulpiride may have anti-dyskinetic effects. If so, \(d\)-sulpiride is of interest as an adjuvant to \(l\)-DOPA in the pharmacotherapy of Parkinson's patients, counteracting the development of oro-facial dyskinesias. However, it should be realized that the specificity of \(d\)-sulpiride will be limited to lower doses, because higher doses can affect dopamine \(D_2\) receptors as well.

Furthermore, \(dl\)-sulpiride may be of interest in the pharmaco-therapeutic treatment of schizophrenia, having therapeutic effects (\(l\)-sulpiride) while counteracting the development of oro-facial dyskinesias (\(d\)-sulpiride). Indeed, \(dl\)-sulpiride chronically administered induces less oro-facial dyskinesias than classical neuroleptics \([24]\) and references therein. In sum, \(d\)-sulpiride (10–50 ng) administered into the nucleus accumbens inhibited DPI-induced oral behaviours and jaw muscle EMG changes, in a specific manner, for: \(d\)-sulpiride did not affect the relative dopamine \(D_2\) receptor occupancy and, thus, the degree of antagonism of \(l\)-sulpiride. This study was supported by the Dutch Society for Scientific Research (NWO), No. 900-548-119.

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