Effects of Alpha-2 Adrenoceptor Agonists and Antagonists on Circling Behavior in Rats with Unilateral 6-Hydroxydopamine Lesions of the Nigrostriatal Pathway

PHILIPPE CHOPIN, FRANCIS C. COLPAERT and MARC MARIEN
Centre de Recherche Pierre Fabre, Cedex, France
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ABSTRACT

The present study examined the influence of alpha-2 adrenoceptor ligands on circling behavior in rats with unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway. The alpha-2 adrenoceptor agonists, clonidine and UK 14304, inhibited both the ipsilateral rotation induced by the indirect dopaminergic agonist, methylenephendate, and the contralateral circling induced by the direct dopaminergic agonist, apomorphine. In contrast, the alpha-2 adrenoceptor antagonists, idalfoxan and enantiomer efaroxan, enhanced the circling induced by either methylenephendate or apomorphine. The facilitating activity of efaroxan was stereoselective because the (+)-enantiomer mimicked the effect of (−)-efaroxan, whereas the (−)-enantiomer was essentially inactive, thus indicating a mediation by alpha-2 adrenoceptors. Upon administration alone, the above-mentioned compounds did not modify spontaneous circling behavior, except for UK 14304, which decreased, and (+)-efaroxan, which slightly increased, the ipsilateral rotation. We conclude that activation and antagonism of alpha-2 adrenoceptors inhibit and enhance, respectively, the circling behavior evoked by both direct and indirect dopaminergic agonists. Although a modulation of dopamine release may be involved in some of these drug effects, the effects on apomorphine-induced circling indicate an influence of alpha-2 adrenoceptor compounds on nigrostriatal neurotransmission at sites downstream from the dopaminergic neurons themselves. These findings support the notion of a potential benefit of alpha-2 adrenoceptor antagonists in the treatment of Parkinson’s disease.

Two major dopamine systems in the rat brain, the nigrostriatal projection from the substantia nigra pars compacta (A9) to the caudate-putamen (striatum), and the mesolimbic projection from the ventral tegmental area (A10) to the septum, nucleus accumbens, and other limbic regions, play important roles in the control of locomotion and posture (Fallon and Moore, 1978; Levitt and Moore, 1979; Arnt, 1987). Adrenergic pathways are also involved in the control of locomotion, although they appear to play a more subtle, modulatory role than the dopaminergic systems. The precise nature of the influence of adrenergic networks on dopamine projections remains poorly understood (Tassin et al., 1994; Antelman and Caggiula, 1994; Marien et al., 1994; Nutt et al., 1994).

One model that has been extensively used in the evaluation of the role of ascending noradrenergic and dopaminergic pathways in the control of motor behavior is the circling behavior in unilateral nigra-lesioned rats. This model is widely used to evaluate the potential antiparkinson activity of experimental therapeutic agents, and has been termed “the hemi-parkinsonian rat” (Costall and Naylor, 1975; Silberman, 1993). The model involves the unilateral injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle. This causes extensive loss of dopamine cells in the ipsilateral substantia nigra, pars compacta, and in the ventral tegmental area (Ungerstedt, 1971; Pycock, 1980; Carman et al., 1991). The resulting imbalance in dopamine innervation between the striata produces a postural asymmetry such that the animal rotates away from the nonlesioned side, i.e., the side of greater dopamine activity (Ungerstedt, 1971). Administration of dopamine-releasing agents, such as methylenephenidate, exacerbates the dopamine imbalance that favors the nonlesioned nigrostriatal projection and thus produces ipsilateral rotations (i.e., toward the lesioned side) (Ungerstedt, 1971; Carman et al., 1991). In contrast, direct agonists such as apomorphine evoke contralateral rotation reflecting an action at supersensitive, denervated dopamine receptors within the striatum of the lesioned side (Ungerstedt, 1971; Pycock, 1980; Carman et al., 1991).

In Parkinson’s disease, in addition to the degeneration of the nigrostriatal dopamine pathway, the locus ceruleus and the noradrenergic pathways are also implicated. There are marked reductions in post-mortem concentrations of noradrenaline in several brain structures (Hornykiewicz and Kish, 1986) and a modification of the density of alpha-2

ABBREVIATIONS: 6-OHDA, 6-hydroxydopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.
adrenoceptors in the prefrontal cortex (Cash et al., 1984). The efficacy of alpha-2 adrenoceptor antagonists to attenuate some of the parkinsonian symptoms in reserpinized rats (Colpaert, 1987) and in monkeys treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Colpaert et al., 1991) suggests that the locus ceruleus-noradrenergic dysfunction has a key role in the pathophysiology and progression of Parkinson’s disease (Colpaert, 1987, 1994). Thus, lesions of the locus ceruleus decrease the release of striatal dopamine in rats (Marien et al., 1994) and retard the spontaneous recovery from parkinsonian deficits in MPTP-treated monkeys (Mavridis et al., 1991b). Since alpha-2 adrenoceptor antagonists increase locus ceruleus-noradrenergic activity (e.g., noradrenaline release) by blockade of the inhibitory alpha-2 autoreceptor (Van Veldhuisen et al., 1993), together these findings provide a rationale for evaluating alpha-2 adrenoceptor antagonists in animal models of Parkinson’s disease and in patients with this disorder. Moreover, recent clinical trials indicate a beneficial effect of the alpha-2 adrenoceptor antagonist idazoxan in Parkinson’s disease patients (Ruzicka et al., 1997; Peyro-Saint-Paul et al., 1997).

In the present study, the effects of the alpha-2 adrenoceptor antagonists, idoxoxan, efaroxan, and its enantiomers, and the alpha-2 adrenoceptor agonists, clonidine and UK 14304 were measured on spontaneous circling behavior and on circling behavior induced by the dopaminomimetic compounds, apomorphine or methylphenidate, in rats with unilateral 6-OHDA-induced lesions of the nigrostriatal pathway. In this model, d-amphetamine is commonly used as the dopamine-releasing agent (Carman et al., 1991; Mavridis et al., 1991a; Hudson et al., 1993; Schwarting and Huston, 1996). In the present study, methylphenidate was used instead, because d-amphetamine enhances both the release of dopamine and noradrenaline, whereas methylphenidate appears to preferentially increase the release of dopamine alone (McMillen, 1983).

Materials and Methods

Animals. Animals were handled and cared for in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 1996) and the European Directive no. 86/609. The experimental protocol was carried out in compliance with French regulations and with local ethical committee guidelines for animal research. Male Sprague-Dawley rats [ICO:OFA-SD (IOPS.Caw), IFFA Credo, Domaine des Oncins, France] weighing 280 to 300 g at the time of surgery were housed singly with free access to food and water in a room maintained at 21 °C on a light/dark cycle with lights on at 7:00 AM.

Surgery and Lesion Verification. One hour before operation, rats were injected i.p. with 25 mg/kg desipramine to prevent concurrent damage of noradrenergic pathways by 6-OHDA infusions. Fifty minutes later, animals were anesthetized with ketamine (60 mg/kg i.p.) and placed in a David Kopf small animal stereotaxic apparatus (model 900) with the incisor bar fixed at −3.3 mm. 6-OHDA (8 μg/2 μl of saline containing 0.2% ascorbic acid) was unilaterally infused into the medial forebrain bundle through a 30-gauge stainless steel cannula at a rate of 0.5 μl/min using a microliter syringe pump (model CMA 100; Carnegie-Medicen, France). The stereotoxic coordinates, based on the atlas of Paxinos and Watson (1986) were −3.0 mm posterior, −1.6 mm lateral, and −8.5 mm ventral from bregma. The cannula was left in place for 2 min after the end of injection to prevent reflux and to allow for toxin diffusion. To assess the extent of the nigrostriatal dopaminergic denervation at 2 weeks posturing, rats were injected s.c. with 0.04 mg/kg apomorphine, and the number of contralateral rotations were recorded during a 1-h period immediately after injection. The criteria for the inclusion of animals in subsequent experiments was a minimum of 10 contralateral rotations during a 10-min period.

Rotational Behavior Testing. To measure the activity of compounds on spontaneous circling behavior, rats were placed in a cylindrical cage (240 mm in diameter, 300 mm high) and the number of rotations, both ipsilateral and contralateral, was recorded using an automatic rotometer (Rota-Count-8; Columbus Instruments) during a 1-h period immediately after drug or vehicle injection. To study the effects of alpha-2 adrenoceptor agonists and antagonists on circling behavior induced by the dopaminergic compounds, rats were placed in the cylindrical cages, and circling behavior was assessed for a 1-h period immediately after an administration of 0.04 mg/kg s.c. apomorphine or 2.5 mg/kg s.c. methylphenidate. Animals were injected i.p. with the alpha-2 adrenoceptor compounds or vehicle 5 min before the administration of dopaminergic drugs. The total number of animals tested per dose was five.

Drugs. The following compounds were synthesized at the Center de Recherche Pierre Fabre (Castres, France): UK 14304 tartrate (5-bromo-6-[2-imidazoline-2-yl amino] quinoxaline), idoxoxan tartrate (2-[2-(1,4-benzodioxanyl)-2-imidazoline], (−)-efaroxan hydrochloride (2-[2-(2-ethyl-2,3-dihydrobenzofuranyl]-2-imidazoline), (−)-efaroxan hydrochloride, and (±)-efaroxan hydrochloride. The other drugs used were clonidine hydrochloride (Sigma, Saint Quentin Fallavier, France), desipramine hydrochloride (Sigma), 6-OHDA hydrochloride (6-hydroxydopamine; Sigma), ketamine hydrochloride (Clorkeutm 1000, Vetoquinol), methylphenidate hydrochloride (Ciba-Geigy Co.), and R(−)-apomorphine hydrochloride (Research Biochemicals Inc., Illkirch, France). 6-OHDA was freshly prepared in saline containing 0.2% ascorbic acid. All other drugs were dissolved in distilled water. An injection volume of 10 ml/kg was used throughout. Doses refer to the free base, and were selected from the geometrical series 0.01, 0.04, 0.16, 0.63, 2.5, and 10 mg/kg.

Statistics. All results were compared using a Kruskal-Wallis nonparametric one-way analysis of variance corrected for ties followed by a two-tailed Mann-Whitney U test (GB-STAT; Friedman, 1991). Results were, however, expressed as the mean ± S.E.M. in spite of the probable non-normality of the distribution of scores, because it was felt that these parameters provide a clearer indication for most tests.

Results

Rats that had undergone the unilateral 6-OHDA lesioning procedure were screened for their circling response to a challenge dose (0.04 mg/kg s.c.) of apomorphine. All rats showed robust contralateral rotation (> 100 turns/h) and were thus used for further testing.

Effects on Spontaneous Circling Behavior. Rats treated with vehicle showed a low level of spontaneous ipsilateral rotation presumably reflecting the basal level of dopamine activity of the nonlesioned side (Tables 1 and 2). Apomorphine dose-dependently induced a contralateral rotation (H = 17.69, P < .001), whereas methylphenidate induced a robust ipsilateral rotation (H = 21.92, P < .001) (Table 1). The doses of 0.04 mg/kg s.c. of apomorphine and 2.5 mg/kg s.c. of methylphenidate were selected as the standard challenge doses for the drug interaction studies because they induced a consistent and pronounced rotation, such that either a potentiation or inhibition of their action by test compounds could potentially be detected.

The alpha-2 adrenoceptor agonist, UK 14304, significantly decreased spontaneous ipsilateral circling (H = 15.02,
P = .005) in a dose-dependent manner with a minimal significant dose of 0.16 mg/kg i.p. Clonidine (0.01–0.63 mg/kg i.p.) was without significant effects on ipsilateral rotation (H = 2.67, N.S.) (Table 1).

Spontaneous ipsilateral rotation was significantly increased by (+)-efaroxan from 0.16 to 10 mg/kg i.p. (H = 21.50, P = .002) (Table 2). In general, an increase of ipsilateral rotation reflects the activity of dopamine-releasing agents such as methylphenidate. However, the magnitude of effects induced by (+)-efaroxan (45–60 turns/h) was not dose-dependent and was mild in comparison to the effect of methylphenidate (up to 435 turns/h) (see Tables 1 and 2). The racemate (±)-efaroxan (H = 7.94, N.S.), its (−)-enantiomer (H = 2.69, N.S.) and idazoxan (H = 6.56, N.S.) were without significant effects on ipsilateral rotation (Table 2).

### Table 1

**Effects of dopaminergic compounds and alpha-2 adrenergic agonists on spontaneous circling behavior**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>No. of contralateral turns/h</th>
<th>No. of ipsilateral turns/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle s.c.</td>
<td>1.6 ± 0.7</td>
<td>18.1 ± 1.9</td>
</tr>
<tr>
<td>Methylphenidate, 0.63 mg/kg s.c.</td>
<td>1.9 ± 1.5</td>
<td>179.1 ± 26.2**</td>
</tr>
<tr>
<td>Methylphenidate, 2.5 mg/kg s.c.</td>
<td>1.3 ± 0.5</td>
<td>189.1 ± 32.5**</td>
</tr>
<tr>
<td>Methylphenidate, 10 mg/kg s.c.</td>
<td>0.4 ± 0.2</td>
<td>435.4 ± 52.5**</td>
</tr>
<tr>
<td>Vehicle s.c.</td>
<td>3.4 ± 1.3</td>
<td>15.2 ± 2.4</td>
</tr>
<tr>
<td>Apomorphine, 0.04 mg/kg s.c.</td>
<td>218.6 ± 49.1***</td>
<td>4.2 ± 1.4***</td>
</tr>
<tr>
<td>Apomorphine, 0.16 mg/kg s.c.</td>
<td>375.8 ± 57.5***</td>
<td>0.8 ± 0.4***</td>
</tr>
<tr>
<td>Apomorphine, 0.63 mg/kg s.c.</td>
<td>600.6 ± 37.3***</td>
<td>0.5 ± 0.2***</td>
</tr>
<tr>
<td>Vehicle i.p.</td>
<td>3.6 ± 1.1</td>
<td>15.4 ± 4.9</td>
</tr>
<tr>
<td>Clonidine, 0.01 mg/kg i.p.</td>
<td>1.4 ± 0.5</td>
<td>14.0 ± 3.1</td>
</tr>
<tr>
<td>Clonidine, 0.04 mg/kg i.p.</td>
<td>1.2 ± 0.7</td>
<td>13.0 ± 3.3</td>
</tr>
<tr>
<td>Clonidine, 0.16 mg/kg i.p.</td>
<td>4.4 ± 3.4</td>
<td>9.8 ± 3.5</td>
</tr>
<tr>
<td>Clonidine, 0.63 mg/kg i.p.</td>
<td>6.6 ± 1.9</td>
<td>17.8 ± 3.7</td>
</tr>
<tr>
<td>Vehicle i.p.</td>
<td>2.8 ± 1.8</td>
<td>17.0 ± 1.8</td>
</tr>
<tr>
<td>UK 14304, 0.01 mg/kg i.p.</td>
<td>1.6 ± 0.8</td>
<td>14.2 ± 4.2</td>
</tr>
<tr>
<td>UK 14304, 0.04 mg/kg i.p.</td>
<td>2.6 ± 0.5</td>
<td>16.0 ± 5.0</td>
</tr>
<tr>
<td>UK 14304, 0.16 mg/kg i.p.</td>
<td>1.4 ± 0.7</td>
<td>3.6 ± 1.0*</td>
</tr>
<tr>
<td>UK 14304, 0.63 mg/kg i.p.</td>
<td>3.4 ± 1.2</td>
<td>2.6 ± 1.2*</td>
</tr>
</tbody>
</table>

The racemate (±)-efaroxan (H = 11.69, P = .04) and its (−)-enantiomer (H = 20.75, P < .001) both dose-dependently increased methylphenidate-induced rotation with a minimal significant dose of 0.63 mg/kg, respectively. The dose-response curves had an inverted U-shape and the max-

### Table 2

**Effects of alpha-2 adrenergic antagonists on spontaneous circling behavior**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>No. of contralateral turns/h</th>
<th>No. of ipsilateral turns/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg, i.p.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>4.8 ± 1.5</td>
<td>23.2 ± 5.1</td>
</tr>
<tr>
<td>Idazoxan, 0.63</td>
<td>2.4 ± 1.9</td>
<td>19.8 ± 4.7</td>
</tr>
<tr>
<td>Idazoxan, 2.5</td>
<td>3.2 ± 2.2</td>
<td>27.8 ± 1.9</td>
</tr>
<tr>
<td>Idazoxan, 10</td>
<td>3.0 ± 1.8</td>
<td>12.8 ± 2.4</td>
</tr>
<tr>
<td>Vehicle</td>
<td>1.4 ± 0.7</td>
<td>21.8 ± 3.5</td>
</tr>
<tr>
<td>(+)-Efaroxan, 0.01</td>
<td>1.8 ± 1.4</td>
<td>17.4 ± 3.3</td>
</tr>
<tr>
<td>(+)-Efaroxan, 0.04</td>
<td>2.0 ± 0.8</td>
<td>22.2 ± 7.8</td>
</tr>
<tr>
<td>(+)-Efaroxan, 0.16</td>
<td>2.6 ± 1.4</td>
<td>43.8 ± 4.7***</td>
</tr>
<tr>
<td>(+)-Efaroxan, 0.63</td>
<td>3.4 ± 0.2</td>
<td>55.4 ± 3.8***</td>
</tr>
<tr>
<td>(+)-Efaroxan, 2.5</td>
<td>3.6 ± 1.8</td>
<td>44.4 ± 10.2***</td>
</tr>
<tr>
<td>(+)-Efaroxan, 10</td>
<td>0.4 ± 0.2</td>
<td>59.6 ± 12.8**</td>
</tr>
<tr>
<td>(−)-Efaroxan, 0.63</td>
<td>0.8 ± 0.6</td>
<td>27.4 ± 7.5</td>
</tr>
<tr>
<td>(−)-Efaroxan, 2.5</td>
<td>4.4 ± 3.4</td>
<td>30.6 ± 3.4</td>
</tr>
<tr>
<td>(−)-Efaroxan, 10</td>
<td>1.4 ± 0.7</td>
<td>30.6 ± 5.2</td>
</tr>
<tr>
<td>(±)-Efaroxan, 0.16</td>
<td>2.6 ± 1.4</td>
<td>15.6 ± 1.9</td>
</tr>
<tr>
<td>(±)-Efaroxan, 0.63</td>
<td>2.8 ± 0.5</td>
<td>23.4 ± 7.2</td>
</tr>
<tr>
<td>(±)-Efaroxan, 2.5</td>
<td>3.6 ± 1.8</td>
<td>27.4 ± 4.0</td>
</tr>
<tr>
<td>(±)-Efaroxan, 10</td>
<td>0.4 ± 0.2</td>
<td>34.2 ± 5.4</td>
</tr>
</tbody>
</table>

Spontaneous contralateral rotation was not significantly modified by the alpha-2 adrenergic agonists clonidine (H = 7.29, N.S.), UK 14304 (H = 2.91, N.S.), nor by the alpha-2 adrenergic antagonists idazoxan (H = 1.67, N.S.), (±)-efaroxan (H = 7.76, N.S.), (−)-efaroxan (H = 0.73, N.S.), and (±)-efaroxan (H = 6.16, N.S.) (Tables 1 and 2).

**Effects on Methylphenidate-Induced Circling.** The alpha-2 adrenergic agonists clonidine (H = 19.47, P < .001) and UK 14304 (H = 17.38, P = .002) dose-dependently decreased methylphenidate-induced rotation with minimal significant doses of 0.04 and 0.16 mg/kg i.p., respectively (Fig. 1).

Idazoxan (H = 23.73, P < .001) dose-dependently enhanced methylphenidate-induced rotation at a minimal significant dose of 0.16 mg/kg i.p. The maximal effect of idazoxan was achieved with the dose of 2.5 mg/kg. In contrast, the dose of 10 mg/kg i.p. of idazoxan significantly decreased methylphenidate-induced rotation (Fig. 1).

The racemate (±)-efaroxan (H = 11.69, P = .04) and its (−)-enantiomer (H = 20.75, P < .001) both dose-dependently increased methylphenidate-induced rotation with a minimal significant dose of 0.63 and 0.16 mg/kg, respectively. The dose-response curves had an inverted U-shape and the max-
imal effect of either compound was observed with the dose of 2.5 mg/kg (Fig. 1). In contrast, (−)-efaroxan did not show any significant activity on the circling behavior induced by methylphenidate (Fig. 1: H = 4.17, N.S.).

**Effects on Apomorphine-Induced Circling.** Clonidine (H = 18.91, P < .001) and UK 14304 (H = 1891, P < .001) dose-dependently decreased apomorphine-induced rotation with minimal significant doses of 0.04 and 0.01 mg/kg i.p., respectively (Fig. 2).

The apomorphine-induced rotation was enhanced by idazoxan (H = 10.45, P = .03), with a minimal significant dose of 0.63 mg/kg. The maximal effect of idazoxan was observed with the dose of 2.5 mg/kg. The racemate (±)-efaroxan (H = 9.70, P = .05) and its (+)-enantiomer (H = 10.05, P = .04) dose-dependently enhanced apomorphine-induced rotation with a minimal significant dose of 0.63 mg/kg. In contrast, (−)-efaroxan did not show any significant activity on the circling behavior induced by apomorphine (Fig. 2: H = 3.79, N.S.) (Fig. 2). Note that the dose-response curves of the alpha-2 adrenoceptor antagonists idazoxan and (±)-efaroxan had an inverted U shape (Fig. 2).

Statistical comparison between the effects of (±)-efaroxan on methylphenidate- versus apomorphine-induced circling indicated a significantly greater effect on methylphenidate-induced responses at the doses of 0.63 mg/kg and 2.5 mg/kg of (±)-efaroxan (U = 6.0, p < .05).

**Discussion**

After injection of vehicle, rats with unilateral 6-OHDA lesions of the nigrostriatal dopaminergic pathway showed a low level of spontaneous ipsilateral rotation, which presumably reflects the basal level of dopamine activity of the nonlesioned side. The present study used this unilateral nigral lesion model for examining the influence of alpha-2 adrenoceptor agonists and antagonists on spontaneous rotation behavior and on the circling behavior induced by the dopamine mimetic agents apomorphine and methylphenidate.

The alpha-2 adrenoceptor agonist, clonidine, was inactive on spontaneous circling, both contralateral and ipsilateral, whereas UK 14304 tested at the same doses (0.01–0.63 mg/kg) significantly decreased spontaneous ipsilateral circling in a dose-dependent manner without having significant effects on contralateral rotation. Higher doses of clonidine were not investigated because they produce almost total depression of behavior (e.g., Pellow et al., 1985) and begin to lose their specificity for alpha-2 adrenoceptors (Delina-Stula et al., 1979). The difference in the effects of UK 14304 and clonidine on ipsilateral rotation may be related to the relative intrinsic activities and/or receptor selectivity of these compounds. In vitro, clonidine acts as a partial agonist (Medgett et al., 1978), whereas UK 14304 has been shown to be a full agonist at alpha-2 adrenoceptors (Grant and Scrutton, 1980; Armah, 1988). In vivo, clonidine is less effective than UK 14304 in reducing the outflow of noradrenaline as measured by microdialysis in the rat cortex (Van Veldhuizen et al., 1993). Moreover, UK 14304 is a high-efficacy agonist, whereas clonidine is an alpha-2 adrenoceptor agonist with lower intrinsic activity in terms of the maximal magnitude of its behavioral effects in reducing the righting reflex and muscle tone in rats (Colpaert 1986a, b; Roach et al., 1983). In addition, clonidine exerts agonist actions at alpha-1 adrenoceptors (Haeseler, 1974; Moroni et al., 1983) and histamine H2 receptors (Bugajski et al., 1980), whereas UK 14304 shows a superior selectivity for the alpha-2 adrenoceptor.

The alpha-2 adrenoceptor antagonists, idazoxan, (±)-efaroxan, and its (−)-enantiomer, were without significant activity on spontaneous contralateral and ipsilateral rotations. On the other hand, spontaneous ipsilateral rotation was significantly increased by (±)-efaroxan. The doses of these drugs were chosen because they have been shown to be optimal for blocking alpha-2 adrenoceptor-mediated effects in the rat central nervous system in vivo, e.g., antagonism of the behavioral effects of alpha-2 adrenoceptor agonists (Colpaert 1986a; Millan et al., 1994). (±)-Efaroxan has been found to be more effective than idazoxan in most in vivo rodent models (Martel et al., 1996; Tellez et al., 1997). However, the magnitude of the effect induced by (±)-efaroxan on spontaneous ipsilateral rotation was mild in comparison to that of methylphenidate. The weak effects of (±)-efaroxan and the absence of effects of the other alpha-2 adrenoceptor antagonists on spontaneous rotation may suggest that the tonic influence of alpha-2 adrenoceptors on nigrostriatal transmission in vivo is weak. Alternatively, a poor efficacy of alpha-2 adrenoceptor antagonists on spontaneous rotation may be related to counteraction by the intrinsic partial agonist properties of alpha-2 adrenoceptors.
these compounds at alpha-2 adrenoceptors (Colpaert 1986a, b). This is consistent with the clear inhibitory effect of the alpha-2 adrenoceptor agonist UK 14304 on spontaneous ipsilateral rotation, and indicates that the nigrostriatal dopamine system is indeed sensitive to a negative modulation by alpha-2 adrenoceptors. This is also coherent with the inhibitory effect of alpha-2 adrenoceptor agonists on striatal dopamine synthesis, release, and metabolism in vivo (Marien et al., 1994; Yavich et al., 1997).

In rats with unilateral 6-OHDA lesions of the nigrostriatal dopaminergic pathway, methylphenidate, a dopamine-release agent, dose-dependently evoked ipsilateral turning presumably due to the release of dopamine from and inhibition of its reuptake (Hudson et al., 1993) into intact, contralateral striatal terminals. The alpha-2 adrenoceptor agonists, clonidine and UK 14304, dose-dependently decreased methylphenidate-induced ipsilateral rotation, whereas the alpha-2 adrenoceptor antagonists, idazoxan and (±)-efaroxan, potentiated the circling behavior evoked by methylphenidate with inverted U-shaped dose-response curves. Moreover, the facilitating activity of efaroxan was stereoselective because the (+)-enantiomer mimicked the effect of (±)-efaroxan, whereas the (-)-enantiomer did not show any significant activity. The clear stereoselectivity of this effect is strong evidence for a mediation by alpha-2 adrenoceptors because (+)-efaroxan has a higher potency for these sites than the (-)-enantiomer (Flamez et al., 1997). Apomorphine, a direct dopamine receptor agonist, dose-dependently induced contralateral turning presumably due to an action at supersensitive, denervated dopamine receptors within the striatum resulting from the unilateral 6-OHDA lesion of the nigrostriatal dopaminergic pathway (Ungerstedt, 1971; Pycock, 1980; Carman et al., 1991). The alpha-2 adrenoceptor agonists, clonidine and UK 14304, dose-dependently decreased apomorphine-evoked contralateral rotation, whereas the alpha-2 adrenoceptor antagonists, idazoxan and (±)-efaroxan, increased apomorphine-induced rotation with inverted U-shaped dose-response curves. This activity of efaroxan was also stereoselective because the (+)-enantiomer mimicked the effect of (±)-efaroxan, whereas the (-)-enantiomer did not show any significant activity, again strongly suggesting a mediation of this effect by alpha-2 adrenoceptors. The effects of (+)-efaroxan was 2 to 3 times higher in methylphenidate-treated rats in comparison to those with apomorphine. The more efficacious effect of (+)-efaroxan on methylphenidate may suggest that noradrenaline modulation predominates in the intact nigrostriatal pathway. In contrast to the present results, clonidine has been shown in a previous study to enhance circling activity induced by both amphetamine and apomorphine (Pycock et al., 1977). However, this study was performed using clonidine (a partial agonist) in mice (instead of rats) lesioned in the striatum rather than in the nigrostriatal pathway. Furthermore, animals were only observed for 1 min rather than 60 min in the present work.

The bell shape of the dose-response curves of alpha-2 adrenoceptor antagonists reported in this study is reminiscent of that observed in the rat with (+)-efaroxan on cortical acetylcholine outflow as measured in vivo by microdialysis (Tellez et al., 1995), with idazoxan and yohimbine on the loss of the righting reflex in behavioral studies (Colpaert, 1986a), and on cortical high voltage spindles in electroencephalogram studies (Yavich et al., 1994). Although the inverted U shape of the dose-response curve has been attributed to partial agonist actions at alpha-2 adrenoceptors (Colpaert, 1986a) and to actions at non-alpha-2 receptors (Yavich et al., 1994), the precise mechanisms underlying this phenomenon are not known for certain. The alpha-2 adrenoceptor agonists, clonidine and UK 14304, have high affinities for the I1-imidazoline receptor and moderate affinities for the I2-imidazoline receptor (Molderings et al., 1993; Miralles et al., 1993). The alpha-2 adrenoceptor antagonist, idazoxan, shows a high (nanomolar) affinity for the I1-imidazoline subtype (Wikberg et al., 1991), whereas efaroxan, which has some affinity for the I1-imidazoline receptor, is essentially inactive on the I2-imidazoline subtype (De Vos et al., 1991; Carlisle et al., 1995; Flamez et al., 1997). Because the imidazoline receptor affinity of these compounds does not appear to correlate with their activity in circling behavior, the I1- and I2-imidazoline receptors are not likely implicated in the effects of the alpha-2 adrenoceptor ligands reported in this study.

Based on molecular cloning and ligand binding studies, alpha-2 adrenoceptors have been subdivided into the subtypes alpha-2A, alpha-2B, and alpha-2C adrenoceptors (Bylund et al., 1992; Bylund, 1995). However, the alpha-2 adrenoceptor agonists and antagonists that are currently available, including the compounds used here, are not sufficiently selective for discriminating between the receptor subtypes in vivo studies (Kendall, 1996; Sallinen et al., 1997). Thus, it is not possible to affiliate our present results with any one alpha-2 adrenoceptor subtype in particular.

Previous studies have indicated that drugs that selectively interact with central noradrenergic mechanisms produce little or no turning activity by themselves and have only modulatory effects on dopamine-dependent circling behavior (Pycock, 1980). The location of the alpha-2 adrenoceptors involved and the identity of the central dopamine system(s) with which they interact are not known for certain, and are possibly multiple. An interaction at the level of the substantia nigra, pars compacta, is possible in view of its noradrenergic input, high density of alpha-2 adrenoceptors, and the respective inhibitory and excitatory influence of alpha-2 adrenoceptor agonists and antagonists thereon (Jones and Moore, 1977; Collingridge et al., 1979; Unnerstall et al., 1984; Grenhoff and Svensson, 1988). An inhibitory influence of alpha-2 adrenoceptors on striatal dopamine release has been indicated by several in vivo studies (see Marien et al., 1994; Yavich et al., 1997). The facilitating effects of alpha-2 adrenoceptor antagonists on apomorphine-induced rotation may result from an interaction with the dopamine system at the level of the striatum or further downstream. Note that the noradrenergic innervation of the striatum is exceedingly sparse and of unclear origin (Jones and Moore, 1977; Levitt and Moore, 1979). The influence of noradrenergic neurotransmission on nigrostriatal dopamine function may also be the consequence of interactions occurring in structures other than the striatum or the substantia nigra. Noradrenaline-dopamine interactions have been reported in the ventral tegmental area, the nucleus accumbens, the septum, or the prefrontal cortex (Hervé et al. 1982; Taghzouti et al., 1991; Tassin et al., 1994; Antelman and Caggiula, 1994). Considering the involvement of the nucleus accumbens in locomotor behavior (Freed and Yamamoto, 1985) and the fairly dense noradrenergic innervation of this region, this nucleus may represent a target or substrate of alpha-2 adrenoceptor an-
that dopaminergic nigrostriatal pathway is only one of the neural elements within the extensive basal ganglia-thalamocortical circuits that are involved in the regulation of motor and complex behavioral activity (Stoof et al., 1996). Furthermore, the action of alpha-2 adrenoceptor compounds on dopamine neurotransmission may also result in part from transsynaptic effects on other neurotransmitters. Neurone


References


alpha 2-adrenoceptor antagonists reduce, whereas alpha-2 adrenoceptor antagonists increase, the release of acetylcholine (Moroni et al., 1983; Tellez et al., 1997), glutamate (Pralong and Magistretti, 1995; Bickler and Hansen, 1996), and serotonin (Yoshioka et al., 1995; Hertel et al., 1997) in several regions of the central nervous system of the rat. Because all of these neurotransmitters can modulate directly or indirectly the dopamine-dependent circling beha

In conclusion, these data may be relevant to Parkinson’s disease because in this disease, in addition to degeneration of the nigrostriatal dopamine pathway, the locus ceruleus and the noradrenergic pathways are implicated. Moreover, clinical trials indicate a beneficial effect of the alpha-2 adrenoceptor antagonist idazoxan in Parkinson’s disease patients (Ruzicka et al., 1997; Peyro-Saint-Paul et al., 1997). The ability of alpha-2 adrenoceptor antagonists to enhance the effect of the direct dopamine agonist apomorphine on circling behavior in rats indicates a facilitating influence of these compounds on nigrostriatal neurotransmission, apparently in this case at a site postsynaptic to the dopamine neurons. In addition, an enhancement of spontaneous and methylphenidate-induced ipsilateral circling suggests also a presynaptic facilitation of nigrostriatal dopamine transmission. Given the evidence for a negative modulatory influence of alpha-2 adrenoceptors on nigrostriatal dopamine transmission in vivo (Marien et al., 1994; Yavich et al., 1997), the effects of alpha-2 adrenoceptor ligands on circling behavior in the unilateral nigral lesioned rat model can be considered to be related to the intrinsic activity of these compounds as agonists at the alpha-2 adrenoceptor(s). Thus, the rank of order of efficacy (magnitude of effect) of compounds to attenuate rotation (either spontaneous or dopaminergic drug induced) parallels their relative efficacy as agonists at alpha-2 adrenoceptors in vitro (Medgett et al., 1978; Grant and Scruton, 1980; Armah, 1988) and in vivo (Roach et al., 1983; Colpaert 1986a, b; Van Veldhuijzen et al., 1993) UK 14304 (high-efficacy agonist) > clonidine (partial agonist) > idazoxan > (+)-efaroxan (antagonist with low intrinsic agonist activity). This relationship would predict that compounds with minimal intrinsic agonist activity at alpha-2 adrenoceptors would exhibit superior facilitatory influence on nigrostriatal dopamine transmission in vivo. As such, the present findings confirm that the circling behavior in rats with unilateral 6-OHDA lesions of the nigrostriatal dopaminergic pathway is an animal model capable of demonstrating activity of compounds working through dopaminergic and nondopaminergic mechanisms. Inasmuch as this “hemi-parkinsonian rat” model reveals a facilitatory effect of alpha-2 adrenoceptor antagonists on nigrostriatal dopamine transmission, the present results support the notion of a potential benefit of alpha-2 adrenoceptor antagonists, devoid of intrinsic agonist activity, for the symptomatic treatment of Parkinson’s disease.

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