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

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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, methylphenidate, and the contralateral circling induced by the direct dopaminergic agonist, apomorphine. In contrast, the alpha-2 adrenoceptor antagonists, idazoxan and (+)-efaroxan, enhanced the circling induced by either methylphenidate 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 dopamine 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 methylphenidate, 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 (Ruzzicka et al., 1997; Peyro-Saint-Paul et al., 1997).

In the present study, the effects of the alpha-2 adrenoceptor antagonists, idoxazol, efaxoxan, 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/EEC. 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-OPA-SD (IOPS.Caw), IPPA Crédo, 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 ± 1°C and 60 ± 5% humidity. There was a 12:12-h 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). The stereotaxic 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 postur-
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 adrenoceptor 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 ± 32.8**</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>606.0 ± 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>

### TABLE 2

Effects of alpha-2 adrenoceptor 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, 0.04</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, 0.63</td>
<td>2.6 ± 1.4</td>
<td>15.6 ± 1.9</td>
</tr>
<tr>
<td>(±)-Efaroxan, 2.5</td>
<td>3.8 ± 1.8</td>
<td>23.4 ± 7.2</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 adrenoceptor agonists clonidine (H = 7.29, N.S.), UK 14304 (H = 2.91, N.S.), nor by the alpha-2 adrenoceptor 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).

**Fig. 1.** Effects of i.p. administration of alpha-2 adrenoceptor agonists and antagonists on methylphenidate-induced ipsilateral rotation. Rats (five per group) were injected i.p. with either the alpha-2 adrenoceptor compound or vehicle 5 min before the administration of methylphenidate (2.5 mg/kg s.c.). The number of rotations was recorded during a 1-h period immediately after the administration of methylphenidate. Results are expressed as a percentage of control (methylphenidate alone). Mean turns per hour in the control groups were between 195 and 258. *P < .05, **P < .01 compared to respective vehicle group (Mann-Whitney U test).
nal 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 non-lesioned 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 (Haeusler, 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 and antagonists on apomorphine-induced circling. Rats (five per group) were injected i.p. with either the alpha-2 adrenoceptor compounds or vehicle 5 min before the administration of apomorphine (0.04 mg/kg s.c.). The number of rotations was recorded during a 1-h period immediately after the administration of apomorphine. Results are expressed as a percentage of control (apomorphine alone). Mean turns per hour in the control groups were between 272 and 316. *P < .05, **P < .01 compared to respective vehicle group (Mann-Whitney U test).

**Fig. 2.** Effects of i.p. administration of alpha-2 adrenoceptor agonists and antagonists on apomorphine-induced contralateral rotation. Rats (five per group) were injected i.p. with either the alpha-2 adrenoceptor compounds or vehicle 5 min before the administration of apomorphine (0.04 mg/kg s.c.). The number of rotations was recorded during a 1-h period immediately after the administration of apomorphine. Results are expressed as a percentage of control (apomorphine alone). Mean turns per hour in the control groups were between 272 and 316. *P < .05, **P < .01 compared to respective vehicle group (Mann-Whitney U test).
these compounds at \( \text{alpha}-2 \) adrenoceptors (Colpaert 1986a, b). This is consistent with the clear inhibitory effect of the \( \text{alpha}-2 \) adrenoceptor agonist UK 14304 on spontaneous ipsilateral rotation, and indicates that the nigrostriatal dopamine system is indeed sensitive to a negative modulation by \( \text{alpha}-2 \) adrenoceptors. This is also coherent with the inhibitory effect of \( \text{alpha}-2 \) 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, methylenidate, a dopamine-releasing 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 \( \text{alpha}-2 \) adrenoceptor agonists, clonidine and UK 14304, dose-dependently decreased methylenidate-induced ipsilateral rotation, whereas the \( \text{alpha}-2 \) adrenoceptor antagonists, idazoxan and (±)-efaroxan, potentiated the circling behavior evoked by methylenidate 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 \( \text{alpha}-2 \) 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 \( \text{alpha}-2 \) adrenoceptor agonists, clonidine and UK 14304, dose-dependently decreased apomorphine-evoked contralateral rotation, whereas the \( \text{alpha}-2 \) 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 \( \text{alpha}-2 \) adrenoceptors. The effects of (±)-efaroxan was 2 to 3 times higher in methylenidate-treated rats in comparison to those with apomorphine. The more efficacious effect of (±)-efaroxan on methylenidate 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 \( \text{alpha}-2 \) 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 \( \text{alpha}-2 \) adrenoceptors (Colpaert, 1986a) and to actions at non-\( \text{alpha}-2 \) receptors (Yavich et al., 1994), the precise mechanisms underlying this phenomenon are not known for certain. The \( \text{alpha}-2 \) adrenoceptor agonists, clonidine and UK 14304, have high affinities for the \( I_1 \)-imidazoline receptor and moderate affinities for the \( I_2 \)-imidazoline receptor (Molderings et al., 1993; Miralles et al., 1993). The \( \text{alpha}-2 \) adrenoceptor antagonist, idazoxan, shows a high (nanomolar) affinity for the \( I_2 \)-imidazoline subtype (Vikberg et al., 1991), whereas efaroxan, which has some affinity for the \( I_1 \)-imidazoline receptor, is essentially inactive on the \( I_2 \)-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 \( I_1 \) and \( I_2 \)-imidazoline receptors are not likely implicated in the effects of the \( \text{alpha}-2 \) adrenoceptor ligands reported in this study.

Based on molecular cloning and ligand binding studies, \( \text{alpha}-2 \) adrenoceptors have been subdivided into the subtypes \( \text{alpha}-2A \), \( \text{alpha}-2B \), and \( \text{alpha}-2C \) adrenoceptors (Bylund et al., 1992; Bylund, 1995). However, the \( \text{alpha}-2 \) 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 \( \text{alpha}-2 \) 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 \( \text{alpha}-2 \) adrenoceptor involved and the identity of the central dopaminergic 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 \( \text{alpha}-2 \) adrenoceptors, and the respective inhibitory and excitatory influence of \( \text{alpha}-2 \) 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 \( \text{alpha}-2 \) 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 \( \text{alpha}-2 \) 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 \( \text{alpha}-2 \) adrenoceptor an-
agonist effects in this model. In addition, it has been shown that the 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 α2-adrenoceptor compounds on dopamine neurotransmission may also result in part from transsynaptic effects on other neurotransmitters. Neurotransmitter interactions between noradrenergic, dopaminergic, glutamatergic, and cholinergic systems are involved in locomotor activity in rodents (Svensson et al., 1995). Several studies have revealed that α2-adrenoceptor agonists reduce, whereas α2-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 behavior in rats with unilateral 6-OHDA lesions of the nigrostriatal dopaminergic pathway (see Pycock, 1980; Gerlach and Riederer, 1996), they could conceivably represent targets of α2-adrenoceptor drug action. The effect of α2-adrenoceptor antagonists on other neurotransmitter systems in the circuitry are indeed not known at present and remain to be examined in future investigations.

In conclusion, these data may be relevant to Parkinson’s disease because in this disease, in addition to degeneration of the nigrostriatal dopaminergic pathway, the locus ceruleus and the noradrenergic pathways are implicated. Moreover, clinical trials indicate a beneficial effect of the direct dopamine agonist apomorphine on circling ability of Parkinson’s disease patients (Ruzicka et al., 1997; Peyro-Saint-Paul et al., 1997). The ability of α2-adrenoceptor antagonists to enhance the effect of the direct dopamine agonist apomorphine on circling behavior in rats indicates a facilitatory 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 α2-adrenoceptor antagonist idazoxan in Parkinson’s disease patients (Ruzicka et al., 1997; Peyro-Saint-Paul et al., 1997), the effects of α2-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 α2-adrenoceptor(s). Thus, the rank of order of efficacy (magnitudes of effect) of compounds to attenuate the antiparkinson activity.

References


Collingridge GL, James TA and MacLeod NK (1979) Neurochemical and electrophysiological evidence for a projection from the locus coeruleus to the substantia nigra. Nature 280:44.


Grant JA and Scrutton MC (1980) Interaction of selective α-adrenoceptor antagonists


Moldering GJ, Moura D, Fink K, Böni H and Göthert M (1993) Binding of α2-adrenoceptor antagonists and α2-adrenoceptors agonists in mice: Influence on locomotor, hypothermic and neurochemical effects of dexmedetomidine, a subtype- 


Taghioui K, Le Moal M and Simon H (1991) Suppression of noradrenergic inner- 


Tassin JP, Trovero F, Blanc G, Hervé D and Glowinski J (1994) Interactions between noradrenaline and dopamine neurotransmission in the rat prefrontal cortex and their consequences on dopaminergic subcortical function, in Noradrenergic Mechan- 

anisms in Parkinson’s Disease (Briley M and Marien M eds) pp107–125, CRC Press, Boca Raton.


Ungerstedt U (1971) Post-synaptic supersensitivity after 6-hydroxydopamine-in- 


Wakenberg RJS, Uhlén S and Chhablani V (1991) Medetomidine stereoisomers deline- 


Yavich L, Sirvio J, Haapalinna A and Riekkinen P (1994) Some unusual effects of 

α2-adrenergic drugs on cortical high voltage spindles in rats. Eur Neuropsycho- 


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