Dopamine receptors in the substantia nigra are involved in the regulation of muscle tone

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ABSTRACT The aim of the present study was to localize the dopamine receptors involved in the regulation of muscle tone. A strategy was used whereby the effects on muscle tone of injecting the irreversible dopamine receptor antagonist N-ethoxy carbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) in discrete brain regions were assessed. Increases in muscle tone were measured as changes in electromyographic activity of the gastrocnemius and tibialis muscles of conscious, unrestrained rats. No increases in muscle tone were found after injections of EEDQ into the anterior and posterior striatum, which produced marked reductions in dopamine receptor concentration. The effects on muscle tone of injecting EEDQ into the substantia nigra pars reticulata were also assessed. Large increases in muscle tone were observed associated with inactivation of either D1 or D2 dopamine receptors in the substantia nigra. The increased muscle tone was not reduced by subcutaneous administration of apomorphine, despite the presence of a normal population of striatal dopamine receptors. These findings provide evidence that dopamine receptors in the substantia nigra play an important role in the regulation of muscle tone. Further, they challenge the hypothesis that the muscle rigidity of Parkinson disease results primarily from loss of striatal dopamine receptor stimulation.

The association of the characteristic symptoms of Parkinson disease—bradykinesia, tremor, and rigidity—with degeneration of nigrostriatal neurons (1) has led to the hypothesis that the neurochemical mechanism underlying these symptoms is a loss of dopamine release in the striatum. The success of L-dihydroxyphenylalanine (l-dopa) in alleviating the motor symptoms of Parkinson disease is attributed to augmentation of dopamine stores and the maintenance of striatal dopamine receptor stimulation (2). Experimental findings have also shown increased muscle tone (muscle rigidity) associated with reductions in striatal dopamine after lesioning of nigrostriatal neurons by the neurotoxin 6-hydroxydopamine (3) or after treatment with reserpine (3, 4).

Although these findings have been interpreted as support for the striatal dopamine hypothesis, they have not taken into account that nigral dopaminergic neurons release dopamine from both their dendrites (5) and their terminals, so that dopamine concentration in the substantia nigra, as well as within the striatum, will be reduced in Parkinson disease and after reserpine or 6-hydroxydopamine treatment. The aim of the present study was to investigate the role of striatal and nigral dopamine receptors in the regulation of muscle tone. The irreversible dopamine receptor antagonist N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was injected into these regions, and the effects on muscle tone, measured as changes in the electromyographic (EMG) activity of the antago nistic muscles of the hind limb, were recorded. The area and extent of the resulting dopamine receptor inactivation were assessed using quantitative autoradiography so that the area(s) associated with changes in muscle tone could be identified.

METHODS

EMG Measurements. Male Sprague-Dawley rats were anesthetized (sodium pentobarbitone at 45 mg/kg and sodium methohexitone at 10 mg/kg, i.p.) and placed in a stereotaxic frame. A pair of stainless steel electrodes was implanted into the gastrocnemius and anterior tibialis muscles, and a fifth wire (earth) was laid on the surface of the tibialis muscle. The five wires were threaded under the skin and joined to a five-pin socket attached with dental cement to the surface of the skull. After recovery from surgery, the animal was connected via a headset containing an amplifier (6) to a Grass polygraph (model 7D). The EMG signal was amplified, filtered (10 Hz–10 kHz), rectified, and integrated over 10-sec periods; the resultant signal was recorded at 10 Hz for 20-min periods on a computerized recording system (CODAS; Dataq, Akron, OH). EMG is expressed as mean tonic EMG activity (mV/10 sec) (3). Phasic activity resulting from animal movement was excluded from analysis.

Intracerebral Injections of EEDQ. Bilateral intrastriatal injections of EEDQ (1 μmol in 1 μl; Sigma) dissolved in dimethyl sulfoxide (DMSO) were made in conscious animals via guide cannulae (7). Control rats were injected with 1 μl of DMSO. Bilateral intranigral injections of EEDQ were made into the substantia nigra pars reticulata (SNr) via 27-gauge stainless steel needles, at the same time the EMG electrodes were inserted. Rats were killed 10 or 24 hr after intracerebral injection, as described in the text. The coordinates of the three striatal injection sites were anterior sites: A, 1.0 mm; L, 2.5 mm; D, −5.0 and −7.4 mm; posterior site: P, −1.0 mm; L, 4.0 mm; V, −5.0 mm; and for the nigral site: P, −5.3 mm; L, 2.4 mm, V, −7.9 mm, according to the atlas of Paxinos and Watson (8). Selective protection of either D2 or D1 receptors was achieved by subcutaneous injections of raclopride (75 μmol/kg) or SCH 23390 (1.7 μmol/kg), respectively, given 1 hr before EEDQ, as described (9, 10).

Quantitative Autoradiography. After measurement of EMG activity, animals were killed by decapitation, the brains were removed, and sagittal sections (20 μm) were cut by a cryostat. Thaw-mounted sections were incubated with either the D1 receptor ligand [3H]SCH 23390 (3 nM) or the D2 ligand [3H]sulpiride (15 nM) for 1 hr at room temperature (7). Nonspecific binding was defined using 1 μM unlabeled SCH 23390 (D1) and 1.7 μM sulpiride (D2), respectively. In experiments involving intranigral EEDQ injections, sections were incubated with the D2 ligand [125I]iodosulpiride according to the method of Morelli et al. (11). Sections were exposed to tritium-sensitive film (Hyperfilm; Amersham) for 5 days.

Abbreviations: EEDQ, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; EMG, electromyographic; DMSO, dimethyl sulfoxide; GABA, γ-aminobutyric acid; 5-HT, 5-hydroxytryptamine.

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(\(^{[125]}\)iodosulpiride), 3 weeks (\(^{[3]}\)H)sulpiride), or 8 weeks (\(^{[3]}\)H)SCH 23390). Autoradiograms were analyzed using a computerized densitometry system (MD20; Flinders Imaging, Adelaide, South Australia), and optical density values of selected brain regions were converted to fmol/mg of tissue by reference to tritium and \(^{125}\)I standards (Amersham). Receptor concentration was calculated from the average optical density of two or three sections from each rat.

**RESULTS**

**Inactivation of Central Dopamine Receptors.** Initial experiments demonstrated that an intraperitoneal injection of EEDQ (60 \(\mu\)mol/kg), a dose previously shown to produce marked reduction in dopamine receptor concentration throughout the brain (9, 10), was associated with significant increases in EMG activity of both gastrocnemius and tibialis muscles. These increases occurred 2 hr after injection, reached a maximum of 287% in gastrocnemius and 284% in tibialis of vehicle-injected control values, and persisted for 48 hr. After EEDQ injection, striatal D\(_1\) and D\(_2\) dopamine receptor concentrations were reduced to 12.1% \(\pm\) 3.5% and 11.8% \(\pm\) 1.9% of control values, respectively. The findings established that inactivation of central dopamine receptors was associated with increased muscle rigidity, and experiments were carried out next to localize the site of the dopamine receptors involved.

**Inactivation of Striatal Dopamine Receptors.** EEDQ or DMSO was injected bilaterally into one of the three striatal sites described in Methods, and EMG activity was recorded for 8–24 hr postinjection. No increases in EMG activity were observed, despite quantitative autoradiographic confirmation of significant dopamine receptor loss at all injection sites (anterior sites: D\(_1\), 19.2% \(\pm\) 2.4%; D\(_2\), 23.4% \(\pm\) 3.6%; posterior site: D\(_1\), 21.8% \(\pm\) 1.4%; D\(_2\), 29.7% \(\pm\) 5.2% compared with vehicle-injected controls). Fig. 1 shows the extent of D\(_1\) receptor loss 10 hr after EEDQ injection into each of the three sites, compared with a DMSO-injected control.

**Inactivation of Nigral Dopamine Receptors.** To investigate the hypothesis that nigral dopamine receptors were involved in the regulation of muscle tone, bilateral injections of EEDQ were made into the substantia pars reticulata. Ten hours after injection there was a significant increase in EMG activity in the gastrocnemius (218%) and anterior tibialis (138%) muscles compared with vehicle-treated controls (Table 1). This effect persisted for at least 24 hr. Behaviorally, the animals were akinetic; they exhibited a hunched posture and increases in muscle tone (rigidity) determined by limb palpation. These changes were associated with reductions in D\(_1\) and D\(_2\) receptor concentrations, averaged for the whole of the substantia nigra, to 14.6% \(\pm\) 2.3% and 24.3% \(\pm\) 6.4%, respectively, of vehicle-injected controls, assessed 10 hr after injection of EEDQ.

To determine if receptor inactivation along the needle track contributed to increases in EMG activity, experiments were conducted in which EEDQ was injected at sites dorsal to the nigra (coordinates: P, –5.3 mm; L, 2.4 mm; V, –6.2 mm). These injections had no effect on EMG activity, which remained at control levels.

EEDQ inactivates both D\(_1\) and D\(_2\) receptors (12, 13), so it is not possible to conclude if one or both receptor subtypes are

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**Fig. 1.** Representative autoradiographs of D\(_1\) receptor binding in sagittal brain sections after intrastriatal injections of EEDQ or DMSO. D\(_1\) receptor distribution in striatum and substantia nigra of a DMSO-injected animal (A) and after intrastriatal injection of EEDQ into the dorsoanterior (B), ventroanterior (C), or lateroposterior (D) striatum is shown. Injection coordinates of the three sites were anterior sites: A, 1.0 mm; L, 2.5 mm; D, –5.0 or –7.4 mm; posterior site: P, –1.0 mm; L, 4.0 mm; V, –5.0 mm.
Table 1. EMG activity in anterior tibialis and gastrocnemius muscles

<table>
<thead>
<tr>
<th>Group*</th>
<th>Injection</th>
<th>n</th>
<th>Tibialis</th>
<th>Gastrocnemius</th>
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<tbody>
<tr>
<td>A</td>
<td>Vehicle</td>
<td>8</td>
<td>0.282 ± 0.032</td>
<td>0.207 ± 0.027</td>
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<td></td>
<td>EEDQ</td>
<td>8</td>
<td>0.388 ± 0.047</td>
<td>0.452 ± 0.027†</td>
</tr>
<tr>
<td></td>
<td>EEDQ + 1 μmol</td>
<td>6</td>
<td>0.275 ± 0.059†</td>
<td>0.319 ± 0.059</td>
</tr>
<tr>
<td></td>
<td>Apo</td>
<td>6</td>
<td>0.252 ± 0.072</td>
<td>0.313 ± 0.115</td>
</tr>
<tr>
<td>B</td>
<td>Vehicle</td>
<td>7</td>
<td>0.277 ± 0.037</td>
<td>0.200 ± 0.045</td>
</tr>
<tr>
<td></td>
<td>EEDQ + SCH</td>
<td>7</td>
<td>0.456 ± 0.041†</td>
<td>0.388 ± 0.054†</td>
</tr>
<tr>
<td></td>
<td>EEDQ + SCH + 1 μmol Apo</td>
<td>7</td>
<td>0.400 ± 0.051†</td>
<td>0.374 ± 0.057†</td>
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<tr>
<td></td>
<td>EEDQ + SCH + 10 μmol Apo</td>
<td>7</td>
<td>0.498 ± 0.039†</td>
<td>0.490 ± 0.043†</td>
</tr>
<tr>
<td>C</td>
<td>Vehicle</td>
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<td>0.225 ± 0.063</td>
<td>0.261 ± 0.053</td>
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<tr>
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<td>0.467 ± 0.039†</td>
<td>0.517 ± 0.034†</td>
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<tr>
<td></td>
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<td>0.471 ± 0.045†</td>
<td>0.423 ± 0.080</td>
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<tr>
<td></td>
<td>EEDQ + RAC + 10 μmol Apo</td>
<td>5</td>
<td>0.470 ± 0.042†</td>
<td>0.453 ± 0.049†</td>
</tr>
</tbody>
</table>

Apo, apomorphine; SCH, SCH 23390; RAC, raclopride.
*Group A, EMG activity 10 hr after intranigral injection of EEDQ; group B, EMG activity 24 hr after intranigral injection of EEDQ and pretreatment with SCH 23390; group C, EMG activity 24 hr after intranigral injection of EEDQ and pretreatment with raclopride.
†P < 0.01 compared with vehicle-injected control.
‡P < 0.05 compared with EMG activity prior to apomorphine injection, by Bonferroni’s t statistic.

involved in the regulation of muscle tone. To identify the subtype, rats were injected with raclopride or SCH 23390 1 hr prior to intranigral injection of EEDQ to prevent inactivation of D2 or D1 receptors, respectively (9, 10, 12). After pretreatment with SCH 23390 and 24 hr after intranigral injection of EEDQ, there were significant increases in EMG activity (Fig. 2) associated with a selective reduction in D2 receptor binding of 43% compared with controls. D1 receptors were maintained at control levels by SCH 23390 pretreatment. Significant increases in EMG activity were also observed 24 hr after EEDQ injection, following raclopride pretreatment. These increases were associated with a selective 40% loss of D1 receptors in the absence of changes in D2 receptor concentration (Fig. 2). In both these experiments, quantitative autoradiography of nigral D1 and D2 dopamine receptors confirmed that increased EMG activity was associated with a large area of receptor loss (Fig. 3).

To investigate if EMG increases were due to nonspecific effects of EEDQ or effects at other receptors, rats were injected with both raclopride and SCH 23390 prior to nigral EEDQ injection to protect both dopamine receptor subtypes (Fig. 2). Twenty-four hours after this treatment, no changes in EMG activity were observed and the animals were behaviorally indistinguishable from vehicle-injected controls. The effectiveness of the antagonist pretreatments was confirmed by quantitative autoradiographic analysis, which showed that nigral D1 and D2 receptor concentrations were not changed from control levels (D1, 92.7% ± 6.8%; D2, 93.9% ± 17.3% of vehicle-injected control values). These results clearly implicate inactivation of nigral dopamine receptors in the regulation of EMG activity.

Effects of Apomorphine on Increased EMG Activity. In most experiments, administration of the mixed D1/D2 dopamine agonist apomorphine (1 and 10 μmol/kg, s.c.) did not reduce the increased EMG activity associated with reductions in nigral dopamine receptor concentration (Table 1). For example, after selective inactivation of nigral D2 (Table 1, group B) or D1 (Table 1, group C) receptors, the significantly increased EMG activity was not reduced by either dose of apomorphine. Quantitative autoradiographic analysis confirmed that striatal dopamine receptors were unaffected by the experimental procedures (see Fig. 3). However, in the group receiving intranigral injection of EEDQ alone (Table 1, group A), increased EMG activity was reduced by apomorphine exclusively in the tibialis. Inspection of the individual data for the EEDQ group showed that apomorphine reduced EMG activity in three of six rats and that they had less loss of nigral dopamine receptors than the three nonresponding rats.

**DISCUSSION**

The results of the present study are consistent with the hypothesis that D1 and D2 dopamine receptors in the substantia nigra play an important role in the regulation of muscle tone. Inactivation of these receptors by EEDQ consistently resulted in an akinetic state and a large increase in EMG activity in both gastrocnemius and anterior tibialis muscles. No increases in
EMG activity were observed after dopamine receptor loss in the striatum. Doses of apomorphine of up to 10 \( \mu \text{mol/kg} \) failed to reduce the EMG increases associated with large losses of nigral dopamine receptors, despite the demonstration that striatal dopamine receptors were not reduced. The failure of apomorphine to reduce EMG activity is interesting and may result from its interaction with the small remaining population of nigral dopamine receptors being insufficient to reverse the functional effects after EEDQ injection. This hypothesis is supported by the finding that in one experiment (Table 1, group A) apomorphine did reduce EMG activity in three of six rats after intranigral injection of EEDQ. The magnitude of their response to apomorphine appeared to be related to the size of the population of dopamine receptors remaining in the substantia nigra. We have previously shown apomorphine to be effective in reducing increased EMG activity associated with dopamine loss after 6-hydroxydopamine lesions, when the population of dopamine receptors was intact (3). Overall, the findings support an important role for nigral dopamine receptors in muscle tone regulation and suggest that the magnitude of nigral dopamine receptor stimulation is an important determinant of EMG activity.

Besides dopamine receptors, EEDQ also inactivates \( \alpha \)-adrenoceptors (14), 5-hydroxytryptamine (5-HT) receptors (12), and muscarinic receptors (15), so it was possible that the increases in EMG activity were attributable to inactivation of these nondopamine receptors or to nonspecific effects. This possibility was investigated by pretreating rats with both raclopride and SCH 23390 prior to intranigral injections of EEDQ to achieve selective protection of D2 and D1 receptors. No increase in EMG activity was seen in the pretreated rats at times when increased EMG activity was consistently observed in nonpretreated rats (Fig. 2). This finding clearly implicates dopamine receptor inactivation in EMG increases and confirms that inactivation of nondopamine receptors in the substantia nigra has no effect on EMG activity. Although high doses of SCH 23390 have been reported to protect 5-HT\(_{2c}\) receptors partially from inactivation by EEDQ (12), the low dose used in the present study (1.7 \( \mu \text{mol/kg} \)) selectively protected D1 receptors because of the 10-fold greater affinity of SCH 23390 \emph{in vivo} for D1 compared with 5-HT\(_{2c}\) receptors (16). Further, subsequent studies showed that protection of 5-HT\(_{2c}\) receptors by pretreatment with mesulergine did not prevent increases in EMG activity after intranigral injection of EEDQ (A.D.C., unpublished observations). Overall, these findings exclude a role for 5-HT\(_{2c}\) receptors and support the conclusion that nigral dopamine receptors are involved in the regulation of muscle tone.

The injection volume used in our study was large (1 \( \mu \text{l} \)), and leakage from the injection site could have resulted in dopamine receptor inactivation in dorsal areas surrounding the injection track, including the superior colliculus, a major projection area of the nigra involved in motor control. This possibility was ruled out by observations that EEDQ injections dorsal to the nigra did not affect EMG activity.

The substantia nigra provides both inputs to the striatum, via dopaminergic neurons originating in the pars compacta, and receives outputs from the striatum to the pars reticulata. These include a direct inhibitory pathway, mediated by D1 receptors, and an indirect pathway, mediated by D2 receptors, via the subthalamic nucleus (reviewed in ref. 17). It has been shown in monkeys rendered parkinsonian by treatment with the dopamine neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine that imbalance of these nigral inputs results in akinesia and increased muscle tone (18). These effects were associated with increases in the activity of nigrothalamic ef-
ferents and increased inhibition of the thalamus andthalamo
cortical neurons.

Our results suggest that dopamine receptor stimulation in
the nigra plays an important role in the regulation of motor
control. Dopamine is released from the dendrites of dopa
minergic neurons (5) and therefore can regulate the activity of
dopaminceptive neurons in the substantia nigra. Autoradi-
ographic and immunohistochemical studies have shown sub-
stantial concentrations of D1 and D2 receptors in the nigra (19,
20). D2 receptors are found in the pars reticulata and the pars
compacta of the substantia nigra, where they are located on
the cell bodies (19) and the dendrites (20) of dopaminergic
neurons and nondopaminergic neurons (20). Their role as
autoreceptors is well documented (21), but they also have
effects on nondopaminergic neurons, inhibiting γ-aminobutyric
typtic acid (GABA) release (22) and regulating the rate of firing
efferent nigral GABAergic neurons (23). D1 receptors are located on
the terminals of striatonigral GABAergic neurons, and their activation increases GABA release from these neurons
(24). Increases in EMG activity were observed after selective inactivation of either dopamine receptor subtype alone, indicat-
ing that both receptor subtypes are involved in the regula-
tion of muscle tone, as shown for many other behavioral and
electrophysiological effects mediated by dopamine receptors
(reviewed in ref. 25). We hypothesize that a decrease in either
nigral receptor subtype leads to increased firing of GABAergic
nigrothalamic neurons, which has been associated with in-
creases in muscle tone (18). The hypothesis that both D1 and
D2 receptors are involved in muscle tone regulation is con-
sistent with clinical reports that the D2 agonist bromocriptine
is more effective in controlling the symptoms of Parkinson dis-
ease, if coadministered with L-dopa (26).

The findings of the current study challenge the view that
dopamine agonists reduce increased muscle tone by interact-
ing only with striatal dopamine receptors, which is widely held
to be the basis of L-dopa’s therapeutic effects in the treatment of Parkinson disease. The hypothesis that nigral dopamine
receptors play an important role in the regulation of muscle
tone is compatible with existing knowledge concerning the
neurochemistry and treatment of Parkinson disease. For ex-
ample, it is well known that there is a marked decrease in nigral, as well as striatal, dopamine concentration in Parkinson
disease (27).

The success of L-dopa therapy, therefore, may be primarily
dependent on replacement of dopamine at nigral sites. This
view is consistent with the findings of experimental studies
showing that the behavioral effects of L-dopa correlated better
with nigral rather than striatal concentrations of dopamine
and were blocked by intranigral administration of SCH 23390
(28, 29).

The failure of dopamine receptor inactivation in large areas
of the striatum to result in increased muscle tone raises impor-
tant questions about the relative roles of nigral and striatal
dopamine receptors in motor control. However, our findings
do not rule out a role for striatal dopamine receptors in the
regulation of muscle tone because the receptor reserve in the
striatum may be larger than the reductions in D1 and D2
receptor concentration of 75–80% achieved in the present
study.

In conclusion, the hypothesis that nigral dopamine receptors
play a key role in the regulation of muscle tone has important
implications for understanding the neurochemical changes
underlying the symptoms of Parkinson disease and the rational
development of new drugs to treat it. Significantly, the nigral
hypothesis may explain the limited success of transplantation
into the striatum for the treatment of this disabling condition
(30).

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