0026-895X/89/010125-07$02.00/0

MOLECULAR PHARMACOLOGY, 34:125-131

Differences in Dopamine Receptor Reserve for *N-n*-Propylnorapomorphine Enantiomers: Single Unit Recording Studies after Partial Inactivation of Receptors By *N*-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline

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Received May 31, 1988; Accepted September 13, 1988

SUMMARY

Previous studies with (*S*)-(+)-*N-n*-propylnorapomorphine (NPA), an enantiomer of the potent dopaminergic agonist (*R*)-(−)-NPA, showed that this compound had a complex pharmacological profile. For example, (*S*)-(+)NPA had weak dopaminergic agonist potency, in that it could slow and ultimately stop firing of substantia nigra and ventral tegmental area dopamine neurons. However, antagonist properties were also evident inasmuch as immediate pretreatment with a low dose of (*S*)-(+)-NPA caused a significant rightward shift of the (*R*)-(−)-NPA dose-response curve. This dual agonist-antagonist nature of (*S*)-(+)-NPA suggested a low intrinsic efficacy for (*S*)-(+)-NPA. To test this hypothesis, we conducted dose-response studies for the inhibition of rat substantia nigra dopamine cell firing by (*R*)-(−)- and (*S*)-(+)-NPA 1 day after partial irreversible dopamine receptor inactivation with 6 mg/kg *N*-ethoxycarbonyl-2-ethoxy-1-2-dihydroquinoline (EEDQ) in an ethanol vehicle. This degree of inactivation resulted in a significant, parallel, rightward shift of the (*R*)-(−)-NPA dose-response curve relative to ethanol-treated control rats, with no loss of maximal response. After pretreatment with the same dose of EEDQ, however, the maximal response to (*S*)-(+)-NPA was reduced by 22% with no shift on the dose axis. The dose-response curves for control and EEDQ-treated groups were subjected to Furchgott analysis to determine per cent response versus fractional occupancy relationships for each drug. A steep hyperbolic relationship was revealed for (*R*)-(−)-NPA. Fifty per cent and maximal (>95%) inhibitions of cell firing occurred at 3.5% and approximately 32% receptor occupancies, respectively. Hence, for (*R*)-(−)-NPA there is about a 68% receptor reserve in this model. The same analysis for (*S*)-(+)-NPA yielded an occupancy versus response plot that was more shallow and linear. Half-maximal effects occurred at 66% occupancy and the maximal response (96% inhibition) required 94% occupancy, indicating few spare receptors for (*S*)-(+)-NPA. Because the ratio of fractional occupancies at a given level of response is a measure of relative efficacy, we calculated a relative efficacy of (*S*)-(+)- to (*R*)-(−)-NPA of 0.05 (at the 50% response level). This confirms that (*S*)-(+)-NPA indeed has a much lower intrinsic efficacy than the (*R*)-(−)-antipode and may account for the previously reported antagonist property of the (+)-form under certain treatment conditions.

In 1956, Nickerson (1) and Stephenson (2) independently challenged the traditional assumptions of theories concerning the relationship between receptor occupancy and tissue response. Before their introductions of the concept of spare receptors, it was implicitly held that this relationship was linear and that per cent response was equal to the percentage of receptors bound by an agonist. Receptor reserve theory now holds that the per cent response is not necessarily equivalent to the fraction of receptors occupied. Indeed, for many ligand-receptor systems, only a small proportion of receptors needs to be occupied by an agonist to elicit a full response. The amount of receptor occupancy required to elicit a given response, in turn, was determined to be a function of the intrinsic efficacy of the compound (2).

Only recently have investigators begun to appreciate the contribution of spare receptors to agonist actions in the nigrostriatal dopamine neuronal system (3–5). The firing rates of substantia nigra pars compacta dopamine neurons are endogenously regulated by the direct actions of dopamine at autoreceptors (6) and by the activity of a striatonigral "feedback loop," which is thought to be GABAergic in nature (7). Low doses of systemically administered or microiontophoretically applied dopaminergic agonists are believed to inhibit substantia nigra dopamine neuron firing by direct effects on somatodendritic dopamine autoreceptors (6). Clearly, the size of the autoreceptor pool will be important in determining the levels

This work was supported by National Institutes of Health Grant NS 23541.

ABBREVIATIONS: GABA, γ-aminobutyric acid; EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; NPA,*N-n*-propylnorapomorphine; GBL, γ-butyrolactone.

of response these cells make to dopamine agonists with differing intrinsic efficacies.

Receptor reserve analysis of central nervous system catecholamine receptors has been facilitated by use of the irreversible receptor inactivator EEDQ (8–10). *In vivo* administration of EEDQ has allowed quantification of cerebral cortex α_2-adrenergic (10) and nigrostriatal terminal dopamine autoreceptor reserves (3). More recently, the presence of somatodendritic autoreceptor reserve on substantia nigra dopamine cells was suggested by *in vivo* extracellular electrophysiologic studies after EEDQ administration (5). In the present study, we used *in vivo* systemic administration of EEDQ to inactivate central dopamine receptors. For both EEDQ and vehicle-treated rats, we then monitored the activity of nigral dopamine neurons during the administration of the potent dopaminergic agonist (*R*)-(−)-NPA or its less potent enantiomer (*S*)-(+)NPA. (*S*)-(+)NPA was of particular interest because it had been shown previously to exhibit both antagonist properties in behavioral studies (11) and weak agonist properties, by slowing dopamine cell firing after intravenous administration (12, 13). Consequently, the receptor reserves for those receptors that regulate dopamine cell responses to agonist administration were calculated for both NPA enantiomers by applying the analytical methods of Furchgott and Burstyn (14) and Minneman and Abel (15), as modified by Meller *et al.* (3) for *in vivo* experiments. In addition, we used this methodology to compute the relative efficacy of (*S*)-(+)NPA versus the more potent enantiomer (*R*)-(−)-NPA. Our results indicate that the former compound has a much lower intrinsic efficacy than the later drug. We conclude, therefore, that virtually all receptors must be occupied for (*S*)-(+)-NPA to elicit a full response, whereas occupancy of only a small fraction of available receptors is required for (*R*)-(−)-NPA to yield maximal inhibition of cell firing.

Methods

Extracellular single unit recording techniques. Twenty four hours before each experimental session, male Sprague-Dawley rats (Charles River, Wilmington, MA), initial weights between 250 and 380 g, were pretreated with either 6 mg/kg EEDQ (2 mg/ml, intraperitoneally) in a vehicle of ethanol and water (1:1) or an equivalent volume (3 ml/kg) of the vehicle. On the following day, animals were anesthetized with chloral hydrate (400 mg/kg, intraperitoneally) and restrained in a stereotaxic apparatus. Extracellular, single-unit recordings of dopamine neurons in the substantia nigra pars compacta were carried out using standard methods as previously described (13, 16). Dopamine neurons were tentatively identified by established electrophysiological criteria (6, 13, 16), and the recording site was later verified by iontophoresis of blue dye from the electrode, followed by histologic examination of brain sections to determine location of the blue spot.

Extracellular action potentials were amplified, filtered, and displayed on an oscilloscope screen. Impulses were counted over 10-sec intervals and the accumulated sums were simultaneously printed by a strip printer and graphed as a rate histogram by a pen recorder. After a stable 3- to 5-min baseline rate was recorded, doses of (*R*)-(−)-NPA or (*S*)-(+)-NPA were injected into a lateral tail vein at 1-min intervals so that each successive dose doubled the previous cumulative dose. Only one cell was recorded from each animal to avoid residual drug effects.

Dose-response analysis. Dose-response relationships for (*R*)-(−)- and (*S*)-(+)-NPA-induced inhibitions of nigral dopamine cell firing were determined for EEDQ- and vehicle-treated animals. The mean firing rate for the 60-sec period after each drug dose was compared with the mean rate during the initial 5-min baseline period for that cell. Response, expressed as a percentage of baseline rate, was plotted as a function of the log dose. The resulting curves were analyzed using the simultaneous curve-fitting computer program ALLFIT (17), as modified for the Apple II computer by Teicher (MED-65, *ALLFIT*, Biomedical Computing Technology Information Center, Nashville, TN, 1983).

Receptor reserve calculation and analysis. The receptor reserve for those receptors mediating intravenous agonist effects on dopamine neuron firing was calculated by the method of Furchgott and Burstyn (14) as recently adapted for systemic drug administration by Meller *et al.* (3). The concentrations of agonist required to yield specific levels of response are compared before and after irreversible receptor inactivation, as related by the formula:

$$\frac{1}{[A]} = \frac{1}{q} \cdot \frac{1}{[A']} + \frac{1-q}{q\,K_A}$$

where $[A]$ is the concentration (dose, in this case) of agonist needed to produce a given response, $[A']$ is the dose that produces the same degree of effect after partial receptor inactivation, q represents the fraction of receptors not inactivated by the pretreatment, and K_A represents the equilibrium dissociation constant for the agonist. To determine values of q and K_A, we constructed a plot of $1/[A]$ versus $1/[A']$ for equieffective doses at five levels of response (30, 40, 50, 60, and 70% of the control maximum response). Linear regression analysis of these points yields a straight line with a slope of $1/q$ and a y intercept equal to $(1-q)/q\,K_A$. Rearranging the latter term gives a K_A equal to (slope−1/y intercept). Under the *in vivo* conditions of our studies, equilibrium was not attained, therefore a "pseudo-K_A" was determined by this method, as was used by Meller *et al.* (3) in similar *in vivo* studies of agonist efficacies and receptor reserve in the dopamine system. It is acknowledged that this pseudo-K_A value is not a constant as are K_A values derived from *in vitro* equilibrium ligand-receptor binding studies. Nevertheless, we concur with Meller that this pseudo-K_A can be used as a valid parameter in calculations of occupancy-response relationships (see below).

In addition to the Furchgott method of analysis, we also used the approach of Minneman and Abel (15) to estimate a value for q when our EEDQ pretreatment did not reduce the maximal response to the agonist. Under these conditions, the ratio of a pair of equieffective doses (such as ED_{50} values) for groups treated with vehicle and the inactivator provides a useful estimate of q.

Finally, fractional receptor occupancy (f) was determined at various agonist doses by substituting the K_A value derived above into the following equation (from the law of mass action):

$$f = \frac{RA}{RT} = \frac{[A]}{[A] + K_A}$$

where $[RA]$ is the concentration of receptor bound by agonist and $[RT]$ is the total concentration of receptors available to interact with agonist (14). The fractional occupancy at a given dose was then plotted against the percentage of maximal response obtained at that dose in the dose-response curve. Receptor reserve was obtained by subtraction of the per cent receptor occupancy at maximal response from 100.

Materials. (*R*)-(−)-NPA and (*S*)-(+)NPA (Research Biochemicals Inc., Natick, MA) were dissolved in distilled water. EEDQ (Aldrich Chemical Co., Milwaukee, WI) was dissolved in 100% ethanol and diluted with distilled water so that the final ethanol/water ratio was 1:1.

Results

Pretreatment with EEDQ produced obvious behavioral changes. As previously reported (9), most of the animals became cataleptic. Although we did not attempt to quantify this effect in a systematic way, variability was evident and symptoms ranged from mild rigidity to profound immobility. EEDQ treat-

ment did not affect the baseline firing rates of nigral dopamine neurons, inasmuch as there was no significant difference among the experimental and control groups (Table 1).

(*R*)-(−)NPA. Fig. 1 displays representative recordings for the inhibition of substantia nigra dopamine cell firing by (*R*)-(−)-NPA for both vehicle- and EEDQ-pretreated rats. Pretreatment with EEDQ (6 mg/kg, intraperitoneally) caused a significant rightward shift of the (*R*)-(−)-NPA dose-response curve without a reduction in the maximum attainable response, i.e., 100% inhibition of cell firing (Fig. 2). ED_{50} values for the two curves, calculated by ALLFIT, were 0.27 ± 0.02 and 0.79 ± 0.04 μg/kg for vehicle and EEDQ-treated groups, respectively, representing a 3-fold shift in the position of the EEDQ curve on the dose axis ($P < 0.05$). ALLFIT analysis revealed no change in the slopes of the two curves.

The fraction of active receptors, q, remaining after irreversible inactivation was calculated both by the method of Minneman and Abel (15), in which q was estimated by the equation $q = [A]/[A']$, and by the method of Furchgott and Burstyn (14), in which q is the reciprocal of the slope of the plot of $1/[A]$ versus $1/[A']$. By the Minneman and Abel technique, q was determined to be 0.35 (i.e., 35% of active receptors remaining; based on a ratio of ED_{50} doses). In good agreement with the above, a value of 0.37 was obtained when q was determined by the method of Furchgott and Burstyn. We conclude, therefore, that the 6 mg/kg dose of EEDQ inactivated approximately 65% of the receptor population involved in the inhibition of dopamine cell firing by (*R*)-(−)-NPA. Conversely, it follows that a maximal inhibitory response could still be obtained with only 35% of the total receptors intact, suggesting at least a 65% receptor reserve for (*R*)-(−)-NPA.

Furchgott analysis also provided a pseudo-K_A value for (*R*)-(−)-NPA equal to 7.7 μg/kg. This K_A value was used in subsequent calculations of fractional occupancies at various levels of response. Fractional occupancy values (i.e. $[A]/[A] + K_A$), calculated for each of the doses in the control dose-response series, were plotted against the percentage of maximal response attained at that dose. This occupancy versus response plot was steep and hyperbolic (Fig. 3), again suggesting a substantial receptor reserve for the potent agonist (*R*)-(−)-NPA. A level of 50% of maximum response was found to occur at only 3.5% receptor occupancy, and maximal levels of response (>95% inhibition) were obtained when only 32% of the receptors were bound by the agonist, based upon extrapolation from the occupancy-response curve. By extension, then, and in agreement

TABLE 1

Baseline firing rates of substantia nigra pars compacta dopamine neurons

Rates are expressed as spikes per 10 sec, ± standard error. There were no significant differences among the groups (ANOVA, $p = 0.902$).

	(R)-(−)-NPA	(S)-(+)-NPA
	spikes/10 sec	
Control	42.5 ± 8.4	40.0 ± 4.9
EEDQ-treated	39.1 ± 6.5	36.6 ± 4.3

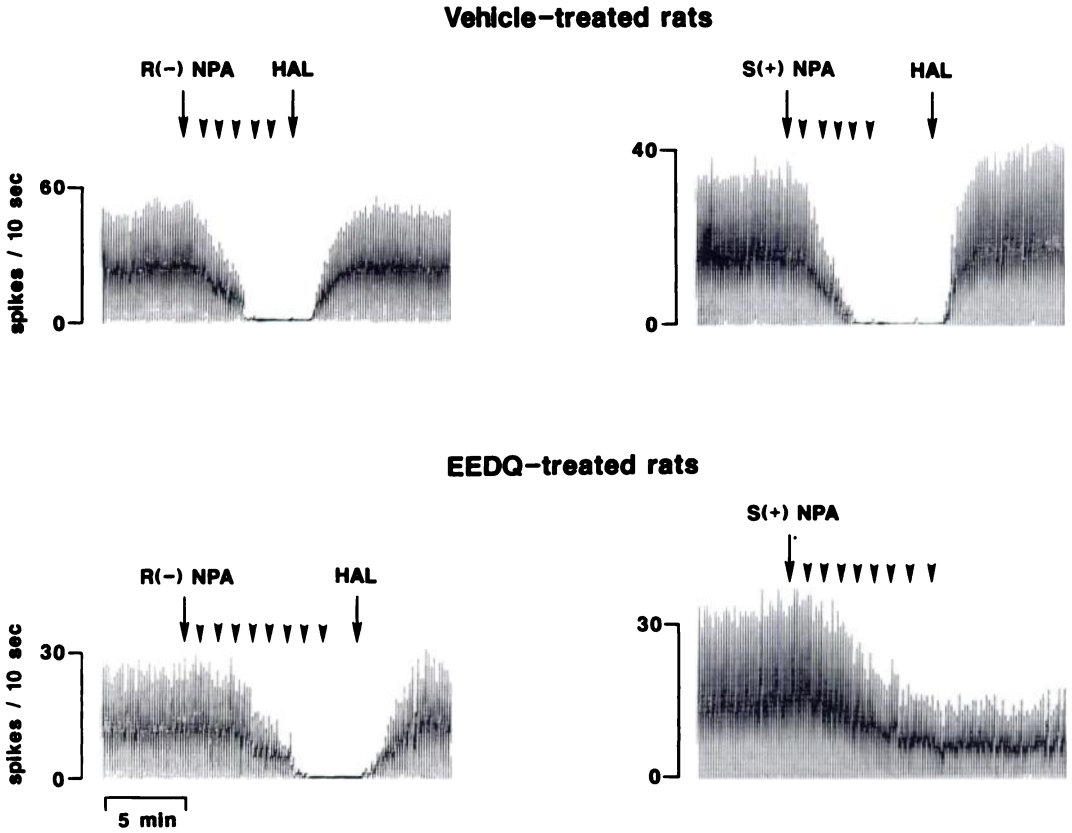

Fig. 1. Rate histogram plots of the inhibition of substantia nigra pars compacta dopamine cell firing by intravenous administration of increasing doses of (*R*)-(−)-NPA and (*S*)-(+)-NPA to rats pretreated with either an ethanol vehicle (*upper panel*) or 6 mg/kg EEDQ (*lower panel*). NPA enantiomers were given at 1-min intervals such that each dose doubled the previous cumulative dose. For (*R*)-(−)-NPA, doses were 0.039, 0.078, 0.156 μg/kg, etc., to a possible cumulative dose of 10 μg/kg; for (*S*)-(+)-NPA, doses were 10, 20, 40 μg/kg, etc., to a possible cumulative dose of 2560 μg/kg. In three of the four cases, haloperidol (*HAL*, 0.2 mg/kg) was administered to reverse the inhibitory effect of the agonist.

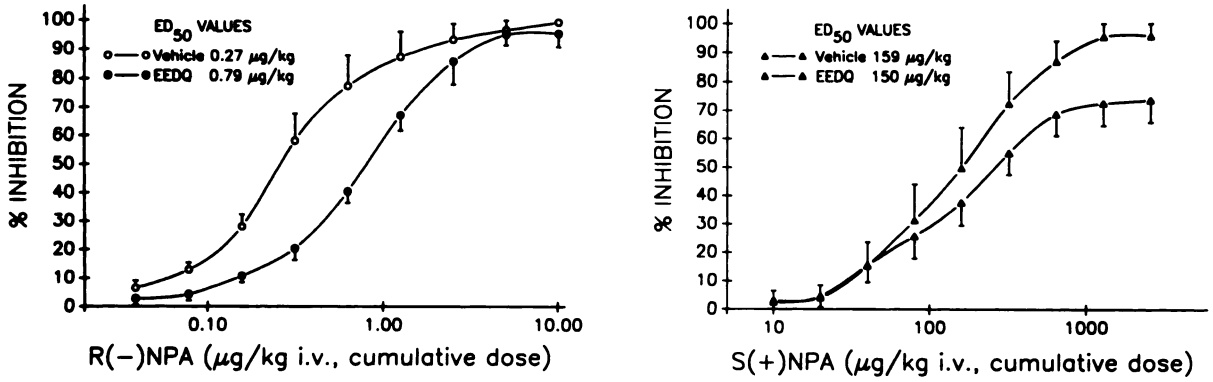

Fig. 2. Cumulative log dose-response curves for inhibition of substantia nigra pars compacta dopamine cell firing by NPA enantiomers (see legend to Fig. 1 for doses) in vehicle and EEDQ-pretreated rats. *Left*, effects of (*R*)-(−)-NPA in animals given vehicle or EEDQ (6 mg/kg) treatment 1 day before electrophysiological studies. ED_{50} values were significantly different ($p < 0.05$), but maximal responses were equivalent. *Right*, effects of (*S*)-(+)-NPA in similarly treated groups of rats. The maximal response in EEDQ-treated animals was reduced to 74 ± 8% inhibition, whereas in the vehicle-treated rats, a full (96 ± 4%) inhibitory response was attained. ED_{50} values for (*S*)-(+)-NPA were nearly identical in both control and EEDQ-treated groups. Each *point* represents the mean ± standard error of the mean for 5 or 6 cells for (*R*)-(−)-NPA, and for 6 to 11 cells for (*S*)-(+)-NPA.

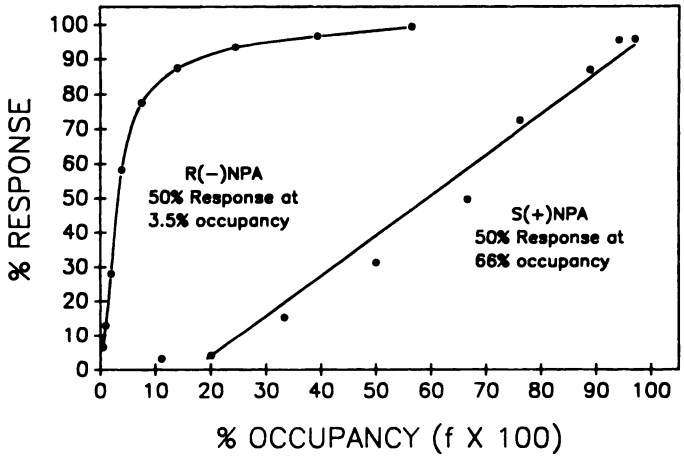

Fig. 3. Occupancy-response relationships for (*R*)-(−)-NPA and (*S*)-(+)-NPA determined by the formula $f = [A]/K_A+[A]$. Pseudo-K_A values for (*R*)-(−)-NPA (7.7 μg/kg) and (*S*)-(+)-NPA (80 μg/kg) were derived from plots of $1/[A]$ versus $1/[A']$ for each enantiomer. The response (per cent inhibition) attained at each dose in the dose-response series is plotted against the calculated per cent receptor occupancy ($f \times 100$). Estimated receptor occupancies at the ED_{50} values for each drug are indicated.

with the above estimate, an approximately 68% receptor reserve exists for (*R*)-(−)-NPA in this model.

(*S*)-(+)-NPA. Log dose-response curves for inhibition of dopamine cell firing by (*S*)-(+)-NPA were again constructed for both vehicle- and EEDQ-pretreated animals, as shown in Figs. 1 and 2. In vehicle-treated rats, dopamine cell firing was nearly but not always completely (95.7 ± 4.3%) suppressed by a cumulative dose of 2560 μg/kg (*S*)-(+)-NPA. The ALLFIT-derived ED_{50} was 159 ± 13 μg/kg. However, in EEDQ-pretreated animals, the maximal attainable response was reduced by 22% (i.e., to 26% of the baseline firing rate, or 74% inhibition). Furthermore, there was no change in the ED_{50} value (150 ± 14 μg/kg) when calculated as a function of the maximal attainable response for the EEDQ-treated group. The slopes, ED_{50} values, and minimum responses (inhibition of firing at the lowest dose) could be constrained by ALLFIT analysis to share common values for the control and EEDQ pretreatment dose-response curves with no significant loss of goodness of fit. The *q* value calculated by Furchgott analysis of the (*S*)-(+)-NPA dose-responses curves from control and EEDQ-treated rats was 0.86. This suggests that only about 14% of the receptors initially available to and stimulated by (*S*)-(+)-NPA were inactivated by EEDQ. This value contrasts with that reported above for (*R*)-(−)-NPA, for which it appeared that EEDQ inactivated about 65% of available receptors. Thus, although it appears that a smaller proportion of receptors for (*S*)-(+)-NPA were inactivated relative to that determined for the (*R*)-(−)-form, this smaller proportional loss of receptors resulted in a reduction in the maximal response attainable for (*S*)-(+)-NPA, which was not observed for (*R*)-(−)-NPA. (*S*)-(+)-NPA, therefore, appears to have nearly full intrinsic activity under control conditions, in that a 96% suppression of firing was attainable in vehicle-treated animals. The low intrinsic efficacy of (*S*)-(+)-NPA, on the other hand, is revealed when the size of the receptor pool is reduced by irreversible inactivation with EEDQ.

Furchgott analysis of the data derived from (*S*)-(+)-NPA dose-response curves yielded a pseudo-K_A equal to 80 μg/kg. Based upon this value for K_A, the occupancy versus response relationship for (*S*)-(+)-NPA was determined (as above), and this plot is displayed in Fig. 3. In contrast to the steep, hyperbolic curve generated for (*R*)-(−)-NPA, (*S*)-(+)-NPA exhibits a shallow and more linear relationship ($r = 0.989$) with 50% response at 66% occupancy and the maximal observed response (i.e., 96% inhibition) occurring at nearly full (94%) receptor occupancy. Hence, few spare receptors are present for (*S*)-(+)-NPA.

Discussion

The *in vitro* methods of Furchgott and Burstyn (14) have been used successfully for *in vivo* determinations of receptor reserve at striatal presynaptic dopamine receptors (autoreceptors) (3). We have applied this same mathematical approach to estimate the receptor reserve to NPA enantiomers for those dopamine receptors that regulate the firing rates of substantia nigra dopamine neurons. Our findings for the level of receptor reserve for (*R*)-(−)-NPA are in close agreement with the previously reported results of Meller *et al.* (3) who evaluated (*R*)-(−)-NPA as an agonist at terminal autoreceptors of the nigrostriatal dopamine neurons. Their studies, using the *in vivo* GBL model of Walters and Roth (18) as a measure of response, showed that EEDQ inactivation of dopamine receptors likewise

revealed a steep hyperbolic relationship between per cent response and fractional dopamine autoreceptor occupancy for (*R*)-(−)-NPA. In that model, 50% response to (*R*)-(−)-NPA occurred at 3.8% receptor occupancy in control animals, and a 70% receptor reserve for the agonist was revealed. Despite the differences in experimental approaches, our results are very similar, with 50% response occurring at an estimated 3.5% receptor occupancy and a receptor reserve for (*R*)-(−)-NPA estimated to be about 68%.

On the other hand, our results differ from those of Meller and associates (3) with respect to the extent of receptor inactivation following 24-hr pretreatment with EEDQ. In their striatal GBL paradigm, pretreatment with as little as 1.5 mg/kg EEDQ reduced the maximal agonist response to (*R*)-(−)-NPA to 75% of control. In our experiments, however, preinjection of a 4-fold higher dose of EEDQ (6 mg/kg, intraperitoneally) resulted in a parallel shift to the right of the (*R*)-(−)-NPA dose-response curve with no significant reduction in the maximal effect. These quantitative differences could indicate either that the autoreceptor pool influencing nigral dopamine neuron firing rates is much larger or that it is less sensitive to EEDQ inactivation than are terminal autoreceptors regulating dopamine synthesis. Most likely, this finding indicates that, whereas the GBL model effectively isolates presynaptic autoreceptor actions, our approach using dopamine cell firing rates as an endpoint could be confounded by multiple effectors (somatodendritic autoreceptors, as well as striatonigral feedback loops, perhaps). Furthermore, this could suggest that some other influences less sensitive to EEDQ inactivation may be operational in determining ultimate firing rates of substantia nigra dopamine cells.

The levels of inactivation for (*R*)-(−)-NPA obtained in the present study are quantitatively more like those of Bergstrom *et al.* (5) for the inhibition of nigral dopamine cell firing by systemic administration of the agonist apomorphine in rats pretreated with 6 mg/kg EEDQ. When those investigators compared the inhibitory responses to apomorphine in EEDQ-treated rats with those of normal rats, they reported that EEDQ caused a nonsignificant rightward shift of the apomorphine dose-response curve with no decline in maximal effect. With the same dose of EEDQ, however, we did find a significant 3-fold shift to the right of the agonist dose-response curve in EEDQ-pretreated animals, compared with vehicle-treated controls (ED_{50} values, 0.79 ± 0.04 μg/kg and 0.27 ± 0.02 μg/kg, respectively; $p < 0.05$ by Student's *t* test). It is likely that our use of vehicle-treated rather than normal controls accounts for the different results. Because others have previously shown that ethanol administration may significantly alter important parameters of dopamine neuron physiology, including firing rates (19), dopamine metabolism (20), and dopamine release (21), we pretreated control animals with an equivalent volume (3 ml/kg) of 50% ethanol vehicle. When we compared these results with those from naive (unpretreated) rats from a previous series of experiments, the ethanol-treated controls were found to be significantly more sensitive to the rate-depressant effects of both (−)- and (+)-NPA than were untreated animals (12, 13).

Previous studies from this laboratory have suggested a complex pharmacological profile for (*S*)-(+)-NPA. Although (*S*)-(+)-NPA was shown to be a very weak agonist in inhibiting dopamine neuron activity (12, 13), it also appeared to have a weak antagonist effect, in that pretreatment with the drug interfered with the effect of the more potent enantiomer (*R*)-(−)-NPA (13). In studies by others, (*S*)-(+)-NPA has been characterized as a dopaminergic agonist *in vitro* (22), yet as a dopamine antagonist *in vivo* (11). Similar presynaptic agonist and postsynaptic antagonist effects have been reported in the literature for other dopaminergic compounds (23–25). It may be possible to reconcile the pharmacological actions of (*S*)-(+)-NPA and these other compounds on the basis of receptor reserve. The presence of a substantial population of receptors (presumably autoreceptors in this case) would permit even low intrinsic efficacy agonists to elicit full or nearly full responses in some tissues. Thus, such compounds could exhibit intrinsic activities of 1 despite weak biological stimulus properties once they bind to the receptor. Conversely, a smaller pool of available receptors in other tissues (3, 5) could favor the expression of antagonist properties by such low efficacy agents because occupation of receptors by such drugs would provide a minimal stimulus but block access by the more efficacious endogenous transmitter. Hence, (*S*)-(+)-NPA may be like other "autoreceptor-selective" agonists that are capable of eliciting full agonist responses at autoreceptors, where the receptor pool is large, yet are at best only weak to moderate partial agonists when receptor number is reduced (3, 5). Compared with (*R*)-(−)-NPA (with an arbitrary efficacy of 1.0), the relative efficacy (ratio of fractional occupancies at 50% response) of (*S*)-(+)-NPA is 0.05. This value is comparable to the previously reported (3) relative efficacies of EMD 23448 (0.19), (+)-3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine (0.12), and (−)-3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine (0.05).

The difference in *q* values (decimal fraction of functional receptors remaining after irreversible inactivation) between (*R*)-(−)- and (*S*)-(+)-NPA was not anticipated and prompts further discussion. The accuracy of the estimates obtained using Furchgott analysis is maximized when ideal *in vitro* experimental conditions are used. Our methods, however, deviate from ideal in several regards. The *in vivo* systemic administration of both the inactivator EEDQ and the agonists introduce some variability because they do not provide for equilibrium conditions and lead to unknown concentrations of these drugs at the receptor sites. In addition, most studies using these analytical methods are performed so that the responses of each isolated tissue sample are studied before and after receptor inactivation. Our methods require that the analysis be performed between *groups* of animals rather than within individuals, hence a single *q* value is determined for each drug rather than a series of individual *q* values, which could be averaged and compared statistically. Given these constraints, some variability might be expected between the *q* values experimentally derived for the two NPA enantiomers, and this difference might not be wholly reflective of differences in pharmacologic mechanisms.

Despite the above reservations concerning the accuracy of *in vivo* estimates of *q*, the difference in *q* values for (*R*)-(−)- and (*S*)-(+)-NPA reported here does raise several theoretical concerns. One possible explanation for the difference is that the two NPA enantiomers are recognizing slightly different binding domains or different conformational states of a single receptor type and that EEDQ differentially obstructs these critical regions within the receptor. Alternatively, it could be postulated that the two compounds may be inhibiting nigral dopamine

firing rates through distinct receptor populations, which have different liabilities to EEDQ inactivation. The latter suggestion seems remote because we have previously shown that the dopaminergic antagonist haloperidol blocked the actions of both stereoisomers of NPA, indicative of a common dopamine receptor mechanism (13). Addressing the former possibility, differences in the "fit" of each enantiomer at the active site of the dopamine receptor could contribute to the difference in q values. Because the inactivation by EEDQ is probably more subtle than the bulky steric obstruction that occurs when alkylating ligands covalently bind to receptors, the access by or critical recognition of all compounds may not be uniformly affected. If, for instance, the sites within the receptor affected by EEDQ were more critical for recognition of (*R*)-(−)-NPA than (*S*)-(+)-NPA, it would appear that fewer receptors for (*S*)-(+)-NPA were inactivated.

Finally, other factors that deviate from ideal conditions, and which could contribute to variable q values, are the complexity of our endpoint and the dual agonist and antagonist nature of responses to (*S*)-(+)NPA. Limbird (26) notes that one of the assumptions made in the Furchgott approach is that the agonist elicits a single response and does so by interacting with one receptor population. This suggests another possible explanation for the apparently different levels of inactivation for (*R*)-(−)- and (*S*)-(+)-NPA, which is consistent with our hypothesis regarding the mixed actions of low intrinsic activity agonists. The firing rates of nigral dopamine neurons are believed to be regulated by the complex interplay of several influences. In the simplest model, local activation of somatodendritic autoreceptors (6), as well as a postsynaptic striatonigral ("long-loop") feedback circuit, (7) mediates the inhibitory actions of dopamine agonists on neuronal firing rate. Highly efficacious compounds [full agonists such as (*R*)-(−)-NPA, for example] act as agonists at both somatodendritic autoreceptors and striatal postsynaptic sites. On the other hand, lower efficacy agents [(*S*)-(+)-NPA, or other partial agonists], although displaying agonist activity at high density sites (autoreceptors), are expected to behave as antagonists at lower density striatal postsynaptic targets (3, 4). Because blockade of striatal dopamine receptors has previously been shown to result in an increase in nigral dopamine cell firing rates (27), one might predict that the combination of conflicting agonist and antagonist effects at these two important sites could account for an apparently lower level of receptor inactivation for (*S*)-(+)-NPA when using inhibition of firing rate as a functional measure of response. Specifically, receptor inactivation by EEDQ would diminish the rate-slowing agonist actions of (*S*)-(+)-NPA at the autoreceptor while still allowing the rate-increasing antagonist effects at striatal targets. The net sum of these opposing actions could blunt the expected diminution of firing rate by (*S*)-(+)-NPA when given intravenously to EEDQ-pretreated animals. This would confound our results to some extent and give the erroneous impression of fewer receptors being inactivated for (*S*)-(+)-NPA than was evident for (*R*)-(−)-NPA.

With regard to its mechanism, EEDQ is reported to promote inactivation of receptor proteins by the "activation" of carboxyl groups, followed by the formation of an internal cross-link with a nearby amine group (28). Radioligand binding studies have shown that the prior administration of an appropriate dopamine antagonist such as (−)-sulpiride or haloperidol prevented the loss of dopaminergic binding sites by EEDQ (29). It would be attractive to similarly validate the use of EEDQ as a dopamine receptor inactivator in our functional studies by demonstrating that pretreatment with a suitable reversible antagonist could prevent the loss of physiological response caused by the irreversible inactivating agent. Although such receptor "protection" methods are easily performed under *in vitro* assay conditions, this approach is not as cleanly transferrable to our *in vivo* experimental conditions, in which residual antagonist used to protect receptors cannot be washed from the tissue and could modify responses to the agonist 24 hr later. For example, 1 mg/kg haloperidol given before EEDQ was shown (29) to spare about 87% of striatal D-2 receptors from inactivation in *in vitro* binding studies. Yet, based upon a 4–5-hr half-life for haloperidol in rat brain (30), one would anticipate that pharmacologically significant levels of this antagonist would persist in brain 24 hours later, when our agonist dose-response studies were performed. Allowing a clearance period longer than 24 hr would further complicate interpretation because regeneration of dopamine receptors has been shown to ensue within 24–48 hr after EEDQ inactivation (31). Consequently, use of receptor protection techniques for *in vivo* physiological studies would be more complex and less practical than under *in vitro* assay conditions.

In summary, this report suggests that receptor inactivation by *in vivo* administration of EEDQ can provide a useful means for studying the contribution of spare receptors to drug effects in the central nervous system, provided that appropriate caution is exercised in the interpretation of values obtained using complex *in vivo* systems. Furthermore, the similarity of the results obtained from two different models of dopamine autoreceptor activation, i.e., the GBL model (3) and the inhibition of dopamine cell firing model (present study), strengthens the concept of a significant autoreceptor reserve for potent agonists in the nigrostriatal dopamine system. Lastly, these results support an explanation for the disparate pharmacological actions (agonist as well as antagonist properties) of certain dopaminergic compounds, including (*S*)-(+)-NPA, when examined under various "assay" conditions. Lower efficacy dopaminergic drugs may display antagonist effects in postsynaptic tissues lacking receptor reserve yet still act as agonists in tissues having many spare receptors.

Acknowledgments

The authors wish to thank Dr. G. Jagadeesh for his assistance with ALLFIT analysis of dose-response curves and for his fruitful discussions regarding Furchgott analysis.

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