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Relationship Between Receptor Occupancy and Response at Striatal Dopamine Autoreceptors

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SUMMARY

The irreversible dopamine (DA) receptor antagonist *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was used to determine the extent of receptor reserve at DA autoreceptors regulating *in vivo* tyrosine hydroxylase activity. Rats were treated with vehicle or EEDQ (1 × 0.5–2 × 6 mg/kg, subcutaneously) and, 24 hr later, dose response curves were generated for DA agonist reversal of γ-butyrolactone-induced striatal L-3,4-dihydroxyphenylalanine (L-DOPA) accumulation. Double reciprocal plots were obtained of equieffective doses of agonist required to elicit response at several levels of effect before and after partial irreversible receptor inactivation. A pseudo-dissociation constant (pseudo-K_A, in units of dose) and the fraction of receptors remaining active (q) were determined; these values were then used to calculate the relationship between receptor occupancy and response. The ED_{50} (1 μg/kg) for the full DA receptor agonist *N*-propylnorapomorphine (NPA) was shifted 2.8-, 4.8-, and 11.3-fold to the right after partial irreversible receptor blockade which left the fraction of receptors remaining active (q) at 0.37, 0.17 and 0.058, respectively. Corresponding maximal reversal of L-DOPA accumulation was 100, 77, and 58%, indicating a nonlinear relationship between receptor occupancy and response for NPA and the presence of a large receptor reserve; maximal and half-maximal responses were calculated to require occupancy of 30 and 3.8% of the total receptor pool, respectively. Dose response curves were also obtained for the DA autoreceptor-selective agents EMD 23,448 and (+)- and (−)-3-PPP before and after EEDQ treatment. In controls, EMD 23,448 and (+)-3-PPP, like NPA, completely reversed striatal γ-butyrolactone-induced L-DOPA accumulation, whereas the maximal effect of (−)-3-PPP was 52% reversal. After EEDQ treatment (6 mg/kg), EMD 23,448 and (+)-3-PPP showed relatively small shifts in ED_{50} values. Furchgott analysis demonstrated that all three atypical agents are partial agonists at the DA autoreceptor with efficacies of 0.19 (EMD 23,448), 0.12 [(+)-3-PPP], and 0.05 [(−)-3-PPP] relative to NPA. The presence of a larger receptor reserve at pre-versus postsynaptic D_2 DA receptors and the partial agonist character of drugs such as EMD 23,448 and the enantiomers of 3-PPP may account for their autoreceptor selectivity.

At least two distinct types of DA receptors, D_1 (mediating stimulation of adenylate cyclase activity) (1) and D_2 (either not coupled or mediating inhibition of cyclase activity) (1, 2) are localized to various sites in the rat striatum. D_1 receptors are found on postsynaptic cells (3). Whereas some striatal D_2 receptors occur postsynaptically on cholinergic interneurons, where their activation leads to inhibition of both neuronal activity and release of ACh (4), others ("autoreceptors") are localized to terminals of nigrostriatal dopaminergic neurons and mediate inhibition of both synthesis (5, 6) and release (7) of DA. These autoreceptors have pharmacological characteristics identical to those of postsynaptic D_2 receptors based on the relative affinities and selectivities of DA receptor *antagonists* at the two sites (6, 8). Substantial behavioral (9), electrophysiological (10), and biochemical (8, 11–13) evidence suggests, however, that DA *agonists* are more potent at D_2 autoreceptors than D_2 postsynaptic sites. Thus, DA agonist-induced sedation has been proposed to occur as a result of selective activation of presynaptic receptors by low doses of apomorphine and other drugs (9). A strong correlation exists between the ED_{50} values for induction of sedation (a presynaptic response) and stereotyped behavior (a postsynaptic response); however, the ED_{50} values for the former effect are generally 10 times lower than for the latter (9). The ED_{50} for DA autoreceptor-mediated inhibition of nigrostriatal DA cell firing by apomorphine is similarly about 5–10 times lower than for elicitation of postsynaptic responses (10). Various DA agonists are also more potent in inhibiting autoreceptor-mediated DA release (8, 11) or synthesis (12, 13) than in eliciting several postsynaptic behavioral (9, 12) or biochemical (8, 13) responses. Furthermore, several new agents have been described, such as 3-PPP (14, 15) and

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ABBREVIATIONS: DA, dopamine; EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; L-DOPA, L-3,4-dihydroxyphenylalanine; 3-PPP, 3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine; EMD 23,448, 3-[4-(4-phenyl-1,2,3,6-tetrahydropyridyl-1)-butyl]indole; NPA, *R*-(−)-*N*-*n*-propylnorapomorphine; GBL, γ-butyrolactone; NSD-1015, *m*-hydroxybenzylhydrazine; ACh, acetylcholine; EDTA, ethylenediaminetetraacetate.

EMD 23,448 (16–18) whose selectivity for DA autoreceptors is much greater than for conventional agonists such as apomorphine (12–15).

A possible explanation of these findings, consistent with receptor occupancy theory (19), is that the greater potency of agonists at D_2 pre- than D_2 postsynaptic receptors may be due to the presence of a larger receptor reserve at the former relative to the latter site. The greater potency at autoreceptors may therefore be viewed as a shift in the dose response curve to the left in the presence of a receptor reserve relative to its absence. Furthermore, the very pronounced autoreceptor selectivity of compounds such as 3-PPP and EMD 23,448 may be explained if it is further hypothesized that these drugs possess low relative intrinsic efficacies (i. e., are partial agonists), such that in the *absence* of a substantial receptor reserve they are unable to elicit a measurable response (19).

The estimation of receptor reserve and relative efficacies of agonists by the methods developed by Furchgott and Bursztyn (20) requires the determination of dose-response relationships before and after partial irreversible receptor inactivation. Hamblin and Creese (21) recently reported that EEDQ irreversibly inactivates DA receptor-binding sites in rat brain. We have utilized this agent to study the pharmacology of striatal D_1 and D_2 receptor-binding ligands *ex vivo* (22, 23) and the repopulation kinetics and receptor reserve of α_2-adrenergic receptors in rat brain cortex (24, 25). We have also used it in preliminary experiments to support the suggestion that a substantial receptor reserve exists at striatal DA autoreceptors (26).

These nerve terminal autoreceptors have been studied for many years by the *in vivo* GBL method described by Roth (6). This method takes advantage of the ability of GBL to effect a cessation of DA neuronal cell firing, thereby eliminating the modulatory feedback effects of both postsynaptic and somatic (cell body) DA receptors on tyrosine hydroxylation. *In vivo* GBL treatment produces a large increase in DA synthesis which can be prevented by treatment with DA agonists; the latter effect is presumed to occur via inhibitory DA autoreceptors, since it is selectively blocked by pretreatment with DA receptor antagonists (6). The present studies have combined this technique and partial irreversible receptor inactivation with EEDQ in order to examine the relationship between receptor occupancy and response at the autoreceptor for a number of DA agonists.

Experimental Procedures

Materials. EEDQ, NSD-1015, and GBL were purchased from Aldrich Chemical Co. (Milwaukee, WI). NPA was obtained from Research Biochemicals Inc. (Wayland, MA). EMD 23,448 was a gift of E. Merck (Darmstadt, West Germany). The (+)- and (−)-enantiomers of 3-PPP were a gift of Dr. S.-O. Ogren, Astra Lakemedel (Sodertalje, Sweden). All reagents and chemicals were of the highest purity commercially available.

Drug treatments. Male Sprague-Dawley rats (200–400 g, Taconic Farms, Germantown, NY) were treated with various subcutaneous doses of EEDQ or vehicle 24 hr before determination of agonist dose response curves for reversal of GBL-induced elevation of striatal L-DOPA levels. Multiple EEDQ injections were separated by 24 hr. Groups of pretreated rats were administered various subcutaneous doses of an agonist or its vehicle 5 min before GBL (750 mg/kg, intraperitoneally); NSD-1015 (100 mg/kg, i.p.) was given 5 min after GBL. All animals were sacrificed by decapitation 30 min after NSD-1015 and striata were immediately dissected in the cold and stored at −70° until assayed. Basal L-DOPA levels (after decarboxylase inhibition with NSD-1015) for both vehicle and EEDQ-treated groups were determined in control (i.e., vehicle-pretreated) animals, since EEDQ pretreatment alone was previously shown to elevate L-DOPA levels (26), presumably because of reduced feedback inhibition resulting from partial DA receptor inactivation.

High pressure liquid chromatographic analysis of L-DOPA. L-DOPA levels were determined essentially as described previously (26) with minor modifications. Striata were homogenized in 0.6 ml of perchloric acid containing 0.4 mM $NaHSO_3$. One hundred ng of α-methyl DOPA were added as internal standard and an aliquot was transferred to tubes containing acid-washed alumina and 0.9 ml of a solution of 0.6 M Tris hydrochloride buffer (pH 8.6), 0.6 mM $NaHSO_3$, and 0.04 mM EDTA. After mixing for 15 min the contents were allowed to settle and the supernatant was discarded. The alumina was washed three times with a solution containing 6 mM Tris-HCl and 1 mM $NaHSO_3$ (pH 8.6); extraction of catechols was carried out by shaking with 0.4 ml of 0.1 M perchloric acid for 30 min at 4°. The mixture was centrifuged and aliquots of the supernatant were injected onto a C_{18} Biophase ODS column (5 μm diameter, 4.6 mm i.d. × 25 cm; Bioanalytical Systems, Inc., West Lafayette, IN). L-DOPA was quantitated by high pressure liquid chromatography with electrochemical detection on a Bioanalytical Systems LC-304B chromatograph using a glassy carbon electrode set at 0.65 V. The mobile phase was 0.15 M monochloroacetate buffer (pH 3.05) containing 0.8 mM Na_2EDTA.

Computer analysis of dose response curves. Dose response curves for DA agonist reversal of GBL-induced striatal L-DOPA accumulation after EEDQ or vehicle pretreatment were simultaneously analyzed for best fit using the ALLFIT computer program of DeLean *et al.* (27) as modified by Teicher (available as MED-65, Biomedical Computing Technology Information Center, Nashville, TN). This iterative program utilizes a four-parameter logistic equation to simultaneously analyze several curves, and provides statistical tests of the goodness of fit after the curves are constrained to share one or more parameters. The four parameters are: 1) response at zero dose, 2) slope factor, 3) 50% of maximally effective dose or ED_{50}, and 4) response at "infinite" dose. In practice, the response at zero dose for all curves was set to zero; where appropriate, the maximum response for control (i.e., vehicle pretreatment) curves was also set to a constant (100%). Curves were first analyzed without constraints and then by successively constraining them to share a common slope factor, ED_{50}, or maximal response. The best fit was that analysis which permitted one or more parameters to be shared without a significant increase in the residual variance (27).

Receptor reserve analysis. Furchgott and Bursztyn (20) described a method for determining the apparent dissociation constant (K_A) of an agonist using an irreversible receptor antagonist. They obtained the following relationship for the effect elicited by a full agonist before and after irreversible receptor inactivation:

$$\frac{1}{[A]} = \frac{1}{q[A']} + \frac{1-q}{qK_A}$$

where $[A]$ is the concentration of agonist necessary to produce a specific level of response before inactivation, $[A']$ is the concentration needed to produce the *same* response after inactivation, and q is the fraction of receptors left intact (i.e., *not* inactivated).

In the present *in vivo* studies the determination of both the pseudo-dissociation constant, pseudo-K_A (see below), and the fraction of receptors not inactivated, q, was made by plotting the reciprocals of the equieffective agonist doses after inactivation, $1/[A']$, against the reciprocals of the doses of agonist before inactivation, $1/[A]$. The equieffective doses were determined, whenever possible, at five levels of response (corresponding to 30, 40, 50, 60, and 70% of the maximum effect after EEDQ treatment) (20) from the ALLFIT-derived best fit dose response curves. The resulting straight line had a slope of $1/q$ and pseudo-K_A equal to (slope−1)/y intercept.

In instances when partial irreversible receptor inactivation did not

reduce the maximum response, this method was not experimentally useful; nevertheless, the fraction of active receptors, q, could still be estimated by the method of Minneman and Abel (28).

The pseudo-K_A value in units of dose was used to calculate fractional receptor occupancy (f) at a particular dose [A] from the law of mass action:

$$f = [RA]/[R_T] = [A]/K_A + [A]$$

where [RA] is the concentration of receptor-agonist complex and [R_T] is the initial or total concentration of active receptors. Fractional receptor occupancy at a particular dose was then plotted against fractional response at that dose (obtained from the control best fit dose response curve). The degree of receptor reserve (for a full agonist) was estimated from the relationship:

$$\% \text{ receptor reserve} = 100 - \% \text{ receptor occupancy required for maximal response}$$

It should be noted that the method of Furchgott and Bursztyn (20) as originally described was restricted to *in vitro* application under steady state conditions, where a constant agonist concentration could be maintained by superfusion and, thus, an apparent *equilibrium* dissociation constant could be calculated. We have extended their method to *in vivo* situations following bolus drug injection, where no equilibrium exists. Therefore, a pseudo-K_A value has been calculated which is *not* a constant and can indeed be shown to depend on where in the drug's time course the dose-response curves are generated. Clearly, the ED_{50} for an agonist is also dependent on the time after drug treatment chosen for analysis. It can be shown, however, that the pseudo-K_A/ED_{50} ratio is a *constant* which is *independent* of the point in the time course chosen for determination of dose response. Thus, although the pseudo-K_A value is not by itself a valid parameter, it can nevertheless be used to determine the relationship between fractional receptor occupancy and response, since the K_A/ED_{50} ratio is a measure of relative efficacy (19).

Determination of relative efficacies. The relative efficacies of two agonists were obtained from plots of receptor occupancy versus response (19, 20). The efficacy of a particular agonist relative to the standard full agonist NPA was obtained from the ratio of the fraction of receptors required to be occupied by NPA over that required to be occupied by the second agonist in order to elicit the same level of response (19, 20).

Results

Effect of partial irreversible receptor inactivation by EEDQ on NPA-mediated reversal of GBL-induced elevation of L-DOPA. ALLFIT-derived best fit dose response curves for NPA reversal of GBL-induced elevation of striatal L-DOPA levels in vehicle- and EEDQ-treated rats are shown in Fig. 1. Partial irreversible receptor inactivation with a low dose of EEDQ (0.5 mg/kg) produced an initial rightward shift in the dose response curve without a reduction in maximal response, suggesting the presence of a receptor reserve. Larger and/or multiple doses produced increasing rightward shifts and progressive reductions in maximal response. Using the method of Furchgott and Bursztyn (20), the linear double reciprocal plot shown in Fig. 2 was obtained on analysis of the vehicle and EEDQ (2 × 6 mg/kg) curves. A q value of 0.058 (i.e., 5.8% active receptors remaining) and a pseudo-K_A value of 24.4 μg/kg were obtained. Parameters derived from analysis of all the NPA dose response curves are shown in Table 1. The receptor reserve can be estimated at >62% since maximal response can still be obtained with only about 38% of the receptor pool intact (Table 1). The pseudo-K_A value in units of dose was similar in

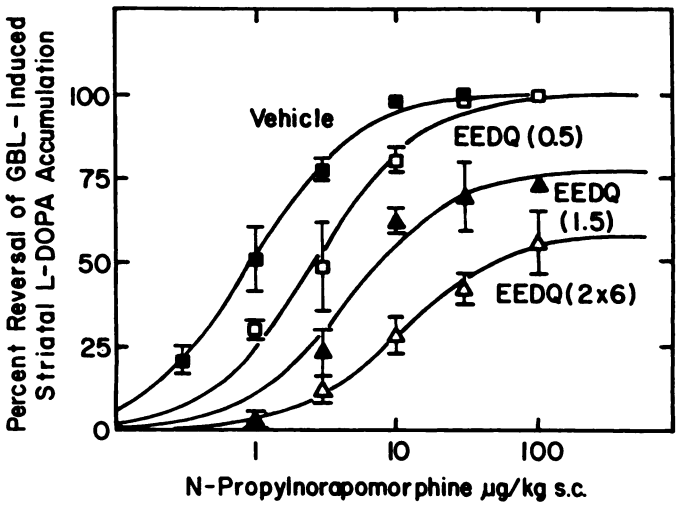

Fig. 1. Dose response curves (ALLFIT) for NPA reversal of GBL-induced L-DOPA accumulation in rat striatum 24 hr after treatment with vehicle or the indicated doses of EEDQ. ALLFIT analysis indicated that the curves could share a common slope factor (1.16; normalized for each curve as a function of its own maximum response) without a significant increase in the residual variance ($F(1,11) = 3.56$; $p > 0.05$). All animals were treated with NSD-1015 (100 mg/kg) to inhibit DOPA decarboxylase (30 min before sacrifice). NPA (various doses) and GBL (750 mg/kg) were administered 40 and 35 min before sacrifice, respectively. Each point is the mean ± standard error of four to five animals.

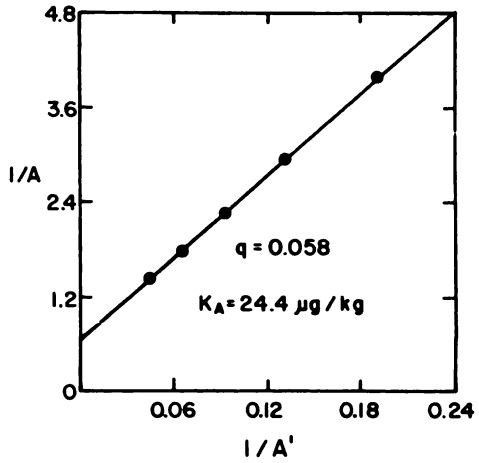

Fig. 2. Double reciprocal plot of the equieffective doses of NPA required for reversal of GBL-induced L-DOPA accumulation in vehicle (A)- and EEDQ- treated (A') animals. Doses were obtained at five levels of effect (corresponding to 30, 40, 50, 60, and 70% of the maximal response in EEDQ (2 × 6 mg/kg)-treated rats from the data shown in Fig. 1.

both instances where it could be calculated by the method of Furchgott and Bursztyn (20) (17 and 24 μg/kg, Table 1).

As we noted earlier (see Experimental Procedures), these studies were carried out *in vivo*, where the concentration of agonist at the receptor is not known and, indeed, is impossible to determine. It has therefore been proposed that receptor occupancy versus response should be evaluated in such *in vivo* studies by comparing experimentally determined q values with maximal response after varying degrees of irreversible receptor inactivation (29). Since q is a dimensionless parameter obtained as 1/slope from a plot of the reciprocals of equieffective agonist doses, few assumptions need be made regarding its use for this purpose (29). Alternatively, we have developed a rationale for utilizing pseudo-K_A values to determine receptor occupancy (see Experimental Procedures). We have therefore used the

TABLE 1

Dose response analysis parameters for NPA reversal of GBL-induced striatal L-DOPA accumulation

Data shown were derived from the dose response curves for NPA reversal of GBL-induced L-DOPA accumulation in Fig. 1. The values of q and K_A were determined as described in the text using the methods of Furchgott and Bursztyn (20) or estimated using the method of Minneman and Abel (28).

Treatment (mg/kg, SC)	ED_{50}	Percentage of maximum reversal	ED_{50} shift	q	Pseudo-K_A
	μg/kg		-fold		μg/kg
Vehicle	0.96	100			
EEDQ (1 × 0.5)	2.70	100	2.8	0.38	
EEDQ (1 × 1.5)	4.59	77	4.8	0.17	17
EEDQ (2 × 6)	10.81	58	11.3	0.058	24

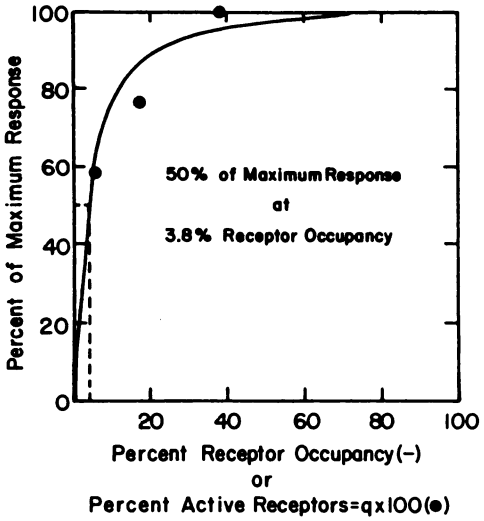

Fig. 3. Maximal response for NPA reversal of GBL-induced L-DOPA elevation as a function of receptor occupancy (—) or the percentage of intact receptors remaining after EEDQ treatment (●). Receptor occupancy was calculated from the law of mass action (fractional occupancy = $[A]/K_A + [A]$) using the pseudo-K_A value (24.4 μg/kg) obtained as in Fig. 2 and the ALLFIT-derived vehicle dose response curve (Fig. 1).

pseudo-K_A value determined as in Fig. 2 to calculate fractional receptor occupancy as a function of response; the hyperbolic relationship obtained (Fig. 3) demonstrates that a receptor reserve exists at the autoreceptor regulating DA synthesis in striatal dopaminergic terminals. For comparison, we have also plotted q values (from Table 1) as a function of response (*solid circles* in Fig. 3). The excellent correspondence obtained between these methods suggests that it is valid to use pseudo-K_A values in dose units (and the mass action equation) to determine the relationship between occupancy and response.

The DA autoreceptor reserve for the full agonist NPA can be estimated from Fig. 3 to be about 70%, since 95% of the maximal response (experimentally indistinguishable in these studies from 100%) is obtained at 30% receptor occupancy.

Effect of EEDQ on reversal of GBL-induced L-DOPA elevation by the selective presynaptic agonists EMD 23,448 and (+)- and (−)-3-PPP. Figure 4 shows the dose response curves obtained for EMD 23,448 reversal of L-DOPA accumulation after vehicle or EEDQ (6 mg/kg) treatment. Complete reversal was obtained in control animals whereas EEDQ treatment shifted the ED_{50} about 3-fold and reduced the maximum response to 42.5% of control. Similar results were obtained with (+)-3-PPP (Fig. 5): complete reversal was obtained in controls, whereas EEDQ (6 mg/kg) produced a small shift (1.7-fold) in the ED_{50} and reduced the maximal response to 52.8% of control. Thus, both EMD 23,448 and (+)-3-PPP appear to have full *intrinsic activity*, i.e., in control striatum they elicit maximal response indistinguishable from that produced by the full D_2 agonist NPA. In contrast, (−)-3-PPP elicited less than maximum possible tissue response in control animals (52%) (Fig. 6). EEDQ treatment (0.5 mg/kg) depressed the maximal response (to 21%) without significantly shifting the ED_{50} (1.0 mg/kg). Furthermore, a dose of EEDQ (2 mg/kg) which virtually abolished the ability of (−)-3-PPP to elicit a response (<5% of maximum; Fig. 6) only slightly reduced the ability of NPA to maximally reverse L-DOPA levels (Fig. 1; note dose response after comparable dose of 1.5 mg/kg EEDQ). These results qualitatively suggest large differences in efficacy between NPA and these autoreceptor-selective agents.

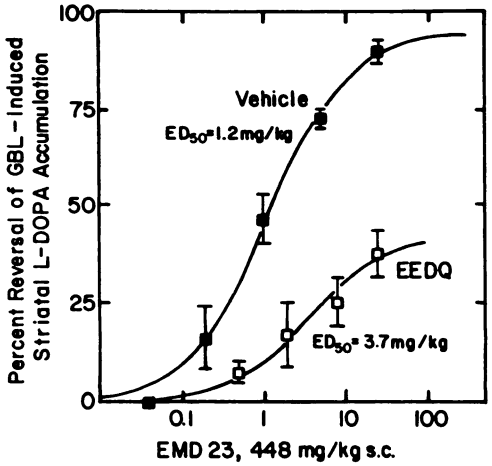

Fig. 4. Dose response curves for EMD 23,448 reversal of GBL-induced L-DOPA elevation in rat striatum 24 hr after treatment with vehicle or EEDQ (6 mg/kg). ALLFIT-derived maximal responses were 95 and 43% for the vehicle and EEDQ curves, respectively. Each point is the mean ± standard error of four to eight rats.

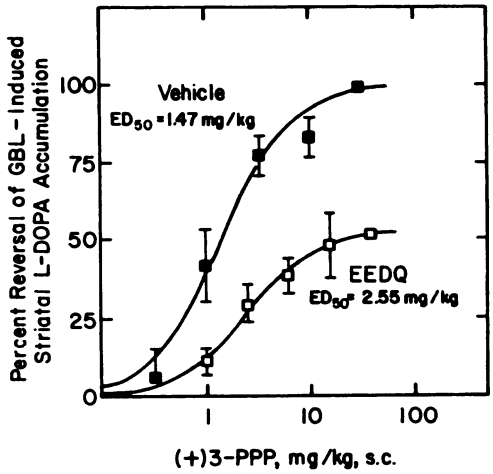

Fig. 5. Dose response curves for (+)-3-PPP reversal of GBL-induced striatal L-DOPA elevation after treatment with vehicle or EEDQ (6 mg/kg). Each point is the mean ± standard error of three to seven animals.

Relative efficacies of the agonists. Analysis of the data from each pair of dose response curves (Figs. 4–6) by the double reciprocal method of Furchgott and Bursztyn (20), followed by calculation of the receptor occupancy versus response relationships, yielded the plot shown in Fig. 7. The data points shown

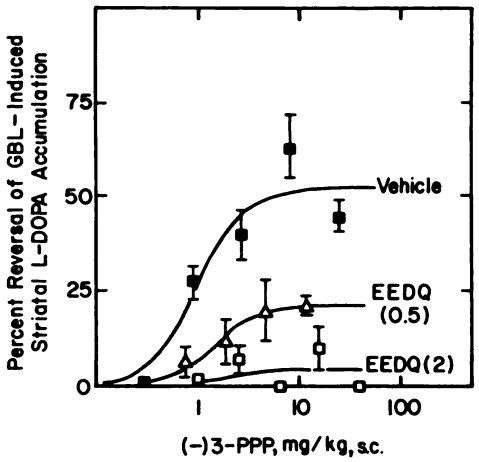

Fig. 6. Dose response curves for (−)-3-PPP reversal of GBL-induced striatal L-DOPA elevation after treatment with vehicle or the indicated doses of EEDQ 24 hr before. Each point is the mean ± standard error of four to seven animals.

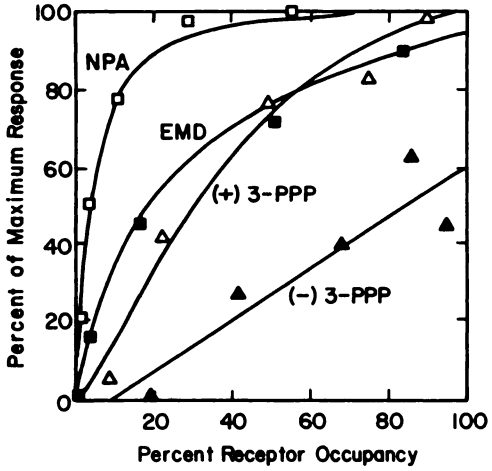

Fig. 7. Maximum response as a function of receptor occupancy for each of the DA agonists. Each *solid curve* was obtained from the corresponding vehicle ALLFIT dose response curve and the calculated pseudo-K_A value (as described in the legend to Fig. 3), except for (−)-3-PPP which is a least squares fit through the data points. The *symbols* represent values calculated for the actual data points in the experimental dose response analyses (Figs. 1 and 4–6).

were obtained from the doses used experimentally; the *solid lines* are the theoretical curves obtained using the ALLFIT-derived dose response curves. Although NPA demonstrates a steep hyperbolic relationship between receptor occupancy and response (50% response at 3.8% occupancy), both EMD 23,448 and (+)-3-PPP show much shallower relationships (50% response at 18 and 30% receptor occupancy, respectively). For (−)-3-PPP, however, the relationship is essentially linear, and 50% response requires occupancy of virtually all the receptors (Fig. 7). The relative efficacies of the agonists, determined from the ratio of the fraction of receptors occupied by the full agonist (NPA) to that occupied by each of the other agonists in order to elicit the same response (19, 20), are listed in Table 2 and demonstrate that the autoreceptor-selective agents are indeed partial agonists at the DA autoreceptor. Because of the large autoreceptor reserve (i.e., highly efficient coupling between receptor occupancy and response), EMD 23,448 and (+)-3-PPP demonstrate full intrinsic *activity* (as opposed to *efficacy*) even though they are weak-to-moderate partial DA agonists; (−)-3-PPP, in contrast, whose relative efficacy is only about 5% that of NPA (Table 2), shows intrinsic activity of about 0.5.

TABLE 2

Relative efficacies of DA agonists to reverse GBL-induced striatal L-DOPA accumulation

Values were derived from the data shown in Fig. 7. The ratio of the fraction of receptors required to be occupied by NPA over that fraction required to be occupied by each of the other agonists, at the 50% response level, yielded the relative efficacy (19, 20).

Agonist	Relative efficacy
NPA	1.0
EMD 23,448	0.19
(+)-3-PPP	0.12
(−)-3-PPP	0.05

Discussion

The method of Furchgott and Bursztyn (20) has been successfully applied over many years to determine receptor reserve and/or relative efficacy of agonists at a variety of receptors mediating diverse physiological responses. Recent examples include examination of opiate receptors in normal and morphine-tolerant guinea pig ileum myenteric plexus (30), α_1-adrenergic receptors in rat vas deferens (31), α_2-adrenergic receptors in canine saphenous vein (32), β-adrenergic receptors in C_6 glioma cells (33), and muscarinic receptors in cultured chick heart cells (34). These and all other such studies examined physiological responses *in vitro*. In this report the method of Furchgott and Bursztyn (20) has been extended to provide quantitative estimates of relative efficacies of agonists *in vivo*. We have provided a rationale for this application of the method, but additional assumptions may be required over those assumed *in vitro*. For example, it is assumed that EEDQ treatment does not affect accessibility of the drug to the receptor (i.e., no effect on central nervous system penetration, clearance, etc.). Nevertheless, obtaining the identical order of relative efficacy both qualitatively and quantitatively (NPA > EMD 23,448 > (+)-3-PPP > (−)-3-PPP) (compare Figs. 1, 4–6, and Table 2) suggests that the Furchgott method applied *in vivo* may yield reasonably accurate estimates of relative efficacy.

These studies utilizing EEDQ would not be possible if *in vivo* EEDQ treatment affected DA synthesis in GBL-treated animals by mechanisms not related to DA autoreceptor function. We have previously shown that this is not the case since L-DOPA concentrations are maximally increased to the same extent by GBL in control and EEDQ-treated rats (26); if EEDQ had affected DA synthesis by a non-receptor mechanism, identical levels of L-DOPA could not have been achieved. Furthermore, in animals treated with the decarboxylase inhibitor NSD-1015 alone, L-DOPA levels were significantly higher in EEDQ-treated striata than in controls (26), consistent with the idea that EEDQ produces a partial blockade of striatal DA receptors (pre and post), thereby reducing both autoreceptor and postsynaptic negative feedback regulation of DA synthesis. Finally, EEDQ (10 μM) also has no effect on tyrosine hydroxylase activity *in vitro*.[1]

Utilizing the full D_2 DA agonist NPA and the method of partial irreversible receptor inactivation developed by Furchgott and Bursztyn (20), we have found a receptor reserve of approximately 70% at the striatal DA autoreceptor (Figs. 1–3).

[1] K. Y. Lee, E. Meller, and M. Goldstein, unpublished observations.

Furthermore, relative to NPA, the autoreceptor-selective agents EMD 23,448 and (+)- and (−)-3-PPP were found to be partial agonists at this receptor. Although other hypotheses may be entertained (see below), the presynaptic selectivity of DA agonists in general, and these agents in particular (see the introduction), may be explained on the basis of current receptor occupancy theory if we hypothesize a differential receptor reserve at pre- versus postsynaptic D_2 receptors (19). A full agonist (e.g., NPA) will demonstrate substantially greater potency in the presence of a receptor reserve (i.e., efficient coupling between receptor occupancy and response) relative to its absence (Fig. 1, Table 1). For a partial agonist, however, the major effect of a large reserve is to increase the maximal tissue response to the agonist (19). Indeed, in the presence of a large receptor reserve, even for a partial agonist with low-moderate efficacy [e.g., EMD 23,448 or (+)-3-PPP, Table 2], the maximal tissue response may be indistinguishable from that elicited by a full agonist (Refs. 19 and 20; Figs. 4 and 5). A very weak partial agonist [e.g., (−)-3-PPP, Table 2) will demonstrate submaximal response even in the presence of a large receptor reserve; as the fraction of active receptors is diminished (e.g., by partial irreversible inactivation), the tissue response falls dramatically until it may be experimentally undetectable (Fig. 6). Agents which are very weak partial agonists may indeed appear to be *antagonists* in systems where coupling between receptor occupancy and response is poor and inefficient. In this regard it is interesting to note that (−)-3-PPP has been reported to act as an *antagonist* in some behavioral and biochemical models of *postsynaptic* D_2 receptor function (14, 15). This suggests that these postsynaptic D_2 receptor systems have little or no receptor reserve.

A number of other hypotheses may be entertained to explain the autoreceptor selectivity of DA agonists. The simplest is that, notwithstanding the pharmacological similarity between D_2 pre- and postsynaptic receptors (6, 8), they are different molecular entities. A second possibility is the recent suggestion by Seeman *et al.* (35) that D_2 autoreceptors operate *in vivo* in the high affinity (R_H) agonist state, whereas postsynaptic D_2 receptors operate in the low affinity (R_L) state. This suggestion was based on a correlation of the dissociation constants of the agonists for high and low affinity sites in ^{3}H-spiperone binding assays (K_H and K_L) with literature IC_{50} values for inhibition of ^{3}H-DA (presynaptic) and ^{14}C-ACh (postsynaptic) release, respectively. However, it is difficult to reconcile this hypothesis with the effects of bromocriptine and EMD 23,488; although neither compound discriminates high and low affinity states of the D_2 receptor (17, 36), bromocriptine is 8 times more potent in inhibiting ^{3}H-DA than ^{14}C-ACh release (11) and 25 times more potent in eliciting behaviors believed to be mediated by presynaptic than postsynaptic DA receptors (37). EMD 23,488 likewise has much greater potency at pre- than postsynaptic receptors (16, 17).

Carlsson (38), attempting to explain the unusual properties of 3-PPP based on receptor theory, elaborated the hypothesis that the intrinsic efficacy of an agonist derives in part from the responsivity of its receptor, which he suggested is determined by the previous agonist occupancy of that receptor. This hypothesis is partially related to our own as described above. However, whereas we have focused on hypothesizing differences in the efficiency of receptor-coupled response at pre- and postsynaptic receptors as the basis for the selective action of these agents, Carlsson's hypothesis (38) is directed toward understanding how the local environment of a particular receptor (in terms of availability of agonist supply) might influence the efficiency of receptor-effector coupling. This focus may have resulted from a desire to explain another puzzling feature of the action of drugs such as 3-PPP and EMD 23,488. Although these agonists are very weak at eliciting postsynaptic responses in normal animals, they *are* effective in animals with DA receptors rendered supersensitive by 6-hydroxydopamine or chronic neuroleptic treatment (14, 15). Carlsson's proposal (38) suggests that, since these treatments reduce the availability of DA at receptor sites, the responsivity of the receptor to agonist increases. From our perspective, since it has been repeatedly shown that the main effect of either chronic neuroleptic treatment or 6-hydroxydopamine lesion is to increase the density of D_2 receptors, we suggest that up-regulation of these receptors would, in effect, *create a receptor reserve where none had existed before.* Thus, receptor occupancy theory may explain, in part, why DA agonists with low intrinsic efficacy, ineffective at normosensitive receptors, would elicit substantial response at supersensitive sites. Changes in receptor reserve (i.e., receptor-effector coupling efficiency) may be a general mechanism for alterations in agonist-induced responses at receptors undergoing up- or down-regulation in response to various treatments.

In conclusion, we have demonstrated a large receptor reserve at DA autoreceptors regulating DA synthesis in striatal nerve terminals, and drugs such as (+)- and (−)-3-PPP and EMD 23,488 have been found to be partial agonists at these D_2 receptors. The marked autoreceptor selectivity of these agents has been hypothesized to result from a differential receptor reserve at pre- versus postsynaptic striatal sites. Analogous studies examining the relationship between receptor occupancy and response at postsynaptic D_2 sites (utilizing agonist-induced elevation of striatal ACh levels as the response parameter) should provide an adequate test of this hypothesis.

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