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Interactions between D1 and D2 dopamine receptor family agonists and antagonists: the effects of chronic exposure on behavior and receptor binding in rats and their clinical implications

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Summary. Functional interactions between dopamine receptor subtypes may affect behavioral and biochemical responses which serve as models for neuropsychiatric illnesses and the clinical effects of drug therapy. We evaluated the effects of chronic exposure to the selective D1 receptor antagonist SCH 23390, and the selective D2 receptor antagonist metoclopramide, on spontaneous and drug-induced behavior and receptor density in rats, and then determined how these effects would be modified by concurrent administration of antagonists or agonists [SKF 38393, LY 171555 (quinpirole)] selective for the complementary receptor subtype. Administered alone, both the D1 and D2 antagonists had acute cataleptic effects to which animals became tolerant following chronic treatment, but the selective antagonists had opposing effects on spontaneous locomotor activity. Both antagonists produced equivalent, supersensitive behavioral responses to apomorphine, and resulted in an increase in D2 receptor density. Coadministration of the D1 and D2 antagonists had a synergistic effect on catalepsy, attenuated the effects on spontaneous locomotor activity observed with either drug alone, and had an additive effect on both apomorphine-induced stereotypic behavior and D2 receptor proliferation. On the other hand, when either selective antagonist was combined with the agonist selective for the complementary receptor subtype, both D2 receptor proliferation and behavioral supersensitivity were completely blocked. Combined antagonist-agonist treatments had opposing effects on the development of tolerance to antagonist-induced catalepsy. D2 – but not D1 – receptor densities were correlated with animals' behavioral responses to apomorphine. There results support and extend the notion that complex functional interactions between D1 and D2 receptor families occur

within the central nervous system, and suggest that novel effects might be derived from combined administration of receptor selective agonists and antagonists.

Keywords: D_1D_2 dopamine receptor, behavior chronic stereotypy, locomotion, catalepsy, supersensitivity

Introduction

An increasing body of evidence suggests that the D1 and D2 families of dopamine receptors interact functionally in ways that may be of potential importance in the pharmacotherapy of neuropsychiatric disorders (Gerfen and Engber, 1992; Gomez-Mancilla and Bedard, 1991; Seeman and Niznik, 1990; Seeman et al., 1989, 1987). These interactive effects, which appear to be both facilitatory and antagonistic, have been most thoroughly investigated in acute studies of the behavioral or biochemical effects of receptor selective agonists (Martin-Iverson, 1991; Braun and Chase, 1988; Walters et al., 1987; Arnt et al., 1987; Molloy and Waddington, 1988). While it is important to characterize functional interactions which are manifest acutely – i.e. following a single dose of drugs – chronic studies may be more important in predicting the consequences of receptor-selective drug combinations in a clinical setting.

This is particularly true in the case of dopamine antagonist therapy, in which side effects and drug efficacy are often manifest after long-term receptor blockade. There have been a number of studies evaluating the effects of chronic exposure to selective antagonists, however these studies have largely focused on contrasting the effects of D1 or D2 selective antagonists administered independently (Duffy et al., 1992; Vaccheri et al., 1987; Esposito and Bunney, 1989; Lublin et al., 1993; LaHoste and Marshall, 1991; Grebb et al., 1990). Only a limited number of studies have examined the chronic effects of D1 and D2 antagonists administered in combination (Schettini et al., 1992; Hess et al., 1988; Marin et al., 1993; Parashos et al., 1989), and there has been, to date, no systematic investigation of all of the potential interactions between dopamine receptor subtypes which could accompany chronic drug exposure. How, for example, might the behavioral or biochemical effects of chronic D2 receptor blockade be modified by concurrent stimulation – or inhibition – of the D1 receptor? How might concurrent manipulation of D2 receptor function modify the effects of chronic D1 receptor blockade?

Dopaminergic agents are used to treat a wide variety of neuropsychiatric disorders. Dopamine antagonists constitute the principal drug therapy for schizophrenia, and are used in the treatment of affective illnesses and hyperkinetic extrapyramidal disorders; dopamine agonists remain the first-line treatment for Parkinson's disease. Because selective agonists administered in combination have proven to behave in novel, unpredictable and potentially useful ways (Braun and Chase, 1986; Walters et al., 1987; Mashurano and Waddington, 1986; Arnt and Perregaard, 1987), we would predict that combinations of antagonists or of antagonists and agonists may have similar unanticipated and potentially significant effects that might impact upon the clinical treatment of the disorders outlined above. Antagonist

or agonist-antagonist interactions could affect tolerance to primary drug effects, as well as the emergence of acute and chronic side effects – e.g. sedation, parkinsonism or tardive dyskinesia with the use of antagonists, and the emergence of dyskinesias which accompany the adminstration of agonists.

In addition to having an impact upon clinical phenomena, interactions between receptor subtypes might be of theoretical importance as well. The distribution of dopamine receptors in the brain is inhomogeneous, and the families of D1 and D2 receptors have been shown to be associated with distinct cortical and subcortical pathways (Gerfen, 1992). Of potential significance is the concept that selective modulation of distinct neuronal networks – for example, activation of a D1 receptor sensitive and concurrent inhibition of a D2 receptor sensitive circuit – might represent a potential therapeutic strategy. Evaluating the effects of such manipulations could conceivably lead to the rational design of drugs used to treat neurological or pyschiatric illnesses.

The present studies were designed to evaluate the chronic effects of selective antagonists administered alone and to see how these effects are modified by concurrent administration of agonists or antagonists selective for the complementary receptor subtype. Three behaviors were evaluated which in turn reflect 1) unstimulated activity of endogenous dopamine neurons (spontaneous locomotor activity), 2) responses to dopamine receptor stimulation (apomorphine-induced stereotypic behavior), and 3) responses to dopamine receptor blockade (antagonist-induced catalepsy). Behaviors were studied before and after chronic treatment, after which animals were sacrificed and striatal D1 and D2 receptor binding was measured.

Materials and methods

Animals

Male Sprague Dawley rats (Taconic Farms, NY), weighing 250–270 grams at the outset were allowed free access to food and water and housed 5 to a cage in a temperature controlled room illuminated on a 12 hour light/dark cycle. Animals were maintained under these conditions throughout the experiment except during periods of behavioral testing.

Initial evaluation of apomorphine sensitivity

Following a week-long period of acclimatization, all animals were tested for behavioral responses to apomorphine (675 μg/kg). Rats were individually housed and acclimated to observation cages for at least one hour prior to drug injection. Apomorphine hydrochloride (Sigma Chemical Co., St. Louis, MO) was dissolved in distilled water immediately before subcutaneous administration. Behaviors were observed in a series of consecutive 15-second intervals evenly distributed throughout a 60 minute period of observation which began five minutes following drug injection. Intensity of oral stereotypies was scored using a modification of the Ernst scale (Klawans et al., 1983). Cumulative Ernst scores (the sum of interval scores derived throughout the period of observation) were calculated for each animal. On the basis of these scores, animals were assigned to one of 6 treatment groups, such that each group was equivalent with respect to apomorphine sensitivity.

Chronic treatments

Following a week-long drug-free interval, groups of ten animals each were treated for 28 days with one of the following drug regimens: SCH 23390 (Research Biochemicals International, Natick, MA), 1 mg/kg; metoclopramide (A. H. Robins, Richmond, VA), 20 mg/kg; SCH 23390 + metoclopramide; SCH 23390 + LY 171555 (quinpirole, Eli Lilly and Co., Indianapolis, IN), 1.5 mg/kg; metoclopramide + SKF 38393 (Research Biochemicals International), 8 mg/kg; and saline. All drugs were delivered intraperitoneally at the above doses twice daily.

Behavioral observation and scoring

Catalepsy was evaluated 60 minutes following the first and last doses of the above drug combinations. Animals were placed on a vertical wire grid and the amount of time which elapsed until animals had descended, placing at least two paws on the floor of the cage, was recorded. Immobility scores were derived by averaging the results of two trials, separated by an interval of five minutes.

Spontaneous locomotor activity occurring within the home cage was evaluated 18 hours after the first and last doses of these drug combinations. Animals were observed in four separate minute-long observation periods evenly distributed over the course of one hour. In each period, activity was scored on a scale of 0 to 3 (0 – inactivity; 1 – motor activity observed less than 50% of time; 2–activity observed more than 50% of the time; 3 – continuous motor activity) in four separate minute-long observation periods evenly distributed over a period of one hour, and a mean behavioral score was derived for each animal.

At the end of a 72-hour drug-free interval which followed the chronic treatment period, the animals' stereotypic behavioral response to 675 µg/kg of apomorphine s.c. was again evaluated, according to the protocol outlined above.

D1 and D2 receptor binding

At the completion of a one-week washout period following the administration of the final test dose of apomorphine, animals were decapitated, brains were removed and the dorsal striata excised and prepared for assay of D1 and D2 receptor binding. Striatal samples excluded the nucleus accumbens and globus pallidus, and were obtained as a continuous block extending from +2.0 mm to −1.4 mm relative to Bregma (Paxinos and Watson, 1986). Samples were frozen at −70 C until assay. All samples were analyzed on the same day and utilized the same standards. Samples were individually thawed in 1/20 w/v of buffer (in mM: Tris-HCl 50, NaCl 120, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, pH 7.4 at 37 C). After homogenization with 10 strokes of a motor-driven Teflon pestle, samples were diluted to 1/40 w/v buffer and preincubated at 37 C for 10 min. The samples were then centrifuged (15,000 rpm, 10 min, 4 C). The pellets were resuspended in the same volume of buffer by vigorous vortexing and recentrifuged. The last step was repeated twice.

Assays were carried out at a single concentration of labeled ligand on each individual sample following previously described methods (Billard et al., 1984; Leysen et al., 1978), with only slight modifications. [^{3}H]spiperone was purchased from Amersham and [^{3}H]SCH23390 from NEN. (+)butaclamol, ketanserin and SCH23390 were purchased from Research Biochemicals International. Incubation was performed in glass test tubes at 37 C for 30 min in a final volume of 600 µl, conditions in which equilibrium is established [per kinetic experiments of the referenced papers (Billard et al., 1984; Leysen et al., 1978)].

For the D2 receptor assay the incubation mixture was composed of 300 µl of the membrane suspension (final tissue dilution of 1/200 w/v, 0.2–0.4 mg of protein per ml as determined by the method of Lowry (Lowry et al., 1951), 100 µl of [^{3}H]spiperone (final concentration of 0.25 nM), 100 µl of ketanserin (final concentration of 100 nM) and either

100 µl of buffer (including 1% of ethanol) or 100 µl of unlabeled (+)butaclamol (final concentration of 1 µM) for non-specific binding. For the D1 binding assay, the incubation suspension consisted of 300 µl of the same membrane suspension, 100 µl of [^{3}H]SCH23390 (final concentration of 0.5 nM), 100 µl of ketanserin (final concentration of 50 nM) and either 100 µl of buffer or 100 µl of unlabeled SCH23390 (final concentration of 100 nM) for nonspecific binding. Each incubation was performed in duplicate. For both receptor assays, the incubation period was terminated by filtration through GF/C Brandel filters, presoaked for 30 min with 0.01% polyethyleneimine. Filters were washed three times with 4 ml of ice cold buffer (Tris-HCl 50 mM, pH 7.4) and placed in plastic vials with 5 ml scintillation fluid (Aquasol) and counted the following day in a beta scintillation counter (Beckman LS 5000 TA, Fullerton, CA). In each case, no more that 15% of the total counts bound were non-specific, as determined by addition of excess unlabelled competitive ligand.

Statistical analysis

All behavioral evaluations were carried out by a single rater, unaware of the animals' treatment status. Animals were tested at the same time of day for each behavior evaluated. Data were analyzed for statistical significance using one-way factorial and repeated-measures analyses of variance in combination with least significant difference and Ryan-Einot-Gabrial-Welsch multiple range tests.

Comparison of D1 and D2 binding parameters were performed blind to the treatment group. Group differences were estimated by ANOVA. In case of significant overall difference between groups, post-hoc analyses were performed using least significant difference multiple range tests.

Results

Catelepsy

Both the selective D1 and D2 antagonists, when administered alone, induced moderate degrees of catalepsy, measured as duration of immobility on a wire grid (Fig. 1, Table 1), following administration of the first dose. However, in both cases continued treatment resulted in tolerance to the cataleptogenic effects of these agents: immobility scores following the final dose were in both instances significantly lower than those obtained at the outset.

On the other hand, combined administration of the antagonists appeared to have a synergistic effect, producing significantly higher levels of cataleptic immobility upon administration of the first dose which did not show evidence of tolerance following continued treatment (Fig. 1, Table 1).

When the D1 antagonist was administered together with the selective D2 family agonist, animals again showed a moderate cataleptic response acutely and developed tolerance following chronic exposure. However, when the D2 antagonist was administered together with the D1 agonist, although animals continued to display moderate degree of catalepsy following the first dose, they did not show evidence of tolerance with chronic treatment.

Spontaneous locomotor activity

Spontaneous locomotor activity (Fig. 2, Table 1) observed 18 hours after the initial dose of any of the drug combinations did not differ significantly from that observed in control animals. Chronic exposure, however, produced sig-

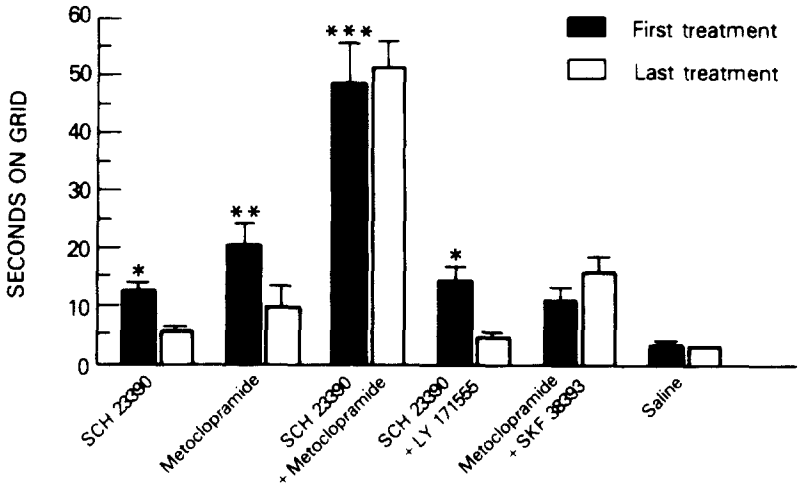

Fig. 1. Cataleptic responses observed 60 minutes after administration of the first (black bars) and last (white bars) treatment with the drug combinations indicated (N = 10, each group). Values represent the number of seconds which elapsed between the time animals were placed on a vertical wire grid and descended, placing at least two paws on the floor of the cage. Bars represent group means ± SD. *$p < 0.01$ vs. post-treatment values within the same treatment group. **$p < 0.05$ vs. post-treatment values within the same treatment group. ***$p < 0.01$ vs. all other values except post-treatment values within the same group

Table 1. Qualitative summary of the effects of chronic exposure to dopamine agonists and antagonists

Chronic treatment	Cataleptic response		Spontaneous motor activity		Stereotypic behavior	D1 Receptor density	D2 Receptor density
	First dose	Last dose	First dose	Last dose	Post treatment	Post treatment	Post treatment
D1 Antagonist	+	— 0	+	— ++	+	0	+
D2 Antagonist	+	— 0	+	— 0	+	0	+
D1 Antagonist + D2 Antagonist	++	— ++	+	— +	++	0	++
D1 Antagonist + D2 Agonist	+	— 0	+	— ++	0	0	0
D2 Antagonist + D1 Agonist	+	— +	+	— 0	0	0	0

Catalepsy and spontaneous locomotor activity following first and last treatments are summarized. Stereotypy scores represent changes vs. pre-treatment baseline. D1 and D2 receptor densities are compared to values derived for saline-treated animals

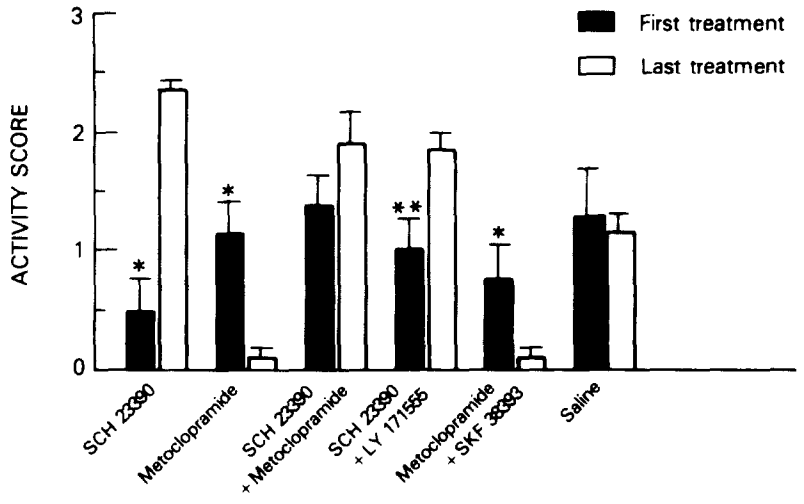

Fig. 2. Spontaneous locomotor activity occurring within the home cage, evaluated 18 hours after the first (black bars) and last (white bars) treatment with the drug combinations indicated (N = 10, each group). Activity was scored on a scale of 0 (inactivity) to 3 (continuous motor activity) in four separate minute-long observation periods over one hour and a mean behavioral score was derived. Bars represent group means ± SD. *$p < 0.01$ vs. post-treatment values within the same treatment group. **$p < 0.05$ vs. post-treatment values within the same treatment group

nificant changes in this behavior. Treatment with the selective D1 and D2 antagonists had distinct and contrasting consequences when these were administered independently: chronic exposure to the D1 antagonist resulted in significantly higher levels of activity 18 hours after the final dose, while chronic exposure to the D2 antagonist resulted in a significant reduction in spontaneous locomotor activity.

Combined administration of the selective antagonists, however, appeared to cancel the effects observed in animals treated with either individual antagonist: spontaneous locomotor activity 18 hours after either the first or last dose of the combination of D1 and D2 antagonists did not significantly differ from activity observed in control animals (Fig. 2, Table 1).

The effects of the selective antagonists were, on the other hand, unaffected by concurrent administration of the complementary receptor-specific agonists (Fig. 2, Table 1).

Apomorphine-induced stereotypic behavior

The behavioral responses of animals to 675 μg/kg apomorphine (Fig. 3, Tables 1, 2) suggest that functional interactions between dopamine receptor subtypes affect the emergence of behavioral supersensitivity during the course of chronic treatment.

Both the selective D1 and D2 antagonists, when administered alone, produced augmented responses to apomorphine; scores obtained in animals treated with either of these drugs were significantly higher than those obtained in saline-treated controls. (Control animals showed an increase of

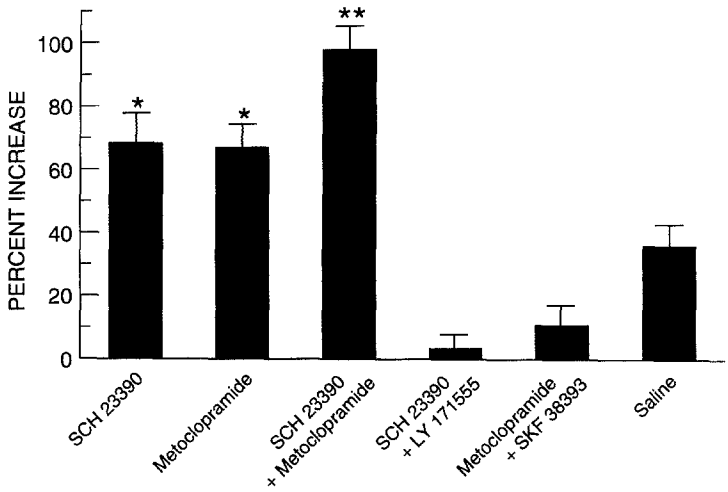

Fig. 3. Percent increases in stereotypic behavioral responses to apomorphine (675 μg/kg) following chronic drug exposure. Ernst scores obtained prior to treatment are compared with those obtained in the same animals following chronic treatment with the drug combinations indicated (N = 10, each group). Bars represent percent changes within each group (mean ± SD). *$p < 0.01$ vs. saline; metoclopramide + SKF 38393; SCH 23390 + LY 171555; and metoclopramide + SCH 23390. **$p < 0.01$ vs. all other groups

Table 2. Stereotypic behavior elicited by apomorphine prior to and following chronic treatment with selective agonists and antagonists

Group	Pre-treatment	Post-treatment
SCH 23390	18.5 ± 2.3	30.1 ± 3.7*
Metoclopramide	17.8 ± 2.7	29.5 ± 2.7*
SCH 23390 + Metoclopramide	19.7 ± 2.6	40.8 ± 2.2**
SCH 23390 + LY 171555	19.3 ± 2.4	19.8 ± 2.6
Metoclopramide + SKF 38393	20.5 ± 2.6	21.6 ± 3.1
Saline	17.4 ± 2.4	23.2 ± 2.7

Stereotypic behaviors elicited by 675 μg/kg apomorphine s.c. were scored on the modified Ernst scale. Treatment groups (N = 10) are identified by the the drugs to which animals were chronically exposed. Values represent mean Ernst scores ± standard errors. *$p < 0.05$ vs. pre-treatment baseline; **$p < 0.01$ vs. pre-treatment baseline

approximately 33% over their own baseline, a phenomenon which is typically seen and attributed to a difference in absolute CNS dose due to the relative rates of brain and body growth.)

Combined administration of the selective antagonists appeared to have an additive effect. Chronic co-admininstration of these compounds produced behavioral scores which were significantly higher than those induced by either antagonist alone.

However, when either selective antagonist was combined with an agonist selective for the complementary receptor subtype (i.e. SCH 23390 plus LY 171555, or metoclopramide plus SKF 38393) the development of behavioral

supersensitivity was completely blocked. The effect of concurrent D2 receptor stimulation in blocking behavioral supersensitivity induced by the D1 receptor antagonist (the effect of SCH 23390+ LY 171555 vs. SCH 23390 alone) was more robust than the effect of concurrent D1 receptor stimulation on D2 receptor blockade (metoclopramide + SKF38393 vs. metoclopramide alone). In fact, the combination of SCH 23390 and LY 171555 appeared to produce subsensitive behavioral responses when Ernst scores in this group of animals were compared to controls, although this difference did not attain statistical significance.

Striatal D1 and D2 receptor binding

While chronic treatments appeared to have no significant effects on D1 receptor density (Tables 1, 3), there was a trend toward an increase in D1 receptor number following chronic treatment with SCH 23390 (Fig. 4).

On the other hand, chronic drug treatments significantly affected D2 receptor density (Tables 1, 3, Fig. 5). Chronic exposure to both SCH 23390 alone and metoclopramide alone resulted in D2 receptor upregulation.

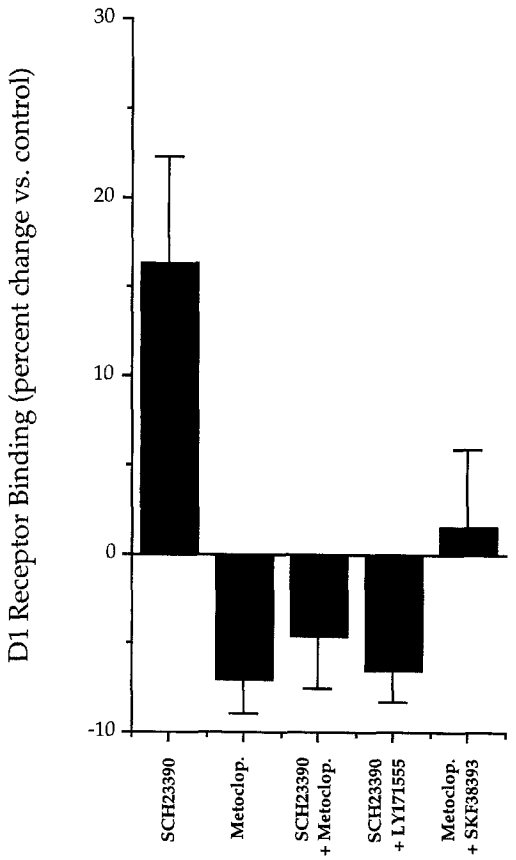

Fig. 4. D1 receptor binding as a function of chronic treatment. Bars represent the percent change in D1 receptor density in the various treatment groups vs. values measured in control animals (mean ± SD; N = 10, each group)

Table 3. Analysis of variance

Source	df	Sum squares	Mean square	F-test
D1 Receptor				
Between groups	5	4.62E + 08	9.25E + 07	0.227
Within groups	54	2.20E + 10	4.07E + 08	NS
Total	59	2.25E + 10		
D2 Receptor				
Between groups	5	2.65E + 09	5.31E + 08	4.05
Within groups	52	6.82E + 09	1.31E + 08	p = 0.003
Total	57	9.47E + 09		

D1 and D2 receptor densities in animals following chronic exposure to selective agonists, antagonists or saline are evaluated. Grouping factor = chronic treatment

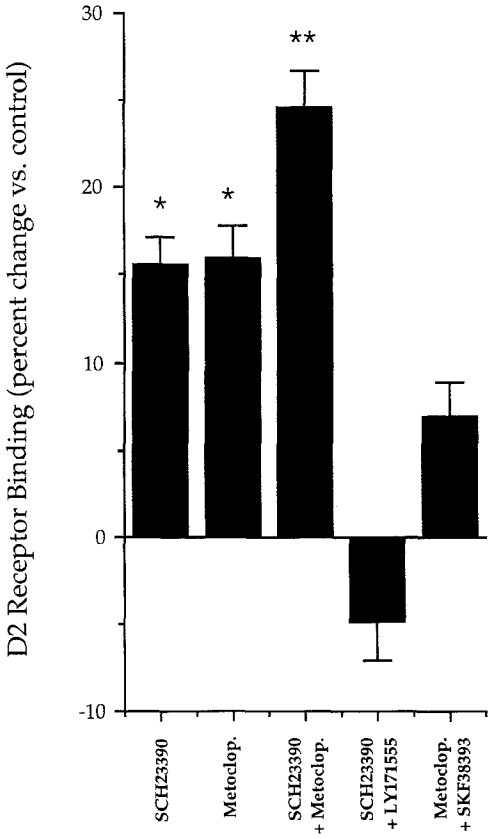

Fig. 5. D2 receptor binding as a function of chronic treatment. Bars represent the percent change in D2 receptor density in the various treatment groups vs. values measured in control animals (mean ± SD). *$p < 0.025$, **$p < 0.005$ vs. saline

The combination of D1 and D2 antagonists appeared to have an additive effect. D2 receptor density was greater in this treatment group than in those treated with either selective antagonist independently, although these differences did not reach statistical significance.

In contrast, the combinations of antagonist and complementary receptor agonist – in both cases – blocked the D2 receptor upregulation observed in animals chronically exposed to either antagonist alone (Fig. 5, Table 1).

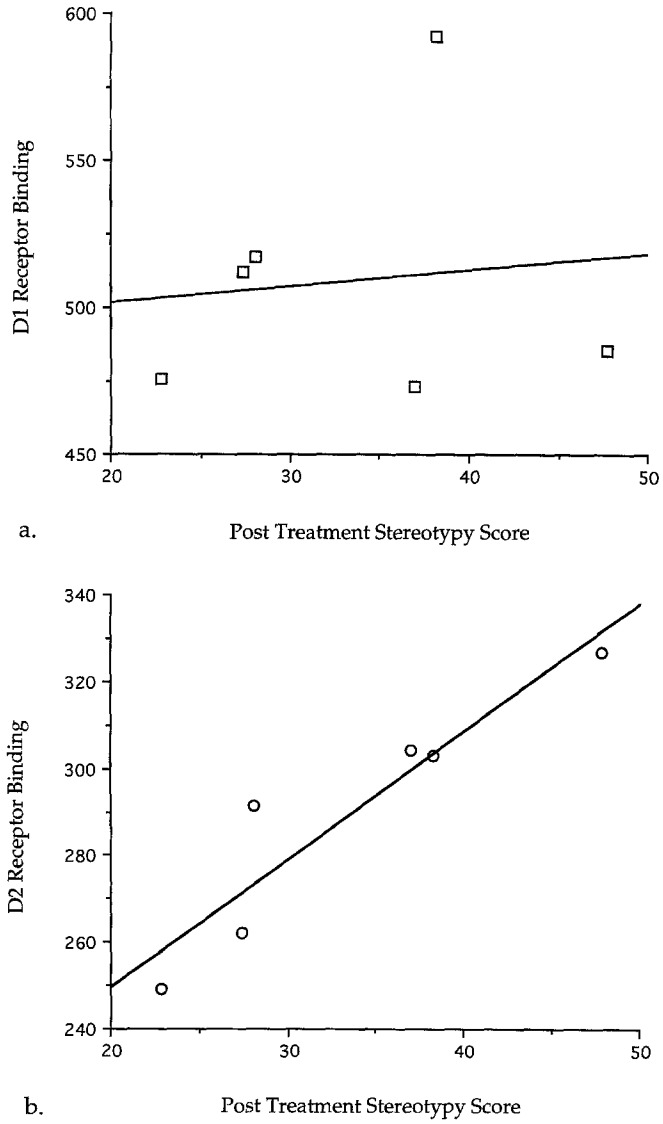

Fig. 6. Correlations between mean post-treatment Ernst scores and D1 (**a**) and D2 (**b**) receptor densities (dpm/mg protein) derived for each of the six treatment groups. Behavioral scores were significantly correlated with D2 receptor density ($F = 28.29$, $p = 0.006$), but not with D1 receptor density ($F = 0.052$, NS)

Relationship between receptor binding and behavioral measures

The effects of chronic drug exposure on D2 receptor density and behavioral responses to apomorphine were congruent (see Table 1). While D1 receptor density was not correlated ($r = 0.115$, $p = NS$, Fig. 6a), D2 receptor density was significantly correlated with measures of behavioral responsiveness (post treatment Ernst scores) across treatment groups ($r = 0.936$, $P < 0.006$, Fig. 6b). Neither D1 or D2 receptor densities correlated with measures of catelepsy or spontaneous locomotor activity.

Discussion

The foregoing results illustrate that complex functional interactions between receptor subtypes accompany chronic exposure to selective dopamine agonists and antagonists. The effects of selective D1 or D2 receptor blockade on behavior and dopamine receptor density appear to be significantly altered either by concurrent stimulation or by blockade of the complementary receptor subtype. These observations may be of heuristic value in attempting to model the complexity of dopamine receptor interactions in the CNS.

Selective antagonists administered alone

The finding that chronic treatment with either the selective D1 or D2 antagonist resulted in behavioral supersensitivity to apomorphine is consistent with several previous studies (Vaccheri et al., 1987; Parashos et al., 1989; Marin et al., 1993; LaHoste and Marshall, 1992, but see also Chipkin et al., 1987). Similarly, the observation that acute administration of either the D1 or D2 antagonists induces catalepsy, and that continued administration results in tolerance has been reported previously (Verma and Kulkarni, 1992; Meyer et al., 1992), although tolerance to the cataleptogenic effects of D1 receptor blockade following chronic treatment is more controversial and has not been consistently reported (Marin et al., 1993; Hess et al., 1988).

We observed an increase in baseline spontaneous locomotor activity following chronic D1 receptor blockade, and a decrease following chronic blockade of the D2 receptor, both of which have been reported previously (Smialowski, 1989; Waddington and Gamble, 1980; Campbell et al., 1993). In the present study, these effects appear to be unequivocally related to treatment chronicity (since they were not observed following the first dose), and do not appear to represent the direct effects of the drugs themselves, since the serum half lives of both SCH 23390 and metoclopramide are between one and two hours and thus changes in motor activity observed after eighteen hours are not likely to be due to the presence of active drug in the brain.

The effects of chronic D2 receptor blockade which we observed – proliferation of D2 receptors without a significant effect on D1 receptor binding – are generally consistent with previous observations (Parashos et al., 1987; LaHoste and Marshall, 1991; Stefanini et al., 1991; LaHoste and Marshall, 1989; McGonigle et al., 1989; See et al., 1989). The trend towards a *decrease* in

D1 receptor density which we observed has been noted previously (Laruelle et al., 1992). While we detected a trend toward an increase in D1 receptor density following chronic D1 receptor blockade, variances were high and the effect did not attain statistical significance. Previous studies have found either increases (Parashos et al., 1987; Lappalainen et al., 1992; McGonigle et al., 1989; Hess et al., 1986; Giorgi et al., 1993; Cheetham et al., 1995) or no change (Lappalainen et al., 1990) in D1 receptor binding following chronic D1 antagonist exposure.

However, upregulation of the D2 receptor by chronic D1 antagonist exposure noted in the present study was unexpected. Previous studies (Duffy et al., 1992; McGonigle et al., 1989; Hess et al., 1988; Lappalainen et al., 1990) noted no apparent effect of chronic treatment with SCH 23390 on D2 receptor density. These studies, however, generally used lower doses of SCH 23390 and shorter durations of treatment. In addition, the drug-free interval prior to sacrifice was longer in the present study suggesting that the effects on D2 receptor might develop over a longer time-course following the discontinuation of D1 antagonist treatment, and may thus have been missed in earlier studies. Replication of these findings will be necessary.

Selective antagonists administered in combination

The acute effects of combined antagonist treatment on catalepsy appeared to be truly synergistic – i.e. immobility scores were more than twice those observed in animals treated with either antagonist alone. This phenomenon has been previously documented (Parashos et al., 1989; Verma and Kulkarni, 1992). Unlike animals treated with either selective antagonist alone, however, those receiving D1 and D2 antagonists concurrently showed no evidence of tolerance following chronic treatment. It is uncertain whether or not D1 and D2 receptor antagonists exert their effects via discrete mechanisms or through a single final common pathway. Nevertheless, the failure of these animals to adapt suggests that tolerance developing in the course of chronic receptor blockade may involve compensatory mechanisms taking place in the complementary receptor system, or the postsynaptic processes to which it is coupled, which may fail to develop when the complementary receptor is blocked. It should be mentioned, however, that changes in absolute intensity or duration of catalepsy – which we used as indices of tolerance – can clearly be affected by other factors which although they may be drug induced, do not constitute classical development of tolerance to drugs themselves (Sanberg et al., 1988). For example, intensity can be affected by changes in blood pressure as well as sensitivity to exteroceptive stimuli or other environmental influences. Furthermore, the effects we saw may not be due entirely to the drugs' actions at D1 and D2 receptors, but might be mediated through secondary effects on other systems – e.g. GABA or acetylcholine – which are known to affect the cataleptic response.

In contrast, combined antagonist treatment appeared to offset the independent effects of D1 or D2 receptor antagonists on spontaneous locomotor activity. Activity in animals treated with the combination of antagonists did

not differ significantly from that observed in control animals. The chronic effects of either D1 or D2 receptor inhibition on spontaneous locomotor activity may similarly require that the complementary receptor system remain pharmacologically unblocked and subject to stimulation by endogenous dopamine at levels which could be dictated (i.e. "reset") by blockade of a single receptor subtype. On the other hand, the present results may simply represent an additive phenomenon, with independent, opposing effects of chronic D1 and D2 blockade canceling one another out. The notion that some chronic effects of selective receptor blockade can be attenuated by simultaneous inhibition of the complementary receptor is nevertheless supported by prior observations (Marin et al., 1993; Parashos et al., 1990, 1987).

The additive effect of concurrent D1 and D2 receptor blockade on the supersensitive behavioral response to apomorphine is a novel observation. Other studies have reported either no effect (Parashos et al., 1989) or a diminished behavioral response (Marin et al., 1993) following combined treatment with D1 and D2 receptor antagonists. Differences between these results and our own could represent differences in the drugs utilized, doses selected or treatment parameters employed. Nevertheless, the behavioral results we observed were paralleled by an similar effect of combined D1 and D2 antagonist exposure on D2 receptor binding. These results – taken together with the results of combined agonist and antagonist treatment discussed below – suggest that the behavioral and biochemical consequences of chronic antagonist exposure might be partially attenuated by mechanisms which are coupled to the complementary receptor system, and that this process may be interrupted when the complementary receptors are blocked – in this case resulting in maximal D2 receptor proliferation and peak behavioral responses to apomorphine.

Combined treatment with agonists and antagonists

Concurrent treatment with either antagonist and the *agonist* selective for the complementary receptor subtype produced unexpected results, appearing to profoundly affect the emergence of behavioral supersensitivity, tolerance to catalepsy, and increases in D2 receptor density.

While chronic treatment with either antagonist alone significantly augmented the animals' subsequent behavioral response to apomorphine, the development of behavioral supersensitivity was blocked, in either case, by coadministration of the complementary receptor agonist. In parallel, antagonist-induced D2 receptor proliferation was prevented by concurrent stimulation of the complementary receptor subtype. A similar effect of D1 agonist treatment on behavioral supersensitivity and D2 receptor proliferation induced by chronic haloperidol exposure has been reported previously (Marin and Chase, 1993).

These results suggest that the behavioral and biochemical consequences of chronic antagonist exposure may depend upon a degree of plasticity in the complementary receptor system, the intraneuronal processes to which it is coupled, or the brain systems which it modulates. Specifically, the emergence

of behavioral supersensitivity or D2 receptor proliferation might be partially attenuated by ongoing stimulation of the complementary receptor subtype by endogenous dopamine. Augmentation of this process by direct stimulation of the complementary receptor with a selective agonist appears to inhibit – just as blockade appeared to augment – dopamine receptor proliferation and the emergence of behavioral supersensitivity.

The physiological consequences of the two agonist/antagonist combinations did not appear to be equivalent, however. Blunting of antagonist-induced supersensitivity by complementary receptor stimulation was more pronounced when the D2 agonist LY 171555 was combined with the D1 antagonist SCH 23390. The combined agonist/antagonist treatments had differing effects on catalepsy as well: concurrent administration of the D2 agonist did not interfere with the development of tolerance to D1 antagonist-induced catalepsy, while concurrent administration of the D1 agonist and D2 antagonist metoclopramide did appear to block the development of tolerance induced by D2 receptor blockade.

Relationship between changes in receptor density and behavior

Changes in animals' behavioral response to apomorphine – the induction, augmentation and blockade of emergent supersensitivity – were correlated with, and may be primarily mediated by, changes in D2 receptor density. Thus, our results suggest that the effects of chronic neuroleptic treatment may be linked to changes induced in the D2, rather than D1, family of receptors. This relationship, however, only pertains to apomorphine-induced stereotypic behavior. Changes in D2 receptor density do not correlate with tolerance to cataleptogenic effects, or to the effect of chronic treatment on spontaneous locomotor activity.

The idea that behavioral consequences of neuroleptic exposure, serving as models for the chronic extrapyramidal side effects of these drugs, are mediated principally through changes in D2 receptor function has been suggested by a number of previous studies (Jenner et al., 1985; Murugaiah et al., 1984), although this notion is not universally supported (LaHoste and Marshall, 1992). Changes in D2 receptor density may account for the dyskinetic side effects of dopamine agonist treatment as well. Bedard and co-workers (Bedard et al., 1993) have shown that chronic bromocriptine administered to drug naive MPTP treated monkeys did not induce dyskinesia. Subsequent analysis of brains from these monkeys revealed a down regulation of D2 receptors. This suggests a pivotal role for D2 receptors in contributing to hyperkinetic behaviors induced by chronic exposure to both dopamine agonists and antagonists.

Alternatively, the behavioral effects we observed might be coupled to changes in the D1 system as well, but mediated via increases in the sensitivity of mechanisms coupled to the D1 receptor rather than changes in receptor binding per se. Such effects – e.g. drug-induced increases in the activity of dopamine sensitive adneyate cyclase, responsiveness of cyclase to GTP, or in the expression of G-protein mRNA, which are unaccompanied by changes in

D1 receptor number – have been documented previously (Jenner et al., 1983; Schettini et al., 1992; Groppetti et al., 1986; Parenti et al., 1986).

Since neither D1 nor D2 receptor binding was correlated with changes in spontaneous locomotor activity or catalepsy, the effects of chronic treatment on these behaviors may have been manifest via the "downstream" postsynaptic mechanisms outlined above or, alternatively, via changes manifest presynaptically.

It should be noted that only striatal receptors were evaluated in the present study. All of the behavioral changes seen, including tolerance to catalepsy, modification of spontaneous locomotor activity, and altered responses apomorphine, might also be related to *extrastriatal* D1 or D2 receptor function. This possibility is supported by previous studies demonstrating that effects of systemically administered antagonists on receptor binding may be regionally inhomogeneous (Lappalainen et al., 1990, 1992; Memo et al., 1987).

Anatomical substrates of antagonist or agonist-antagonist interactions

What anatomic substrate might account for the functional interactions between systemically administered agonists and antagonists? These could take place at the level of the individual neuron or within the same local neuronal field where chronic stimulation or inhibition of receptors might have simple facilitatory or antagonistic effects. For example, D1 and D2 receptors have been shown to have local interactive effects on immediate early gene expression (Paul et al., 1992), neuronal adenylate cyclase activity (Stoof and Kebabian, 1981), and regulation of G-protein synthesis (Schettini et al., 1992).

On the other hand, interactions between D1 and D2 receptors need not be local. The distribution of dopamine receptor subtypes in the brain is inhomogeneous, and the families of D1 and D2 receptors have been shown to be associated with distinct cortical and subcortical pathways. For example, D1 receptors are more abundant in certain regions of the neocortex where they may be selectively associated with corticostriatal glutamatergic projections (Boyson et al., 1986; McGeer et al., 1977). Within the striatum, D1 and D2 receptors appear to be associated with distinct subsets of intrinsic output neurons, projecting to nigral and pallidal targets respectively (Gerfen, 1992; Hallett, 1993).

In light of these inhomogeneities, selective stimulation or blockade of D1 and D2 receptors might theoretically represent selective activation or inhibition of activity in discrete neuronal circuits. For example, chronic D1 receptor stimulation and D2 receptor blockade might result in long-term activation of corticostriatal and striatonigral pathways and concomitant suppression of striatopallidal outflow. It may be that selective modulation of discrete neuronal networks – rather than local facilitatory or antagonistic interactions – might represent the authentic substrate for interactions between receptor subtypes, and may be responsible for the long-term consequences of chronic exposure.

Whether these complex, interactive effects operate at the level of individual neurons, local circuits or within distributed brain systems, the possibility of producing unique behavioral or biochemical effects by combined treatment with D1 and D2 agonists or antagonists might be of value in the design of future drug therapies for neuropsychiatric illnesses.

Clinical considerations

The behaviors evaluated in the present study have served as animal models for clinical features which accompany dopamine antagonist treatment – both primary antipsychotic effects and extrapyramidal side effects of chronic antagonist exposure, for example.

Behavioral supersensitivity in response to systemic apomorphine and long-term changes in dopamine receptor sensitivity may be predictive of potential chronic extrapyramidal side effects (EPS), such as tardive dyskinesia, dystonia or Tourette-like features (Klawans et al., 1983; Janssen et al., 1966; Janssen and Awouters, 1994). Catalepsy may predict the development of acute parkinsonian side effects of neuroleptic treatment (Elliott et al., 1990; Hornykiewicz, 1975), and tolerance to these cataleptogenic effects may predict habituation to the acute EPS observed clinically. Alterations in spontaneous locomotor activity may serve as a behavioral measure of changes in limbic dopamine transmission which accompany chronic neuroleptic exposure (Robbins and Koob, 1985; Costall and Naylor, 1975; Kelly et al., 1975).

In the present study, both D1 and D2 antagonists administered alone induced behavioral supersensitivity, D2 receptor upregulation, and catalepsy (to which tolerance readily developed), suggesting that these treatments might have similar potential for the induction of acute EPS (which would be expected to habituate over time), as well as the emergence of chronic EPS. The contrasting effects on spontaneous locomotor activity, however, suggest that these drugs may have different effects on limbic dopamine transmission in the course of chronic treatment – which could represent significant differences in the primary antipsychotic effects of these compounds.

The combined administration of D1 and D2 antagonists produced significant augmentation of the behavioral responses to apomorphine and proliferation of D2 receptors which were observed in animals treated with the selective antagonists independently. Moreover, combined treatment had a synergistic effect on catalepsy to which tolerance did not develop in the course of chronic treatment. These findings suggest that such combinations could have the potential for the development of more severe acute EPS (which may not habituate) as well as more significant chronic EPS. If these behavioral and biochemical endpoints are at least partially reliable as predictors of clinical responses, the use of such combinations might represent a significant risk and their clinical evaluation might be approached with these caveats in mind.

On the other hand, the coadministration of antagonists and complementary receptor agonists in either case attenuated D2 receptor proliferation and blocked the development of behavioral supersensitivity – but did not alter the

primary effects of antagonist treatment on spontaneous locomotor activity. These observations suggest that combined D1–D2 agonist-antagonist treatment of psychotic disorders might be less likely to induce chronic extrapyramidal side effects, without significantly altering antipsychotic potential. Testing the effect of such drug combinations on conditioned avoidance responses – which may serve as more reliable models of antipsychotic efficacy – will be necessary in order to support, extend or modify these hypotheses. In addition to the treatment of psychosis, pharmacotherapy of hyperkinetic disorders in which symptoms respond to dopamine receptor blockade – e.g. Tourette's syndrome, choreatic disorders or stuttering – might in theory be more effectively treated, with fewer attendant side effects, utilizing novel combinations of D1 and D2 receptor selective compounds.

Finally, the notion advanced above – that concurrent administration of D1 and D2 receptor agonists and antagonists may make it possible to selectively stimulate and/or attenuate activity in discrete neuronal circuits – may impact upon the treatment of extrapyramidal disorders for which relatively detailed pathophysiological models currently exist. If this notion is correct – that is, if such agonist-antogonist combinations were to make it possible to selectively modify activity in direct and indirect striatal motor circuits, or to differentially regulate outflow through stiatonigral and striatopallidal pathways – it would prove to be of value in the rational design of pharmocotherapies for disorders such as Parkinson's disease or dystonia.

Conclusions

The foregoing results support the idea that complex functional interactions between D1 and D2 receptor families occur within the central nervous system. Behavioral and biochemical effects of selective receptor blockade are significantly altered by concurrent stimulation – or inhibition – of the complementary receptor subtype. These effects may reflect local facilitatory or antagonistic processes or may represent activation or inhibition of neuronal circuits with which the receptors may be selectively associated. Combined administration of antagonists and agonists for the complementary receptor subtype had unexpected consequences which could prove to be useful in a clinical setting. For example, agonist-antagonist combinations may affect parameters which serve as models for the unwanted side effects of long-term neuroleptic treatment without affecting those which may reflect the primary antipsychotic actions of these drugs. Attention to receptor interactions may become increasingly important as more selective agonists and antagonists become available – particularly those which will discriminate between subtypes of the D1 (D1a and D1b) and D2 (D2, D3, D4) dopamine receptor families themselves.

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