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Chronic Treatment with Dopamine Receptor Antagonists: Behavioral and Pharmacologic Effects on D_1 and D_2 Dopamine Receptors

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Rats were treated for 21 d with the selective D_1 dopamine receptor antagonist SCH23390, the selective D_2 dopamine receptor antagonist spiperone, the nonselective dopamine receptor antagonist *cis*-flupentixol, or a combination of SCH23390 and spiperone. In addition, a group of rats received L-prolyl-L-leucyl-glycinamide (PLG) for 5 d after the 21 d chronic spiperone treatment. Chronic treatment with SCH23390 resulted in a significant increase in D_1 dopamine receptor density with no change in the D_2 dopamine receptor density. Conversely, spiperone treatment resulted in a significant increase in D_2 dopamine receptors and no change in D_1 dopamine receptor density. PLG treatment had no effect. SCH23390 plus spiperone treatment resulted in a significant increase in both D_1 and D_2 dopamine receptor densities. However, although *in vitro cis*-flupentixol has an equal affinity for D_1 and D_2 dopamine receptors, only the D_2 dopamine receptor density increased after chronic treatment with *cis*-flupentixol.

***In vivo* treatment with the protein-modifying reagent *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), which irreversibly inactivates D_1 and D_2 dopamine receptors, was used to investigate the paradoxical, selective D_2 dopamine receptor up-regulation induced by *cis*-flupentixol treatment. *In vivo* treatment with *cis*-flupentixol before EEDQ administration prevented the D_1 and D_2 dopamine receptor reductions induced by EEDQ. However, *cis*-flupentixol protected, in a dose-dependent manner, a greater percentage of D_2 dopamine receptors than of D_1 dopamine receptors from EEDQ-induced modification. These data indicate that, *in vivo*, *cis*-flupentixol preferentially interacts with D_2 dopamine receptors and could explain why only D_2 dopamine receptors were up-regulated following chronic treatment with *cis*-flupentixol.**

Rats were tested for their cataleptic response to the administered drug over the course of the chronic drug treatment. Catalepsy scores of rats receiving spiperone decreased over the course of treatment, with a significant reduction in catalepsy occurring by treatment day 5. The profound catalepsy observed in rats receiving SCH23390 did not change over the 21 d of treatment. Rats receiving *cis*-flupentixol demonstrated tolerance to its cataleptogenic effects, with a significant reduction in catalepsy observed by treatment day 7. During the 3 week treatment, the time between drug injection and a full cataleptic response to *cis*-flupentixol increased from 20 to 60 min, suggesting a tolerance to the D_2, but not D_1, dopamine receptor antagonism by *cis*-flupentixol. A group of rats treated with SCH23390 for 21 d received a final acute injection of spiperone on day 22. These rats were as tolerant to the cataleptogenic effects of spiperone as were those rats tested on day 21 of chronic spiperone treatment. Conversely, rats challenged with an acute dose of SCH23390 after 21 d spiperone treatment were profoundly cataleptic. Thus, D_2 dopamine receptor up-regulation is not necessary for the development of behavioral tolerance to D_2 dopamine receptor antagonism, whereas behavioral tolerance to D_1 dopamine receptor antagonism was not observed in these studies in spite of a D_1 dopamine receptor up-regulation.

Two distinct dopamine receptor subtypes coexist in the CNS (Kebabian and Calne, 1979; Creese et al., 1983): D_1 dopamine receptors mediate the stimulation of adenylate cyclase activity (Hyttel, 1978), while D_2 dopamine receptors inhibit the activity of this enzyme (Stoof and Kebabian, 1981; Onali et al., 1984; Battaglia et al., 1985). Although many neuroleptics block both dopamine receptor subtypes, previous research has focused on the role of D_2 dopamine receptors in the mechanism of action of neuroleptic drugs. This was due primarily to the high correlation between the affinities of neuroleptic drugs at the D_2 dopamine receptor and their clinical dosages as antipsychotic agents (Creese et al., 1976; Seeman et al., 1976). In addition, selective D_2 dopamine receptor antagonists such as sulpiride are antipsychotic. Investigations of the D_1 dopamine receptor were hampered because of the lack of compounds selective for this receptor.

Tardive dyskinesia is a major complication of long-term neuroleptic drug administration used in treating schizophrenia. This disorder is characterized by abnormal movements of the facial muscles, tongue, and extremities (for review, see Baldessarini and Tarsy, 1980). To provide an animal model of tardive dyskinesia, rodents have been chronically treated with neuroleptic

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drugs (Tarsy and Baldessarini, 1974). The chronic treatment of rodents with neuroleptics does not usually result in the obvious dyskinetic movements of tardive dyskinesia, but does result in an increased sensitivity to the motor stimulatory effects of dopamine agonists, accompanied by an increase in dopamine receptor density. For example, chronic treatment with the D_2 dopamine receptor-selective antagonist haloperidol results in an increase in D_2 dopamine receptor density (Burt et al., 1977). It has been suggested that "pharmacological" denervation of dopamine receptors occurs during chronic antagonist treatment, which results in the supersensitive response to subsequent agonist administration (Creese and Sibley, 1981).

In humans and in rodents, the acute administration of neuroleptics results in Parkinsonian or cataleptic extrapyramidal motor side effects, respectively. However, tolerance develops to the extrapyramidal side effects of the classic neuroleptics, such as haloperidol or spiperone, after several weeks of repeated administration (Klett and Caffey, 1972; Ezrin-Waters and Seeman, 1977; Hess et al., 1986a). Similar to the receptor subtype-selective up-regulation seen following chronic D_2 dopamine receptor blockade, chronic treatment with the D_1 dopamine receptor-selective antagonist SCH23390 leads to a selective up-regulation in D_1 dopamine receptors (Creese and Chen, 1985). However, rats receiving chronic SCH23390 treatment demonstrate no tolerance to the cataleptogenic effects of this drug (Hess et al., 1986a). Paradoxially, long-term treatment with antagonists such as fluphenazine or flupentixol, which have equal affinities for the D_1 and D_2 dopamine receptors *in vitro,* has been reported to up-regulate *only* D_2 dopamine receptors (Murugaiah et al., 1984; Mackenzie and Zigmond, 1985; Boyson et al., 1986). It is unclear whether tolerance develops to the cataleptogenic effects of the mixed D_1/D_2 dopamine receptor antagonists (Murugaiah et al., 1984).

Because D_1 and D_2 dopamine receptors interact in the modulation of behavior (Arnt and Hyttel, 1985; Breese and Mueller, 1985; Molloy and Waddington, 1985; Hess et al., 1986a), it is possible that the simultaneous antagonism of D_1 and D_2 dopamine receptors by drugs such as flupentixol may modulate or prevent the predicted D_1 dopamine receptor up-regulation. Alternatively, the paradoxical effects of mixed D_1/D_2 dopamine receptor antagonists may be due to differences in the interactions of these drugs at D_1 and D_2 dopamine receptors *in vivo.* For example, D_2 dopamine receptors may be situated in a manner that allows a more rapid drug equilibration and prolonged blockade *in vivo.*

In order to investigate these phenomena further, we have assessed the effects of chronic treatment with a variety of dopamine receptor antagonists on D_1 and D_2 dopamine receptor densities and have determined the time course of the cataleptogenic effects of these drugs throughout the chronic treatment regimen. We have also examined the dopamine receptor-blocking properties of the mixed D_1/D_2 dopamine receptor antagonist flupentixol at D_1 and D_2 dopamine receptors *in vivo* using the protein-modifying reagent *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). Peripherally administered EEDQ has been shown to irreversibly inactivate a number of neurotransmitter receptors, including D_2 (Hamblin and Creese, 1983; Norman et al., 1987) and D_1 (Hamblin and Creese, 1983; Hess et al., 1987a) dopamine receptors, S_2 serotonin (Battaglia et al., 1986), and α_2-adrenergic receptors (Adler et al., 1985). EEDQ-induced inactivation of these receptors appears to occur at the ligand-recognition site, as EEDQ-induced inactivation can be prevented by prior administration of receptor-selective antagonists (Meller et al., 1985; Battaglia et al., 1986; Hess et al., 1987a). Meller et al. (1985) have demonstrated that flupentixol administration prior to EEDQ administration will "protect" both D_1 and D_2 dopamine receptors from *in vivo* EEDQ-induced modification. We have examined the time course of the interaction of flupentixol at D_1 and D_2 dopamine receptors *in vivo* by assessing flupentixol protection of D_1 and D_2 dopamine receptors from EEDQ-induced inactivation.

Additionally, we have investigated the possible desensitizing effects of the compound L-prolyl-L-leucyl-glycinamide (PLG) on chronic neuroleptic-induced D_2 dopamine receptor up-regulation. PLG is thought to be a hypothalamic factor that inhibits the release of melanocyte-stimulating hormone (MSH) from the pituitary (Nair et al., 1971). This tripeptide has been reported to reverse the increased D_2 dopamine receptor density that occurs after chronic D_2 dopamine receptor blockade in rats (Chiu et al., 1981, 1985). It has been suggested that PLG may be useful in ameliorating the symptoms of tardive dyskinesia in man (Bhargava, 1984).

Materials and Methods

Chronic drug treatment and behavioral testing. Male Sprague-Dawley rats (180 gm; Simonsen, Gilroy, CA) were maintained in group cages on ad lib food and water and a 12 hr light/dark cycle (6 AM/6PM). Rats were injected daily (s.c.) for 21 d at 10 AM with the following drugs dissolved in saline and a minimal amount of 0.1 *N* HCl (5.0 μl HCl/ml saline): 0.5 mg/kg SCH23390 ($n = 12$), 1 mg/kg *cis*-flupentixol ($n = 6$), 0.2 mg/kg spiperone ($n = 12$), 0.5 mg/kg SCH23390 plus 0.2 mg/kg spiperone ($n = 6$). In addition, 6 control rats were injected with the saline vehicle. Rats were tested on days 1, 3, 5, 7, 14, and 21 for a cataleptic response to their administered drug. Also, on day 22, half the group of chronic spiperone-treated animals were injected with 0.5 mg/kg SCH23390 and half the group of chronic SCH23390-treated animals were injected with 0.2 mg/kg spiperone and observed for cataleptic responses. To test for catalepsy, observations were made every 20 min for 2 hr following drug injection. Rats were individually placed in a box (20 × 20 × 30 cm) with a small bar diagonally placed across one corner, 10 cm above the floor. The rat's front paws were placed on the bar and the time taken for the rat to remove both paws was measured, with a cutoff time of 120 sec (Sanberg et al., 1984). Two days following final drug injection, the rats were killed by decapitation and their brains rapidly removed to chilled saline. The striata were dissected, frozen in liquid nitrogen, and stored (1–2 weeks) at −70°C until radioligand binding assay. Those rats receiving an acute antagonist challenge on day 22 were not used in the radioligand binding assays.

To examine the effects of PLG on neuroleptic-induced D_2 dopamine receptor up-regulation, rats were injected daily (s.c.) with saline ($n = 12$) or 0.2 mg/kg spiperone ($n = 12$) for 21 d. On the 5 d following this chronic treatment, half the rats receiving saline ($n = 6$) and spiperone ($n = 6$) treatment were administered saline (s.c.). The remaining 6 animals from each group were injected daily (s.c.) with 20 mg/kg PLG for 5 d. Two days following final drug injection, rats were killed and striata dissected as described above.

Radioligand binding assays. ^{3}H-SCH23390 saturation analyses to determine D_1 dopamine receptor density and ^{3}H-spiperone saturation analyses, to determine D_2 dopamine receptor density were performed in parallel in the striata of each rat. Flupentixol competitions for 0.25 nM ^{3}H-SCH23390 or 0.1 nM ^{3}H-spiperone were performed in parallel in control rat striata. Tissue from each animal was homogenized (Tekmar Tissumizer setting 7, 10 sec) individually in 40 volumes of ice-cold 50 mM Tris-HCl (pH 7.4 at 37°C). The tissue was centrifuged (35,000 × *g,* 10 min) and washed once more in the same buffer, followed by a final resuspension in assay buffer consisting of 50 mM Tris-HCl (pH 7.4 at 37°C), 5 mM $MgSO_4$, and 0.5 mM EDTA. Incubations for each radioligand were initiated by adding tissue (2 mg wet weight tissue/tube) to duplicate tubes containing ^{3}H-antagonists to yield a 2.5 ml final assay volume. All saturation analyses included 6 concentrations of radioligand. Competition and saturation studies of ^{3}H-SCH23390 (0.125–2 nM) were conducted in the presence or absence of 100 nM *cis*-flupentixol

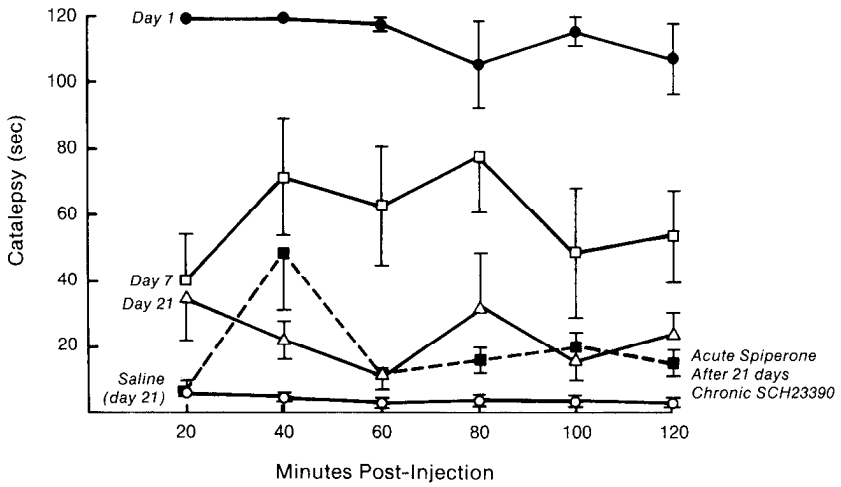

Figure 1. Cataleptic response of rats treated chronically with spiperone ($n = 6$) or saline ($n = 6$). Spiperone treatment days 1, 7, and 21, and saline treatment day 21 are presented. Additionally, data from rats treated with SCH23390 for 21 d and then challenged with an acute injection of spiperone on day 22 are presented ($n = 6$). All drug-treated rats were significantly more cataleptic than saline-treated rats on all testing days ($p < 0.001$). Catalepsy scores on days 7 and 21 of spiperone treatment and acute spiperone challenge after chronic SCH23390 treatment were significantly lower than scores on spiperone treatment day 1 ($p < 0.001$). Catalepsy scores from acute spiperone challenge after chronic SCH23390 treatment did not differ significantly from those after day 21 of spiperone treatment. Data represent means ± SEM, with a maximum catalepsy score cutoff of 120 sec.

in order to distinguish the specific from nonspecific binding. All ^{3}H-spiperone assays (0.025–4 nM) were conducted in the presence of 40 nM ketanserin to preclude binding of ^{3}H-spiperone to S_2 serotonin receptors; nonspecific binding was defined by 1 μM (+)butaclamol. Competition experiments included 21 concentrations of flupentixol. Tubes were incubated for 40 min at 37°C, filtered over Whatman GF/C glass-fiber filters that were then washed rapidly with 15 ml (3 × 5 ml) of ice-cold Tris buffer and counted by scintillation spectroscopy at an efficiency of 50%.

In vivo *flupentixol specificity.* Male Sprague-Dawley rats (160–180 gm; Simonsen) were injected (i.p.) with EEDQ (8 mg/kg) freshly dissolved in ethanol/water (1:1 vol/vol) or vehicle alone. To determine whether EEDQ had the same EC_{50} for D_1 and D_2 dopamine receptors, a dose–response for EEDQ was performed, in which rats received 1 mg/kg, 2 mg/kg, or 4 mg/kg EEDQ. For protection experiments testing the specificity of flupentixol *in vivo,* rats were injected (s.c.) with 0.25, 0.5, 0.75, or 1 mg/kg flupentixol dissolved in saline, or the saline vehicle 40 min before EEDQ (8 mg/kg) injection. Additionally, a second group of rats was injected with 0.5 mg/kg flupentixol or vehicle 20, 40, 60, 80, 100, or 120 min prior to EEDQ (8 mg/kg) injection. Four hours after EEDQ treatment, rats were decapitated, the striata removed, frozen rapidly in liquid nitrogen, and stored (−70°C) for 2–4 weeks. Saturation analyses of ^{3}H-antagonist binding were performed in the striata as described above, with the following tissue preparation to facilitate flupentixol washout: Tissue from each animal was individually homogenized in 40 volumes of ice-cold 50 mM Tris-HCl (pH 7.4 at 37°C). The tissue was centrifuged (35,000 × *g,* 10 min) and resuspended to 10 mg tissue wet weight/ml in this same buffer, followed by a 10 min incubation (37°C). The incubation was stopped by the addition of 30 ml ice-cold 50 mM Tris-HCl. Tissue was centrifuged (35,000 × *g,* 10 min) and washed once more in this buffer, followed by a final resuspension in assay buffer consisting of 50 mM Tris-HCl (pH 7.4 at 37°C), 5mM $MgSO_4$, and 0.5 mM EDTA. The saturation analyses were then completed as described above with ^{3}H-SCH23390 and ^{3}H-spiperone binding performed in parallel on striata from each rat.

Data analysis. ^{3}H-Antagonist saturation data were analyzed by the method of Scatchard (1949). A weighted, nonlinear, least-squares curve-fitting program, LIGAND (Munson and Rodbard, 1980) was used in the computer analysis of competition curves. All data were fitted while constraining the nonspecific binding parameter defined by 100 nM *cis*-flupentixol or 1 μM (+)butaclamol. Data obtained in the catalepsy-testing sessions were analyzed using arcsine transform analysis of variance with repeated measures and the Newman-Keuls test.

Materials. ^{3}H-SCH23390 (87 Ci/mmol) and ^{3}H-spiperone (88 Ci/mmol) were obtained from Dupont–New England Nuclear (Boston, MA). EEDQ was obtained from Aldrich Chemical Co. (Milwaukee, WI), and L-prolyl-L-leucyl-glycinamide from Sigma Chemical Co. (St. Louis, MO). The following drugs were generous gifts from the following sources: butaclamol, Ayerst Laboratories (Montreal, Canada); *cis*-flupentixol, Dr. J. Hyttel of H. Lundbeck and Co. (Denmark); SCH23390, Drs. Iorio and Barnett of Schering Corp. (Bloomfield, NJ); spiroperidol, Dr. J. Leysen, Janssen Pharmaceutica (Beerse, Belgium). Other reagents were obtained from standard commercial sources.

Results

Flupentixol competition for ^{3}H-SCH23390 or ^{3}H-spiperone binding

Computer-fitted curves for *cis*-flupentixol competition for 0.25 nM ^{3}H-SCH23390 binding modeled best to a single homogenous population of binding sites with a K_i of 0.69 ± 0.01 nM ($n = 3$). Computer-fitted curves for *cis*-flupentixol competition for 0.1 nM ^{3}H-spiperone binding modeled best to a single homogeneous population of binding sites with a K_i of 0.88 ± 0.03 nM ($n = 3$). The affinity of flupentixol for D_1 dopamine receptors was not significantly different from that of flupentixol for D_2 dopamine receptors ($p > 0.05$).

Behavioral effects of chronic drug administration

The overall catalepsy score, collapsed over each 2 hr session of each daily treatment for rats treated with the D_2 dopamine receptor-specific antagonist spiperone, decreased over the course of the 21 d treatment (Fig. 1). A significant reduction in the overall catalepsy score recorded on day 1 of spiperone treatment occurred by day 5 ($p < 0.01$) and progressively increased over the 21 d period ($p < 0.001$). However, complete tolerance to the cataleptogenic effects of spiperone was not observed: the overall catalepsy score on testing day 21 of rats treated chronically with spiperone was significantly greater ($p < 0.01$) than the day 21 catalepsy scores of rats treated chronically with saline.

In contrast to the rats treated with spiperone (Fig. 1) and other classic neuroleptics, in which tolerance to their cataleptogenic effects develops after chronic treatment (Ezrin-Waters and Seeman, 1977), rats receiving chronic administration of the D_1 dopamine receptor-selective antagonist SCH23390 for 21 d demonstrated no tolerance to its cataleptogenic action over the 21 d administration ($p > 0.05$) (Fig. 2). The cataleptic effects of SCH23390 did diminish over the 2 hr testing session, but the time course of this response did not change from that observed on day 1 over the 21 d treatment period.

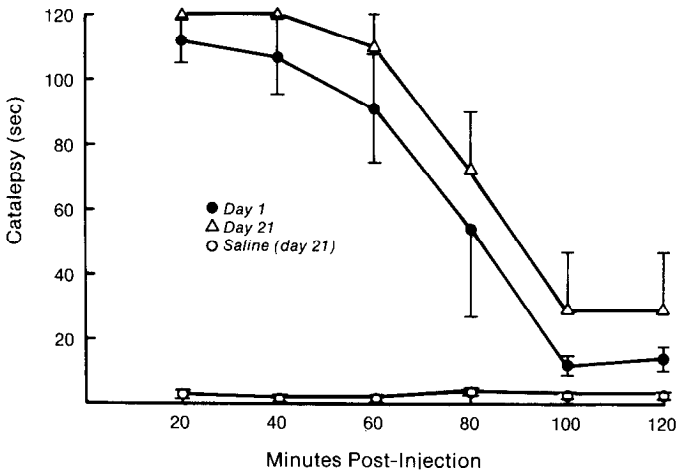

Figure 2. Cataleptic response of rats treated chronically with SCH23390 ($n = 6$) on days 1 and 21 of treatment, or saline ($n = 6$) on day 21 of treatment. SCH23390-treated rats were significantly more cataleptic than saline-treated rats ($p < 0.001$). The response to SCH23390 treatment did not change over the 21 d treatment regimen. Data represent means ± SEM, with a maximum catalepsy score of 120 sec.

Rats receiving chronic administration of the mixed D_1/D_2 dopamine receptor antagonist flupentixol for 21 d demonstrated tolerance to its cataleptogenic action over the 21 d administration period, with a significant reduction ($p < 0.01$) in the overall catalepsy score collapsed over each 2 hr session occurring by day 7 of treatment (Fig. 3). This was accompanied by a change in the pattern of catalepsy observed during the 2 hr catalepsy scoring session over the duration of *cis*-flupentixol treatment. Over the 3 week treatment, in the first 60 min of the scoring session, these animals appeared to become significantly less cataleptic ($p < 0.01$) after *cis*-flupentixol administration. That is, the time between injection and a full cataleptogenic response to *cis*-flupentixol increased from 20 to 60 min over the 21 d administration period. In contrast, the pattern of catalepsy observed

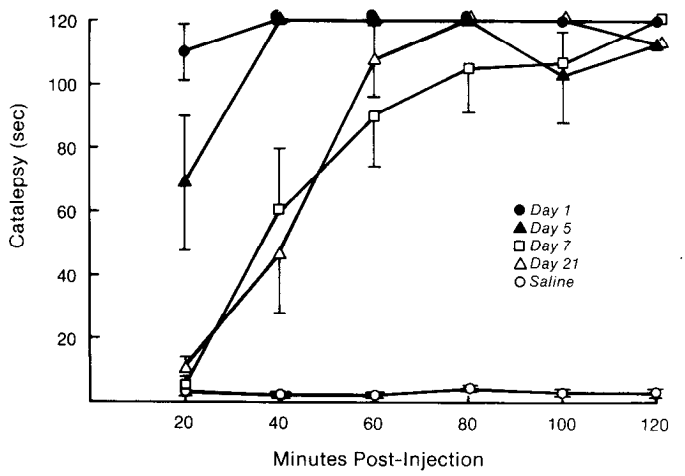

Figure 3. Cataleptic response of rats treated chronically with *cis*-flupentixol ($n = 6$) on days 1, 5, 7, and 21, or saline ($n = 6$) on day 21. Flupentixol-treated rats were significantly more cataleptic than saline-treated rats on all days tested ($p < 0.001$). Catalepsy scores on days 7 and 21 were significantly lower ($p < 0.01$) than scores on day 1, owing to a reduction in catalepsy scores at 20, 40, and 60 min after flupentixol injection ($p < 0.05$). Data represent means ± SEM, with a maximum catalepsy score of 120 sec.

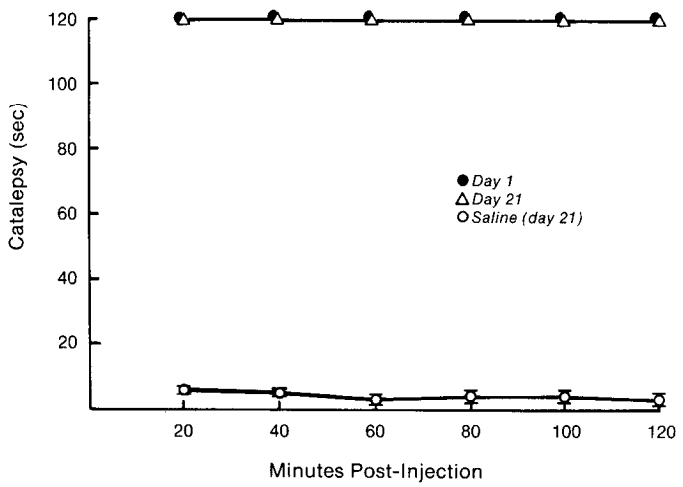

Figure 4. Cataleptic response of rats treated chronically with SCH23390 plus spiperone ($n = 6$) on days 1 and 21 of treatment, or saline ($n = 6$) on day 21 of treatment. SCH23390 plus spiperone-treated rats were significantly more cataleptic than saline-treated rats ($p < 0.001$). The response to SCH23390 plus spiperone treatment did not change over the 21 d treatment regimen. Data represent means ± SEM, with a maximum catalepsy score of 120 sec.

over the 2 hr catalepsy-scoring session did not change for the 21 d duration of either SCH23390 or spiperone treatment. Those rats receiving SCH23390 plus spiperone demonstrated no tolerance to the cataleptic effect of this combination of D_1 and D_2 dopamine receptor antagonists (Fig. 4).

When rats were treated with SCH23390 for 21 d and then administered an acute injection of spiperone, tolerance to the cataleptogenic effects of spiperone was observed (Fig. 1). In fact, the catalepsy scores, collapsed over the 2 hr session following acute challenge with spiperone after chronic 21 d SCH23390 treatment, were not different ($p > 0.05$) from the scores of chronic spiperone-treated rats on spiperone injection day 21. Conversely, rats challenged with an acute injection of SCH23390

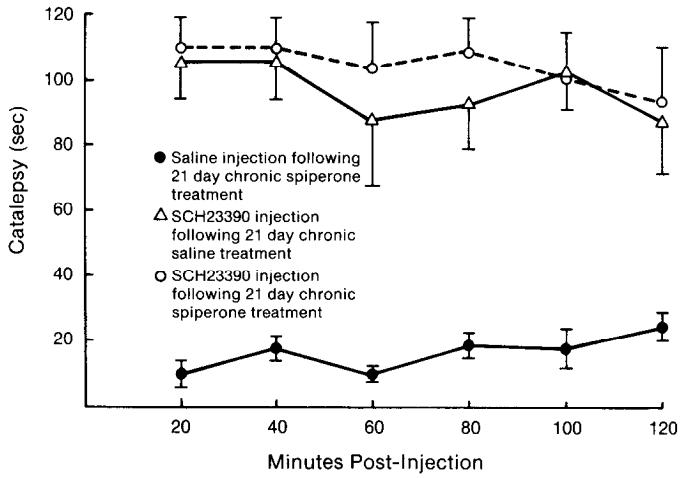

Figure 5. Cataleptic response of rats treated chronically with spiperone for 21 d and challenged with an acute injection of either saline ($n = 6$) or SCH23390 ($n = 6$) and rats treated with saline for 21 days and challenged with SCH23390. Catalepsy scores for a final SCH23390 injection were significantly greater than for the final saline injection ($p < 0.001$). Catalepsy scores for chronic 21 d spiperone treatment and chronic saline treatment with a final SCH23390 challenge did not differ significantly. Data represent means ±SEM, with a maximum catalepsy score of 120 sec.

Table 1. Effects of 21 d chronic drug treatments on striatal D_1 and D_2 dopamine receptor binding parameters

Chronic drug treatment	^{3}H-SCH23390 binding		^{3}H-Spiperone binding	
	B_{max} (pmol/gm tissue)	K_d (nM)	B_{max} (pmol/gm tissue)	K_d (nM)
Control	53.5 ± 1.3	0.44 ± 0.03	21.7 ± 0.7	0.08 ± 0.01
Flupentixol	56.1 ± 1.8	0.44 ± 0.03	27.9 ± 0.8***	0.10 ± 0.01
Spiperone	54.7 ± 1.7	0.46 ± 0.07	26.8 ± 0.6***	0.10 ± 0.02
SCH23390	62.0 ± 2.0*	0.41 ± 0.01	23.6 ± 0.6	0.08 ± 0.01
SCH23390 + spiperone	63.6 ± 2.2**	0.46 ± 0.01	28.6 ± 0.8***,[a]	0.11 ± 0.01

Data represent means ± SEM (n = 6) for the affinities (K_d) and densities (B_{max}) of D_1 and D_2 dopamine receptors, determined by saturation analyses of ^{3}H-SCH23390 and ^{3}H-spiperone binding, respectively. Rats were treated with *cis*-flupentixol (1mg/kg per day), spiperone (0.2mg/kg per day), SCH23390 (0.5mg/kg per day) or spiperone (0.2mg/kg per day) plus SCH23390 (0.5mg/kg per day) for 21 d. Asterisks denote values significantly greater than control values: * $p < 0.01$, ** $p < 0.005$, *** $p < 0.001$.

[a] SCH23390 plus spiperone value significantly greater than spiperone alone ($p < 0.01$); 1-way ANOVA and Duncan's test.

after 21 d spiperone treatment were as profoundly cataleptic as rats that had received the 21 d chronic SCH23390 treatment (Fig. 5).

Effect of chronic drug treatment on striatal dopamine receptor radioligand binding

Consistent with previous reports by this laboratory (Creese and Chen, 1985; Hess et al., 1986a), chronic treatment with SCH23390 resulted in a highly significant increase (+16%) in D_1 dopamine receptor-specific ^{3}H-SCH23390 binding ($p < 0.01$), but no significant change in D_2 dopamine receptor-specific ^{3}H-spiperone binding was observed ($p > 0.05$) (Table 1). Chronic treatment with the D_2 dopamine receptor-selective antagonist spiperone resulted in a no significant change in ^{3}H-SCH23390 binding ($p > 0.05$), while ^{3}H-spiperone binding was significantly increased by 24% ($p < 0.001$) (Table 1). Chronic treatment with SCH23390 plus spiperone resulted in a significant increase in both ^{3}H-SCH23390 (+19%) and ^{3}H-spiperone binding (+32%) (Table 1). The increase in ^{3}H-spiperone binding in the animals treated with SCH23390 plus spiperone was significantly greater ($p < 0.05$) than that observed in ^{3}H-spiperone binding in rats treated with spiperone alone. However, the increase observed in ^{3}H-SCH23390 binding in rats treated with SCH23390 plus spiperone did not differ significantly from that in ^{3}H-SCH23390 binding in rats treated with SCH23390 alone. Chronic treatment with the mixed D_1/D_2 dopamine receptor antagonist *cis*-flupentixol resulted in a significant increase in ^{3}H-spiperone binding *only* (+24%) ($p < 0.001$), with no change observed in ^{3}H-SCH23390 binding (Table 1). The K_ds of ^{3}H-SCH23390 binding and of ^{3}H-spiperone binding were unchanged by chronic drug treatment in any experimental condition (Table 1).

Effects of PLG administration following D_2 dopamine receptor up-regulation

The chronic 21 d spiperone treatment resulted in a significant 20% increase in ^{3}H-spiperone binding ($p < 0.01$). Administration of PLG for 5 d following chronic spiperone treatment did not affect the antagonist-induced D_2 dopamine receptor up-regulation (Table 2). In addition, 5 d PLG administration following 21 d saline treatment did not affect the B_{max} of the normosensitive D_2 dopamine receptor population. In all treatments, the K_d of ^{3}H-spiperone binding was unchanged from control values (Table 2).

Effects of in vivo *flupentixol pretreatment on EEDQ-induced reductions of D_1 and D_2 dopamine receptors*

^{3}H-SCH23390 and ^{3}H-spiperone binding were markedly reduced by *in vivo* EEDQ treatment in a dose-dependent manner. *In vivo* EEDQ-induced reductions in D_1 and D_2 dopamine receptors were comparable at all doses of EEDQ; EEDQ has an apparent EC_{50} of approximately 1.5 mg/kg (i.p.) for both the D_1 and D_2 dopamine receptors (Table 3). The magnitude of the EEDQ-induced reductions in D_1 and D_2 dopamine receptor binding could be reduced by treating rats with flupentixol before EEDQ administration. However, at all doses, flupentixol protected D_2 dopamine receptor binding to a greater extent than it did D_1 dopamine receptor binding when flupentixol was injected 40 min before 8 mg/kg EEDQ administration (Table 4). In fact, administration of 0.25 mg/kg flupentixol prior to EEDQ treatment protected 66% of D_2 dopamine receptors, while only 21% of D_1 dopamine receptors were protected by this dose. Admin-

Table 2. Effects of L-prolyl-L-leucyl-glycinamide (PLG) treatment on striatal D_2 dopamine receptor binding after 21 d spiperone administration

Treatment (d)	B_{max} (pmol/gm tissue)	K_d (nM)
Saline (26)	21.7 ± 0.5	0.068 ± 0.011
Saline (21) followed by PLG (5)	21.1 ± 0.6[a]	0.072 ± 0.002
Spiperone (21) followed by saline (5)	26.13 ±*	0.072 ± 0.002
Spiperone (21) followed by PLG (5)	27.18 ± 0.8*,[a]	0.075 ± 0.003

Data represent means ± SEM (n = 6) for the affinity (K_d) and density (B_{max}) of D_2 dopamine receptors, determined by saturation analyses of ^{3}H-spiperone binding. Rats were treated with saline or spiperone (0.2 mg/kg) for 21 d and then treated with either saline or PLG (20 mg/kg) for the 5 d following chronic treatment. Asterisk denotes values significantly different from saline treatment ($p < 0.01$).

[a] Values not significantly different ($p > 0.05$) from comparable non-PLG-treated group.

Table 3. Dose-dependent reductions in striatal D_1 and D_2 dopamine receptor binding after peripheral administration of EEDQ

	^{3}H-SCH23390 binding		^{3}H-Spiperone binding	
EEDQs dose	K_d (nM)	B_{max} (% reduction) (pmol/gm tissue)	K_d (nM)	B_{max} (% reduction) (pmol/gm tissue)
Control	0.47 ± 0.02	89.3 ± 1.3	0.095 ± 0.005	28.1 ± 0.2
1 mg/kg	0.48 ± 0.02	61.8 ± 5.3 (−31%)	0.103 ± 0.010	20.2 ± 1.6 (−28%)
2 mg/kg	0.51 ± 0.02	34.2 ± 2.2 (−62%)	0.101 ± 0.005	11.5 ± 0.7 (−59%)
4 mg/kg	0.49 ± 0.05	16.3 ± 1.2 (−82%)	0.123 ± 0.007	6.6 ± 0.3 (−77%)

Data represent means ± SEM (n = 4–5) for affinities (K_d) and densities (B_{max}) of D_1 and D_2 dopamine receptors determined by ^{3}H-SCH23390 and ^{3}H-spiperone saturation analyses. Values in parentheses represent percentage loss of control receptor densities. Radioligand binding assays for both D_1 and D_2 dopamine receptors were performed in parallel on tissue from individual rats.

istration of 1 mg/kg flupentixol was necessary to achieve a degree of protection of the D_1 dopamine receptor comparable to that afforded the D_2 dopamine receptor with 0.25 mg/kg flupentixol. A dose of 0.75 mg/kg flupentixol saturated the D_2 dopamine receptor, since the degree of protection reached a plateau at this dose, with 86% of D_2 dopamine receptors protected from irreversible modification by EEDQ. Because the flupentixol–dopamine receptor interaction is a reversible process, with some flupentixol free and some bound at any given instant, it is not surprising that 100% protection was never achieved, especially when using a close to saturating dose of EEDQ, as in these experiments.

To determine whether the differential protection was a time-dependent phenomenon based on the accessibility of the drug to each dopamine receptor subtype (i.e., flupentixol had more rapid accessibility to D_2 dopamine receptors than to D_1 dopamine receptors *in vivo*), a time course of flupentixol protection of D_1 and D_2 dopamine receptors from EEDQ-induced inactivation was measured. Protection of D_2 dopamine receptors by 0.5 mg/kg flupentixol was between 82 and 97% at all time points tested except the 20 min point, when flupentixol had probably not yet reached steady-state levels. The protection afforded D_1 dopamine receptors by flupentixol did not appear to differ from the time course of flupentixol protection of D_2 dopamine receptors within the time period that had been examined for the catalepsy measures. Maximal protection of D_1 dopamine receptors, which occurred 60 min after flupentixol administration, never exceeded 54% of control levels (Table 5). Furthermore, during the second hour of the experiment, protection of D_1 dopamine receptors declined to approximately 45%, while protection of D_2 dopamine receptors was ~97% during the second hour and had only declined to 82% protection at the 120 min point. In some instances, the K_d of ^{3}H-SCH23390 and ^{3}H-spiperone binding was significantly greater than control values, presumably because of residual flupentixol remaining after the wash procedures, as EEDQ itself does not alter the affinity of ^{3}H-SCH23390 or ^{3}H-spiperone binding (Table 3).

Discussion

As we have previously reported (Creese and Chen, 1985; Hess et al., 1986a), chronic treatment with the D_1 receptor-selective antagonist SCH23390 resulted in a significant and selective increase in the D_1 dopamine receptor density. Likewise, consistent with previous reports, chronic treatment with the D_2 dopamine receptor-selective antagonist spiperone selectively increased the D_2 dopamine receptor density. However, chronic treatment with the mixed D_1/D_2 receptor antagonist flupentixol resulted in the up-regulation of D_2 dopamine receptors *only* while the D_1 dopamine receptor density was unchanged, consistent with previous reports (Murugaiah et al., 1984; Mackenzie and Zigmond, 1985). As we have demonstrated herein, flupentixol has comparable affinities for the D_1 and D_2 dopamine receptors *in vitro*. Since many investigators have reported an interaction between D_1 and D_2 dopamine receptors in the regulation of behavioral (Arnt and Hyttel, 1985; Molloy and Waddington, 1985; Breese and Mueller, 1985; Hess et al., 1986a) and electrophysiological (Carlson et al., 1987) responses, the lack of an increase in D_1 dopamine receptor density following chronic flupentixol treatment may be due to a modulatory receptor interaction occurring with concomitant D_1 and D_2 dopamine receptor blockade. To

Table 4. Effects of flupentixol pretreatment on *in vivo* EEDQ-induced reductions in striatal D_1 and D_2 dopamine receptor densities

	^{3}H-SCH23390 binding		^{3}H-Spiperone binding	
Drug treatment	K_d (nM)	B_{max} (% protection) (pmol/gm tissue)	K_d (nM)	B_{max} (% protection) (pmol/gm tissue)
Control	0.42 ± 0.05	63.1 ± 1.9	0.06 ± 0.003	21.5 ± 0.9
EEDQ (8 mg/kg)	0.53 ± 0.04	2.9 ± 0.1**	0.094 ± 0.005	1.3 ± 0.1**
Flupentixol (0.25 mg/kg)	0.52 ± 0.04	15.4 ± 1.3 (21%)**	0.10 ± 0.005	14.6 ± 0.3 (66%)**
Flupentixol (0.5 mg/kg)	0.72 ± 0.08	20.9 ± 1.1 (30%)**	0.17 ± 0.006	17.6 ± 2.3 (81%)*
Flupentixol (0.75 mg/kg)	0.68 ± 0.05	28.6 ± 2.9 (43%)**	0.18 ± 0.012	18.7 ± 1.3 (86%)
Flupentixol (1 mg/kg)	0.72 ± 0.06	40.5 ± 3.8 (62%)**	0.18 ± 0.009	18.6 ± 0.9 (86%)

Data represent means ± SEM (n = 4–7) for affinities (K_d) and densities (B_{max}) of D_1 and D_2 dopamine receptors determined by ^{3}H-SCH23390 and ^{3}H-spiperone saturation analyses. Values in parentheses represent percentage protection of the receptor-inactivating action of EEDQ by flupentixol, calculated using total receptors inactivated by EEDQ and subtracting receptors remaining after 8 mg/kg EEDQ treatment from control values and from receptors protected by flupentixol. EEDQ (8 mg/kg) was administered (i.p.) 40 min after flupentixol injection (s.c.). Asterisks represent values significantly lower than control values: * $p < 0.05$, ** $p < 0.001$.

Table 5. Time course of flupentixol protection of striatal D_1 and D_2 dopamine receptors from EEDQ-induced modification *in vivo*

	^{3}H-SCH23390 binding		^{3}H-Spiperone binding	
	K_d (nM)	B_{max} (% protection) (pmol/gm tissue)	K_d (nM)	B_{max} (% protection) (pmol/gm tissue)
Control	0.64 ± 0.07	71.1 ± 2.1	0.104 ± 0.010	22.2 ± 1.1
EEDQ	0.69 ± 0.14	2.4 ± 0.5	0.328 ± 0.105	1.8 ± 0.4
Time after flupentixol administration (min)				
20	1.15 ± 0.12*	27.7 ± 3.9 (37%)**	0.28 ± 0.02	15.0 ± 0.8 (65%)**
40	1.12 ± 0.12	29.0 ± 1.4 (39%)**	0.37 ± 0.08*	20.4 ± 0.9 (91%)
60	1.16 ± 0.18*	39.8 ± 0.6 (54%)**	0.26 ± 0.02	19.7 ± 0.3 (88%)
80	0.87 ± 0.05	29.8 ± 1.8 (40%)**	0.32 ± 0.04	21.5 ± 1.0 (97%)
100	1.00 ± 0.10	34.9 ± 3.1 (47%)**	0.28 ± 0.02	21.4 ± 0.6 (96%)
120	1.36 ± 0.19*	33.2 ± 2.9 (45%)**	0.24 ± 0.02	18.5 ± 1.3 (82%)*

Data represent means ± SEM (n = 4–6) for affinities (K_d) and densities (B_{max}) of D_1 and D_2 dopamine receptors determined by ^{3}H-SCH23390 and ^{3}H-spiperone saturation analyses. Values in parentheses represent percentage protection by flupentixol of EEDQ-induced receptor inactivation. EEDQ (8 mg/kg) was administered (i.p.) 20, 40, 60, 80, 100, or 120 min after flupentixol injection (0.5 mg/kg, s.c.). Asterisks indicate values significantly different from control values: * $p < 0.05$, ** $p < 0.01$.

test this hypothesis, rats received simultaneous treatment with a D_1 receptor-selective antagonist (SCH23390) and a D_2 dopamine receptor-selective antagonist (spiperone) to mimic the potential dual-receptor subtype-blocking activity of the mixed D_1/D_2 dopamine receptor antagonist flupentixol. Simultaneous D_1 and D_2 dopamine receptor antagonism resulted in an increase in the density of *both* D_1 and D_2 dopamine receptors using the drug doses employed in this study. Thus, simultaneous D_1 and D_2 dopamine receptor antagonism does not appear to reduce the ability of D_1 dopamine receptors to up-regulate.

The increase in D_2 dopamine receptors observed after SCH23390 plus spiperone treatment was significantly greater than the increase in D_2 dopamine receptors after spiperone treatment alone. Thus, chronic blockage of D_1 dopamine receptors, which alone does not result in a statistically significant D_2 dopamine receptor up-regulation, potentiated the D_2 dopamine receptor up-regulation resulting from chronic D_2 dopamine receptor blockade. Saller and Salama (1986) have recently demonstrated that SCH23390 reduces the *in vivo* increase in dopamine metabolites elicited by D_2 dopamine receptor blockade. Thus, it is possible that the potentiation in D_2 dopamine receptor up-regulation is due to a reduction in dopamine release onto D_2 receptors caused by the D_1 dopamine receptor blockade by SCH23390. The reduction in dopamine release would result in an effective increase in D_2 dopamine receptor blockade by spiperone. Alternatively, another possibility is that the enhanced D_2 dopamine receptor up-regulation after SCH23390 plus spiperone treatment may be the result of additional direct D_2 dopamine receptor blockade by SCH23390. Although SCH23390 has minimal D_2 dopamine receptor affinity in *in vitro* radioligand binding assays (Hess et al., 1986b), any small, additional direction D_2 dopamine receptor blockade caused by SCH23390 *in vivo* (Stoof and Kebabian, 1981; Andersen and Nielsen, 1986) on top of the large blockade by spiperone may have been sufficient to induce the greater D_2 dopamine receptor up-regulation. Clearly, more extensive experimentation is necessary before this question can be answered.

An hypothesis to explain the differential effect of chronic flupentixol administration on D_1 and D_2 dopamine receptors is that, in contrast to its equal affinity at D_1 and D_2 dopamine receptors *in vitro, in vivo* flupentixol interacts differentially with D_1 and D_2 dopamine receptors. Flupentixol protection of D_1 and D_2 dopamine receptors from EEDQ-induced inactivation was used to test this hypothesis. To insure that EEDQ did not have a greater potency at D_1 or D_2 dopamine receptors *in vivo,* an EEDQ dose–response experiment assessing the effects of EEDQ on D_1 and D_2 dopamine receptors was performed: *in vivo* administration of EEDQ induced comparable percentage reductions in D_1 and D_2 dopamine receptors. That EEDQ induced equal reductions in D_1 and D_2 dopamine receptor binding at all doses administered *in vivo* is not surprising. The mechanism of action of EEDQ is thought to be due to the creation of highly reactive mixed carbonic anhydrides from carboxyl groups that, in turn, interact with nucleophilic groups such as free α-amino groups (Belleau et al., 1968). EEDQ also has the capacity to modify enzymes such as serine proteases (Belleau et al., 1968) and adenylate cyclase (Hess et al., 1987a), in addition to neurotransmitter receptors. In fact, we have demonstrated that *in vitro* the carboxyl groups of the adenylate cyclase molecule appear to be as sensitive to EEDQ-induced alterations as those carboxyl groups modified at the D_1 dopamine receptor. Thus, EEDQ-induced modifications seem to be dependent on the availability of carboxyl groups in a given protein. That is, EEDQ-induced reductions in the D_1 and D_2 dopamine receptor density may be due to nonreceptor-specific carboxyl group modifications rather than to any specific "affinity" EEDQ might have for these receptors.

Since EEDQ induces comparable percentage reductions at D_1 and D_2 dopamine receptors, it may be used as a tool to assess the relative interactions of drugs administered *in vivo* at these receptors. Treatment with flupentixol before the peripheral administration of EEDQ protected both D_1 and D_2 dopamine receptors from irreversible blockade by EEDQ, as previously demonstrated (Meller et al., 1985). However, at all doses of flupentixol tested and at all time points after flupentixol injection, flupentixol protected a greater percentage of D_2 dopamine receptors than D_1 dopamine receptors. That is, *in vivo,* flupentixol demonstrated a preference for the D_2 dopamine receptor. The lipophilicity of flupentixol, which can result in free ligand depletion, could mask such a small, but functionally significant, difference in the determination of flupentixol affinities *in vitro.* Conversely, *in vivo* drug–receptor interactions, assessed from

the use of drug treatment followed by EEDQ treatment, would reveal these small differences in affinity provided the drug was administered in a *subsaturating* dose. The *in vivo* procedure assesses *relative* drug interactions at 2 or more receptors *simultaneously* by measuring fractional occupancy. Thus, small differences in the affinity of a drug for 2 receptors would become obvious because fractional occupancy would never be equal at the different receptors, provided the drug is not at saturating concentrations for the examined receptors. Although this method of drug-induced protection from EEDQ-induced modification *in vivo* is not suitable for the quantitative determination of drug affinities at receptors, it should provide, with ease, a good reflection of relative drug interactions at specific receptors *in vivo*. These results are consistent with the results of P. H. Andersen (personal communication), who reports that when using *in vivo* radioligand binding for a direct quantitative assessment of *in vivo* receptor affinity, flupentixol *in vivo* has a higher affinity for D_2 dopamine receptors than for D_1 dopamine receptors. In our experiments, flupentixol appears to equilibrate at each dopamine receptor subtype within 40 min after injection, so the behavioral experiments were not confounded by any possible time-dependent differences in the accessibility of the drug to the receptor. Thus, it seems from these results, as well as from the report of P. H. Andersen (personal communication), that chronic flupentixol treatment does not block a sufficient percentage of D_1 dopamine receptors to induce up-regulation. These results are noteworthy, as they demonstrate that, just as behavioral drug studies may not always provide an accurate assessment of specific drug–receptor interactions (e.g., the once-accepted hypothesis that apomorphine-induced stereotypies were due solely to D_2 dopamine receptor activation), extrapolating specific *in vivo* drug–receptor interactions from *in vitro* radioligand binding data may be imprecise.

Twenty-one day chronic spiperone treatment, followed by 5 d PLG treatment, had no effect on the D_2 dopamine receptor density. Previous studies (Bhargava, 1984; Chiu et al., 1985) have reported a PLG-induced reversal in the D_2 dopamine receptor up-regulation following chronic neuroleptic treatment. Although the experiments described in those reports were fundamentally similar to the protocol described herein, those investigators administered haloperidol rather than spiperone; the difference in chronic drug treatment may account for the striking differences in the putative D_2 dopamine receptor density-regulatory properties of PGL. PLG may also exert its regulatory effects, preventing neuroleptic-induced D_2 dopamine receptor up-regulation, if it is administered prior to neuroleptic treatment, as previous reports have suggested (Chiu et al., 1981, 1985). Clearly, the prophylactic effects of PLG on dopamine receptor up-regulation require further examination.

As we have previously reported (Hess et al., 1986a), after chronic treatment with SCH23390 for 21 d, rats demonstrated no tolerance to the cataleptogenic effects of SCH23390. The decrease observed in the cataleptic response to SCH23390 over the 2 hr testing session (Fig. 2) was probably due to rapid metabolism of the drug, rather than a tolerance phenomenon, since it was present on the first administration of the drug. It might be suggested that the prolonged catalepsy response to acute SCH23390 injection following 21 d chronic spiperone treatment results from a modulatory effect of the increased D_2 dopamine receptor density (compare Figs. 2 and 5). However, rats treated with saline for 21 d also demonstrated a prolonged catalepsy response to acute SCH23390 injection (Fig. 5). These results suggest that the differences observed in SCH23390-induced catalepsy over the 2 hr testing sessions may reflect the behavioral variability seen when different groups of rats are tested on different days. In contrast to the pattern of catalepsy observed over the course of chronic SCH23390 treatment, rats became progressively tolerant to the cataleptic effects of spiperone; behavioral tolerance to these effects became obvious on treatment day 5, and the rats became progressively more tolerant over the 21 d treatment period. Tolerance was not observed to the combination of SCH23390 plus spiperone, with maximal catalepsy scores recorded at all time points on all testing days. Rats treated chronically with flupentixol appeared to become less sensitive to the immediate cataleptic effects of this drug over the course of treatment, but continued to demonstrate profound catalepsy in the second hour after flupentixol administration. Since the EEDQ protection experiments indicated that flupentixol has a greater D_2 than D_1 dopamine receptor-blocking activity, it is possible that the behavioral tolerance to flupentixol observed during the first hour after drug administration is a reflection of the development of behavioral tolerance to the D_2 dopamine receptor antagonism by flupentixol.

The discovery of antagonist drug-induced receptor up-regulation provided an attractive explanation for the behavioral tolerance to chronic antagonist administration (for review, see Creese and Sibley, 1981). However, the results described herein demonstrate that this mechanism cannot explain the tolerance or lack of tolerance of the cataleptic response to the chronic administration of dopamine receptor antagonists. It is not yet clear why rats become tolerant to the cataleptogenic effects of D_2 dopamine receptor antagonists, but do not become tolerant to the D_1 dopamine receptor antagonist SCH23390. Tolerance to chronic spiperone administration occurs concomitant with a D_2 dopamine receptor up-regulation but does not occur to chronic SCH23390 administration in spite of a similar concomitant D_1 dopamine receptor increase. Conversely, in spite of a normal complement of D_2 dopamine receptors following chronic SCH23390 treatment, rats are just as tolerant to spiperone as they are following chronic spiperone administration. It is possible that *in vivo* SCH23390 may interact with D_2 dopamine receptors to induce tolerance to an acute spiperone injection after chronic SCH23390 treatment. However, we have previously demonstrated that treatment with SCH23390 (0.5 mg/kg, s.c.) before peripheral administration of EEDQ protects D_1 dopamine receptors from irreversible blockade by EEDQ, but that neither D_2 nor S_2 serotonin receptors were protected from EEDQ-induced modifications by SCH23390 (Hess et al., 1986a). This indicates that SCH23390 at the dose administered in this study interacts *in vivo* exclusively with D_1 dopamine receptors. Thus, behavioral tolerance to an acute injection of spiperone following 21 d chronic SCH23390 treatment is probably not due to any nonspecific effects of SCH23390 at the D_2 dopamine receptor. It appears then that D_2 dopamine receptor up-regulation is not necessary for the induction of behavioral tolerance. Although the D_2 dopamine receptor recognition site may not be up-regulated, alterations in its second-messenger system may have occurred. For example, in a previous study we demonstrated that chronic SCH23390 treatment increased the ability of GTP to activate adenylate cyclase in rat striatal homogenate (Hess et al., 1986a).

Many studies have demonstrated that D_1 and D_2 dopamine receptors are interactive in their modulation of electrophysiological responses, as well as motor behavior. It has recently been suggested that drug-induced changes in the tonic electrical activity of the basal ganglia, particularly the globus pallidus, may

reflect drug-induced behavioral effects (Carlson et al., 1987). That is, agonist-induced increases in pallidal electrical activity seem to correlate approximately with agonist-induced increases in behavioral responses, while the converse is also true. Thus, an increase in D_1 dopamine receptors induced by chronic SCH23390 treatment may result in an overall increase in the tonic activity of the basal ganglia owing to the enhanced action of endogenous dopamine at the up-regulated D_1 dopamine receptors. This overall activation imparted by increased D_1 dopamine receptor activity may be great enough to impart what appears to be behavioral tolerance to a D_2 dopamine receptor antagonist essentially by "overriding" the D_2 antagonist signal. These results parallel our earlier findings, where the behavioral effects of the D_2 receptor agonist LY171555 were potentiated after a selective D_1 dopamine receptor up-regulation (Hess et al., 1986a). In fact, we have also previously demonstrated that after D_1 dopamine receptor up-regulation, rats exhibit an increase in spontaneous locomotor activity (Hess et al., 1986a). Following D_2 dopamine receptor up-regulation, however, neither an increase nor decrease in spontaneous activity has been observed (Tarsy and Baldessarini, 1974). Thus, enhanced D_1 dopamine receptor stimulation following D_1 dopamine receptor up-regulation seems to promote an overall increase in activity levels, which may result from the enhanced effectiveness of endogenous dopamine at the up-regulated D_1 dopamine receptors. Conversely, D_2 dopamine receptor supersensitivity due to neuroleptic treatment is only apparent when the animal is challenged with a dopamaine receptor agonist. These results support the hypothesis (Barone et al., 1986; Waddington, 1986) that D_1 dopamine receptor activation may provide a tonic background activation that may be equated with an overall level of arousal. In this model, additional D_2 dopamine receptor stimulation may be associated with the development of overt stereotyped behavior, which may be regulated by the level of D_1 dopamine receptor activation, with greater D_1 dopamine receptor activation yielding more intense agonist-induced D_2 dopamine receptor-mediated stereotypy.

It has previously been suggested that the symptoms of schizophrenia may be due in part to "overactive" D_1 dopamine receptors (Memo et al., 1983; Hess et al., 1987b). Hence, administration of an antipsychotic drug with a potent *in vivo* D_1 dopamine receptor antagonist component may be beneficial in certain cases (Andersen et al., 1986). Such a mixed D_1/D_2 dopamine receptor antagonist would reduce what may be supranormal tonic activity by blocking D_1 dopamine receptors, while specific "stereotypical" symptoms would be controlled by the D_2 dopamine receptor antagonist component. While many antipsychotic drugs have equal affinities at D_1 and D_2 dopamine receptors *in vitro,* it is not clear that these drugs are potent antagonists at D_1 dopamine receptors *in vivo,* as exemplified by the flupentixol/EEDQ experiments presented herein. Because the 2 dopaminergic systems seem to interact in a synergistic manner (Braun et al., 1986; Waddington et al., 1986), it is possible that much lower doses of a drug having equal affinities at D_1 and D_2 dopamine receptors *in vivo* than a D_2 dopamine receptor-selective antagonist could be used to ameliorate the totality of schizophrenic symptoms, possibly reducing the subsequent incidence of tardive dyskinesia.

Tolerance develops to the extrapyramidal side effects of classic neuroleptics that act primarily at D_2 dopamine receptors. However, since tolerance to the cataleptogenic effects of SCH23390 was not observed, chronic SCH23390 administration may induce unremitting Parkinsonian extrapyramidal side effects in clinical trials. That tolerance to the cataleptogenicity of SCH23390 plus spiperone treatment was not observed in this study also suggests that a complete D_1 plus D_2 dopamine receptor blockade may also produce unremitting Parkinsonian side effects in the clinical setting. However, the doses of the drugs used in this study were rather high and the testing extended only over 21 d. It would be useful to test treatments using lower doses of SCH23390 plus spiperone over a longer test period to determine whether the development of tolerance to the extrapyramidal side effects of stimultaneous D_1 and D_2 dopamine receptor blockade can occur.

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