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Studies on D1 and D2 Dopamine Receptor Involvement in Conditioned Taste Aversions

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ASIN, K. E. AND W. E. MONTANA. *Studies on D1 and D2 dopamine receptor involvement in conditioned taste aversions.* PHARMACOL BIOCHEM BEHAV **32**(4) 1033–1041, 1989.—This series of studies investigated the ability of compounds selective for either the D1 or D2 dopamine receptor to induce a conditioned taste aversion (CTA) in thirsty rats. Neither the D1 antagonist SCH23390 (0.12–0.60 mg/kg) nor the D2 antagonist haloperidol (0.125–0.375 mg/kg) were able to induce CTAs to a saccharin solution. In contrast, the D1 agonist SKF38393 produced a dose-dependent taste aversion which was stereoselective to the (R−) enantiomer. The aversion to (R,S)-SKF38393 was not blocked by pretreatment with either SCH23390 or haloperidol, suggesting that the aversion is not mediated through stimulation of either dopamine receptor subtype. The D2 dopamine receptor agonist quinpirole was also found to produce a dose-dependent CTA. This aversion was blocked by injections of haloperidol and was attenuated following injections of domperidone, suggesting involvement of peripheral dopamine receptors in the aversion. Pretreatment with SCH23390 failed to affect the quinpirole-induced CTA, providing additional evidence that the D1 and D2 dopamine receptor subtypes can function independently of one another in the control of behavior. Finally, it does not appear that the area postrema is importantly involved in these taste aversions since lesions of this brain region did not affect the CTAs induced by either SKF38393 or quinpirole.

Dopamine D1 D2 Quinpirole SKF38393 Haloperidol LY171555 Area postrema
Conditioned taste aversions

THE conditioned taste aversion (CTA) paradigm has been used extensively to detect aversive properties of drugs, and dopamine agonists have been among the most frequently studied compounds. Thus, both indirect (e.g., amphetamine, cocaine, methylphenidate) and direct (e.g., apomorphine) dopamine agonists have been reported to potently inhibit the consumption of a novel fluid after being paired with it [e.g., (5, 7, 12, 16, 45)].

Recent evidence suggests that the D1 and D2 subtypes of dopamine receptors in the brain may be differentially (although sometimes synergistically) involved in the control and expression of certain behaviors [e.g., (2, 4, 8, 29, 35)]. At present, dopaminergic agonists which have been demonstrated to produce CTAs result in either direct or indirect stimulation of both the D1 and D2 dopamine receptors. In contrast to the effects of agonists in taste aversion studies, however, when D2 dopamine antagonists have been examined for their ability to induce CTAs, reports generally have been negative [(17,19), but see (5)]. The ability of selective D1 agonists or antagonists, and the ability of a selective D2 agonist, to induce taste aversions have not yet been studied. There are indications that the aversive, CTA-inducing properties of amphetamine and apomorphine (which result in both D1 and D2 stimulation) are at least partially mediated via stimulation of the D2 dopamine receptor, since these CTAs can be attenuated by the D2 antagonist pimozide (21,42) (the effects of a D1 antagonist were not studied). However, many of the behavioral effects of drugs with selective affinity for the D1 or D2 dopamine receptor can be blocked by either D1 or D2 selective compounds. For example, it has been reported that injections of D1 antagonists inhibit the stereotypy produced by injections of the selective D2 dopamine receptor agonist RU24213 (32,43) and that the catalepsy produced by SCH23390 is antagonized by D2 receptor agonists (30,36). It would therefore be of interest to investigate: 1) whether CTAs could be induced by selective D1 or D2 receptor agonists or antagonists; and 2) whether any such agonist-induced CTAs could be blocked by injections of selective dopamine receptor antagonists.

This series of studies was undertaken in order to examine the ability of compounds selective for either the D1 dopamine receptor (SKF38393) (48) or the D2 dopamine receptor (quinpirole) (50) to induce a conditioned taste aversion in rats. We furthermore studied possible D1/D2 interactions in the development of these aversions by examining their reversal by selective dopamine receptor antagonists, active either centrally and/or peripherally. Finally, we investigated one possible neuronal substrate for the agonist-induced CTAs by examining selective dopamine receptor agonist-induced taste aversions in rats with lesions of the area postrema. Portions of these data have been published in abstract form (3).

GENERAL METHOD

Subjects were adult, male Sprague-Dawley derived rats weigh-

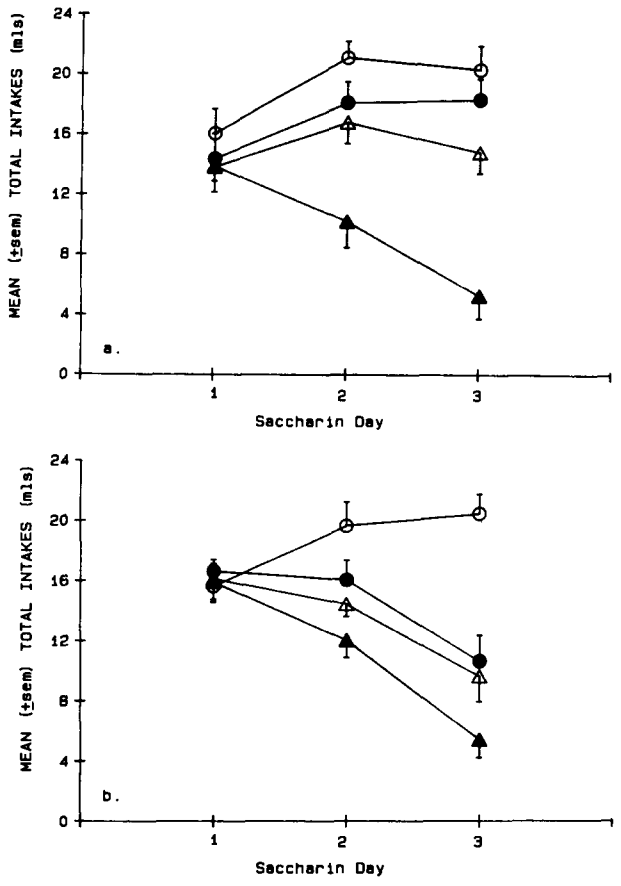

FIG. 1. Induction of conditioned taste aversions by selective dopamine receptor agonists. (a) Mean one-hour saccharin intakes for animals injected immediately after the drinking period (Days 1 and 2) with 0 (○), 1.0 (●), 5.0 (△) or 20.0 mg/kg (▲) (R,S)-SKF38393. (b) Saccharin intakes for animals injected with 0 (○), 0.075 (●), 0.15 (△) or 0.60 (▲) mg/kg quinpirole. There were 6–8 animals/group.

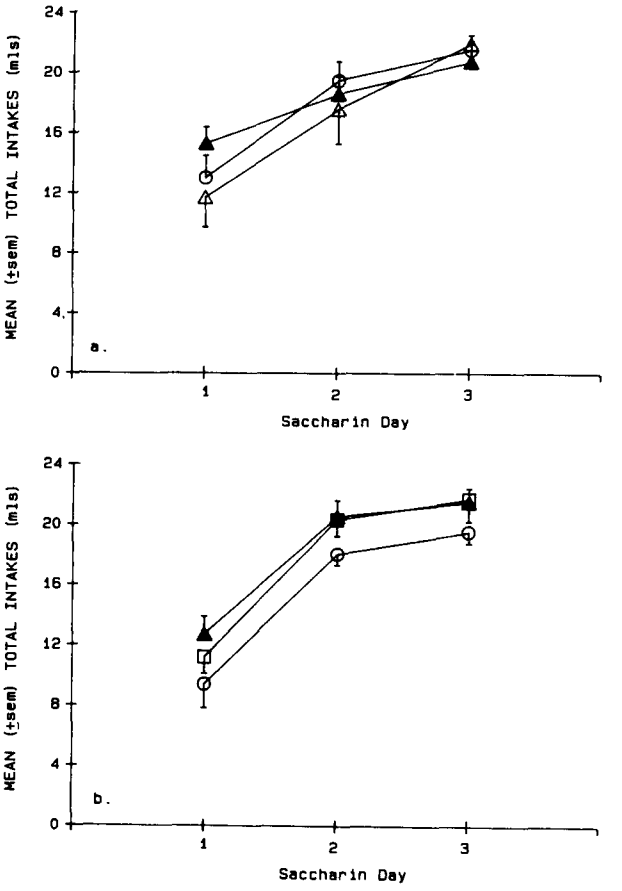

FIG. 2. Failure of D1 or D2 dopamine receptor antagonists to induce a CTA. (a) Mean one-hour saccharin intakes in animals treated immediately after the drinking period (Days 1 and 2) with either 0 (○), 0.24 (△) or 0.60 (▲) mg/kg of the D1 antagonist SCH23390. (b) Saccharin intakes for rats treated immediately after the drinking period with either 0 (○), 0.125 (□) or 0.375 (△) mg/kg of the D2 antagonist haloperidol. There were 8–12 rats/group.

ing approximately 250–350 g. Rats were experimentally naive and were only used in a single experiment. Animals were individually housed in wire mesh cages and were maintained on a 12:12 light:dark schedule, with food available ad lib.

Procedure

The procedure for inducing CTAs using a one-bottle test was as follows: Rats were maintained on a 23-hr water deprivation schedule with water available from graduated drinking tubes for 1 hr per day on days 1, 2, 4, 5, 7, and 8. On the 3rd day of the schedule, rats were presented with a 0.1% saccharin Na solution instead of water. Although the solution was available for 60 min, 40-minute intakes were recorded and animals were divided into experimental groups matched for these intakes. After one-hour access to saccharin, the solution was removed, and animals were injected with the appropriate treatment (vide infra). In experiments where agonist/antagonist interactions were studied, the antagonist was injected immediately following the drinking period and the agonist was injected 15–20 min later. A second saccharin/drug conditioning trial was given three days later (Day 6) and a final saccharin presentation was made on Day 9. Sixty-minute intakes were recorded daily. No drug treatment was found to alter subsequent water intakes so these data are not presented.

The paradigm for studying CTAs using a two-bottle test was essentially the same as that described above, except that on Day 9 of the deprivation period rats were simultaneously presented with one bottle of the saccharin solution and one bottle of tap water. (The position of the bottles was counterbalanced across groups.) Only the saccharin solution and water intakes on this day are presented.

Area Postrema Lesion Methods

Subjects were adult, male rats, similar to those described above. Rats were anesthetized with sodium pentobarbital (50 mg/kg) and were placed in the ear bars of a stereotaxic instrument. The neck was then flexed ventrally and an incision was made above the posterior border of the occipital bone. Following retraction of the overlying muscles, the atlanto-occipital membrane was punctured, allowing visualization of the obex with the aid of a dissecting microscope. Under visual guidance, a thermocautery lesion of the AP was made. Control animals underwent similar surgery except that the AP was not cauterized. Rats were allowed a three-week postoperative recovery period prior to testing, by which time body weights had stabilized.

Following CTA testing, rats were sacrificed with an overdose

of sodium pentobarbital and were then perfused transcardially with isotonic saline followed by a 10% formalin solution. Brains were removed from the skull and stored in formalin for at least 1 week prior to histological analysis. Brains were then cut into 64-μ thick sections throughout the extent of the lesion, stained with cresyl violet, and examined microscopically to determine the extent of damage to the AP and nearby structures.

Compounds

SKF38393 (the racemate and its enantiomers), SCH23390 (all from Research Biochemicals, Natick, MA) and quinpirole (Eli Lilly, Indianapolis, IN) were dissolved in distilled water; haloperidol (Sigma, St. Louis, MO) and domperidone (Janssen Pharmaceuticals, Beerse, Belgium) were dissolved in a minimal amount of acetic acid and brought up to volume with distilled water. All compounds were injected in a volume of 1 ml/kg body weight except 20 mg/kg SKF38393, which was injected as 2 ml/kg body weight. The dopamine receptor agonists were injected intraperitoneally, while the antagonists were injected subcutaneously.

EXPERIMENT I. ABILITY OF SELECTIVE D1 AND D2 DOPAMINE AGONISTS AND ANTAGONISTS TO PRODUCE CONDITIONED TASTE AVERSIONS: EFFECTS OF SKF38393, QUINPIROLE, SCH23390 AND HALOPERIDOL

Results

Saccharin intakes following (R,S)-SKF38393 (0–20 mg/kg) are shown in Fig. 1a. A two-way analysis of variance (ANOVA) (Dose × Days, with repeated measures on the Days factor) indicated significant Dose, $F(3,24)=10.26$, $p<0.001$, and Day, $F(2,48)=3.35$, $p<0.05$, effects and a significant Dose × Days interaction, $F(6,48)=8.02$, $p<0.001$, indicating that SKF38393-treated rats showed a decline in saccharin intakes across days compared to controls. Post hoc comparisons (Newman-Keuls procedure using the Satterthwaite approximation for degrees of freedom) (54) indicated that intakes of the animals injected with the highest dose (20 mg/kg) differed from controls across both test days, $7.4 \leq F(4,67) \leq 9.9$, $p<0.01$, and that the intakes of animals treated with 5 mg/kg differed significantly from control intakes on the second test day, $F(3,67)=3.9$, $p<0.01$.

The effects of various doses of quinpirole (0–0.60 mg/kg) on the establishment of a CTA are shown in Fig. 1b. Statistical analysis of group saccharin intakes indicated significant Dose, $F(3,24)=7.4$, $p<0.005$, and Day, $F(2,48)=21.4$, $p<0.001$, effects and a significant Dose × Day interaction, $F(6,48)=9.9$, $p<0.001$. Post hoc comparisons for data collected on the first test day indicated significant reductions in saccharin intakes for animals injected with either 0.15 mg/kg, $F(3,64)=3.8$, $p<0.01$, or 0.60 mg/kg, $F(4,64)=5.5$, $p<0.01$, quinpirole compared to control animals. Post hoc comparisons for intakes on the second test day indicated that the vehicle-treated group differed significantly from all quinpirole-treated groups, 0.075 mg/kg: $F(2,64)=6.8$, $p<0.01$; 0.15 mg/kg: $F(3,64)=7.6$, $p<0.01$; 0.60 mg/kg: $F(4,64)=10.5$, $p<0.01$. As indicated in Fig. 1b, the intakes of all quinpirole-treated rats declined across days compared to controls.

Injections of the D1 dopamine receptor antagonist SCH23390 (22,25) at a dose of 0.12 mg/kg failed to induce a significant taste aversion compared to vehicle injections. A two-way (Dose × Day) ANOVA with repeated measures on the Days factor indicated a significant effect of Days, $F(2,28)=7.3$, $p<0.01$, but neither a significant Dose effect nor a Dose × Days interaction ($F<1.0$) (data not shown). In another experiment using higher drug doses (0, 0.24 or 0.60 mg/kg), the ANOVA (Dose × Days)

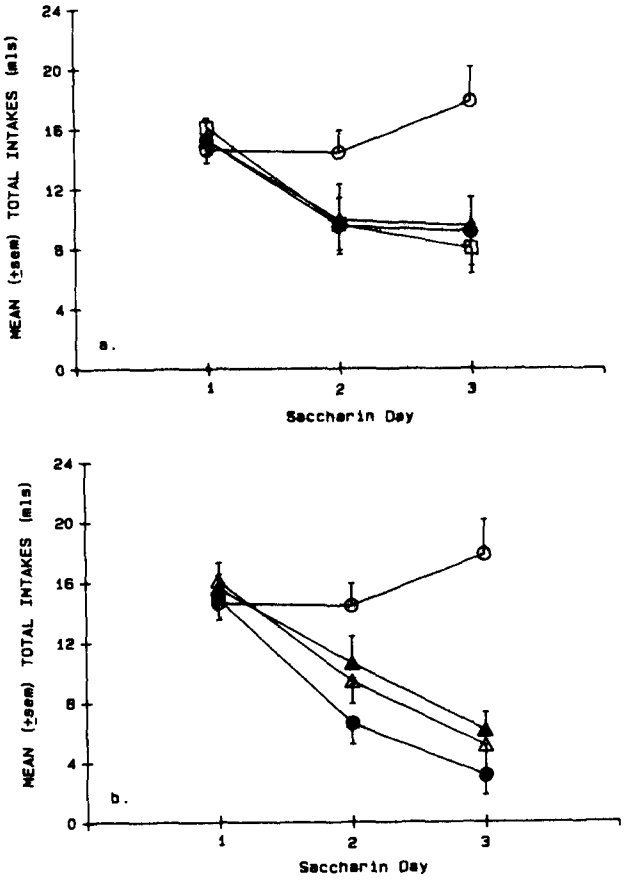

FIG. 3. (a) Injections of SCH23390 fail to affect the acquisition of a (R,S)-SKF38393-induced CTA. Rats were treated with vehicle or the antagonist immediately after access to the saccharin solution and were then injected with either vehicle or (R,S)-SKF38393 (20 mg/kg) 15–20 min later. The four treatment groups were: Vehicle + vehicle (○), vehicle + SKF38393 (□), SCH23390 (0.12 mg/kg) + SKF38393 (▲), and SCH23390 (0.24 mg/kg) + SKF38393 (●). (b) SCH23390 pretreatment fails to affect conditioning of a taste aversion to quinpirole (0.60 mg/kg). The four treatment groups were: Vehicle + vehicle (○), vehicle + quinpirole (●), SCH23390 (0.12 mg/kg) + quinpirole (△), and SCH23390 (0.24 mg/kg) + quinpirole (▲). There were 8–9 rats in each treatment group.

(with repeated measures on the Days factor) also indicated a significant effect of Days, $F(2,56)=56.8$, $p<0.001$, but neither a significant Dose effect, $F(2,28)<1$, nor a significant Dose × Days interaction, $F(4,56)=1.8$, $p>0.10$. As shown in Fig. 2a, all treatment groups showed equivalent increases in saccharin intakes across days.

Mean total saccharin intake for rats injected with the D2 antagonist haloperidol are shown in Fig. 2b, where it may be seen that fluid intakes increased similarly across days in all groups. As expected, the Days × Dose ANOVA (with repeated measures on the Days factor) indicated a significant Days effect, $F(2,54)=134.0$, $p<0.001$, but neither a significant Dose effect, $F(2,27)=2.1$, $p>0.10$, nor a significant Days × Dose interaction, $F(4,54)<1.0$.

EXPERIMENT II. D1 DOPAMINE ANTAGONIST EFFECTS ON D1 AND D2 DOPAMINE AGONIST-INDUCED CTAS: ONE- AND TWO-BOTTLE TESTS

Results

The effects of SCH23390 (0.12 or 0.24 mg/kg) on agonist-

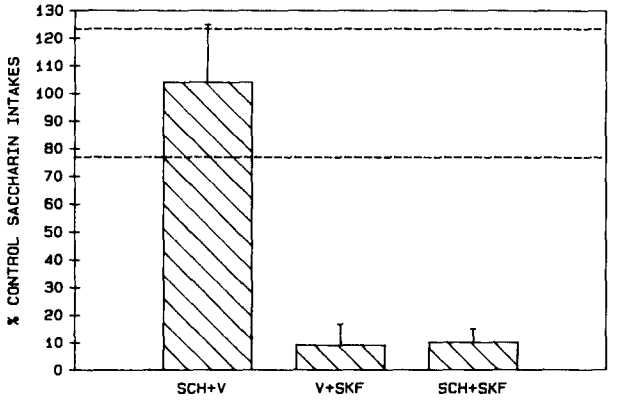

FIG. 4. Pretreatment with SCH23390 (0.60 mg/kg) fails to affect the acquisition of a (R,S)-SKF38393-induced CTA using a two-bottle test. The data are expressed as mean (±sem) group saccharin intakes as percent saccharin intakes of the control group. Total fluid intakes (water + saccharin) did not differ between groups. Abbreviations: SCH: SCH23390; V: Vehicle; SKF: (R,S)-SKF38393. Mean (±sem) intake of the control group is indicated by the dotted line. There were 8–9 rats/group.

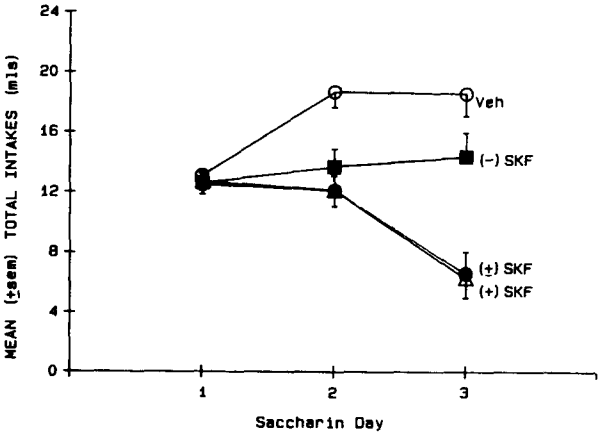

FIG. 5. Enantiomeric selectivity of the effects of SKF38393 on the induction of a CTA. Rats were injected with either vehicle, (−)-SKF38393 (10 mg/kg), (+)-SKF38393 (10 mg/kg), or the racemate (±)-SKF38393 (20 mg/kg) immediately after the drinking period. There were 12 or 13 animals/group. Only injections of the (R−) (+) enantiomer resulted in a significant CTA (see text).

induced CTAs in two-bottle tests may be seen in Fig. 3a. When the effects of the D1 antagonist on an SKF38393-induced CTA were analyzed, the multivariate ANOVA (MANOVA) (using the profile technique to handle the days factor) indicated that in comparison to vehicle-treated animals, the agonist produced a significant suppression of intakes relative to baseline, $F(2,27)=7.1$, $p<0.003$, but that neither the low nor the high dose of the antagonist had any statistically reliable effect on the induction of a CTA by SKF38393 ($F<1.0$). Similar results were obtained for the effects of these doses of SCH23390 on a quinpirole-induced CTA (Fig. 3b). The MANOVA indicated that quinpirole produced a significant suppression of intakes relative to baseline, $F(2,27)=12.8$, $p<0.001$, but the effects of SCH23390 on the quinpirole-induced CTA did not approach statistical significance ($F<1.0$). In a separate series of experiments we examined the effects of a higher dose of SCH23390 (0.60 mg/kg) on SKF38393- or quinpirole-induced CTAs. Even at this high dose, the D1 antagonist failed to attenuate the induction of either taste aversion. Significant effects of the agonists were obtained for both SKF38393, $F(4,42)=0.023$, $p<0.02$, and quinpirole, $F(4,82)=16.0$, $p<0.001$, but pretreatment with SCH23390 did not confer any protection from the CTA-inducing properties of either compound ($F<1.0$) (data not shown).

The failure of SCH23390 to block the D1 agonist-induced CTA was indeed surprising given the in vivo and in vitro pharmacology of the two compounds. We therefore decided to investigate whether some effect of SCH23390 on a (R,S)-SKF38393-induced CTA might become apparent using a two-bottle procedure, which is generally more sensitive than is a one-bottle test. The results of this study, expressed as percent saccharin intakes of the control group, are shown in Fig. 4. Although total fluid intakes (saccharin + water) on the test day did not differ between groups ($F<1.0$), a one-way ANOVA conducted on saccharin intakes on the two-bottle test day indicated a significant group effect, $F(3,31)=10.3$, $p<0.001$. Post hoc comparisons indicated that saccharin intakes were sharply suppressed in the (R,S)-SKF38393-treated group compared to vehicle-injected controls, $t(15)=3.6$, $p<0.01$, and that this suppression was not attenuated by pretreatment with SCH23390, $t(15)=0.15$, $p>0.50$. Also of interest is the observation that even in a two-bottle test, injections of SCH23390 failed to induce a CTA compared to injections of vehicle alone, $t(16)=0.11$, supporting the generality of our results using a one-bottle test.

EXPERIMENT III. ENANTIOMERIC SELECTIVITY OF SKF38393'S ABILITY TO INDUCE A CONDITIONED TASTE AVERSION: STUDIES COMPARING (R)-, (S)-, AND (R,S)-SKF38393

Results

Fluid intakes across the three saccharin presentation days were analyzed using a MANOVA [(R)-SKF38393 × (S)-SKF38393 × (Days)]; the Days factor was handled using the profile technique. The analysis indicated an overall significant (R−) × (Days) interaction term, $F(2,44)=22.6$, $p<0.001$. There appeared to be a trend for the (S−) enantiomer to produce a CTA on its own, but the MANOVA indicated that neither the (S−) × (Days) interaction, $F(2,44)=2.5$, $p<0.10$, nor the (R−) × (Days) interaction, $F(2,44)=1.9$, $p<0.16$, reached the 0.05 level of significance. Thus, only the (R−) enantiomer of SKF38393, either alone or in combination with the (S−) enantiomer, led to the development of a significant conditioned taste aversion; furthermore, the presence of the (S−) enantiomer did not significantly modify the potency of the (R−) enantiomer. Mean total intakes for the four groups are shown in Fig. 5. As may be seen, the animals receiving either the (R−) enantiomer alone or in the racemate showed reduced consumption of saccharin across days compared to either the vehicle group or the group receiving only the (S−) enantiomer.

Although there was a trend for the (S−) enantiomer to affect subsequent saccharin consumption (individual group comparisons using *t*-tests indicated that the (S−) enantiomer significantly reduced intakes compared to controls on the first, $t(22)=3.1$, $p<0.01$, but not second, $t(22)=1.9$, $p>0.05$, test day, the effect was not significant when intakes were analyzed using a MANOVA, the most appropriate test for these data. Furthermore, the intakes of the two groups of animals injected with the (R−) enantiomer were indistinguishable from one another and were significantly lower than the intakes of rats injected with either vehicle or (S−)-SKF38393 on the second test day, $t(23)=3.6$, $p<0.01$. In any case, even if the (S−) enantiomer has some aversive properties, they are significantly less than those of the (R−)

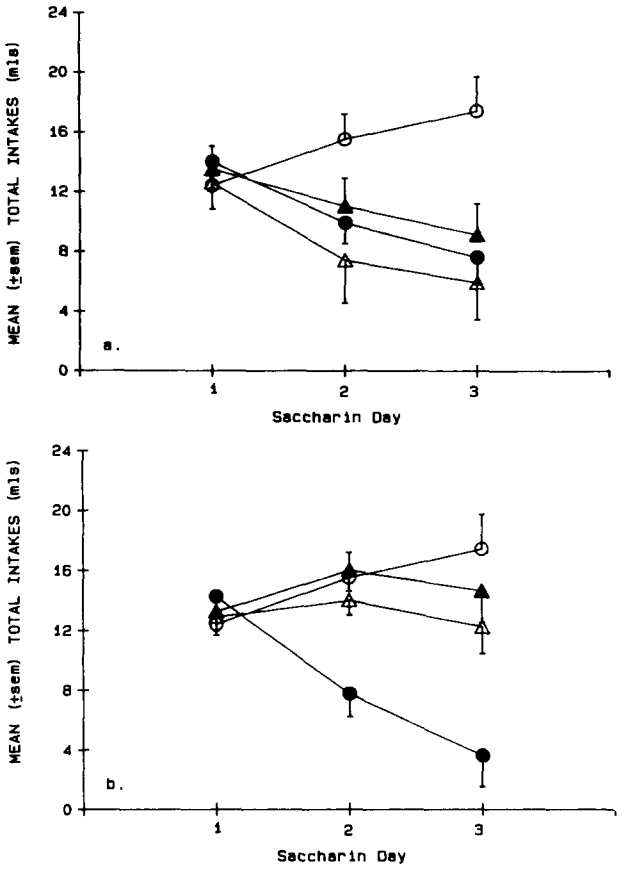

FIG. 6. (a) Haloperidol pretreatment fails to significantly affect a (R,S)-SKF38393-induced (20 mg/kg) CTA. The four treatment groups were: Vehicle + vehicle (○), vehicle + SKF38393 (△), haloperidol (0.12 mg/kg) + SKF38393 (▲), and haloperidol (0.375 mg/kg) + SKF38393 (●). (b) Haloperidol successfully blocks the acquisition of a quinpirole-induced (0.6 mg/kg) CTA. The four treatment groups were: Vehicle-vehicle (○), vehicle + quinpirole (●), haloperidol (0.125 mg/kg) + quinpirole (△), and haloperidol (0.375 mg/kg) + quinpirole (▲). There were 7–8 animals/group.

enantiomer. Our results suggest that (R,S)-SKF38393 may have actions at non-D1 dopamine receptor sites and that the aversive properties of this compound are primarily mediated through non-D1 mechanisms.

EXPERIMENT IVA. EFFECTS OF THE CENTRALLY ACTIVE D2 ANTAGONIST, HALOPERIDOL, ON THE ESTABLISHMENT OF A CTA BY (R,S)-SKF38393 AND QUINPIROLE

Results

The effects of haloperidol pretreatment on the establishment of CTAs by SKF38393 and quinpirole are shown in Fig. 6a and b. The MANOVA conducted on the effects of haloperidol indicated that, in comparison to vehicle-treated rats, (R,S)-SKF38393 produced a significant suppression of intakes relative to baseline, $F(2,26)=17.0$, $p<0.001$, but neither the low dose, $F(2,26)=1.3$, $p<0.29$, nor the high dose ($F<1.0$) of haloperidol significantly modified the SKF38393-induced CTA (Fig. 6a).

With regard to haloperidol's effects on a quinpirole-induced CTA, the MANOVA indicated a significant effect of quinpirole, $F(2,26)=14.6$, $p<0.001$, and a significant attenuation of the

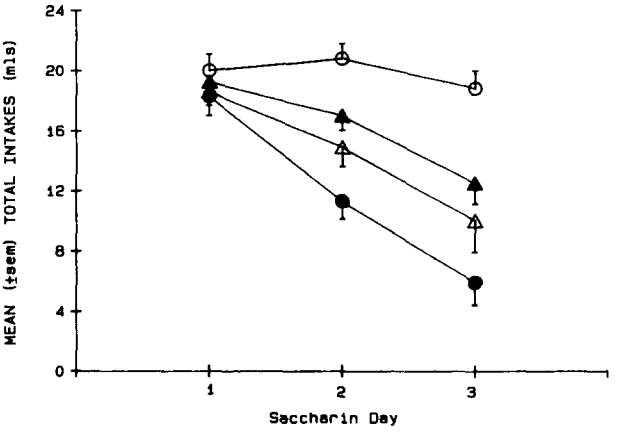

FIG. 7. Domperidone dose-dependently attenuates the induction of a CTA by quinpirole (0.6 mg/kg). Rats were injected with domperidone (or vehicle) immediately after the drinking period and 15–20 min before the second injection. The four treatment groups were: Vehicle + vehicle (○), vehicle + quinpirole (●), domperidone (2 mg/kg) + quinpirole (△), and domperidone (12 mg/kg) + quinpirole (▲). There were 8–9 rats/group.

quinpirole effect by both the low dose, $F(2,26)=3.4$, $p<0.05$, and the high dose, $F(2,26)=8.2$, $p<0.005$, of haloperidol. Individual comparisons indicated that, relative to baseline scores, the effects of haloperidol pretreatment at either dose were statistically reliable across both days of testing compared to the group receiving vehicle + quinpirole treatment, $5.0 \leq F(1,27) \leq 16.9$, $p<0.03$, for all comparisons. As may be seen in Fig. 6b, the higher dose of haloperidol produced a virtually complete blockade of quinpirole's effects.

EXPERIMENT IVB. EFFECTS OF THE PERIPHERAL D2 ANTAGONIST DOMPERIDONE ON A QUINPIROLE-INDUCED CTA

Results

The results of this experiment may be seen in Fig. 7. A MANOVA conducted on saccharin intakes indicated that quinpirole treatment produced a suppression of intakes from baseline compared to vehicle-injected controls, $F(2,28)=13.45$, $p<0.001$. There was a trend for the low dose of domperidone (3 mg/kg) to attenuate the quinpirole-induced CTA, but the effect did not achieve statistical significance at the 0.05 level, $F(2,28)=2.3$, $p<0.12$. A significant effect was obtained for the higher dose of domperidone, $F(2,28)=4.8$, $p<0.02$, and analysis of contrasts indicated significant attenuation of quinpirole's effect on both the first, $F(1,29)=10.0$, $p<0.004$, and second, $F(1,29)=5.1$, $p<0.03$, test days.

EXPERIMENT V. INVESTIGATIONS OF ONE POSSIBLE NEURONAL SUBSTRATE OF THE AGONIST-INDUCED CTAS: EFFECT OF AREA POSTREMA LESIONS

Results

Only data from rats with virtually complete destruction of the area postrema were included in the statistical analysis. In most animals there was minimal to extensive damage to the solitary tract and to the nucleus of the solitary tract; in about 30% of the animals there was damage to the nucleus gracilus. The extent of damage for the smallest and largest AP lesion is shown in Fig. 8.

Group intakes across the three days of saccharin presentations were analyzed with multivariate statistics using the profile tech-

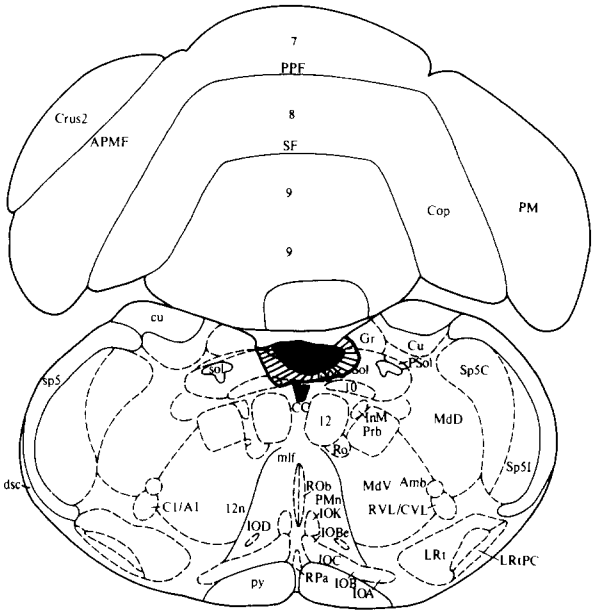

FIG. 8. The least (darkened area) and greatest (hatched area) amount of tissue damage following cauterization of the area postrema in rats. Figure modified from (41).

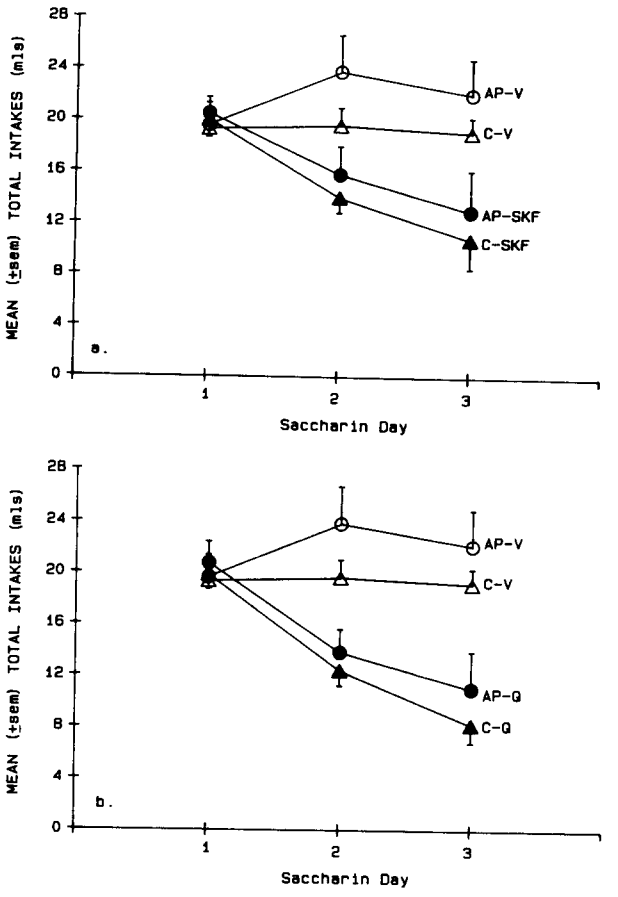

FIG. 9. Lesions of the area postrema fail to affect the acquisition of either (a) (R,S)-SKF38393- or (b) quinpirole-induced CTAs in rats. Abbreviations: AP: Area postrema-lesioned; C: Control (i.e., "sham"-operated); V: Vehicle; SKF: (R,S)-SKF38393 (20 mg/kg); Q: Quinpirole (0.60 mg/kg). There were 10 rats in each of the three lesioned groups and 14–17 rats in each of the three operated control groups.

nique (i.e., drug × lesion × days); data from quinpirole and SKF38393 groups were analyzed separately.

The results of the area postrema study are presented in Fig. 9a and b. Analysis of the data from rats treated with (R,S)-SKF38393 indicated an effect of SKF38393 across both test days, $F(2,44) = 19.4$, $p<0.001$, but neither the Lesion effect nor the Lesion × Drug interaction approached significance ($F<1.0$ in both cases). With regard to quinpirole, statistical analysis also indicated a significant overall drug effect across both test days, $F(2,46) = 31.8$, $p<0.001$, and the absence of either a significant Lesion effect or Lesion × Drug interaction ($F<1.0$).

GENERAL DISCUSSION

The series of studies reported here provides new information regarding the aversive properties of compounds selective for the D1 or D2 dopamine receptor subtype.

In the first experiment we were able to demonstrate that injections of either the D1 agonist (R,S)-SKF38393 or of the D2 agonist quinpirole can induce a CTA to a saccharin solution in rats. Studies which have examined potential reinforcing or aversive properties of these selective agonist compounds using the conditioned place preference paradigm have reported a place aversion to an environment paired with SKF38393 (10 mg/kg) and a place preference to an environment paired with quinpirole (1 mg/kg) (21). Additionally, self-administration studies have reported that quinpirole will sustain such behavior, whereas SKF38393 will not (55). Thus, quinpirole, but not SKF38393, appears to have reinforcing as well as aversive properties, which liken it to other CNS stimulants.

Our results also indicate that conditioned nausea is unlikely to be a prerequisite for the induction of a CTA by dopamine agonists since the selective D1 dopamine receptor agonist SKF38393 fails to produce emesis in dogs (48). Previously, Goudie *et al.* (17) demonstrated that injections of antiemetics do not affect the acquisition of an amphetamine-induced CTA and other evidence, recently reviewed (18), also support this conclusion.

Our failure to obtain a haloperidol-induced CTA in the first experiment is consistent with the findings of other investigators (15) who have reported the inability of haloperidol (1 mg/kg), pimozide (1 mg/kg) or chlorpromazine (20 mg/kg) to induce taste aversions when injected after the drinking trial. Haloperidol has been reported to be relatively selective for the D2 dopamine receptor (23,24) and has been demonstrated to block pergolide-induced rotation in rats with unilateral nigrostriatal bundle lesions at approximately 1/200 the dose needed to block SKF38393-induced rotation (1). Haloperidol has the property of being relatively fast-acting compared to other D2 antagonists, which allows its injection following the drinking period rather than prior to it. This is an important advantage since neuroleptics have been shown to reduce unconditioned drinking [e.g., (13,56)], which may confound the interpretation of results from CTA studies.

The results of our studies also indicate that injections of SCH23390 at doses well above those needed to produce catalepsy fail to induce significant CTAs using either one- or two-bottle tests. These new findings provide additional evidence for the similar behavioral profiles of the D1 antagonist SCH23390 and classic neuroleptic drugs with D2 receptor properties. Since SCH23390 produces behavioral effects (i.e., catalepsy) within 15 min after injection, it is unlikely that its inability to induce a CTA is due to a prolonged CS-UCS interval. Our failure to induce a CTA with either a D1 or D2 antagonist suggests that the affective

state produced by them either is nonaversive, fails to be conditionable to ingested stimuli, or is not easily discriminable by animals, as suggested by drug discrimination studies [e.g., (9)]. Neither haloperidol (0.2 mg/kg) nor SCH23390 (0.05 mg/kg) have been shown to be aversive (or reinforcing) using a conditioned place preference paradigm (28).

In the present study we found that only the R-enantiomer was capable of inducing a significant CTA. There was a nonsignificant trend for (S−) SKF38393 to induce a CTA and others have reported behavioral effects of this enantiomer (34). Previous studies have reported enantiomeric selectivity of (R−) SKF38393 in producing behavioral effects (4,31) and only the (R−) enantiomer is capable of displacing ^{3}H-piflutixol binding in the striatum (39).

Although there was stereospecificity to (R,S)-SKF38393's effects on the development of a CTA, injections of neither the D1 nor the D2 antagonist were capable of attenuating the agonist-induced CTAs. The failure of SCH23390 to affect a SKF38393-induced CTA was unexpected since, to our knowledge, this is the first demonstration of a (R,S)-SKF38393-induced behavior that has not been blocked by the antagonist; however, there is preliminary biochemical evidence supporting non-D1 receptor mediated effects of SKF38393 on DA synthesis in vitro (38). Those results, as well as ours, emphasize the need for caution when interpreting the behavioral effects of (R,S)-SKF38393 with regard to neurochemical substrates in the absence of enantiomeric and/or antagonist studies. Injections of the D1 antagonist also failed to influence a quinpirole-induced CTA, suggesting minimal involvement of D1 receptors in the acquisition of this aversion. Since other behavioral effects of SCH23390 are extremely long in duration (47), it is unlikely that its failure to reverse the effects of the agonist compounds on taste aversion learning is due to its having a short time course of action.

Several authors have reported that in rats with normosensitive dopamine receptors, D1 or D2 agonist-induced behaviors that contain a large locomotor component (e.g., stereotypy or general locomotor activity) can be blocked nonselectively by injections of either a D1 or D2 antagonist (vide infra). However, our results, and those of others, suggest that more subtle agonist-induced behaviors are more sensitive to the appropriate, selective receptor antagonist. Thus, although D1 antagonists inhibit D2 agonist-induced stereotypy (32,43), SCH23390 does not interfere with the interoceptive cues produced by quinpirole, as demonstrated in the current study and in drug discrimination tasks (53). In parallel fashion, although metoclopramide inhibits some aspects of locomotor activation by SKF38393 (33), haloperidol is unable to block the discriminative stimulus properties of SKF38393 (11). Similarly, SCH23390, but not the D2 antagonist sulpiride, is capable of attenuating SKF38393-induced changes in EEG activity (40).These results suggest that blockade of either the D1 or D2 dopamine receptor may render an animal incapable of demonstrating behaviors requiring a large motor output, whether induced by a D1 or D2 agonist. Agonist effects with minimal motoric requirements, however, are more selectively affected by the appropriate D1/D2 antagonist. [Some evidence indicates that D2 antagonists may actually *enhance* some of the behavioral effects of D1 stimulation with minimal motoric demands (i.e., perioral movements) (46).]

In the present study we demonstrated that the quinpirole-induced CTA most likely involves stimulation of the D2 dopamine receptor since injections of either haloperidol or domperidone were able to antagonize development of the CTA. The attenuation of the quinpirole taste aversion by haloperidol was not likely to be due to a general associative impairment of neuroleptic-treated rats since the drug did not affect the induction of a CTA in (R,S)-SKF38393-treated rats. The failure of haloperidol to affect the D1 receptor agonist-induced CTA indicates that D2 receptors do not contribute to the actions of SKF38393 in this behavior.

Our studies with domperidone suggest that D2 receptors lying outside of the blood-brain barrier are at least partially involved in the aversive properties of quinpirole. Based on behavioral and biochemical data, domperidone is believed to cross the blood-brain barrier poorly, if at all (10, 27, 35, 44). Although there is evidence to suggest that systemic (SC) injections of the compound may influence DA turnover in central sites (olfactory tubercle, striatum) (14) eight hours or more after injection, quinpirole elicits behavioral changes in less than 15 min (personal observations). Therefore, it is unlikely that the delayed CNS effects of domperidone would be responsible for its attenuation of the quinpirole-induced CTA. Using doses of domperidone which were similar to those of the present study, Pratt and Stolerman (42) were unable to block the establishment of an apomorphine-induced CTA. However, injections of pimozide did attenuate the aversion, suggesting central D2 mediation of apomorphine's effects.

One possible neuronal substrate for the CTA produced by quinpirole is the area postrema, which lies outside of the blood-brain barrier and has been shown, in dog, to contain D2 dopamine receptors (49) and to be involved in the emetic actions of apomorphine [e.g., (52)]. Additionally, since the area postrema has been shown to be importantly involved in the induction of some taste aversions but not in others [e.g., (6)], we decided to compare the ability of (R,S)-SKF38393 and quinpirole to produce CTAs in rats with and without AP lesions. Lesions of this brain region were found to be without effect on the induction of CTAs by either agonist compound. Area postrema lesions also fail to affect the acquisition of CTAs following systemic injections of apomorphine or amphetamine (6,51). Since these compounds would be expected to act as agonists at both D1 and D2 dopamine receptor subtypes, a lesion of the AP might "selectively" attenuate D2 effects of the drugs without affecting any D1 receptor-mediated contribution to the CTA. However, our finding that AP lesions fail to affect a quinpirole-induced CTA suggests that the structure is not importantly involved in the aversive actions of D2 agonists following systemic injections in rats. Since the gut has been shown to be sensitive to compounds with affinity for the D2 receptor [e.g., (20,26)], it is possible that at least some of the aversive properties of quinpirole are mediated through stimulation of these receptors. However, the almost complete reversal of the quinpirole-induced CTA by haloperidol, compared to only an attenuation of the CTA by the relatively high dose of domperidone, suggests that there is some centrally-mediated D2 contribution to quinpirole's effects.

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REFERENCES

1. Arnt, J.; Hyttel, J. Inhibition of SKF38393 and pergolide-induced circling in rats with unilateral 6OHDA lesion is correlated to dopamine D-1 and D2 receptor affinities *in vitro*. J. Neural Transm. 67:225–240; 1986.
2. Arnt, J.; Perregaard, J. Synergistic interaction between dopamine D-1 and D-2 receptor agonists: Circling behaviour of rats with hemitransection. Eur. J. Pharmacol. 143:45–53; 1987.
3. Asin, K. E.; Montana, W. E. Studies on selective D1/D2 dopamine

agonist-induced conditioned taste aversions in rats. Soc. Neurosci. Abstr. 13:449; 1987.
4. Asin, K. E.; Montana, W. E. Rotation following intranigral injections of a selective D1 and D2 agonist in rats. Pharmacol. Biochem. Behav. 29:89–92; 1988.
5. Berger, B. D. Conditioning of food aversions by injections of psychoactive drugs. J. Comp. Physiol. Psychol. 81:21–26; 1972.
6. Berger, A. D.; Wise, C. D.; Stein, L. Area postrema damage and bait shyness. J. Comp. Physiol. Psychol. 82:475–479; 1973.
7. Cappell, H.; LeBlanc, A. E. Conditioned aversion to saccharin by single administrations of mescaline and d-amphetamine. Psychopharmacologia 22:352–256; 1971.
8. Clark, D.; White, F. J. Review: D1 dopamine receptor—the search for a function: A critical evaluation of the D1/D2 dopamine receptor classification and its functional implications. Synapse 1:347–388; 1987.
9. Colpaert, F. C.; Niemegeers, C. J. E.; Janssen, P.A. J. Theoretical and methodological considerations on drug discrimination learning. Psychopharmacology (Berlin) 46:169–177; 1976.
10. Costall, B.; Fortune, D. H.; Naylor, R. J. Neuropharmacological studies on the neuroleptic potential of domperidone (R33812). J. Pharm. Pharmacol. 31:344–347; 1979.
11. Cunningham, K. A.; Callahan, P. M.; Appel, J. B. Dopamine D1 receptor mediation of the discriminative stimulus properties of SKF38393. Eur. J. Pharmacol. 119:121–125; 1985.
12. D'Mello, G. D.; Goldberg, D. M.; Goldberg, S. R.; Stolerman, I. P. Conditioned taste aversion and operant behavior in rats: Effects of cocaine, apomorphine and some long-acting derivatives. J. Pharmacol. Exp. Ther. 219:60–68; 1981.
13. Falk, J. L.; Burnidge, G. K. Drug antagonism and water intake. Physiol. Behav. 5:193–198; 1970.
14. Farah, J. M.; Demarest, K. T.; Moore, K. E. A comparison of domperidone and haloperidol effects on different dopaminergic neurons in the rat brain. Life Sci. 33:1561–1566; 1983.
15. Gale, K. Catecholamine-independent behavioral and neuro-chemical effects of cocaine in rats. In: Sharpe, C., ed. Mechanism of tolerance and dependence. Washington, DC: U.S. Government Printing Office; 1984:323–332.
16. Goudie, A. J.; Dickins, D. W.; Thornton, E. W. Cocaine-induced conditioned taste aversions in rats. Pharmacol. Biochem. Behav. 8:757–761; 1978.
17. Goudie, A. J.; Stolerman, I. P.; Demellweek, C.; D'Mello, G. D. Does conditioned nausea mediate drug-induced conditioned taste aversions? Psychopharmacology (Berlin) 78:277–281; 1982.
18. Grant, V. L. Do conditioned taste aversions result from activation of emetic mechanisms? Psychopharmacology (Berlin) 93:405–415; 1987.
19. Grupp, L. A. Effects of pimozide on the acquisition, maintenance, and extinction of an amphetamine-induced taste aversion. Psychopharmacology (Berlin) 53:235–242; 1977.
20. Harrington, R. A.; Hamilton, C. W.; Brogden, R. N.; Linkewich, J. A.; Romankiewicz, J. A.; Heel, R. C. Metoclopramide. An updated review of its pharmacological properties and clinical use. Drugs 25:451–494; 1983.
21. Hoffman, D. C.; Beninger, R. J. Selective dopamine D1 and D2 receptor agonists produce conditioned taste aversions. Soc. Neurosci. Abstr. 13:835; 1987.
22. Hyttel, J. SCH23390—the first selective dopamine D-1 antagonist. Eur. J. Pharmacol. 91:153–154; 1983.
23. Hyttel, J. Biochemical challenge and biological markers for schizophrenia. Int. J. Neurosci. 31:265–266; 1986.
24. Hyttel, J.; Christensen, A. V. Biochemical and pharmacological differentiation of neuroleptic effect on dopamine D1 and D2 receptors. J. Neural Transm. 18:157–164; 1983.
25. Iorio, L. C.; Barnett, A.; Leitz, F. H.; Houser, V. P.; Korduba, C. A. SCH23390, a potential benzazepine antipsychotic with unique interactions on dopaminergic systems. J. Pharmacol. Exp. Ther. 226: 462–468; 1983.
26. Kusunoki, M.; Taniyama, K.; Tanaka, C. Dopamine regulation of (^{3}H)acetylcholine release from guinea-pig stomach. J. Pharmacol. Exp. Ther. 234:713–719; 1985.
27. Laduron, P. M.; Leysen, J. E. Domperidone, a specific *in vitro* dopamine antagonist, devoid of *in vivo* central dopaminergic activity. Biochem. Pharmacol. 28:2161–2165; 1979.
28. Leone, P.; DiChiara, G. Blockade of D-1 receptors by SCH23390 antagonizes morphine- and amphetamine-induced place preference conditioning. Eur. J. Pharmacol. 135:251–254; 1987.
29. Longoni, R. L.; Spina, L.; DiChiara, G. Permissive role of D-1 receptor stimulation for the expression of D-2 mediated behavioral responses: A quantitative phenomenological study in rats. Life Sci. 41:2135–2145; 1987.
30. Meller, E.; Kuga, S.; Friedhoff, A. J.; Goldstein, M. Selective D2 dopamine receptor agonists prevent catalepsy induced by SCH23390, a selective D1 antagonist. Life Sci. 36:1857–1864; 1985.
31. Molloy, A.G.; Waddington, J. L. Dopaminergic behaviour stereospecifically promoted by the D1 agonist R-SK&F38393 and selectively blocked by the D1 antagonist SCH23390. Psychopharmacology (Berlin) 82:409–410; 1984.
32. Molloy, A. G.; Waddington, J. L. The enantiomers of SK&F83566, a new selective D-1 dopamine antagonist, stereospecifically block stereotyped behaviour induced by apomorphine and by the selective D-2 agonist RU24213. Eur. J. Pharmacol. 116:183–186; 1985.
33. Molloy, A. G.; Waddington, J. L. Sniffing, rearing and locomotor responses to the D-1 dopamine agonists R-SK&F38393 and to apomorphine: Differential interactions with the selective D-1 and D-2 antagonists SCH23390 and metoclopramide. Eur. J. Pharmacol. 108:305–308; 1985.
34. Molloy, A. G. Waddington, J. L. Assessment of grooming and other behavioural responses to the D-1 dopamine receptor agonist SK&F38393 and its R- and S-enantiomers in the intact adult rat. Psychopharmacology (Berlin) 92:164–168; 1987.
35. Molloy, A. G.; O'Boyle, K. M.; Pugh, M. T.; Waddington, J. L. Locomotor behaviors in response to new selective D-1 and D-2 dopamine receptor agonists, and the influence of selective antagonists. Pharmacol. Biochem. Behav. 25:249–253; 1986.
36. Morelli, M.; DiChiara, G. Catalepsy induced by SCH23390 in rats. Eur. J. Pharmacol. 1178:179–185; 1985.
37. Niemegeers, C. J. E. Antiemetic specificity of dopamine antagonists. Psychopharmacology (Berlin) 78:210–213; 1982.
38. Novak, E. A.; Salab, R. S.; Clark, D.; Galloway, M. P. Regulation of dopamine (DA) synthesis in striatal slices: A model for DA autoreceptors. Soc. Neurosci. Abstr. 13:1641; 1987.
39. O'Boyle, K. M.; Waddington, J. L. Selective and stereospecific interactions of R-SK&F38393 with ^{3}H-piflutixol but not ^{3}H-spiperone binding to striatal D-1 and D-2 receptors: comparisons with SCH23390. Eur. J. Pharmacol. 98:433–436; 1984.
40. Ongini, E.; Caporali, M. G.; Massotti, M. Stimulation of dopamine D-1 receptors by SKF38393 induces EEG desynchronization and behavioral arousal. Life Sci. 37:2327–2333; 1985.
41. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. Orlando, FL: Academic Press; 1986.
42. Pratt, J. A.; Stolerman, I. P. Pharmacologically specific pretreatment effects on apomorphine-mediated conditioned taste aversions in rats. Pharmacol. Biochem. Behav. 20:507–511; 1984.
43. Pugh, M. T.; O'Boyle, M.; Molloy, A. G.; Waddington, J. L. Effects of the putative D-1 antagonist SCH23390 on stereotyped behaviour induced by the D-2 agonist RU24213. Psychopharmacology (Berlin) 87:308–312; 1985.
44. Reyntjens, A. J.; Niemegeers, C. J. E.; VanNueten, J. M.; Laduron, P.; Heykants, J.; Schellekens, H. L.; Marsboom, R.; Jagenau, A.; Broekaert, A.; Janssen, P. A. J. Domperidone, a novel and safe gastrokinetic anti-nauseant for the treatment of dyspepsia and vomiting. Arzneimittelforschung 28:1194–1196; 1978.
45. Riley, A. L.; Zellner, D. A. Methylphenidate-induced conditioned taste aversion: An index of toxicity. Physiol. Psychol. 6:354–358; 1978.
46. Rosengarten, H.; Schweitzer, J. W.; Friedhoff, A. J. Induction of oral dyskinesias in naive rats by D1 stimulation. Life Sci. 33:2479–2482; 1983.
47. Schulz, D. W.; Staples, L.; Mailman, R. B. SCH23390 causes persistent antidopaminergic effects in vivo: Evidence for longterm occupation of receptors. Life Sci. 36:1941–1948; 1985.
48. Setler, P. E.; Sarau, H. M.; Zirkle, C. L.; Saunders, H. L. The central effects of a novel dopamine agonist. Eur. J. Pharmacol. 50:419–430; 1978.
49. Stefanini, E.; Clement-Cormier, Y. Detection of dopamine receptors in the area postrema. Eur. J. Pharmacol. 74:257–260; 1981.

50. Tsuruta, K. E.; Frey, A.; Grewe, C. W.; Cote, T. E.; Eskay, R. I.; Kebabian, J. W. Evidence that LY141865 specifically stimulates the D2 dopamine receptor. Nature 292:463–465; 1981.
51. Van der Kooy, D.; Swerdlow, N. R.; Koob, G. F. Paradoxical reinforcing properties of apomorphine: Effects of nucleus accumbens and area postrema lesions. Brain Res. 259:111–118; 1983.
52. Wang, S. C.; Borison, H. L. the vomiting center. A critical experimental analysis. Arch. Neurol. Psychiatry 63:928–941; 1950.
53. Weathersby, R. T.; Appel, J. B. Dopamine D2 receptor mediation of the discriminative stimulus properties of LY171555 (quinpirole). Eur. J. Pharmacol. 132:87–91; 1986.
54. Winer, B. J. Statistical principles in experimental design. New York: McGraw-Hill; 1971.
55. Woolverton, W.; Goldberg, L. I.; Ginos, J. Z. Intravenous self-administration of dopamine receptor agonists by rhesus monkeys. J. Pharmacol. Exp. Ther. 230:678–683; 1984.
56. Xenakis, S.; Sclafani, A. The effects of pimozide on the consumption of a palatable saccharin-glucose solution in the rat. Pharmacol. Biochem. Behav. 15:435–442; 1981.