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5-HT_3 Receptor Antagonists Block Cocaine-Induced Locomotion via a PCPA-Sensitive Mechanism

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SVINGOS, A. L. AND R. HITZEMANN. *5-HT_3 receptor antagonists block cocaine-induced locomotion via a PCPA-sensitive mechanism.* PHARMACOL BIOCHEM BEHAV 43(3) 871-879, 1992.—We report results in rats pretreated with (±)-zacopride (0.03 mg/kg, IP), ICS 205-930 (0.1 mg/kg, IP), and MDL 72222 (1.0 mg/kg, IP) 15 min before challenge with (−)-cocaine (10.0 mg/kg, IP). At a dose of 10 μg/kg, zacopride significantly inhibited (approximately 50%) cocaine-induced locomotion. We also investigated whether or not 5-hydroxytryptamine$_3$ (5-HT_3) antagonists block the cocaine binding site on the dopamine transporter and/or affect the ability of dopamine to regulate this binding site. In well-washed striatal membranes, neither zacopride nor ICS 205-930 (10^{-9}-10^{-5} M) inhibited [^{3}H]2β-carbomethoxy-3β-(4-fluorophenyl)tropane ([^{3}H]WIN 35,428) (0.3 nM) binding. Furthermore, neither of these compounds affected the ability of dopamine to block WIN 35,428 binding. To determine if 5-HT is required for the 5-HT_3 antagonist effect, we examined the interaction between cocaine and zacopride in rats pretreated with *p*-chlorophenylalanine (PCPA) (3 days × 100 mg/kg/day). PCPA pretreatment shifted the cocaine dose-response curve to the right and blocked the ability of zacopride to reverse cocaine-induced activity.

Cocaine Serotonin 5-HT_3 antagonists Behavior Rat Dopamine transporter PCPA

BEHAVIORAL data suggest that 5-hydroxytryptamine$_3$ (5-HT_3) receptor antagonists are potent inhibitors of some but not all drug-induced behaviors associated with activation of brain dopamine systems. For example, ICS 205-930 and MDL 72222 block place preference induced by either nicotine or morphine but not by amphetamine administration (10). Costall et al. (14) found that GR 38032F (a 5-HT_3 antagonist whose structure does not contain a tropane moiety) blocked the hyperactivity caused by acute amphetamine administration but did not block the stereotypical behaviors induced by chronic intoxication. Ondansetron, GR 65630, ICS 205-930 and MDL 72222 blocked the hyperactivity induced by DiMe-C7, a substance P analog and dopamine activator (18). In mice, Reith (34) observed that the 5-HT_3 receptor antagonists, zacopride and ICS 205-930, block the hyperactivity induced by an acute cocaine injection. In contrast, the 5-$HT_{1,2}$ antagonist, methysergide, failed to block cocaine-induced behaviors. Paris and Cunningham (29) found that while 5-HT_3 antagonists inhibit unconditioned cocaine-induced behaviors the discriminative stimulus effects of cocaine remain intact.

One explanation for some of the paradoxical results noted above is that 5-HT_3 antagonists of differing structure may have somewhat different mechanisms of action, perhaps by interacting with different subclasses of 5-HT_3 receptors. However, voltage-clamp studies suggest that from a functional perspective all 5-HT_3 receptor sites are identical (31). An alternative explanation is that 5-HT_3 antagonists affect only certain behaviors and that the specificity is associated with either the principle neurotransmitters and/or brain regions involved in the behavior. All behaviors noted above have been shown to involve brain dopamine although the relative involvement of the mesolimbic and nigrostriatal systems may differ. In this regard, 5-HT_3 receptors have been located in both the mesolimbic (A10) and nigrostriatal (A9) dopamine pathways (21). Sorenson et al. (39) found that 5-HT_3 antagonists decrease dopamine firing rates both in A10 and A9. Similarly, 5-HT_3 antagonists block dopamine release in both the nucleus accumbens and striatum (5,9). It should be noted that under some conditions 5-HT_3 antagonists appear to have postsynaptic actions. Tyers et al. (42) found that the hyperactivity normally caused by infusion of dopamine into the nucleus accumbens is inhibited by ICS 205-930.

In the present study, we have a) investigated the effects of multiple 5-HT_3 antagonists on cocaine-induced hyperactivity,

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b) investigated some alternative mechanisms of 5-HT_3 action, and c) investigated the requirement of endogenous serotonin for the 5-HT_3 antagonist inhibition of cocaine-induced locomotion. The 5-HT_3 antagonists (±)-zacopride, ICS 205-930, and MDL 72222 were used to behaviorally assess the effectiveness of structurally different compounds. ICS 205-930 and MDL 72222 are tropane derivatives; zacopride is not.

[^{3}H]2β-Carbomethoxy-3β-(4-fluorophenyl) tropane ([^{3}H]-WIN 35,428), a high-affinity phenyltropane cocaine analog, was used to determine if 5-HT_3 antagonists inhibit cocaine receptor binding to the striatal dopamine transporter. Madras et al. (27) have shown that the specific dopamine uptake blockers (mazindol, GBR 12909, LU 19-005) inhibit striatal [^{3}H]WIN 35,428 binding by 90%. They have also shown that cocaine and cocaine congeners fully displaces WIN 35,428 binding. We employed [^{3}H]WIN 35,428 to examine if 5-HT_3 antagonists inhibit cocaine induced locomotion by a) directly blocking cocaine's ability to bind with the cocaine receptor and/or b) interacting with the dopamine binding sites on the transporter. To determine the role of endogenous 5-HT, animals were pretreated with *p*-chlorophenylalanine (PCPA) for 3 days (100 mg/day) to reduce endogenous 5-HT levels by 90% (1) and then challenged with zacopride and cocaine.

METHOD

Materials

[^{3}H]WIN 35,428 (87.0 Ci/mM) was obtained from Du Pont-New England Nuclear (Boston, MA). (±)-Zacopride was generously supplied by Wyeth-Ayerst (Princeton, NJ). ICS 205-930 and MDL 72222 were obtained from Research Biochemicals, Inc. (Natick, MA). (−)-Cocaine and PCPA were obtained from Sigma Chemical Co. (St. Louis, MO).

Drug Treatment

Male Sprague-Dawley rats (Taconic Farms) weighing between 250–300 g were used. Animals were maintained on a 12 L : 12 D schedule and provided with food and water ad lib. Animals were divided into five subgroups on the basis of pretreatment with saline, zacopride (0.03 mg/kg, IP), ICS 205-930 (0.1 mg/kg, IP), or MDL 72222 (3.0 mg/kg, IP). The 5-HT_3 antagonists were administered 15 min before either cocaine (10 mg/kg, IP) or saline injection (n = 8–12 animals/group). All drugs were brought into solution with saline except ICS 205-930 and MDL 72222, to which glacial acid was added. The pH was then adjusted to 5.5. The control vehicle for these groups reflected this difference. The doses of the 5-HT_3 antagonists were based upon dose-response curves for each antagonist.

In another set of experiments (n = 6/group), animals were pretreated with PCPA (100 mg/kg, IP) daily for 3 days. One group of animals were pretreated with (±)-zacopride (0.03 mg/kg, IP) and challenged with 10.0 mg/kg cocaine. The control groups consisted of one group that received saline pretreatment and a 10.0-mg/kg cocaine challenge and one group that was pretreated and challenged with saline. A second group of animals was pretreated with zacopride (0.03 mg/kg, IP) and challenged with 3.0 mg/kg cocaine. The control groups were the same as indicated above, with changes reflecting differences in cocaine dosages.

Measurement of Behavior

An open-field, Plexiglas, four-quadrant arena (94 × 94 × 38 cm) with a one-way mirrored top was used for manual observation. Animals are acclimated to the arena for 0.5 h prior to injection. Hyperactive locomotion was defined as locomotion that exceeded the pace of normal locomotion based upon the number of quadrant crossovers. Measurements were taken every 10 min for a 4-min period. Observations were made between 9:00 a.m. and 1:00 p.m. All trials lasted 1 h, were run double blind, and were recorded on videocamera.

Binding Assay

Binding assays were performed as described elsewhere (26,27). Briefly, animals were decapitated and brains quickly removed. The caudate putamen was dissected and homogenized in 10 vol ice-cold sodium phosphate (10 mM) and sucrose (0.32 M) buffer (pH 7.4 at 4°C). The homogenate was centrifuged at 17,500 × g for 20 min. The resulting pellet was resuspended in 40 vol buffer and the entire wash procedure was repeated twice. The Lowry et al. (25) method was used to determine protein concentration. Assay tubes contained buffer or buffer plus test drug (0.15 ml), [^{3}H] WIN 35,428 (0.05 ml, 0.3 nM), and tissue (0.7 ml) to a final volume of 0.9 μm. Nonspecific binding was determined with cocaine (30 ml). All incubations were performed at 0–4°C and terminated after 2 h by rapid filtration over Whatman (Maidstone, England) GF/B filters presoaked in 0.1% bovine serum albumin. The filters were washed twice with 10 ml ice-cold buffer, put into minivials, and 5 ml Scintiverse-E added. Radioactivity was counted on a Beckman LKB liquid scintillation counter (Beckman Instruments, Fullerton, CA). All experiments were conducted in triplicate, and each experiment was the average of three experiments.

Statistical Analysis

The behavioral data were analyzed using a multivariate analysis of variance (MANOVA), followed by posthoc analysis. Estimates of IC_{50} values for the binding data were analyzed by the EBDA software program (28).

RESULTS

Behavioral Data

Analysis of the data for animals pretreated with saline, (±)-zacopride (0.03 mg/kg), ICS 205-930 (0.1 mg/kg), or MDL 72222 (3.0 mg/kg) followed 15 min later by injection with saline or cocaine revealed significant differences among groups for the pretreatment × treatment × time interaction, $F(12, 224) = 13.89$, $p < 0.0001$, and pretreatment × treatment interaction, $F(3, 56) = 57.43$, $p < 0.00001$ (Fig. 1). Collapsing across time, increased locomotor activity was observed in saline-cocaine- as compared to saline-saline-treated animals [139 ± 32 vs. 12 ± 3 square crossings/50 min [(mean ± SD)] ($p < 0.0001$). Pretreatment with (±)-zacopride (0.03 mg/kg), ICS 205-930 (0.1 mg/kg), or MDL 72222 (3.0 mg/kg) significantly attenuated cocaine-induced locomotion. Total square crossings for the 5-HT_3 antagonist-pretreated groups were zacopride 29 ± 9, ICS 205-930 32 ± 9, and MDL 72222 32 ± 11. All 5-HT_3 antagonist-saline-treated groups showed increased activity when compared to the saline-saline group ($p < 0.05$ for all comparisons, Duncan's multiple-range test). There were no significant differences between the 5-HT_3 antagonist-saline- vs. antagonist-cocaine-treated groups except zacopride-pretreated animals, where the cocaine-treated group showed lower activity than the saline-treated group (15 ± 7 vs. 29 ± 9 square crossings/50 min, $p < 0.05$).

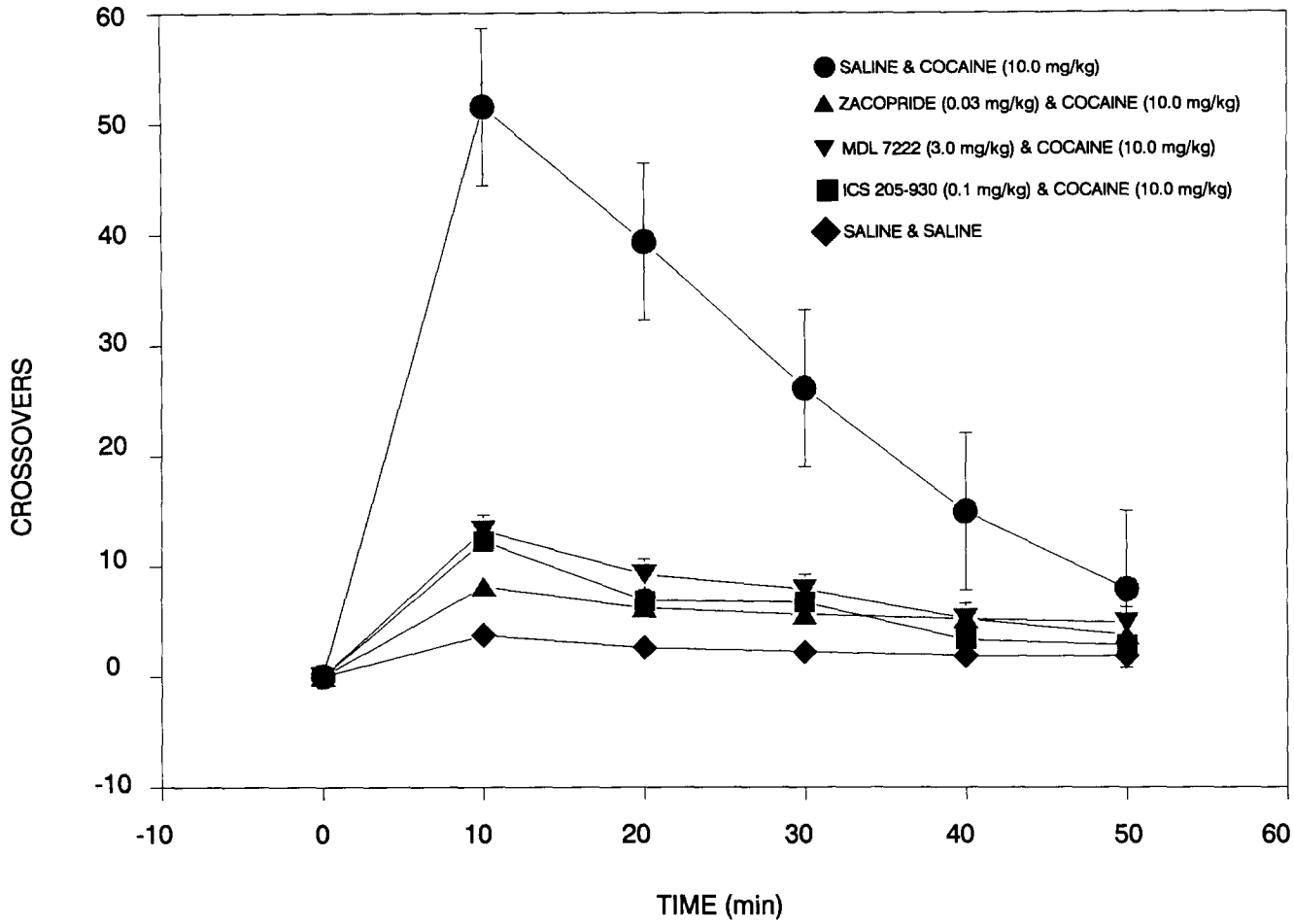

FIG. 1. Time course of animals pretreated with zacopride (0.03 mg/kg), ICS-205, 930 (0.1 mg/kg), MDL 72222 (3.0 mg/kg), or saline and then challenged with cocaine (10.0 mg/kg). Results are means ± SEM. A significant attenuation of cocaine-induced hyperactivity was seen in groups pretreated with zacopride, ICS-205, 930, and MDL 72222 ($p < 0.001$).

The zacopride dose-response data (Fig. 2) revealed a significant pretreatment (0.1, 0.03, and 0.1 mg/kg) × treatment (saline or cocaine) × time interaction, $F(8, 152) = 15.32$, $p < 0.00001$, and a significant pretreatment × treatment interaction, $F(2, 38) = 15.49$, $p < 0.00001$. Collapsing across time, 0.01 mg/kg zacopride significantly attenuated the cocaine-induced increase of ambulation; the 0.03 and 0.1 mg/kg zacopride × cocaine data did not differ from each other, but both caused a significantly greater inhibition of the cocaine effect as compared to the 0.01-mg/kg group ($p < 0.05$ for all comparisons, Duncan's multiple-range test).

Animals were pretreated either with saline or PCPA (100 mg/kg, IP, × 3 days) (pretreatment$_1$) prior to administration of saline or zacopride (0.03 mg/kg, IP) (pretreatment$_2$); 15 min later, animals were administered saline or cocaine (10 mg/kg, IP) (treatment) and open-field behavior (time) was monitored as described above. The pretreatment$_1$ × pretreatment$_2$ × treatment × time interaction was significant, $F(4, 224) = 9.92$, $p < 0.01$; the pretreatment$_1$ × pretreatment$_2$ × treatment interaction across time was also significant, $F(1, 56) = 32.11$, $p < 0.001$. PCPA- × saline- × cocaine-treated animals in comparison to saline- × saline- × cocaine-treated animals showed a 70% decrease in activity (Fig. 3). PCPA-treated animals were primarily engaged in nonlocomotor stereotyped behaviors. The residual locomotor activity in PCPA-pretreated animals was resistant to the effects of zacopride ($p > 0.05$, Duncan's multiple-range test).

In a separate series of experiments, the dose of cocaine was lowered to 3.0 mg/kg. Collapsing across time, the pretreatment$_1$ × pretreatment$_2$ × treatment interaction was significant, $F(1, 50) = 9.9$, $p < 0.003$. In the saline × saline-pretreated groups, 3.0 mg/kg cocaine had no significant effect on activity compared to the saline-treated group ($p > 0.05$, Duncan's multiple-range test) (Fig. 4). After PCPA pretreatment, cocaine significantly increased activity ($p < 0.05$, Duncan's multiple-range test) compared to non-PCPA-treated animals. There was no significant difference in activity between the PCPA- × zacopride- × cocaine- and the PCPA- × saline- × cocaine-treated groups ($p > 0.05$, Duncan's multiple-range test).

5-HT$_3$ Antagonists, Cocaine Binding Sites, and the Dopamine Transporter

Cocaine displaced specifically bound [^{3}H]WIN 35,428 (0.3 nM) in a concentration-dependent manner (IC_{50} = 106 ± 18

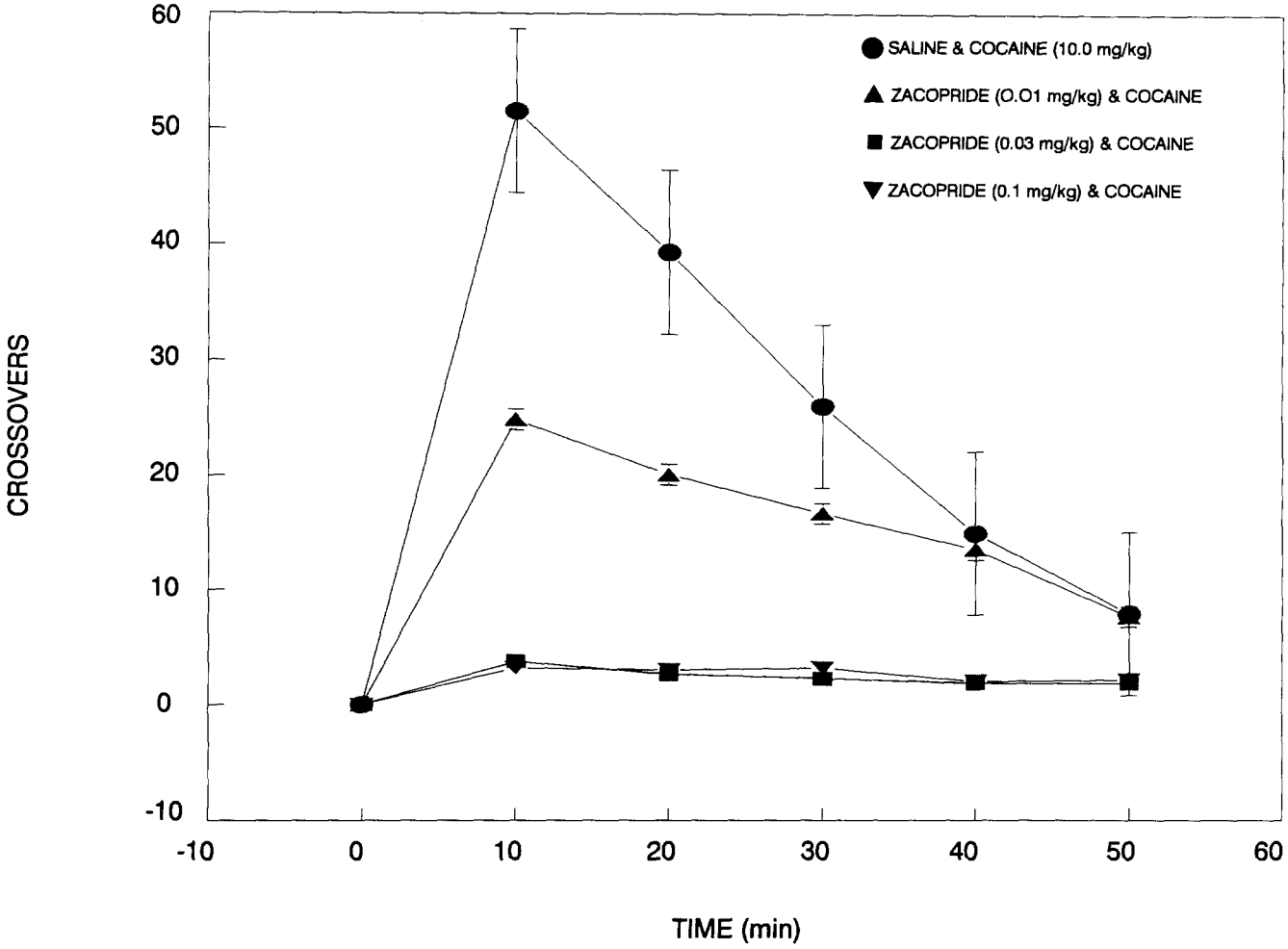

FIG. 2. Dose-response curve for (±)-zacopride. Zacopride 0.01 mg/kg IP reduced cocaine-induced locomotor activity by approximately 50%, while 0.03 and 0.1 mg/kg did not differ from saline-treated animals ($p < 0.05$). Results are means ± SEM.

nM) (Fig. 5). Neither zacopride (100 pM–50 μM) nor ICS 205-930 (5 nM–50 μM) inhibited cocaine binding to [^{3}H]WIN 35,428 (Fig. 5). (±)-Zacopride and ICS 205-930 were chosen by binding assays because of their relatively higher receptor affinities compared to other 5-HT$_3$ antagonists and for comparison between nontropane and tropane compounds.

Dopamine inhibited in a dose-dependent manner [^{3}H]WIN 35,428 binding (IC_{50} = 4.7 ± 0.05 μM) (Fig. 6). Figure 6 shows that over a wide range of concentrations (5 nM–500 mM) neither (±)-zacopride nor ICS 205-930 blocked or potentiated the dopamine effect on [^{3}H]WIN 35,428 binding.

DISCUSSION

Behavioral Data

The present study investigated the effects of 5-HT$_3$ antagonists on dopamine-associated cocaine-induced behaviors (19,20,36). Our behavioral data corroborate those of Reith et al. (34), demonstrating that 5-HT$_3$ antagonists block the increased locomotor activity induced by acute cocaine administration. These results do not seem to be associated with nonspecific sedative qualities of the antagonists because it has been shown that 5-HT$_3$ antagonists did not attenuate caffeine-induced hyperactivity (34). Nor does it appear that the 5-HT$_3$ antagonists directly affect 5-HT or dopamine turnover. For example, Koulu et al. (23) found that acute administration of 5-HT$_3$ antagonists produced no changes in the levels of 5-HT, dopamine, or the amine metabolites within the striatum, nucleus accumbens, and substantia nigra.

Our data differ from those of Reith (34) in that it was found that (±)-zacopride inhibited cocaine-induced locomotion at lower doses than had previously been reported (0.03 vs. 0.1 mg/kg). Although we did not examine doses lower than 0.03 mg/kg, the marked potency of this dose suggests that doses as low as 0.01 mg/kg may be effective. The discrepancy in dose potency may be due to a species difference, although the same dose of ICS 205-930 (0.1 mg/kg) was effective in both mice and rats. The difference in effective 5-HT$_3$ antagonist dosage may also be due to differences in cocaine dosages (10.0 vs. 25.0 mg/kg) or route of administration (IP vs. SC). It is of interest that the behavioral potency of the 5-HT$_3$ antagonists reflects their relative binding potencies (za-

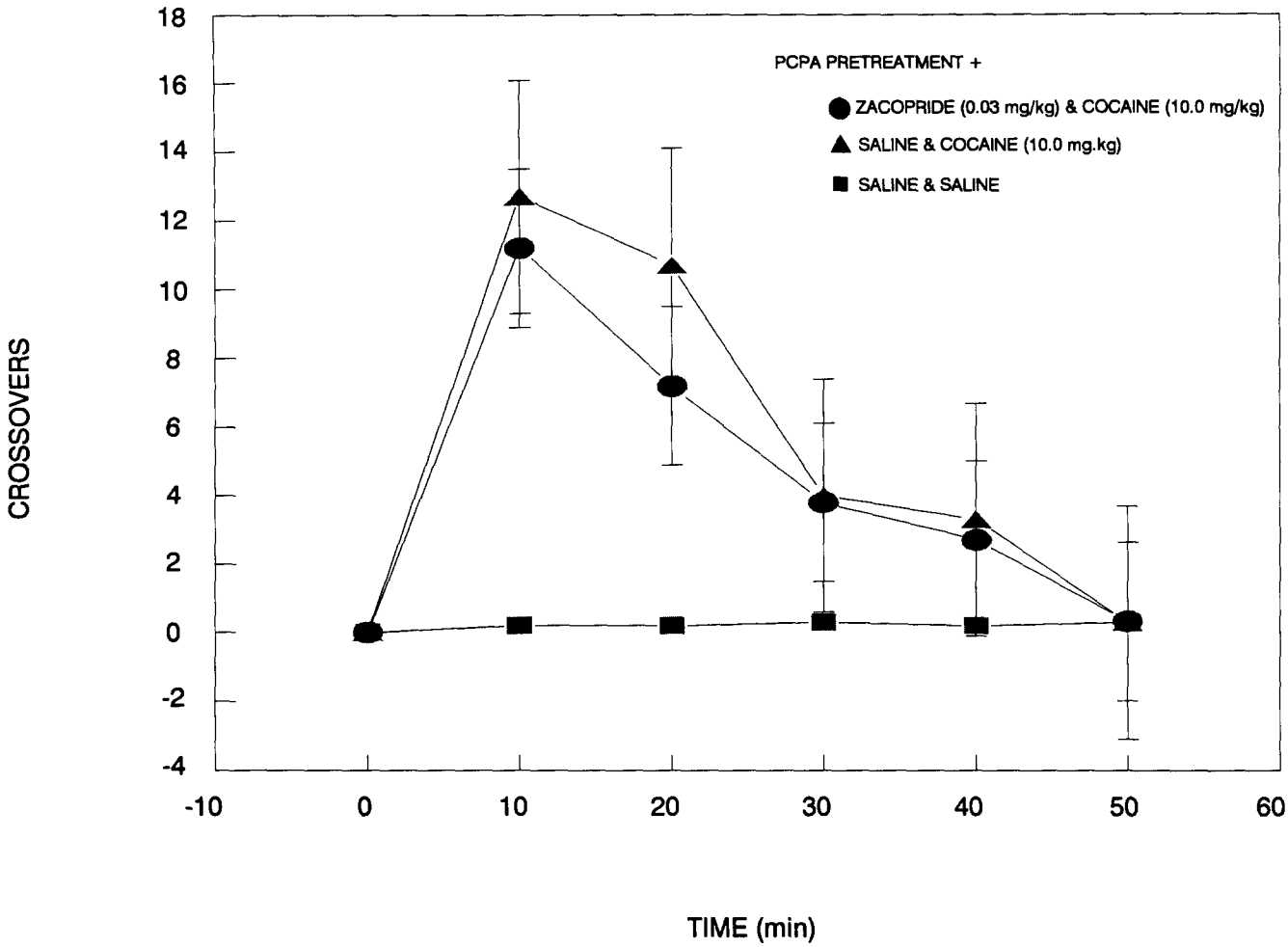

FIG. 3. Response of rats pretreated with PCPA (100 mg/kg, IP, ×3) to a) saline pretreatment and cocaine (10.0 mg/kg), b) zacopride (0.03 mg/kg) pretreatment and cocaine (10.0 mg/kg), or c) saline pretreatment and saline. Results are means ± SEM. The cocaine- and zacopride-pretreated groups were significantly different from the saline group ($p < 0.001$). *p*-Chlorophenylalanine (PCPA)-treated animals appeared more sensitive to cocaine administration as they exhibited less locomotor activity and more stereotypical behavior than those not pretreated with PCPA.

copride being the most potent, MDL 72222 being the least) (10,13,19).

The PCPA experiments show that in the absence of endogenous 5-HT, 5-HT$_3$ antagonist pretreatment did not significantly inhibit cocaine-induced locomotion. It has been previously suggested that endogenous 5-HT is necessary for cocaine's actions (16). Studies using the axonal flow inhibitor τ-butyrolactone (τ-BL) show that intact serotonergic circuitry is also essential for cocaine's effects (7). Our data corroborate those of others suggesting that animals pretreated with PCPA are more sensitive to cocaine administration (40). Those challenged with 10.0 mg/kg cocaine exhibited a slight but significant increase in locomotor activity, accompanied by stereotypical activity in excess of that seen in non-PCPA-treated animals. Those challenged with 3.0 mg/kg cocaine exhibited some stereotypical activity, accompanied by a modest but significant increase in locomotor activity. In non-PCPA-treated animals, administration of 3.0 mg/kg cocaine produced no significant changes in any unconditioned behavior. The mechanisms of the PCPA effects on cocaine-induced behavior are not clear. However, it has been reported that PCPA pretreatment alters the sensitivity of 5-HT cell bodies and receptors to cocaine (16,40).

Binding Data

It has been hypothesized that 5-HT$_3$ receptors presynaptically regulate dopamine release (10,12). One possible site of this regulation is at the dopamine transporter. To investigate if 5-HT$_3$ antagonists interact with cocaine and/or dopamine binding to the dopamine transporter, competition experiments were conducted. Previous experiments have shown that [^{3}H]GR 65630 binding is inhibited by high (μM) concentrations of cocaine; similarly, ^{3}[H]cocaine binding is inhibited by concentrations of 5-HT$_3$ antagonists more than 10,000 times higher than required for binding at the 5-HT$_3$ receptor (21,26). Our results indicate that the 5-HT$_3$ antagonists (±)-zacopride and ICS 205-930 (in concentrations up to 10 μM) do not affect

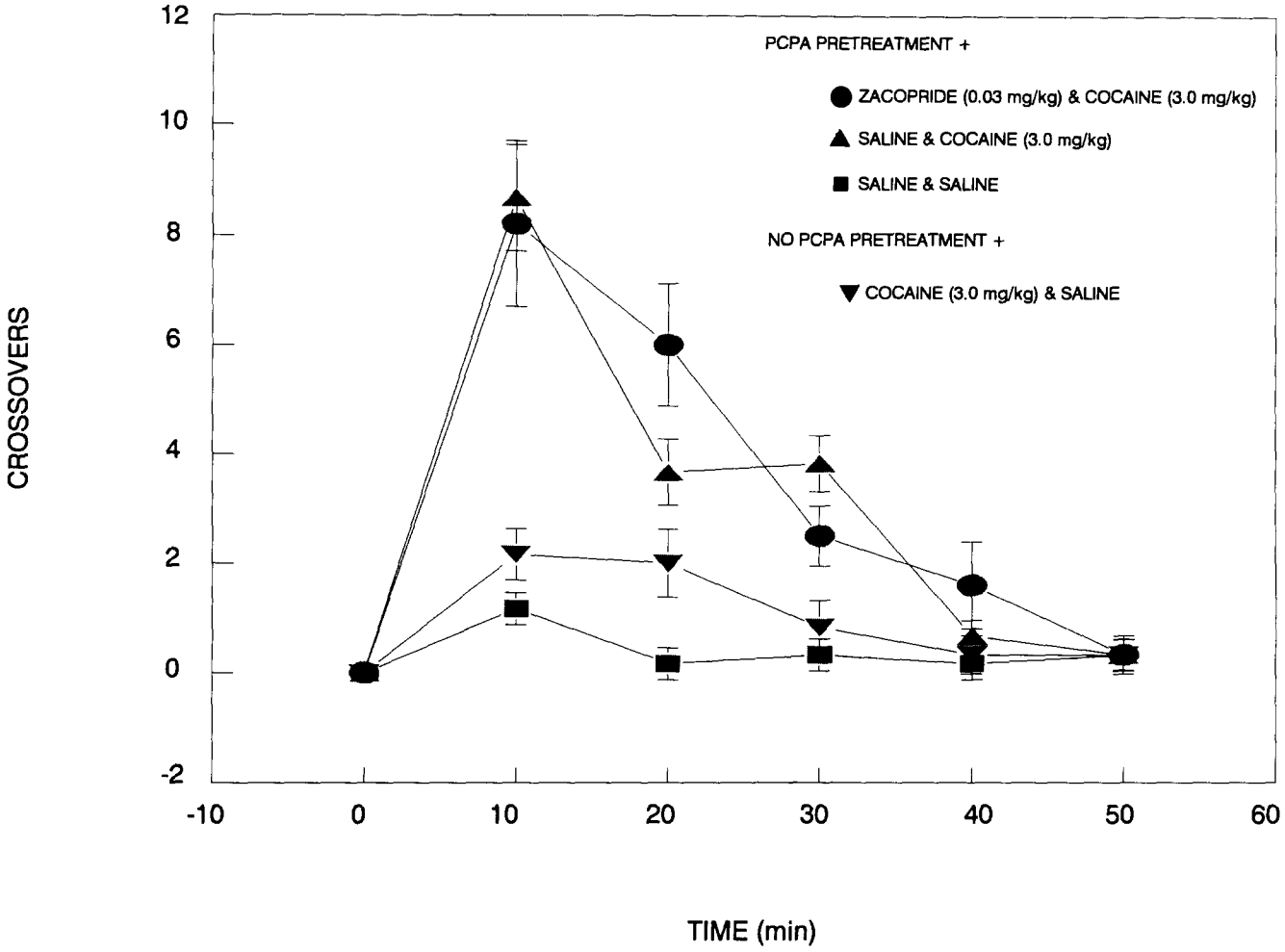

FIG. 4. Response of rats pretreated with *p*-chlorophenylalanine (PCPA) (100 mg/kg, IP, ×3) to a) saline pretreatment and cocaine (3.0 mg/kg), b) zacopride (0.03 mg/kg) pretreatment and cocaine (0.03 mg/gk), or c) saline pretreatment and saline. The fourth group was not pretreated with PCPA and challenged with 3.0 mg/kg cocaine. The PCPA-treated groups challenged with cocaine differed significantly in response from the saline- and non-PCPA-pretreated groups ($p < 0.005$). The results are means ± SEM.

^{3}[H]WIN 38,428 bindings or the ability of dopamine to alter this binding. From these results, it can be inferred that the interaction between cocaine and 5-HT_3 antagonist binding does not occur at the site of the dopamine transporter or that the interaction occurs at a site insensitive to WIN 38,428 binding.

The question remains as to whether or not there are cocaine-insensitive dopamine transport sites that are sensitive to the 5-HT_3 antagonists. For example, Madras et al. (26) have shown that both cocaine congeners and dopamine uptake inhibitors have a high affinity for ^{3}[H]cocaine, while dopamine uptake inhibitors bind only to a subclass of ^{3}[H]WIN 35,428-labeled sites. Kinetic analysis in primates and rodents revealed two binding components for cocaine and WIN 35,428, whereas dopamine has a single binding component (8,26). Recently, in the rabbit single binding sites were shown for both WIN 38,428 and cocaine (3). As previously suggested, it can be inferred from this data that cocaine and cocaine congeners (including WIN 38,428) bind to a subpopulation of dopamine transporter sites (24,27). Cloning of the dopamine transporter has shown it to be sensitive to both cocaine and WIN 38,428, revealing binding profiles characteristic of synaptosomal uptake studies (22,38). It has yet to be determined if dopamine transporters are homogeneous throughout the brain. For example, Cass et al. (11) suggested that after acute and chronic cocaine administration the sensitivity of the dopamine transporter differs among anatomic sites.

The lack of competitive interaction among 5-HT_3 antagonists, cocaine, and dopamine may also be attributed to 5-HT_3 receptor subtypes and/or heterogeneous binding sites and kinetics among various antagonists. For example, 5-HT_3 receptors have also been delineated based upon tissue-specific antagonist affinity, as well as species differences (30,35). It has recently been shown that the R isomer of zacopride binds to a high affinity site (10 nM) in rat cortex and NG 108 cells (4,17). This site is poorly recognized by the S isomer, as well as other 5-HT_3 antagonists. The racemic form of zacopride was not tested. The association of the 5-HT_3 receptor with ligand-gated ion channels implies that particular subunit compositions may determine channel characteristics based upon its

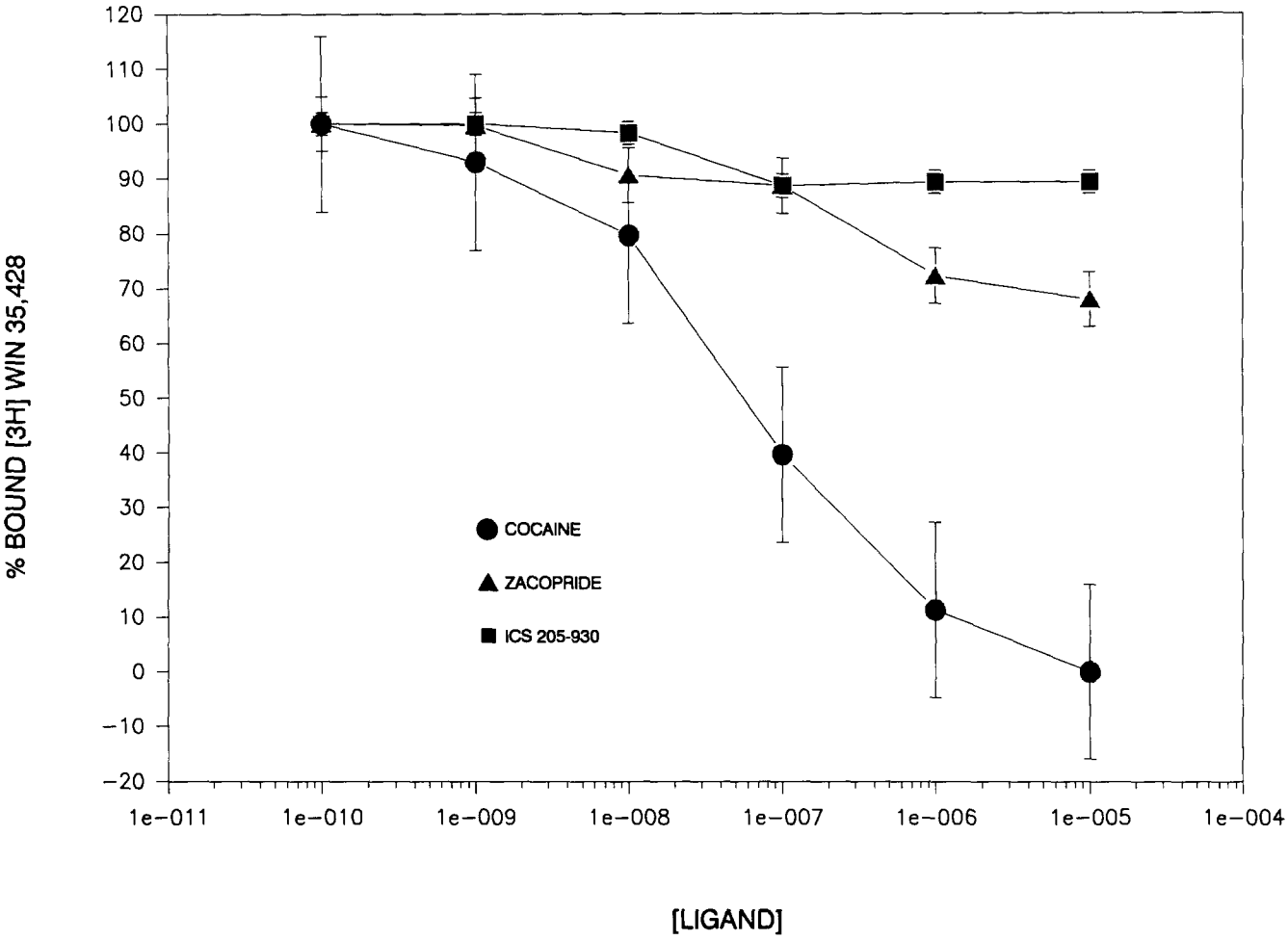

FIG. 5. Displacement of [^{3}H]WIN 35, 428 (0.3 nm) binding by (−) cocaine (10^{-10}–10^{-5}M), (±)-zacopride (10^{-10}–10^{-5}M), or ICS-205, 930 (10^{-10}–10^{-5}M). Cocaine, but not the 5-HT$_3$ antagonists, significantly inhibited [^{3}H]WIN 35,428 binding ($p < 0.001$, Duncan's multiple-range test). Each curve is the mean of three to four individual experiments, each performed in triplicate.

multimeric structure. Although multiple forms of 5-HT$_3$ have not been definitively illustrated, the presence of 5-HT$_3$ subclasses would not be incompatible with our data.

In collaboration with Strecker and McNeish, we have found using microdialysis that zacopride does not inhibit either baseline or cocaine-induced dopamine release in the nucleus accumbens (41). Although cocaine and amphetamine have some differing mechanisms of action (33), it is of interest to note that our results parallel those of Carboni et al. (10), who found that amphetamine-induced dopamine release was not blocked by 5-HT$_3$ receptor antagonism. However, with other central stimulants 5-HT$_3$ antagonists do effect dopamine release in the nucleus accumbens. For example, microdialysis studies reveal that 5-HT$_3$ antagonists inhibit morphine-, nicotine-, ethanol-, and phenylbiguanide-induced dopamine release (10,12). The lack of cocaine, amphetamine, and 5-HT$_3$ interaction suggested from microdialysis studies is surprising because it has been postulated that the locomotor component of cocaine administration is associated with the nucleus accumbens (18).

Binding and lesion studies have demonstrated that after cocaine administration the nucleus accumbens displays characteristics distinct from those of the striatum. In terms of the action of cocaine in the dopamine transporter, it has been shown that exposure to cocaine decreases both [^{3}H]mazindol and [^{3}H]GBR 12935 binding in the nucleus accumbens but does not alter binding in the striatum (2,37). Sharpe et al. (37) have shown that after cocaine withdrawal decreased [^{3}H]mazindol binding is seen in the nucleus accumbens but not in the striatum. It has also been shown that destruction of the nucleus accumbens attenuates cocaine self-administration (32). Studies using in vivo electrochemistry reveal that the nucleus accumbens is more sensitive to systemic cocaine administration than the striatum (11). Based upon [^{3}H]mazindol binding, Cass et al. (11) suggested that this greater sensitivity may be due to fewer dopamine transporter complexes in the nucleus accumbens. Therefore, further study of the interaction between 5-HT$_3$ receptors, cocaine, and the dopamine transporter, specifically in the nucleus accumbens, seems warranted.

In the present study, we provided further evidence that 5-HT$_3$ receptor antagonists attenuate the locomotor activity induced by acute cocaine administration. To substantiate that 5-HT$_3$ antagonism of cocaine-induced behaviors is serotonin

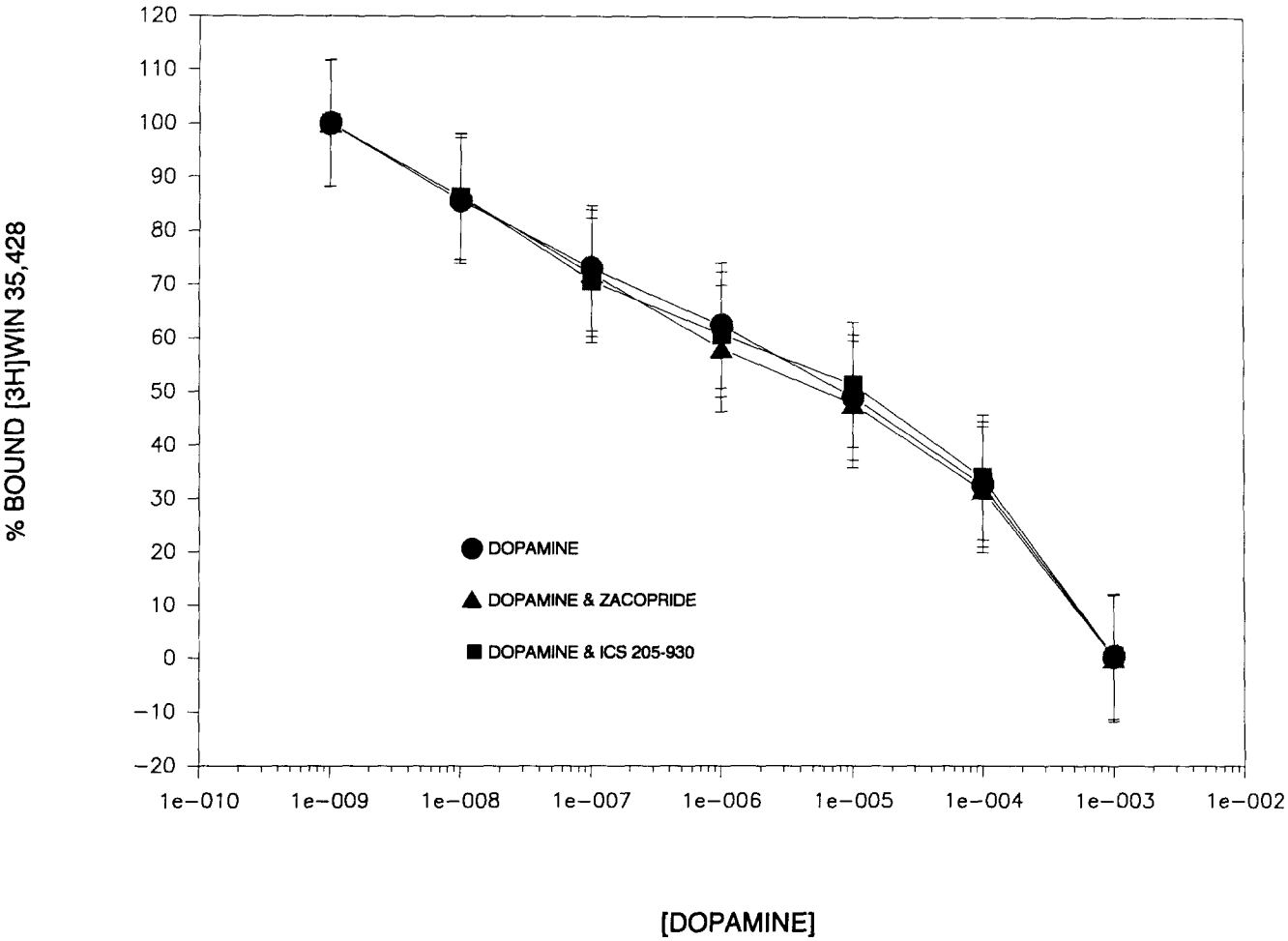

FIG. 6. Displacement of [^{3}H]WIN 35,428 (0.3 nm) binding by dopamine (10^{-9}–10^{-3}M), dopamine and (±)-zacopride (10^{-9}–10^{-3}M), and dopamine and ICS 205-930 (10^{-9}–10^{-3}M). Binding in the three groups was not significantly different from one another. Each curve is the mean of three to four experiments, each performed in triplicate.

dependent, behavioral experiments were conducted on PCPA-treated animals. Our results indicate that serotonin is necessary for 5-HT$_3$ antagonists to attenuate cocaine-induced behaviors. These data are of interest because Broderick (6) has shown using in vivo voltammetry that synaptic concentrations of 5-HT in the nucleus accumbens decrease after subcutaneous cocaine administration. Although our data and those of Broderick (6) are seemingly paradoxical, both studies emphasize the importance of 5-HT in the mechanisms of cocaine action. To investigate possible mechanisms for 5-HT$_3$ efficacy, binding studies were conducted. Our results revealed that 5-HT$_3$ antagonists do not inhibit dopamine or cocaine binding to the dopamine transporter in the striatum. Other data suggest that 5-HT$_3$ antagonists do not affect extracellular dopamine concentrations after cocaine administration (41). It is, of course, possible that 5-HT$_3$ antagonist/cocaine/dopamine interactions occur at sites for dopamine transport or release that could not be measured because of temporal and anatomic limitations to the methods employed.

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