Pharmacology Biochemistry and Behavior, Vol. 41, pp. 519–527, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Repeated Administration of Low Doses of Cocaine Enhances the Sensitivity of 5-HT_2 Receptor Function

NISSAR A. DARMANI,*[1] BILLY R. MARTIN*[2] AND RICHARD A. GLENNON*†

Departments of Pharmacology/Toxicology and Medicinal Chemistry†*
Virginia Commonwealth University/Medical College of Virginia, Richmond, VA 23298-0613

Received 23 September 1991

DARMANI, N. A., B. R. MARTIN AND R. A. GLENNON. *Repeated administration of low doses of cocaine enhances the sensitivity of 5-HT_2 receptor function.* PHARMACOL BIOCHEM BEHAV 41(3) 519–527, 1992.—The acute and chronic effects of cocaine were evaluated on the 5-hydroxytryptamine (5-HT)-receptor 5-HT_2 mediated behavioral function, the head-twitch response (HTR), in mice. In a recent study, we reported that the (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI)-induced HTR was dose dependently reduced by cocaine via indirect stimulation of serotonergic 5-HT_{1A} and adrenergic α_2 receptors. In the present investigation, the HTR was evoked by the nonselective 5-HT agonist 5-methoxy-*N*,*N*-dimethyltryptamine hydrogen oxolate (5-MeO-DMT). Cocaine by itself failed to produce HTR but dose dependently inhibited the 5-MeO-DMT-induced behavior. Cocaine's effects were not due to 5-HT_3 antagonism since acute administration of the more potent 5-HT_3 antagonist (ICS-205,930) failed to produce or modify the 5-MeO-DMT-induced behavior. During withdrawal from chronic cocaine treatment (5–20 mg/kg), 5-MeO-DMT-induced HTR was enhanced. Depending upon the cocaine dose used, the induced supersensitivity persisted up to 172 h following cessation of cocaine treatment. The mechanisms of cocaine-induced supersensitivity were further investigated using the more selective 5-HT_2 agonist DOI. Withdrawal from a low-dose (0.03–1.25 mg/kg) chronic cocaine treatment caused the DOI-induced HTR to increase, whereas withdrawal from a 5- and 10-mg/kg cocaine regimen had no significant effect. The maximal effect persisted up to 36 h following termination of cocaine treatment. Relative to vehicle-exposed controls, withdrawal from cocaine treatment enhanced the inhibitory potency of the 5-HT_{1A} agonist (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin HBr (8-OH-DPAT) on DOI-induced HTR. Moreover, the 5-$HT_{1B/1C}$ agonist 1-(3-trifluoromethylphenyl)-piperazine HCl (TFMPP) and the 5-HT_3 agonist *m*-chlorophenylbiguanide HCl (mCPBG) failed to modify DOI-induced HTR in chronically vehicle-exposed mice but both agents reduced the induced behavior in the cocaine-treated group. Unlike changes in the sensitivity of serotonergic receptor subtypes, cocaine exposure failed to modify α_2 adrenoceptor sensitivity because clonidine was equipotent in inhibiting DOI-induced behavior in both groups treated chronically with vehicle and cocaine. Thus, the present study demonstrates a serotonergic component of cocaine's action in that low-dose chronic exposure enhances the functional sensitivity of serotonergic 5-HT_{1A}, 5-HT_2, and 5-HT_3 receptors.

Cocaine Clonidine DOI ICS-205,930 mCPBG 8-OH-DPAT TFMPP Supersensitivity 5-HT_{1A}, 5-HT_2, and 5-HT_3 receptors

A major pharmacological effect of the stimulant cocaine is to potentiate the effects of dopamine (DA), norepinephrine (NE), and serotonin (5-hydroxytryptamine, 5-HT) by blocking their neuronal uptake. Recent reviews indicate that increased activity of the dopaminergic system is thought to be the basis of many of the behavioral and pharmacological effects of cocaine (7,28). For example, acute administration of cocaine in rodents produces a dose-dependent increase in spontaneous locomotor activity and a significant elevation in the concentration of extracellular dopamine in the nucleus accumbens of freely moving conscious rats (29). Moreover, continued daily injections of cocaine can induce a parallel increase in both extracellular DA levels and locomotor activity. In such chronic studies, a significant correlation was found between enhanced locomotor activity and brain cocaine concentration (42). In addition, cocaine analogs with greater affinities for DA uptake sites (e.g., WIN 35428) are more potent in stimulating locomotor activity (43).

In the studies cited above, large doses of cocaine (10–25 mg/kg) were used to induce enhanced locomotion. However,

[1] Present address: Department of Pharmacology, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501.
[2] Requests for reprints should be addressed to Billy R. Martin, Department of Pharmacology/Toxicology, Box 613, MCV Station, Virginia Commonwealth University, Richmond, VA 23298-0613.

other reports indicate that cocaine at very low doses (0.1–0.75 mg/kg) depresses motor activity (20). Cocaine analogs (e.g., norcocaine, pseudococaine), which possess greater affinity for serotonergic uptake sites, have also been reported to inhibit spontaneous locomotor activity (43). The motor depressant effects of cocaine may be due to increased concentration of synaptic 5-HT (20) because cocaine is known to inhibit 5-HT uptake more potently than catecholamine uptake (45). Indeed, inhibition of 5-HT synthesis and receptor blockade have been reported to potentiate cocaine-induced motor activity; conversely, 5-HT precursor treatment antagonizes cocaine-induced locomotor stimulation (40). In a similar manner, increased serotonergic activity tends to reduce cocaine self-administration (5,6), The notion that cocaine at low doses may have selective effects on the serotonergic system is further highlighted by a recent electrophysiological study (11). The latter investigators reported that cocaine can inhibit 5-HT neurons at doses (ID_{50} = 0.5 mg/kg, IV) that can result in only a 60–70% inhibition of activity of NE or DA neurons.

To date, there is a lack of systematic investigation on the mechanism of action of cocaine on the serotonergic system. This is partly due to existence of large number of 5-HT receptors both in the periphery and in the CNS. Indeed, up to four distinct types of 5-HT receptors (5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4) have been identified by radioligand binding studies [for recent reviews, see (21,47)]. The situation is further complicated because 5-HT_1 sites appear to consist of several subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, and 5-HT_{1E}), and such heterogeneity may also exist in the 5-HT_3 class of receptor sites (44). Moreover, the various 5-HT receptors may modulate one another's function (1,14,15,22). A variety of serotonergic behavioral models have been used to determine the effects of drugs on the various 5-HT receptor subtypes. One of the most popular behavioral models to study 5-HT_2 receptor function is the head-twitch response (HTR) in rodents. Although HTR can be produced by different neurotransmitter mechanisms (25), the head-twitch produced by selective or nonselective (direct and indirect) 5-HT_2 receptor agonists is antagonized by selective 5-HT_2 receptor antagonists (14,35). Recently, Tadano and coworkers (50) reported that the HTR induced by the indirect 5-HT agonist *p*-hydroxyamphetamine (*p*-OHA) was inhibited by cocaine pretreatment. These authors suggested that cocaine's inhibitory effect was due to inhibition of *p*-OHA-induced release of 5-HT from nerve endings. Following this report, we investigated the effects of cocaine on HTR induced by direct agonists.

METHOD

Animals and Drugs

Male albino ICR mice from Dominion laboratories (Dublin, VA) weighing 16–20 g at the beginning of the experiments were used throughout the study. Animals were housed on a 12 L:12 D cycle with access to food and water at a room temperature of 22 ± 1°C. The behavioral experiments were conducted between 10:00 a.m. and 6:00 p.m. The following drugs were obtained from RBI (Natick, MA): (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl [(±)-DOI)], (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin HBr (8-OH-DPAT), 1-(3-trifluoromethylphenyl)-piperazine HCl (TFMPP), 5-methoxy-*N*,*N*-dimethyltryptamine hydrogen oxolate (5-MeO-DMT) clonidine HCl, and ICS-205,930. Cocaine HCl was obtained from the National Institute on Drug Abuse and *m*-chlorophenylbiguanide HCl (mCPBG) was synthesized in our own facilities. The 5-HT_3 antagonist ICS-205,930 was initially dissolved in methanol and then diluted in distilled water. Unless stated otherwise, all remaining drugs were dissolved in distilled water and given at a volume of 10 ml/kg.

Acute Studies

To habituate animals to the test environment, each mouse was randomly transferred 10 min prior to treatment to a 40 × 25 × 16 cm plastic cage lined with a thin layer of sawdust. Mice were then injected IP with 5-MeO-DMT (either 0, 2, 4, 8, 16, or 32 mg/kg, n = 6 per dose). The cumulative HTR frequency was scored every 2 min for the first 10 min following 5-MeO-DMT injection. To investigate the possible acute inhibitory effects of cocaine on HTR, a dose of 8.0 mg/kg 5-MeO-DMT was chosen to induce the behavior. Mice were pretreated with cocaine (either 0.0, 1.25, 2.5, 5.0, 10.0, or 20.0 mg/kg, IP, n = 6 per dose) 10 min prior to 5-MeO-DMT administration. To determine whether cocaine inhibition of the induced HTR was due to 5-HT_3 antagonism, mice were pretreated with ICS-205,930 (either 2, 4, or 8 mg/kg, IP, n = 6) 40 min prior to administration of 5-MeO-DMT. In both experiments, HTR frequency was scored following 5-MeO-DMT administration in 2-min intervals as described above.

Chronic Studies

Mice were treated IP with either 0, 5, 10, or 20 mg/kg cocaine twice daily for 13.5 days. Following the last injection, animals were randomly divided into four different groups. Each group contained both vehicle- (n = 5–10) and cocaine-treated (n = 5–10) mice. Different groups of mice were then challenged with 5-MeO-DMT (8.0 mg/kg, IP) either 5, 29, 77, or 177 h following cessation of chronic cocaine treatment. The 5-MeO-DMT-induced HTR frequency was scored following each injection as described for the acute studies. When the latter study indicated that lower doses of cocaine may produce greater effects, DOI was used instead of 5-MeO-DMT to further investigate the chronic effects of cocaine on HTR behavior. In addition to its greater 5-HT_2 selectivity, DOI is a more potent agonist with a longer duration of action (12).

The acute effects of cocaine on DOI-induced HTR have already been reported (16). For chronic studies, mice were treated twice daily for 13.5 days with cocaine (either 0, 0.03, 0.1, 0.5, 1.25, 2.5, 5.0, or 10.0 mg/kg, IP, n = 6–14 per group). Twelve hours following cessation of chronic treatment, each mouse was allowed to habituate to the test environment 20 min prior to an injection of DOI (2.5 mg/kg, IP). HTR frequency was scored for the first 20 min at 2-min intervals following DOI injection. The 1.25-mg/kg cocaine dose caused the greatest increase in the induced behavior. This dose was chosen to further study the effects of chronic cocaine treatment on DOI-induced HTR. Thus, a large number of mice were treated chronically with 1.25 mg/kg cocaine or vehicle. These animals were divided into three different groups. Each group comprised of vehicle- (n = 4–5) and cocaine- (n = 6–12) treated animals. Different groups of mice were challenged with DOI (2.5 mg/kg, IP) either 12, 36, or 74 h following cessation of chronic treatment. The DOI-induced HTR score was recorded as described above.

To investigate the possible mechanisms through which supersensitivity occurs in the 5-HT_2 receptor system (i.e., cocaine-induced increase in HTR frequency), the effects of various agonists that stimulate selective receptors that may

modulate HTR frequency were tested on chronically cocaine-treated mice. For these experiments, mice were chronically treated either with vehicle or cocaine as above. Twelve hours following cessation of chronic treatment, different groups of mice were injected with different drugs in the following manner: 1) both chronic vehicle-control (n = 7) and cocaine-treated control mice (n = 7) received an IP injection of distilled water and 10 min later were injected with DOI (2.5 mg/kg, IP) and the induced behavior was recorded; 2) a second group of chronically vehicle- (n = 6) and cocaine- (n = 7) treated mice received an initial injection of 8-OH-DPAT (0.25 mg/kg, IP) and 10 min later both groups received DOI (2.5 mg/kg, IP); 3) a third group of chronically vehicle- (n = 6) and cocaine-treated mice (n = 7) received an initial injection of TFMPP (1.25 mg/kg, IP) and 5 min later DOI (2.5 mg/kg, IP); 4) a fourth group of chronically vehicle- (n = 7) and cocaine-treated mice received clonidine (0.05 mg/kg) 30 min prior to DOI injection (2.5 mg/kg, IP); 5) the final groups of chronically vehicle- (n = 7) and cocaine- (n = 7) pretreated mice received mCPBG (5.0 mg/kg, IP) 30 min prior to DOI injection. In every case, DOI-induced behavior was recorded as described earlier. Doses of all drugs used in these experiments were based on our previous published and unpublished findings.

Statistics

Data were analyzed by one way of analysis of variance (ANOVA) and posthoc analysis by Dunnett's t-test to compare vehicle controls with different drug treatment or Scheffe test to compare different doses of the same drug.

RESULTS

Acute Studies

The nonselective 5-HT agonist 5-MeO-DMT caused a dose-dependent increase in HTR frequency up to 16 mg/kg (Fig. 1). Further increase in dose did not significantly increase the head-twitch score in the 10-min observation period. The maximal HTR frequency was obtained between the 2- to 4-min observation period and thereafter declined so that little activity was seen between 8–10 min (data not shown). Thus, the drug is absorbed quickly from the IP sites and possesses a short duration of action.

At the doses used (10–40 mg/kg), cocaine by itself failed to elicit head-twitch behavior (Fig. 2); however, cocaine pretreatment caused a dose-dependent decrease in 5-MeO-DMT-induced HTR (Fig. 3). The lowest cocaine dose tested (1.25 mg/kg) significantly decreased ($p < 0.05$) the 5-MeO-DMT-induced HTR score by 34%. The highest cocaine dose (20 mg/kg) inhibited the induced response by 93%. The dose of cocaine that produced a 50% inhibition (ID_{50}) was calculated to be 1.95 mg/kg with a 95% confidence limit of 0.75–5.1 mg/kg. The potent 5-HT_3 receptor antagonist ICS-205,930 (2–8 mg/kg) by itself did not evoke the behavior nor affect the 5-MeO-DMT-induced HTR (Fig. 4).

Chronic Studies

Dose–response and time course of chronic cocaine treatment on 5-MeO-DMT-induced HTR. When challenged 5 h following termination of chronic cocaine treatment, the 5-MeO-DMT-induced HTR score exhibited a bell-shaped dose-response curve (Fig. 5), that is, relative to chronic vehicle control (14 ± 1 HTR's) the 5- and 10-mg/kg chronic cocaine pretreatment significantly increased ($p < 0.05$) 5-MeO-DMT-induced HTR by 163 (37 ± 5) and 140% (34 ± 3), respectively. However, the 20-mg/kg chronic cocaine dose regimen caused a 49% increase in the 5-MeO-DMT-induced behavior that did not reach statistical significance (Fig. 5). When challenged 29 h following cessation of chronic cocaine treatment, the 5-MeO-DMT-induced score was only significantly different from vehicle control at the 5-mg/kg cocaine dose (a 74% increase, $p < 0.05$). The 10- or 20-mg/kg cocaine regimen had no significant effect (Fig. 5). Similarly, 77 h following withdrawal from chronic cocaine treatment the

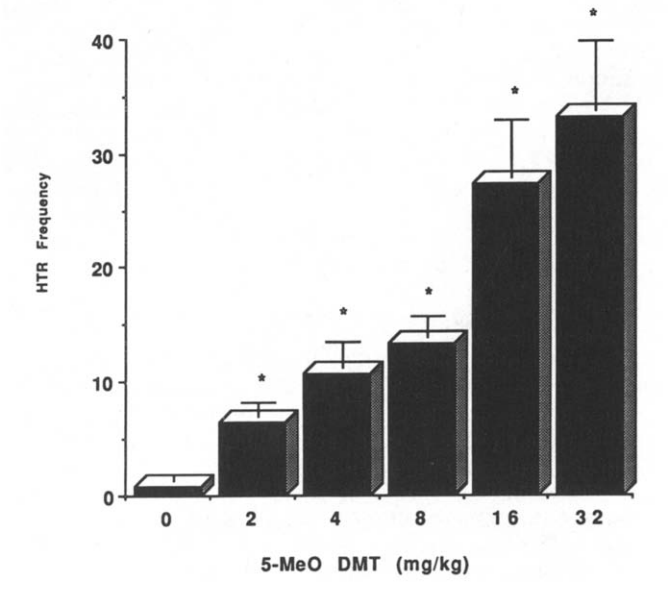

FIG. 1. Dose responsiveness of 5-MeO-DMT induction of the HTR in mice. Behavior was scored for 10 min immediately after an IP injection. Results are given as means ± SEM (n = 6 per dose). *Significantly different from vehicle control at $p < 0.05$.

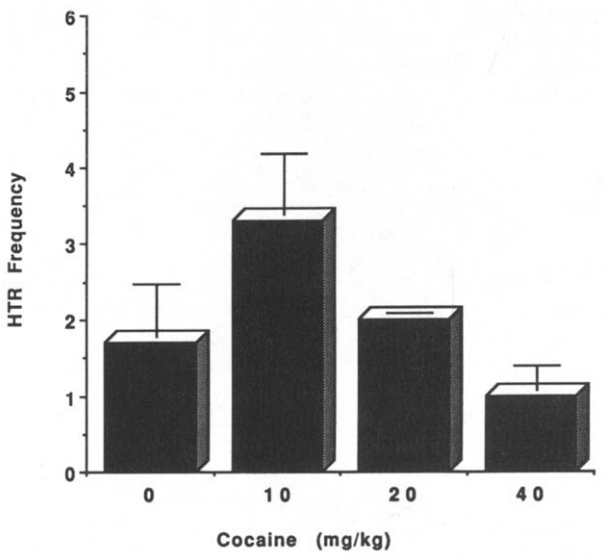

FIG. 2. Evaluation of cocaine to produce the HTR. Behavior was scored for 30 min immediately after an IP injection. Results are given as means ± SEM (n = 6 per group).

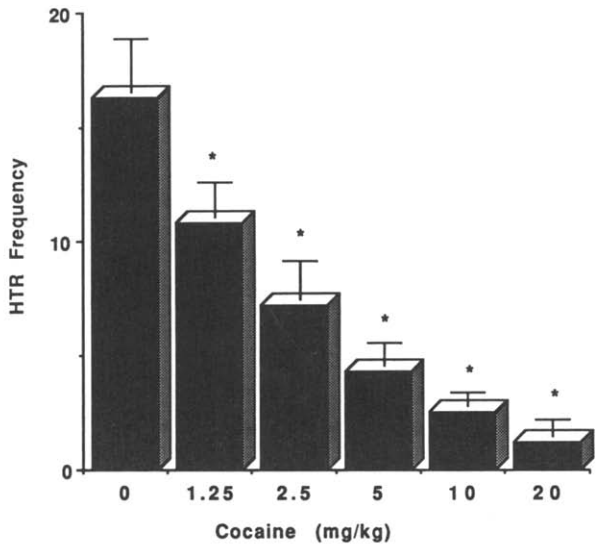

FIG. 3. Dose-dependent inhibitory effects of cocaine on head-twitch frequency induced by 5-MeO-DMT. Mice were pretreated with either vehicle or different doses of cocaine 10 min prior to 8.0 mg/kg 5-MeO-DMT administration. Induced behavior was scored for 10 min immediately following 5-MeO-DMT injection. Data are presented as means ± SEM (n = 6). *Significantly different from vehicle control at $p < 0.05$.

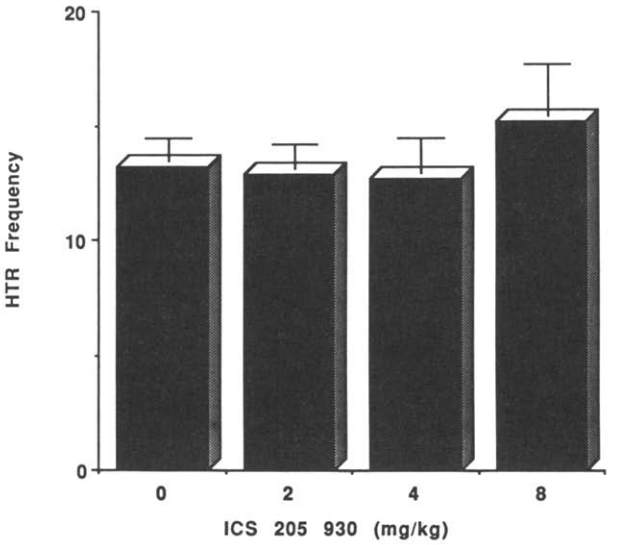

FIG. 4. Effect of ICS-205,930 on the head-twitch frequency induced by 5-MeO-DMT. Mice were either pretreated with an acute injection of vehicle or ICS-205,930 40 min prior to administration of 8.0 mg/kg 5-MeO-DMT. Behavior was scored for 10 min immediately following 5-MeO-DMT injection. Results are given as mean ± SEM (n = 6 per group).

5-MeO-DMT challenge caused a significant increase (81%, $p < 0.05$) in the 5-mg/kg cocaine-treated group. However, 172 h following termination of chronic cocaine treatment 5-MeO-DMT challenge produced significant increases in the 5- (80%, $p < 0.05$) and 10- (112%, $p < 0.05$) mg/kg cocaine groups relative to chronic vehicle regimen. Although the 20-mg/kg cocaine dose also exhibited an increase in HTR score (53%), it did not reach statistical significance (Fig. 5).

Dose-response effects of chronic cocaine treatment on DOI-induced HTR. Similar to 5-MeO-DMT studies, chronic exposure to different doses of cocaine caused a bell-shaped dose-response curve for DOI-induced HTR 12 h following cessation of chronic cocaine treatment. Relative to chronic vehicle regimen, the lowest chronically administered cocaine dose tested (0.03 mg/kg) caused a significant increase (42%, $p < 0.05$) in the DOI-induced HTR score (Fig. 6). The maximal increase was produced by the 1.25-mg/kg cocaine dose regimen (76%, $p < 0.05$). On further increase in cocaine dose, the maximal score was dose dependently decreased so that the 5- and 10-mg/kg chronic cocaine treatment regimen had no significant effect on DOI-induced behavior (Fig. 6).

Time-response studies following chronic administration of 1.25 mg/kg cocaine (twice daily for 13.5 days). When challenged with 2.5 mg/kg DOI 12 h following cessation of chronic cocaine treatment, the 5-HT_2 agonist produced a 77% increase in HTR score (58 ± 2) relative to chronically vehicle treated control (33 ± 2) (Fig. 7). Such a significant increase persisted 36 h following withdrawal from chronic cocaine treatment. However, when challenged with DOI 74 h after termination of chronic cocaine treatment HTR frequency was only increased by 26% and did not reach the 95% degree of significance.

Contribution of possible modulatory receptors on the DOI-induced head-twitch behavior following withdrawal from chronic cocaine treatment. Acute IP DOI administration (2.5 mg/kg 10 min after a single injection of vehicle) 12 h following cessation of chronic vehicle treatment produced 33 ± 1 head-twitches in the 20-min observation period. When either 8-OH-DPAT (0.25 mg/kg) or clonidine (0.05 mg/kg) were administered instead of the acute injection of vehicle prior to DOI, these agents significantly reduced ($p < 0.05$) the DOI-induced HTR score by 56 and 48%, respectively (Fig. 8a). In this paradigm, acute administration of either TFMPP (1.25 mg/kg) or mCPBG (5 mg/kg) had no significant effect on DOI-induced HTR frequency (Fig. 8a).

In chronically cocaine-treated mice (1.25 mg/kg, twice daily for 13.5 days), an IP injection of DOI (2.5 mg/kg) 10 min after an acute vehicle administration following a 12-h withdrawal from chronic cocaine treatment produced 57 ± 3 HTR's (Fig. 8b). Thus, chronic cocaine administration caused a 73% increase in DOI-induced HTR score relative to chronically vehicle-exposed animals (Fig. 8a, b). In such cocaine-pretreated animals, prior acute administration of previously stated doses of either 8-OH-DPAT, clonidine, TFMPP, or mCPBG significantly reduced ($p < 0.05$) the DOI-induced behavior by 72, 44, 40, and 33%, respectively (Fig. 8b).

DISCUSSION

Recent acute behavioral (20), biochemical (45), and electrophysiological studies (11) indicate that cocaine at lower doses may have selective effects on the serotonergic system. The present study addressed the possibility of such a notion by using the most widely employed behavioral model for 5-HT_2 receptor function, the HTR in mice. The readily quantifiable nature of this behavior in mice makes it a potentially valuable indicator of central serotonergic receptor activity and sensitivity.

Since the early 1980s, it has been known that stimulation of the noradrenergic receptor system can modulate the HTR induced by 5-HT agonists [see (25) for a review]. In particular, excitation of noradrenergic α_2 receptors inhibit 5-HT_2-induced

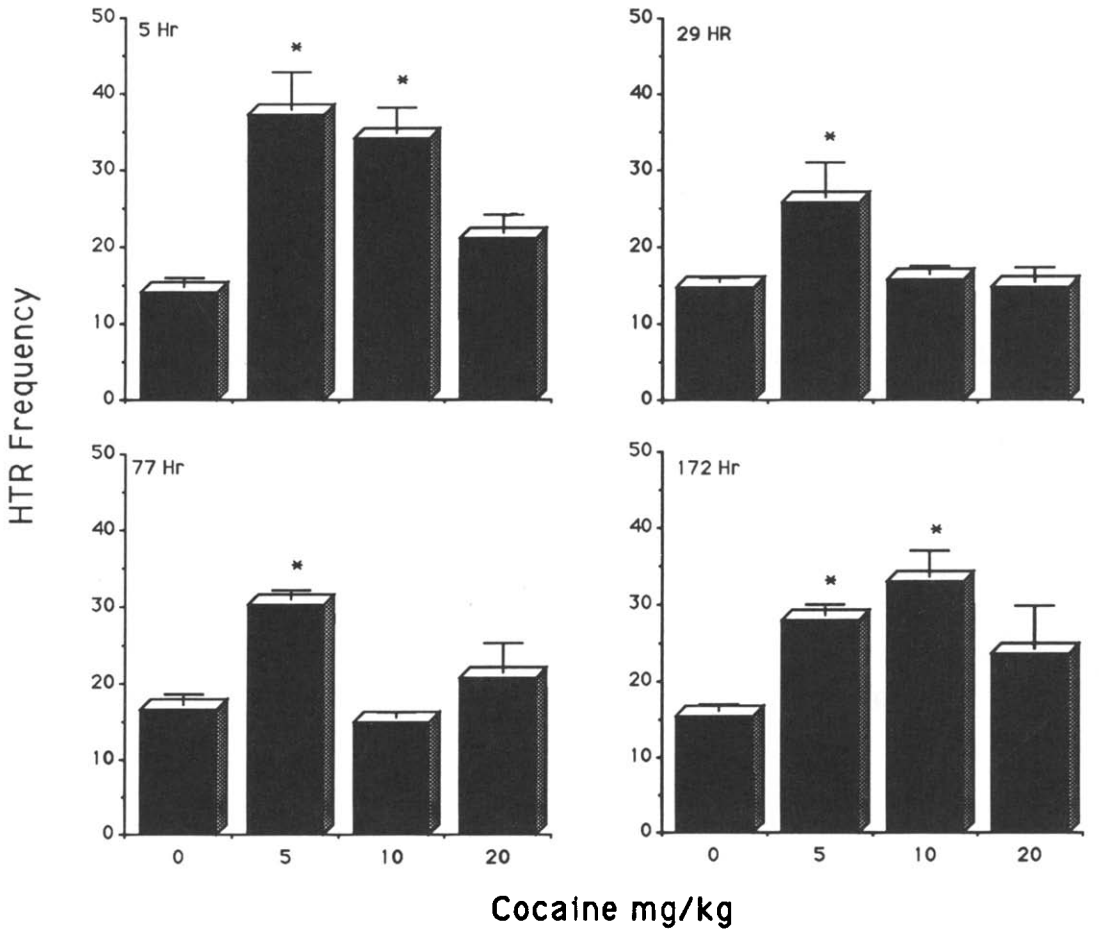

FIG. 5. Time-dependent effects of withdrawal from chronic administration of cocaine (twice daily for 13.5 days, IP) on 5-MeO-DMT-induced HTR. After termination of cocaine treatment, separte groups of mice were challenged with 5-MeO-DMT (8.0 mg/kg, IP) at the indicated time intervals and the induced-HTR was scored for 10 min. Data are presented as means ± SEM (n = 6 per group). *Significantly different from vehicle respective controls at $p < 0.05$.

HTR. More recently, we and others reported that there also exists a functional interaction between 5-HT_{1A} and 5-HT_2 receptors (1,14,22) such that simultaneous costimulation of 5-HT_{1A} receptors are inhibitory to 5-HT_2-induced head-twitch behavior. Observation of HTR, therefore, not only allows one to investigate the effects of serotonergic drugs on 5-HT-receptor function, but also enables one to demonstrate the modulatory effects of other central neurotransmitters on HTR induced by serotonergic agonists. For this reason, the behavior has been referred to as a "gut bath for the brain" (25). Since cocaine mainly affects central DA, NE, and 5-HT function, the HTR provides a behavioral model through which the serotonergic and other effects of cocaine can be investigated.

Using this model, we recently showed that acute cocaine administration dose dependently inhibits the HTR induced by the "more selective" 5-HT_2 agonist DOI (16). This inhibition in 5-HT_2 receptor-mediated function was due to indirect stimulation of the inhibitory adrenergic α_2 and serotonergic 5-HT_{1A} receptors. Although DOI also possesses high affinity for 5-HT_{1C} receptors, our antagonist studies showed that both ketanserin and spiperone are equipotent in inhibiting DOI-induced HTR, thus supporting the role of 5-HT_2 receptors in the induction of the head-twitch behavior (14). Moreover, in the same study, the 5-$HT_{1B/1C}$ agonist TFMPP failed to induce HTR. In the present investigation, acute cocaine administration failed to produce head-twitch behavior but dose dependently inhibited the HTR produced by the nonselective 5-HT agonist 5-MeO-DMT. Cocaine appears to be six times more effective in inhibiting 5-MeO-DMT than DOI-induced HTR [ID_{50} = 1.95 and 11.8 mg/kg, respectively (16)]. It is possible that 5-MeO-DMT by itself contributes to the indirect inhibitory effects of cocaine by stimulating both the excitatory 5-HT_2 as well as the inhibitory 5-HT_{1A} receptors. Such a notion for the less robust effects of 5-MeO-DMT relative to DOI-induced HTR has already been suggested (14). Cocaine also possesses weak 5-HT_3 antagonist properties (44). The potent 5-HT_3 antagonist ICS-205,930 failed to produce HTR and did not affect the 5-MeO-DMT-induced behavior, thus indicating that the 5-HT_3 antagonist properties of cocaine following its acute administration do not contribute to the decrease in 5-MeO-DMT-induced HTR. We (14) and Arnt and Hyttel (1) previously ruled out the possible modulatory effects of acute ICS-205,930 administration on DOI-induced HTR. Thus, it seems possible that the acute inhibitory effects of cocaine on 5-MeO-DMT-induced HTR probably involve similar inhibitory mechanisms to those described for DOI-induced HTR (16).

In the present investigation, our initial chronic cocaine

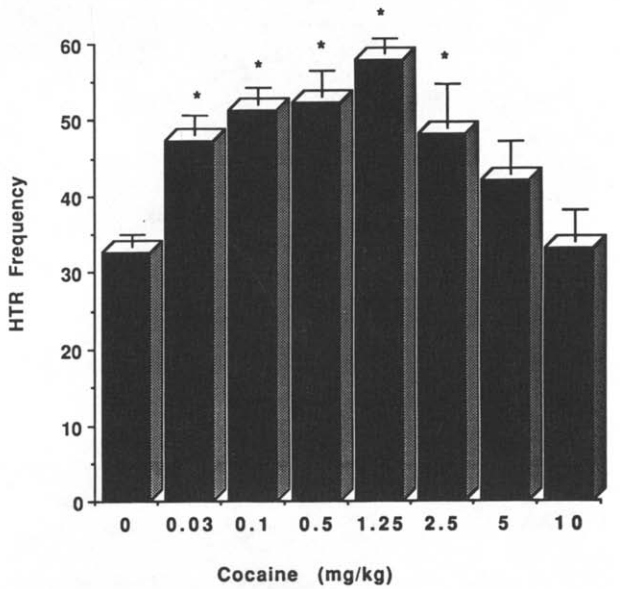

FIG. 6. Chronic effects of cocaine on DOI-induced HTR. Mice were treated either with vehicle or cocaine (twice daily for 13.5 days, IP). Twelve hours following termination of the chronic treatment, animals were challenged with 2.5 mg/kg DOI (IP), and the induced HTR was scored for the next 20 min. Results are given as means ± SEM (n = 6–14). *Significantly different from vehicle control at $p < 0.05$.

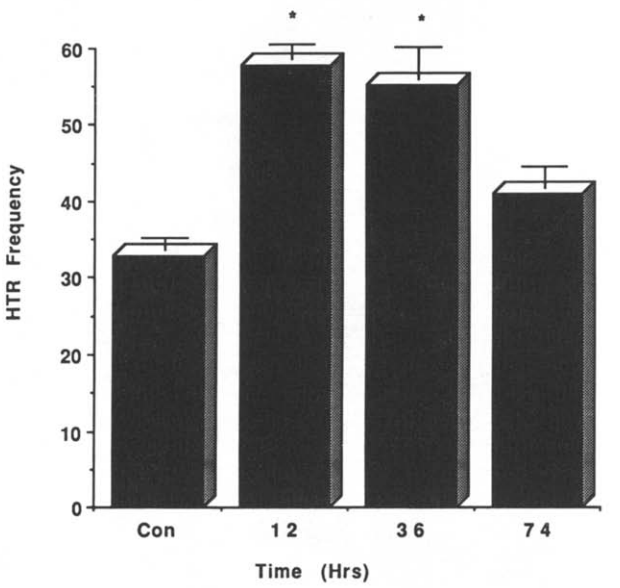

FIG. 7. Time-dependent effects of chronic administration of cocaine (1.25 mg/kg) on DOI-induced HTR. Mice were treated either with vehicle (Con) or cocaine twice daily for 13.5 days. At the indicated time intervals following cessation of chronic treatment, different groups of mice were challenged with DOI (2.5 mg/kg, IP) and the induced HTR was scored for the first 20 min following DOI injection. Results are given as means ± SEM (n = 6–14). *Significantly different from vehicle controls.

studies indicated a supersensitivity to 5-HT_2 receptor function 5 h after cessation of repeated administration of moderate to high doses of cocaine. However, higher cocaine doses did not affect the induced behavior. Furthermore, this supersensitivity persisted up to 172 h following termination of the 5-mg/kg cocaine regimen, whereas the 10-mg/kg dose had significant effects at the 5 and 172 h test sessions only. These initial chronic studies indicated that chronic cocaine exposure not only causes supersensitivity in 5-HT_2 receptor function but that lower cocaine doses may also affect 5-HT function.

Due to the nonselective nature of 5-MeO-DMT, the more selective 5-HT_2 receptor agonist DOI was used to further investigate the chronic effects of cocaine. Recently, we reported that chronic administration of low doses of cocaine (0.5 and 1.0 mg/kg) significantly increased aggressive behavior in mice in contrast to high-dose cocaine exposure, which nonselectively reduced the aggressive repertoires (12). Thus, the chronic effects of a wide range of cocaine doses was studied. As with the aggression studies, differential effects were observed with low and high doses of cocaine. Chronic cocaine exposure at lower doses (0.03–1.25 mg/kg) caused significant dose-dependent increases in DOI-induced HTR frequency, whereas the 5- and 10-mg/kg cocaine regimen had no apparent effect. Therefore, the 1.25-mg/kg cocaine dose, which produced maximal supersensitivity, was used to further study the chronic effects of cocaine exposure. The time-response studies indicated that supersensitivity persisted up to 36 h following cessation of cocaine treatment and no effect was observed after 74 h. Thus, depending upon the 5-HT agonist used to induce HTR, distinct differences in the induced supersensitivity was observed following withdrawal from the same chronic cocaine regimen. These differential effects may be due to: 1) the selective and nonselective nature of the agonist used to induce the HTR because 5-MeO-DMT stimulates both the inhibitory 5-HT_{1A} and the excitatory 5-HT_2 receptors, 2) changes in the sensitivity of 5-HT_{1A} receptors can occur following chronic cocaine exposure that may affect the 5-MeO-DMT-induced HTR frequency, 3) chronic cocaine treatment may alter the pharmacokinetic properties of the two agonists.

The accepted monoamine receptor adaptation theory specifies that chronic agonist exposure causes downregulation whereas persistent deprivation of receptor stimulation (e.g., denervation or persistent antagonist treatment) induces upregulation of the appropriate receptors. The 5-HT_2 receptor system does not fully follow this adaptation dogma in that chronic exposure to both agonists and antagonists cause downregulation of 5-HT_2 receptor density (3,4,33,34,37,52). Furthermore, behavioral models of 5-HT_2 receptor activation, although not entirely consistent, lend support to the unpredictable response hypothesis. For example, following withdrawal from chronic administration of either agonists (13) or antagonists (39,49) the 5-HT_2 receptor system exhibits supersensitivity. Moreover, chemical denervation of 5-HT neurons has no effect on 5-HT_2 receptor density, but does induce supersensitivity in its function, that is, an increase in HTR frequency [for a review, see (8)]. However, withdrawal from chronic administration of very high doses of mianserin induces downregulation of 5-HT_2 receptor function (3). Although some 5-HT uptake inhibitors downregulate the 5-HT_2 site, the effect is not universal and appears to correlate more closely with the affinity for 5-HT_2 receptor rather than for 5-HT uptake site (8).

Thus, in the present investigation the increase in DOI-induced HTR frequency following withdrawal from chronic administration of small doses of cocaine can be due to either a prolonged increase or decrease in synaptic 5-HT concentration in those CNS structures involved in the production of

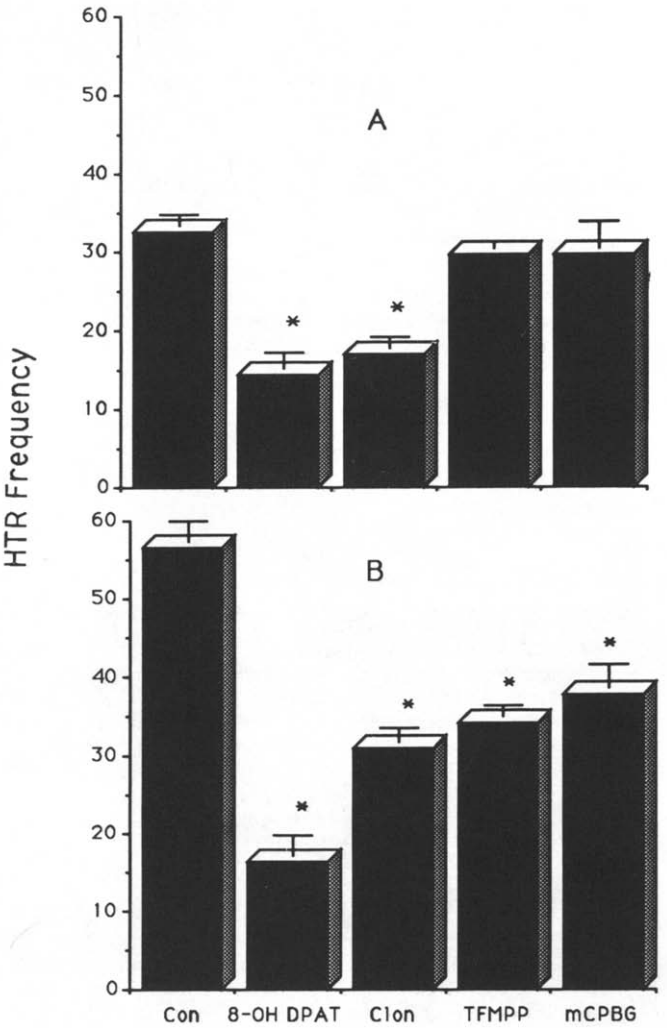

FIG. 8. Effects of acute administration of clonidine, 8-OH DPAT, or mCPBG on DOI-induced HTR in (A) chronically vehicle exposed and (B) cocaine-treated mice (1.25 mg/kg, twice daily for 13.5 days IP). For experimental details, see the Methods section. Data are presented as means ± SEM (n = 6–7). *Significantly different from vehicle controls.

HTR. Subsequent to the initial elevation in synaptic concentration of DA, 5-HT, and NE, acute cocaine administration has been reported to decrease transmitter synthesis by a compensatory feedback mechanism (18,40). Furthermore, chronic cocaine exposure is reported to decrease brain 5-HT content (46), probably via inhibition of uptake of its precursor tryptophan and reduction in the activity of tryptophan hydroxylase (32,51). Thus, the cocaine-induced supersensitivity to DOI may be due to a persistent decrease in synaptic 5-HT concentration. Whether the cocaine-induced supersensitivity is due to an increase in 5-HT_2 receptor density is not yet known. However, a recent study of 5-HT_2 receptor signal transduction mechanisms in hippocampal tissue indicates that repeated administration of low doses of cocaine in monkeys increases receptor-effector coupling efficiency (17). This enhanced coupling may be involved in the sensitization of the head-twitch behavior.

Although chronic administration of cocaine increases DA content in the CNS (29,46), a review of the literature suggests that DA has no modulatory role in HTR induced by 5-HT agonists (25). Except for a short-term increase in NE content, chronic cocaine administration does not modify the concentrations of NE and its major metabolite 3-methoxy-4-hydroxyphenylglycol in the CNS (30,46). Thus, it was of interest to investigate whether any changes in the functional sensitivity of the inhibitory adrenergic α_2 and serotonergic 5-HT_{1A} receptors can occur following chronic low-dose cocaine exposure, which may also modify the DOI-induced HTR frequency. The 5-HT_{1A} agonist 8-OH-DPAT was more effective in inhibiting the DOI-induced HTR in chronically cocaine-treated animals than to vehicle-exposed controls. These data suggest that 5-HT_{1A} receptor function has become more sensitive following chronic cocaine exposure. There is evidence of supersensitivity to other 5-HT receptor agonists. For example, the 5-$HT_{1B/1C}$ agonist TFMPP and the 5-HT_3 agonist mCPBG had no effect on chronically vehicle-exposed animals, whereas in cocaine-exposed mice these agents significantly reduced the DOI-induced HTR. At higher doses, TFMPP also possesses affinity for 5-HT_{1A} sites (48) and can partially inhibit DOI-induced HTR via stimulation of these inhibitory receptors (1). However, TFMPP at lower doses in the present study and in other published findings (14) had no significant effect on DOI-induced behavior. Thus, it is reasonable to suggest that inhibition of DOI-induced HTR by such a low dose of TFMPP in chronically cocaine-pretreated mice is due to stimulation of a "more sensitive" 5-HT_{1A} receptor site. Similar to the lack of effect of the 5-HT_3 antagonist ICS-205,930 [(1,16), present investigation], the potent 5-HT_3 agonist mCPBG failed to affect the induced head-twitch behavior in chronically vehicle-exposed mice. However, in the cocaine-treated group mCPBG significantly reduced the DOI-induced HTR score. mCPBG has no affinity for other 5-HT-receptor subtypes (31). Whether this effect is via stimulation of possible supersensitive 5-HT_3 receptors or via other mechanisms is not yet known. The α_2 agonist clonidine was equipotent in inhibiting the DOI-induced HTR in both vehicle and chronically cocaine-exposed mice. Thus, it appears that chronic administration of low doses of cocaine enhances the sensitivity of 5-HT_{1A}, 5-HT_2, and possibly 5-HT_3 receptors without affecting α_2 adrenoceptor function. This lack of change in α_2 adrenoceptor sensitivity correlates with the lack of effect on central NE concentration following chronic cocaine exposure (30,46).

In the present study, chronic cocaine exposure at higher doses did not modify DOI-induced HTR. At these high doses, cocaine probably affects these receptor mechanisms, as well as other modulatory neurotransmitter systems. For example, high-dose cocaine exposure modifies central benzodiazepine receptors (36), as well as GABA release (27), which are involved in the modulation of head-twitch behavior (25). The selective effect of chronic low- relative to high-dose cocaine exposure in isolated aggressive mice has already been discussed (12). Moreover, human addicts abuse cocaine at such low doses whereas high doses (such as 10 mg/kg) would certainly be fatal in man. Thus, the absence of direct serotonergic effects following chronic administration of high doses of cocaine is probably one important reason for the lack of research effort in regard to this important component of cocaine's actions.

In animal studies, conditions that increase serotonergic activity such as dietary tryptophan or fluoxetine treatment (a 5-HT uptake blocker) reduce cocaine self-administration (5,6). Interestingly, acute administration of the 5-HT_{1A} partial

agonist buspirone also produces similar effects (23) and upon chronic administration inhibits the development of cocaine-induced stereotyped behaviors (53). Furthermore, repeated administration of the 5-HT_2 receptor antagonist ritanserin has been shown to reduce the preference and consumption of cocaine in a dose-dependent manner (38). As discussed in the Introduction section, cocaine inhibits serotonergic dorsal raphe neuronal activity indirectly via stimulation of impulse-modulating 5-HT_{1A} receptors. Similar to the present investigation, chronic cocaine exposure was reported to induce sensitization of this inhibitory 5-HT_{1A} receptors (10). However, unlike this in vivo extracellular electrophysiological model, their recent in vitro model has shown development of tolerance (2). Thus, the in vivo functional interaction between 5-HT_{1A} and 5-HT_2 sites and cocaine-induced changes in the sensitivity of these receptors may have relevance in the ability of these two receptor systems in diminishing cocaine self-administration.

Drugs that can alter the effects of cocaine on serotonergic receptor function in animals may help to provide new insights for the better clinical treatment of cocaine abuse and management of its symptoms. For example, lithium, which has been used prophylactically for the treatment of several types of depression (26,41), can also reverse the effects of cocaine on tryptophan uptake and 5-HT synthesis in rats (32) and has been reported to block cocaine-induced euphoria and craving in humans (9,19). Furthermore, unlike chronic cocaine-induced augmentation, chronic lithium administration is shown to attenuate the function of 5-HT_{1A}, 5-HT_2, and α_2 receptors (24). Thus, application of serotonergic drugs in clinical setting and realization of serotonergic receptor subtype interaction may provide a new avenue in the clinical management of cocaine abuse.

ACKNOWLEDGEMENTS

This research was supported by grant DA-02396 from the National Institute on Drug Abuse.

REFERENCES

1. Arnt, J.; Hytell, J. Facilitation of 8-OH DPAT-induced forepaw treading of rats by the 5-HT_2 agonist DOI. Eur. J. Pharmacol. 161:45–51; 1989.
2. Black, W. E.; Lakoski, J. M. In vitro electrophysiology and dorsal raphe neurons in subchronic cocaine treated rats: Development of tolerance to acute cocaine administration. Mol. Cell. Neurosci. 1:84–91; 1990.
3. Blackshear, M. A.; Sanders-Bush, E. Serotonin receptor sensitivity after acute and chronic treatment with mianserin. J. Pharmacol. Exp. Ther. 221:303–308; 1982.
4. Buckholtz, N. S.; Zho, D.; Freedman, D. X. Serotonin agonist administration downregulates rat brain serotonin$_2$ receptors. Life Sci. 42:2439–2445; 1988.
5. Carroll, M. E.; Lac, S. T.; Asencio, M.; Kargh, R. Fluoxetine reduces intravenous cocaine self-administration in rats. Pharmacol. Biochem. Behav. 35:237–244; 1990.
6. Carroll, M. E.; Lac, S. T.; Asencio, M.; Kargh, R. Intravenous cocaine self-administration in rats is reduced by dietary tryptophan. Psychopharmacology (Berl.) 100:239–300; 1990.
7. Clouet, D.; Asghar, K.; Brown, R. Mechanisms of cocaine abuse and toxicity. NIDA Monograph 88. Washington, DC: U.S. Government Printing Office; 1988.
8. Conn, P. J.; Sanders-Bush, E. Central serotonin receptors: Effector systems, physiological roles and regulation. Psychopharmacology (Berl.) 92:267–277; 1987.
9. Cronson, A. J.; Flemenbaum, A. Antagonism of cocaine highs by lithium. Am. J. Psychiatry 135:856–857; 1978.
10. Cunningham, K. A.; Asprodini, E. K.; Bernau, N. A.; Richard, C. A.; Lakoski, J. M. Enhanced inhibitory responses of serotonin neurons in the dorsal raphe nucleus (DRN) after repeated cocaine exposure. Soc. Neurosci. Abstr. 13 (part 3): 1651; 1987.
11. Cunningham, K. A.; Lakoski, J. M. The interaction of cocaine with serotonin dorsal raphe neurons: Single unit extracellular recording studies. Neuropsychopharmacology 3:41–50; 1990.
12. Darmani, N. A.; Hadfield, M. G.; Carter, W. H.; Martin, B. R. Acute and chronic effects of cocaine on isolation-induced aggression. Psychopharmacology (Berl.) 102:37–40; 1990.
13. Darmani, N. A.; Martin, B. R.; Glennon, R. A. Withdrawal from chronic treatment with (±)-DOI causes supersensitivity to 5-HT_2 receptor-induced head-twitch behavior in mice. Eur. J. Pharmacol. 186:115–118; 1990.
14. Darmani, N. A.; Martin, B. R.; Pandey, U.; Glennon, R. A. Do functional relationships exist between 5-HT_{1A} and 5-HT_2 receptors? Pharmacol. Biochem. Behav. 36:901–906; 1990.
15. Darmani, N. A.; Martin, B. R.; Pandey, U.; Glennon, R. A. Pharmacological characterization of ear-scratch response in mice as behavioral model for selective 5-HT_2-receptor agonists and evidence for 5-HT_{1B} and 5-HT_2 receptor interactions. Pharmacol. Biochem. Behav. 37:95–99; 1990.
16. Darmani, N. A.; Martin, B. R.; Pandey, U.; Glennon, R. A. Inhibition of 5-HT_2 receptor mediated head-twitch response by cocaine via indirect stimulation of adrenergic α_2 and serotonergic 5-HT_{1A} receptors. Pharmacol. Biochem. Behav. 38:353–357; 1991.
17. Farfel, G. M.; Wainwright, M. S.; Salti, H. I.; Klevin, M. S.; Woolverton, W. L.; Seiden, L. S.; Perry, B. D. Neurotransmitter receptor effector alterations in rhesus monkey brain following repeated cocaine injections. In: Harris, L. S., ed. Problems of drug dependence. NIDA Research Monograph. Bethesda, MD: NIDA; 1991 (in press).
18. Galloway, M. P. Regulation of dopamine and serotonin synthesis by acute administration of cocaine. Synapse 6:63–72; 1990.
19. Gawin, F. H.; Kleber, H. D. Cocaine abuse treatment: Open pilot trial with desipramine and lithium carbonate. Arch. Gen. Psychiatry 41:903–909; 1984.
20. George, F. R. Cocaine produces low dose locomotor depressant effects in mice. Psychopharmacology (Berl.) 99:147–150; 1989.
21. Glennon, R. A. Serotonin receptors: Clinical implications. Neurosci. Biobehav. Rev. 14:35–47; 1990.
22. Glennon, R. A.; Darmani, N. A.; Martin, B. R. Multiple populations of serotonin receptors may modulate the behavioral effects of serotonergic agents. Life Sci. 48:2493–2498; 1991.
23. Gold, L. H.; Balster, R. L. The effects of buspirone and geopirone on cocaine self-administration in rhesus monkeys. In: Harris, L. S., ed. Problems of drug dependence. NIDA Research Monograph. Bethesda, MD: NIDA; 1991 (in press).
24. Goodwin, G. M.; De Souza, R. J.; Wood, A. J.; Green, A. R. Lithium decreased 5-HT_{1A} and 5-HT_2 receptor and α_2-adrenoceptor mediated function in mice. Psychopharmacology (Berl.) 90: 482–487; 1986.
25. Handley, S. L.; Singh, L. Neurotransmitters and shaking behavior: More than a "gut bath" for the brain. Trends Pharmacol. Sci. 7:324–328; 1986.
26. Heninger, G. R.; Charney, D. S.; Sternberg, D. E. Lithium carbonate augmentation of antidepressant treatment: An effective treatment for treatment refractory depression. Arch. Gen. Psychiatry 40:1335–1342; 1983.
27. Ikeda, M.; Dohi, T.; Tsujimoto, A. Inhibition of gamma aminobutyric acid release from synaptosomes by local anesthetics. Anesthesiology 58:495–499; 1983.
28. Johanson, C. E.; Fischman, M. W. The pharmacology of cocaine related to its abuse. Pharmacol. Rev. 41:3–52; 1989.
29. Kalivas, P. W.; Duffy, P. Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. Synapse 5:48–58; 1990.

30. Karoum, F.; Suddath, R. L.; Wyatt, R. J. Chronic cocaine and rat brain catecholamines: Long term reduction in hypothalamic and frontal cortex dopamine metabolism. Eur. J. Pharmacol. 186:1-8; 1990.
31. Kilpatric, G. J.; Butler, A.; Burridge, J.; Oxford, A. W. 1-(m-Chlorophenyl)-biguanide, a potent high affinity 5-HT_3 receptor agonist. Eur. J. Pharmacol. 182:193-197; 1990.
32. Knap, S.; Mandell, A. J. Narcotic drugs: Effects in the serotonin biosynthetic systems of the brain. Science 177:1209-1211; 1972.
33. Leysen, J. E.; Pauwels, P. J. Central and peripheral 5-HT_2 receptors: Role in physiological versus pathological conditions. In: Paoletti, R.; Vanhoute, P. M.; Brunello, N.; Maggi, F. M., eds. Serotonin: From cell biology to pharmacology and therapeutics. Dordrecht: Kluer Academic Press; 1990:323-329.
34. Leysen, J. E.; Van Gompel, P.; Gommeren, W.; Woestenborghs, R.; Janssen, P. A. G. Down-regulation of serotonin-S_2 receptor sites in rat brain by chronic treatment with the serotonin-S_2 antagonist, ritanserin and setoperone. Psychopharmacology (Berl.) 88: 434-444; 1986.
35. Lucki, I.; Nobler, M. S.; Frazer, A. Differential actions of serotonin antagonists on two behavioral models of serotonin receptor activation in the rat. J. Pharmacol. Exp. Ther. 228:133-139; 1984.
36. McAllister, K.; Goeders, N.; Dworkin, S. Chronic cocaine modifies brain benzodiazepine receptor densities. Natl. Inst. Drug Abuse Monogr. Ser. 81:101-108; 1988.
37. McKenna, D. J.; Nazarali, A. J.; Himeno, A.; Saavedra, J. M. Chronic treatment with (±)DOI, a psychotomimetic 5-HT_2 agonist, down regulates 5-HT_2 receptors in rat brain. Neuropsychopharmacology 2:81-87; 1989.
38. Meert, T.; Clinke, G.; Gelders, Y.; DeSmedt, G. Ritanserin, a new therapeutic approach for drug abuse. In: Harris, L. S., ed. Problems of drug dependence. NIDA Research Monograph. Bethesda, MD: NIDA; 1991 (in press).
39. Mogilnicka, E.; Klimek, V. Miaserin, danitracen and amitriptyline withdrawal increases the behavioral responses of rats to L-5-HTP. Commun. J. Pharm. Pharmacol. 31:704-705; 1979.
40. Pradhan, S. N.; Bhattacharyya, A. K.; Pradhan, S. N. Serotonergic manipulation of the behavioral effects of cocaine in rats. Commun. Psychopharmacol. 2:481-486; 1978.
41. Prien, R. F.; Kupfer, D. J.; Mansky, P. A.; Small, J. G.; Tanson, V. B.; Voss, C. B.; Johanson, W. E. Drug therapy in the prevention of recurrences in unipolar and bipolar disorders. Arch. Gen. Psychiatry 41:1096-1104; 1984.
42. Reith, M. E. A.; Benuck, M.; Lajtha, A. Cocaine disposition in the brain after continuous or intermittent treatment and locomotor stimulation in mice. J. Pharmacol. Exp. Ther. 243:281-287; 1987.
43. Reith, M. E. A.; Meisler, B. E.; Lajtha, A. Locomotor effects of cocaine, cocaine congeners and local anesthetics in mice. Pharmacol. Biochem. Behav. 23:831-836; 1985.
44. Richardson, B. P.; Engle, G.; Donatch, P.; Stadler, P. A. Identification of serotonin m-receptor subtypes and their specific blockade by a new class of drugs. Nature (London) 316:126-131; 1985.
45. Ritz, M. C.; Lab, R. J.; Goldberg, S. R.; Kuhar, M. J. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237:1219-1223; 1987.
46. Roy, S. N.; Bhattacharyya, A. K.; Pradhan, S.; Pradhan S. N. Behavioral and neurochemical effects of repeated administration of cocaine in rats. Neuropharmacology 17:559-564; 1978.
47. Schmidt, A. W.; Peroutka, S. J. 5-Hydroxytryptamine receptor families. FASEB J. 3:2242-2249; 1989.
48. Schoeffter, P.; Hoyer D. Interaction of arylpiperazines with 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} receptors. Do discriminatory 5-HT_{1B} receptor ligands exist? Naunyn Schmiedebergs Arch. Pharmacol. 339:675-683; 1989.
49. Stoltz, J. F.; Marsden, C. A.; Middlemiss, D. N. Effects of chronic antidepressant treatment and subsequent withdrawal on ^{3}H-5-hydroxytryptamine and ^{3}H-spiperone binding in rat frontal cortex and serotonin mediated behaviour. Psychopharmacology (Berl.) 80:150-155; 1983.
50. Tadano, T.; Satoh, S.; Satoh, N.; Kisara, K.; Arai, Y.; Kim, S. K.; Kinemuchi, H. Potentiation of para-hydroxytryptamine-induced head-twitch response by inhibition of monoamine oxidase type A in the brain. J. Pharmacol. Exp. Ther. 250:254-260; 1989.
51. Taylor, D.; Ho, B. T. Effect of short- and long-term treatment with cocaine on rat brain tryptophan hydroxylase. Res. Commun. Chem. Pathol. Pharmacol. 15:805-808; 1976.
52. Twist, E. C.; Mitchel, S.; Brazel, C.; Stahl, S. M.; Campbell, I. C. 5-HT_2 receptor changes in rat cortex and platelets following chronic ritanserin and clorgyline administration. Biochem. Pharmacol. 39:161-166; 1990.
53. Tucker, S. M.; Dimen, K. R.; Ji, Z.; Bowman, E. R.; Aceto, M. D. Buspirone blocks the development of cocaine-induced stereotyped behavior in rats. Pharmacologist 33:P270; 1991.