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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

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

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

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DARMANI, N A , B R MARTIN, U PANDEY AND R A. GLENNON *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**(2) 353–357, 1991 —Cocaine inhibits the 5-HT_2-mediated (±)-DOI-induced head-twitch response (HTR) in mice in a dose-dependent manner In order to investigate the possible inhibitory mechanism(s) of cocaine on 5-HT_2 receptor function, we studied the effects of the selective adrenergic α_2 receptor antagonist yohimbine and the β-adrenergic/5-HT_1 receptor antagonist alprenolol, and the 5-HT_3 antagonist ICS 205-930 on the inhibitory action of cocaine on the (±)-DOI-induced HTR Neither yohimbine (0 1 and 0 5 mg/kg) nor alprenolol (10 mg/kg) pretreatment had any significant effect on the (±)-DOI-induced HTR However, both antagonists prevented the inhibitory effects of cocaine on the (±)-DOI-induced HTR The 5-HT_3 antagonist ICS 205-930 neither produced HTR nor decreased the (±)-DOI-induced HTR frequency The present results suggest that cocaine inhibits 5-HT_2 receptor function by increasing the synaptic concentration of norepinephrine and serotonin via inhibition of their uptake and thus indirectly stimulating the respective inhibitory adrenergic α_2 and serotonergic 5-HT_{1A} receptors Furthermore, cocaine's 5-HT_3 antagonist properties appear not to play a role in the inhibition of head-twitch behavior

Cocaine DOI Yohimbine Alprenolol ICS 205-930 Head-twitch 5-HT_{1A} receptor 5-HT_2 receptor α_2-Adrenoceptor

ALTHOUGH cocaine produces numerous central and peripheral effects, the drug is commonly referred to as a psychomotor stimulant primarily because cocaine can stimulate the central nervous system [for a recent review, see (11)]. At low doses, cocaine induces a response consisting of increases in exploration, locomotion, grooming and rearing (20,22). At higher doses, locomotor activity decreases and the behavioral pattern becomes stereotyped (17). The majority of evidence indicates that these neurobehavioral effects of cocaine involve an increase in the activity of dopaminergic neurons (11) However, more recent studies indicate that cocaine, at very low doses, inhibits locomotor activity through potentiation of serotonergic receptor activity via selective inhibition of serotonin uptake (7). This is consistent with the report that cocaine possesses a 5–10-fold higher affinity for serotonin (5-HT) transporter than for dopamine (DA) and norepinephrine (NE) transporter sites (19)

Detailed studies on the mechanism of action of cocaine on serotonergic models of behavior are lacking. The few existing studies indicate that high doses of cocaine induce some signs of the serotonin syndrome, such as lateral head-weaving and straub tail in rats (3) and inhibit head-twitch response in mice (23). The full repertoire of the serotonin syndrome consists of the above symptoms as well as tremor and hypertonicity. 5-HT-mediated behaviors are important pharmacological tools for examining the effects of drugs on 5-HT receptor function. Several types of 5-HT binding sites (5-HT_1, 5-HT_2, 5-HT_3) have been identified by radioligand binding studies (8). In addition, 5-HT_1 receptors appear to consist of a number of subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_{1E}) of which 5-HT_{1A} sites are perhaps best studied. One of the most often used models of 5-HT_2 receptor function is the stereotypic twitch-like movements of the head (head-twitch response) induced in rodents by direct- or indirect-acting 5-HT agonists (6,13) Although head-twitch response (HTR) can be mediated by multiple neurotransmitter systems (9), the head-twitch induced by 5-HT agonists is antagonized by selective 5-HT_2 receptor antagonists (6,13). In fact, there is a significant correlation between antagonist potency and 5-HT_2 site affinity (15). The serotonin syndrome is a behavioral phenomenon that is

[1]Requests for reprints should be addressed to Dr Nissar A Darmani, Department of Pharmacology/Toxicology, MCV, Box 613, Richmond, VA 23298

distinct from the HTR and is induced by stimulation of 5-HT_{1A} receptors by selective (e.g., 8-OH DPAT) and nonselective (e.g., 5-MeO DMT) 5-HT agonists in rodents (21). As might be expected, selective 5-HT_2 receptor antagonists such as ketanserin do not inhibit the serotonin syndrome.

Recently, the indirect 5-HT agonist p-hydroxyamphetamine (p-OHA) was reported to induce the HTR in mice and the induced behavior was inhibited by cocaine pretreatment (23). The cocaine effect was hypothesized to be due to inhibition of p-OHA-induced release of 5-HT from nerve endings However, this mechanism cannot account for the inhibitory effects of cocaine on the head-twitch behavior induced by direct-acting 5-HT_2 agonists (present study) Cocaine possesses weak 5-HT_3 antagonist properties (18), as well as the ability to inhibit the uptake of monoamines Previously, it has been reported that noradrenergic mechanisms can modulate the HTR induced by 5-HT agonists In particular, stimulation of adrenergic α_2 receptors inhibits the induced behavior (9,10). Furthermore, recent evidence also indicates that stimulation of 5-HT_{1A} sites also modulates the behavior induced by selective 5-HT_2 agonists (6). Thus monoamine interaction or 5-HT_3 antagonism may explain the inhibitory effects of cocaine on the HTR induced by 5-HT_2 selective direct-acting agonists The purpose of the present study was to investigate whether monoamine interaction and/or 5-HT_3 antagonism can explain the inhibitory action of cocaine on the HTR. To this end, we used the putative 5-HT_2 selective agonist (±)-DOI to induce HTR in mice. To investigate the mechanism of inhibition of (±)-DOI-induced HTR by cocaine, we pretreated the animals with various drugs including: yohimbine (an α_2-antagonist), alprenolol (a 5-HT_1/β adrenoceptor antagonist) and ICS 205-930 (a 5-HT_3 antagonist). These drugs were administered either alone or in combination with cocaine.

METHOD

Male albino mice (ICR), weighing 16–20 g, were used throughout the study The mice were housed in groups of five on a 12-hour light/dark cycle with ad lib supply of food and water. To habituate the animals to the test environment, each animal was randomly transferred 30 min prior to treatment to a 40 × 25 × 16 cm plastic cage lined with sawdust. Mice were injected intraperitoneally with various doses of (±)-DOI (0, 0 32, 0 63, 1.25, 2.5, 5.0 and 10.0 mg/kg) or H_2O (N = 14–15) and the head-twitch response was scored for the first 30 min following injection Total mean scores (±SEM) over the 30-minute interval were calculated.

To see whether cocaine itself could induce the HTR, mice were injected intraperitoneally with increasing doses of the stimulant (0, 2 5, 5, 10, 20 and 40 mg/kg, n = 6) The animals were observed for 30 min following cocaine injection. In order to investigate the possible effects of cocaine on the (±)-DOI-induced HTR, the animals were pretreated with different doses of cocaine (0, 2.5, 5, 10, 20 and 40 mg/kg, IP) 10 min prior to (±)-DOI administration (2 5 mg/kg, IP, n = 6) The HTR frequency was scored for 30 min following (±)-DOI injection. To determine a possible contribution of 5-HT_3 antagonist properties of cocaine in inhibition of HTR, the selective 5-HT_3 antagonist ICS 205-930 (2, 4 and 8 mg/kg) was given intraperitoneally 40 min prior to injection of (±)-DOI. The HTR frequency was scored as described above

Two doses of the α_2 antagonist yohimbine (0.1 and 0.5 mg/kg) were mixed with either cocaine solution (20 mg/kg, n = 4–5) or distilled water (n = 5) and were administered intraperitoneally to two different groups of mice 10 min prior to (±)-DOI injection (IP) For this α_2-adrenergic interaction study, two doses of (±)-DOI, 1.0 and 2.5 mg/kg were used to induce HTR in mice and the behavior was scored for 20 or 30 min postinjection [(±)-DOI] respectively. The β-blocker alprenolol, which also possesses 5-HT_1 antagonist properties, was used to investigate the possible contribution of 5-HT_1 receptors towards the cocaine-induced inhibition of head-twitch behavior. Alprenolol (10 mg/kg, n = 5) or water (n = 5) were administered subcutaneously to two different groups of mice 20 min prior to an intraperitoneal injection of cocaine (20 mg/kg). Then 10 min later the animals were further treated intraperitoneally with 1.0 mg/kg (±)-DOI. The HTR frequency was scored for the next 20 min. For cocaine controls, the same procedure was repeated, except the animals received an intraperitoneal injection of water instead of cocaine.

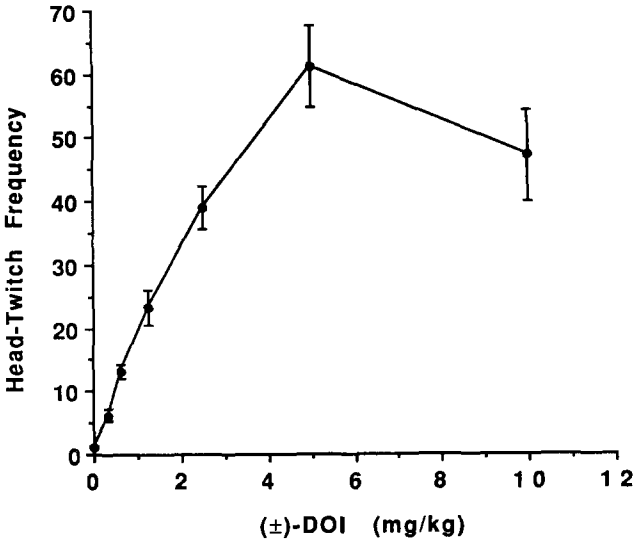

FIG 1 The effect of (±)-DOI administration (IP) on the production of head-twitch response in mice The behavior was observed for 30 minutes immediately after injection Results are given as means ± SEM A portion of these data were previously published (6)

The drugs used were obtained from the following sources (±)1-(2,5-dimethyoxy-4-iodophenyl)-2-aminopropane HCl [(±)-DOI HCl] and 3-tropanyl-indole-3-carboxylate (ICS 205-930) from Research Biochemicals, Inc (Natick, MA); yohimbine hydrochloride and (−)alprenolol tartrate from Sigma and cocaine hydrochloride from the National Institute on Drug Abuse. Unless otherwise stated, all drugs were dissolved in distilled water and given at a volume of 10 ml/kg

Statistical Analysis

Data were analyzed by one-way analysis of variance and post hoc analysis by Dunnett *t*-test.

RESULTS

The phenylisopropylamine 5-HT_2 agonist (±)-DOI produces a dose-dependent increase in HTR frequency up to 5 mg/kg (Fig. 1) At higher doses (e.g., 10 mg/kg), (±)-DOI produces a frequency of HTR that is not significantly different than that produced by the 5 mg/kg dose. At the doses employed, cocaine by itself did not elicit the head-twitch behavior (data not shown), however, cocaine pretreatment caused a dose-dependent decrease in (±)-DOI-induced HTR (Fig. 2). The lowest dose of cocaine tested (2 5 mg/kg) significantly ($p<0.05$) reduced the DOI-in-

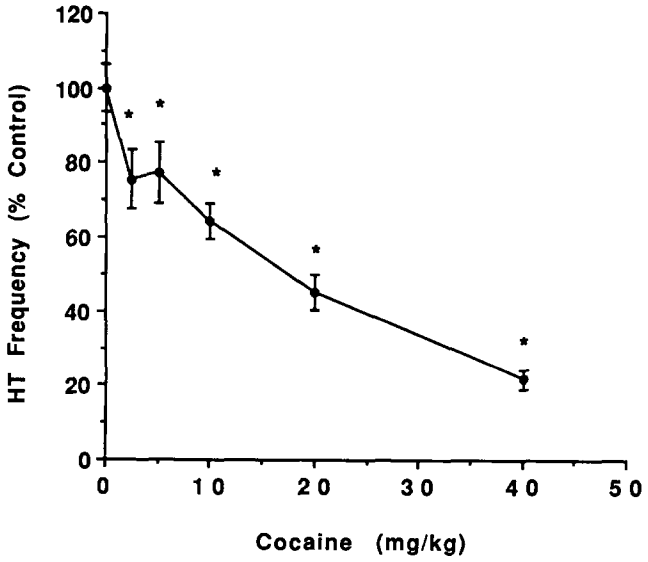

FIG 2 Dose-dependent inhibitory effect of cocaine on head-twitch frequency induced by 2 5 mg/kg of (±)-DOI Data are presented as percent control head-twitch response (±SEM) produced by 2 5 mg/kg (±)-DOI in the absence of cocaine Cocaine was administered 10 min prior to DOI *Significantly different from control at $p<0$ 05, n=6 at each dose

duced head-twitch response by 25%; at the highest dose tested (40 mg/kg), cocaine decreased the induced HTR frequency by 80% (ID_{50}=11.8 mg/kg; 95% confidence limit 6.4–21.4 mg/kg). The α_2-adrenoceptor antagonist yohimbine (at 0.1 and 0.5 mg/kg) induced no significant change in (±)-DOI-induced (2.5 mg/kg) HTR ($p>0.05$) (Fig. 3a). Pretreatment with cocaine (20 mg/kg) plus yohimbine (0.1 or 0.5 mg/kg) also caused no significant effect on (±)-DOI (2.5 mg/kg)-induced HTR (Fig. 3a). Because 2 5 mg/kg of (±)-DOI might be considered to be a moderately high dose, the experiment was repeated using a lower (i.e , 1 mg/kg) dose of (±)-DOI. The results of the second experiment (Fig. 3b) were virtually the same as the first (Fig. 3a).

At a dose of 10 mg/kg, alprenolol had no effect on (±)-DOI-induced HTR (Fig 4). Although a combination of alprenolol (10 mg/kg) and cocaine (20 mg/kg) reduced the (±)-DOI-induced HTR by about 30% (Fig. 4), the reduction in HTR was not statistically significant because of interanimal variability. Consistent with the results shown in Fig. 2, cocaine reduced (±)-DOI-induced HTR by 55–60% in all of the above three drug interaction studies (Figs. 3a, b, 4).

At doses of 2 to 8 mg/kg, the 5-HT_3 antagonist ICS 205-930 had no significant effect ($p>0.05$) on (±)-DOI-induced HTR; furthermore, by itself, ICS 205-930 did not produce the HTR (data not shown).

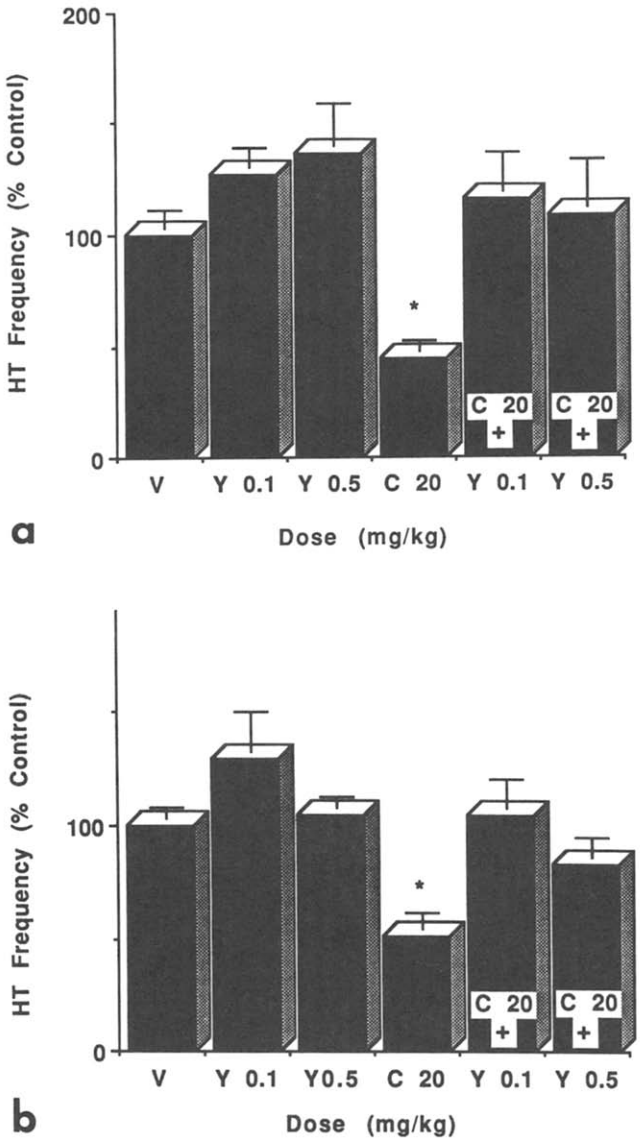

FIG 3 The effects of prior administration of either water vehicle (V), the α_2 antagonist yohimbine (Y, 0 1 and 0 5 mg/kg), cocaine alone (C, 20 mg/kg), or a combination of cocaine (20 mg/kg) and yohimbine (0 1 and 0 5 mg/kg) on the head-twitch frequency induced either by 2 5 mg/kg (a) or 1 0 mg/kg (±)-DOI (b) The data are presented as percent head-twitch response induced by the respective (±)-DOI doses in the absence of cocaine and yohimbine (instead of cocaine or yohimbine animals received a dose of vehicle=V) *Significantly different from control at $p<0$ 05, n=6 at each dose

DISCUSSION

Cocaine can increase synaptic levels of 5-HT by inhibiting 5-HT uptake by serotonergic nerve endings (19). Because drugs which increase synaptic concentrations of 5-HT induce head-twitch behavior in rodents (4), cocaine might be expected to enhance HTR. However, it has recently been reported that cocaine inhibits the HTR when the behavior is induced by the indirect 5-HT agonist p-hydroxyamphetamine (p-OHA) (23). It was suggested that cocaine inhibits the p-OHA uptake into the serotonergic nerves which prevents 5-HT release and thus reduces the frequency of HTR induced by p-OHA. Although this mechanism may partly explain the inhibition of p-OHA-induced HTR, it cannot account for the inhibitory effect of cocaine on HTR induced by the direct acting 5-HT_2 selective agonist (±)-DOI.

(±)-DOI produces HTR in mice by what is believed to be a 5-HT_2 agonist mechanism (6) This effect is dose-dependently attenuated by 5-HT_2 antagonists (6) and by cocaine (Fig 2). Although cocaine possesses little affinity for 5-HT_2 receptors, it is a weak 5-HT_3 antagonist (18). However, the present results using ICS 205-930, together with the results of Arnt and Hyttel (2), rule out the possibility that the HTR involves a 5-HT_3 mechanism.

The HTR induced by 5-HT agonists can be attenuated by α_2-

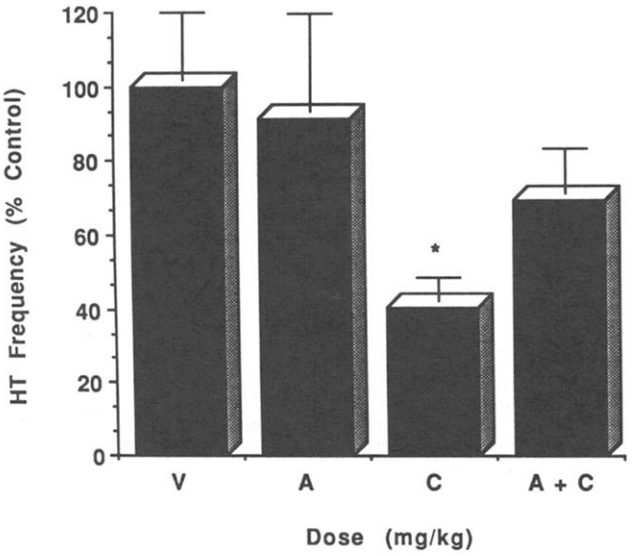

FIG 4 Effects of prior administration of water vehicle (V), alprenolol (A, 10 mg/kg), or cocaine (C, 20 mg/kg) alone, and a combination of alprenolol (10 mg/kg) and cocaine (20 mg/kg) on the head-twitch frequency produced by 1.0 mg/kg (±)-DOI The data are presented as percent control head-twitch response produced by 1 0 mg/kg (±)-DOI in the absence of alprenolol and cocaine *Significantly different from control at $p<0$ 05, n = 6 at each dose

adrenoceptor agonists (9). Additionally, cocaine is known to increase synaptic levels of norepinephrine (16). The selective α_2-adrenoceptor antagonist yohimbine has been shown to increase 5-HT agonist-induced HTR (9). In the present study, yohimbine (0.1 and 0 5 mg/kg) produced a slight, but not statistically significant, increase in (±)-DOI-induced HTR (Fig. 3). Arnt and Hyttel (1) have reported that yohimbine, at similar doses, has no effect on 5-HTP-induced HTR. As shown in Fig. 3, yohimbine attenuates the inhibitory effect of cocaine on (±)-DOI-induced HTR. These results suggest that cocaine-induced increases in synaptic norepinephrine levels can account for the reduction in (±)-DOI-induced HTR.

β-Adrenoceptor antagonists are reported to have no effect on 5-HT agonist-induced HTR (9) In the present investigation, alprenolol failed to antagonize (±)-DOI-induced HTR (Fig. 4). However, alprenolol prevented the reduction in (±)-DOI-induced HTR by cocaine. That is, whereas 20 mg/kg of cocaine reduces (±)-DOI-induced HTR by 60% (Fig. 3b), a combination of cocaine (20 mg/kg) and alprenolol (10 mg/kg) failed to cause a significant ($p>0.05$) change. In light of this seemingly paradoxical finding, it should be noted that certain nonselective β-adrenoceptor antagonists, including alprenolol, bind with high affinity at 5-HT_{1A} sites (2) and can act as 5-HT_{1A} antagonists. We have previously demonstrated a functional relationship between 5-HT_{1A} receptor activation and (±)-DOI-induced HTR (6); Arnt and Hyttel (2) have reported similar findings. Stimulation of 5-HT_2 receptors results in a marked HTR; simultaneous costimulation of 5-HT_{1A} receptors (by the 5-HT_{1A}-selective agonist 8-OH DPAT, or the nonselective 5-HT agonist 5-MeO DMT) results in an apparent antagonism of the HTR. Because cocaine can act as an indirect 5-HT agonist (by virtue of its ability to increase synaptic concentrations of 5-HT), and because its effect on (±)-DOI-induced HTR is attenuated by the 5-HT_{1A} antagonist alprenolol, its inhibitory effects are likely to involve a 5-HT_{1A} mechanism

In support of this indirect 5-HT_{1A} agonist effect, Cunningham and Lakoski (5) have recently reported that cocaine inhibits the spontaneous activity of dorsal raphe neurons through indirect stimulation of 5-HT_{1A} receptors by increasing synaptic 5-HT concentration via inhibition of its uptake.

In summary, it appears that cocaine inhibits the head-twitch behavior induced by the selective 5-HT_2 agonist (±)-DOI by at least two mechanisms: i.e., cocaine inhibits uptake of both 5-HT and NE, thereby increasing their synaptic concentrations by which they in turn stimulate serotonergic 5-HT_{1A} and adrenergic α_2 receptors respectively, leading to the inhibition of serotonergic 5-HT_2 receptor-mediated HTR. Although cocaine is a weak 5-HT_3 antagonist and can also inhibit dopamine uptake, neither 5-HT_3 [Arnt and Hyttel (2), present investigation] nor dopaminergic mechanisms (9) appear to be involved in the modulation of HTR. However, the mechanism of inhibition of cocaine may be more complex since the drug at high doses affects other central neurotransmitters such as benzodiazepine (14) and GABA (12) function, and selective agents at these sites can modulate HTR frequency (9).

ACKNOWLEDGEMENT

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