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The Stimulatory and Inhibitory Components of Cocaine's Actions on the 5-HTP-Induced 5-HT_{2A} Receptor Response

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DARMANI, N. A. AND S. L. REEVES. *The stimulatory and inhibitory components of cocaine's actions on the 5-HTP-induced 5-HT_{2A} receptor response.* PHARMACOL BIOCHEM BEHAV **55**(3) 387–396, 1996.—Previously we have shown that cocaine attenuates the 5-HT_{2A} receptor-mediated head-twitch response (HTR) in mice produced by the 5-$HT_{2A/C}$ direct agonist (±)-1(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). This inhibition appears to be due to cocaine-induced indirect stimulation of the inhibitory serotonergic 5-HT_{1A} and noradrenergic α_2 receptors via the inhibition of reuptake of synaptic serotonin (5-HT) and norepinephrine (NE), respectively. In the present study, we investigated the effects of cocaine, its phenyltropane analogue WIN 35428, and the selective 5-HT (sertraline), NE (nisoxetine) and dopamine (DA) (GBR 12935) reuptake inhibitors on the 5-hydroxytryptophan (5-HTP)-induced HTR. We utilized two experimental protocols where cocaine or the cited drugs were administered either after (protocol 1) or prior (protocol 2) to 5-HTP injection. Cocaine in both protocols produced a dose-dependent enhancement in the 5-HTP-induced HTR (ED_{50} 4.68 ± 1.21 and 3.55 ± 1.31, respectively). Sertraline was more potent (ED_{50} 2.64 ± 1.1 and 2.1 ± 1.54, respectively) in enhancing the induced behavior and dose by dose produced greater (3 to 10 times) HTRs than cocaine. On the other hand, nisoxetine dose dependently and completely attenuated the induced behavior (ID_{50} 3.33 ± 1.32 and 1.72 ± 1.34, respectively), whereas GBR 12935 only at high doses (ID_{50} 15.34 ± 1.52 and 11.91 ± 1.3, respectively) decreased the induced response. The inability of cocaine to induce as many HTRs as sertraline appears to lie in its ability to also indirectly stimulate the inhibitory 5-HT_{1A} and α_2 receptors because the stimulant caused greater enhancement in the 5-HTP–induced HTRs in the presence of their corresponding antagonists [S(−)-UH 301 and yohimbine, respectively]. WIN 35428 was more potent (ED_{50} 2.87 ± 1.3 and 1.79 ± 1.1 for protocols 1 and 2, respectively) in stimulating the 5-HTP–induced HTR and produced a bell-shaped dose–response curve. The results indicate that cocaine enhances the 5-HTP–induced HTR via the inhibition of synaptic 5-HT reuptake. The stimulant also simultaneously attenuates the induced behavior by indirect simulation of the serotonergic 5-HT_{1A} and noradrenergic α_2 receptors via inhibition of reuptake of the corresponding monoamines. **Copyright © 1996 Elsevier Science Inc.**

Head-twitch response 5-Hydroxytryptophan 5-HT_{2A} receptor Cocaine Sertraline GBR 12935 WIN 35428 Yohimbine S(−)-UH 301

COCAINE abuse in the USA has reached epidemic proportions; however, as yet no clinical study has indicated an effective modality for the treatment of cocaine addiction (59). Cocaine prevents the reuptake of dopamine (DA), norepinephrine (NE), and serotonin (5-HT) by binding to their reuptake sites and enhances the synaptic concentrations of these monoamines (50). Multiple regression analysis of transporter affinity of cocaine and structurally related compounds suggest that inhibition of DA transporter is most closely involved with cocaine's reinforcement mechanisms, whereas inhibition of 5-HT transporter appears to be negatively related to its reinforcement (34,51).

Although the catecholaminergic components of cocaine's actions have been studied for decades, only recently the serotonergic effects of cocaine has become the focus of intense study. In vitro electrophysiological findings show that the threshold cocaine dose required to affect the serotonergic neuronal activity in the raphe is three times lower (45,61) than for the noradrenergic system in the locus coeruleus (55) or for the dopaminergic system in the ventral tegmental area (35). Monoamine transporter affinity studies (51) suggest that cocaine possesses a 5–10-fold higher affinity for the 5-HT transporter than for the DA and NE transporters. In vivo electrophysiological studies also support the more potent ef-

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fects of cocaine on the serotonergic system (10,36). Moreover, serotonergic lesions of specific brain loci tend to increase (37), whereas conditions that increase the synaptic 5-HT function [such as 5-HT precursor loading (6) or selective serotonin reuptake inhibitors (5,24,49) or direct 5-HT agonist quipazine (39)] dose dependently reduce cocaine self-administration in animals. In addition, 5-HT_2 antagonists attenuate cocaine self-administration by rats (40,41). Clinically, serotonin reuptake inhibitors may block cocaine euphoria and craving (43,52) and use of such drugs in combination with serotonin precursors is useful in attenuating cocaine-induced psychiatric disorders (44).

Up to seven different 5-HT receptor sites (5-HT_{1-7}) have been identified and many of these receptors consist of several subtypes (30). The 5-HT_2 receptors consists of three different subtypes (5-HT_{2A-C}). The head-twitch response (HTR) in rodents is considered as a specific behavioral model for the activation of the serotonergic 5-HT_{2A} receptors (22,54). The HTR produced by the 5-HT agonists can be dose dependently attenuated by 5-HT_{2A} receptor antagonists. Moreover, antisense 5-HT_{2A} receptor oligonucleotides prevent the production of HTR (2). Cocaine can also dose dependently attenuate the HTR produced by direct 5-HT_{2A} agonists such as DOI and 5-MeO DMT (11–13,15). However, cocaine lacks affinity for 5-HT_{2A} receptors, and its ability to inhibit the DOI-induced HTR is thought to be via indirect stimulation of the inhibitory adrenergic α_2- and serotonergic 5-HT_{1A} receptors. The HTR can be also produced indirectly by the use of 5-HT precursor 5-hydroxytryptophan (5-HTP) (9). Thus, following its systemic administration, 5-HTP enters the CNS and undergoes decarboxylation to form 5-HT (27). This leads to functional increases in the synaptic concentration of 5-HT (21), which then results in the production of HTR (9,17). It is of interest to elucidate the influence of serotonergic and nonserotonergic components of cocaine's actions on the production of 5-HTP–induced head-twitch behavior. Therefore, we investigated the influence of cocaine, its phenyltropane analogue, WIN 35428, and the selective 5-HT, NE, and DA reuptake inhibitors [sertraline (42), nisoxetine (58), and GBR 12935 (46), respectively] on the production of 5-HTP–induced HTR.

METHOD

Animals and Drugs

Albino ICR male mice, weighing 25–30 g, were used throughout the study. Animals were housed in groups of five on a 12 L:12 D cycle at a room temperature of 22 ± 1°C with free access to food and water. All experiments were performed between 0800 and 1700 h. Nisoxetine HCl, GBR 12935 2HCl, and S(−)-UH 301 HCl were purchased from Research Biochemicals, Inc. (Natick, MA). 5-Hydroxy-L-tryptophan and yohimbine HCl were bought from Sigma Chemical Co. (St. Louis, MO). Cocaine HCl and WIN 35428 were obtained from the National Institute on Drug Abuse. Sertraline HCl and carbidopa were generously donated by Pfizer (Groton, CT) and Merck Sharp and Dhome (West Point, PA) respectively. 5-Hydroxytryptophan was dissolved in a small volume of concentrated HCl and then further diluted by distilled water and back titrated to pH 5 by the addition of NaOH. All other drugs were dissolved in distilled water.

Measurement of HTR

The HTR is a very distinctive behavior in mice and usually cannot be mistaken for such behaviors as head shakes (lateral movement of the head from side to side) or head jerks (up and down jerking). The HTR was scored cumulatively by a trained observer using a multiple tally counter. The observer was blind to drug treatment. Naive animals were used for different experiments.

Experimental Protocols

On the test day, the animals were transferred to the experiment room and were allowed to acclimate for at least 1 h prior to experimentation. The fume hood was turned on to produce a constant white noise during the experimental procedures. In the initial experiments, the dose–response effects of 5-hydroxytryptophan (5-HTP) for the production of head-twitch response (HTR) in mice was determined. Thus, at zero time, mice were pretreated with carbidopa (10 mg/kg, IP) to prevent the conversion of 5-HTP to 5-HT in the periphery (25,28). The animals were then allowed to habituate to the test environment in plastic holding cages (40 × 25 × 26 cm) lined with wood chippings. Twenty minutes later, different groups of carbidopa-treated mice received varying doses of 5-HTP (10, 20, 37.5, 75, 150, and 300 mg/kg, IP, n = 5–10) and were individually transferred to plastic observation cages with similar dimensions as the holding cages. The frequency of 5-HTP–induced HTR (mean ± SEM) for each mouse was individually scored for the first 30 min immediately following 5-HTP injection at 5-min intervals cumulatively. In addition, the total observation period for some 5-HTP doses (37.5, 75, 150, and 300 mg/kg) were extended to 60 min. From these initial experiments, we decided to utilize a 37.5 mg/kg dose of 5-HTP, as the HTR inducer for the investigation of the interactions of cocaine and other selective monoamine reuptake blockers on the production of 5-HTP–induced HTR.

We have previously reported that cocaine by itself does not induce the HTR (15). The effects of cocaine on the 5-HTP–induced HTR were investigated under two different experimental conditions. In the first protocol, at zero time a large group of mice received carbidopa (10 mg/kg, IP) and at 20 min 5-HTP (37.5 mg/kg, IP). At 30 min different groups of these treated mice received varying doses of cocaine (0, 0.63, 1.25, 2.5, 5, 10, and 20 mg/kg, IP, n = 11–15). The HTR frequency was scored cumulatively for the next 30 min immediately following cocaine or vehicle injection at 5-min intervals as described previously. In these studies, cocaine dose dependently enhanced the 5-HTP–induced HTR. Because literature suggests that cocaine can inhibit the uptake of tryptophan in the CNS (32), a second experimental protocol was used to see whether such interaction can occur with tryptophan metabolite 5-HTP. Thus, at zero time a large group of mice received carbidopa (10 mg/kg, IP) and at 10 min different groups of these treated mice received varying doses of cocaine (0, 0.63, 1.25, 2.5, 5, 10, and 20 mg/kg, IP, n = 6–8). At 20 min, each mouse received 5-HTP (37.5 mg/kg, IP) and the HTR frequency (mean ± SEM) was recorded for the next 30 min as described previously. To determine the effects of the phenyltropane analogue of cocaine, WIN 35428, and the selective monoamine reuptake blockers (sertraline, nisoxetine, and GBR 12935) on the 5-HTP–induced HTR, each drug (0, 0.63, 1.25, 2.5, 5, 10, and 20 mg/kg, IP, n = 5–8) was studied individually under the cited two experimental conditions except these drugs replaced cocaine.

In the present study, the selective norepinephrine reuptake inhibitor, nisoxetine, dose dependently reduced the 5-HTP–induced HTR. Furthermore, cocaine can indirectly attenuate the DOI-induced HTR via the activation of the inhibitory adrenergic α_2 and serotonergic 5-HT_{1A} receptors (15). To fur-

ther investigate such inhibitory mechanisms of cocaine's actions on the 5-HTP–induced HTR, we carried out the following two antagonist studies with their appropriate control groups. The control group received carbidopa (10 mg/kg, IP) at zero min and 5-HTP (37.5 mg/kg, IP) at 20 min. This control group was then divided into three subgroups, which then received either distilled water, 10, or 20 mg/kg cocaine at 30 min. The HTR score was then recorded immediately following the last treatment for the next 30 min as described earlier. In the first antagonist group, a large group of mice was treated at zero time with a single intraperitoneal injection containing a mixture of carbidopa (10 mg/kg) and the α_2 adrenoceptor antagonist, yohimbine (0.05 mg/kg). Ten minutes later, these mice were injected with 5-HTP (37.5 mg/kg, IP). The yohimbine treated group were similarly divided into three subgroups and these subgroups received a further injection of either distilled water, 10, or 20 mg/kg of cocaine (n = 5–6, IP) at 30 min. The HTR score was recorded for the next 30 min immediately following the last injection as described above. To determine the inhibitory 5-HT_{1A} component of cocaine's action, the above antagonist experiment was repeated except yohimbine was replaced by the silent 5-HT_{1A} receptor antagonist S(−)-UH 301 and only one dose of cocaine (10 mg/kg) was tested in combination with S(−)UH 301 (2 mg/kg, IP).

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA), and post hoc analysis was performed by Dunnett's t-test and Scheffe's F-test. The ED_{50} (the effective dose that enhances the HTR frequency by 50%) and ID_{50} (the inhibitory dose that attenuates the maximal HTR frequency by 50%) values were calculated by the use of a computerized program (Graph Pad InPlot, San Diego, CA). For the calculation of ED_{50} values, the 20 mg/kg dose of the drugs was not included because some agents produced bell-shaped dose–response effect.

RESULTS

5-HTP produced a dose- and a time-dependent increase in the HTR frequency in mice (Fig. 1). Figure 2 represents the effects of cocaine on the 5-HTP (37.5 mg/kg)-induced HTR under the conditions of experimental protocol 1 where 5-HTP was administered 10 min prior to cocaine. Cocaine dose dependently increased the total frequency of 5-HTP–induced HTR. However, significant effects, $F(6, 82) = 25$, $p < 0.0001$, were only observed at the 5, 10, and 20 mg/kg doses of cocaine [91, 230, and 300% increase over control (42 HTRs), respectively]. In experimental protocol 2, cocaine was injected 10 min prior to administration of 5-HTP. The stimulant also dose dependently increased the 5-HTP–induced HTR in protocol 2 (Fig. 2). Again, only the 5, 10, and 20 mg/kg doses of cocaine significantly, $F(6, 42) = 8.8$, $p < 0.0001$, enhanced the total HTR frequency relative to vehicle treated control (45 HTRs) (116, 211, and 164% increase, respectively). The corresponding HTR frequencies for the vehicle controls and the varying doses of cocaine in protocols 1 and 2 were not significantly ($p > 0.5$) different from each other. Cocaine's ED_{50} values in experimental protocols 1 and 2 were computed to be 4.68 ± 1.21 and 3.5 ± 1.31 mg/kg, respectively.

Figure 3 represents the effects of the phenyltropane analogue of cocaine, WIN 35428, on the 5-HTP–induced HTR under the conditions of experimental protocol 1. WIN 35428 dose dependently enhanced the 5-HTP–induced HTR frequency. However, only the 10 and 20 mg/kg doses of WIN 35428 exhibited significant effects [455 and 337% increase over vehicle control group (27 HTRs), $F(6, 37) = 6.64$, $p < 0.001$]. The effects of WIN 35428 on the 5-HTP–induced HTR in experimental protocol 2 exhibited a bell-shaped dose–response curve (Fig. 3). At 5 mg/kg dose, WIN 35428 maximally increased, $F(6, 35) = 5.44$, $p < 0.0005$, the 5-HTP–induced HTR score [281% of control (33 HTRs)], whereas its 2.5 and 10 mg/kg doses caused 206 and 172% enhancement over control values ($p < 0.05$). Other tested doses of WIN 35428 (0.63, 1.25, and 20 mg/kg) had no significant effect relative to vehicle-treated control. The corresponding HTRs for controls and for the different doses of WIN 35428 in experimental protocols 1 and 2 were not significantly different from each other. The ED_{50} values for WIN 35428 in experimental protocols 1 and 2 were found to be 2.87 ± 1.3 and 1.79 ± 1.1 mg/kg, respectively.

Figure 4 exhibits the effects of the 5-HT selective uptake inhibitor sertraline on the 5-HTP–induced HTR in experimental protocol 1. Sertraline dose dependently increased the HTR frequency and significant effects were produced by the 2.5, 5, 10, and 20 mg/kg doses of sertraline [300, 445, 693, and 603% of control (29 HTRs), respectively, $F(6, 53) = 12.2$, $p < 0.0001$]. Sertraline also produced a dose-dependent enhancement in the 5-HTP–induced HTR under the experimental protocol 2 (Fig. 4). Significant effects were produced by the 2.5, 5, 10, and 20 mg/kg doses of sertraline [540, 692, 828, and 1560% of control (25 HTRs), respectively, $F(6, 29) = 24.6$, $p < 0.0001$]. When the corresponding controls and the corresponding doses of sertraline in the experimental protocols 1 and 2 were directly compared, only the 20 mg/kg dose of sertraline exhibited a significant difference, $F(13, 82) = 20.9$, $p < 0.0001$, in that it produced a greater HTR frequency in protocol 2 vs. protocol 1. The ED_{50} values for sertraline in experimental protocol 1 and 2 were found to be 2.64 ± 1.1 and 2.1 ± 1.54 mg/kg, respectively.

Figure 5 represents the effects of the selective norepinephrine uptake inhibitor, nisoxetine, on the 5-HTP–induced HTR in experimental protocol 1. Nisoxetine dose dependently attenuated the HTR frequency and significant reductions, $F(6, 55) = 9.3$, $p < 0.0001$, were observed at the 5, 10, and 20 mg/kg doses [72, 75, and 86% decrease relative to vehicle control (29 ± 3 HTRs), respectively]. Under the experimental protocol 2, nisoxetine attenuated the 5-HTP–induced HTR score in a similar manner (Fig. 5). Thus, the 5, 10, and 20 mg/kg doses of nisoxetine produced significant reductions [76, 86, and 98% reduction relative to vehicle control (43 ± 4 HTRs), $F(6, 28) = 5.2$, $p < 0.001$]. When the corresponding controls and the corresponding doses of nisoxetine in the two experimental protocols were directly compared, none of the treatment doses were significantly different from each other. The ID_{50} dose for nisoxetine in the experimental protocols 1 and 2 were 3.33 ± 1.32 and 1.72 ± 1.34 mg/kg, respectively.

The effects of the selective dopamine reuptake inhibitor, GBR 12935, on the 5-HTP–induced HTR under the experimental protocol 1 is presented in Fig. 6. GBR 12935 at smaller doses (0.63–2.5 mg/kg) tended to enhance the 5-HTP–induced HTR, but significance was not attained. Larger doses of GBR 12935 tended to attenuate the 5-HTP–induced HTR and a significant, $F(6, 53) = 5.8$, $p < 0.0001$, reduction was observed only at the 20 mg/kg dose (62% reduction relative to vehicle control). In the experimental protocol 2, GBR 12935 dose dependently attenuated the 5-HTP–induced HTR and significant reductions were observed at the 10 and 20 mg/Kg doses [60 and 61% reduction, $F(6, 28) = 5.4$, $p < 0.0008$] relative to vehicle control (43 ± 4 HTRs) (Fig. 6). When the first

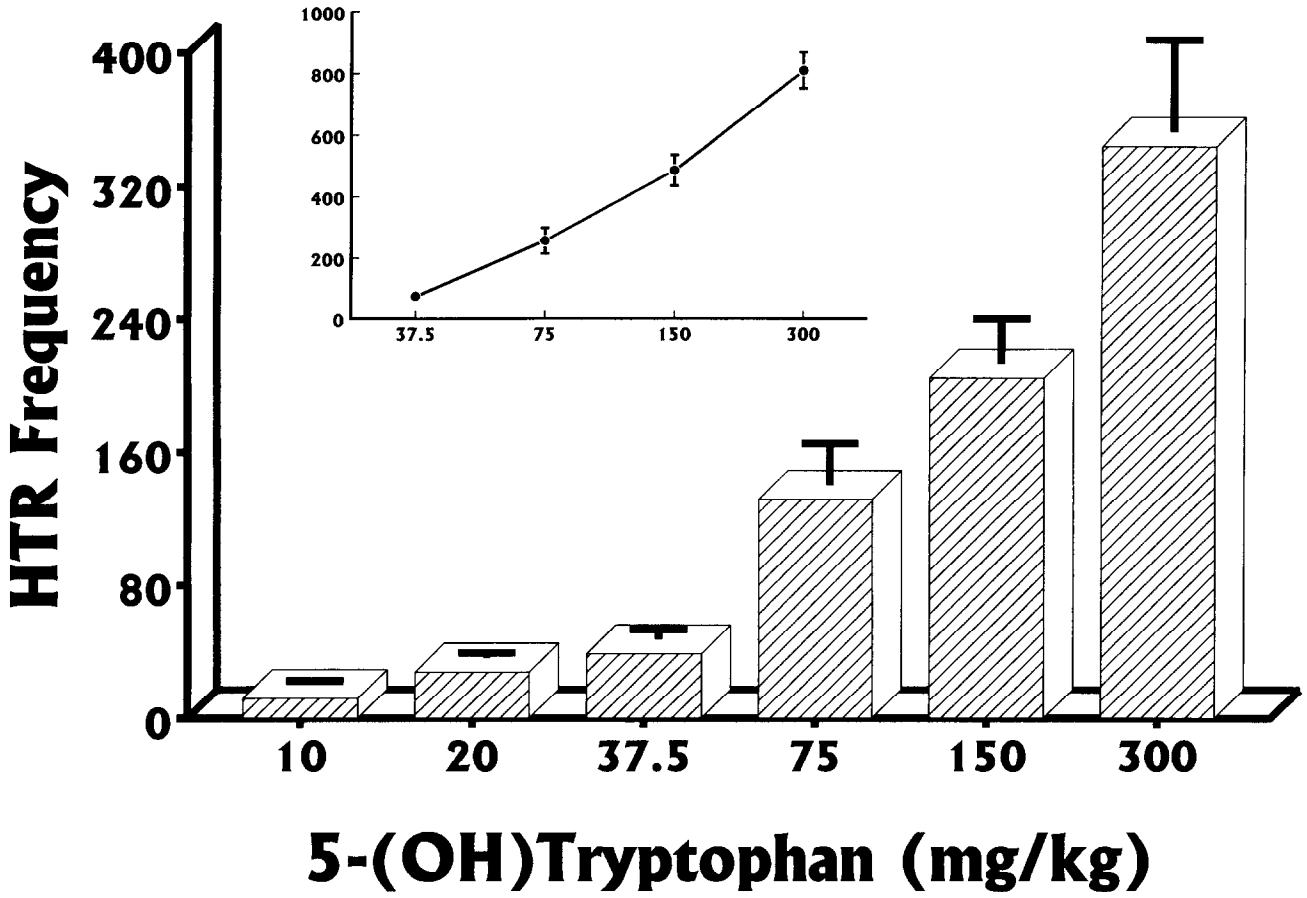

FIG. 1. The dose–response effect (mean ± SEM, n = 5–10) of 5-hydroxy-L-tryptophan [5-(OH) tryptophan = 5-HTP] on the production of head-twitch response (HTR) in mice in the presence of carbidopa (10 mg/kg). The behavior was observed for 30 min immediately following 5-HTP injection. The inset represents the same experiment except the HTR frequency was observed for 60 min following 5-HTP injection.

and second experimental protocols were compared directly, neither the controls nor the respective GBR 12935 doses were significantly different from each other. The ID_{50} doses for GBR 12935 in protocols 1 and 2 were found to be 15.34 ± 1.52 and 11.92 ± 1.3 mg/kg, respectively.

To further identify inhibitory mechanisms of cocaine on the 5-HTP–induced HTR, we utilized the α_2 antagonist, yohimbine, and the silent 5-HT_{1A} antagonist S(−)-UH 301. Yohimbine (0.05 mg/kg) by itself had no significant effect (Fig. 7). Cocaine-only treated groups (10 and 20 mg/kg) significantly enhanced, $F(5, 25) = 12.1$, $p < 0.0001$, the 5-HTP–induced HTR (142 and 155% increase relative to its vehicle control (36 ± 6 HTRs)). Combined yohimbine (0.05 mg/kg)- and cocaine (10 and 20 mg/kg)-treated mice exhibited significantly greater increases (192 and 198%, respectively) relative to the yohimbine-only–treated control group (49 ± 6 HTRs). In addition, combined yohimbine plus cocaine-treated animals also produced significantly greater HTR frequencies than cocaine-only–treated control groups. S(−)-UH 301 (2 mg/kg) by itself had no significant effect on the 5-HTP–induced HTR relative to vehicle-treated control group (Fig. 8). Combined S(−)-UH 301- and cocaine (10 mg/kg)-treated group produced significantly greater HTR frequencies than S(−)-UH 301-only or cocaine-only–treated control groups [313 and 128% increase, respectively, $F(3, 17) = 12.1$, $p < 0.0002$ (Fig. 8)].

DISCUSSION

Similar to the previous findings (26,28), the intraperitoneal administration of the immediate 5-HT precursor, 5-HTP, in the presence of carbidopa (a peripheral decarboxylase inhibitor) caused a dose- and a time-dependent increase in the frequency of HTR in mice. One important finding of the present study is that systemic injection of cocaine following 5-HTP administration (i.e., experimental protocol 1) enhanced the frequency of 5-HTP–induced HTR in a dose-dependent manner. Thus, the present behavioral study supports the previously published biochemical findings in that cocaine increases the synaptic concentration of 5-HT (4,7,17). At first sight, the present results appears to be in direct contradiction of our previously published findings, as prior cocaine treatment was reported to attenuate the HTR produced by DOI (11–13,15). Thus, the effects of cocaine prior to administration of 5-HTP was investigated in the experimental protocol 2 for two reasons: 1) to simulate the experimental conditions under which cocaine pretreatment attenuated the DOI-induced HTR, and 2) to see whether acute cocaine administration affects 5-HTP conversion to serotonin because the stimulant can inhibit the uptake of the 5-HTP precursor, tryptophan, and the activity of the 5-HT rate-limiting enzyme tryptophan hydroxylase (32). Indeed, it is known that acute cocaine admin-

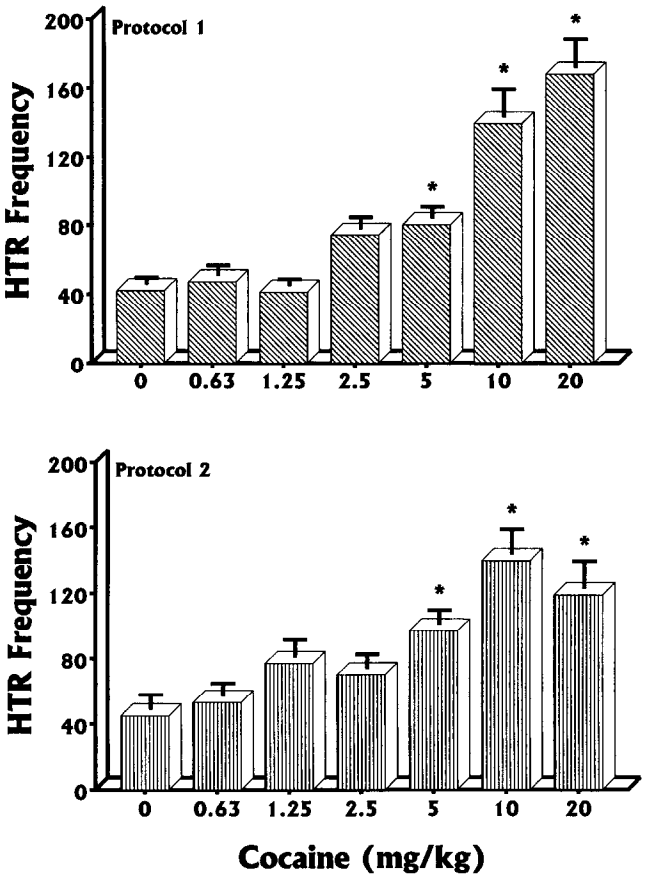

FIG. 2. Dose-dependent stimulatory effects of the cited doses of cocaine on the 5-HTP–induced head-twitch response (HTR) (mean ± SEM) in mice. Protocol 1—animals were injected at 0 min with carbidopa (10 mg/kg), at 20 min with 5-HTP (37.5 mg/kg), at 30 min with cocaine (n = 11–15 per group), or vehicle (n = 13) and were then observed for the next 30 min. Protocol 2—animals were injected at 0 min with carbidopa (10 mg/kg), at 10 min with cocaine (n = 6–8 per group), or vehicle (n = 7), at 20 min with 5-HTP (37.5 mg/kg), and were then observed for the next 30 min. *Significantly different between vehicle control and the indicated doses of cocaine, $F(6, 82) = 25$, $p < 0.0001$, and $F(6, 42) = 8.8$, $p < 0.0001$ for protocols 1 and 2, respectively.

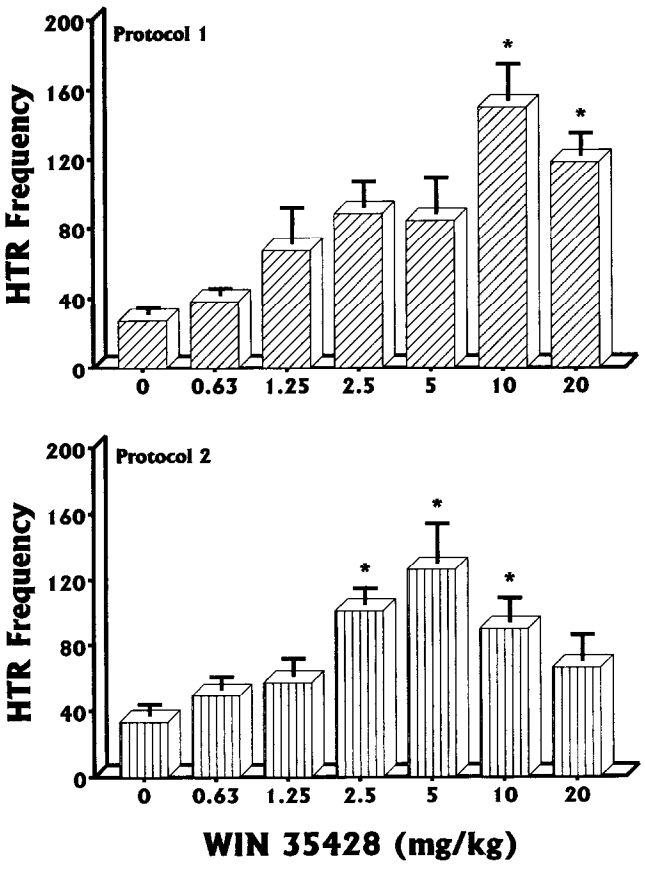

FIG. 3. Dose-dependent bell-shaped dose–response effect of the cited doses of WIN 35428 on the 5-HTP–induced head-twitch response (HTR) (mean ± SEM) in mice. Drug injection schedule for experimental protocols 1 and 2 were the same as Fig. 2 except WIN 35428 replaced cocaine. *Significant difference between vehicle control and the indicated doses of WIN 35428, $F(6, 37) = 6.64$, $p < 0.001$, and $F(6, 35) = 5.44$, $p < 0.0005$ for protocols 1 and 2, respectively.

istration reduces serotonin turnover (18,20). In the experimental protocol 2, cocaine essentially produced a similar enhancement as in the protocol 1. If cocaine would have either inhibited 5-HTP uptake or its conversion to 5-HT, then one would have expected a dose-dependent inhibition of 5-HTP–induced HTR rather than the observed enhancement in the induced behavior. In support of this notion, in a procedure similar to the present protocol 2 drug administration schedule, it was shown that cocaine potently attenuates the HTR produced by p-hydroxyamphetamine via the inhibition of its uptake into serotonergic neurons (56).

To clarify the effects of different components of cocaine's actions we also evaluated the effects of selective monoamine reuptake inhibitors on the production of 5-HTP–induced HTR. The selective serotonin reuptake inhibitor, sertraline, dose dependently potentiated the 5-HTP–induced HTR in both experimental protocols 1 and 2. Relative to protocol 1, sertraline in protocol 2 produced a greater enhancement (1.2 to 2.6 times) in HTR frequency and significant enhancing effect was observed at the highest dose tested (20 mg/kg). However, its ED_{50} values in the two experimental protocols were similar. Thus, these results suggest that sertraline may not affect either the uptake of 5-HTP or its conversion to 5-HT, and the greater HTR frequencies observed in protocol 2 vs. 1 is probably due to animals being exposed to sertraline for a longer duration. The present data supports the previously published findings in that oral administration of sertraline 1 h prior to 5-HTP (100 mg/kg, IP) enhanced the HTR frequency with an ED_{50} of 2.6 mg/kg, which mirrors the present results (33). The latter authors have also shown that sertraline is several times more potent the than other selective and nonselective serotonin reuptake blockers in potentiating the 5-HTP–induced HTR. In this respect, sertraline also appears to be more potent than cocaine and produced relatively greater HTR frequencies (3 to 10 times). In an in vivo microdialysis study Gartside et al. (21) have shown that the 5-HTP–induced increase in extracellular 5-HT in the rat hypothalamus occurs largely via exocytosis because the 5-HT release was mainly calcium dependent and was attenuated by the 5-HT_{1A} receptor selective agonist, 8-OH DPAT. Because 5-HT_{1A} receptor serves as a somatodendritic autoreceptor on the cell

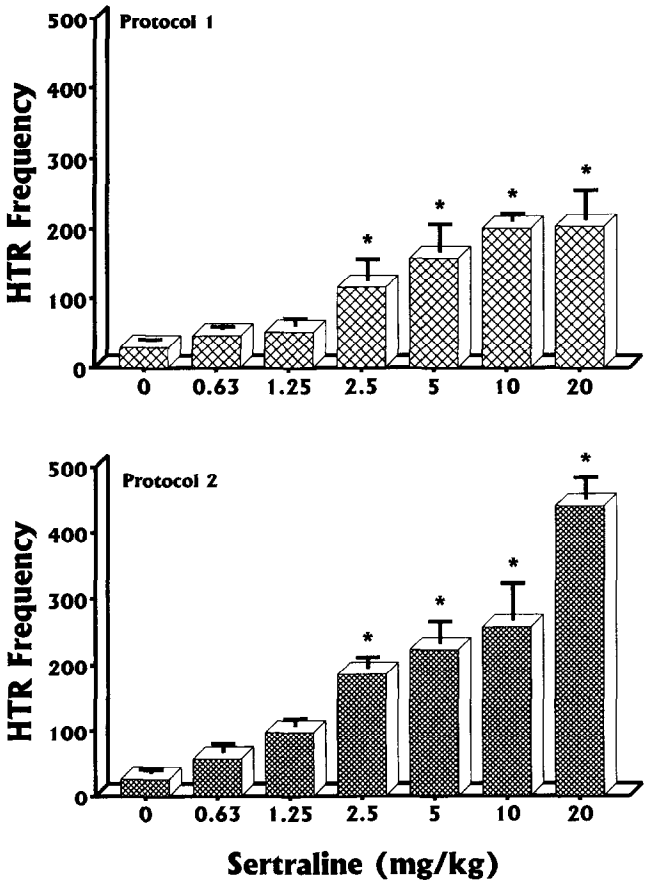

FIG. 4. Dose-dependent stimulatory effects of the cited doses of sertraline on the 5-HTP–induced head-twitch response (HTR) (mean ± SEM) in mice. Drug injection schedule for experimental protocols 1 and 2 were the same as for Fig. 2 except sertraline replaced cocaine. *Significant difference between vehicle control and the indicated doses of sertraline, $F(6, 35) = 12.2$, $p < 0.0001$, and $F(6, 29) = 24.6$, $p < 0.0001$ for protocols 1 and 2, respectively).

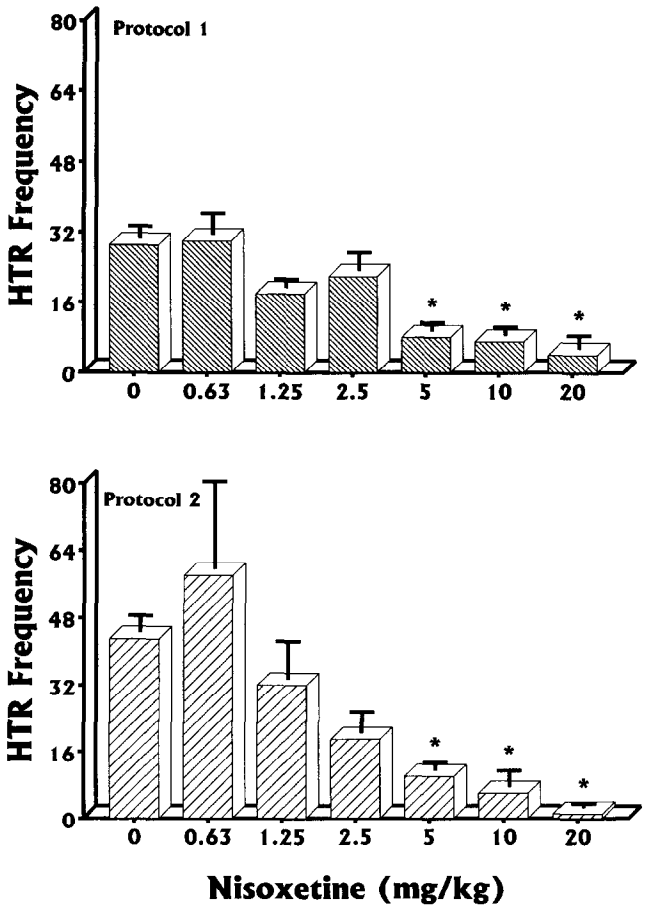

FIG. 5. Dose-dependent inhibitory effects of the cited doses of nisoxetine on the 5-HTP–induced head-twitch response (HTR) (mean ± SEM) in mice. Drug injection schedule for experimental protocols 1 and 2 were the same as for Fig. 2 except nisoxetine replaced cocaine. *Significant difference between vehicle control and the indicated doses of sertraline, $F(6, 55) = 9.3$, $p < 0.0001$, and $F(6, 28) = 5.2$, $p < 0.001$, for protocols 1 and 2, respectively.

bodies of 5-HT neurons and its activation decreases neuronal firing [review: (19)], the above findings suggest that 5-HT neurons are the most probable source of the increased extracellular 5-HT following peripheral injection of 5-HTP. Because 8-OH DPAT can also inhibit the HTR produced by either direct (14) or indirect (16,23) 5-HT_{2A} agonists, it was interesting to see whether cocaine can induce greater HTR score in the presence of a selective and silent 5-HT_{1A} antagonist such as S(−)-UH 301 (3). Combined S(−)-UH 301 and cocaine pretreatment caused a greater enhancement in 5-HTP–induced HTR relative to exposure of either drug alone. Moreover, S(−)-UH 301 is reported to increase the 5-HTP–induced HTR (3), and in the present study the antagonist tended to enhance the HTR frequency but did not attain significance. These results can be interpreted as serotonin neurons may induce a ceiling effect in the extent to which cocaine-induced 5-HT reuptake inhibition increases the synaptic concentration of 5-HT by activating the impulse modulating somatodendritic 5-HT_{1A} receptors. This would, therefore, slow their firing rate resulting in less serotonin being made and released. Indeed, currently in vivo biochemical evidence exists for such a ceiling effect for several 5-HT reuptake blockers (e.g., sertraline, citalopram) and presence of 5-HT_{1A} antagonists such as S(−)-UH 301, penbutolol, or methiothepin abolish the ceiling effect and enhance the ability of 5-HT reuptake blockers to further increase the synaptic concentration of 5-HT (28,29,31). Moreover, S(−)-UH 301 can enhance the ability of sertraline or cocaine to increase the frequency of 5-HTP–induced HTR in a time-dependent manner via the above mechanism [(16) and present study].

Relative to sertraline, the inability of cocaine to induce greater HTR scores probably resides in the fact that it also inhibits the reuptake of NE and DA, which may affect the overall frequency of the 5-HTP–induced behavior. To test this hypothesis, animals were treated with the selective NE inhibitor, nisoxetine, instead of cocaine. Both in protocols 1 and 2, nisoxetine dose dependently attenuated the HTR frequency, being more effective in protocol 2, indicating the longer exposure of animals to the drug. Because cocaine's indirect stimulation of the inhibitory postsynaptic adrenergic α_2 receptors is partly responsible for the attenuation of the DOI-induced HTR, which can be reversed by pretreatment with the α_2 adrenoceptor antagonist, yohimbine (15), this in-

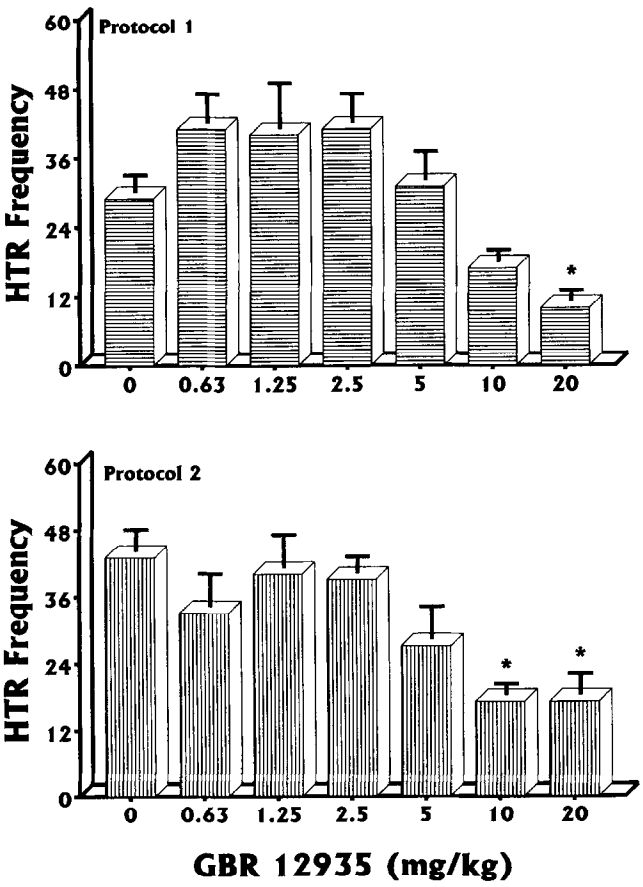

FIG. 6. Effect of the cited doses of GBR 12935 on the 5-HTP–induced head-twitch response (HTR) (mean ± SEM) in mice. Drug injection schedule for experimental protocols 1 and 2 were the same as for Fig. 2 except GBR 12935 replaced cocaine. *Significant difference between vehicle control and the indicated doses of GBR 12935, $F(6, 53) = 5.8$, $p < 0.0001$, and $F(6, 28) = 5.4$, $p < 0.0008$, for protocols 1 and 2, respectively.

hibitory mechanism was evaluated on the 5-HTP–induced HTR. Yohimbine by itself had no effect on the 5-HTP–induced HTR. In the presence of yohimbine cocaine produced significantly greater HTR scores relative to cocaine-only–treated control groups, thus suggesting that the noradrenergic α_2 receptor inhibitory component of cocaine's actions is also operative on the 5-HTP–mediated HTR. Indeed, a recent in vivo microdialysis study has shown that 5-HT release from the serotonergic nerve terminals in the rat hippocampus is attenuated by α_2 adrenoceptors (57). Thus, these results, in part, may explain why dose for dose cocaine produces fewer HTRs than sertraline.

Although it is generally accepted that dopaminergic mechanisms may not modulate the HTR [for review, see (26)], more recently Schreiber et al. (54) have suggested a permissive role for the dopamine D_1 receptors in modulating head twitches because dopamine agonists did not produce the behavior, whereas dopamine D_1 antagonists attenuated the DOI-induced HTR. In the present study, the selective dopamine reuptake blocker, GBR 12935, attenuated the 5-HTP–induced HTR only at the highest doses tested (10 and 20 mg/kg) in both

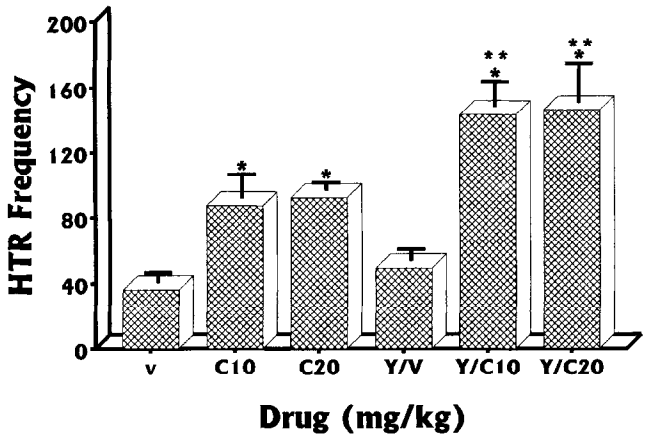

FIG. 7. The effect of cocaine (C) (10 and 20 mg/kg, $n = 6$–8 per group) and a combination (Y/C) of cocaine (C) (10 and 20 mg/kg, $n = 6$–8) and yohimbine (Y) (0.05 mg/kg) and their respective controls (v = vehicle) (V and Y/V) on the 5-HTP-induced head-twitch response (HTR). *Significantly different from corresponding vehicle controls. **Significantly different from cocaine-only treated control, $F(5, 25) = 12.1$, $p < 0.0001$.

experimental protocols 1 and 2. This inhibitory effect is probably due to loss of specificity of GBR 12935 because at high doses it can also increase the endogenous levels of NE (7).

The above findings are supported by a recent single-dose study (17) that has shown that 5-HTP alone (100 mg/kg) or in rats pretreated with either 32 mg/kg GBR 12909 (another selective DA reuptake inhibitor) or 10 mg/kg desipramine (a NE reuptake inhibitor) failed to produce head twitches. However, 5-HTP administered to cocaine (17.8 mg/kg)- or fluoxetine (5.6 mg/kg)-pretreated animals caused robust HTR frequencies. The selective 5-HT reuptake inhibitor, fluoxetine, was also more potent than cocaine. The inability of rats to exhibit head twitches in 5-HTP-only–treated group is probably

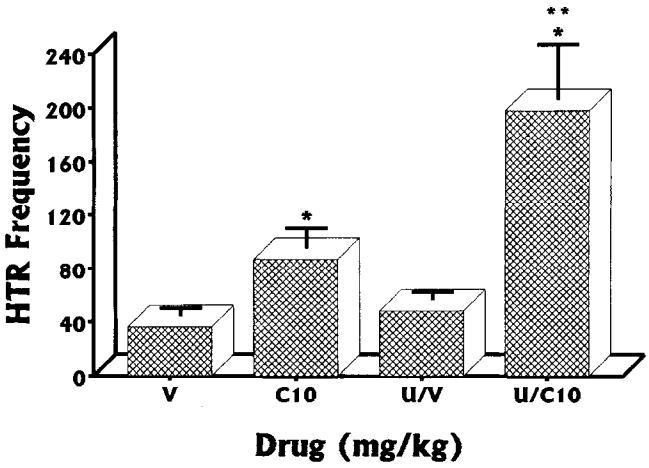

FIG. 8. The effect of cocaine (C) (10 mg/kg, $n = 6$–8) and a combination (U/C) of cocaine (C) (10 mg/kg) and S(−)-UH 301 (U) (2 mg/kg) and their respective controls (V = vehicle) (V and U/V) on the 5-HTP–induced head-twitch response (HTR). *Significantly different from corresponding vehicle controls. **Significantly different from cocaine-only treated control, $F(3, 17) = 12.1$, $p < 0.0002$.

due to the absence of carbidopa because the latter group (38) and others (1) have previously reported robust HTR frequencies with such a dose of 5-HTP in the presence of carbidopa.

Relative to cocaine, its phenyltropane analogue, WIN 35428, is a more potent inhibitor of monoamine reuptake (47) and a powerful inhibitor of raphe impulse activity via indirect stimulation of the somatodendritic 5-HT_{1A} autoreceptors (10). In the present study, WIN 35428 was also more potent than cocaine in enhancing the 5-HTP–induced HTR. Its maximal effects occurred at lower doses relative to cocaine and exhibited a bell-shaped dose–response curve. This effect was more pronounced in experimental protocol 2. On the descending limb of the latter dose–response curve, WIN 35428, at a 20 mg/kg dose, had no significant effect relative to vehicle control group. The bell-shaped dose–response curve can be explained in terms of its more potent inhibitory effects on monoamine reuptake. Thus, at high doses its combined inhibitory indirect stimulation of 5-HT_{1A}, noradrenergic α_2, and possibly dopamine receptors overrides its indirect stimulatory action on 5-HT_{2A} receptors to mediate the head-twitch behavior. Moreover, even cocaine tended to exhibit bell-shaped dose–response effect in protocol 2, which can also be explained by the above notion because animals had longer exposure to cocaine in this protocol. Although the maximal HTR score produced by WIN 35428 is similar to cocaine, its ED_{50} values in both experimental protocols 1 and 2 are in line with the ED_{50} values of sertraline than with cocaine. Moreover, these ED_{50} values are in general agreement with previoiusly published affinity studies in that relative to cocaine both WIN 35428 and sertraline possess higher affinity (10 to 30 times, respectively) for the serotonergic uptake sites (33,47,48,51). This, again, complements the greater potency of WIN 35428 relative to cocaine. WIN 35428 has a weak local anesthetic effect compared to cocaine (8), the impressive potency of WIN 35428 to suppress the electrical activity of dorsal raphe (10) supports the notion that local anesthetic actions do not account for the observed effects of cocaine on 5-HT neuronal activity and its behavioral effects.

The specificity of selective uptake inhibitors to discriminate between different monoamine uptake sites can be lost at high doses. The respective ED_{50} and ID_{50} values for sertraline and nisoxetine in the present study are in the concentration range that are selective for their corresponding effects (33,58). Although GBR componds are 25 to 2000 times more selective for dopamine uptake sites (60), GBR 12935 can lose specificity at high doses (7). Because GBR 12935 affected the head-twitch behavior only a high doses, the present results indicate no specific role for endogenous dopamine in the modulion of 5-HTP–induced HTR. Although at low doses cocaine may affect all three monoamine uptake sites (49), its tropane analog WIN 35428 appears to be 38 to 58 times more potent on serotonergic relative to catecholaminergic uptake sites (51). The ED_{50} value of WIN 35428 in the present study is in general agreement with these findings.

Although as yet we cannot provide an exact explanation as to why cocaine attenuates the DOI- but potentiates the 5-HTP–induced HTR, we can offer some suggestions: 1) DOI-type drugs are partial agonists of 5-HT_{2A} receptors (53) and are probably more responsive to the inhibitory components of cocaine's actions; and 2) serotonin is a full but nonselective 5-HT_{2A} receptor agonist and, therefore, would tend to attenuate the HTR frequency to some degree via simultaneous activation of the inhibitory 5-HT_{1A} receptors (14). Thus, any further enhancement in central 5-HT levels by cocaine may not lead to further increase in stimulation of the inhibitory receptors. The inhibitory noradrenergic component of cocaine may not be functional at low doses of the stimulant because it has lower affinity for the noradrenergic relative to serotonergic uptake sites. In addition, in protocol 2 of the present study, cocaine at the highest doses tested (10–20 mg/kg) caused a bell-shaped dose–response curve, whereas its tropane analog, WIN 35428, produced the later effect in both protocols.

In summary, based on the published biochemical findings, our results suggest that cocaine simultaneously inhibits the reuptake of 5-HT, NE, and DA. The increase in synaptic concentration of 5-HT enhances the frequency of the 5-HTP–induced head-twitch behavior. Concomitantly, cocaine seems to attenuates the frequency of the induced behavior via indirect stimulation of the inhibitory serotonergic 5-HT_{1A} and noradrenergic α_2 receptors.

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