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Cocaine and Selective Monoamine Uptake Blockers (Sertraline, Nisoxetine, and GBR 12935) Prevent the *d*-Fenfluramine-Induced Head-Twitch Response in Mice

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DARMANI, N. A. *Cocaine and selective monoamine uptake blockers (sertraline, nisoxetine, and GBR 12935) prevent the d-fenfluramine–induced head-twitch response in mice.* PHARMACOL BIOCHEM BEHAV **60**(1) 83–90, 1998.—Serotonin release subsequent to 5-HT precursor loading mainly occurs via exocytosis. Acute cocaine or sertraline administration promote the ability of 5-HT precursors (e.g. L-tryptophan) to induce the 5-HT_{2A} receptor-mediated head-twitch response (HTR) in rodents. The 5-HT releaser, *d*-fenfluramine, at behaviorally active doses, can induce the head-twitch response in rodents by releasing cytoplasmic 5-HT via the serotonin uptake carrier working in reverse. The purpose of the present study was to utilize the *d*-fenfluramine–induced HTR to determine the serotonergic and nonserotonergic components of cocaine's actions on the *d*-fenfluramine–sensitive pool of cytoplasmic 5-HT. Because a dramatic differential potentiation in HTR frequency is obtained when cocaine is administered prior relative to after L-tryptophan injection, the effects of varying doses of cocaine and the selective serotonin (sertraline), dopamine (DA) (GBR 12935), and norepinephrine (NE) (nisoxetine) uptake blockers were investigated on the *d*-fenfluramine–induced behavior in two experimental protocols. Thus, each uptake inhibitor was administered either 10 min following (protocol 1) or 10 min prior to (protocol 2) *d*-fenfluramine injection. All the tested uptake inhibitors attenuated the *d*-fenfluramine–induced HTR in a dose-dependent manner in both experimental protocols. However, their order of potency in either protocol 1 (nisoxetine > GBR 12935 > cocaine > sertraline) or protocol 2 (cocaine > GBR 12935 > nisoxetine = sertraline) does not agree with in vitro affinity of these drugs for the 5-HT transporter. In addition, the potency order for cocaine and nisoxetine in protocol 1 was significantly reversed in protocol 2. The inhibitory effects of the cited drugs on the *d*-fenfluramine–induced HTR are discussed in terms of: 1) high doses of selective monoamine uptake blockers may not exhibit as much selectivity for their target uptake sites as indicated by in vitro tests; and 2) possible pharmacokinetic interactions between *d*-fenfluramine and the monoamine uptake blockers. © 1998 Elsevier Science Inc.

d-Fenfluramine Cocaine Sertraline Nisoxetine GBR 12935 Head-twitch response 5-HT_{2A} receptor

COCAINE is a nonselective monoamine uptake blocker and it therefore increases the concentrations of both catecholamines [(norepinephrine (NE) and dopamine (DA)] and the indolamine, serotonin (5-hydroxytryptamine = 5-HT), in their corresponding neuronal terminal fields (5,21,34,50). It is well accepted that the reinforcing effects of cocaine are due to its ability to inhibit the uptake of DA [review, (33)]. More recent studies suggest that blockade of 5-HT transporter may alter cocaine reinforcement (38,51). Indeed, increases in serotonergic activity either via the administration of the initial 5-HT precursor L-tryptophan (6,42) or the selective 5-HT uptake inhibitor fluoxetine (7,49) or the direct 5-HT agonist quipazine (41) can attenuate the indices of cocaine self-administration. In addition, 5-HT_{2A} receptor antagonists enhance the rate increasing stimulant effects of low to intermediate doses of cocaine and attenuate the rate decreasing effects of larger doses of the stimulant (29). Cocaine also appears to inhibit the uptake of L-tryptophan and the activity of the 5-HT synthesis rate-limiting enzyme L-tryptophan hydroxylase (35). Consequently, cocaine reduces serotonin turnover (2,20,21).

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Stimulation of 5-HT_{2A} receptors have a facilitatory effect on DA efflux in the striatum (31,44,54) and nucleus accumbens (45) but attenuates DA release in the medial prefrontal cortex (55). Up to seven different 5-HT receptor sites (5-HT_{1-7}) have been identified and many of these receptors possess several subtypes (30). The 5-HT_2 receptors consist of three different subtypes (5-HT_{2A-C}). In this laboratory the effects of cocaine have been studied on the head-twitch response (HTR) in mice. Drug-induced HTR has not only been shown to have considerable potential as a quantitative probe on in vivo 5-HT_{2A} receptor function but also as a means of unravelling neurotransmitter interactions (27,28,56). Acute cocaine pretreatment can dose dependently reduce the HTR produced by the selective and nonselective direct 5-HT_{2A} receptor agonists such as DOI or 5-MeO DMT (11–13,15). However, the stimulant lacks affinity for 5-HT_{2A} receptors and its ability to attenuate the DOI-induced HTR is thought to be due to cocaine's indirect stimulation of the inhibitory adrenergic α_2- and serotonergic 5-HT_{1A} receptors. On the other hand, acute cocaine exposure significantly potentiates the HTR produced by the 5-HT precursors such as 5-hydroxytryptophan (5-HTP) (16,17) or L-tryptophan (10). It appears that the discussed inhibitory components of cocaine's actions are simultaneously operative on the 5-HT precursor-induced HTR, but still the stimulant potentiates the induced head twitches. Indeed, relative to respective control groups (i.e., in the absence of appropriate antagonists), cocaine in the presence of either α_2- or 5-HT_{1A} antagonists potentiates the 5-HTP–induced HTR to a greater degree. Moreover, the selective NE uptake inhibitor, nisoxetine, was shown to potently prevent the HTR produced by L-tryptophan (10) or 5-HTP (16).

The HTR in rodents can be also induced by 5-HT releasers [(9,39,57,63); present study]. In vivo microdialysis studies suggest that both physiological and precursor-induced release of 5-HT mainly occurs via exocytosis probably from a small vesicular pool of recently synthesized 5-HT (1,22,26). On the other hand, 5-HT releasers such as *d*-fenfluramine or parachloroamphetamine (PCA), at behaviorally active doses, mainly cause release of cytoplasmic 5-HT into the synapse through the 5-HT uptake carrier working in reverse (3,25,47,62). Indeed, several in vivo microdialysis studies have shown that *d*-fenfluramine can increase the extracellular 5-HT concentration in different loci of the rat brain (37,47,53). The latter studies also show that pretreatment with selective serotonin uptake blockers (citalopram or fluoxetine) prevent the *d*-fenfluramine–induced 5-HT release. Moreover, such uptake inhibitors prevent the hyperthermia (46) but not always the serotonin syndrome (39) induced by the cited 5-HT releasers. Thus, the initial aim of the present study was to investigate whether the selective (sertraline)- and nonselective (cocaine)–5-HT uptake blockers can prevent the *d*-fenfluramine–induced HTR in mice. In addition, because relative to serotonin precursors, *d*-fenfluramine releases 5-HT from a different presynaptic pool, it was of interest to determine whether the different monoamine components of cocaine's actions on *d*-fenfluramine–induced HTR resembles the discussed ability of cocaine to attenuate the HTR produced by direct 5-HT_{2A} agonists or its ability to potentiate the frequency of 5-HT precursor-induced HTR. Thus, the effects of cocaine and selective 5-HT, NE, and DA uptake blockers [sertraline (43), nisoxetine (60), GBR 12935 (48)] were studied. Because cocaine was shown to dramatically enhance the HTR frequency to different degrees when it was administered prior to vs. after L-tryptophan injection (10), the effects of the cited uptake blockers were individually studied in two experimental protocols by administrating each drug either 10 min prior (protocol 2) or after (protocol 1) *d*-fenfluramine injection.

METHOD

Animals and Materials

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. *d*-Fenfluramine HCl, nisoxetine HCl, and GBR 12935 2HCl were purchased from Research Biochemicals, Inc. (Natick, MA). Cocaine HCl was obtained from the National Institute on Drug Abuse. Sertraline HCl was generously donated by Pfizer (Groton, CT). All drugs were dissolved in distilled water and were administered intraperitoneally at a volume of 10 ml/kg of body weight.

Experimental Protocols

On the test day animals were transferred to the experiment room and were allowed to acclimate for at least 1 h prior to experimentation. In the initial experiments, the dose–response effects of *d*-fenfluramine for the production of HTR in mice were determined. Thus, mice were individually placed in the observation cages (40 × 25 × 26 cm) lined with wood chippings 30 min prior to an intraperitoneal (IP) administration of *d*-fenfluramine (1.25, 2.5, 5, 10, and 20 mg/kg, n = 5–8 per group). Each mouse was individually observed immediately following injection and the HTR score (mean ± SEM) was recorded cumulatively at 5-min intervals for the next 30 min. A dose of 2.5 mg/kg *d*-fenfluramine produced a robust frequency of HTR and was, therefore, used as the HTR inducer for the investigation of interactions of cocaine and other selective monoamine uptake blockers with *d*-fenfluramine on the induced HTR.

The effects of cocaine on the *d*-fenfluramine–induced HTR were investigated under two experimental conditions. In protocol 1, at zero time a large group of mice received *d*-fenfluramine (2.5 mg/kg, IP) and at 10 min varying doses of cocaine (0, 5, 10, and 20 mg/kg, IP, n = 5–10 per group). The

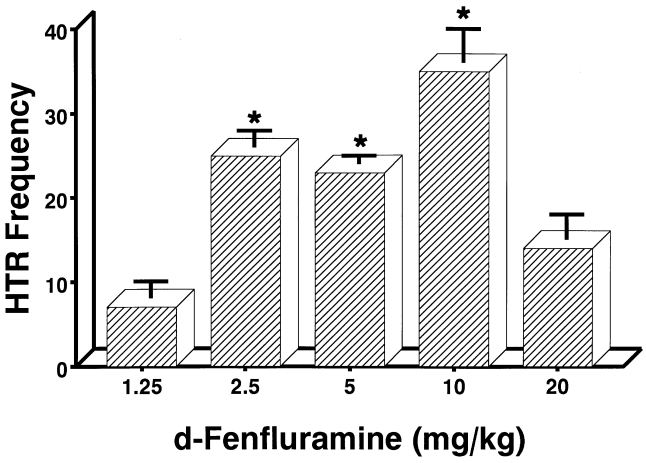

FIG. 1. The dose–response effect (mean ± SEM, n = 5–8) of *d*-fenfluramine on the production of HTR. The behavior was observed for 30 min immediately following injection. *Significantly different from vehicle control, $F(5, 32) = 22.6$, $p < 0.0001$.

HTR frequency was scored cumulatively for the next 30 min immediately following cocaine or vehicle injection at 5-min intervals as described previously. In experimental protocol 2, the mice were injected at zero time with varying doses of cocaine (0, 0.125, 0.625, 1.25, 2.5, 5, and 10 mg/kg, IP, n = 6–9 per group). At 10 min, each mouse received *d*-fenfluramine (2.5 mg/kg, IP) and the HTR frequency (mean ± SEM) was recorded for the next 30 min as described above. To determine the effects of sertraline, nisoxetine, and GBR 12935 on the *d*-fenfluramine–induced HTR, each drug (0 to 20 mg/kg, n = 5–10 per group) was studied individually under the cited two experimental protocols except that these drugs replaced cocaine.

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} (inhibitory dose that attenuates the maximal HTR frequency by 50%) values were calculated by the use of a computerized

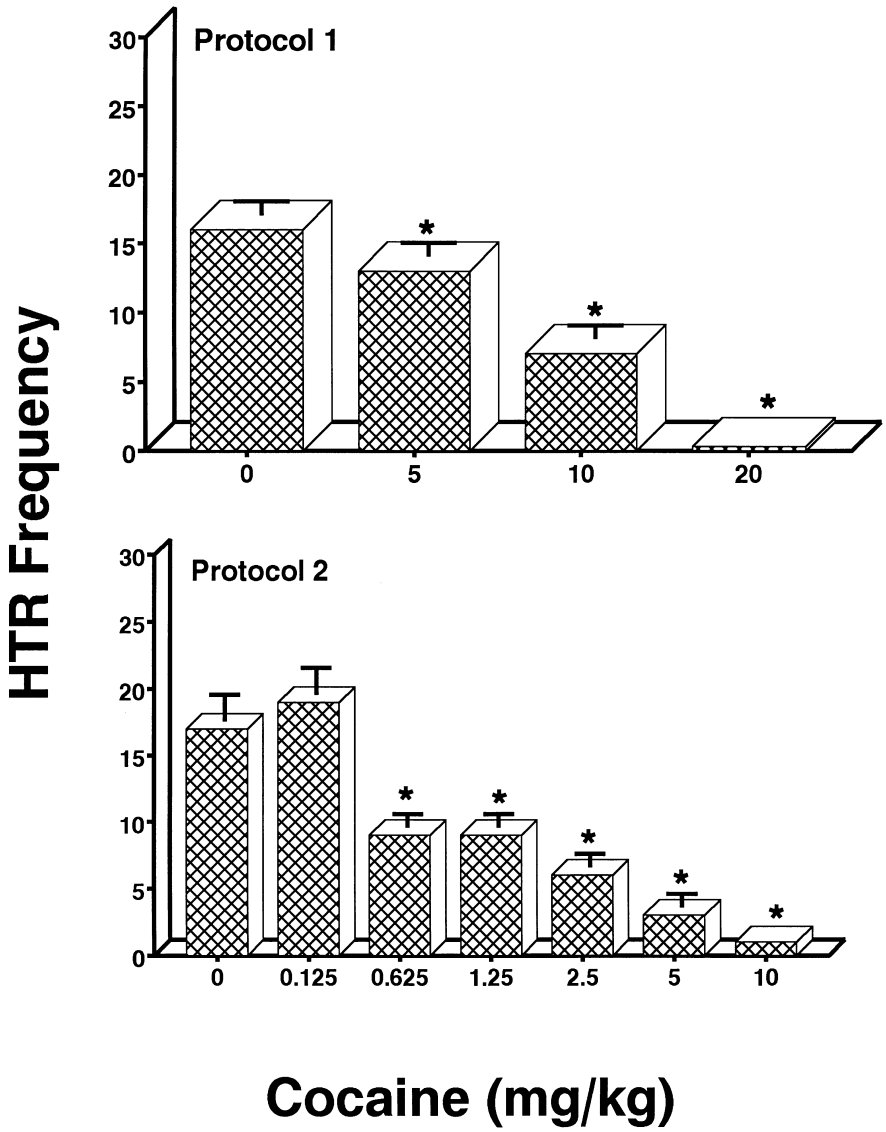

FIG. 2. Dose-dependent inhibitory effects of cocaine on the frequency of *d*-fenfluramine (2.5 mg/kg, IP)-induced HTR (mean ± SEM) in mice. Protocol 1—animals were injected at 0 min with *d*-fenfluramine, at 10 min with either vehicle (n = 10) or the cited doses of cocaine (n = 5–6) per group and were then observed for the next 30 min. Protocol 2—animals were injected at 0 min with either vehicle (n = 9) or different doses of cocaine (n = 5–6 per group), at 10 min with *d*-fenfluramine and were then observed for the next 30 min. *Significant difference between vehicle control and the indicated doses of cocaine, $F(3, 21) = 71.53$, $p < 0.0001$, and $F(6, 37) = 20.98$, $p < 0.0001$, for protocols 1 and 2, respectively.

program (Graph Pad Inplot, San Diego, CA). Differences among mean ID_{50} values were also resolved by one-way analysis of variance followed by post hoc analysis.

RESULTS

d-Fenfluramine produced a bell-shaped dose-dependent increase, $F(5, 32) = 22.6$, $p < 0.0001$, in the HTR frequency in the 30-min observation period (ED_{50} = 4.76 ± 2.47 mg/kg) (Fig. 1). Although a robust HTR score (25 ± 2) was obtained at 2.5 mg/kg dose, a maximal effect was seen at 10 mg/kg (35 ± 4 HTRs). At 5 mg/kg, *d*-fenfluramine produced a mean HTR score similar to its 2.5 mg/kg dose. However, the smallest and largest doses tested (1.25 and 20 mg/kg, respectively) failed to significantly enhance the HTR score relative to the vehicle control group. The top panel in Fig. 2 represents the effect of cocaine on the *d*-fenfluramine (2.5 mg/kg)-induced HTR under the conditions of experimental protocol 1, where *d*-fenfluramine was administered 10 min prior to cocaine. Cocaine administration dose dependently attenuated the *d*-fenfluramine–induced HTR with an ID_{50} of 7.69 ± 1.6 mg/kg. Indeed, significant reductions, $F(3, 21) = 71.53$, $p < 0.0001$, were observed at the 5, 10, and 20 mg/kg doses of cocaine [20, 54, and 99% decrease relative to vehicle control (16 ± 1 HTRs)]. In experimental protocol 2, cocaine was administered 10 min prior to *d*-fenfluramine injection. In the latter protocol, the stimulant induced greater inhibitory effects on the *d*-fenfluramine–induced HTR (bottom panel, Fig. 2). Thus, the effects of lower doses of cocaine (0.125 to 10 mg/kg) were investigated. Significant, $F(6, 37) = 20.98$, $p < 0.0001$, reductions [45%, 43, 66, 82, and 94% decrease relative to vehicle control (17 ± 2 HTRs), respectively] occurred at the 0.625, 1.25, 2.5, 5, and 10 mg/kg doses of the stimulant (ID_{50} = 1.59 ± 1.64 mg/kg). Cocaine ID_{50}s in protocols 1 and 2 were significantly different ($p < 0.05$).

The top graph in Fig. 3 shows the inhibitory effects of the selective 5-HT uptake inhibitor sertraline on the *d*-fenfluramine–induced HTR under the conditions of experimental protocol 1. Sertraline attenuated the induced HTR with an ID_{50} of 10.4 ± 1.2 mg/kg. Significant, $F(3, 22) = 72.79$, $p < 0.0001$) reductions [19, 44, and 98% decrease relative to control (16 ± 1 HTRs)] were observed at the three tested doses of sertraline (5, 10, and 20 mg/kg, respectively). In experimental protocol 2, the cited doses of sertraline, $F(3, 21) = 17.19$, $p < 0.0001$, decreased the *d*-fenfluramine–induced HTR by 20, 41, and 74% of The vehicle-treated control group, respectively (ID_{50} = 9.4 ± 1.1 mg/kg) (lower graph in Fig. 3). Significant differences were observed at the 10 and 20 mg/kg doses. Sertraline's ID_{50}s in protocols 1 and 2 were not different from each other ($p > 0.05$).

The upper panel in Fig. 4 represents the inhibitory effects of the selective NE uptake inhibitor nisoxetine on the *d*-fenfluramine–induced HTR. Nisoxetine dose dependently inhibited the induced behavior with an ID_{50} of 2.94 ± 1.41 mg/kg. Significant, $F(4, 24) = 59.9$, $p = 0.0001$, reductions [(42, 83, 65, and 73% decrease relative to vehicle-treated control (16 ± 1 HTRs)] occurred at 2.5, 5, 10, and 20 mg/kg doses of nisoxetine. Nisoxetine was less effective in attenuating the induced behavior under the conditions of experimental protocol 2 (ID_{50} = 9.02 ± 1.42). Indeed, nisoxetine significantly, $F(3, 18) = 25.4$, $p < 0.0001$, reduced [72 and 66% decrease relative to vehicle control (15 ± 1)] the induced HTR at 10 and 20 mg/kg only (lower panel in graph 4). Nisoxetine's ID_{50}s in protocols 1 and 2 were signficiantly different ($p < 0.02$).

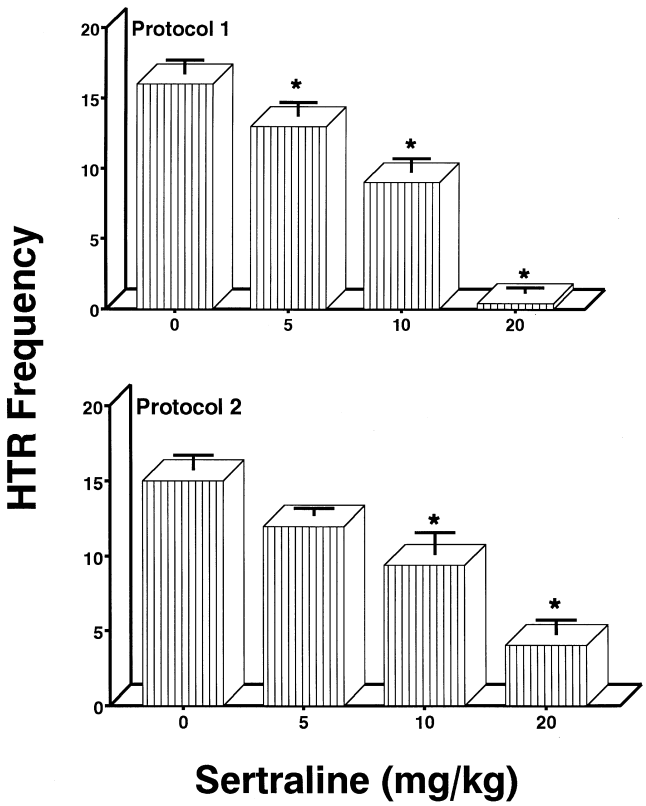

FIG. 3. Dose-dependent inhibitory effects of the selective 5-HT uptake inhibitor, sertraline, on the frequency of *d*-fenfluramine (2.5 mg/kg, IP)-induced HTR (mean ± SEM) in mice. Protocol 1—animals were injected at 0 min with *d*-fenfluramine, at 10 min with either vehicle (n = 10) or the cited doses of sertraline (n = 5) and were then observed for the next 30 min. Protocol 2—animals were injected at 0 min with either vehicle (n = 9) or different doses of sertraline (n = 5), at 10 min with *d*-fenfluramine and were then observed for the next 30 min. *Significant difference between vehicle control and the indicated doses of sertraline, $F(3, 22) = 72.79$, $p < 0.0001$, and $F(3, 21) = 17.19$, $p < 0.0001$, for protocols 1 and 2, respectively.

Figure 5 shows the inhibitory effects of the selective DA uptake inhibitor GBR 12935 on the *d*-fenfluramine-induced HTR in experimental protocols 1 (upper panel) and 2 (lower panel). In protocol 1, GBR 12935 dose dependently attenuated the induced response with and ID_{50} of 4.77 ± 1.2 mg/kg. Significant, $F(4, 25) = 47.9$, $p < 0.0001$) reductions (30, 54, 60, and 94% decrease relative to vehicle control (16 ± 1 HTRs)] were obtained at 2.5, 5, 10, and 20 mg/kg doses of GBR 12935. In protocol 2, GBR 12935 had a similar inhibitory potency (ID_{50} = 3.73 ± 1.42). Significant, $F(4, 22) = 38.86$, $p < 0.0001$, reductions (47, 93, and 93% decrease relative to vehicle control (15 ± 1 HTRs)] were obtained following 5, 10, and 20 mg/kg doses. GBR 12935 ID_{50}s in protocols 1 and 2 were not significantly different from each other.

DISCUSSION

Intraperitoneal administration of *d*-fenfluramine produced a dose-dependent increase in the HTR frequency up to 10 mg/kg. However, further increase in the dose reduced the maximal attainable HTR score. Such bell-shaped dose response is

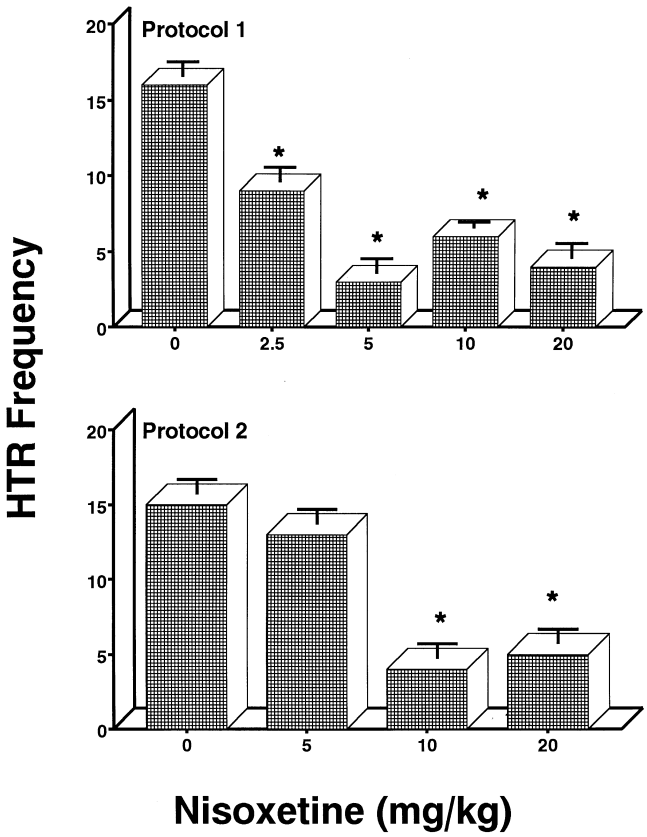

FIG. 4. Dose-dependent inhibitory effects of the selective NE uptake inhibitor, nisoxetine, on the frequency of *d*-fenfluramine (2.5 mg/kg, IP)-induced HTR in mice. Protocol 1—mice were injected at 0 min with *d*-fenfluramine, at 10 min with either vehicle ($n = 10$) or the cited doses of nisoxetine ($n = 4$–6 per group) and were then observed for the next 30 min. Protocol 2—mice were injected at 0 min with either vehicle ($n = 9$) or different doses of nisoxetine ($n = 4$–5 per group), at 10 min with *d*-fenfluramine and were then observed for the next 30 min. *Significant difference between vehicle control and the indicated doses of nisoxetine, $F(4, 24) = 59.9$, $p < 0.0001$, and $F(3, 18) = 25.4$, $p < 0.0001$, for protocols 1 and 2, respectively.

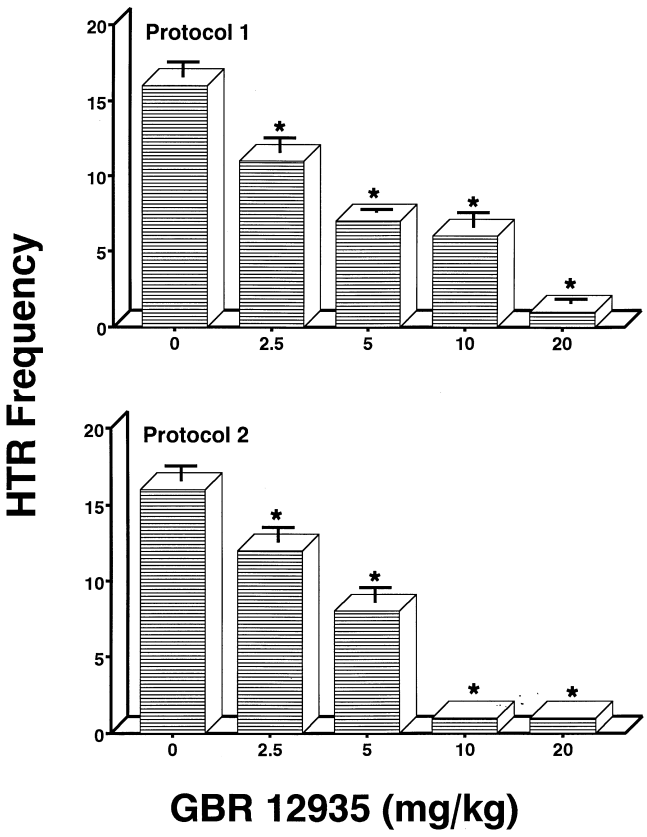

FIG. 5. Dose-dependent inhibitory effects of the selective DA uptake inhibitor, GBR 12935, on the frequency of *d*-fenfluramine (2.5 mg/kg, IP)-induced HTR in mice. Protocol 1—animals were injected at 0 min with *d*-fenfluramine, at 10 min with either vehicle ($n = 10$) or the cited doses of GBR 12935 ($n = 5$ per group) and were then observed for the next 30 min. Protocol 2—animals were injected at 0 min with either vehicle ($n = 9$) or different doses of GBR 12935 ($n = 4$–5 per group), at 10 min with *d*-fenfluramine and were then observed for the next 30 min. *Significant difference between vehicle control and the indicated doses of GBR 12935, $F(4, 25) = 47.9$, $p < 0.0001$, and $F(4, 22) = 38.86$, $p < 0.0001$, for protocols 1 and 2, respectively).

not particular to *d*-fenfluramine; it has also been shown for other nonselective 5-HT_{2A} agonists (64). At higher doses such agents also stimulate postsynaptic 5-HT_{1A} receptors that induce the serotonin syndrome and can interfere with the production of HTR [see (14) for a detailed discussion]. In addition, 5-HT_{1A} and 5-HT_{2A} receptor subtypes functionally interact with each other, and even at behaviorally inactive doses selective 5-HT_{1A} agonists reduce the HTR frequency produced by 5-HT_{2A} agonists [review, (23)]. Although 0.5 μM *d*-fenfluramine selectively releases 5-HT, at higher concentrations [10–20 mg/kg ∼ 2–6 μM (19,58)], it can cause overflow from noradrenergic and dopaminergic synaptosomes (18,24, 25) as well as releasing NE in the rodent brain (32). Thus, in this study the effects of cocaine and the selective 5-HT, NE, and DA uptake inhibitors (sertraline, nisoxetine, and GBR 12935, respectively) were studied on the HTR produced by a 2.5 mg/kg dose of *d*-fenfluramine.

Injection of either cocaine or the cited selective monoamine uptake blockers following *d*-fenfluramine administration (i.e., protocol 1) attenuated the induced HTR in a dose-dependent manner. Among the compounds tested, nisoxetine appeared to be the most potent HTR inhibitor with the following potency order: nisoxetine > GBR 12935 > cocaine > sertraline. However, only sertraline's ID_{50} was significantly ($p < 0.05$) greater than nisoxetine. These in vivo ID_{50} values suggest that the cited agents probably nonselectively attenuate the *d*-fenfluramine–induced HTR. Unlike the present ID_{50} data, in vitro receptor affinity binding results show 25–1750 times selectivity for sertraline, nisoxetine, and GBR 12935 for their target monoamine uptake sites (4,50,52), whereas cocaine exhibits relatively similar potency for the 5-HT, DA, and NE transporters (51). Recent in vivo microdialysis studies have shown that although 5-HT uptake blockers and GBR 12935 preferentially but not exclusively increase the concentrations of their target amine, nisoxetine does not show any selectivity for NE (8,40). Because induction of serotonergic behaviors by 5-HT releasers are completely dependent upon 5-HT release (39), and 5-HT release occurs via the 5-HT uptake carrier working in reverse (3,25,47), even slight nonselectivity of the "specific" monoamine uptake blockers may have significant effects on the release of 5-HT and the catechola-

mines. Another mechanism via which at least cocaine and nisoxetine may attenuate the *d*-fenfluramine–induced HTR is via the discussed potentiation of endogenous synaptic NE, which may subsequently stimulate the inhibitory adrenergic α_2-receptors. The potent ability of GBR 12935 in reducing the *d*-fenfluramine–induced HTR is rather surprising because it does not affect the HTR produced by 5-HTP or L-tryptophan (10,16). This further suggests the possible in vivo nonselectivity of such agents. The relatively large ID_{50} of sertraline for inhibition of the induced behavior probably reflects the atypical uptake inhibition properties of *d*-fenfluramine because, unlike the classical 5-HT uptake blockers, *d*-fenfluramine behaves as a competitive substrate at the 5-HT carrier (65).

Prior administration of cocaine (i.e., protocol 2) produces a significantly lesser degree of potentiation in L-tryptophan-induced HTR relative to its injection after the administration of the 5-HT precursor (i.e., protocol 1). In the present study, cocaine in protocol 2 was approximately five times more potent ($p < 0.05$) than in protocol 1 in attenuating the *d*-fenfluramine–induced HTR. Moreover, cocaine appeared to be the most potent agent in protocol 2 and its ID_{50} was also significantly less than sertraline ($p < 0.01$) and nisoxetine ($p < 0.05$) with the following potency order: cocaine > GBR 12935 > nisoxetine = sertraline. A possible reason for cocaine's ID_{50} differences in the two experimental protocols is probably that in protocol 2 cocaine had a greater exposure time, and thus may further enhance the endogenous synaptic levels of NE, which could inhibit the induced behavior more potently. However, nisoxetine is a more potent NE uptake inhibitor and its ability to reduce HTR in protocol 1 was significantly ($p < 0.05$) reduced in protocol 2. A second possibility is that cocaine is less potent at the monoamine uptake sites, and consequently, its displacement from these sites is easier. Thus, cocaine's influence on the cascade of events induced by *d*-fenfluramine is less than the cited selective uptake blockers if it is given after *d*-fenfluramine. These differential alterations in cocaine and nisoxetine potency order cannot easily be explained from the present behavioral results and requires biochemical studies for further elucidation of mechanisms of interactions of these drugs with *d*-fenfluramine.

The discussed lack of correlation between the present ID_{50} values and the published affinity data suggest possible roles for other mechanisms for attenuation of *d*-fenfluramine–induced HTR. Some selective reuptake blockers are biotransformed to less active (e.g., sertraline), more active (e.g., zimelidine), or to nonselective (e.g., zimelidine) metabolites (36). However, to the author's knowledge the activity and selectivity of metabolites of both GBR 12935 and nisoxetine are not yet known. Pharmacokinetic interactions may provide another mechanism for the observed differential potency orders. Indeed, selective monoamine uptake blockers such as nisoxetine, GBR 12909, and fluoxetine enhance the plasma levels of cocaine (61). However, pharmacokinetic interactions between *d*-fenfluramine and the cited uptake blockers have not yet been investigated. A final possibility is perturbation of the interior acidic proton gradient in vesicles of monoaminergic neurons. In this regard, basic psychostimulants such as cocaine and *d*-fenfluramine reduce the vesicular pH gradient across the storage vesicles and alter the release of monoamines (59). Because all drugs used in the present report are basic, it is probable that differences in injection schedule may differentially affect the HTR frequency in the two protocols via the latter mechanism.

It seemingly appears that cocaine modulates the *d*-fenfluramine–induced HTR broadly via mechanisms similar to cocaine's attenuation of HTR induced by direct $5\text{-}HT_{2A}$ agonists (11,12,13,15) rather than potentiation of 5-HT precursor-induced HTR (10,16). In the case of direct $5\text{-}HT_{2A}$ agonist-induced HTR, inhibition is due to the potentiation of endogenous synaptic NE and 5-HT levels via the inhibition of reuptake of the cited exocytotically released monoamines. As discussed in the introductory paragraphs, these, in turn, inhibit the induced behavior by stimulating the inhibitory adrenergic α_2- and serotonergic $5\text{-}HT_{1A}$ receptors. However, in the *d*-fenfluramine model, in addition to the latter possible mechanisms, cocaine also prevents the *d*-fenfluramine–induced 5-HT release. It should be stressed that in 5-HT precursor-induced HTR, the inhibitory α_2 and $5\text{-}HT_{1A}$ components of cocaine's actions are also concomitantly operative on the induced HTR, but the enhanced stimulation of $5\text{-}HT_{2A}$ receptors override these inhibitory modulations (see introductory paragraphs).

In summary, the present data show that *d*-fenfluramine can produce the HTR in mice in a dose-dependent manner. Both selective (sertraline, nisoxetine, and GBR 12935) and nonselective (cocaine) monoamine uptake blockers attenuated the *d*-fenfluramine–induced HTR. However, their order of potency in inhibiting the induced behavior does not follow their affinity order for the 5-HT transporter. In addition, the potency order for cocaine and nisoxetine was reversed when the latter agents were tested 10 min prior to (i.e., protocol 2) relative to 10-min post (protocol 1) *d*-fenfluramine injection. A single mechanism cannot fully explain the attained results, and the observed alterations may probably involve several mechanisms including nonselectivity of action at monoamine transporters (pharmacodynamic) and/or pharmacokinetic interactions as well as possible alterations of vesicular pH.

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