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The Mechanism by Which the Selective 5-HT_{1A} Receptor Antagonist S-(−)UH 301 Produces Head-Twitches in Mice

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DARMANI, N. A. AND S. L. REEVES. *The mechanism by which the selective 5-HT_{1A} receptor antagonist S-(−)UH 301 produces head-twitches in mice.* PHARMACOL BIOCHEM BEHAV **55**(1) 1–10, 1996.—Electrophysiological studies indicate that certain 5-HT_{1A} receptor antagonists increase the basal firing rate of some but not all raphe neurons by antagonizing the inhibitory endogenous serotonin tone operating on the somatodendritic pulse-modulating presynaptic 5-HT_{1A} autoreceptors. This effect should enhance the synaptic concentration of 5-HT (5-hydroxytryptamine) in serotonergic terminal fields, which may then activate postsynaptic 5-HT receptors. However, in vivo microdialysis studies show that generally such 5-HT_{1A} antagonists by themselves do not increase the basal 5-HT release but potentiate the ability of serotonin reuptake blockers to increase the neuronal serotonin terminal output in the rat brain via the above mechanism. The purpose of the present study was to determine whether antagonism of the proposed endogenous serotonin tone on the 5-HT_{1A} autoreceptors can potentiate the activity of other postsynaptic serotonin receptors. To this end, we utilized the head-twitch response (HTR) in mice as an in vivo model of postsynaptic 5-HT_{2A} receptor function. The selective and silent 5-HT_{1A} receptor antagonist, S-(−)UH 301, by itself, in a dose-dependent manner, produced the HTR in normal but not in reserpinized animals. The 5-HT_{2A} antagonist, SR 46349B, completely prevented S-(−)UH 301-induced HTR. Pretreatment with S-(−)UH 301 also potentiated 5-hydroxytryptophan (5-HTP)-induced HTR both in normal and in the reserpinized mice. At low doses (0.06–0.25 mg/kg), the 5-HT_{1A} selective agonist, 8-OH DPAT, significantly but partially inhibited 5-HTP-induced HTR. However, further attenuation was not observed following the administration of larger doses of 8-OH DPAT. Depending upon the dose used, S-(−)UH 301 pretreatment not only antagonized but also broke through the inhibitory effect of 8-OH DPAT on 5-HTP-induced HTR. The selective (sertraline) and nonselective (cocaine) serotonin reuptake blockers potentiated the ability of 5-HTP to induce the head-twitch behavior in mice. Pretreatment with S-(−)UH 301 enhanced the potentiating effect of serotonin reuptake blockers on the 5-HTP-induced HTR. These results suggest that an endogenous 5-HT tone via the discussed mechanism controls the terminal field synaptic activity of serotonergic neurons in mice. In addition, disinhibition of pulse-modulating 5-HT_{1A} autoreceptors by S-(−)UH 301 can potentiate the synaptic effects of serotonin reuptake blockers as well as the serotonin precursor 5-HTP. However, a more firm general conclusion regarding antagonism of presynaptic 5-HT_{1A} receptors leading to indirect functional enhancement of other postsynaptic serotonergic receptors can only be made when the above hypothesis is further tested with other selective 5-HT_{1A} receptor antagonists (such as WAY 100 635), which we were unable to obtain. The present study is the first report to show that a selective 5-HT_{1A} antagonist by itself can produce a serotonin-mediated function via indirect stimulation of another serotonin receptor subtype in mice.

Head-twitch response 5-HT_{2A} receptor 5-HT_{1A} receptor Cocaine Sertraline Reserpine
5-Hydroxytryptophan 8-OH DPAT S-(−)UH 301 SR 46349B

IN vivo microdialysis studies show that intraperitoneal administration of 5-hydroxytryptophan (5-HTP) increases the extracellular concentration of serotonin [5-hydroxytryptamine (5-HT)] in the rat brain by three- to fourfold (23,55,71). The enzyme responsible for the conversion of 5-HTP to 5-HT is L-aromatic aminoacid decarboxylase (31). The newly made 5-HT from serotonergic terminals may be released: a) by spill over from intraneural stores (29); b) by means of 5-HT reuptake carrier-proteins operating in reverse (48); or c) via exocytosis (23). Serotonin reuptake blockers (such as citalopram, fluoxetine, or sertraline) can increase the basal levels of extracellular 5-HT in the various loci of rat brain by one- to sixfold [review: (22)]. In addition, depending upon the dose of 5-HTP administered, such agents potentiate the increase in the extra-

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cellular concentration of 5-HTP–induced 5-HT by 8–16-fold in the rat hypothalamus (23,55,71).

Several studies have shown that systemic administration of reuptake blockers [clomipramine (1); fluvoxamine (8); sertraline (40); citalopram (41); fluoxetine (24)] significantly and preferentially enhance the extracellular 5-HT concentration in the rat raphe relative to frontal cortex. Artigas (6) has explained this differential effect in terms of: a) serotonergic activity in the raphe nuclei is largely enhanced by reuptake blockers because this area has the highest density of 5-HT transporter in both the rat and human brain (12,39); b) cell bodies and dendrites of 5-HT–containing neurons have a very high density of pulse-modulating 5-HT_{1A} autoreceptors that exert a negative control on both cell firing and 5-HT release from nerve terminals (63,66); and c) the excess 5-HT outside cells produced by the blockade of the transporter in the raphe nuclei activates 5-HT_{1A} autoreceptors and slows down the firing rate of 5-HT neurons and their terminal release. Indeed, some 5-HT_{1A} antagonists such as S-(−)-UH 301, penbutolol, and (−) tertatolol can potentiate the ability of citalopram to increase serotonin terminal output in vivo (36,58). Although, 5-HT_{1A} antagonists by themselves enhance the basal raphe electrical activity (3,42), they do not generally appear to potentiate the release of basal levels of 5-HT even though several studies suggest that the inhibitory pulse-modulating 5-HT_{1A} autoreceptors on the raphe are tonically activated by endogenous 5-HT [(3,10,18–20,42,58), present study]. Thus, disinhibition of 5-HT_{1A} autoreceptors may enhance the synaptic release of serotonergic neurons, which then should enhance the function of other postsynaptic 5-HT receptors. To this end, we have investigated the mechanisms of action of the putative selective and silent 5-HT_{1A} antagonist, S-(−)UH 301 (9), on the production of head-twitch response (HTR) in mice because this agent can enhance the frequency of the 5-HTP–induced HTR (9). The HTR can be produced either by direct 5-HT_{2A} agonists (selective or nonselective), 5-HT precursors (5-HTP or tryptophan) or 5-HT releasers (e.g., fenfluramine) [review: (32)]. The HTR in rodents is considered as a specific behavioral model for the activation of the postsynaptic serotonergic 5-HT_{2A} receptors (32,62).

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 ad lib supply of food and water. S(+)-fenfluramine, ICI 118551 HCl, (±)8-OH DPAT HBr, (±)-propranolol HCl, S-(−)UH 301 HCl were purchased from Research Biochemicals, Inc. (Natick, MA). Atenolol, 5-Hydroxy-L-tryptophan, and reserpine were bought from Sigma Chemical Co. (St. Louis, MO). Cocaine HCl was obtained from the National Institute on Drug Abuse. Sertraline HCl, carbidopa, and SR 46349B were generously donated by Pfizer (Groton, CT), Merck Sharp and Dome (West Point, PA), and Sanofi Recherche (Montpellier, France), respectively. 5-Hydroxytryptophan was dissolved in a small volume of concentrated HCl and was then further diluted by distilled water and back titrated to pH 5 by the addition of NaOH. Reserpine was suspended in distilled water following sonication. All other drugs were dissolved in distilled water. Drugs were administered intraperitoneally at a volume of 10 ml/kg.

Experimental Protocols

To determine any direct effect of S-(−)UH 301, animals were allowed to habituate to test environment in plastic holding cages (40 × 25 × 26 cm) lined with wood chippings for 30 min prior to treatment. Then mice were injected with varying doses of S-(−)UH 301 (0, 0.5, 1, or 2 mg/kg, IP, n = 4–6), and the HTR frequency for each mouse (mean ± SEM) was scored cumulatively at 5-min intervals for 30 min immediately following injection. To demonstrate that S-(−)UH 301-induced HTR is a 5-HT_{2A} receptor-mediated phenomenon, another group of mice were treated with the selective 5-HT_{2A} antagonist, SR 46349B (2 mg/kg, IP n = 4). Twenty minutes later, these mice were treated with S-(−)UH 301 (2 mg/kg, IP) and the HTR frequency was scored for the next 30 min as described earlier.

Next, the mechanisms by which S-(−)UH 301 may increase 5-HTP–induced HTR was investigated. We first determined the effect of 8-OH DPAT on the 5-HTP–induced HTR in the following manner: at zero time mice were treated with carbidopa (10 mg/kg, IP) to prevent conversion of 5-HTP to 5-HT in the periphery (30,33). At 10 min, different groups of carbidopa-treated mice received varying doses of 8-OH DPAT (0, 0.06, 0.25, and 1 mg/kg, IP, n = 5–6). At 20 min, each mouse received an intraperitoneal injection of 37.5 mg/kg 5-HTP (15) and the induced HTR was scored for the next 30 min as described previously. In a similar manner, the effect of S-(−)UH 301 (0, 1, and 2 mg/kg, IP, n = 5–7) was investigated on the 5-HTP–induced HTR. The effects of other nonselective 5-HT_{1A}/β adrenoceptor antagonists propranolol (0, 2.5, 5, and 10 mg/kg, IP, n = 5) and alprenolol (0, 5, and 10 mg/kg, IP, n = 5) were also investigated in the above protocol. For obvious reasons, we also investigated the possible effects of the selective β_1 (atenolol, 0, 5, and 10 mg/kg, IP) and β_2 (ICI 118551, 0 and 5 mg/kg, IP, n = 5) adrenoceptor antagonists in the latter protocol. To determine whether S-(−)UH 301 can prevent the inhibitory effect of 8-OH DPAT on the 5-HTP–induced HTR, we used the following injection schedule: at zero time different groups of mice were treated (IP) with carbidopa (10 mg/kg) plus varying doses of S-(−)UH 301 (0, 0.5, and 1 mg/kg, n = 4–6). At 10 min, the mice were treated with 8-OH DPAT (1 mg/kg, IP). At 20 min, each mouse received 5-HTP (37.5 mg/kg, IP) and the induced HTR was scored as described earlier. We further investigated the effects of S-(−)UH 301 on the 5-HTP–induced HTR in the presence of sertraline or cocaine. Thus, at zero time two large groups of mice received (IP) either carbidopa alone (10 mg/kg) or a mixture of carbidopa (10 mg/kg) and S-(−)UH 301 (2 mg/kg). At 20 min, each mouse received 5-HTP (37.5 mg/kg, IP) and at 30 min different groups of these treated mice received varying doses of sertraline (0, 2.5, and 10 mg/kg, IP, n = 6–8). The HTR frequency was scored for the next 20 min following the last injection. The effect of cocaine (0 and 10 mg/kg, IP, n = 11–15) was investigated in a similar manner to sertraline.

In the third group of experiments, we investigated whether intact synaptic vesicles are necessary for S-(−)UH 301 to produce HTR. Thus, mice were reserpinized (2 mg/kg, IP) 24 and 48 h prior to experimentation according to our previous findings (13). The reserpinized animals were then divided into four groups (n = 5–6). On the test day, the first two groups received an intraperitoneal injection of either S-(−)UH 301 (2 mg/kg) or vehicle. The HTR frequency was scored for the next 30 min as before. The remaining two reserpinized groups were injected with carbidopa (10 mg/kg) at zero time on the test day. Then at 10 min, the latter two groups received either

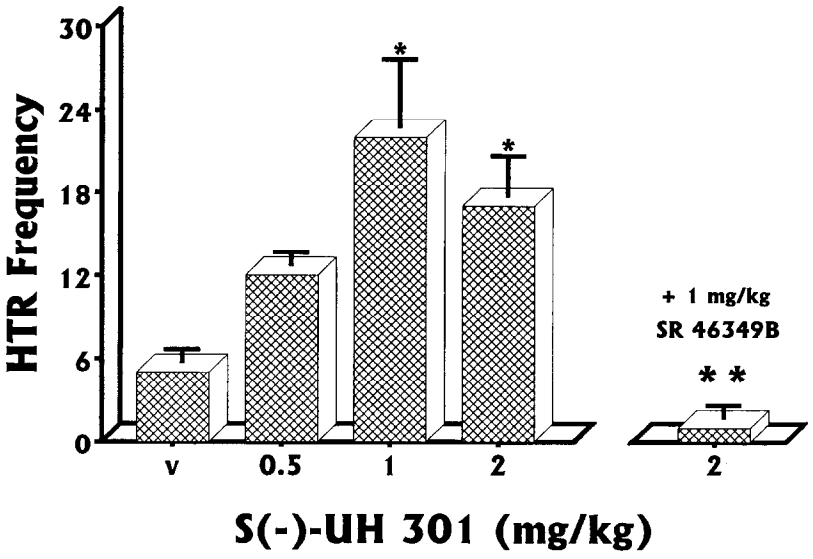

FIG. 1. The dose–response effect (mean ± SEM, n = 4–6) of the 5-HT_{1A} selective antagonist S-(−)UH 301 on the production of HTR in mice. The behavior was observed for 30 min immediately following the S-(−)UH 301 injection. Column to the right represents the inhibitory effect of the selective 5-HT_{2A} antagonist, SR 46349B (2 mg/kg, n = 4), on the frequency of the HTR produced by S-(−)UH 301 (2 mg/kg). *Significantly different from the vehicle control at $p < 0.05$ by the Dunnett's t-test. **Significantly different from the 2 mg/kg S-(−)UH 301 group at $p < 0.05$ by the Fisher's PLSD test.

S-(−)UH 301 (2 mg/kg, IP) or vehicle. At 20 min, both groups received 5-HTP (37.5 mg/kg, IP) and the induced HTR was scored for the next 30 min following the last injection.

Another mechanism by which 5-HT can be released from presynaptic nerve terminals is via the 5-HT reuptake carrier proteins working in reverse (48). At behavioral effective doses (45,69), *d*-fenfluramine causes 5-HT release via the latter mechanism (27). Thus, the effect of S-(−)UH 301 in combination with *d*-fenfluramine was investigated. Our preliminary dose–response studies (data not shown) indicated that *d*-fenfluramine at a dose of 2.5 mg/kg (IP) produces a robust frequency of HTR. This dose was used in the subsequent experiments. First group of animals (n = 5) received an intraperitoneal injection of distilled water at zero time and 10 min later *d*-fenfluramine. The induced HTR was scored for the next 30 min as before. The second group received the same drug injection protocol except S-(−)UH 301 (2 mg/kg, n = 5) was administered instead of its vehicle at zero min. The third animal group received S-(−)UH 301 (2 mg/kg) at zero time and at 10 min distilled water.

Statistics

The data were analyzed by one-way analysis of variance (ANOVA) and post hoc analysis by the Dunnett's t-test or Fisher's PLSD test. A p-value of < 0.05 was necessary to achieve statistical significance.

RESULTS

Intraperitoneal injection of S-(−)UH 301 caused a dose-dependent increase in the HTR frequency in the 30-min observation period (Fig. 1). Significant effects were seen at doses of 1 mg/kg, $F(3, 14) = 8.17$, $p < 0.002$, or greater. The greatest HTR frequency occurred at 1 mg/kg. Although S-(−)UH 301 produced a lower mean HTR frequency at 2 mg/kg, it was not significantly different from the 1 mg/kg mean HTR score. The 5-HT_{2A} selective antagonist SR 46349B at 1 mg/kg completely prevented the S-(−)UH 301 (2 mg/kg)-induced HTR, $F(1, 8) = 35.34$, $p < 0.0003$ (Fig. 1).

The 5-HT precursor 5-HTP (37.5 mg/kg) produced a robust HTR frequency (35 ± 3) in the 30-min observation period. The 5-HT_{1A} selective agonist, 8-OH DPAT, caused a dose-dependent attenuation in the 5-HTP–induced HTR score (Fig. 2A). However, the inhibition was not complete. Thus, significant reductions occurred at 0.06, 0.25, and 1 mg/kg doses of 8-OH DPAT [34, 42, and 48%, respectively; $F(3, 19) = 4.05$, $p < 0.02$]. Unlike 8-OH DPAT, S-(−)UH 301 enhanced the 5-HTP–induced HTR in a dose-dependent manner (Fig. 2B). However, significant enhancement was mainly observed at 2 mg/kg [100% increase, $F(2, 14) = 5.09$, $p < 0.02$]. Although S-(−)UH 301 at 1 mg/kg caused a greater mean HTR frequency (37% increase, $p > 0.05$) than vehicle control, it did not attain significance. Pretreatment with S-(−)UH 301 dose-dependently antagonized the inhibitory effect of 8-OH DPAT (1 mg/kg) on the 5-HTP–induced HTR (Fig. 2C). Thus, S-(−)UH 301 at 0.5 mg/kg prevented the 8-OH DPAT-induced inhibition, and at 1 mg/kg it actually enhanced [100% increase relative to control; $F(2, 13) = 4.49$, $p < 0.03$] the 5-HTP–induced HTR frequency in the presence of 8-OH DPAT (1 mg/kg). Figure 3 represents the effects of the mixed 5-HT_1/β adrenoceptor antagonists (propranolol and alprenolol) and the selective β_1- and β_2-adrenoceptor antagonists (atenolol and ICI 118551, respectively) on the 5-HTP–induced HTR. Alprenolol and ICI 118551 had no significant effect, whereas propranolol and atenolol at high doses [5 and 10 mg/kg; $F(3, 16) = 4.62$, $p < 0.02$, and $F(3, 16) = 3.95$, $p < 0.028$, respectively] attenuated the 5-HTP–induced HTR.

Figure 4A exhibits the effect of the selective 5-HT reuptake inhibitor, sertraline, on the 5-HTP–induced HTR in the 20-min observation period. At the doses tested (2.5 and 10 mg/kg), sertraline caused significant enhancements in the 5-HTP–induced behavior (200 and 400% increase relative to vehicle control (21 ± 2 HTRs) respectively, $F(6, 53) = 8.84$, $p < 0.0001$. Figure 4B represents the effects of sertraline and its vehicle in the presence of S-(−)UH 301 (2 mg/kg). Sertraline (2.5 and 10 mg/kg) caused 209–324% increase, $F(2, 13) = 13.3$, $p < 0.0007$, in 5-HTP–induced HTR relative to its corresponding control. When the corresponding sertraline vehicle controls in the presence and absence of S-(−)UH 301 in the latter protocols were directly compared, there was a 57% increase in the 5-HTP–induced HTR frequency in S-(−)UH 301-treated group. Nonetheless, this increase did not attain significance ($p > 0.05$). However, both the 2.5 and 10 mg/kg doses of sertraline produced significantly greater HTR frequencies in the presence of S-(−)UH 301 relative to its absence [62 and 37%, respectively, $F(5, 43) = 21.24$, $p < 0.001$]. Figure 5A represents the effect of cocaine (10 mg/kg) and its vehicle on the 5-HTP–induced HTR in the 20-min observation period. Cocaine significantly enhanced the 5-HTP–induced HTR frequency by 158%, $F(1, 9) = 10.99$, $p < 0.009$, relative to its vehicle control. In the presence of S-(−)UH 301 (2 mg/kg), cocaine enhanced the 5-HTP–induced HTR frequency by 370%, $F(1, 8) = 21.89$, $p < 0.0016$, relative to cocaine's vehicle control group (Fig. 5B). Moreover, cocaine produced a greater HTR score in the presence of S-(−)UH 301 [131% increase; $F(3, 17) = 17.9$, $p < 0.0001$ relative to its absence (Fig. 5)].

In the reserpinized mice, S-(−)UH 301 (2 mg/kg) failed to induce a significant degree of head-twitch behavior relative to vehicle-treated control group (Fig. 6). However, relative to its vehicle-treated control, S-(−)UH 301 (2 mg/kg) caused 133% increase, $F(1, 8) = 8.3$, $p < 0.02$, in the 5-HTP–induced HTR score in the reserpinized mice (Fig. 6). Figure 7 represents the effect of S-(−)UH 301 on the *d*-fenfluramine-induced HTR. *d*-Fenfluramine (2.5 mg/kg) produced 19 ± 2 HTRs in the 30-min observation period. Larger doses of *d*-fenfluramine produced greater HTR scores (data not shown). At 2 mg/kg, S-(−)UH 301 failed to significantly enhance the *d*-fenfluramine-induced HTR frequency (22 ± 4 HTRs, $p > 0.05$).

DISCUSSION

One important finding of the present study is that intraperitoneal administration of the silent and selective 5-HT_{1A} antagonist, S-(−)UH 301, produces head-twitches in mice in a dose-dependent manner. We (14) and others [e.g. (62)] have previously shown that selective 5-HT_{2A} receptor antagonists dose dependently attenuate the HTR induced by the 5-$HT_{2A/C}$ selective agonist, DOI (1-2,5-dimethoxy-4-iodophenyl)-2-aminopropane), in mice and rats. In the present study, a low dose of the 5-HT_{2A} selective antagonist, SR 46349B (57), completely attenuated the S-(−)UH 301-induced head-twitches. SR 46349B can also prevent the 5-HTP (57)- and DOI (62)-induced HTR. Thus, it is reasonable to assume that the S-(−)UH 301-induced HTR is also a 5-HT_{2A} receptor-mediated phenomenon. However, S-(−)UH 301 only possesses high affinity for 5-HT_{1A} receptors and lacks appreciable affinity for the 5-HT_{2A} or other monoamine receptors except for the dopamine D_2 sites (34). In addition, the role of D_2 sites in the production of S-(−)UH 301-induced HTR seems to be negligible because D_2 selective agonists do not induce the behavior, whereas D_2 antagonists attenuate the DOI-induced HTR (62). Thus, it appears that S-(−)UH 301 produces the HTR indirectly by enhancing the serotonergic function in the terminal fields because the head-twitch behavior is produced via postsynaptic 5-HT_{2A} receptors [review: (32)].

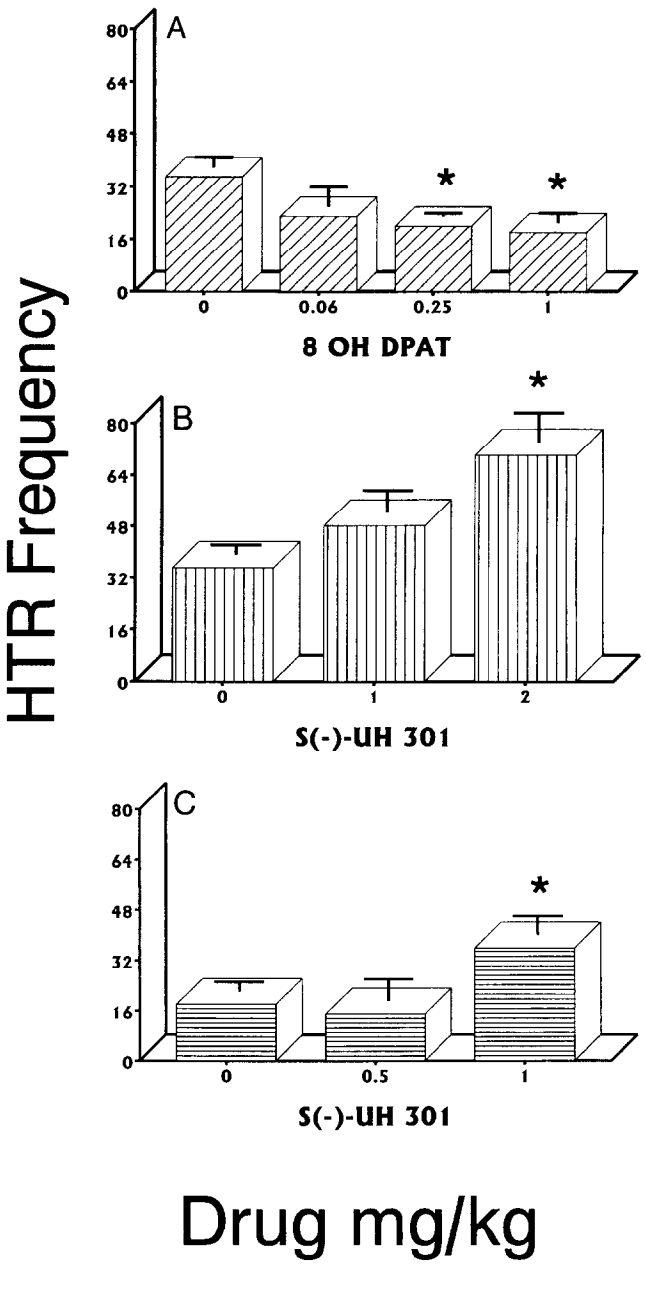

FIG. 2. The respective inhibitory and sitmulatory dose–response effects of the selective 5-HT_{1A} agonist 8-OH DPAT (panel A) and the selective 5-HT_{1A} antagonist S-(−)UH 301 (panel B) on the frequency of the 5-HTP (37.5 mg/kg)-induced HTR in mice. Panel C represents the effect of the cited doses of S-(−)UH 301 on the frequency of the 5-HTP (37.5 mg/kg)-induced HTR in the presence of 1 mg/kg 8-OH DPAT. Results are given as means ± SEM (n = 4–7 per group). *Significantly different from the corresponding controls at $p < 0.05$.

General support for the above notion comes from the in vivo electrophysiological studies. For example, several 5-HT_{1A} antagonists such as S-(−)UH 301 (3), (−)tertatolol (42) and WAY 100635 (18,20,24) can significantly increase the firing rate of some but not all raphe neurons in rats and cats. Further-

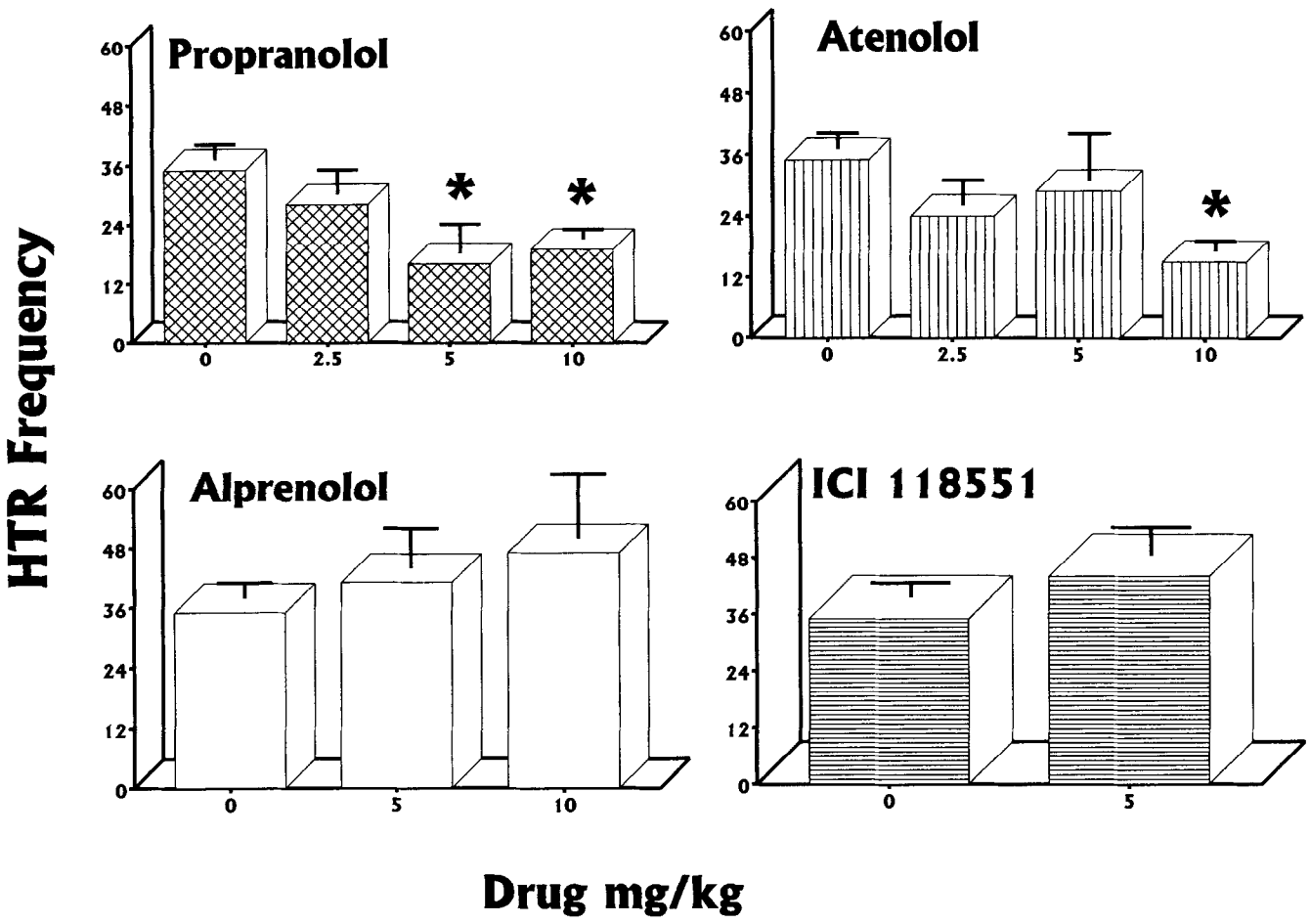

FIG. 3. The effects of the cited doses of propranolol, atenolol, alprenolol and ICI 118551 on the frequency of the 5-HTP (37.5 mg/kg)-induced HTR. The results are given as means ± SEM (n = 5 per group). *Significantly different from the vehicle control at $p < 0.05$ by the Dunnett's t-test.

more, their in vivo administration blocks the inhibitory effect of the 5-HT_{1A} selective agonist, 8-OH DPAT, on the firing rate of raphe neurons. However, it is noteworthy that in the in vivo microdialysis studies to date, the selective and silent 5-HT_{1A} antagonists [e.g. S-(−)UH 301 and WAY 100135] have not increased 5-HT release or turnover when administered alone (54,59). Thus, these biochemical findings suggest either: a) a lack of 5-HT tone on somatodendritic 5-HT_{1A} pulse-modulating autoreceptors (54,64); or b) as supported by the discussed electrophysiological studies, only a proportion of the raphe neurons are affected by endogenous 5-HT tone, and as such, the biochemical techniques are not yet sufficiently sensitive to detect accurately small changes in brain 5-HT concentrations. In addition, to enhance 5-HT detection most in vivo microdialysis studies are performed in the presence of 5-HT reuptake blockers, and as such these agents may alter the effects of drugs that either increase or decrease 5-HT release (46). The cited biochemical and electrophysiological studies were conduced in rats and cats. There is little known about serotonergic tone in mice. The presence of tone on the pulse-modulating 5-HT_{1A} autoreceptors in mice is indicated by the present study in that S-(−)UH 301 failed to induce head-twitches in reserpinized mice but dose dependently produced the behavior in normal mice. Indeed, reserpine removes endogenous 5-HT tone from 5-HT_{1A} autoreceptors (35).

S-(−)UH 301 is reported to potentiate the HTR induced by the 5-HT precursor 5-HTP (9). Thus, our second goal was to determine whether such an effect can occur in mice and attempt to elucidate the mechanism(s) of such enhancement. In the present study, S-(−)UH 301 pretreatment dose dependently enhanced the frequency of 5-HTP-induced HTR in mice. Furthermore, the selective 5-HT_{1A} agonist, 8-OH DPAT, at very low doses (0.06–0.25 mg/kg) significantly but partially inhibited (34–42% inhibition) the 5-HTP-induced HTR. However, further significant reduction was not obtained following administration of larger doses of 8-OH DPAT. Interestingly, S-(−)UH 301 at 0.5 mg/kg prevented the inhibitory effect of 8-OH DPAT (1 mg/kg) on 5-HTP-induced HTR, and at 1 mg/kg it actually enhanced the behavior in the presence of 8-OH DPAT. These results suggest that 8-OH DPAT-induced attenuation in 5-HTP-mediated HTR occurs via the activation of the inhibitory pulse-modulating 5-HT_{1A} autoreceptors. Indeed, low doses of 8-OH DPAT also partially prevented the 5-HTP-induced increase in the extracellular concentration of 5-HT in the rat hypothalamus via such a mechanism (23). We (14) and others [e.g., (4)] have previously shown that complex functional interactions occur between 5-HT_{1A} and 5-HT_{2A} receptors in that 5-HT_{1A} agonists can also attenuate the HTR produced by direct 5-HT_{2A} agonists, and this inhibition can be prevented by 5-HT_{1A} antagonists (16). However, the exact

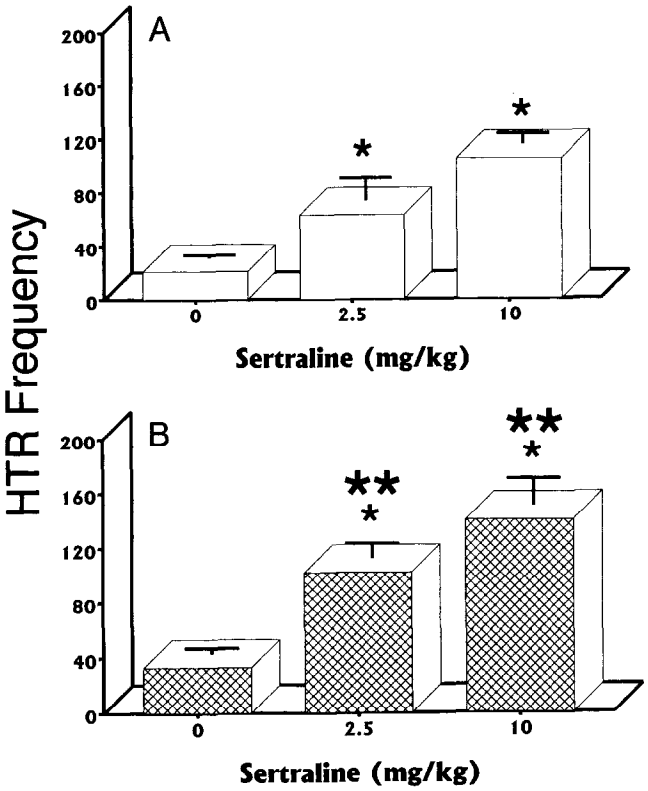

FIG. 4. The effects of the cited doses of sertraline in the absence (panel A) and presence (panel B) of S-(−)UH 301 (mg/kg) on the frequency of the 5-HTP (37.5 mg/kg)-induced HTR. The results are given as means ± SEM (n = 6–8). *Significantly different from the corresponding vehicle controls at $p < 0.05$ by the Dunnett's t-test. **Significant difference between the corresponding doses of sertraline in the absence and presence of S-(−)UH 301 at $p < 0.05$ by the Fisher's PLSD test.

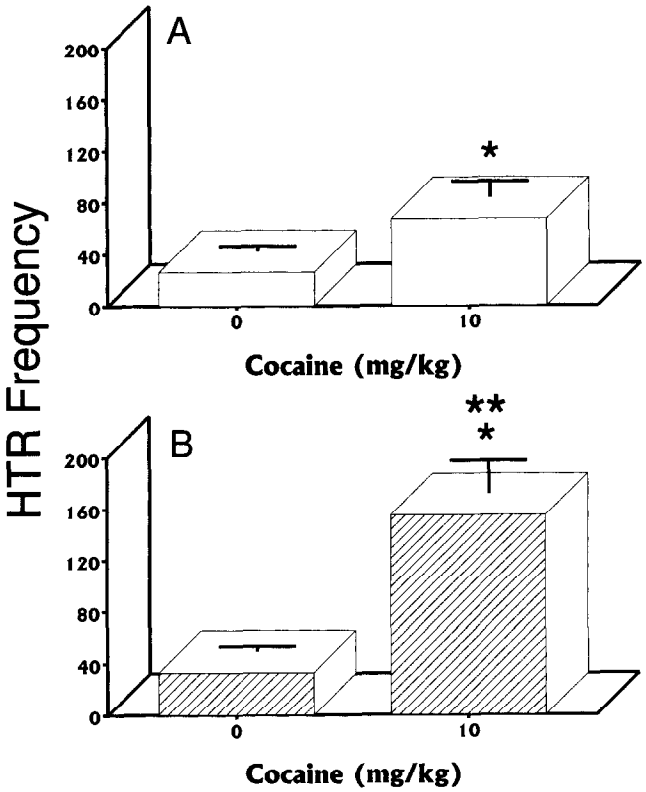

FIG. 5. The effects of cocaine (10 mg/kg) in the absence (panel A) and presence (panel B) of S-(−)UH 301 (2 mg/kg) on the frequency of the 5-HTP (37.5 mg/kg)-induced HTR. The results are given as means ± SEM (n = 11–15). *Significantly different from the corresponding vehicle controls at $p < 0.05$ by the Dunnett's t-test. **Significant difference between corresponding doses of cocaine in the absence and presence of S-(−)UH 301 (2 mg/kg) at $p < 0.05$ by Fisher's PLSD test.

site of this interaction is as yet unknown although both pre- (16) and postsynaptic (62) interaction sites have been suggested.

Systemic administration of the nonselective 5-HT_{1A}\β adrenoceptor antagonist propranolol does not effectively antagonize the 8-OH DPAT-induced decrease in: a) 5-HT release (63); b) raphe cell firing (11,20); or c) 5-HTP–mediated HTR (28). However, propranolol possesses high affinity for 5-HT_{1A} sites and antagonizes the effects of 8-OH DPAT in the functional models of postsynaptic 5-HT_{1A} receptors [review: (32)]. In the present study, systemic administration of propranolol significantly attenuated 5-HTP–induced head-twitch behavior up to 57%. Propranolol has been shown not to affect 5-HTP–induced HTR in mice (28), but can attenuate 5-HTP–induced wet-dog shakes (a behavior equivalent to HTR in mice) in rats (4). The latter anomaly is probably due to the very short duration of time in which the HTR was scored in mice. Our results suggest that propranolol may act as a partial agonist on the presynaptic 5-HT_{1A} autoreceptors following its systemic administration. Indeed, from stimulus generalization studies, Glennon et al. (25) have concluded that the two β-blockers tested (propranolol and pindolol) possess 5-HT_1 agonist properties. On the other hand, in the present study alprenolol (also a mixed 5-HT_{1A}/β antagonist) and ICI 118551 (a β_2 selective antagonist) did not affect the 5-HTP–induced HTR. Although presently the β_1 selective antagonist, atenolol, partially attenuated the induced behavior at the highest dose tested, it is generally accepted that selective β antagonists by themselves do not affect the 5-HTP–induced HTR (30,33). However, in the latter reports both β_1 and β_2 selective agonists potentiated the 5-HTP–induced HTR, and this effect was antagonized by their corresponding selective β-adrenoceptor antagonists. Thus, it is considered that activation of β_1- and β_2-adrenoceptors may have a facilitatory role on the induced behavior. We are unable to offer an explanation as to why high-dose atenolol attenuates the 5-HTP–induced HTR, it may be possible that atenolol abolishes the possible enhancing effects of endogenous norepinephrine on β_1 receptors. Because many nonselective β-blocking agents can also bind both 5-HT_{1A} and 5-HT_{1B} sites, the overall outcome of experimental results may be influenced by the combined effects of these receptors. Although alprenolol, cyanopindolol, propranolol, and pindolol are usually thought as β-blockers possessing 5-HT_1 antagonist action, they can display potent agonist activity in cell lines containing 5-HT_{1B} receptors (53,70). These in vitro studies suggest that the agonist activity of an agent not only depends upon its intrinsic activity but also on the concentration of receptors present on a given tissue as well as the coupling efficiency of a receptor to its signal transduction mechanism(s). Although functional studies suggest that a differential receptor reserve

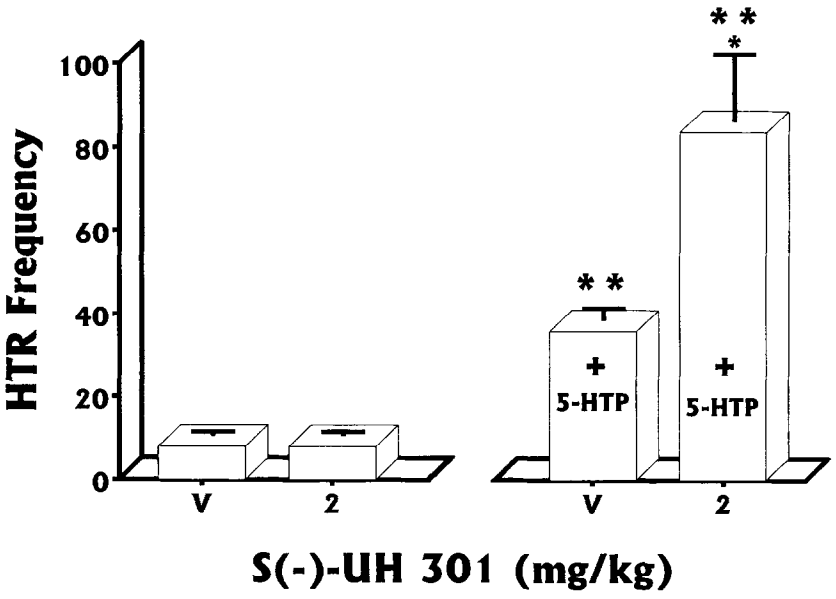

FIG. 6. The left panel exhibits the ability of S-(−)UH 301 (2 mg/kg) at its vehicle (V) to induce the HTR in reserpinized mice. The right panel shows the potentiating effect of S-(−)UH 301 (2 mg/kg) relative to its vehicle on the ability of 5-HTP (37.5 mg/kg) to produce the HTR in the reserpinized mice. The results are given as means ± SEM (n = 5–6). *Significantly different from the corresponding vehicle controls at $p < 0.05$ by the Dunnett's t-test. **Significant difference between the corresponding treatment groups in the absence and presence of 5-HTP.

for 5-HT_{1A} receptors exists at various sites in the brain (50), neither the relative intrinsic activity of the discussed agents nor the coupling efficiency of 5-$HT_{1A/B}$ receptors in different parts of the brain are known. However, it has been shown that such agents can be either inactive or act as agonist or antagonist on various functional models of 5-HT_{1A}- and 5-HT_{1B}-receptors (11,21,25,26,37,43,51,61). Thus, it appears that these agents have different degrees of action ranging from agonist to pure antagonist. This notion is supported by the fact that isamoltane (a β-blocker with high affinity for 5-$HT_{1A/B}$ sites) by itself

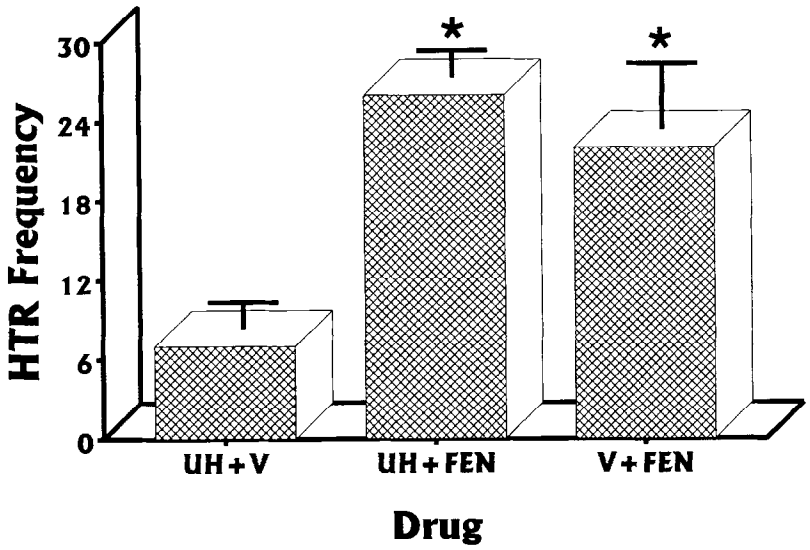

FIG. 7. The effect of S-(−)UH 301 (UH) (2 mg/kg) pretreatment on the d-fenfluramine (FEN) (2.5 mg/kg)-induced HTR (middle column). The left and right columns represent the ability of S-(−)UH 301 and d-fenfluramine to induce the HTR in the corresponding vehicle (V)-treated control groups. The results are given as means ± SEM. *Significantly different from non-d-fenfluramine-treated control group.

produces wet-dog shakes in rats (56). This effect was blocked by the tryptophan hydroxylase inhibitor p-chlorophenylalanine. Isamoltane-induced wet-dog shakes do not seem to be due to β-adrenoceptor blocking action of isamoltane since selective β-adrenoceptor antagonists did not affect the frequency of the induced behavior. In contrast, alprenolol and the 5-HT_2 antagonist ritanserin potently attenuated its frequency. Thus, the latter report further supports our proposed hypothesis that blockade of pulse-modulating 5-HT_{1A} receptors may enhance the function of other postsynaptic 5-HT receptors. Furthermore, blockade of terminal 5-HT_{1B} autoreceptors may produce a similar effect, as proposed by Rényi et al. (56). Indeed, nerve terminal 5-HT_{1B} autoreceptor activation in vivo also mediate suppression of 5-HT biosynthesis, which is independent of the prevailing level of 5-HT neuronal firing (37,38). However, unlike selective 5-HT_{1A} receptor agonists, 5-HT_{1B} agonists do not attenuate the DOI-induced HTR (4,14).

From in vivo microdialysis studies Gartside et al. (23) have suggested that the 5-HTP–induced increase in 5-HT overflow occurs only via exocytotic release process because the release mechanism is mostly Ca^{2+}- and 5-HT_{1A} autoreceptor-dependent. However, behavioral studies suggest that production of HTR and the serotonin syndrome (a postsynaptic 5-HT_{1A} receptor behavioral model) are not totally dependent upon an exocytotic release mechanism because the 5-HT releaser, parachloroamphetamine, can induce these behaviors in reserpinized rats where 5-HT synthetic capacity remains intact (49,52), but 5-HT storage capacity is abolished and brain 5-HT content is reduced more than 90% (47,65). These authors suggest that 5-HT is stored in a large storage pool (80–90%) and a small functional pool. The smaller pool apparently contains the newly synthesized 5-HT located in the cytoplasm, which is preferentially released by the 5-HT releasers. Indeed, the irreversible tryptophan hydroxylase inhibitor, parachlorophenylalanine, prevents both the synthesis of new 5-HT as well as the production of the cited behaviors in both reserpinized (2,47) and normal rats (67). The present study further complements and extends these findings in that 5-HTP also produced the HTR in reserpinized mice, thus suggesting that the 5-HT synthetic capacity also remains intact in these animals. Moreover, S-(−)UH 301 pretreatment significantly enhanced (133%) the 5-HTP–induced HTR in reserpinized mice. This indicates that the newly made 5-HT exerts a negative tone on either 5-HT release or conversion of 5-HTP to 5-HT (or both) via the activation of pulse-modulating 5-HT_{1A} autoreceptors. However, this inhibition is only partial, probably because of the nonphysiological concentration of 5-HTP in serotonergic nerves following 5-HTP administration. Thus, in addition to Gartside et al.'s exocytotic release hypothesis controlling 5-HTP–induced serotonin release in normal animals, the present study suggests that 5-HT_{1A} autoreceptors may also control the release of 5-HT via a nonexocytotic mechanism because it occurs in reserpinized animals. Indeed, it has recently been shown that acute reserpine treatment elevates 5-HT synthesis via the removal of endogenous 5-HT, which exerts a negative tone on raphe cell activity (35). Moreover, pretreatment with 8-OH DPAT was shown to reduce the reserpine effect. Furthermore, Theoharides et al. (68) have demonstrated that nonexocytotic 5-HT release from mast cell occurs via an unknown process that is not sensitive to amitriptyline but still requires metabolic energy and Ca^{2+}, two criteria often cited as indicators of an exocytotic process. The role of serotonin reuptake carrier proteins operating in reverse to release the 5-HTP–induced increase in the neuronal 5-HT also appears doubtful because serotonin reuptake blockers by design should prevent the release of the newly made 5-HT.

Although *d*-fenfluramine at low doses (0.2–0.5 mg/kg) can cause 5-HT release via a Ca^{2+}-dependent exocytotic mechanism, at higher behaviorally active doses (1–5 mg/kg) it mainly causes 5-HT release via a carrier-mediated but Ca^{2+}- and neuronal firing-independent release mechanism (22,27,60,69). The later factors are probably the main reasons why S-(−)UH 301 did not enhance the *d*-fenfluramine-induced HTR in the present study.

As discussed in the introduction, 5-HT reuptake blockers enhance the extracellular concentration of brain 5-HT, and this effect can be potentiated by some 5-HT_{1A} receptor antagonists. Thus, another aspect of the current study was to determine whether S-(−)UH 301 can enhance the effects of 5-HT reuptake blockers on the 5-HTP–induced HTR. In accordance with previous reports (5,17,44,45), both the selective (sertraline) and nonselective (cocaine) 5-HT reuptake blockers enhanced the 5-HTP–induced HTR in the present study. Moreover, presence of S-(−)UH 301 significantly increased the potentiating effects of both sertraline and cocaine on the 5-HTP–mediated head-twitch behavior. These results once again support the general notion that serotonin neurons may induce a ceiling effect in the extent to which 5-HT reuptake blockers can increase the synaptic concentration of 5-HT by activating the pulse-modulating somatodendritic 5-HT_{1A} receptors; this would, therefore, slow their firing rate, resulting in less serotonin being made and released (22). Terminal 5-HT_{1B} autoreceptors may also participate in the overall negative feedback-induced self-limiting effect of serotonin reuptake blockers. Indeed, local perfusion of serotonergic nerve terminal fields with the 5-$HT_{1A/B}$ antagonist (−)penbutolol, has been reported to augment the S-(−)UH 301-induced potentiation of 5-HT output response to subcutaneous citalopram (36).

In summary, it appears that the pulse modulating 5-HT_{1A} autoreceptors are tonically active and control serotonergic neuronal terminal field activity because: a) the putative selective and silent 5-HT_{1A} antagonist, S-(−)UH 301, by itself produces the HTR in normal mice; b) S-(−)UH 301 by itself did not induce the HTR in reserpinized mice where there is little endogenous 5-HT tone; c) S-(−)UH 301 enhanced the 5-HTP–induced HTR both in normal and in reserpinized mice; d) S-(−)UH 301 can also potentiate 5-HTP–induced wet-dog shakes in rats (9); e) S-(−)UH 301 pretreatment antagonized the inhibitory effect of low doses of 8-OH DPAT on the 5-HTP–induced HTR; f) S-(−)UH 301 potentiated the enhancing effect of 5-HT reuptake blockers on the 5-HTP–induced HTR, and g) Rényi et al. (56) have shown that the β-blocker with 5-$HT_{1A/1B}$ antagonist action, isamoltane, by itself can induce wet-dog shakes in rats that can be inhibited by PCPA and ritanserin but not by selective β-blockers. Thus, the present and published results suggest that blockade of 5-$HT_{1A/B}$ autorecptors may increase the concentration of 5-HT in serotonergic terminal fields, which then can lead to functional activation of other postsynpatic 5-HT receptors. This notion has important clinical implications, as proposed by Artigas (6). Indeed, a preliminary clinical report has shown that pindolol caused a dramatic and rapid improvement in the antidepressant efficacy of selective serotonin reuptake blockers (7). In addition, this study may also help to explain the anxiolytic effects of some β-blockers as well as 5-HT_{1A} agonists.

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NOTE ADDED IN PROOF

While this manuscript was at press, another study reported that the selective and silent 5-HT$_{1A}$ antagonist, WAY 100635, by itself produced ear-twitch behavior in guinea pigs, an effect that was antagonized by the 5-HT$_{2A}$ receptor antagonist ketanserin (Mundey, M. K.; Fletcher, A.; Marsden, C. A. Effects of 8-OH DPAT and 5-HT$_{1A}$ antagonists WAY 100135 and WAY 100635, on guinea pig behaviour and dorsal raphe 5-HT neurone firing. Br. J. Pharmacol. 117:750–756; 1996). The latter report provides further support for our conclusions.