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The Head-Twitch Response in the Least Shrew (*Cryptotis parva*) Is a 5-HT_2- and Not a 5-HT_{1C}-Mediated Phenomenon

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DARMANI, N. A., O. B. MOCK, L. C. TOWNS AND C. F. GERDES. *The head-twitch response in the least shrew* (Cryptotis parva) *is a 5-HT_2- and not a 5-HT_{1C}-mediated phenomenon.* PHARMACOL BIOCHEM BEHAV **48**(2) 383-396, 1994.—Our initial studies suggested that the 5-$HT_{2/1C}$ agonist (±)-1-(2,5-dimethoxy-4-iodophenyl-2-aminopropane [(±)-DOI] produces both the head-twitch response (HTR) and the ear-scratch response (ESR) in mice via stimulation of 5-HT_2 receptors. However, challenge studies revealed that these behaviors are produced via two different receptors (possibly 5-HT_2 and 5-HT_{1C}). Due to a lack of selective agents one cannot designate a particular response for the activation of a specific receptor. The purpose of the present study was to investigate such behaviors in the least shrew, which is more sensitive to (±)-DOI than rodents. IP injection of (±)-DOI in shrews produced a dose-dependent (bell-shaped) and time-dependent increase in the HTR frequency. The (±)-DOI-induced HTR was equipotently and completely attenuated by the 5-$HT_{2/1C}$ antagonists ketanserin and spiperone. The 5-HT_{1C} antagonist with 5-HT_2 agonist action, lisuride, also produced the HTR in a bell-shaped dose- and time-dependent fashion. Central injections of both (±)-DOI (0.2 μg) and lisuride (0.5 μg) also induced the behavior. Both peripheral and central administration of lisuride failed to produce the ESR. (±)-DOI significantly induced the ESR only at the highest dose tested (2.5 mg/kg, IP). Centrally administered (±)-DOI (0.2 μg) produced more ESRs relative to vehicle controls; however, the difference did not attain significance. At low doses (0.31 and 0.63 mg/kg), (±)-DOI had no effect on locomotor activity, but it significantly attenuated the behavior at larger doses. Both low and high doses of lisuride increased the motor activity. Spiperone dose-dependently suppressed locomotion, whereas ketanserin had no effect. The present results suggest that the HTR is a 5-HT_2 receptor-mediated event and changes in locomotor activity do not affect the induced HTR.

Head-twitch response Ear-scratch response Locomotor activity DOI Lisuride Ketanserin Spiperone

APPLICATION of molecular techniques and utilization of biochemical and behavioral models have helped to identify multiple functional receptor sites for the neurotransmitter serotonin (5-hydroxytryptamine [5-HT]) [for review see (37)]. The serotonergic functional sites include: 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4 receptors. Some of these receptors (e.g., 5-HT_1) are heterogenous and consist of a number of subsites (e.g., 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_{1E}). Specific behavioral responses associated with the activation of particular serotonergic receptor sites have often been used to provide evidence that the radiolabeled serotonergic recognition sites are functional receptors. One such behavioral model is the head-twitch response (HTR) in rodents [for review see (15)]. The HTR involves a twitch-like movement of the head in the mouse, but rats exhibit simultaneous twitching of their head and upper trunk. Numerous authors have tentatively concluded that the HTR is a 5-HT_2 receptor-mediated phenomenon [for review see (15)]. This conclusion is based on the fact that 5-HT precursors and 5-HT_2 receptor "selective" (e.g., DOI, DOM [an analogue of DOI]) and nonselective agonists (e.g., LSD, 5-MeO DMT) produce the behavior. Furthermore, "selective" and nonselective 5-HT_2 receptor antagonists dose-dependently attenuate the induced response.

Corne and Pickering (7) considered the HTR as an animal behavior correlate of hallucination in man. Glennon and coworkers (17) were first to present evidence that the mechanism

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of action of hallucinogens is via direct activation of serotonergic 5-HT_2 receptors. Indeed, the latter authors have shown a direct correlation between 5-HT_2 site affinity and both discrimination-derived ED_{50} values and human hallucinogenic potencies (16). However, both the indolamine and the phenylethylamine hallucinogens as well as many other 5-HT_2 receptor agonists (and antagonists) also possess high affinity for the serotonergic 5-HT_{1C} site. In fact, as yet no selective agonist or antagonist exists for either the 5-HT_{1C} or 5-HT_2 receptors. There is a remarkable similarity between the structure–affinity relationships of various hallucinogens for the 5-HT_{1C} and 5-HT_2 sites (31). These authors have speculated that 5-HT_{1C} sites may also (or alternatively) play a role in hallucination.

At present, the most effective agent to differentiate the 5-HT_2 and 5-HT_{1C} receptors is spiperone. Spiperone has antagonist activity at both receptors but possesses 1000 to 2000 times higher affinity for the 5-HT_2 versus 5-HT_{1C} site. On the other hand, ketanserin exhibits 10–50-fold selectivity for the 5-HT_2 site [for review see (37)]. The hallucinogen (±)-1-(2,5-dimethoxy-4-iodophenyl-2-aminopropane [(±)-DOI] is an agonist and possesses similar affinity for 5-HT_2 and 5-HT_{1C} sites. Recently, Darmani et al. (10) concluded that the DOI-induced HTR is a 5-HT_2-mediated event because spiperone and ketanserin were equipotent in inhibiting the induced behavior. Using the same antagonists and similar reasoning, the latter authors further suggested that the DOI-induced ear-scratch response (ESR) in mice is also a 5-HT_2-mediated phenomenon (11). However, these DOI-induced behaviors exhibit differential adaptation mechanisms following either acute or chronic exposure to either DOI or ketanserin (8,9). These studies appear to suggest that the DOI-induced behaviors are probably produced via stimulation of two different serotonergic receptors (probably 5-HT_{1C} and 5-HT_2), and due to a lack of selective agents, one cannot designate a particular behavior for a specific receptor site.

Sanders-Bush and coworkers (29) have pharmacologically characterized phosphoinositide (PI) turnover as a biochemical model for the activation of signal transduction systems for both the 5-HT_2 (in the rat cerebral cortex) and 5-HT_{1C} (in the rat choroid plexus) receptors. The hallucinogens LSD and DOM exhibit partial agonist activity for the activation of PI turnover for both 5-HT_2 and 5-HT_{1C} receptors. The nonhallucinogenic congener of LSD, lisuride, appears to be a partial agonist at 5-HT_2 and an antagonist at the 5-HT_{1C} site (3). Gerber et al. (12) have postulated from behavioral studies that lisuride is a 5-HT_2 receptor antagonist because by itself it does not produce HTR but antagonizes the LSD-induced head-twitch behavior in the rat. These contradictory biochemical and behavioral results have helped to further mask the enigma of "whether the HTR is a 5-HT_{1C} or a 5-HT_2 mediated event" (8).

Our preliminary studies indicated that, relative to rodents, the least shrew (*Cryptotis parva*) is extremely sensitive to DOI. Shrews belong to the order Insectivora and the family Soricidae. Members of this family are probably not too distantly removed in structure and habit from the earliest placental mammals (5), and there is good evidence that the primates are early and direct descendants of Insectivora ancestors (6). The musk shrew (*Suncus murinus*) has recently been used to study the antiemetic effects of 5-HT_3 receptor antagonists (33). Because of the unique sensitivity of least shrews to DOI, the purpose of the present study was to investigate whether in the shrew the HTR is a 5-HT_{1C}- or a 5-HT_2-mediated phenomenon.

METHODS

Animals and Drugs

Shrews (*Cryptotis parva*) were bred and maintained in the animal facilities of the Kirksville College of Osteopathic Medicine as described by Mock (26). Both male and female (3.5–4.5 g, 30–60 days old) shrews were used throughout the study. The animals were kept on a 14 : 10-h light–dark cycle at a room temperature of 21 ± 1°C in open-top clear polycarbonate cages (20 × 18 × 21 cm) lined with heat-treated dry loam soil. Depending upon the size of the litter, three to six littermates were housed per cage. A wooden nestbox (5.5 × 5.5 × 9 cm) containing dry grass, a food bowl, and a licktube water bottle were placed in each cage. Nestboxes were small to discourage urination and defecation within its confines. The entrance to the box was 1.8 cm in diameter and located 1.0 cm above the floor to prevent the removal of grass clippings from the nest area. Animals were fed twice daily. In the morning, five to six mealworms (*Tenebrio sp*) were given per animal, and in the evening each shrew was offered a 6-g mixture consisting of two-thirds dry cat food (PMI Nutrition Cat Formula) and one-third canned cat food (Kozy Kitten) in sufficient water to give the mixture a paste-like consistency. All experiments were performed between 0900 and 1700.

The following drugs were purchased from Research Biochemicals Inc. (Natick, MA): (±)-DOI HCl, ketanserin tartrate, and spiperone hydrochloride. The (+)- and (-)-DOI isomers were provided by Prof. R. A. Glennon. Lisuride hydrogen maleate (Berlex Laboratories, Cedar Knolls, NJ) was donated by Prof. Sanders-Bush. Unless otherwise indicated, all drugs were dissolved in distilled water and given IP at a volume of 10 ml/kg.

Measurement of HTR and Spontaneous Locomotor Activity

The HTR is a very distinctive behavior and usually cannot be mistaken for other head movements such as head shakes (lateral movements of the head from side to side) or head jerks (up and down jerkings). The HTR exhibited by the shrew is similar to the head-twitch behavior in mice in that only movement of the head is involved in the induced behavior. However, the speed and frequency with which the HTR occurs in response to small doses of DOI is much greater in the shrew. The HTR was counted continuously and recorded cumulatively at 5-min intervals immediately following DOI injection for a total of 30 min by trained observers using multiple tally counters. The animals were videotaped during the HTR scoring procedure and their locomotor activity was analyzed from videotapes in the following manner: the bottom of the observation cage was divided by two dark lines into four equal quadrants. Animal movement from one quadrant to another was counted via a tally counter cumulatively at 5-min intervals for the duration of the observation period.

Experimental Protocol

Peripheral administration.

Agonist studies. To habituate the shrews to the test environment each animal was randomly selected and transferred 30 min prior to treatment to a 20 × 18 × 21-cm clean clear plastic holding cage. For agonist dose–response studies different groups of shrews were injected IP with varying doses of either (±)-DOI (0.31, 0.63, 1.25, and 2.5 mg/kg; n = 10–12), lisur-

ide (0.63, 1.25, and 2.5 mg/kg; n = 4), or vehicle (distilled water; n = 4–8). Immediately following injection, each shrew was placed in the observation cage and the HTR frequency was directly counted cumulatively every 5 min for the next 30 min. For each group, mean HTR scores (± SEM) in each 5-min interval were calculated. For the determination of locomotor activity each of the 30-min observation periods was videotaped and later analyzed. Using the above protocol, the potency of $S(+)$ and $R(-)$ isomers of DOI was determined in different groups of shrews at a dose of 0.31 mg/kg (n = 4–5, IP). Following completion of each behavioral observation the holding and observation cages were thoroughly washed with tap water. The locomotor effects of the cited agonists were determined cumulatively at 5-min intervals (mean ± SEM) from videotapes (for details see above).

Antagonist Studies. Varying doses of the 5-HT$_{2/1C}$ antagonists ketanserin (0, 0.05, 0.25, and 1.0 mg/kg, IP; n = 7–10) or spiperone (0, 0.05, 0.33, and 1.0 mg/kg, IP; n = 7–10) were administered to different groups of shrews 30 min prior to an IP injection of a 0.63-mg/kg dose of (±)-DOI. For each shrew the HTR frequency was counted beginning immediately following (±)-DOI injection and recorded at 5-min intervals for 30 min as described for the agonist studies. The 30-min HTR observation periods were videotaped and the spontane-

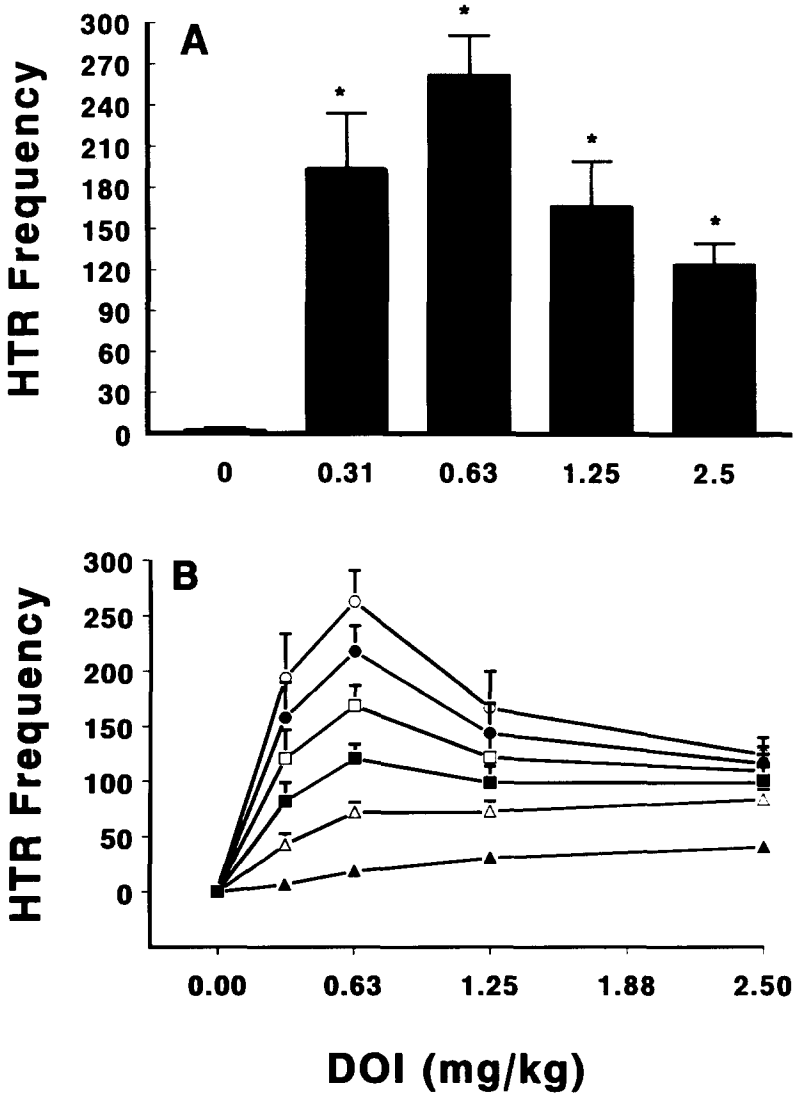

FIG. 1. (A) The dose-response effect of (±)-DOI (IP) on the production of total head-twitch response (HTR) in shrews observed for 30 min immediately after injection. Results are given as means (± SEM). *Significally different from control by Dunnett's t test at $p < 0.05$. (B) The dose- and time-response effects of (±)-DOI on the production of HTR. The behavior was scored cumulatively immediately following injection at succeeding 5-min intervals for a period of 30 min. Time intervals: 0–5, ▲; 5–10, △; 10–15, ■; 15–20, □; 20–25, ●; 25–30, ○. The mean HTR in each time-period is significantly different from vehicle control ($p < 0.05$).

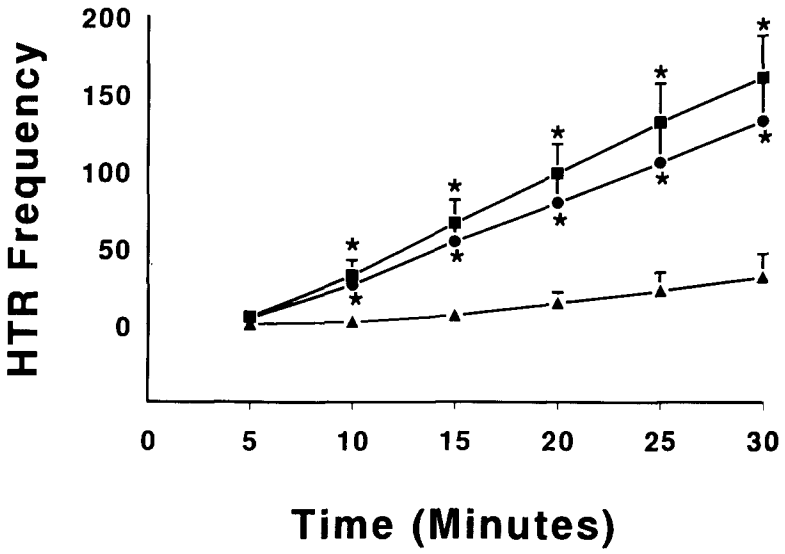

FIG. 2. Time-dependent effects of IP administration (0.31 mg/kg) of DOI isomers [*R*(−), ■; *S*(+), ▲; *RS*(±), ●] on the production of HTR in shrews scored cumulatively at 5-min intervals for a period of 30 min immediately following injection. Results are given as means (± SEM) for each time period. *Significantly different ($p < 0.05$) from the *S*(+)-DOI isomer.

ous locomotor activity for each shrew was analyzed as described previously.

Central administration.

Agonist studies. A subsequent goal of the research protocol was to determine whether the HTR in shrews is a centrally or a peripherally mediated phenomenon. Thus, injection of (±)-DOI or lisuride was made directly into the brain. Because of the small size and shape of the shrew skull, injections into specific forebrain structures could not be made under rigid stereotaxic control. The results of pilot experiments, however, showed that injections of small volumes of fluid could reliably be made into the lateral ventricles and that the injected substances spread throughout the ventricular system within 10–30 min.

Each shrew was anesthetized with ether, and the hair in the appropriate part of the scalp was trimmed with clippers. Injections were made under a dissecting microscope using a hand-held 28-g needle connected via fluid-filled polyethylene tubing to a 10-μl Hamilton syringe. The needle was inserted through the skin, muscle, and skull at a position 13 mm caudal to the tip of the nose and 1.5 mm lateral to the midsagittal midline; the tip of the needle was inserted to a depth of 2 mm below the skin surface. Lisuride (0.5 μg in 2 μl, $n = 5$), (±)-DOI (0.2 μg in 2 μl, $n = 4$) or distilled water (2 μl, $n = 5$) was injected over 10–20 s into the right lateral ventricle of different groups of shrews. The needle was withdrawn after an additional 20–30 s. Each shrew was placed in the observation cage and the HTR frequency was counted cumulatively every 5 min for the next 30 min as described previously. At the conclusion of the observation period the animals were sacrificed by ether anesthesia and then decapitated. The skinned heads were placed in 10% formalin for several days. The fixed brains were removed from the skull and the site of injection on the cortical surface was noted relative to the midline and the olfactory sulcus. Each brain was then sectioned on a vibratome to further document the site of injection.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA), and post hoc analysis was performed by Dunnett's *t* test.

RESULTS

HTR: Agonist Dose- and Time-Response Studies

The 5-$HT_{2/1C}$ agonist (±)-DOI produced dose-dependent bell-shaped dose-response (Fig. 1A) and time-response effects (Fig. 1B). In the 30-min observation period a maximal increase in the total HTR score (263 ± 28) was produced following administration of a 0.63-mg/kg dose of the hallucinogen. Fewer HTRs were observed following administration of lower (0.31 mg/kg) and higher (1.25 or 2.5 mg/kg) doses of (±)-DOI (194 ± 40, 167 ± 33, and 125 ± 15, respectively) (Fig. 1A). Further, for each (±)-DOI dose a time-dependent cumulative increase in the HTR frequency in the succeeding 5-min observation intervals was seen (Fig. 1B). The shape of the dose-response curve for the first two time intervals (i.e., the 5- and 10-min cumulative scores) is such that the cumulative HTR score increased for doses up to 0.63 mg/kg and further increase in the dose did not cause a change in the frequency of the induced behavior. However, the 15-min cumulative score attained the appearance of a bell-shaped dose-response curve which became progressively more prominent at the 30-min time period (Fig. 1B). Central administration of (±)-DOI (0.2 μg per animal) produced 87 ± 30 HTRs, whereas the vehicle-injected control group exhibited 1 ± 1 HTR. When isomers of DOI were tested simultaneously at a dose of 0.31 mg/kg (IP) in different groups of shrews, the *R*(−) isomer produced the highest frequency of HTRs (160 ± 27) in the 30-min observation period, followed by the racemic mixture (±)-DOI (132 ± 30), and both produced significantly greater HTR scores than the *S*(+) isomer (31 ± 15) (Fig. 2).

The 5-HT$_2$ receptor agonist with 5-HT$_{1C}$ antagonist effect, lisuride, also produced a dose-dependent bell-shaped dose-response curve (Fig. 3A). Lisuride produced a maximal effect at a 1.25-mg/kg dose (49 ± 13 HTRs), whereas the 0.63- (28 ± 10) and the 2.5-mg/kg (38 ± 10) doses of the agent produced lower HTR frequencies on the ascending and descending limbs of the dose-response curve, respectively. The cited doses of lisuride were most effective in producing the HTR between the 5th and 15th min of observation (Fig. 3B). Following the 15th-min observation interval the HTR frequency increased only slightly. Central administration of lisuride (0.5 μg per animal) also produced a good frequency of HTRs (38 ± 9) relative to the vehicle-injected group (1 ± 1).

HTR: Antagonist Studies

The 5-HT$_{2/1C}$ antagonist ketanserin dose-dependently attenuated the HTR produced by a 0.63-mg/kg dose of (±)-DOI with a 50% inhibition dose (ID$_{50}$) of 0.14 (0.05–0.4) mg/kg (Fig. 4A). Increasing doses of ketanserin (0.05, 0.25, and 1.0 mg/kg) successively reduced the (±)-DOI-induced HTR by 22% ($p > 0.05$), 77% ($p < 0.05$), and 90% ($p < 0.05$),

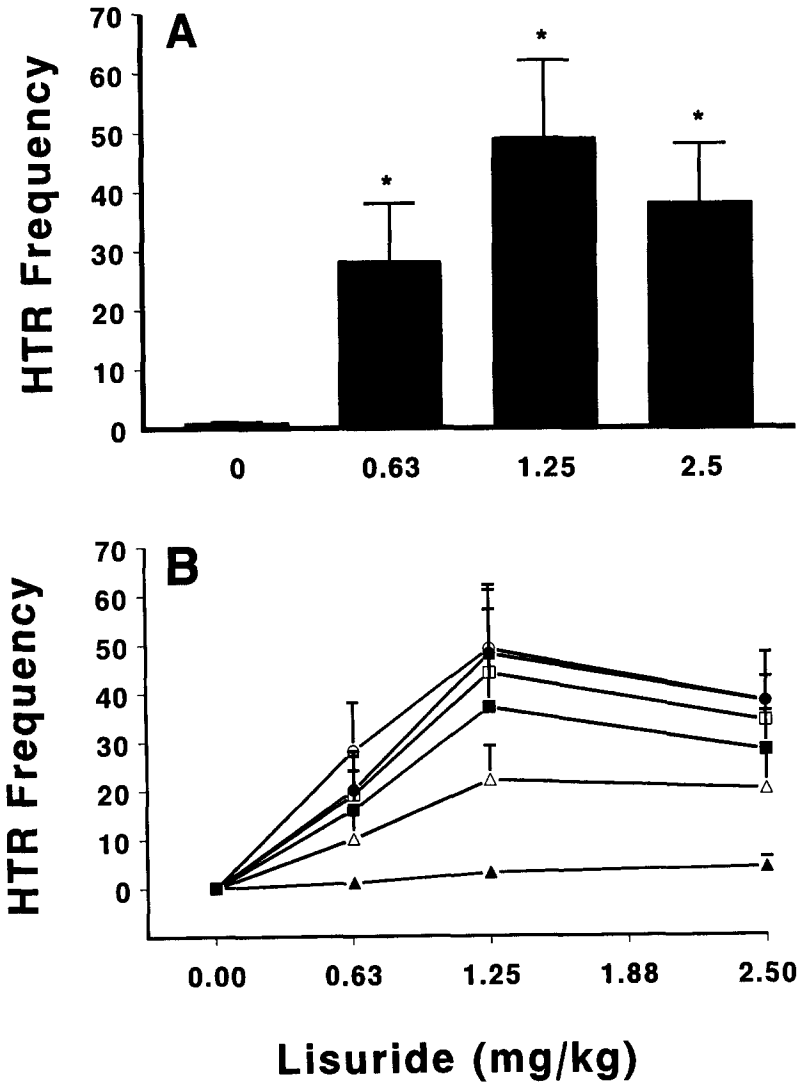

FIG. 3. (A) The dose-response effect of lisuride on the production of HTR in shrews. The behavior was observed for 30 min following injection, and results are given as means (± SEM). *Significantly different from vehicle control by Dunnett's t test at $p < 0.05$. (B) The dose- and time-response effects of lisuride on the production of HTR. The response was scored cumulatively at 5-min intervals immediately following injection for a period of 30 min. Time intervals: 0–5, ▲; 5–10, △; 10–15, ■; 15–20, □; 20–25, ●; 25–30, ○. The 1.25-mg/kg dose exhibited a significant difference from vehicle control from the 5–10-min observation interval and persisted throughout the observation period. The 2.5- and 0.63-mg/kg doses attained significance from the 10–15- and 15–20-min observation intervals, respectively.

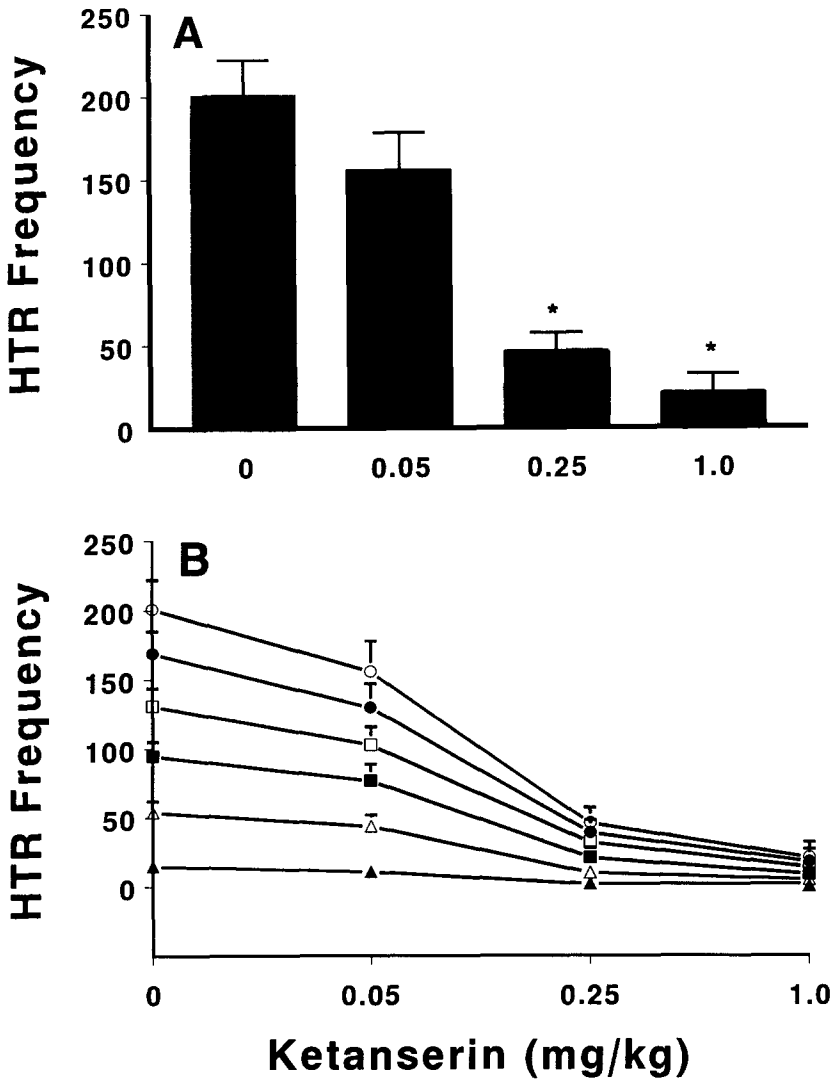

FIG. 4. (A) Dose-dependent inhibitory effect of ketanserin on the head-twitch frequency (mean ± SEM) induced by 0.63 mg/kg (±)-DOI. The cited doses of ketanserin were administered IP 30 min prior to (±)-DOI injection. The HTR frequency was scored for 30 min immediately following (±)-DOI administration. *Significantly different from control by Dunnett's t test at $p < 0.05$. (B) Dose- and time-response inhibition of (±)-DOI-induced (0.63 mg/kg) HTR (mean ± SEM) by the cited doses of ketanserin recorded at 5-min intervals following administration of (±)-DOI in the above treatment schedule. Time intervals: 0–5, ▲; 5–10, △; 10–15, ■; 15–20, □; 20–25, ●; 25–30, ○. The 0.05-mg/kg dose of ketanserin had no significant effect on the induced behavior at any time point. The 0.25- and 1.0-mg/kg doses of ketanserin significantly reduced the (±)-DOI-induced HTR from the first 5-min scoring interval ($p < 0.05$), and this inhibition persisted throughout the 30-min observation.

respectively. The inhibitory effect of a particular dose of ketanserin on the induced behavior appears to be constant at succeeding 5-min scoring intervals—that is, the 0.05-mg/kg dose of ketanserin inhibited the induced behavior between 19% and 27% at each time interval throughout the 30-min observation period (Fig. 4B). In a similar manner, the 0.25-mg/kg dose attenuated the induced behavior between 76% and 87%, whereas the highest ketanserin dose tested (1.0 mg/kg) was 89% to 97% effective in reducing the HTR score at any given time. The other 5-$HT_{2/1C}$ antagonist, spiperone (which also possesses affinity for dopamine and other 5-HT receptors), also dose-dependently reduced the (±)-DOI-induced (0.63 mg/kg) HTR score (ID_{50} = 0.15 [0.05–0.46] mg/kg) (Fig. 5A). At a 0.05-mg/kg dose, spiperone reduced the induced behavior by 24% ($p > 0.05$), and larger doses of the antagonist (0.33 and 1.0 mg/kg) reduced the HTR score by 70% to 91% ($p < 0.05$), respectively. Like ketanserin, any given dose of spiperone produced a constant inhibitory effect

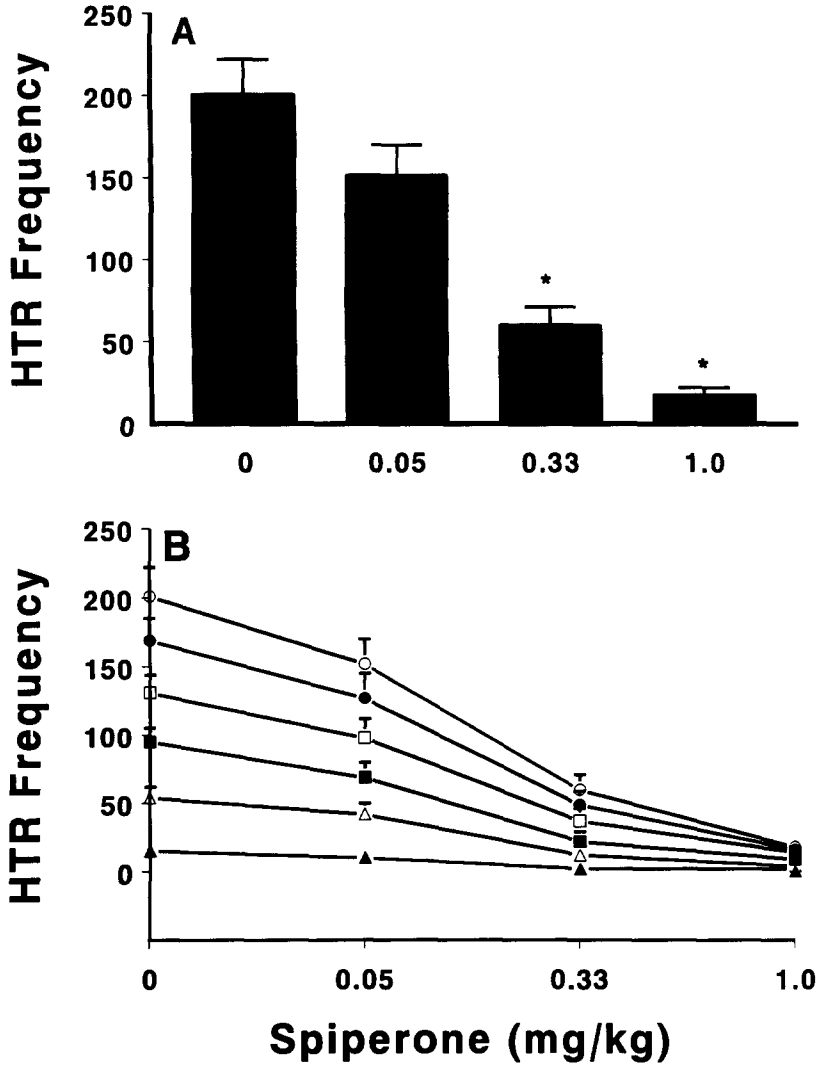

FIG. 5. (A) Dose-dependent inhibition of (±)-DOI-induced HTR by spiperone. Different doses of spiperone (0, 0.05, 0.33, and 1.0 mg/kg; IP) were administered 30 min prior to an IP injection of (±)-DOI (0.63 mg/kg). The HTR frequency was recorded for 30 min following (±)-DOI injection. *Significantly different from vehicle control by Dunnett's t test at $p < 0.05$. (B) Dose- and time-response inhibitory effects of spiperone on the (±)-DOI-induced HTR in the above treatment schedule. The HTR frequency (mean ± SEM) was recorded cumulatively at 5-min intervals for 30 min immediately following (±)-DOI injection. Time intervals: 0–5, ▲; 5–10, △; 10–15, ■; 15–20, □; 20–25, ●; 25–30, ○. The 0.05-mg/kg dose of spiperone had no significant effect on the HTR frequency, whereas the larger doses significantly ($p < 0.05$) reduced the behavior from the first time interval. This inhibition persisted throughout the 30-min observation period.

on the (±)-DOI-induced HTR score at succeeding 5-min observation intervals (Fig. 5B).

Locomotor Activity: Agonist and Antagonist Studies

Low doses of (±)-DOI (0.31 and 0.63 mg/kg) had no significant effect on the spontaneous locomotor activity of the shrews at any given time interval during the 30-min observation period (Fig. 6). The $R(-)$ and $S(+)$ isomers of DOI also failed to alter the spontaneous locomotor activity at a 0.31-mg/kg dose (Fig. 7). Relative to vehicle-treated animals, the 1.25-mg/kg dose of (±)-DOI generally attenuated the locomotor activity throughout the observation period; however, the reduction (52%, $p < 0.05$) was only significant at the 25–30-min observation interval (Fig. 6). The 2.5-mg/kg dose of (±)-DOI produced significantly greater inhibition, which was apparent from the 5–10-min observation interval (53%, $p < 0.05$) and persisted until the termination of the observation schedule (70%, $p < 0.05$) (Fig. 6). Ketanserin pretreatment (0.05–1.0 mg/kg) had no significant effect on the locomo-

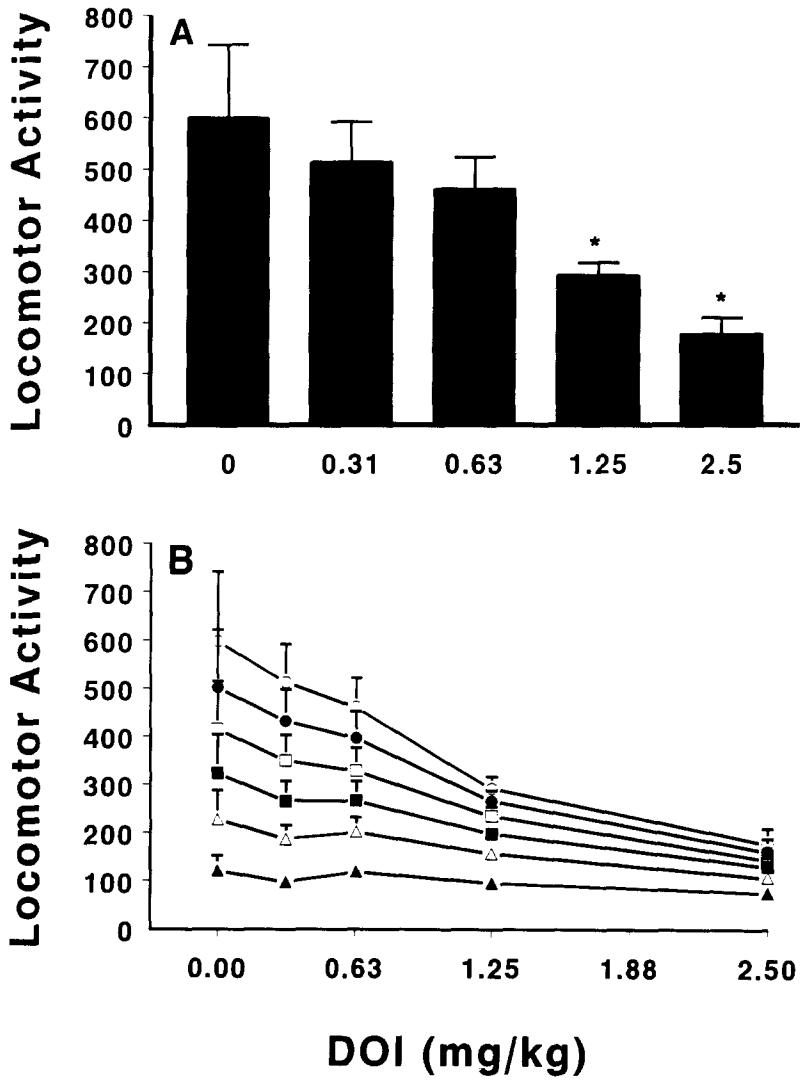

FIG. 6. (A) Dose-dependent inhibitory effect of (±)-DOI (0, 0.31, 0.63, 1.25, and 2.5 mg/kg; IP) on the spontaneous locomotor activity of shrews recorded for 30 min immediately following injection. Results are shown as mean ± SEM. *Significantly different from vehicle control by Dunnett's t test at $p < 0.05$. (B) The dose- and time-response effects of the cited doses of (±)-DOI on the spontaneous locomotor activity recorded cumulatively at 5-min periods for 30 min following injection of (±)-DOI. Time intervals: 0–5, ▲; 5–10, △; 10–15, ■; 15–20, □; 20–25, ●; 25–30, ○. The 2.5-mg/kg dose of (±)-DOI significantly ($p < 0.05$) reduced motor activity from the second time interval and persisted throughout the observation period. The 1.25-mg/kg dose attained significant inhibition at the 25–30-min observation interval. The smaller doses of (±)-DOI had no significant effect.

tor activity of (±)-DOI-treated (0.63 mg/kg) shrews relative to water-treated controls (Fig. 8). However, spiperone pretreatment (0.05–1.0 mg/kg) significantly reduced the locomotor activity of (±)-DOI-treated (0.63 mg/kg) animals at 0.33- and 1.0-mg/kg doses ($p < 0.05$) (ID_{50} = 0.23 [0.06–0.92] mg/kg) (Fig. 9). The inhibitory effect was apparent from the first observation interval (63% and 79%, respectively) and persisted throughout the duration of the experiment (Fig. 9B).

At 0.63 mg/kg, lisuride potently increased ($p < 0.05$) the spontaneous locomotor activity of the shrews by 156% (Fig. 10A). Further increase in dose (1.25 and 2.5 mg/kg) did not produce greater effects. Each cited lisuride dose produced a time-dependent increase in the cumulative mean score of the locomotor activity which attained significance following the 10–15-min observation interval and persisted throughout the duration of the experiment (Fig. 10B).

Other Observed Behaviors

Shrews also exhibit another behavior that is similar to the discussed ESR in mice following administration of (±)-DOI. However, unlike mice, shrews exhibit a greater frequency of

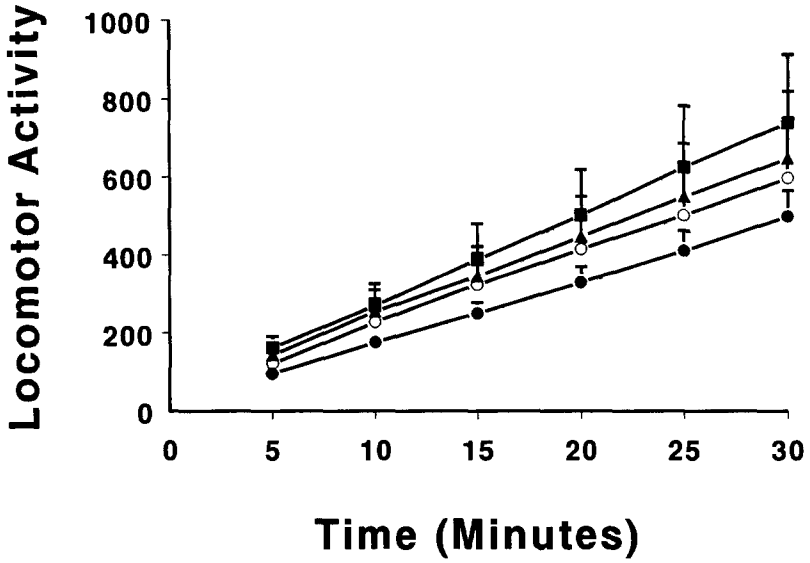

FIG. 7. The effect of IP administration of vehicle and isomers of (±)-DOI (0.31 mg/kg) on the spontaneous locomotor activity (mean ± SEM) of shrews. The activity was recorded for 30 min at 5-min intervals immediately following injection (vehicle, ○; *R*(−)-DOI, ■; *S*(+)-DOI, ▲; (±)-DOI, ●).

ESRs as a normal behavioral repertoire. Further, small doses of (±)-DOI (IP) do not produce a significantly greater frequency of ESR relative to vehicle control (4 ± 1) in the 30-min observation period. However, the 2.5-mg/kg dose of (±)-DOI produced significantly greater responses (54 ± 21, $p < 0.05$). Central injection of (±)-DOI (0.2 μg per animal) produced 44 ± 8 ESRs relative to the vehicle-injected group (24 ± 9, $p > 0.05$). Both peripheral (0.625–2.5 mg/kg) and central (0.5 μg per animal) administration of lisuride failed to produce any ESRs.

DISCUSSION

The phenylalkylamine hallucinogens such as DOI and DOM are considered to be fairly selective 5-HT$_{2/1C}$ agonists (13) or partial agonists (29). DOI is reported to have either a similar (21) or up to 40-fold higher (31) affinity for 5-HT$_2$ versus 5-HT$_{1C}$ sites. Therefore, it is reasonable to assume that the DOI-induced HTR is either a 5-HT$_2$- or a 5-HT$_{1C}$-mediated response. In the present investigation, IP administration of (±)-DOI in the least shrew produced head-twitch behavior. The induced response is bell-shaped and dose- and time-dependent. Relative to mice (10) and rats (2), shrews not only produced a several-fold greater frequency of HTRs per unit time but also exhibited maximal effect at a (±)-DOI dose that is 3 to 8 times lower than those required by rodents. As in rodents (18), the DOI-induced HTR in shrews is centrally mediated because central injection of the hallucinogen (0.2 μg per animal) produced a good frequency of HTRs. The DOI-induced HTR appears to be stereoselective in that the *R*(−)-DOI is 5 times as potent as the *S*(+) enantiomer. Mice exhibit similar responses, except that the potency difference between the DOI isomers is twofold for the HTR (10) and sixfold for the ESR (11). These potency variations in behavioral observations are consistent with published radioligand studies in that the *R*(−) isomers of such phenylalkylamine hallucinogens typically bind at 5-HT$_2$ and 5-HT$_{1C}$ receptors with 2–10 times the affinity of their *S*(+) enantiomers [for review see (14)]. Similar potency variations have also been reported for their ability to activate PI hydrolysis via stimulation of 5-HT$_2$ and 5-HT$_{1C}$ receptors in the cerebral cortex and the choroid plexus, respectively (29).

In the present study, the 5-HT$_{2/1C}$ antagonists ketanserin and spiperone equipotently and completely attenuated the (±)-DOI-induced HTR in a dose-dependent fashion. These agents have also been reported to reduce the (±)-DOI-induced HTR at identical doses in mice (10). Although shrews exhibit several times more sensitivity to (±)-DOI, these animals are only as sensitive as mice to the inhibitory effects of ketanserin and spiperone. While both ketanserin and spiperone possess similar affinity for the 5-HT$_2$ receptor, these agents exhibit differential affinity for the 5-HT$_{1C}$ site. Ketanserin possesses a 10–50-fold higher affinity for the 5-HT$_2$ relative to the 5-HT$_{1C}$ receptor, whereas spiperone exhibits up to 2000-fold selectivity for the 5-HT$_2$ versus the 5-HT$_{1C}$ site [for review see (37)]. Therefore, the present results and the cited antagonist studies suggest that the HTR is a 5-HT$_2$ receptor-mediated phenomenon because if it was a 5-HT$_{1C}$-mediated event, ketanserin would have been considerably more potent in inhibiting the (±)-DOI-induced behavior. Darmani et al. (11) used similar reasoning to postulate that the DOI-induced ESR in mice is also a 5-HT$_2$-mediated response. However, more detailed challenge studies following acute or chronic exposure to either ketanserin or (±)-DOI indicated that the HTR and ESR are produced by activation of two different receptors, possibly via 5-HT$_2$ and 5-HT$_{1C}$ sites (8,9). Therefore, these studies could not definitely designate a particular behavior for activation of a specific receptor site. Alternatively, both behaviors could be mediated via one 5-HT receptor, and differences in the microenvironment of the cell membrane in different brain regions could possibly affect the manner in which the site is regulated. In addition, these differences in regulation could reflect differential coupling of the same 5-HT receptor to different effector mechanisms (1).

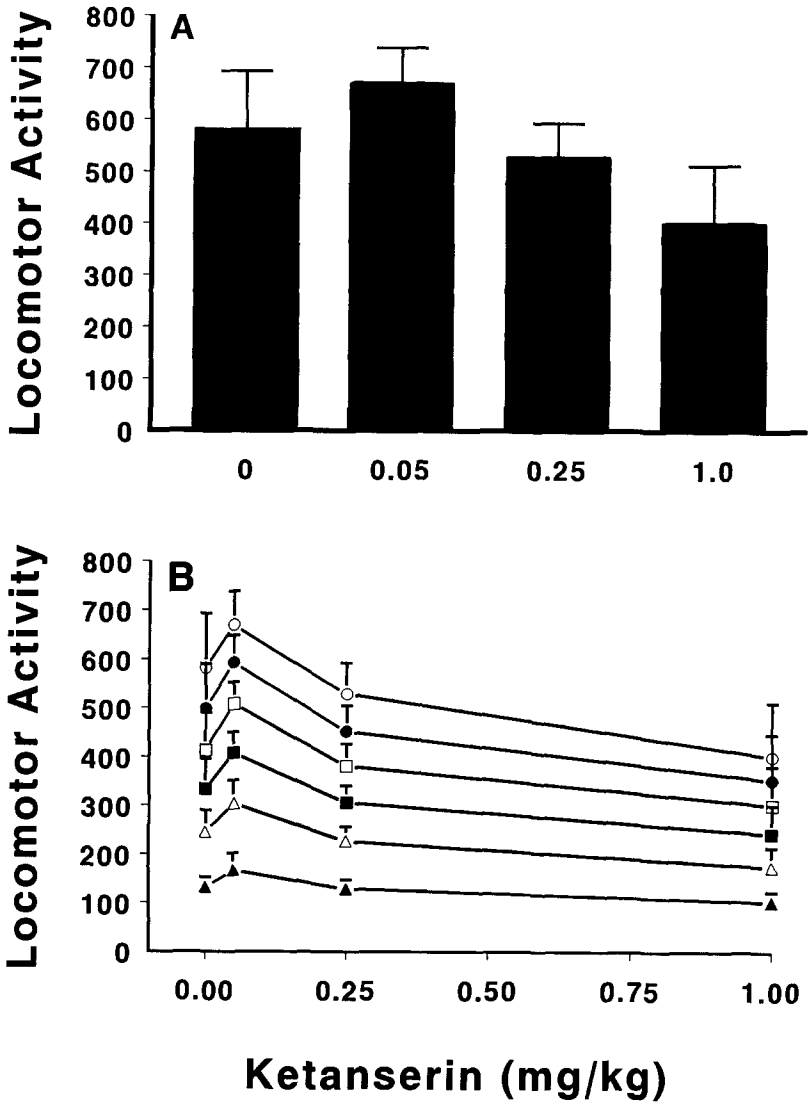

FIG. 8. (A) The effect of 30 min prior treatment with vehicle or several doses of ketanserin (0.05, 0.25, and 1.0 mg/kg; IP) on the spontaneous locomotor activity of shrews following administration of (±)-DOI (0.63 mg/kg, IP). Locomotor activity was recorded for 30 min immediately following (±)-DOI injection. (B) Time- and dose-response effects (cumulative) for the above treatment schedule at 5-min intervals for the 30-min observation period. Time intervals: 0–5, ▲; 5–10, △; 10–15, ■; 15–20, □; 20–25, ●; 25–30, ○.

From the above discussion, it appears that agents which have differential agonist or antagonist affinity for both 5-HT_2 and 5-HT_{1C} sites may not resolve the enigma as to whether the HTR is a 5-HT_2- or a 5-HT_{1C}-mediated event. Some novel compounds appear to possess better selectivity than (±)-DOI for the 5-HT_{1C} versus the 5-HT_2 site (28); however, the receptor profile of their pharmacological activity has not been fully investigated. Application of such agents in behavioral studies such as the present investigation may not help to clarify the cited enigma because possible stimulation of other receptors by such agents may modulate the HTR frequency (19). As discussed in the introductory section, lisuride possesses agonist activity at the 5-HT_2 site but antagonizes the effects of the 5-HT_{1C} receptor (3). Therefore, lisuride provided an excellent opportunity to resolve the above enigma. Similar to (±)-DOI, IP injections of lisuride in shrews produced HTR in a bell-shaped, dose-response and time-response manner. However, lisuride was relatively less potent than (±)-DOI, as it produced fewer HTRs when administered either centrally or peripherally. Consistent with its lower behavioral potency, it also exhibits lower affinity for the 5-HT_2 site [for review see (37)]. Therefore, it is reasonable to suggest that HTR is a 5-HT_2 receptor-mediated phenomenon. However, the present notion is in contradiction with the HTR study of Gerber et al. (12) in which those authors concluded that lisuride is a 5-HT_2 receptor antagonist because by itself lisuride failed to produce HTR in rats but antagonized the 5-HT-induced HTR. With the present state of knowledge regarding the 5-HT receptor

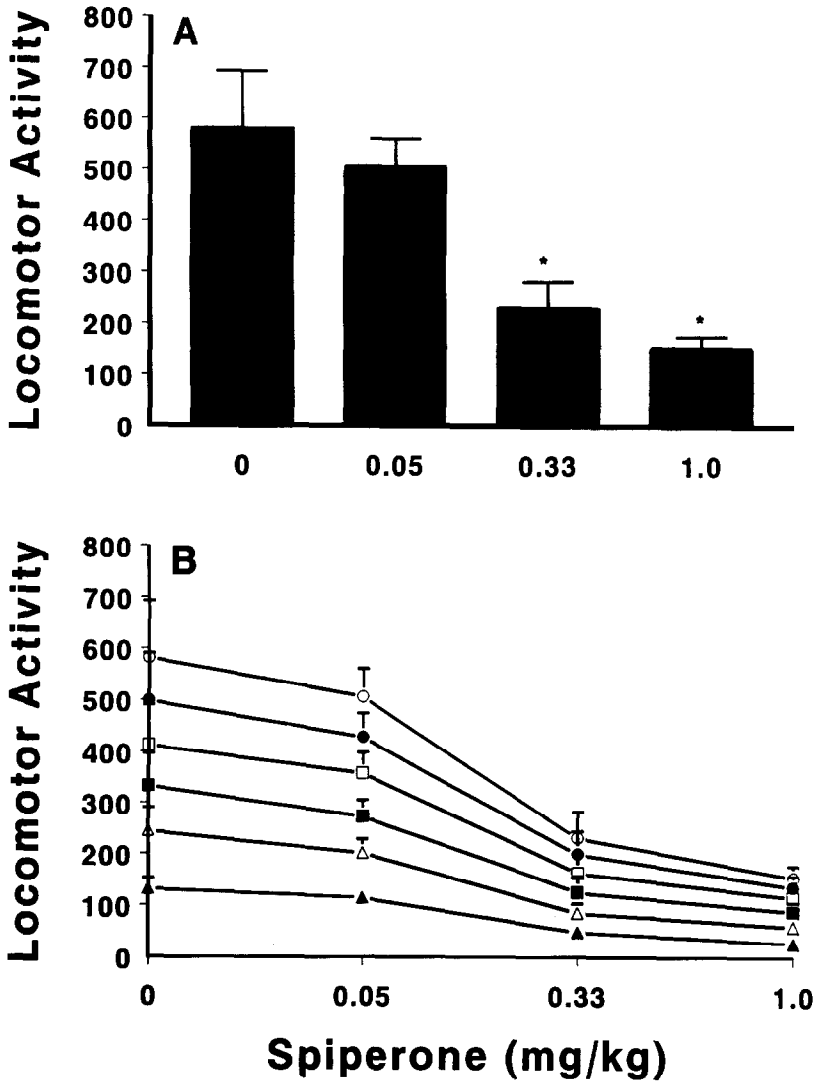

FIG. 9. (A) The inhibitory effect of 30 min prior treatment of spiperone (0, 0.05, 0.33, and 1.0 mg/kg; IP) on the spontaneous locomotor activity of (±)-DOI-treated (0.63 mg/kg) shrews. Locomotor activity was recorded for 30 min immediately following (±)-DOI injection. *Significantly different from control by Dunnett's t test at $p < 0.05$. (B) Dose- and time-response effects of spiperone in the above treatment schedule. The motor activity is shown at 5-min intervals for the 30-min observation period. Time intervals: 0-5, ▲; 5-10, △; 10-15, ■; 15-20, □; 20-25, ●; 25-30, ○. At low dose (0.05 mg/kg), spiperone had no effect on the locomotor activity but significantly reduced activity at higher doses from the first 5-min scoring interval which persisted throughout the 30-min observation period.

subtype interactions, this contradiction appears to be less ambiguous. Several investigators have now shown that simultaneous costimulation of 5-HT$_{1A}$ receptors potently inhibits the HTR produced by "selective" and nonselective 5-HT$_2$ receptor agonists (2,10,35). The exact mechanisms by which this 5-HT receptor interaction occurs have not yet been elucidated. However, this phenomenon can explain why lisuride and LSD are sometimes referred to as agonists and other times as antagonists. Both lisuride and LSD possess high affinity for 5-HT$_{1A}$ receptors [for review see (37)], and thus, both induce the 5-HT syndrome in rats via activation of the 5-HT$_{1A}$ receptor (12). The 5-HT syndrome consists of several behaviors, including hindleg abduction, forepaw treading, lateral head-weaving, tremor, rigidity, and straub tail. LSD produces HTR via activation of the 5-HT$_2$ receptor but can also inhibit the HTR produced by other 5-HT agonists (12). Therefore, it appears that the inhibitory effect of both LSD and lisuride on the 5-HT-induced HTR occurs via simultaneous stimulation of the inhibitory 5-HT$_{1A}$ receptor. Indeed, lisuride can induce the head-twitch behavior when it is directly injected into the medial prefrontal cortex of rats (18). In the latter study, lisuride was less potent than LSD and the $R(-)$ and $S(+)$ isomers of DOM. This is consistent with the relatively lower affinity of lisuride for the 5-HT$_2$ site [for review see (37)]. It appears that

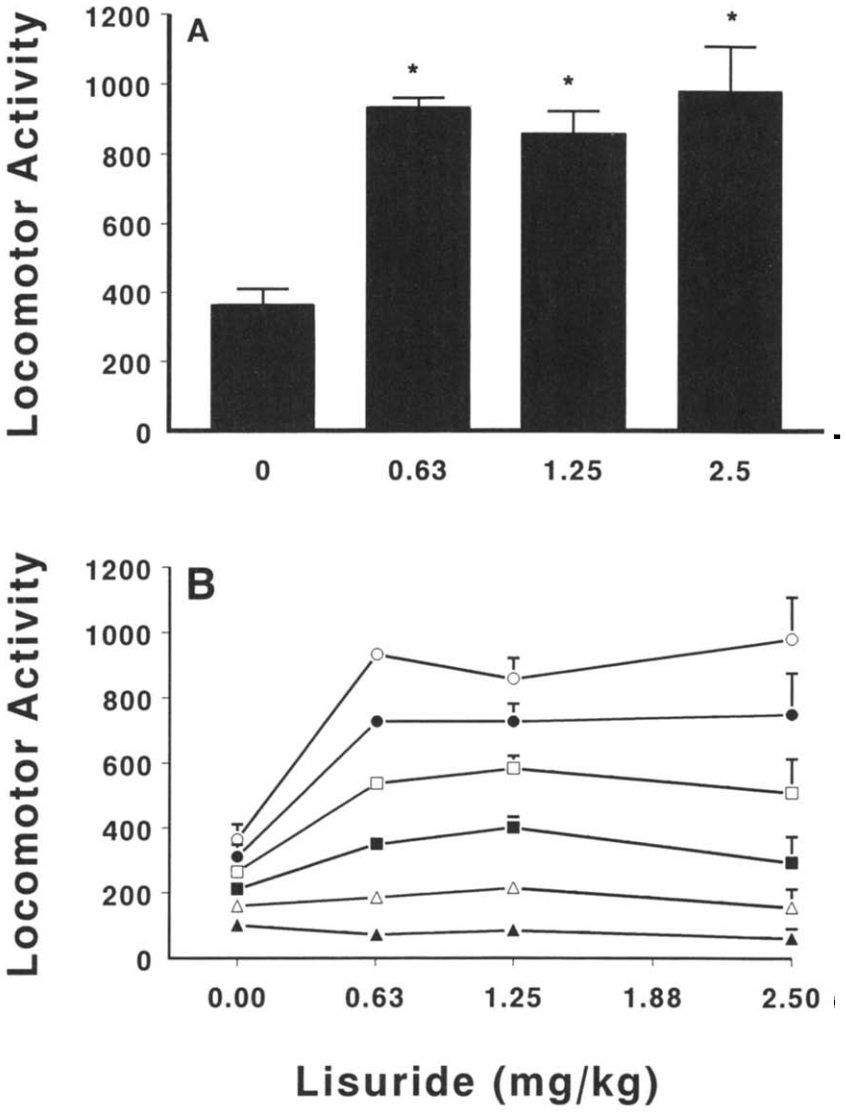

FIG. 10. (A) Stimulatory effect of several doses of lisuride (0, 0.63, 1.25, and 2.5 mg/kg; IP) on the spontaneous locomotor activity of shrews recorded for 30 min immediately following injection. Results are shown as mean ± SEM. *Significantly different from vehicle by Dunnett's t test at $p < 0.05$. (B) Time–response effects of the cited doses of lisuride on the locomotor activity of shrews recorded at 5-min intervals for 30-min duration immediately following injection of lisuride or vehicle. The 1.25-mg/kg lisuride dose exhibited significance of difference from the 10–15-min interval, whereas the lower and higher (0.63 and 2.5 mg/kg) doses of the agent were different ($p < 0.05$) from vehicle controls from the 15–20-min observation period.

peripheral administration of lisuride to rats causes more overt stimulation of the inhibitory 5-HT_{1A} than the stimulatory 5-HT_2 receptor. Indeed, published literature shows that rats are particularly more sensitive to 5-HT_{1A} agonists [for review see (15)]. The behavioral difference between the peripheral and central routes of administration of lisuride in rats suggests that the inhibitory 5-HT_{1A} receptors are probably not located within the vicinity of the stimulatory 5-HT_2 receptors. The ability of shrews to produce HTR following peripheral administration of lisuride probably resides in the fact that these animals are not very sensitive to 5-HT_{1A} agonists (unpublished findings). Although further studies are required, the present results suggest that the ESR may be a 5-HT_{1C}-mediated response because lisuride does not produce the behavior, whereas (±)-DOI can induce the behavior following both peripheral (high doses) and central administration.

Felines exhibit limb flicking and abortive grooming following peripheral administration of hallucinogens, and these behaviors are also considered to be animal models of hallucinogenic activity (22). Since lisuride has generally been thought to lack hallucinogenic activity in man (30) but can produce the cited behaviors in the cat, the agent is considered as a false positive in this model of hallucination (23). However, some clinical studies have reported that lisuride can produce halluci-

natory episodes (4,32). The dogma that lisuride is not hallucinogenic is probably due to limitation on the dose of lisuride administered to humans because of its dopamine-induced emetic action. Although it is only speculation, the ability of lisuride to produce HTR in shrews and limb flicking in cats may suggest that lack of full hallucinatory action of lisuride in man may reside in the potent effects of lisuride on 5-HT$_{1A}$ receptors, which may reduce the hallucinatory effectiveness of 5-HT$_2$ receptors. Support for this notion comes from the previously discussed animal studies where simultaneous costimulation of 5-HT$_{1A}$ receptors dose-dependently attenuates the 5-HT$_2$ receptor-mediated HTR.

Though thus far the effects of lisuride have been discussed in regard to HTR and serotonergic systems, it also possesses potent dopaminergic activity. Similar to the dopaminergic agonist apomorphine, lisuride administration in rodents causes increased locomotion (20). In the present study, lisuride at a low dose (0.63 mg/kg) significantly increased spontaneous locomotor activity in shrews by 156%. Administration of larger doses of lisuride did not lead to further increases in locomotor activity. Shrews are normally very active animals, and unlike rodents, they do not usually come to rest in the observation arena following acclimation. It is interesting to note that the lisuride-induced maximal increase in locomotor activity does not seem to impair the ability of the agent to produce maximal head-twitching because the latter behavior occurs following administration of larger doses of the agonist. Indeed, assessment of various dopaminergic agonists has revealed a lack of correlation between dopamine-mediated behaviors and blockade of 5-HT-induced head-twitches in rats (12). Unlike lisuride, (±)-DOI significantly reduced spontaneous locomotor activity in shrews in a dose- and time-dependent manner. However, the smaller doses of (±)-DOI which lie on the ascending limb of the HTR dose-response curve, including the maximal effective dose (0.63 mg/kg), had no significant depressive action on locomotion. In the rat, however, small doses of (±)-DOI are reported to produce more potent locomotor depressive action (24,25,34). Furthermore, the latter studies show that ketanserin pretreatment prevents the suppressive effects of (±)-DOI. In the present study, spiperone dose-dependently attenuated the spontaneous locomotor activity in (±)-DOI-treated (0.63 mg/kg) shrews during the 30-min period in which the HTR was scored. Spiperone has also been reported to reduce locomotor activity in rodents (27). Although at doses tested no overt cataleptic effect was observed in the shrew, spiperone can induce catalepsy in rodents (36). Thus, we cannot rule out the possibility that spiperone may reduce the HTR nonspecifically via sedation or via a mild cataleptic effect. However, in the present study similar doses of ketanserin had no significant effect on locomotion but potently reduced the HTR score. Therefore, the present results, along with previously published data, suggest that changes in locomotion do not affect the frequency of HTR.

In summary, the present results and previously published data suggest that the HTR is most probably a 5-HT$_2$ receptor-mediated phenomenon. Because of possible species differences in the sensitivity of 5-HT receptor subtypes and their interaction, some agonists such as lisuride do not produce the behavior in every species. Finally, simultaneous changes in locomotor activity produced by the HTR inducers appear not to affect the head-twitch frequency.

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