0022-3565/92/2622-0692$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 262, No. 2
Printed in U.S.A.

Behavioral Evidence for Differential Adaptation of the Serotonergic System after Acute and Chronic Treatment with (±)-1-(2,5-Dimethoxy-4-Iodophenyl)-2-Aminopropane (DOI) or Ketanserin[1]

NISSAR A. DARMANI,[2] BILLY R. MARTIN and RICHARD A. GLENNON

Departments of Pharmacology/Toxicology and Medicinal Chemistry. Virginia Commonwealth University/Medical College of Virginia, Richmond, Virginia

Accepted for publication April 8, 1992

ABSTRACT

The 5-hydroxytryptamine (5-HT)$_2$ agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and antagonist ketanserin were evaluated for acute and chronic effects on the 5-HT$_2$ receptor-mediated head-twitch response (HTR) in mice. A single dose of DOI resulted in tolerance to the DOI-induced HTR at 24 hr but supersensitivity at 48 hr. This apparent supersensitivity persisted up to 144 hr after the first DOI injection. Chronic once daily DOI administration significantly reduced the HTR frequency (days 2–6), which then returned slowly to control levels by treatment day 13. A 48-hr withdrawal from this chronic regimen produced a similar supersensitivity to that observed after a single DOI injection upon a 48-hr challenge. This effect also persisted up to 144 hr after cessation of chronic treatment. Acute pretreatment with a single injection of ketanserin significantly reduced the DOI-induced HTR frequency when tested 24 and 48 hr, but not 120 hr, after injection of the antagonist. After withdrawal from chronic ketanserin treatment, the DOI-induced HTR was significantly reduced at 24 hr but significantly increased at 48 hr. This enhanced effect subsided when mice were tested with DOI 72 hr after cessation of chronic antagonist treatment. These data suggest that the serotonergic system adapts to chronic exposure of either agonists or antagonists in a fashion distinctly different from that exhibited by other monoamine neurotransmitter systems.

Advances in radioligand binding techniques and the availability of synthetic tritiated compounds (such as [^{3}H]-5-HT and [^{3}H]spiperone) in the 1970s sparked a continuing flurry of interest that has led to the present classification of the serotonergic receptor system. To date at least four different populations of 5-HT receptor binding sites have been identified (Glennon, 1990; Schmidt and Peroutka, 1989). These populations include 5-HT$_1$, 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$ sites. In addition, 5-HT$_1$ receptors can be subdivided into 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$ and 5-HT$_{1D}$ sites. Moreover, 5-HT$_3$ receptors may also be heterogeneous and consist of a number of subsites (Richardson *et al.*, 1985). The molecular, biochemical and physiological correlates for some of these receptors have recently been reviewed (Schmidt and Peroutka, 1989). The 5-HT receptor system appears to be involved in the control/modulation of a variety of normal physiological processes such as appetite, blood pressure, sexual behavior and sleep. In addition, alteration in 5-HT receptor function has been linked to central nervous system disorders such as aggression, anxiety, depression, migraine and neurodegenerative disorders including Parkinson's disease, Huntington's disease and Alzheimer's disease (for a review see Glennon, 1990).

The 5-HT$_2$ receptor site is probably the most widely studied site in the 5-HT receptor family. Agents which activate these receptors are thought to possess hallucinogenic activity (Glennon *et al.*, 1984). In fact, a significant correlation ($r > 0.9$) appears to exist between 5-HT$_2$ binding affinities and human hallucinogenic activities for various analogs. Moreover, the latter authors have shown that there appears to be a similar correlation ($r > 0.9$) between 5-HT$_2$ receptor affinity and production of certain behaviors in rodents. In rodents, 5-HT$_2$ selective and nonselective drugs can induce a stereotypic twitch-like movement of the head which is generally referred to as the HTR. This effect is thought to be due to stimulation of central 5-HT$_2$ receptors. The HTR can be produced by a variety of different neurotransmitters, hormones and environmental conditions (*e.g.*, immersion of rodents in ice-cold water).

Received for publication September 9, 1991.
[1] Supported by National Institute on Drug Abuse Grants DA02396 and DA 01642.
[2] Present address: Department of Pharmacology, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; HTR, head-twitch response; DOI, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane.

However, the head-twitch behavior produced by 5-HT agonists is antagonized by 5-HT$_2$-selective antagonists (for a review see Handley and Singh, 1986). Furthermore, Ortmann *et al.* (1982) reported that a direct relationship exists between 5-HT$_2$ site affinity and antagonist potency for the inhibition of the induced behavior.

Monoamine adaptation theory implies that persistent agonist stimulation by the endogenous neurotransmitter or prolonged exposure to synthetic agonists normally results in receptor down-regulation. On the other hand, chronic deprivation of receptor excitation (*e.g.*, denervation or persistent antagonist exposure) tends to induce up-regulation of receptors. Several radioligand binding studies designed to investigate the adaptive changes in the 5-HT$_2$ receptor system after chronic exposure to different 5-HT$_2$ antagonists, such as mianserin (Blackshear and Sanders-Bush, 1982), ritanserin and setoperone (Leysen *et al.*, 1986; Twist *et al.*, 1990b) have revealed down-regulation of 5-HT$_2$ receptors. This paradoxical antagonist-induced down-regulation does not follow the monoamine adaptation dogma. Some behavioral studies tend to confirm the chronic effects of the "selective" antagonists on the 5-HT$_2$ receptor function. For example, HTR frequency induced by the nonselective 5-HT agonist 5-methoxy-N,N-dimethyltryptamine has been reported to be reduced after chronic exposure to mianserin (Blackshear and Sanders-Bush, 1982). Other behavioral studies, however, indicate induction of supersensitivity after withdrawal from chronic 5-HT$_2$ antagonist treatment (Mogilnicka and Klimek, 1979; Stoltz *et al.*, 1983). Studies of 5-HT-stimulated phosphoinositide hydrolysis, a cellular response that is linked to the 5-HT$_2$ receptor recognition site, indicate either a decrease (Conn and Sanders-Bush, 1986; Sanders-Bush *et al.*, 1989; Kendall and Nahorski, 1985) or an increase (Twist *et al.*, 1990a) in 5-HT-induced inositol phosphate turnover after chronic antagonist treatment.

In addition, chronic administration of a more selective 5-HT$_2$ receptor agonist, such as DOI, has also been shown to decrease 5-HT$_2$ receptor density (Bukholtz *et al.*, 1988; McKenna *et al.*, 1989). Moreover, Leysen and Pauwels (1990) have reported recently that prolonged exposure to DOM (an analog of DOI) not only reduces 5-HT$_2$ receptor capacity in rat frontal cortex, but can also reduce 5-HT-induced inositol phosphate hydrolysis in vascular muscle cells in culture. In a recent communication it was reported that once daily chronic DOI administration caused an initial down-regulation of 5-HT$_2$ receptor function (a decrease in HTR frequency) which returned slowly to control levels upon daily agonist administration (Darmani *et al.*, 1990a). Moreover, evidence was provided for supersensitivity after withdrawal from the chronic agonist regimen.

Thus, chronic administration of either 5-HT$_2$ receptor agonists or antagonists can induce a decrease in 5-HT$_2$ receptor number. Therefore, induction of a supersensitive state after withdrawal from such treatments is a paradox. On the basis of published biochemical and behavioral data, we hypothesized that the 5-HT$_2$-induced HTR does not follow the accepted monoamine theory of receptor adaptation. The purpose of the present investigation was to examine the validity of the above hypothesis.

Methods

Animals and drugs. Male albino ICR mice (Dominion Laboratories, Dublin, VA), weighing 16 to 18 g at the beginning of the experiments, were used throughout the study. The animals were kept on a 12-hr light/dark cycle at a room temperature of 22 ± 1°C with *ad libitum* supply of food and water. All of the behavioral experiments were performed between 10:00 A.M. and 6:00 P.M. Ketanserin tartrate and DOI HCl were purchased from Research Biochemicals, Inc. (Natick, MA). DOI was dissolved in distilled water, whereas ketanserin was dissolved in HCl acid, back titrated to pH 5.2 with sodium bicarbonate solution and diluted further with distilled water. Drugs were injected i.p. in a volume of 10 ml/kg.

Measurement of HTR. The HTR is a very distinctive behavior and usually cannot be mistaken for such behaviors as head-shakes (lateral movement of the head from side to side) or head-jerks (up and down jerking). The HTR was scored during 2-min intervals by trained observers using multiple tally counters.

Behavioral experiments with agonists. In order to habituate the mice to the test environment, each animal was transferred randomly 20 min before treatment to a 40 × 25 × 16 cm plastic cage lined with a thin layer of sawdust. To determine the acute effects of DOI, mice (n = 12) were injected with a 2.5-mg/kg i.p. dose of DOI. This dose has been shown previously to produce a robust HTR in mice (Darmani *et al.*, 1990b). The HTR was scored cumulatively at 2-min intervals for the next 20 min after DOI injection. The data obtained after the first injection were used as control for subsequent injections of DOI. In order to investigate the possible development of either acute tolerance or supersensitivity by a single dose of DOI, mice were injected with a single 2.5-mg/kg dose of DOI. These animals were then divided into four groups which were then challenged with a second dose of DOI (2.5 mg/kg) either 48 (n = 11), 72 (n = 12), 144 (n = 12) or 192 (n = 12) hr after the initial single acute DOI injection. After injection of the challenge dose, the DOI-induced behavior was scored for each animal for the next 20 min as described previously.

To investigate the effects of repeated agonist administration, mice were injected with DOI (2.5 mg/kg i.p., n = 14), once daily for 13 days and the induced-HTR frequency was scored as described above after each daily injection. The results from day 1 were used as control. In order to investigate the effectiveness of DOI after withdrawal from such a prolonged treatment, mice were treated once daily for 13 days with either distilled water (n = 19) or 2.5 mg/kg of DOI (n = 34). After the 13th day, the treatment was stopped and the mice were divided into four groups. Each group contained vehicle (n = 4–5)- and DOI (n = 10–14)-treated mice. Different groups of mice were then challenged with DOI (2.5 mg/kg) either 48, 96, 144 or 240 hr after cessation of chronic treatment. The behavior was scored after the challenge dose of DOI as described for the acute studies. The chronically water-exposed groups were then pooled and their mean score ± S.E.M. was used as controls. Two other groups of animals were treated in a similar manner with either water (n = 19) or DOI (n = 14) for 13 days. Both groups were challenged with DOI 48 hr later (*i.e.*, day 15) and the induced behavior was scored. The chronically DOI-exposed group received further injections of DOI on days 16, 17 and 18. The induced-HTR was scored after each injection. Treatment was ceased for 5 days before these animals received another challenge dose of DOI (2.5 mg/kg) on day 23. The induced behavior was again recorded.

Behavioral experiments with antagonists. For the determination of the acute effects of antagonist treatment on DOI-induced HTR, mice were injected with either vehicle (n = 8) or ketanserin (1.0 mg/kg i.p., n = 18). These animals were divided into three groups, each of which consisted of a number of vehicle (n = 2–3)- and ketanserin (n = 6)-treated mice. These groups of mice then received an injection of DOI (2.5 mg/kg i.p.) at either 24, 48 or 120 hr after the injection of ketanserin or its vehicle. The induced behavior was scored for the next 20 min at 2-min intervals after the DOI injection. The DOI-induced HTR scores for vehicle-pretreated groups were pooled, and their mean score (±S.E.M.) was used as control. To investigate the effects of repetitive administration of ketanserin, mice were injected once daily for 5 days with either ketanserin (1.0 mg/kg i.p., n = 18) or vehicle (n = 8). Animals were divided into three groups as described for acute studies and then received an injection of DOI (2.5 mg/kg i.p.) at either

24, 48 or 72 hr after cessation of ketanserin treatment. The induced behavior was scored and analyzed similar to that described for the acute studies.

Statistics. Data were analyzed by one-way analysis of variance and a 2-way analysis of variance with repeated measures. Post-hoc analysis was done by either Dunnett's t test or Scheffe's F test.

Results

Effects of a challenge dose of DOI at various time intervals after its first injection. In the first 20 min after a single administration, DOI (2.5 mg/kg) produced 37 ± 2 HTRs (fig. 1). When the animals were challenged with a second 2.5-mg/kg DOI dose 48 hr later, the total HTR frequency was significantly increased by 51% ($P < .05$). The cumulative increase in HTR score attained significance ($P < .05$) as early as the 6th min of the observation period and persisted throughout the experiment. When a second group of animals was challenged with DOI 72 hr after the initial injection, the total HTR score was still significantly ($P < .05$) increased (46% greater than on day 1). A third group of animals received the challenge DOI dose 144 hr after the first injection and produced 48 ± 3 HTRs (a 29% increase over day 1, which was statistically significant, $P < .05$). However, the cumulative HTR frequency was only significantly different from day 1 values for observation time intervals 16 to 20 min (data not shown). Animals that were injected with the second DOI dose 192 hr after the first injection produced a maximal frequency of behavior that was similar to day 1 control (fig. 1). It is interesting to note that their cumulative HTR scores between the 2- to 6-min time intervals were significantly lower than their corresponding values on day 1. Recording of cumulative HTR score in such repetitive short-time intervals reveals significant differences between control and treated groups which would be unnoticed if the total frequency of the behavior was counted in one 20-min period.

Effects of repeated once daily injections of DOI for 13 days. A single injection of DOI on day 1 produced a total of 39 ± 2 head twitches in the 20-min observation period (fig. 2). When these mice were challenged with the same DOI dose 24 hr later (day 2), the total HTR score was significantly reduced by 41% (*i.e.*, 23 ± 1, $P < .05$). This significant decrease was

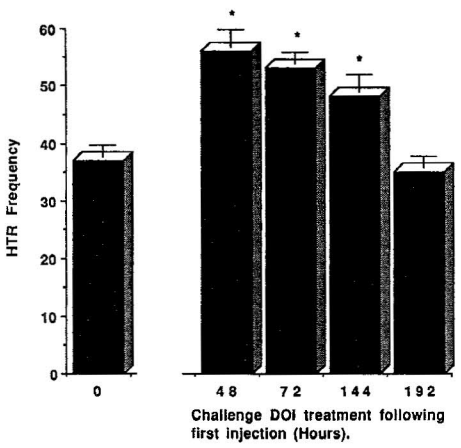

Fig. 1. Time-dependent effects of a challenge dose of DOI (2.5 mg/kg) on the induced HTR frequency at 48, 72, 144 or 192 hr after its single administration. Results are given as the frequency of behavior (mean ± S.E.M.) produced within 20 min after its injection. *Significantly different from control by Dunnett's t test at $P < .05$.

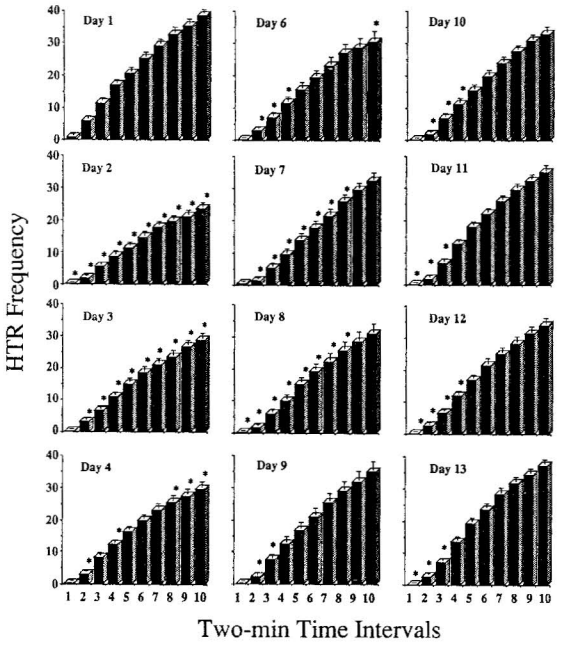

Fig. 2. Effects of 13 daily DOI injections (2.5 mg/kg once a day) on the induced HTR frequency. Results are shown as the cumulative mean ± S.E.M. HTR score at 2-min intervals for the first 20 min after DOI injection for each treatment day. *Significantly different from corresponding 2-min time intervals on day 1 (Dunnett's t test, $P < .05$). On day 5 the HTR score for the stated time intervals were not significantly different from respective controls and therefore was not included in the figure. However, in both published and unpublished findings from our laboratory, the animals exhibited significant reductions in HTR frequency on treatment day 5.

apparent from the onset of the observation period. During the daily observation period, the total HTR frequencies on days 3 and 4 (30 ± 2 and 29 ± 2 HTRs, respectively) were significantly less than that on day 1. As can be seen in figure 2, the differences relative to day 1 control were apparent from the onset and persisted throughout the observation period. On day 5 the pattern and intensity of responding was not significantly different from that observed on day 1. However, on day 6 the total HTR score (31 ± 3) was significantly ($P < .05$) less than that on day 1. On this day, the cumulative scores for some time intervals were not significantly different from the corresponding time periods on day 1. From day 7 to day 13, the daily DOI injections produced the same total HTR frequency that was observed on day 1. However, on day 7, the cumulative HTR scores between 2- to 16-min observation periods were significantly different from the corresponding scores on day 1. The duration of this difference was reduced slowly with increasing daily DOI injections so that on day 13 the cumulative HTR frequency was only significantly different from the corresponding day 1 controls at time intervals of 0 to 6 min (fig. 2).

Effects of a challenge dose of DOI after the 13-day DOI treatment regimen. Forty eight hours after cessation of chronic treatment, a challenge dose of DOI produced 57 ± 2 HTRs in the 20-min observation period which was 68% greater ($P < .05$) than that produced by DOI in the vehicle-treated controls (34 ± 1) (fig. 3). Different groups of DOI-treated animals were challenged with DOI 96, 144 or 240 hr after cessation of chronic treatment. The challenge injection of DOI produced significant ($P < .05$) increases in HTR at 96 and 144 hr, but not at 240 hr (fig. 3). The significant difference in cumulative HTR count between the vehicle-exposed controls

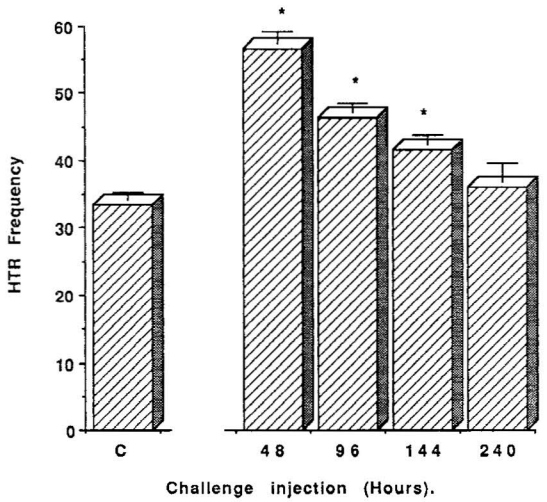

Fig. 3. Effects of a challenge dose of DOI (2.5 mg/kg) on HTR frequency at various time intervals after cessation of a 13-day DOI treatment (2.5 mg/kg/day). Results are given as the total HTR frequency observed immediately after DOI injection. These HTR scores are compared with the HTR frequency produced by 2.5 mg/kg of DOI after cessation of daily vehicle treatment (C) by the Dunnett's *t* test (*P < .05).

and DOI-pretreated animals was generally apparent from the third observation time interval, which then persisted throughout the duration of the observation period. The DOI challenge 240 hr after cessation of chronic agonist treatment produced a total HTR frequency that was not significantly different from control. However, the HTR counts were significantly lower than vehicle controls between the 3rd- and 8th-min observation time intervals. In order to examine further the effects of DOI during this withdrawal period, the chronically DOI-treated group that was challenged 48 hr after cessation of chronic treatment was treated further with once daily DOI injections on days 16, 17 and 18. On these days the HTR frequencies for the DOI-exposed group were significantly reduced relative to day 15 DOI-exposed animals (table 1). However, the HTR frequency on these days was not significantly different from water-exposed animals on day 15. After day 18, the DOI treatment was discontinued for 5 days. Thus, on day 23 these mice were again challenged with DOI and exhibited a significant increase in HTRs.

Effects of acute administration of ketanserin on DOI-induced HTR. A single injection of ketanserin (1 mg/kg) administered 24 hr before DOI resulted in only 25 ± 2 head twitches which was 32% less ($P < .05$) than that in a corresponding vehicle-pretreated group (fig. 4A). Furthermore, a greater degree of reduction (57% of control) in HTR frequency (21 ± 1, $P < .05$) was observed when ketanserin-treated mice were injected with DOI 48 hr after the antagonist injection. However, when DOI was injected 120 hr after the ketanserin injection, the behavior was similar to that observed in the control group (fig. 4A). Because the HTR frequency elicited by a challenge dose of DOI can be either decreased or increased when DOI is administered 24 or 48 hr after its initial administration, respectively, it was of interest to determine the possible effects of a challenge dose of DOI in animals which were treated initially with DOI and then 1.5 hr later with ketanserin (1.0 mg/kg). The challenge dose of DOI 24 hr after the combination DOI-ketanserin treatment resulted in significantly reduced behavior (23 ± 1 HTRs) relative to vehicle-treated controls (fig. 4B). This degree of reduction (62% of control) is similar to that

TABLE 1

Effects of repeated once-daily DOI treatment 2 days after withdrawal from a 13-day chronic DOI (2.5 mg/kg, once daily) treatment[a]

Two groups of mice were either chronically treated with vehicle or DOI for 13 days. On day 15 both groups received a challenge dose of DOI (2.5 mg/kg) and the induced-HTR frequency was recorded for both groups. The chronically DOI-treated group was further injected with 2.5 mg/kg of DOI on days 16, 17 and 18. The treatment was ceased for 5 days and on day 23 these treated animals were again injected with DOI (2.5 mg/kg). After each injection the induced behavior was recorded in 2-min intervals for 20 min as described under "Methods."

	2-min Observation Time Intervals									
	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	16–18	18–20
Day 15 Vehicle (N = 19)	0.1 ± 0.1	2.7 ± 0.4	7.3 ± 0.4	11.7 ± 1.2	15.8 ± 0.6	20 ± 0.8	23.9 ± 0.9	26.9 ± 0.9	30.4 ± 0.9	35.5 ± 1.0
Day 15 DOI (N = 14)	0.3 ± 0.1	3.8 ± 0.4*	11.2 ± 0.9*	18.0 ± 1.2*	25.7 ± 1.3*	32.7 ± 1.5*	38.9 ± 1.3*	45.7 ± 1.4*	51.7 ± 1.6*	56.7 ± 1.8*
Day 16 DOI (N = 14)	0.4 ± 0.1	2.9 ± 0.3	8.3 ± 0.6	13.4 ± 0.9	19.0 ± 1.2	23.0 ± 1.3**	27.2 ± 1.3**	31.3 ± 1.3**	34.8 ± 1.4**	36.9 ± 1.4**
Day 17 DOI (N = 14)	0.6 ± 0.2	3.6 ± 0.4	8.6 ± 0.8	14.2 ± 0.8	19.7 ± 1.2	24.1 ± 1.3**	28.7 ± 1.5**	32.3 ± 1.6**	36.6 ± 1.6*,**	40.1 ± 1.7*,**
Day 18 DOI (N = 14)	0.3 ± 0.2	3.0 ± 0.3	8.1 ± 0.7	13.3 ± 1.1	17.9 ± 1.5**	22.9 ± 1.6**	26.7 ± 2.0**	31.2 ± 2.1**	35.8 ± 2.2**	39.6 ± 2.5**
Day 23 DOI (N = 14)	0.9 ± 0.3	8.0 ± 0.5*,**	15.8 ± 0.8*	23.3 ± 1.0	30.3 ± 1.2*	36.2 ± 1.1	42.4 ± 1.3*	47.0 ± 1.4*	52.8 ± 1.5*	57.1 ± 1.9*

[a] The HTR score for each 2-min interval for the various treatments was analyzed by one-way analysis of variance. Dunnett's *t* test was used to determine the significance of difference ($P < .05$) between chronically vehicle-treated mice on day 15 and the different DOI-treated groups (*). The Scheffe F test was used to compare the DOI-treated group on day 15 with other treatment days (**$P < .05$).

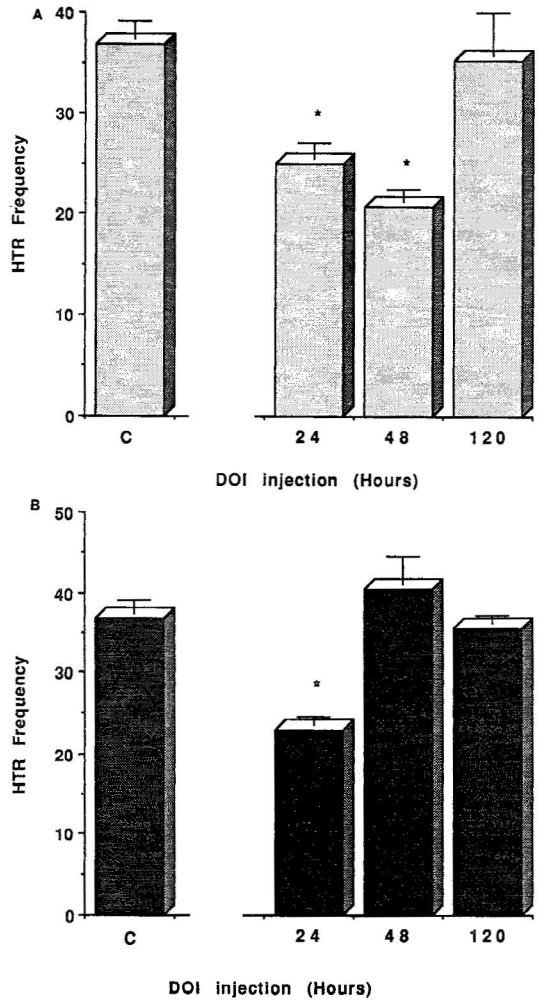

Fig. 4. Effects of DOI administration (2.5 mg/kg) after either a single administration of ketanserin (1.0 mg/kg) (A) or a combination of DOI (2.5 mg/kg) and ketanserin (1.0 mg/kg) injection (B) (DOI was administered first and 1.5 hr later ketanserin was injected. The DOI challenge dose was then administered at the indicated time intervals after ketanserin injection). The total score (mean ± S.E.M.) obtained at 20 min after the challenge DOI injection was compared by the Dunnett's *t* test with the HTR frequency induced by DOI in vehicle-pretreated controls (C). *Significantly different at $P < .05$.

observed with DOI in the ketanserin pretreated group (68% of control) (fig. 4A). However, unlike the reduction in the frequency in the ketanserin pretreated group, the ketanserin- + DOI-treated animals exhibited no reduction ($P < .05$) in HTR count (40 ± 4) when they were challenged 48 hr later with DOI after the initial treatment (fig. 4B). Similar to the ketanserin pretreated group, DOI- + ketanserin-treated animals produced a frequency of behavior similar to that of vehicle control when they were challenged with DOI 120 hr after their initial respective treatments (fig. 4, A and B).

Effects of chronic ketanserin administration on DOI-induced HTR. When administered 24 hr after cessation of chronic ketanserin treatment, DOI produced a frequency of behavior (22 ± 2 HTRs) which was significantly less (61% of control) than that induced in respective vehicle control (36 ± 1) (fig. 5). The degree of significance was apparent from the 4th min of observation ($P < .05$). However, when DOI was administered 48 hr after cessation of the chronic ketanserin regimen, the HTR frequency (56 ± 3) was significantly increased by 56% relative to that of control (fig. 5). The significance of difference was apparent from the 6th min and persisted throughout the 20-min observation period ($P < .05$). When another group of chronically ketanserin-treated mice was injected with DOI 72 hr after termination of antagonist treatment, the animals produced only 34 ± 2 HTRs which was not significantly different from the corresponding controls (fig. 5).

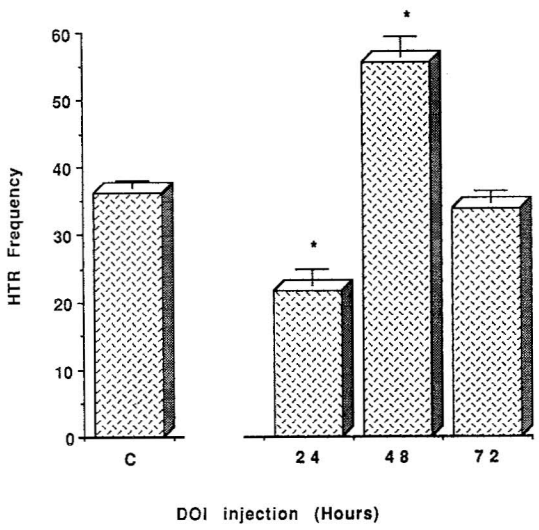

Fig. 5. Effects of DOI administration (2.5 mg/kg) after cessation of daily treatment with ketanserin (1.0 mg/kg/day for 5 days). Results are presented as total HTR score (mean ± S.E.M.) produced by DOI during the 20 min after its injection. *Significantly different from HTR score produced by DOI after daily vehicle exposure (C) ($P < .05$).

Discussion

It is now well established that DOI, a phenylisopropylamine, can induce the HTR both in mice (Darmani *et al.*, 1990b) and in rats (Arnt and Hytell, 1989), presumably *via* stimulation of 5-HT_2 receptors. The most important finding of the present investigation is that, when a challenge dose of DOI was administered to mice 48 hr after an initial injection, a significant increase occurred in the frequency of the induced behavior. This supersensitivity persisted up to 6 days after the initial single DOI injection. However, the degree of supersensitivity decreased with time so that at 8 days after the initial injection the induced HTR frequency was not significantly different from the initial values on day 1. Supersensitivity did not occur if mice were challenged with DOI within 24 hr after the initial injection. In fact, a significant decrease in HTR frequency was observed after a 24-hr challenge dose which is consistent with published reports (Darmani *et al.*, 1990a).

As was reported previously (Darmani *et al.*, 1990a), daily DOI injections in the present investigation also reduced the HTR up to 6 days, which then slowly returned to day 1 control frequency by day 13. In addition, the present study shows that, although the total cumulative counts from day 7 to day 13 are not significantly different from day 1, other 2-min observation-interval cumulative scores are significantly different from corresponding controls. The times at which differences occur slowly diminish from the 16- to 18-min observation period on day 7 to the 2- to 6-min period on day 13. These results highlight the advantage of persistently monitoring the induced behavior at short intervals, and further indicate significant reductions in the functional sensitivity of 5-HT_2 receptors, which would

be unnoticed if the behavior was recorded in one 20-min observation interval. However, the mechanism responsible for this shift in the effect period is unclear.

A challenge dose of DOI 48 hr after cessation of daily DOI injections induced a degree of supersensitivity similar to that observed after a 2-day challenge dose after a single injection of DOI. This supersensitive state also persisted for a similar length of time and disappeared completely 10 days (240 hr) after cessation of chronic treatment. The supersensitivity condition can revert to normal sensitivity if animals are reinjected with DOI. For example, upon DOI challenge 48 hr after the chronic DOI regimen HTR was reduced to water control values when these mice were reinjected with DOI on days 16, 17 and 18. In addition, the supersensitive state was once again evident on day 23 (*i.e.*, when persistent DOI treatment had ceased on day 18 and animals were subsequently challenged with DOI on day 23). Thus, the induction of a supersensitive state is a reversible process, and its recurrence is dependent upon the time lag between the challenge dose and the last DOI injection after cessation of treatment.

Several investigators have reported significant reductions in 5-HT$_2$ receptor density (22–43%) after chronic DOI treatment (Buckholtz *et al.*, 1988; McKenna *et al.*, 1989; Leysen *et al.*, 1989). This reported reduction in receptor capacity does not correlate with the supersensitivity exhibited in the present investigation, when mice were challenged with DOI 48 hr after cessation of chronic agonist treatment. Thus, the present results suggest that down-regulation of 5-HT$_2$ receptors can lead to an increase in HTR frequency. Similar changes between receptor density and function also appeared to occur after a single DOI injection. For example, several investigators have reported that administration of a single, moderate dose of DOI does not affect 5-HT$_2$ receptor content in the rat frontal cortex (McKenna *et al.*, 1989; Buckholtz *et al.*, 1988). However, in the present behavioral investigation, a second DOI injection 24 hr after its initial administration reduced HTR frequency significantly by 41%. Moreover, 48 hr after the initial DOI injection, a challenge dose of the drug increased the HTR score by 51%. Thus, the change in receptor function induced by a single injection of DOI does not appear to follow the reported lack of change in 5-HT$_2$ receptor density.

The present data underscore the complexity of the adaptive mechanisms of the 5-HT system. Leysen *et al.* (1989) reported that administration of 5-HT$_2$ agonists in rats can cause a rapid decrease in HTR frequency and down-regulation of 5-HT$_2$ receptors. Furthermore, they showed that a rapid desensitization of 5-HT-induced inositol phosphate formation occurs in vascular muscle cells in culture upon prolonged agonist exposure (Leysen and Pauwels, 1990). This reduction in 5-HT$_2$ receptor transduction returned to normal levels within hours after termination of agonist exposure, whereas 5-HT$_2$ receptor number recovered slowly to normal levels by 6 to 10 days. Thus, on agonist exposure, the ability of 5-HT$_2$ receptor systems to induce reversible up- or down-regulation of its function in relatively short periods of time suggest that changes in the sensitivity of the transduction mechanism may also have profound effects on the induction of behavioral response. Therefore, it is possible that desensitization and sensitization produced by daily administration of DOI could involve a combination of receptor/effector uncoupling as well as alterations in the the number of 5-HT$_2$ receptors.

Acute administration of antagonists appears to reduce both 5-HT$_2$ receptor density and affinity (Blackshear and Sanders-Bush, 1982; Blackshear *et al.*, 1986; Leysen *et al.*, 1985, 1986). Behavioral studies also suggest a decrease in 5-HT$_2$ receptor-mediated behaviors after antagonist treatment (Blackshear and Sanders-Bush, 1982; Ortman *et al.*, 1982; Smith *et al.*, 1990). Our present results provide further evidence for such a decrease in 5-HT$_2$ receptor function, inasmuch as acute administration of ketanserin significantly reduced the HTR frequency when the behavior was induced by DOI 24 to 48 hr after antagonist injection. This effect was not apparent when mice were injected with DOI 120 hr after antagonist administration. However, the effects of a combination of DOI and ketanserin pretreatment are not additive. In addition, no significant effect was observed when animals were challenged with DOI either 48 or 120 hr after initial agonist/antagonist pretreatment. It appears that the agonist administration before ketanserin injection prevents the long-lasting effects of acute ketanserin treatment. The fact that a challenge dose of DOI 48 hr after a single agonist injection induces supersensitivity and subsensitivity after acute antagonist administration suggests that these opposing effects offset each other.

Chronic administration of 5-HT$_2$ receptor antagonists or antidepressants with affinity for 5-HT$_2$ sites tends to reduce 5-HT$_2$ receptor density (Blackshear and Sanders-Bush, 1982; Blackshear *et al.*, 1986; Kendall and Nahorski, 1985; Leysen *et al.*, 1986; May *et al.*, 1986; Sanders-Bush *et al.*, 1989; Twist *et al.*, 1990b). However, the chronic effects of such agents on 5-HT$_2$ receptor behavioral function are less clear. For example, chronic exposure has been reported to either attenuate (Blackshear and Sanders-Bush, 1982) or enhance (Friedman *et al.*, 1983; Mogilnicka and Klimek, 1979; Stoltz *et al.*, 1983) 5-HT$_2$-induced HTR. Induction of supersensitivity by agonists in the presence of reduced 5-HT$_2$ receptor number after withdrawal from such chronic antagonist exposure is once again a paradox. Although direct evidence is lacking, it is possible that chronic antagonist exposure can differentially affect receptor capacity and signal transduction. It has been known for some years that acute administration of 5-HT$_2$ antagonists inhibit 5-HT-stimulated phosphoinositide hydrolysis (Conn and Sanders-Bush, 1987). In addition, more recently the latter investigators have suggested that, after acute antagonist treatment, adaptation of the transduction system more accurately correlates with behavior rather than changes in 5-HT$_2$ receptor density (Smith *et al.*, 1990). Prolonged exposure to antidepressants (*e.g.*, amitriptyline, imipramine or desipramine) or 5-HT$_2$ receptor antagonists such as mianserin also tend to reduce 5-HT-induced phosphoinositide turnover (Kendall and Nahroski, 1985; Conn and Sanders-Bush, 1986; Godfrey *et al.*, 1988; Sanders-Bush *et al.*, 1989). However, recently Twist *et al.* (1990a) have reported that chronic ritanserin exposure produces a differential increase in 5-HT-induced phosphoinositide hydrolysis and a concomitant decrease in 5-HT$_2$ receptor density.

To account for the anomalous adaptation of 5-HT$_2$ receptor density after chronic exposure to antagonists, Leysen *et al.* (1989) proposed that 5-HT$_2$ receptors normally exist in a supersensitive state and cannot develop further supersensitivity. Our current results argue against the inability of development of supersensitivity after chronic administration of either 5-HT$_2$ agonists or antagonists. Furthermore, the present investigation along with other published data suggest that acute administration of either agonists or antagonists affect the functional sensitivity of 5-HT$_2$ receptors rather than their density. On

chronic administration, agonists or antagonists can independently affect both receptor density and sensitivity. Such an hypothesis can explain our present behavioral results where discontinuation of repeated administration of both agonists and antagonists induced supersensitivity in the presence of the previously reported reduced receptor density. Furthermore, depending upon the properties of serotonergic drugs, the present hypothesis suggests that serotonergic drugs can have the ability to change independently receptor sensitivity and density in the same or opposite directions. Thus, the behavioral outcome from agonist and antagonist administration will probably depend upon a combination of changes in receptor sensitivity and receptor density.

References

ARNT, J. AND HYTELL, J.: Facilitation of 8-OH-DPAT-induced forepaw treading of rats by the 5-HT_2 agonist DOI. Eur. J. Pharmacol. **161:** 45–51, 1989.

BLACKSHEAR, M. A., MARTIN, L. L. AND SANDERS-BUSH, E.: Adaptive changes in the 5-HT_2 binding site after chronic administration of agonists and antagonists. Neuropharmacology **25:** 1267–1271, 1986.

BLACKSHEAR, M. A. AND SANDERS-BUSH, E.: Serotonin receptor sensitivity after acute and chronic treatment with mianserin. J. Pharmacol. Exp. Ther. **221:** 303–308, 1982.

BUCKHOLTZ, N. S., ZHOU, D. AND FREEDMAN, D. X.: $Serotonin_2$ agonist administration down-regulates rat brain $serotonin_2$ receptors. Life Sci. **42:** 2439–2445, 1988.

CONN, P. J. AND SANDERS-BUSH, E.: Regulation of serotonin-stimulated phosphoinositide hydrolysis: Relation to serotonin 5-HT_2 binding site. J. Neurosci. **6:** 3669–3675, 1986.

CONN, P. J. AND SANDERS-BUSH, E.: Central serotonin receptors: Effector systems, physiological roles and regulation. Psychopharmacology **92:** 267–277, 1987.

DARMANI, N. A., MARTIN, B. R. AND GLENNON, R. A.: Withdrawal from chronic treatment with (±)-DOI causes supersensitivity to 5-HT_2 receptor-induced head-twitch behavior in mice. Eur. J. Pharmacol. **186:** 115–118, 1990a.

DARMANI, N. A., MARTIN, B. R., PANDEY, U. AND GLENNON, R. A.: Do functional relationships exist between 5-HT_{1A} and 5-HT_2 receptors? Pharmacol. Biochem. Behav. **36:** 901–906, 1990b.

FRIEDMAN, E., COOPER, T. B. AND DALLOB, A.: Effects of chronic antidepressants on serotonin receptor activity in mice. Eur. J. Pharmacol. **89:** 69–76, 1983.

GLENNON, R. A.: Serotonin receptors: Clinical implications. Neurosci. Biobehav. Rev. **14:** 35–47, 1990.

GLENNON, R. A., TITLER, M. AND MCKENNEY, J. D.: Evidence for 5-HT_2 involvement in the mechanism of action of hallucinogenic agents. Life Sci. **35:** 2505–2511, 1984.

GODFREY, P. P., MCCLUE, S. J., YOUNG, M. M. AND HEAL, D. J. L.: 5-Hydroxytryptamine-stimulated inositol phosphate phospholipid hydrolysis in the mouse cortex has pharmacological characteristics compatible with mediation via 5-HT_2 receptors but this response does not reflect altered 5-HT_2 function after 5,7-dihydroxytryptamine lesioning or repeated antidepressant treatments. J. Neurochem. **50:** 730–738, 1988.

HANDLEY, S. L. AND SINGH, L.: Neurotransmitters and shaking behavior: More than a "gut bath" for the brain. Trends Pharmacol. Sci. **7:** 324–328, 1986.

KENDALL, D. A. AND NAHORSKI, S. R.: 5-Hydroxytryptamine-stimulated inositol phospholipid hydrolysis in rat cerebral cortex slices: Pharmacological characterization and effects of antidepressants. J. Pharmacol. Exp. Ther. **233:** 473–479, 1985.

LEYSEN, J. E., GOMMEREN, W., VAN GOMPEL, P., WYNATS, J., JANSSEN, P. F. M. AND LADURON, P. M.: Receptor-binding properties *in vitro* and *in vivo* of ritanserin: A very potent and long acting serotonin-S_2 antagonist. Mol. Pharmacol. **27:** 600–611, 1985.

LEYSEN, J. E., JANSSEN, P. F. M. AND NIEMEGEERS, C. J. E.: Rapid desensitization and down-regulation of 5-HT_2 receptors by DOM treatment. Eur. J. Pharmacol. **163:** 145–149, 1989.

LEYSEN, J. E. AND PAUWELS, P. J.: Central and peripheral 5-HT_2 receptors: Role in physiological *versus* pathological conditions. *In* Serotonin: From Cell Biology to Pharmacology and Therapeutics, ed. by R. Paoletti, P. M. Vanhoute, N. Brunello and F. M. Maggi, pp. 323–329, Kluwer Academic Press, Dordrecht, 1990.

LEYSEN, J. E., VAN GOMPEL, P., GOMMEREN, W., WOESTENBORGHS, R. AND JANSSEN, P. A. G.: Down regulation of serotonin-S_2 receptor sites in rat brain by chronic treatment with the serotonin-S_2 antagonists, ritanserin and setoperone. Psychopharmacology **88:** 434–444, 1986.

MAY, P. C., MORGAN, D. G. AND FINCH, C. E.: Regional serotonin receptor studies. Chronic methysergide treatment induces a selective and dose-dependent decrease in serotonin-2 receptors in the mouse cerebral cortex. Life Sci. **38:** 1741–1747, 1986.

MCKENNA, D. J., NAZARLI, A. J., HIMENO, A. AND SAAVEDRA, J. M.: Chronic treatment with (±) DOI, a psychotomimetic 5-HT_2 agonist, down regulates 5-HT_2 receptors in rat brain. Neuropsychopharmacology **2:** 81–87, 1989.

MOGILNICKA, E. AND KLIMEK, V.: Mianserin, danitracen and amitriptyline withdrawal increases the behavioral responses of rats to L-5-HTP. Commun. J. Pharm. Pharmacol. **31:** 704–705, 1979.

ORTMANN, R., BISCHOFF, S., RADEKE, E., BUECH, O. AND DELII-STULA, A.: Correlation between different measures of antiserotonin activity of drugs. Naunyn-Schmeideberg's Arch. Pharmacol. **321:** 265–270, 1982.

RICHARDSON, B. P., ENGLE, G., DONATSCH, P. AND STADLER, P. A.: Identification of serotonin m-receptor subtypes and their specific blockade by a new class of drugs. Nature (Lond.) **316:** 126–131, 1985.

SANDERS-BUSH, E., BREEDING, M., KNOTH, K. AND TSUTSUMI, M.: Sertraline-induced desensitization of the serotonin 5-HT_2 receptor transmembrane signaling system. Psychopharmacology **99:** 64–69, 1989.

SCHMIDT, A. W. AND PEROUTKA, S. J.: 5-Hydroxytryptamine receptor families. Fed. Am. Soc. Exp. Biol. J. **3:** 2242–2249, 1989.

SMITH, R. L., BARRETT, R. J. AND SANDERS-BUSH, E.: Adaptation of brain 5-HT_2 receptors after mianserin treatment: Receptor sensitivity, not receptor binding, more accurately correlates with behavior. J. Pharmacol. Exp. Ther. **254:** 484–488, 1990.

STOLTZ, J. F., MARSDEN, C. A. AND MIDDLEMISS, D. N.: Effects of chronic antidepressant treatment and subsequent withdrawal of ^{3}H-5-hydroxytryptamine and ^{3}H-spiperone binding in rat frontal cortex and serotonin receptor mediated behavior. Psychopharmacology **80:** 150–155, 1983.

TWIST, E. C., BRAMMER, M. J., STEPHENSON, J. D., CORN, T. H. AND CAMPBELL, I. C.: The effect of chronic ritanserin and clorgyline administration on 5-HT_2 receptor linked inositol phosphate hydrolysis. Biochem. Pharmacol. **40:** 2111–2116, 1990a.

TWIST, E. C., MITCHELL, S., BRAZELL, C., STAHL, S. M. AND CAMPBELL, I. C.: 5-HT_2 receptors changes in rat cortex and platelets following chronic ritanserin and clorgyline administration. Biochem. Pharmacol. **39:** 161–166, 1990b.

Send reprint requests to: Professor B. R. Martin, Department of Pharmacology/Toxicology, P.O. Box 613, MCV Station, Richmond, VA 23298.