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# Further Evidence for Enigmas in Adaptation Mechanisms for the DOI-Induced Behaviors

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DARMANI, N. A. *Further evidence for enigmas in adaptation mechanisms for the DOI-induced behaviors.* PHARMACOL BIOCHEM BEHAV 43(3) 765–770, 1992.—The acute and chronic effects of the 5-hydroxytryptamine$_{2/1C}$ (5-HT$_{2/1C}$) receptor agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and the antagonist ketanserin were evaluated on the DOI-induced 5-HT$_2$ receptor-mediated ear-scratch response (ESR) in mice. A challenge dose of DOI (2.5 mg/kg) administered 24 h following its first injection reduced the ESR frequency by 80–97%. The ESR score attained first injection value when the time lag between the first and the second injection was greater than 72 h. On the other hand, a single administration of ketanserin (1.0 mg/kg) caused no significant effect at 24 or 48 h but significantly reduced (51%, $p < 0.05$) the DOI-induced ESR 120 h following its injection. Chronic once-daily DOI injections reduced the ESR score by 80–97% throughout the treatment regimen. Following cessation from chronic treatment, the DOI-induced ESR frequency returned to control levels in a time-dependent manner. Repeated ketanserin administration significantly reduced the DOI-induced ESR score by 46% when tested 24 or 48 h following cessation of antagonist administration but had no effect at 78 h. Recently, we reported that 48 h following either a single DOI injection or termination from repeated DOI or ketanserin administration the DOI-induced head-twitch response (HTR) in mice exhibited supersensitivity. Thus, it appears that the DOI-induced behaviors exhibit differential adaptation mechanisms following either agonist or antagonist exposure. These studies further support our hypothesis that serotonergic drugs may have the ability to change independently the 5-HT-receptor sensitivity (signal transduction) and receptor density in the same or opposite directions. The present study also suggest that the DOI-induced behaviors are probably produced via stimulation of two receptors (5-HT$_2$ and 5-HT$_{1C}$). However, due to lack of selective agents one cannot designate a particular behavior for a specific receptor site.

Ear-scratch response  Acute  Chronic  Adaptation  DOI  Ketanserin

SEROTONIN [5-hydroxytryptamine (5-HT)] acts as a neurotransmitter both in the periphery and CNS. The concept of multiple functional 5-HT receptors was born in the 1950s and nearly three decades later Peroutka and Snyder (33) radiochemically defined two serotonin binding sites in the rat cortex. In the last decade, research interest in this field has been overwhelming and up to four major classes of 5-HT receptors have been recognized (5-HT$_1$, 5-HT$_2$, 5-HT$_3$, and 5-HT$_4$) (16). In addition, some of these receptors appear to be heterogeneous and consist of a number of subtypes. For example, the 5-HT$_1$ class of receptors appears to consist of at least five subtypes (5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, 5-HT$_{1D}$, and 5-HT$_{1E}$). Anatomic, biochemical, and behavioral correlates have been reported for some of these sites (17,22,37).

Serotonergic-induced behaviors are often used to study 5-HT receptor function. Administration of the phenylethylamine- [e.g., (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)] or the indolamine- (e.g., 5-MeO DMT or LSD) hallucinogens to mice result in the induction of the head-twitch response (HTR). This effect is mediated via the stimulation of 5-HT$_2$ receptors and can be attenuated by 5-HT$_2$ receptor-selective antagonists (10,17). A more curious but much less investigated behavioral repertoire, the ear-scratch response (ESR) or hind-limb scratching, is also produced when mice are injected with phenylethylamine-type hallucinogens (24). The indolamine hallucinogens, however, do not produce the ESR (11,44). Indeed, such agents [e.g., LSD (4) and 5-MeO DMT (11)] inhibit the phenylethylamine-induced ESR in a dose-dependent manner. Using different 5-HT$_2$ receptor-selective agonists and antagonists, Darmani et al. (11) postulated that, similar to the HTR, the DOI-induced production of ESR is also a 5-HT$_2$ receptor-mediated phenomenon. These authors further suggested that the inability of the indolamine hallucinogens to produce ESR and their potent inhibitory effects on the phenylethylamine-induced scratching resides in the fact that the former agents are nonselective agonists and stimulate a number of 5-HT receptor subtypes including the 5-HT$_{1B}$ sites. Simultaneous costimulation of 5-HT$_{1B}$ receptors by the indolamine hallucinogens or by the "more selective" 5-HT$_{1B}$ agonists (such as TFMPP or RU 24969) dose

dependently inhibit the induced ESR (11). The phenylethylamine hallucinogens only bind to the 5-$HT_{1C}$ and 5-$HT_2$ receptors (16).

Although the acute effects of ESR-inducing drugs have been studied to some extent, no chronic study has been undertaken to investigate the adaptation mechanisms of this unique behavior. Monoamine receptor regulation theory suggests that chronic deprivation of postsynaptic receptor stimulation (e.g., by a loss of presynaptic input or receptor blockade) results in an increase in receptor number (disuse supersensitivity) while persistent receptor excitation leads to a decrease in receptor density (downregulation). Numerous studies suggest that the 5-$HT_2$ receptor adaptation system does not follow this pattern of events. For example, chronic exposure to 5-$HT_2$ receptor antagonists tends to decrease rather than increase 5-$HT_2$ receptor number (3,28,43). Ironically, chronic administration of 5-$HT_2$ receptor agonists also decreases 5-$HT_2$ receptor density (5,29). Moreover, repeated administration of these agonists or antagonists causes behavioral subsensitivity (7,8,27,32), while withdrawal from such chronic treatments leads to behavioral supersensitivity, that is, an increase in HTR frequency to a challenge dose of a 5-$HT_2$ agonist (7,8,32,40). The aim of the present investigation was to determine whether chronic exposure to a 5-$HT_2$ receptor agonist (DOI) or antagonist (ketanserin) can induce similar adaptation mechanisms in the induction of ESR in mice.

## METHOD

### *Subjects*

Male albino mice of the ICR strain, bred in the animal facilities of the Kirksville College of Osteopathic Medicine, were used. Animals weighed 16–20 g at the beginning of the experiments and were housed in groups of six on a 12 L : 12 D cycle at a room temperature of 22 ± 1°C with ad lib supply of food and water. All experiments were performed between 8:00 a.m. and 5:00 p.m.

### *Agonist Studies*

To habituate mice to the observation environment, each mouse was randomly transferred 20 min prior to treatment to a 45 × 25 × 20-cm plastic cage. To determine the acute effects of DOI, mice ($n$ = 13) were injected with a 2.5-mg/kg IP dose of DOI. This dose has previously been shown to produce a robust ESR frequency in mice (11). The ESR frequency was scored cumulatively at 2-min intervals for the next 20 min following DOI injection. These data were used as control for subsequent injections of DOI. To investigate the possible development of either acute tolerance or supersensitivity by a single injection of DOI, a large number of mice ($n$ = 39) were injected with a 2.5-mg/kg dose of DOI. These animals were then divided into three groups that were then challenged with a second dose of DOI (2.5 mg/kg) either 48 ($n$ = 13), 72 ($n$ = 13), or 144 ($n$ = 13) h following the initial single DOI administration. Following injection of the challenge dose, the DOI-induced behavior was scored for each animal for the next 20 min as described previously.

For the investigation of the effects of repeated agonist administration, mice were injected with DOI (2.5 mg/kg, IP, $n$ = 8) once daily for 4 days and the ESR frequency was scored as described above following each daily injection. Following the last day of observation, animals were divided into two groups. The different groups of mice were then challenged with DOI (2.5 mg/kg) either 48 (day 6, $n$ = 4) or 72 (day 7, $n$ = 4) h following cessation of daily treatment. The results from day 1 were used as control. Recently, Darmani et al. (7,8) have shown that persistent once-daily injections of DOI for 13 days caused an initial reduction in HTR score that then returned to day 1 control level following the sixth day of DOI treatment. To investigate such a possible adaptation mechanism for the ESR, another group of mice were injected with DOI (2.5 mg/kg, IP, $n$ = 13) once daily for 13 days and the induced-ESR frequency was scored as described above following each daily injection. The results from day 1 were used as control. To investigate the effects of a challenge dose of DOI following withdrawal from such a prolonged treatment, mice were treated once daily for 13 days with either distilled water ($n$ = 18) or 2.5 mg/kg DOI ($n$ = 30). After the 13th day, the treatment was stopped and mice were divided into three groups. Each group contained vehicle- ($n$ = 6) and DOI- ($n$ = 10) treated mice. Different groups of mice were then challenged with DOI (2.5 mg/kg) either 48, 96, or 144 h following cessation of chronic treatment. The behavior was scored following the challenge dose of DOI as described for the acute studies. The chronically water-exposed groups were then pooled and their mean score ± SEM was used as control.

### *Antagonist Studies*

The acute effects of antagonist treatment on DOI-induced ESR was investigated by pretreating mice with either vehicle ($n$ = 6) or ketanserin (1.0 mg/kg, IP, $n$ = 18). These animals were divided into three groups, each of which consisted of a number of vehicle- ($n$ = 2) and ketanserin-treated mice ($n$ = 6). These groups of mice then received an injection of DOI (2.5 mg/kg, IP) at either 24, 48, or 120 h following injection of ketanserin or its vehicle. The induced behavior was scored for the next 20 min following DOI injection. The DOI-induced ESR scores for vehicle-pretreated groups were pooled, and their mean (± SEM) was used as control. To investigate the effects of repetitive administration of ketanserin, mice were injected for 5 days with either ketanserin (1.0 mg/kg, IP, $n$ = 18) or vehicle ($n$ = 6). Animals were divided into three groups as described for the acute studies and then received an injection of DOI (2.5 mg/kg, IP) at either 24, 48, or 72 h following cessation of chronic treatment. The DOI-induced ESR was recorded as for the acute studies.

### *Drugs*

The following drugs were obtained from Research Biochemicals (Natick, MA): DOI HCl and ketanserin tartrate. Distilled water was used as vehicle for DOI whereas ketanserin was dissolved in acidified distilled water. Both drugs were given at a volume of 10 mg/kg.

### *Statistical Analysis*

Data were analyzed by one-way analysis of variance (ANOVA) and a two-way ANOVA with repeated measures. Post-hoc analysis was done by either Dunnett's $t$-test or Scheffe's $f$-test.

## RESULTS

### *Agonist Studies*

A single dose of 2.5 mg/kg DOI produced 46 ± 5 ESRs in the 20-min observation period (Fig. 1). When such treated mice were challenged 48 h later with a second 2.5-mg/kg DOI dose, the mean ESR frequency (9 ± 3) was significantly re-

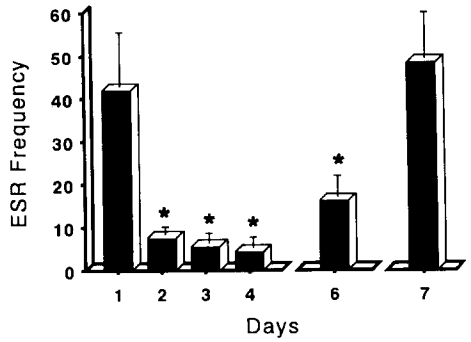


FIG. 1. Time-response effects of a challenge dose of DOI (2.5 mg/kg) on the induced ESR frequency at 48, 72, and 144 h following its single injection (0). Results are given as means (±SEM) ESR frequency produced in the 20-min observation period following its injection. *Significantly different from control (0) ($p < 0.05$, $n = 13$ per group).

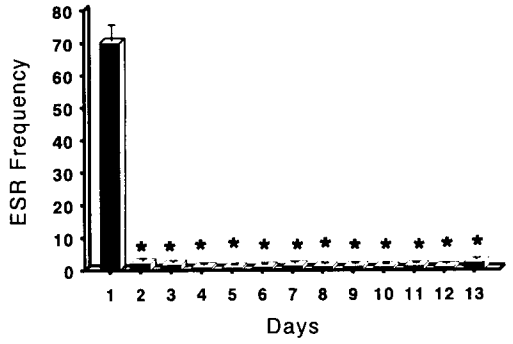


FIG. 3. Effects of acute (day 1, $n = 13$) and chronic once-daily (days 2-13, $n = 13$) DOI injections (2.5 mg/kg) on the induced ESR frequency. Results are presented as mean (±SEM) obtained in the 20-min observation period immediately following injection. *Significantly different from day 1 control.

duced by 80% of day 1 control ($p < 0.05$). A second DOI dose (2.5 mg/kg) administered 72 h following the first injection produced 28 ± 4 ESRs (39% reduction relative to day 1, $p < 0.05$). Following 144 h pause between the first and second injections, the DOI challenge dose produced a frequency of behavior (47 ± 6) not significantly different from that following a single injection of the hallucinogenic agent (Fig. 1).

In an initial preliminary chronic study, a single injection of DOI (2.5 mg/kg) produced 42 ± 13 ESRs. Upon a second injection on day 2, the mean ESR frequency (8 ± 2) was significantly reduced by 80% ($p < 0.05$) (Fig. 2). Upon further DOI challenge on days 3 and 4, the mean ESR scores were further reduced by 84 and 91%, respectively. When such treated mice (2.5 mg/kg DOI treatment, once daily for 4 days) were challenged with DOI (2.5 mg/kg) 48 h (i.e., day 6) following the last injection, the mean ESR score was 16 ± 5 (a 62% reduction relative to day 1 control) (Fig. 2). When mice were challenged 72 h following termination of the 4-day repeated-treatment regimen, the ESR score (49 ± 11) returned to day 1 control level. Because the number of animals ($n = 4$-8) in this preliminary chronic experiment was relatively small, and because recent reports (7,8) revealed that the DOI-induced head-twitch score was initially reduced by daily DOI treatment and then returned to control levels upon persistent treatment, the above experiment was repeated. Mice ($n = 13$) were treated with DOI (2.5 mg/kg) once daily for 13 days. The initial injection on day 1 produced 70 ± 5 ESRs (Fig. 3). The subsequent once-daily DOI injections (treatment days 2-13) only produced 1-2 ESRs in the corresponding 20-min observation periods (i.e., 97-98% reduction relative to day 1 control, $p < 0.05$). In order to study the effects of a challenge dose of DOI (2.5 mg/kg) following cessation of chronic treatment, mice were treated either with distilled water ($n = 18$) or 2.5 mg/kg DOI ($n = 30$) once daily for 13 days. Different groups of vehicle-exposed mice ($n = 6$) were injected with DOI (2.5 mg/kg) either 48, 96, or 144 h following cessation of chronic vehicle treatment. In these test schedules, the mean DOI-induced ESR scores did not significantly vary from each other and were therefore combined to give one control group (51 ± 4 ESRs, $n = 18$) (Fig. 4). The chronically DOI-exposed mice were similarly divided into three groups. These different groups of mice ($n = 10$) received a challenge DOI injection either 48 ($n = 10$), 96 ($n = 10$), or 144 ($n = 10$) h following

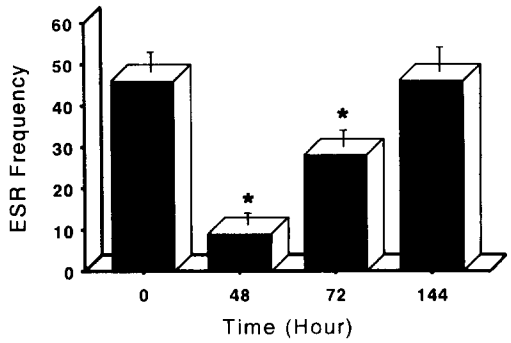


FIG. 2. Effects of acute (day 1, $n = 8$) and repeated once-daily injections (days 2-4, $n = 8$) of DOI (2.5 mg/kg) on the mean (±SEM) ESR frequency. Animals were divided into two groups and were further treated with one additional injection of DOI either 24 (day 5, $n = 4$) or 48 h (day 6, $n = 4$) following cessation of once-daily treatment. *Significantly different from day 1 control ($p < 0.05$).

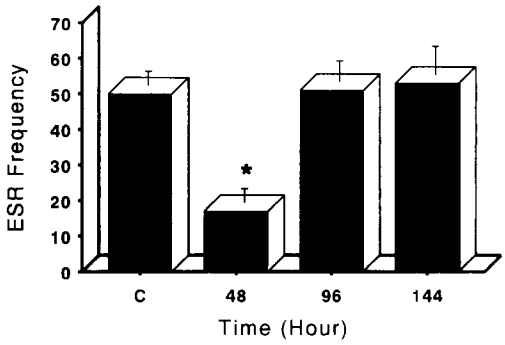


FIG. 4. Time-response effects of a challenge dose of DOI (2.5 mg/kg) on the ESR frequency at 48, 96, and 144 h following cessation of a 13-day DOI treatment (2.5 mg/kg/day). Results are given as the total ESR frequency observed in the 20-min observation period immediately following DOI injection. These ESR scores are compared with the mean ESR frequency produced by 2.5 mg/kg DOI following cessation of chronic once-daily vehicle treatment (C) by Dunnett's $t$-test (*$p < 0.05$, $n = 10$-18).

cessation of chronic DOI regimen. The 48-h challenge group exhibited a 67% reduction in ESR frequency relative to vehicle-exposed controls. The 96- and 144-h challenge groups produced mean frequencies of behavior (51 ± 6 and 53 ± 8) not significantly different from vehicle control (Fig. 4).

*Antagonist Studies*

A single administration of DOI (2.5 mg/kg) 24 h following an injection of acidified distilled water (vehicle control) produced 55 ± 8 ESRs in the 20-min observation period (Fig. 5). In the ketanserin- (1.0 mg/kg) pretreated mice, the same DOI challenge test produced a mean ESR score (48 ± 9) slightly less than in the vehicle control group ($p > 0.05$). When DOI was administered 48 h following the single injection of ketanserin, the mean ESR score (39 ± 10) was further reduced (relative to vehicle control) but did not reach the 95% significance. However, when DOI was injected 120 h following the single injection of ketanserin the ESR score (27 ± 8) was significantly reduced by 51% (Fig. 5). Twenty-four hours following cessation of repeated ketanserin administration, the 2.5-mg/kg DOI dose produced 31 ± 7 ESRs, 46% less ($p < 0.05$) than that produced in the chronically vehicle-exposed group (58 ± 6) (Fig. 6). A similar degree of reduction was apparent when ketanserin-exposed mice were challenged with DOI 48 h after termination of chronic ketanserin treatment. The DOI-induced ESR score returned to control values 78 h following cessation of chronic ketanserin injections (43 ± 9, $p > 0.05$).

## DISCUSSION

The isopropylamine hallucinogens such as DOI induce the ESR in mice via the stimulation of 5-$HT_2$ receptors (11). The behavior seems to be species specific in that such selective 5-$HT_{2/1C}$ agonists induce the behavior in mice only (12) and the effect is centrally mediated (24). Mice and rats also exhibit the behavior following either central or spinal administration of several peptides (e.g., substance P), neurophysiological hormones, and some excitatory amino acids (13,20,23, 30,31,39). Induction of ESR by these agents appears to involve alteration in serotonergic activity because pretreatment

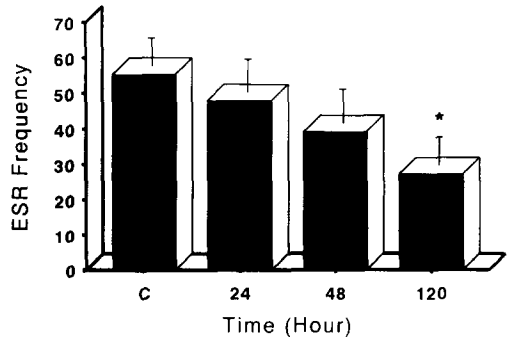


FIG. 5. Time-response effects of DOI (2.5 mg/kg)-induced ESR following a single injection of ketanserin (1.0 mg/kg). The mean score (±SEM) obtained in the 20-min observation period following the challenge DOI injection was compared by Dunnett's *t*-test with the ESR frequency produced by DOI (2.5 mg/kg) in vehicle-pretreated controls (C). *Significantly different from vehicle control at $p < 0.05$ ($n = 6$).

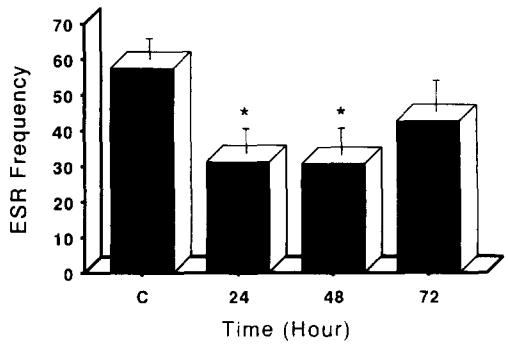


FIG. 6. Time-response effects of DOI administration (2.5 mg/kg) on the ESR frequency following termination of daily treatment with ketanserin (1.0 mg/kg/day for 5 days). Results are given as mean ESR score (±SEM) obtained in the 20-min observation period immediately following DOI injection. *Significantly different from ESR frequency produced by DOI (2.5 mg/kg) following daily vehicle treatment (C) ($p < 0.05$, $n = 6$).

with peptide antagonists attenuates serotonergically induced ESR (14), whereas coinjection of peptide and serotonergic agonists have additive effects (31). Moreover, modification of serotonergic function alters the ability of such agents to produce ESR (15,21). Furthermore, central injection of such agents also induces the HTR in rodents and 5-$HT_2$ receptor antagonists attenuate the induced behavior (19). In the rat, serotonergic agonists with high affinity for 5-$HT_{1D}$ receptors can produce ESR via peripheral mechanisms, and 5-$HT_{2/1C}$ agents inhibit the induced behavior (1).

One important finding of the present study is that a single injection of DOI reduced the ESR score by 80–97% when mice were challenged with the same dose 24 h later. A similar refractoriness to a second injection of mescaline and other phenylethylamines has already been reported (24). The present study extends these observations in that the induced refractoriness slowly dissipates with time so that the ESR frequency attains first injection score when the time lag between the first and second injections is over 72 h. This pattern of adaptation completely differs from that exhibited by the head-twitch behavior, where a challenge dose of DOI 24 h following the first injection has been shown to reduce the HTR frequency by only 41% (8). Second, these authors have shown that the HTR score was increased by over 50% when the second DOI dose was administered 48 h (or more) following its initial injection. This supersensitivity persisted up to 8 days following the first DOI administration. A single injection of a moderate dose of DOI does not reduce the 5-$HT_2$ receptor density when tested up to 48 h following its injection (5,9,29). Thus, a near total decrease in ESR score by a second DOI dose 48 h following its first injection in the presence of no apparent change in receptor parameters appears to be a paradox.

Many studies have shown that acute antagonist administration can reduce both the affinity and density of 5-$HT_2$ receptors (2,3,25,28). A significant change in density without alteration in 5-$HT_2$ receptor affinity 48 h following an acute injection of ketanserin has been reported (35). Published behavioral reports support these binding studies (3,8,38). In the present investigation, a time-dependent decrease in the DOI-induced ESR score was apparent and became significantly different from control levels 120 h following the single ketanserin injection. However, Darmani et al. (8) have shown that the

DOI-induced HTR score following a similar treatment schedule exhibited significant reduction at 24 and 48 but not 120 h after antagonist administration.

These agonist- and antagonist-induced differential adaptation mechanisms for the ESR and HTR suggest that: a) the 5-$HT_2$ receptors can adapt themselves via different mechanisms or b) the two behaviors are produced by different 5-HT receptor subtypes. Because DOI has been reported to have a similar (16) or 40-fold higher (41) affinity for 5-$HT_2$ over 5-$HT_{1C}$ receptors, it is probable that the ESR and HTR are produced via selective stimulation of these two different sites. Because no selective agonist or antagonist exists for either of these sites, no definitive conclusion can be drawn. However, there are several antagonists with partial selectivity for these sites. For example, spiperone has a 2000-fold selectivity for 5-$HT_2$ vs. 5-$HT_{1C}$ whereas ketanserin is only 50 times more selective (36). Thus, it would be expected that acute pretreatment with these antagonists would exhibit differential inhibitory dose-response effects on these DOI-induced behaviors. However, both antagonists appear to be equipotent in inhibiting the DOI-induced ESR and HTR (10,11).

Following a single administration, once-daily repeated administration of the same DOI dose for 4 or 13 days reduced the ESR score by 80–98%. Upon cessation from such repeated injection schedules, the DOI-induced ESR score exhibited a time-dependent (72–96 h) return to control values. Withdrawal from repeated once-daily ketanserin treatment significantly reduced the DOI-induced ESR score up to 48 h following the last injection of the antagonist. Thus, the present study suggests that the mechanisms that allow adaptation of the ESR following chronic exposure to either agonist or antagonist cause a downregulation in ESR function. This agrees well with the multitude of published data that chronic exposure to both agonists and antagonists decreases 5-$HT_2$ receptor density (2,3,5,26,28,29,34,43). However, the near complete reduction in the ESR frequency following repeated agonist exposure does not agree with the reported 22–55% reduction in 5-$HT_2$ receptor density following similar DOI treatments (5,9,29). In addition, the adaptation mechanisms for the DOI-induced HTR following such repeated daily injections is reported to be an initial 41% reduction in HTR frequency, which slowly returned to 95% control by day 13 of the treatment schedule (8). Moreover, withdrawal from similar chronic exposure to either DOI or ketanserin was shown to induce HTR supersensitivity to a challenge dose of DOI. Thus, again like the acute studies, the ESR and HTR behaviors appear to be differentially adapted following chronic exposure to agonists or antagonists. As discussed in the introductory section, the 5-$HT_2$ receptor adaptation mechanisms appear not to follow the monoamine adaptation dogma because prolonged exposure to both agonists and antagonists induces a decrease in receptor number. Moreover, recent acute antagonist studies indicate that changes in the signal transduction mechanisms more accurately correlate with 5-$HT_2$ receptor-induced behavior rather than receptor density (38). In addition, although prolonged exposure to 5-$HT_2$ antagonists tends to reduce phosphoinositide (PI) turnover when determined up to 48 h following the last antagonist injection (6,18), recently Twist et al. (42) have shown a differential increase in the 5-HT-induced PI turnover and a concomitant decrease in 5-$HT_2$ receptor density 72 h following cessation of antagonist treatment. Furthermore, prolonged agonist exposure has been reported to induce a rapid decrease in PI turnover that returned to control levels within hours, whereas the reduced 5-$HT_2$ receptor density attained control values after several days (27). These biochemical studies suggest that postreceptor mechanisms are of paramount importance to the overall adaptation mechanisms of the 5-$HT_2$ receptor system. The present and cited studies support our recent hypothesis that "serotonergic drugs may have the ability to change independently receptor sensitivity and density in the same or opposite directions" (8). The present investigation further suggests the possibility that either 5-$HT_2$ receptors can adapt themselves differentially in different parts of the CNS or the DOI-induced ESR and HTR in mice are produced by two different 5-HT (5-$HT_2$ and 5-$HT_{1C}$) receptor sites. These supposals can explain the ability of DOI and ketanserin pretreatment (acute and chronic) to produce differential adaptations in the functional responses of DOI-induced ESR and HTR in the presence of paradoxical changes in 5-$HT_2$ receptor density in the CNS.

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