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Temporal Differential Adaptation of Head-Twitch and Ear-Scratch Responses Following Administration of Challenge Doses of DOI

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DARMANI, N. A. and C. F. GERDES. *Temporal differential adaptation of head-twitch and ear-scratch responses following administration of challenge doses of DOI.* PHARMACOL BIOCHEM BEHAV **50**(4) 545–550, 1995.—Previously, we reported that administration of the 5-$HT_{2A/C}$ receptor agonist, DOI [(±)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane], can simultaneously produce the head-twitch response (HTR) and the ear-scratch response (ESR) in mice. Our recent studies have indicated that the HTR is a 5-HT_{2A} receptor-mediated phenomenon, whereas the ESR is probably a 5-HT_{2C} receptor-mediated event. The HTR and ESR exhibit subsensitivity to a challenge dose of DOI (2.5 mg/kg) administered 24 h after its acute or termination of its chronic (2.5 mg/kg, once daily for 13 days) administration. When the dose interval for the challenge dose of DOI was increased to 48 h, both the acute- and chronically treated mice exhibited a simultaneous supersensitive HTR response and a subsensitive ESR effect. The purpose of the present study was to investigate the dose-response effects of lower challenge doses of DOI 48 h following their respective first injections as well as determining the effects of repeated DOI injections at 2-h intervals for 8 h. Thus, in the present study, initial administration of DOI produced a dose- and time-dependent increase in the mean frequencies of both HTR and ESR. Significant HTRs were observed after administration of the lowest tested dose of DOI (0.25 mg/kg), whereas a robust frequency of ESR was only evident at 1 mg/kg or greater doses of DOI. A 48-h challenge administration of lower doses of DOI (0.25 and 0.5 mg/kg) did not significantly affect their respective first injection HTR scores. However, larger challenge doses of DOI (1 and 2.5 mg/kg) produced supersensitivity in the mean HTR score (+46% and +40%, respectively, $p < 0.05$) and subsensitivity in the mean ESR frequency (−92% and −67%, respectively, $p < 0.05$) relative to their first injection control values. All administered doses of DOI (0.25, 0.5, or 1 mg/kg) eventually significantly reduced their first injection (control) HTR scores when injected repeatedly at 2-h intervals. Significant HTR reductions were attained quicker for the larger DOI doses. It appears that a mouse needs to receive either cumulatively or in a single injection about 1 mg/kg dose of DOI prior to exhibiting a significant reduction in the HTR score in response to further administration of DOI. As with their initial first injection ESR scores, repeated administration of lower doses of DOI (0.25 and 0.5 mg/kg) did not produce a significant effect. However, the 1 mg/kg challenge dose of DOI, 2 h following its first injection, nearly completely attenuated its first injection control ESR score. Further repeated injections of DOI at 2-h intervals did not cause additional alteration in the mean ESR score. The present results indicate that adaptation mechanisms for the DOI-induced HTR and ESR are different, and this difference is probably a reflection of the adaptation mechanisms of the 5-HT_{2A}- and 5-HT_{2C}-receptor function.

Head-twitch response Ear-scratch response Adaptation 5-HT_{2A} 5-HT_{2C} Receptor DOI

THE CONTINUOUS explosion of publications regarding 5-hydroxytryptamine (5-HT) receptors has prompted the serotonin club to propose a new nomenclature system that incorporates the more recent findings (12). According to the new proposal, the 5-HT_1 receptor subtypes now consists of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F} sites. At least three 5-HT_2 receptor subtypes have been recognized (5-HT_{2A-C}). The 5-HT_{2A} refers to the classical 5-HT_2 receptor, 5-HT_{2B} (formerly 5-HT_{2F}) mediates serotonin's contractile action in the fundus, and the 5-HT_{2C} receptor corresponds to

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the previously known 5-HT_{1C} site. Other serotonergic receptor subclasses include the 5-HT_3, 5-HT_4, 5-HT_5, and 5-HT_6 groups (12,15).

Only a few behavioral correlates expressing the functional activation of particular serotonergic receptor subtypes have been critically evaluated (10). Specifically, animal models expressing activation of the serotonergic 5-HT_{2A} receptor subtype have been used to study its adaptation mechanisms following treatment with serotonergic agonists or antagonists [e.g., (5,6)]. The head-twitch response (HTR) is a 5-HT_{2A}-mediated event, whereas the ear-scratch response appears to be a 5-HT_{2C}-induced behavior (9). The 5-$HT_{2A/C}$ agonist, DOI, is reported to have either a similar (11) or up to 40-fold higher (20) affinity for 5-HT_{2A} vs. 5-HT_{2C} sites. DOI at 2.5 mg/kg (IP) can simultaneously induce both behaviors in mice (7,8) and in least shrews (*Cryptotis parva*) (9). However, lower doses of DOI (< 1 mg/kg) can only produce a robust frequency of HTR in the cited animals. Moreover, the 5-HT_{2C} antagonist with 5-HT_{2A} agonist action, lisuride (4), only induces the head-twitch behavior in least shrews (9).

Recently, we reported that the HTR and ESR exhibit differential adaptation mechanisms to a challenge dose of DOI (2.5 mg/kg) following acute or chronic (once daily for 13 days) pretreatment with a 2.5 mg/kg dose of DOI (5,6). For example, a challenge dose of DOI administered in mice 24 h following its first injection reduced the ESR frequency by 80–90%. The ESR score attained first injection value when the time lag between the first and the second injection was greater than 72 h. Chronic once-daily DOI injections reduced the ESR frequency in a similar manner, and the induced behavior returned to control levels in a time-dependent manner following cessation of treatment. On the other hand, the HTR exhibited supersensitivity when a challenge dose of DOI was administered 48 h (or greater) following either a single DOI injection or termination from repeated DOI administration. The purpose of the present study was to further investigate the adaptation mechanisms of the cited behaviors using lower doses of DOI. Thus, the present study determined: a) the dose-response effects of lower doses of DOI 48 h following their initial administration, and b) the effects of repeated 2-h administration of lower doses of DOI on the cited behaviors.

METHOD

Subjects and Drugs

Male albino ICR mice (Sprague-Dawley, Indianapolis, IN), weighing 18-22 g, were used throughout the study. The animals were kept in groups of five to 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 0800 and 1700 h.

Measurements of HTR and ESR

The HTR is a very distinctive head-twitching behavior in mice 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 frequency was scored at 5-min intervals by trained observers using multiple tally counters. The ESR is a rapid scratching movement of the head, neck, or lateral area by either hind limb. The ESR episodes were also scored by a tally counter. An ESR episode produced by a particular hind limb consisted of one or more repetitive scratches with less than a 2-s rest in between. If the interval between consecutive scratches by a particular hind limb was greater than 2 s, then the scratches were considered as separate episodes. If scratches were produced by alternative hind legs, then each scratch was considered as a separate episode.

Experimental Protocol

The initial goal of the study was to determine the lowest DOI dose necessary to induce a significant change in the cited behaviors when mice were challenged with corresponding doses of DOI 48 h later. To habituate the mice to the test environment, each animal was transferred randomly 20 min before treatment to a 40 × 25 × 26 cm plastic cage lined with a thin layer of wood chippings. Initially, mice were injected intraperitoneally with varying doses of DOI (0.25, 0.5, 1, and 2.5 mg/kg, n = 5-7). The HTR and ESR frequencies were scored cumulatively at 5-min intervals for the next 20 min

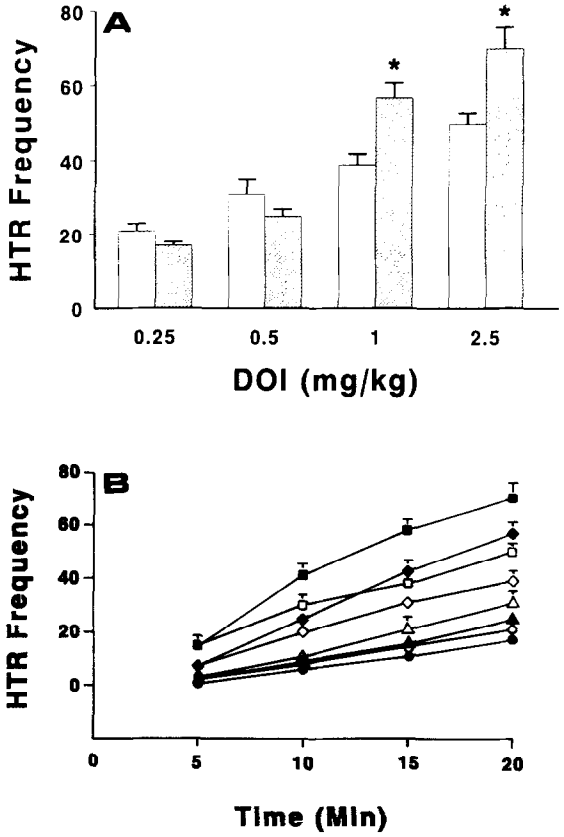

FIG. 1. (A) The dose-response effect of DOI on the production of total head-twitch response (HTR) in mice observed for 20 min immediately after injection. Results are given as means (± SEM). Blank columns represent DOI's first injection dose-response effects, whereas hatched columns depict the effects of challenge doses of DOI administered 48 h after their respective initial administration. *Significantly different from respective first injection 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 20 min. Open symbols represent first injection control HTR scores, whereas closed symbols depict the HTR frequencies for the challenge doses of DOI administered to their respective groups 48 h later. (DOI doses in mg/kg are: ○, ● = 0.25; △, ▲ = 0.5; ◇, ◆ = 1; □, ■ = 2.5). For statistical details, see the text.

after DOI administration. The data obtained were used as control for subsequent injection of the corresponding doses of DOI 48 h later. Thus, 48 h following the initial test, each group received their corresponding challenge doses of DOI and the cited behaviors were recorded as described above. The second goal of the study was to investigate the effects of repeated DOI administration at 2-h intervals. Different groups of mice were injected with corresponding doses of DOI (0.25, 0.5, and 1 mg/kg, $n = 5$) at 0, 2, 4, 6, and 8 h repeatedly, and the cited behaviors were recorded as described previously. The data obtained at 0 h was considered as control.

(±)-DOI HCl was purchased from Research Biochemicals Inc., Natick, MA. DOI was dissolved in distilled water and given intraperitoneally (IP) at a volume of 10 ml/kg.

Statistical Analysis

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

RESULTS

The 48-h Challenge Study

The 5-HT$_{2A/C}$ agonist, DOI, produced a dose- and time-dependent increase in the HTR frequency following its administration in mice (Fig. 1). Relative to its lowest dose (0.25 mg/kg), the 1 and 2.5 mg/kg doses of DOI produced significantly greater ($p < 0.05$) total HTR frequencies in the 20-min observation period as shown by both Dunnett's *t*-test and Scheffe's *F*-test (Fig. 1A). The significant effect was apparent from the first 5-min observation interval and persisted throughout the experiment (Fig. 1B). Similar statistical differences between the lowest (0.25 mg/kg) and higher doses of DOI (1 and 2.5 mg/kg) were observed when these mice were injected with respective challenge doses of the agonist 48 h later (Fig. 1). When data from the first injection and the 48-h challenge doses of DOI were directly compared for each respective dose, the total HTR scores were not different for the lower doses of DOI (0.25 and 0.5 mg/kg) (Fig. 1A). However, the 1 and 2.5 mg/kg challenge DOI doses produced significantly greater effects (46% and 40%, respectively, $p < 0.05$) relative to their respective first-injection control values (Fig. 1). The significant increases were apparent from the 15-min observation period for the 1 mg/kg dose and from the 10-min observation interval for the 2.5 mg/kg DOI dose (Fig. 1B).

DOI also induced a dose- and time-dependent increase in the ESR frequency (Fig. 2). However, lower doses of DOI (0.25 and 0.5 mg/kg) did not produce a robust effect relative to previously reported water-treated control mice (8). The 1 and 2.5 mg/kg doses of DOI produced robust frequencies of ESR in the 20-min observation period (54 ± 10 and 85 ± 21, respectively) (Fig. 2A). Relative to the lowest dose, the 1 mg/kg DOI dose produced significantly greater ESR in the 15-min time interval following injection, and the effect persisted throughout the observation period (Fig. 2B). Its 2.5 mg/kg dose produced significant effects earlier (i.e., at the 10-min interval), which also persisted throughout the duration of the observation (Fig. 2B). Relative to the first-injection control ESR score, the respective challenge doses of DOI (> 1 mg/kg) administered 48 h following its initial administration produced significantly fewer responses. Thus, the 2.5 mg/kg challenge dose of DOI significantly attenuated its initial total ESR score by 67% (85 ± 21 vs. 28 ± 13) (Fig. 2A). The induced reduction attained significance from the 15-min observation

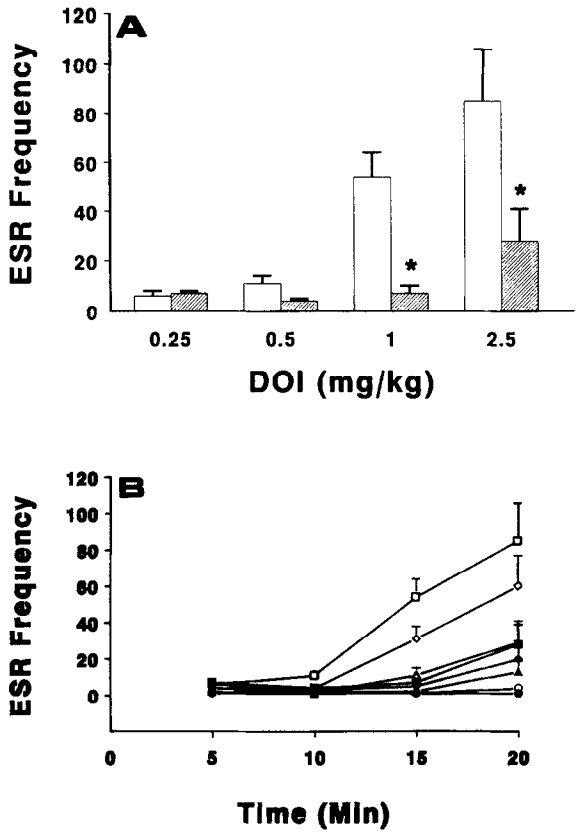

FIG. 2. (A) The dose-response effect of DOI on the production of total ear-scratch response (ESR) in mice observed for 20 min immediately following its injection. Results are given as means (± SEM). Blank columns represent DOI's first injection dose-response effects, whereas hatched columns depict the effects of challenge doses of DOI injected 48 h following their initial administration. *Significantly different from respective first injection control by Dunnett's *t*-test at $p < 0.05$. (B) The dose- and time-response effects of DOI on the production of ESR. The behavior was scored cumulatively immediately following injection at succeeding 5-min intervals for a period of 20 min. Open symbols represent first injection control HTR scores, whereas closed symbols depict the ESR frequencies for the challenge doses of DOI administered to their respective groups 48 h later. (DOI doses in mg/kg are: ○, ● = 0.25; △, ▲ = 0.5; ◇, ◆ = 1; □, ■ = 2.5). For statistical details, see the text.

interval (Fig. 2B). The 1 mg/kg challenge dose of DOI produced less than 10% (7 ± 3, $p < 0.05$) of its respective first injection value (54 ± 10) (Fig. 2A). This reduction also attained significance from the 15-min observation interval (Fig. 2B). The lower challenge doses of DOI (< 1 mg/kg) did not induce a significant effect relative to their respective first injection control groups (Fig. 2).

Repeated 2-h DOI Administration Study

Differential reductions in HTR frequency were observed when varying doses of DOI (0.25, 0.5 and 1 mg/kg) were repeatedly administered to different groups of mice at 2-h intervals (Fig. 3). The 0.25 mg/kg DOI dose produced 25 ± 3 HTRs (control) in the 20-min observation period following its first injection. The second 0.25 mg/kg DOI injection did not alter the mean total control HTR frequency. Its third and

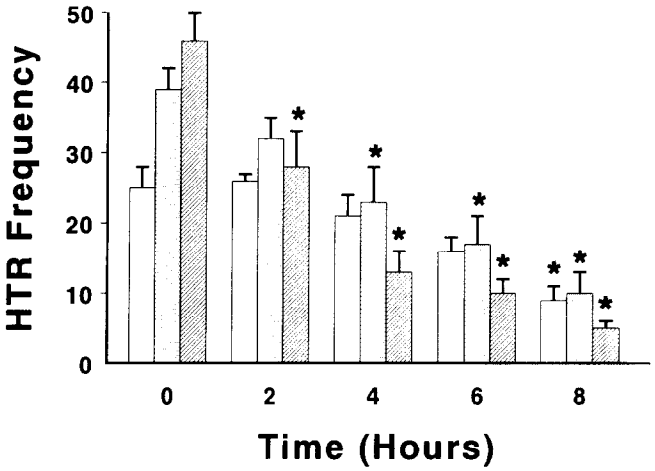

FIG. 3. Dose-response effects of 2-h repeated intraperitoneal administration of varying doses of DOI (clear column = 0.25; dotted column = 0.5, and slanted rule column = 1 mg/kg) on the production of total head-twitch response (HTR). The HTR was scored cumulatively at 5-min intervals immediately following each injection for 20 min. The mean HTR scores for the first injection of the cited doses of DOI (i.e., at zero time) were considered as respective controls for their subsequent 2-h injections. *Significantly different from their respective zero time controls by Dunnett's t-test at $p < 0.05$. For further statistical details, see the text.

fourth injections attenuated the control HTR frequency (16% and 36%, respectively, $p > 0.05$) but did not attain significance. However, its fifth injection significantly reduced the control HTR score by 64%. The 0.5 mg/kg DOI dose attenuated its initial (control) HTR score (39 ± 3) significantly (41%, $p < 0.05$) following its third repetitive injection (23 ± 5, $p < 0.05$). Its fourth and fifth injections further attenuated the control HTR score by 56% (17 ± 4) and 74% (10 ± 3), respectively. The second 1 mg/kg dose of DOI attenuated its first (control) injection score (46 ± 4) by 39% (28 ± 5, $p < 0.05$). Its subsequent third, fourth, and fifth injections further attenuated the control HTR score by 72 (13 ± 3), 78 (10 ± 2), and 90% (5 ± 2), respectively.

Unlike the HTR adaptation, the first injection of lower doses of DOI (0.25 and 0.5 mg/kg) did not produce a robust ESR effect, and subsequent repetitive injections of their respective doses failed to produce a significant alteration in the induced behavior (Fig. 4). However, at 1 mg/kg dose, the second DOI injection nearly totally attenuated (3 ± 1) its initial control injection score (38 ± 10). Its subsequent third, fourth, and fifth injections did not further reduce the ESR score.

DISCUSSION

In the present investigation, the 5-$HT_{2A/C}$ agonist, DOI, simultaneously and in a dose- and time-dependent manner produced the HTR and ESR in male albino ICR mice (Sprague-Dawley, Indianapolis, IN). These mice appear to be more sensitive to DOI relative to our previously stocked male albino ICR mice (Dominion Laboratories, Dublin, VA). Thus, in the present investigation, the dose-response effects of DOI for the induction of HTR and ESR have shifted to the left relative to our previous reports (7,8). However, the ESR/HTR ratio (= 1-2) for the 2.5 mg/kg dose of DOI appears to be the same, although greater frequencies of both behaviors were exhibited by the present mice. On the other hand, the ESR/HTR ratio to the cited dose of DOI is less than 0.3 in male albino ICR mice obtained from Hilltop (Scottdale, PA) (unpublished findings). As with our previously published data (7,8), the present mice exhibited significant HTR frequencies to both low and high doses of DOI within the first 5-min observation period, whereas substantial ESR score accumulated from the 5-min observation interval only to larger doses of DOI. As discussed in the introduction, DOI is known to possess either a similar or up to 40-fold higher affinity for the 5-HT_{2A} vs. 5-HT_{2C} sites. Initially, we considered both DOI-induced behaviors to be a 5-HT_{2A} receptor-mediated phenomenon because ketanserin and spiperone pretreatment equipotently attenuated these behaviors (7,8). We reasoned, if induction of either behavior is due to activation of 5-HT_{2C} sites, then ketanserin would have been considerably more potent in attenuating the DOI-induced responses because it has a 10-50-fold higher affinity for the 5-HT_{2A} relative to 5-HT_{2C} receptor, whereas spiperone exhibits up to 2000-fold selectivity for the 5-HT_{2A} vs. 5-HT_{2C} sites (21). However, more detailed challenge studies following acute or chronic exposure to either ketanserin (1 mg/kg) or DOI (2.5 mg/kg) indicated that the HTR and ESR are produced by activation of two different receptors, possibly via 5-HT_{2A} and 5-HT_{2C} sites (5,6). Our subsequent acute study in the least shrew (*Cryptotis parva*) appears to confirm this hypothesis because the 5-HT_{2C} antagonist with 5-HT_{2A} agonist action, lisuride (4), can induce the HTR but not the ESR (9). Although further studies are required, the present and published findings suggest that the HTR is a 5-HT_{2A}-mediated phenomenon and the ESR is possibly a 5-HT_{2C}-induced event.

One of the important findings of the present study is that a certain challenge dose of DOI administered 48 h after its initial injection produces significantly more HTRs. The DOI dose necessary to produce this supersensitivity is about 1 mg/kg (146% of first injection, $p < 0.05$). A larger dose of DOI (2.5 mg/kg) did not further increase the supersensitive response

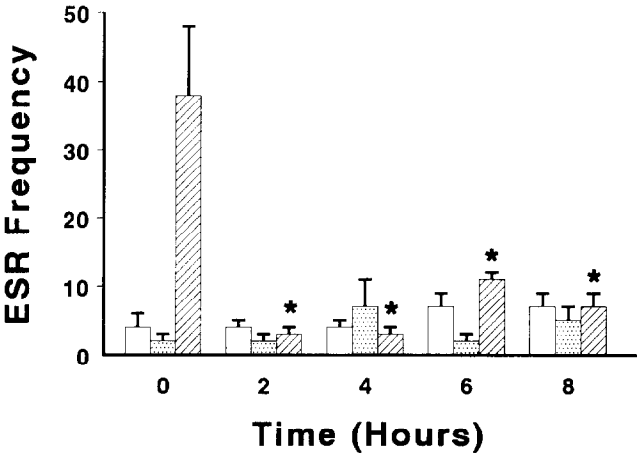

FIG. 4. Dose-response effects of 2-h repeated intraperitoneal injections of varying doses of DOI (open column = 0.25; dotted column = 0.5, and slanted rule column = 1 mg/kg) on the production of total ear-scratch response (ESR). The ESR was scored cumulatively at 5-min intervals immediately following each injection for 20 min. The mean ESR scores for the first injection of the cited doses of DOI (i.e., at zero time) were considered as respective controls for their subsequent 2-h injections. *Significantly different from their respective zero time controls by Dunnett's t-test at $p < 0.05$. For further statistical details, see the text.

(140% of its first injection). Previously, we had shown a 51% increase in HTR frequency to a 2.5 mg/kg challenge dose of DOI in ICR mice obtained from Dominion Laboratories (Dublin, VA) (6). Lower challenge doses of DOI (0.25 and 0.5 mg/kg) administered 48 h following their initial injection, however, failed to induce HTR supersensitivity. When mice were injected with DOI (0.25, 0.5, or 1 mg/kg) repeatedly at 2-h intervals for 8 h, all the cited doses of DOI eventually significantly attenuated the HTR score. However, the reduction only became significant when mice had cumulatively received more than 1 mg/kg DOI. Thus, its 0.25 mg/kg dose significantly reduced the mean HTR score following its fifth injection, whereas its 0.5 and 1 mg/kg doses produced significant reductions following their respective third and second injections. Therefore, it appears that mice have an internal mechanism that inactivates serotonergic 5-HT_{2A} receptor function when they receive more than 1 mg/kg of the hallucinogen.

Leysen et al. (13) have shown that repeated administration of DOM (an analog of DOI) at 8-h intervals for 24 h in rats causes a rapid decrease in HTR frequency and downregulation of central 5-HT_{2A} receptors. These authors further reported that a rapid desensitization of 5-HT-induced phosphoinositide (PI) formation occurs in vascular muscle cells in culture upon short-term DOM exposure (14). This reduction in 5-HT_{2A} receptor transduction mechanism returned to normal levels within hours after termination of agonist exposure, whereas 5-HT_{2A} receptor density recovered slowly to control level by 6 to 10 days. More recently, Akiyoshi et al. (1) have reported that when rat cerebral granular cells are exposed to DOI, a rapid and time-dependent desensitization of PI turnover occurs. The desensitization was detected within 30-min of DOI preincubation and reached a maximum decrement at 8-h exposure. Because a single injection of a moderate dose of DOI does not alter the central 5-HT_{2A} receptor density (3,16), it appears that alterations in PI hydrolysis has profound effects on the sensitivity of the DOI-induced behaviors. Indeed, drugs can exhibit differential effects on 5-HT_{2A}-receptor density and PI hydrolysis, as shown by Sanders-Bush's group, where a single dose of mianserin produced a significant decrease in the number of 5-HT_{2A} receptor sites (44%) 24 h after treatment, but loss of sites recovered 36 h postinjection and, therefore, receptor loss could not account for mianserin's continued antagonism of both PI hydrolysis and behavior (20). Thus, the observed HTR supersensitivity to a challenge dose of DOI 48 h following its first injection may also be due to alterations in signal transduction mechanisms.

Administration of low doses of DOI (0.25 and 0.5 mg/kg) did not produce a significant frequency of ESR. However, at moderate to high doses (1 and 2.5 mg/kg) the hallucinogen produced robust behavior. The inability of lower doses of DOI to induce the ESR is probably a reflection of its lower affinity for the 5-HT_{2C} site. A moderate challenge dose of DOI (1 mg/kg) administered either 2 or 48 h following its initial injection only produced less than 10% of the initial ESR score. Further injections of DOI at 2-h intervals failed to induce additional attenuation in the ESR frequency. At present, there is a lack of information regarding the adaptation mechanisms of serotonergic 5-HT_{2C} receptors. Available biochemical evidence indicates that a single injection of DOI does not alter 5-HT_{2C} receptor affinity nor its density in the rat spinal cord (17). However, chronic administration of agonists (such as quipazine or DOI) can reduce 5-HT_{2C} receptor density and 5-HT_{2C}-mediated PI turnover (2,17,19). To our knowledge, the effect of a challenge dose of DOI on the 5-HT_{2C}-mediated PI turnover following its initial administration has not been studied. It appears reasonable to suggest that the rapid reduction in the ESR score in mice is possibly due to desensitization of PI turnover response, whereas long-term DOI exposure could induce both a downregulation of 5-HT_{2C} receptors and subsensitivity of PI signal transduction mechanism.

In summary, the present and published results indicate that administration of DOI in mice simultaneously produces the HTR and ESR in a dose- and time-dependent manner. The induced behaviors appear to adapt differentially following administration of moderate to high challenge doses of DOI after its initial administration. Depending upon the dose interval, the HTR can exhibit either subsensitivity or supersensitivity, whereas the ESR adaptation reveals subsensitivity only.

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