Pharmacology Biochemistry & Behavior, Vol. 37, pp. 95–99. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Pharmacological Characterization of Ear-Scratch Response in Mice as a Behavioral Model for Selective 5-HT_2-Receptor Agonists and Evidence for 5-HT_{1B}- and 5-HT_2-Receptor Interactions

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Received 17 April 1990

DARMANI, N. A., B. R. MARTIN, U. PANDEY AND R. A. GLENNON. *Pharmacological characterization of ear-scratch response in mice as a behavioral model for selective 5-HT_2-receptor agonists and evidence for 5-HT_{1B}- and 5-HT_2-receptor interactions*. PHARMACOL BIOCHEM BEHAV **37**(1) 95–99, 1990.—(±)1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane [(±)-DOI], a phenylisopropylamine hallucinogen, is a 5-HT_2-receptor agonist. The drug induced a dose-dependent increase in ear-scratch response (ESR) in mice, and the R(−)-isomer was more than 6 times as potent as its S(+)-enantiomer. The induced behavior was potently inhibited by selective 5-HT_2-receptor antagonists such as ketanserin and spiperone. The (±)-DOI-induced ESR is also inhibited by stimulation of 5-HT_1-receptors and the inhibition seems to be through a 5-HT_{1B}-receptor mechanism. Thus, taken together, the present investigation indicates that ESR is due to selective stimulation of 5-HT_2-receptors and that simultaneous costimulation of 5-HT_{1B}-receptors inhibits the induced behavior. The study further suggests that the inability of the indolealkylamine hallucinogens to induce ESR is due to simultaneous excitation of 5-HT_{1B}-receptors which are inhibitory to induction of ESR. Moreover, the data suggest possible inhibitory control mechanisms through 5-HT_1-receptor subtypes to provide a damping mechanism to reduce excessive 5-HT_2-receptor excitation due to exogenous drug stimulation or pathological conditions.

Ear-scratch Interaction 5-HT_{1A}-receptor 5-HT_{1B}-receptor 5-HT_2-receptor

ADMINISTRATION of hallucinogenic phenalkylamines to mice results in stereotypic twitch-like movements of the head, referred to as the head-twitch response. This effect is believed to be mediated via a serotonergic 5-HT_2-receptor mechanism and can be attenuated by pretreatment of the animals with 5-HT_2-selective antagonists (8). Indolealkylamine hallucinogens such as 5-methoxy-N,N-dimethyltryptamine (5-MeO DMT) and (+)lysergic acid diethylamide (LSD) also elicit this response (8). A much less investigated behavioral response produced in mice by phenalkylamine hallucinogens is a rapid scratching movement of the head and/or neck area by a hindlimb. This effect, variously referred to as the scratch reflex, scratch-reflex stereotypy, or the ear-scratch response (ESR), was first reported for mescaline by Deegan and Cook (5) in 1958. The ESR is produced by other hallucinogenic phenalkylamines such as 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) and its ethyl and isopropyl homologs DOET and DOIP, but not by the inactive (as a hallucinogen) tertiary butyl homolog DOTB (10). The effect is reported to be stereoselective with the R(−)-enantiomers being the active isomers (12,15). Curiously, indolealkylamine hallucinogens do not produce the ESR and can, in fact, antagonize the ESR produced by, for example, DOM (1, 3, 15). Yim *et al.* (15) have suggested that production of the ESR may be a useful predictor of hallucinogenic activity for phenalkylamines but that it is not universally applicable because indolealkylamine hallucinogens appear to be inactive. For a recent review of the ESR, see Glennon (7).

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Because no detailed pharmacological investigation of the ESR has been reported, and because it is known to be elicited only by certain classes of hallucinogens, the purpose of the present investigation was (a) to further characterize the receptor system(s) involved in the ESR elicited by hallucinogenic phenalkylamines, and (b) attempt to explain why indolealkylamine hallucinogens do not produce this effect but are able to antagonize the ESR produced by phenalkylamine hallucinogens.

METHOD

Male albino (ICR) mice, weighing 16–18 g, were purchased from Dominion Laboratories (Dublin, VA). The animals were housed in groups of five on a 12-hour light-dark cycle and were allowed free access to food and water. In order to habituate the animals to the test environment, each animal was randomly transferred 30 minutes prior to treatment to a $40 \times 25 \times 16$ cm plastic cage lined with a thin layer of sawdust. Mice were injected intraperitoneally with doses of (±)-DOI (0.63, 1.25, 2.5 and 5 mg/kg) or distilled water (6 mice per group). The ear-scratch response (ESR) was scored every 2 minutes for the first 30 minutes following injection. Total mean scores (±S.E.M.) over the 30-minute period, as well as the mean scores (±S.E.M.) in each 2-minute interval were calculated. A dose of 2.5 mg/kg (±)-DOI was found to produce a reliable frequency of the induced behavior. Throughout the study, mice were never used more than once.

In order to determine which isomer of (±)-DOI was the more potent, each isomer was tested at a dose of 2.5 mg/kg. For drug interaction studies, (±)-DOI was used at a dose of 2.5 mg/kg and different doses of the other drugs were given in a random manner. Doses of 5-MeO DMT (0, 2, 4 and 8 mg/kg) were coadministered with a dose of 2.5 mg/kg of (±)-DOI. The drugs were injected together via the intraperitoneal route (4 mice per group). The selective 5-HT_{1A} agonist 8-OH DPAT was administered intraperitoneally at doses of 0, 0.5, 1 and 2.5 mg/kg 10 minutes prior to injection of (±)-DOI (6–8 animals per group). The selective 5-HT_{1B}/5-HT_{1C} agonist TFMPP (0.313, 0.625 and 1.25 mg/kg) was injected intraperitoneally 5 minutes prior to administration of (±)-DOI (5–7 animals per group). The more selective 5-HT_{1B} agonist RU 24969 (0, 1.25 mg/kg) was injected intraperitoneally ten minutes prior to administration of (±)-DOI. Doses of the 5-HT_2 antagonists ketanserin (0, 0.063, 0.125, 0.25 and 1 mg/kg subcutaneously) and spiperone (0, 0.05, 0.125 and 0.5 mg/kg IP) were administered 30 minutes prior to (±)-DOI injection (4 animals per group). The dosing schedules were based on our previous studies. In each case, the ear-scratch response was recorded in 2-minute intervals for 30 minutes after (±)-DOI injection as described above.

The following drugs were obtained from Research Biochemicals (Natick, MA): (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl [(±)-DOI], (±)-8-hydroxy-2-(di-n-propylamino)tetralin HBr (8-OH DPAT), 1-(3-trifluoromethylphenyl) piperazine HCl (TFMPP), ketanserin tartrate and spiperone. 5-Methoxy-3-(1,2,3,5-tetrahydropyridyl)indole succinate (RU 24969) was a gift from Roussel-Uclaf. The (+) and (−) isomers of DOI were previously synthesized in our facilities. Unless otherwise stated, all drugs were dissolved in distilled water and given at a volume of 10 ml/kg.

Statistical Analysis

Data were analyzed by one-way analysis of variance and post hoc analysis by Dunnett *t*-test. Whenever possible, ED_{50} values were calculated by method of Finney (6).

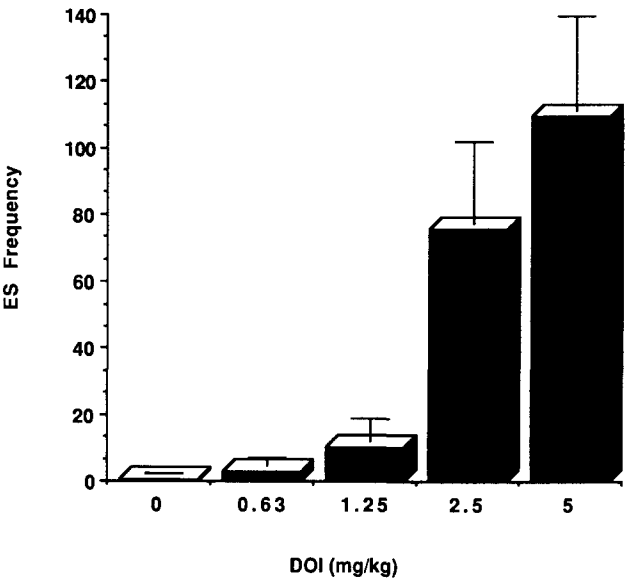

FIG. 1. The effect of (±)-DOI administration (IP) on the induction of ear-scratch response (ESR) in mice (N = 6). The behavior was scored for 30 minutes immediately after injection. Results are given as means ± S.E.M.

RESULTS

The phenalkylamine hallucinogen (±)-DOI induced a dose-dependent increase in the ear-scratch response (ESR) when mice were observed for 30 minutes after injection (Fig. 1). Maximal effects were produced within 12 minutes after the (±)-DOI treatment and persisted up to 26 minutes after postinjection (Fig. 2). Thereafter, the frequency of the induced behavior declined but persisted beyond the 30-minute observation period. The R(−)-isomer induced six times more ESR (137 ± 47) than the S(+)-

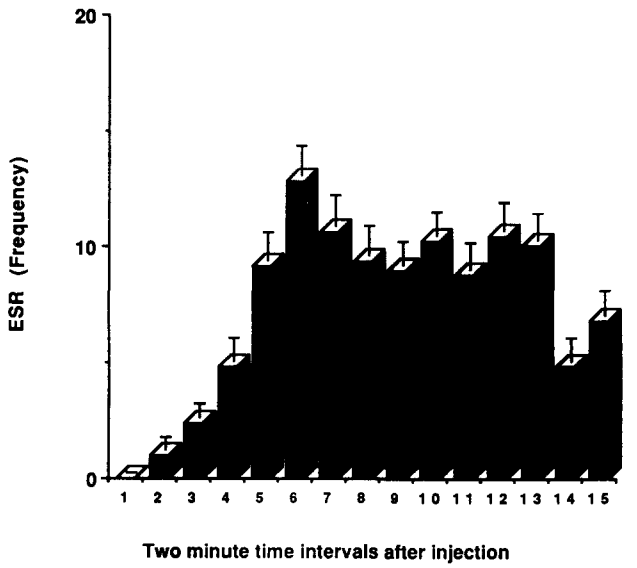

FIG. 2. The time course of ESR frequency in mice observed at two-minute intervals for 30 minutes after an intraperitoneal injection of 5 mg/kg (±)-DOI (N = 6). Results are given as means ± S.E.M. at each time point.

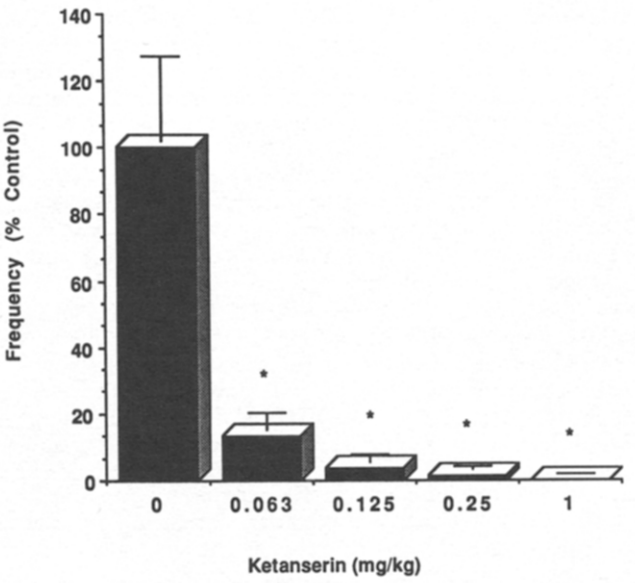

FIG. 3. Dose-dependent inhibitory effect of ketanserin on ESR frequency induced by 2.5 mg/kg (±)-DOI (N=4). Data (means±S.E.M.) are presented as percent of ESR produced by 2.5 mg/kg of (±)-DOI in the absence of ketanserin. *Significantly different from control at $p<0.05$.

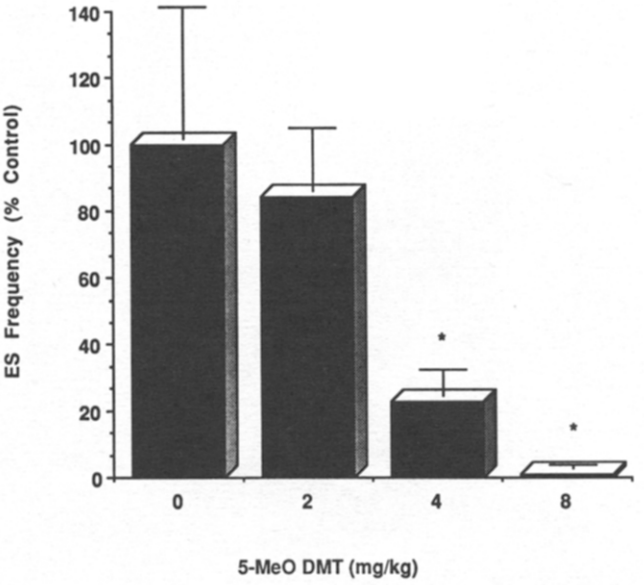

FIG. 5. Effects of the nonselective 5-HT agonist 5-MeO DMT on (±)-DOI-induced ESR (2.5 mg/kg, N=4) in mice. Data (means±S.E.M.) are presented as percent of ESR produced by 2.5 mg/kg (±)-DOI in the absence of 5-MeO DMT. *Significantly different from control at $p<0.05$.

isomer (20±11) when tested at 2.5 mg/kg. Due to limited quantities of the optical isomers, a full dose-response effect study was not practical. The selective 5-HT_2-receptor antagonist ketanserin potently inhibited ESR induced by 2.5 mg/kg (±)-DOI (Fig. 3). In a similar manner, spiperone, another 5-HT_2 antagonist, also inhibited the response (Fig. 4). In both cases doses of 0.05 to 0.06 mg/kg nearly completely inhibited the induced response. The nonselective 5-HT agonist 5-MeO DMT reduced the ESR induced by 2.5 mg/kg (±)-DOI in a dose-dependent manner [ID_{50}=3.1 (1.95–4.77) mg/kg] (Fig. 5). The selective 5-HT_{1A} agonist 8-OH DPAT did not cause a significant change in the (±)-DOI-induced behavior (Fig. 6). The 5-HT_{1B}/5-HT_{1C} agonist TFMPP dose dependently reduced the (±)DOI-induced response [ID_{50}=0.63 (0.42–0.97) mg/kg] (Fig. 7). Similarly, an equivalent dose of the more selective 5-HT_{1B} agonist RU 24969 (1.25 mg/kg) inhibited the effect to a similar extent (Fig. 7). Due to the availability of

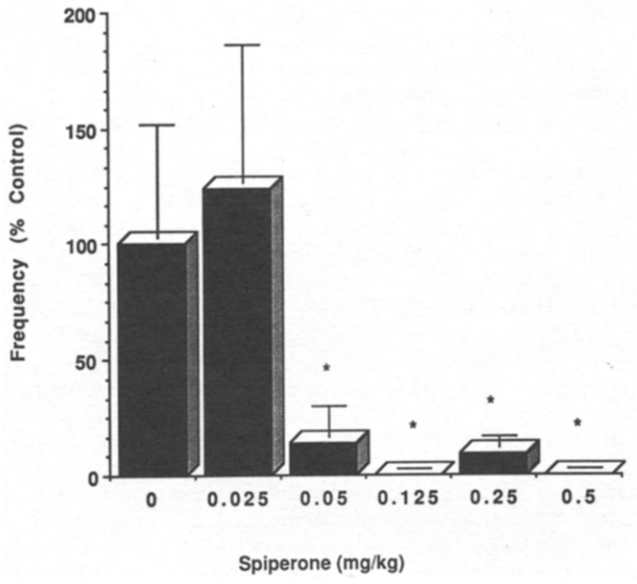

FIG. 4. Dose-dependent inhibition of (±)-DOI-induced ESR (2.5 mg/kg) by spiperone (N=4). Data (means±S.E.M.) are presented as percent of ESR produced by 2.5 mg/kg of (±)-DOI in the absence of spiperone. *Significantly different from control at $p<0.05$.

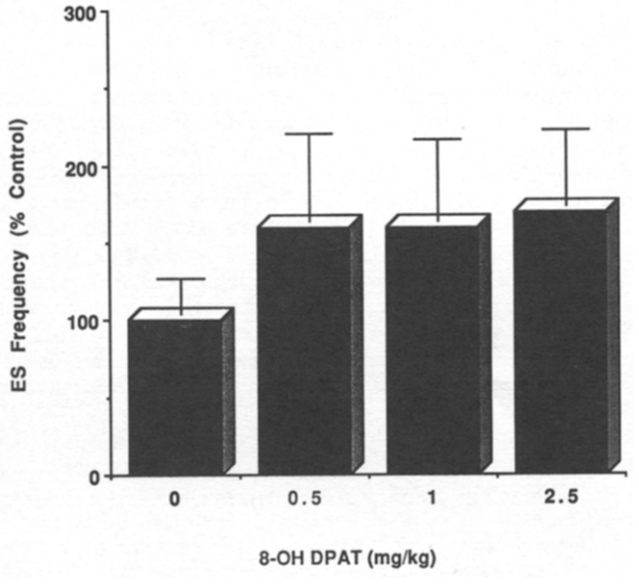

FIG. 6. Effects of the selective 5-HT_{1A} agonist 8-OH DPAT on ESR frequency induced by 2.5 mg/kg (±)-DOI (N=6–8). Data (mean±S.E.M.) are presented as percent of ESR produced by 2.5 mg/kg of (±)-DOI in the absence of 8-OH DPAT. *Significantly different from control at $p<0.05$.

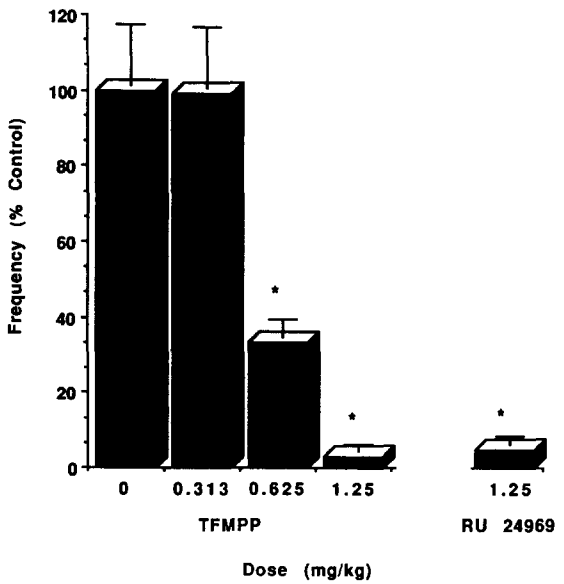

FIG. 7. Effects of the selective 5-HT_{1B}/5-HT_{1C} agonist TFMPP and the selective 5-HT_{1B} agonist RU 24969 on (±)-DOI-induced ESR (2.5 mg/kg, N = 5–7). Data (mean ± S.E.M.) are presented as percent of ESR produced by 2.5 mg/kg of (±)-DOI in the absence of TFMPP and RU 24969. *Significantly different from control at $p<0.05$.

limited quantities of RU 24969, the drug was only tested at one dose. Apart from (±)-DOI and its isomers, none of the drugs tested induced ESR when administered alone (data not shown).

DISCUSSION

Phenalkylamine hallucinogens such as DOI and DOM are considered to be fairly selective 5-HT_2 agonists (9), or at least partial agonists (8). Therefore, it is reasonable to hypothesise that ESR is a 5-HT_2-mediated phenomenon. The R(−)-isomers of phenalkylamine hallucinogens typically bind at 5-HT_2 receptors with two to ten times the affinity of their S(+)-enantiomers (8). This difference in affinity is consistent with the present observation that the R(−)-isomers of DOI (this study) and DOM (12) are more potent than their S(+)-enantiomers in producing the ESR. Also consistent with this hypothesis is the ability of the 5-HT_2 antagonists ketanserin and spiperone to attenuate (±)-DOI-induced ESR. Both ketanserin and spiperone are also dopamine antagonists, however, the ESR does not appear to be dopaminergically mediated; for example, there is no evidence that the response is produced by the indirect-acting dopaminergic agonist amphetamine (5). The phenalkylamine hallucinogens also bind at 5-HT_{1C} sites (14) and there is evidence that DOM is a 5-HT_{1C} agonist (2). Both ketanserin and spiperone are 5-HT_{1C} antagonists; spiperone, however, possesses up to a 2000-fold selectivity for 5-HT_2 versus 5-HT_{1C} sites (13) and it might be expected that ketanserin would be considerably more potent than spiperone if the DOI-induced ESR was a 5-HT_{1C}-mediated event. Although we cannot rule out the possibility that spiperone is acting nonspecifically to induce motor depressant effects, both agents were able to significantly and potently attenuate the ESR at very low, and at nearly identical doses.

The indolealkylamine hallucinogens 5-MeO DMT and LSD do not elicit the ESR in mice. Both of these agents bind at 5-HT_2-receptors (9); however, unlike the phenalkylamines DOM and DOI, 5-MeO DMT and LSD are nonselective serotonergic agents (9). That is, they bind at multiple populations of 5-HT_1-receptors including 5-HT_{1A}-, 5-HT_{1B}-, and 5-HT_{1C}-receptors (9).

Recently, we proposed that simultaneous costimulation of 5-HT_{1A}-receptors has an inhibitory effect on a 5-HT_2-receptor-mediated function (head-twitch response) when induced by (±)-DOI (4). Therefore, we investigated the likelihood of a similar inhibitory relationship in the ESR by examining the effects of various nonselective and selective 5-HT agonists on the ESR induced by (±)-DOI. Similar to (±)-DOI-induced head-twitch behavior (4), the (±)-DOI-induced ESR was dose dependently inhibited by the nonselective 5-HT agonist 5-MeO DMT. However, unlike the head-twitch behavior (4), ESR was not inhibited by the selective 5-HT_{1A} agonist 8-OH DPAT. Moreover, the induced behavior appeared to be increased by 8-OH DPAT, however, the increase was not significant due to interanimal variability. Thus, the 5-MeO DMT-induced inhibition of (±)-DOI-induced ESR does not appear to involve a 5-HT_{1A} mechanism. Both the 5-HT_{1B}/5-HT_{1C} agonist TFMPP and the 5-HT_{1B} agonist RU 24969 completely inhibited the (±)-DOI-induced ESR. By themselves, these agents do not elicit ESR, and at comparable doses they are not effective in inhibiting head-twitch behavior [(4) and unpublished findings respectively].

Thus, it appears that the 5-MeO DMT-induced inhibition of ESR in mice may involve a 5-HT_{1B} mechanism. Although both the head-twitch response and ESR can be simultaneously induced by the selective 5-HT_2 agonist (±)-DOI (in the same mice), and both effects can be modulated by 5-HT_1 agonists, the inhibitory "control" mechanisms seemingly involve different populations of 5-HT_1 receptors, i.e., 5-HT_{1A}- and 5-HT_{1B}-receptors respectively. These results could also account for the inability of 5-MeO DMT and LSD to produce the ESR. That is, being nonselective 5-HT agonists, these agents are able to act at 5-HT_{1B}- as well as at 5-HT_2-receptors and can conceivably inhibit their own behavior.

Why should there be such an inhibitory switching mechanism for the termination of 5-HT_2-receptor-mediated effects? Recently, it has been suggested that the 5-HT_2-receptors may receive little impetus from endogenous 5-HT and may consequently exist in a supersensitive state (11). If this hypothesis is correct, then our studies suggest that the 5-HT_{1A}/5-HT_{1B}-receptor subtypes may provide a damping mechanism to reduce excessive 5-HT_2-receptor excitation due to exogenous drug stimulation or pathological conditions. Thus, this control mechanism may prevent the reported detrimental effects produced after 5-HT_2-receptor stimulation (11).

In summary, we have demonstrated (a) that racemic DOI and both of its optical isomers induce ESR, (b) that R(−)-DOI is more potent than S(+)-DOI in this regard, (c) that, consistent with a 5-HT_2 mechanism, ketanserin is capable of attenuating (±)-DOI-induced ESR, (d) that the nonselective 5-HT agonist 5-MeO DMT dose-dependently attenuates the (±)-DOI-induced effect, (e) that neither the 5-HT_{1B}/5-HT_{1C} agonist TFMPP nor 5-HT_{1B} agonist RU 24969 produce ESR, but that both produce a similar antagonism of (±)-DOI-induced ESR, (f) that the selective 5-HT_{1A} agonist 8-OH DPAT did not affect the (±)-DOI-induced effect. Furthermore, (±)-DOI-induced ESR appears to be a 5-HT_2- and not a 5-HT_{1C}-mediated effect, on the basis that the 5-HT_{1B}/5-HT_{1C} agonist TFMPP does not induce the effect and because spiperone is essentially equipotent with ketanserin in antagonizing (±)-DOI-induced ESR. Thus, taken together, we propose that the ESR is a 5-HT_2-receptor-mediated phenomenon and simultaneous costimulation of 5-HT_{1B}-receptors has a modulatory role on the 5-HT_2-receptor-induced ESR. Furthermore, this hypothesis accounts for the ability of 5-MeO DMT to antagonize DOI- (or DOM-) induced ESR, and for the inability of indolealkylamine hallucinogens to produce the ESR in mice.

ACKNOWLEDGEMENTS

This work was supported, in part, by DA-02396 and DA-01642.

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