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Effects of LSD, Ritanserin, 8-OH-DPAT, and Lisuride on Classical Conditioning in the Rabbit

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WELSH, S. E., W. J. KACHELRIES, A. G. ROMANO, K. J. SIMANSKY AND J. A. HARVEY. *Effects of LSD, ritanserin, 8-OH-DPAT, and lisuride on classical conditioning in the rabbit.* PHARMACOL BIOCHEM BEHAV **59**(2) 469–475, 1998.—*d*-Lysergic acid diethylamide (LSD), an agonist at the 5-$HT_{2A/2C}$ and 5-HT_{1A} receptors, has previously been demonstrated to enhance associative learning as measured by accelerated acquisition of the rabbit's classically conditioned nictitating membrane (NM) response. The present study examined further the role of these receptors in the action of LSD. LSD (30 nmol/kg, IV) significantly enhanced conditioned response (CR) acquisition to both tone and light conditioned stimuli (CSs), while the 5-HT_{1A} receptor agonists 8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT; 50 and 200 nmol/kg) and lisuride (0.3–30 nmol/kg) had no effect. Ritanserin (6.7–6700 nmol/kg, SC), a selective 5-$HT_{2A/2C}$ receptor antagonist, retarded acquisition of CRs to both tone and light CSs in a dose-dependent manner. Ritanserin (6.7–670 nmol/kg, SC) also dose dependently antagonized the enhancement of CR conditioning produced by LSD (30 nmol/kg, IV) to both tone and light CSs. We conclude that the enhancement of CR acquisition by LSD was due to an action at the 5-$HT_{2A/2C}$ receptor. These results suggest that the 5-$HT_{2A/2C}$ receptor plays an important role in learning. © 1998 Elsevier Science Inc.

Learning Classical conditioning Nictitating membrane Blink reflex Rabbit Serotonin 5-HT_2A/2C receptor Lysergic acid diethylamide Ritanserin

STUDIES employing classical conditioning of the rabbit's NM response have demonstrated that LSD and other hallucinogenic drugs such as MDA (*d*,l-methylenedioxyamphetamine), MDMA (*d*,l-methylenedioxymethamphetamine), and DOM (*d*,l-2,5-dimethoxy-4-methylamphetamine) enhance associative learning at doses comparable to those producing reliable psychedelic effects in humans (9,14,29,30,36). It is generally agreed that these drugs are partial agonists at 5-HT_2 receptors (11,12,33,34), and that their central actions, as measured in drug discrimination paradigms, are blocked by 5-HT_2 antagonists (3,7,8). However, there is disagreement as to whether hallucinogenic drugs produce their predominant action at the 5-HT_{2A} (31,38,39) or 5-HT_{2C} (6,33) receptor subtype.

These data suggest that LSD, the prototypic drug in this series, may also be enhancing associative learning through an action on 5-HT_2 receptors. However, this assumption has not been tested, nor have actions of LSD at other receptors been ruled out. It is known that LSD has equivalent affinities for the serotonin 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors with K_i values of 1.3, 2.5, and 4.3 nM, respectively (17,41). However, it is not known whether a selective 5-$HT_{2A/2C}$ receptor antagonist would block the effects of LSD on learning or whether 5-HT_{1A} receptor agonists would also enhance learning.

Five experiments were carried out to define further the receptors through which LSD enhances the acquisition of CRs. The first experiment examined the effects of ritanserin on CR acquisition to a tone CS. Ritanserin is a potent serotonin antagonist with high affinity for both 5-HT_{2A} (K_i = 0.24 nM) and 5-HT_{2C} (K_i = 0.6 nM) receptors and with substantially lower affinities for other serotonergic and nonserotonergic receptors, for example, K_i = 470 nM for the 5-HT_{1A} receptor (16,17). A second experiment determined whether ritanserin would block the enhancement of CR acquisition produced by LSD. A third experiment compared the effects of LSD and ritanserin on the acquisition of CRs to both tone and light CSs. The fourth experiment examined the effects of lisuride on CR acquisition to tone and light CSs. Lisuride is a nonhallucinogenic ergot that resembles LSD in having equivalent affinities for the 5-HT_{1A} and 5-HT_{2A} receptors (K_i values of 1.2 and 3.3 nM, respectively), but differs from LSD in having a somewhat lower affinity (K_i = 12 nM) for the 5-HT_{2C} receptor (17). Finally, a fifth experiment measured the effects of the highly

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selective 5-HT_{1A} receptor agonist, 8-OH-DPAT, on CR acquisition (16).

METHOD

Animals

New Zealand White albino rabbits of both sexes, weighing 1.8 to 2.2 kg upon arrival, were housed individually with free access to rabbit chow and water under a 12 L:12 D cycle in an AAALAC-approved colony maintained at 22 ± 1°C. Rabbits were given 5 days of adaptation to the laboratory before initiation of experiments. All animal experiments were carried out in accordance with the National Institute of Health guide for the care and use of Laboratory Animals.

Drugs

LSD (d-lysergic acid diethylamide tartrate), a gift of NIDA, was dissolved in sterile, nonpyrogenic distilled water, and lisuride hydrogen maleate (Schering AG) was dissolved in sterile physiological saline. LSD, lisuride, and their vehicle controls were injected into the marginal ear vein by means of a syringe pump (Model 355, Sage Instruments) in a volume of 0.4 ml/kg and at a rate of 3 ml/min, 20–30 min prior to behavioral testing. 8-OH-DPAT [8-hydroxy-2-(dipropylamino)tetralin hydrobromide] and ritanserin (Research Biochemicals Int., Natick, MA) were injected subcutaneously, between the scapulae. 8-OH-DPAT was dissolved in deionized nitrogenated water, and ritanserin was dissolved in a minimum quantity of acetic acid and the pH adjusted to 5.0 with NaOH. 8-OH-DPAT and its vehicle were injected in a volume of 2 ml/kg, 30 min prior to testing, while ritanserin and its vehicle were injected in a volume of 4 ml/kg, 1 h prior to testing.

Apparatus for Conditioning Studies

The apparatus, IBM PC-AT and ASYST software for stimulus control and data acquisition, have been described in detail elsewhere (30). Briefly, each animal was placed in a Plexiglas restrainer and fitted with a headmount that supported a potentiometer that was coupled directly to a suture placed in the right NM. Movements of the NM were transduced to DC voltages and digitized every 5 ms with a resolution of 0.03 mm of NM movement per analog-to-digital count. A response was defined as a 0.5 mm or greater extension of the NM and its onset latency was calculated from the time at which the response first deviated from baseline by at least 0.03 mm. The headmount also supported a 2-mm diameter metal tube positioned 5–7 mm from the center of the right cornea for delivery of a 100-ms airpuff unconditioned stimulus (US) at a pressure of 200 g/cm^2 as measured at the end of the metal tube. A speaker mounted in front and above the rabbit was used to deliver an 800-ms, 1-kHz, 75 or 85 dB tone CS while an 800-ms interruption of the house light at 10 Hz served as the light CS. For experiments with lisuride, the US was a 100-ms, 3-mA, 60-Hz electric shock delivered paraorbitally as described previously (14).

Conditioning of the Rabbit's Nictitating Membrane Response

One day prior to conditioning sessions, rabbits received one 60-min adaptation session, during which time no stimuli were presented and no drug was injected. Two types of conditioning procedures were employed involving the use of either 5 or 10 daily 60-min sessions. For the 5-day conditioning procedure, each daily session consisted of 60 pairings of the tone CS and US. For the 10-day conditioning procedure, each daily session consisted of 30 pairings of the tone CS and US and 30 pairings of the light CS and US as described previously (14). For both procedures, offset of the 800-ms CSs occurred simultaneously with US onset and the intertrial interval was 60 s (range 55–65 s). Responses were scored as CRs if they occurred within 800 ms of CS onset and as unconditioned responses (URs) if they occurred after US onset.

Five experiments were carried out with separate sets of experimentally naïve rabbits. A first set of animals was injected with ritanserin, 60 min prior to each of five conditioning sessions, at doses of 0 (vehicle, $n = 11$), 6.7 ($n = 12$), 20 ($n = 12$), 67 ($n = 11$), 670 ($n = 6$), and 6700 nmol/kg ($n = 6$), i.e., 0.003–3.0 mg/kg. A second set of animals was injected with vehicle or ritanserin and 1 h later with vehicle or LSD (30 nmol/kg; 14 μg/kg as the salt) prior to each of five conditioning sessions. This provided five groups of animals: saline + saline ($n = 9$); saline + LSD ($n = 11$); ritanserin (6.7 nmol/kg) + LSD ($n = 11$); ritanserin (67 nmol/kg) + LSD ($n = 10$); and ritanserin (670 nmol/kg) + LSD ($n = 10$). Conditioning sessions began 20–30 min after the second injection of vehicle or LSD (i.e., 80–90 min after the first injection of vehicle or ritanserin). A third set of rabbits was injected with vehicle or ritanserin (1000 nmol/kg; 0.48 mg/kg) and 1 h later with vehicle or LSD (30 nmol/kg), 20–30 min prior to each of 10 conditioning sessions that employed both tone and light CSs. This provided four groups of animals: vehicle + vehicle ($n = 12$); vehicle + LSD ($n = 12$); ritanserin + vehicle ($n = 12$); and ritanserin + LSD ($n = 12$). A fourth set of animals was injected with lisuride, 20–30 min prior to each of 10 conditioning sessions, at doses of 0 (vehicle, $n = 12$), 0.3 ($n = 11$), 3.0 ($n = 12$), and 30

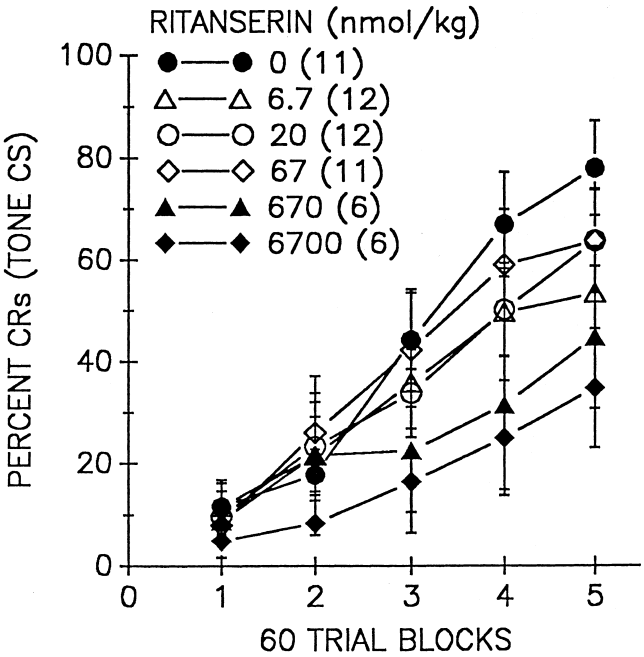

FIG. 1. Effect of ritanserin on CR acquisition to a tone CS across 5 conditioning days. Each conditioning day involved 60 pairings of a tone CS and US. Ritanserin or its vehicle were injected SC, 60 min prior to each conditioning session. Each point represents the mean percentage of CRs and vertical bars one SEM Numbers in parentheses indicate the number of animals per group.

nmol/kg ($n = 12$), i.e., 0.14–14 μg/kg as the salt. Finally, a fifth set of animals was injected with 8-OH-DPAT 30 min prior to each of five conditioning sessions, at doses of 0 (vehicle, $n = 12$), 50 ($n = 8$), and 200 nmol/kg ($n = 8$), i.e., 16 and 66 μg/kg as the salt.

Data Analysis

Data were analyzed by repeated measures ANOVA using the SYSTAT statistical package (43). Follow-up tests for significant effects were carried out by Dunnett's *t*-test for multiple comparisons (44). Statistical significance was set at $p < 0.05$.

RESULTS

Ritanserin Retarded CR Acquisition and Antagonized the Enhancement of Acquisition Produced by LSD

Ritanserin produced a dose-dependent retardation of CR acquisition to a tone CS across the five conditioning sessions (Fig. 1). The analysis of variance yielded a significant effect of drug dose, $F(5, 52) = 5.0$, $p < 0.05$, and a follow-up analysis indicated that animals receiving the 670 and 6700 nmol/kg doses of ritanserin exhibited significantly fewer CRs than controls ($p < 0.05$).

Figure 2 presents the effects of LSD given alone or in combination with ritanserin on the acquisition of CRs. There was a significant difference in the rate of CR acquisition to the tone CS between the different injection groups across the five days of acquisition, $F(16, 184) = 1.88$, $p < 0.05$. As seen in Fig. 2, LSD enhanced CR acquisition and ritanserin antagonized this effect of LSD in a dose-dependent manner. For example, on the last day of conditioning, animals receiving LSD alone demonstrated a significantly higher percentage of CRs (86 ± 3) compared with vehicle controls (49 ± 11). Animals receiving LSD and the lowest dose of ritanserin (6.7 nmol/kg) also demonstrated a significantly higher percentage of CRs (80 ± 6) on the last conditioning session than controls. In contrast, the percentage of CRs demonstrated by animals receiving LSD and either the 67 or 670 nmol/kg dose of ritanserin (71 ± 9 and 65 ± 10) was not significantly different from that of controls. To compare the effects of ritanserin given alone (Fig. 1) or prior to LSD (Fig. 2) as a dose–response function, we calculated the mean percentage of CRs across the five conditioning sessions for each dose of ritanserin, LSD, or ritanserin + LSD, and expressed these values as a percentage of the mean value for their respective vehicle controls (Fig. 3). It can be seen that ritanserin, at a dose of 67 nmol/kg, had no significant effect on CR acquisition when given alone (closed circle, Fig. 3), but did partially block the effects of LSD (closed diamond, Fig. 3).

We next examined the effects of ritanserin on CR acquisition to both tone and light CSs, to extend the generality of the

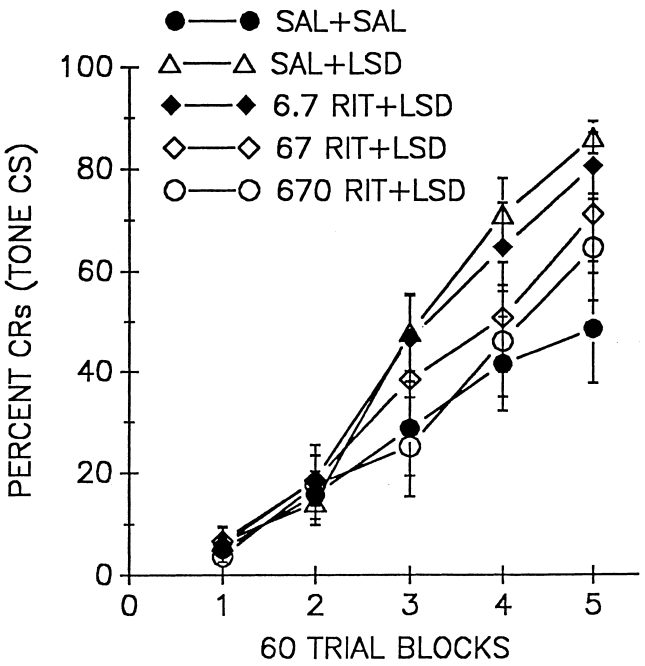

FIG. 2. Antagonism by ritanserin of the effects of LSD on CR acquisition to a tone CS across 5 conditioning days. Data are presented as in Fig. 1. Ritanserin (6.7, 67, 670 nmol/kg) or its vehicle were injected SC, 1 h prior to the IV injection of LSD (30 nmol/kg). Conditioning sessions were carried out 20–30 min after injection of LSD. Each point represents the mean percentage of CRs of 9–11 animals and vertical bars 1 SEM.

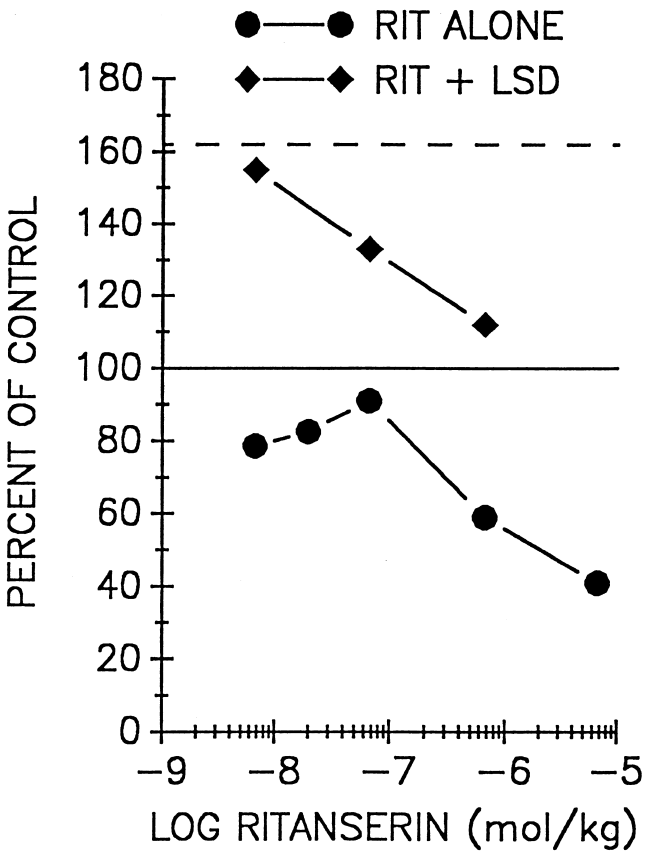

FIG. 3. Dose–response relationships for ritanserin, LSD, and ritanserin + LSD. The dose–response curve for ritanserin given alone (closed circles) is taken from the data of Fig. 1 and for ritanserin's antagonism of LSD effects (closed diamonds) from the data in Fig. 2. All points represent average CR frequencies expressed as a percentage of the corresponding vehicle control. The horizontal dotted line represents the percentage increase in CR acquisition demonstrated by animals receiving LSD alone.

effects obtained. Using this procedure, LSD (30 nmol/kg) enhanced ($p < 0.001$) and ritanserin (1000 nmol/kg) retarded ($p < 0.01$) CR acquisition to both tone and light CSs (Fig. 4). The enhancement of CR acquisition to the tone and light CSs produced by the 30 nmol/kg dose of LSD was antagonized completely by the 1000 nmol/kg dose of ritanserin.

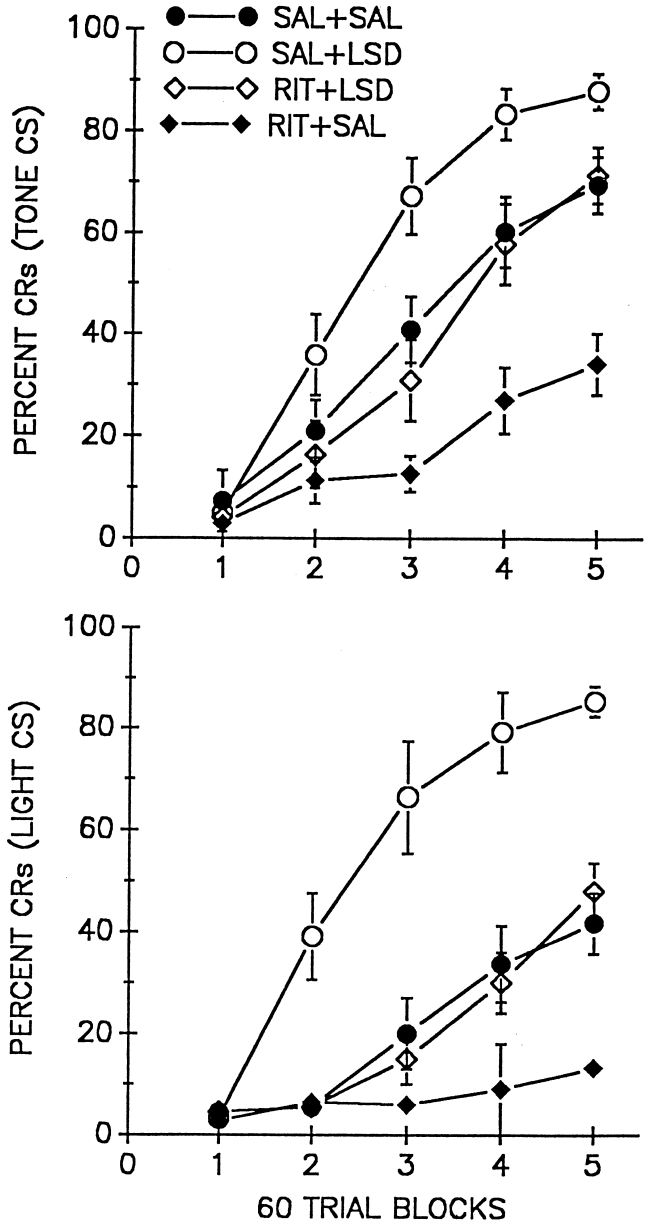

FIG. 4. Antagonism by ritanserin (1000 nmol/kg) of the effects of LSD (30 nmol/kg) on CR acquisition to tone (top panel) and light (bottom panel) CSs across 10 conditioning days. Each conditioning day involved 30 pairings of a tone CS and US and 30 pairings of a light CS and US. Data are presented as the average of 60 conditioning trials for each modality (average of 2 conditioning days). Ritanserin or its vehicle were injected SC, 60 min prior to the IV injection of LSD or its vehicle. Conditioning trials were begun 20–30 min after injection of LSD or its vehicle. Each point represents the mean percentage of CRs of 12 animals and vertical bars 1 SEM. In some cases the SEM is not seen because it fell within the size of the symbol.

Lisuride and 8-OH-DPAT Had No Effect on CR Acquisition

As shown in Fig. 5, all groups of animals in the lisuride study demonstrated a significant acquisition of CRs to the tone and light CSs across conditioning days, $F(9, 387) = 146.2$, $p < 0.001$. There was no significant effect of lisuride on the acquisition of CRs to the tone and light CSs, $F(3, 43) = 1.82$, $p > 0.15$, and no significant interaction between drug dose and acquisition days, $F(27, 387) = 1.96$, $p > 0.10$, or drug dose and CS modality, $F(3, 43) = 0.76$, $p > 0.80$. As shown in Fig. 6, there was also no significant effect of 8-OH-DPAT on CR acquisi-

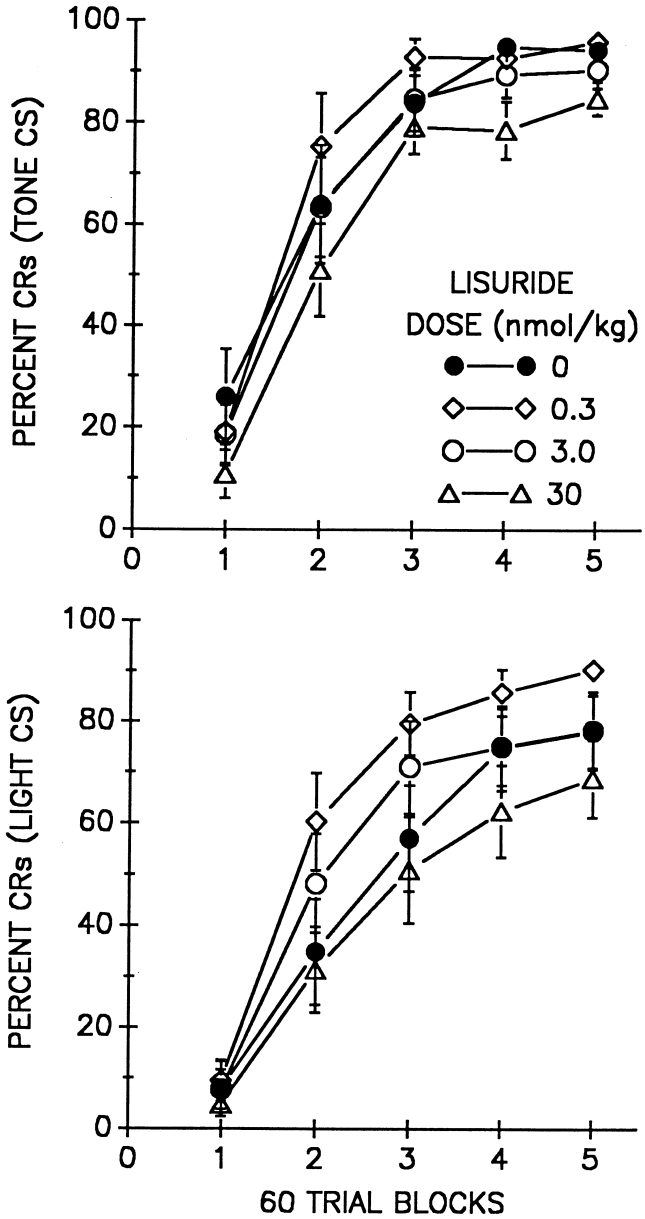

FIG. 5. Effects of lisuride on CR acquisition to tone (top panel) and light (bottom panel) CSs across 10 conditioning days. Data are presented as in Fig. 4. Lisuride was injected IV, 20–30 min prior to each conditioning session. Each point represents the mean of 11–12 animals, and vertical bars 1 SEM.

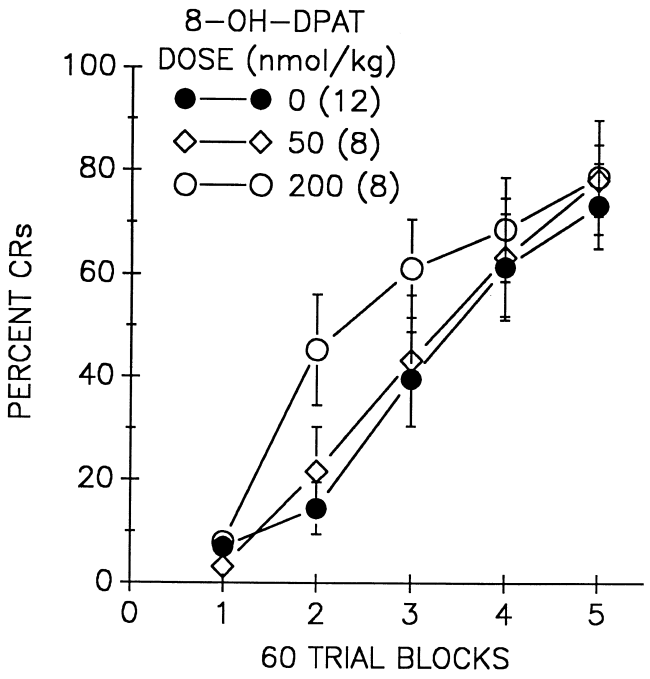

FIG. 6. Effect of 8-OH-DPAT on CR acquisition to a tone CS across 5 conditioning days. 8-OH-DPAT or its vehicle were injected SC, 30 min prior to each conditioning session. Each daily session consisted of 60 pairings of a tone CS and airpuff US. Each point represents the mean percentage of CRs and vertical bars 1 SEM. Numbers in parentheses indicate the number of animals per group.

tion to a tone CS, $F(2, 25) = 1.44$, $p > 0.26$, nor was there any significant drug × days interaction, $F(8, 100) = 0.84$, $p > 0.57$.

DISCUSSION

LSD is a partial agonist, having equal affinities for the serotonin 5-HT_{1A} (K_i, 1.3 nM), 5-HT_{2A} (K_i, 2.5 nM), 5-HT_{2C} (K_i, 4.3 nM), and the dopamine D_2 receptor (K_i, 6.4 nM) (17,41). Several drugs were employed in the present study to identify the receptors through which LSD produces its enhancement of CR acquisition. The 5-HT_{1A} receptor does not appear to be involved in this action of LSD because 8-OH-DPAT had no effect on CR acquisition at doses of 50 or 200 nmol/kg (i.e., 16 and 66 μg/kg, SC). 8-OH-DPAT is a 5-HT agonist with high and selective affinity for the 5-HT_{1A} receptor (K_i, 2.8 nM) compared with the 5-HT_{2A} (K_i, > 10,000) or 5-HT_{2C} (K_i, 7,800) receptor subtypes (16,23). This conclusion was further supported by the absence of any effect of lisuride (0.3 to 30 nmol/kg; 0.14–14 μg/kg, IV) on CR acquisition to either tone or light CSs. Lisuride, a nonhallucinogenic ergot, resembles LSD in having equivalent affinities for the serotonin 5-HT_{1A} (K_i, 1.2 nM) and 5-HT_{2A} (K_i, 3.3 nM) and for the dopamine DA_2 (K_i, 0.94 nM) receptors, but differs from LSD in having a lower affinity (K_i, 12 nM) for the 5-HT_{2C} receptor (17). These affinities not only confirm the results with 8-OH-DPAT in indicating that activation of the 5-HT_{1A} receptor does not enhance CR acquisition but also suggest the lack of involvement of agonist actions at the dopamine D_2 receptor. This might appear to suggest that the enhancement of CR acquisition produced by LSD was due to an action at the 5-HT_{2A} receptor. However, such a conclusion must be tempered by the fact that lisuride has less than a fourfold greater affinity for the 5-HT_{2A} compared with the 5-HT_{2C} receptor. It is also possible that the enhancement of learning produced by LSD requires the activation of both the 5-HT_{2A} and the 5-HT_{2C} receptor subtypes.

The absence of significant effects of 8-OH-DPAT and lisuride on CR acqusition were not due to the range of doses used in this study. Lisuride produced decrements in operant responding in the rat with an ED_{50} of 16 μg/kg, IP (21), while 8-OH-DPAT retarded acquisition at a dose of 62 μg/kg, IP in the rat (20). Other studies in rats and mice are in general agreement with the view that enhancement of CR acquisition is produced by agonists at the 5-$HT_{2A/2C}$ but not at the 5-HT_{1A} receptor (13). For example, the 5-HT_2 agonists 1-[m-trifluoromethylphenyl]piperazine and quipazine increased acquisition of the conditioned avoidance response (1) while 5-HT_{1A} agonists either had no effect, for example, tandospirone (26) or retarded acquisition, for example, buspirone (1) and 8-OH-DPAT (20).

The conclusion that activation of the 5-$HT_{2A/2C}$ receptor enhances learning is consistent with what is known concerning the distribution and function of these receptors in brain. The binding of the 5-$HT_{2A/2C}$ agonist, [^{125}I]-DOI, is similar to that seen for [^{125}I]-LSD (2), and the binding sites are located within brain structures known to be importantly involved in learning, for example, layers 1 and 4 of the neocortex in the rat (2,24,25,40) as well as in the rabbit (32). More importantly, serotonin has an excitatory effect on both 5-HT_{2A} and 5-HT_{2C} receptors in rat cortex (18,35). The activation of 5-HT_2 receptors in cortex may alter activity in cholinergic neurons because these receptors have been located on cholinergic neurons in layer 4 of rat cortex (27). In agreement with this view, decreases in cholinergic and serotonergic activity have been reported to produce a synergistic decrement in learning in experimental animals (28). While these data suggest that the enhancement of learning produced by the activation of some 5-HT_2 receptors may be due to an increase in cholinergic activity within cerebral cortex, it has also been reported that serotonin inhibits potassium stimulated release of acetylcholine through an action at the 5-HT_3 receptor (19). Thus, the precise mechanisms involved in the interactions of serotonergic and cholinergic activity in cognitive processes (4,22,37) remains unclear.

Of great interest in this study was the finding that ritanserin retarded the acquisition of classically conditioned CRs to both tone and light CSs in a dose-dependent manner. Ritanserin has also been reported to block classical conditioning in humans (15). The low doses of ritanserin required to produce a significant retardation of CR acquisition (670 nmol/kg) or to block the enhancement of CR acquisition produced by LSD (67 nmol/kg) are consistent with the high affinity of ritanserin for the 5-HT_{2A} (K_i = 0.24 nM) and 5-HT_{2C} (K_i = 0.6 nM) receptors (16,17). The precise behavioral mechanisms by which ritanserin produced a retardation of CR acquisition is not clear, and could be due to a combination of effects on learning and/or performance variables.

The ability of ritanserin to retard CR acquisition is shared by another selective 5-$HT_{2A/2C}$ receptor antagonist, pizotifen, which was also reported to retard acquisition of the rabbit's NM response (10). However, d-2-bromolysergic acid diethylamide (BOL), an ergot derivative of LSD, and a selective 5-$HT_{2A/2C}$ receptor antagonist, was reported to have no significant effect on the rate of CR acquisition to tone and light CSs (14). These findings suggest the existence of two types of serotonin antagonists: neutral antagonists such as BOL; and antagonists such as ritanserin and pizotifen that have actions op-

posite to that of 5-$HT_{2A/2C}$ agonists such as LSD, DOM, MDA, and MDMA. Although, both ritanserin and pizotifen are considered to be reasonably selective 5-$HT_{2A/2C}$ antagonists, it is possible that they retarded CR acquisition through an action at some other receptors. In this case, the antagonism of LSD's effects on learning by ritanserin might be due to a physiological antagonism and not to a direct, pharmacological antagonism at the 5-HT_{2A} or 5-HT_{2C} receptor. For example, although 5-HT_1A agonists did not produce an enhancement of CR acquisition, it remains possible that antagonists at the 5-HT_{1A} or at other receptors might have also modified the actions of LSD. However, the data presented in Figs. 1, 2, and 3 argue against this interpretation. Ritanserin, at the dose of 67 nmol/kg, had no significant effect on CR acquisition when given alone (Figs. 1 and 3) but blocked the enhancement of CR acquisition produced by LSD (Figs. 2 and 3). The alternative interpretation is that ritanserin and pizotifen may be acting as inverse agonists at the 5-$HT_{2A/2C}$ receptor and the antagonism of LSD by ritanserin was due to a pharmacological antagonism. Studies in transfected cell lines, in which LSD and 5-HT enhance PI hydrolysis, have reported the existence of inverse agonist actions at the serotonin 5-HT_{2C} receptor (5,33,42). Although ritanserin has not been examined, pizotifen has been characterized as an inverse agonist and BOL as a neutral antagonist (42). An action of ritanserin as an inverse agonist at the 5-$HT_{2A/2C}$ receptor in vivo, would suggest that activity at this receptor is normally required for the occurrence of optimal learning.

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REFERENCES

1. Alhaider, A. A.; Ageel, A. M.; Ginawi, O. T.: The quipazine- and TFMPP-increased conditioned avoidance response in rats: Role of 5-HT_{1C}/5-HT_2 receptors. Neuropharmacology 32:1427–1432; 1993.
2. Appel, N. M.; Mitchell, W. M.; Garlick, R. K.; Glennon, R. A.; Teitler, M.; De Souza, E. B.: Autoradiographic characterization of (±)-1-(2,5-dimethoxy-4-[^{125}I]iodophenyl)-2-aminopropane ([^{125}I]DOI) binding to 5-HT_2 and 5-HT_{1C} receptors in rat brain. J. Pharmacol. Exp. Ther. 255:843–857; 1990.
3. Arnt, J.: Characterization of the discriminative stimulus properties induced by 5-HT_1 and 5-HT_2 agonists in rats. Pharmacol. Toxicol. 64:165–172; 1989.
4. Arranz, B.; Eriksson, A.; Mellerup, E.; Plenge, P.; Marcusson, J.: Effect of aging in human cortical preand postsynaptic serotonin binding sites. Brain Res. 620:163–166; 1993.
5. Barker, E. L.; Westphal, R. S.; Schmidt, D.; Sanders-Bush, E.: Constitutively active 5-hydroxytryptamine$_2$C receptors reveal novel inverse agonist activity of receptor ligands. J. Biol. Chem. 269:11687–11690; 1994.
6. Burris, K.; Breeding, M.; Sanders-Bush, E.: LSD, but not its nonhallucinogenic congeners, is a potent 5-HT_{1C} receptor agonist. J. Pharmacol. Exp. Ther. 258:891–896; 1991.
7. Colpaert, F.; Meert, T.; Niemegeers, C.; Janssen, P.: Behavioral and 5-HT antagonist effects of ritanserin: A pure and selective antagonist of LSD discrimination in rat. Psychopharmacology (Berlin) 86:45–54; 1985.
8. Cunningham, K.; Appel, J.: Neuropharmacological reassessment of the discriminative stimulus properties of LSD. Psychopharmacology (Berlin) 91:67–73; 1987.
9. Gimpl, M. P.; Gormezano, I.; Harvey, J. A.: Effects of LSD on learning as measured by classical conditioning of the rabbit nictitating membrane response. J. Pharmacol. Exp. Ther. 208:330–334; 1979.
10. Ginn, S. R.; Powell, D. A.: Pizotifen attenuates classical eye-blink and heart rate conditioning in rabbits. Physiol. Psychol. 14:36–41; 1986.
11. Glennon, R. A.: Do classical hallucinogens act as 5-HT_2 agonists or antagonists? Neuropsychopharmacology 3:509–517; 1990.
12. Glennon, R. A.; Teitler, M.; Sanders-Bush, E.: Hallucinogens and serotonergic mechanisms. NIDA Res. Monogr. 119:131–135; 1996.
13. Harvey, J. A.: Serotonergic regulation of associative learning. Behav. Brain Res. 73:47–50; 1996.
14. Harvey, J. A.; Gormezano, I.; Cool, V. A.: Effects of d-lysergic acid diethylamide, d-2-bromolysergic acid diethylamide, *d*,l-2,5-dimethoxy-4-methylamphetamine and *d*-amphetamine on classical conditioning of the rabbit nictitating membrane response. J. Pharamcol. Exper. Ther. 221:289–294; 1982.
15. Hensman, R.; Guimaraes, F. S.; Wang, M.; Deakin, J. F.: Effects of ritanserin on aversive classical conditioning in humans. Psychopharmacology (Berlin) 104:220–224; 1991.
16. Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Hartig, P. R.; Martin, G. R.; Mylecharane, E. J.; Saxena, P. R.; Humphrey, P. P. A.: VII International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol. Rev. 46:157–203; 1994.
17. Leysen, J. E.: 5-HT_2-receptors: Location, pharmacological, pathologic and physiological role. In: Langer, S. Z.; Brunello, N.; Racagni, G.; Mendlewicz, J., eds. Serotonin receptor subtypes: Pharmacological significance and clinical implications. Int. Acad. Biomed. Drug Res., vol. 1. Basel: Karger; 1992:31–43.
18. Marek, G. J.; Aghajanian, G. K.: Excitation of interneurons in piriform cortex by 5-hydroxytryptamine: Blockade by MDL 100,907, a highly selective 5-HT_{2A} receptor antagonist. Eur. J. Pharmacol. 259:137–141; 1994.
19. Maura, G; Andrioli, G. C.; Cavazzani, P.; Raiteri, M.: 5-Hydroxytryptamine3 receptors sited on cholinergic axon terminals of human cerebral cortex mediate inhibition of acetylcholine release. J. Neurochem. 58:2334–2337; 1992.
20. Meneses, A.; Hong, E.: Modification of 8-OH-DPAT effects on learning by manipulation of the assay condition. Behav. Neural Biol. 61:29–35; 1994.
21. Mokler, D. J.; Rech, R. H.: Behavioral effects of intracerebroventricular administration of LSD, DOM, mescaline or lisuride. Pharmacol. Biochem. Behav. 21:281–287; 1984.
22. Nordberg, A.: Neuroreceptor changes in Alzheimer disease. Cerebrovasc. Brain Metab. Rev. 4:303–328; 1992.
23. Olivier, B.; Mos, J.; Tulp, M. Th. M.; van der Poel, A. M.: Animal models of anxiety and aggression in the study of serotonergic agents. In: Langer, S. Z.; Brunello, N.; Racagni, G.; Mendlewicz, J., eds. Serotonin receptor subtypes: Pharmacological significance and clinical implications. Int. Acad. Biomed. Drug Res., vol. 1. Basel: Karger; 1992:67–79.
24. Pazos, A.; Cortes, R.; Palacios, J. M.: Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. Brain Res. 346:231–249; 1985.
25. Pompeiano, M.; Palacios, J. M.; Mengod, G.: Distribution of the serotonin 5-HT_2 receptor family mRNAs: Comparison between 5-HT_{2A} and 5-HT_{2C} receptors. Brain Res. Mol. Brain Res. 23: 163–178; 1994.
26. Quartermain, D.; Clemente, J.; Shemer, A.: The 5-HT_{1A} agonist tandospirone disrupts retention but not acquisition of active avoidance learning. Pharmacol. Biochem. Behav. 48:805–807; 1994.
27. Quirion, R.; Richard, J.; Dam, T. V.: Evidence for the existence of serotonin type-2 receptors on cholinergic terminals in rat cortex. Brain Res. 333:345–349; 1985.
28. Richter-Levin, G.; Segal, M.: Age-related cognitive deficits in rats are associated with a combined loss of cholinergic and serotonergic functions. Ann. NY Acad. Sci. 695:254–257; 1993.
29. Romano, A. G.; Harvey, J. A.: MDMA enhances associative and nonassociative learning in the rabbit. Pharmacol. Biochem. Behav. 47:289–293; 1994.

30. Romano, A. G.; Bormann, N. M.; Harvey, J. A.: A unique enhancement of associative learning produced by methylenedioxyamphetamine. Behav. Pharmacol. 2:225–231; 1991.
31. Sadzot, B.; Baraban, J.; Glennon, R.; Lyon, R.; Leonhardt, S.; Jan, C.; Titeler, M.: Hallucinogenic drug interactions at human brain 5-HT_2 receptors: Implications for treating LSD-induced hallucinogenesis. Psychopharmacology (Berlin) 98:495–499; 1989.
32. Salt, G. L.; Aloyo, V. J.; Harvey, J. A.: Autoradiographic characterization of (+)[^{125}I]DOI binding to 5-HT_{2A} and 5-HT_{2C} receptors in the rabbit brain. Soc. Neurosci. Abstr. 22:1775; 1996.
33. Sanders-Bush, E.; Breeding, M.: Choroid plexus epithelial cells in primary culture: A model of 5-HT_{1C} receptor activation by hallucinogenic drugs. Psychopharmacology (Berlin) 105:340–346; 1991.
34. Sanders-Bush, E.; Burris, K.; Knoth, K.: LSD and DOM are partial agonists at serotonin receptors linked to phosphoinositide hydrolysis. J. Pharmacol. Exp. Ther. 246:924–928; 1988.
35. Sheldon, P. W.; Aghajanian, G. K.: Excitatory responses to serotonin (5-HT) in neurons of the rat piriform cortex: Evidence for mediation by 5-HT_{1C} receptors in pyramidal cells and 5-HT_2 receptors in interneurons. Synapse 9:208–218; 1991.
36. Siegel, S.; Freedman, D. X.: Effects of LSD-25 on classical trace conditioning. Pharmacol. Biochem. Behav. 30:427–431; 1988.
37. Steckler, T.; Sahgal, A.: The role of serotonergic-cholinergic interactions in the mediation of cognitive behaviour. Behav. Brain Res. 67:165–199; 1995.
38. Teitler, M.; Leonhardt, S.; Appel, N. M.; De Souza, E. B.; Glennon, R. A.: Receptor pharmacology of MDMA and related hallucinogens. Ann. NY Acad. Sci. 600:626–638; 1990.
39. Titeler, M.; Lyon, R.; Glennon, R.: Radioligand binding evidence implicates the brain 5-HT_2 receptor as a site of action for LSD and phenylisopropylamine hallucinogens. Psychopharmacology (Berlin) 94:213–216; 1988.
40. Watts, S. W.; Gackenheimer, S. L.; Gehlert, D. R.; Cohen, M. L.: Autoradiographic comparison of [^{125}I]LSD-labeled 5-HT_{2A} receptor distribution in rat and guinea pig brain. Neurochem. Int. 24: 565–574; 1994.
41. Watts. V. J.; Lawler, C. P.; Fox, D. R.; Neve, K. A.; Nichols, D. E.; Mailman, R. B.: LSD and structural analogs: Pharmacological evaluation at D_1 dopamine receptors. Psychopharmacology (Berlin) 118:401–409; 1995.
42. Westphal, R. S.; Sanders-Bush, E.: Reciprocal binding properties of 5-hydroxytryptamine type 2C receptor agonists and inverse agonists. Mol. Pharmacol. 46:937–942; 1994.
43. Wilkinson, L.: SYSTAT: The system for statistics. Evanston, IL: SYSTAT, Inc.; 1990.
44. Winer, B. J.: Statistical principles in experimental design. New York: McGraw-Hill; 1971.