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Operant Response Suppression Induced With Systemic Administration of 5-Hydroxytryptophan Is Centrally Mediated

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ENGLEMAN, E. A., J. M. MURPHY, F. C. ZHOU, M. H. APRISON AND J. N. HINGTGEN. *Operant response suppression induced with systemic administration of 5-hydroxytryptophan is centrally mediated.* PHARMACOL BIOCHEM BEHAV **52**(3) 525–529, 1995.—Intracerebroventricular (ICV) administration of selective serotonergic agents was used to examine the extent of central mediation of 5-HTP-induced operant response suppression in rats. ICV administration of LY53857 (1.0, 3.75, or 7.5 μg/5 μl/5 min) dose dependently blocked response suppression induced with systemically administered 5-HTP (25 mg/kg, IP), whereas ICV 0.9% saline (5 μl over 5 min) had no significant effect on 5-HTP-induced response suppression. ICV ketanserin (7.5 μg/5 μl/5 min) also blocked response suppression induced with systemically administered 5-HTP. ICV administration of the $5\text{-HT}_{2A/2C}$ receptor agonist DOI (80 μg/5 μl/5 min) induced significant periods of response suppression in this model, which was blocked with LY53857 (1.0 mg/kg, IP) pretreatment. These data demonstrate that central administration of $5\text{-HT}_{2A/2C}$ antagonists potently attenuate operant response suppression induced with systemically administered 5-HTP or DOI and are in agreement with previous findings suggesting central mediation of 5-HTP-induced operant response suppression.

Serotonin Behavior 5-Hydroxytryptophan Operant responding Operant conditioning Depression Intracerebroventricular administration 5-HT_2 receptors Rats Serotonin agonists Serotonin antagonists

FOR MORE than 30 years it has been known that administration of the serotonin (5-HT) precursor 5-hydroxytryptophan (5-HTP) to rats or pigeons trained to respond for food reinforcement on operant tasks induces a transient period of response suppression (1,2). Aprison et al. (4) were able to correlate the changes in behavior emitted by pigeons following 5-HTP (IP) administration with corresponding changes in brain 5-HT levels measured in the telencephalon and diencephalon. Chronic or acute pretreatment of rats with certain antidepressants or 5-HT antagonists was found to reduce the period of response suppression (8,9). A positive correlation was found between the percent blockade of the 5-HTP induced effect and the affinity of these agents for 5-HT receptors (8). Desipramine, a selective NE uptake inhibitor having little affinity for serotonergic uptake sites or receptors, displayed little or no capacity to block the 5-HTP effect; however, acute administration of the selective 5-HT uptake inhibitor fluoxetine potentiated the effect (8,12).

Although some investigators have suggested that 5-HTP-induced response suppression is essentially a peripheral effect (11,15), Aprison, Hingtgen, and others have provided at least four pieces of evidence that suggest that 5-HTP-induced response suppression (as measured in the current study) is centrally mediated: a) operant response suppression occurring after systemic administration of 5-HTP is associated with increased levels of free 5-HT in telencephalic and diencephalic brain regions but not in peripheral tissues including lung, blood, and liver (4); (b) iproniazid, a MAO inhibitor with higher central than peripheral activity, potentiates the 5-HTP effect which does not normalize until brain, but not periph-

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eral, MAO activity returns to normal (4); (c) direct injections of 5-HTP into the lateral hypothalamus can induce response suppression (10); and (d) central nervous system lesions of the 5-HT system, produced with the selective 5-HT neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) injected directly into the dorsal and median raphe nuclei, potentiate the response suppression induced with systemically administered 5-HTP (5). In addition, systemic administration of 5-HTP has been found to increase extracellular levels of 5-HT in rat hypothalamus (7,14). These studies together suggest a central mediation of 5-HTP-induced response suppression possibly in the diencephalic or cortical brain regions.

In the current study, central vs. peripheral mediation of 5-HTP-induced response suppression was determined by using ICV administration of selective antagonists for 5-HT receptors that, when administered systemically, block 5-HTP-induced response suppression. The extent of blockade of the systemically induced 5-HTP response suppression with central administration of the blocker provides an indication of central vs. peripheral mediation of the effect. In addition, DOI, a selective 5-$HT_{2A/2C}$ receptor agonist was administered ICV or systemically to induce response suppression that was also blocked with a 5-$HT_{2A/2C}$ antagonist.

METHOD

The behavioral methods used were similar to those previously described (5,10). Male Wistar and Sprague-Dawley rats were purchased from Harlan Industries, Indianapolis, IN. Upon receipt, the rats were weighed, numbered, handled, and kept in separate home cages on a 12 L : 12 D cycle (lights on at 0600 h). They were fed rodent laboratory chow #5001 (Purina Mills Inc., St. Louis, MO), ad lib until reaching their adult free feeding weight, at which time they were food deprived to 80% of that weight. Rats were trained to press a lever for 0.10 ml of sweetened milk (Borden Eagle Brand) on a variable interval 1-min schedule of reinforcement (VI 1′) in an operant chamber.

Behavioral Apparatus and Methods

The operant chamber was a large modular test cage (25 × 27 × 30 cm) with an aluminum grid floor and three interchangeable panels in the front wall (Coulbourn Instruments, Lehigh Valley, PA, model E10-10). A stainless steel lever was positioned 1.5 cm above the cage floor in the right front panel of the cage with a cue light bank 2.5 cm above the lever. A liquid reinforcement module was inserted in the center panel 4 cm to the left of the lever 1.5 cm above the floor. Behind the front panel, a 0.10 ml cup extended down into a reservoir containing milk. When the unit was activated, the milk was presented through a hole in the bottom of a 2.0 × 2.0 × 2.5 cm magazine. With each lever press during an operant session the green cue light was illuminated for 0.10 s as a cue indicating successful lever activation. When a reinforcement was presented, the magazine was illuminated with a white light for the duration of dipper activation (4 s) to serve as a cue for consumption of the reinforcement. A white house light positioned 1.5 cm below the top of the chamber in the center panel was on through out the sessions except during minute 16 when saline or drugs were administered. The entire operant chamber was enclosed in a soundproof outer chamber with an observation hole.

Computer programs were written in the SKED computer language (State Systems Inc., Kalamazoo, MI) and executed on a Digital PDP11/73 computer with the Micro RSX operating system version 3.0 (Digital Equipment Corp., Maynard, MA) to implement the appropriate schedules of reinforcement. Sessions were 15, 30, 60, or 180 min in duration. Programs were written to calculate the period of response suppression by counting the number of lever presses in the first 15 min of the session, multiplying that result by two and comparing the product with the total number of presses for the entire session. At the point in time when these two numbers were equal, 30 min was subtracted from the total elapsed time to that point in the session to calculate response suppression time (5).

Once the rats were consistently emitting the lever pressing response, they were given short sessions (15 or 30 min) with successively longer variable interval schedules of reinforcement (VI 5,″ VI 15,′ VI 30,′ VI 1′). Repeated 60 min VI 1′ sessions were given until the rats reached stable baseline performance such that the total number of lever presses did not differ by more than ± 15% from session to session. Rats were then acclimated to the injection procedure by removing the animals 15 min after the start of the session, administering 0.5 ml of a 0.9% saline solution intraperitoneal (IP), and returning them to the apparatus. This procedure was repeated until the pre- and postinjection response rates were not significantly different.

The following agents were used in behavioral experiments and surgical procedures. L-5-hydroxytryptophan (L-5-HTP) was purchased from Sigma Chemical Company Inc., St. Louis, MO. (±)-1-(2,5 dimethoxy-4-iodophenyl)-2-aminopropane (DOI), (±)-8-hydroxydipropylaminotetralin hydrobromide (8-OH-DPAT), and 3-[2-(4-fluorobenzoyl)-1-piperdinyl]-2,4(1H,^{3}H)-quinazolinedione tartrate (ketanserin) were purchased from Research Biochemicals Inc., Natick, MA. 6-Methyl-1-(1-methylethyl)-ergoline-8β-carboxylic acid 2-hydroxy-1-methylpropyl ester, maleate salt (LY53857) was provided by Eli Lilly & Company, Indianapolis, IN. Pentobarbital (Abbott Labs, Chicago, IL) and Atropine Sulfate (Sigma Chemical Company Inc., St. Louis, MO) were used in surgical procedures.

Systemic Administration of 5-HT Agonists and Antagonists

All drugs were made fresh daily in 0.9% saline and were administered between 0900 and 1200 h. A minimum 72-h washout period was allowed between days of drug injection. IP injections of 0.9% saline, L-5-HTP (25 mg/kg), or DOI (1.0 mg/kg) were administered after 15 min of baseline responding. Subsequently, LY53857 (1.0 mg/kg, IP) was injected 1 h prior to sessions of DOI-induced response suppression as previously described (8).

Intraventricular Administration of 5-HT Agents

Eight rats were implanted with unilateral indwelling cannulae (described in the following section) for lateral ventricular microinjection. DOI (80 μg) was administered 15 min into an operant session through microinjection cannulae connected to a 10 μl Hamilton syringe via 40 cm of polyethylene tubing. The Hamilton syringe was mounted on a syringe pump (Sage Instruments Inc., Cambridge, MA; model 355) that was calibrated to provide steady administration of injectate over the desired period. The volume for ICV injections was 5.0 μl, and the injection rate was 1.0 μl per minute for all injections. After 15 min of baseline responding, rats were removed from the operant chamber, dummy cannulae were removed, and microinjection cannulae were carefully inserted into the guide can-

nulae. The syringe pump was turned on and the rats were allowed to move about freely in their home cages during the injection period. When the injection was completed, the syringe pump was turned off and injection cannulae remained in place for 1 min, after which the rats were immediately placed back into the operant chamber and the session was resumed.

In experiments where central administration of 5-HT antagonists was used to attenuate response suppression, the response suppression was initially induced with systemic administration of L-5-HTP (25 mg/kg, IP), or DOI (1.0 mg/kg, IP). After a minimum 72-h washout period, LY53857 (1.0, 2.5, or 7.5 μg), ketanserin (7.5 μg), or 0.9% saline was injected ICV in a volume of 5 μl over a 5-min period (as described above) immediately prior to operant sessions in which response suppression was induced with systemic administration of 25 mg/kg L-5-HTP or 1.0 mg/kg DOI.

Stereotaxic Implantation of Microinjection Cannulae

Microinjection guide (22 gauge), injection (28 gauge), and dummy (28 gauge) cannulae sets were purchased from Plastics One Inc. (Roanoke, VA). The injection cannulae were cut such that when inserted into the guide, the tip extended 1.0 mm below the bottom of the guide cannulae, whereas the dummy cannulae were flush with the bottom of the guide cannulae. Rats were anesthetized with 60 mg/kg pentobarbital IP and were given 1.0 mg/kg atropine IP prior to surgery. While anesthetized, the scalp in the incision area was shaved with electric shears and the rat was placed in a David Kopf stereotaxic instrument (David Kopf Instruments, Tujunga, CA; model 900). A sagittal incision was made in the midline and the skull surface was exposed. Cannulae were implanted unilaterally at coordinates AP −1.0, ML ±1.5, DV −2.5 mm measured from bregma (13). Three or four stainless steel screws were tightly screwed into the skull at positions around the cannulae implant sites. Cranioplastic cement was mixed and applied over the open surface of the skull covering both the screws and the guide cannulae. The incision was closed around the implant and the dummy cannulae were inserted. Rats were returned to their home cages and 7 days recovery time was allotted before beginning intraventricular drug administration.

Histology

Upon completion of ICV injection sessions, rats were anesthetized with a lethal dose of pentobarbital (150 mg/kg) and 5.0 μl of India ink or trypan blue was injected through the guide cannulae over a 5-min period. All rats were decapitated with a guillotine and the brains were quickly removed and placed in 10% formalin for at least 48 h. Brains were blocked free hand with a razor blade and mounted for coronal sectioning on a microtome. The blocks were frozen in liquid carbon dioxide and Tissue Tek mounting media was used to mount the tissue to the stage of the microtome. Sections (25 μm) were cut coronally through the tissue and every third section was mounted on gelatin-coated glass slides (25 × 7 × 1 mm). After 24 h the slides were stained with either neutral red or cresyl violet and coverslipped. The sections were viewed with the aid of a dissection microscope and areas marked with the microinjected dye were examined to determine if the dye entered the ventricular system by comparison with a rat brain atlas (13).

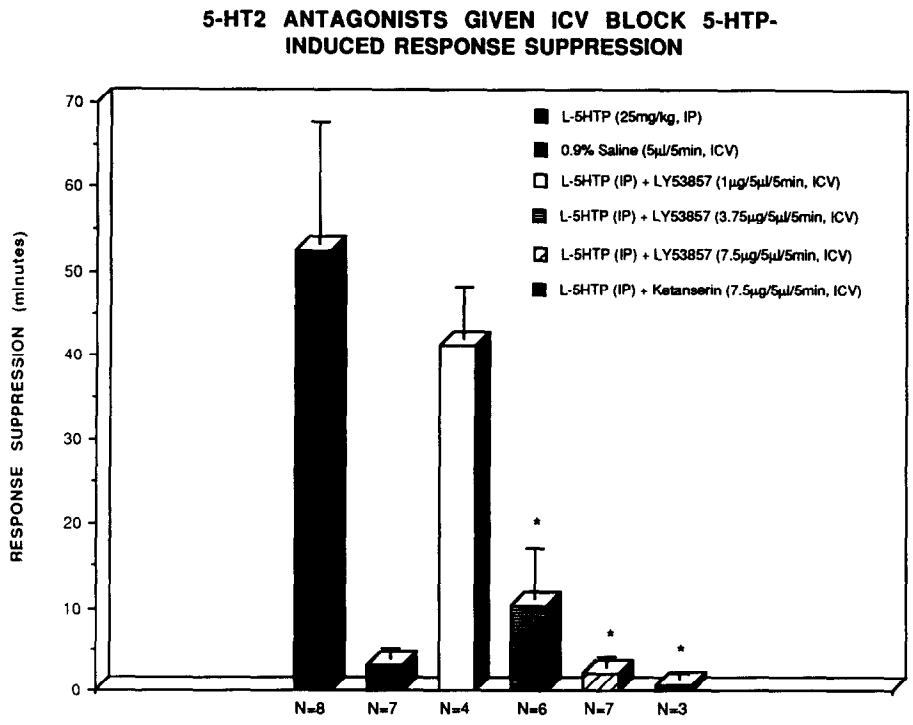

FIG. 1. Intraventricular administration of LY53857 (1.0, 3.75, or 7.5 μg/5 μl/5 min) or ketanserin (7.5 μg/5 μl/5 min) attenuates mean response suppression induced with L-5-HTP (25 mg/kg, IP). Values are mean ± SEM. *Significantly different from 5-HTP, paired *t*-test.

RESULTS

LY53857 (1.0, 3.75, or 7.5 μg/5 μl/5 min) administered ICV immediately prior to sessions in which response suppression was induced with 25 mg/kg L-5-HTP (IP), dose dependently reduced the period of suppression (paired t-tests: 3.75 μg $t = 3.80, p = 0.013$ and 7.5 μg $t = 3.37, p = 0.015$; Fig. 1). Similarly, ketanserin (7.5 μg/5 μl/5 min) given ICV prior to 25 mg/kg L-5HTP (IP) induced response suppression, attenuated the 5-HTP effect (paired t-test: $t = 7.45, p = 0.018$; Fig. 1).

ICV administration of DOI (80 μg/5 μl/5 min) induced a significant period of response suppression (29 ± 10 min, mean ± SEM, $n = 4$) after a 15-min period of baseline responding. Preadministration with LY53857 (1.0 mg/kg, IP) 1 h prior to ICV DOI (80 μg/5 μl/5 min) significantly reduced (93%) the period of DOI-induced response suppression (see Fig. 2) to 2 ± 1 min ($t = 2.45, p = 0.046, n = 4$, paired t-test). LY53857 (1.0 mg/kg, IP) did not significantly affect mean response rates in the 15-min period of baseline responding prior to ICV DOI administration (542 ± 193 and 477 ± 157; mean ± SEM responses with and without LY53857 preadministration, respectively). Similarly, LY53857 (15 μg/5 μl/5 min) given ICV immediately prior to DOI-induced response suppression (1.0 mg/kg) significantly reduced the mean period of DOI-induced response suppression in the same animals ($t = 7.61, p = 0.0047, n = 4$, paired t-test; see Fig. 3). A delay in the onset of the DOI-induced response suppression after ICV LY53857 was seen in many of the sessions (see Fig. 4).

Histological analysis of sections after dye injection revealed that the dye coated the ventricular system with the darkest staining in the lateral ventricle into which the stain was injected. The stain penetrated up to 1.0 mm into brain areas in contact with the ventricles. Stain was also found along the cannulae tracks from the implants for some rats.

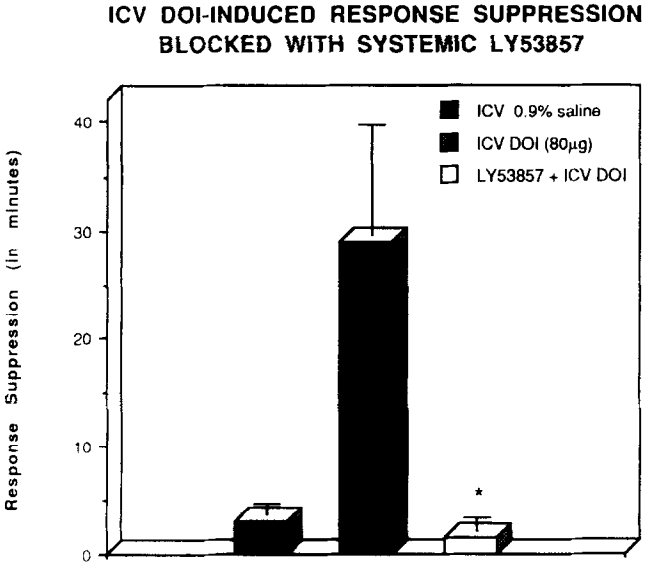

FIG. 2. Systemic administration of 1.0 mg/kg LY53857 1 h prior to sessions attenuated the period of response suppression induced with 80 μg DOI given ICV. *Significantly different from 80 μg DOI alone, $n = 4$, paired t-test.

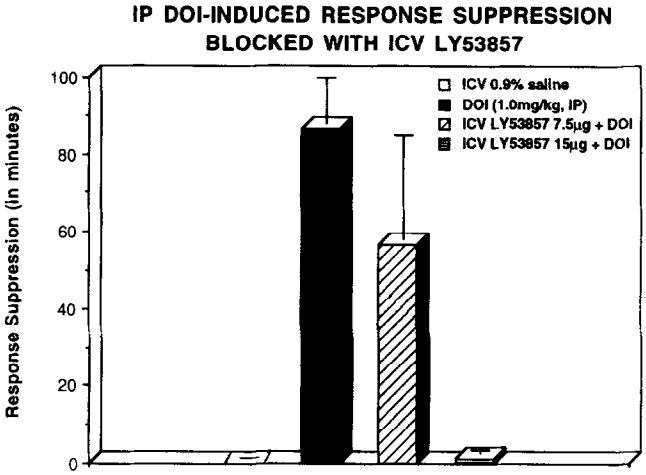

FIG. 3. Response suppression induced with systemic administration of 1.0 mg/kg DOI was attenuated by preadministration with LY53857 given ICV. *Significantly different than DOI 1.0 mg/kg alone, $n = 4$, paired t-test.

DISCUSSION

Systemic administration of the 5-HT precursor 5-HTP has long been used to produce disruptions in certain operant behaviors (1,2). In the current study, a central mediation of this effect was demonstrated using ICV administration of selective serotonergic agents. Operant response suppression induced with systemic administration of L-5-HTP (25 mg/kg, IP) was dose dependently blocked by ICV administration of LY53857 (Fig. 1). Similarly, ICV ketanserin (7.5 μg), which has high affinity and selectivity for 5-HT_{2A} receptors, also blocked the response suppression induced with systemic 5-HTP (Fig. 1). All dosages of centrally administered LY53857, which significantly blocked the 5-HTP effect, were lower than the concentration necessary to block response suppression when administered systemically (8).

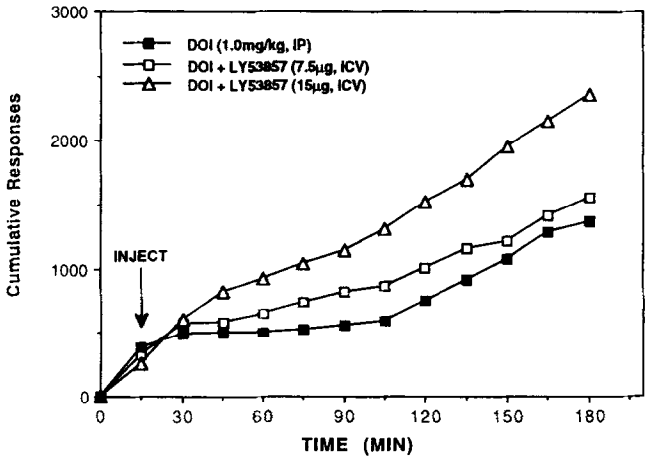

FIG. 4. Cumulative response records for a representative rat responding on a VI 1′ schedule. This rat received IP injections of 1) 1.0 mg/kg DOI, 2) 1.0 mg/kg DOI after 7.5 μg LY53857 ICV, or 3) 1.0 mg/kg DOI after 15 μg LY53857 ICV.

ICV administration of the 5-HT_{2A} and 5-HT_{2C} agonist (±)DOI (80 μg/5 μl/5 min) induced a mean 29 ± 10 min period of response suppression that was blocked with preadministration of LY53857 (1.0 mg/kg, IP) 1 h prior to the behavioral session (Fig. 2). Periods of response suppression induced with systemic administration of either 5-HTP or DOI were similarly attenuated with LY53857 (1.0 mg/kg, IP) (8). The dose of DOI administered centrally (0.25 mg/kg) in this experiment was found not to induce significant periods of response suppression in this model when administered systemically (6). The fact that this effect is blocked by peripheral injections of the 5-HT_{2A} and 5-HT_{2C} antagonist, LY53857, suggests that it is likely mediated through 5-HT_{2A} and/or 5-HT_{2C} receptors, and that systemically administered LY53857 may also be producing its behavioral effects through a central mechanism. Initially, ICV LY53857 (7.5 μg/5 μl/5 min) did not consistently attenuate response suppression induced with systemically administered 1.0 mg/kg DOI. The fact that this dose of LY53857 does not block the DOI effect, as it does L-5-HTP, suggests that DOI may be having some peripheral effects. Alternatively, because DOI is a highly potent agonist and binds 5-HT_{2A} and 5-HT_{2C} receptors with much higher affinity than 5-HT and no high affinity reuptake system exists to deactivate it as there is for 5-HT, it was reasoned that higher dosages of ICV LY53857 may be necessary to block this effect. Indeed, at 15 μg administered ICV, LY53857 completely attenuated response suppression induced with 1.0 mg/kg DOI administered systemically (Fig. 3). A delay in onset of response suppression induced with DOI was evident in most animals in this experiment (see Fig. 4). This would suggest that LY53857 was having effects on the DOI-induced suppression even at the lower dosages used in this experiment.

In summary, the current data demonstrate that operant response suppression induced with systemic administration of 5-HTP or DOI can be blocked with central administration of 5-$HT_{2A/2C}$ antagonists. In addition, central administration of DOI, a selective 5-$HT_{2A/2C}$ agonist, induced operant response suppression. These data support previous studies (4,5,10) suggesting that 5-HTP-induced operant response suppression, as measured in this study, is centrally mediated through 5-HT_2 receptors.

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