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Systemic Injection of *p*-Chloroamphetamine Eliminates the Effect of the 5-HT_3 Compounds on Learning[1]

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HONG, E. AND A. MENESES. *Systemic injection of* p-*chloroamphetamine eliminates the effect of the 5-HT_3 compounds on learning.* PHARMACOL BIOCHEM BEHAV **53**(4) 765–769, 1996. – There is evidence that 5-HT_3 antagonists enhance learning and memory; however, their mechanisms of action are unknown. The aim of the present work was to investigate further the role of 5-HT_3 receptors involved in learning, using the specific 5-HT_3 agonist 1-(*m*-chlorophenyl)-biguanide (mCPBG) and the 5-HT_3 antagonists ondansetron and tropisetron. *p*-Chloroamphetamine (PCA) pretreatment was used to determine whether pre- or postsynaptic 5-HT_3 receptors are involved in learning. The posttraining intraperitoneal (IP) injection of each drug was analyzed on a lever-press response on autoshaping, which is an associative learning task. The results showed that mCPBG impaired retention of the conditioned response (CR), whereas tropisetron and ondansetron improved it. In other animals, PCA alone did not affect CR but was able to block the effects of the 5-HT_3 ligands. The present data suggest that the actions of 5-HT_3 compounds could be due to their interaction with presynaptic 5-HT_3 receptors.

Serotonin 5-HT_3 receptors Learning Rats

THE 5-HYDROXYTRYPTAMINE (5-HT) neurons project to many brain areas (12); some of them are involved in learning and memory processes (26). Serotonergic neurotransmission involves the action of multiple 5-HT receptors types and subtypes 5-$HT_{1A\text{-}1F}$, 5-$HT_{2A\text{-}2C}$, and 5-$HT_{3\text{-}7}$ (10). Some experimental findings suggest that 5-HT_3 antagonists improve learning (3), and such an effect has been obtained with both systemic and intraamygdala administrations (3). There is also evidence that the 5-HT_3 receptor increases the magnitude and duration of long-term potentiation (LTP), a form of synaptic plasticity supposedly related to learning and memory (24). It is unclear whether pre- or postsynaptic 5-HT_3 receptors are involved. Autoradiographic studies have shown that 5-HT_3 receptors localized in the amygdala, hippocampus, and entorhinal cortex are reduced when 5,7-dihydroxytryptamine (5,7-DHT) is administered into the dorsal raphe nucleus (13), suggesting that such receptors are localized presynaptically. We have previously found that the 5-HT_{1A} agonist 8-OH-DPAT (18) and the 5-HT uptake inhibitor fluoxetine (20) enhanced retention of conditioned responses in the autoshaping learning task, whereas the 5-HT_{1B} agonist TFMPP impaired it (9). The activation of 5-HT_{1A} and 5-HT_{1B} presynaptic receptors mediates the effect of 8-OH-DPAT (17) and TFMPP (9), respectively. In contrast, the effect of fluoxetine is due to the stimulation of multiple 5-HT receptors of a postsynaptic nature (20). In the present work, we hoped to determine the role of 5-HT_3 receptors in the retention of conditioned response on the autoshaping task. Autoshaping has been useful to detect the facilitation effect of *d*-amphetamine (21) and the impairment of scopolamine (20).

The receptor nomenclature used in this report is recommended by the Serotonin Club Nomenclature Committee (10). The research was supported by CONACYT Grant 4367-M9406.

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METHOD

Subjects

Male Wistar rats (12 weeks old) were collectively housed in a temperature- and light-controlled room under a 12 L : 12 D cycle (light on at 0700 h). Water and food were provided ad lib for 1 week. After that period, their body weights were reduced to 85% by gradually reducing the food intake during 7 days.

Apparatus

Operant chambers for rats with standard sound-attenuation were used. Chambers were 25 cm wide, 29 cm long, and 25 cm high. A retractable lever was mounted 4 cm above the floor and 10 cm from the right and left walls. The lever required a force of 10 g for operation. A food magazine for rat pellets (BioServ, Frenchtown, NJ) was located 5 cm to the right of the lever and 3 cm above the floor. A house light was located in the right top corner. Solid-state programming equipment was used for control and recording (Coulbourn Instruments, Lehigh Valley, PA).

Autoshaping Training

Each rat was placed into an experimental chamber and allowed to habituate to the experimental environment until the animal found and ate 30 food pellets (45 mg each). Immediately thereafter, the program began. This consisted in the presentation of an illuminated retractable lever for 8 s [conditioned stimulus (CS)] followed by delivery of a food pellet [unconditioned stimulus (US)], each 60 s. When the animal pressed the CS, the lever was retracted, the light was turned off, and a food pellet (US) was immediately delivered; this action was considered a conditioned response (CR). The increase or decrease in percentage of CR in treated groups relative to vehicle animals was considered to be an enhancement or impairment of retention of the CR. The first session consisted of 10 trials and the second session, 20. All compounds were injected immediately after the first autoshaping session and rats were tested 24 h later. The results shown correspond to the second autoshaping session.

Drug Treatment

The drugs used were: *p*-chloroamphetamine (PCA × 2 consecutive days), on days 7 and 8 before the first autoshaping session, and 1-(*m*-chlorophenyl)-biguanide (mCPBG) (Research Biochemical, Wayland, MA), tropisetron (Sandoz Pharma Ltd., Basel, Switzerland), and ondansetron (Glaxo, Greeford, Middlesex, UK). All drugs were dissolved in saline and injected (IP) in a volume of 1 ml/kg.

Measurements and Analysis

CR were transformed to a percentage of total trials of the second session. Multiple group comparisons were made using analysis of variance followed by Dunnett's *t*-test (e.g., vehicle vs. several dose treatments, or PCA vs. agonist, antagonists, or the combination of the neurotoxin plus other drugs) or Student's *t*-tests (e.g., vehicle vs. PCA pretreatment). In all statistical comparisons, $p < 0.05$ was used as the criterion for significance. *n* per group was 8, and animals were used only once.

Experiment 1: Effects of mCPBG, Ondansetron, or Tropisetron Administration After Training

The purpose of this experiment was to determine the effects of posttraining injection of the compounds in retention of the CR. Animals were injected immediately after the first session, with vehicle or mCPBG (1 or 10 mg/kg), tropisetron (0.001, 0.01, or 0.1 mg/kg) or ondansetron (0.01, 0.01, or 1.0 mg/kg). Rats were placed in their home cage and tested the next day.

Experiment 2: Effects of 5-HT_3 Compounds Plus PCA Administration

The purposes of these experiments were to determine whether the effects of mCPBG could be altered by 5-HT_3 antagonists and to investigate the mechanism of action of 5-HT_3 agonists and antagonists, with respect to the location of the receptors with which they interact. Therefore, several groups of rats were injected with ondansetron or tropisetron immediately after the first autoshaping session, and 10 min later received mCPBG. Other animals were pretreated consecutively with PCA (10 mg/kg) on days 7 and 8 before the autoshaping test; this group was compared with a control group receiving only vehicle. These PCA-pretreated groups were injected with mCPBG, tropisetron, or ondansetron immediately after the first autoshaping session and tested 24 h later.

RESULTS

Experiment 1: Effects of mCPBG, Ondansetron, or Tropisetron Administration After Training

The results show that the control group exhibited 11 ± 1% of CR, whereas administration of mCPBG significantly decreased the rate of the CR [$F(2, 23) = 5.1$, and $p < 0.05$] (Fig. 1A). In contrast, a significant increase in CR was observed after tropisetron [$F(3, 39) = 3.6$, and $p < 0.05$] (Fig. 1B) and ondansetron [$F(3, 31) = 4.2$, and $p < 0.05$] (Fig. 1C) administration. A further analysis with Dunnett's *t*-test revealed that tropisetron increased significantly the rate of the CR at doses of 0.01 and 0.1 mg/kg, whereas ondansetron produced a similar effect at doses of 0.1 and 1.0 mg/kg (Fig. 1).

Experiment 2: Effects of 5-HT_3 Compounds Plus PCA Administration

Data for vehicle- and PCA-treated animals during the first and second autoshaping sessions (Table 1) show that there were no significant differences in both sessions [$F(1, 31) = 0.01$ and $p > 0.05$]. Figure 2A shows that tropisetron [$F(3, 31) = 16.2$, and $p < 0.05$] and ondansetron [$F(3, 31) = 12.2$ and $p < 0.05$] significantly blocked the decrement in the CR induced by mCPBG injection. Tropisetron (0.01 mg/kg) or ondansetron (0.1 mg/kg) significantly antagonized mCPBG-induced impairment. The PCA injection did not affect CR by itself, but prevented the effect provoked by mCPBG (Fig. 2B), producing 2 ± 1% of CR [$F(3, 31) = 5.6$ and $p < 0.05$]. PCA administration diminished the increment in CR produced by tropisetron (Fig. 2C), as this combination elicited 13 ± 2% of CR [$F(3, 31) = 5.8$ and $p < 0.05$]. The increase in the CR induced by ondansetron was also significantly diminished [$F(3, 31) = 5.6$ and $p < 0.05$] by PCA injection, resulting in a score of 10 ± 3% (Fig. 2D).

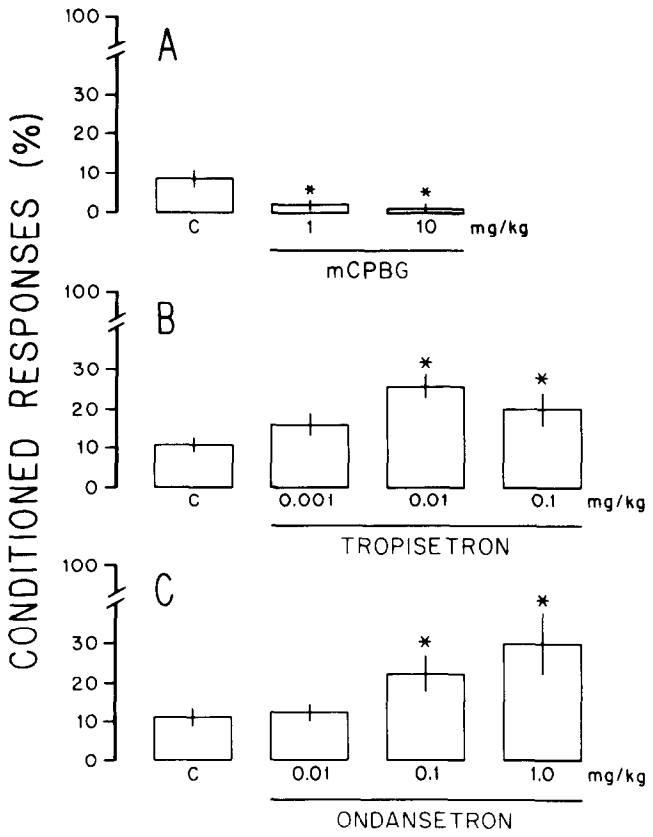

FIG. 1. Effect of acute posttraining administration (IP) of mCPBG (A), tropisetron (B), and ondansetron (C) on autoshaping task in fasting animals. Data are plotted as a percentage of control conditioned responses (CR%). All rats received injection immediately after the first training session. Values represent the mean ± SEM of eight different animals. *Dunnet's t-test < 0.05 vs. vehicle-injected controls.

DISCUSSION

Validity of the Behavioral Model

The autoshaping procedure has been previously used to assess learning (8,9,17–21). In this experimental assay, the training session itself results in significant increases of the CR when comparing the trained group (10 ± 2%) with the untrained one (0.6 ± 0.6%) (unpublished observations). Slight modifications to the procedure may result in variable data. For instance, when animals did not receive training to the food magazine, they displayed a lower percentage of the CR (3 ± 2%), whereas control animals that were trained with the food magazine showed 8 ± 2% of the CR (20) in the corresponding autoshaping session. A similar score to the latter was obtained in the present work in the case of PCA-treated animals and their respective controls (Table 1), indicating high reliability in the scores of control values. It should be noted that during the autoshaping training, animals learn to emit an active behavior (e.g., lever-press responses) as a result of the association between CS and US; such an association can be modified by drugs and training (17–21). The difference in CR between the first and second autoshaping sessions is modest (and usually not significant), thereby implying that the conditions of the test are adequate to detect whether a drug alters learning. Thus, under similar experimental conditions, the posttraining injection of scopolamine produced a signifi-

TABLE 1

EFFECT OF PCA ON AUTOSHAPING LEARNING TASK (CR%) IN RATS

	Session of PCA Administration	
	First	Second
Control	8 ± 2	11 ± 2
PCA (10 mg/kg × 2 days)	7 ± 2	12 ± 3

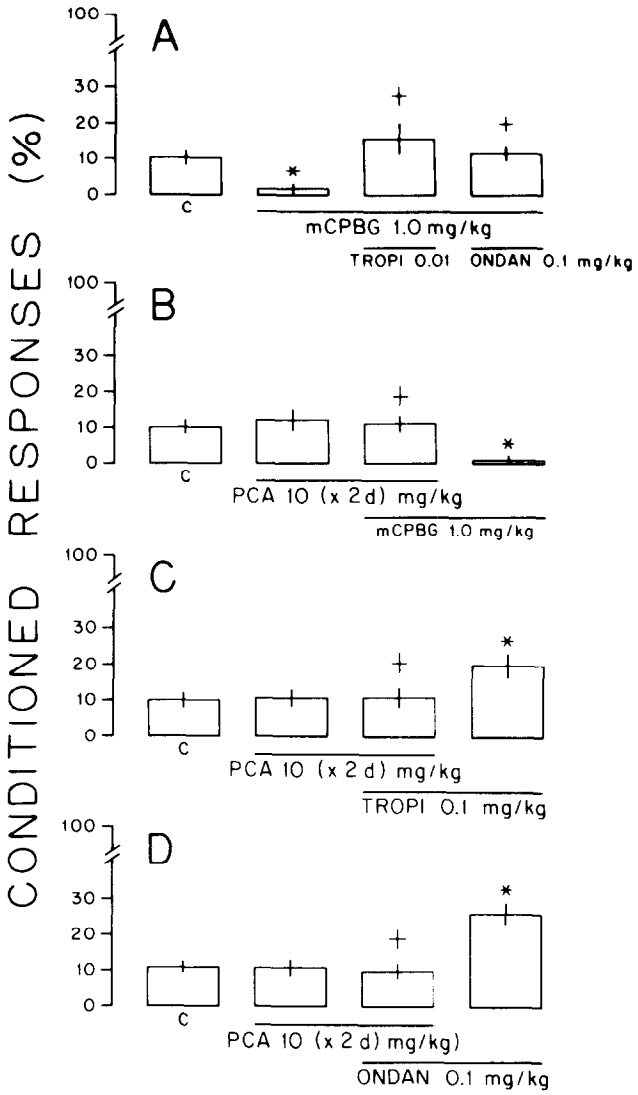

FIG. 2. Effects of posttraining injection of tropisetron, ondansetron (A), and PCA (B) on the impairment induced by posttraining injection of mCPBG on CR of autoshaping task in fasting animals. Also shown are the effects of tropisetron (C) or ondansetron (D) in normal or PCA-pretreated rats. *Dunnet's t-test < 0.05 vs. vehicle-injected controls; $^+$PCA vs. 5-HT$_3$ drugs.

cant decrease of CR (4 ± 1%) (20), whereas *d*-amphetamine induced a large increment (28 ± 6%) (21). It has been noticed that the use of 10 trials detects drug-induced changes in CR better (unpublished results). Furthermore, deteriorated animals (aged or hypertensive) compared to healthy young or normotensive rats clearly showed decreased learning (8,19).

Effects of 5-HT$_3$ Receptor Drugs

The present findings indicate that the 5-HT$_3$ agonist mCPBG impaired retention of the CR. In contrast, the administration of tropisetron and ondansetron improved it. The impairment effect induced by mCPBG injection was prevented by the two 5-HT$_3$ antagonists. It should be noted that according to the recent classification, mCPBG is considered to be a very potent and selective agonist at 5-HT$_3$ receptors, and is more active than phenylbiguanide or 2-methyl-5-HT [see (10) for a review]. In this connection, the doses of mCPBG reported to provoke behavioral effects (7,10) are similar to those used in the present work. With regard to the doses of antagonist drugs, they are consistent with 5-HT$_3$-receptor involvement. Thus, the doses of 0.01 mg/kg tropisetron and 0.1 mg/kg ondansetron produced increase in learning; lower doses only produced a weak inhibition of mCPBG-induced decrement in the CR. Interestingly, the individual effects of the 5-HT$_3$-receptor agonist and antagonist drugs were significantly decreased by PCA pretreatment.

Although we did not perform neurochemical determination, there is evidence that PCA treatment (under the same schedule used here) significantly reduced the 5-HT levels in some cerebral areas (1). Admittedly, such treatment does not alter all serotonergic innervation, as some neurotoxic amphetamines, such as PCA, cause extensive degeneration of fine 5-HT axons in forebrain, but groups of beaded 5-HT axons and serotonergic cell bodies in the brainstem are unaffected (14). This could explain why vehicle- and PCA-treated animals did not show significant differences in the CR (present data). Furthermore, it has been reported that *p*-chlorophenylalanine (PCPA) administration alone does not alter learning (17); indeed, several authors found that 5-HT depletion induced by PCA, PCPA, or 5,7-DHT impaired, improved, or had no effect on learning (1,2,6). Such discrepancies may be attributed to differences in the behavioral tasks, doses of drugs, pharmacologic treatments, and environmental manipulations employed in such studies (1,6). The fact that rats received drugs after the first training session allows us to suggest that present results cannot be attributed to unspecific effects, because at the time of the session test, the effect of the drugs had vanished completely (16,18). Autoradiographic studies revealed that 5-HT$_3$ receptors localized in the amygdala, hippocampus, and entorhinal cortex are reduced when 5,7-DHT is administered into dorsal the raphe nucleus (13). This finding suggests that these 5-HT$_3$ receptors could be located presynaptically (13), possibly located on the soma, axon, and/or nerve terminals of the GABAergic interneurones (22). In the present work, PCA pretreatment blocked the effects of mCPBG, tropisetron, and ondansetron, implying that activation and blockade of 5-HT$_3$ presynaptic receptors may be involved in the enhancement and impairment of learning. However, previous information suggests that postsynaptic 5-HT$_3$ receptors as well could be involved in learning and memory processes [e.g., the blockade of 5-HT$_3$ enhanced LTP (24)].

Diverse effects have been observed when 5-HT$_3$ antagonists are administered in the amygdala, including an improvement of learning and anxiolysis (3). Therefore, it is possible that 5-HT$_3$ presynaptic receptors localized in (at least) the amygdala and hippocampus could be involved in learning, without excluding other neurotransmission systems. In fact, electrophysiologic studies have shown that 5-HT$_3$ receptors acting as heteroreceptors in nuclei raphe, septum, hippocampus, nucleus magnocellularis, and cortex (5) modulate the activity of several neurotransmitters (12). For instance, the stimulation of 5-HT$_3$ modulates glutamatergic neurotransmission (22,23). In addition, the inhibition of neural firing through autoreceptors involves somatodendritic 5-HT$_{1A}$ or 5-HT$_{1B}$ receptors, whereas the facilitatory effect of 5-HT release is mediated by 5-HT$_3$ receptors (5,15).

The role of 5-HT receptors in learning and memory still needs to be established; however, the present and other results suggest that 5-HT$_{1A}$ (17,18), 5-HT$_{1B}$ (9), and 5-HT$_3$ presynaptic receptors are involved in such processes. For instance, the stimulation of 5-HT$_{1B}$ or 5-HT$_3$ presynaptic receptors impaired retention of the CR, whereas the activation of 5-HT$_{1A}$ autoreceptors enhanced it. Therefore, it is possible that an inverse relationship between learning and 5-HT activity involving such receptors exists. This possibility is consistent with the findings that the electrical stimulation of the dorsal raphe or intrahippocampal injection of serotonin impaired learning on avoidance and Y-maze tasks (1). This view, however, should be taken with caution, as there is evidence that fluoxetine pretreatment enhanced learning by favoring the interaction of 5-HT with various postsynaptic receptors (20).

In conclusion, the present work strongly suggests that central 5-HT$_3$ receptors, probably located in presynaptic neurons, participate in learning and memory, though further work using 5-HT$_3$ agonists and antagonists in PCA-pretreated animals will be required to confirm this suggestion.

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