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Manipulation of Serotonin Protects Against an Hypoxia-Induced Deficit of a Passive Avoidance Response in Rats

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STREK, K. F., K. R. SPENCER AND V. J. DENOBLE. *Manipulation of serotonin protects against an hypoxia-induced deficit of a passive avoidance response in rats.* PHARMACOL BIOCHEM BEHAV **33**(1) 241–244, 1989.—The 5-HT antagonists ketanserin, mianserin, methysergide, and cyproheptadine and the 5-HT uptake inhibitors fluoxetine and zimeldine were evaluated for their ability to protect against an hypoxia-induced performance deficit in a passive avoidance (PA) task. The ability to retain a PA response was found to decrease as the oxygen concentration decreased with the largest retention deficit occurring at 6.5% O_2. The 5-HT_2 selective antagonists ketanserin (0.01–10.0 mg/kg SC) and mianserin (0.05–10.0 mg/kg SC) administered one minute after PA training produced dose-dependent increases in retention latencies following exposure to a 6.5% oxygen environment. Peak effective doses (PED) for ketanserin and mianserin were 3.0 mg/kg SC and 0.05 mg/kg SC, respectively. In contrast, methysergide (0.05–30.0 mg/kg SC) and cyproheptadine (0.05–7.0 mg/kg SC), antagonists that show less affinity for the 5-HT_2 receptor subtype, were not effective in preventing the hypoxia-induced amnesia. Inhibition of 5-HT reuptake by fluoxetine (0.01–1.0 mg/kg SC) produced dose-dependent increases in retention latencies with a PED of 0.05 mg/kg SC while zimeldine (0.1–10.0 mg/kg SC), another 5-HT reuptake inhibitor, had no effect on the amnesia. The results of this study suggest that modification of 5-HT after exposure to hypoxia can ameliorate a performance deficit in an animal model of learning and memory.

Serotonin Amnesia Passive avoidance Hypoxia Rats

THE role of serotonin (5-HT) in the processes underlying learning and memory has been the subject of several laboratory investigations. Activation of the serotonergic nervous system has been shown to interfere with passive avoidance retention (PA) (12), active avoidance acquisition (18) and retention of a brightness discrimination (22). In contrast, interference with serotonergic activity has been shown in most instances to enhance both retention and acquisition in similar procedures (6,22). When these experiments were conducted, the pharmacology of the various 5-HT receptors was not well delineated and it was not possible to test compounds that had nonselective vs. selective 5-HT receptor activity in these learning and memory tests. A recent series of reports has demonstrated that administration of a number of serotonergic receptor antagonists both nonselective (metergoline, methysergide) and 5-HT_2 selective (pirenperone, ketanserin and mianserin) can enhance the memory of a previously learned inhibitory avoidance response in mice (2, 3, 17). Further, the results showed that the enhanced retrieval was specific to post-training drug administration and that compounds given before training produced dose-dependent impairment of retention (3). These studies provide strong support for the notion that blockade of the 5-HT_2 receptor subtype after avoidance training can enhance memory retrieval in mice. However, the actions of 5-HT antagonists on experimentally-induced amnesia have not been studied. It is well documented that exposure to hypoxia induces alterations in neurotransmitter functions which are directly dependent upon oxygen synthesis. For example, synthesis of catecholamines and 5-HT are decreased when the oxygen concentrations in inspired air are reduced to 9.4% (8,10). Similarly, when animals are maintained in low-level oxygen environments and then trained in a learning test, there is a resulting amnesia displayed during retention testing (4, 11, 21). To extend the previously mentioned findings with posttraining administration of 5-HT antagonists resulting in enhanced retention of an avoidance response (2,3), the present experiment examined the effects of posttraining administration of a number of 5-HT antagonists as well as 5-HT uptake inhibitors on hypoxia-induced PA retention deficits in rats.

METHOD

Animals

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Kingston, NY) weighing 125–135 g at the time of their

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arrival were used. The animals were housed four per cage 45(L) × 20.0(H) × 24.0(W) cm with food and water provided ad lib. They were maintained on a 12-hr light/dark cycle (lights on from 0600 to 1800 hr) at a room temperature of 22 ± 1°C with a relative humidity of 50 ± 10%.

Apparatus

The experimental sessions were conducted in a two-compartment PA box. One compartment, made of clear plastic with a perforated clear plastic floor, measured 21(L) × 24.5(H) × 17(W) cm and was illuminated with a 60-watt incandescent light bulb placed 36 cm above the floor. The other compartment, made of black plastic, measured 30.5(L) × 20.3(H) × 21.5(W) cm and had a floor made of 4-mm stainless steel rods spaced 1.2 cm apart. A Coulbourn Instruments Grid Floor Shocker was connected to the steel rods providing a scrambled footshock. The two compartments were separated by a solenoid-operated side door (Lafayette Instrument Co., Lafayette, IN). A Coulbourn Instruments Electronic Counter, activated by the opening or closing of the slide door, recorded acquisition and retention latencies. These latencies were defined as the time, in seconds, it took an animal to enter (all four paws on the grid floor) the dark compartment.

For memory disruption, rats were exposed to an hypoxic environment for 30 min immediately prior to PA training. The hypoxia chamber was constructed of clear plastic, measured 32.5(L) × 22.5(H) × 23(W) cm, and was continuously perfused with a gas mixture of oxygen and nitrogen. The flow rate was adjusted such that the gas turnover in the chamber was 15 liters per min. To determine the effects of different oxygen concentrations on PA retention, the rats were exposed to gas mixtures containing known percentages of oxygen (21%, 10%, 9%, 8%, 7% or 6.5%) supplemented with nitrogen for 30 min prior to PA training.

Passive Avoidance Training

Passive avoidance training began by placing the rat into the clear compartment of the two compartment PA box. Following a 10-sec delay, the slide door was raised providing access to the dark compartment. Once the rat moved completely into the dark compartment, the slide door was lowered and, after a 10-sec delay, a 1.5-mA shock was applied to the grid floor for 3 sec. This was followed by an additional 10-sec period at the end of which the rat received another 3-sec shock (1.5 mA). The rats were immediately removed from the dark compartment after receiving the second shock, injected with vehicle or test compound and returned to their home cage. Rats not entering the dark compartment within 90 sec were removed from the study. Of the animals tested, 3% were removed for not entering the dark compartment within the alloted time (90 sec) during acquisition training.

A retention test was given four hr later. It proceeded in the same manner as the training session except no shock was applied to the grid floor when the rats entered the dark compartment. During the retention test, the rats were provided access to the dark compartment for 300 sec.

To determine if the compounds were producing nonspecific effects (e.g., ataxia, sedation), a group of rats were given the highest dose of each compound and then placed into the clear compartment of the PA box. The procedure was the same as for the acquisition training except no shock was applied to the grid floor. All animals were dosed 15 min prior to being placed in the PA box.

Drug Preparation and Administration

Ketanserin (0.01–10.0 mg/kg, Janssen, Belgium), mianserin HCl (0.05–10.0 mg/kg, Organon, Holland), methysergide maleate (0.05–30.0 mg/kg, Sandoz, Hanover, NJ) and cyproheptadine HCl (0.05–7.0 mg/kg, Merck Sharp and Dohme, West Point, PA) were all suspended in 0.25% methylcelluose. Fluoxetine HCl (0.01–1.0 mg/kg, Eli Lilly and Co., Indianapolis, IN) and zimeldine HCl (0.10–10.0 mg/kg, Astra Pharmaceutical Products, Inc., Worcester, MA) were dissolved in 0.85% saline. All compounds were administered subcutaneously (SC) in a volume of 1 ml/kg of body weight 1 min following PA training.

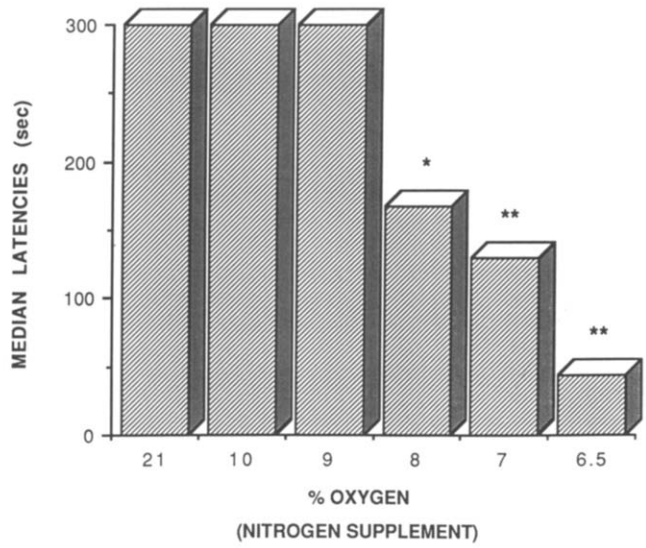

FIG. 1. The median passive avoidance (PA) retention latencies of rats are shown as a function of percent oxygen. Rats were exposed for 30 min to various concentrations of oxygen immediately prior to PA training (normal air contains 21% oxygen). PA retention was measured 4 hr later. Each bar represents the median retention latency (sec) obtained for a minimum of 15 rats and a maximum 300-sec cutoff. *Significantly different from 21% oxygen, $p<0.05$. **Significantly different from 21% oxygen, $p<0.025$.

Data Analysis

Overall significance was calculated using the Kruskal-Wallis one-way analysis of variance (ANOVA). Post hoc, median retention latencies were compared for vehicle controls and each treated group with a Mann-Whitney U-test. Data for different doses were accumulated over several days with both nonhypoxic and hypoxic vehicle control groups included with each day's testing.

RESULTS

The retention latencies for the PA response following hypoxia decreased as the oxygen concentration in the hypoxia chamber was reduced. That is, the median PA response retention latencies of rats treated with vehicle and exposed to normal air (21% oxygen) or moderate hypoxia (9–10% oxygen) were at the maximal level of 300 sec. However, when oxygen concentrations were further reduced to 8%, 7% or 6.5% oxygen, PA retention latencies were reduced to 167, 130 and 43 sec, respectively (Fig. 1). Lower oxygen concentration (6% oxygen) produced death in several rats, therefore the 6.5% oxygen concentration was selected for its ability to produce maximal memory disruption without producing mortality.

Ketanserin and mianserin, 5-HT_2 selective antagonists, produced dose-dependent increases in retention latencies with peak effective doses (PED) of 3.0 mg/kg SC (median retention latency =

TABLE 1

MEDIAN RETENTION LATENCIES OF A PASSIVE AVOIDANCE RESPONSE IN RATS TREATED WITH VEHICLE OR TEST COMPOUND FOLLOWING EXPOSURE TO 6.5% OXYGEN

	Dose (mg/kg, SC)[1]											
Drug	0	0.01	0.03	0.05	0.10	0.3	1.0	3.0	7.0	10.0	18.0	30.0
Ketanserin	47	134†	132*	—	64	103	160†	217†	—	61	—	—
Mianserin	51	—	—	267†	179	212†	234†	61	—	69	—	—
Methysergide	41	—	—	156†	27	60†	25	57	—	48	55	53
Cyproheptadine	42	—	—	79	17	81	34	26	15	—	—	—
Fluoxetine	29	61†	—	134†	89†	43†	30	—	—	—	—	—
Zimeldine	44	—	—	—	100	108	42	65	—	25	—	—

[1]Each dose was tested in a minimum of 11 rats.
*Significantly different from vehicle, $p<0.05$.
†Significantly different from vehicle, $p<0.025$.
—Doses not tested.

217 sec) and 0.05 mg/kg SC (median retention latency = 267 sec), respectively (Table 1). Methysergide and cyproheptadine, at doses at which they were less selective for the 5-HT_2 receptor subtype (1.0–30.0 mg/kg SC and 0.05–7.0 mg/kg SC, respectively), produced no significant increase in retention latencies compared to vehicle-treated rats exposed to hypoxia (Table 1). However, low doses of methysergide (0.05 and 0.3 mg/kg SC) which have been shown to have more selectivity for the 5-HT_2 receptor, produced significant increases in the latency with median retention latencies of 156 sec at 0.05 mg/kg SC and 60 sec at 0.3 mg/kg SC.

The 5-HT uptake inhibitor, fluoxetine, also produced latency increases at doses ranging from 0.01 to 0.3 mg/kg SC with a PED of 0.05 mg/kg SC (median retention latency = 134 sec). In contrast, zimeldine, also a 5-HT uptake inhibitor, did not significantly increase latencies at doses ranging from 0.1 to 10.0 mg/kg SC (Table 1).

The increased latencies seen in some of these compounds were not a result of nonspecific drug effects on behavior in general (e.g., producing ataxia or nyctophobia) since nonshocked rats injected with the highest dose of each compound showed no significant increases in retention latencies as compared to vehicle-treated shock controls.

DISCUSSION

The main result from the present research is that manipulations of 5-HT following exposure to hypoxia can reduce hypoxia-induced amnesia in a PA retention test. Previous investigations have shown that exposure to hypoxia can produce a rapid onset of reversible brain dysfunction that results in memory loss similar to that seen in senile dementia (16,20). Hypoxia-induced neurotransmitter changes may provide a mechanism through which impaired performance is mediated since oxygen is used in the synthesis of many neurotransmitters. For example, norepinephrine, dopamine and epinephrine are synthesized from the combination of tyrosine and oxygen and 5-HT is synthesized from tryptophan and oxygen. Tyrosine hydroxylation is impaired when the oxygen concentration is reduced in a brain striatal synaptosome preparation suggesting that the availability of oxygen can regulate the synthesis of catecholamines in brain (9). Hypoxia, therefore, may selectively effect neurons that store neurotransmitters that utilize oxygen for synthesis (catecholamines and serotonin). Behaviorally, animals maintained in hypoxia before and/or after acquisition of a PA response show retention deficits when tested 24 hr later (4, 11, 21).

In the present experiment, retention of the PA response was tested 4 hr after training and hypoxia exposure produced similar-effects to those previously reported; that is, a profound amnesia for the PA response (4, 11, 12). In addition, the median entry latency was shown to vary as a function of the chamber's oxygen concentration indicating a functional relationship between oxygen availability and memory formation.

Posttraining administration with some drugs that alter the activity of 5-HT at its receptor sites prevented the hypoxia-induced PA retention deficit. While the effects of serotonergic drugs on retention of an inhibitory response have been documented (2,3), this is the first report of some serotonergic agents preventing an hypoxia-induced amnesia of a single trial PA response. Specifically, posttraining administration of the 5-HT_2 selective antagonists, ketanserin and mianserin (7), significantly increased retention latencies. However, the facilitory or inhibitory effect of 5-HT antagonists have been found to be dependent on time of drug administration. Pretraining treatment of ketanserin and mianserin produced decreases in retention latencies of a PA response in mice when tested 48 hr later, while posttraining administration of these compounds produced increases in the retention latencies (3). While these animals were tested under conditions of extinction, the results of the posttraining administration of 5-HT antagonists are consistent with the present study in which the rats were exposed to hypoxia. The combined results of these studies suggest that alteration of serotonergic function can facilitate memory information processing as well as prevent the effects of hypoxia-induced amnesia.

Protection against an hypoxia-induced amnesia appears to be 5-HT_2 selective since methysergide and cyproheptadine, both of which have pharmacological effects at 5-HT_1 and 5-HT_2 receptor sites (5,19), when administered at a variety of doses, produced no changes in retention latencies. However, at low doses, methysergide increased retention latencies. Receptor binding studies have shown that at nanomolar concentrations, methysergide has a high affinity for the 5-HT_2 receptor site (5,19), therefore, the effectiveness of low doses of methysergide used in this study may be related to 5-HT_2 selectivity when given in low doses. However, the effect of methysergide and cyproheptadine on both 5-HT_1 and 5-HT_2 receptor sites may explain their inability to protect against the hypoxia-induced deficits and suggests that 5-HT_2 antagonists are more effective in this model.

Stimulation of the serotonergic system, either through pharmacological manipulation of 5-HT (12,22) or through direct electrical stimulation (13), has been implicated in disrupting the learning or memorial process. To determine if increased levels of 5-HT effects retention of a PA response in animals exposed to hypoxia, 5-HT uptake inhibitors, fluoxetine and zimeldine were tested. In the present study, fluoxetine produced dose-dependent increases in

retention latencies of animals exposed to hypoxia. Posttraining administration of fluoxetine increased retention latencies and facilitated retrieval of a PA response in mice when tested one week following training (15). However, administration of fluoxetine has also been shown to increase corticosterone levels which has also been implicated in improving memory retention (14), therefore, it is not clear whether the increases in retention latencies are due to increases of 5-HT or that of increased corticosterone levels. In contrast, zimeldine produced no significant changes in retention latencies although it has been previously reported to facilitate the retrieval of a PA response in mice 24 hr after training when administered prior to retention testing (1). While fluoxetine and zimeldine both are specific 5-HT uptake inhibitors and both have previously been shown to facilitate the retrieval of a PA response, it is not clear why fluoxetine was effective in protecting against the hypoxia-induced amnesia and zimeldine was not unless the enhanced release of corticosterone by fluoxetine is responsible for preventing the hypoxia-induced amnesia.

Taken together, the results of this study are in agreement with that of previous findings suggesting a role for 5-HT in learning and memory and that alterations in 5-HT are effective in preventing hypoxia-induced memory deficits.

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