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Differential Effect of 5-Hydroxytryptophan on Approach and Avoidance Behavior in Rats

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PARK, W. K., J. N. HINGTGEN AND M. H. APRISON. *Differential effect of 5-hydroxytryptophan on approach and avoidance behavior in rats.* PHARMACOL BIOCHEM BEHAV **38**(1) 191–194, 1991.—The current hypersensitive postsynaptic serotonin receptor theory of depression developed and expanded by Aprison and Hingtgen was based on an animal model of behavior in which food-reinforced approach behavior was suppressed following 5-hydroxytryptophan (5-HTP) administration. In this paper, data are presented to show that when the same animal is taught to emit, alternatingly, approach and avoidance behavior, and the serotonin precursor, 5-HTP, is administered, only the approach behavior is affected. Adult, male Wistar rats were trained on Sidman avoidance (RS20:SS10) and food-reinforced approach (VI 1) schedules. During the first part of this study, rats received separately 50-min sessions for approach and avoidance responding. For the second part, both schedules were given in the same experimental chamber. In the third part, 10-min alternating approach and avoidance components were combined in the same 50-min sessions. Significant behavioral suppression of approach responding was observed following administration of L-5-HTP (50 mg/kg IP), as well as after D,L-5-HTP (25 and 50 mg/kg IP) in a dose-dependent relationship, whereas no significant effect was seen for Sidman avoidance responding during this type of session. These results support the role of serotonin in food-reinforced approach behavior and suggest that suppression of Sidman avoidance behavior may be mediated by other neurotransmitter systems.

5-Hydroxytryptophan (5-HTP) 5-Hydroxytryptamine (5-HT) or serotonin Approach behavior Avoidance behavior Postsynaptic serotonin receptors Animal model of depression Theory of depression

SINCE 1959, Aprison and co-workers have been involved in the study of the quantitative interaction between changes in specific neurotransmitters, including their biosynthetic and degradative enzymes, and changes in behavior (1–4, 8–10, 12, 13, 19, 20). This extensive series of neurochemical/behavioral experiments with animals included the study of the role of cerebral serotonin in approach behavior. These studies led to the conclusion that 5-HTP-induced increases in the concentration of 5-HT within key synapses in the telencephalon and diencephalon are related to behavioral suppression of food-reinforced operant behaviors. More recent research has provided data which indicated specifically the importance of the lateral hypothalamus (14,15) in this type of behavioral suppression.

It has been established that 5-HTP in a 50 mg/kg dose given to animals working on food-reinforced operant schedules does not significantly affect catecholamine (CA) levels [see (7) for review]. On the other hand, Sidman avoidance behavior may be more sensitive to CA perturbations (23,24). Taken together, these data strongly suggest that suppression of approach and avoidance behavior is not mediated by the same mechanisms. We now wanted to compare the effect of both the L- and D,L-forms of the immediate precursor of 5-HT, 5-HTP, on Sidman avoidance as well as on approach behavior. It has been shown that when D,L-5-HTP is given to rats working on food-reinforced approach schedules, only the L-5-HTP form has a major effect on approach behavior, e.g., 25 mg/kg L-5-HTP is as effective as 50 mg/kg D,L-5-HTP (21). Further, it was of great interest to administer the 5-HTP to the same animal emitting both behaviors to test whether increased cerebral 5-HT affects Sidman avoidance behavior in an animal whose approach behavior is markedly affected. Before one can do these studies, it was necessary to develop a procedure to train rats to emit both behaviors alternately in a single box during a complete behavioral session. The experiments described in this paper were designed to study the effect of 5-HTP in the same rat working on approach and avoidance schedules of reinforcement.

METHOD

Behavioral Training

Male Wistar rats (250–300 g) were trained on two behavioral paradigms, an approach schedule (8) and a Sidman avoidance

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schedule (22), using three different procedures. All rats were maintained at 80% free-feeding weights (15 g food/day) during the study; free access to water was also provided. The rats were housed one per cage in a room with 12-hour light/dark cycles.

In the first method, the approach conditioning apparatus (Apparatus I) was constructed of 0.3 cm Plexiglas with interior dimensions (l/w/h) of 21/15/12 cm, respectively. A dipper-feeding device was mounted on the center of the front panel of each box with an aluminum brass lever located at right side, and 5 cm above the floor. The grid floor for the chamber was constructed of parallel brass rods at 1.5-cm intervals (8). Rats were trained to press a lever for 0.15 ml sweetened condensed milk on a variable interval 1 min (VI 1) schedule so that reinforcements were delivered an average of once per minute during stable lever pressing performance. The Sidman avoidance apparatus was of similar construction, but without the dipper. A grid floor provided foot shock with a response shock interval of 20 s (RS20) and a shock-shock interval 10 s (SS10). Shocks were given every 10 s unless the lever was pressed to postpone the impending shock for 20 s. The shock duration was 0.5 s with 1.0 mA intensity. The entire behavioral unit was placed in a sound-insulated outer compartment. The animals were trained 5 times a week for daily 60 min sessions for approach and 45-min sessions for avoidance until they could perform at stable baseline levels.

After a series of L-5-HTP and D,L-5-HTP injections (see below), the same group of rats was trained in Apparatus II (Model E10-10, Coulbourn Co.) with contingencies controlled by computer programming, the whole unit being placed in a sound-proof cage. The interior dimensions were similar to Apparatus I. The behavioral schedules remained the same, but now each behavioral component was given one contingency at a time (60 min of approach followed by 45 min of avoidance) in the same chamber. While either schedule was in effect, the house light was on all the time.

Following additional L-5-HTP or D,L-5-HTP injections, the same rats were trained in the same box (Apparatus II) on a combination approach/avoidance schedule. The approach lever was on the left side with a green color cue light; a lever for Sidman avoidance was mounted on the right side with red color cue light. This schedule consisted of an alternating contingency of 10-min approach and 10-min avoidance responding during a 50-min session.

Injection Procedures

After the lever pressing was stabilized, the saline (0.9%) injections were given in the same volume as 5-HTP. All IP injections of D,L-5-HTP (25 and 50 mg/kg) and L-5-HTP (50 mg/kg) were given 15 min after the start of each session. 5-HTP was dissolved in 0.9% saline and the pH was adjusted to 7.4. The 5-HTP and vehicle injections were always administered at a designated time (10 a.m.) each day. At least 5 days were allowed between the drug injections.

Recordings of cumulative lever pressing during the session were obtained for each rat. Behavioral depression was calculated as the time from the point of injection of 5-HTP to the point at which a rat doubled the number of cumulative responses that occurred during the 15 minutes of the control session (8).

For the combination schedule, 5-HTP was administered IP 5 min before the session started. The percent decrease in response rate was calculated by comparing the responses after injection to the previous day's control responses for approach and avoidance behavior.

All data were subjected to statistical analysis using the Student's *t*-test and analysis of variance where appropriate.

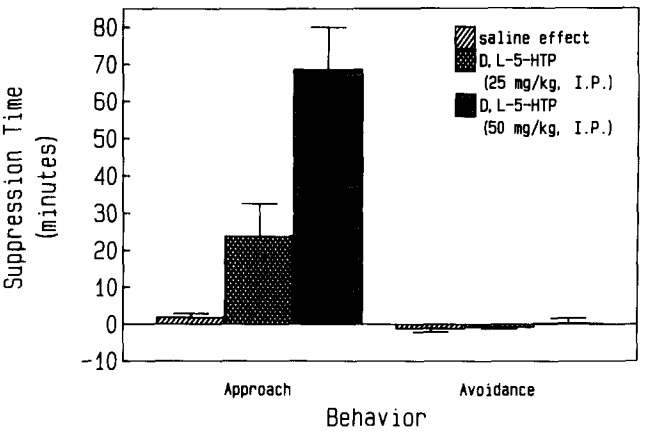

FIG. 1. 5-HTP-induced behavioral suppression (min) of approach responding (VI 1) and Sidman avoidance responding (SS10:RS20) measured in apparatus I (separate behavioral chamber used for each type of behavior, see text). Means ± S.E.M. (n = 9). Approach rates were significantly decreased compared to control rates ($p<0.01$).

RESULTS

The average response suppression time following 5-HTP administration for a group of rats (N = 9) on either VI (approach) or Sidman (avoidance) schedules in Apparatus I is given in Fig. 1. Food-reinforced approach responding was significantly depressed by 5-HTP administration. Injections of 25 mg/kg (IP) of D,L-5-HTP resulted in 24 min (mean) of suppression time, whereas a dose of 50 mg/kg (IP) of D,L-5-HTP produced 69 min (mean) of suppression time. No significant change was seen in the avoidance response rate after these same doses.

Using Apparatus II, significant behavioral suppression of approach behavior was induced by 5-HTP: D,L-5-HTP (50 mg/kg, IP) resulted in 26 min (mean) of behavioral suppression; L-5-HTP at the same dose (50 mg/kg, IP) resulted in 77 min (mean) suppression (see Fig. 2). Again, no significant suppression of avoidance responding was observed.

Average baseline response rates for the 10-min approach and 10-min avoidance periods during the combination sessions are given in Fig. 3. Although both the average approach and avoidance responding was increased during the session, statistical analysis of these data indicate that the means of the first, the third and the fifth periods of all approach sessions are not significantly different from each other ($F = 0.69$). The difference in mean response rates between the second and the fourth sessions of avoidance responding also was found not to be significant. Furthermore, the mean response rates of the first 10-min approach and 10-min avoidance sessions are similar to each other, and not significantly different from the next 20 minutes of approach and avoidance responding (3rd and 4th periods).

In the experiments for the combination approach/avoidance schedule where the serotonin precursor are injected, the overall decrease of control rate was 75% with D,L-5-HTP (50 mg/kg, IP) and L-5-HTP (25 mg/kg, IP) when six rats were pooled together for approach responses (see Fig. 4). No significant decrease in avoidance responding was seen.

DISCUSSION

The early psychoneuropharmacological studies from this laboratory on the effect of elevating cerebral serotonin levels involved animals trained to emit one type of food-reinforced approach

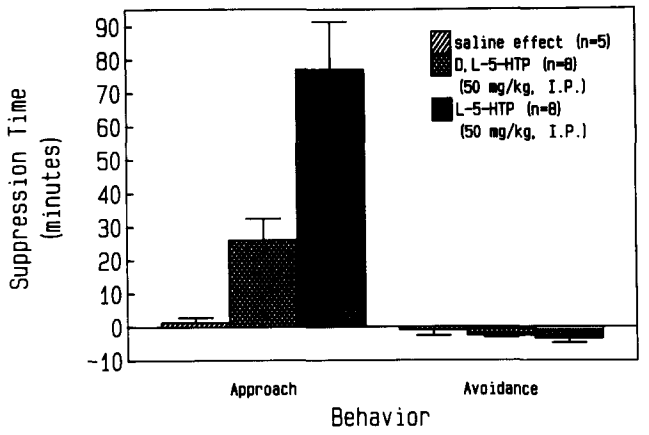

FIG. 2. 5-HTP-induced behavioral suppression (min) of approach responding (VI 1) and Sidman avoidance responding (SS10:RS20) as measured in apparatus II (computerized apparatus in which the same rats were trained in the same box for the approach and avoidance responding one contingency at a time). Means ± S.E.M. (n = 8). Approach rates were significantly decreased compared to control rates ($p<0.01$).

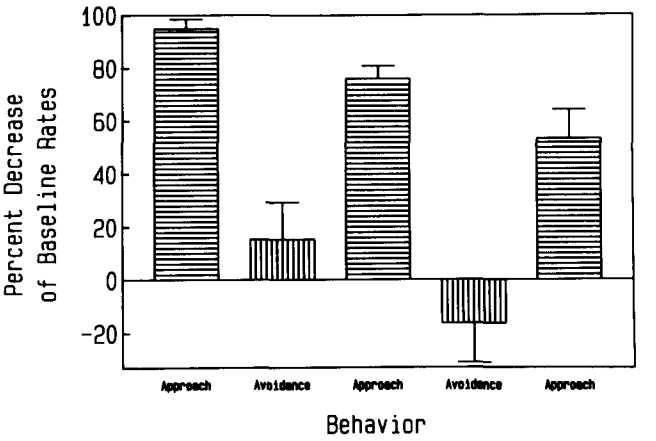

FIG. 4. The percent decrease of the approach and avoidance response rate on the combination schedule following either D,L-5-HTP (50 mg/kg) or L-5-HTP (25 mg/kg) IP (means ± S.E.M.; n = 6). Approach rates were significantly decreased compared to control rates ($p<0.01$).

behavior (1–6). Therefore, it was of great theoretical interest to learn what would happen to an animal performing two different behaviors when only the serotonin system was affected by injecting the immediate precursor, i.e., L-5-HTP or D,L-5-HTP (2–7). A new procedure was developed to train rats on both approach and avoidance schedules which involved three components: (a) approach and avoidance schedules given in separate operant chambers; (b) approach and avoidance schedules given during a separate session but in the same operant chamber; and (c) a combination approach/avoidance schedule given during the same session as alternating 10-min approach and avoidance components. Using this procedure, it was possible to successfully train the rats to make the transition to emit both behaviors in a single apparatus during the same 50-min session. In all of these procedures, a significant 5-HTP-induced behavioral suppression of approach behavior was seen, whereas no significant effect on Sidman avoidance responding was noted.

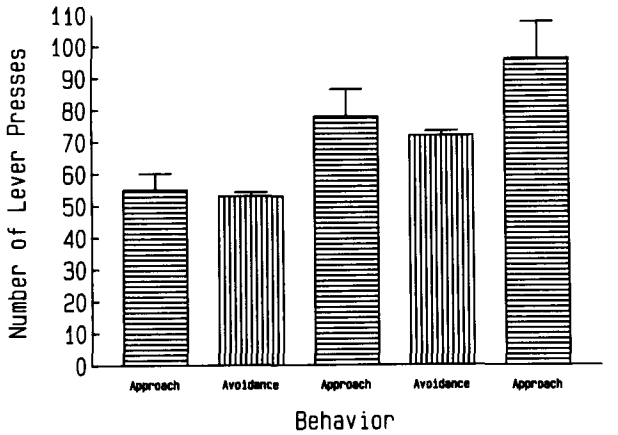

FIG. 3. The average baseline response of the combination approach avoidance behavioral model was measured during 10-min periods, each of alternating schedules of approach and avoidance responding during a 50-min session. (n = 7; number of trials = 9; means ± S.E.M.)

Neurochemical data (7, 16, 17) have shown that 5-HT synthesized from 5-HTP can cause the release of [^{3}H]-5-HT previously taken up into preparations of nerve endings isolated from the telencephalon of pigeons and rats. Doses of 5-HTP used in this laboratory do not affect the release of [^{3}H]-norepinephrine or [^{3}H]-dopamine when the synaptosomes were labelled with these transmitters (16,17). Furthermore, after 5-HTP injections, the levels of 5-HTP, 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) were significantly higher in the nerve ending fraction isolated from the telencephalon and diencephalon of pigeons and rats given 50 mg/kg D,L-5-HTP in comparison to control values (7, 16, 17). All these data provide direct biochemical evidence to indicate that the injected 5-HTP can accumulate within serotonergic neurons and significantly raise the levels of 5-HT within their respective nerve endings as well as increase the release of 5-HT into the synaptic cleft. More recent studies also have supported the role of central 5-HT mechanisms in the 5-HTP effect [see (14) for references].

The present data support the idea that the neuronal pathways utilized by the rat to emit approach behavior are sensitive to the cerebral serotonin levels at key synapses (5–7). Further, since in the present experiments the rats have learned to emit two types of behavior, approach and avoidance, the data also support the conclusion that normal key serotonergic synapses do not appear to be present in the neuronal pathways utilized to emit Sidman avoidance behaviors, or, if present, only have such synapses that are not as sensitive to elevations in released synaptic 5-HT. Therefore, these data suggest other systems, such as the catecholamine neurotransmitter system (4, 23, 24) and/or the cholinergic neurotransmitter system, are implicated in avoidance types of behaviors (11).

Tanaka et al. (24) demonstrated that Sidman avoidance was decreased markedly when cerebral norepinephrine (NE) was depleted in the telencephalon and brain stem by 200 mg/kg (IP) α-methyl tyrosine (α-MT), an inhibitor of tyrosine hydroxylase, which also reduced the content of cerebral 5-HT by 23%. Administration of L-dopa (100 mg/kg, IP) reversed the effect of α-MT on avoidance responding and restored NE levels in the brain. They also used 300 mg/kg (PO) p-chlorophenylalanine (PCPA), an inhibitor of tryptophan hydroxylase, as a 5-HT depletor, and demonstrated an increase in the avoidance response rate. This PCPA effect was partially antagonized by D,L-5-HTP (50 mg/kg,

IP), while brain 5-HT levels were restored to 60% of normal. While such a level of 5-HT in brain may be too low to permit normal functioning of this important neurotransmitter system, these findings suggested to Tanaka et al. (24) the existence of a catecholaminergic excitatory system and serotonergic inhibitory system associated with Sidman type avoidance behavior. In light of the Tanaka et al. (24) conclusion, it is very interesting that Steranka, Barrett and Sanders-Bush (23) reported when employing p-chloroamphetamine (PCA) in rats displaying Sidman avoidance behavior, this drug produces an amphetamine-like facilitatory effect on behavior which is independent of its effects on 5-HT mechanisms, but is related to the changes in the metabolism of cerebral catecholamines. Further consideration should also be given to other systems, since studies have indicated that the main effect of muscarinic-cholinergic stimulation on operant avoidance behavior appear to be depressant, while cholinergic blockade or nicotinic stimulation results in facilitation (11).

One can hypothesize that the suppression of food-reinforced approach behavior is mediated by 5-HT postsynaptic receptors, but Sidman avoidance responding is not. When PCPA depleted the brain 5-HT levels, there was a facilitation on Sidman avoidance responding. It is of great interest to note that Fleisher et al. (9) from this laboratory reported that PCPA alone did not change the approach response, whereas 5-HTP administered after PCPA potentiated behavioral suppression. These results suggest that supersensitive 5-HT postsynaptic receptors play a role in suppression of food-reinforced approach behavior, but may not play such a key role in avoidance behavior.

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