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Tryptamine Impairs the Acquisition of a One-Way Active Avoidance Task

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FLETCHER, P. J. *Tryptamine impairs the acquisition of a one-way active avoidance task.* PHARMACOL BIOCHEM BEHAV 32(1) 317–321, 1989.—The effects of intraperitoneal administration of tryptamine to rats pretreated with iproniazid, on the acquisition of an unsignalled one-way active avoidance task, were examined. Tryptamine at 2.5 and 5 mg/kg significantly increased the number of trials required to perform this task to a 9/10 consecutive avoidances criterion, without affecting escape performance. The iproniazid pretreatment had no affect on acquisition, or any other performance variable, of the task. Tryptamine did not significantly affect the avoidance response, or escape response latencies; further tryptamine did not alter gross locomotor activity measured as photocell counts. These results suggest that the acquisition deficit was not the result of nonassociative effects such as changes in response capability, general activity level or nociception. The acquisition deficit induced by tryptamine may involve a direct stimulation of central 5-HT receptors since it was not induced by systemically administered 5-HT, was reversed by the 5-HT antagonists methysergide and metergoline, but was not affected by depletion of brain 5-HT, with PCPA, or by the dopamine antagonist haloperidol. Possible behavioural mechanisms for the action of tryptamine are discussed.

Tryptamine 5-Hydroxytryptamine Active avoidance Rats

CENTRAL administration, or peripheral injection of tryptamine [often in combination with a monoamine oxidase inhibitor (MAOI)], induces a variety of physiological and behavioural changes [reviewed in (9)]. In many cases these effects are similar to those induced by 5-hydroxytryptamine (5-HT) or 5-HT agonists. Examples include hyperactivity and the 5-HT syndrome in rats (17,18), myoclonus in guinea pigs (15), a behavioural syndrome involving caudally directed biting and scratching (13), changes in cardiovascular function (12), reduced food intake (5) and conditioned taste aversion (8). In other cases tryptamine has been shown to enhance the effects of 5-HT or 5-HT agonists. Thus, tryptamine at concentrations which are ineffective in inducing physiological or behavioural changes was found to enhance the effects of 5-HT on neuronal firing rate (10), locomotor activity (6), the biting and scratching syndrome (14) and the behavioural syndrome elicited by tryptophan plus tranylcypromine (17,18). Further studies have shown also that tryptamine and 5-HT may induce quite different physiological and behavioural changes. Injection of tryptamine into the preoptic area of the hypothalamus in the rat induces hyperthermia, whereas 5-HT injected into the same area induces hypothermia (2,3). Moreover, these thermic effects of tryptamine and 5-HT were differentiated further in terms of their susceptibility to 5-HT antagonists (2). Since tryptamine is present in the mammalian nervous system (26), these results have led to several theories concerning the possible functional role of endogenous tryptamine [reviewed in (9)]. Thus, it has been suggested that tryptamine may act directly on the brain 5-HT system, may be a modulator of 5-HT function, or may be a neurotransmitter in its own right.

A previous study has reported that peripherally-injected tryptamine to rats pretreated with the MAOI, iproniazid, disrupts conditioned avoidance responding without changing gross behaviour (29). Avoidance responding is affected also by manipulations of 5-HT function (21). A consistent finding in this area is that *p*-chloroamphetamine (PCA) impairs the ability of rats to acquire an active avoidance task (22–24). This effect of PCA has been attributed to the 5-HT-releasing action of this compound, and cannot be explained in terms of changes in motor performance, nociception or shock-induced motivation (22,24).

The present experiments were undertaken, therefore, to examine the effects of tryptamine (in combination with iproniazid) on the acquisition of an active avoidance task, and to examine the possible relationship, if any, with 5-HT systems. The avoidance procedure used was an unsignalled one-way active avoidance task similar to that described by Ogren (22).

METHOD

Animals

Male Wistar rats (240–300 g) were used. The animals were group-housed under standard laboratory conditions, and maintained on a 12-hour light/dark cycle. Food and water were freely available except during testing.

Avoidance Testing

Testing was carried out in a shuttlebox (Coulbourn Instruments, Lehigh Valley, PA) measuring 36×17×21 cm and divided into two identical compartments by a 4-cm hurdle.

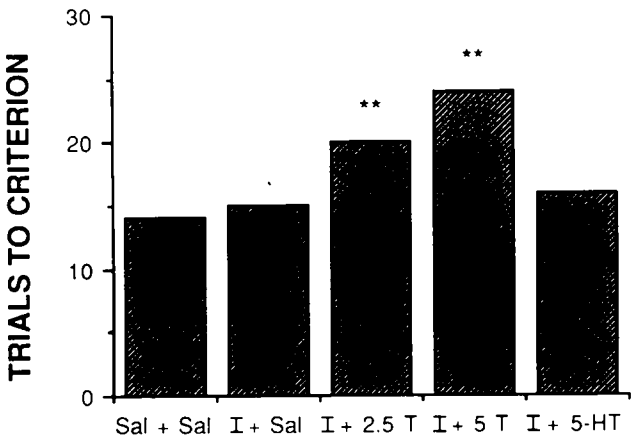

FIG. 1. Median number of trials to a criterion of 9 out of 10 avoidance responses for groups of rats (n=7) treated with saline, iproniazid (I), 2.5 and 5 mg/kg tryptamine (T) (plus I), and 5 mg/kg 5-HT (plus I). *Different from saline, $p<0.05$ (Mann-Whitney U-test); **different from saline, $p<0.01$ (Mann-Whitney U-test).

The floor of each compartment consisted of stainless steel bars spaced 15 mm apart. The floor of one compartment could be electrified by a shock generator built in this laboratory.

A procedure similar to that described by Ogren (22) was used. Seven minutes following drug treatment (see below) each rat was allowed to explore freely the shuttlebox for 3 minutes, whereupon it was placed in the "shock" compartment for the first trial. Five seconds later a scrambled, constant current shock (0.5 mA) was delivered to the floor for a period of 5 seconds. The rat could escape the shock by traversing the hurdle to reach the safe compartment. The shock could be avoided by entering the safe compartment within 5 seconds of being placed in the "shock" compartment. Following either escape or avoidance responses the animal was allowed to remain in the safe compartment for 20 seconds, and then removed to a holding cage for a further 10 seconds. The next trial was then initiated. Any rat failing to escape from the shock was placed in the safe compartment for 20 seconds and then in the holding cage for 10 seconds before begining the following trial. For each animal a maximum of 30 trials was conducted. A criterion of 9 out of 10 consecutive avoidance responses was adopted to define acquisition of the task (22). On each trial response latencies were recorded using a hand-held stop-watch.

Activity Testing

Locomotor activity was measured in four Plexiglas cages (40×40×23 cm) positioned in an infra-red photobeam recording device (Opto-Varimex, Columbus Instruments, OH). Interruption of any of the 12×12 photobeams positioned around the base of the cages was recorded by an Apple II Plus microcomputer through a microprocessor. The total number of beam interruptions served as the measure of locomotor activity.

Four groups (n=8 each) of rats were used. Each group received two injections spaced 24 hr apart: group 1—saline + saline; group 2—100 mg/kg iproniazid + saline; group 3—100 mg/kg iproniazid + 2.5 mg/kg tryptamine; group 4—100 mg/kg iproniazid + 5 mg/kg tryptamine. Seven minutes following the injection each rat was placed in the apparatus and locomotor activity was recorded for the following 30 minutes.

Effects of PCPA on Brain 5-HT Levels

Since PCPA was used in one experiment to deplete brain 5-HT content 12 rats were used to determine the extent of the 5-HT depletion. Six rats were treated orally with 400 mg/kg PCPA ethyl ester (in 3 ml/kg distilled water), and the other six received water only. Three days later the rats were decapitated and their brains removed. Amine levels were determined using high-performance liquid chromatography with electrochemical detection according to the method described by Sloley *et al.* (28).

Drugs

All drugs except *para*-chlorophenylalanine (PCPA) were administered intraperitoneally in a volume of 1 ml/kg; doses refer to the salts. Tryptamine HCl, iproniazid phosphate and serotonin creatinine sulphate (Sigma Chemical Co., St. Louis, MO) were dissolved in 0.9% saline. Methysergide bimaleate (Sandoz, Canada) was dissolved in distilled water. Metergoline (Farmitalia, Torino, Italy) was dissolved in 1% ascorbic acid; haloperidol (Janssen, Belgium) was dissolved in 1% tartaric acid. PCPA ethyl ester (Sigma) was dissolved in distilled water and administered orally in a volume of 3 ml/kg. All animals with the exception of the control animals in the dose response experiment were pretreated with 100 mg/kg iproniazid phosphate 24 hours prior to testing. Iproniazid is a long-lasting MAOI; for example, a single intraperitoneal injection of 50 mg/kg iproniazid inhibited MAO activity in rat brain by approximately 90% 18 hr later (27).

Statistics

The results from the avoidance experiments were analysed by a Kruskall-Wallis analysis of variance by ranks; following a significant H value, post hoc comparisons were made using the Mann-Whitney U-test (two-tailed).

RESULTS

Effect of Tryptamine on Avoidance Behaviour

Tryptamine in combination with iproniazid induced an increase in the median number of trials required to reach the acquisition criterion as shown in Fig. 1. Statistical analysis revealed a significant treatment effect [Kruskall-Wallis, $H(4)=13.5$, $p<0.01$]; pair-wise testing showed that animals treated with 2.5 and 5 mg/kg tryptamine required more trials to reach criterion compared to saline-treated controls. Animals receiving iproniazid treatment alone did not differ from control animals. Therefore, the observed deficit in rats treated with iproniazid plus tryptamine can be attributed to the tryptamine treatment. Injection of 5 mg/kg 5-HT in combination with iproniazid did not significantly change the median number of trials to criterion.

Table 1 shows several other aspects of behaviour in the avoidance task. No significant treatment effects were observed on measures of the trial number on which the first avoidance response was observed, $H(4)=0.7$, $p>0.1$, escape latencies during the first 10 trials, $H(4)=0.5$, $p>0.1$, and avoidance latencies throughout the testing, $H(4)=6.9$,

TABLE 1

MEDIAN (AND RANGE) RESPONSE PARAMETERS FOR GROUPS TREATED WITH SALINE, IPRONIAZID, IPRONIAZID + TRYPTAMINE (T), AND IPRONIAZID + 5-HT

Group	First Avoidance (Trial No.)	No. Shocks	Response Failures	Escape[1] Latencies	Avoidance[2] Latencies
Sal + Sal	3 (2–5)	3 (2–7)	0 (0)	2.2 (1.1–3.6)	0.9 (0.7–2.1)
Sal + Ipron	4 (2–6)	5 (3–8)	0 (0–1)	1.7 (0.7–2)	2.1 (1.4–3.7)
Ipron + 2.5 mg/kg T	5 (1–8)	9* (3–11)	0 (0–6)	1.6 (0.6–2)	1.9 (0.8–2.9)
Ipron + 5 mg/kg T	4 (3–9)	9* (4–18)	0 (0–1)	1.7 (1.3–3.2)	2.1 (0.9–3.4)
Ipron + 5 mg/kg 5-HT	4 (3–8)	6 (3–7)	0 (0)	2.5 (1.7–2.6)	1.6 (1.1–2.5)

[1]On trials 1–10.
[2]On all trials; latency from shock onset.
*$p<0.01$ compared to Sal + Sal (Mann-Whitney, U-test).
n=7 per group.

$p>0.1$. Tryptamine significantly increased the number of shocks received, $H(4)=10.5, p<0.05$. Only 3 animals showed response failures, one from the iproniazid alone treatment group, and one each from the tryptamine-treated groups.

Three animals were treated with iproniazid and 10 mg/kg tryptamine. These animals were observed to lie prone on the grid floor, and generally failed to escape from the shock. This experiment was thus discontinued.

Effects of Pretreatments on the Tryptamine-Induced Acquisition Deficit

The effects of 1 mg/kg metergoline and 5 mg/kg methysergide injected 1 hr and 30 min respectively prior to 5 mg/kg tryptamine on the median number of trials to criterion are shown in Fig. 2. A significant treatment effect was observed, $H(5)=15.3$, $p<0.01$. Pair-wise testing showed that tryptamine significantly increased the number of trials to criterion, and that this effect was blocked by both 5-HT antagonists.

Figure 3 illustrates the effects of 0.1 mg/kg haloperidol (1-hr pretreatment) and 400 mg/kg PCPA (administered orally 3 days prior to tryptamine injection) on the acquisition deficit induced by tryptamine. Again a significant treatment effect was found, $H(5)=19.3$, $p<0.005$. Tryptamine significantly increased the median number of trials to criterion, but this effect was not blocked by haloperidol or PCPA. Neither treatment alone increased the number of trials to criterion. Treatment with PCPA significantly reduced whole brain 5-HT levels from 395 (±25) ng/g to 23 (±3) ng/g, $t(10)=209$, $p<0.001$. Noradrenaline and dopamine levels were not significantly altered by the PCPA treatment.

Effects of Tryptamine on Locomotor Activity

Figure 4 illustrates the effects of 2.5 and 5 mg/kg tryptamine in combination with iproniazid on locomotor activity. One-way analysis of variance did not show a significant main effect of treatment on activity, $F(3,28)=0.7, p>0.1$. Thus, none of the treatments altered motor activity relative to controls.

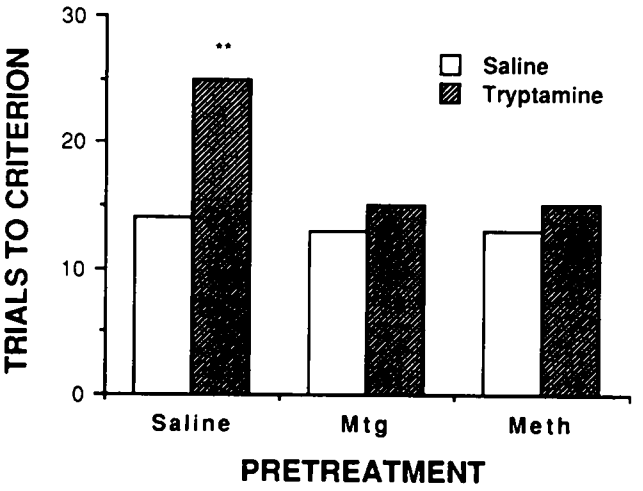

FIG. 2. Effects of 1-hr pretreatment with 1 mg/kg metergoline (Mtg) and 30 min pretreatment with 5 mg/kg methysergide (Meth) on the median number of trials to criterion for groups of rats treated with saline or 5 mg/kg tryptamine (T) (plus iproniazid). (n=7–9 per group.) **Different from appropriate saline-treated group, $p<0.01$.

DISCUSSION

The results of these experiments show that 2.5 and 5 mg/kg tryptamine administered to iproniazid-treated rats impaired the acquisition of a one-way active avoidance task. This deficit was not associated with impaired escape performance, although a higher dose of tryptamine (10 mg/kg) did affect escape responses. The acquisition deficit induced

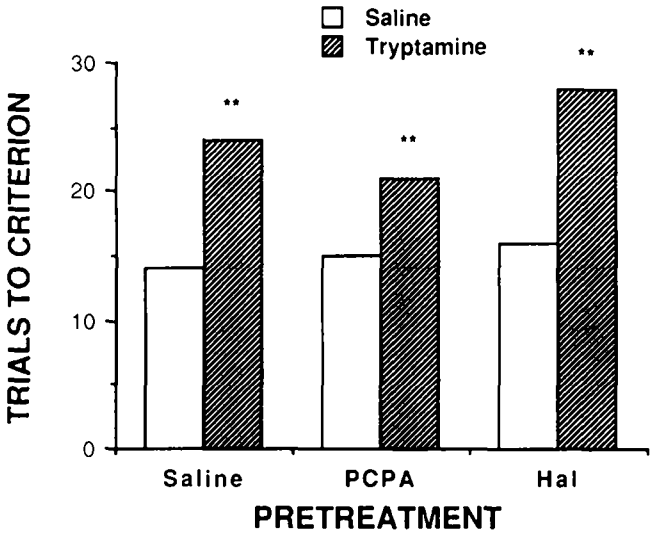

FIG. 3. Effects of 400 mg/kg PCPA (3-day pretreatment) and 1-hr pretreatment with 0.1 mg/kg haloperidol (Hal) on the median number of trials to criterion for groups of rats treated with saline or 5 mg/kg tryptamine (T) (plus iproniazid). (n=5–9 per group.) **Different from appropriate saline-treated group, $p<0.01$.

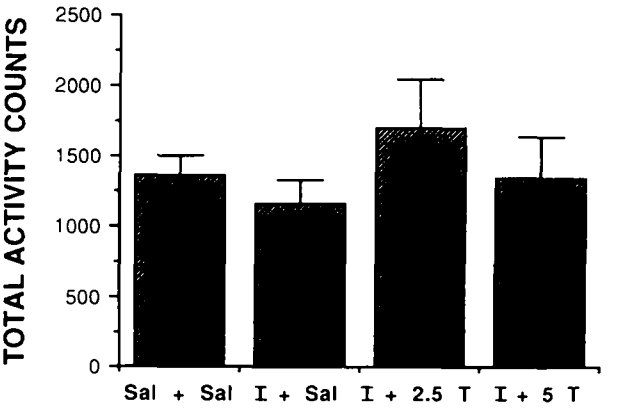

FIG. 4. Effects of saline, iproniazid (I), 2.5 and 5 mg/kg tryptamine (T) (plus iproniazid) on locomotor activity. Values represent the mean (±SEM) total number of photocell counts for each group (n=8) over a 30-minute period.

by tryptamine was prevented by pretreatment with the 5-HT receptor antagonists metergoline and methysergide, suggesting that it may be mediated via an action at 5-HT receptors. It is likely that this action involves a direct stimulation of 5-HT receptors. Depletion of brain 5-HT levels with PCPA failed to affect the tryptamine-induced acquisition deficit thus ruling out the possibility that tryptamine interacts with presynaptic 5-HT mechanisms, at least under the conditions employed here. The failure of systemically administered 5-HT, which does not cross the blood-brain barrier, to affect avoidance behaviour indicates that the effect of tryptamine is not mediated by 5-HT receptors located peripherally. This, together with the observation that peripherally administered tryptamine, in iproniazid-treated rats, results in significant levels of tryptamine in the brain, suggests the behavioural effects of tryptamine are mediated centrally (7). Recent autoradiographic studies have demonstrated the existence of specific high affinity [^{3}H]-tryptamine binding sites, particularly in areas such as the nucleus accumbens and striatum which receive dense dopaminergic input (1, 16, 25). However, since the effect of tryptamine was not blocked by haloperidol, at a dose which did not affect avoidance acquisition in its own right, tryptamine does not appear to interact with the dopaminergic system.

When interpreting the effects of drug-induced deficits in avoidance learning it is necessary to consider that nonspecific effects of the drug can account for the observed deficits. Two such effects which should be considered in the present context are alterations in locomotor activity, and pain sensitivity. Indeed tryptamine has been shown previously to affect both activity level (17,18) and nociception (13). Observation of the animals in the present study did not reveal any obvious motor abnormalities, except for a slight flattening of posture in animals treated with 5 mg/kg tryptamine. Tryptamine-treated rats did not differ from control animals in terms of the trial on which the first avoidance response was observed; nor was the avoidance latency significantly altered by tryptamine. These findings indicate that tryptamine-treated rats were capable of making correct avoidance responses. In a test of gross motor activity, tryptamine did not affect general activity level. Thus, changes in general motor activity, or response capability are unlikely to underlie the acquisition deficit. Examination of escape latencies showed that tryptamine did not affect the latency to escape from shock. Therefore since tryptamine-treated animals were as responsive to shock as control animals, a change in nociceptive threshold cannot account for the effects of tryptamine.

The effect of tryptamine on avoidance acquisition is mediated via serotonergic systems and does not appear to involve obvious nonassociative effects. In these respects the effect of tryptamine appears to resemble the effect of PCA. It has been suggested that PCA may affect avoidance acquisition by interfering with arousal, attentional and perceptual processes so that the animal is unable to attend appropriately to the relevant stimuli required to acquire the task (22). Possibly tryptamine impairs avoidance acquisition in a similar way. Previously tryptamine (plus iproniazid) was noted to affect conditioned avoidance responding in a fashion similar to hallucinogenic compounds such as dimethyltryptamine, mescaline and LSD, which also appear to interact with serotonergic systems (29). Further, tryptamine appears to induce in rats (20), cats (11), dogs (20) and humans (19) pharmacological and behavioural changes which are similar to those induced by LSD and other hallucinogens. Normal acquisition of the avoidance task employed in the present experiments would appear to depend upon the animal developing an association between the shock environment, and the subsequent delivery of shock. However, if tryptamine (plus iproniazid) possesses hallucinogenic-like activity, this associative process may be impaired by the animal failing to attend properly to the stimulus context, thus resulting in an increase in the number of acquisition trials. Further work is required to elucidate the behavioural mechanism by which tryptamine disrupts avoidance acquisition.

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