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BRIEF COMMUNICATION

Evidence that Taste Aversion Learning Induced by *l*-5-Hydroxytryptophan Is Mediated Peripherally

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ERVIN, G. N., R. B. CARTER, E. L. WEBSTER, S. I. MOORE AND B. R. COOPER. *Evidence that taste aversion learning induced by l-5-hydroxytryptophan is mediated peripherally.* PHARMACOL BIOCHEM BEHAV 20(5) 799–802, 1984.—Rats learned to avoid a saccharin solution if their initial consumption of it was followed by intraperitoneal (IP) administration of 25 mg/kg *l*-5-hydroxytryptophan (*l*-5-HTP); this taste aversion learning did not occur in rats pretreated with 50 mg/kg (IP) of the aromatic *l*-amino acid decarboxylase inhibitor RO 4-4602 (benserazide). RO 4-4602 antagonized the *l*-5-HTP-induced elevation of 5-hydroxytryptamine (5-HT) in the mesentery but significantly increased the *l*-5- HTP-induced elevation of 5-HT in the brain. These results indicate that *l*-5-HTP-induced taste aversion is correlated with peripheral, but not central, elevation of 5-HT.

l-5-Hydroxytryptophan 5-Hydroxytryptamine Taste aversion learning
Peripheral aromatic *l*-amino acid decarboxylation Rat

INJECTION of *l*-5-HTP has a variety of behavioral effects, including the reduction of operant responding maintained by food [1,13] or water [6,7] reinforcement. While Aprison and Hingtgen [1] suggested that these effects are mediated centrally (since the temporal pattern of the disruption of operant behavior is correlated with increased levels of 5-HT in the brain after 5-HTP administration), others [6, 7, 15] have argued that they might be mediated peripherally. The main evidence for this is that the disruptive effects of 5-HTP are antagonized by the peripheral 5-HT receptor blocker, xylamidine tosylate, or by the inhibition of peripheral aromatic *l*-amino acid decarboxylase (AADC; E.C. 4.1.1.28) by carbidopa (MK 486) or RO 4-4602 (benserazide) at doses which reportedly block peripheral, but not central, AADC [3,4].

Recently, it was demonstrated that the administration of 5-HTP after the consumption of a novel flavor led to an aversion for that flavor [16]; this suggests that 5-HTP produces its disruptive behavioral effects via a mechanism also leading to taste aversion (i.e., illness). In this experiment, we demonstrate that systemic administration of *l*-5-HTP at a dose reported to disrupt operant responding [6,7] also produces learned taste aversion and, in addition, that this effect is due to an action in the periphery (i.e., outside of the blood-brain barrier).

METHOD

Subjects

All subjects were naive male Long Evans rats (Charles Rivers Laboratories, Wilmington, MA) weighing 343±5.3 (mean±S.E.M.) grams (12 weeks of age) at the beginning of experiments. Rats were group-housed at 23°C with ad lib food and water and a 12-hour light-dark cycle (0600–1800 hours light).

Taste Aversion Learning Paradigm

One week prior to the conditioned taste aversion procedure [12], rats were housed singly with ad lib food and water. On Day 0, rats were weighed and deprived of water, but not food. On the following 8 days, rats were weighed and deprived of food at 1000 hours. At 1100 hours, rats were presented with fluid for 15 minutes in 100 ml calibrated drinking tubes with bent stainless steel spouts (Hazelton Systems,

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Inc., Aberdeen, MD). Then, fluid was removed and food was returned to each cage. On Days 1, 2, 3 and 4, rats were presented with water for 15 minutes. On Day 5, rats received a pretreatment of 50 mg/kg RO 4-4602 (benserazide; N(DL-seryl-)-N-(2,3,4-trihydroxyl-benzyl)hydrazine; 2 ml/kg; IP; generously provided by Dr. W. E. Smith, Hoffman-LaRoche, Nutley, NJ), its vehicle solution (double-distilled H_2O; 2 ml/kg), or 0.9% NaCl (2 ml/kg) 3.75 hours before drinking. On Day 5, rats were weighed and food-deprived as usual, but were presented with 0.25% sodium saccharin solution instead of water. Thirty seconds after the 15-minute access to saccharin, rats were injected with 25 mg/kg *l*-5-HTP (2 ml/kg; IP; Sigma Chemical Co., St. Louis, MO), the 5-HTP vehicle (double-distilled H_2O; pH 2.4; 2 ml/kg), or 0.9% NaCl (2 ml/kg). The different drug pretreatment and treatment combinations are listed in Fig. 1. On Days 6 and 7, all rats were presented with water for the usual 15-minute period. On Day 8, all rats were again offered the 0.25% sodium saccharin solution instead of water for 15 minutes. Within group comparisons of saccharin intakes on Day 8 and water intakes on Day 7 were made with a two-way matched-pair *t*-test. Significantly less saccharin intake was considered evidence for learned taste aversion resulting from treatments given on Day 5.

5-HT Assays

A separate group of rats of similar weight from the same supplier was used for the measurement of 5-HT 40 minutes after administration of 25 mg/kg *l*-5-HTP or an equi-volume injection of vehicle alone. Some rats receiving *l*-5-HTP had been pretreated 4 hours previously with 50 mg/kg RO 4-4602. Rats were decapitated and whole brains were quickly removed, weighed and homogenized in 7 ml of 5% trichloroacetic acid. The peritoneum was opened and the mast-cell-rich mesentery surrounding the intestines was removed. The mesentery sample weighed approximately 1 gram and was homogenized in 7 ml of 5% trichloroacetic acid. 5-HT in the brain and mesentery was separated using Dowex columns and assayed fluorometrically [2].

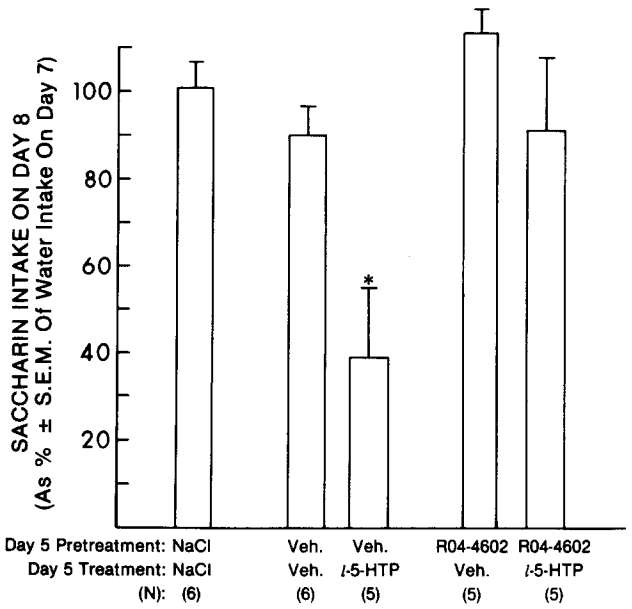

FIG. 1. Each bar represents saccharin intake on Day 8 as % ±S.E.M. (brackets) of H_2O intake on Day 7 by the number of rats given in parentheses below the bar. Treatments and pretreatments were given on Day 5 only. As pretreatments, rats were administered 50 mg/kg RO 4-4602, its vehicle (double-distilled H_2O, 2 ml/kg), or 0.9% NaCl (2 ml/kg) 3.75 hours before being offered 0.25% sodium saccharin for the first time. As treatments, rats were administered 25 mg/kg *l*-5-HTP, its vehicle (double-distilled H_2O, pH 2.4, 2 ml/kg), or 0.9% NaCl (2 ml/kg) 30 seconds after 15-minute access to saccharin. All injections were IP. For each group, H_2O consumption on Day 7 and saccharin consumption on Day 8 (±S.E.M.), respectively, was: NaCl/NaCl (13.8±0.64 and 14.0± 0.84 ml), vehicle/vehicle (16.0±1.79 and 14.4±0.92 ml), vehicle/*l*-5-HTP (15.8±0.91 and 6.2±2.24 ml, $p<0.01$). RO 4-4602/vehicle (15.4± 0.35 and 17.6±0.76 ml) and RO 4-4602/*l*-5-HTP (13.8±0.95 and 12.6±2.13 ml). *Saccharin intake on Day 8 was significantly different from water intake on Day 7, $p<0.01$, by Student's *t*-test.

RESULTS

Figure 1 shows the saccharin consumption on Day 8 resulting from pretreatments before and treatments immediately after initial saccharin consumption on Day 5. In Fig. 1, mean saccharin consumption on Day 8 is expressed as a percentage (±S.E.M.) of mean water intake on Day 7 for each group. Actual fluid intakes on Days 7 and 8 are given in the legend to Fig. 1. Only vehicle pretreated rats treated with *l*-5-HTP displayed taste aversion learning as evidenced by the finding that the vehicle/*l*-5-HTP group drank significantly less saccharin on Day 8 than water on Day 7 ($p<0.01$). Taste aversion learning induced by the *l*-5-HTP treatment did not occur in those rats pretreated with RO 4-4602.

The effects of *l*-5-HTP (with or without RO 4-4602 pretreatment 4 hours earlier) on 5-HT levels in brain and mesentery 40 minutes after injection are shown in Table 1. Compared to vehicle treatment, *l*-5-HTP treatment elevated 5-HT in both the brain ($p<0.001$) and the mesentery ($p<0.001$). RO 4-4602 pretreatment significantly antagonized the *l*-5- HTP-induced increase in 5-HT in the mesentery (RO 4-4602/*l*-5-HTP versus *l*-5-HTP alone, $p<0.05$) and significantly potentiated the *l*-5-HTP-induced increase in 5-HT in the brain (RO 4-4602/*l*-5-HTP versus *l*-5-HTP alone, $p<0.02$).

DISCUSSION

Systemic administration of 25 mg/kg *l*-5-HTP is aversive to rats as demonstrated in the present experiment by taste aversion learning, or the "Garcia effect" [5], consistent with previous reports [8,16]. Pretreatment with 50 mg/kg RO 4-4602, which blocked *l*-5-HTP-induced taste aversion learning (Fig. 1), attenuated the *l*-5-HTP-induced increase in 5-HT in the mesentery, but potentiated the *l*-5-HTP-induced increase in 5-HT in the brain (Table 1). These behavioral and neurochemical effects of RO 4-4602 pretreament suggest: (1) *l*-5-HTP-induced taste aversion learning is initiated by events peripheral to the blood-brain barrier; and (2) elevation of brain 5-HT to the extent achieved in this experiment (175%) is not a sufficient unconditioned stimulus to produce taste aversion learning. These data also extend our preliminary observations that administration of 5-HT by the intraperitoneal route produces taste aversion learning and that xylamidine tosylate (BW 545C), an antagonist of peripheral 5HT receptor sites [9], antagonizes *l*-5-HTP-induced taste aversion learning [8].

The majority of endogenous peripheral 5-HT is found within the gut, and gut 5-HT is within the enterochromaffin cells [10] and within the enteric nervous system [11]. *l*-5-HTP-induced increases in 5-HT may act there to produce

TABLE 1

THE EFFECTS OF l-5-HTP (WITH OR WITHOUT RO 4-4602 PRETREATMENT) ON 5-HT LEVELS

Treatment	Brain 5-HT			Mesentery 5-HT		
	μg/g	%	(N)	μg/g	%	(N)
Vehicle	0.384 ± 0.012	100 ± 3.1	(8)	0.259 ± 0.025	100 ± 9.8	(7)
l-5-HTP (25 mg/kg)	0.544 ± 0.037	142 ± 9.5*	(8)	0.537 ± 0.061	207 ± 23.6 *	(6)
RO 4-4602 (50 mg/kg) + l-5-HTP (25 mg/kg)	0.671 ± 0.028	175 ± 7.3*‡	(7)	0.371 ± 0.034	143 ± 13.1†§	(7)

Values are the mean ± S.E.M. of the number of observations in parentheses. Rats were treated with vehicle or l-5-HTP and pretreated with RO 4-4602 at the same doses and time intervals as those for Fig. 1.

*Different from vehicle group, $p<0.001$.

†Different from vehicle group, $p<0.01$.

‡Different from l-5-HTP group, $p<0.02$.

§Different from l-5-HTP group, $p<0.05$.

aversive effects relayed neuronally to the brain to affect behavior. *l*-5-HTP administration also elevates 5-HT levels within the serum [14] and elevated circulating 5-HT may stimulate the area postrema and thereby affect behavior. There are, of course, a number of other peripheral sites and brain sites outside the blood-brain barrier where *l*-5-HTP might act pharmacologically to produce aversive effects [10]. Localization of the aversive cues to one or more sites awaits further study.

These findings have relevance to animal studies on the observed behavioral effects of systemically administered *l*-or *dl*-5-HTP. For example, systemic administration of *l*- or *dl*-5-HTP decreases operant responding of rats for food or water [7,13]. Others [1] have suggested that the operant disruption by 5-HTP is centrally mediated since the behavioral disruption temporally correlated more closely with central than with peripheral 5-HTP-induced increases in 5-HT levels; however, Carter *et al.* [6,7] demonstrated that *l*-5-HTP- (25 mg/kg, IP) induced decreases in operant responding for water were prevented by pretreatment with RO 4-4602 (50 mg/kg, IP). Carter *et al.* [6,7] assumed that this dose of RO 4-4602 only inhibited peripheral AADC activity. Since doses of *l*-5-HTP and RO 4-4602 and the dosing schedule which they used were the same as used here (Table 1), it does appear that the disruption of operant responding was correlated with increases in 5-HT levels in the periphery, but not in the central nervous system.

In conclusion, systemic administration of a dose of *l*-5-HTP reported to be behaviorally effective in reducing responding in appetitive operant tasks produced conditioned taste aversion learning in the rat. The aversive properties of *l*-5-HTP as well as the *l*-5-HTP-induced rise in 5-HT in the periphery, but not in the brain, were antagonized by pretreatment with a peripheral decarboxylase inhibitor, suggesting that the aversive *l*-5-HTP-induced effects are mediated peripheral to the blood brain barrier. These findings have clear implications for studies of the behavioral actions of peripherally administered *l*-5-HTP.

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