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Central and Peripheral Components of the Inhibitory Actions of 5-HTP on Ethanol Consumption in the Rat

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ZABIK, J. E., J. E. SPRAGUE AND K. BINKERD. *Central and peripheral components of the inhibitory actions of 5-HTP on ethanol consumption in the rat.* PHARMACOL BIOCHEM BEHAV 47(3) 547-551, 1994.—When administered under a backward conditioning paradigm, 5-HTP administration resulted in a decrease in ethanol intake followed by a persistently decreased ethanol consumption. A central component to this inhibitory effect was suggested by the inability of xylamidine to significantly reduce the initial inhibitory effect of 5-HTP. The persistent rejection was prevented by xylamidine. Methysergide reduced the initial as well as the persistent effects of 5-HTP. In studies utilizing a forward conditioning paradigm, 5-HTP and lithium were each effective in developing CTAs to ethanol and saccharin. Only the ethanol-5-HTP pairing showed a persistent aversion. A peripheral component to the actions of 5-HTP was suggested by xylamidine blocking the CTAs induced by 5-HTP. Xylamidine also prevented the persistent ethanol avoidance induced by 5-HTP, but was ineffective in antagonizing lithium-induced CTAs. These results suggest central as well as peripheral components associated with the inhibitory effects of 5-HTP on ethanol consumption. Central actions appear to mediate the initial inhibitory effects while peripheral actions appear to be associated with persistent avoidance of ethanol following 5-HTP treatment.

Serotonin 5-Hydroxytryptophan Ethanol Conditioned taste aversion Peripheral Central
Saccharin Lithium chloride Xylamidine 5-HTP Backward conditioning Forward conditioning

CENTRAL infusion of 5-hydroxytryptophan (5-HTP), the immediate precursor to serotonin (5-HT) (10), or 5-HT itself (6) has been shown to reduce ethanol consumption. Intraperitoneal administration of 5-HTP has also consistently resulted in a decreased ethanol intake (3,4,9), presumably through conversion to 5-HT in the central nervous system. Studies conducted in this laboratory have shown that a single dose of 5-HTP, administered under a backward conditioning paradigm to rats chronically consuming ethanol as their only source of fluid, significantly reduced ethanol consumption (12,14). In addition to this effect, many of the 5-HTP-treated rats continued to reject ethanol during posttreatment days. When ethanol was the only drinking fluid available, approximately 25% of the rats died if an alternate drinking fluid was not made available (12). The persistent avoidance of ethanol leading ultimately to death was suggestive of an avoidance conditioned by the 5-HTP-ethanol pairing. To evaluate this possibility, a subsequent study utilizing the forward conditioning taste aversion (CTA) paradigm was conducted, and it was demonstrated that 5-HTP was capable of inducing a CTA when administered following the ingestion of ethanol or other novel fluids such as saccharin or tartaric acid (13). Even under CTA conditions, the rats that received the ethanol-5-HTP pairing exhibited persistent refusal to drink ethanol on subsequent retest days. This effect with ethanol appeared to be uniquely serotonergic in nature because the LiCl-ethanol pairing failed to result in similar persistent effects.

Although 5-HTP has been shown to decrease ethanol ingestion as well as induce CTAs to ethanol, it has not been clear if the effects are mediated by central and/or peripheral mechanisms. Attempts to demonstrate whether the inhibitory effects of 5-HTP on ethanol ingestion are due to a central phenomenon or whether the effects are peripherally mediated have met with limited success. In one study (5), it was demonstrated that Ro 4-4602, an aromatic L-amino acid decarboxylase inhibitor usually thought to act peripherally, attenuated the abil-

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ity of 5-HTP to reduce ethanol consumption under free-choice conditions in rats. Relying on a report (7) that Ro 4-4602 significantly reduced the conversion of 5-HTP to 5-HT in the brain, it was argued that 5-HTP acted centrally to reduce ethanol consumption.

Similar discrepancies have made it difficult to determine the site of action of 5-HTP in CTA studies. In one study (1), it was reported that the ability of 5-HTP to induce a CTA to saccharin involved a peripheral mechanism because xylamidine, a peripheral 5-HT antagonist (8), was shown to attenuate the CTA. In another study (2), where Ro 4-4602 was used to antagonize 5-HTP-induced elevations in 5-HT, a peripheral site of action was also proposed because Ro 4-4602 prevented the learning of a conditioned aversion to saccharin. In this study, biochemical data indicated that Ro 4-4602 antagonized the conversion of 5-HTP to 5-HT in the periphery but not the CNS. However, a central mechanism for 5-HTP inducing conditioned taste aversion has also been suggested (11) by the inability of either Ro 4-4602 or xylamidine to attenuate the CTA induced by 5-HTP to either saccharin or other novel fluids.

Because of the discrepancies in the literature concerning the site(s) of action of 5-HTP, the present studies were designed using forward and backward conditioning paradigms to determine whether central as well as peripheral mechanisms were operative in the decreased ethanol drinking following 5-HTP administration: that is, was the initial reduction in ethanol intake due to central elevation of 5-HT and the persistent reduction observed due to a CTA development of peripheral origin.

METHOD

Animals

Male Sprague–Dawley rats, weighing 200–225 g upon arrival, were used in these studies. Rats used in Experiment 1 (backward conditioning study) were purchased from Harlan Sprague–Dawley, Inc. (Indianapolis, IN). Rats used in Experiment 2 (forward conditioning study) were purchased from Laboratory Supply Co. (Indianapolis, IN). Upon arrival, the animals were housed singly in stainless steel cages measuring 23.5 × 21 × 28 cm and maintained at a constant room temperature of 23°C with a 14 L : 10 D cycle. Food and tap water were available ad lib while the animals were allowed to acclimate for at least 1 week prior to experimental use. Fluid was provided in 100-ml Richter tubes so that fluid consumption could be monitored.

Drugs

L-5-HTP was purchased from Sigma Co. (St. Louis, MO.) and was administered as a suspension prepared by homogenization in distilled water with one drop of Tween 80 per 5 ml of water. Xylamidine was provided as gift by Burrows Wellcome Research Lab Co. (Langly Court, Beckenham, Kent, England) and was dissolved in distilled water by sonication. Methysergide was provided by Sandoz Co. (East Hanover, NJ.) and was similarly dissolved. Reagent grade lithium chloride was purchased from Fisher Scientific Co. (Fair Lawn, NJ) and was dissolved in normal saline to give a solution of 3 mEq/ml. The saccharin solution (0.1%) was prepared from sodium saccharin dihydrate purchased from J. T. Baker Chemical Co. (Phillipsburg, PA) Ethanol solutions (12% v/v) were prepared by diluting 190 proof ethyl alcohol, USP, with distilled water. All treatment agents were administered IP in a volume of 2 ml/kg of body weight.

Design of Experiment 1

In the first experiment, a backward conditioning paradigm was used to evaluate the inhibitory effect of 5-HTP on ethanol consumption. A total of 63 rats, randomly divided into eight experimental groups, were used. All rats were placed on 23 h of fluid deprivation with access to water for 1 h daily from 100-ml Richter tubes. At the end of the 1 h, the tubes were removed and the amount of fluid consumed was recorded to the nearest ml. Once the daily water consumption stabilized, the water was replaced by a 12% v/v ethanol solution. After 14 days, the daily ethanol consumption stabilized and the experimental treatment was started. Baseline values were determined for each rat by calculating the average ethanol consumption based on the last 10 days of consumption.

On the treatment day, 2 h before the drinking session, two groups of rats received an IP injection of saline (SAL), two groups received xylamidine (XYL, 3 mg/kg), two groups received methysergide (MET 10, 10 mg/kg), and two groups received methysergide (MET 20, 20 mg/kg). One hour after the first injection, one of each of the above groups received a second IP injection of L-5-HTP (5-HTP, 50 mg/kg) and the remaining groups received saline. The ethanol consumption for each rat on the treatment day was compared with its baseline value to determine if the drug treatment affected ethanol consumption.

Design of Experiment 2

In this study, using a forward conditioning paradigm, a total of 73 rats were initially deprived of water for 23 h per day as described in Experiment 1. One day after the water consumption had stabilized, the rats were subdivided into 12 groups; six of the groups were offered a solution of saccharin (0.1%) and the remaining six groups were offered a solution of ethanol (12% v/v) during the 1-h drinking period. This day was called novel day. These fluids served as the conditioned stimulus (CS). Immediately following the 1-h consummatory period, the fluids were removed and each group of rats received the first of two injections. Three groups from each novel fluid received an IP injection of SAL and the remaining three groups from each novel fluid received an IP injection of XYL. One hour later, each of the SAL or XYL subgroups received a second injection of either saline, 5-HTP, or lithium chloride (LiCl, 3 mEq/kg). Lithium or 5-HTP served as the unconditioned stimulus (UCS). All rats received water for the next 2 days (1 h each day). On the following day, called retest day, each rat was again offered its respective novel fluid to drink.

Statistics

Data were statistically evaluated using the two-tail paired Student's *t*-test. In the pretreatment studies, baseline was compared to treatment, and in the CTA studies, novel day was compared to retest day 1. Comparisons were considered statistically significant at $p < 0.05$.

RESULTS

The effects of 5-HTP on ethanol consumption, under the backward conditioning paradigm, are presented in Table 1. The rats in this study had access to ethanol as their sole drinking fluid for 14 days before any drug injections were made.

TABLE 1

EFFECT OF XYLAMIDINE OR METHYSERGIDE ON THE ABILITY OF L-5-HTP TO DECREASE ETHANOL CONSUMPTION IN THE RAT

Drugs	N	Baseline	Drug Day	% of Baseline
SAL-SAL	5	39.1 ± 0.6	38.8 ± 4.3	99.2
SAL-5-HTP	10	41.4 ± 1.1	18.2 ± 4.3*	44.0
XYL-SAL	8	40.0 ± 1.9	47.7 ± 3.4	119.3
MET(10)-SAL	6	39.9 ± 1.4	37.7 ± 3.4	94.5
MET(20)-SAL	8	38.8 ± 2.2	32.1 ± 2.8	82.7
XYL-5-HTP	8	43.4 ± 2.7	31.4 ± 2.4*	72.4
MET(10)-5-HTP	10	42.1 ± 1.7	31.5 ± 3.2*	74.8
MET(20)-5-HTP	8	39.5 ± 1.1	34.0 ± 4.3	86.1

Data are expressed as ml consumed body weight (mean ± SEM). *Statistically different from baseline ($p < 0.05$).

The average baseline volumes for each group of rats ranged from 38.8 to 43.4 ml/kg. By this time, the daily ethanol intake had reached a stable, reproducible value. As evidenced by daily weight gain, the rats had adapted to the 23-h deprivation schedule by consuming approximately half of a normal daily fluid intake as a solution of ethanol in the 1-h drinking period. When administered following saline, 5-HTP resulted in a significant reduction (56.0%) in ethanol consumption. This inhibitory effect of 5-HTP was not significantly antagonized by prior administration of XYL. The 10 mg/kg dose of MET was also ineffective, but the 20 mg/kg dose did attenuate the 5-HTP reduction in ethanol intake. Neither XYL nor either dose of MET significantly influenced ethanol intake in the absence of 5-HTP treatment. A persistent refusal to drink ethanol was observed following administration of the saline–5-HTP pairing. Two of the rats persisted in drinking low amounts of ethanol (data not shown). A persistent refusal to drink ethanol, following the administration of 5-HTP in ethanol-drinking rats, was reported previously (12,13). In the current study, these two rats were allowed access to water after 7 days and began normal drinking. Based on previous reports (12,13), these rats would have died if not given an alternate drinking fluid. Rats treated with XYL before 5-HTP also reduced their ethanol intake on the day of injection; however, all of the rats resumed normal ethanol drinking on subsequent test days. Likewise, the rats treated with MET prior to 5-HTP also resumed normal drinking on subsequent days.

The effects of XYL on the ability of 5-HTP or LiCl to condition an aversion to either ethanol or saccharin, under a forward conditioning paradigm, are presented in Table 2. The intake of either saccharin or a solution of ethanol on novel day is presented for each group of animals. The intake of ethanol ranged from a low of 15.3 ml/kg to a high of 19.0 ml/kg, and the intake of a 0.1% solution of saccharin ranged from a low of 54.8 ml/kg to a high of 69.9 ml/kg. The administration of 5-HTP (when paired with SAL) resulted in a significant reduction in ethanol as well as saccharin intake on retest day, indicative of a conditioned taste aversion (CTA).

TABLE 2

EFFECT OF XYLAMIDINE ON THE ABILITY OF L-5-HTP AND LiCl TO CONDITION AN AVERSION TO EITHER ETHANOL OR SACCHARIN

Drugs	N	Novel Day	Retest Day	% of Novel
		Ethanol (12% v/v)		
SAL-SAL	6	15.3 ± 1.1	16.2 ± 1.5	105.9
SAL-5-HTP	4	18.3 ± 2.5	5.0 ± 3.8*	27.3
SAL-Li	4	16.5 ± 1.1	9.8 ± 2.2*	59.4
XYL-SAL	4	15.8 ± 1.6	17.2 ± 2.3	108.9
XYL-5-HTP	7	15.7 ± 1.8	14.7 ± 1.1	93.6
XYL-Li	5	19.0 ± 2.5	12.6 ± 3.8*	66.3
		Saccharin (0.1%)		
SAL-SAL	6	65.1 ± 4.9	68.5 ± 7.8	105.2
SAL-5-HTP	8	69.9 ± 3.4	43.1 ± 10.0*	61.7
SAL-Li	6	69.2 ± 6.3	55.4 ± 3.8*	80.1
XYL-SAL	8	62.7 ± 2.2	66.0 ± 1.8	105.3
XYL-5-HTP	7	54.8 ± 5.1	51.3 ± 3.6	93.6
XYL-Li	8	61.7 ± 3.0	50.3 ± 4.1*	81.5

Data expressed as ml consumed/kg body weight (mean ± SEM).
*Statistically different from novel day ($p < 0.05$).

Lithium likewise resulted in significantly reduced intake of ethanol as well as saccharin. XYL, administered before 5-HTP, prevented the development of a CTA to either ethanol or saccharin. XYL pretreatment had no effect on the ability of LiCl to condition aversions to either saccharin or ethanol.

DISCUSSION

The inhibitory effects of 5-HTP on ethanol ingestion have been documented under a variety of experimental procedures including ICV (6,10) and IP administration (3,4,9). One of the first studies to ascribe a central site of action to the inhibitory effects of 5-HT on ethanol consumption was based on ICV administration of 5-HT itself (6). Others have likewise suggested that 5-HTP acted centrally to decrease ethanol ingestion (5), although the results/conclusions were hampered by a lack of biochemical data to support the proposed central site of action of Ro 4-4602.

The results of our backward conditioning study, using MET and XYL in an attempt to block the effects of 5-HTP pretreatment on ethanol ingestion, support the conclusions of a central site of action; XYL (the peripherally active 5-HT antagonist) was ineffective but MET (the centrally as well as peripherally active 5-HT antagonist) was capable of attenuating the effects of 5-HTP on ethanol ingestion. However, although the reduction in ethanol intake produced by 5-HTP was not blocked by XYL, the persistent refusal to consume ethanol, usually observed following 5-HTP administration (12,13), was attenuated. MET, presumably because of its ability to act peripherally as well as centrally, prevented the persistent refusal to drink ethanol as well. These combined effects of XYL and MET on ethanol intake following 5-HTP treatment suggest that 5-HTP was exerting central as well as peripheral inhibitory actions on ethanol ingestion. Central actions mediated the initial decrease in ethanol intake, and peripheral actions mediated the posttreatment ethanol avoidance.

With regard to the posttreatment ethanol avoidance, prior studies conducted in this laboratory documented the ability of 5-HTP to condition an aversion not only to saccharin but to ethanol as well (13). The first clue that an inhibitory action of 5-HTP on ethanol ingestion was associated with a CTA came when rats persisted in avoiding ethanol for long periods of time following administration of a single dose of 5-HTP (12). In some rats, the avoidance was of such magnitude that the rats died rather than ingest ethanol. The present results, utilizing the forward conditioning paradigm, extend this observation by suggesting a peripheral nature to the CTA induced by 5-HTP. XYL effectively prevented the CTA induced by 5-HTP to either saccharin or ethanol. The work of others (1,2) likewise supports a peripheral mechanism in the ability of 5-HTP to condition an aversion: in these cases, a CTA to saccharin was blocked by either Ro 4-4602 (2) or XYL (1). However, in another study (11), a CTA to saccharin was induced by 5-HTP, but neither Ro 4-4602 nor XYL were able to block it; thus, the effect was interpreted as being of central origin. Differences in methodology (11) may have affected these results; 5-HTP was paired with saccharin over several days, followed by use of a two-bottle choice paradigm to evaluate the ability of XYL to block the observed CTA. The multiple pairings may have resulted in a CTA of such magnitude that XYL was unable to block its occurrence.

The present studies were undertaken to determine if the inhibitory effects of 5-HTP on ethanol ingestion were due to central, peripheral, or combined effects. The results of the two studies utilizing both forward and backward conditioning paradigms indicate that the effects of 5-HTP on ethanol ingestion are not merely centrally or peripherally mediated but in fact represent a blending of the two. The 23-h fluid-deprivation paradigm used in this study provided no opportunity to the rat to ingest an alternate fluid if ethanol was not consumed. Even under this severe motivational state, 5-HTP treatment reduced ethanol consumption and resulted in long-term avoidance of ethanol beyond the day on which a single dose of 5-HTP was administered. The rats used in this study consumed ethanol for many reasons: certainly, they were motivated by thirst due to the 23-h fluid restriction as well as food deprivation, because rats deprived of fluid normally restrict their food intake as well. It is possible the rats may have ingested ethanol for its reinforcing property as well. The inhibitory effects of 5-HTP observed in this study could therefore be the result of an interaction with any one or more of these. With respect to the fluid deprivation, we have shown it not to be a factor in the effects of 5-HTP on fluid intake: in an earlier study (12) the ingestion of distilled water under identical deprivation conditions was not affected by administration of 5-HTP. In the present study, the CTA induced by 5-HTP to ethanol under the forward conditioning paradigm appears to be serotonergic in nature because the CTA induced by lithium chloride, under identical experimental conditions, to either saccharin or ethanol was unaffected by XYL.

In conclusion, the present studies indicate the inhibitory effects of 5-HTP appear to be mediated via the serotonergic system. Further, central actions of 5-HTP appear to mediate direct effects on ethanol consumption, and peripheral effects associated with CTA appear to be associated with the long-term refusal to drink ethanol. Additional studies are required to determine the specific mechanism(s) by which 5-HTP acts to decrease ethanol ingestion.

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