Pharmacology Biochemistry & Behavior, Vol. 17, pp. 435–443, 1982. Printed in the U.S.A.

Effects of Central and Peripheral Pretreatment with Fluoxetine in Gustatory Conditioning[1]

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Received 12 March 1982

LORDEN, J. F. AND W. B. NUNN. *Effects of central and peripheral pretreatment with fluoxetine in gustatory conditioning*. PHARMAC. BIOCHEM. BEHAV. **17**(3) 435–443, 1982.—The administration of fluoxetine, a relatively specific serotonin uptake inhibitor, an hour prior to a taste-drug pairing was shown to attenuate the acquisition of taste aversions in a dose-dependent manner. Desipramine which is less effective than fluoxetine in blocking the reuptake of serotonin was also less potent in reducing the magnitude of taste aversions. Depletion of forebrain serotonin by lesions of the dorsal and median raphe nuclei or of norepinephrine by lesions of the dorsal noradrenergic bundle failed to prevent the pretreatment effect produced by either fluoxetine or desipramine. Rats with raphe lesions consistently consumed less of the taste paired with lithium than did control animals; however, this decreased intake occurred under both drug and saline pretreatment conditions, suggesting an increased sensitivity to the taste-lithium pairing rather than a diminution of the pretreatment effect. Rats with dorsal bundle lesions failed to differentiate between drug and saline pretreatment, consuming similar amounts under both conditions. These findings as well as the observation that intraventricular administration of fluoxetine did not produce a pretreatment effect suggest that forebrain serotonergic systems are not the critical site of action for the production of pretreatment effects by monoamine uptake inhibitors. Instead, the hypothesis that the peripheral effects of fluoxetine have a stimulus value that acts by way of an associative mechanism to attenuate gustatory conditioning must be considered.

Taste aversion Pretreatment effect Fluoxetine Desipramine Raphe lesions
Dorsal noradrenergic bundle lesions

MANIPULATION of serotonin levels has been shown to alter the behavior of rats in the conditioned taste aversion paradigm [23, 24, 25]. In this paradigm, the ingestion of a novel-tasting fluid is paired with a drug injection. Following the taste-drug pairing, animals reject the taste cue [13]. Taste aversion learning is generally conceptualized as a form of Pavlovian conditioning in which a taste cue serves as the conditioned stimulus (CS) and gastrointestinal distress or other drug effects are presumed to act as unconditioned stimuli (UCS). Rejection of the CS is enhanced in animals depleted of forebrain serotonin by either electrolytic or 5,7-dihydroxytryptamine (5,7-DHT) lesions of the dorsal and median raphe nuclei [24]. Administration of 5-hydroxytryptophan (5-HTP) immediately prior to the conditioning trial in which a taste is paired with the injection of lithium chloride (LiCl) attenuates the acquisition of an aversion [25]. The effects of serotonin depletion and repletion in the taste aversion paradigm parallel those obtained with these treatments in other behavioral tests. Reduction of brain serotonin levels decreases the flinch-jump threshold in rats and administration of 5-HTP restores both serotonin levels and pain thresholds to normal [14, 34, 41].

In the flinch-jump test, central decarboxylation of 5-HTP appears necessary to alter sensitivity to footshock. Administration of a peripheral decarboxylase inhibitor does not disrupt the effects of 5-HTP [42]. However, in rats with central serotonin depletion, destruction of forebrain catecholamine neurons does prevent the normalization of pain thresholds by 5-HTP [42]. The locus of the 5-HTP effect in the taste aversion paradigm has yet to be determined. The similarity of the behavioral results obtained with the taste aversion and flinch jump paradigms suggests that central serotonin normally inhibits responsiveness to a variety of noxious stimuli. It is known that the magnitude of a taste aversion is related to the drug dose used [13]. Administration of 5-HTP could reduce taste-drug associations by effectively reducing the consequence of an LiCl injection. Increases in central serotonin levels produced with 5-HTP might decrease sensitivity to the noxious effects of the LiCl used to induce a taste aversion. However, the fact that the taste aversion paradigm is a learning paradigm complicates the analysis of the 5-HTP effect.

In Pavlovian conditioning, factors other than temporal contiguity between the CS and UCS may affect the acquisi-

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tion of the conditioned response. Previous experience with the CS, UCS, or other stimulus may alter the formation of the CS-UCS connection [27,38]. In the taste aversion paradigm, pretreatment with the UCS can block or attenuate the acquisition of an aversion. Disruption of the acquisition of a taste aversion has been reported with pretreatment-training intervals ranging from 30 min to ten days [4,5]. Moreover, the pretreatment effect can be seen in animals preexposed to a drug other than that used as the UCS (e.g., [7, 8, 37]). This observation effectively rules out drug tolerance as an explanation for the phenomenon. Recent evidence tends to support the idea that associative factors underlie the pretreatment effect [2, 4, 31]. Thus, it seems reasonable to conjecture that 5-HTP may disrupt the acquisition of a conditioned taste aversion by altering the association between taste and consequence rather than by producing a decrease in responsiveness to aversive stimuli, as seems likely in studies of pain thresholds.

The experiments reported here were designed to further explore the role of central serotonergic neurons in the taste aversion paradigm. In particular the role of forebrain serotonin in the pretreatment effect was examined. The experiments were designed to determine whether drug-induced increases in the availability of central serotonin could directly alter taste aversion learning or whether a more complicated mechanism involving associative learning needs to be postulated to account for the 5-HTP induced attenuation of taste aversions.

EXPERIMENT 1

Administration of 5-HTP prior to taste-drug conditioning trials has been shown to retard taste aversion learning in both serotonin-depleted and intact rats [25]. The finding that rats with raphe lesions displayed attenuation of taste aversion learning may be due to the decarboxylation of 5-HTP in catecholaminergic neurons [42]. One strategy for determining whether or not central decarboxylation of 5-HTP is necessary to attenuate gustatory conditioning would be to administer a peripheral decarboxylase inhibitor prior to 5-HTP pretreatment. However, this would result in two pretreatments rather than one and the administration of this additional drug could by itself attenuate an aversion if associative factors account for pretreatment effects. For this reason, fluoxetine, a highly specific serotonin uptake inhibitor was tested for its ability to block the acquisition of a taste aversion [21,41]. Fluoxetine was chosen to circumvent the problem of the decarboxylation of 5-HTP by catecholaminergic neurons. If fluoxetine proved effective in attenuating taste aversions, it could then be tested in rats with selective depletions of central serotonin. The effectiveness of fluoxetine was compared with that of another uptake inhibitor, desipramine, a more potent inhibitor of norepinephrine than serotonin uptake [16].

METHOD

Subjects

Thirty-five male Long-Evans hooded rats (275–350 g) bred in the laboratory were housed in individual cages in a colony room maintained at 21–25°C. A 12-hr light-dark cycle was in effect throughout the experiment. Wayne Lab Blox were available at all times throughout the experiment.

Training

The rats were kept on a 23-hr water deprivation schedule. Water was available for 10 min/day in 50 ml graduated centrifuge tubes equipped with spouts and stoppers. At the end of the 10-min drinking period, the tubes were removed and replaced with standard water bottles that remained for an additional 50 min. When water intake had stabilized, a single pretreatment and conditioning trial took place.

On the training day, the rats were unsystematically assigned to one of five treatment groups (n=7/group). All rats received a pretreatment injection one hour prior to the usual drinking period. At the time of the 10 min drinking period, all rats were presented with 50 ml of sucrose (50 g/l) rather than water. Immediately following the 10 min drinking period the rats were injected intraperitoneally with either 0.15 M lithium chloride (LiCl) at a dose of 12 cc/kg or an equivalent volume of physiological saline. Water bottles were then placed on the cages for the usual 50-min drinking period.

Group FLX_{10}/LiCl received an injection of 10 mg/kg of fluoxetine HCl (Lilly) as a pretreatment and LiCl following the sucrose presentation. A second group (FLX_5/LiCl) was treated like FLX_{10}/LiCl except that a 5 mg/kg dose of fluoxetine was administered as a pretreatment. Group DMI/LiCl was also treated like FLX_{10}/LiCl except that this group received a 10 mg/kg injection of desipramine HCl (USV Pharmaceuticals). Group SAL/LiCl received an injection of physiological saline equivalent in volume to the fluoxetine injection received by the FLX_{10}/LiCl group. Finally, Group FLX_{10}/SAL received 10 mg/kg of fluoxetine as a pretreatment and an injection of physiological saline following sucrose presentation.

Testing

The rats were given a three day recovery period on the 23-hr deprivation schedule. Sucrose and water were then presented on alternate days during the 10-min drinking period. Four test presentations of sucrose were made.

Statistical Analysis

The water and sucrose consumption data from the extinction trials were analyzed separately in two-way (Group × Trials) repeated measures analyses of variance. Training trial sucrose consumption was analyzed by a one-way ANOVA. Comparisons among means were made with the Newman-Keuls test.

RESULTS AND DISCUSSION

Prior to the administration of LiCl, the groups did not differ reliably in their consumption of sucrose (Fig. 1). However, in the four extinction trials that followed, a significant Group × Trial interaction was obtained, $F(12,90)=5.24$, $p<0.0001$. Throughout the extinction trials, all groups that received LiCl injections consumed less sucrose than the FLX_{10}/SAL group. Furthermore, all groups that received fluoxetine or DMI as a pretreatment drank more sucrose than the SAL/LiCl group.

On the first extinction trial, the LiCl treated groups did not differ. However, as the aversions began to extinguish on the second sucrose test trial, both the FLX_5/LiCl and DMI/LiCl groups drank less sucrose than the FLX_{10}/LiCl group ($p<0.05$). The FLX_5 and DMI/LiCl groups never differed reliably. By the third test trial, the sucrose aversion had extinguished in all but the SAL/LiCl group which con-

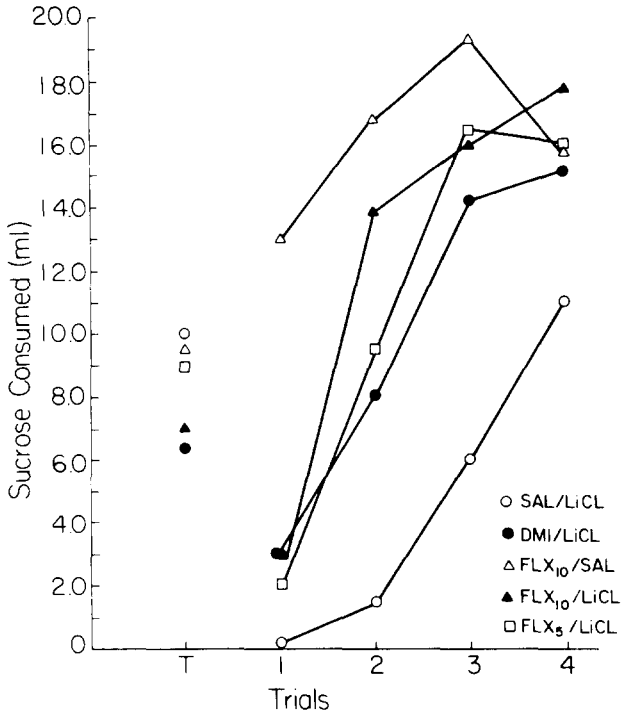

FIG. 1. Sucrose consumed during the training trial (T) and four extinction trials by all groups in Experiment 1.

tinued to consume less sucrose than all the other groups ($p<0.05$).

The sucrose aversions declined rapidly, but the data indicate that pretreatments with fluoxetine, like 5-HTP, can attenuate taste aversions in a dose dependent manner. Furthermore, desipramine was also effective in attenuating taste aversions although fluoxetine appeared more potent. The differences observed among pretreatment groups cannot be attributed to overall differences in fluid intake. A significant Group × Trial interaction was obtained for water consumption, $F(20,150)=1.99$, $p<0.01$. However, water consumption varied reliably among groups only on the first day following the training trial. At that time, water consumption was depressed in each of the LiCl-treated groups in comparison with the SAL group ($p<0.05$, for all).

EXPERIMENT 2

The greater potency of fluoxetine in comparison with desipramine observed in Experiment 1 suggested that the pretreatment effect might depend on a specific effect on central serotonergic systems such as, a reduction in the aversive effects of the UCS by increasing the availability of 5-HT at central synapses. However, if at the doses used, fluoxetine and desipramine had effects which were discriminable by the animals, then an associative process such as blocking might account for the effects observed. Since uptake inhibitors have not previously been studied in the taste aversion paradigm, Experiment 2 used fluoxetine and desipramine as unconditioned stimuli in order to determine whether rats are able to detect the effects of these compounds.

METHOD

Subjects

Twenty-one male Long-Evans hooded rats (335–450 g) bred in the laboratory were used as subjects. The rats were housed individually in standard cages in a light and temperature controlled colony room as described above.

Training

The rats were acclimated to the 23-hr water deprivation schedule described in Experiment 1. After 7 days on the deprivation schedule, rats were assigned unsystematically to one of three treatment groups (n=7/group). On the training day a sucrose solution (50 g/l) rather than water was presented during the 10-min drinking period. Immediately following the removal of the sucrose, the fluoxetine (FLX) group received an injection of 10 mg/kg of fluoxetine HCl; the desipramine (DMI) group, 10 mg/kg of desipramine HCl; and the saline (SAL) group, an equivalent volume of isotonic saline. The drugs were administered intraperitoneally at a concentration of 5 mg/cc. Water was available as usual for 50 min.

Testing

A three-day recovery period during which the rats were maintained on the deprivation schedule preceded the test trials. On the fourth day after the sucrose-drug pairing, sucrose was presented again during the 10-min drinking period. On the fifth and sixth days, water and sucrose were presented, respectively.

Data Analysis

Fluid consumption during the 10-min drinking period was analyzed by analysis of variance. Separate analyses were conducted for the training trial sucrose presentation, the sucrose extinction trials and the water trials.

RESULTS AND DISCUSSION

All groups consumed similar quantities of sucrose on the training day (Fig. 2). Both fluoxetine and desipramine produced conditioned taste aversions. The analysis performed on the sucrose extinction data revealed a significant drug treatment effect, $F(2,18)=12.26$, $p<0.0004$. Extinction trial sucrose consumption in both the FLX and DMI groups was significantly lower than that of the saline-treated group ($p<0.05$ for both). Fluoxetine was also found to be more potent than desipramine in suppressing sucrose consumption ($p<0.05$). Analysis of the water consumption data did not reveal any reliable effects on overall fluid consumption which would account for the differences in extinction trial sucrose consumption.

The fact that fluoxetine and desipramine can be used to condition taste aversions indicates that rats can detect the effects of these drugs. Furthermore, the detectability of these compounds was correlated with their effectiveness in attenuating taste aversions in a pretreatment paradigm. Thus, the pretreatment effects observed in Experiment 1 may have been the result of associative learning. The stimulus properties of the drugs rather than their specific effects on central serotonergic neurons mediating the response to noxious stimuli may have caused the attenuation of the sucrose aversions.

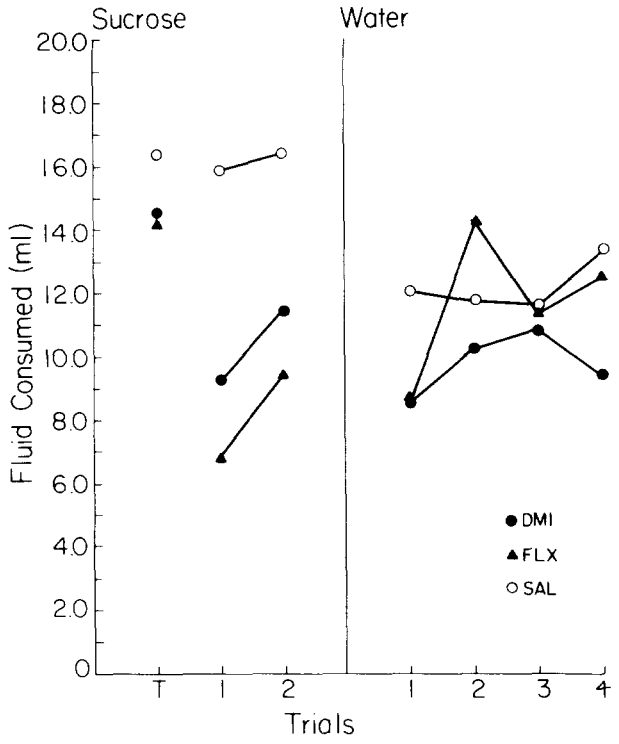

FIG. 2. Training trial (T) and extinction trial sucrose consumption for all groups in Experiment 2 are presented in the left panel. The right panel shows water consumption during recovery (water trials 1–3) and extinction (Trial 4).

EXPERIMENT 3

In order to determine whether the effects of fluoxetine or desipramine were mediated by forebrain serotonin systems that appear to be important in determining flinch-jump thresholds, rats with lesions of the dorsal and median raphe nuclei were tested for pretreatment effects in the taste aversion paradigm. Rats with lesions of dorsal noradrenergic bundle were included in the design as a control for the specificity of any lesion effects. Previous research has shown that these lesions do not impair the acquisition of taste aversions induced by LiCl [29,32]. The effects of the lesions were examined in a 3×3 (Lesion × Pretreatment) factorial design. The effects of fluoxetine and desipramine were compared to saline pretreatment. Infusions of the vehicle used to dissolve the neurotoxins were used as a control for the lesions.

METHOD

Subjects

Eighty-one male hooded rats weighing between 235 and 445 g at the time of surgery were used. The animals were maintained under the same colony conditions described in Experiment 1.

Surgery

Animals were assigned to one of three surgical groups (n=27/group). Animals in the raphe group received infusions of the serotonin neurotoxin 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT, Regis Chemical Co.) into the dorsal and median raphe nuclei. Six micrograms of the free base dissolved in 1 μl of a physiological saline-0.02% ascorbic acid vehicle were infused at each site at a rate of 0.2 μl/min. Stereotaxic coordinates for the dorsal raphe lesion were: 1 mm anterior to lambda; 1.2 mm lateral in the midline; and 6.7 mm ventral to the surface of the skull. The coordinates for the median raphe lesions were: 1.0 mm anterior to lambda; 1.5 mm lateral to the midline, and 8.6 mm ventral to the surface of the skull. For both lesions, the incisor bar was set at 3.5 mm above the interaural line, and the cannula was held at a 10° angle from the midline. A second group of rats was given infusions of the catecholamine neurotoxin 6-hydroxydopamine hydrobromide (6-OHDA, Regis Chemical Co.) into the dorsal noradrenergic bundle (DB). A 4 μg (free base) dose of the neurotoxin at a concentration of 10 μg/μl was delivered bilaterally at the rate of 0.2 μl/min. For this lesion the skull was flat between lambda and bregma and the cannula was aimed 1.3 mm anterior to the ear bar zero; 1.1 mm lateral to the midline; and 6.4 mm ventral to the surface of the skull [20]. The vehicle control group received infusions of the physiological saline-ascorbic acid vehicle equal in volume to those used for the lesions. Fourteen rats received infusions at the raphe and 13 at the DB coordinates. Ether was used as the anesthetic for all surgery and infusions were made through 30 ga stainless steel cannulas.

Training

Following a three week recovery period during which all rats had ad lib access to food and water, a 23-hr water deprivation schedule was instituted as in Experiment 1. After drinking had stabilized, nine rats in each surgical group were assigned to one of three drug pretreatment groups: fluoxetine, desipramine, or saline. One hour prior to the 10-min drinking period, pretreatment injections of 10 mg/kg fluoxetine and desipramine were given intraperitoneally. An equivalent volume of saline was administered to the control group. Sucrose (50 g/l) was then presented to all rats during the 10-min drinking period and followed immediately by IP injections of 0.15 M LiCl (12 cc/kg).

Behavioral Tests

Following the drug treatment and a three-day recovery period, testing began. Three four-day sequences of taste presentations were used. On the first and third day of each series, water was presented during the 10-min drinking period; and on the second and fourth days, 0.9% saline and sucrose, respectively. Water was always available for the following 50 min.

Chemical Assays

At the completion of the behavioral testing, all subjects were sacrificed by decapitation, the brains removed, and dissected into right and left telencephalon. The telencephalic dissection was made by lifting the occipital and ventral regions of the cortex to expose the corona radiata and the columns of the fornix. Bilateral cuts were made through the diencephalon using the corona radiata as guides. A cut through the anterior commissure separated the telencephalon from the diencephalon and the left and right hemispheres were divided by a midsagittal cut. The telencephalic sections were then frozen in liquid nitrogen and weighed. Norepi-

TABLE 1

EFFECTS OF RAPHE AND DORSAL BUNDLE LESIONS ON FOREBRAIN MONOAMINE LEVELS

Group	n	5-HT	NE	DA
Raphe				
Fluoxetine	9	0.238 ± 0.129*	0.225 ± 0.035	0.793 ± 0.155
Desipramine	6	0.278 ± 0.228*	0.244 ± 0.050	0.876 ± 0.323
Saline	8	0.244 ± 0.130*	0.150 ± 0.040	0.518 ± 0.320
Dorsal Bundle				
Fluoxetine	8	0.557 ± 0.149	0.096 ± 0.047*	0.474 ± 0.228
Desipramine	7	0.788 ± 0.116	0.108 ± 0.081*	0.783 ± 0.282
Saline	7	0.667 ± 0.215	0.082 ± 0.050*	0.672 ± 0.369
Vehicle				
Fluoxetine	8	0.688 ± 0.182	0.208 ± 0.081	0.752 ± 0.225
Desipramine	7	0.672 ± 0.115	0.237 ± 0.104	0.718 ± 0.275
Saline	6	0.489 ± 0.204	0.189 ± 0.092	0.689 ± 0.324

Values are expressed as micrograms of amine/g tissue (M±SD).
Abbreviations: 5-HT=serotonin; NE=norepinephrine; DA=dopamine.
*Differs significantly from corresponding vehicle-treated groups, $p<0.05$.

nephrine, dopamine, and serotonin levels were assayed by fluorometric techniques [15].

Statistical Analyses

Separate Lesion × Drug pretreatment analyses of variance were performed on the total 10-min consumption of water, sucrose, and saline. Analytical comparisons were made using Dunn's test. Pre-LiCl sucrose consumption and norepinephrine, dopamine, and serotonin levels were also analyzed in separate 3×3 ANOVAS.

RESULTS

Chemical Assays

In the 69 animals that survived surgery, significant reductions in forebrain serotonin were noted only in the raphe lesion groups (Table 1). Dorsal bundle lesions depleted telencephalic norepinephrine. Dopamine levels were unaffected by either the raphe or dorsal bundle lesions. One animal in the raphe group and two in the dorsal bundle group showed either no depletion or a unilateral depletion. These three animals were dropped from further consideration.

TABLE 2

TRAINING TRIAL SUCROSE CONSUMPTION

Group	n	Sucrose Consumed
Raphe		
Fluoxetine	9	11.3 ± 3.2*
Desipramine	6	10.7 ± 3.7*
Saline	8	16.4 ± 2.2
Dorsal Bundle		
Fluoxetine	8	10.6 ± 3.8*
Desipramine	7	11.6 ± 4.7*
Saline	7	17.7 ± 4.5
Vehicle		
Fluoxetine	8	12.6 ± 5.3
Desipramine	7	9.3 ± 4.5
Saline	6	15.7 ± 2.7

Values are in milliliters (M±SD).
*Differs from Saline group of the same lesion type, $p<0.05$.

Behavioral Tests

On the training day, pretreatment with either fluoxetine or desipramine resulted in a significant depression of sucrose drinking in comparison with saline pretreatment, $F(2,55)=13.57$, $p<0.0001$, (Table 2). This effect was observed in both lesion and vehicle groups but was most pronounced in the animals with lesions.

During extinction, a Lesion × Pretreatment interaction was obtained (Fig. 3, top) in sucrose drinking, $F(4,55)=459$, $p<0.01$. The vehicle-treated animals consumed more sucrose during extinction if pretreated with fluoxetine or desipramine than with saline ($p<0.05$, for both). This was also true for the raphe groups ($p<0.05$ for both comparisons). However, the raphe animals consistently drank less sucrose than the vehicle groups during these trials ($p<0.05$, for all). No significant differences in sucrose consumption were found between fluoxetine and desipramine pretreatment groups for either the raphe or vehicle groups. Unlike the raphe and vehicle groups, rats with dorsal bundle lesions consumed similar amounts of sucrose under all three pretreatment conditions. The dorsal bundle group differed from the raphe group only in the saline pretreatment conditions ($p<0.05$) and never differed significantly from the vehicle group.

Saline consumption (Fig. 3, middle panel) was similar in all groups and was stable over the three test presentations. Water consumption was depressed in all groups during the recovery period following the lithium injections. By the be-

ginning of the extinction sequence, however, water intake had returned to baseline levels and showed no significant change over the six presentations. Water intake was comparable in all groups (Fig. 3, bottom panel). Thus, the differences in sucrose consumption were not due to an overall difference in fluid consumption or to an altered response to novel taste cues.

DISCUSSION

The effect of raphe lesions as seen in this study is similar to that previously reported [23, 24, 25]. Raphe lesions appeared only to enhance taste aversions and not to block the pretreatment effect. Sucrose consumption was below normal in rats with raphe lesions under saline as well as drug pretreatment. Thus, the attenuation of taste aversion learning which follows fluoxetine or desipramine pretreatment does not appear to depend on forebrain serotonergic neurons. However, other central serotonergic pathways may have mediated the effects of fluoxetine. The descending projections of the serotonergic nuclei B1, B2 and B3 are involved in the response to painful stimuli. There is evidence that these projections inhibit pain signals at the level of the spinal cord [1,12]. Even in rats with large depletions of forebrain 5-HT, pretreatment with fluoxetine might increase the suppressive effect of serotonin in this descending system and reduce the animal's responsiveness to the aversive consequences of the LiCl injection.

Alternatively, animals with raphe lesions may have developed receptor supersensitivity. Chronic depletion of a neurotransmitter following a lesion can result in an increase in receptor number [6,18]. Thus, in rats with raphe lesions, pretreatment with fluoxetine may have increased the availability of any remaining 5-HT in a system made supersensitive to 5-HT. The animals in the raphe groups might have shown the same degree of disruption of taste aversion learning as intact animals, despite lower absolute levels of forebrain serotonin.

The fluoxetine pretreatment effect might also be mediated by the area postrema. This brainstem region contains chemoreceptors sensitive to bloodborne toxins, projects to an emetic area [39] and has been implicated in gustatory conditioning [3,30]. Serotonin is present in the area postrema [33] and is known to decrease activity in area postrema neurons [19]. Fluoxetine pretreatment, therefore, might decrease the sensitivity of area postrema chemoreceptors to toxins such as LiCl. The origin of the 5-HT projection to the area postrema has not yet been determined. Thus, the failure of the raphe lesions to block the fluoxetine pretreatment effect might be attributable to a sparing of the 5-HT terminals in the region or receptor supersensitivity if the projection is removed.

The data presented in Fig. 3 suggest that rats with dorsal bundle lesions may have a deficit in the acquisition of taste aversions. However, consumption of sucrose on the first extinction trial was significantly reduced in the saline pretreated dorsal bundle group in comparison with training trial sucrose consumption (paired t, $p<0.05$). This is in keeping with the failure of other investigators [29,31] to observe disruption of LiCl-induced aversions with dorsal bundle lesions. The apparent inability of rats with dorsal bundle lesions to distinguish between saline and drug pretreatment may be due to the attentional deficit that these animals display in other learning tasks [29]. Rats with dorsal bundle lesions have been shown to be more distractable than normal

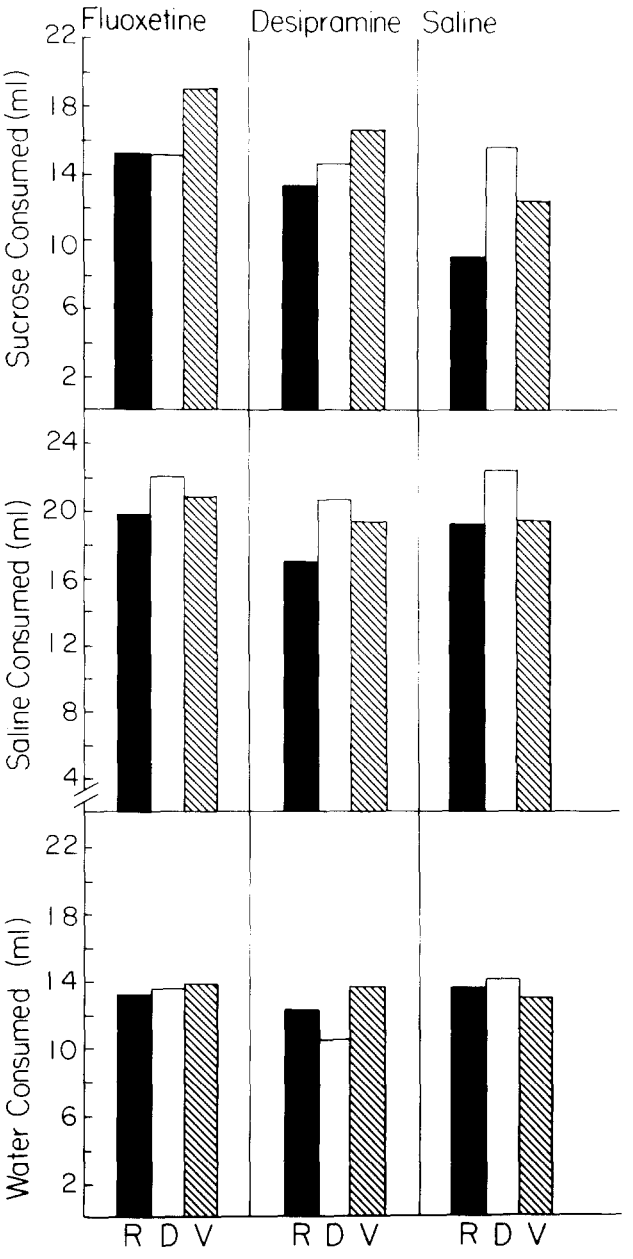

FIG. 3. Sucrose, saline and water consumption during extinction for all groups in Experiment 3. Consumption is averaged over three trials for sucrose and saline and over six trials for water.

[28] and are unable to ignore stimuli that are unrelated to the conditions of reward [26]. The injection procedure, even if not followed by drug effects, undoubtedly provides a variety of novel stimuli. If animals with dorsal bundle lesions sample more cues than normal, as suggested by Mason and Iverson [29], then even a control injection procedure should provide stimuli that are available to the associative processes that may underly the pretreatment effect.

EXPERIMENT 4

The failure of raphe lesions to block fluoxetine's effects

as a pretreatment drug in gustatory conditioning may be attributable to the sparing of a critical central 5-HT pathway or to the development of receptor supersensitivity. However, it is also possible that both fluoxetine and desipramine act peripherally to produce the pretreatment induced suppression of taste aversion learning. Pretreatment with fluoxetine or other drugs by intracerebral or intraventricular administration offers the most direct test of this possibility.

METHOD

Sixteen adult male rats (360–460 g), maintained as described above, were prepared under barbiturate anesthesia with chronic 23 ga cannulas stereotaxically aimed at the lateral cerebral ventricle. The coordinates [20] used for the implantation procedure were: 0.75 mm posterior to bregma, 1.5 mm lateral to the sagittal suture, and 4.5 mm ventral to the surface of the skull. After recovery from surgery, the rats were put on the 23-hr water deprivation schedule used in Experiments 1–3. One week later, a single sucrose-lithium chloride training trial was conducted as in Experiments 1 and 3. Fifteen to 30 min prior to the sucrose presentation, however, eight rats received intraventricular injections 10 μg of fluoxetine HCl, dissolved in 1 μl of physiological saline. The dose was chosen to produce an obvious sedative-like effect in the animals without disrupting their ability to drink during the sucrose training trial. The remaining eight rats received only 1 μl of saline. After a recovery period of three days, the rats were tested for the acquisition of a sucrose aversion with presentations of sucrose alternating daily with water during the 10-min drinking period. A total of four sucrose presentations were made.

At the completion of the behavioral tests, the rats were perfused with formalin. Frozen sections were cut through the forebrain and stained with cresyl violet to verify the cannula placements.

RESULTS AND DISCUSSION

Fluoxetine had a suppressive effect on the training trial consumption of sucrose ($t=3.67$, $df=14$, $p<0.01$) (Fig. 4) and the animals appeared sedated when injected with LiCl. However, fluoxetine administered intraventricularly did not alter the acquisition or extinction of a conditioned aversion to sucrose. No reliable differences in sucrose or water intake were found during the test trials.

Examination of the brains after sacrifice confirmed the placement of the cannulas in the ventricles. Furthermore, the depressed sucrose consumption during the training trial also indicates that the fluoxetine was delivered effectively. It is of course possible that a different population of neurons mediates fluoxetine's suppressive effects on training trial sucrose drinking and its effects on conditioning and that the intraventricular administration of fluoxetine reached only the former. However, the failure of fluoxetine to alter sucrose aversions under these conditions strongly suggests that direct action of the drug on central serotonergic neurons is not critical in the pretreatment effect.

GENERAL DISCUSSION

Fluoxetine and desipramine have both been shown to attenuate LiCl-induced taste aversions when administered prior to the taste-LiCl pairing. The efficacy of these two drugs in producing a pretreatment effect was correlated with their ability to induce taste aversions when used as a UCS.

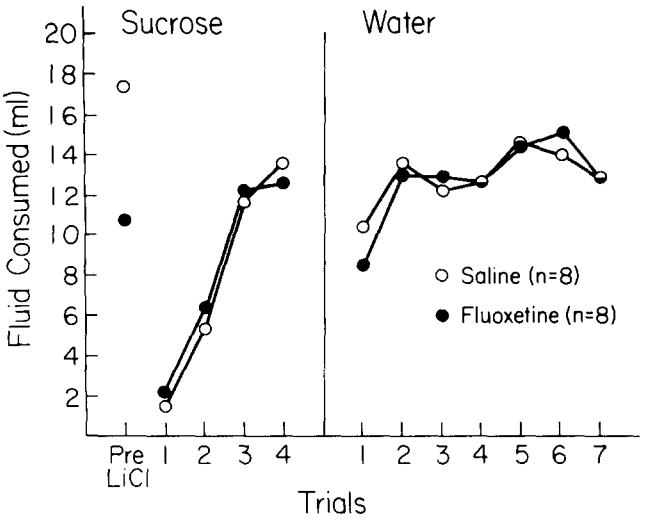

FIG. 4. Sucrose consumption during the training (Pre LiCl) and extinction trials (1–4) for animals pretreated with intraventricular, fluoxetine or saline in Experiment 4 (left panel). Water consumption during the recovery period (Trials 1–3) and the extinction trials (4–7) is presented in the right panel.

Lesions of the dorsal and median raphe or of the dorsal noradrenergic bundle did not block the pretreatment effects produced by fluoxetine or desipramine. Furthermore, intraventricular administration of fluoxetine did not attenuate taste aversion learning. These findings suggest that fluoxetine and perhaps desipramine do not act centrally to produce the pretreatment effect. It also seems likely that 5-HTP which attenuates taste aversions when used as a pretreatment drug [25] probably does not do so by acting on central serotonergic pain mechanisms to reduce the aversiveness of the UCS. Rather, when systemically administered, the effects of these drugs, presumably at peripheral sites, serve as stimuli in an associative process.

The neural basis of the pretreatment effect has not been examined previously. However, evidence is accumulating to suggest that many drugs with central nervous system effects do not produce taste aversions by actions solely on specific neuronal systems in the brain which directly mediate other effects of these drugs. For example, central injections of LiCl are ineffective in producing taste aversions [35]. Massive depletion (80% or more) of central norepinephrine and dopamine is necessary to produce even an attenuation of the effectiveness of amphetamine as an aversion-inducing agent [22,32]. Similarly, fenfluramine, a drug with actions on central serotonergic neurons [9], can be used to produce taste aversions even after raphe lesions similar to those used in the present study [23]. Thus, it appears likely that the peripheral effects of these compounds are conveyed centrally by a route such as the vagus nerve. Interruption of the vagus has been shown to seriously impair the acquisition of learned aversions when intragastric copper sulfate is used as a UCS [10]. Alternatively, bloodborne drugs or toxins may act on chemosensitive circumventricular organs such as the area postrema that lie outside the blood-brain barrier. Lesions of the area postrema have been shown to disrupt LiCl and methylscopalamine-induced aversions [3,30].

At the behavioral level several hypotheses have been advanced to account for the pretreatment phenomenon. Sev-

eral investigators [31,40] have shown that associations formed between the injection procedure and the drug effect are important in the production of the pretreatment effect. When trials in which non-reinforced presentation of drug administration cues were interspersed with preexposure trials, the pretreatment effect was reduced. Presumably, the drug injection procedure was no longer a reliable predictor of the UCS. Thus, Poulos and Cappell [31] suggest that the pretreatment effect is essentially a case of the blocking paradigm first described by Kamin [17]. Substantial support for the stimulus blocking explanation of the preexposure effect has also been provided by Batson and Best [2] in experiments demonstrating that salient environmental cues associated with drug preexposure can be used to disrupt CTA learning. An explanation of this type most readily accounts for the effect which is observed after multiple preexposures usually occurring at least one day before the taste aversion conditioning trial.

In the present studies only a single pretreatment trial was used and this took place immediately prior to sucrose-LiCl pairing. Investigators using proximal UCS preexposure [4,11] report that this is an effective means of disrupting CTA learning. Stimuli that would ordinarily produce backward conditioning instead attenuate learning. The proximal UCS preexposure effect has been shown to have a temporal gradient which is related to drug dose. Animals show the preexposure effect even if tested while under the influence of the preexposure drug injection, ruling out the possibility that this effect depends on a generalization decrement produced by training and testing animals under different drug conditions [4].

The proximal preexposure effect differs from the multi-trial effect in that it is not disrupted by treatments which maintain the contingency between drug treatment and taste [4]. If a taste cue is paired with the preexposure trials in the multi-trial paradigm, the preexposure effect is reduced when another novel taste cue is then paired with the UCS. This does not appear to be the case when a single proximal preexposure trial is used, suggesting that different mechanisms may account for the two preexposure phenomena.

Best and Domjan [4] have argued that proximal UCS preexposure either reduces the effectiveness of the conditioning UCS, thereby producing a smaller unconditioned response, or UCS preexposure makes the CS less available for association. In the first case, the preexposure effect may be due to priming [36] or an opponent process effect [36]. In the context of the priming model, the preexposure drug trial primes or represents the UCS in short-term memory. If the UCS is then repeated while the representation is still in short term memory, the effectiveness of the second presentation is reduced. The opponent process model suggests that the learning decrement produced by UCS preexposure results from an opponent aftereffect of the original drug effect. The opponent aftereffect is thought to block the reinstatement of the initial drug effect when the taste-UCS pairing occurs.

Both the priming and opponent process explanations of the proximal preexposure effect are best-suited to those experiments in which the same drug is used for both the preexposure and the conditioning trials. Otherwise, it must be assumed that the opponent aftereffects or primed representations are rather non-specific. This explanation cannot be completely ruled out, however, since it is some consequence of the drug administration which serves as the UCS in taste aversion learning. For some drugs or treatments (e.g., LiCl, apomorphine, radiation), the important consequence is most likely gastrointestinal distress. But for many of the other drugs used in the taste aversion paradigm, it is more difficult to specify the nature of the stimulus that constitutes the UCS and it is possible that the UCS is some undifferentiated event. However, asymmetries have been demonstrated with the pretreatment effect. Equipotent doses of morphine will attenuate morphine-induced aversions but will not block the effectiveness of tetrahydrocannabinol as a UCS and will only slightly interfere with diazepam-induced aversions [37]. Furthermore, lesions of the area postrema [3,30], which block LiCl and methylscopolamine-induced aversions do not impair amphetamine-induced aversions. These facts are at least suggestive of heterogeneity in the UCS. Thus, the present experiments favor the explanation that it is the availability of the CS for association which is affected by the preexposure trial [4]. This mechanism assumes that some salient experience such as a drug injection prevents or reduces the access of the CS to an attentional or short term memory system. The preexposure treatment reduces the opportunity of the CS to become associated with the UCS during the conditioning trial. These events do not appear to depend on intact forebrain serotonergic systems; however, the data presented here suggest a disturbance of this type of processing in animals with lesions of the dorsal noradrenergic bundle.

ACKNOWLEDGMENTS

This research was supported in part by Grants BNS 77-15251 and BNS 80-14675 from the National Science Foundation. The authors gratefully acknowledge the gift of fluoxetine by the Lilly Company and of desipramine by USV Pharmaceuticals. Part of the research reported here was submitted to the Graduate School of the University of Alabama in Birmingham by the second author in partial fulfillment of the requirements for the Master of Arts degree.

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