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Cyproheptadine Prevents the Initial Occurrence of Successive Negative Contrast

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GRIGSON, P. S. AND C. F. FLAHERTY. *Cyproheptadine prevents the initial occurrence of successive negative contrast.* PHARMACOL BIOCHEM BEHAV **40**(2) 433–442, 1991.—Rats shifted from 32% to 4% sucrose make fewer licks for 4% sucrose than rats having only experienced the lower reward. In Experiment 1, the occurrence of this contrast effect was prevented by the administration of the nonspecific serotonin antagonist cyproheptadine (3.0 or 6.0 mg/kg). The results of Experiments 2 and 3 demonstrated that the contrast-reducing action of cyproheptadine was not mediated by the antiserotonergic properties of the drug since systemic administration of the serotonin synthesis inhibitor, PCPA (150 or 300 mg/kg), failed to influence either the occurrence of contrast or the attenuation of contrast by cyproheptadine. The results of Experiment 4 indicated that the contrast-reducing action of cyproheptadine was not mediated by the antihistaminergic properties of the drug since the antihistamine, pyrilamine (6 or 12 mg/kg), also failed to prevent the occurrence of contrast. Finally, the contrast-reducing action of cyproheptadine was not due to rate-dependent and/or appetite stimulating effects since cyproheptadine did not serve to increase lick frequency in rate-dependent controls.

Successive negative contrast Cyproheptadine Serotonin Pyrilamine PCPA

RATS shifted from a 32% to a 4% sucrose solution consume less 4% sucrose than rats having experienced only 4% sucrose. This successive negative contrast effect is accompanied by an increase in activity (19), probably searching (unpublished data from this laboratory), and is not thought to be associated with stress or conflict initially since corticosterone levels are not elevated (18) and benzodiazepines are ineffective in preventing the occurrence of contrast on the first postshift day (23). On the second postshift day, possibly after it has been determined that the previously received reward is no longer available, the presence of "stress" or "conflict" seems indicated. That is, corticosterone levels are elevated (18) and benzodiazepines effectively attenuate contrast when administered on the second postshift day (24).

In order to determine the pharmacological mechanisms which underlie successive negative contrast, the potential contrast-reducing actions of a number of drugs thought to have "anxiolytic" properties have been evaluated on the first and the second postshift day, see Table 1. Anxiolytic agents such as chlordiazepoxide (CDP) and ethanol have no effect on contrast when administered on the first postshift day (3, 23, 24), but these drugs, along with midazolam, reduced contrast when administered on the second postshift day (2–4, 22, 24). Other agents with some anxiolytic properties, such as sodium amobarbital and morphine, reduced contrast when administered on either the first or the second postshift day, but these effects were numerically small (17, 21, 37).

The evidence regarding the contrast-reducing properties of serotonergic (5-HT) compounds (also thought to exert some anxiolytic actions) has focused primarily on the second postshift day (i.e., during recovery from contrast) and is largely negative. Only buspirone (5-HT_{1A} agonist) and ritanserin (5-HT_2 antagonist) have been investigated on the first postshift day, and neither are effective in preventing the occurrence of contrast. Buspirone, gepirone (a second 5-HT_{1A} agonist), ritanserin, ketanserin (a second 5-HT_2 antagonist), and methysergide (a nonspecific 5-HT antagonist) have been investigated on the second postshift day, and all failed to promote recovery from contrast (22).

Surprisingly, two other drugs with antiserotonergic properties have not only been found effective in promoting the recovery from contrast when administered on the second postshift day, but do so with a potency equal to that of the benzodiazepines. Becker (2) reported that the nonspecific serotonin antagonists cyproheptadine and cinanserin exerted potent contrast-reducing actions when administered on the second postshift day.

While the contrast-reducing action of the benzodiazepines and ethanol, when administered on the second postshift day, strongly implicates a role for gamma-aminobutyric acid (GABA) in the recovery from contrast, little is known about the mechanism which mediates the initiation of contrast on the first postshift day. Given the robust contrast-reducing action of cyproheptadine when administered on the second postshift day, and the possibility that this effect may be mediated by a number of different mechanisms [cyproheptadine binds to a number of different receptors in the central nervous system (33, 36, 42)], Experiment 1 was designed to investigate the extent to which cyproheptadine might prevent the occurrence of contrast when administered on the first postshift day.

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TABLE 1

EFFICACY OF COMPOUNDS TESTED IN THE SUCCESSIVE NEGATIVE CONTRAST PARADIGM ON THE FIRST AND/OR THE SECOND POSTSHIFT DAY

Category	Compound	Postshift Day 1	Postshift Day 2	Refs.
Anxiolytics	Chlordiazepoxide	inactive	active	(23,24)
	Midazolam	not tested	active	(2,22)
	Ethanol	inactive	active	(3,4)
Agents with some anxiolytic properties	Sodium Amobarbital	active (small effect)	active (small effect)	(17,21)
	Morphine	active (small effect)	active (small effect)	(37)
Nonselective serotonin antagonists	Cyproheptadine	not tested	active	(2)
	Cinanserin	not tested	active	(2)
	Methysergide	not tested	inactive	(2)
Serotonin 5-HT1A agonists	Buspirone	inactive	inactive	(22)
	Gepirone	not tested	inactive	(22)
Serotonin 5-HT2 antagonists	Ritanserin	inactive	inactive	(22)
	Ketanserin	not tested	inactive	(22)
Histamine (H1) antagonist	Pyrilamine	not tested	inactive	(2)
Cholinergic (M) antagonist	Scopolamine	inactive	inactive	(2,25)

EXPERIMENT 1

METHOD

Subjects

The subjects were thirty-six male Sprague-Dawley rats purchased from Blue Spruce at 90 days of age. Following two weeks of adaptation to the colony room, the subjects were deprived to 82% of their free-feeding body weight which was subsequently maintained by a once per day feeding. The animals were housed in suspended stainless steel cages under a 14/10-hour light/dark cycle with water available ad lib. Testing began approximately 5 hours into the light phase.

Apparatus

Testing was conducted in six Plexiglas chambers located in a room adjacent to the colony room. The chambers measured $30 \times 25 \times 25$ cm. One 1.5-cm diameter hole was centered on one wall of each apparatus, 6 cm above the hardware cloth floor. Solutions were delivered by means of graduated cylinders with metal spouts. The cylinders were attached to motors that inserted or withdrew the spouts from the access hole. Licks were recorded through a contact relay circuit and microprocessors.

Procedure

The subjects were assigned to one of two groups. The first group received five minutes daily access to a 32% sucrose solution beginning with the first lick for 10 consecutive days (preshift phase). These rats were then shifted to five minutes daily access to a 4% sucrose solution for 4 additional days (postshift phase). The second group served as the unshifted controls receiving five minutes daily access to the 4% sucrose solution beginning with the first lick on all 14 days of the experiment. The subjects were run in six sets of six animals, with the unshifted controls being run first. The latency to make the first lick and the total number of licks made in the five-minute period were recorded daily.

Following the ten-day preshift phase, the unshifted controls (group 4-4) and the shifted subjects (group 32-4) were matched separately by terminal lick frequency (the average lick frequency for day 9 and day 10) and assigned to one of three drug conditions. The drug conditions were as follows:

1) *Cypro 3*: Six unshifted controls and six shifted subjects were injected intraperitoneally (IP) with 3.0 mg/kg cyproheptadine;

2) *Cypro 6*: Six unshifted controls and six shifted subjects were injected IP with 6.0 mg/kg cyproheptadine;

3) *PEG*: Six unshifted controls and six shifted subjects were injected IP with an equal volume of the vehicle, 5% polyethylene glycol (PEG).

On the first postshift day (day 11) the subjects were removed from their home cages, weighed, given the appropriate drug treatment, and returned to their home cage for 30 minutes. Thereafter, the subjects were transported to the experimental room for a 5-minute access period to the 4% sucrose solution. Recovery from contrast was evaluated over the succeeding postshift period (days 12–14). The running order throughout the experiment was consistent with that appropriate for the injection schedule on the first postshift day.

Cyproheptadine (Sigma Chemical Company, St. Louis, MO) was mixed with 5% PEG (1 ml PEG in 19 ml distilled water) immediately prior to testing, and was refrigerated between injections. Sucrose solutions were prepared daily from commercial grade cane sugar and tap water [weight of sucrose/(weight of sucrose + water)] 24 hours prior to their use and were presented at room temperature.

Data Analysis

Data (from all experiments) were evaluated by analysis of variance. Post hoc analyses were conducted with Fisher's least significant difference (lsd) test. Since latency data are inherently

skewed, latency data were transformed to a log10 latency prior to statistical analysis.

RESULTS

Preshift

Latency. Analysis of the terminal preshift data [(day 9 + day 10)/2] indicated that rats initiated licking more quickly for 32% (mean = 0.36) than for 4% sucrose (mean = 0.70), $F(1,24) = 4.47$, $p<0.045$.

Lick frequency. Analysis of the terminal lick frequency data [(day 9 + day 10)/2] indicated that rats licked more for 32% sucrose (mean = 1548) than for 4% sucrose (mean = 1289), $F(1,30) = 13.35$, $p<0.001$.

Postshift

Latency. Analysis of the latency data from the terminal preshift period through day 14 yielded results similar to the preshift data—rats shifted from 32% to 4% sucrose continued to initiate licking more quickly than rats having only experienced the 4% sucrose solution, $F(1,30) = 13.06$, $p<0.001$. This effect of preshift condition (32% or 4% sucrose) on the latency to initiate responding was not influenced by drug treatment (Solution × Drug, $F<1.0$), did not vary across the four-day postshift period [Solution × Day, $F(4,80) = 1.25$, $p>0.05$], and was not altered by drug treatment across days (Solution × Drug × Day, $F<1.0$).

Lick frequency. Analysis of lick frequency scores from the terminal preshift period through day 14 indicated that rats shifted from 32% to 4% sucrose made fewer licks for 4% sucrose than the unshifted controls overall, $F(8,116) = 2.43$, $p<0.02$. Subsequent analysis indicated that this contrast effect was reliable on the first two postshift days in the PEG-treated controls, but was eliminated on the first postshift day by both doses of cyproheptadine, see Fig. 1. Contrast was reliable on the second postshift day in subjects that had been treated on the first postshift day with either dose of cyproheptadine, and on the third postshift day, in the subjects that had been treated with 6.0 mg/kg cyproheptadine. Recovery from contrast was complete by the fourth postshift day in all three treatment groups ($p<0.05$).

DISCUSSION

The administration of cyproheptadine (3.0 or 6.0 mg/kg) on the first postshift day eliminated negative contrast. Relative to the weak contrast-reducing action of either sodium amobarbital or morphine, and the ineffectiveness of either CDP or ethanol, these data represent the first instance whereby a psychoactive drug fully prevented the occurrence of contrast when administered on the first postshift day.

Cyproheptadine may prevent the occurrence of contrast via at least one of three mechanisms. Cyproheptadine is a serotonergic antagonist which binds to both 5-HT_1, and 5-HT_2 receptors, though it is 40–400 times less active at the 5-HT_1 receptor subtype (33, 36, 42). Cyproheptadine has also long been recognized as an antihistaminergic and an anticholinergic agent and some reports indicate that cyproheptadine binds with almost equal affinity to these three receptor sites (33, 39, 42).

Although the contrast-reducing actions of cyproheptadine are probably not mediated by serotonergic inhibition (buspirone, gepirone, ritanserin, ketanserin, and methysergide fail to attenuate contrast), a role of 5-HT in several animal models of conflict and/or anxiety remains suggestive (7, 12, 13, 28, 41, 43). For this reason, a more direct investigation of this possibility

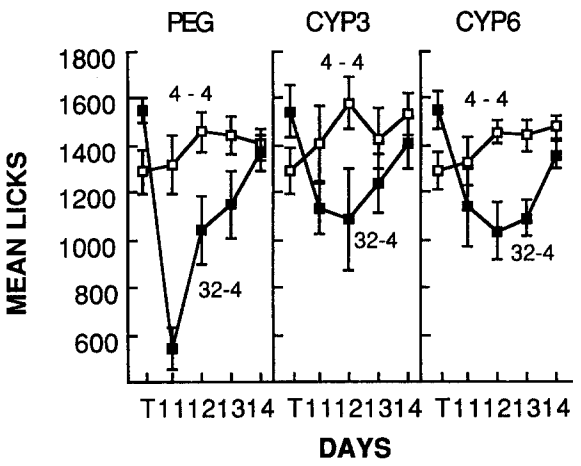

FIG. 1. Mean licks for the terminal preshift period (T: average lick frequency for day 9 and day 10) and the postshift period for shifted (32-4) and unshifted (4-4) subjects. Rats received an IP injection of either 5% polyethylene glycol (PEG), 3 mg/kg cyproheptadine (CYP 3), or 6 mg/kg cyproheptadine (CYP 6) 30 minutes prior to the first postshift period (day 11). Both doses of cyproheptadine statistically eliminated contrast on the first postshift day.

was necessary. Specifically, if serotonin plays an important role in the initiation of contrast, and if the contrast-reducing action of cyproheptadine is mediated by serotonergic inhibition, depletion of brain serotonin following the administration of the serotonin synthesis inhibitor, para-chlorophenylalanine methyl ester hydrochloride (PCPA), should also serve to prevent the occurrence of contrast on the first postshift day.

EXPERIMENT 2

METHOD

Subjects

The subjects were 36 male Sprague-Dawley rats purchased from Harlan Sprague-Dawley Inc. at 90 days of age. All conditions were as described in Experiment 1.

Apparatus

The apparatus was the same as described in Experiment 1.

Procedure

Subjects were assigned to one of two sucrose conditions. The shifted group received five minutes daily access to 32% sucrose beginning with the first lick for 12 days and was then shifted to 4% sucrose on day 13. The unshifted controls received five minutes daily access to 4% sucrose beginning with the first lick on all 13 days of the experiment. The subjects were run in six sets of six animals, with the unshifted controls being run first. The latency to make the first lick and the total number of licks made in a five-minute period were recorded daily.

Following eight days of acquisition the unshifted controls (group 4-4) and the shifted subjects (group 32-4) were matched separately by lick frequency (the average lick frequency for days 6, 7, and 8) and assigned to one of three drug condi-

tions. The drug conditions were as follows:

1) *PCPA 150*: Six shifted subjects and six unshifted controls were injected IP with PCPA (150 mg/kg) two hours following testing on preshift days 8 and 9;

2) *PCPA 300*: Six shifted subjects and six unshifted controls were injected IP with PCPA (300 mg/kg) two hours following testing on preshift day 8, and saline two hours following testing on preshift day 9;

3) *SAL*: Six shifted subjects and six unshifted controls were injected IP with an equal volume of 0.9% saline two hours following testing on preshift days 8 and 9.

The acquisition phase was extended from 10 days, which is the standard procedure, to 12 days because PCPA treatment suppressed intake of sucrose, particularly 32% sucrose. On the first postshift day (day 13) all subjects were transported to the experimental room for a five-minute access period to the 4% sucrose solution. Following this session, the subjects were individually transported to another room where they were immediately sacrificed by decapitation and the striatum was removed for reverse-phase High Performance Liquid Chromatography (HPLC) analysis of serotonin and dopamine.

PCPA (Sigma Chemical Company, St. Louis, MO) was mixed in saline by gentle warming immediately prior to administration and refrigerated between injections. Sucrose solutions were prepared as described in Experiment 1.

HPLC Analysis

Dissection of the brain. The methods for brain dissection and HPLC analysis were consistent with those reported by De Vito and Wagner (10). The rats were sacrificed by decapitation and their brains were rapidly removed and placed on the dorsal surface. The brains were dissected with the initial coronal slice taken approximately 2.0 mm anterior to the hypothalamus. The next slice was taken directly anterior to the hypothalamus. The striatum was then removed from the caudal surface of this slice of brain, based on its distinct morphological appearance. The caudate putamen included tissue dorsal to the anterior commissure, ventral to the corpus callosum, and medial to the external capsule. The isolated striatum was then stored in liquid nitrogen until assayed.

Biochemical determinations. Concentrations of serotonin and dopamine in tissue were determined by HPLC with electrochemical detection. Striatal tissue (30–50 mg) was homogenized in 10 vol. (w/v) of 0.4 N perchloric acid and then centrifuged at a temperature of 4 degrees centigrade for 20 minutes at 15,000 × g. The supernatant was assayed on a BAS HPLC system, equipped with a Spectra-Physics model SP8770 dual piston pump. The sample was delivered through a high-pressure valve, fitted with a 20-microliter sample loop, onto a Biophase ODS C-18 reverse phase column (Bioanalytical Systems, West Lafayette, IN; 5 μm, 250 × 4.6 mm i.d.). The detector (LC-4B; BAS) was set at a range of 50 nA for dopamine and 10 nA for serotonin, and the sample was oxidized with a +0.72 V potential between the glassy carbon electrode and the Ag/AgCl reference electrode. The filtered and degassed mobile phase consisted of 0.10 M citric acid, 0.10 M sodium phosphate dibasic, and 10% methanol (v/v). The mobile phase was pumped in at a rate of 1.0 ml/min. Quantification was against external standards for dopamine or serotonin.

RESULTS

Three subjects (one from the unshifted controls and two from the shifted group) were dropped from the experiment due to failure to lick following treatment with 300 mg/kg PCPA.

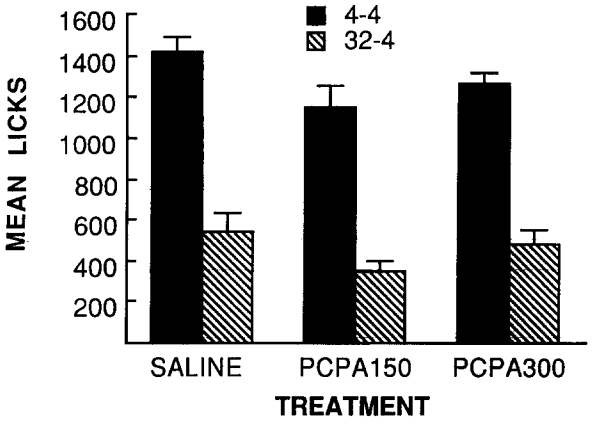

FIG. 2. Mean lick frequency for 4% sucrose in shifted (32-4) and unshifted (4-4) subjects on the first postshift day (day 13). Rats were injected IP with either saline (day 8 and day 9), 150 mg/kg PCPA (day 8 and day 9), or 300 mg/kg PCPA (day 8 and saline on day 9). Treatment with PCPA failed to alter contrast relative to the saline-treated controls.

Preshift

Latency. Analysis of the latency data from the terminal pre-PCPA period [(day 6 + 7 + 8)/3] indicated that the latency to initiate responding did not differ between subjects receiving either 32% or 4% sucrose ($F<1.0$). Analysis of the latency data across days 9–12 (i.e., following PCPA administration, but prior to the shift) evidenced a reliable Solution × Day interaction, $F(3,80) = 4.29$, $p<0.007$. Subsequent analysis indicated that rats initiated licking more slowly for 32% than for 4% sucrose on the ninth, but not on subsequent preshift days.

Lick frequency. Lick frequency for 4% and 32% sucrose differed early in acquisition, but not on the terminal acquisition day prior to PCPA administration, $F(7,189) = 8.71$, $p<0.0001$. The administration of PCPA led to a decline in lick frequency for sucrose over the 4-day period preceding reward downshift (day 9–day 12), Drug, $F(2,27) = 18.03$, $p<0.0001$.

Analysis with the lsd test indicated that the suppressive effect of two administrations of 150 mg/kg PCPA were greater than a single administration of 300 mg/kg PCPA ($p<0.05$). Specifically, lick frequency for 4% sucrose was reliably reduced by two doses of PCPA 150 (mean = 1185), but not by a single dose of PCPA 300 (mean = 1320), relative to the saline-treated controls (mean = 1368). Lick frequency for 32% sucrose, on the other hand, was reliably reduced by both PCPA 150 (mean = 748) and PCPA 300 (mean = 880) relative to the saline-treated control (mean = 1368). Finally, treatment with PCPA 150 and PCPA 300 decreased the number of licks made for 32% sucrose well below the number made for 4% sucrose [Solution × Drug, $F(2,27) = 4.51$, $p<0.02$]. The sucrose condition did not interact with drug treatment across days 9–12 [Solution × Drug × Day, $F<1.0$].

Postshift

Latency. There was no effect of solution, $F(1,27) = 1.15$, $p<0.10$, or drug condition (Drug, $F<1.0$) on the latency to initiate licking.

Lick frequency. Rats shifted from 32% to 4% sucrose made

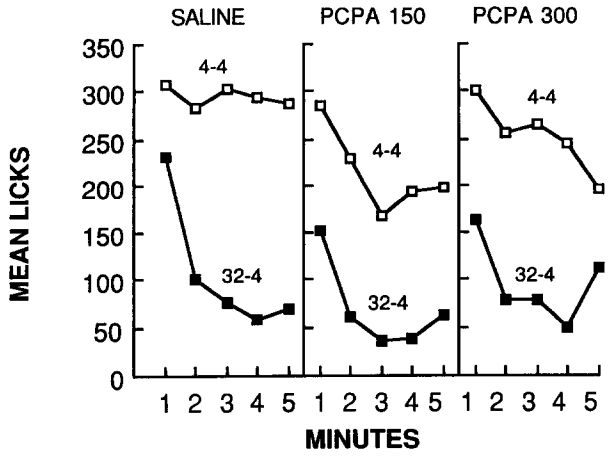

FIG. 3. Mean lick frequency for 4% sucrose in shifted (32-4) and unshifted (4-4) subjects across the five-minute access period on the first postshift day (day 13). Rats were injected IP with saline (day 8 and day 9), 150 mg/kg PCPA (day 8 and day 9), or 300 mg/kg PCPA (day 8 and saline on day 9). Minute by minute analysis of lick frequency on the first postshift day indicated that PCPA failed to alter contrast.

reliably fewer licks for the 4% sucrose solution (mean = 451.1) than did the 4% controls (mean = 1309.1), $F(1,26) = 185.53$, $p<0.0001$. Although PCPA treatment reliably influenced lick frequency, $F(2,26) = 8.16$, $p<0.002$, [subjects treated with PCPA 150 made fewer licks than either the saline or PCPA 300-treated subjects ($p<0.05$)], the Solution × Drug interaction was not reliable, indicating that successive negative contrast was not influenced by PCPA treatment ($F<1.0$), see Fig. 2.

Minute by minute analysis of the five-minute access period on the first postshift day showed that PCPA did not alter the profile of the contrast effect across the access period [Solution × Drug × Minute, $F(8,108) = 1.63$, $p>0.05$], see Fig. 3.

Because PCPA-treated rats were making fewer licks for 32% sucrose prior to reward downshift than were being made for 4% sucrose by unshifted PCPA-treated controls, we further examined degree of contrast in terms of a shift ratio (lick frequency on the first postshift day/lick frequency during the terminal preshift period). The shift ratio was then compared to the shift ratio for the saline-treated controls and for a population of approximately 400 untreated rats tested in our laboratory (26).

The shift ratio determined for the shifted subjects treated with either dose of PCPA did not differ from that determined for the shifted, saline-treated controls [saline = 0.350; PCPA 150 = 0.343, PCPA 150 vs. saline, $t<1.0$; PCPA 300 = 0.495, PCPA 300 vs. saline, $t(8) = 1.46$, $p<0.18$] or from that of the 400 untreated rats [population mean = 0.358; PCPA 150 vs. pop. mean, $t<1.0$; PCPA 300 vs. pop. mean, $t(3) = 1.6$, $p<0.2$].

HPLC Analysis

Serotonin. The level of serotonin in the striatum was not altered by reward downshift (Solution, $F<1.0$). PCPA treatment (both doses) led to an approximate 90% depletion of serotonin in the striatum in both shifted and unshifted subjects, $F(2,27) = 153.29$, $p<0.0001$. The Solution × Drug interaction did not approach statistical reliability ($F<1.0$), see Table 2 (top, left panel).

Dopamine. The level of dopamine in the striatum was not altered by reward downshift ($F<1.0$), the drug condition, $F(2,27) = 1.62$, $p>0.05$, or the Solution × Drug interaction ($F<1.0$), see Table 2 (top, right panel).

DISCUSSION

Contrary to the findings of Experiment 1, a magnitude of reward effect was not evidenced during the acquisition period in either the latency or the lick frequency measure. Although this finding may be due to the short acquisition period prior to PCPA administration (eight rather than ten days), it is probably simply due to chance. Occasionally one group of rats makes almost as many licks for 4% sucrose as are made for 32% sucrose by a separate group of rats. Although there is no obvious reason for this, we approach this finding with little concern since reward downshift leads to a contrast effect regardless of the presence or absence of a magnitude of reward effect during the preshift phase.

The disruptive effect of PCPA treatment on preshift lick frequency also deserves some mention. Since most reports indicate few side effects of PCPA treatment (6,32) and, as stated, PCPA typically increases rather than decreases food intake (11,29), this finding was unexpected. Some evidence suggests that this inhibitory effect of PCPA on sucrose intake may be mediated by the peripheral consequences of PCPA administration, rather than by 5-HT depletion per se. Specifically, Coupar and Taylor (8) reported that treatment with PCPA led to an increase in glucose absorption in the rat lumen. Although this effect was small, it is

TABLE 2

STRIATAL SEROTONIN AND DOPAMINE (μg NEUROTRANSMITTER/g TISSUE ± SEM) IN SHIFTED (32-4) AND/OR UNSHIFTED (4-4) RATS FOLLOWING IP PCPA IN EXPERIMENTS 2 AND 3 AND IP POLY-ETHYLENE GLYCOL (PEG) OR CYPROHEPTADINE (CYPRO) IN EXPERIMENT 3

	Serotonin		Dopamine	
		Experiment 2		
	4-4	32-4	4-4	32-4
Saline	0.75 (0.04)	0.76 (0.08)	15.9 (0.28)	15.0 (1.64)
PCPA 150	.08 (0.01)	0.08 (0.01)	14.2 (0.84)	12.5 (1.5)
PCPA 300	0.16 (0.03)	0.09 (0.008)	12.4 (1.51)	13.0 (3.76)
		Experiment 3		
	PEG	CYPRO	PEG	CYPRO
Saline	0.8 (0.12)	0.6 (0.06)	6.44 (0.91)	7.08 (0.95)
PCPA 150	0.14 (0.05)	0.15 (0.05)	6.8 (0.5)	7.2 (1.18)

possible that it is related to the suppressed sucrose consumption. Further, treatment with PCPA increases urine output which reportedly leads to negative water-salt balance (30). This state of negative water-salt balance may also have contributed to the decrease in intake of 32% sucrose.

The level of 5-HT depletion determined in the striatum was consistent with the findings of Koe and Weissman (32). If 5-HT played an important role in contrast, a depletion of this magnitude would have been likely to prevent the occurrence of contrast. For example, a 90% depletion of brain 5-HT following PCPA administration was sufficient to increase food intake in free-feeding rats (11), an 82.5% depletion of brain 5-HT was sufficient to increase punished responding in a modified Vogel test (38), and a 74% depletion of brain 5-HT following PCPA treatment was sufficient to increase social interaction in rats in an unfamiliar testing environment (15). To the contrary, however, an approximate 90% depletion of brain 5-HT (as reflected in the striatum) did not alter the occurrence of successive negative contrast.

Dopamine levels in the striatum were not reliably altered by either PCPA treatment or reward downshift. This finding is consistent with other evidence which negates a role for dopamine in contrast. For example, contrast was not altered by treatment with the dopamine antagonists haloperidol or chlorpromazine (unpublished data from this laboratory), contrast was not antagonized by buspirone, which also acts at the dopamine autoreceptors to inhibit synthesis and release of dopamine (22), nor was contrast produced by the dopamine antagonist pimozide (unpublished observations from this laboratory). Thus, although a great deal of evidence points to a mediating role for dopamine in reward (14), the available evidence does not support a role for dopamine in contrast.

EXPERIMENT 3

In spite of the evidence to the contrary, the possibility that 5-HT plays a critical role either in the occurrence of contrast or in the contrast-reducing action of cyproheptadine was investigated more fully by examining the extent to which cyproheptadine might prevent the occurrence of contrast in PCPA-treated subjects. Since the results of Experiment 2 indicated that PCPA treatment disrupted sucrose intake, making comparison between shifted and control subjects difficult, no unshifted subjects were included in this experiment. This was not regarded as a problem since the degree of contrast could be evaluated in terms of a shift ratio and compared to the shift ratio of the population mean. Additionally, since some rats treated with the 300 mg/kg dose of PCPA had to be discarded from Experiment 2, only the lower dose of PCPA was used in this experiment.

METHOD

Subjects

The subjects were twenty-four male Sprague-Dawley rats purchased from Harlan Sprague-Dawley Inc. at 90 days of age. All conditions were the same as described in Experiment 1.

Apparatus

The apparatus was the same as described in Experiment 1.

Procedure

Following three weeks of adaptation to the colony room, the subjects were deprived to 82% of their free-feeding body weight. All subjects received five minutes daily access to the 32% sucrose solution beginning with the first lick for 12 days. On Day 13 all subjects were shifted to the 4% sucrose solution. The subjects were run in four groups of six subjects. The latency to make the first lick and the total number of licks made in a five-minute period were recorded daily.

Following eight days of acquisition, the subjects were matched by lick frequency for days 6, 7, and 8 and assigned to one of two drug conditions. The drug conditions were as follows:

1) *PCPA*: Twelve subjects were injected IP with 150 mg/kg PCPA two hours following testing on days 8 and 9;

2) *Saline*: Twelve subjects were injected IP with an equal volume of 0.9% saline two hours following testing on preshift days 8 and 9.

Following the 12-day preshift phase, subjects were matched by terminal lick frequency (the average lick frequency determined for preshift days 11 and 12) and assigned to either a PEG or a cyproheptadine (3.0 mg/kg) treatment group. On the first postshift day (day 13) each subject was removed from its home cage, weighed, injected IP with either PEG or cyproheptadine, and returned to its home cage for 30 minutes. Following this pretreatment period, subjects were transported to the experimental room for a five-minute access period to the 4% sucrose solution and were then returned to their respective home cages. All subjects were sacrificed by decapitation 48 hours following the first postshift day and the striatum was removed for HPLC analysis of serotonin and dopamine. The running order throughout the experiment was consistent with that appropriate for the injection schedule on the first postshift day.

PCPA, cyproheptadine, and the sucrose solutions were prepared and maintained as previously described.

HPLC Analysis

Dissection of the brain and HPLC analysis were the same as described in Experiment 2.

RESULTS

Preshift

Latency. Analysis of the latency data across days 9–12 indicated that the administration of PCPA (150 mg/kg) had no effect on the latency to initiate licking 32% sucrose ($F<1.0$).

Lick frequency. The administration of PCPA led to a small, but reliable decline in lick frequency over the four-day period preceding reward downshift (day 9–day 12), $F(1,20)=12.03$, $p<0.002$. This suppressive effect did not change across days ($F<1.0$), indicating that lick frequency for 32% sucrose in the PCPA-pretreated subjects did not return to the level of the saline-pretreated controls by the end of the preshift period.

Postshift

Latency. Analysis of the latency to initiate licking on the first postshift day indicated that latency was not altered by PCPA pretreatment (Pre, $F<1.0$), cyproheptadine treatment (Drug, $F<1.0$), or the interaction between the pretreatment condition (SAL or PCPA) and drug treatment (PEG or cyproheptadine) ($F<1.0$).

Lick frequency. Intake declined following the shift from 32% to 4% sucrose in the PEG-treated controls. The mean shift ratio for the saline-pretreated, PEG-treated controls was 0.58, which did not differ reliably from the population mean (mean = 0.358), $t(5)=2.16$, $p<0.08$. The mean shift ratio for the PCPA-pre-

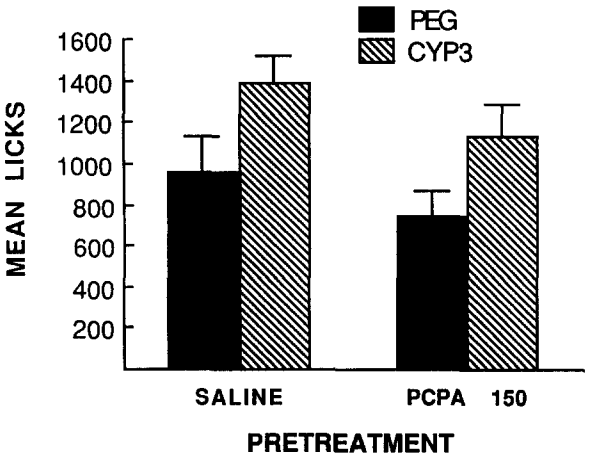

FIG. 4. Mean lick frequency for 4% sucrose on the first postshift day (day 13) in saline- or PCPA-pretreated subjects shifted from 32% to 4% sucrose. Rats were injected IP with either 5% polyethylene glycol (PEG) or 3 mg/kg cyproheptadine (CYP 3) 30 minutes prior to the first postshift period. Cyproheptadine treatment increased lick frequency in rats shifted from 32% to 4% sucrose relative to PEG-treated controls.

treated, PEG-treated controls was 0.49, which also did not differ reliably from the population mean, $t(5)=1.88$, $p<0.12$. Thus pretreatment with PCPA once again failed to reliably alter the magnitude of the reduction in lick frequency following reward downshift.

The subjects treated with cyproheptadine (3.0 mg/kg) made reliably more licks than the PEG-treated controls [Drug, $F(1,20)=7.43$, $p<0.01$]. This "contrast-reducing" action of cyproheptadine was not altered by PCPA pretreatment (Pre × Drug, $F<1.0$), see Fig. 4. Analysis of the data in terms of shift ratios again reflected similar results. Specifically, the mean shift ratio for the saline-pretreated, cyproheptadine-treated subjects was 0.84, which was reliably higher than the population mean, $t(5)=7.28$, $p<0.001$, and the mean shift ratio for the PCPA-pretreated, cyproheptadine-treated subjects was 0.77, which was also reliably higher than the population mean, $t(5)=4.46$, $p<0.007$.

Minute-by-minute analysis of the five-minute access period on the first postshift day also indicated that cyproheptadine-treated subjects made reliably more licks than the PEG-treated controls following the shift from 32% to 4% sucrose [Drug × Minute, $F(4,80)=3.47$, $p<0.01$]. Subsequent analysis indicated that cyproheptadine-treated subjects made reliably more licks in the second through the fifth minute of access ($p<0.05$). Finally, this facilitatory effect of cyproheptadine on lick frequency over the five-minute access period did not differ between the saline- or the PCPA-pretreated subjects ($F<1.0$).

HPLC Analysis

Serotonin. Pretreatment with PCPA led to an 80% reduction in striatal serotonin, $F(1,22)=51.58$, $p<0.0001$. Striatal serotonin was not altered by cyproheptadine administration, $t(10)=1.55$, $p>.05$, see Table 2 (bottom, left panel).

Dopamine. Although the levels of DA in the striatum were lower than those reported in Experiment 2 overall, neither pretreatment with PCPA ($F<1.0$) nor cyproheptadine administration altered striatal dopamine ($t<1.0$), see Table 2 (bottom, right panel).

DISCUSSION

Shifting rats from 32% to 4% sucrose led to a precipitous decline in lick frequency in the saline- and the PCPA-pretreated subjects. This suppressive effect was offset by the administration of 3.0 mg/kg of cyproheptadine in both groups. Thus cyproheptadine exerted its "contrast-reducing" action in subjects with an approximate 80% reduction in brain 5-HT (as reflected in the striatum). The results of this experiment lend further support to the contention that brain 5-HT does not play a role in the initiation of successive negative contrast, nor in the contrast-reducing action of cyproheptadine.

EXPERIMENT 4

The most likely explanation remaining involves the possibility that cyproheptadine prevents the occurrence of contrast via inhibition of either acetylcholine or histamine. The possibility that cyproheptadine's actions might be mediated by cholinergic inhibition is unlikely since the administration of the cholinergic antagonist, scopolamine, failed to alter contrast when administered on either the first or the second postshift day (2,25). Further, disruption of hippocampal, cholinergic function following the intradentate administration of the neurotoxin colchicine or electrolytic lesions of the septum also failed to alter successive negative contrast (20,27).

The possibility that the contrast-reducing effect of cyproheptadine is mediated by antihistaminergic action is interesting since the decrease in fluid intake, as well as the increase in activity or "arousal" (19) on the first postshift day, could be mediated not only by serotonin, but also by histamine (34,35). Thus the first part of the final experiment was designed to investigate the possibility that the contrast-reducing action of cyproheptadine is mediated by antihistaminergic action. If this is the case, the contrast-reducing action of cyproheptadine should be mimicked by the administration of the antihistamine pyrilamine on the first postshift day.

The second part of this experiment was included as a control to test for the possibility that the selective increase in lick frequency in the shifted subjects following cyproheptadine administration was not due to its "contrast-reducing" action per se, but due to rate-dependent and/or appetite-stimulating effects associated with cyproheptadine administration.

METHOD

Subjects

The subjects were forty-six male Sprague-Dawley rats purchased from Harlan Sprague-Dawley Inc. at 90 days of age. They were deprived to 82% of their free-feeding body weight and maintained as described in Experiment 1.

Apparatus

The apparatus was the same as described in Experiment 1.

Procedure

Following two weeks of adaptation to the colony room, subjects were deprived to 82% of their free-feeding body weight and assigned to one of three groups. The first group received five minutes daily access to a 32% sucrose solution beginning with the first lick for a ten-day preshift period. They were then shifted to five minutes daily access to a 4% sucrose solution for a two-day postshift period. The second group served as the unshifted

controls receiving five minutes daily access to the 4% sucrose solution beginning with the first lick on all 12 days of the experiment. The third group, which served as rate-dependent controls, received five minutes daily access to a 2% sucrose solution for the entire 12 days of the experiment. Each day, the rate-dependent controls (group 2-2) were run first, followed by the unshifted controls (group 4-4), and the shifted subjects (group 32-4). The latency to make the first lick and the total number licks made in the five-minute period were recorded daily.

Following the ten-day preshift phase, the unshifted controls (group 4-4) and the shifted subjects (group 32-4) were matched separately by terminal lick frequency (the average lick frequency for day 9 and day 10) and assigned to one of three drug conditions as follows:

1) *Pyril 6*: Six unshifted controls and six shifted subjects were injected IP with 6.0 mg/kg pyrilamine 30 minutes prior to the first postshift period. Pyrilamine was mixed immediately prior to testing and was maintained in the refrigerator between injections;

2) *Pyril 12*: Six unshifted controls and six shifted subjects were injected IP with 12.0 mg/kg pyrilamine 30 minutes prior to the first postshift period;

3) *Saline*: Six unshifted controls and six shifted subjects were injected IP with an equal volume of 0.9% saline 30 minutes prior to the first postshift period (day 11).

Following the ten-day preshift phase, the rate-dependent controls (group 2-2) were matched and assigned to one of two drug conditions. The drug conditions were as follows:

1) *Cypro 3*: Five subjects were injected IP with 3.0 mg/kg cyproheptadine 30 minutes prior to the first postshift period. Cyproheptadine was mixed as described in Experiment 1 and refrigerated between injections;

2) *PEG*: Five subjects were injected IP with an equal volume of vehicle, 5% polyethylene glycol, 30 minutes prior to the first postshift period.

On the first postshift day the subjects were removed from their home cages, weighed, given the appropriate drug treatment, and returned to their home cages for 30 minutes. At the end of the 30-minute period the subjects were transported to the experimental room for a five-minute access period to the appropriate postshift solution (either 2% or 4% sucrose). The latency to make the first lick and the number of licks made in the five-minute period were recorded and the subjects were returned to their home cages. Recovery from contrast was evaluated over the succeeding postshift period (day 12). The running order throughout the experiment was consistent with that appropriate for the injection schedule on the first postshift day.

Sucrose solutions were prepared and maintained as described in Experiment 1.

RESULTS

Preshift

Latency. Analysis of the terminal preshift data [(day 9 + day 10)/2] indicated that rats did not initiate licking more quickly for 32% than for 4% sucrose, $F(1,30) = 1.13$, $p > 0.05$.

Lick frequency. Analysis of lick frequency over the same terminal preshift period indicated that rats made reliably more licks for 32% than for 4% sucrose, $F(1,30) = 14.76$, $p < 0.0006$.

Postshift

Latency. Analysis of the latency data over the terminal preshift period through day 12 reflected a reliable magnitude of reward effect. That is, rats having prior experience with 32% sucrose initiated licking faster than the unshifted controls overall

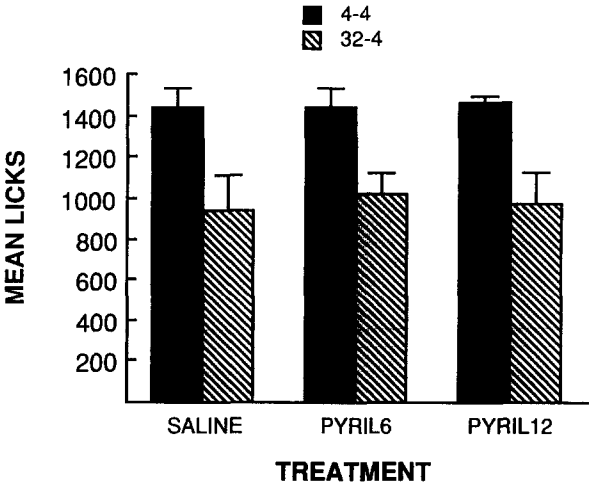

FIG. 5. Mean lick frequency for 4% sucrose in both shifted (32-4) and unshifted (4-4) rats on the first postshift day (day 11). Rats were injected IP with either saline (SALINE), 6.0 mg/kg pyrilamine (PYRIL 6), 12.0 mg/kg pyrilamine (PYRIL 12) 30 minutes prior to the first postshift period. Treatment with pyrilamine failed to attenuate contrast in rats shifted from 32% to 4% sucrose.

[Solution, $F(1,30) = 5.29$, $p < 0.02$]. As reported in Experiment 1, this magnitude of reward effect was not altered by reward downshift [Solution × Day, $F(2,30) = 2.08$, $p < 0.10$].

Lick frequency. Rats shifted from 32% to 4% sucrose made reliably fewer licks for 4% sucrose than the unshifted controls [Solution, $F(1,30) = 9.2$, $p < 0.005$]. This contrast effect was not altered by the administration of either dose of pyrilamine (Solution × Drug × Day, $F < 1.0$), see Fig. 5.

Rate-dependent controls. The 2% control subjects served as acceptable rate-dependent controls for group 32–4 since the lick frequency on the first postshift day (day 11) for 2% sucrose by the 2% control subjects (mean = 999) did not differ from the lick frequency for 4% sucrose by rats shifted from 32% to 4% sucrose (mean = 940) (Sol, $F < 1.0$). Further, the administration of 3.0 mg/kg cyproheptadine did not alter lick frequency in the 2% control subjects on the first postshift day (mean = 940) (Drug, $F < 1.0$).

DISCUSSION

Successive negative contrast was not altered by the administration of either dose of pyrilamine on the first postshift day, suggesting that the contrast-reducing action of cyproheptadine is not mediated by antihistaminergic activity. In addition, the finding that cyproheptadine administration failed to alter lick frequency in the 2% control subjects (which were licking at approximately the same rate as the shifted subjects) indicates that the reduction in contrast following cyproheptadine administration in shifted subjects is not related to the level of lick frequency per se.

GENERAL DISCUSSION

Rats shifted from 32% to 4% sucrose demonstrated a reliable suggestive negative contrast effect in all experiments. Cyproheptadine prevented the occurrence of contrast when administered on the first postshift day, as was the case when administered on the second postshift day in an earlier study (2). Further, the results of Experiment 3 demonstrated that the contrast-reducing action of cyproheptadine was reliable beginning with the second

minute of access to the postshift solution.

The robust and immediate nature of the contrast-reducing action of cyproheptadine is both unique and compelling since no other psychoactive drug has been found as effective when administered on the first postshift day. As a result, cyproheptadine serves as the first pharmacological tool available for investigating the potential mechanisms mediating the initial occurrence of contrast.

Contrast requires that the animal accurately detect and identify the value of the available solution (4% sucrose), remember the previously received reward level (32% sucrose), compare the two levels of reward, and reject the available stimulus if it is determined to be inferior to the previously received stimulus.

The administration of cyproheptadine on the first postshift day did not appear to alter accurate detection of the postshift solution (4% sucrose) since cyproheptadine-treated rats decreased lick frequency when shifted from 32% to 4% sucrose to a level not different from the unshifted controls. Cyproheptadine treatment also did not appear to disrupt the memory for the value of the previously received reward (32% sucrose) since rats demonstrating a magnitude of reward effect in latency continue to do so following cyproheptadine treatment. Cyproheptadine administration could have interfered with either the comparison between the two levels of reward or simply the response to that comparison. This distinction cannot be made from the available data.

Although it is not yet known at what point in the process cyproheptadine prevents the occurrence of contrast on the first postshift day, the evidence in this report indicates that it does not do so via inhibition of serotonin, histamine, or acetylcholine. The contrast-reducing action of cyproheptadine on the first postshift day was not mimicked by the serotonin synthesis inhibitor PCPA, the antihistamine pyrilamine, or the anticholinergic agent scopolamine (2,25).

An alternative system by which cyproheptadine might exert its contrast-reducing actions is the GABA/benzodiazepine/chloride ionophore receptor complex. This possibility is intriguing for a number of reasons. First, the response profile for cyproheptadine in negative contrast was similar to that following corticomedial amygdala lesions (5), and the corticomedial amygdala (including the central nucleus), is a region known to be replete with GABA and benzodiazepine receptor sites (31). Second, the response profile following cyproheptadine administration was not unlike that found with the ICV administration of the GABA agonist muscimol (unpublished data from this laboratory). Finally, evidence is available to suggest that cyproheptadine binds to both "picrotoxin-sensitive" TBPS receptor sites and benzodiazepine receptor sites (1, 9, 40). Although contradictory evidence has also been reported (42), the possibility that cyproheptadine may prevent the occurrence of contrast via this complex is currently under investigation.

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