ELSEVIER

Pharmacology Biochemistry and Behavior, Vol. 53, No. 2, pp. 347-354, 1996

0091-3057/96 $15.00 + .00

0091-3057(95)02033-6

# $H_1$, $H_2$, and $H_3$ Receptors Contribute to Drinking Elicited by Exogenous Histamine and Eating in Rats

F. SCOTT KRALY,[1] MELISSA E. KEEFE, ROBERT A. TRIBUZIO, YOUNG-MEE KIM, JARED FINKELL AND CHRISTOPHER J. BRAUN

*Department of Psychology, Colgate University, Hamilton, NY 13346*

Received 10 February 1995; Revised 22 May 1995; Accepted 30 May 1995

KRALY, F. S., M. E. KEEFE, R. A. TRIBUZIO, Y.-M. KIM, J. FINKELL AND C. J. BRAUN. *$H_1$, $H_2$, and $H_3$ receptors contribute to drinking elicited by exogenous histamine and eating in rats.* PHARMACOL BIOCHEM BEHAV 53(2) 347-354, 1996. – Roles for $H_1$, $H_2$, and $H_3$ receptor subtypes for drinking elicited by exogenous histamine and drinking elicited by eating was examined in adult male Sprague-Dawley rats. Drinking elicited by SC 5 mg/kg histamine was: (a) inhibited approximately 30% by $H_1$ antagonism using IP 1 mg/kg dexbrompheniramine (DXB); (b) inhibited approximately 30% by $H_2$ antagonism using IP 16 mg/kg cimetidine (C); (c) inhibited approximately 40% by $H_3$ antagonism using SC 10 mg/kg thioperamide (Th); (d) inhibited approximately 80% by combined $H_1$ and $H_2$ antagonism using IP DXB plus IP C; (e) inhibited approximately 85% by combined $H_1$ and $H_3$ antagonism using IP DXB plus SC Th; (f) inhibited approximately 70% by combined $H_2$ and $H_3$ antagonism using IP C plus SC Th; and (g) abolished by combined $H_1$, $H_2$, and $H_3$ antagonism using IP DXB plus IP C plus SC Th. For rats eating pellets and drinking after 24-h food deprivation: (a) systemic injections of DXB, C, and Th, sufficient to abolish drinking elicited by SC histamine, inhibited water/food ratio (W/F) by approximately 20%; (b) ICV injections (through a chronic cannula in a lateral ventricle) of 50 μg DXB plus 100 μg C plus 60 μg Th inhibited W/F by approximately 20%. For rats drinking after IG infusion (through a chronic gastric catheter) of 2 ml 1800 mOsm/kg NaCl: (a) systemic injections of DXB, C, and Th, sufficient to abolish drinking elicited by SC histamine, inhibited water intake by approximately 70%; (b) IP DXB alone and IP C alone failed to inhibit water intake; (c) IP Th alone inhibited water intake by approximately 20%; (d) IP DXB combined with IP C inhibited water intake by approximately 55%. The results demonstrate the involvement of $H_1$, $H_2$, and $H_3$ receptors for drinking elicited by exogenous histamine, and our findings extend the evidence for a role for endogenous histamine and $H_1$, $H_2$, and $H_3$ receptor subtypes for drinking elicited by eating, including drinking elicited by gastrointestinal osmotic consequences of eating that can increase systemic plasma osmolality.

Histamine Drinking Food-related drinking Histaminergic $H_1$ receptors $H_2$ receptors $H_3$ receptors

PHARMACOLOGICAL antagonism of $H_1$ and $H_2$ receptors for histamine inhibits drinking elicited by exogenous histamine (9,11) and drinking elicited by eating (10,11) in rats. The recent discovery of agonists and antagonists selective for the $H_3$ receptor subtype (2,21), together with reports (4,16) that the $H_3$ agonist *R*-α-methylhistamine is dipsogenic and that the $H_3$ antagonist thioperamide can attenuate drinking elicited by exogenous histamine (4), requires study of the contribution of $H_3$ receptors and the relation of the $H_3$ subtype to the contributions of $H_1$ and $H_2$ receptors for drinking elicited by histamine and food-related drinking.

We examined (a) the ability of pharmacological antagonism of $H_3$ and/or $H_1$ and/or $H_2$ receptors to inhibit drinking elicited by SC histamine, and (b) the ability of combined $H_1$, $H_2$, and $H_3$ antagonism to inhibit drinking elicited by eating and drinking elicited by IG infusion of NaCl. Our findings extend the evidence supporting a role for endogenous histamine and $H_1$, $H_2$, and $H_3$ receptor subtypes for drinking behavior in the rat.

## EXPERIMENT 1

Histamine injected SC increases water intake in a dose-related manner (13), and such drinking is inhibited by IP or

[1] To whom requests for reprints should be addressed.

SC injection of antagonists to $H_1$ (9), $H_2$, (13) or $H_3$ receptors (4,16). We have examined for the first time in a single repeated-measures experiment the relative contributions of $H_1$, $H_2$, and $H_3$ receptor subtypes by measuring the ability of various combinations of drugs selectively antagonistic to each of these subtypes to inhibit drinking elicited by SC histamine.

## METHOD

### *Animals*

Twelve Sprague-Dawley albino male rats (275-350 g) were housed individually in conventional stainless steel cages with a wire mesh floor on a 12 L : 12 D cycle. Rats had continuous access to pelleted chow on the floor of the cage and to tap water available through a stainless steel spout attached to a glass graduated cylinder mounted on the wall of the cage. Experimental protocols were approved by the Institutional Animal Care and Use committee.

### *Procedure*

Twelve rats were tested repeatedly at intervals of 2-5 days in the following paradigm. Food was removed and rats were weighed at 30 min prior to the start of the test. At 10 min prior to the start of the test rats received IP and/or SC injections of vehicle solution(s) or receptor antagonist drug(s). The test was initiated with a SC injection of 5 mg/kg histamine (or 0.9% NaCl on tests to establish baseline for drinking). Latency to initiate drinking was recorded. Water intake was recorded for a 1-h test by reading from the graduated water bottles. Food was returned at the end of the test. Each rat was tested 17 times in this paradigm. The first and the last test in this sequence determined baseline for drinking; on these tests all injections were 0.9% NaCl. The remaining 15 tests were tests on which SC histamine was preceded by vehicle solution(s) or histamine antagonist drug(s). On the first of these 15 tests, all rats received SC histamine preceded 10 min by vehicle injection. On the second of these 15 tests, rats received SC histamine preceded 10 min by a histamine antagonist drug. On the third of these 15 tests, all rats again received SC histamine preceded by vehicle injection. Thus, as this series of 15 tests proceeded, each antagonist drug(s) plus histamine test was bracketed (i.e., one before and one after) by two histamine plus vehicle tests. The volume of vehicle solution(s) injected was matched to volume of drug solution injected on the subsequent drug test. The pharmacological antagonism of histamine receptors was accomplished in the following order across these 15 tests: $H_3$, $H_1$, $H_2$, $H_1 + H_3$, $H_1 + H_2$, $H_2 + H_3$, $H_1 + H_2 + H_3$.

### *Drugs*

Selection of drugs and choice of dosages for each drug were carefully determined based upon published findings. A 5-mg/kg SC dose of histamine was chosen because it elicits a rapid and robust drinking response that is less than the maximal drinking response (13). Dexbrompheniramine (DXB) is an $H_1$ antagonist that has been used extensively in the study of food-related drinking in the rat (11); a 1-mg/kg IP dose of DXB was used because it is just subthreshold for inhibition of drinking elicited by SC 20 mg/kg histamine, a dose of histamine that elicits a maximal drinking response (9). Cimetidine (C) is an $H_2$ antagonist that has been used extensively in the study of food-related drinking in the rat (11); a 16-mg/kg IP dose of C was used because it is just subthreshold for inhibition of drinking elicited by SC 20 mg/kg histamine (9). The $H_3$ antagonist thioperamide (Th) is a relatively new compound that has only recently been used in the study of drinking in the rat (4); 10 mg/kg Th given systemically can selectively abolish drinking elicited by the $H_3$ agonist *R*-$\alpha$-methylhistamine (4,16) and can reduce approximately by half drinking elicited by IP 4 mg/kg histamine (4). The histamine diphosphate, dexbrompheniramine maleate, and cimetidine were purchased from Sigma Chemical Co. The thioperamide was purchased from Research Biochemicals International. The histamine, DXB, and Th were prepared in 0.9% NaCl. The C was prepared in vehicle as described elsewhere (9).

### *Data Analysis*

Data for latency to initiate drinking did not meet assumptions necessary for parametric statistical analysis. Therefore, median (instead of mean) values are reported in Table 1 for latency to drink, and nonparametric statistical tests (Friedman's test, Wilcoxon test) were used for within-group comparisons. Data for water intake were first examined with analysis of variance (ANOVA). ANOVA showed that there were no differences for water intake on the two tests (at the beginning and at the end of the sequence of tests) for determining baseline for drinking. ANOVA also showed that there were no differences for water intake on the eight histamine tests that were preceded by injections of vehicle solutions. Therefore, the results of the two baseline tests were averaged (i.e., mean of the raw data for each rat) and these values were used to determine change from baseline scores for water intake presented in Fig. 1. In addition, the data (latency to drink and water intake) for the eight histamine tests (no antagonist drug) were averaged for subsequent statistical analysis and presentation in Fig. 1 and Table 1. The measures presented in Fig. 1 were examined with ANOVA and then with a simple contrasts procedure (using the overall error term) to make multiple comparisons of drug treatment vs. SC histamine. ANOVA was also used to examine data presented in Fig. 2 to compare the water intake after *combined* drug treatments vs. the sum (for each rat) of the water intake scores for each drug treatment. Alpha level was chosen as 0.05.

## RESULTS

The latency to initiate drinking after SC histamine was significantly increased (Table 1) by the $H_2$ antagonist C ($p < 0.02$), the $H_3$ antagonist Th ($p < 0.005$), combined antago-

TABLE 1

MEDIAN LATENCY TO INITIATE DRINKING AFTER 5 mg/kg SC HISTAMINE

| Treatment | Latency to Drink (min) |
|---|---|
| Vehicle | 3.8 |
| $H_1$ | 4.0 |
| $H_2$ | 6.0 |
| $H_3$ | 6.9 |
| $H_1 + H_2$ | 4.0 |
| $H_1 + H_3$ | 5.2 |
| $H_2 + H_3$ | 12.8 |
| $H_1 + H_2 + H_3$ | >60 |
| Baseline | >60 |

Baseline treatment was 0.9% NaCl injections only (i.e., no SC histamine).

nism of $H_1$ and $H_3$ receptors using DXB and Th ($p < 0.02$), combined antagonism of $H_2$ and $H_3$ receptors using C and Th ($p < 0.005$), and combined $H_1$, $H_2$, and $H_3$ antagonism using DXB, C, and Th ($p < 0.005$). The latency to drink was sufficiently increased to not be different ($p > 0.20$) from latency under baseline conditions (i.e., no histamine) following only the combined DXB, C, and Th treatment (Table 1).

The $H_1$ antagonist DXB appeared to inhibit water intake elicited by SC histamine by approximately 30% (Fig. 1), but this effect was not statistically significant ($p > 0.20$). Similarly, the $H_2$ antagonist C also appeared to inhibit water intake elicited by histamine by approximately 30% (Fig. 1), but this effect was not statistically significant ($p > 0.20$). The $H_3$ antagonist Th inhibited ($p < 0.005$) water intake by approximately 40% (Fig. 1). Combined antagonism of $H_1$ and $H_2$ receptors using DXB plus C inhibited ($p < 0.001$) water intake by approximately 80%; combined antagonism of $H_2$ and $H_3$ receptors using C plus Th inhibited ($p < 0.05$) water intake by approximately 70%; combined antagonism of $H_1$ and $H_3$ receptors using DXB plus Th inhibited ($p < 0.001$) water intake by approximately 85% (Fig. 1). Combined antagonism of $H_1$, $H_2$, and $H_3$ receptors inhibited ($p < 0.001$) water intake by approximately 95% (Fig. 1); drinking was essentially abolished by this treatment because water intake was not different from baseline water intake ($p > 0.20$).

Receptor antagonist drugs appeared to be additive in their effects upon drinking elicited by histamine (Fig. 2): the inhibition of water intake produced by combined DXB and C was not significantly different ($p > 0.10$) from the sum of the effects on water intake produced by DXB alone plus those produced by C given alone. Similarly, the inhibition of water intake produced by combined C and Th was not significantly different ($p > 0.20$) from the sum of the effects on water intake produced by C alone plus those produced by Th given alone. Likewise, the inhibition of water intake produced by combined DXB and Th was not significantly different ($p > 0.20$) from the sum of the effects on water intake produced by DXB alone plus those produced by Th given alone. Finally, the inhibition of water intake produced by combined DXB, C, and Th was not significantly different ($p > 0.20$) from the sum of the effects on water intake produced by DXB alone plus those produced by C alone plus those produced by Th given alone (Fig. 2).

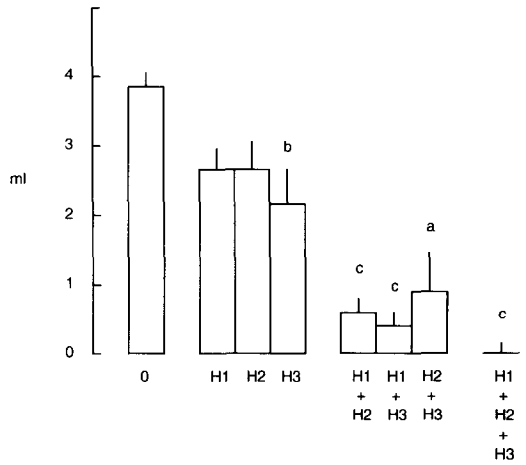


FIG. 1. Mean ± SE 1-h water intake (as ml change from baseline conditions, i.e., systemic injections of 0.9% NaCl) for 12 rats receiving SC injection of 5 mg/kg histamine (all bars) preceded by IP or SC vehicle solutions (0) or $H_1$ antagonist dexbrompheniramine alone ($H_1$), $H_2$ antagonist cimetidine alone ($H_2$), $H_3$ antagonist thioperamide alone ($H_3$), dexbrompheniramine plus cimetidine ($H_1 + H_2$), dexbrompheniramine plus thioperamide ($H_1 + H_3$), cimetidine plus thioperamide ($H_2 + H_3$), or dexbrompheniramine plus cimetidine plus thioperamide ($H_1 + H_2 + H_3$). $^a p < 0.05$; $^b p < 0.005$; $^c p < 0.001$ vs. 0 open bar.

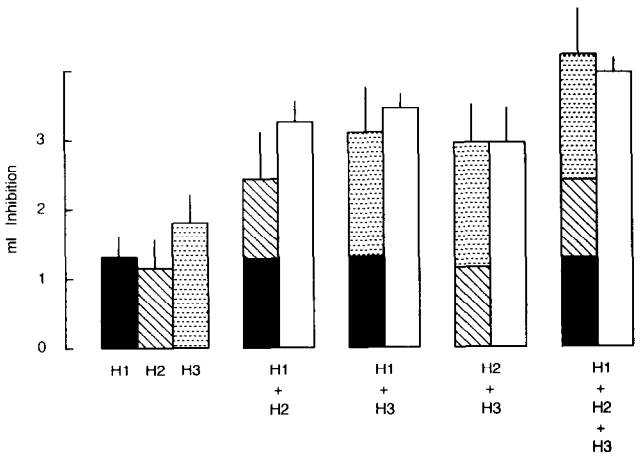


FIG. 2. Mean ± SE ml inhibition of 1-h water intake for 12 rats receiving SC injection of 5 mg/kg histamine (all bars) preceded by IP or SC $H_1$ antagonist dexbrompheniramine alone ($H_1$), $H_2$ antagonist cimetidine alone ($H_2$), $H_3$ antagonist thioperamide alone ($H_3$), dexbrompheniramine plus cimetidine ($H_1 + H_2$), dexbrompheniramine plus thioperamide ($H_1 + H_3$), cimetidine plus thioperamide ($H_2 + H_3$), or dexbrompheniramine plus cimetidine plus thioperamide ($H_1 + H_2 + H_3$). All open bars and single-filled bars represent data collected in the various treatments of the experiments. The bars with multiple-filled types represent the sums of the data collected for treatments $H_1$ (gray bar), $H_2$ (cross-hatched bar), and $H_3$ (stippled bar).

## DISCUSSION

These results demonstrate the involvement of $H_1$, $H_2$, and $H_3$ receptor subtypes for drinking elicited by SC histamine. The findings that the 1-mg/kg dose of $H_1$ receptor antagonist DXB and the 16-mg/kg dose of $H_2$ receptor antagonist C are subthreshold (when given alone) for statistically significant inhibition of drinking elicited by histamine are consistent with earlier work from our laboratory using a larger (20 mg/kg) dose of histamine (9). The ability of the $H_3$ antagonist Th (SC) to inhibit drinking elicited by SC histamine is consistent with a recent report of the results of experiments using IP Th and IP histamine (4).

In apparent contrast to earlier work (9), however, is our finding that the effects of these various histamine receptor antagonist drugs, when given in various combinations, appear to be additive in nature. For example, 1 mg/kg DXB and 16 mg/kg C (given IP) are each reported to be subthreshold for inhibition of water intake elicited by 20 mg/kg SC histamine—a dose that elicits the maximal drinking response (9). When these subthreshold doses of DXB and C are combined prior to administration of SC 20 mg/kg histamine, however, water intake can be abolished (9), revealing a synergistic relation between these $H_1$ and $H_2$ antagonist drugs. The results reported here using the same doses of DXB and C, but a dose of histamine (5 mg/kg) that is well below the dose (20 mg/kg) for

a maximal drinking response (13), reveal that 1 mg/kg DXB and 16 mg/kg C (given IP) are each subthreshold for statistically significant inhibition of drinking elicited by 5 mg/kg SC histamine, but that their effects when given in combination appear to be additive, not synergistic. These findings demonstrate that the relative contributions made by $H_1$ and $H_2$ receptors for drinking elicited by exogenous histamine depend upon the dose of exogenous histamine employed. The fact that combined $H_1$ and $H_2$ antagonism vs. a smaller (5 mg/kg) dose of SC histamine reveals apparently additive receptor-mediated effects, whereas the combined $H_1$ and $H_2$ antagonism vs. a larger (20 mg/kg) dose of histamine reveals apparently synergistic receptor-mediated effects, may reflect the involvement of several mechanisms by which SC histamine elicits drinking. There is evidence that the degree of involvement of two such mechanisms (e.g., gastric vagal afferent, renal renin–angiotensin) can be dose dependent (11), and therefore, it is reasonable to speculate that the dose-related activation of different mechanisms could explain dose-related additive vs. synergistic effects of receptor antagonist drugs for $H_1$ and $H_2$ receptors.

Our finding that antagonism of $H_3$ receptors has effects on water intake that are additive to those induced by antagonism of $H_1$ or $H_2$ or $H_1$ plus $H_2$ receptors is news, as is the finding that combined $H_1$, $H_2$, and $H_3$ antagonism can abolish drinking elicited by SC histamine. These findings together demonstrate that to achieve complete pharmacological antagonism of the dipsogenic ability of exogenous histamine, it is likely to be necessary (under at least some conditions) to pharmacologically block each of the three identified subtypes of receptors for histamine.

## EXPERIMENT 2

Combined pharmacological antagonism of $H_1$ and $H_2$ histamine receptors using IP DXB plus C inhibits drinking by approximately 25% for rats eating and drinking after 24-h food deprivation (10). This measure of the relative contribution of endogenous histamine to food-related drinking may represent an underestimate given the finding in Experiment 1 that blockade of $H_3$ receptors may be required for complete blockade of the dipsogenic effects of exogenous histamine under some experimental conditions. Therefore, this experiment evaluated the effect of combined systemic pharmacological antagonism of $H_1$, $H_2$, and $H_3$ receptors (demonstrated in Experiment 1 to be sufficient to abolish drinking elicited by SC histamine) upon drinking elicited by eating in 24-h food-deprived rats.

### METHOD

#### *Procedure*

Twelve Sprague–Dawley albino male rats (275–350 g), maintained as described for Experiment 1, were used in this test for drinking. Rats were deprived of food for 24 h before the time for testing at the midpoint of the 12-h light phase. Water remained available throughout food deprivation. After 24-h deprivation from food rats were weighed and then offered food to initiate a test. Rats were free to eat and drink for a 1-h period while the experimenter recorded water intake by reading from the graduated water bottles. To initiate a test, a measured amount of pelleted chow (Purina) was placed on the floor of the cage 10 min after a rat received its IP and SC injections. A clean sheet of paper was placed beneath each cage to collect spilled crumbs of food. Food intake was determined by collecting remaining pellets from the cage floor and spillage from the paper at the end of 1 h; the difference between the food placed into the cage minus the collected food and spillage was taken to be 1-h food intake. The water/food ratio (W/F) was calculated as the 1-h water intake divided by the 1-h food intake. The latency to drink was measured as the time from the offering of food pellets to the first lick of the water spout. Rats were tested twice in this paradigm: (a) following IP and SC vehicle solutions, and (b) following IP 1 mg/kg DXB plus 16 mg/kg C plus SC 10 mg/kg Th. A simple counterbalancing procedure was used to control for sequence of testing; half of the rats received test a prior to test b, the other half received the tests in reverse order. Tests were performed 4 days apart.

#### *Data Analysis*

Planned within-group comparisons were made with a matched-pairs $t$-test (two-tailed). Alpha level was chosen as 0.05.

### RESULTS

Combined systemic injection of DXB, C, and Th (Fig. 3) inhibited food intake ($p < 0.05$) and water intake ($p < 0.001$), and reduced W/F ($p < 0.05$) by 17% without significant effect on latency to drink ($p > 0.05$). It is interesting to note that 5 of the 12 rats tested did not have their food intake inhibited by the combination of these three drugs. Of these rats, four of five drank less water after histamine antagonists than after vehicle injections and all five rats had a reduced W/F after histamine antagonists.

### DISCUSSION

The combined antagonism of $H_1$, $H_2$, and $H_3$ receptors using systemic DXB, C, and Th appeared to nonspecifically inhibit ingestive behavior: both food and water intakes were significantly inhibited (Fig. 3). The inhibitory effect upon water intake appeared to be proportionately greater, however, resulting in a significant reduction of the W/F. This approxi-

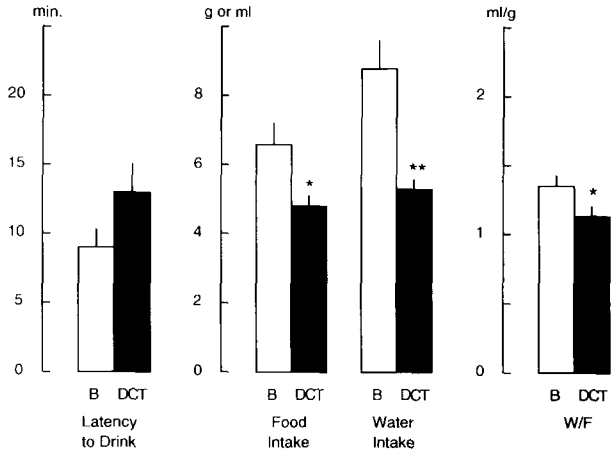


FIG. 3. Mean ± SE latency to initiate drinking (min), 1-h food intake (g), water intake (ml), and water/food ratio (W/F, ml/g) for 24-h food-deprived rats ($n = 12$) eating and drinking after systemic injections of vehicle solutions (B, open bars) or 1 mg/kg dexbrompheniramine plus 16 mg/kg cimetidine plus 10 mg/kg thioperamide (DCT, black bars). *$p < 0.05$; **$p < 0.001$ vs. paired open bar.

mately 20% inhibition of W/F induced by simultaneous antagonism of three subtypes of histamine receptors is similar to the magnitude of inhibition of food-related drinking induced by combined $H_1$ and $H_2$ antagonism for rats tested eating and drinking under identical experimental conditions (10).

## EXPERIMENT 3

Inhibition of food-related drinking induced by systemic injections of DXB, C, and Th could be, at least in part, attributable to the effects of DXB or C or Th on histamine receptors in the brain, because IP DXB and SC Th penetrate the blood-brain barrier (2,3) and IP cimetidine could have access to histamine receptors in areas of the brain not protected by the blood-brain barrier (19). Therefore, we examined the ability of ICV injections of DXB, C, and Th to affect drinking elicited by eating after 24-h food deprivation.

### METHOD

*Surgery*

Sprague-Dawley male albino rats (275-350 g), maintained as described for Experiment 1, received surgical implantation of a stainless steel guide cannula (22 ga; Plastics One) aimed for a lateral cerebral ventricle. Stereotaxic surgery was performed under general anesthesia (IP 50 mg/kg pentobarbital sodium, Nembutal; Abbott Labs.). The chronic cannula was secured with cranioplastic cement to four screws in the skull. Rats received prophylactic doses (SC 1 mg gentamicin sulfate; TechAmerica) daily for the first 7 postoperative days. Rats were permitted 2 weeks to recover from surgery before the beginning of testing. Two methods were used for verification of placement of cannula. First, prior to any other experimental procedures, each rat was tested for 30 min for drinking in response to a bolus injection of 100 ng angiotensin II (Peninsula) in 1 $\mu$l 0.9% NaCl delivered through a 28-ga injector inserted into the cannula. If a rat failed to drink rapidly (within 5 min) or failed to drink a considerable amount (greater than 5 ml) of water it was discarded from the study. Second, following all testing the brain of each rat was examined at autopsy. A 1-$\mu$l injection of India ink was delivered through the cannula immediately after perfusion of the anesthetized rat. The brain was then removed from the skull and sliced with a razor to determine whether ink was distributed throughout the ventricular system and whether it was confined to the ventricles. If a rat passed the angiotensin test for verification of placement but subsequently failed the India ink test, its data were discarded from the study.

*Procedure*

Fourteen rats were tested eating and drinking after 24-h food deprivation according to the procedures described for Experiment 2. Instead of receiving injections systemically, however, rats received ICV injections through the indwelling cannula. With the rat being held in the experimenter's hands, the injections (approximately 0.5 $\mu$l/s) were made using a 28-ga injector cannula that had replaced the obdurator in the cannula. After injections were completed, the obdurator replaced the injector and the rat was returned to its home cage. The test was initiated 10 min later when rats were offered food pellets. For ICV administration of DXB, C, and Th, we chose a dose of DXB (50 $\mu$g) that when given alone ICV has no apparent effect upon drinking elicited by SC histamine, but when combined with ICV 100 $\mu$g C can abolish drinking elicited by systemic histamine (12). We chose a 100-$\mu$g dose of C that when given alone ICV can inhibit drinking elicited by SC histamine by approximately 75% (12). We chose 60 $\mu$g Th, a dose that when given ICV can selectively abolish drinking elicited by the ICV $H_3$ agonist *R*-$\alpha$-methylhistamine (16). All rats were tested twice: (a) after ICVT 4 $\mu$l vehicle solution, and (b) after ICV 50 $\mu$g DXB plus 60 $\mu$g Th in 2 $\mu$l plus 100 $\mu$g C in 2 $\mu$l. Half of the rats received test a prior to test b, the other half received the tests in reverse order. Tests were performed 4 days apart. Data were analyzed as described for Experiment 2.

### RESULTS

Combined ICV injections of DXB, C, and Th failed to affect latency to drink ($p > 0.20$) and food intake ($p > 0.20$). Water intake was not significantly inhibited ($p > 0.10$), but W/F was decreased ($p < 0.05$) by 21% (Fig. 4).

### DISCUSSION

The W/F was selectively inhibited by ICV combined antagonism of $H_1$, $H_2$, and $H_3$ receptors. This finding suggests that at least part of the effects (in Experiment 2) of combined systemic injections of DXB, C, and Th upon drinking behavior could be due to the central effects of one or more of these drugs. Because combined ICV injections of DXB and C failed in an earlier study (12) to selectively inhibit drinking behavior in this experimental paradigm, however, the results of this experiment would appear largely attributable to the effects of ICV Th. When Th is given alone ICV, however, it also fails to inhibit food-related drinking in rats (16). It appears, therefore, that only when ICV Th is combined with ICV DXB and/or C is a role for histamine receptors in brain revealed for drinking elicited by eating pellets after food deprivation.

## EXPERIMENT 4

Ingestion of pellets has numerous postprandial physiological consequences including hypovolemia (1,18,20), increased plasma renin activity (17,20), and increased systemic plasma

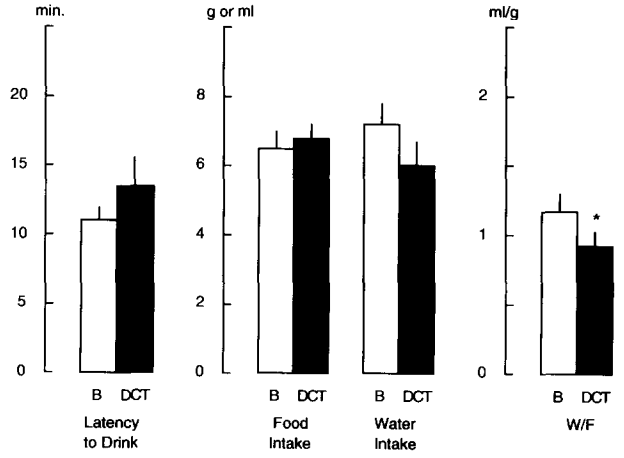


FIG. 4. Mean ± SE latency to initiate drinking (min), 1-h food intake (g), water intake (ml), and water/food ratio (W/F, ml/g) for 24-h food-deprived rats ($n = 14$) eating and drinking after ICV injections of vehicle solutions (B, open bars) or 50 $\mu$g dexbrompheniramine plus 100 $\mu$g cimetidine plus 60 $\mu$g thioperamide (DCT, black bars). *$p < 0.05$ vs. paired open bar.

osmolality (1,5). Increased plasma osmolality induced by IP hypertonic NaCl can elicit drinking that can be inhibited by systemic pharmacological antagonism of histamine receptors (6). We examined the ability of systemic injections of DXB and/or C and/or Th to inhibit drinking elicited by IG infusion of 2 ml 1800 mOsm/kg NaCl, a treatment that is sufficient to elicit increase in systemic plasma osmolality at the initiation of drinking in rats (14). We used IG rather than IP administration of NaCl in an attempt to mimic, at least in part, the means by which osmotic properties of ingested food can elicit postprandial increases in plasma osmolality.

## METHOD

### *Surgery*

Sprague–Dawley male rats (275–350 g), maintained as described for Experiment 1, received under general anesthesia surgical implantation of a medical-grade silicone catheter (0.030 in. i.d., 0.065 in. o.d.; Silastic) in the stomach. The beveled tip of the catheter (15 mm) was threaded through a puncture wound on the greater curvature of the stomach where it was anchored with 4-0 surgical silk. The catheter was anchored a second time with suture during the closing of the wound (using 4-0 silk) on the abdominal wall. The distal end of the catheter was tunneled beneath the skin to the nape of the neck where it was externalized and anchored. A steel plug closed the distal end of the catheter. The incision in the skin was closed with wound clips.

### *Procedure*

Thirty-seven rats equipped with a gastric catheter were tested for drinking according to the following procedures. The rat was removed from its cage and weighed, and food was removed from the cage. The IP and/or SC injections of drug(s) or vehicle solution(s) were made 10 min prior to the IG infusion of NaCl, which initiated the test for drinking. For IG infusions, the plug was removed from the gastric catheter and the catheter was attached to a length of tubing attached to a syringe. With the rat resting comfortably on the experimenter's arm or chest, a 2-ml infusion (2 ml/30 s) was delivered through the gastric catheter. The tubing was removed and the plug was replaced in the catheter. The rat was immediately returned to its home cage and the test was begun. With the rat having free access to tap water from the graduated bottle with spout, water intake was recorded for 1 h. Food was returned to the cage at the end of each 1-h test.

The volume (2 ml) and concentration of NaCl to be delivered into the stomach were carefully selected. The 2-ml 1800 mOsm/kg NaCl has been used previously (14) in studying the effects of IG osmotic loads on drinking and body fluid balance in rats; it is above threshold for increase in systemic plasma osmolality at the initiation of drinking (14) and it is below the threshold for irritation of the gastric mucosa (22).

Rats were first tested after IG infusion of 2 ml 290 mOsm/kg (i.e., 0.9%) NaCl to establish baseline conditions for drinking in this paradigm. Subsequent tests to examine for the effects of a specific drug included rats being tested twice in response to 1800 mOsm/kg NaCl. Each pair of tests consisted of one test on which the effects of drug(s) were assessed and the other test in which rats received an equivolume control IP or SC injection of vehicle solution(s). A simple counterbalancing procedure was used for each pair of tests with half of the rats receiving the drug treatment(s) on the first of the two tests, and the other half of the rats receiving the drug treatment(s) on the second of the two tests. The dosages and combinations of histamine receptor antagonist drugs were the same as those described for Experiment 1.

### *Data Analysis*

Planned within-group comparisons were performed with the matched-pairs *t*-test (two-tailed). When data did not meet assumptions necessary for parametric analysis (i.e., data for latency to drink), nonparametric statistical procedures (Friedman's test; Wilcoxon test) were used and the median rather than the mean was used as a descriptive statistic. Alpha level was 0.05.

## RESULTS

The IG infusion of 2 ml 1800 mOsm/kg NaCl significantly shortened the latency to initiate drinking ($p < 0.05$) and increased 1-h water intake ($p < 0.001$) compared to baseline conditions (i.e., IG 290 mOsm/kg NaCl) (Fig. 5). The combined systemic injections of DXB, C, and Th significantly increased latency to initiate drinking ($p < 0.05$) and inhibited water intake ($p < 0.005$) by 70% (Fig. 5). Water intake after IG 1800 mOsm/kg NaCl was not abolished by the histamine receptor antagonists, however, because intake after DXB, C, and Th was greater than intake under baseline conditions ($p < 0.05$).

The injection of DXB alone failed to inhibit ($p > 0.10$) water intake after IG 1800 mOsm/kg NaCl (Fig. 6). The injection of C alone also failed to inhibit ($p > 0.20$) water intake after IG 1800 mOsm/kg NaCl (Fig. 6). The combined injections of DXB plus C inhibited ($p < 0.005$) by 55% water intake after IG NaCl (Fig. 6). The injection of Th alone inhibited ($p < 0.05$) by 22% water intake after IG NaCl (Fig. 6).

## DISCUSSION

Whereas DXB alone and C alone failed to significantly inhibit drinking elicited by IG hypertonic NaCl, DXB combined with C inhibited such drinking by approximately 50%

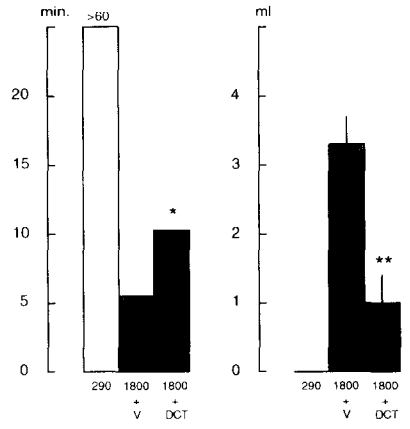


FIG. 5. Median latency to initiate drinking (min) and mean ± SE 1-h water intake (ml) for six nondeprived rats receiving IG 2 ml 290 mOsm/kg NaCl (290, open bars), 1800 mOsm/kg NaCl preceded by systemic injections of vehicle solutions (1800 + V, gray bars), or 1800 mOsm/kg NaCl preceded by systemic injections of 1 mg/kg dexbrompheniramine plus 16 mg/kg cimetidine plus 10 mg/kg thioperamide (1800 + DCT, black bars). *$p < 0.05$; $p < 0.005$ vs. 1800 + V bar.

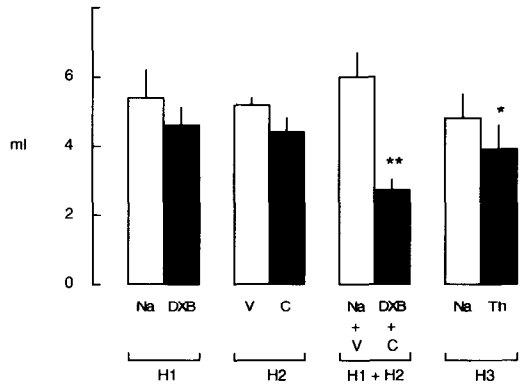


FIG. 6. Mean ± SE 1-h water intake for nondeprived rats receiving IG infusion of 2 ml 1800 mOsm/kg NaCl (all bars) preceded by IP or SC injections of 0.9% NaCl (Na) or vehicle (V) solutions (open bars) or histamine receptor antagonist drugs (black bars), including the $H_1$ antagonist dexbrompheniramine (DXB; 1 mg/kg IP, $n = 9$), the $H_2$ antagonist cimetidine (C; 16 mg/kg IP, $n = 8$), the $H_1$ and $H_2$ antagonists dexbrompheniramine (1 mg/kg IP) and cimetidine (16 mg/kg IP) given in combination (DXB + C; $n = 10$), and the $H_3$ antagonist thioperamide (Th; 10 mg/kg SC, $n = 9$). $*p < 0.05$; $**p < 0.005$ vs. paired open bar.

(Fig. 6). The Th alone inhibited drinking elicited by IG hypertonic NaCl by approximately 20% (Fig. 6). When DXB, C, and Th were given together, drinking elicited by IG NaCl was inhibited by approximately 70% (Fig. 5). These findings roughly parallel the findings of Experiment 1 in which it was demonstrated that the effects of DXB, C, and Th were approximately additive in their effects upon drinking elicited by exogenous histamine. These findings reveal a role for endogenous histamine and histamine $H_1$, $H_2$, and $H_3$ receptor subtypes in mediating drinking elicited by the gastrointestinal osmotic consequences of eating, in particular when those consequences are sufficient to increase systemic plasma osmolality at the initiation of drinking.

## GENERAL DISCUSSION

The results of Experiment 1 demonstrate that the ability of exogenous histamine to elicit drinking in the rat depends upon, at least under some experimental conditions, the activation of each of the three identified receptor subtypes for histamine. This finding makes clear that in pharmacological attempts to prevent the dipsogenic action of systemic exogenous or endogenous histamine, those attempts should include manipulations that simultaneously block $H_1$, $H_2$, and $H_3$ receptors. Such combined systemic antagonism of three histamine receptor subtypes can abolish drinking elicited by SC histamine; however, such pharmacological antagonism (using these three drugs) may result in nonspecific inhibition of behavior under some experimental conditions. Apparent nonspecific inhibition of ingestive behavior was evident in Experiment 2: combined systemic antagonism of $H_1$, $H_2$, and $H_3$ receptors inhibited eating as well as drinking behavior, making interpretation of results difficult. Although both eating and drinking were inhibited by combined DXB, C, and Th, the effects upon drinking behavior were more pronounced, as was evident in the significant, approximately 20% inhibition of W/F.

This 20% inhibition of drinking (elicited by eating pellets after 24-h food deprivation) caused by combined systemic antagonism of $H_1$, $H_2$, and $H_3$ receptors is very similar to the inhibition of such drinking caused by combined systemic antagonism of $H_1$ and $H_2$ receptors (10). This suggests that these earlier assessments (10), which predate the discovery of the $H_3$ receptor subtype (2), of the proportion of food-related drinking that is attributable to endogenous histamine were not underestimates of the contribution to food-related drinking for rats eating after food deprivation. It is important to note, however, that the relative contribution of a histaminergic control to food-related drinking may depend upon the conditions. For example, when nondeprived rats eat and drink spontaneously at the beginning of the dark phase, combined systemic $H_1$ and $H_2$ antagonism inhibits food-related drinking by approximately 60% (15).

Earlier study of the contribution of endogenous histamine to food-related drinking found that ICV administration of $H_1$ and/or $H_2$ antagonists fails to reproduce the effects that such drugs have upon food-related drinking when the drugs are given systemically (12). This finding, together with other considerations regarding endogenous histamine's functional roles (e.g., its peripheral vasodilatory and gastric secretory functions) in the neuroendocrine consequences of eating, supports the hypothesis of peripheral endogenous histamine involvement in food-related drinking (11). The finding in Experiment 3, however, that ICV administration of combined $H_1$, $H_2$, and $H_3$ antagonists can decrease W/F in rats eating after food deprivation suggests a role for histamine receptors in the brain. Although such an hypothesis is not unrealistic given the wide distribution of subtypes of receptors for histamine in the rodent brain (19), further work is necessary to examine for the direct involvement of histamine in the brain in food-related drinking.

Among the consequences of eating that are known to be related to drinking behavior is increased systemic plasma osmolality (1,5). Should endogenous histamine mediate an osmotic signal for thirst, as suggested by earlier work (6), then systemic antagonism of $H_1$, $H_2$, and $H_3$ receptors should inhibit drinking elicited by a small gastric osmotic load that could at least partially mimic the gastrointestinal osmotic consequences of eating (7,8) and increase systemic plasma osmolality (14). The results of Experiment 4 demonstrate that endogenous histamine and $H_1$, $H_2$, and $H_3$ receptor subtypes are involved in mediating drinking elicited by gastrointestinal osmotic load, in particular a load that is sufficient to elicit an increase in systemic plasma osmolality at the time that drinking is initiated (14). Further work is required to determine the mechanism(s) by which a gastrointestinal osmotic load indirectly activates peripheral receptors for histamine or the mechanism(s) by which a subsequent increase in systemic plasma osmolality indirectly activates peripheral and/or central receptors for histamine.

In summary, the results of our experiments demonstrate the utility of combined pharmacological antagonism of systemic or brain $H_1$, $H_2$, and $H_3$ receptors for evaluating the dipsogenic effects of exogenous histamine and for assessing a role for endogenous histamine in drinking elicited by eating in rats. Our results extend the evidence supporting a role for endogenous histamine in food-related drinking, including drinking under those conditions in which eating can increase systemic plasma osmolality.

## ACKNOWLEDGEMENTS

This research was supported by a grant from the National Institutes of Health (NS19133) to F.S.K. We thank Dr. Matthew Berent for his assistance.

## REFERENCES


1. Almli, R. C.; Gardina, J. Ad libitum drinking of rats and vascular osmolality changes. Physiol. Behav. 12:1043–1049; 1974.
2. Arrang, J.-M.; Garbarg, M.; Lancelot, J.-C.; Lecomte, J.-M.; Pollard, H.; Robba, M.; Schunack, W.; Schwartz, J.-C. Highly potent and selective ligands for histamine $H_3$-receptors. Nature 327:117–123; 1987.
3. Bowman, W. C.; Rand, M. J. Textbook of pharmacology. 2nd ed. Oxford, England: Blackwell; 1980.
4. Clapham J.; Kilpatrick, G.J. Histamine $H_3$ receptor-mediated modulation of water consumption in the rat. Eur. J. Pharmacol. 232:99–103; 1993.
5. Deaux, E.; Sato, E.; Kakolewski, J. W. Emergence of systemic cues evoking food-associated drinking. Physiol. Behav. 5:1177–1179; 1970.
6. Goldstein, D. J.; Halperin, J. A. Mast cell histamine and cell dehydration thirst. Nature 267:250–252; 1977.
7. Houpt, T. R. Patterns of duodenal osmolality in young pigs fed solid food. Am. J. Physiol. 261:R569–R575; 1991.
8. Jodal, M.; Hallback, D. A.; Lundgren, O. Tissue osmolality in intestinal villi during luminal perfusion with isotonic electrolyte solutions. Acta Physiol. Scand. 102:94–107; 1978.
9. Kraly, F. S. A probe for a histaminergic component of drinking in the rat. Physiol. Behav. 31:229–232; 1983.
10. Kraly, F. S. Histamine plays a part in induction of drinking by food intake. Nature 301:65–66; 1983.
11. Kraly, F. S. Drinking elicited by eating. In: Epstein, A. N.; Morrison, A., eds. Progress in psychobiology and physiological psychology. vol. 14. New York: Academic Press; 1990:67–133.
12. Kraly, F. S.; Arias, R. L. Histamine in brain may have no role for histaminergic control of food-related drinking in the rat. Physiol. Behav. 47:5–9; 1990.
13. Kraly, F. S.; June, K. R. A vagally mediated histaminergic component of food-related drinking in the rat. J. Comp. Physiol. Psychol. 96:89–104; 1982.
14. Kraly, F. S.; Kim, Y.-M.; Dunham, L. M.; Tribuzio, R. A. Drinking after intragastric NaCl without increase in systemic plasma osmolality in rats. Am. J. Physiol. Reg. Integ. Comp. Physiol. (in press).
15. Kraly, F. S.; Specht, S. M. Histamine plays a major role for drinking elicited by spontaneous eating in rats. Physiol. Behav. 33:611–614; 1984.
16. Kraly, F. S.; Tribuzio, R. A.; Kim, Y.-M.; Keefe, M. E.; Finkell, J. Histamine $H_3$ receptors contribute to drinking elicited by eating in rats. Physiol. Behav. 58:1099–1109; 1995.
17. Mann, J. F. E.; Johnson, A. K.; Ganten, D. Plasma angiotensin II: Dipsogenic levels and angiotensin-generating capacity of renin. Am. J. Physiol. 238:R372–R377; 1980.
18. Nose, H.; Morita, M.; Yawata, T.; Morimoto, T. Continuous determination of blood volume on conscious rats during water and food intake. Jpn. J. Physiol. 36:215–218; 1986.
19. Pollard, H.; Bouthenet, M. L. Autoradiographic visualization of the three histamine receptor subtypes in the brain. In: Schwartz, J.-C.; Haas, H. L., eds. The histamine receptor. New York: Wiley-Liss; 1992:179–192.
20. Rowland, N. E.; Caputo, F. A.; Fregly, M. J. Body fluid shifts and elevation in plasma renin activity accompany meal-associated drinking in rats. Fed. Proc. 46:1434; 1987.
21. Schwartz, J. C.; Arrang, J. M.; Garbarg, M.; Pollard, H. A third histamine receptor subtype: Characterisation, localisation and functions of the $H_3$-receptor. Agents Actions 30:13–23; 1990.
22. Takeuchi, K.; Yamakuni, H.; Nobuhara, Y.; Okabe, S. Functional and morphological alterations in the rat stomach following exposure to hypertonic NaCl solution. Jpn. J. Pharmacol. 42: 549–560; 1986.