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Food Intake Suppression by Histidine[1]

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SHEINER, J. B., P. MORRIS AND G. H. ANDERSON. *Food intake suppression by histidine.* PHARMACOL BIOCHEM BEHAV **23**(5) 721–726, 1985.—Following injection of histidine (as l-histidine monohydrochloride, 500 mg/kg, IP) rats showed a suppression of total food intake within the first 2 hours of a 12 hour daily feeding period but not if the rats were adapted to a 4 hour daily feeding period. Furthermore, rats adapted to a nocturnal as compared to a diurnal 12 hour feeding period showed a greater response (50% vs. 20% suppression of feeding) to histidine. Overall, within an experiment, food intake suppression correlated with the histidine dose (0, 125, 250, 375 and 500 mg/kg; for mean response $r(3)=0.90$, $p<0.05$) although the lowest dose measured to be effective in a cross-over design experiment was 375 mg/kg. No differential effect upon protein or carbohydrate intake was observed in any of the studies. The effects of injections of 250 and 500 mg/kg histidine on food intake were associated with significant elevations of brain histidine and histamine. We conclude that histidine, possibly by changes in brain histidine and histamine, influences total food intake but not macronutrient selection.

Histidine Food intake Protein Carbohydrate

DIETARY protein and amino acids are factors influencing the food intake of rats. Deficiencies or excesses of either protein or amino acids, as well as amino acid imbalances, cause food intake suppression [11,15]. Although the mechanisms by which amino acids affect feeding behaviour have not been defined, in recent years considerable interest in the direct effects of amino acids on the nervous system has arisen from the known relationship between certain amino acids and neurotransmitter synthesis. Tryptophan, tyrosine and histidine exert precursor control over the synthesis of 5-hydroxytryptamine, the catecholamines and histamine, respectively [2]. All of these neurotransmitters are putatively involved in food intake regulating mechanisms [15], however, the evidence for histamine involvement is relatively limited compared to that for the catecholamines and 5-hydroxytryptamine [26,31].

Evidence for histamine involvement is twofold. First, the highest concentrations of brain histamine and histidine decarboxylase are found in the hypothalamus [27], a brain structure known to be associated with food intake regulation [17]. Second, changes in brain histidine and histamine are associated with food intake suppression. Decreases in brain histidine by feeding a histidine deficient diet [23,25], or of histamine by injection of a brain histidine decarboxylase inhibitor [19], result in decreased food intake by rats. On the other hand, increases following intracerebral injections of histidine or histamine inhibit feeding in rats [6], and intraventricular injections of histamine inhibit feeding in cats [5].

Of greater physiological significance to the regulation of feeding may be the normal diet-induced variations in histidine availability. Brain histidine concentration is not sufficient to saturate histidine decarboxylase, the enzyme which converts histidine to histamine [27]. Thus histidine administration either intraperitoneally [4,28] or by diet [10] increases brain histidine and/or histamine.

Apart from histamine formation, the other pathways of cerebral histidine metabolism are protein biosynthesis and formation of oligopeptides such as homocarnosine [7]. The exact function of homocarnosine in the brain is not known, however, there are indications that this metabolite has a predominantly inhibitory action on some neurons [30]. Furthermore, in a series of protein malnutrition studies, it has been demonstrated that brain homocarnosine synthesis is responsive to histidine availability [7, 8, 9]. Because of this relationship of histidine availability to homocarnosine and particularly to histamine synthesis, it seemed reasonable to predict that histidine injections would affect feeding behaviour of rats.

Accordingly, the present study examined the effect of histidine administration on both total food intake and macronutrient selection in a self-selection feeding paradigm. It was shown that histidine suppressed total food consumption with equal suppression of protein and carbohydrate intakes. These effects upon feeding were accompanied by changes in brain histidine and histamine. In addition, some of the methodological considerations required in a behavioural assay of this nature are illustrated.

METHOD

Male Wistar rats (Woodlyn Farms, Guelph, Ontario) 90 to 100 grams in weight were randomly assigned to individual wire-meshed galvanized cages. Environmental conditions of light (lights on 0700–1900 hr) and temperature (24±2°C) were controlled and water was supplied ad lib.

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For each of the five feeding experiments conducted the animals were allowed to select from 10% and 60% casein diets for two weeks. Prior to the treatments they were given 10 days to adapt to consuming their daily food intake within 4 hours (for experiment one) or within 12 hours (for experiments two through five.) The rats were then divided into matched groups of similar daily total food intake, protein intake and selected protein concentration. The following day the rats were fasted from the beginning of the light period (0700 hr) until 1845 hr. At this time groups of rats were injected intraperitoneally with either 0.9% saline (0.75 ml/200 g body weight), l-histidine (as monohydrochloride, dissolved in an equivalent volume of 0.9% saline) or an equimolar solution of l-alanine in 0.9% saline. Sixty minutes later the rats had access to the 10% and 60% casein diets and food consumption was measured by weighing the food cups at 2 hours and at the end of the feeding period.

Experiment 1

Total food intake and macronutrient selection during a 4 hour daily feeding period (1900–2300 hr) was measured in two groups of ten rats each. One group was injected with histidine (500 mg/kg body weight), while the other received saline.

Experiment 2

To examine feeding behaviour under conditions which mimicked the animal's normal daily feeding pattern, a second experiment was conducted with three groups of eight rats each. Prior to a 12 hour nocturnal feeding period (1900–0700 hr), one group received 500 mg/kg (2.6 mmol/kg) of histidine, while another group received saline. One additional group was introduced and received alanine (2.6 mmol/kg) prior to feeding, as a control for possible non-specific effects of histidine nitrogen.

Experiment 3

To examine the circadian responsiveness to histidine treatments, the magnitude of the feeding response to 500 mg/kg of histidine was compared in one set of rats adapted to a nocturnal feeding period (1900–0700 hr) and in a second set of rats adapted to a diurnal feeding period (0700–1900 hr). Again saline controls were used and group size was eleven animals in each.

Experiment 4

This experiment examined the effect of graded doses (0, 125, 250, 375 and 500 mg/kg) of histidine on food intake and selection during a 12 hour nocturnal feeding period. On each of the four days of injection, two groups of eleven rats were formed and one group received histidine while the other received alanine. The order of histidine dose administered was from lowest (i.e., 125 mg/kg on the first day of injection) to highest (i.e., 500 mg/kg on the final day of injection). A minimum of three days was allowed between injections; rats were randomized into control and treatment groups for each day of injection.

Experiment 5

Since experiments one through four were carried out using group comparisons, a final experiment was designed to examine the effect of histidine, injected at 375 and 500 mg/kg, on food intake and selection by employing a repeated measures design. On the first treatment day, four rats selected at random, were injected with 500 mg/kg (2.6 mmol/kg) of histidine, and another four with an equimolar alanine solution. On the following day the rats which had previously received a histidine injection were administered alanine and vice versa. A separate group of 8 animals administered 375 mg/kg (2.0 mmol/kg) of histidine or alanine (2.0 mmol/kg), was carried through the same procedure. The experiment lasted six days with each rat receiving a total of three histidine and three alanine injections.

TABLE 1

EFFECT OF HISTIDINE ON 4 HOUR FOOD INTAKE AND SELECTION IN RATS (EXPERIMENT 1)*

Treatment	Food Selected: Total g	Protein† g	CHO‡ g	PC§
		0–2nd hours		
Saline	9.5±0.49¶	2.6±0.29	6.9±0.50	26.2±2.8
Histidine	8.2±0.44	2.3±0.26	5.9±0.31	26.1±2.1
		2nd–4th hours		
Saline	8.0±0.54	1.8±0.22	6.2±0.44	20.9±2.2
Histidine	7.6±0.68	2.1±0.25	5.5±0.55	26.5±2.2
		Total		
Saline	17.6±0.77	4.5±0.49	13.1±0.81	24.0±2.5
Histidine	15.8±0.74	4.4±0.45	11.4±0.79	25.9±1.9

*L-histidine (500 mg/kg) was injected one hour before food was provided.

†Total protein intake (g) = 0.6 × intake of 60% casein diet (g) + 0.1 × intake of 10% casein diet (g).

‡Carbohydrate (CHO)intake (g) is expressed as non-protein intake (g) = total food intake (g) − protein intake (g). Since fat, vitamin and mineral contents are fixed at 0.165 g/g, carbohydrate content is the only variable in, and the major constituent of, the non-protein intake.

§Protein concentration (PC) is the percentage (%) of protein energy in the food selected from the diets.

$$PC = \frac{\text{Protein intake (g)} \times 4 \text{ kcal/g Protein}}{\text{Total food intake (g)} \times 4.25 \text{ kcal/g Food}} \times 100\%$$

¶Mean±SEM, n=10. Histidine and saline groups were not significantly different ($p>0.05$, $df=18$, Student's t-test).

Experiment 6

The last experiment, comprising three groups of fifteen animals injected with saline, 250 or 500 mg/kg histidine, was conducted to determine the effect of histidine injection on brain levels of histidine, homocarnosine and histamine. At various times (0, 0.5, 1.5, 3.5 and 12 hr) during their regular 12 hour nocturnal feeding period, five animals from each of the three groups were sacrificed by decapitation and brains were then removed for assay of histidine, homocarnosine and histamine content.

The basal diet contained cornstarch (83.5%), corn oil (10%), minerals (Teklad Test Diets, TD-67233) (4%) and vitamins (2.5%) [20]. To obtain the desired protein concen-

TABLE 2

EFFECT OF HISTIDINE ON 12 HOUR FOOD INTAKE AND SELECTION IN RATS (EXPERIMENT 2)*

Treatment	Food Selected Total g	Protein g	CHO g	PC
		0–2nd hours		
Saline	4.9±0.44†	2.5±0.33	2.4±0.22	45.9±5.1
Alanine	4.5±0.47	2.3±0.29	2.3±0.36	47.9±4.0
Histidine	2.5±0.56‡	1.3±0.22‡	1.2±0.17‡	49.0±6.5
		3rd–12th hours		
Saline	11.1±0.78	5.1±0.48	6.0±0.86	44.4±4.5
Alanine	11.8±0.77	5.9±0.75	6.0±0.88	47.0±5.3
Histidine	13.0±1.30	4.7±0.44	8.3±1.10	34.6±3.2
		Total		
Saline	16.1±0.71	7.6±0.72	8.4±0.89	44.9±4.4
Alanine	16.4±0.74	8.1±0.88	8.3±1.10	47.2±4.9
Histidine	15.5±1.70	6.1±0.58	9.5±1.50	40.0±3.7

*L-histidine (500 mg/kg) was injected one hour before food was provided.

†Mean±SEM, n=8.

‡Histidine group significantly different from alanine or saline groups ($p<0.05$; ANOVA followed by Duncans test).

Abbreviations as given in Table 1.

TABLE 3

CIRCADIAN EFFECTS ON FOOD INTAKE AND SELECTION IN RATS FOLLOWING HISTIDINE ADMINISTRATION (EXPERIMENT 3)*

Treatment	Food Selected Total g	Protein g	CHO g	PC
		0–2nd hours		
Dark				
Saline	5.1±0.55†	2.6±0.34	2.5±0.30	49.0±2.9
Histidine	2.7±0.37¶	1.3±0.23¶	1.3±0.18¶	44.5±3.3
Light				
Saline	5.6±0.40	3.1±0.23	2.6±0.17	50.9±1.6
Histidine	4.5±0.30‡	2.4±0.21‡	2.1±0.15‡	49.0±2.7
		3rd–12th hours		
Dark				
Saline	13.5±0.89	7.3±0.56	6.2±0.46	50.3±2.1
Histidine	13.7±0.85	6.9±0.47	6.7±0.58	48.0±1.9
Light				
Saline	8.2±0.69	4.2±0.29	4.0±0.52	49.1±2.6
Histidine	8.3±0.59	4.2±0.37	4.1±0.40	47.8±3.1

*L-histidine (500 mg/kg) was injected one hour prior to the onset of the light (0700–1900 hr) and dark (1900–0700 hr) feeding periods.

†Mean±SEM, n=11.

‡Histidine group significantly different from saline group ($p<0.05$, Student's t-test).

¶Histidine group significantly different from saline group ($p<0.01$, Student's t-test).

Abbreviations as given in Table 1.

tration of the 10% and 60% casein diets, casein was substituted at the expense of cornstarch. All diets were isocaloric containing 4.25 kcal/g, based on the Atwater factors of 4, 9, 4 kcal/g for carbohydrate, fat and protein, respectively.

For brain histidine and homocarnosine analysis the following procedure was followed. Brain halves were weighed and homogenized in 2 vol (wv) of 0.2 M sucrose. The homogenate was treated with 15% sulphosalicylic acid to a final concentration of 7.5% and centrifuged (at 4°C, 20,000 g) for 15 minutes. The protein-free supernatant was diluted with 0.2 M sodium citrate buffer (pH 2.2) and histidine and homocarnosine concentrations were determined on 300 μl of the supernatant containing 50 mg of brain tissue, using a Beckman Spinco Automated Analyzer, Model 116/119 (Beckman Instruments Co., Palo Alto, CA as described by Enwonwu and Worthington [7].

Brain histamine was assayed flourometrically after its isolation by ion-exchange chromatography, according to the method of Lewis *et al.* [12] with the following modification. For the NaCl elution step, the columns were eluted with five 5 ml aliquots of 0.3 M NaCl instead of 0.2 M NaCl/pH 6.5. This elution procedure was found to increase recovery of histamine from the column. Mean recovery was 66±3%. The values reported have been corrected for these recoveries. Brain halves were randomly assigned to histidine or histamine determinations so that each assay had equal numbers of right and left hemispheres.

Statistical analysis was by analysis of variance followed by Duncan's new multiple-range test, by repeated measures analysis, and by Student's t-test [29,32]. In all analyses, 0.05 was taken as the acceptable level of statistical significance.

RESULTS

Experiment 1

Histidine (500 mg/kg), compared to saline injection, had no effect on total food, protein or carbohydrate intakes at any time within the 4 hour nocturnal feeding period (Table 1).

Experiment 2

During the first 2 hours of a 12 hour nocturnal feeding period, total food, protein and carbohydrate intakes of the histidine-treated (500 mg/kg) animals were reduced 50% below those of the saline-treated controls (Table 2: total $F(2,21)=9.68$, $p<0.01$, protein $F(2,21)=4.74$, $p<0.05$, CHO $F(2,21)=5.20$, $p<0.05$). Selected dietary protein concentration was not significantly affected. There were no significant differences in the feeding parameters measured between rats injected with saline or alanine. For the remainder of the feeding period (3–12 hr), the histidine treated rats tended to consume more food than either the saline or alanine treated rats (Table 2). Thus mean daily total food intake, protein intake and protein concentration were similar for the three groups.

Experiment 3

Confirming the observations of experiment two, histidine (500 mg/kg) administered to rats adapted to a 12 hour nocturnal feeding period suppressed total food, protein and carbohydrate intakes by 50% within the first 2 hours of feeding (Table 3: total ($t=3.64$), protein ($t=3.12$), CHO ($t=3.19$); all

TABLE 4

HISTIDINE DOSE ON FOOD INTAKE AND SELECTION IN RATS, GROUP COMPARISONS (EXPERIMENT 4)*

Dose (mg/kg histidine)	Food Selected Total g	Protein g	CHO g	PC
0	3.3±0.37†	1.4±0.22	1.9±0.36	43.0±5.7
125	3.5±0.63	1.5±0.29	2.0±0.44	39.5±6.0
0	4.1±0.67	1.4±0.20	2.7±0.66	40.1±5.7
250	3.2±0.59	1.3±0.34	1.9±0.39	34.7±6.4
0	4.5±0.79	1.2±0.31	3.3±0.70	28.3±5.8
375	2.9±0.52	0.9±0.24	2.0±0.45	24.8±6.0
0	3.4±0.64	1.0±0.21	2.4±0.32	32.0±6.9
500	1.2±0.52‡	0.4±0.17‡	0.8±0.25‡	33.0±7.5

*L-histidine (125, 250, 375 and 500 mg/kg) was injected one hour before food was provided.

†Mean±SEM, n=11.

‡Histidine group significantly different from saline group ($p<0.05$, Student's t-test).

Abbreviations as given in Table 1.

$p<0.01$, $df=20$). However, the same dose of histidine administered to rats adapted to a 12 hour diurnal feeding period was less effective, with only a 20% reduction in total food, protein and carbohydrate consumption occurring (Table 3: total ($t=2.50$), protein ($t=2.20$), CHO ($t=2.10$); all $p<0.05$, $df=20$). In both feeding periods, selected protein concentration was not affected by histidine administration, indicating that macronutrient preference was not altered, consistent with the results of the second experiment.

Experiment 4

Histidine administered at 500 mg/kg again uniformly reduced total food, protein and carbohydrate intakes with no change in selected protein concentration after two hours of feeding (Table 4: total ($t=2.74$), protein ($t=2.48$), CHO ($t=2.96$); all $p<0.05$, $df=20$). Lower doses did not appear to be effective, however, food intake suppression (percent) correlated strongly with the dose of histidine administered (for mean response, $r(3)=0.90$, $p<0.05$). Food consumption during the remainder of the feeding period (3–12 hr) was not different at any dose level between the histidine-injected rats and their saline-injected controls (data not shown).

Experiment 5

Evidence for food intake suppression by injections of histidine below 500 mg/kg was confirmed in this repeated measures design. In addition, further analysis of the data revealed that this inhibition was restricted to the first hour of feeding (Table 5). During this period histidine treatments of 375 and 500 mg/kg uniformly suppressed total food, protein and carbohydrate consumption by approximately 28% and 65%, respectively. (for 1st hour 375 and 500 mg/kg, respectively: total ($F(1,7)=8.44$ and 33.60), protein ($F(1,7)=6.11$ and 24.60), CHO ($F(1,7)=6.06$ and 30.60); $p<0.05$ and $p<0.01$, respectively). A less effective but still significant suppression was sustained after 2 hours of feeding, when total food,

TABLE 5

EFFECT OF HISTIDINE ON FIRST 2 HOUR FOOD INTAKE AND SELECTION IN RATS, REPEATED MEASURES DESIGN (EXPERIMENT 5)*

Treatment	Food Selected Total g	Protein g	CHO g	PC
		1st hour		
Alanine	4.1±0.55†	1.6±0.25	2.4±0.41	37.3±4.0
Histidine (375 mg/kg)	2.9±0.57‡	1.1±0.19‡	1.8±0.43‡	35.5±4.3
Alanine	4.4±0.72	2.4±0.46	2.1±0.28	46.5±4.3
Histidine (500 mg/kg)	1.6±0.49§	0.9±0.29§	0.8±0.21§	39.3±6.4
		2nd hour		
Alanine	2.3±0.41	0.8±0.13	1.5±0.29	31.4±2.8
Histidine (375 mg/kg)	2.1±0.37	0.7±0.15	1.4±0.28	25.5±3.9
Alanine	2.1±0.26	0.9±0.10	1.2±0.23	35.9±5.0
Histidine (500 mg/kg)	2.0±0.26	0.9±0.16	1.1±0.16	35.2±5.5
		0–2nd hour		
Alanine	6.3±0.68	2.4±0.31	3.9±0.49	36.0±3.6
Histidine (375 mg/kg)	5.0±0.57‡	1.8±0.23‡	3.2±0.45‡	34.3±4.1
Alanine	6.6±0.82	3.2±0.51	3.4±0.39	45.6±3.0
Histidine (500 mg/kg)	3.6±0.46§	1.8±0.30§	1.8±0.21§	43.1±5.2

*L-histidine (375 and 500 mg/kg) was injected one hour before food was provided. Treatments were repeated on every second day for six consecutive days.

†Mean±SEM, n=8.

‡Histidine group significantly different from alanine group ($p<0.05$, ANOVA, repeated measures).

§Histidine group significantly different from alanine group ($p<0.01$, ANOVA, repeated measures).

Abbreviations as given in Table 1.

protein and carbohydrate intakes were reduced by 20% and 45% following injections of 375 and 500 mg/kg, respectively. (for 0-2nd hour 375 and 500 mg/kg, respectively: total ($F(1,7)=10.12$ and 38.70), protein ($F(1,7)=7.98$ and 30.70), CHO ($F(1,7)=8.36$ and 32.10); $p<0.05$ and $p<0.01$, respectively). As noted in previous experiments, selected protein concentration was not affected by histidine administration.

Experiment 6

One hour after injections of histidine, i.e., at the onset of feeding, brain histidine was significantly increased (Table 6: $F(2,12)=34.01$, $p<0.01$). After injections of 250 and 500 mg/kg, brain concentrations were roughly four and nine times, respectively, the control value. The concentrations then declined rapidly, but significant elevations were sustained for the first 30 minutes of feeding after injections of 250 mg/kg, $F(2,12)=12.36$, $p=0.01$, and for 1.5 hours of feed-

TABLE 6

TIME RESPONSE OF BRAIN HISTIDINE AND HISTAMINE CONCENTRATIONS TO GRADED DOSES OF HISTIDINE (EXPERIMENT 6)*

	Hours of Feeding				
Dose (mg/kg histidine)	0†	0.5	1.5	3.5	12
Brain Histidine (nmoles/g)					
0	62.4± 7.4‡§	55.7± 5.0§	50.9± 7.6§	41.5± 3.5	53.3±5.5
250	246.5±42.0¶	152.3±20.2¶	77.0±16.5§	61.0± 9.8	47.8±4.7
500	563.1±61.8**	216.5±33.9¶	180.6±26.3¶	88.4± 7.2	44.0±2.3
Brain Histamine (ng/g)					
0	34.4± 1.5§	37.0± 3.4§	42.9± 4.2§	42.5± 4.5	44.1±4.8
250	52.1± 3.8¶	63.3± 6.9¶	66.6± 6.1§	55.2±10.4	39.4±4.0
500	82.3± 9.1**	95.0±20.6¶	106.8± 6.3¶	52.7± 5.2	50.0±7.0

*Effect of graded doses of l-histidine on brain histidine and histamine concentrations at various times throughout the feeding period.

†0 hr=1 hr after histidine injection.

‡Mean±SEM, n=5. Means with different superscripts are significantly different ($p<0.05$; ANOVA followed by Duncans test.)

ing following injections of 500 mg/kg, $F(2,12)=13.77$, $p<0.01$.

Subsequent to histidine injections of 500 mg/kg, brain histamine was significantly increased for approximately 2 hours into feeding (Table 6). At this dose the concentration steadily increased from the onset until 1.5 hours of feeding, at which time the level was roughly two and one half times the control value. Histidine administered at 250 mg/kg caused statistically significant brain histamine elevations at the onset and at 0.5 hours of feeding (0 hr: $F(2,12)=17.63$, $p<0.01$; 0.5 hr: $F(2,12)=4.57$, $p<0.05$; 1.5 hr: $F(2,12)=23.95$, $p<0.01$).

Brain homocarnosine was not affected by histidine injections at any dose level. The brain homocarnosine levels (calculated as the average value over all the time points, mean±SEM) were 58.2±2.7, 60.1±2.3 and 58.5±3.9 nmoles/g for the saline, 250 mg/kg and 500 mg/kg injected rats, respectively.

DISCUSSION

The results of the present experiments show that food intake by rats was decreased, but food selection was not altered, by histidine injections. Under conditions of the experimental design, this anorexia was brought about by 375 mg/kg of histidine, approximately one-third the normal daily intake of rats [21], and also exhibited circadian rhythmicity. These effects upon feeding were accompanied by changes in brain histidine and histamine.

In this study consumption of protein and carbohydrate, as well as total food, was measured for two reasons. First, the intake of these macronutrients is regulated by animals provided food choices [20]. Second, stimulation of neurochemical systems may result in the adjustment of food choice, of total food intake, or both [1,15]. For example, dietary treatments including carbohydrate or tryptophan which increase brain 5-hydroxytryptamine turnover, or the injection of serotonin agonists [16], result in the rat decreasing carbohydrate consumption relative to protein in the next meal. In contrast to the effect of histidine observed here, tryptophan alone in small amounts (15 mg/kg) does not affect food intake in the next hour of feeding, but decreases the proportion of carbohydrate consumed relative to protein [16].

Experimental design was an important determinant of the observed behavioural response to histidine administration. For example, histidine suppressed food intake by rats allowed access to food over a 12 hour feeding period, but no such effects were detected in the intakes of rats restricted to a 4 hour feeding period. This decreased sensitivity of behavioural assays in which animals have access to food for only brief periods of time may be explained by such factors as abnormal animal behaviour [3] and modified brain neurotransmission [24]. Thus use of a feeding paradigm in which the rat's normal feeding pattern received only a minimal disruption [13,14] appears to constitute a more sensitive approach to the detection of changes in feeding behaviour, at least after amino acid supplementation. Likewise, the choice of experimental design was important in reducing experimental error to maximize the opportunity of identifying treatment effects. The more refined repeated measures design, as opposed to a group comparison design, enabled us to establish that 375 mg/kg of histidine affected food intake. Whether or not a lower dose of histidine is effective has not been determined by this design.

Another important aspect of the experimental design was the length of the time interval between injection and food introduction. One hour was selected as the interval between histidine injection and food access. This interval was based on the observation that 60 minutes after intraperitoneal administration of 500 mg/kg histidine to rats, hypothalamic histidine levels are maximal and brain histamine concentration rises steadily until peaking at three hours following injection [28]. Thus it was assumed that any alteration of feeding behaviour induced by histidine would be maximized within the time frame spanning the start of the feeding period (60 min post injection) to the end of the first two hours of feeding (3 hr post injection). Confirmation of this assumption was pro-

vided by the suppressed feeding response to histidine injections and the occurrence of elevated brain histidine and histamine with the same temporal relationship just described. However it is conceivable that the magnitude of the anorexic response to histidine administration may be more readily seen with smaller doses by measuring food intake after just 30 minutes or 1 hour of feeding and/or by decreasing the interval between histidine injections and food access. The dramatic effects of 375 mg/kg histidine in the first hour of feeding (Table 5) illustrates this point.

The enhanced anorexic action of histidine observed during the night may be explained by the association of three factors related to circadian variation in brain histamine. First, during a 12 hour light-dark cycle rat hypothalamic histamine levels are maximal during the light period (presumably because of minimal release) and reach minimum levels during the dark period (presumably because of maximal release) [22]. Second, in rats and mice hypothalamic histamine concentration and motor activity are inversely related [18]. Third, an increased rate of synthesis of brain histamine during a period of increased motor activity has been shown in mice injected with histidine [26]. Therefore one would expect a greater response to histidine administration during the night (a period of increased motor activity) because of a higher rate of conversion of histidine to histamine and maximal histamine release.

Although the histidine induced changes observed in feeding behavior, brain histidine, and brain histamine are consistent with previous suggestions of a possible role for brain histamine in food intake regulation [5,15], the specific mechanisms involved are not known at this time. Nevertheless, it is possible to conclude from the present study that histidine influences the total amount of food consumed, but not protein and carbohydrate selection.

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