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Effects of essential amino acids on food and water intake of rats

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This study examined the effects of selected groups of essential amino acids (EAAs), given by gavage, on short-term food and water intake. Amino acid groups were selected on the basis of their common physiologic functions in relation to current hypotheses on the role of amino acids in food intake control, and the quantities given were based on the proportions in 1.5 g of the EAA content of albumin. The complete EAA mixture (1.5 g) suppressed food intake by an average of 60 and 37% during the 1st and 2nd h of feeding, respectively, but had no influence on feeding in the subsequent 12 h. Total daily (14 h) intake was decreased by 9%. With the exception of the aromatic amino acid (Phe + Tyr + Trp, 0.34 g) group, all groups significantly decreased food intake by a comparable magnitude (32%) during the 1st h. In this time period, rats given the EAAs, Arg + Met + Val (0.38 g), and Arg + His + Lys (0.44 g) mixtures increased their water intake, whereas intake by rats given the Phe + Tyr + Trp + Thr (0.46 g) and Ile + Leu + Val (0.45 g) mixtures was unchanged. Thus, the food intake suppression caused by EAAs was not accounted for by an equal effect of its component amino acid groups. As well, food intake suppression by amino acid groups was not explained by increased water consumption, nor was it simply related to the quantity of nitrogen provided by the treatment.

Key words: food intake, water intake, essential amino acids.

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On a examiné les effets de groupes sélectionnés d'acides aminés essentiels (AAE), administrés par gavage, sur l'absorption à court terme d'eau et de nourriture. Les groupes d'acides aminés retenus l'ont été d'après leurs fonctions physiologiques communes, compte tenu de l'hypothèse actuelle sur le rôle des acides aminés dans le contrôle de l'absorption de nourriture, et administrés à raison de 1,5 g de la teneur en AAE de l'albumine. Le mélange complet d'AAE (1,5 g) a supprimé l'absorption de nourriture de 60 et 37% en moyenne durant la 1re et la 2e h de prise alimentaire, respectivement, mais il n'a pas eu d'influence sur cette dernière dans les 12 h subséquentes. L'absorption quotidienne totale (14 h) a été diminuée de 9%. À l'exception du groupe des acides aminés aromatiques (Phe + Tyr + Trp, 0,34 g), tous les groupes ont diminué significativement et de manière comparable (32%) l'absorption de nourriture durant la 1re h. Pendant ce temps, l'absorption d'eau des rats ayant reçu les mélanges d'AAE, Arg + Met + Val (0,38 g) et Arg + His + Lys (0,44 g), a augmenté, alors que celle des rats ayant reçu les mélanges Phe + Tyr + Trp + Thr (0,46 g) et Ile + Leu + Val (0,45 g) est demeurée inchangée. Ainsi, la suppression de l'absorption de nourriture par les AAE reflète celle de ses groupes constitutifs. De même, la suppression de l'absorption de nourriture par les divers groupes d'acides aminés ne peut être expliquée par une plus grande absorption d'eau ni simplement reliée à la quantité d'azote fournie par le traitement.

Mots clés : absorption de nourriture, absorption d'eau, acides aminés essentiels.

[Traduit par la Rédaction]

Introduction

The appetite-suppressive effect of protein is well recognized. However, the exact role of its constituent amino acids in food intake regulation is poorly defined. Both the essential amino acid (EAA) and nonessential amino acid (NEAA) components of albumin account for its food intake suppressive effect (Anderson et al. 1994a). In a detailed investigation of the NEAAs, food intake after amino acid preloads was observed to be strongly and negatively associated with the quantity administered (Anderson et al. 1994b). No particular combination or specific NEAA appeared to exert an effect out of line with this general association.

However, the EAAs have been the most frequently cited and investigated component of protein-induced satiety (Anderson 1988; Li and Anderson 1983). A number of specific hypotheses have been advanced to explain the relationship between EAA intake and feeding behaviour. Considerable recent research has been based on the hypothesis that the aromatic amino acids (AAAs) tyrosine (Tyr), phenylalanine (Phe), and tryptophan (Trp) are more important than others in mediating appetite suppression because they may exert precursor control over neurotransmitter systems known to be involved in food intake regulation (Anderson 1988; Li and Anderson 1983). In addition, increases in plasma EAA concentration are thought to provide signals to the central nervous system (Li and Anderson 1983; Peng et al. 1969). Of these, the branched-chain amino acids (BCAAs) valine (Val), isoleucine (Ile), and leucine (Leu) show the most sustained elevation when rats are maintained on high-protein diets (Anderson et al. 1990; Johnson and Anderson 1982; Glanville and Anderson 1985), whereas arginine (Arg), Val, and methionine (Met) increase most sharply after a meal (Anderson et al. 1994a). Urea and ammonia are also putative signals for food intake control mechanisms (Harper et al. 1956). Thus, it might be predicted that the basic amino acids, because of their high nitrogen content, involvement in the urea cycle (Arg), or role as a precursor to histamine (histidine, His), could provide important appetite signals reflecting protein ingestion.

Traditionally, the effect of dietary protein on food intake is thought to arise from activation of satiety mechanisms. However, the effect of amino acids on food intake may in part be explained by their effect on water intake. Water intake increases after rats consume high-protein diets or are given an acute protein load (Geary 1979; Harri and Brockway 1985), perhaps as

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a result of demands created by amino acids on the regulation of body fluid balance (Hamilton 1973). Protein consumption increases glomerular filtration rate and effective renal plasma flow (Woods et al. 1993). As well, hypertonic solutions can shift water balance and increase water consumption of rats (Booth 1972). Because increased water intake can be expected to reduce stomach capacity, it may be that food intake is limited for this reason. In addition, expansion of the stomach and duodenum contributes to the development of short-term satiety by activating stretch and tension receptors (Geary 1979; Nicholl et al. 1985).

The purpose of this study was to examine the effect of selected groups of EAAs, given in physiologic amounts by gavage, on both short-term food and water intake in rats. The amino acid groups were selected on the basis of their common physiologic functions in relation to current theories on the role of amino acids in food intake control. Four primary groups of amino acids were examined: (i) AAAs; (ii) Arg + Met + Val; (iii) BCAAs; and (iv) basic amino acids.

Materials and methods

Animals

Male Wistar rats (Charles River Farms, St-Constant, Que.) were used for all experiments. Animals were housed individually upon arrival, in suspended wire-mesh stainless-steel cages, in a room with a 12 h light : 12 h dark cycle (lights on at 06:00). The room temperature was maintained at $22 \pm 1°C$. Diet was presented at the onset of the dark period (18:00), and the rats had access to the food cups until 08:00, after which the food was removed. Water was available ad libitum from spouts connected to an automated watering system. In the experiments where water intake was measured, the automated watering system was disconnected and water was supplied from calibrated glass bottles marked to 0.5 mL, which were attached to the front of cages with metal springs. Body masses of rats averaged 150 ± 10 g when they were placed on the laboratory diet and 250 ± 20 g at the beginning of experiments. This study was approved by the University of Toronto Animal Care Committee.

Diet

Rats were fed a 25% protein diet, which provided 4.05 kcal/g (1 cal = 4.1868 J) (Anderson et al. 1994a, 1994b). The individual ingredients of the diet were as follows (per kilogram diet): high protein casein (the casein was 87% protein by nitrogen determination) (287.5 g), cornstarch (515.5 g), corn oil (100 g, 111 mL), cellulose (50 g), choline bitartrate (2 g), mineral mixture AIN-76 (35 g), and vitamin mixture AIN-76A (10 g). Cornstarch and corn oil were obtained from a local supplier (Christian Brothers Restaurant Supplies, Toronto, Ont.). Other ingredients were obtained from Teklad Test Diets (Madison, Wis.). The diet was presented in 250-mL glass food cups (7.6 cm high) equipped with a stainless-steel screen insert and spill-proof lid (4.5-cm opening).

Procedures

Upon arrival, rats were allowed to adapt to the new environment, the experimental diet, and feeding schedule for 8–10 days. In this time period, rats were given water (5 mL/rat) by gavage at least three times to allow them to become accustomed to the treatment procedures involved in amino acid administration. Prior to experimentation, a 2-day adaptation test was conducted to determine whether the gavage procedure affected normal feeding. Rats were divided into two groups, with only one group receiving the water gavage (5 mL) on each day. Rats that received gavage on day 1 received nothing on day 2 and vice versa. Food was presented to all rats 0.5 h after gavage (18:00). Food consumption adjusted for spillage was measured to the nearest 0.1 g after 1, 2, and 14 h. The experiments began when mean food intake of the rats did not differ between the 2 days. Rats that consistently consumed less than 2 g during the first 2 h of feeding

were excluded from experiments to avoid difficulties in detecting reductions of food intake. Food intake measurements were conducted under red light to maintain the light–dark cycles.

Design

Treatments given to rats included whole protein (chicken egg albumin, Sigma Chemical Co., St. Louis, Mo.) or groups of EAAs (formulated on the basis of the amino acid profile of albumin). The composition of the 1.5-g EAA mixture based on the EAA profile of albumin was Arg, 148 mg; cystine (Cys), 71 mg; His, 50 mg; Ile, 113 mg; Leu, 179 mg; lysine (Lys), 246 mg; Met, 73 mg; Phe, 164 mg; threonine (Thr), 124 mg; Trp, 39 mg; Tyr, 134 mg; and Val, 156 mg. Since growing rats were used in all experiments, both conditionally indispensable amino acids (Cys and Tyr) were included. L-Isomers of the purified amino acids were purchased from Sigma Chemical Co. L-Lysine was given in the hydrochloride form. Albumin or the amino acid mixtures were dissolved in distilled water so that the gavaged volume was the same for all treatments (5 mL/rat). The protein or amino acid suspensions were stirred continuously with a magnetic stirrer, quickly taken up by syringe, and administered immediately to the rats. The rats were hand held and did not require any restraint during gavage.

The effects of each treatment on food intake were tested using a crossover design, described as follows. On the 1st day, one half of the rats were gavaged with a protein or amino acid solution, and the remaining rats received distilled water of equal volume. All rats received no gavage on the following day, but on the 3rd day, the treatment order was reversed so that rats previously receiving amino acids were given water and vice versa. Thus, the effect of each treatment was always compared with that after water, using each rat as its own control. Rats were gavaged at 17:30 and food cups were made available at 18:00. When water consumption was a measured parameter, water bottles were removed before gavage and returned to the cages at 18:00. Water intake was measured at 1, 2, and 24 h.

Experiment 1

To confirm previous observations (Anderson et al. 1994a) and to establish a reference point in this series of experiments, the effect of chicken egg albumin, or the EAA components of albumin, on food intake of rats was determined. Twenty-two rats were randomized into two groups. One group received 1.5 g albumin protein and the other, 1.5 g of EAA.

Experiment 2

The effect of amino acids that serve as precursors of neurotransmitters known to play a role in feeding mechanisms was examined. The neutral amino acids Phe, Tyr, Trp, and Thr (Li and Anderson 1983) were included in this study, and food and water intake were measured. First (experiment 2a), the four amino acids were given as a group (0.46 g), and their impact on feeding was compared with that of 1.5 g EAA, which was given as a treatment to a separate group of rats. Then, only Phe + Tyr + Trp (0.34 g) were tested as a group (experiments 2b and 2c). Finally, the impact of deleting these three AAAs from the original EAA mixture was determined (experiment 2d). Rats were tested with either the complete EAA mixture (1.5 g) or with the EAA mixture devoid of Phe + Tyr + Trp (1.16 g).

Experiment 3

The effect of a mixture of three amino acids, Arg, Met, and Val (0.38 g), which showed the largest increases in their plasma concentrations after rats were gavaged with 1.5 g of a mixture of EAA (Anderson et al. 1994a), on food and water intake of rats was determined. Food intake was quantitated after rats were given the mixture (0.38 g) or water in experiment 3a. This was repeated with water intake as an additional parameter in experiment 3b.

Experiment 4

The effects of either branched-chain or basic amino acids on food and water intake were investigated. In experiment 4a, rats were given a BCAA mixture (0.45 g) containing Ile, Leu, and Val or a mixture (0.44 g) of Arg, His, and Lys as treatment by gavage. Experiment

TABLE 1. Experiment 1. Effect of egg albumin and EAA mixture on food intake

Treatment	Food intake (g)		
	0–1 h	1–2 h	2–14 h
Albumin (1.5 g)			
Control	3.7±0.3	2.2±0.3	18.9±0.6
Albumin	2.3±0.3	0.9±0.2	16.7±0.3
MDS[a]	−1.4±0.2**	−1.3±0.3*	−2.2±0.8*
EAA (1.5 g)			
Control	3.7±0.3	2.6±0.2	19.3±0.9
EAA	1.3±0.2	1.7±0.3	20.1±0.7
MDS[a]	−2.4±0.2**	−0.9±0.2*	0.8±0.4

NOTE: Values are mean ± SEM, $n = 11$. *$p < 0.05$; **$p < 0.01$.
[a]MDS, mean difference score (food intake after treatment minus food intake after control).

TABLE 3. Experiment 2a. EAAs, neurotransmitter precursor amino acids, and water intake

Treatment	Water intake (mL)		
	0–1 h	1–2 h	2–24 h
EAA (1.5 g)			
Control	1.1±0.4	2.1±0.4	27.0±1.4
EAA	3.0±0.3	1.9±0.7	22.4±1.4
MDS[a]	1.9±0.4**	−0.2±0.7	−4.6±2.1*
Phe + Tyr + Trp + Thr (0.46 g)			
Control	0.6±0.2	1.7±0.3	27.5±1.7
Phe + Tyr + Trp + Thr	1.0±0.2	0.9±0.2	24.3±1.4
MDS[a]	0.4±0.2	−0.8±0.4	−3.2±1.8

NOTE: Values are mean ± SEM, $n = 13$. *$p < 0.05$; **$p < 0.01$.
[a]MDS, mean difference score (water intake after treatment minus water intake after control).

TABLE 2. Experiment 2a. EAAs, neurotransmitter precursor amino acids, and food intake

Treatment	Food intake (g)		
	0–1 h	1–2 h	2–14 h
EAA (1.5 g)			
Control	3.3±0.3	2.3±0.3	17.2±0.9
EAA	1.0±0.2	1.5±0.2	19.0±0.7
MDS[a]	−2.3±0.2**	−0.8±0.3*	1.8±0.6*
Phe + Tyr + Trp + Thr (0.46 g)			
Control	3.0±0.3	2.4±0.4	17.1±0.7
Phe + Tyr + Trp + Thr	2.2±0.3	2.5±0.4	17.0±0.6
MDS[a]	−0.8±0.3*	0.1±0.5	−0.1±0.4

NOTE: Values are mean ± SEM, $n = 13$. *$p < 0.05$; **$p < 0.01$.
[a]MDS, mean difference score (food intake after treatment minus food intake after control).

TABLE 4. Experiment 2. AAAs and food intake

Treatment	Food intake (g)		
	0–1 h	1–2 h	2–14 h
Experiment 2b			
Control	4.1±0.4	2.1±0.2	16.7±0.6
Phe + Tyr + Trp	4.0±0.5	2.2±0.3	16.3±0.5
MDS[a]	−0.1±0.4	0.1±0.5	−0.4±0.6
Experiment 2c			
Control	4.2±0.4	2.7±0.4	17.3±0.9
Phe + Tyr + Trp	4.3±0.3	2.6±0.4	17.4±1.3
MDS[a]	0.1±0.5	−0.1±0.4	0.1±0.6

NOTE: Phe, Tyr, and Trp (0.34 g) was given in a 5-mL suspension. Values are mean ± SEM, $n = 13$.
[a]MDS, mean difference score (food intake after treatment minus food intake after control).

4b was conducted to confirm the observed effect of BCAA and basic amino acids on food intake, to compare their effects with that of the complete EAA mixture, and to determine their effects on water consumption. The treatments were EAA (1.5 g), Ile + Leu + Val (0.45 g), or Arg + His + Lys (0.44 g).

Experiment 5

Since only the basic amino acid group consistently both suppressed food intake and increased water intake, individual basic amino acids were tested to further determine whether the effects were attributable generally to all basic amino acids and whether food suppression was always in conjunction with an increased water intake. Rats were given either Lys (0.25 g), Arg (0.15 g), or His (0.10 g) as treatment. Their food intake and water intake were measured. To further determine if food intake suppression after basic amino acids was attributable to Lys, a fourth group was included that received His + Arg (0.2 g).

Statistical analysis

Results are expressed as mean ± standard error of the mean ($x ±$ SEM). Treatment effects were expressed as mean difference scores (intake after treatment minus intake after control). As each rat served as its own control, statistical evaluation of the differences in food intake was performed by paired t test. All statistical analyses were performed by SAS Institute Software (Cary, N.C.) on an IBM compatible system. Effects of treatments were considered statistically significant at $p < 0.05$.

Results

Experiment 1. Protein, EAAs, and food intake

Albumin protein (1.5 g) significantly suppressed food intake of rats during the 1st h, the 2nd h, and the remaining 12 h of feeding (Table 1). Compared with the water control treatment, food intake was reduced by 1.4 g or 38% ($p < 0.01$), 1.3 g or 59% ($p < 0.05$), and 2.2 g or 12% ($p < 0.05$), respectively. Over the entire 14-h period, food intake was reduced by 4.9 g or 20% ($p < 0.01$). EAAs (1.5 g) significantly depressed food intake by 2.4 g or 65% during the 1st h and by 0.9 g or 35% during the 2nd h of feeding (Table 1). During the remaining 12 h, food intake was not affected. Over the entire feeding period (0–14 h), food intake was reduced by 2.5 g or 10% ($p < 0.01$).

Experiment 2. Neurotransmitter precursor amino acids, and food and water intake

Effects of the four neutral amino acids (experiment 2a) given as a group on food and water intake are given in Tables 2 and 3, respectively. Consistent with the results of experiment 1, 1st-h food intake was reduced by 70% ($p < 0.01$) after rats were given the complete EAA mixture. The magnitude of decrease was much less (0.8 g or 27%) when rats were given Phe + Tyr + Trp + Thr ($p < 0.05$). In addition, the

TABLE 5. Experiment 2c. AAAs and water intake

Treatment	Water intake (mL)		
	0−1 h	1−2 h	2−24 h
Control	1.5±0.5	1.8±0.4	26.3±1.1
Phe + Tyr + Trp	2.1±0.5	1.2±0.2	27.7±1.4
MDS[a]	0.6±0.7	−0.6±0.4	1.4±1.1

NOTE: Phe, Tyr, and Trp (0.34 g) was given in a 5-mL suspension. Values are mean ± SEM, $n = 13$.

[a]MDS, mean difference score (water intake after treatment minus water intake after control).

TABLE 6. Experiment 2d. Effect of EAAs minus AAAs on food intake

Treatment	Food intake (g)		
	0−1 h	1−2 h	2−14 h
	EAA (1.5 g)		
Control	3.0±0.3	1.8±0.4	17.5±0.8
EAA	1.0±0.3	1.0±0.2	17.6±0.6
MDS[a]	−2.0±0.2**	−0.8±0.2*	0.1±0.6
	EAA − AAA (1.16 g)		
Control	3.3±0.5	1.9±0.3	18.6±0.7
EAA − AAA	1.1±0.2	1.2±0.2	19.2±0.8
MDS[a]	−2.2±0.4**	−0.7±0.4	0.6±0.1

NOTE: Values are mean ± SEM, $n = 10$. *$p < 0.05$; **$p < 0.01$.

[a]MDS, mean difference score (food intake after treatment minus food intake after control).

TABLE 7. Experiment 3. Plasma amino acid responders and food intake

Treatment	Food intake (g)		
	0−1 h	1−2 h	2−14 h
	Experiment 3a		
Control	3.6±0.3	2.6±0.4	16.0±0.5
Arg + Met + Val	2.4±0.3	2.4±0.3	16.8±0.9
MDS[a]	−1.2±0.3*	−0.2±0.4	0.8±0.8
	Experiment 3b		
Control	4.0±0.3	3.6±0.4	17.1±0.9
Arg + Met + Val	2.4±0.3	3.1±0.4	17.5±0.4
MDS[a]	−1.6±0.3*	−0.5±0.5	0.4±0.8

NOTE: Arg, Met, and Val (0.38 g) was given in a 5-mL suspension. Values are mean ± SEM, $n = 13$. *$p < 0.01$.

[a]MDS, mean difference score (food intake after treatment minus food intake after control).

appetite-suppressing effect of the precursor amino acids mixture was not sustained in the 2nd h of feeding (Table 2).

Water intake after the EAA mixture was increased significantly (173%) in the 1st h (Table 3). In contrast, intake remained stable after Phe + Tyr + Trp + Thr. Interestingly, rats significantly decreased their water intake during 2−24 h after EAA treatment.

The three AAAs (0.34 g) as a group (experiments 2b and 2c) did not affect either food or water intake (Tables 4 and 5). Their absence in the EAA mixture did not alter its strong food intake suppression (experiment 2d) (Table 6). First-hour food

TABLE 8. Experiment 3c. Plasma amino acid responders and water intake

Treatment	Water intake (mL)		
	0−1 h	1−2 h	2−24 h
Control	1.4±0.4	1.8±0.5	27.8±1.0
Arg + Met + Val	2.1±0.3	1.5±0.3	27.0±1.5
MDS[a]	0.7±0.3*	−0.3±0.5	−0.8±1.4

NOTE: Arg, Met, and Val (0.38 g) was given in a 5-mL suspension. Values are mean ± SEM, $n = 13$. *$p < 0.05$.

[a]MDS, mean difference score (water intake after treatment minus water intake after control).

TABLE 9. Experiment 4a. Effect of branched-chain and basic amino acids on food intake

Treatment	Food intake (g)		
	0−1 h	1−2 h	2−14 h
	BCAAs (0.45 g)		
Control	3.6±0.3	2.5±0.3	16.1±0.5
Ile + Leu + Val	2.5±0.3	2.8±0.3	17.6±0.6
MDS[a]	−1.1±0.5*	0.3±0.4	1.5±0.5*
	Arg + His + Lys (0.44 g)		
Control	3.0±0.3	2.4±0.4	14.7±0.5
Arg + His + Lys	2.2±0.3	2.5±0.4	16.9±0.4
MDS[a]	−0.8±0.3**	0.1±0.5	2.2±0.7*

NOTE: Values are mean ± SEM, $n = 13$. *$p < 0.05$; **$p < 0.01$.

[a]MDS, mean difference score (food intake after treatment minus food intake after control).

intake was equally reduced by the complete EAA mixture and by the mixture without the AAAs. Although suppression in food intake in the 2nd h was statistically significant only after 1.5 g of EAA, no difference in food intake was found during the last 12 h of feeding.

Experiment 3. Large plasma responders

The Arg + Met + Val combination suppressed only 1st-h feeding ($p < 0.01$, Table 7). In both experiments (3a and 3b), the magnitude of reduction in food intake was also comparable, ranging between 1.2 and 1.6 g (33−40%). Measurement of water intake (experiment 3c) revealed that the three amino acids when given together caused a transient 50% (1st h only) increase in drinking ($p < 0.05$, Table 8).

Experiment 4. Branched chain amino acids

The BCAAs (experiment 4a) suppressed food intake of rats during the 1st h of feeding by 1.1 g or 31% ($p < 0.05$, Table 9). No difference in food intake was observed during the 2nd h. During the last 12 h, there was a rebound in food intake (an increase of 1.5 g or 9.3%, $p < 0.05$). A very similar change in the temporal feeding pattern was observed after rats were given a mixture containing three basic amino acids. First-hour feeding was reduced by 27% ($p < 0.01$) and a significant rebound occurred in the last 12 h of feeding (15% increase).

In experiment 4b, the BCAAs and basic amino acids also caused comparable reductions (34 and 32%, respectively) in 1st-h food intake. However, there was no rebound on food intake in the 2- to 14-h period (Table 10).

TABLE 10. Experiment 4b. Effect of branched-chain and basic amino acids on food intake

Treatment	Food intake (g)		
	0−1 h	1−2 h	2−14 h
EAA (1.5 g)			
Control	3.9±0.4	1.8±0.5	17.6±0.5
EAA	2.3±0.3	1.2±0.2	19.0±0.5
MDS[a]	−1.6±0.4**	−0.6±0.6	1.4±0.8
BCAA (0.45 g)			
Control	4.7±0.5	1.7±0.4	16.7±0.9
Ile + Leu + Val	3.1±0.4	2.5±0.3	17.3±0.8
MDS[a]	−1.6±0.3**	0.8±0.3	0.6±0.4
Arg + His + Lys (0.44 g)			
Control	4.1±0.7	1.9±0.7	18.4±0.7
Arg + His + Lys	2.8±0.4	2.2±0.3	18.7±0.7
MDS[a]	−1.3±0.5*	0.3±0.4	0.3±0.6

NOTE: Values are mean ± SEM, $n = 10$. *$p < 0.05$; **$p < 0.01$.
[a]MDS, mean difference score (food intake after treatment minus food intake after control).

TABLE 11. Experiment 4b. Effect of branched-chain and basic amino acids on water intake

Treatment	Water intake (mL)		
	0−1 h	1−2 h	2−24 h
EAA (1.5 g)			
Control	2.6±0.4	1.4±0.3	25.8±1.4
EAA	4.8±0.7	1.2±0.2	24.1±1.8
MDS[a]	2.2±0.7*	−0.2±0.3	−1.7±1.5
BCAA (0.45 g)			
Control	2.4±0.4	1.6±0.3	23.9±2.0
Ile + Leu + Val	1.7±0.3	2.2±0.3	23.7±2.1
MDS[a]	−0.7±0.6	0.6±0.5	−0.2±1.4
Arg + His + Lys (0.44 g)			
Control	2.2±0.5	1.5±0.2	24.6±1.0
Arg + His + Lys	5.1±0.7	1.7±0.3	22.1±2.1
MDS[a]	2.9±1.0*	0.2±0.5	−2.5±1.1*

NOTE: Values are mean ± SEM, $n = 10$. *$p < 0.05$.
[a]MDS, mean difference score (water intake after treatment minus water intake after control).

Rats given the complete EAA mixture and the basic amino acids mixture increased water intake during the 1st h by 2.2 mL (85%) and 2.9 mL (132%), respectively. Water intake of the Arg + His + Lys group significantly decreased (10%) during the last 22 h (Table 11).

Experiment 5. Individual basic amino acids

When tested individually, only one of the three basic amino acids affected feeding. In the 1st h of feeding, Lys but not Arg or His significantly suppressed food intake (22%, $p < 0.05$; Table 12). Furthermore, Arg and His given together had no significant effect on food intake at any time period.

Water intake was significantly affected only when rats were given treatments containing His. When given alone, His increased water intake in the 1st h of feeding (40%, $p < 0.05$; Table 13). Arg and His when given together increased water

TABLE 12. Experiment 5. Individual basic amino acids and food intake

Treatment	Food intake (g)		
	0−1 h	1−2 h	2−14 h
Control	5.8±0.5	3.2±0.4	18.0±1.0
Lys (0.25 g)	4.5±0.4	3.9±0.5	18.5±1.0
MDS[a]	−1.3±0.7*	0.7±0.5	0.5±0.6
Control	2.8±0.3	1.8±0.3	19.8±0.6
Arg (0.15 g)	2.7±0.3	1.9±0.3	20.4±0.4
MDS[a]	−0.1±0.4	0.1±0.5	0.6±0.4
Control	3.7±0.3	2.6±0.2	18.7±0.5
His (0.1 g)	3.8±0.4	3.0±0.6	18.1±0.7
MDS[a]	0.1±0.3	0.4±0.5	−0.6±0.4
Control	5.7±0.6	2.6±0.4	18.7±0.8
Arg + His (0.2 g)	5.1±0.5	2.9±0.4	18.4±1.2
MDS[a]	−0.6±0.6	0.3±0.5	−0.3±0.7

NOTE: Values are mean ± SEM, $n = 10$. *$p < 0.05$.
[a]MDS, mean difference score (food intake after treatment minus food intake after control).

TABLE 13. Experiment 5. Individual basic amino acids and water intake

Treatment	Water intake (mL)		
	0−1 h	1−2 h	2−24 h
Control	2.2±0.2	2.0±0.3	29.3±1.6
Lys (0.25 g)	2.8±0.4	1.9±0.3	26.2±2.0
MDS[a]	0.6±0.4	−0.1±0.4	−3.1±1.7
Control	1.7±0.3	1.2±0.3	27.9±0.9
Arg (0.15 g)	2.4±0.3	0.8±0.2	27.4±1.1
MDS[a]	0.7±0.4	−0.4±0.3	−0.5±0.6
Control	2.0±0.4	2.0±0.2	26.1±0.9
His (0.1 g)	2.8±0.4	2.0±0.2	25.5±1.1
MDS[a]	0.8±0.3*	0.0±0.2	−0.6±0.7
Control	1.9±0.4	2.2±0.3	29.4±1.3
Arg + His (0.2 g)	3.2±0.5	1.2±0.2	28.4±1.6
MDS[a]	1.3±0.4*	−1.0±0.2*	−1.0±1.3

NOTE: Values are mean ± SEM, $n = 10$. *$p < 0.05$.
[a]MDS, mean difference score (water intake after treatment minus water intake after control).

intake in the same time period by 68% ($p < 0.05$) but decreased water intake by 45% in the 2nd h of feeding.

Discussion

The present study suggests that the depression in food intake brought about by a protein meal is caused by the EAAs other than the aromatics. With the exception of the AAA subgroup, all amino acid groups tested caused significant food intake suppression of comparable magnitude during the 1st h of feeding. Feeding suppression was not secondary to increased water intake, since food intake suppression after amino acid treatments did not occur exclusively in conjunction with an increase in water intake.

As previously observed (Anderson 1988; Anderson et al. 1994a, 1994b), amino acid loads reduced food intake by an amount greater than what could be accounted for by their energy content. In these studies, the 1.5-g amino acid loads were in a physiological range, equivalent to approximately 25% of the

total amino acids that the rats would normally consume daily from the 25% casein diet, or the equivalent nitrogen load in only 6 g of food, the amount a rat eats in the first 2 h of the night feeding. The quantity for separate EAA groups was about 0.45 g, which is equivalent to what the rat would consume if it ate 1.8 g of the habitual diet. The reduction in total energy intake in the 1st h was generally 2- to 3-fold greater than could be accounted for by the energy content of the preload.

That AAAs did not affect food intake was surprising in view of the current hypothesis regarding the role of precursor amino acids in food intake regulatory mechanisms (Li and Anderson 1983). According to the precursor hypothesis, provision of Phe, Tyr, and Trp will enhance the synthesis of brain catecholamines and serotonin, which are monoamines known to be involved in food intake control (Anderson 1988; Baile 1974). The results of this study do not indicate that the AAAs given by gavage play a role in the satiety response induced by EAA mixtures, and thus do not provide evidence that diet-induced fluctuations in availability of these amino acids account for the satiety effect of protein. It could be argued that the quantities of amino acids administered in the present studies were not sufficient to increase their concentrations in brain. However, the aromatics were given in a dose of 1.3 g/kg body mass, which is an amount comparable with that in previous studies in which significant increases in brain amino acid concentrations were observed when the treatment failed to alter food intake of rats (Bialik et al. 1989; Ng and Anderson 1992). Perhaps the lack of effect of the AAAs may be explained by the route of administration. The hypothesized effects of precursor amino acids on food intake have been based primarily on studies in which the precursor amino acids were administered intraperitoneally (Fernstrom 1983; Li and Anderson 1983; Anderson et al. 1988). Phenylalanine injected intraperitoneally at only 60–100 mg/kg body mass decreased food intake by 30–40% during the next 2 h of feeding, but phenylalanine given intragastrically in amounts up to 720 mg/kg body mass had no effect (Bialik et al. 1989). Similarly, Trp and Tyr given intraperitoneally at 100 mg/kg body mass suppressed food intake by 33–45% over a 2-h feeding period. When given intragastrically at this dose, neither Trp nor Tyr affected food intake (Ng and Anderson 1992).

The BCAAs were grouped together on the basis of the hypothesis that they cause feeding responses after protein ingestion (Anderson et al. 1990). Their levels in plasma and brain are proportional to dietary protein content (Anderson et al. 1990; Glanville and Anderson 1985; Johnson and Anderson 1982; Peters and Harper 1981), they are energy substrates for the brain (Brosnan et al. 1985), and food intake suppression after a high protein diet is associated with their accumulation in plasma (Anderson et al. 1994a). The fact that BCAAs significantly suppressed food intake also suggests that a role for the neurotransmitter 5-hydroxytryptamine (5-HT) in the feeding response to EAA loads seems unlikely. Based on amino acid uptake mechanisms at the blood–brain barrier, BCAAs are predicted to decrease brain 5-HT concentration by competing for brain uptake with Trp. Thus, Trp availability to the brain is reduced, and brain 5-HT concentration is reduced (Li and Anderson 1983), rather than increased, which is associated with the inhibition of feeding (Luo and Li 1990).

Another mechanism by which protein ingestion is hypothesized to suppress food intake is based on the ammonia and urea produced by the rapid catabolism of amino acids that are consumed in excess of requirements (Harper et al. 1956; Katuhiko 1975). Therefore, in the present study, three basic amino acids were grouped together because of their high nitrogen content and thus potential role in the production of ammonia. Although His is a basic amino acid as well as the precursor of the neurotransmitter histamine, His was included in this group rather than in the precursor amino acid group in order to bring the total load of the basic group (0.44 g) to match that of the BCAA (0.45 g) and precursor (0.46 g) groups. Total nitrogen content was doubled in the basic amino acids group (0.11 g) compared with BCAA (0.05 g) and precursor (0.04 g) groups. Since this group did not cause larger reductions in food intake than the BCAA group, it seems unlikely that the feeding response was related to the total nitrogen provided by the treatment. In support of this argument are the data showing that Lys, but not Arg, suppressed food intake (Table 12) while both treatments had similar nitrogen content (0.04 g).

A previous study that attempted to elucidate the role of individual NEAAs on food intake found a strong inverse correlation between the amount of nitrogen in the treament and food intake in the 1st h after treatment (Anderson et al. 1994b). There was little evidence that any one NEAA or group of NEAAs accounted in any disproportionate way to this general association. In contrast, the present study failed to describe a statistically significant correlation ($r = 0.52$, ns) between nitrogen content of the individual treatment groups and food intake. Thus it may be that nitrogen content is not necessarily a primary determinant of the effect of EAAs on food intake and that their impact on food intake regulatory systems is different from the NEAAs.

Elevated plasma and brain amino acid concentrations have been hypothesized to play a role in appetite control (Li and Anderson 1983; Peng et al. 1969; Peng and Harper 1970). Therefore, in experiment 3, the Arg, Met, and Val combination was selected on the basis that these three amino acids exhibited the largest concentration increase in plasma 60 min after rats were gavaged with a 1.5-g mixture of EAAs (Anderson et al. 1994a). Although this combination significantly suppressed 1st-h feeding, its effect was no stronger than that induced by the administration of the BCAA group or the basic amino acid group. Thus, an increase in plasma concentration does not appear to be a good predictor of the potency of amino acids on short-term food intake.

In the present studies, no attempt was made to control for osmotic effects of the intragastric loads. Thus, it could be argued that short-term satiety and food intake suppression was caused by gastric distention with activation of gastrointestinal mechano- or osmo-receptors (Houpt et al. 1979; Pappas et al. 1989). All of the amino acid solutions used were hypertonic, and their osmolarity was proportional to the amount of amino acid present, since the volume was held constant at 5 mL. However, results arising from the present studies as well as that of others (Anderson et al. 1994a) suggest that appetite suppression seen after the gavage of a solution containing three or four amino acids is unlikely the result of osmotic inhibition. For instance, the BCAA group and the basic amino acid group produced a comparable degree of food intake suppression in the 1st h of feeding, even though the solution containing the BCAA was more hypertonic than the one containing the basic amino acids (715 vs. 572 mosmol/L). As well, the solution containing the three AAAs was hypertonic (385 mosmol/L) but did not influence food intake. The dissociation between tonicity and food intake is further supported by the findings that
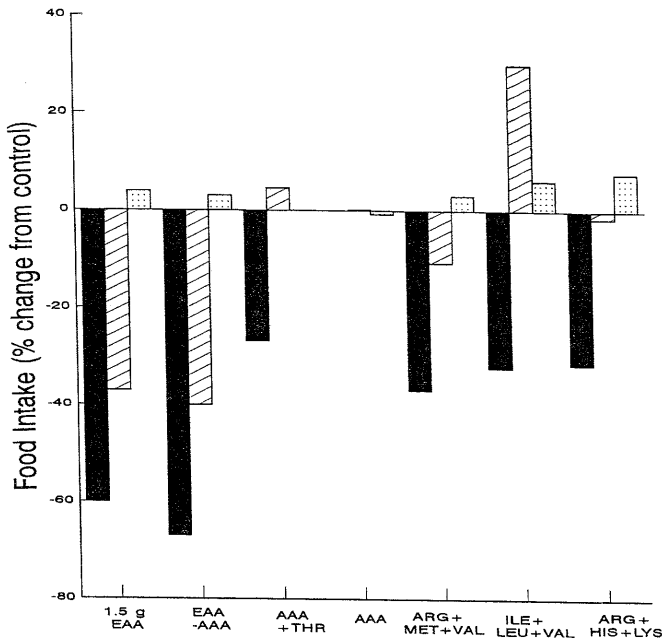
FIG. 1. Effects of EAAs on food intake of rats. Response is shown as the mean result of reported experiments. AAAs, 0.34 g; EAA – AAA, 1.16 g; AAA+Thr, 0.46 g; Arg+Met+Val, 0.38 g; Ile+Leu+Val, 0.45 g; Arg+His+Lys, 0.44 g. Food intake was measured during 0–1 h (closed bars), 1–2 h (hatched bars), and 2–14 h (stippled bars).

the Lys solution (270 mosmol/L), which was isotonic, significantly decreased food intake. Furthermore, we have found that a sodium chloride solution with an osmolarity of 650 mosmol/L did not affect food intake in the 1st h of feeding, and that the decrease in food intake after a glucose solution with an osmolarity of 1300 mosmol/L was comparable with that achieved after the gavage of an isotonic (280 mosmol/L) glucose solution (E.T.S. Li, unpublished data). It is also relevant to note that the effect of hypertonic solutions on food intake is very short term. Booth (1972) concluded that feeding in the first 30 min after gavage of a hypertonic load was inhibited, but noted that subsequent food intake suppression was derived from that part of the load that has already been absorbed, rather than being due to the osmotic load in the gut. In the present study, food and water intake were measured beginning 30 min after the preload was administered.

Over the range of treatment doses used in these studies, a decrease in food intake was not simply related to the quantity of amino acid given by gavage, suggesting that composition of the mixture was an important factor. This is evident from results of experiment 2 (Table 6). One group contained the complete EAA mixture in 1.5 g and the other contained a mixture of EAA without the AAAs in 1.16 g. Despite a different amount (0.34 g) being given, the degree of food intake suppression observed was comparable between these two groups, suggesting that not all amino acids are required to induce the maximum suppression of food intake in the 1st h and also strengthening the argument that the AAAs have little effect on short-term food intake. Further evidence of the importance of composition can be obtained by comparing the feeding response after treatment with the Phe + Tyr + Trp and Arg + Met + Val groups. The quantity of amino acid given was identical, yet the latter suppressed food intake by 50% of that observed
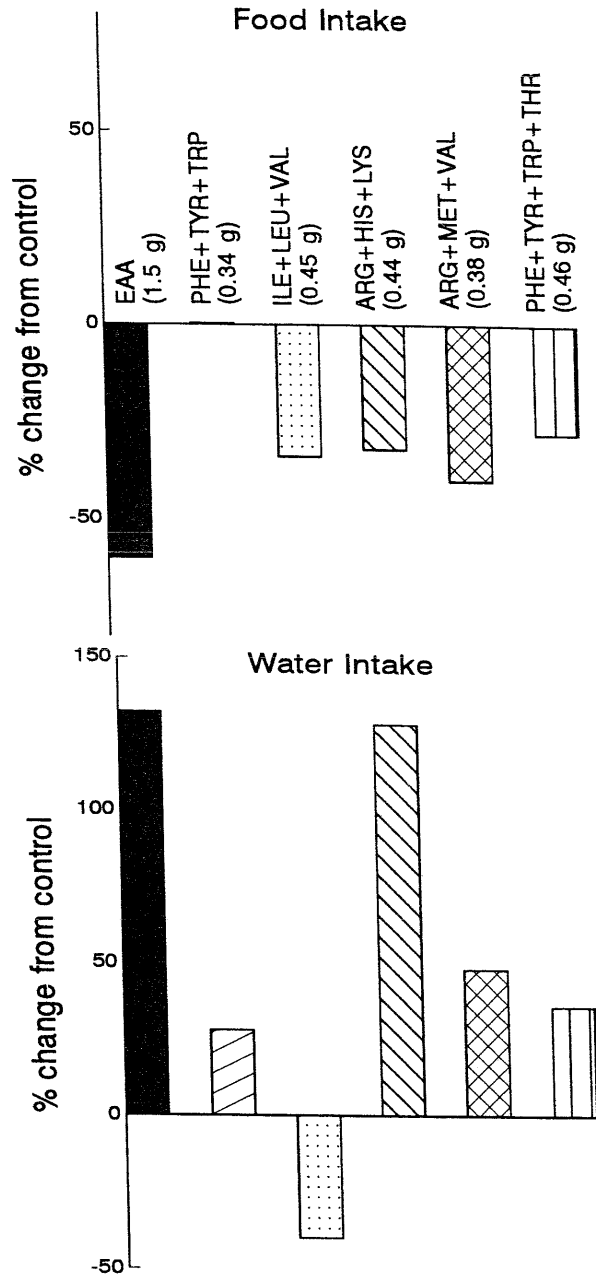

FIG. 2. Summary of the effects of EAAs on food and water intake during the 1st h of feeding. Results shown are the means of reported experiments.

after the complete EAA mixture, while the AAA group had no effect (Fig. 1).

No consistent effects of amino acid treatments on water intake were observed, nor were changes in water intake necessarily accompanied by changes in food intake (Fig. 2). The basic amino acid group (0.44 g) increased water consumption to a magnitude that was comparable with that seen after the 1.5-g EAA mixture (129 vs. 130%). However, both the four precursor amino acids and the BCAA groups suppressed food intake without affecting water consumption. The data in this study provide some evidence that the basic amino acids may be the major activator of increased water intake occurring after rats are given protein or amino acid mixtures by gavage. Although basic amino acids as a group decreased food intake

accompanied by increased water intake, the study on the individual basic amino acids revealed that an increase in water intake occurs after Arg and His but not after Lys administration. In contrast, only Lys but not Arg and His affected food intake. Together these data suggest that food intake suppression after EAA treatment is unlikely to be caused by increased water intake.

In summary, food intake suppression occurring after rats were gavaged with an EAA mixture was not accounted for by any one of the selected amino acid groups. Differences observed in feeding behaviour after treatment with the selected groups were not readily explained by increased water intake, by nitrogen content, or by osmolarity of the amino acid loads. Furthermore, the results do not support the hypothesis that those EAAs that serve as precursors for neurotransmitters known to be involved in food intake regulation are of greater influence on food intake than those with no known neurochemical linkage.

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