Pharmacology Biochemistry & Behavior, Vol. 28, pp. 525–528. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

BRIEF COMMUNICATION

Acceptability by Rats of Aqueous Solutions of Amino Acid Analogues

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Received 4 May 1987

TEWS, J. K., J. J. REPA AND A. E. HARPER. *Acceptability by rats of aqueous solutions of amino acid analogues.* PHARMACOL BIOCHEM BEHAV **28**(4) 525–528, 1987.—Preferences differed widely when rats were offered choices between water and solutions of various natural amino acids and structurally related analogues. They avoided the branched-chain amino acid valine but preferred solutions of its isomer norvaline and of norleucine. The hydrochloride forms of ornithine and arginine were preferred to water at concentrations up to about 100 mM and avoided at 410 mM; homoarginine·HCl was never preferred and was avoided at 39 and 78 mM. Rats were indifferent to taurine and β-alanine at most concentrations but refused these amino acids at high concentrations (205 and 410 mM, respectively). In conjunction with earlier observations on feeding behavior in response to dietary additions of amino acids, the results show that selections by rats between water and amino acid solutions cannot be used to predict choices among amino acid-containing diets.

Amino acid analogues Branched-chain amino acids Cationic amino acids Choice Drinking Rats Taste

SOME of our recent work has involved the use of a variety of amino acid analogues in experiments designed to study the effects of such agents on feeding behavior and tissue amino acid patterns [5, 8, 9] and on transport of physiologically important amino acids from the blood into the brain [6,7]. When added to diets containing an appropriate mixture of indispensable and dispensable amino acids, these analogues often cause marked reductions in consumption of the diet. However, in diet choice studies acceptance or avoidance of the amino acid-containing diets may depend on the alternate food which is also available.

Previous reports have shown that rats will select between water and solutions of amino acids [2,13], and that preference or avoidance can occur at greatly different concentrations of the various amino acids. Therefore, in order to determine if the taste of a given amino acid may be important in determing food intake or selection of diets containing that amino acid, we have identified the concentrations at which rats prefer or avoid drinking solutions of several amino acids. These included valine and the branched-chain amino acid isomers, norvaline and norleucine; the cationic amino acids ornithine, arginine and homoarginine (each as the hydrochloride); and taurine and β-alanine. The results, along with earlier observations, imply that avoidance or selection of a given amino acid-supplemented diet cannot be predicted solely on the basis of taste preferences for that amino acid in solution.

METHOD

Adult male rats of the Sprague-Dawley strain (7 rats per group, initially weighing 343±2 g) were fed a stock diet ad lib and were offered choices between water and increasing concentrations of the tested amino acids [2,13]. Each rat had access for 24 hr to 2 bottles, one containing tap water and the other an aqueous solution of the amino acid (L-form where pertinent). The control rats received water in both bottles, with one being designated as the "experimental" bottle. Tests were run for 6 days after which the concentrations of amino acids were increased 2- or 2.5-fold and the tests repeated. The results thus represent 42 selections for each concentration. In order to avoid influence from preferences by rats for drinking from bottles at a particular location [13], the bottles containing the amino acids or the "experimental" water were placed an equal number of times during each test in each of the two possible positions on the cages. Fresh solutions (room temperature) were prepared daily, either by diluting pre-measured, frozen concentrated solutions, or by dissolving an appropriate weight of the crystalline amino acid in tap water. Daily intakes were determined by weighing the bottles at the beginning and end of each 24 hr period;

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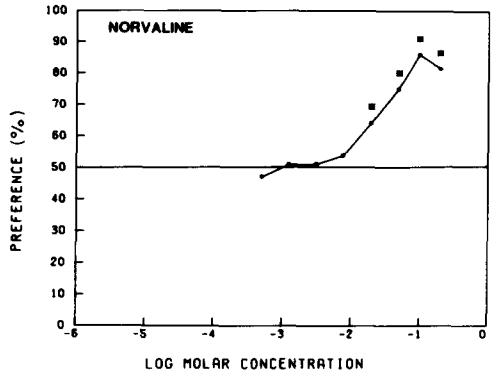
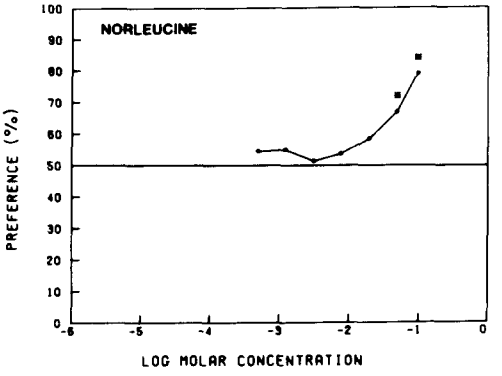
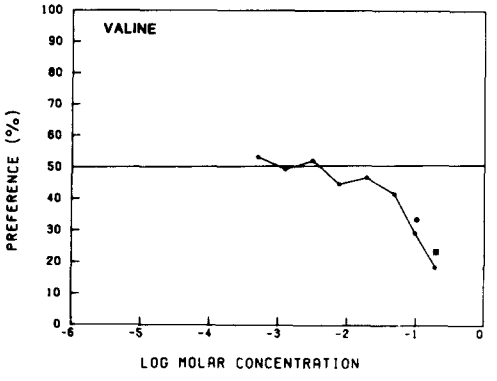
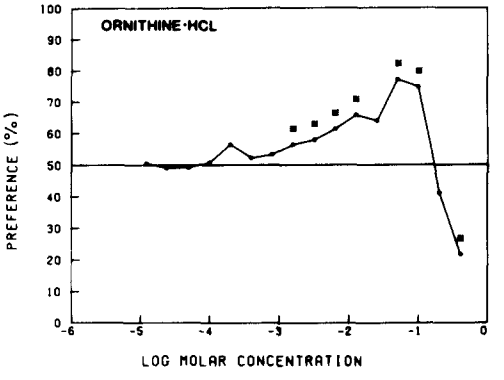
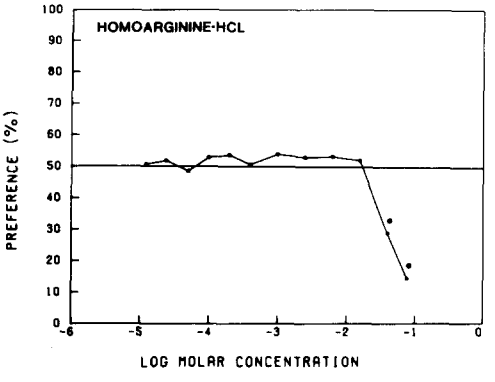
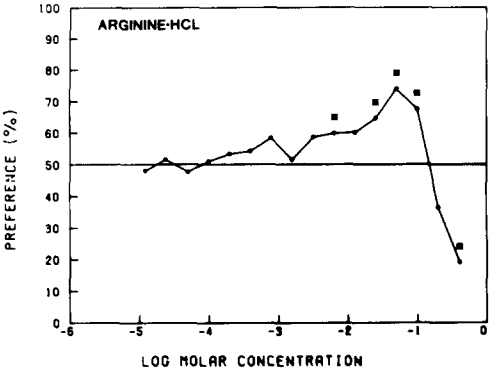

NORVALINE
PREFERENCE (%)
LOG MOLAR CONCENTRATION

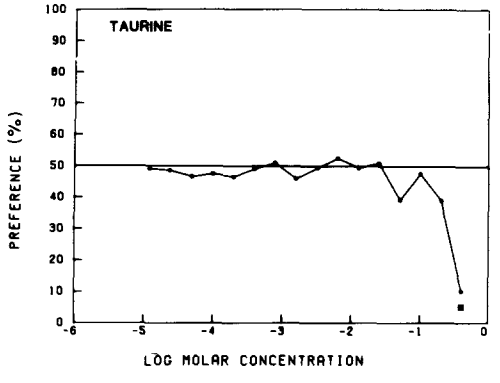

NORLEUCINE
PREFERENCE (%)
LOG MOLAR CONCENTRATION

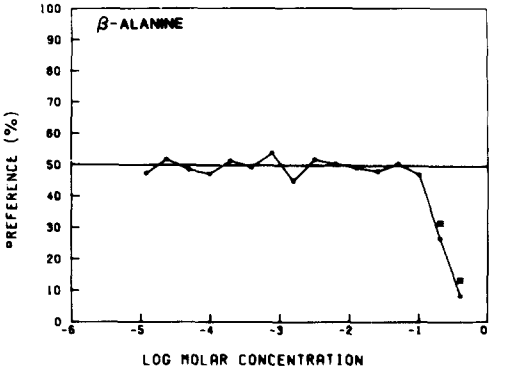

VALINE
PREFERENCE (%)
LOG MOLAR CONCENTRATION


ORNITHINE·HCL
PREFERENCE (%)
LOG MOLAR CONCENTRATION


HOMOARGININE·HCL
PREFERENCE (%)
LOG MOLAR CONCENTRATION


ARGININE·HCL
PREFERENCE (%)
LOG MOLAR CONCENTRATION


TAURINE
PREFERENCE (%)
LOG MOLAR CONCENTRATION


β-ALANINE
PREFERENCE (%)
LOG MOLAR CONCENTRATION

corrections for minor losses due to dripping and evaporation were made after weighing similar bottles which were inaccessible to the rats. Bottles were washed daily.

Statistical Analysis

Duncan's multiple range test was performed with SAS computer programs [3] in order to identify the concentrations of amino acids at which selections by the rats differed from those of water.

RESULTS

Acceptability by rats of the different amino acids differed widely with concentration. They preferred both norvaline and norleucine to water at and above concentrations of 19.5 and 49 mM, respectively (Fig. 1). At 98 and 196 mM the rats consumed 86% and 82%, respectively, of their total fluid from the norvaline solution; 79% of total intake was from the 98 mM solution of norleucine. Higher concentrations of these amino acids could not be tested because of poor solubility. Valine, the naturally occurring, branched-chain isomer of norvaline, was never preferred and at concentrations of 98 and 196 mM, respectively, the rats drank only 29% and 18% of their total intake from that amino acid solution.

The rats showed a significant preference for ornithine·HCl starting at the low concentration of 1.6 mM, and they drank at least 75% of their total intake from the amino acid solution at 51 and 102 mM. However, at higher concentrations the rats no longer preferred solutions of this amino acid; at 410 mM they consumed only 22% of their fluid from the ornithine solution. Consumption of arginine·HCl showed a similar pattern of preferences (60% of total intake at 6.4 mM and 74% at 51 mM), followed by avoidance at the highest concentration which was tested (19% of total intake at 410 mM). There was again a contrast between the rats' acceptance of these natural basic amino acids and their behavior towards homoarginine·HCl. The rats never preferred solutions of this analogue and clearly avoided it at the highest concentrations which were tested (39 and 78 mM).

Preferences for taurine and β-alanine, amino acids having certain structural similarities to GABA, were also determined. Neither of these amino acids was preferred during any choice period and only at the relatively high concentration of 410 mM was taurine significantly avoided (10% of total intake); β-alanine was avoided at 205 and 410 mM (26% and 8% of total intake, respectively).

DISCUSSION

One of the most obvious findings in these and earlier studies of taste perception [2,13] is that rats often respond differently to isomeric forms of amino acids. Thus, behavior toward valine and norvaline differed as did that toward GABA, α-amino-n-butyric acid and α-aminoisobutyric acid in our earlier study [13]. The difference between norvaline and valine is not necessarily due to the branched structure of valine, as rats avoided isoleucine but not leucine [2]. We found that they preferred the isomer norleucine at least as much as did other rats selecting leucine [2]. As another example, alanine was strongly preferred over a wide range of concentrations [2] while its isomer β-alanine was either without effect or was avoided. As with GABA [13], rats were indifferent to β-alanine and taurine at all but the highest concentrations which were tested. Solutions of lysine·HCl were never preferred [2] while the homologous ornithine was chosen over a 60-fold range of concentrations. There was a similar difference in preferences for the arginine and homoarginine homologues.

Another consistent observation has been that preference or avoidance of an amino acid solution is not a guide to the feeding behavior of rats consuming a diet containing the same amino acid in crystalline form. However, a complicating factor may be that the sensory qualities of crystalline and dissolved amino acids are not necessarily identical, especially when diets may contain other amino acids. Rats offered a choice between a non-protein diet and one containing an amino acid mixture including homoarginine will select the latter diet [11]; this choice occurs despite the fact that rats avoid drinking 39 and 78 mM solutions of homoarginine·HCl, concentrations below that present in the diet (1.92%, equivalent to 85 mM). However, other rats will avoid diets containing norleucine at concentrations (85 mM) which the rats clearly prefer in aqueous solutions. These contrasting dietary choices were obtained with rats fed diets containing unusual amino acid mixtures. The results may be related less to taste than to the inability to produce severe depressions in food intake and growth of rats by adding homoarginine to a diet low in lysine (lysine imbalance) [9], versus the ease with which these effects are produced by feeding a diet low in valine and containing added amounts of the large neutral amino acid analogue norleucine (valine imbalance) [5]. These imbalanced diets accentuate the physiological effects of low intake of the indispensable lysine or valine. For example, homoarginine and norleucine, added to the diets to create the imbalances, are potent competitors which decrease normal rates of transport of lysine and valine, respectively, across the blood-brain barrier [6,7], and can selectively deplete brain (and sometimes muscle) pools of basic and large neutral amino acids [5–9]. The preference for the homoarginine-containing rather than the non-protein diet may reflect the fact that, even when a diet containing protein or amino acids is severely deficient in lysine, animals can maintain body weight for extended periods; this conservation of amino acid does not occur when other amino acids are limiting in the diet [1], so that animals will lose weight when fed a diet severely deficient in valine.

Our earlier studies showed that the avoidance of dietary GABA cannot be explained on the basis of any unusual sen-

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FIG. 1. Selection by rats between bottles containing water and amino acid solutions at the indicated concentrations. The line at 50% represents the theoretical water intake (e.g., the control water/water group) which assumes intake is equally divided between the 2 bottles. Actual intakes were 49.9% and 50.1% of the total volume (mean of 966 values). Results show the volume of amino acid solution consumed, expressed as percent of the total intake. Symbols show concentrations at which intakes of amino acid solution were significantly different from the volume of water chosen by the corresponding control group ($p<0.05$).

sitivity to the taste of solutions of this amino acid [13]. Furthermore, olfactory bulbectomized rats, in which the senses of odor and taste are lost or damaged, continue to avoid a GABA-containing diet although they can no longer distinguish between control diets with or without added lemon extract, a flavor almost completely avoided by the control rats [12]. Both control and bulbectomized rats refuse to eat a threonine-imbalanced diet and consume less of a diet containing added threonine than of the control diet. Another instance of contrasting behavior is the preference by control rats for an alanine-containing diet while bulbectomized rats are indifferent to it. Thus, in this latter case odor/taste of the diet may have been important, especially as normal rats strongly prefer solutions of alanine [2] which tastes sweet to humans [4].

Disparities between the observations on choices from diets and from solutions show that preferences for amino acid solutions are not predictors of choices among amino acid-containing diets.

ACKNOWLEDGEMENTS

We thank David B. Piper for assistance with data summation, and Emmanuel Maurice and the Statistical Consulting Service of the College of Agricultural and Life Sciences for assistance with the statistical analysis. This work was supported in part by Grant AM-10747 from the National Institutes of Health, Bethesda, MD.

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