Pharmacology Biochemistry and Behavior, Vol. 2, pp. 481–485. ANKHO International Inc., 1974. Printed in the U.S.A.

Dietary Aversion Established by a Deficient Load: Specificity to the Amino Acid Omitted From a Balanced Mixture[1]

P. C. SIMSON

School of Biological Sciences, University of Sussex

AND

D. A. BOOTH[2]

Department of Psychology, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, England

(Received 25 February 1974)

SIMSON, P. C. AND D. A. BOOTH. *Dietary aversion established by a deficient load: specificity to the amino acid omitted from a balanced mixture.* PHARMAC. BIOCHEM. BEHAV. 2(4) 481–485, 1974. – Rats become averse to the odor of a protein-free diet presented after gastric administration of loads devoid of histidine and isoleucine but otherwise balanced in amino acid composition. The specific sensory aversion may in these cases explain the intake decrement seen shortly after loading. The suppression of intake shortly after a threonine-devoid load was, in contrast, not allied with an acquired aversion under the experimental conditions used. There were signs of aversion or anorexia following loads devoid of methionine, valine, phenylalanine or lysine. A complete balanced load, and to a lesser extent tryptophan- and perhaps glycine-devoid loads, induced a preference for the associated odor over an odor paired with saline intubation. Omission of leucine or arginine from the balanced mixture produced neither preference nor aversion. The results support a suggestion that reduced synthesis of a brain protein is aversion-inducing.

Amino acid deficiency Olfactory aversion Histidine Isoleucine Threonine Tryptophan
Amino acids and appetite

AVERSION to the odor or taste of a protein-free diet can be induced by presenting the flavored diet to a food-deprived rat immediately after it has been gastrically loaded with an amino acid mixture which is balanced in composition except for the omission of histidine [1, 3, 11, 12, 13]. Intake of the odorized diet begins to be depressed after the odor has been presented for about 2 hr following the histidine-devoid load. The evidence is that this is not a general anorexia but an early expression of the acquired stimulus-specific aversion [13].

The present experiments addressed the question whether the omission of any other amino acid from the balanced mixture would also induce an acquired odor-specific aversion. A sensitive and unambiguous measure of acquired stimulus control was provided by presenting the rat with a choice between diet having the odor paired with the amino acid load and diet having an odor paired with a control load. The generality of a 2-hr latency for the suppression of intake under single-stimulus conditions was also explored, by measuring intakes for several hours on loading days. The effects of omitting each of eleven amino acids, including all those essential for the rat, and also of a balanced load (which has a net preference-inducing effect relative to a control load [1, 11, 12]) were examined.

METHOD

Animals, Diets and Loads

Male albino rats were supplied at 80–100 g body-weight by Oxford Laboratories Animal Colonies. On arrival, they were placed in groups of 4–6 in large mesh cages in a room maintained at 20–22° with a normal 12-hr/12-hr lighting cycle. The rats had free access to tap water and pellets of autoclaved Small Animals Diet (Spillers, London).

Proetin-free diets were based on a partially hydrolysed starch preparation which was palatable and low in glucose

[1] Work supported by a grant from the Medical Research Council, U.K.

[2] Reprint requests should be addressed to David Booth, Department of Psychology, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, England.

and maltose content (maltodextrin MD05, Manbré, London). The diets contained 5% of salt mixture [10]; 0.025% vitamin mixture [10] without sucrose diluent but with 4-aminobenzoic acid added, to 0.002% of diet; 5% maize oil; and MD05 to 100% by weight. The mixture was odorized shortly before use by adding benzyl acetate or geraniol (10 μl/100 g).

The balanced amino acid mixture was, in proportions by weight of L isomers: leucine 11.9, isoleucine 5.5, valine 5.2, methionine 4.3, threonine 6.5, tryptophan 3.5, phenylalanine 6.8, lysine hydrochloride 12.8, arginine hydrochloride 7.8, histidine hydrochloride 2.9, glycine 5.5 and monosodium glutamate 27.5. In the deficient mixtures, the amino acid specified was completely omitted. The mixtures were intubated in 9 times their weight of water. The control gastric load was 2.7% sodium chloride solution, approximately iso-osmotic to the amino acid loads.

Initial Treatment

Within 7 days of arrival, rats were rehoused individually using the same cages. Before odor presentation, the rats were deprived of food overnight and then given unodorized protein-free diet ad lib for 54 hr. After the rats had had 24 hr on this diet, a gastric load of casein hydrolyzate in 25% solution was given at a dose of 15 g/kg. Finally, the rats were deprived of food for 18 hr over the night preceding the first odor access. Water was freely available at all times.

Load and Odor Pairing

Each rat was given a gastric load (50 ml/kg) of amino acids or saline at about 11.00 hr and was immediately presented with an odorized diet. Half the rats in a group were given amino acids on this first day; half had them on the following day. Generally, half of each of these subgroups had one odor on the first day and the other on the second. Thus sets of four rats were internally counterbalanced for differential effects of specific pairing between load and odor or of specific sequence of loads or odors. Rats were run in squads of about twelve, with a pair of rats from each of a variety of amino acid groups being included in each squad.

In Experiment 1, odorized diet was presented for 4 hr. In Experiment 2, it remained for 6 hr after the gastric load. In both cases, intake was measured every 2 hr.

The rats then remained food-deprived overnight and the same general procedure was applied the following day to provide the second pairing.

Preference Testing

After overnight food deprivation following the second odor presentation, the rats were given access to Small Animals Diet for 6 hr and then deprived of food overnight once again. At 11.00 hr the following day, they were presented for 3 hr with two samples of protein-free diet, each containing one of the odors. Intakes of both odorized diets were measured hourly.

Analysis of Intake Effects

To reduce variability arising from individual differences in basal intake response, intake differences were assessed by a ratio score. Differences both between single-stimulus intakes on the loading days and between intakes in the preference test were expressed as the intake of odorized diet paired with amino acid load minus the intake of control odorized diet, this difference being divided by the sum of the two intake values.

On the null hypothesis, the group mean ratio should not differ from zero. The statistical significance of a load-induced intake depression or an acquired odor preference was determined by two-tailed correlated *t*-test.

RESULTS

Ratios of intake difference to intake sum following gastric loads are given in Table 1. A negative value indicates lower intake on the day of amino acid administration. As in previously reported experiments, food intake was depressed at 2–4 hr after administration of a histidine-devoid load, but not in the first 2 hr period. No other amino acid load produced as large an effect consistently between the two experiments. Intake was however reduced in the first 2 hr after administration of a threonine-devoid load, in the single experiment in which that load was tested. Consistently negative intake ratios followed loading with the isoleucine-devoid mixture, once approaching statistical significance; the effects of this load could be worth further investigation, as might also the large but variable depression at 4–6 hr after the methionine-devoid load. The pattern of statistically reliable effects was not altered by combining data from Experiments 1 and 2 for the 0–4 hr period.

The food-intake ratios in the preference test showed similar patterns when calculated over the first hour of choice, over 0–2 hr or over the whole 3 hr of the test; the 3 hr ratios are given in Table 2. The results of previous work were again replicated in the groups given balanced or histidine-devoid mixtures. The odorized diet paired with the balanced mixture was preferred to the odorized diet paired with the control load, although not always with statistical significance. An aversion developed to odors paired with histidine deficiency.

This aversion-inducing effect proved not to be unique to the histidine-devoid load. In parallel to the actual or possible depressions of intake on the day of intubation, aversions were also acquired to odorized diets paired with isoleucine-devoid and methionine-devoid loads (Table 2). Omission of valine, phenylalanine or lysine also produced negative mean ratios, differing significantly from the balance-induced positive ratios, although not differing significantly from zero.

Omission of tryptophan left a mixture still capable of inducing a preference, although one considerably weaker than that induced by the complete balanced amino acid mixture. The one experiment on a glycine-devoid load produced a mean intake ratio in the direction of preference, although not with statistical significance (Table 2).

DISCUSSION

The rats in these experiments spent most of several days without any protein or amino acid intake, and indeed were deprived of all nutrient for 18-hr periods. On the other hand, a large amino acid load was administered at one stage of initial treatment. It is therefore likely that, before odor-paired loading, some amino acids were in short supply and yet substantial levels of degradative hepatic enzymes were still present. Under such conditions, administration of an

TABLE 1
INTAKES ON DAYS OF GASTRIC LOADING

Amino acid load	Experiment	Number of rats	Food intake ratio* 0–2 hr Mean	SE	2–4 hr Mean	SE	4–6 hr Mean	SE
Balanced	1	8	−0.01	2.17	−0.16	0.15	–	
	2	8	−0.14	0.23	−0.11	0.12	0.31	0.17
Histidine-devoid	1	8	0.06	0.11	−0.25†	0.06	–	
	2	8	0.19	0.09	−0.38†	0.14	−0.21	0.19
Isoleucine-devoid	1	4	−0.44	0.07	−0.02	0.15	–	
	2	4	−0.12	0.12	−0.15	0.23	0.13	0.31
Valine-devoid	1	4	0.06	0.15	−0.09	0.02	–	
	2	4	−0.18	0.22	−0.21	0.09	0.21	0.19
Methionine-devoid	1	4	0.02	0.13	0.08	0.19	–	
	2	4	−0.05	0.06	0.03	0.08	−0.52	0.28
Phenylalanine-devoid	1	4	−0.01	0.18	0.09	0.11	–	
	2	4	−0.22	0.09	0.28	0.19	−0.27	0.34
Lysine-devoid	1	4	−0.09	0.15	0.17	0.15	–	
	2	4	0.00	0.24	−0.16	0.15	−0.03	0.21
Threonine-devoid	1	6	−0.19†	0.07	0.04	0.09	–	
Leucine-devoid	1	4	−0.03	0.13	−0.03	0.43	–	
	2	6	−0.07	0.09	0.02	0.22	0.08	0.12
Tryptophan-devoid	1	4	−0.02	0.21	0.16	0.22	–	
	2	4	0.03	0.02	−0.38	0.21	0.04	0.04
Arginine-devoid	2	8	−0.13	0.15	0.11	0.06	0.04	0.16
Glycine-devoid	2	7	0.02	0.16	0.08	0.16	0.21	0.15

*The ratio is: $\frac{\text{Intake on amino acid day} - \text{Intake on saline day}}{\text{Sum of intakes}}$

†$p<0.05$

TABLE 2

PREFERENCE TEST INTAKE RATIOS

Amino acid load on conditioning day	Experiment 1 Number of rats	Food intake ratio* Mean	SE	Experiment 2 Number of rats	Food intake ratio Mean	SE
Balanced	8	0.36†	0.12	8	0.35	0.15
Histidine-devoid	8	−0.53†	0.16	8	−0.18‡	0.09
Isoleucine-devoid	4	−0.54†,‡	0.08	4	−0.24‡	0.21
Valine-devoid	4	0.05	0.22	4	−0.29‡	0.21
Methionine-devoid	4	−0.01	0.31	4	−0.51‡	0.18
Phenylalanine-devoid	4	−0.04‡	0.14	4	0.25	0.34
Lysine-devoid	4	−0.22‡	0.29	4	0.15	0.14
Threonine-devoid	6	0.11	0.20		–	
Leucine-devoid	4	0.25	0.24	6	−0.04	0.23
Tryptophan-devoid	4	0.20†	0.07	4	−0.02	0.10
Arginine-devoid		–		8	0.08	0.15
Glycine-devoid		–		7	0.32	0.15

†$p<0.05$, for null hypothesis of zero mean
‡$p<0.05$, for difference from mean of balance-loaded group, by two-tailed uncorrelated t-test
*The ratio is: $\frac{\text{Amino acid-paired odor} - \text{Saline-paired odor}}{\text{Sum}}$

amino acid mixture lacking a single amino acid could induce or accentuate a critical imbalance or deficiency in the free amino acid pattern of plasma or of receptor tissue which appears to be located in the brain [5].

In the present experiments, the omission of isoleucine and perhaps of methionine, like the omission of histidine, established an acquired aversion to a sensory cue experienced close in time to the absorption of the otherwise balanced amino acid mixture. The results were consistent with aversion having been acquired sufficiently rapidly and strongly to suppress intake of the sole available diet within a few hours, yet with no initial intake suppression as should be evinced by a direct satiating or anorexigenic effect. Mixtures devoid of certain other essential amino acids also showed signs of inducing an aversion, but this did not appear to be strong enough to suppress intake reliably on the day of administration.

A threonine-devoid load had a different pattern of effects under these conditions, at least so far as the present data go. A prompt depression of intake was not paralleled by a acquired aversion. The depressed intake of threonine-deficient and threonine-imbalanced diets might therefore arise from direct anorexigenic or over-satiating effects, as has been proposed to be the case for all poorly accepted deficient or imbalanced diets [6,7]. Absorption towards the end of a meal may be sufficiently rapid to activate such direct suppression of appetite parenterally.

Nevertheless, the effects of the also frequently used isoleucine or histidine deficiency or imbalance remain susceptible to the alternative explanation in terms of an acquired sensory aversion. This mechanism had been postulated by some workers on amino acid imbalance [8,15] and was demonstrated to exist in the present experiments and earlier results obtained using similar methods [1, 11, 13]. In most experiments on suppression of the intake of imbalanced or deficient diets, the texture or taste of the diet contrasts sufficiently strongly with the protein-free control diets to serve as a sensory basis for acquired aversion. Thus the suppression of intake by the specific aversion mechanism need not be affected by ablation of the olfactory bulbs [4]. Indeed, under conditions which almost certainly eliminate such taste and texture cues – choice between imbalanced and corrected diets – the strong aversion takes considerably longer to develop [8]. Under such conditions, olfactory markers might have to be provided by the animal itself, or some very slight cue unintentionally provided by the experimenter may be detectable. The advantage of separation of amino acid administration from dietary ingestion, as in the present experiments, is that it permits direct control of the sensory associations available to the rat and the relative timing of postingestive and sensory events. Without such experimental control we are unlikely to elucidate the relative importance of direct anorexia or satiety on one hand and acquired aversion on the other, let alone their physiological mechanisms.

Amino acid recycling in the liver is sufficiently rapid to make hepatic protein synthesis relatively insensitive to deficiencies in the supply of individual amino acids in vivo. Tryptophan is the only amino acid for which it is established that supply is critical [9]. The tryptophan-devoid load in the present experiments not only failed to induce an aversion; it induced a weaker form of the prefer-

ence induced by the balanced amino acid load. This weighs against a major role for the liver in the acquisition of the aversion. It is also against a nonspecific effect on brain biochemistry, in so far as this might be elicited by effects of circulating tryptophan on cerebral serotonin [14].

The effects of a mixture devoid of the dispensable amino acid glycine differed little if at all from the effects of the complete amino acid mixture. The relative effectiveness of the omission of any of a number of indispensable amino acids which are likely to be in short supply in the deprived rat is additional support for a proposal that acquisition of the aversion is mediated by disruption of the synthesis of a protein with rapid turnover, presumably in the brain [2].

REFERENCES

1. Booth, D. A. and P. C. Simson. Food preference acquired by association with variations in amino acid nutrition. *Q. Jl exp. Psychol.* **23**: 135–145, 1971.
2. Booth, D. A. and P. C. Simson. Aversion to a cue acquired by its association with effects of an antibiotic in the rat. *J. comp. physiol. Psychol.* **84**: 319–323, 1973.
3. Booth, D. A. and P. C. Simson. Gustatory aversion induced by an amino acid deficient load. *Physiol. Psychol.* in press, 1974.
4. Leung, P. M. B., D. A. Larson and Q. R. Rogers. Food intake and preference of olfactory bulbectomized rats fed amino acid imbalanced or deficient diets. *Physiol. Behav.* **9**: 553–557, 1972.
5. Leung, P. M. B. and Q. R. Rogers. Food intake: Regulation by amino acid pattern. *Life Sci.* **8**: 1–9, 1969.
6. Leung, P. M. B. and Q. R. Rogers. Importance of prepyriform cortex in food-intake response of rats to amino acids. *Am. J. Physiol.* **221**: 929–935, 1971.
7. Leung, P. M. B. and Q. R. Rogers. Effect of amygdaloid lesions on dietary intake of disproportionate amounts of amino acids. *Physiol. Behav.* **11**: 221–226, 1973.
8. Leung, P. M. B., Q. R. Rogers and A. E. Harper. Effect of amino acid imbalance on dietary choice in the rat. *J. Nutr.* **95**: 483–492, 1968.
9. Munro, H. N. Adaptation of mammalian protein metabolism to amino acid supply. *Proc. Nutr. Soc.* **28**: 214–225, 1969.
10. Rogers, Q. R. and A. E. Harper. Amino acid diets and maximal growth in the rat. *J. Nutr.* **87**: 267–273, 1965.
11. Simson, P. C. and D. A. Booth. Olfactory conditioning by association with histidine-free or balanced amino acid loads in rats. *Q. Jl exp. Psychol.* **25**: 354–359, 1973.
12. Simson, P. C. and D. A. Booth. Effect of CS-US interval on the conditioning of odour preferences by amino acid loads. *Physiol. Behav.* **11**: 801–808, 1973.
13. Simson, P. C. and D. A. Booth. The rejection of a diet which has been associated with a single administration of an histidine-free amino acid mixture. *Br. J. Nutr.* **31**: 285–296, 1974.
14. Wurtman, R. J. and J. D. Fernstrom. Brain serotonin content: Physiological regulation by plasma neutral amino acids. *Science* **178**: 414–416, 1972.
15. Zahler, L. P. and A. E. Harper. Effects of dietary amino-acid pattern on food preference behavior of rats. *J. comp. physiol. Psychol.* **81**: 155–161, 1972.