Rebalancing essential amino acids intake by self-selection in the rat

BY GILLES FROMENTIN AND STYLIANOS NICOLAIDIS

Laboratoire de Neurobiologie des Régulations, CNRS URA 1860, Collège de France, 11 Place Marcellin Berthelot, F75231 Paris Cedex 05, France

(*Received 19 April 1995 – Revised 24 July 1995 – Accepted 16 August 1995*)

The purpose of the present study was to assess whether rats are capable of selecting the right proportions of two diets that are individually inappropriate in terms of essential amino acid composition to satisfy their amino acid requirements. Rats were offered a choice of one protein-free regimen and another devoid of only one essential amino acid (either threonine or isoleucine) set up in such a way as to provide amino acid balance if they were consumed in 1/3 and 2/3 proportions respectively. Preliminary experiments had established that all our diets were aversive by themselves except for the 60 g casein/kg diet. Rats did reach almost the necessary proportion with, according to published standards (National Research Council, 1978), some excess in isoleucine intake. In addition, given access to two aversive diets that were each nutritionally inadequate, rats showed no aversion and gained body weight when they had the opportunity to consume both of them. Beyond the capacity that rats have of rebalancing their micronutrient intake, the present experiment brings out the idea that the imbalance-induced aversion: preference ratio may be completely upset when this omnivore has access to more than one feed.

Dietary choice: Preference: Conditioned taste aversion

Specific hungers for dietary micronutrients have been reported for many years. For instance, it has been shown that rats made deficient in Na, Ca or vitamins of the B group show a preference for feed containing the needed substance (Harris *et al.* 1933; Scott & Quint, 1946; Richter, 1956; Rodgers, 1967). The feed amino acid pattern plays a role in the regulation of feed intake and dietary choice in rats. It has been known for many years that if a diet is protein-free or not properly balanced in amino acid composition, the two main effects are (1) a decrease in feed intake and (2) a loss of body weight (Harper *et al.* 1970). After a neurobiochemical phase of rapid recognition of the deficiency, the animal develops a subsequent conditioned taste aversion for the taste characteristic of the deficient feed (reviewed by Harper *et al.* 1970 and Gietzen, 1993).

Specific hungers for essential amino acids have also been demonstrated. Halstead & Gallagher (1962) concluded that the rat posseses the ability to discriminate between two essential amino acid solutions that differ with respect to presence or absence of threonine only and to prefer the non-devoid solution. Similarly, Rogers & Harper (1970) have shown that when rats fed on a histidine-imbalanced diet have the choice of drinking from two bottles, one containing water and the other containing a histidine solution, they choose to drink more of the histidine solution.

The dietary self-selection technique has been widely used to demonstrate the existence of specific hungers in deficient animals and the choice-related regulation of nutrient intake in normal animals. Using a self-selection feeding method, Yamamoto *et al.* (1985) attempted to show that rats were able to adjust the intake of several essential amino acids by offering the choice between one aversive diet devoid or imbalanced in the essential amino acid and a non-aversive well-balanced diet. This methodology always induced a preference for the non-aversive, balanced diet.

The present investigation was undertaken to determine whether (a) rats have the ability to adjust precisely their intake of an essential amino acid when offered a self-selection between two inappropriate and therefore aversive diets, the first being deficient in a given amino acid but otherwise well balanced and the second being a protein-free diet supplemented with the missing amino acid, and (b) rats are able to reverse the aversive property that each diet possesses when consumed by itself into a non-aversive property when both diets are consumed jointly. For this purpose, the amino acid supplementation of the protein-free diet was calculated so that the rats could rebalance their amino acid intake by eating the protein-free, amino acid-supplemented diet and the devoid diet in the proportions of 33% and 67% respectively, according to the composition of diets used in most investigations of this type (Gietzen *et al.* 1992). This capacity for rebalancing was investigated using two essential amino acids, threonine and isoleucine.

METHODS

Animals

The subjects were male Wistar rats (Ifa-Credo) weighing 200–320 g at the start of the experiments. They were individually housed in stainless steel wire cages at $22 \pm 2°$, on a 12:12 h light–dark cycle (06.00–18.00 hours). All rats were naive to both the diets and choice paradigms used in these experiments. All experimental procedures conformed to the guidelines of the National French Animal Care Committee.

Procedure

For the first 3 d the rats were fed *ad libitum* on a chow diet (extra-labo M25 from Pietrement, 77482 Provins Cedex, France) and water in their individual cages where all experiments took place (habituation period). During the ten following days the rats were fed *ad libitum* on a diet containing 60 g casein/kg to prevent intake and storage of excessive amounts of amino acids (preparation period) (Harper, 1959; Harper *et al.* 1970). During the next 8 d the rats were always given a choice between two diets depending on the experiment. The various diets are described subsequently and also detailed in Table 1 (self-selection period). Both feed containers were refilled with fresh feed and the position of the containers was alternated daily between either side of the cage to prevent side choice effects. Feed consumption and body weight were measured daily at 17.00 hours. All the diets were moistened (33·3% water, 66·6% powder) to prevent spillage. Water was always given *ad libitum*.

Composition of diets

The composition of the diets is shown in Table 1. Briefly, all the diets contained (g/kg): cellulose 20, lipids 50 (rapeseed oil 30 and peanut oil 20), mineral mixture 45, vitamin mixture 10. Carbohydrate varied between 668 and 875 g/kg DM and the rest was protein. The carbohydrate fraction was a mixture of maize starch and glucose (1:1, w/w) and its proportion was calculated to make the weight of the DM to 1 kg. This composition was chosen according to Centre National d'Etudes et de Recommandations sur la Nutrition et l'Alimentation (CNERNA), recommendations (Potier de Courcy *et al.* 1989). The specific compositions of the various diets were as follows:

P6%, the low-protein diet, contained 60 g casein/kg;
THR-DEV, the threonine-devoid diet did not contain threonine but otherwise was well balanced for the other indispensable amino acids (Gietzen *et al.* 1992);

Table 1. *Composition of the diets used in the experiments (g/kg diet)*

Diet...	P6%	THR-DEV	COR	P0%	P0%+ 1·2% THR	P0%+ 1·2% SER	P0%+ 2·6% ILEU	ILEU-DEV
Casein*	60	—	—	—	—	—	—	—
Non-essential amino acids†	—	77	77	—	—	—	—	77
Essential amino acids‡	—	124	124	—	—	—	—	113·4
L-Methionine	0·5	—	—	—	—	—	—	—
L-Threonine	—	—	6	—	12	—	—	—
L-Serine	—	—	—	—	—	12	—	—
L-Isoleucine	—	—	—	—	—	—	26	—
Salt mixture§	45	45	45	45	45	45	45	45
Vitamin mixture‖	10	10	10	10	10	10	10	10
African peanut oil¶	20	20	20	20	20	20	20	20
Rapeseed oil¶	30	30	30	30	30	30	30	30
Cellulose**	20	20	20	20	20	20	20	20
Maize starch††	407·2	337	334	437·5	431·5	431·5	424·5	347·3
Glucose‡‡	407·2	337	334	437·5	431·5	431·5	424·5	347·3
Total§§	1000	1000	1000	1000	1000	1000	1000	1000

* Vitamin-free, ICN Biomedicals, OH, USA.

† Provided (g/kg diet): glutamic acid 30, L-glycine 10, L-arginine 10, L-alanine 3·5, L-asparagine 10, L-proline 10, L-serine 3·5 (Degussa, Ridgefield Park, NJ, USA).

‡ Provided (g/kg diet): THR-DEV: L-methionine 10, L-cystine 6, L-histidine 12, L-lysine 15, L-isoleucine 15, L-leucine 21, L-phenylalanine 15·5, L-tryptophan 4, L-valine 16, L-tyrosine 9·5; ILEU-DEV: L-methionine 8·5, L-cystine 6, L-histidine 7·6, L-lysine 22, L-threonine 12, L-leucine 19, L-phenylalanine 12·5, L-tryptophan 2·8, L-valine 14·5, L-tryosine 8·5 (Degussa).

§ Provided (g/kg diet): calcium phosphate 17·1, potassium phosphate 10·8, calcium carbonate 8·1, magnesium sulphate 4·0, sodium chloride 3·16, magnesium oxide 0·9, ferric sulphate 0·39, zinc sulphate 0·23, manganese sulphate 0·23, cupric sulphate 0·046, sodium fluoride 0·037, potassium chromate 0·002, potassium iodide 0·002, ammonium molybdate 0·001, cobalt carbonate 0·001, sodium selenite 0·001.

‖ Provided (/kg diet): retinyl acetate 5 mg, cholecalciferol 62·5 μg, D,L-α-tocopherol acetate 5 g, menadione 1 mg, thiamin 10 mg, riboflavin 10 mg, nicotinic acid 45 mg, D-calcium pantothenate 30 mg, pyridoxine HCl 10 mg, inositol 50 mg, s-biotin 0·2 mg, pteroylmonoglutamic acid 2 mg, cyanocobalamin 0·0135 mg, ascorbic acid 100 mg, *p*-amino benzoic acid 50 mg, choline chloride 750 mg.

¶ Lesieur Aliment, 59412 Coudekerque Branche Cedex, France.

** Medias Filtrants Durieux, 77202 Marne la Vallée, France.

†† Cerestar, 59482 Hanbourdin Cedex, France.

‡‡ Roquette frères, 59022 Lille, France.

§§ All ingredients were purchased and/or prepared by the atelier de préparation des aliments expérimentaux, Institut National de la Recherche Agronomique, Jouy en Josas, 78 France.

COR, the threonine-corrected diet, was identical to THR-DEV except that it was supplemented with threonine (6 g/kg) in order to correct the balance of the amino acid mixture;

ILEU-DEV, the isoleucine-devoid diet, was deficient in isoleucine instead of threonine but otherwise well balanced for the other indispensable amino acids (Naito-Hoopes *et al.* 1993);

P0%, was protein free;

P0% + 1·2% THR, was a protein-free diet but contained added threonine (12 g/kg);

P0% + 1·2% SER, was protein free but contained added serine, (12 g/kg);

P0% + 2·6% ILEU, was protein free but contained added isoleucine, (26 g/kg).

Data analysis

All results are shown as means with their standard errors of all daily intakes for all rats. Statistical significances of variations in the response variable, i.e. feed intake, were determined by a one-way ANOVA using STAT-GRAPH (Statistical Graphic Corporation, Rockville, MD, USA), introducing diets, days and groups as factors. Statistical significance was set at $P < 0.05$. When differences were detected ($P < 0.05$), differences between individual means were determined using a post-hoc test (Scheffé, $P < 0.05$).

RESULTS: PRELIMINARY AND CONTROL EXPERIMENTS

All the choices observed in the main experiments could be interpreted only after establishing the relative palatabilities of the diets used in those experiments. Therefore, several preliminary trials were performed to check, under our experimental conditions, the various preferences or aversions, namely (a) that protein-free diets containing one essential amino acid are indeed aversive, (b) that rats prefer a protein-free diet, either with or without a single amino acid other than threonine, rather than a threonine-devoid diet, (c) that rats, given a choice between a diet previously devoid of, but now corrected for, one essential amino acid and a protein-free diet enriched with this amino acid, prefer the former over the latter.

Expt 1. Aversion for a protein-free diet containing 12 g threonine/kg

Six male albino rats (210–230 g initial weight) pre-fed for 10 d on the low-protein diet (P6 %) were switched to a protein-free diet containing 12 g threonine/kg (P0 % + 1·2 % THR) for the following 8 d.

During the 10 d when P6 % was offered the average feed intake was 379 (SEM 4·1) kJ and the daily body-weight gain was +0·73 (SEM 0·3) g. During the following 8 d when P0 % + 1·2 % THR was offered the average feed intake was 297 (SEM 16·0) kJ and the daily body-weight loss was −2·38 (SEM 0·21) g (Fig. 1). During the first 2 d of the experimental period the daily feed intake was not different from the pre-feeding period. However, over the entire 8 d of the choice period, there was a gradual decrease in feed intake of the P0 % + 1·2 % THR diet ($F(8,45)$ 4·35, $P = 0.0006$; Fig. 1). An average 20 % decrease in feed intake was observed at the end of the experiment.

Expt 2. Aversion for a protein-free diet containing 26 g isoleucine/kg

This experiment was identical to Expt 1 except that six male albino rats (235–265 g initial weight) were switched to a protein-free diet containing 26 g isoleucine/kg (P0 % + 2·6 % ILEU) instead of the threonine of the previous experiment.

During the 10 d when P6 % was offered the average feed intake was 387 (SEM 9·0) kJ and the daily body-weight gain was +2·55 (SEM 0·57) g. During the following 8 d when P0 % + 2·6 % ILEU was offered the average feed intake was 260 (SEM 7·1) kJ and the daily body-weight loss was −3·42 (SEM 0·29) g (Fig. 1). As early as the very first choice day and for the entire 8 d choice period there was a gradual decrease in feed intake of P0 % + 2·6 % ILEU ($F(8,45)$ 11·57, $P < 0.0001$). At the end of the experiment the average decrease in intake of P0 % + 2·6 % ILEU was 37·8 % (Fig. 1). The decrease in intake of P0 % + 2·6 ILEU was more pronounced than the decrease in intake of P0 % + 1·2 % THR in the previous experiment ($F(1,94)$ 9·87, $P = 0.002$), especially during the first 3 d of the choice period.

Expt 3. Preference for a protein-free diet over a diet devoid of threonine

Six male albino rats (280–350 g initial weight) pre-fed for 10 d on P6 %, were given a choice between a protein-free diet (P0 %) and a threonine-devoid diet (THR-DEV).

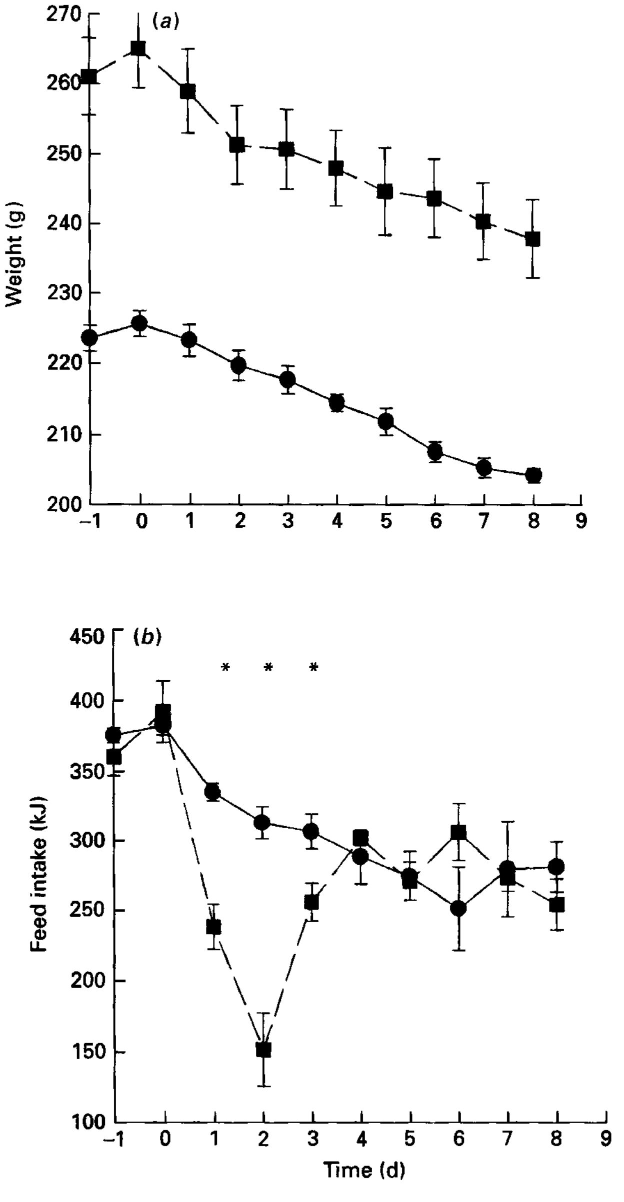

Fig. 1. Changes in (*a*) body weight and (*b*) feed intake of rats fed on either a protein-free diet containing 12 g threonine/kg (●), or a protein-free diet containing 26 g isoleucine/kg (■). Until day 0 rats were fed on a low-protein diet (60 g casein/kg). Values are means for six rats, with their standard errors indicated by vertical bars. * Mean values were significantly different between groups (Scheffé test, $P < 0.05$).

During the 10 d when P6 % was offered the average feed intake was 356 (SEM 10·9) kJ and the daily body-weight gain was +2·05 (SEM 0·43) g. During the following 8 d when a choice between P0 % and THR-DEV was offered the average total feed intake was 317 (SEM 9·9) kJ and the daily body-weight loss was −3·06 (SEM 0·07) g. There was a decrease in the daily total feed intake throughout the experimental period ($F(7,40)$ 2·4, $P = 0.041$; Fig. 2). As

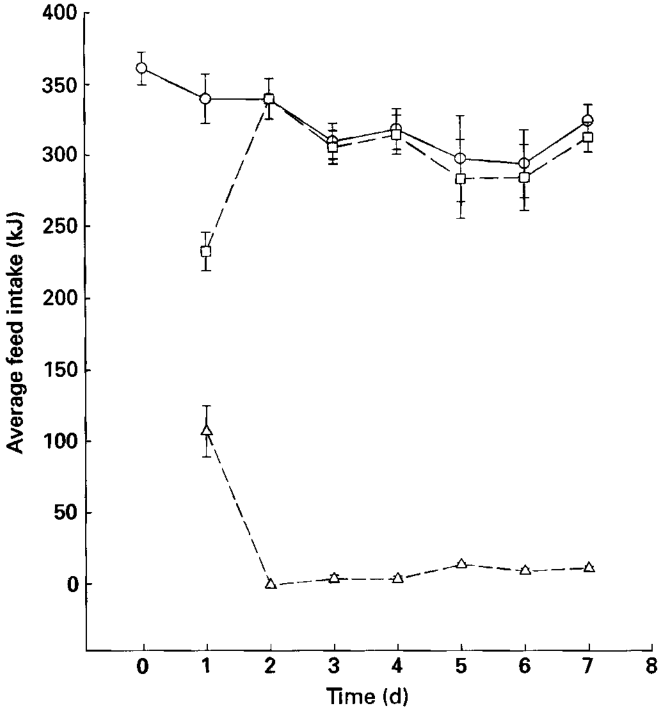

Fig. 2. Feed intake of rats given a choice between a protein-free diet (□) and a diet devoid of threonine (△). (○), Total feed intake. Until day 0, rats were fed on a low-protein diet (60 g casein/kg), and the choice began on day 1. Values are means for six rats, with their standard errors indicated by vertical bars.

early as the second choice day the intakes of THR-DEV and P0 % reached a plateau until the end of the experiment. The preference for P0 % began on the very first choice day and was different from no choice (50 %) ($P = 0{\cdot}009$). Considering the entire 7 d experimental choice period rats preferred P0 % and, except for the first choice day, they ate almost exclusively from it (93·1 (SEM 1·2) % of the total intake; Fig. 3).

Expt 4. Preference for a diet containing 12 g serine/kg over a diet devoid of threonine

The purpose of this experiment was to test whether the results recorded in Expt 3 would be altered if another amino acid, different from threonine, was added to the protein-free diet. The replacement of threonine by serine was guided by the fact that both threonine and serine are tasteless.

Six male albino rats (215–250 g initial weight) pre-fed on P6 %, were given a choice between THR-DEV and a protein-free diet containing 12 g serine/kg (P0 % + 1·2 % SER).

During the 10 d when P6 % was offered the average feed intake was 415 (SEM 8·6) kJ and the daily body-weight gain was + 1·92 (SEM 0·37) g. During the following 8 d when a choice between THR-DEV and P0 % + 1·2 % SER was offered the average total feed intake was 385 (SEM 7·5) kJ and the daily body-weight loss was − 1·67 (SEM 0·23) g. There was a decrease in the total daily feed intake throughout the experimental period ($F(7, 40)$ 2·51, $P = 0{\cdot}39$; Fig. 4). Rats preferred P0 % + 1·2 % SER (+ 77·14 (SEM 0·6) %) rather than THR-DEV (Fig. 3). This preference ($P < 0{\cdot}0001$) began on the very first choice day and remained unchanged throughout the 8 d choice period.

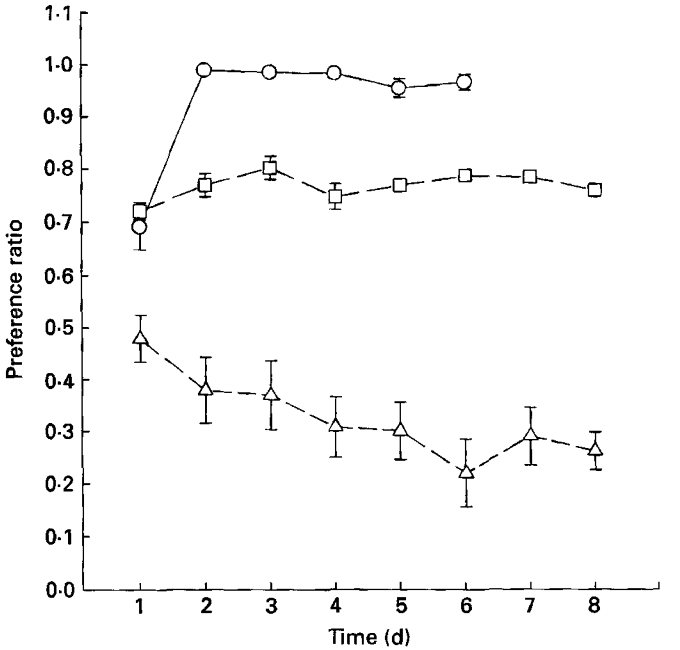

Fig. 3. Preference ratio values for rats given the choice between a protein-free diet containing a single amino acid, and a diet devoid of a single amino acid. Until day 0 rats were fed on a low-protein diet (60 g casein/kg) and the choice began on day 1. (□), Preference ratio = intake of serine-containing diet/total intake × 100. Rats were given a choice between a protein-free diet containing 12 g serine/kg and a diet devoid of threonine. (○), Preference ratio = protein-free diet intake/total intake × 100. Rats were given a choice between a protein-free diet and a diet devoid of threonine. (△), Preference ratio = intake of threonine-containing diet/total intake × 100. Rats were given a choice between a protein-free diet containing 12 g threonine/kg and a threonine-corrected diet. Values are means for six rats, with their standard errors indicated by vertical bars.

Statistical comparison of Expts 3 and 4

The rats in Expt 4 which had to choose between THR-DEV and P0% + 1·2% SER ate more of the two diets than the rats that had to choose between THR-DEV and P0% ($F(1, 82)$ 47·88, $P < 0·001$). The difference was due to the fact that less of THR-DEV was eaten (88·2 (SEM 3·5) kJ) when it was presented in conjunction with P0% alone than when it was presented with P0% + 1·2% SER (21·5 (SEM 4·2) kJ) ($F(1, 82)$ 85·70, $P < 0·0001$). Curiously body-weight loss was similar in both groups despite the lower total feed intake by the rats in Expt 3.

Expt 5. Preference for a threonine-corrected diet over a protein-free diet containing 12 g threonine/kg

Six male rats (210–320 g initial weight) pre-fed on P6%, were given a choice between a threonine-corrected diet (COR) and P0% + 1·2% THR.

During the 10 d when P6% was offered the average feed intake was 421 (SEM 7·4) kJ and the daily body-weight gain was +3·83 (SEM 0·3) g. During the following 8 d when a choice between COR and P0% + 1·2% THR was offered the average total feed intake was 455 (SEM 10·3) kJ and the daily body-weight gain was +5·15 (SEM 0·25) g. The total daily feed intake of the two diets was not different throughout the pre-feeding and the experimental periods (Fig. 5). On the overall experimental period rats preferred COR rather than P0% + 1·2% THR ($F(1, 190)$ 114, $P < 0·001$). On the fourth choice day the average

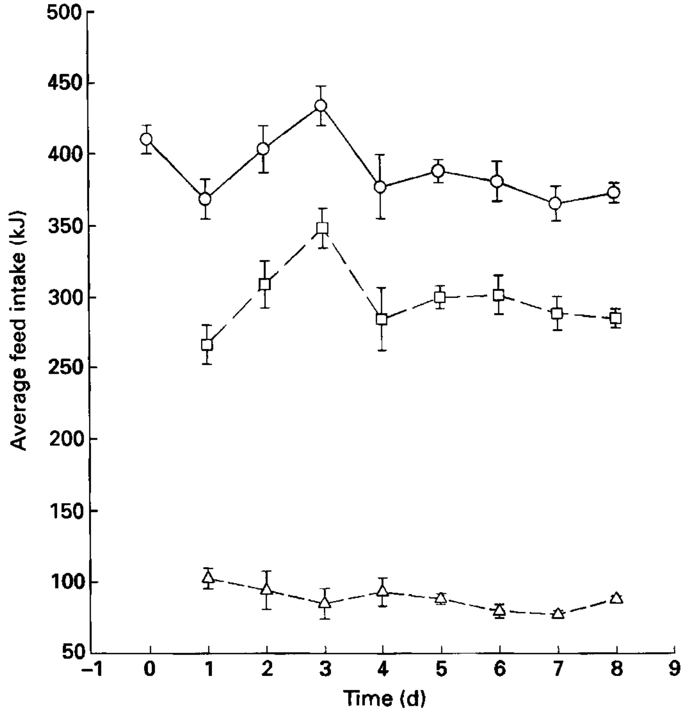

Fig. 4. Feed intake of rats given a choice between a protein-free diet containing 12 g serine/kg (□) and a diet devoid of threonine (△). (○), Total feed intake. Until day 0, rats were fed on a low-protein diet (60 g casein/kg), and the choice began on day 1. Values are means for six rats, with their standard errors indicated by vertical bars.

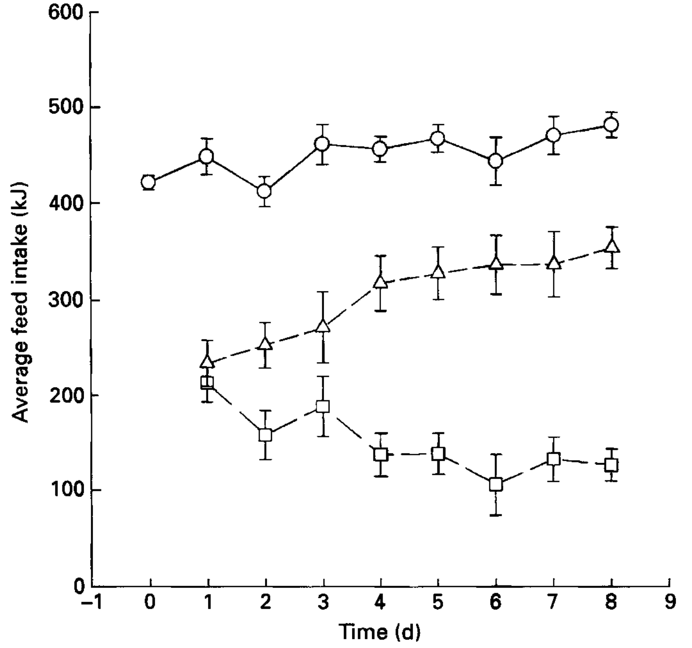

Fig. 5. Feed intake of rats given a choice between a protein-free diet (□) and a threonine-corrected diet (△). ○), Total feed intake. Until day 0 rats were fed on a low-protein diet (60 g casein/kg). The choice period began on day 1. Values are means for six rats, with their standard errors represented by vertical bars.

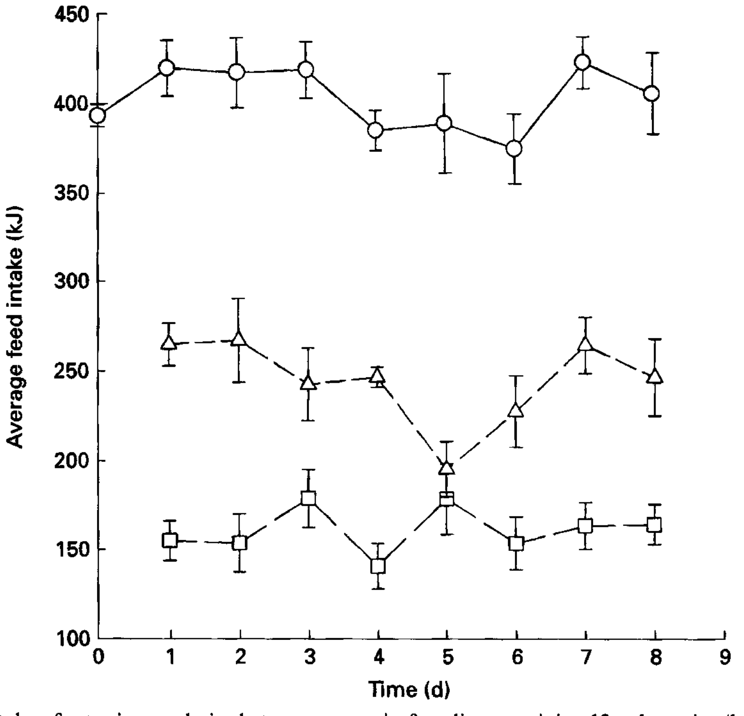

Fig. 6. Feed intake of rats given a choice between a protein-free diet containing 12 g threonine/kg (□) and a diet devoid of threonine (△). (○), Total feed intake. Until day 0 rats were fed on a low-protein diet (60 g casein/kg). The choice period began on day 1. Values are means for twelve rats, with their standard errors indicated by vertical bars.

proportion of P0% + 1·2% THR (31·1 (SEM 5·8)%) in the total intake, was different from no choice (50%) ($P < 0.0001$) and remained unchanged throughout the experimental period (Fig. 2).

RESULTS: MAIN EXPERIMENTS

The purpose of the main experiments was to test whether the rat had the ability to combine two of the deficient diets explored in the preliminary experiments in such a way that the self-imposed mixture resulted in (a) an amino acid-balanced diet and therefore (b) the turning of two aversive diets in a non-aversive mixture.

Expt 6. Choice between a protein-free diet containing 12 g threonine/kg and a diet devoid of threonine

Twelve male albino rats (210–275 g initial weight) were pre-fed on P6% and then given a choice between THR-DEV and P0% + 1·2% THR. The threonine-supplementation of the protein-free diet was calculated so that if the rats ate 33% P0% + 1·2% THR and 67% THR-DEV, the resulting mixture would provide a diet well balanced for amino acids according to the composition of diets used in most investigations of this type (Gietzen *et al.* 1992).

During the 10 d when P6% was offered the average feed intake was 393 (SEM 9·0) kJ and the daily body-weight gain was +2·66 (SEM 0·58) g. During the following 8 d when a choice between P0% + 1·2% THR and THR-DEV was offered the average total feed intake was 402 (SEM 8·8) kJ and the daily body-weight gain was +4·33 (SEM 0·25) g. The total daily feed intake of the two diets was not different throughout the experimental period compared with the pre-feeding period (Fig. 6) During the choice period the variations of both

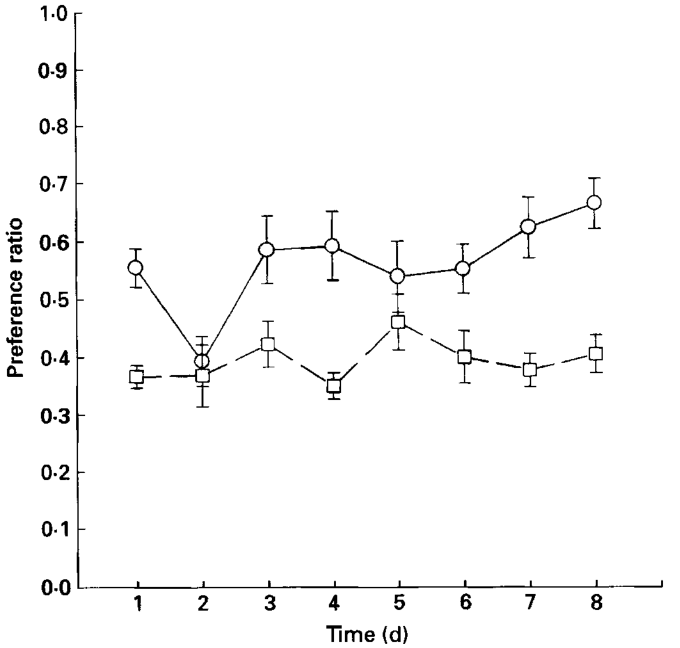

Fig. 7. Preference ratio values for rats given a choice between a protein-free diet and a diet containing a single essential amino acid. Until day 0 rats were fed on a low-protein diet (60 g casein/kg), and the choice period began on day 1. (□), Preference ratio = intake of threonine-containing diet/total intake × 100. Rats were given a choice between a protein-free diet containing 12 g threonine/kg and a diet devoid of threonine. (○), Preference ratio = intake of isoleucine-containing diet/total intake × 100. Rats were given a choice between a protein-free diet containing 26 g isoleucine/kg and a diet devoid of isoleucine. Values are means for twelve rats, with their standard errors represented by vertical bars.

P0% + 1·2% THR intake and THR-DEV intake were not significant from one day to another. On the overall 8 d choice period rats ingested P0% + 1·2% THR in a proportion averaging 39·5 (SEM 1·40)% instead of the expected 33% ($P < 0.0001$) (Fig. 7). This proportion was also different from no choice (50%) ($P < 0.0001$). A proportion of 29 (SEM 0·02)% was observed during the very first choice day and did not vary from one day to another on the subsequent choice period, despite changes such as those on the fourth choice day that did not reach statistical significance.

Discussion. Rats were able to adjust the proportion of the two diets in such a way that the self-imposed mixture provided a relatively balanced diet. This adjustment was reached as early as the very first choice day and did not change during the seven subsequent days. However, rats ate more of the essential amino acid than expected according to the composition used in most investigations of this sort (Gietzen *et al.* 1992). As a result, the average threonine intake was 4·7 g/kg diet against the 4·0 g/kg diet expected. Given that intake of 1 kg P0% + 1·2% THR provided 12 g threonine, an intake of 100 g of the well-equilibrated mixture (THR-DEV 66·6% and P0% + 1·2% THR 33·3%) should have provided 0·4 g threonine.

Expt 7. Choice between a protein-free diet containing 26 g isoleucine/kg and a diet devoid of isoleucine

The aim of this experiment was to see whether the results we obtained with threonine would extend to another essential amino acid, namely isoleucine.

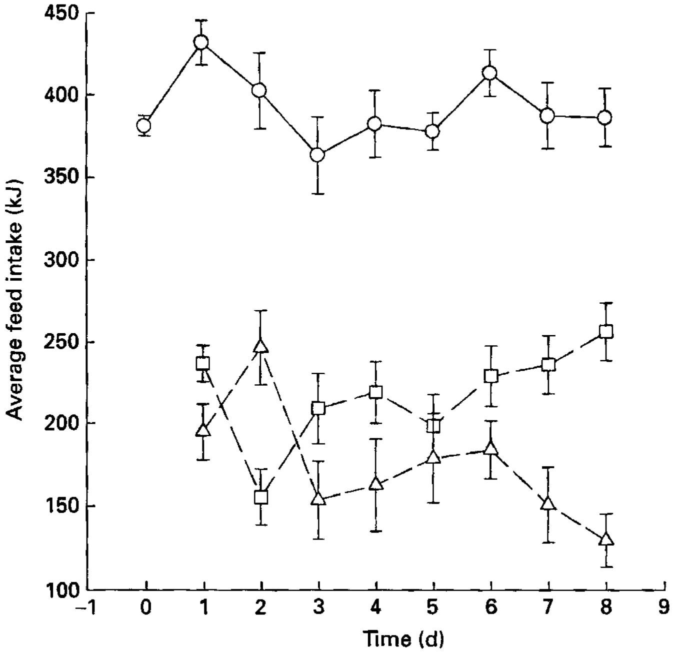

Fig. 8. Feed intake of rats given a choice between a protein-free diet containing 26 g isoleucine/kg (□), and a diet devoid of isoleucine (△). (○), Total feed intake. Until day 0 rats were fed on a low-protein diet (60 g casein/kg). The choice period began on day 1. Values are means for twelve rats, with their standard errors represented by vertical bars.

This experiment was similar to Expt 6 except that isoleucine replaced threonine. Twelve male albino rats (220–340 g initial weight) pre-fed on P6 %, were given a choice between an isoleucine-devoid diet (ILEU-DEV), and P0 % + 2·6 % ILEU. The isoleucine-supplementation of the protein-free diet was calculated so that if the rats ate 33 % P0 % + 2·6 % ILEU and 67 % ILEU-DEV, the resulting mixture would provide a diet well balanced for amino acids according to the composition of diets used in most investigations of this type (Naito-Hoopes *et al.* 1993).

During the 10 d when P6 % was offered the average feed intake was 381 (SEM 6·2) kJ and the daily body-weight gain was + 3·52 (SEM 0·43) g. During the following 8 d when a choice between P0 % + 2·6 % ILEU and ILEU-DEV was offered the average total feed intake was 393 (SEM 11·2) kJ and the daily body-weight gain was + 2·12 (SEM 0·57) g. The total daily feed intake of the two diets throughout the choice period was not different from that during the pre-feeding period (Fig. 8). During the choice period the variations from one day to another of intakes of both P0 % + 2·6 % ILEU and ILEU-DEV were not significant. On the overall experimental period rats ingested P0 % + 2·6 % ILEU in a proportion of 53·65 (SEM 5) % instead of the expected 33 % ($P < 0.001$) (Fig. 7). This proportion was also different from no choice (50 %) ($P = 0.003$).

Statistical comparisons. In Expt 6 the rats which had to choose between THR-DEV and P0 % + 1·2 % THR ate as much of the two diets as the rats in Expt 7 which had to choose between ILEU-DEV and P0 % + 2·6 % ILEU. However, less of THR-DEV was eaten by rats of Expt 6 than ILEU-DEV by rats of Expt 7 ($F(1, 182)$ 42·145, $P < 0.0001$).

Discussion. On each day of the experimental period rats ate the same proportion of the two diets offered to them. Rats ate more of the essential amino acid than expected according to the comparison used in most investigations of this sort (Gietzen *et al.* 1992). The average isoleucine intake was 14 g/kg diet against the 8·6 g/kg diet expected. Given that 1 kg P0% + 2·6% ILEU provided 26 g isoleucine, an intake of 100 g of the well-equilibrated mixture (ILEU-DEV 66·6% and P0% + 2·6% ILEU 33·3%) should have provided 0·86 g isoleucine.

GENERAL DISCUSSION

The present experiment shows that the rat has the ability to adjust its intake of dietary essential amino acids in order to optimize the quality of the protein ingested in terms of appropriate composition of essential amino acids. By achieving such a choice the animal not only balances its amino acid composition but also turns two aversive diets into one mixture that is no longer aversive.

As shown in our preliminary experiments, except for P6% all of the diets used in the present studies are both aversive and nutritionally inappropriate. Under our experimental conditions protein-free diets containing one essential amino acid (threonine in Expt 1 and isoleucine in Expt 2) are rather aversive. In all instances when rats were offered these diets they decreased their feed intake and lost weight during the 8 d of the experimental period. Our preliminary findings are in agreement with results in the literature. It is well-established that rats fed *ad libitum* on a protein-free or threonine-devoid or isoleucine-devoid diet show a drastic depression in their feed intake and a continuous loss of weight (Rogers & Leung, 1977). Sidransky & Miloslav Rechcigl (1962) compared the feed intakes of young rats fed on a protein-free diet or a threonine-devoid diet. A reduction in feed intake certainly occurred when these rats were fed on the protein-free diet but this hypophagia was less pronounced than when they were fed on the threonine-devoid diet (20% *v.* 50%) and weight losses were comparable in both groups. Leung *et al.* (1968) also showed that rats preferred a protein-free diet rather than a threonine-imbalanced diet. In Leung's experiment, also, rats lost weight during the entire 8 d experimental period. In agreement with these observations, our Expt 3 showed that, when offered a choice between a protein-free diet and a threonine-devoid diet, rats strongly preferred (93%) a protein-free diet. In all cases decreasing feed intake seems to be a way of preserving animals from pathologic consequences which are presumably more drastic when a threonine-devoid diet rather than a protein-free diet is fed.

Expt 6 showed that the decreased intake of the protein-free diet containing 12 g threonine/kg was not the result of a random choice. Whenever rats were offered another choice with obvious preferences to be made, they performed the appropriate choice. When offered a choice between a threonine-corrected diet and P0% + 1·2% THR rats selected the threonine-corrected diet, which is a nutritionally adequate diet, rather than the protein-free diet. These results agree with the observations of Leung *et al.* (1968) who found that rats prefer almost exclusively the corrected diet rather than a protein-free diet. Other choices are less obvious; when in Expt 4 rats were offered a choice between a diet devoid of threonine and a protein-free diet containing an amino acid other than threonine such as serine added in the same percentage as in the threonine version, they chose the latter. Yet, addition of serine does not replace threonine in the amino acid synthesis pathway and this preference cannot be accounted for by taste differences, not only because serine is tasteless but also because rats eat the same daily amount of P0% whether it is enriched or not with serine (Expts 3 and 4).

The main experiments show that the choice made by rats rebalances the amino acid composition of the mixture. However, the notion of rebalancing may vary depending on whether the ideal diet composition comes from one source or another. Concerning the balance of threonine, the 4·7 g/kg of this essential amino acid that the rats self-administered seems to be excessive with regard to the ideal amino acid composition that most of the previous investigators in this field have used (Leung & Rogers, 1985; Gietzen *et al.* 1992). However, according to the National Research Council (1978) standard the ideal mixture should rather have provided 5 g/kg threonine, an amount which is much closer to what our rats self-administered. As for the isoleucine rebalance, the 14 g/kg diet intake appears quite excessive, whether the ideal composition is considered to be closer to that used in the literature or that of the National Research Council (5 g/kg diet). In both cases the difference is not large and the excess of isoleucine is far from being toxic (Harper *et al.* 1970). The relatively excessive isoleucine intake cannot be accounted for by some taste improvement, first because the added amount of isoleucine (26 g/kg total diet) was too modest to change significantly the entire mixture's taste and second, if anything, the taste of isoleucine is rather bitter according to Schiffman & Engelhard's (1976) sensory analysis in humans (see also Fig. 1(*b*). The question of whether the essential amino acid requirements established by the National Research Council or others are more judicious than those chosen by the rat itself is open.

Reiterating some basic notions, we see that ingestion serves both quantitative and qualitative needs of the economy. The former insures the energy requirement by adjusting the intakes of macronutrients that will fuel energy production. The latter provides the right amount of micronutrients which will play a structural and functional role in the sophisticated machinery that does much more than providing energy. The adjustment of intake of macro- and micronutrients seems to obey distinct mechanisms, which although different, are interdependent. In omnivores, micronutrients usually, but not always, accompany the macronutrients. In this respect the essential amino acid-deficiency-induced anorexia indicates that a deficiency in an essential amino acid is more prejudicial than no amino acid at all and more prejudicial than insufficient fuel. In the course of producing the right mixture these animals experience new preference–aversion ratings of the coupled diets that are obviously different from the ratings they would have experienced in response to each of the conjugated diets if they were consumed separately. This finding raises the possibility that the sensory characteristics of a food may be totally different depending on whether each food's micronutrient composition is balanced or not and, if not, on whether their presentation is paired or not. In this respect the present experiments also show the remarkable finding that two individual aversive diets can result in a combination that is no longer aversive and this phenomenon may be responsible for unexpected adjustments of feed intake that deserve to be further explored.

REFERENCES

Gietzen, D. W. (1993). Neural mechanisms in the responses to amino acid deficiency. *Journal of Nutrition* **123**, 610–625.

Gietzen, D. W., McArthur, L. H., Thiesen, J. C. & Rogers, Q. R. (1992). Learned preference for the limiting amino acid in rats fed a threonine deficient diet. *Physiology and Behavior* **51**, 909–914.

Halstead, W. C. & Gallagher, B. (1962). Autoregulation of amino acids in the albino rat. *Journal of Comparative and Physiological Psychology* **55**, 107–111.

Harper, A. E. (1959). Sequence in which the amino-acids of casein become limiting for the growth of the rat. *Journal of Nutrition* **67**, 109–122.

Harper, A. E., Benevenga, N. J. & Wohlhueter, R. M. (1970). Effects of ingestion of disproportionate amounts of amino acids. *Physiological Reviews* **80**, 428–458.

Harris, L. J., Hargreaves, F. & Ward, A. (1933). Appetite and choice of diet. The ability of the vitamin B deficient rat to discriminate between diets containing and lacking the vitamin. *Proceedings of the Royal Society, Series B* **113**, 161–190.

Leung, P. M. B. & Rogers, Q. R. (1985). Effect of amino imbalance and deficiency on dietary choice patterns of rats. *Physiology and Behavior* **37**, 747–758.

Leung, P. M. B., Rogers, Q. R. & Harper, A. E. (1968). Effect of amino acid imbalance on dietary choice. *Journal of Nutrition* **95**, 483–492.

Naito-Hoopes, M., Gietzen, D. W., McArthur, L. H. & Rogers, Q. R. (1993). Learned preference and aversion for complete and isoleucine-devoid diet. *Physiology and Behavior* **53**, 485–494.

National Research Council (1978). Nutrient requirements of the laboratory rat. In *Nutrient Requirements of Laboratory Animals*. Washington, DC: National Academy of Sciences.

Potier de Courcy, G., Durand, G., Abraham, J. & Gueguen, L. (1989). Recommendations of the feeding conditions of the laboratory animals (rats and mice). CNERNA (Centre National d'Etudes et de Recommandations sur la Nutrition et l'Alimentation). *Science des Aliments* 209–217.

Richter, C. P. (1956). Salt appetite in mammals: its dependence on instinct and metabolism. In *l'Instinct dans le Comportement des Animaux et de l'Homme*, pp. 577–629 [M. Autuori, editor]. Paris; Masson et Cie.

Rodgers, W. (1967). Specificity of specific hungers. *Journal of Comparative and Physiological Psychology* **64**, 49–58.

Rogers, Q. R. & Harper, A. E. (1970). Selection of a solution containing histidine by rats feeding on an histidine imbalanced diet. *Journal of Comparative and Physiological Psychology* **72**, 66–71.

Rogers, Q. R. & Leung, P. M. B. (1977). The control of food intake: when and how are amino acids involved? In *Chemical Senses and Nutrition*, pp. 213–249, [M. R. Kare, editor]. New York: Academic Press.

Schiffman, S. S. & Engelhard, H. H. (1976). Taste of dipeptides. *Physiology and Behavior* **7**, 523–535.

Scott, E. M. & Quint, E. (1946). Self selection of diet: appetites for B vitamins. *Journal of Nutrition* **37**, 285–291.

Sidransky, H. & Miloslav Rechcigl, J. R. (1962). Chemical pathology of acute amino acid deficiencies: comparison of morphologic and biochemical changes in young rats fed protein-free or threonine diets. *Journal of Nutrition* **78**, 269–277.

Yamamoto, Y., Suzuki, M. & Murumatsu, K. (1985). Self selection of dietary threonine in the rat and the effect of taste stimuli on its selection. *Agricultural and Biological Chemistry* **49**, 2849–2859.

Printed in Great Britain