

**Dietary GABA and Food Selection by Rats (42229)**

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*Abstract.* To obtain further information pertaining to amino acid-induced alterations in feeding behavior, studies were performed to examine the food choices made by rats fed low protein diets made more or less aversive by the addition of various amino acids. When rats were allowed to choose between two diets, they preferred a low protein control, threonine-imbalanced or nonprotein diet to one containing 2.5%  $\gamma$ -aminobutyric acid (GABA). Acceptance increased when GABA content was lowered to 1.5%; rats preferred this diet when the alternative diet was made sufficiently aversive. There were large individual differences among rats selecting from pairs of unacceptable diets. Avoidance of, or preference for, a given diet is clearly affected by the relative aversive qualities of the offered pair of diets. © 1986 Society for Experimental Biology and Medicine.

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Many studies have shown that disproportionate amounts of amino acids in the diet can depress food intake and growth of rats (1). Such effects are apparently often accompanied by alterations in competition for amino acid transport from blood into brain (2). Studies on the ability of small neutral amino acids to alter food intake showed unexpectedly that dietary  $\gamma$ -aminobutyric acid (GABA) also reduces food intake and growth of rats fed a low protein diet (3); similar effects were subsequently found in lean and genetically obese mice (4), hamsters (4), and cats (5). However, there is no evidence to suggest that modification of transport of amino acids into brain by GABA is critical in its ability to alter feeding behavior. At present the mechanism by which GABA reduces food intake is unknown. The results with rats suggest that this avoidance of GABA-containing diets is quite strong; it is not overcome by adding an acceptable flavoring agent or by feeding the amino acid in the presence of a palatable food (6), implying that taste is probably not a critical factor. Even a low dietary level of GABA (1.5%) reduces food intake slightly. This effect on food intake occurs despite the fact that a single loading dose of GABA is rapidly cleared from the body, presumably via hepatic and renal GABA-aminotransferase activity as well as by urinary excretion (3).

These earlier studies have utilized single diets only and therefore no information is available concerning the relative aversive qualities of GABA-containing diets. In order

to investigate this point we have allowed rats to choose between GABA-containing diets and others already known to induce aversions of varying severities. We have also examined to what extent changes in the concentration of dietary GABA will alter subsequent food choices.

**Materials and Methods.** Male rats of the Sprague-Dawley strain (King, Oregon, Wis.) initially weighing 60-65 g were housed individually in metal cages with wire-mesh bottoms in a room lighted from 0700 to 1900 hr and maintained at about 24°C. The animals were fed *ad libitum* for the first 3-4 days the basal (control) diet in powder form, which contained (in grams): casein, 6.0; L-methionine, 0.2; L-threonine, 0.1; corn oil, 5; mineral mixture, 5 (7); vitamin mixture, 0.5 (7); choline chloride, 0.2; and equal amounts of cornstarch and glucose monohydrate to make 100 g; this diet is limiting in threonine. When the rats weighed about 70 g they were randomly divided into groups of six each and were offered choices *ad libitum* between various pairs of diets. Each of the diets used in the choice studies was prepared as an agar gel and tinted with food colors to permit easy identification (7); the diets were made by mixing 1 kg diet with 30 g agar dissolved in about 1100 ml boiling water. Amino acid supplements were added at the expense of the carbohydrates; casein, methionine, and threonine were omitted from the nonprotein diet. The diets were isocaloric (4.05 kcal/g dry diet). The refrigerated gel diets were cut into small blocks which were

weighed and randomly placed in the cages each day.

Body weights, food intakes, and food choices were recorded daily. Food consumption was calculated after daily determinations of differences between the dry weights of the initial and uneaten portion of the offered, agar gel diets; diets were dried to a constant weight in a vacuum oven.

The arcsin  $\sqrt{\%}$  transformation was applied to the data to determine if the amounts chosen from each of the two available diets were significantly different from 50% of the total daily intake. Bartlett's test was then applied to the residuals to determine homogeneity of variance (8) before performing analysis of variance (ANOVA) by the general linear models procedure with correction for repeated measures as necessary (9). When variances were heterogeneous, the *t* test for samples with nonhomogeneity of variance was utilized (8). Further details are given in the legends to the figures.

**Results.** These experiments were designed to determine (i) the approximate concentration of GABA at which rats will no longer clearly avoid selecting a diet containing this amino acid; and (ii) if a choice of diets can be offered such that a GABA-supplemented diet would be selected preferentially over certain other aversive diets.

In our initial experiment we examined the selections made by rats allowed choices between the agar gel forms of the nonprotein diet, the control low protein diet, or this diet containing 4.5% GABA, a level previously shown to induce striking depressions in food intake and growth (3). The results (not shown) indicated that, after the first 4 days, the rats completely avoided the GABA diet when the choice was between this and the basal diet; during a 9-day period these rats chose a total of only 6.4 g of the GABA diet while selecting an average of 82 g of the basal diet ( $P < 0.001$  for the difference between intakes of the two diets). Rats offered a choice between the GABA and nonprotein diets also ate little or none of the former diet after the fourth day. Total consumption for 9 days was 6.2 g of the GABA diet and 47 g of the nonprotein diet ( $P < 0.001$ ).

In the following experiment the GABA concentration was reduced to 2.5% of the diet, a level which also reduces food intake and

growth in a nonchoice situation. The diet choices were again between pairs of diets prepared as agar gels: (i) basal (control) vs a 2.5% GABA-supplemented diet; (ii) basal vs threonine imbalance (the imbalanced diet contained 4.5% of a threonine-devoid mixture of the other indispensable L-amino acids plus cystine; this mixture was equimolar in valine, isoleucine, leucine, phenylalanine, arginine-HCl, lysine-HCl, and histidine, and half this molarity in tryptophan, methionine and cystine); (iii) basal vs nonprotein; (iv) nonprotein vs threonine imbalance; (v) nonprotein vs 2.5% GABA; (vi) 2.5% GABA vs threonine imbalance. The rats again showed a strong preference for the basal instead of the GABA diet (Fig. 1A). At this level of GABA, most of the rats ate some of the GABA diet on each day of the experiment. Overall, they chose to eat only about 15% of their total 10-day intake from the GABA diet ( $P < 0.001$ ). One rat ate the GABA diet exclusively during the final 3 days of the experiment but only in amounts which maintained body weight, while two others ate none of this diet during the same period. The rats also showed a strong preference for the basal diet instead of either the threonine-imbalanced diet (Fig. 1B) or the nonprotein diet (Fig. 1C). The rats chose from the imbalanced or nonprotein diets only 8 or 6%, respectively, of their total 10-day intake ( $P < 0.001$  when compared with the corresponding value for the basal diet).

The rats did not make markedly different choices between two diets when both were unacceptable. There was a statistically nonsignificant trend toward a preference for the threonine-imbalanced diet rather than the nonprotein diet as the experiment progressed (Fig. 1D). However, selections by individual rats varied considerably [ $F(4,36) = 23.54$ ,  $P < 0.001$ ]; for example, one of the rats chose none of the nonprotein diet after Day 2, whereas another rat ate only the nonprotein diet on all but Day 2. Of the total intake for the 10-day experiment 27% was selected from the nonprotein diet (NS). Except for Day 1, the rats tended to choose greater amounts of the nonprotein than of the GABA diet (Fig. 1E). These rats selected 73% of their total intake from the nonprotein diet ( $P < 0.05$ ). Although Fig. 1B shows that the rats almost completely avoided the threonine-imbalanced

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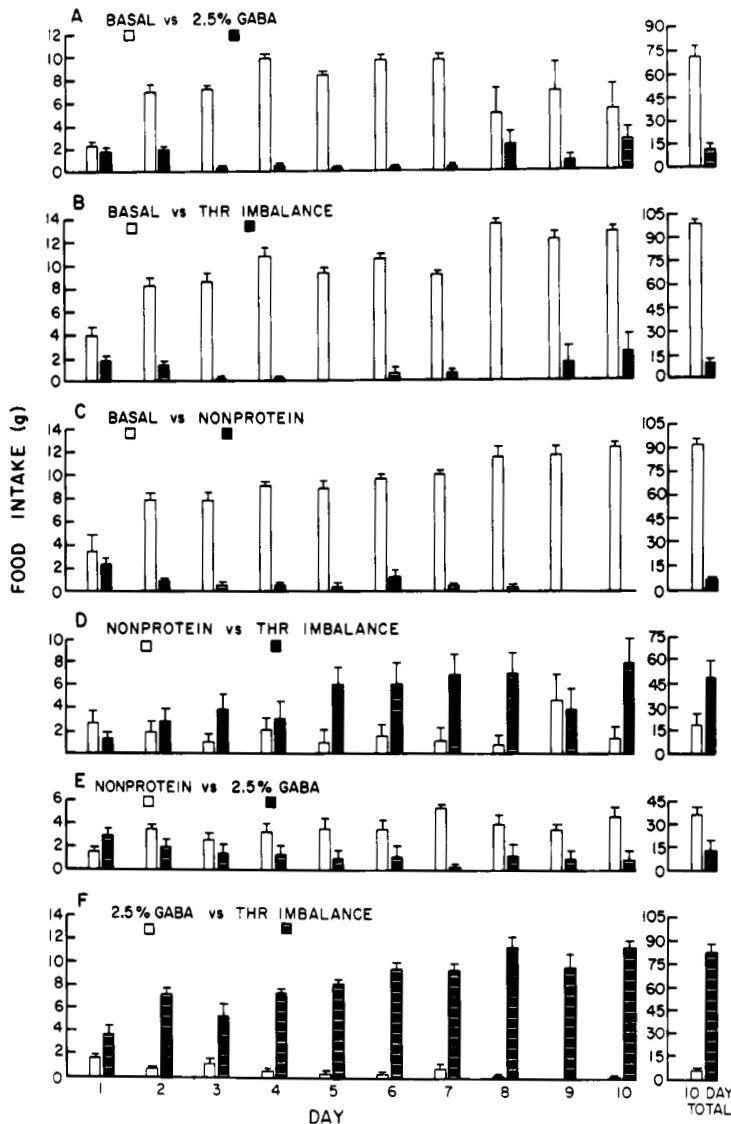


FIG. 1. Daily food intakes of rats offered choices between a basal (control) diet or diets containing 2.5% GABA, a mixture of indispensable amino acids (threonine-imbalanced diet), or no protein. The amino acid mixture added to produce the imbalance is described in the text. Choices are shown by the sets of two bars each; results are expressed as means  $\pm$  SE for six rats per group (five in group D). Variances were nonhomogeneous for all but group D; significant differences between amounts of food chosen by groups A-C, E, F were determined with the *t* test as described under Materials and Methods. Initial body weight was  $70.0 \pm 0.4$  g. Final weights were Group A,  $92.3 \pm 2.7$ ; B,  $108.7 \pm 2.6$ ; C,  $98.8 \pm 1.9$ ; D,  $85.6 \pm 6.3$ ; E,  $61.2 \pm 2.2$ ; and F,  $103.5 \pm 3.1$ .

diet when a more acceptable diet was also available, Fig. 1F clearly shows that, when the choice was between the imbalanced and the GABA-supplemented diet, except for Day 1, they selected far more of the imbalanced diet

than of the GABA-supplemented diet; during the 10-day experiment the rats chose less than 7% of their total intake from the GABA diet ( $P < 0.001$ ). After Day 2 some rats completely refused the GABA diet.

Only the rats which had to select from the nonprotein and GABA-supplemented diets (Fig. 1E) did not grow; final body weights are given in the legend to Fig. 1.

Although the rats apparently found the 2.5% GABA diet to be somewhat more acceptable than one containing 4.5% GABA, the former diet was also largely avoided. Therefore, a further choice study was carried out with GABA at 1.5% of the diet, a concentration which induces only small reductions in food intake and growth. In this trial (Fig. 2) the choices were between a diet containing 1.5% GABA and (i) the basal diet; (ii) a nonprotein diet; (iii) the threonine-imbalanced diet described above; or (iv) the threonine-imbalanced diet plus 1.5% GABA. When rats had access to both the 1.5% GABA and the basal diet, they usually chose more of the basal diet, but almost always ate some of the GABA-supplemented diet as well (Fig. 2A); only rarely did a rat refuse to eat any of the GABA diet. During the 10-day experiment the rats selected a total of 27 and 73% of their intakes from the experimental

and basal diets, respectively ( $P < 0.005$ ). Thus, under these conditions the threshold for avoidance of dietary GABA probably occurs at some concentration slightly below 1.5% of the diet.

When the rats chose between the GABA-supplemented diet and the nonprotein diet, the daily average intakes of the former diet were always higher (Fig. 2B); however, the difference between total intakes was not significant because selections by individual rats varied greatly [ $F(5,45) = 36.45$ ,  $P < 0.001$ ]. For example, two of the rats completely avoided the nonprotein diet after the second day while two others ate such small amounts of the GABA diet that they showed a net weight loss. During the 10-day experiment this group of rats chose a total of 64% of their food from the GABA-supplemented diet and 36% from the nonprotein diet; there was no significant change over time in the diet choices.

Overall, the rats showed little preference for either diet when they had to choose between the 1.5% GABA-supplemented and threonine-

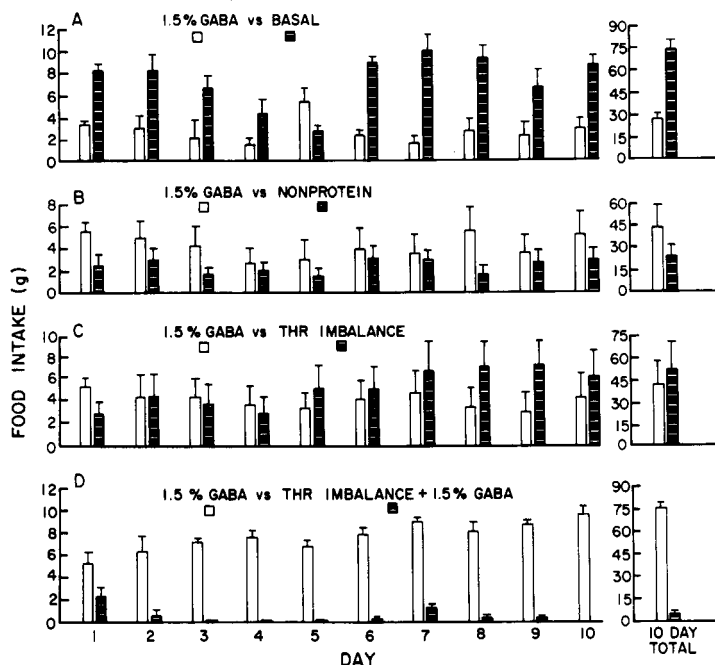


FIG. 2. Daily food intakes of rats offered choices between a diet containing 1.5% GABA and a control or nonprotein diet or one having a threonine imbalance. Results are expressed as means  $\pm$  SE for six rats per group; variances were nonhomogeneous for groups A and D. Other details are given in Fig. 1. The mean initial weight of the rats was  $70.1 \pm 0.3$  g. Final body weights were Group A,  $103.2 \pm 4.2$ ; Group B,  $78.8 \pm 7.4$ ; Group C,  $94.0 \pm 3.0$ ; and Group D,  $102.7 \pm 4.4$  g, respectively.

imbalanced diets; they consumed a total of 44 and 56%, respectively, from these diets (Fig. 2C). However, there were large differences between individual rats, as each one usually selected most of its total intake from one or the other of the two diets throughout the 10-day experiment [ $F(5,45) = 66.41, P < 0.001$ ]. Selections did not change significantly over time. When GABA (1.5%) was also included in the threonine-imbalanced diet, rats ate almost exclusively the diet containing GABA alone, especially after Day 2 (Fig. 2D). During the 10-day experiment the rats chose 94% of their total food from the 1.5% GABA diet ( $P < 0.001$ ).

Only the rats choosing between the non-protein and GABA-supplemented diets (Fig. 2B) grew poorly; final body weights are shown in the legend to Fig. 2.

**Discussion.** The results of these studies confirm and extend our earlier observations that dietary GABA, when added to a low protein diet, can strongly reduce food intake and growth of rats and other animals (3-5). These effects of GABA differ from those seen when similar or even larger concentrations of other small neutral amino acids are added to a low protein diet. For example, supplementation with serine, alanine, and glycine in amounts equimolar to 4.5% GABA does not depress food intake and growth of rats if appropriate amounts of the indispensable amino acids are provided by the diet (2). Sauberlich (10) found that low protein diets containing 5% of amino acids such as alanine, glutamate, proline, serine, and threonine had either no effect on growth or decreased weight gain by less than 30%. Only a few of the tested amino acids produced growth depressions as severe as we have found with 4.5% GABA (e.g., methionine, the only amino acid which caused a weight loss; and histidine, tryptophan, tyrosine, and phenylalanine which depressed growth by 65 to 80%). We have found that adult rats offered a choice between a low protein diet supplemented with alanine (equimolar to 2.5% GABA) and the corresponding control diet selected 75% of their food from the former diet. However, when these rats selected from the control diet and one supplemented with threonine, they chose only about 25% of their food from the latter diet (11). Thus, clear differences occurred in the feeding behavior of rats choos-

ing between a control diet and one containing supplements of various small neutral amino acids.

The present studies also showed that, when rats were allowed to choose between two diets, the one containing 2.5 or 4.5% GABA was avoided even when the other diet was inadequate. In general, the results suggest that these GABA-containing diets were the least preferred of all the diets. The preference for the inadequate, nonprotein diet occurred despite the fact that even the 4.5% GABA diet will support growth of those few rats that will eat it under normal conditions. Similarly, when rats were allowed to choose between a non-protein diet and one with a severe amino acid imbalance they again avoided the latter diets which also can support growth (1). As the rats preferred the threonine-imbalanced diet to the one containing 2.5% GABA, our results suggest that this GABA diet is even more aversive than the imbalanced diet used in these studies. In essence, the degree of aversion to the threonine-imbalanced diet depends upon the alternate choice available.

The results also show that rats could be induced to select almost exclusively the 1.5% GABA diet when the aversive characteristics of the alternate diet were augmented. In this situation the rats consumed enough of the 1.5% GABA diet to grow as well as those choosing between the control and GABA diets, but eating little of the latter diet. However, rats selecting from the 1.5% GABA and non-protein diets grew poorly, presumably because their intake of the GABA (protein-containing) diet was clearly low. It has also been possible to induce rats to eat a 4.5% GABA diet in single diet studies in which the animals were adapted to a high protein diet; the 4.5% GABA diet was also eaten when rats were fed a cellulose-diluted, GABA-containing diet or were housed in the cold and therefore had to increase their food consumption in order to maintain body weight and survive (6). It is not known if rats will choose a 4.5% GABA diet under otherwise normal conditions if the alternate diet is even more aversive.

A striking aspect of these studies is the extent to which the rats showed individual behavior, especially those represented in Fig. 1D, 2B, and 2C. Such results imply that a diet containing GABA becomes unacceptable to dif-

ferent rats at different concentrations of the amino acid. However, it is not clear why rats almost invariably avoid consuming GABA-supplemented diets under normal conditions. Furthermore, rats will not consume normal amounts of a highly palatable diet or one containing an acceptable flavoring agent when such diets are supplemented with GABA (6). Our results indicate that this aversion to GABA is not because the amino acid is inherently toxic. The deleterious effects of GABA on food intake and growth occur despite the fact that the amino acid is rapidly cleared from the body by excretion in the urine and by metabolism to CO<sub>2</sub> (3). Odor does not appear to be critical (11). Studies in which GABA was fed in a high protein diet (3) or was injected intraperitoneally (unpublished results) suggest that the taste of this amino acid is also not important.

Direct effects on the central nervous system seem unlikely because GABA does not cross the blood-brain barrier (12). It is possible that this amino acid causes the animal to feel ill. The gut contains GABA receptors and transport sites; motility can be affected by blockade of the receptors (13). The release of gastrin and somatostatin from rat antral mucosa *in vitro* can also be modified by GABA (14). Regardless of the mechanism by which dietary GABA acts to depress food intake, our studies imply that there are distinct differences in the means by which dietary amino acids modify feeding behavior.

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