Importance of prepyriform cortex in food-intake response of rats to amino acids

PHILIP M.-B. LEUNG AND QUINTON R. ROGERS

Department of Physiological Sciences, School of Veterinary Medicine, University of California,
Davis, California 95616

Leung, Philip M.-B., and Quinton R. Rogers. Importance of prepyriform cortex in food-intake response of rats to amino acids. Am. J. Physiol. 221(3): 929-935. 1971.—Bilateral electrolytic lesions were placed in the prepyriform cortex of male 250- to 250-g rats and their food intake as affected by amino acid imbalance, deficiency, and excessive protein was examined. All rats with such lesions and groups of intact controls were fed in turn a basal, imbalanced (with either threonine or isoleucine as the limiting amino acid), or deficient diet (devoid of either threonine or isoleucine), and finally the low- or high-protein (6% or 75% casein) diet. No change in food intake was observed in rats fed the control diet after the placement of the lesions. However, animals with lesions in certain areas of the anterior prepyriform cortex showed a significant increase in food intake as compared to the intact controls fed the imbalanced or deficient diets, but the decrease in food intake was still evident in rats fed the high-protein diet. Intact animals select a protein-free diet over an imbalanced diet, whereas the rats with lesions in certain areas of the anterior prepyriform cortex chose the imbalanced diet over the protein-free diet. The altered choice between the basal and the imbalanced or deficient diets of the rats with lesions in the critical prepyriform cortical areas suggests that these areas are involved in the regulation of food intake of rats fed amino acid-imbalanced or deficient diets.

food-intake regulation; amino acid imbalance and deficiency; protein; prepyriform lesions; dietary choice

AMINO ACID IMBALANCES or amino acid deficiencies (diets devoid of one of the indispensable amino acids) depress food intake and cause weight loss of young and adult animals (7, 12, 13). An amino acid imbalance is most readily created by the addition of a surplus of all but one of the indispensable amino acids to a low-protein diet or to diets containing free amino acids as the protein source (7, 12). One of the earliest biochemical or metabolic effects of an amino acid imbalance or deficiency is the change in plasma amino acid pattern, especially the lowering of the most limiting amino acid shortly after the animals are fed such diets (5, 15). It has been postulated that these alterations in plasma amino acid patterns affect, directly or indirectly, a food-intake regulatory mechanism which ultimately curtails the voluntary intake of the imbalanced or deficient diets (8).

The probable involvement of the central nervous system in the regulation of food intake of animals fed amino acid-imbalanced diets has been demonstrated in our previous studies (12), in which the food-intake depression of rats fed amino acid-imbalanced diets was prevented by the infusion of a small amount of the most limiting amino acids into the carotid artery, but was not prevented when infused into the jugular vein of the animals. These experiments suggest that some area of the brain must be sensitive to the concentration of the growth-limiting amino acid in the blood.

Although it has been suggested that food intake of animals is regulated primarily by hypothalamic mechanisms (1, 17), extrahypothalamic neural areas have also been reported to be involved in the regulation of food intake (6, 18). Studies from our laboratory and others (13, 24) have shown that the intact ventromedial hypothalamic nuclei (ablation of which causes hyperphagia) are not essential in the regulation of food intake of animals fed amino acid-imbalanced or deficient diets. That is, lesions in these areas did not prevent the deleterious effects of amino acid imbalance (13, 24) or deficiency (13) on food intake of animals in the dynamic (13) or static (24) phase of hyperphagia. Dietary excesses of individual amino acids and high levels of dietary protein also depressed food intake of animals (2, 7). The inhibition of food intake of rats fed such diets has also been shown to be independent of the ventromedial hypothalamic satiety areas (10, 11, 24). Also “finickiness” or the low palatability of the amino acid or the high-protein diet has been shown not to be the cause for the sensitivity of hypothalamic hyperphagic rats to such diets (13, 24).

Thus, it becomes apparent that areas outside the ventromedial hypothalamic nuclei are responsible for providing satiety signals leading to the curtailment of food intake of animals fed diets containing disproportionate amounts of amino acids. In recent years, the various neural circuits interlinking the hypothalamus with other key brain areas has been defined in detail by morphological studies (20, 21). Especially pertinent to us are the connections of the prepyriform cortex and the amygdala to the lateral hypothalamic “feeding area.” The present studies were undertaken to examine extrahypothalamic influence, in particular, the prepyriform cortical area, on the food intake regulation of animals fed amino acid-imbalanced or deficient diets or diets containing a high level of protein.

MATERIAL AND METHODS

Male, Sprague-Dawley rats, 147 animals weighing 230-250 g, were used in the experiments. The rats were fed commercial rations (Purina laboratory chow) for 3 days to allow them to adapt to the environment before feeding the purified experimental basal diets containing only free amino
acids as the protein source. In experiment I, a threonine basal diet, in which threonine was the most limiting amino acid, was fed prior to the lesion placements, while in experiment II, the isoleucine-basal diet (isoleucine is the most limiting amino acid) was used. Food intake of animals consuming the basal diets were measured for at least 7 days (at such time it became relatively constant) before bilateral electrolytic lesions were placed in the prepyriform cortex of the animals according to the stereotaxic coordinates of De Groot (3). The lesions were produced by passing a direct current of 2 mA for 15 sec through the tip of a stainless steel electrode insulated with Epoxylite except at the tip. Animal groups are denoted by anterior (A) coordinates, e.g. A8.6 is one group name (see Tables 3 and 4 for detailed coordinates and number of animals used in each experiment). All animals (in both experiments) were fed the appropriate basal diets immediately after the placement of lesions and allowed to recover until their food intake returned approximately to their preoperative levels. Then daily food intake was measured for at least another 7 days before the start of the dietary switching process involving feeding consecutively the imbalanced, the deficient, or the high-protein diets. Two groups of intact rats of about the same size and age (15 and 10 animals in experiments I and II, respectively) served as controls and followed the same dietary substitutions as the animals with lesions. The dietary switching schemes in both experiments are shown in Table 1. The complete composition of the threonine- or isoleucine-basal, imbalanced, and deficient diets has been reported elsewhere (12, 13). All experimental diets except those involving proteins contained free L-amino acids as the protein source. The imbalanced diets were prepared by adding a mixture of indispensable amino acids (9.8% and 4.9%, respectively, for threonine and isoleucine imbalanced mixtures) lacking the most limiting amino acid, threonine or isoleucine, to the respective basal diets. The threonine- or isoleucine-deficient diet had the same composition as the threonine- or isoleucine-imbalanced diet, except that one free amino acid (threonine or isoleucine) was completely deleted from the amino acid diet.

All animals were individually housed in an air-conditioned room of approximately 24°C. They had free access to water and experimental diets at all times. Daily food intake was carefully measured and corrected for any spillage.

At the end of the experiment, animals with lesions were anesthetized with sodium pentobarbital (Diabutal) and killed by perfusion through the hearts with 0.9% saline followed by 10% Formalin. After the brains were completely fixed, they were embedded in paraffin and sections of 7-μm thickness were cut through all areas with lesions. Every 14th section was mounted and stained with alum hematoxylin and eosin and examined to locate the intended structures followed mainly the atlas of König and Klippel (9). Since intact animals select a protein-free diet over an amino acid-imbalanced diet (16), some of the animals with prepyriform lesions, which did not diminish their food intake of the imbalanced diets, were tested to see if they would distinguish between a protein-free diet and the threonine- or isoleucine-imbalanced diet.

### RESULTS

The food intake of intact rats (five per group) (about the same size and age of the animals used in the dietary substitution studies), fed only the threonine or isoleucine-deficient diet is shown in Table 2. Marked food intake depressions were observed in animals fed the threonine- or isoleucine-deficient diet (33% and 19% of the basal control values, respectively). The food intake of rats fed the deficient diets remained low at all times even after prolonged feeding (5 weeks).

The average daily food intake (7 days) of all groups of animals with lesions in various prepyriform cortical areas, consuming the threonine-basal diet before and after the operation prior to the dietary switching process was similar (Table 3). The intact controls, likewise, showed no difference in food intake during the same experimental periods. The average daily food intake patterns, expressed as percentages of respective base-line control values (averages of basal diet consumptions 4 days prior to the dietary substitutions) of the groups A8.6, A9.0, A9.4, A9.6 and the intact controls, as affected by the ingestion of the threonine-imbalanced or deficient diet are shown in Fig. 1. The base-line control

### Table 1. Sequence of diets fed to intact rats and rats with prepyriform lesions

<table>
<thead>
<tr>
<th>Day</th>
<th>Animal Groups</th>
<th>Th</th>
<th>Thr</th>
<th>Thr</th>
<th>Thr</th>
<th>Def.</th>
<th>Def.</th>
<th>Def.</th>
<th>Def.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Intact</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>A8.6, A9.0, A9.4, A9.6 (L 4.0)</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>A9.0, A9.8 (L 3.0) A10.0, A10.2</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II Intact</td>
<td>A9.9, A10.2</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* See footnote 1. † See footnote 2.

### Table 2. Average daily food intake of intact rats as affected by amino acid-deficient diets

<table>
<thead>
<tr>
<th>Dietary Regimen</th>
<th>Food Intake, g/day</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Threonine basal</td>
<td>16.2</td>
<td>17.6</td>
</tr>
<tr>
<td>Threonine deficient</td>
<td>5.4</td>
<td>5.6</td>
</tr>
<tr>
<td>Isoleucine basal</td>
<td>14.8</td>
<td>15.6</td>
</tr>
<tr>
<td>Isoleucine deficient</td>
<td>2.8</td>
<td>2.0</td>
</tr>
</tbody>
</table>

No. of rats per group = 5. See ref. 13 for amino acid-deficient diet.

### Table 3. Average daily food intake of intact rats as affected by amino acid-deficient diets

<table>
<thead>
<tr>
<th>Group</th>
<th>Food Intake, g/day</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>A8.6</td>
<td>16.2</td>
<td>17.6</td>
</tr>
<tr>
<td>A9.0</td>
<td>5.4</td>
<td>5.6</td>
</tr>
<tr>
<td>A9.4</td>
<td>14.8</td>
<td>15.6</td>
</tr>
<tr>
<td>A9.6</td>
<td>2.8</td>
<td>2.0</td>
</tr>
</tbody>
</table>

No. of rats per group = 5. See ref. 13 for amino acid-deficient diet.
values for these groups were 21.3, 22.7, 19.3, 17.6, and 18.5 g/day, respectively. The food intake of all groups of animals with lesions except group A9.6, exhibited similar initial depression in food intake of about 40-50% as did the intact controls. When the threonine-deficient diet was fed later, the groups A9.8 (L 4.0 and 3.0) with 1st day food-intake of 69.2 ± 4.5 and 65.9 ± 3.5, respectively, again exhibited significant elevation in food consumption (P < .01 in both cases) compared with that of the intact controls (51.6 ± 2.6). Figure 3 illustrates the food-intake patterns of some of the individuals or groups of animals with lesions in A9.6, A9.8 (L 4.0 and 3.0), or A10.0 area that showed little or no depression in food intake of the threonine-imbalanced or deficient diet.

At the end of the substitution of the threonine diets, the intact controls and animals with prepyriform lesions were fed the isoleucine-basal diet for 7 days before they were switched to the isoleucine-imbalanced or deficient diet (see dietary scheme of experiment I in Table 1). Again, all animals with lesions except those in groups A9.6 and A9.8 (L 1.4.0 and 3.0) reduced markedly their food intake of the isoleucine-imbalanced or deficient diet as did the intact controls. These results are not shown here.

The food intake of animals in groups A8.6, A9.0, A9.4, and A9.8 (L 4.0) as well as the intact controls fed the 75%
P, M.-B. LEUNG AND Q. R. ROGERS

However, all animals with lesions showed a slight decline in food intake of the isoleucine-deficient diet by the end of the feeding period.

Histological materials of animals in both experiments I and II that exhibited little or no depression in food intake of the imbalanced or deficient diets were carefully examined.

TABLE 4. Average daily food intake of rats with prepyriform lesions fed isoleucine-imbalanced or deficient diet

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>No. of Animals</th>
<th>Isoleucine basal</th>
<th>Isoleucine imbalanced, days</th>
<th>Isoleucine deficient, days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-operative</td>
<td>Post-operative</td>
<td></td>
</tr>
<tr>
<td>A9.81</td>
<td>7</td>
<td>21.6</td>
<td>20.5</td>
<td>84.4 ± 5.9</td>
</tr>
<tr>
<td>(L3. D0)</td>
<td></td>
<td></td>
<td></td>
<td>105.9</td>
</tr>
<tr>
<td>A10.2</td>
<td>5</td>
<td>19.0</td>
<td>20.2</td>
<td>84.1 ± 4.9</td>
</tr>
<tr>
<td>(L3. D0)</td>
<td></td>
<td></td>
<td></td>
<td>109.9</td>
</tr>
<tr>
<td>Intact</td>
<td>10</td>
<td>22.8</td>
<td>22.9</td>
<td>57.0 ± 6.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>80.3</td>
</tr>
</tbody>
</table>

* See ref. 12. † Values for ingestion are derived from daily average of 7-day periods. ‡ Respectively, anterior, lateral, and vertical depth coordinates in millimeters according to the stereotaxic coordinates of deGroot (3).

3 Composition of diet (in percentage): casein, 6; corn oil, 5; minerals (23), 5; vitamins (vitamin diet fortification mixture, Nutritional Biochemicals Corporation, Cleveland, Ohio), 1.0; choline chloride, 0.1; L-methionine, 0.3; and starch and sucrose in 2-to-1 ratio to make up to 100%.

3 Composition: same as 6% casein basal diet except casein was added at a level of 75% of diet at the expense of the carbohydrate and no methionine was added.

FIG. 4. Food intake of prepyriform lesioned rats fed 75% casein diet (see Table 3 for the number of animals used).

FIG. 5. Cross sections of representative prepyriform cortical lesions (A 9.6 and A 9.8, L 3.0) in animals that exhibit little or no depression in food intake of the imbalanced or deficient diets. A: from group A9.6. B: from group A9.8 (L 3.0).
and compared with those of other animals that showed the usual depression in food intake of such diets to locate the effective lesions in the cortical areas of the prepyriform cortex. Representative sections showing the areas of effective lesions are presented in Fig. 5. Figure 6 illustrates the composite representation of these areas (shaded). An example of non-effective lesions is shown in Fig. 7.

The results of the dietary choice studies involving the threonine- or isoleucine-imbalanced diet and a protein-free diet are shown in Fig. 8. Intact animals (five rats per group), as observed previously (16), selected the protein-free diet over the imbalance diets; whereas, after the first day of feeding, animals with lesions in A10.0 areas of the prepyriform cortex chose the threonine-imbalanced or the isoleucine-imbalanced diet over the protein-free diet. Also, animals with lesions in A9.8 area likewise selected the isoleucine-imbalanced diet over the protein-free diet.

**DISCUSSION**

Our results indicate that the extent to which rats discriminate between the threonine- or isoleucine-basal and the imbalanced or deficient diets is either abolished or diminished in animals with lesions in the anterior portion (A9.6–A9.8 L 4.0 and 3.0) of the prepyriform cortex. This is the first instance, to our knowledge, that the prepyriform cortex is involved in the discrimination of these diets.

---

**FIG. 6.** Outline of cross section of anterior prepyriform cortex showing effective bilateral lesions (shaded areas), identified according to stereotaxic atlas of König and Klippel (9). A: A9.6, B: A9.8.

**FIG. 7.** Cross sections of sample prepyriform cortical lesions in animals that showed similar food-intake depression of imbalanced or deficient diets as did intact controls (from group A9.6).
cortical area has been shown to be important in the regulation of food intake of animals fed a diet deficient in a single nutrient. These results are consistent with our previous suggestion (12), that some part of the brain or central nervous system reacts to the metabolic response caused by feeding amino acid-imbalanced diets and, therefore, is important in the regulation of food intake. It would appear that the signal is the circulating level of the growth-limiting amino acid since the infusion of small amounts of the limiting amino acid into the carotid artery prevented the food-intake decrease of rats ingesting amino acid-imbalanced diets, whereas a similar infusion into the jugular vein produced no effect on food intake. Our previous studies have also shown (13) that animals with hypothalamic lesions, even in their dynamic phase of hyperphagia, reduced their intake of the amino acid-imbalanced or deficient diets. Thus, the ventromedial hypothalamic nuclei (satiety center) does not appear to be responsible for the curtailment of the voluntary intake of animals fed amino acid-imbalanced or deficient diets. Similar food-intake depression of such diets was observed when obese hyperphagic rats were brought back to their dynamic phase of hyperphagia, and these effects were not due to the finickiness of these animals to such diets (13). Scharrer et al. (24) have also reported inhibition in food intake of hypothalamic hyperphagic rats fed amino acid-imbalanced diets during their static phase of hyperphagia.

The fact that lesions in the prepyriform cortical areas did not alter the food intake of animals consuming the threonine-or isoleucine-basal diet, which was relatively balanced with respect to their amino acid components, and that lesions in the critical prepyriform areas (A9.6 and A9.8, in particular) raised the food intake of animals fed amino acid-imbalanced or deficient diets, indicates that this portion of the prepyriform cortex may be involved in metering the balance of amino acids in the blood (diet), especially one deficient with respect to growth. Since the animals with effective prepyriform lesions that failed to curtail the food intake of the imbalanced or deficient diets exhibited marked depression in the consumption of the high-protein diet (balanced pattern of amino acids in excess), it would appear that the specific prepyriform area in question may be responsive in particular to an amino acid deficit but not to a surplus of amino acids. Other mechanisms must therefore be involved under situations of amino acid excess.

The delay and thereafter slow decline in food intake of animals with prepyriform lesions (A9.6 and A9.8) and fed the deficient diets (see especially the results of the rats fed the isoleucine-deficient diet in Table 9) may be a result of secondary effects, since a deficient diet, when force-fed, causes the development of pathological lesions (25, 26). These secondary noxious effects could serve to trigger other alternative homeostatic protective mechanisms, inhibiting the further consumption of large quantities of the deficient diets.

Although the effective prepyriform lesions are adjacent to or, in some rats, infringe upon the lateral olfactory tracts, it is highly unlikely that olfactory deficit is the cause of the differences observed between the food intake of the intact and animals with prepyriform lesions fed amino acid-imbalanced or deficient diet since olfactory bulbectomized rats reduced their food intake just as much as intact animals fed the same imbalanced or deficient diets (11). The aversion of the intact animals toward the imbalanced or deficient diets cannot be explained solely on the basis of taste characteristics of such diets because of the presence of large quantities of free amino acids in both the imbalanced and corrected diets (13). That is, both normal and hyperphagic (finicky) rats drastically reduce their food intake of the isoleucine-imbalanced or deficient diet, and yet a small amount of additional isoleucine increases markedly their consumption of the same diet (13). Even though the prepyriform-lesioned animals exhibited an altered choice, these animals did not consume indiscriminately the experimental diets with different taste characteristics, but effectively chose the imbalanced diet. It, therefore, seems unlikely that the increase in food intake of the animals with the effective prepyriform lesions is a result of the abolishment of the sense of smell or taste.

It still appears likely, therefore, that the preference of the intact animals for the protein-free diet, and their rejection of the imbalanced diet is based on the different metabolic responses of the two diets (13). That is, the ingestion of the imbalanced diet would give rise to an abnormal plasma amino acid pattern, while the protein-free diet, though nutritionally inadequate, results in restoration of plasma amino acid pattern to normal (15).

Learned aversions under unfavorable gustatory or specific vitamin-deficiency conditions have been reported in dietary choice studies (4, 22). However, it is difficult to envisage that the increase in food intake of the imbalanced or deficient diets, observed in animals with effective prepyriform lesions as compared with the intact controls, is a result of the abolishment of the learning process, since the lesions did not cause the prepyriform-damaged animals to consume the experimental diets without any preference and the rats did decrease their intake of the high protein diet. The most plausible explanation for the enhanced food intake of the rats fed the imbalanced or deficient diets is that the effective prepyriform lesions destroy or damage a receptor or part of the food intake regulatory mechanism that normally responds to the imbalanced amino acid patterns. This is in agreement with our previous observations that animals will choose a nutritionally adequate diet with balanced amino acid constituents over the protein-free diet (16); and is consistent with the results of the present experiment in which the animals with prepyriform lesions rejected the protein-free diet which is nutritionally inferior, and selected the amino acid imbalanced diet.

Extrahypothalamic neural areas such as the amygdaloid complex have also been reported to affect the regulation of food intake of animals (6, 19). It has been suggested that these areas may be involved in inhibitory mechanisms directed towards the lateral hypothalamic feeding area since it has been shown that a close functional relationship exists between the hypothalamus and the rhinencephalic complex including the prepyriform cortex (21). The critical anterior prepyriform cortical areas may well send signals which inhibit the lateral hypothalamic feeding area in the face of amino acid imbalance or deficiency and thus affect the voluntary intake of such diets. The destruction of certain areas in the medial amygdala with bilateral electrolytic lesions prevented the depression in food intake of rats fed amino acid-imbalanced diets but not that of the completely deficient diet (unpublished observations). Work is continu
ing in an effort to determine the extent of the involvement of the amygdaloid complex in the mechanism inhibiting food intake of rats fed amino acid-imbalanced or deficient diets.

The exact mechanism whereby the satiety signal is mediated or modulated in animals fed amino acid-imbalanced or deficient diet awaits elucidation.

REFERENCES