Effect of ammonia via prepyriform cortex on regulation of food intake in the rat

KATUHIKO NODA AND KAZUMASA CHIKAMORI
Faculty of Education and School of Medicine, Tokushima University, Tokushima, Japan

NODA, Katuhiko, and Kazumasa Chikamori. Effect of ammonia via prepyriform cortex on regulation of food intake in the rat. Am. J. Physiol. 231(4): 1263-1266. 1976. - Studies were made on whether ammonia, which is an obligatory intermediate of amino acid metabolism, depresses the food intake of rats fed a low-casein (basal) diet containing imbalanced amino acid mixtures (imbalanced diets). Bilateral lesions in the prepyriform cortex caused normalization of food intake of rats fed amino-acid-imbalanced diets, confirming the work of Leung and Rogers (Am. J. Physiol. 221: 929-935, 1971). Unlike normal rats, animals with prepyriform cortical lesions consumed as much of a diet containing 3% NH₄Cl as did the basal diet. However, like normal rats, they rejected a diet containing a mixture of keto acids. Unilateral injection of NH₄Cl into prepyriform cortical areas reduced the food intake to a greater extent than injection of NaCl into these areas or injection of NH₄Cl into other parts of the brain. These results suggest that ammonium ions influence the appetite through their effect on prepyriform cortical areas.

amino acid imbalance; keto acid; appetite

RECENT FINDINGS (9) SUGGEST a possible relation between elevation of the blood ammonia level and reduction of food intake in rats fed amino acid-imbalanced diets. Russek (13) suggested that some receptors of ammonia may affect feeding behavior. Leung and Rogers (7) reported that when rats were fed amino acid-imbalanced diets or amino acid-deficient diets their food intake was normalized by lesions of certain areas of the anterior pyriform cortex, and they suggested that appetite might be controlled by the level of some growth-limiting amino acid in the blood. However, it was found that food intake of rats was not affected by decreases in the plasma concentrations of certain growth-limiting amino acids (9, 16).

The present study deals with the role of ammonia in the regulation of food intake via the prepyriform cortex. The results obtained indicate that ammonia may depress food intake in rats fed amino acid-imbalanced diets through its action on prepyriform cortical areas.

METHODS AND MATERIALS

Male Wistar-strain rats, weighing about 250 g, were kept in individual cages in an animal room at 23 ± 1°C with a 12-h light-dark cycle. They were maintained on a commercial diet (Oriental Yeast Co., Ltd.).

In experiment I, bilateral lesions were made in prepyriform cortical areas (A9.8, L3.5, D8.0) of rats, according to the stereotaxic coordinates of DeGroot (1), by application of a direct current of 2.0 mA for 15 s from the tip of an electrode. The electrode was insulated with enamel except at the tip. After the operation animals were given a commercial diet for 5-7 days to allow recovery from stress and damage due to the operation and then they were fed the basal diet for 5 days. After that, they were given an amino acid-imbalanced diet, an ammonia-containing diet, or keto acid-containing diet for a few hours or for 5 days.

The precise composition of the basal diet and imbalanced diets has been reported elsewhere (9). The basal (control) diet contained 5% casein, 0.3% L-methionine, and 0.2% L-threonine as nitrogen sources. The imbalanced diets were made by adding imbalanced amino acid mixtures to the basal diet. The ammonia- and keto acid-containing diets were prepared by adding 3% NH₄Cl and 3% of a mixture of keto acids (2% α-ketoglutarate, 0.5% α-ketobutyrate, and 0.5% pyruvate), respectively, to the basal diet at the expense of carbohydrates.

Preliminary experiments showed that food intake and food selection of rats with lesions in A9.8, L3.5, D6.0-7.5 (upper parts of the prepyriform cortical areas) were the same as those of intact rats (unpublished observations). Therefore, some animals in each lot were sham-operated (lesions in A9.8, L3.5, D6.0) as controls and were given the same diets as animals with lesions in prepyriform cortical areas. All rats had free access to the experimental diets and water. Food intake was measured daily in the case of 5-day observation periods and every 2 h in the short-term experiments.

Rats with lesions were also tested to see whether they could distinguish the basal diet from the ammonia-containing diet. For this, rats with lesions were given the two diets at the same time, and the amount of each diet consumed was measured every day and compared with the intakes of control rats. In this experiment untreated rats were used as controls. In experiment I, each group contained five rats.

In experiment II, ammonia (NH₄Cl) or NaCl was injected unilaterally into the brain to examine the direct effect of ammonia on food intake mediated by prepyriform cortical areas. In this experiment, rats that had been kept on the commercial diet were fasted overnight. Then they were lightly anesthetized with ethyl ether, and 2% NaCl or NaCl solution was injected by a microsyringe at a dose of 1.0 µl/100 g body wt. The solutions...
were placed into the prepyriform cortical area (A9.8, L3.5, D3.0). As a sham operation the same dose of NH₄Cl solution was injected into another part of the brain (A9.8, L3.5, D3.0) of some rats. This is referred to as "misdirected NH₄Cl" in Fig. 4. The location of this sham operation was different from that in experiment I (A9.8, L3.5, D6.0), because we wanted to avoid the possibility that the injected ammonia might diffuse to prepyriform cortical areas. Thirty minutes after injection of NH₄Cl or NaCl, the rats were given access to the commercial diet, and their food intake was measured every 2 h for 6 h.

After this they were given the commercial diet ad libitum for 4 days and then fasted overnight; their 6-h food intakes were again measured on day 5 (postrecovery) to check that they did not show anorexia or excessive food intake due to brain damage during the injection. (No rats showed any abnormalities.)

At the end of the experiments, the brains were fixed, embedded in paraffin wax, and sectioned to determine the locations of the lesions. In experiment I, the locations of lesions were compared with the illustration of Leung and Rogers (7) and the atlas of König and Klippel (3), and animals with lesions in incorrect locations were excluded. In experiment II it was impossible to identify the exact sites of the injections, so all animals were included in the effective number.

Results are expressed as means ± SE of the values for the rats in each group. The significance of differences between values for each group was evaluated by the t test for unpaired samples (14).

RESULTS

Experiment I. The mean daily food intake of the basal diet of rats with bilateral lesions in the prepyriform cortex was about the same as that of rats without lesions: i.e., expressed as the mean food intake ± SE, it was 21.5 ± 1.4 g/day for rats with lesions and 23.7 ± 0.8 g/day for those without lesions. The food intakes of rats fed amino acid-imbalanced and ammonia-containing diets, expressed as a percentage of their respective intakes of the basal diet in the previous 5 days, are summarized in Fig. 1. The intakes of the imbalanced and ammonia-containing diets by rats with prepyriform cortical lesions were practically the same as those of the basal diet, whereas the intakes of these diets by sham-operated rats were about 70–80% of the basal diet. Thus, bilateral lesions in the prepyriform cortical areas caused normalization of food intake in rats fed the amino acid-imbalanced and ammonia-containing diets.

Intact animals can distinguish between the basal and imbalanced diets and select the basal diet (10). Therefore, we examined whether rats with prepyriform cortical lesions could also distinguish between the basal and unfavorable diets. The ammonia-containing diet was used as the unfavorable diet. As shown in Fig. 2, intact rats ate the basal diet almost exclusively in preference to the ammonia-containing diet. On the other hand, rats with lesions did not show any clear preference for the basal diet, and their intakes of the two diets were not statistically significant except on day 4.

Experiment II. The results of experiment I show that lesions of the prepyriform cortical areas of rats prevent decreased food intake of the ammonia-containing diet. Therefore, we next examined the effect on food intake of ammonia injection (as NH₄Cl) into the prepyriform cortical areas. As controls we examined the effects of NaCl injection into the same areas (to test the effect of Cl⁻ ion on the prepyriform cortical areas) and of NH₄Cl injection into another part of the brain (to test the nonspecific toxic effect of ammonia on the brain). The latter is named the "sham-operated" group and results are la-
FOOD INTAKE REGULATION BY AMMONIA

FIG. 4. Mean 6-h food intakes after unilateral injection of NH$_4$Cl or NaCl into prepyriform cortex or another area (A 9.8, L 3.5, D 3.0) of brain (misdirected NH$_4$Cl). Bars show SE of mean values for numbers of rats shown in parentheses.

were not due to physical damage of the brain during injection. The food intakes of the NH$_4$Cl, NaCl, and sham-operated (misdirected NH$_4$Cl) groups were 32, 67, and 59%, respectively, of their postrecovery intakes measured on day 5 after the injections. The food-intake depression in the control and misdirected NH$_4$Cl groups may have been due to the stress and injury of operation.

DISCUSSION

Ammonia, which is inevitably liberated during amino acid metabolism, has a neurotoxic effect at high levels, so that it must be kept below a toxic level in living cells by sensitive mechanisms. Russek (13) reported that intraportal infusion of ammonium salts or glycine depressed the food intake of fasted dogs. Moreover, Harper et al. (2) and Russek (13) showed that glycine depressed appetite owing to its metabolic conversion to ammonia. Therefore, the above authors and we (9) have suggested that ammonia may influence some appetite-controlling mechanisms related to protein or amino acid intake.

To explain the effect of amino acid imbalances in depressing food intake, Harper and his co-workers (8, 12) proposed an aminostatic hypothesis: i.e., that the plasma amino acid pattern may regulate the appetite of experimental animals. Leung and Rogers (7) suggested that this pattern of amino acids might be detected in the prepyriform cortical areas and serve as a signal for inhibitory mechanisms to depress food intake of rats fed amino acid-imbalanced diets. In the present study, injection of ammonia into the prepyriform cortex reduced food intake more than its injection into another part of the brain, indicating that local injection of a minute amount of ammonia into the prepyriform cortex was not due to a nonspecific toxic effect of ammonia on the brain.

Bilateral lesions of the ventral amygdala (5) or hypothalamus (6), or surgical section of the olfactory bulb (4) or the vagus and celiac plexus (10), do not affect depression of food intake due to amino acid imbalance. Therefore, the normalization of intake of imbalanced diets in rats with lesions of prepyriform cortical areas does not seem to be due to altered sensitivity to the palatability of the diet.
Panksepp and Booth (11) reported that injection of amino acids into the dorsolateral perifornical hypothalamus depressed food intake more than injection of urea, NaCl, or glucose into the same area. Some amino acids change neural firing rates (15), but it is uncertain whether amino acids per se act as an appetite-depressing signal or whether their effects are due to their metabolites. The present work shows that an appetite-controlling mechanism in the prepyriform cortical areas is regulated by the level of ammonia rather than the level of keto acids, which are also intermediates in amino acid metabolism. The results do not exclude the possibility of the direct effect of an amino acid(s) itself on appetite, and further studies are needed on the possible role of amino acids per se and on the exact neural pathways involved in the reduction of food intake.

Received for publication 8 December 1975.

REFERENCES