Dorsomedial Hypothalamic Lesions Alter Intake of an Imbalanced Amino Acid Diet in Rats

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ABSTRACT Within 3 h of ingesting an imbalanced amino acid diet (IAAD), rats show attenuated intake. The associated conditioned taste aversion can be ameliorated by giving the serotonin receptor blocker, tropisetron (TROP). A recent c-fos study indicated that the dorsomedial hypothalamic nucleus (DMN) may be activated 2–3 h after ingestion of IAAD. In Experiment 1, DMN-lesioned rats (DMNL) or sham-operated (SHAM) rats were injected with saline (SAL) or TROP just before introduction of IAAD. By 3 h, SAL-DMNL rats consumed more (P < 0.01) of the IAAD than did the SAL-SHAM rats. Thereafter, over the next 21 h, the intake of the SAL-DMNL group returned to control levels. TROP treatment enhanced the intake of the treated groups; the TROP and the lesion effect were additive (P < 0.01). By d 4 of receiving the IAAD, the DMNL groups were eating less than SHAM rats (P < 0.05). The data suggest that the DMN may be involved in the early detection of the amino acid deficiency induced by IAAD, is not involved in the TROP effect and is necessary for proper long-term adaptation to an IAAD. J. Nutr. 128: 1213–1217, 1998.

KEY WORDS: body weight • serotonin receptors • feeding • rats • essential amino acid deficiency

Rats are able to detect imbalances in a diet's content of essential amino acids (Harper et al. 1970, Hrupka et al. 1997). When given a choice between an imbalanced amino acid diet (IAAD) and a protein-free diet, the rats invariably choose the protein-free diet (Rogers and Leung 1977), showing the profound conditioned taste aversion to these diets that develops. There appear to be at least three phases (Gietzen 1993) in the rat's response to an IAAD. The first phase is recognition by the rat that the diet is imbalanced (first 3 h after ingesting an IAAD); this is accompanied by an initial hypophagia. The second phase follows the recognition phase and occurs when a serotonin-mediated conditioned taste aversion develops (3–6 h after ingesting an IAAD); hypophagia continues (Erecius et al. 1996). The third phase occurs in the absence of an alternate dietary choice when the animal adapts to the IAAD (requires several days) and gains body weight.

The anterior piriform cortex (APC) plays an important role in the rat's ability to recognize an IAAD, because lesioning this area permanently normalizes the animal's intake of an IAAD (Leung and Rogers 1971, Noda and Chikamori 1976), and injection of the limiting amino acid into the APC increases food consumption from the usual 45–50% to 75–85% of baseline intake (Beverly et al. 1990a and 1990b).

One area containing projections from the APC is the medial amygdala. Lesioning this area attenuates the hypophagia caused by an IAAD (Leung and Rogers 1973), probably by eliminating the development of a conditioned taste aversion (Meliza et al. 1981, Terry-Nathan et al. 1995) which, as noted above, is the second phase of the anorectic response to an IAAD (Gietzen 1993). This second phase also has a peripheral serotoninergic (5-HT) component, because the IAAD-induced hypophagia can be attenuated by prior administration of a serotonin (5-HT) receptor blocker that acts in the periphery (Erecius et al. 1996, Hrupka et al. 1991, Jiang and Gietzen 1994, Terry-Nathan et al. 1995).

More recently, a study using c-fos expression as an indicator of neural activity (Wang et al. 1996) demonstrated that the dorsomedial hypothalamic nucleus (DMN), an area shown to affect both ingestion and body weight regulation (Bellinger et al. 1979, Bellinger 1987, Bellinger and Bernardis 1991, Bellinger et al. 1994, Dalton et al. 1981), was activated 2 h after rats ingested an IAAD. No other hypothalamic areas showed changes in c-fos expression in this study. The DMN has neural connections to the APC (see below), medial nucleus of the amygdala (Luiten and Room 1980) and hindbrain areas (Bernards and Bellinger 1987, Luiten et al. 1987, ter Horst and Luiten 1986) that send and receive peripheral information. The c-fos data and the anatomical connections of the DMN suggested that it might be involved in one of the following: Phase 1, the early recognition that the diet induces amino acid deficiency or Phase 2, development of conditioned taste aversion to the diet with its peripheral 5-HT3 component, or...
both phases. Last, as noted above, rats eating IAAD demonstrate adaptation (Phase 3); over a period of days, they eat more of the IAAD and gain body weight.

In light of the c-fos expression data in the DMN of rats fed IAAD, it was of interest to determine whether DMNL rats would, like intact rats, recognize that they were consuming an IAAD, respond like intact rats to a 5-HT3 receptor blocker and later show normal feeding and body weight adaptation to the IAAD.

METHODS AND MATERIALS

General procedures. Male Sprague-Dawley rats (Harlan Industries, Houston, TX) were housed individually in a light-cycle–controlled (12-h light:dark cycle with lights out at 0900 h) and temperature-controlled (23°C) room. Upon arrival, the rats consumed a nonpurified pelleted diet [HarlanTeklad Mouse/Rat Diet (24% protein), Harlan Industries] and water ad libitum for 8 d. At the end of this period, the rats were anesthetized with ketamine (90 mg/kg body weight) and xylazine (9 mg/kg body weight) and body weights were recorded. The rats were then placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA) and bilateral electrolytic lesions (0.25 mm diameter, sparsely varnished, stainless steel electrodes with tip bare 0.2 mm; DC current 1.0 mA for 7 s) were placed in the area of the DMN using the coordinates: anterior–posterior, +3.9 mm; lateral, 0.5 mm; depth, 2.4 mm dorsal to ear bar zero. Surgical procedures have been described in detail elsewhere (Bellinger and Bernardis 1991). Sham operations (SHAM) were performed by inserting the electrode to a depth of 1.0 mm dorsal to the DMN. Nonpurified diet intake, corrected for spillage, was recorded daily for 8 d. The rats were then given a purified low protein basal diet for 7 d (Hammer et al. 1990), which supplies L-amino acids (Ajinomoto, Teaneck, N.J.) as the protein equivalent at ~50% of requirements for amino acids, and in which isoleucine was the growth-limiting amino acid. The basal diet was used because rats demonstrate a dramatic and reliable suppression of food intake when subsequently switched to an isoleucine-imbalanced diet (Hammer et al. 1990, Harper et al. 1970).

Food intake of all diets, corrected for spillage, was measured daily and body weights were recorded at various times throughout the studies. Water was available for ad libitum consumption.

After rats were given the basal diet for 2 d, food was removed at 0800 h and the rats were injected with saline (SAL). Food cups were returned at 0900 h. This sequence was repeated during the next 3 d. At the end of this period, the rats were injected intraperitoneally with SAL at 0800 h. Food was presented at 0900 h; then 3-, 6-, 12- and 24-h intakes were measured. Food intake at each measurement time was corrected for spillage, which was collected on a pad below each cage. These measurements were repeated the next day.

The following day, at 0800 h, rats from the SHAM and DMNL groups were injected intraperitoneally once either with SAL or with 9 mg/kg body weight (Hammer et al. 1990) of the 5-HT3 receptor antagonist tropisetron (TROP; Research Biochemicals International, Natick, MA). A 2 × 2 factorial design was used with TROP and SAL as the drug conditions and DMNL and SHAM operations as the surgery conditions. At 0900 h, the rats were presented with an isoleucine IAAD (Hammer et al. 1990) and food intake recorded as described above. Daily consumption of the IAAD was measured for six additional days.

At the end of the study, the rats were killed by using CO2 and their brains saved for histological examination as previously described (Bellinger 1987, Bellinger and Williams 1983). Briefly, frozen brain sections were cut 8 μm thick and stained by the Klüver-Barrera method (Luxol fast blue and cresyl echt violet). Only those rats with correctly placed lesions were used for statistical analysis.

After eliminating those rats with misplaced lesions, the group sizes were: SAL-SHAM, n = 9; TROP-SHAM, n = 10; SAL-DMNL, n = 8; and TROP-DMNL, n = 12.

Statistical methods. Cumulative hourly intakes were analyzed by one-way ANOVA without repeated measurements, whereas daily intakes were analyzed by two-way ANOVA with a repeated-measures design. Data found significant by ANOVA were further analyzed using Duncan’s Multiple Range test (Alder and Roessler 1964). In some cases, data were transformed into percentages before statistical analysis. Statistical significance was accepted at the P < 0.05 level. Statistical analyses were performed with the use of the ABstat computer program, release 6.51, (Anderson-Bell, Parker, CO).

RESULTS

Histology revealed that the principal sites of bilateral destruction were located in the DMN (Fig. 1). The lesions did not extend laterally beyond the fornix or dorsal to the mammo-thalamic tracts and were dorsal to the ventromedial hypothalamic nucleus.

As expected, the DMNL rats were hypophagic when they consumed the nonpurified diet (8 d mean: SAL-SHAM, 19.7 ± 1.0 g vs. SAL-DMNL, 16.7 ± 1.9 g, P < 0.001; TROP-SHAM, 20.0 ± 1.7 g vs. TROP-DMNL, 16.5 ± 1.4 g, P < 0.001) and basal diet (SAL-SHAM, 21.6 ± 1.4 g vs. SAL-DMNL, 14.3 ± 1.9 g, P < 0.001; TROP-SHAM, 20.7 ± 0.7 g vs. TROP-DMNL, 14.6 ± 0.5 g, P < 0.001) compared with the SHAM rats. The hourly cumulative intake (g) of the basal diet differed among the groups with the DMNL groups eating less (P < 0.05) than the SHAM groups starting with the 3-h measurement (Fig. 2). When the hourly basal intake was reanalyzed as basal diet consumed per 100 g of body weight (data not shown), the DMNL rats indicated significant (P < 0.05) hypophagia at every measurement point. Therefore, to compare DMNL with SHAM rats, it was necessary to ex-
press the data as a percentage of each rat’s own basal diet intake.

On d 1 of ingesting the IAAD, cumulative intake differed significantly (P < 0.01) among the DMNL and SHAM groups (Fig. 3). The SAL-DMNL group consumed more IAAD than did the SAL-SHAM rats during the 0–3 h measurement period. During the remainder of d 1, the cumulative intake of the IAAD did not differ significantly between the SAL groups. The lesion and TROP effects were additive during the 0–3 h period, with the TROP-DMNL group eating significantly more (P < 0.01) of the IAAD than the TROP-SHAM group. The cumulative intake of both TROP groups did not differ from 6 to 24 h. During d 1 of ingesting the IAAD, both TROP groups consumed significantly (P < 0.01) more of the IAAD than did the SAL groups at every measurement point. Exactly the same significant pattern emerged when the data were reanalyzed (data not shown) as amount of IAAD consumed per 100 g body weight as a percentage of basal diet consumed per 100 g body weight.

When the rats’ daily intakes of the IAAD were compared, there were significant (P < 0.01) group differences (Fig. 4). By d 2, the 24-h intake of all groups, except the TROP-DMNL group, did not differ significantly. The latter group’s consumption of the IAAD was less than that of the other three groups, reaching significance (P < 0.01) in comparison with the SHAM groups. The intake of the TROP-DMNL group remained below that of the other groups from d 2 to 7; the TROP-DMNL group even ate significantly (P < 0.05) less than the SAL-DMNL on d 4. By d 2, and through d 7, the 24-h intake of the SHAM groups was similar (P > 0.1), but significantly (P < 0.05–0.01) greater than that of the DMNL groups. During this period the SHAM groups showed better adaptation to the IAAD than did the DMNL groups.

The body weights of the four groups did not differ (P > 0.1) at the time of surgery (SAL-SHAM, 172.3 ± 4.3 g; TROP-SHAM, 170.9 ± 2.0 g; SAL-DMNL, 172.4 ± 2.8 g; TROP-DMNL, 169.6 ± 4.3 g). All groups gained weight while consuming the nonpurified diet. However, the SHAM groups weighed more than the DMNL groups by the day the groups were switched to basal diet (SAL-SHAM, 223.6 ± 5.0 g vs. SAL-DMNL, 208.4 ± 4.6 g, P < 0.05; TROP-SHAM, 223.2 ± 3.3 g vs. TROP-DMNL, 205.1 ± 4.7 g, P < 0.01). All groups gained weight when consuming the basal diet, but the DMNL groups still weighed less (P < 0.01) than the SHAM groups on the day of TROP and SAL injection (SAL-SHAM, 236.2 ± 4.8 g vs. SAL-DMNL, 211.6 ± 6.2 g; TROP-SHAM, 240.8 ± 3.6 g vs. TROP-DMNL, 217.3 ± 4.7 g).

The body weights (Fig. 5) of the groups, expressed as a percentage of their weight on the day they received TROP or SAL, differed (P < 0.01) over the 7 d of ingesting the IAAD.
During the first 2 d, the two SAL-injected groups lost weight compared with either their own starting body weight or with that of the TROP groups. Although the TROP groups also lost weight on d 1 and 2, they lost less weight than the SAL groups. By d 3, and through d 7, the DMNL groups weighed less. On d 1, the DMNL rats lost weight that was expressed as a percentage of body weight at the time of injections (initial body weight). DMNL, dorsomedial hypothalamic lesion; SHAM, sham operation. 

DISCUSSION

Small electrolytic or excitotoxin (kainic acid or ibotenic acid, which cause only loss of cell bodies and not axons of passage) lesions in the DMN of rats produce animals that are hypophagic, hypodipsic and show reduced body weight and linear growth (both 70--85% of SHAM), but have normal body composition when compared with SHAM rats (see Bernardis and Bellinger 1987 for a review). Once the rat attains the lesion-induced lower body weight, its food and water consumption decreases commensurately with its body weight (Bernardis and Bellinger 1987).

Interest in a role for the DMN in the responses to amino acid deficiency, as induced by an IAAD, arose from a recent c-fos mapping study (Wang et al. 1996). Two hours after ingesting the IAAD, Fos-like immunoreactivity in the DMN increased abruptly and significantly over both the corrected and basal diet groups and remained elevated through 3 h. No changes in c-fos expression were seen in the other hypothalamic areas examined, including the ventromedial hypothalamic nucleus, lateral hypothalamic area or paraventricular nucleus. Outside the hypothalamus, Fos immunoreactivity was observable 2 h after IAAD presentation in the APC and infralimbic cortex. The time course for c-fos expression suggests that the DMN may also be a component of the recognition system, or of the integrative APC neural responses to acute amino acid deficiency, or both (Wang et al. 1996).

These data provide additional support for a role for the DMN in the early detection of the amino acid deficiency induced by IAAD. SAL-treated DMNL rats showed an attenuation of the food intake suppression normally observed during the first 3 h of ingesting an IAAD. Thus the lesion interfered with the early recognition of an amino acid deficiency (Phase 1) caused by eating an IAAD. However, after 3 h, both SAL-DMNL and SAL-SHAM groups consumed similar amounts of the IAAD. This indicates that the major effect of the lesion occurred during the initial 3 h of diet ingestion. Apparently the lesion did not affect the onset of Phase 2, i.e., development of aversion with continued reduction of IAAD intake (Gietzen 1993, Hammer et al. 1990, Harper and Peters 1989, Hrupka et al. 1991, Terry-Nathan et al. 1995), which may occur as late as 4 h after consuming IAAD (Simson and Booth 1973) and is attenuated by TROP for over 12 h (Erecius et al. 1996). The TROP alleviated, but did not fully correct the aversive Phase 2 response, resulting in the rats consuming more of the IAAD than the SAL-SHAM group, which experienced both Phase 1 and 2. Thus it is quite possible that TROP-treated SHAM rats still recognized an imbalanced amino acid diet (Phase 1) and responded with a lower-than-maximal food consumption (usually ~85% of basal, see Hammer et al. 1990). On the other hand, treatment of the DMNL rats with TROP apparently caused an additive lesion and drug effect during the first 3 h of ingesting the IAAD. This could occur if the lesion initially interfered with the recognition phase (Phase 1), while the TROP blocked any signals pertaining to nausea or malaise that were subsequently generated in, or routed through the periphery (Phase 2).

Further support for our suggestion that the DMN may be part of the early recognition system comes from recent findings that a decreased concentration of the limiting amino acid, isoleucine, was found in the DMN after introduction of an IAAD. Moreover, the concentrations of the excitatory amino acids, glutamate and aspartate, were reduced in DMN by 2.5 h after diet presentation (Gietzen et al. 1998).

In addition to the above-mentioned data, anatomical findings also lend support to the possible involvement of the DMN in recognizing a diet as amino acid deficient. The APC has indirect neuronal connections with the DMN; it has efferent connections with the amygdala cortex and secondarily with the central nucleus of the amygdala (Ottersen 1982, Price et al. 1987). The amygdala in turn sends efferent fibers to the DMN (Luiten and Room 1988, Luiten et al. 1987, Luskin and Price 1983). The APC also has efferent connections with the bed nucleus of the stria terminalis (Swanson 1987), which in turn has both efferent (Luiten and Room 1988, Swanson 1987) and afferent (ter Horst and Luiten 1986) connections with the DMN. Finally, the APC sends efferent fibers to the infralimbic cortex (Luskin and Price 1983), which sends neurons to the DMN and amygdala (Hurley et al. 1991, Saper 1985). Therefore, there are a number of pathways by which the APC can reach and possibly influence the DMN.

The DMN also has direct efferent connections with the posterior piriform cortex (Saper 1985), which in turn projects to the APC (Luskin and Price 1983). Additionally, the DMN has efferent connections with the amygdala (ter Horst and Luiten 1986), insular cortex, infralimbic cortex and retrosplenial cortex (Saper 1985). These four areas in turn send fibers to the APC (Hurley et al. 1991, Luskin and Price 1983, Swanson 1987). The insular cortex can also be reached by the DMN indirectly by way of its connections with the lateral hypothalamus, which in turn projects to the insular cortex (Swanson 1987). Thus there are several routes by which information processed in the DMN could reach and possibly influence the APC.
The 24-h intake of the IAAD by the TROP-treated groups decreased on d 2. Interestingly, on this day, the intake of the TROP-DMNL group was suppressed compared with all other groups. Whether this is a compensatory response for their initial high intake on d 1, or whether the anti-aversive effects of TROP had diminished is unclear. All groups increased their intake of the IAAD on d 3, the beginning of Phase 3. Notably, by d 3, the two DMNL groups were consuming less of the IAAD than their SHAM counterparts. This occurred despite the fact that consumption was calculated as a percentage of the DMNL rats’ basal diet intake to account for the inherent lesion-induced hypophagia. Thus the DMNL rats were taking relatively less of the IAAD than the controls, suggesting decreased adaptation to the IAAD in the DMNL rats. The reason for this is uncertain and will require further study.

The DMNL lesion-induced attenuation of the normal hypophagia seen during the first 3 h after ingestion of IAAD, early c-fos expression in the DMN and a reduction in the concentration of the limiting amino acid in this area suggest a possible role for the DMN in the earliest sensing of IAAD, along with the previously recognized APC (Beverly et al. 1990a and 1990b, Gietzen and Jhanwar-Uniyal 1996, Leung and Rogers 1971). Clearly, the role of the DMN, introduced for the first time here, must be considered in future studies of the mechanisms underlying the responses of animals to IAAD.

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LITERATURE CITED


