Lesions of tuberomammillary nuclei induce differential polydipsic and hyperphagic effects

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Abstract

This study aimed to examine the function of the tuberomammillary complex in water and food intake of Wistar rats. The results show that lesions restricted to tuberomammillary subnuclei: caudal ventral tuberomammillary nucleus (E1), rostral ventral tuberomammillary nucleus (E2), medial ventral tuberomammillary nucleus (E3) or medial dorsal tuberomammillary nucleus (E4), induce a strong and persistent polydipsia with specific characteristics for each nucleus. Interestingly, the distribution of tuberomammillary hyperdipsia throughout the day was similar to that in non-lesioned animals, in contrast to the lack of rhythmicity observed in rats with anodic lesion to median eminence. This polydipsia appears to be independent of food intake, as food deprivation for 22 h did not significantly reduce the water intake. Finally, lesions in ventral tuberomammillary nuclei E1 and E2 induce hyperphagia, confirming a possible role for the tuberomammillary complex in food intake. This increase in food intake is not observed after lesions in medial subnuclei E3 and E4. These results are interpreted in terms of the hypothalamic systems involved in the consumption of both food and water.

Introduction

Polydipsic behaviours have been observed after the electrolytic lesions of various brain regions, including the amygdala (Grossman & Grossman, 1963), septum (Harvey & Hunt, 1965), locus coeruleus (Osumi et al., 1975), raphe nuclei (Coscina et al., 1972), medial hypothalamus (Sclafani & Grossman, 1969) and posterior hypothalamus (Tejedor del Real et al., 1972; Morales & Puerto, 1986).

With respect to the hypothalamus, the tuberomammillary system is found in the ventral region and on both sides of the mammillary recess (Watanabe et al., 1984; Inagaki et al., 1988). This anatomical complex, which comprises various subnuclei (designated E1, E2, etc.), is present in all animal species and is most developed in humans (Köhler et al., 1985). Various neurobiological studies have shown a close relationship between this tuberomammillary system and other brain nuclei, including the supraoptic and paraventricular, implicated in hydromineral regulation (Weiss et al., 1989; Bealer & Crowley, 1999; Brown et al., 2001).

On the other hand, histamine has been shown to play a major role in nutritional behaviour, with a reduction in food intake after its central administration or the use of drugs that potentiate its action (Clineschmidt & Lotti, 1973; Sakata et al., 1990; Machidori et al., 1992; Brown et al., 2001; Eriksson et al., 2001; Masaki et al., 2004). Furthermore, it has been demonstrated that Zucker rats, genetically obese, have sevenfold lower histaminergic levels compared with normal rats (Sakata et al., 1991; Brown et al., 2001). This transmitter is synthesized in tuberomammillary nuclei (Airaksinen et al., 1992; Valdés et al., 2005), which have terminals that project to paraventricular and ventromedial nuclei, closely related to food intake (Orthen-Gambill & Salomon, 1992; Woods et al., 1998; Mollet et al., 2003).

With this background, we hypothesized that the structural and functional properties of this hypothalamic system make it a potential candidate for an important function in the regulation of food and water intake.

The hypothesis was tested by performing a series of individual lesions in each of the different tuberomammillary nuclei, and then studying the water and food intake behaviour of these lesioned animals. The day/night rhythmicity of the food and water intake of the animals was analysed. These results were compared with data obtained using other animal models of polydipsia (electrolytic lesions in median eminence, Rolls, 1970). Finally, each group of animals underwent a prandiality test to examine the implication of non-homeostatic salivary mechanisms in this polydipsia (Epstein et al., 1964; Kissileff & Epstein, 1969; Kissileff, 1969; Ramos & Puerto, 1991).

Materials and methods

Subjects

Male Wistar rats (250–320 g each) from a breeding colony at the University of Granada were randomly assigned to one of seven experimental groups. Four groups had individual lesions in one of the four components of the tuberomammillary system: caudal ventral tuberomammillary (E1), rostral ventral tuberomammillary (E2), medial ventral tuberomammillary (E3) and medial dorsal tuberomammillary (E4); two groups had lesions produced by anodic (ME+) or cathodic (ME–) current in the median eminence; and a final non-lesioned group (NLG) underwent surgery but received no electrolytic lesion.

During the experiment, the animals were individually housed in 30 × 15 × 30 cm methacrylate cages, with unlimited access to food.
and water (Unión Alimentaria Sanders, Madrid, Spain) unless otherwise indicated. The room was maintained on a 12 : 12 h light : dark cycle at approximately 22 °C. All experimental procedures took place during light periods. Use and handling of the animals followed guidelines established by European Community Council Directive 86/609/CEE and Spanish Royal Law 223/1988. All efforts were made to reduce the number of animals used in these experiments, which were performed under veterinary guidance.

Surgical procedure

Tuberomammillary lesions were made under anaesthesia induced by i.p. injection (50 mg/kg) of sodium thiopental (Laboratory Abbot, Madrid, Spain).

Anaesthetized animals were placed in a stereotaxic apparatus (Stoelting Co. 51.600, USA). In the case of the tuberomammillary-lesioned animals, a cathodic current of 1.2 mA was bilaterally applied for 20 s using a lesion generator (Grass Instruments, Quincy, MA, USA). Stereotaxic coordinates, obtained from the stereotaxic atlas of Paxinos & Watson (1986), were as follows. E1: 4.2 mm anterior, 1.2 mm lateral and 1.0 mm dorsal to interaural line; E2: 4.7 mm anterior, 1.2 mm lateral and 0.6 mm dorsal; E3: 4.7 mm anterior, 0.6 mm lateral and 0.4 mm dorsal; and E4: 5.2 mm anterior, 0.2 mm lateral and 0.8 mm dorsal, in all cases with reference to the interaural line.

The surgical procedure for the two ME-lesioned groups was the same as above except for the type of electrolytic lesion. In one group a continuous cathodic current of 1.2 mA was applied for 20 s, as above, and in the other group a continuous anodic current of 1.5 mA was applied for 15 s (Rolls, 1970). In both groups, the stereotaxic coordinates were: 6.44 mm anterior to interaural line, 0.4 mm lateral to midline and 0.2 mm dorsal to interaural line.

The NLG underwent the same surgical procedure as the above groups, but no electric current was applied.

In order to prevent possible infection from the surgery, all animals received i.m. injection of 0.1 cc penicillin (Penilevel retard, Laboratory Level, S.A., Barcelona, Spain) at 250 000 IU/mL.

Experimental procedure

Experiment 1: effects of tuberomammillary lesions on water and food intake

The 38 animals used were randomly assigned to one of seven groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>Lesion Coordinates</th>
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<tbody>
<tr>
<td>E1</td>
<td>4.2 mm A, 1.2 mm L, 1.0 mm D</td>
</tr>
<tr>
<td>E2</td>
<td>4.7 mm A, 1.2 mm L, 0.6 mm D</td>
</tr>
<tr>
<td>E3</td>
<td>4.7 mm A, 0.6 mm L, 0.4 mm D</td>
</tr>
<tr>
<td>E4</td>
<td>5.2 mm A, 0.2 mm L, 0.8 mm D</td>
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<tr>
<td>ME+</td>
<td>4.2 mm A, 1.2 mm L, 1.0 mm D</td>
</tr>
<tr>
<td>ME−</td>
<td>4.7 mm A, 1.2 mm L, 0.6 mm D</td>
</tr>
<tr>
<td>NLG</td>
<td>5.2 mm A, 0.2 mm L, 0.8 mm D</td>
</tr>
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</table>

During the 10 days before surgery, the animals were housed under conditions of ad libitum access to food (Sandermus dry chow, Sanders, S.A., Madrid, Spain) and water. Within this period, at 6 days before surgery (Day −6), the animals were deprived of food for 22 h, although they continued to have ad libitum access to water. After this 22-h period, food was again made available and a record was made of the number of prandial responses and the amount of water and food intake during a 2-h period from 10.00 h to 12.00 h (Ramos & Puerto, 1991). The aim was to examine by this method whether water intake during the 22-h deprivation period was also recorded.

After the surgery, the water and food intake of each experimental group was measured under ad libitum conditions. Because any polydipsic syndrome is always interpreted and discussed in terms of diabetes insipidus, the results obtained during the 16-day experimental period were divided into blocks in order to represent the distinct behavioural phases (triphasic pattern) reported in this syndrome (Hollinshead, 1964; Bakker & Waring, 1976; Huang & Dellman, 1996; Saborio et al., 2000). The aim was to explore both quantitative (water intake volume) and qualitative (evolution of polydipsia) differences among the study groups.

Experiment 2: effects of tuberomammillary lesions on night/day rhythmicity of water and food intake

The procedure used on Days −1 and −2 to analyse the percentage water and food intake during dark phases was repeated on post-lesion Days 17 and 18.

Experiment 3: prandiality and tuberomammillary polydipsia

As on Day −6, the animals were deprived of food for a 22-h period on post-lesion Day 21 and a record was again made of the number of drinking responses and the amount of water and food intake during a 2-h period from 10.00 h to 12.00 h (Ramos & Puerto, 1991). The water intake during the 22-h deprivation period was also recorded.

Histology

On completion of the experiments, all animals were deeply anaesthetized with sodium pentothal (80 mg/kg) and perfused transcardially. The brains were then removed from the skull and placed in 4% formaldehyde for at least 24 h. Coronal sections were cut at 40 nm on a freezing microtome (Leitz 1320, Wetlar, Germany), and a one-in-three series of sections was mounted on gelatin-coated slides and examined under a microscope (stereoscopic microscope UMZ-4F, Olympus, Tokyo, Japan) and microphotographed (Olympus Optical, mod. PM-G).

Results

Histological analysis

Tuberomammillary nuclei

The authors estimated the extent of damage for each animal by reconstructing the location and extent of the lesion on the plates of the rat brain atlas (Paxinos & Watson, 1986). Figures 1 and 2 depict the maximum and minimum degree of damage produced by the lesions to the tuberomammillary nuclei. Histological study of the brains of the animals showed the lesions to be relatively homogeneous in their size and localization.

All lesions were bilateral and complete. However, in a few cases the lesion extended beyond the nucleus. Thus, in two E1 animals the lesions were more extensive and involved the dorsal part of the lateral mammillary area (Figs 1 and 3). However, the behaviour of these animals did not differ according to the extent of E1 damage, therefore no animal was excluded from the study. In two E3 animals, slight damage was observed in the lateral base of the arcuate nucleus, but there was no involvement of adjacent hypothalamic structures and this was not related to performance, therefore neither animal was excluded (Figs 2 and 4). In two E4 animals, there was a small dilatation of the third ventricle, possibly by diffusion of the electric current; in the rostral dimension, more extensive lesions occasionally involved the dorsal part of the dorsomedial hypothalamic nucleus but without affecting behavioural results, so that no animal was excluded (Figs 2 and 4).
Median eminence

The lesions in the ME– group were practically complete (Fig. 5). In two animals, there was slight damage to the most ventral part of the lateral division of the hypothalamic arcuate nucleus, but this was not related to performance (Figs 5 and 6). In the ME+ group, the lesions were very extensive in the caudal–rostral dimension, with significant damage to hypothalamic structures in all cases (e.g. arcuate nucleus,

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ventromedial hypothalamus, dorsomedial hypothalamus) and to posterior regions relevant to this experimental study (E4 and E3), respectively (Figs 5 and 6).

**Experiment 1: effects of tuberomammillary lesions on water and food intake**

**Water intake of tuberomammillary-lesioned animals**

Given the small sample sizes, non-parametric tests were applied (for methodological rationale see Siegel & Castellan, 1988; Hollander & Wolfe, 1999). At baseline (pre-surgery), no significant differences in water intake were observed among any of the study groups, as shown by the Kruskal–Wallis test ($H_{6,38} = 5.85, P = 0.4395$, Day 1) (Fig. 7A).

After the surgery, the Kruskal–Wallis test showed significant differences among the groups during all time-blocks studied (Day 1, $H_{6,38} = 30.29, P = 0.0001$; Day 2, $H_{6,38} = 32.17, P = 0.0001$; Day 3, $H_{6,38} = 31.92, P = 0.0001$; Days 4/7, $H_{6,38} = 28.56, P = 0.0001$; Days 8/11, $H_{6,38} = 29.25, P = 0.0001$; Days 12/16, $H_{6,38} = 26.81, P = 0.0002$) (Fig. 7A).

From post-operative Day 1, groups E3 and E4 significantly differed in water intake from the NLG and ME– group during almost the entire experimental period, as shown by the post hoc Kolmogorov–Smirnov test (E3 vs. NLG, $P < 0.05$; Days 1, 2, 3, 4/7, 8/11, 12/16) (E4 vs. ME–, $P < 0.05$; Days 8/11 and 12/16) (E4 vs. ME–, $P < 0.05$; Days 4/7 and 12/16) (Fig. 7A). The polydipsia of the E4 group was significantly greater than that observed in the ME+ on post-lesion Day 1 ($P < 0.01$).

Although the water intake of E1 and E2 groups did not differ from that of the NLG and ME– group in the first post-surgical days, a trend to a greater intake was observed that reached significance towards the end of the experiment (E1, E2 vs. NLG, $P < 0.05$; Days 4/7, 8/11 and 12/16) (E1 vs. ME–, $P < 0.05$; Days 8/11 and 12/16) (E2 vs. ME–, $P < 0.05$; Days 4/7 and 12/16) (Fig. 7A).

From post-operative Day 4, there was a reduction in the differences in polydipsic behaviour between the ventral tuberomammillary-lesioned groups (E1/E2) and the medial tuberomammillary groups (E3/E4) and ME+ group (E3 vs. E1, $P < 0.05$; Days 1, 2 and 3) (E3 vs. E2, $P < 0.05$; Day 1) (E4 vs. E1, E2, $P < 0.01$; Days 1, 2 and 3) (ME+ vs. E1, $P < 0.01$; Days 1, 2 and 3) (ME+ vs. E2, $P < 0.05$; Day 1; $P < 0.05$; Day 2; $P < 0.05$; Day 3), and in the last 5 days of the experiment (Days 12–16) these differences disappeared.

As expected, the water intake of the ME+ group (Rolls, 1970) was significantly higher than that of the NLG throughout the post-operative period (ME+ vs. ME–, $P < 0.05$; Days 1, 4/7 and 8/11; $P < 0.01$; Days 2, 3 and 12/16) (ME+ vs. NLG, $P < 0.01$; Days 1, 2, 3, 4/7, 8/11, 12/16) (Fig. 7A). The ME– group only showed a higher response than the NLG during the first few post-operative days ($P < 0.05$; Days 2 and 3) (Fig. 7A).

**Food intake of tuberomammillary-lesioned animals**

The mean pre-surgical food intake (baseline value) did not differ among the study groups, as shown by results of Kruskal–Wallis test ($H_{6,38} = 9.05, P = 0.1705$, Day 1) (Fig. 7A).

From post-operative Day 1, groups E3 and E4 significantly differed in water intake from the NLG and ME– group during almost the entire experimental period, as shown by the post hoc Kolmogorov–Smirnov test (E3 vs. NLG, $P < 0.05$) (E4 vs. NLG, $P < 0.01$) (E3 vs. ME–, $P < 0.05$; Days 1, 4/7, 8/11, 12/16) (E4 vs. ME–, $P < 0.01$; Days 1, 2, 3, 4/7, 8/11, 12/16) (Fig. 7A). The polydipsia of the E4 group was significantly greater than that observed in the ME+ on post-lesion Day 1 ($P < 0.01$).

The mean pre-surgical food intake (baseline value) did not differ among the study groups, as shown by results of Kruskal–Wallis test ($H_{6,38} = 23.21, P = 0.0007$, Day 2, $H_{6,38} = 13.24$, J. Mahía and A. Puerto © The Authors (2006). Journal Compilation © Federation of European Neuroscience Societies and Blackwell Publishing Ltd European Journal of Neuroscience, 23, 1321–1331
During post-operative days 1, 2, 3 and 4/7, no differences in food intake were observed between the ventral tuberomammillary groups (E1/E2) and the NLG (Kolmogorov–Smirnov test). From post-operative day 8 (Days 8/11), however, the food intake of groups E1 and E2 was significantly higher (E1, E2 vs. NLG, $P < 0.05$; Days 8/11 and 12/16) (Fig. 7B).

These differences with the NLG were not observed in the medial tuberomammillary-lesioned groups (E3/E4). Moreover, E3 group and especially E4, the most polydipsic group, showed a significantly lower food intake than groups E1 and E2 throughout almost the entire post-operative period, reaching a maximum difference from post-operative day 8 (E1 vs. E3, $P < 0.05$; Days 8/11 and 12/16) (E1 vs. E4, $P < 0.05$; Days 2 and 8/11; $P < 0.01$; Days 1, 3, 4/7 and 12/16) (E2 vs. E3, $P < 0.05$; Days 8/11) (E2 vs. E4, $P < 0.01$; Days 1, 2 and 4/7; $P < 0.05$; Days 8/11 and 12/16).

The group with anodic lesion in median eminence (ME+) showed significant differences in food intake with the NLG, the ME– group and with all tuberomammillary-lesioned groups (ME+ vs. ME–, $P < 0.05$; Days 2 and 3; $P < 0.01$; Days 4/7, 8/11 and 12/16) (ME+ vs. E1, $P < 0.05$; Day 2; $P < 0.01$; Days 3, 4/7, 8/11, 12/16) (ME+ vs. E2, $P < 0.05$; Day 2; $P < 0.01$; Days 3, 4/7, 8/11, 12/16) (ME+ vs. E3, $P < 0.01$; Day/s 1, 2, 3, 4/7, 8/11, 12/16) (ME+ vs. E4, $P < 0.01$; Day/s 1, 2, 3, 4/7, 8/11, 12/16) (Fig. 7B).

However, this hyperphagic behaviour was not observed in the cathodic ME– group except during the last 5 days of the experiment (Days 12/16), when this group consumed more food than the NLG and E4 group (NLG vs. ME–, $P < 0.05$) (E4 vs. ME–, $P < 0.05$).

**Experiment 2: effects of tuberomammillary lesions on night/day rhythmicity of water and food intake**

At baseline (pre-surgery), there were no differences in water or food intake during the 12 h dark cycle among the study groups (Kruskal–Wallis test; Fig. 8A and B) (night/day rhythmicity of water intake $H_{6,38} = 11.45$, $P = 0.0753$) (night/day rhythmicity of food intake $H_{6,38} = 4.11$, $P = 0.6614$). After surgery, significant differences in the rhythmicity of both intakes were observed between the lesioned groups and the NLG (night/day rhythmicity of water intake $H_{6,38} = 22.23$, $P = 0.0011$) (night/day rhythmicity of food intake $H_{6,38} = 27.10$, $P = 0.0001$) (Fig. 8A and B).

The nocturnal rhythmicity of water intake was not changed after the lesion in tuberomammillary-lesioned and ME– groups, and did not significantly differ from that of the NLG [ME– vs. NLG (79.80–81.20%)] [E1 vs. NLG (83.60–81.20%)] [E2 vs. NLG (89.00–81.20%)] [E3 vs. NLG (84.40–81.20%)] [E4 vs. NLG (88.33–81.20%)] (Fig. 8A). In contrast, the lesion clearly interfered with the water intake pattern of the ME+ group, with 55.42% of their water intake occurring during the 12 h dark cycle vs. 81.20% in the NLG (ME+ vs. NLG, $P < 0.01$) (Fig. 8A). This absence of rhythmicity in water consumption by the ME+ group was also found in comparison with the other lesioned groups (Kolmogorov–Smirnov test) [ME+ vs. ME– (55.42–79.80%), $P < 0.01$] [ME+ vs. E1 (55.42–83.60%), $P < 0.01$] [ME+ vs. E2 (55.42–89.00%), $P < 0.01$] [ME+ vs. E3 (55.42–84.40%), $P < 0.01$] [ME+ vs. E4 (55.42–88.33%), $P < 0.01$] (Fig. 8A).

The ME+ group consumed 55.86% of their food during the nocturnal phase vs. 80.60% in the NLG (ME+ vs. NLG, $P < 0.01$), a significantly different pattern from that of the tuberomammillary-lesioned groups [ME+ vs. E2 (55.86–81.80%), $P < 0.01$] [ME+ vs. E3 (55.86–76.60%), $P < 0.01$] [ME+ vs. E4 (55.86–83.83%), $P < 0.01$], with the exception of the E1 group. Although no significant difference was found between the E1 and the ME+ group (65.60–55.86%),
significant differences in percentage nocturnal food intake were observed between the E1 group and the NLG (65.60–80.60%),(E1 vs. NLG, $P < 0.05$) and E4 group (65.60–83.83%), (E1 vs. E4, $P < 0.01$) (Fig. 8B).

**Experiment 3: prandiality and tuberomammillary polydipsia**

No differences in mean water intake frequency were found among study groups on the day after food deprivation in the pre-surgical period (Kruskal–Wallis test) ($H_{6,38} = 1.37, P > 0.9673$) (Fig. 9). No differences in this frequency were found between the lesioned groups and the NLG on the post-surgical day following food deprivation (Day 21) (Kruskal–Wallis test) ($H_{6,38} = 1.47, P > 0.96$) (Fig. 9). The intragroup analysis (Friedman test) showed no difference for any group between the pre-operative and post-operative periods (ME+, $Z = 0.52, P = 0.60$; ME−, $Z = 0.53, P = 0.59$; E1, $Z = 0.00, P = 1.00$; E2, $Z = 0.67, P = 0.50$; E3, $Z = 0.40, P = 0.68$; E4, $Z = 0.00, P = 1.00$; NLG, $Z = 0.64, P = 0.51$).

**Fig. 6.** Photomicrograph of coronal sections showing typical anodic median eminence (ME+) lesion on the left (A) and typical cathodic median eminence (ME−) lesion on the right (B).

**Fig. 7.** (A) Water intake of tuberomammillary-lesioned animals (Experiment 1). The graph shows amounts of water ingested by experimental and control groups throughout different blocks of days. Day −1 shows baseline (pre-surgical) data. (B) Food intake of tuberomammillary-lesioned animals (Experiment 1). The graph shows amounts of food ingested by experimental and control groups throughout different blocks of days. Day −1 shows baseline (pre-surgical) data. Data are shown as means ± SEMs. When the SEM is very small it is not visible on the graph.

**Fig. 8.** (A) Effects of tuberomammillary lesions on night/day rhythmicity of water intake (Experiment 2). The graph shows the percentage of water intake during the nighttime period in experimental and control groups before (Days −2/−1; left-hand graph) and after (Days 17/18; right-hand graph) the surgery. (B) Effects of tuberomammillary lesions on night/day rhythmicity of food intake (Experiment 2). The graph shows the percentage of food intake during the nighttime period in experimental and control groups before (Days −2/−1; left-hand graph) and after (Days 17/18; right-hand graph) the surgery. Data are shown as means ± SEMs. When the SEM is very small it is not visible on the graph. Significance of group differences *$P < 0.05$, **$P < 0.01$. 

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However, the Kruskal–Wallis tests revealed significant differences in the volume of water intake among the different groups after food deprivation on Day 22 ($H_{6,38} = 32.05, P < 0.0001$) (Fig. 10).

The water intake of the ME+ group was drastically reduced during the food deprivation period and was less than that of all other groups, including the NLG (ME+ vs. E4, $P < 0.01$) (ME+ vs. E1, E2, E3, NLG, $P < 0.01$). In contrast, the ME+ group showed a clear polydipsic response on the day before food deprivation, with a higher water intake compared with the NLG (Kolmogorov–Smirnov test) (ME+ vs. NLG, $P < 0.01$) (Fig. 10).

In the case of the tuberomammillary-lesioned groups, although some of them (E1 and E4) reduced their water intake during food deprivation with respect to the previous day (Wilcoxon test) (E1, $Z = 2.02, P = 0.0431$; E4, $Z = 2.20, P = 0.0277$), their intake was significantly higher than that of the NLG (Kolmogorov–Smirnov test), (E1, E2, E3 vs. NLG, $P < 0.025$) (E4 vs. NLG, $P < 0.01$).

At the end of the food deprivation phase (22 h), the food and water intakes of the different experimental and control groups were recorded for a 2-h period both before and after the surgical intervention.

Differences in mean food intake among study groups on the day after food deprivation (Day 22) were only observed in the postsurgical period (Kruskal–Wallis test) ($H_{6,38} = 19.37, P < 0.0036$). Specifically, the group with anodic lesion in median eminence showed a significantly greater food intake compared with the other study groups (Kolmogorov–Smirnov test (ME+ vs. E1, E2, E3, NLG, $P < 0.05$) (ME+ vs. E4, $P < 0.005$) (ME+ vs. ME−, $P < 0.01$).

The intragroup analysis (Wilcoxon test) showed differences in food consumption during the 2 h after food deprivation between the...
pre-operative and post-operative periods in the group with anodic lesion in median eminence (ME+, Z = 2.36, P = 0.01).

No differences in mean water intake were found between the lesioned groups and the NLG on either the pre-surgical or post-surgical day after food deprivation (Kruskal–Wallis test) (H_{6,38} = 12.28, P = 0.0601).

Discussion

The results of this study show that lesions restricted to tuberomammillary subnuclei (E1, E2, E3, or E4) induce polydipsia. In all cases, the increase in water intake was permanent, although the hyperdipsia did not appear to be uniform in all tuberomammillary-lesioned subgroups. In fact, the data showed both quantitative and qualitative differences according to the site of the electrolyte lesion.

Thus, animals with lesions to E3 and E4 tuberomammillary nuclei showed an early polydipsic response from the first post-lesion day, with no modification in food consumption despite the closer anatomical proximity of these medially located nuclei to the ventromedial hypothalamus. Although lesions to ventral tuberomammillary nuclei E1 or E2 also produced a significant rise in water intake, the increase was slower and no significant difference was observed with the non-lesioned group until the fourth post-surgical day (Days 4/7). These differences can be explained according to available anatomical and physiological data, which distinguish between a ventral tuberomammillary region, where most neurons are magnocellular and compact, and a medial tuberomammillary region, where less organized cells of medium size predominate (Köhler et al., 1985; Yang & Hatton, 1997; Hatton & Li, 1998).

This functional dissociation among tuberomammillary groups is also shown in the fact that ‘E1 and E2 ventral polydipsia’ was associated with an increase in food intake, with significant differences from Day 8 (Days 8/11) in comparison with medial tuberomammillary groups and NLG.

It is possible that the high water intake observed after tuberomammillary nucleus lesions is in some way related to an alteration in the brain circuits involved in hydromineral regulation. In fact, abundant anatomical connections have been demonstrated between the tuberomammillary system and brain structures involved in the detection and control of increases in plasma osmolality, in which sodium usually plays an essential role (Knigge et al., 1987; Mckinley & Oldfield, 1994).

Thus, it has been reported that activation of tuberomammillary cells by various osmotic stimuli (dehydration, liquid deprivation, etc.) produces depolarization of magnocellular neurons of the supraoptic/paraventricular complex. This magnocellular activation triggers the release of hormones (vasopressin and oxytocin) that offer an appropriate response in situations that threaten maintenance of an optimal hydromineral balance (Akins & Bealer, 1993; Brown et al., 1993; Bealer & Crowley, 1999). Moreover, pharmacological blockade of tuberomammillary cells significantly decreases the secretion of vasopressin (Kjaer et al., 1994a,b) and oxytocin (Kjaer et al., 1995; Bealer & Crowley, 1999) of the posterior hypophysis in response to the same osmotic stimuli.

Likewise, it is important to note the morphological and anatomical features of tuberomammillary neurons, whose abundant dendritic processes extend throughout the ependymal layer of the ventral surface of the brain and mammillary recess, placing them in an advantageous position to respond to osmotic changes produced in the organism (Watanabe et al., 1984; Reiner et al., 1987).

Furthermore, experimental studies have shown that traumatic or other injuries to the CNS, especially in the posterior hypothalamus area (where tuberomammillary nuclei or their connections may have been damaged), can bring about a major imbalance in the organism’s internal sodium levels, frequently producing polydipsic symptoms in the animals (Cort, 1963; Naitcheff et al., 1975; Fitzsimons, 1979; Morales & Puerto, 1986).

This increase in water intake cannot be attributed to the characteristic tissue irritation of anodic lesions, which would explain the high water intake in the ME+ group (Rolls, 1970). Cathodic lesions (ME–), identical to those applied to the tuberomammillary groups, produced virtually no change in water intake.

The nutritional effects observed in the E1 and E2 groups are in agreement with findings of pharmacological studies related to inhibitors of histamine, a neurotransmitter that is only synthesized in the tuberomammillary complex (Wada et al., 1991) or to drugs that mimic its action and reduce food intake, both in animals (Fukagawa et al., 1989; Itoh et al., 1999; Morimoto et al., 2000; Mollet et al., 2003) and humans (Clineschmidt & Lotti, 1973).

Therefore, the tuberomammillary nutritional effect may be mediated by medial hypothalamic centres, such as the paraventricular and ventromedial nuclei, in which histaminergic tuberomammillary fibres are densely distributed (Panula et al., 1989; Orthen-Gambill & Salomon, 1992; Chailou et al., 2000).

This possibility was recently supported by observations that activation of the histaminergic tuberomammillary system may control the leptin-induced anorectic effect in some of these hypothalamic centres where leptin receptors are abundant (Masaki et al., 2001, 2004). In fact, chronic central treatment with histamine contributed to improve the energetic balance in db/db and obese agouti (A(y)/a) mice (Masaki et al., 2003; Toftegaard et al., 2003). Moreover, the administration of alfa-fluoromethylhistidine to animals attenuated the leptin-induced anorectic response induced by leptin (Yoshimatsu et al., 1999).

In this context, it is well established that pharmacological blockade and histaminergic inhibition significantly increase the food intake and body weight of animals (Morimoto et al., 2000), especially when histaminergic inhibitors are applied to the ventromedial hypothalamus and paraventricular nucleus (Sakata et al., 1990).

Ventral tuberomammillary cells have also been shown to have high levels of CART (cocaine- and amphetamine-regulated transcript), a neuropeptide whose central administration (paraventricular nucleus) significantly reduces the food intake (Dall Vechia et al., 2000).

Unlike the polydipsia induced by anodic median eminence lesions, the normal rhythmicity of water intake was not altered by tuberomammillary lesions. Thus, although polydipsic, the latter animals distributed their water intake throughout the day in a similar manner to non-lesioned animals, confirming findings by Morales & Puerto (1986) after lesions to the posterior hypothalamus. These results suggest that tuberomammillary polydipsia may be a non-polyuria-dependent hyperdipsia, because if the water retention and preservation process had been affected, the polydipsia should also have been observed during the day-night period, as in the animals with anodic lesions to the median eminence. The night/day rhythmicity of water intake of the ME+ group was severely affected after the lesion, suggesting a possible alteration in fluid preservation mechanisms (Hennesy et al., 1977).

This absence of rhythmicity in the water intake was also observed in the food intake of the ME+ animals. Unlike the tuberomammillary-lesioned and non-lesioned animals, the polydipsic animals of the ME+ group showed a similar food intake between daytime and nighttime periods. This hyperphagic effect may be due to the wide extent of the lesion, which may have involved mediobasal-periventricular hypothalamic centres (adjacent to the study area) that critically participate in food intake (Hakansson et al., 1996; Flier & Maratos-Flier, 1998; Choi et al., 1999). These lesioned hypothalamic structures may also include...
ventral tuberomammillary nuclei E1 and E2, which, as shown above (Experiment 1), may form part of a neural circuit involved in nutritional regulation.

This absence of night–day rhythmicity in food intake was also observed in the E1 ventral tuberomammillary nucleus, which showed no statistically significant differences with the ME+ group.

In this context, several recent studies proposed that tuberomammillary histamine may be involved in food consumption during the day, when histamine levels remain high, but not at night when they are low (Haas & Panula, 2003). These results may be explained by a possible relationship between neuronal histamine and circadian food intake rhythms in the animals (Doi et al., 1994; Deurveilher et al., 2002). In fact, some studies showed that a large number of histaminergic tuberomammillary fibres and H1 receptors were localized in the ventromedial and paraventricular hypothalamus, and especially in the suprachiasmatic nucleus, a key area in the control of circadian rhythmicity (Tuomisto et al., 2001; Krout et al., 2002).

In summary, the results of this study (Experiment 2) appear to confirm that the polydipsia of tuberomammillary- and median eminence-lesioned animals widely differ in the rhythmic pattern of their water intake, and suggest that the two types of hyperdipsia are/might be qualitatively different.

Mammillary polydipsia may be explained by some alteration in non-homeostatic mechanisms, especially salivary secretion deficits. This secretory disorder may produce a prandial pattern of water intake, in which the animals increase the frequency of intake and obtain a larger amount of water in order to humidify their oropharyngeal structures, thereby facilitating deglutition (Epstein et al., 1964; Ramos & Puerto, 1991).

The procedure used in this study is especially sensitive to this type of behaviour (Epstein et al., 1964; Kissileff & Epstein, 1969; Kissileff, 1969; Ramos & Puerto, 1991) and permitted the observation that during 2 h after a 22-h food deprivation there were no differences in water intake frequency between the tuberomammillary and control animals in the presence of dry food. These findings are very different from the frequencies obtained in similar studies of animals that were desalivated or whose central salivary nuclei were lesioned (Kissileff & Epstein, 1969; Kissileff, 1969; Ramos & Puerto, 1991).

Furthermore, food deprivation did not block the polydipsia in tuberomammillary-lesioned animals, as these animals, including those with E1 or E2 ventral tuberomammillary lesions, continued to consume greater amounts of water compared with the non-lesioned group.

In contrast, polydipsia was completely abolished in animals with anodic lesions to the median eminence when food was no longer available to them. This result is important, because it suggests that animals with anodic lesion in the median eminence retain the capacity (in cases of deprivation) for water retention. If this is so, the polydipsia described cannot be wholly explained by excessive liquid losses via urine. This interpretation is supported by the studies of Smith & McCann (1962), who showed the presence of hyperdipsia in diabetic animals (anodic electrolytic lesion to median eminence) despite being nephrectomized.

These results may lead to a reconsideration of this animal model of diabetes insipidus. Moreover, the fact that no alteration in hydric regulation was observed in animals with cathodic electrolytic lesions to the median eminence, similar lesions to those used in the tuberomammillary groups, suggests that the role of this region in water consumption behaviour remains uncertain.

Finally, it is worth mentioning clinical reports of polydipsia of unexplained origin but associated with different anomalies in posterior regions of the hypothalamus (Fitzsimons, 1979; Robertson, 1987). In this context, anomalous liquid consumption, ranging from 5 to 20 L daily, is relatively frequent among psychiatric patients, especially schizophrenics (6–17%) (Verghese et al., 1993). Post mortem studies of some schizophrenic patients revealed a lower bilateral neuronal density (34%) in the mammillary body region with respect to normal individuals (Briess et al., 1998). In some of these cases, significantly elevated plasma sodium levels were observed (hypernatremia), whereas the value of other electrolytes (K+, Ca) remained within normal ranges (Lerner et al., 2000). This clinical picture of polydipsia is similar to recent observations in soldiers with traumatic injuries to the base of the brain and/or areas adjacent to the third ventricle (Bacic et al., 1999).

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Abbreviations

E1, caudal ventral tuberomammillary nucleus; E2, rostral ventral tuberomammillary nucleus; E3, medial ventral tuberomammillary nucleus; E4, medial dorsal tuberomammillary nucleus; ME+, anodic-lesioned median eminence group; ME−, cathodic-lesioned median eminence group; NLG, non-lesioned group.

References


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