

Faster gastric emptying of a liquid meal in rats after hypothalamic dorsomedial nucleus lesion

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Abstract

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The effects of dorsomedial hypothalamic (DMH) nucleus lesion on body weight, plasma glucose levels, and the gastric emptying of a liquid meal were investigated in male Wistar rats (170-250 g). DMH lesions were produced stereotaxically by delivering a 2.0-mA current for 20 s through nichrome electrodes (0.3-mm tip exposure). In a second set of experiments, the DMH and the ventromedial hypothalamic (VMH) nucleus were lesioned with a 1.0-mA current for 10 s (0.1-mm tip exposure). The medial hypothalamus (MH) was also lesioned separately using a nichrome electrode (0.3-mm tip exposure) with a 2.0-mA current for 20 s. Gastric emptying was measured following the orogastric infusion of a liquid test meal consisting of physiological saline (0.9% NaCl, w/v) plus phenol red dye (6 mg/dl) as a marker. Plasma glucose levels were determined after an 18-h fast before the lesion and on the 7th and 15th postoperative day. Body weight was determined before lesioning and before sacrificing the rats. The DMH-lesioned rats showed a significantly faster ($P < 0.05$) gastric emptying (24.7% gastric retention, $N = 11$) than control (33.0% gastric retention, $N = 8$) and sham-lesioned (33.5% gastric retention, $N = 12$) rats, with a transient hypoglycemia on the 7th postoperative day which returned to normal by the 15th postoperative day. In all cases, weight gain was slower among lesioned rats. Additional experiments using a smaller current to induce lesions confirmed that DMH-lesioned rats had a faster gastric emptying (25.1% gastric retention, $N = 7$) than control (33.4% gastric retention, $N = 17$) and VMH-lesioned (34.6% gastric retention, $N = 7$) rats. MH lesions resulted in an even slower gastric emptying (43.7% gastric retention, $N = 7$) than in the latter two groups. We conclude that although DMH lesions reduce weight gain, they do not produce consistent changes in plasma glucose levels. These lesions also promote faster gastric emptying of an inert liquid meal, thus suggesting a role for the DMH in the regulation of gastric motility.

Key words

- Gastric emptying
- Hypothalamus
- Dorsomedial nucleus

Introduction

The digestive-absorptive process is dependent on the interplay between gastric emptying and small intestine movements. Changes in gastric emptying or intestinal digestion may result in the malabsorption of nutrients leading to malnutrition (1). Gastric emptying is regulated by motor and secretory activities of the fundus, body and antrum of the stomach (2), which may be influenced by stressful stimuli (3). In a previous paper, we demonstrated that lesion of the paraventricular nucleus (PVN) blocked the effects of stressful stimuli on gastric emptying in rats (4). These observations led us to reason that other nuclei might also be involved in the neuronal circuitry regulating gastric emptying. Since lesion of the dorsomedial hypothalamic (DMH) nucleus reduces weight gain and retards growth (5), we investigated the involvement of this nucleus in the control of gastric emptying.

Material and Methods

Experimental design

Two sets of experiments were performed to evaluate the role of the DMH nucleus in gastric emptying. In the first set, the DMH nucleus was lesioned electrolytically using a monopolar nichrome electrode (0.7 mm in diameter and 0.3-mm tip exposure). Gastric emptying, body weight and plasma glucose levels were measured before and after lesioning. In the second set, the DMH and ventromedial hypothalamic (VMH) nuclei were lesioned with a small electrode (0.1 mm in diameter and 0.1-mm tip exposure) in order to produce restricted lesions in the nuclei. In order to assess the influence of lesions occurring outside the DMH nucleus when the 0.3-mm tip electrode was used, complementary lesions of the medial hypothalamus (MH) were produced in a separate

group of rats. In the latter two experimental sets only gastric emptying was determined.

Animals

Adult male Wistar rats (170-250 g) were isolated in individual cages for three days before experimental manipulation. The animals were housed under controlled temperature and lighting conditions, with free access to standard rat chow (Labina, Purina, Campinas, Brazil) and water.

Surgical procedures

After an 18-h fast, the rats were anesthetized with pentobarbital (70 mg/kg) and bilateral electrolytic lesions of the DMH and MH were produced by delivering a 2.0-mA current for 20 s through an insulated nichrome electrode (0.7 mm in diameter and 0.3-mm tip exposure) placed stereotaxically into the nucleus using the following coordinates: incisor bar 3 mm below the interaural line; antero-posterior (AP), 3.3 mm posterior to the bregma; lateral (L), ± 0.6 mm from the sagittal suture for the DMH lesion and ± 0.9 mm for the MH; vertical (H), 9.0 mm from the dura for the DMH and 9.6 mm for the MH (6). In restricted lesions of the DMH and VMH nuclei, the nichrome electrode (0.1 mm in diameter and 0.1-mm tip exposure) was introduced with the following stereotaxic coordinates: for the DMH, the same coordinates as in the previous experiment were used, while for VMH lesions, L was +0.8 mm from the midline and H was 10.0 mm from the dura (6). The electrolytic lesions were produced with a 1-mA current for 10 s. The sham-lesioned rats underwent stereotaxic placement of the electrode 2.0 mm below the dura without any passage of current. All operated animals were allowed to recover from surgery for at least 15 days before being used for gastric emptying studies. Control rats were kept in their home cages during this period.

Blood collection

In the first set of experiments, blood samples were collected from a tail vein between 9:00 and 11:00 a.m. one day before surgery and 7 and 15 days after surgery. The plasma glucose levels were subsequently determined by a colorimetric glucose oxidase method.

Body weights

Body weights were determined one day before surgery and 15 days after surgery, immediately before the determination of gastric emptying at 1:00 p.m. The rats were weighed on an Ohaus precision scale for small animals.

Gastric emptying

Gastric emptying was measured by a previously described technique (7,8) using a test meal of 0.9% (w/v) physiological saline containing phenol red (6 mg/dl) as a marker. The experiments were carried out between 1:00 and 5:00 p.m. The rats received the test meal (2 ml/100 g body weight) orogastrically after a 20-h fast during which only water was available. After administration of the test meal, the rats were returned to their cages for 8.5 min. Control rats remained in their cages for a similar period of time. Subsequently, the rats were anesthetized with ethyl ether (60 s) and their stomachs were exposed by laparotomy and clamped at the pylorus for 30 s and the contents were recovered by aspiration and washing. The phenol red concentration, as a percentage of the gastric contents (percent gastric retention, % GR) 10 min after orogastric administration, was used to indirectly evaluate gastric emptying (7). This evaluation is considered technically correct for gastric motility when the test meal is physiological saline since there is no activation of intestinal receptors and hence no interference with gastric function

(9). At the end of the experiment, the rats were sacrificed by ether inhalation.

Histology

To determine the site and extension of the lesions, the brains were fixed in buffered formalin and embedded in paraffin and 40- μ m thick coronal sections were cut and stained with hematoxylin-eosin. At the end of each experiment, the stained sections were carefully compared with diagrams in the atlas of Paxinos and Watson (6). Only data from rats with bilateral lesions restricted to the DMH or VMH (two rats presented small dorsal arcuate lesions) were included in the analysis. The MH lesions damaged the DMH, VMH, the perifornical area and the lateral hypothalamus (LH) (Figure 1).

Statistical analysis

Single factor analysis of variance (ANOVA) and the Newman-Keuls test (10) were used to analyze the data with the level of significance set at $P < 0.05$.

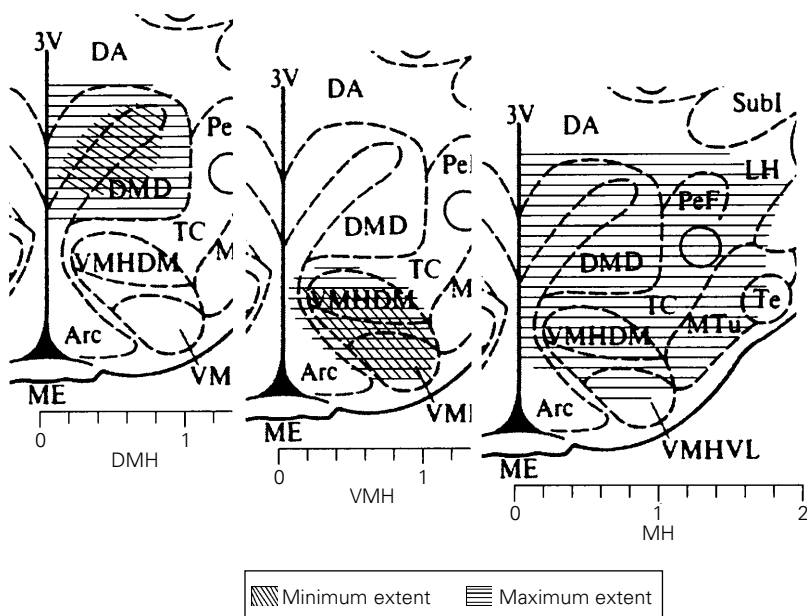


Figure 1 - Diagram of rat brain coronal sections showing the minimum and the maximum extent of lesions in the dorsomedial hypothalamic (DMH) nucleus, ventromedial hypothalamic (VMH) nucleus and medial hypothalamus (MH).

Results

Table 1 shows the body weight and plasma glucose levels before and after surgery. The percent body weight increase in DMH-lesioned rats was significantly lower than in the control rats, and the latter showed values similar to those for the sham group. At 7 days after surgery, the lesioned rats had significantly lower plasma glucose levels than the control and sham groups. By 15 days after surgery, the glucose levels in the lesioned rats were similar to those of the control and sham groups and significantly higher ($P<0.05$) than in lesioned rats 7 days after surgery. The results for gastric emptying are presented in Figure 2. The DMH-lesioned group had a significantly ($P<0.05$) lower gastric retention ($24.7 \pm 1.8\%$ GR) compared to the control ($33.0 \pm 2.1\%$ GR) and sham-lesioned ($33.5 \pm 0.1\%$ GR) rats; the values for the latter two groups were similar.

In the second experiment, only gastric emptying was measured since we simply wished to confirm the results of the first experiment using smaller lesions. The results are shown in Figure 3. The two rats with VMH and dorsal arcuate lesions were included because their gastric retention was the same as that of rats with only VMH lesions. The control and VMH-lesioned rats had similar levels of gastric retention which were significantly higher ($P<0.05$) than those of DMH-lesioned rats. Damage to a large portion of the MH resulted in a gastric reten-

tion which was significantly higher ($P<0.05$) than that observed in the other groups.

Discussion

Gastric emptying is a physiological process probably regulated by several hypothalamic nuclei. Based on the interplay between the paraventricular and the DMH nuclei, we hypothesized that the DMH nucleus may be involved in this regulation. Under conditions of cold-restraint stress, vagal non-adrenergic and non-cholinergic fibers have a relaxing effect on the stomach (11,12) while cholinergic fibers have a stimulatory effect on small intestinal movements (13). The PVN in these circumstances has been reported to activate both divisions of autonomic outflow (14). Our study with PVN lesions demonstrated that non-stressed rats with such lesions showed the same response as those subjected to cold-restraint stress (4). This similarity in response may reflect an increased activity of vagal non-adrenergic and non-cholinergic fibers (11,12) or a diminished activity of cholinergic fibers since vagotomy blocked this effect (4). In the present study, a typical electrolytic lesion of the DMH destroyed the nucleus bilaterally, although a small portion of the ventromedial, perifornical and dorsal regions of the arcuate nucleus were damaged in four animals. As illustrated in Figure 2, lesioned rats had a faster gastric emptying compared to sham and control animals. The surgical procedure apparently did

Table 1 - Increase in body weight and glucose levels in control (C), sham- (Sh) and DMH-lesioned (L) rats.

Data are reported as mean \pm SEM for the number of rats given in parentheses. * $P<0.05$, C vs L; ** $P<0.05$, L vs C and Sh; + $P<0.05$, 7 days after surgery vs 15 days after surgery (Newman-Keuls test).

Group	Weight difference (g)	Weight increase (%)	Glucose levels (mg/dl)		
			1 day before surgery	7 days after surgery	15 days after surgery
Control	173.5-291.0 (8)	38.1 ± 2.6	96.8 ± 12.3 (8)	99.5 ± 7.9 (8)	116.0 ± 5.5 (7)
Sham	201.0-255.5 (12)	27.6 ± 4.0	86.3 ± 2.9 (12)	92.5 ± 4.8 (12)	113.1 ± 3.2 (12)
Lesioned	235.0-266.4 (11)	$14.9 \pm 6.5^*$	83.1 ± 3.3 (11)	74.0 ± 3.2 (11)**	104.0 ± 8.7 (11)+

not interfere with gastric emptying since the control and sham values were not statistically different. In view of the destruction of small portions of the perifornical and arcuate regions of the VMH nucleus, we examined gastric emptying in rats in which the lesions were restricted to the DMH and VMH nuclei. Figure 3 shows that gastric retention was similar after the two types of lesions, thus confirming that small lesions of the VMH and/or of the VMH + arcuate nuclei did not interfere significantly with the results. This finding is corroborated by the observation that VMH lesions resulted in a gastric retention similar to that of control rats (Figure 3). The destruction of a large portion of the MH, thereby damaging the DMH, VMH, the perifornical area and the LH, resulted in pronounced gastric retention (Figure 3) and reinforced the effects observed with DMH lesions. Since the VMH and the dorsal arcuate nuclei do not influence the regulation of gastric emptying, it remains to be determined which nucleus or nuclei are responsible for the effect of MH lesions. It is well known that the intra-PVN administration of corticotrophin releasing factor (CRF) in rats produces all the symptoms of emotional stress (15), including a high gastric retention (14) similar to that seen with MH lesions. Whether the high gastric retention observed reflected PVN activation was not determined. The blockage of GABA_A receptors in the DMH nucleus produces symptoms similar to those of emotional stress (13) since the PVN is the main hypothalamic nucleus that regulates stress-related events (16). Considering the recently described interconnections between the DMH and PVN (17), it is possible that the blocking of GABA_A receptors in the DMH may activate the PVN and lead to the symptoms of emotional stress (13) in a manner similar to the intranuclear injection of CRF, thereby altering gastric emptying (14). On the other hand, lesioning only the DMH removed one of the activators of the PVN

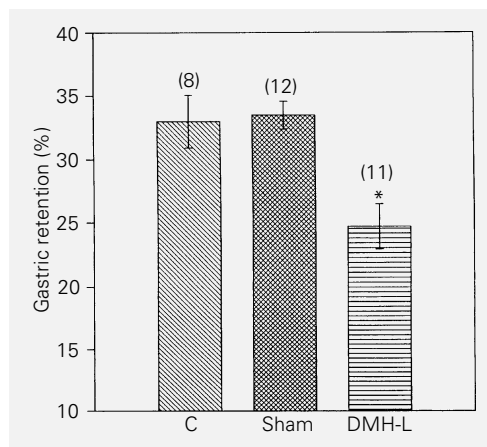


Figure 2 - Percent gastric retention of a liquid meal (0.9% NaCl, w/v) 10 min after orogastric administration. Each column indicates the mean \pm SEM of the number of rats given in parentheses. C, Control; DMH-L, dorsomedial hypothalamic nucleus-lesioned rats. * $P < 0.05$ compared to control and sham groups (Newman-Keuls test).

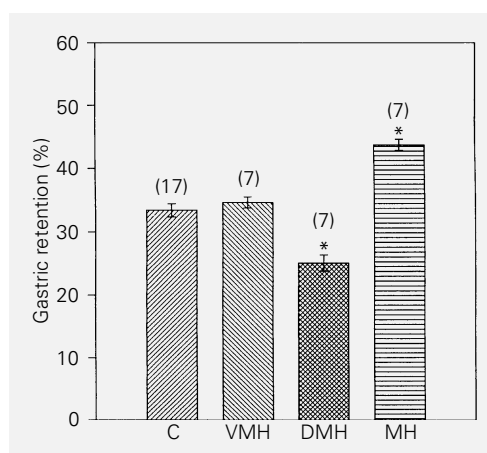


Figure 3 - Effect of hypothalamic lesions on the gastric retention of a liquid meal (0.9% NaCl, w/v) 10 min after orogastric administration. The lesioned structures were the dorsomedial (DMH) and ventromedial hypothalamic (VMH) nuclei and the medial hypothalamus (MH). Each column indicates the mean \pm SEM of the number of rats given in parentheses. * $P < 0.05$, DMH vs C and VMH; MH vs C, VMH and DMH (Newman-Keuls test).

and resulted in diminished gastric retention. DMH lesions have been reported to alter diurnal feeding and weight gain cycles (18). Similarly, the rats employed in the present study showed hypoactivity and loss of appetite along with faster gastric emptying of a liquid meal. The latter effect possibly reflects the diminished stimulation of vagal non-adrenergic and non-cholinergic fibers or an increased parasympathetic activity. The DMH nucleus is involved in the regulation of ingestive behavior (19). Thus, the slower weight gain of DMH-lesioned compared to sham-lesioned and control rats (Table 1) may reflect an altered ingestive behavior coupled with a loss of appetite following the lesion. With regard to glucose levels, only a transient decrease was observed in the lesioned rats on the 7th postoperative day

(Table 1) which returned to normal (control) values by the 15th postoperative day, in agreement with data reported in the literature (5). The reason for the decrease in glucose levels on the 7th day is unclear, but may be associated with a transient hypophagia and/or a transient mild hyperinsulinemia (20). Thus, the DMH appears to be involved in the maintenance of weight gain but has no definite effect on plasma glucose levels. Based on the results obtained for rats with VMH or VMH + arcuate nucleus and/or MH lesions, we conclude that the VMH and dorsal portion of the arcuate nucleus are not involved in the regulation of gastric emptying. The data for gastric retention in the presence of DMH lesions confirmed our hypothesis regarding the involvement of the DMH nucleus in the regulation of this phenomenon. The high gastric retention observed with the MH lesions (without any DMH involvement) shows that activation of the PVN derives from a source external to the MH and that the

DMH is not the only structure responsible for this activation. The DMH is likely to be an important element along with the PVN in a more complex neuronal circuitry in which the locus coeruleus also plays a role (21). The above results support the suggestion that there is a fine balance between the DMH and PVN in the regulation of gastric motility. Further studies are needed to clarify the interplay between these two regulators and their role in the digestive-absorptive process.

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