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Effects of adrenalectomy and adrenal enucleation on liquid gastric emptying in rats

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Abstract

The effects of adrenalectomy and adrenal enucleation on liquid gastric emptying were studied in male Wistar rats that were adrenalectomized, adrenal enucleated (AE) or sham operated (SH). The animals in the first group had free access to a 1% NaCl solution (ADS), while the animals in the second and third groups were divided into two subgroups, which ingested either tap water (AEW, SHW) or 1% NaCl solution (AES, SHS). The gastric emptying study was performed on the 16th post-operative day. Three test meals labeled with phenol red (6 mg/dl) were used (0.9% NaCl, 1.8% NaCl and 5% glucose). Percent gastric retention was determined 10 min after orogastric infusion of the NaCl test meals and 15 min after the glucose meal. Gastric retention of the ADS subgroup was significantly lower ($P<0.01$) (median = 19.8% *vs* 25.5% for SHW, *vs* 31.9% for SHS, *vs* 25.7% for AEW, and *vs* 27.1% for AES) for the 0.9% NaCl test meal and for the 1.8% NaCl test meal (33.5% for ADS *vs* 47.5% for AEW and 50.6% for AES). When 5% glucose was used as a test meal, gastric retention was similar for all subgroups. These results suggest that ablation of the adrenal cortex results in increased gastric emptying of an isosmolar NaCl meal.

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Key words
- Gastric emptying
- Adrenalectomy
- Adrenal enucleation
- Adrenal insufficiency

Adrenal hormone insufficiency or adrenalectomy is known to affect certain gastrointestinal tract functions (1,2). However, the role of these hormones in the regulation of gastric emptying is unclear. This study was therefore designed to examine the effects of adrenalectomy and adrenal enucleation on liquid gastric emptying (GE) in rats.

Male Wistar rats aged 8 weeks (195-240 g) were used after four days of acclimatization to the laboratory (25 ± 3°C and 12-h light/dark cycle). For the experiments, the rats were adrenalectomized (AD), adrenal enucleated (AE) or sham operated (SH).

Under general ethyl ether anesthesia, the retroperitoneal cavity was opened on both sides to expose the adrenal glands, which were then entirely removed (AD group) or partially removed (AE group). In the latter case, the adrenal medulla was removed by slitting the adrenal capsule and gently squeezing out this portion, leaving the remaining adrenocortical tissue in place. In the rats of the SH group, the glands were isolated as described above but were left intact. The rats were housed in individual cages for at least one week after surgery.

Rats of the AD group were housed with

free access to 1% saline (NaCl) drinking solution for 16 days after surgery (henceforth denominated ADS) before the GE test. Animals from the AE and SH groups were divided into two subgroups, depending on the type of liquid offered (tap water - AEW and SHW subgroups, or 1% saline solution - AES and SHS subgroups). Standard rat chow (Labina; Purina, Brazil) was also provided *ad libitum*.

Mean arterial pressure was measured by tail plethysmography (3) on the 14th day after surgery. On the 16th day, after a 24-h fast (solid food withdrawn), blood samples were collected from the abdominal vena cava of 10 rats in each subgroup after laparotomy under ether anesthesia. Samples were analyzed for hematocrit using a Centrimicro centrifuge, for plasma protein levels by refractometry, for plasma glucose levels by a colorimetric assay based on the O-toluidine method, and for plasma sodium and potassium levels by flame photometry. A further subset from each subgroup was used for the GE study. The weight and mean arterial pressure of this subset of rats were also measured.

Three test meals labeled with phenol red (6 mg/dl, 2 ml/100 g animal weight) were used: 0.9% (w/v) NaCl (290 mOsm/kg), 1.8% (w/v) NaCl (584 mOsm/kg), and 5% (w/v) glucose (296 mOsm/kg). Ten rats from each subgroup were used for each test meal. Before assessing GE, the rats were deprived of liquids for 1 h. Percent gastric retention (%GR) was determined 10 min after an orogastric infusion of the NaCl test meals and 15 min after a glucose meal, as previously described (4,5).

The ANOVA test ($k \geq 3$) was used to compare subgroups, and the Tukey test to compare pairs for weight gain (%), mean arterial pressure, hematocrit and plasma parameters. For both tests, an α value of 0.05 was used. For the %GR results, the Kruskal-Wallis test was used ($\alpha = 0.05$) and, when the result was significant, multiple comparison by a rank-sum test ($\alpha = 0.01$) was performed (6).

As previously reported (7), adrenalectomized rats gained less weight than the other groups during the 15-day post-operative period. Rats from the ADS subgroup also had lower plasma protein levels and were hypotensive, hypoglycemic, hyponatremic and hyperkalemic when compared to rats from the SH group. In contrast, AE rats showed a significant weight gain, only slight hypotension but also lower plasma protein levels (Table 1).

ADS rats retained significantly less (median = 19.8%) of the 0.9% NaCl test meal than the animals from the other subgroups (SHW = 25.5%; SHS = 31.9%; AEW = 25.7%, and AES = 27.1%, all median values

Table 1 - Weight gain, mean arterial pressure, hematocrit, plasma protein, glucose, sodium and potassium levels for the subgroups of rats studied.

The data are reported as means ± SEM. The number of animals is given in parentheses. [a]P<0.05 compared to ADS; [b]P<0.05 compared to SHW; [c]P<0.05 compared to SHS (Tukey test). AD, Adrenalectomized; AE, adrenal enucleated; SH, sham operated. The third letter in each abbreviation refers to tap water (W) or 1% saline (S).

	SHW	SHS	ADS	AEW	AES
Weight gain (%)	20.7 ± 0.8[a] (33)	19.8 ± 1.0[a] (36)	12.1 ± 1.2 (37)	30.0 ± 0.6[a,b,c] (54)	28.0 ± 0.6[a,b,c] (54)
Mean arterial pressure (mmHg)	110.9 ± 1.9[a] (40)	115.1 ± 2.4[a] (39)	100.0 ± 2.4 (41)	103.9 ± 2.1[c] (44)	109.6 ± 2.4[a] (39)
Hematocrit (%)	46.2 ± 0.4 (10)	46.7 ± 0.4 (10)	45.6 ± 0.7 (10)	44.7 ± 0.5 (10)	45.2 ± 0.7 (10)
Plasma protein (g/dl)	6.2 ± 0.1[a] (10)	6.0 ± 0.1[a] (10)	5.5 ± 0.1 (10)	5.5 ± 0.1[b,c] (10)	5.4 ± 0.1[b,c] (10)
Plasma glucose (mg/dl)	70.7 ± 4.7 (10)	77.1 ± 3.6[a] (10)	56.5 ± 3.1 (10)	68.5 ± 3.3 (10)	68.2 ± 5.1 (10)
Plasma sodium (mEq/l)	144.3 ± 1.4[a] (10)	141.4 ± 1.8 (10)	137.4 ± 1.5 (10)	144.4 ± 2.4[a] (10)	144.5 ± 0.5[a] (10)
Plasma potassium (mEq/l)	2.9 ± 0.1[a] (10)	2.7 ± 0.1[a] (10)	3.9 ± 0.2 (10)	2.5 ± 0.1[a] (10)	3.0 ± 0.3[a] (10)

- P<0.01). When the 1.8% NaCl test meal was used, the AE subgroups (AEW = 47.5%; AES = 50.6%) exhibited significantly greater retention (P<0.01) than the ADS subgroup (median = 33.5%). For these test meals no difference was observed between the SH and AE subgroups. Furthermore, the animals submitted to the 5% glucose test meal had similar gastric retention (Figure 1).

Stress has been demonstrated to reduce the GE of liquid caloric test meals (8) and this gastric motor response is mediated by the action of corticotropin-releasing hormone (CRH) in the central nervous system (9). In a stress situation, the plasma concentrations of CRH and other hormones, such as adrenocorticotropin (ACTH), cortisol and catecholamines, increase (9); however, this effect is transitory and after 14 days corticosteroid levels are the same in sham-operated and non-operated animals (10). Thus, it is believed that the 16-day period between surgery and the GE test used in these experiments may be considered to be safe.

Variations in blood glucose levels may also affect gastrointestinal motility and GE. The latter is characteristically reduced in hyperglycemia induced by an *iv* glucose infusion (11), and increased after hypoglycemia induced by an *iv* insulin infusion (12,13). These GE changes are not mediated by insulin (14). The ADS subgroup was hypoglycemic when compared to the SHS subgroup (Table 1), and it is possible that this condition contributed to reducing gastric retention of the 0.9% saline test meal in ADS rats.

Gastrointestinal motor and absorptive functions and extracellular fluid volume are tightly interlinked, and changes in blood volume may accordingly modify gastroduodenal flow (15,16). Experimental hypervolemia decreases gastroduodenal flow, whereas experimental hypovolemia has the opposite effect (15,16). It is possible that the ADS rats were hypovolemic 15 days after surgery. First, while hypovolemia is frequent in adrenal insufficiency it may not be detected by the hematocrit test because of mild anemia (2) concomitant with the decrease in plasma volume. Second, some investigators (7) found a larger total urine volume, a lower gain of total water and hyponatremia, related to

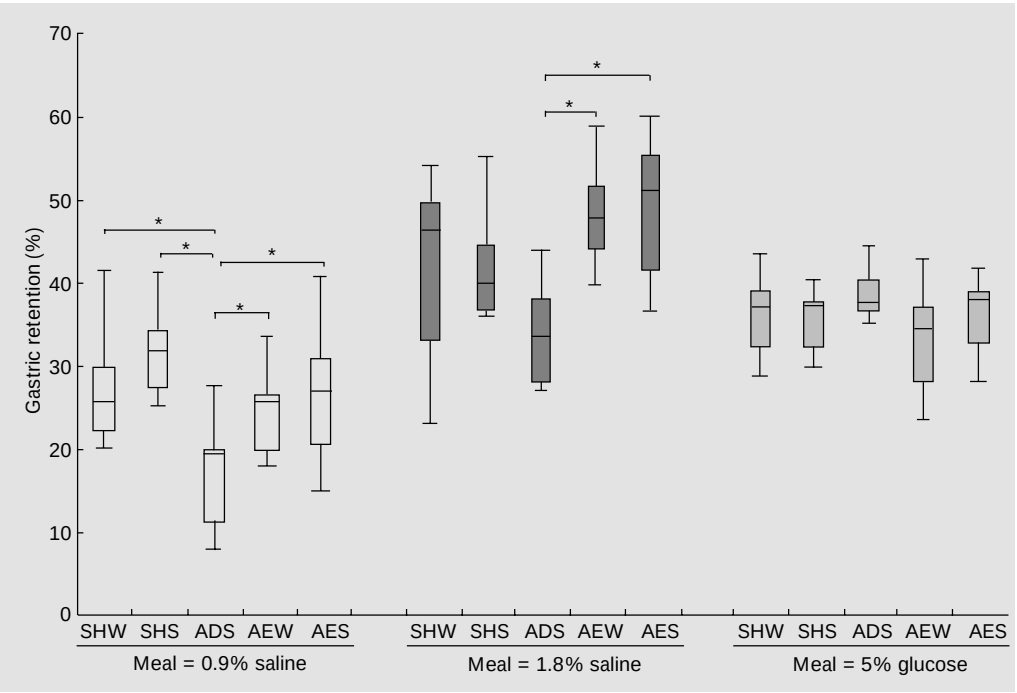

Figure 1 - Gastric retention of three test meals (0.9% saline, 1.8% saline and 5% glucose) in adrenalectomized (AD), adrenal enucleated (AE) and sham-operated (SH) rats. The third letter in each abbreviation refers to tap water (W) or 1% saline (S). The data are reported as box plots for 10 rats/subgroup. *P<0.01 (multiple comparisons test).

greater fecal and urinary excretion of sodium in adrenalectomized animals, 12 days after surgery. Finally, after ablation of the adrenal gland, a decrease in colonic electrolyte and water absorption is common (17). Accordingly, the lower mean arterial pressure observed in the ADS subgroup (Table 1) may also be a sign of hypovolemia, since blood pressure depends on blood volume and vasomotor tonus.

High plasma ACTH levels are common in adrenal insufficiency (2) and after adrenalectomy or adrenal enucleation (18), although following adrenal enucleation this elevation lasts for only about 15 days. This period corresponds to the recovery time allowed for the rats in this experiment. Since the AEW and AES subgroups did not differ significantly from the SH group in any test meal, it is unlikely that ACTH is involved in the observed responses. The involvement of CRH in gastrointestinal transit is also unlikely (9).

The osmolality and the caloric content of the meals are two concurrent factors that may modify the GE. A third factor, the volume, the only natural stimulus increasing GE by distending the gastric smooth musculature, was under control (2 ml/100 g animal weight). The 0.9% saline solution has been demonstrated to be the best meal to explicit the intrinsic gastric motor role in GE, since this isotonic and inert solution is a minimal stimulus to duodenal receptors that slow GE (19); then, the altered %GR observed in the ADS subgroup (with this test meal) suggests that this effect may be secondary to the adrenocortical suppression, since this effect was not seen among the animals of either the AE or SH subgroups. On the other hand, the GE pattern of the ADS subgroup did not show any difference from the SH subgroups when the two other test meals (1.8% saline and 5% glucose) were tested. The hyperosmolality of the first meal and the caloric content of the second may have exerted an inhibitory stimulus on GE (19,20) that overcame the influence of adrenocortical ablation (as revealed by the result of the 0.9% saline test meal experiment).

In conclusion, since we did not observe a difference between the sham-operated and adrenal enucleated groups, the results suggest that the increased gastric emptying of the isosmolar NaCl test meal in adrenalectomized rats reflects the resection of the adrenal cortex.

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