Aortocaval fistula delays gastric emptying of liquid test meal in awake rats

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As a consequence of inducing such changes, the placement of an AV fistula activates complex neurohumoral mechanisms that may restore hemodynamic homeostasis. During the decompensated phase, the exacerbated activity of vasoconstrictors and sodium-retaining agents [e.g., the renin-angiotensin system, arginine vasopressin (AVP), oxytocin (OT), the sympathetic nervous system] overwhelm the vasodilatory and natriuretic influence of atrial natriuretic peptide (ANP), bradykinin, and nitric oxide, leading to avid salt retention. For compensation to occur, the effects of natriuretic substances must prevail over the effects of opposing systems, increasing the renal excretion of fluid and electrolytes (38, 39).

In addition to their vasoactive and diuretic properties, these agents alter the activity of gut smooth muscle and may enhance gastric tonus (16). Moreover, we have shown that blood volume redistribution modifies gastrointestinal motor behavior (14, 40). Taken together, our hypothesis is that the opening of an AV fistula impels the circulation to a hyperkinetic status, which affects the gut motor behavior in virtue of hemodynamic adjustments and multiple neurohumoral changes. Thus this study was designed to verify in awake rats 1) if the hemodynamic changes elicited by an ACf alter the gastric emptying (GE) rate and upper gastrointestinal transit of a liquid test meal; 2) if the autonomic nervous system exerts a role on such phenomenon; and 3) if the establishment of ACf acutely alters the plasma levels of AVP, ANG, OT, ANP, and corticosterone.

MATERIALS AND METHODS

All of the procedures were performed in accordance with the ethical principles for the care and use of laboratory animals of the Brazilian Society for Laboratory Animal Science after approval by the Local Ethics Committee (protocol no. 46/07). Male Wistar rats (230–280 g) were obtained from colonies raised by the Federal University of Ceará and maintained in a temperature-controlled room on a 12-h/12-h light-dark cycle. They were isolated in Bollman’s cages and fasted for 18 h with free access to an oral rehydration solution, consisting of (in mM) 75 Na+, 65 Cl−, 20 K+, 75 glucose, and 10 citrate, to clear the stomach of food residue while maintaining normovolemia and euglycemia.

Surgical procedures. After overnight fasting, the rats were anesthetized with tribromethanol (250 mg/kg ip). After laparotomy, they were subjected to ACf placement (ACf group) or a sham-operated procedure with no ACf placement ( sham-operated group) as previously reported (13). Briefly, the abdominal aorta and inferior vena cava were exposed and dissected together. Using a vascular clamp, the vessels were occluded together below the renal artery, briefly stopping distal blood flow. A disposable needle was then used to puncture the aorta and advanced until it perforated the opposite wall to reach the vena cava lumen. After removal of the needle, the vascular holes were sealed with cyanocrylate glue. Prompt observation of the pulsatile flow of oxygenated blood in the vein was considered confirmation of a successful AV shunt. Separate subgroups of ACf rats were formed according to the extent of the AV shunt created by 26-gauge arteriovenous fistula; gastrointestinal motility; hyperkinetic circulation; intestinal transit; natriuretic hormone

HYPERKINETIC CIRCULATION IS a systemic condition that may originate from an arteriovenous (AV) fistula, either as a congenital malformation or acquired after invasive procedures (e.g., percutaneous renal biopsy), surgeries (e.g., nephrectomy), or trauma (e.g., stab or gunshot wounds) (11, 24). Large AV fistulas are considered a simple and reliable model to elicit congestive heart failure (13). Laboratory animals with an aortocaval fistula (ACF) present hemodynamic and neurohumoral changes that resemble those seen in patients with heart failure, including cardiac hypertrophy (22). These animals have high levels of cardiac output (CO) and mean circulatory filling pressure, in addition to tachy-
Three stainless-steel wires (0.203 mm outer diameter; Teflon-coated; A. M. Systems, Everett, WA) were affixed to the chest muscles and hip muscle of the left paw and then exteriorized at the interscapular region. After they were connected to a bioamplifier (ML132 BioAmp) coupled to a data acquisition system (PowerLab/8SP, ADInstruments), an electrocardiographic signal could be derived to continuously record heart activity. The femoral and common carotid arteries and jugular vein were then cannulated with polyethylene (PE)-50 thermocouple and PE-90 catheters, respectively. The distal ends of the catheters were subcutaneously exteriorized and fixed at the interscapular region. Continuous monitoring of MAP (in mmHg), central venous pressure (CVP; in cmH2O), and heart rate (HR; in beats/min) was performed by connecting the catheters to pressure transducers coupled to a digital system. CO (in ml/min) was estimated using the thermal dilution method (6). Systemic vascular resistance (SVR in dyn·s·cm−5) was calculated as SVR = MAP/C0, and stroke volume (SV in ml/beats) was calculated as SV = CO/HR. After surgery, the rats were subjected to 12, 24, or 48 h of fasting but with free access to the oral rehydration solution. Hemodynamic monitoring was performed just before the gut motility tests.

GE assessment. A dye dilution technique, previously adapted by us (34), was used to evaluate the GE of a liquid (1.5 ml) test meal (0.5 mg/ml of phenol red in 5% glucose solution). After 10, 20, or 30 min of the meal gavage, the rats, respectively named 10, 20, and 30-min postprandial subgroups, were euthanized by an intravenous thiopental overdose.

After laparotomy, the gut was divided into consecutive segments: stomach and small intestine. Each segment volume was calculated by submerging it in a graduated cylinder with 100 ml of 0.1 N NaOH. After homogenization, the proteins in each segment were precipitated with 0.5 ml of 20% trichloroacetic acid. After centrifugation, 3 ml of the supernatant was added to 4 ml of 0.5 N NaOH, and the samples were read by a spectrophotometer at 560 nm to construct dilution curves by plotting the dye concentrations against optic densities. The value of fractional gastric dye recovery was estimated from the following equation:

\[
\text{Gastric dye recovery (in %) = } \frac{\text{Amount of phenol red recovered in stomach}}{\text{Total amount of phenol red recovered from all segment}} \times 100
\]

To exclude the possible influence of gastric acid secretion on the effects of ACF on gut motor behavior, a separate group of rats was subjected to a similar protocol (i.e., laparotomy either followed or not by ACF placement with a 21-G needle). After 24 h, the rats received an intravenous injection (0.1 ml/kg) of omeprazole (20 mg/kg). After basal hemodynamic monitoring, the rats were gavage fed the test meal and euthanized 20 min later for gastric dye recovery analysis as described above.

To verify the influence of blood volume on the effects of ACF on gut motor behavior, we studied a group of rats either previously subjected to tribromoethanol anesthesia (250 mg/kg ip) and laparotomy, or by bilateral subdiaphragmatic vagotomy (19) or celiac ganglionection + splanchnicectomy (12). Two days later, they were anesthetized again and subjected or not (sham operated) to ACF (21 G) placement. Twenty-four hours after the second surgery, the rats were subjected to basal hemodynamic monitoring, followed by gavage feeding with the test meal and euthanized 20 min later for gastric dye recovery analysis.
Statistical analysis. The hemodynamic data that were recorded throughout the studies were pooled as mean MAP, CVP, and HR values into consecutive 10-min intervals: basal (i.e., the first 40 min of monitoring, which included the pharmacological treatments) and post-prandial (i.e., up to 30 min, just after gavage of the test meal). Each subgroup consisted of 6–9 rats. All of the data are expressed as means ± SE with the exception of the gastrointestinal transit index values, which are presented as medians and interquartile ranges. Differences in gastric retention values between groups were assessed by one-way analysis of variance (ANOVA), followed by Tukey’s multiple comparison test compared with the control group. Hemodynamic intragroup data differences between the basal period and successive intervals were compared using one-way repeated-measures ANOVA, followed by the Bonferroni test when appropriate. Values of \( P < 0.05 \) were considered statistically significant. For the analysis of plasma hormone levels, we used the unpaired Student’s \( t \)-test.

RESULTS

In the present study, the following hemodynamic parameters were monitored: MAP, CVP, HR, CO, SV and SVR. During the basal monitoring period, they varied spontaneously, but no difference (\( P > 0.05 \), ANOVA) was found between the baseline mean values recorded throughout the 40 min observation interval, either in control or ACF rats.

The impact of ACF placement on hemodynamic indexes in awake rats is shown in Fig. 1. When compared with the basal mean values of their respective sham-operated group, the values of the 23-G and 21-G ACF rats had higher CVP, CO, HR, and SV levels (\( P < 0.05 \), ANOVA and Tukey’s test) and lower MAP and SVR levels (\( P < 0.05 \), ANOVA and Tukey’s test). When compared with the mean values in the respective

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![Fig. 1. Effects of aortocaval fistula (ACF) and its respective sham operation (control, white bar) on basal hemodynamic values in awake rats. The fistula was created by vascular puncture with a 26-gauge (26 G, light gray bar), 23-gauge (23 G, medium gray bar), or 21-gauge (21 G, black bar) needle. Twenty-four hours after surgery, the animals were subjected to continuous hemodynamic monitoring for 40 min. A: mean arterial pressure (MAP, in mmHg). B: mean central venous pressure (CVP, in cmH₂O). C: cardiac output (CO, in ml/min). D: heart rate (HR, in beats/min). E: systemic vascular resistance (SVR, in dyn·s·cm⁻⁵). F: stroke volume (SV, in ml/beats). Each subgroup consisted of 6–9 rats. The data (means ± SE) are expressed as bars and vertical lines. \*\( P < 0.05 \) vs. their respective controls and between ACF-groups values (ANOVA followed by Tukey’s test).](http://ajpheart.physiology.org/)
sham-operated rats, the values resulting from 26-G ACF placement did not significantly alter MAP, CVP, or HR levels but increased CO levels \((P < 0.05, \text{ANOVA and Tukey’s test; Fig. 1, A, B, and D})\). On the other hand, regarding their respective MAP and SVR values, 21-G and 23-G ACF rats showed lower \((P < 0.05)\) levels compared with those of 26-G ACF subset. On the other hand, both 21-G and 23-G ACF rats showed higher \((P < 0.05)\) values of CVP and HR compared with the respective levels of 26-G ACF subset. Thus we used a 21-G needle to further study the mechanisms that underlie ACF-induced GE delay. Figure 2 shows that 21-G ACF placement significantly increased CVP, HR, CO, and SV levels but decreased MAP and SVR levels, which were manifested as soon as 12 h and persisted for at least 48 h.

Figure 3 shows that ACF placement delayed the GE of a liquid test meal in awake rats. When compared with the values in the respective sham-operated rats, the values in the 21-G ACF group showed no significant difference in fractional gastric dye recovery, but it was enhanced at both 24 and 48 h \((P < 0.05, \text{ANOVA and Tukey’s test; Fig. 3A})\). In rats studied 24 h after AV shunt or sham operation, ACF placement consistently increased fractional gastric dye recovery compared with their respective control values at different postprandial time intervals (i.e., after 10, 20, and 30 min of meal gavage; \(P < 0.05, \text{ANOVA and Tukey’s test; Fig. 3B})\). Moreover, gastric retention caused by ACF placement depended on the dimensions of the AV shunt. When we analyzed all of the data from ACF rats handled with 21-G needles and studied 20 min postprandially, a strong \((r = 0.96)\) and significant \((P < 0.001)\) positive correlation was found between the degree of the AV shunt and respective fractional gastric dye recovery value (Fig. 4).

Gastric retention caused by ACF placement appeared to be unrelated to a putative effect of hyperdynamic circulation on gastric acid secretion. Omeprazole pretreatment did not alter the increase in gastric dye recovery values \((P < 0.05, \text{ANOVA and Tukey’s test})\) elicited by ACF placement (33.5 ± 2.5% vs. 51.4 ± 4.5% in sham-operated and ACF rats, respectively). In contrast, acute bleeding prevented the GE delay in ACF rats (Fig. 5).

Figure 6B shows that bilateral subdiaphragmatic vagotomy prevented gastric retention in ACF rats. When compared with the gastric recovery values in respective sham-operated rats, ACF placement enhanced \((P < 0.05)\) gastric retention in rats previously subjected to coeliac ganglionectomy + splanchnicectomy (32.8 ± 6.7 vs. 54.0 ± 4.0%, respectively; Fig. 6A). Hexamethonium and pirenzepine treatment increased gastric recovery values \((P < 0.05)\) compared with vehicle-treated sham-operated rats (53.5 ± 3.8 and 52.1 ± 3.4 vs. 37.1 ± 2.5%, respectively) but prevented the ACF-induced GE delay.
The plasma levels of ANP, ANG II, and corticosterone are shown in Fig. 7. When experimental groups was compared with their respective sham-operated groups, the establishment of an AV shunt by creating an ACF significantly decreased the plasma levels of ANG II (46.8 ± 12.2 vs. 114.4 ± 15.1 pg/ml; \( P < 0.01 \), unpaired Student’s \( t \)-test), whereas it significantly increased corticosterone (19.2 ± 2.3 vs. 8.7 ± 1.9 \( \mu \)g/dl; \( P < 0.05 \), unpaired Student’s \( t \)-test) and ANP levels (72.9 ± 5.0 vs. 44.2 ± 5.9 pg/ml). On the other hand, ACF establishment did not affect plasma values of OT (2.6 ± 0.5 vs. 3.4 ± 0.3 pg/ml) or AVP (1.3 ± 0.3 vs. 1.4 ± 0.1 pg/ml).

In contrast, ACF placement did not alter the small intestine transit of the liquid test meal (53.5 ± 4.6 vs. 48.7 ± 2.9 and 52.1 ± 3.4 vs. 48.0 ± 3.5%, respectively; Fig. 6, C and D).

The plasma levels of ANP, ANG II, and corticosterone are shown in Fig. 7. When experimental groups was compared with their respective sham-operated groups, the establishment of an AV shunt by creating an ACF significantly decreased the plasma levels of ANG II (46.8 ± 12.2 vs. 114.4 ± 15.1 pg/ml; \( P < 0.01 \), unpaired Student’s \( t \)-test), whereas it significantly increased corticosterone (19.2 ± 2.3 vs. 8.7 ± 1.9 \( \mu \)g/dl; \( P < 0.05 \), unpaired Student’s \( t \)-test) and ANP levels (72.9 ± 5.0 vs. 44.2 ± 5.9 pg/ml). On the other hand, ACF establishment did not affect plasma values of OT (2.6 ± 0.5 vs. 3.4 ± 0.3 pg/ml) or AVP (1.3 ± 0.3 vs. 1.4 ± 0.1 pg/ml).

In contrast, ACF placement did not alter the small intestine transit of the liquid test meal. No significant changes in marker progression in the gut were found between the 21-G ACF and sham-operated rats studied 20 min postprandially, reflected by the median values of the meal’s geometric center [3.3 (2.7–3.4) vs. 3.4 (0.3–4.0), respectively]. No significant changes were observed in the blood pH and gas analysis between sham-operated and 21-G ACF rats (Table 1).

DISCUSSION

The present study showed that ACF placement delayed the GE of a liquid test meal in awake rats. The phenomenon occurred during the decompensated phase of blood volume redistribution, unbalancing cardiovascular function, inducing arterial hypotension and tachycardia, and increasing CVP and CO levels.

The vascular puncture technique that is used to create an ACF elicits hyperkinetic status that clearly depends on the extent of the AV shunt. The ACF placement with a 26-G needle did not change MAP, CVP, or HR levels. In contrast, these indexes changed when the vessels were punctured with wider bevels. Under such conditions, the hemodynamic changes were reliable and noticeable 24 h after surgery. However, none of the effects faded after
48 h, especially the increase in SV, because of the influence of homeostatic factors. Thus we used a 21-G needle and allowed a 24-h interval for the full expression of the hyperkinetic state.

Although the difference in gastric retention between the sham-operated and ACF rats studied 12 h after surgery was not statistically significant, ACF placement delayed GE at 24 h, a phenomenon that persisted for at least 48 h. The gastric dye recovery values in the sham-operated group studied at 12 h were significantly elevated, likely because anesthesia and laparotomy caused gastroparesis (4, 5). Such an hypothesis seems plausible because gastric dye recovery decreased at 24 h and plateaued thereafter. According to Coimbra and Plourde (8), the paralytic ileus is a short-lived phenomenon in rats because their gut motor behavior pattern returns to normal with 12 to 24 h.

The gut motility assessment was performed using a dye dilution technique, a simple and reliable method (27). Nonetheless, the phenol red dye used as a marker is a pH-dependent reagent, which may have biased the present analysis if one considers that AV placement eventually increases gastric acid secretion, thus inhibiting GE via duodenal chemical stimula-
in ACF-induced GE delay. Such involvement seems to be similar. Thus parasympathetic innervation appears to be involved in recovery values in the sham-operated and ACF subgroups were gastric retention caused by ACF placement. In rats previously awake rats previously submitted to sham operation or ACF placement excitatory input to the stomach that sustains gastric motor activity may be understood if one considers that the present data were obtained acutely (i.e., only 24 h after ACF placement) during a stage before full activation of the renin-angiotensin-aldosterone system. Nevertheless, the low ANG II level observed in the present study is consistent with ACF-induced GE delay. In fact, ANG II is considered to have predominantly stimulatory actions on small intestine motility (35).

Another important finding was the increase in the blood levels of corticosterone in ACF rats studied at 24 h, which may be putatively involved in ACF-induced GE delay since laparotomy increases the secretion of corticosterone, thus stressful conditions may delay GE in laboratory animals (29). However, it should be taken into account the specific temporal patterns of gastric retention observed in the present study (i.e., a gradual decrease 12 h after surgery in sham-operated rats while a steady elevation in the ACF group even 48 h later). Moreover, corticosterone, even when administered systemically, does not change GE rate in mice and dogs (23, 28). Thus corticosterone seems not to be directly responsible for the ACF-induced GE delay.

When we consider the complex process that modulates the gastroduodenal flow of liquid meals in awake mammals (26), the ACF-induced GE delay observed in the present study may have resulted from increased gastric relaxation, decreased antral contractility, or enhanced pyloric or duodenal resistance (10). Because an isotonic liquid was used as the test meal, such an effect is unlikely to be mediated through the enhanced intestinal inhibition of GE (i.e., via the “duodenal brake”). Moreover, no difference was found in the marker progression of intestinal transit in sham-operated and ACF rats. Thus we consider that ACF placement increased gastric dye recovery by inhibiting the tonus of the proximal stomach.

<table>
<thead>
<tr>
<th>n</th>
<th>Sham</th>
<th>ACF</th>
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<tbody>
<tr>
<td>pH</td>
<td>7.39 ± 0.02</td>
<td>7.38 ± 0.01*</td>
</tr>
<tr>
<td>Base excess, mmol/l</td>
<td>1.22 ± 0.57</td>
<td>2.00 ± 0.71*</td>
</tr>
<tr>
<td>[HCO$_3^-$], mmol/l</td>
<td>25.43 ± 0.55</td>
<td>26.23 ± 0.65*</td>
</tr>
<tr>
<td>Pco$_2$, mmHg</td>
<td>45.45 ± 2.71</td>
<td>48.03 ± 3.12*</td>
</tr>
<tr>
<td>Pco$_2$, mmHg</td>
<td>62.61 ± 4.95</td>
<td>61.31 ± 4.86*</td>
</tr>
<tr>
<td>SaO$_2$, %</td>
<td>77.10 ± 3.62</td>
<td>78.66 ± 2.81*</td>
</tr>
<tr>
<td>Hct, %</td>
<td>37.93 ± 0.55</td>
<td>40.28 ± 1.08*</td>
</tr>
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Values are means ± SE; n, number of rats per group. ACF, aortocaval fistula; SaO$_2$, arterial O$_2$ saturation. *Not significant, P > 0.05 after unpaired Student’s t-test.
As a fact, ACF establishment delays the GE of a liquid test meal in awake rats and, therefore, its respective inflow to the small intestine, which, in turn, postpones the absorption of fluids and electrolytes by the enteric epithelium. Thus it is conceivable to cogitate that the hyperkinetic circulation elicited by ACF placement alters the gut motor and permeability behavior, lessening the blood volume overload, at least acutely. Moreover, the present GE delay may be associated with the gut dysmotility complaints (i.e., bloating and dyspepsia) reported by patients with heart failure syndrome due to AV fistula (37).

In conclusion, the placement of a large AV infrafrenal shunt (i.e., ACF) induced a hyperkinetic circulation and elicited the GE delay of a liquid test meal in awake rats, phenomena that depended on an intact and functional parasympathetic nerve drive to the gut.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
M.T.B.S., F.G.O., and J.B.L. performed experiments; M.T.B.S., R.C.P.J., F.G.O., and J.B.L., prepared figures; M.T.B.S. and R.C.P.J. drafted manuscript; J.A.-R., R.B.O., P.J.M., and A.A.S. analyzed data; M.T.B.S., R.C.P.J., F.G.O., and J.B.L., interpreted results of experiments; J.A.-R., R.B.O., P.J.M., and A.A.S. conceived and designed of research; J.A.-R., R.B.O., P.J.M., and A.A.S. approved final version of manuscript; P.J.M. and A.A.S. edited and revised manuscript.

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