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Direct *in Vivo* Measurement of Gastric Microvascular Pressures in the Rat

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There are no direct data available on micropressures in the gastric microcirculation in spite of its pivotal role in the development of acute gastric mucosal lesions. Our goal was to develop an *in vivo* method to directly measure intravascular pressure and vessel diameter in various gastric microvessels. This paper describes methods and procedural details of our novel preparation of the exteriorized rat stomach for vascular micropuncture studies. The stomach of the anesthetized rat was fixed with minimal surgery in a temperature-controlled gastric chamber. Two preparations were used, both from the serosal side: a seromuscular preparation to study the circulation of superficial outer muscular layers and a submucosal preparation—following careful dissection of the seromuscular layer—to study the submucosal and deeper mucosal microcirculations. Intravascular hydrostatic pressure was measured with a servo-null micropressure measuring system, while vessel diameter was evaluated on the television screen with videometry. Data (average ± SE) were obtained from muscular arterioles (20.8 ± 0.93 μm; 29.8 ± 1.32 mmHg), venules (23.4 ± 1.61 μm; 18.1 ± 0.61 mmHg), submucosal arterioles (50.9 ± 3.55 μm; 55.4 ± 2.78 mmHg), venules (53.7 ± 2.06 μm; 21.4 ± 0.73 mmHg), and deeper mucosal arterioles (20.2 ± 1.06 μm; 33.8 ± 0.81 mmHg), venules (29.9 ± 1.17 μm; 25.8 ± 0.47 mmHg), at a systemic arterial pressure of 110 ± 2.4 mmHg (n = 10 each from 14 animals). Further experiments demonstrated the applicability of this method to examine the effects of systemic blood pressure reduction and local application of vasoactive agents on the gastric microcirculation. This method is useful for analyzing the microcirculation of the stomach *in vivo* under different experimental conditions.

Key Words: microvascular pressures; micropuncture; intravital microscopy; gastric microcirculation.

INTRODUCTION

Recently, many improved techniques have been established to study the microcirculation in the gastrointestinal tract (Holm-Rutili and Öbrink, 1985; Ohno *et al.*, 1995). However, direct measurement of microvascular pressures in gastric vessels have, up till now, not been made most probably due to difficulties in establishing a physiologically reliable preparation of this tissue for intravital microscopy and micropuncture.

Numerous studies have shown that microcirculatory changes, with emphasis on increased vascular resistances, play an important role in the pathogenesis of gastric mucosal injury (Holm, 1988; Yabana and Yachi, 1988). Indirect measurements or whole-organ techniques, such as reflectance spectrophotometry (Sato *et al.*, 1979), hydrogen gas clearance (Murakami *et al.*, 1982), laser Doppler velocimetry (Holm-Rutili and Öbrink, 1985), and flowmetry (Saita *et al.*, 1984) have particularly been used to evaluate only blood flow changes in the gastric microcirculation. These methods

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are inherently limited, since both blood flow and intravascular pressure measurements are necessary to calculate resistance changes along the vascular tree (Peti-Peterdi *et al.*, submitted for publication).

Consequently, there is a need for a technique, which allows simultaneous studies of basic microvascular parameters in the stomach, such as microvascular pressure and changes in vessel diameter. This paper describes a method for preparation of the exteriorized rat stomach for vascular micropuncture studies in the muscular, submucosal, and deeper mucosal layers of the gastric wall. We modified and improved the intravital microscopic observation methods for rat gastric microcirculation, as first developed by Guth and Rosenberg (Guth and Rosenberg, 1972; Rosenberg and Guth, 1970) to permit vascular micropuncture. We also employed micropressure measuring techniques in our studies similar to that reported by Wiederhielm and coworkers (Wiederhielm *et al.*, 1964).

MATERIALS AND METHODS

Animal Preparation

Male Wistar rats, weighing approximately 200 g, were fasted overnight with free access to water. The rats were anesthetized with thiobutabarbital-Na (Inactin-BYK, Germany), 120 mg/kg body wt intraperitoneally. Animals were placed on a micropuncture table and body temperature was maintained at 37 ± 0.5°C by means of a heating pad controlled by a rectal thermistor probe. Tracheotomy was performed and the trachea was intubated to facilitate spontaneous breathing. Systemic arterial blood pressure was monitored continuously through a PE-50 catheter placed in the left femoral artery by a Statham electromanometer (P23). A long central vein catheter (PE-50) was inserted through the left femoral vein for continuous infusion of Ringer's solution (0.5 ml/h/100g body wt) during surgical preparation and experimental conditions. Systemic arterial blood pressure could be experimentally manipulated by simply removing blood through this previously heparinized cannula. Heparinization of the animal was avoided in order to prevent excessive bleeding during tissue preparation.

Tissue Preparation

The abdomen was opened via a 5-cm midline incision and the stomach gently exteriorized. The surrounding gastric ligaments were cut. For exteriorization and to prevent bleeding from the short gastric artery and vein, a ligature was placed around the gastrosplenic ligament near to fundus before cut. Care was taken to avoid damaging or cutting gastroepiploic vessels running along the greater curvature. Immediately after exposing the stomach, we kept the tissue constantly bathed in warmed Ringer solution to avoid desiccation and irreversible cessation of blood flow in the superficial blood vessels. The animal was positioned on its right side on the micropuncture table and the stomach was placed in a gastric chamber. Appropriate temperature of the vascularly intact and innervated stomach in gastric chamber was maintained with a heating pad and by continuous superfusion (1 ml/min) of a 37°C Ringer solution, delivered via a commercial infusion set. A thermocontroller, having dual thermistor probes placed in the gastric chamber environment and peritoneal cavity, was used to measure the temperature difference, which was kept near to zero. The continuous water jacket and the use of heating pad kept the stomach in a constant environment with a high humidity and a temperature of 37 ± 1°C. In order to stabilize the preparation with the posterior wall facing up, four small pins were inserted into the chamber wall through the esophagus, antrum, forestomach, and corpus (Fig. 1a). However, the esophagus was not tightly fixed, in order to avoid respiration-induced gastric movements and changes in macrovascular parameters (e.g., venous pressure elevation) due to stretch. Care was taken to prevent compression or excessive manipulation of the left gastric artery, vein, and nerves to the stomach.

Vessels in different layers of the gastric wall were approached from the serosal side. A very light rubber ring was mounted on the gastric surface in order to contain a film of fluid which was needed for the micropuncture technique (Figs. 1a and 1b.). The narrow gap between the rubber ring and the preparation was sealed with agar gel. The area inside the ring (working win-

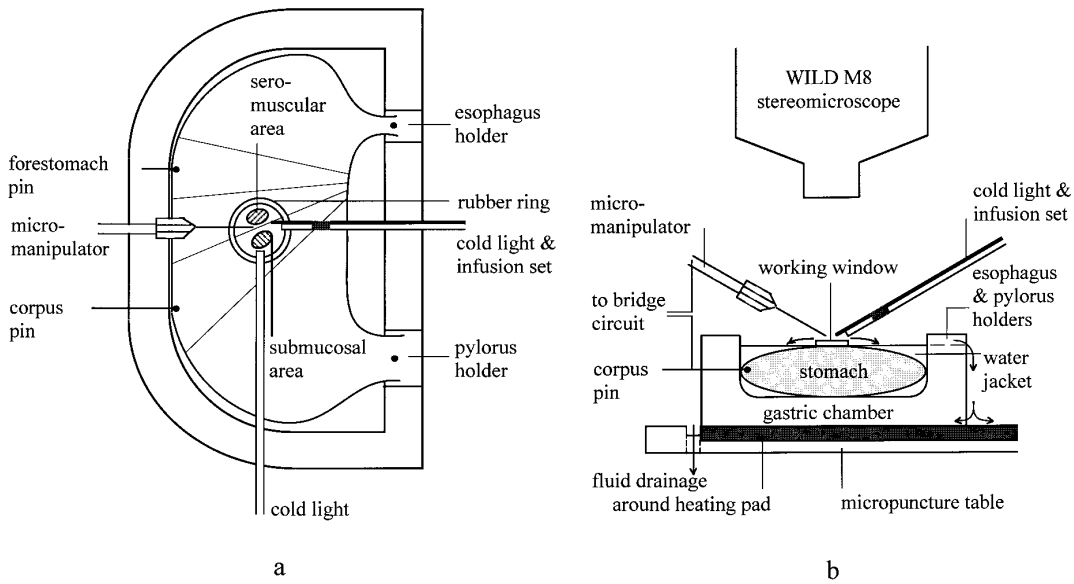

FIG. 1. (a) Diagram of the gastric chamber and the instrument setup. Outside dimensions: 6 × 4 × 2 cm. The seromuscular and submucosal investigation area were supplied by different small arteries (lines). The stomach was fixed with the help of four small pins inserted into the esophagus, forestomach, corpus, antrum, and by creating an esophagus and pylorus holder indentation on the gastric chamber wall toward the animal. (b) Cross-section of the preparation in the gastric chamber together with the instrument setup at the level of the working window. Dimensions of micropuncture table: 25 × 15 × 1 cm. Heating pad: 15.5 × 6 × 1 cm. Pin inserted into the peripheral corpus served as one arm and the micropipette holder as the other arm of the servo-null bridge circuit. The arrows show the direction of fluid flow. See text for details.

dow) was 1 cm in diameter and contained approximately 1 ml of fluid. The tip of the infusion-system was located in a peripheral part of the working window. The superfusate from the working window then supplied the whole gastric chamber with fluid (water jacket), which was drained at the level of esophagus/pylorus holders and down around the heating pad.

The working window consisted of two parts, the seromuscular and submucosal areas, both supplied by different small arteries. The seromuscular area served for muscular blood vessel measurements. For the submucosal preparation, a seromuscular tissue on the posterior wall of the corpus was removed (1 cm in diameter) by careful dissection from an area free of large vessels. If bleeding did occur, it stopped spontaneously in the course of a few minutes and blood was removed by repeated flushing. Thus we were able to visualize the submucosal microcirculation. In a small area of the submucosal preparation, we totally removed the submucosal connective tissue and the superficial part of the muscularis mucosae. In this way, the microvessels in the basal mucosa also became accessible through the remaining thin muscularis mucosae. The preparation procedure required up to 30 min. After completion of the experimental setup, the tissue was allowed to equilibrate for 1 hour before any experiments were attempted. During this time we visually observed that the diameter as well as the amplitude and frequency of vasomotion of blood vessels decreased and stabilized at a constant level.

Measuring Techniques

The preparation, illuminated with an Intralux 4000-1 fiber optic illuminator (Volpi AG, Switzerland), was visualized using a Wild M8 zoom stereomicroscope. Microvascular pressures were measured in microvessels with a modified Wiederhielm servo-null transducer (Model 4A, IPM, CA) (Wiederhielm *et al.*, 1964). With this system, hydrostatic pressures in small blood vessels can be measured using glass micropipettes filled with 2 M NaCl solution. Micropipettes (o.d., 0.85 mm;

i.d., 0.54 mm; Custom Glass Tubing, Drummond Scientific Co., Bromwall, PA) were pulled with a pipette puller (Experimetria Ltd., Hungary) to a tip diameter range of 1–3 μm and sharpened at an angle of 30°. Insertion of the micropipettes into the vessels was performed with a Leitz micromanipulator at a magnification of 100×. Pipette solution contained Evans-blue (Reanal Co., Hungary) to verify proper placement of the pipette into the lumen of the vessel. In addition, each micropipette was calibrated over the range of zero to 200 mmHg in a calibration chamber using a manometer. A pulse pressure, synchronous with the heart, was measured in all arteriolar vessels and was one indicator of a successful impalement. Microvascular pressures and systemic arterial blood pressure were recorded simultaneously on an OH-814/1 (Radelkis Co., Hungary) recorder at sensitivity ranges that resulted in a total system accuracy of ± 1 mmHg. The accuracy of the servonull technique in our system was evaluated before experiments were attempted by penetrating approximately the same medium-sized second-order segments of the submucosal arterial plexus under normal systemic blood pressure (110 ± 1.6 mmHg, mean ± SE). A total of 36 vessels from 5 animals were sampled at different time intervals (1–5 h) for intravascular pressure (54.1 ± 1.9 mmHg) and diameter (45.1 ± 1.9 μm) measurements. The small standard errors of our results warranted the reproducibility of the technique.

Microvessel images were viewed through the microscope with a TV camera (Sony, SSC-M370CE), continuously displayed on a TV monitor (Sony PVM-145E), and recorded on videotape (Sony SLV815VP). Vessel dimensions were determined using the scale of a built in microscope eyepiece and measured in video recordings displayed on the television screen with a caliper. The final magnification on the television screen was up to 1000×, and the estimated accuracy of this method was ± 1 μm.

In a separate series of experiments, we studied the effects of systemic arterial pressure reductions on intravascular pressure and vessel dimension of muscular, submucosal, and deep mucosal arterioles. Systemic blood pressure was lowered by approximately 40 mmHg by removing 0.5–1 ml blood in four steps of 10–20 mmHg. Blood pressure was allowed to stabilize over 5–10 min prior to obtaining data at each level of systemic blood pressure.

Studies were also performed during topical application of acetylcholine (1 μM, Sigma Chemical Co., St. Louis, MO) and epinephrine (0.1 μM, Sigma Chemical Co.) applied directly to the working window by switching the superfusate to another Ringer's solution containing these drugs. Data were recorded prior to application of drugs (control values), during the administration of these vasoactive agents (5–10 min) and after the drugs had been removed by switching back to the normal Ringer's solution (recontrol values).

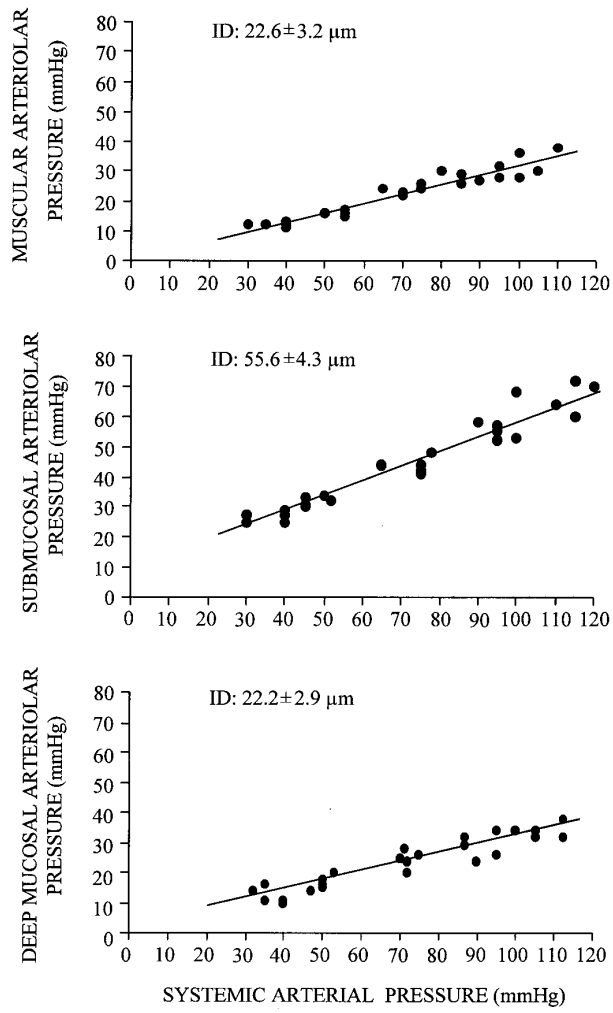

FIG. 2. Intravascular pressures measured in muscular, submucosal, and deep mucosal arterioles, expressed as a function of mean systemic arterial blood pressure. Indicated diameters of each vessel type are averages of internal diameters (ID) when systemic blood pressure was normal (108 ± 2.38 mmHg). A total of 75 vessels from 5 animals were sampled. Lines were obtained from linear regression analysis.

RESULTS

Control microvascular pressure and diameter measurements were performed for up to 5 h with no signs of deterioration of the tissue as judged by the absence of leukocytes sticking and rolling along the walls in venules or progressive dilatation of arterioles and large amplitude vasomotion. Spontaneous gastric muscle contractions and respiratory movements were, in most animals, small enough to allow micropuncture measurements.

In the first series of experiments a total of 60 vessels from 14 animals were examined. Vessel diameters (ID) and microvascular pressures (P_m) were measured in the following six vessel types from the three main gastric vascular units: muscular arterioles (20.8 ± 0.93 μm; 29.8 ± 1.32 mmHg), venules (23.4 ± 1.61 μm; 18.1 ± 0.61 mmHg), submucosal arterioles (50.9 ± 3.55 μm; 55.4 ± 2.78 mmHg), venules (53.7 ± 2.06 μm; 21.4 ± 0.73 mmHg), and deeper mucosal arterioles (20.2 ± 1.06 μm; 33.8 ± 0.81 mmHg), venules (29.9 ± 1.17 μm; 25.8 ± 0.47 mmHg)(n = 10 each). Systemic blood pressure averaged 110 ± 2.4 mmHg.

Figure 2 shows the steady-state pressures in muscular, submucosal, and deep mucosal arterioles as a function of systemic pressure from other studies. Data were recorded from each indicated vascular segment from five animals, separately in each step of systemic pressure reduction, resulted in a total of 75 vessels sampled. Pressures in all arteriolar segments were a linear function of systemic pressure. The equation for the muscular arterioles was

$$P_m = (0.32 \pm 0.02)P_a + (0.135 \pm 1.45),$$

for the submucosal arterioles was

$$P_m = (0.504 \pm 0.03)P_a + (8.13 \pm 2.05),$$

and for the deeper mucosal arterioles was

$$P_m = (0.3 \pm 0.02)P_a + (1.79 \pm 1.72),$$

with r values of 0.959, 0.970, and 0.941, respectively, based on linear regression analysis (P_m, microvascular pressure; P_a, systemic arterial pressure). The initial arteriolar diameters were reduced when blood pressure was lowered. The maximal decrease in diameter was 12.2 ± 0.89, 13.8 ± 1.48, and 10.8 ± 0.93 μm, respectively, as above.

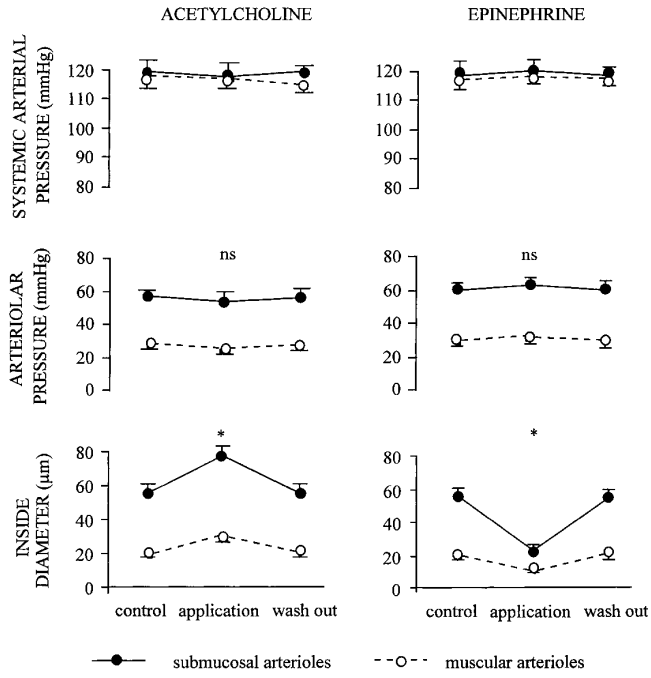

FIG. 3. Response of gastric arterioles in muscular (open circles, dashed line) and in submucosal (solid circles, solid line) layers to topical application of acetylcholine (1 μM) and epinephrine (0.1 μM) on the working window. Data are presented as mean ± SE. (n = 5 each, *$P < 0.05$ based on paired t test; ns, nonsignificant).

In the last series of experiments we studied the local effects of different vasoactive agents on the gastric microcirculation as shown in Fig. 3. Data were obtained from the same segments of both muscular and submucosal arterioles from five animals, before, during, and after application of drugs. Acetylcholine induced a marked dilatation of the muscular (ΔID: +10.2 ± 0.58 μm) and submucosal arterioles (ΔID: +22.0 ± 2.54 μm, $P < 0.05$); however, we observed only slight differences in intravascular pressure (ΔP_m: −2.8 ± 0.44 and −3.2 ± 1.19 mmHg, respectively, ns). Epinephrine markedly constricted the arterioles (ΔID: −9.8 ± 0.85 and −32.2 ± 1.27 μm, $p < 0.05$) and also caused no significant change in intravascular pressure (ΔP_m: +2.2 ± 1.19 and +2.8 ± 0.48 mmHg). All changes in pressures and diameters

were reversible upon return to the normal Ringer's superfusate. Medium-sized venules in the same layers showed no change in these microvascular parameters in response to either drug. Systemic arterial blood pressure was stable and did not change during these experiments.

DISCUSSION

Micropuncture studies are available for several organs including the small intestine (Bohlen and Gore, 1976; Gore and Bohlen, 1977), yet, to our knowledge, there are no data on the gastric intravascular pressures, except for a preliminary report from our laboratory (Peti-Peterdi and Rosivall, 1995).

The original pioneering study for *in vivo* microscopy of the gastric microcirculation originated from the work of Guth and Rosenberg (Guth and Rosenberg, 1972; Rosenberg and Guth, 1970), in which they dissected a portion of muscle and serosa and directly observed the submucosal microcirculation. This method, nonetheless, may lead to difficulties in observation, because of respiratory and peristaltic movements and transillumination problems. Therefore, other techniques were developed (Holm-Rutili and Öbrink, 1985; Ohno *et al.*, 1995) in order to secure and fix the tissue by longitudinally cutting the greater curvature of the stomach. However, this major surgery may adversely influence many gastric functions including the microcirculation.

Our preparation has several advantages over the earlier methods: (i) without disturbing the macroscopic gastrointestinal circulation, the stomach was fixed with only minimal surgery in a special gastric chamber, which also prevented all but minimal movement due to respiration and peristalsis, (ii) a special intravital microscopic technique, permitting continuous intravascular pressure measurements (micropuncture) can be used, (iii) by creating an isolated working window in the same system, the local effect of vasoactive substances can be analyzed, and (iv) due to the extragastric high intensity light sources we did not need to transilluminate the tissues from inside the gastric cavity.

The previously shown series of our experiments clearly demonstrates that both macro- and microcirculatory parameters can be altered and measured using our gastric micropuncture preparation. The tissue environment in the working window can be controlled; the microvessels of the outer muscular, submucosal, and deeper mucosal layers are readily accessible. Furthermore, for future studies, arteries, veins, and nerves to the stomach can be easily manipulated.

The control microvascular pressure and diameter data, measured in the three main segments of the gastric vasculature, correspond well to the physiologic values of similar vascular segments in the systemic or intestinal circulations (Gore and Bohlen, 1977). One exception is the relatively high venous pressure (25.8 $\pm$ 0.47 mmHg) in the mucosal venules. However, further studies are needed to fully understand the significance of this elevated pressure.

In other experiments (Fig. 2.) it was found that intravascular pressures of muscular, submucosal, and deep mucosal arterioles were reduced in a linear fashion with decreases in systemic arterial pressure. The slope of the regression line was almost the same in muscular and deep mucosal arterioles (0.32 $\pm$ 0.02 and 0.3 $\pm$ 0.02), but significantly higher (0.504 $\pm$ 0.03) in submucosal arterioles. Furthermore, the intravascular pressures of the submucosal vessels were twofold higher, compared to the other vessels. Indeed, submucosal arterioles are larger blood vessels, and they are exhibiting macrovascular characteristics. These results are similar to the parameters of small intestinal arterioles with same size (Gore and Bohlen, 1975).

Application of acetylcholine and epinephrine resulted in marked changes in vascular diameter. However, these drugs surprisingly did not cause significant intravascular pressure changes. Our findings are consistent with those reported by Gore (Gore, 1972), measuring the effect of microperfused norepinephrine on mesenteric arterioles. In our studies, as well, there was no significant change in pressures during constriction or dilation. Thus vasoactive substances appear to control vessel diameter and vascular blood flow, while intravascular pressure is kept constant.

In conclusion, the present investigation developed an intravital microscopy technique, permitting continuous quantitative measurements of microvascular pressures and vessel diameters. With this method it has become possible to investigate other basic microvascular parameters in different levels of gastric microcirculation.

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