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Effect of Graded Arterial Occlusion on Ileal Blood Flow Distribution (40634)

RICHARD E. PARKER AND D. NEIL GRANGER

Department of Physiology, College of Medicine, University of South Alabama, Mobile, Alabama 36688

In recent years there has been growing interest in the intestinal microcirculation relative to its ability to regulate blood flow and oxygen delivery. The role of local myogenic and metabolic factors in the regulation of intestinal blood flow has received much attention and support (1, 2). The effects of graded arterial occlusions on blood flow are frequently used to elucidate the mechanism(s) involved in the intrinsic regulation of blood flow. An assumption inherent in previous studies is that the reactive hyperemic response to arterial occlusion occurs uniformally throughout the wall of small intestine (2–4).

While various physiological and pharmacological interventions are known to alter blood flow distribution to the various layers of the intestinal wall the effect of graded arterial occlusion on blood flow distribution has not previously been studied. The present study was designed to examine the effect of graded arterial occlusion (60s, 120s) on blood flow distribution in the small intestine.

Methods. The experimental preparation used in this study is similar, except for minor alterations, to that described in detail in a previous study (3). In brief, six cats previously fasted for 18 hr, were initially anesthetized with 150 mg ketamine HCl. The right femoral vein was cannulated for administering sodium pentobarbital. Systemic arterial pressure was measured with a Statham P23A transducer connected to a femoral artery cannula. A tracheotomy was performed to facilitate breathing and as a means of artificial respiration if the cats failed to breath spontaneously during the experiment. A midline abdominal incision was made, and the greater omentum, lesser omentum, spleen, large intestine, duodenum, jejunum, and a small portion of proximal ileum were extirpated. In some animals the stomach was surgically removed due to excessive distention. Immediately after isolation of the ileal segment and before cannulation of the superior mesenteric artery and vein, heparin (1 mg/kg) was administered intravenously. Body temperature was maintained at 37° by use of an overhead infrared lamp. To minimize evaporation and tissue hydration, all exposed tissue was moistened with saline-soaked gauze and covered with a plastic sheet.

The superior mesenteric (SM) artery was rapidly ligated and cannulated in both distal and proximal directions. The ends of the two cannulas were connected via a Carolina Medical Electronics flow probe with pressure ports. Superior mesenteric arterial pressure was measured through one of the flow probe pressure ports, which was connected to a Statham P23Ac transducer. The signal from the flow probe was used to drive a square-wave electromagnetic flowmeter (Caroline Medical Electronics). A large polyethylene cannula was inserted into the superior mesenteric vein, and venous outflow was passed into a reservoir before being returned to the cat through a jugular vein. The end of the venous outflow cannula was firmly attached to a vertically positioned pulley system. Venous outflow pressure of the intestinal segment was set by adjusting the height of the reservoir and monitored from a T connector located in the venous circuit. Heparinized blood from a freshly sacrificed donor cat was used to prime the extracorporeal venous and arterial circuits. The remaining portion of the mesenteric pedicle was tied off to ensure that venous outflow represented the total organ value. Blood flow distribution within the different layers of the small intestine was determined by use of multiple pulse injections of radiolabeled microspheres (^{85}Sr, ^{169}Yb, and ^{141}Ce, 3M Co., St. Paul, Minn.). The radiolabeled microspheres, with a mean diameter of 15 μm were injected into a rubber port in the mesenteric arterial loop located immediately distal to the flow probe. The microsphere-containing arterial blood entered a mixing chamber before entering the circulation of the small intestine. Blood was with-

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drawn for 4 min at a constant rate (0.51 ml/min) from a rubber port in the arterial loop located immediately distal to the mixing chamber. The total counts in the withdrawn blood was used to calculate absolute blood flow. In general, the ^{141}Ce microspheres (0.2 ml, 5 μCi) were injected into the arterial loop in the control state when systemic arterial pressure, superior mesenteric and venous pressures, and intestinal blood flow were in a steady state. Ten to fifteen minutes after the ^{141}Ce injection, the superior mesenteric artery was occluded proximal to the flow probe for 60 sec, after which the occlusion was released. Fifteen seconds prior to releasing the occlusion 5 Ci (0.2 ml or approximately 40,000 microspheres) of ^{85}Sr microspheres were injected into the occluded arterial loop proximal to the mixing chamber. After releasing the occlusion, blood was withdrawn for 4 min as in the control condition. Ten to fifteen minutes after a new steady state was achieved the arterial loop was occluded for 120 sec and the aforementioned procedure was repeated using ^{169}Yb microspheres. In four preparations, ^{85}Sr, ^{169}Yb, and ^{141}Ce microspheres were injected in random sequence during steady-state blood flow in order to determine if our results were due to differences among the microspheres rather than the experimental intervention.

After the 4-min blood withdrawal (120-sec occlusion) the mucosal–submucosal samples were acquired by scraping (3-cm length) the mucosal–submucosal layers from the remaining tissue with glass microscope slides, and the underlying muscle layers were cut into 3-cm-long sections (10 samples). All mucosal–submucosal and muscularis samples were placed in counting tubes and counted in a three-channel gamma counter (Packard Model 3000). Corrections were made for overlap in the gamma energy spectrums. Control and reactive hyperemic blood flows (BF) in each tissue sample (per gram wet wt) were calculated from the blood withdrawal rates (WR), total counts/min of arterial blood withdrawn during each period (BC), and the counts/min of each isotope in each tissue sample (TC) assuming BF = TC × WR ÷ BC.

In three preparations, small bowel motor action was determined by monitoring intraluminal pressure with a fluid-filled, open-tipped polyethylene catheter connected externally to a pressure transducer. The lumen was filled with enough Tyrodes to maintain a pressure of 10–15 mm Hg. Lumen pressure was continuously monitored under control conditions and during 60- and 120-sec arterial occlusions.

Statistical analyses were performed by conventional methods, i.e., arithmetic mean ± SE, Student's t test.

Results. Under control conditions the systemic arterial pressure was 102.5 ± 6.9 mm Hg and the superior mesenteric blood flow was 28.6 ± 3.5 ml/min/100 g (as measured by the electromagnetic flowmeter) at a venous pressure of 5.0 mm Hg.

Figure 1 depicts the results of a representative experiment in which systemic arterial pressure (P_A), superior mesenteric arterial pressure (P_{MA}), venous pressure (P_V), and superior mesenteric blood flow (Q_B) were continuously monitored. When blood flow (31.0 ml/min/100 g) and the various perfusion pressures were in a steady state, ^{141}Ce

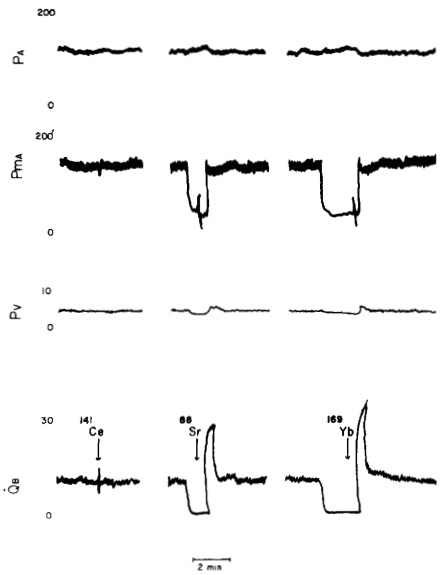

FIG. 1. Reactive hyperemic response of the cat ileum to graded arterial occlusions. Systemic arterial pressure (P_A), superior mesenteric arterial pressure (P_{MA}), venous outflow pressure (P_V), and total intestinal blood flow (Q_B) were continuously monitored. Radiolabeled microspheres were injected for determination of control (^{141}Ce), 60-sec (^{85}Cr), and 120-sec (^{169}Yb) arterial occlusion blood flow distributions.

microspheres were injected for control flow distribution within the various layers of the intestinal wall. Fifteen minutes later the arterial loop was occluded for 60 sec and ^{88}Sr microspheres were injected prior to releasing the occlusion. Upon release of the occlusion blood flow increased to a peak hyperemic level of 2.86 times control (86.1 ml/min/100 g). Fifteen minutes after all parameters returned to control levels the arterial loop was occluded for 120 sec (^{169}Yb microspheres were injected into the loop prior to releasing the occlusion). A peak hyperemic flow of 99.3 ml/min/100 g (3.3 × control) was observed for the 120-sec occlusion. For all experiments, occlusion of the superior mesenteric artery for 60 sec resulted in 2.24 ± 0.15-fold increase in total intestinal blood flow while 120-sec occlusions resulted in 3.06 ± 0.16-fold increase in flow. The duration of the reactive hyperemia for the 60- and 120-sec occlusions were 51.5 ± 2.1 and 76.2 ± 4.2 sec, respectively. Systemic arterial pressure was 109.8 ± 8.0 mm Hg during the arterial occlusions. In four separate experiments we observed no significant difference in the absolute blood flows and the relative blood flow distribution under steady-state conditions when the microspheres were administered in a random sequence. This suggests that the results obtained were due to the experimental interventions rather than differences among the microspheres.

The relative blood flow distribution in the mucosa–submucosal (M-S) and muscularis (M) layers of the intestine under control conditions was 64.5 ± 3.8% for M-S and 35.5 ± 3.8% for M. The cumulative results of the effect of 60- and 120-sec arterial occlusion on hyperemic flow to the mucosa–submucosa and muscularis layers are presented in Table I. The data indicates that 60-sec arterial occlusion causes a 2.10 ± 0.15-fold increase in M-S flow while M blood flow increases to 3.58 ± 0.83 times control. The difference between the M and M-S response to 60-sec arterial occlusion was not significant ($P > 0.10$). The 120-sec arterial occlusion causes a 2.48 ± 0.52-fold increase M-S blood flow while M blood flow increases to 6.30 ± 0.90 times control. Although the M-S response to 120-sec arterial occlusion was not significantly different than that observed after 60-sec occlusions, the M response to 120-sec occlusion was significantly greater than both the 120-sec M-S response ($P > 0.05$) and the 60-sec M response ($P > 0.01$). The results suggest that increasing the duration of the arterial occlusion does not significantly influence the response of the mucosa–submucosa vasculature yet has a significant effect on the muscularis circulation.

TABLE I. EFFECT OF ARTERIAL OCCLUSION ON THE REACTIVE HYPEREMIC RESPONSE IN THE MUCOSA–SUBMUCOSA AND MUSCULARIS LAYER OF THE CAT ILEUM

	Reactive hyperemia (× control)	
Occlusion time (sec)	Mucosa–submucosa[a]	Muscularis[b]
60	2.10 ± 0.15	3.58 ± 0.83
120	2.48 ± 0.52	6.30 ± 0.90*

[a] Absolute blood flow under control conditions = 22.8 ± 1.4 ml/min/100 g.

[b] Absolute blood flow under control conditions = 12.3 ± 1.4 ml/min/100 g.

* $P < 0.05$ When compared to the other layer of the same occlusion time, and when compared to the same layer and a different occlusion time.

An increase in motility and mean lumen pressure (tonus) was observed during 60- and 120-sec arterial occlusion when compared to the resting state. Although the mean lumen pressures achieved during 60- and 120-sec arterial occlusions were not significantly different; motility during 120-sec arterial occlusion (7 ± 1.2 contractions/min) was significantly greater than that observed during 60-sec occlusions (2.8 ± 0.6 contractions/min).

Discussion. The reactions of the intestinal microvasculature to vascular occlusion have been used to delineate the mechanisms involved in the regulation of intestinal blood flow, i.e., metabolic vs myogenic. An inherent assumption in previous studies was that the reactive hyperemic responses of the intestinal vasculature to graded arterial occlusion occur either uniformly in the mucosa, submucosa, and muscularis layers or predominantly in the mucosal layer. The magnitude of the reactive hyperemic response we observed at 60- and 120-sec arterial occlusions closely resemble previously reported values (2–4); however, the findings reported herein suggest that relatively uniform increases in intestinal wall blood flow occurs only for an occlusion period of 60 sec. Increasing the duration of

the arterial occlusion to 120 sec results in a predominant hyperemia in the muscularis layer.

The mechanism(s) by which increasing the duration of arterial occlusions increases the reactive hyperemic response of the muscularis vasculature is not clear but the increase in total intestinal blood flow subsequent to release of an arterial occlusion has been explained by a combination of metabolic and myogenic mechanisms. It is difficult to explain the results by a myogenic mechanism since one would expect the reduced transmural pressure at the microvascular level to be the same, irrespective of the duration of the occlusion. An increased activity of visceral smooth muscle during longer occlusion periods would decrease vascular transmural pressure and thus may account for the enhanced hyperemic response in the muscularis. A more likely explanation for the greater hyperemia in the muscularis is a greater reduction in tissue oxygen tension in this layer after 120-sec arterial occlusion. The results of our studies indicate that arterial occlusions of 60 and 120 sec increase motility; however, a greater increase in motility is observed during 120-sec occlusions. Cessation of flow for 120 sec has also been shown to cause an increased motility in dog ileum (5). A greater oxygen deficit in the muscularis during the period of ischemia may be due to a greater demand for oxygen by the tissue due to increased motility. Chou and Grassmick (6) have recently demonstrated an increased percentage of total wall flow perfusing the muscularis-serosa when motility is increased by manipulation, distention, and physostigmine. Therefore, the greater hyperemic response of the muscularis layer to 120-sec arterial occlusion may be due to an enhanced activity (motility) of visceral smooth muscle.

Irrespective of which mechanism(s) is responsible for the greater response of the muscularis layer to longer arterial occlusion periods, our results indicate that shorter occlusion periods are more suitable for interpretation of a reactive hyperemic response relative to metabolic and myogenic theories since a more uniform increase in intestinal wall blood flow occurs.

Summary. The reactive hyperemic response of the intestinal vasculature to graded arterial occlusion is often used to delineate mechanisms involved in the intrinsic regulation of intestinal blood flow. This study assesses if the reactive hyperemic response to 60- and 120-sec arterial occlusions occur uniformly throughout the wall of the small intestine. Radiolabeled microsphere injections were used to determine blood flow distribution in the mucosa–submucosa and muscularis layers of the small intestine under control conditions and during 60- and 120-sec arterial occlusion. The results of this study suggest that relatively uniform increases in intestinal wall blood flow occurs only for an occlusion period of 60 sec. Increasing the duration of the arterial occlusion to 120 sec results in a predominant hyperemia in the muscularis layer.

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