Effect of Nerve Stimulation on Precapillary Sphincters, Oxygen Extraction, and Hemodynamics in the Intestines of Cats

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SUMMARY The effect of stimulation of the nerves to the intestine on intestinal oxygen uptake and hemodynamics was examined in anesthetized cats. In the normally perfused gut, nerve stimulation results in a reduced vascular conductance followed by a partial escape from neurogenic vasoconstriction. The oxygen extraction ratio increases to an appropriate extent to maintain oxygen uptake at prestimulation levels during the escape plateau. In a gut perfused via a pump, a similar response was seen when pressure was held constant; however, when blood flow was held constant a constriction of precapillary sphincters was unmasked and there was a prolonged decrease in oxygen extraction ratios. The data are compatible with the hypothesis that nerve stimulation results in vasoconstriction of precapillary resistance vessels and precapillary sphincters in the gut. Oxygen consumption of the tissue declines dramatically but because of the reduced blood flow at the site of the sphincters there is a secondary dilation and an increased oxygen extraction as more capillaries are opened at any given time. The resistance vessels show escape during nerve stimulation in situations of constant pressure or flow, and the escape of blood flow due to escape of the resistance vessels is a separate phenomenon unrelated to the escape of the sphincters. The return of oxygen uptake to normal during the escape phase suggests that redistribution of intestinal flow does not occur during vascular escape from neurogenic vasoconstriction. An equation for quantification of the extent of vascular escape is presented.

FOLKOW et al.1 reported that during stimulation of the sympathetic nerves supplying the intestine the vascular resistance to blood flow is markedly elevated, but that in spite of maintained stimulation the blood flow returns toward control levels. This response will be referred to as "vascular escape from neurogenic vasoconstriction" or simply "escape." It originally was proposed that the escape resulted from a redistribution of blood within the intestine whereby blood flow was shifted selectively from the mucosa to the submucosa.1-3 More recently it also has been suggested that the redistribution occurs in the opposite direction, that is, from the deeper layers of the gut wall toward the mucosa.4

Richardson and Johnson,5 however, suggested that the escape was due to relaxation of the same vessels that initially were constricted. Ross6 and Greenway et al.,7-8 using independent techniques, provided evidence that redistribution is unlikely to occur. Escape from vasoconstriction also has been demonstrated for isolated pieces of mesenteric arteries.9,10 In view of these later studies the bulk of evidence supports the concept of a wearing-off of the constrictor influence of norepinephrine on the vascular smooth muscle of the resistance vessels.

The vascular escape, as discussed, refers solely to the response of the resistance vessels, that is, those vessels controlling total blood flow to the intestine. The involvement of the precapillary sphincters, however, does not necessarily parallel that of the resistance vessels. It was therefore the purpose of this study to determine the effect of nerve stimulation on oxygen uptake in the intestine of the cat, with the secondary aim of assessing the likelihood of precapillary sphincter control of oxygen uptake.

Methods

Cats were anesthetized with Na-pentobarbital (25 mg/kg, ip) and small supplements (5 mg) were administered throughout the experiment via a cannula in the foreleg vein. Rectal temperature was monitored and controlled at 37.5°C by the use of a heated table. The right femoral artery was cannulated and systemic blood pressure was monitored.

A laparotomy was performed and the spleen was removed. A cannula was passed via a small vein draining the appendix into the portal vein. The artery running parallel to the vein from the appendix was cannulated and pressure was monitored throughout the experiment. The large intestine was removed with the exception of a small stub at the rectum (about 1.5 cm long). The inferior mesenteric artery and its anastomoses with the superior mesenteric artery were ligated. The small intestine loop was isolated by severing the duodenum at the junction with the stomach and removing a small portion of the intestine to just past the pancreatic veins. In this manner a segment of small intestine is left with normal vascular connections and normal innervation.

The periarterial nerves were stripped carefully from around the superior mesenteric artery. The peripheral end of the nerve bundle was placed in a circular stimulating electrode made of plexiglass with two platinum wires running around the interior of the electrode. The electrode was connected to a Grass S9 stimulator and the stimulus parameters were maintained at 15 V, 1 msec in duration, at a frequency of 8 Hz. This stimulus intensity and duration were used in escape studies by others,9 and vascular
escape in the gut occurs at stimulus frequencies of up to 20 Hz.11

A noncannulating electromagnetic flow probe was placed around the artery to record arterial blood flow (Carolina Medical Electronics, model 501 electromagnetic flowmeter and EP 407 flow probe). A micrometer-controlled screw clamp attached to the artery distal to the flow probe permitted determinations of an occlusive-zero flow.

At the conclusion of the experiments the superior mesenteric artery was cannulated and the flow probe was calibrated in situ. The intestine then was removed, the lumen was cleaned, and the tissue weighed. Parameters were expressed per 100 g of tissue.

Oxygen uptake was calculated from the product of blood flow and arteriovenous difference. Conductance was calculated by dividing the blood flow by the driving blood pressure (SMAP-PVP).

**BLOOD GASES**

Blood was taken from the venous and arterial cannula and analyzed for Po2 and Pco2, pH, hematocrit, and total oxygen content. The method for obtaining multiple small samples of blood, free of contamination by air or cannula fluids, has been described in detail12 and the stability of the blood gases stored in plastic syringes under ice for periods up to 4 hours has been verified.12,13 Blood gases were determined on a Corning 161 pH/blood gas instrument and total oxygen content was determined directly on a Lex O2 Con (Lexington Instruments) total oxygen content analyzer. All determinations can be made on a 1-ml blood sample. The barometric pressure at which the readings were made was 720 ± 2 mm Hg and the temperature was 22°C.

**ARTERIAL LONG CIRCUIT**

In three cats the intestine was prepared as described previously but the blood supply was provided via an arterial long circuit from the femoral artery and pressure or flow could be controlled by fine adjustments of the peristaltic pump. Perfusion pressure was set at the site of the probe. When the nerves were stimulated either pressure or flow could be controlled by fine adjustments of the peristaltic pump. Perfusion pressure was set at the control blood pressure.

**Results**

In the first series of experiments only cats with control hematocrits greater than 30% were included (n = 16). The small intestinal segment studied weighed 64 ± 2.8 (mean ± SE) g and during the control period had a blood flow of 59.3 ± 6.4 ml/100 g per min. Arterial hematocrit was 34.6 ± 1.0%, pH was 7.41 ± 0.02, Pco2 was 27.2 ± 1.0 mm Hg, Po2 was 83 ± 2.9 mm Hg, and oxygen content was 13.7 ± 0.5 ml of O2/100 ml of blood. Portal venous Po2 was 42 ± 2.0 mm Hg, O2 content was 8.6 ± 0.6 ml/100 ml of blood, oxygen uptake was 2.5 ± 0.1 ml of O2/100 g of tissue, and oxygen extraction was 37 ± 4%.

Arterial blood pressure was 124 ± 4.4 mm Hg and portal venous blood pressure was 6.4 ± 0.4 mm Hg.

A stabilization period of at least 30 minutes was allowed before the nerves were stimulated. Figure 1 illustrates the response to nerve stimulation in the intestine (82 g) of one cat. In this particular cat the vascular escape from neurogenic vasoconstriction was not marked. Escape is calculated as: (plateau escaped flow – peak response flow)/(control flow – peak response flow) × 100%. Escape was only 45%; however, oxygen extraction increased sufficiently to return oxygen uptake to control levels. The time taken for the blood flow to reach 75% of the final plateau value (t3M) is used as an index of the rapidity of escape. The t3M escape time was 3.6 minutes in this cat.

Figure 2 shows the mean ± SE of the responses of the small intestine to nerve stimulation in 16 cats. Arterial blood pressure did not change significantly throughout the period of nerve stimulation. Vascular conductance...
changes paralleled flow changes and mean values were within 1% of those shown for flow changes in Figure 2. Initially the blood flow decreased dramatically. Oxygen uptake decreased to a similar extent, thus the observation that oxygen and CO\textsubscript{2} levels in the venous blood were not altered notably. By the time the partial escape values were measured the tissue had increased the efficiency of oxygen extraction, and oxygen uptake was nearly normal even though blood flow was still reduced. This resulted in the reduced levels of oxygen and elevated CO\textsubscript{2} in the venous blood.

During the escape phase, extraction ratios increased to 180% of control, but as flow continued to escape the extraction ratio decreased. The extraction ratio was adjusted according to need and was therefore secondary to flow changes. The mean time to 3/4 escape was 2.8 ± 0.2 minutes (range, 1.8-4.9 minutes) and the mean escape was 59 ± 4.4% (range, 30-82%). Venous oxygen levels remained lower than control and CO\textsubscript{2} levels remained higher than control as a result of the continued reduction in blood flow and the return to normal of intestinal oxygen uptake.

Arterial long circuit: In three cats the superior mesenteric artery received blood via a pump from the femoral artery to permit comparison of the response during constant flow with that seen during constant pressure perfusion. The responses seen on five occasions in the three cats were qualitatively similar to that shown in Figure 3.

Discussion

Stimulation of the nerves supplying the intestine resulted in a marked decrease in vascular conductance which was followed by a return toward control flows in spite of continued nerve stimulation. Oxygen extraction changed to an extent that entirely compensated for the reduced blood flow during the plateau of the escape phase. Maintained oxygen uptake during nerve stimulation was also reported for skeletal muscle\textsuperscript{14} and the liver.\textsuperscript{15} Baker and Mendel,\textsuperscript{17} however, reported that infusion of norepinephrine produced a well maintained decrease in oxygen uptake in the gut. The reason for the different response to norepinephrine and to nerve stimulation is not clear but their control flows were considerably below those reported by others, a point they mention but do not resolve. If flow is already low, then further reduction can lead to decreased oxygen uptake regardless of local vascular compensation (personal observation). Whether the low control flows can completely account for the difference in responses is not clear.

NEUROGENIC CONTROL OF SPHINCTERS

During nerve stimulation with normal arterial supply, the oxygen uptake was maintained as a result of increased oxygen extraction. When the intestines were perfused via an arterial long circuit and blood flow was held constant, a reduction in extraction ratios was evident, indicating a significant effect on precapillary sphincters. Shepherd et al.,\textsuperscript{16} using a constant flow preparation, showed a similar decrease in oxygen extraction during nerve stimulation.

The data of Shepherd et al.\textsuperscript{16} and those reported here...
are compatible with the following hypothesis: In the situation of uncontrolled blood flow, stimulation of the sympathetic nerves to the gut results in constriction of the precapillary resistance vessels, producing a reduced conductance and constriction of the precapillary sphincters which, in turn, effectively eliminates certain capillaries from active circulation for short periods. In spite of a high venous oxygen content the intestinal oxygen consumption is dramatically reduced (Figs. 1 and 2). However, the low blood flow to the gut during this intense constriction results in a secondary dilation of the sphincters so that oxygen extraction increases to nearly double its control level during the escape phase. As flow (the resistance vessels) continues to escape, the quantity of blood passing the sphincters increases and the sphincters constrict slightly. Oxygen extraction is thus lower (147% of control) during the full escape phase than it was (180%) before flow became stable. Oxygen extraction is more fully discussed later. During nerve stimulation in a preparation with constantly maintained blood flow, the vascular conductance is decreased because of activation of the precapillary resistance vessels, and the precapillary sphincters are also constricted, causing a reduction in capillary surface area. In this circumstance, however, blood flow is not reduced and the sphincters do not show a secondary dilation. Accordingly the oxygen extraction ratio is reduced from normal and remains low throughout nerve stimulation.

According to this scheme of events the sphincters vasodilate as a result of local effects of reduced vascular conductance upstream. The local dilation counteracts the direct effect of nerve stimulation. During precapillary constriction the blood flow and blood pressure are both reduced at the site of the sphincters and therefore one could invoke either a myogenic or metabolic hypothesis to account for the dilation.

Metabolic models accounting for this escape hold that decreased flow allows either an accumulation of a dilator metabolite or depletion of oxygen to result in relaxation of the sphincters in low flow states. Conversely, in high flow states the metabolites are washed out or P02 is high enough to permit constriction of the sphincters. Constant flow in the presence of neurogenic constriction results in maintained constriction by washing out of dilator or provision of adequate P02 to permit maintained sphincter constriction. The blood downstream in the capillaries is then rapidly depleted of oxygen supplies and cellular uptake is impaired. Myogenic control of the sphincters is also possible in this case, because changes in the resistance vessel tone influence the transmural pressure at the site of the sphincters. The situation in the constant flow preparation does not clarify the problem. Although flow remains constant it is conceivable that the pressure at the sphincter also is maintained, since both the resistance to flow and the driving pressure are elevated. A similar hypothesis concerning neurogenic constriction of sphincters in skeletal muscle as a flow-dependent phenomenon was discussed by Mellander and Johansson.

Our present study does not distinguish between the metabolic or myogenic hypothesis, but previous data from this laboratory indicate that oxygen uptake in the gut is not correlated with myogenic stimuli or with changes in conductance but rather is related to blood flow. After isovolemic hemodilution the oxygen uptake of the intestines is maintained; this is due in part to increased vascular conductance and in part to increased oxygen extraction. Correlation studies were made and it was determined that oxygen extraction changes in the gut do not correlate with changes in blood pressure or vascular conductance but do correlate very well with changes in portal blood flow. The metabolic hypothesis predicts that oxygen extraction should be linked to blood flow. In another set of experiments’ stimulation of the nerves in the liver produced marked alterations in systemic arterial and portal venous pressure and portal vascular conductance but throughout these changes oxygen extraction remained constant; portal blood flow also did not significantly change. This again suggests that flow is more important in the control of oxygen extraction than are myogenic stimuli. Thus, while the precapillary resistance vessels may primarily be under myogenic local control the sphincters appear to be primarily if not solely under metabolic control.

This statement is not incompatible with the myogenic hypothesis that local control of precapillary resistance vessels tends to control capillary hydrostatic pressure. It should be stressed that the present hypothesis deals only with the precapillary sphincter control of capillary surface area and oxygen extraction. We previously have demonstrated clear separation of overall vascular conductance changes from extraction changes in the gut, as discussed above. It must also be stressed that the process of autoregulation and that of vascular escape from constriction influence are independent of one another, since vascular escape can be elicited in situations in which autoregulation no longer is evident.

Capillary filtration coefficient (CFC) and the “permeability-surface area product” (PS) are methods used to estimate precapillary sphincter activity. CFC is a filtration-dependent phenomenon, whereas PS is diffusion-dependent and must be measured at constant blood flow. PS in the gut was affected by nerve stimulation to a much lesser extent than was CFC, but the interpretation of these data is difficult because blood flow was not controlled. Earlier studies indicate that CFC is decreased and shows no escape from nerve stimulation. The present data showing escape of oxygen extraction would appear to conflict with the earlier data showing no escape of sphincters. This discrepancy cannot be resolved at this time.

OXYGEN EXTRACTION

Although the sphincters can affect oxygen extraction by altering the diffusion distances within the tissue, they are not the sole determinant of the oxygen extraction ratio. As long as the P02 is in excess of the levels that become limiting to cellular metabolism, increasing the demand to supply ratio does not impair cellular uptake of oxygen but simply lowers the oxygen content of the blood; that is, the extraction ratio is increased. During the peak response the sphincters are constricted, tending to reduce the oxygen extraction ratio (Fig. 3); simultaneously the blood flow and oxygen delivery are reduced, and this tends to

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produce a counteracting increase in oxygen extraction ratio. During the later phases of the response the sphincters have dilated because of local factors, permitting a more efficient extraction of oxygen (Fig. 1). It would appear from Figure 3 that the sphincters are not significantly constricted during the escape phase when blood flow is reduced. If they were, the oxygen uptake would have been impaired as it was at constant flow during nerve stimulation.

**RESISTANCE VESSELS**

The phenomenon of vascular escape from neurogenic vasoconstriction is confirmed in the present study at both uncontrolled and constant blood flow. The mean time to 3/4 of full escape was 2.8 minutes (1.8-4.9 minutes) and the mean escape (calculated from an equation shown in Results) was 59% (30-82%). Although the data do not deal directly with the mechanisms of escape of the resistance vessels, the data are compatible with the proposal that escape is simply a reopening of the same vessels that were initially constricted. If escape occurred because of opening of vascular shunts, oxygen uptake from the shunted blood would be reduced, resulting in an overall decrease in intestinal O$_2$ uptake. Oxygen uptake was clearly not impaired in the present studies. Redistribution of blood flow could also not be demonstrated by the rigorous use of tagged microspheres. Recent papers dealing with flow distribution in the gut and mechanisms of vascular escape from neurogenic vasoconstriction should be consulted for detailed discussion of these areas.

**References**

19. Lautt WW: Control of hepatic and intestinal blood flow; effect of isovolemic hemodilution on blood flow and oxygen uptake in the intact liver and intestines. J Physiol (Lond) (in press)
Effect of nerve stimulation on pericapillary sphincters, oxygen extraction, and hemodynamics in the intestines of cats.

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