Effects of Neural Stimuli on Blood Flow through Vasa Vasorum in Dogs

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SUMMARY The purpose of this study was to determine whether neural stimuli alter blood flow through vasa vasorum in the thoracic aorta. We measured flow with microspheres in anesthetized dogs and observed responses to sympathetic stimulation and baroreceptor stimulation. During these interventions, changes in arterial pressure were prevented with propranolol or an arterial reservoir, to minimize indirect effects on vasa vasorum mediated through changes in aortic wall tension. Electrical stimulation of the stellate ganglion at 10 Hz reduced blood flow to vasa vasorum in the thoracic aorta from 11 ± 1.6 (mean ± SE) to 6.8 ± 1.1 ml/min per 100 g (P < 0.05). Aortic diameter, measured with a sonomicrometer technique, did not change during sympathetic stimulation. Thus, the reduction in blood flow through vasa vasorum during sympathetic stimulation appears to be a direct effect, and not the result of constriction of the aorta and compression of vasa. To determine effects of physiological alterations in neurogenic vasoconstrictor activity, we examined responses to stimulation of carotid baroreceptors. When pressure in isolated, perfused carotid sinus baroreceptors was raised from 81 ± 3 to 198 ± 2 mm Hg, blood flow to vasa vasorum of the thoracic aorta increased from 3.7 ± 0.6 to 10 ± 2.2 ml/min per 100 g (P < 0.05). We conclude that vasa vasorum are responsive to neural stimuli, since they constrict during sympathetic stimulation and dilate in response to baroreceptor stimulation.

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NUTRITIONAL needs of the thoracic aorta apparently are met by diffusion from the lumen of the aorta, diffusion from adventitial vessels, and by blood flow through vessels in the aortic wall called vasa vasorum (Geiringer, 1951; Wolinsky and Glagov, 1967). Ligation of the intercostal arteries that give rise to vasa vasorum produces medial necrosis in dogs (Wilens et al., 1965), which indicates that vasa vasorum play a critical role in the nourishment of the thoracic aorta.

Studies of vasa vasorum have been limited primarily to morphological observations. In dog and man, vasa can be identified in the outer portion of the media of the thoracic aorta (Wolinsky and Glagov, 1967). Electron micrographs (unpublished observations, Mark Armstrong, M.D.) indicate that vasa vasorum have their own smooth muscle. The smooth muscle fibers are oriented in relation to the vasa, and not in relation to the aortic media. In addition, nerve fibers, most of which apparently originate in or traverse the left stellate and cervical ganglia (Miller et al., 1964; Mizeres, 1955; McKibben and Getty, 1968), penetrate the aortic media near the vasa (Kienecker and Knoche, 1978).

Recently, we have described a method to measure blood flow through vasa vasorum in the aortic media (Heistad et al., 1978). We found that vasa vasorum provide considerable amounts of blood flow to the outer half of the media of the thoracic aorta in dogs. Vasa are responsive to physiological stimuli, as they dilate during infusion of adenosine and constrict during hemorrhagic hypotension. However, effects of neural stimuli on blood flow through vasa vasorum have not been examined.

In these experiments, we have examined effects of sympathetic nerves and a reflex stimulus on blood flow through vasa vasorum. First, effects of electrical stimulation of the stellate sympathetic ganglion were determined. We then considered the possibility that sympathetic stimulation might affect flow, not by a direct constrictor effect on vasa vasorum but, instead, by constriction of the aorta and compression or distortion of the vasa. To test this possibility, aortic diameter was measured during stellate stimulation. Finally, we examined effects of stimulation of carotid sinus baroreceptors on blood flow through vasa vasorum.

An important part of the design of these studies was that we attempted to keep arterial pressure from changing during the neural stimuli. Because changes in arterial pressure affect blood flow through vasa vasorum (Heistad et al., 1978), prob-
ably in part through changes in aortic diameter, it was important to prevent changes in pressure. Increases in aortic pressure during sympathetic stimulation were minimized by injection of propranolol or with an arterial reservoir, and decreases in pressure in the thoracic aorta during baroreceptor stimulation were prevented by occlusion of the aorta at the level of the diaphragm.

**Methods**

**Preparation of Dogs**

Twenty-six dogs, weighing 12-29 kg, were anesthetized with intravenous chloralose (50 mg/kg) and urethane (500 mg/kg), paralyzed with decamethonium bromide (0.3 mg/kg), and ventilated with a respirator. Cannulae were placed in the left atrium for injection of microspheres, in a brachial and carotid artery for withdrawal of blood samples, and in the other brachial artery for measurement of pressure.

**Measurement of Flow**

Blood flow through vasa vasorum was measured with labeled microspheres. We have described our experimental approach in detail (Heistad et al., 1978).

To measure blood flow through vasa vasorum, we injected microspheres, 9 μm in diameter and labeled with $^{46}$Sc, $^{90}$Nb, $^{85}$Sr, or $^{141}$Ce, into the left atrium. Prior to injection, the vial containing microspheres was agitated vigorously for 5 minutes, which disperses about 98% of the spheres. Approximately 5–15 million spheres were injected during each measurement of blood flow. The microspheres were injected slowly over a period of 10 seconds, and the cannula then was flushed with 5 ml of saline at 37°C during the subsequent 10 seconds. Beginning shortly before injection of microspheres and continuing until 3 minutes after injection, reference blood samples were withdrawn from brachial and carotid arteries at 2.06 ml/min with a Harvard pump. Blood samples were collected from the tubing as well as from withdrawal syringes.

At the end of each study, the dog was killed and segments of ascending and descending aorta were removed. The adventitia was stripped from the samples using a dissecting microscope. Histological examination indicates that essentially all of the adventitia is removed using this method (Heistad et al., 1978). The aortic media then was split into inner one-third and outer two-thirds, the media was split into inner layers (approximately one-third of the wall) and outer layers (approximately two-thirds of the wall). Tissue samples weighed 0.5–2.0 g.

The samples were weighed, placed in plastic tubes, and counted for 10 minutes in a 3-inch well-type γ counter. Reference blood samples were divided into aliquots so that their counting geometry was similar to that of the tissue samples. The energy windows were 800–1500 keV ($^{46}$Sc), 650–800 keV ($^{90}$Nb), 400–600 keV ($^{85}$Sr), and 125–175 keV ($^{141}$Ce). The isotope separation was performed according to standard techniques (Rudolph and Heymann, 1967). Output from the γ counter was punched on paper tape and processed in a PDP-11 computer. Blood flow through vasa vasorum was calculated using the formula: $C_v \times 100 \ RBF/C_r = \text{vasa vaso-rum blood flow in ml/min per 100 g of aorta,}$ where $C_v = \text{counts/g of aortic sample, } RBF = \text{reference blood flow (rate of withdrawal of blood samples from arteries in ml/min), and } C_r = \text{total counts in reference arterial blood.}$ The counts from the two reference blood samples were averaged.

Statistical analysis was performed using analysis of variance and orthogonal contrasts (Snedecor and Cochran, 1972). Orthogonal contrasts were used to compare two values without interventions (during control and recovery periods) with one or two values during interventions.

**Transmural Distribution of Vasa**

The depth to which vasa vasorum penetrate the thoracic aorta is variable (Wolinsky and Glagov, 1967). The depth of penetration of vasa depends on the species, the age of the animal, and the number of aortic lamellae. It is not known, however, whether variation in body weight and number of aortic lamellae in adult animals within a species affects transmural distribution of vasa.

In seven adult dogs (body weight 12–29 kg) we determined the number of lamellae and the transmural distribution of blood flow in the thoracic aorta. Microspheres were injected under normal conditions. A segment of the descending aorta at the level of the first and second intercostal arteries was removed. The aorta was not distended at physiological pressure or fixed at its in vivo length. One part of the aortic segment was fixed in 10% formalin and embedded in paraffin. Transverse sections were stained with hematoxylin and orcein. The number of lamellae was counted in two transverse sections in each dog, in four areas of each vessel about 90° apart, and an average value was calculated; the thickness of the media also was measured. The adventitia was removed from the other part of the aortic segment and, instead of dividing the media into inner one-third and outer two-thirds, the media was split into inner, middle, and outer thirds. The weight of the segments was 0.38 ± 0.10, 0.39 ± 0.10, and 0.36 ± 0.10 g in the inner, middle, and outer segments, respectively. Blood flow was calculated in each of the segments.

Blood flows to the inner, middle, and outer thirds of the aorta were 0.5 ± 0.2, 11.9 ± 3.5, and 12.8 ± 2.2 ml/min per 100 g, respectively. The variability in blood flow to the aorta between different dogs was not significantly correlated with the thickness of the aorta or the number of lamellae, perhaps due in part to the small number of dogs that were studied. Although variation in penetration of vasa...
would affect between-animals comparison of flow, this variation would not affect within-animal comparison of responses that were performed in our studies.

Measurement of Aortic Diameter

Aortic diameter was measured by the sonomicrometer technique (Stegall et al., 1967; Theroux et al., 1974), employing two sonomicrometer transducers that were sutured to the adventitia on opposing sides of the descending aorta. A transducer was made by soldering 0.15-mm diameter stainless steel wires to a 5-mm diameter, 5-mHz piezoelectric crystal. This assembly was coated with epoxy glue to develop a hemispherical lens for signal broadening. The crystal was backed with a polystyrene sewing ring for attachment to the aortic wall. The two transducers were connected to form a dimension gauge. One of the crystals was excited by a 0.2-msec, 200-V pulse producing a sound wave that traveled through tissue or blood at a velocity of 1.5 \times 10^4 mm/sec. The other transducer received the signal, and the gauge then translated the transit time into distance.

The gauge was calibrated in two ways. Prior to the experiments, the in vitro distance between the transducers was measured by a vernier caliper. After the transducers were sutured to the aorta, distance calibration was obtained by inserting a pulse of 1 \mu sec (equivalent to 1.5 mm) into the gauge.

The theoretical resolution of the sonomicrometer technique is a small fraction of the wave length of the sonic signal (less than 0.05 mm). However, the sensitivity of the transducers in vitro is dependent also on the orientation of the two crystals. To evaluate the orientation of the transducers, the oscilloscopic appearance of the signals always was observed to be tracked accurately on the first received spike, regardless of aortic diameter.

To examine further the sensitivity of the method for measuring aortic diameter, the relationship between aortic diameter and aortic pressure was studied under three conditions: normotension, acute hypotension produced by a bolus of chloralose and urethane, and acute hypertension produced by clamping the aorta at the level of the diaphragm. Aortic pressure was measured with a Konigsberg 3.5-mm solid-state transducer in the descending aorta. The aortic dimension was measured at both the nadir of aortic diastolic pressure and at the peak of aortic systolic pressure. These data then were normalized by dividing the measurement by the control end-diastolic measurement and multiplying by 100. The data are plotted in Figure 1.

Stimulation of Sympathetic Nerves

Blood flow was measured in seven dogs. To minimize changes in arterial pressure during stimulation of the stellate ganglion, propranolol (1 mg/kg) was injected intravenously in four dogs several minutes before beginning the experimental protocol. In three dogs, changes in arterial pressure were minimized by allowing blood to equilibrate with a reservoir connected to the arterial circulation through a large catheter in the femoral artery. The left stellate ganglion was decentralized, and the caudal fibers were stimulated electrically at 2 and 10 Hz, 10-15 V, 5 msec. Microspheres were injected four times: during a control period, about 1 minute after beginning sympathetic stimulation at 2 Hz and 10 Hz, and during a recovery period about 15 minutes after stopping sympathetic stimulation. The interval between the two periods of sympathetic stimulation was 15-30 minutes.

Effects of sympathetic stimulation on aortic diameter were examined in two dogs. To minimize changes in arterial pressure during sympathetic stimulation, propranolol (1 mg/kg) was injected intravenously, and a reservoir was connected to the arterial circulation. The left stellate ganglion was stimulated for 2 minutes at 10 Hz, 10 V, 4 msec.

Stimulation of Carotid Baroreceptors

Carotid sinus baroreceptors were stimulated using a preparation described previously (Heistad et al., 1974). Both vagus nerves were cut, and both carotid bifurcations were exposed in 10 dogs. On one side, carotid sinus baroreceptors were denervated. On the other side, the internal carotid artery and all branches of the external carotid artery were ligated. Arterial blood was pumped at 80 ml/min into the common carotid artery. Blood flowed out through a cannula in the external carotid artery and through a Starling resistor to a jugular vein.
Changes in carotid perfusion were made with the Starling resistor.

Systemic arterial pressure, measured in a brachial artery, was prevented from decreasing during baroreceptor stimulation by inflation of a cuff around the aorta, immediately cephalad to the diaphragm. The cuff was more than 2 inches below the segment of descending aorta that was later removed for determination of flow.

Microspheres were injected 3 times: during a control period (when carotid sinus pressure was 81 ± 3 mm Hg), about 2 minutes after raising carotid sinus pressure to 198 ± 2 mm Hg, and during a recovery period about 15 minutes after reducing carotid sinus pressure to 78 ± 2 mm Hg.

Results

Blood Flow to the Aortic Wall

In dogs in which effects of neural stimuli were studied, flow to the outer two-thirds of the media of the ascending and descending aorta was 10 ± 1.9 (mean ± SE) and 13 ± 1.9 ml/min per 100 g, respectively, during control conditions. Blood flow to the inner third of the media of the ascending and descending aorta was only 1 ± 0.6 and 0.5 ± 0.4 ml/min per 100 g, respectively, and was unchanged during neural stimuli.

Responses to Sympathetic Stimulation

In both dogs in which aortic diameter was measured, there was no change in diameter during stellate stimulation when changes in arterial pressure were prevented (Fig. 2). The method was sensitive in detecting changes in aortic diameter: increases or decreases in aortic pressure produced corresponding changes in aortic diameter (Fig. 1). The aortic diameter increased approximately 50% in the two dogs when aortic pressure was raised from 40 mm Hg (diastolic pressure during hypotension) to 164 mm Hg (systolic pressure during hypertension).

Stimulation of the stellate ganglion at 10 Hz reduced blood flow to the outer two-thirds of the media of the ascending and descending aorta to 5 ± 1.0 and 9 ± 1.7 ml/min per 100 g (P < 0.05), respectively (Fig. 3). There was no significant change in mean arterial pressure (Fig. 3), so the decreases in flow during sympathetic stimulation indicate a decrease in conductance.

Responses to Baroreceptor Stimulation

When arterial baroreceptors were stimulated by raising perfusion pressure in an isolated carotid sinus, blood flow to the aortic media increased (Fig. 4). Flow to the ascending and descending aorta increased from 3 ± 0.5 and 4 ± 1 ml/min per 100 g during control conditions to 9 ± 2 and 11 ± 3 ml/min per 100 g (P < 0.05), respectively. Changes in mean arterial pressure were prevented during baro-

Discussion

These studies indicate that vasa vasorum in the thoracic aorta are responsive to neural stimuli. Vasa vasorum constrict during sympathetic stimulation and dilate in response to baroreceptor stimulation.

We should consider the method that was used to measure blood flow through vasa vasorum. As described previously (Heistad et al., 1978), the fundamental assumptions of the microsphere technique appear to be satisfied in measuring flow to baroreceptor stimulation (Fig. 4), so the increase in flow during baroreceptor stimulation indicates an increase in conductance.
the aortic wall: microspheres do not pass through arteriovenous shunts, an excessive number of vessels are not occluded, and uneven distribution of the spheres is avoided by using small spheres. A large number of spheres is injected, because flow to vasa vasorum is not high and the size of tissue samples is small. The technique appears to provide valid and reproducible measurements of blood flow through vasa vasorum.

Under control conditions, the distribution of blood flow to the aortic wall in these studies was similar to that which we observed previously (Heistad et al., 1978). The inner layers of the aorta are virtually avascular (Geiringer, 1951; Wolinsky and Glagov, 1967), and they receive minimal blood flow through vasa vasorum. The outer layers of the aorta receive significant blood flow through vasa vasorum. These studies indicate that there is moderate heterogeneity of blood flow to the outer two-thirds of the aortic media, perhaps related in part to the size of the dog. Because we examined the effects of neural stimuli using a within-animal comparison, heterogeneity of flow to the media would not affect our conclusions.

Blood flow to vasa vasorum under control conditions was lower in the experiments with baroreceptor stimulation (Fig. 4) than in the experiments with sympathetic stimulation (Fig. 3). The lower blood flow in the studies before baroreceptor stimulation may be related to several factors. Afferent input from arterial baroreceptors was interrupted partially by vagotomy and denervation of carotid baroreceptors on one side. Because baroreceptor discharge affects blood flow through vasa vasorum, it seems likely that denervation of aortic baroreceptors and one carotid sinus nerve would reduce flow through vasa. Furthermore, longer duration of the study and lower mean arterial pressure (90 mm Hg before baroreceptor stimulation and 106 mm Hg before stellate stimulation) also may contribute to lower baseline blood flow through vasa vasorum in the experiments with baroreceptor stimulation.

Stimulation of the stellate ganglion usually produces an increase in arterial pressure. We have observed that increases in arterial pressure decrease conductance of vasa vasorum (Heistad et al., 1978). It was important in these studies, therefore, to prevent increases in arterial pressure during stellate stimulation so that direct effects on vasa vasorum could be determined. By minimizing changes in arterial pressure during sympathetic stimulation, we were able to exclude indirect effects of acute hypertension on vasa vasorum.

The source of sympathetic innervation to the aorta has not, to our knowledge, received extensive attention. It appears that sympathetic nerves to the descending, and perhaps the ascending, thoracic aorta originate in the left stellate ganglion, although the left caudal cervical (vertebral) ganglion may also supply nerve fibers (Miller et al., 1964; Mizeres, 1955; McKibben and Getty, 1968). Our studies provide functional evidence that the left stellate ganglion in the dog supplies sympathetic fibers to vasa vasorum in both the ascending and descending aorta.

The reduction in blood flow through vasa vasorum during sympathetic stimulation probably is the result of a direct constrictor effect on vasa. An alternative mechanism that required consideration was that nerve stimulation affects the aorta and reduces the aortic diameter, thereby compressing or distorting the vasa vasorum. This possibility was examined by measuring the aortic diameter during sympathetic stimulation. Gerova et al. (1973) found that, when increases in arterial pressure were prevented, sympathetic stimulation reduced the diameter of the distal abdominal aorta by 8% but did not constrict the proximal abdominal aorta. We found that stellate stimulation did not produce detectable changes in diameter of the thoracic aorta unless arterial pressure changed. The sensitivity of the method in detecting changes in aortic diameter was demonstrated by the measurable changes in diameter during increases or decreases in arterial pressure. It is unlikely, therefore, that changes in conductance of vasa vasorum during sympathetic stimulation are a passive response to constriction of the aorta. We cannot exclude the possibility that small changes in diameter of the aorta occurred during sympathetic stimulation that were not detected with the sonomicrometer method.

In some experiments, propranolol was injected before sympathetic nerves were stimulated. If sympathetic stimuli to the aorta increase metabolism, similar to the effect observed in the myocardium (Mark et al., 1972), one might expect that propranolol would block the metabolic component and unmask vasoconstrictor responses. In this study, however, sympathetic stimulation also produced constriction of vasa vasorum in experiments in which propranolol was not used.
The increase in blood flow through vasa vasorum during baroreceptor stimulation indicates that the vessels are responsive, not only to intense electrical stimulation of nerves, but also to a physiological stimulus. The increase in conductance of vasa vasorum during baroreceptor stimulation presumably is mediated through withdrawal of sympathetic tone. Despite a marked increase in flow during baroreceptor stimulation, blood flow through vasa only returned to normal levels because baseline flow was low. Thus, although vasa are responsive to a reflex stimulus, it is not clear whether baroreceptor stimulation can increase flow above normal levels. It also is unclear whether vasa have a significant level of sympathetic tone under normal conditions.

Because ligation of intercostal arteries, which give rise to vasa vasorum, produces aortic medial necrosis (Wilens et al., 1965), it appears that vasa vasorum are essential for the nourishment of the aorta. These studies indicate that vasa vasorum are responsive to neural stimuli and suggest that neural mechanisms may influence nourishment of the aortic wall.

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