Blood Flow through Terminal Arterial Vessels after Denervation of the Bat Wing

By Mary P. Wiedeman, Ph.D.

ABSTRACT

Previous studies of microcirculation in the denervated wing of the unanesthetized bat showed that major arterial vessels increased markedly in diameter and lost their ability to contract spontaneously. Arteriolar vessels did not show such an increase in diameter, and furthermore their spontaneous contractile activity increased. These observations led to the idea that regulation of blood flow and blood pressure might be continued to some degree in the absence of central nervous control through enhanced activity of arteriolar vessels which just precede the capillary nets.

Therefore a thorough study by direct microscopic observation of the behavior of arterial vessels in the following surgical and pharmacological denervation was made. The data support the hypothesis that loss of tone and loss of contractile activity in the large arterial vessels following denervation produce an increase of tone and activity in the smaller, more distally located vessels, thereby causing a redistribution of the site of regulation of flow through capillary nets. The largest arterial vessels showed an increase in average diameter of 38% after surgical denervation, while the terminal arterioles showed a decrease in average diameter of 24%. Neither phenoxybenzamine (Dibenzyline) nor pentolinium (Ansolysen) were as effective as surgical denervation in producing relaxation of the large arterial vessels. Neither drug inhibited spontaneous contractile activity of the terminal vessels.

ADDITIONAL KEY WORDS

surgical denervation contractile activity residual vascular tone
myogenic response phenoxybenzamine pentolinium methacholine

Identification of sympathetic nerve fibers at the level of terminal arterial vessels has been difficult, and convincing evidence of their distribution to the vascular smooth muscle cells of these vessels is lacking despite recent advances in histological techniques (1-3). Although Ehinger et al. reported that fluorescence studies showed that adrenergic nerve fibers accompany vessels down to the finest precapillary arterioles in rat skeletal muscle (4), it is often difficult to identify vessels correctly in fixed tissue. Nerve fibers adjacent to terminal arterial vessels have been demonstrated in electron micrographs (5), but there is still doubt about their functional significance.

Convincing physiological evidence that precapillary arteriolar vessels are innervated by the sympathetic nervous system is also lacking, while evidence that these terminal vessels are primarily under local control continues to accumulate (6, 7).

In studies of microcirculation involving surgical denervation of blood vessels in bat wings (8-10), microscopic observation of the vessels following denervation revealed that (1) major arterial vessels increased markedly in diameter following denervation; (2) small arterial vessels such as arterioles and terminal arterioles did not show such an increase;
(3) major arterial vessels lost their ability to contract in circumstances which had previously provoked active contraction; and (4) small arterial vessels continued to contract spontaneously after denervation. It appeared that only the large arterial vessels were rendered inactive by denervation, and it was assumed that only they were predominantly under nervous control. The terminal vessels seemed to be dependent on other factors for their activity in that they neither relaxed nor became quiescent following nerve section.

In a more recent study (11) using the bat wing technique in which contractile activity of arteriolar vessels was recorded, another interesting result of denervation was revealed. Arteriolar vessels exhibiting spontaneous contractile activity were found to be more active after denervation of the wing. The duration of bouts of vascular contractions was increased in terminal vessels following removal of central nervous control. This observation suggested that regulation of blood flow and blood pressure might continue to some degree in the absence of central nervous control by activity of arteriolar vessels which just precede the capillary nets. Local factors, such as intraluminal pressure changes or accumulation or washout of metabolites, or both, could be considered as stimuli which would enhance contractile activity and tone of vascular smooth muscle.

Therefore a thorough study of the behavior of arterial vessels following surgical and pharmacological denervation was made. The data support the hypothesis that loss of tone and loss of contractile activity in large arterial vessels following denervation produce an increase of tone and activity in the smaller, more distally located vessels. Such a condition results in a redistribution of the site of regulation of flow and pressure from the large arterial vessels to the terminal arteriolar vessels.

**Methods**

The blood vessels in the wing of the common brown bat (Myotis lucifugus) served as the site for observation. An unanesthetized bat was placed in a holder with one wing extended over a glass plate and held in the desired position with metal clips. The tail membrane was also positioned to permit cannulation of its large vein for intravenous infusion of various drugs. A small area in the wing was denuded to increase visualization of blood vessels when an optical magnification of 1200 diameters was used. The area from which the epidermis had been removed was covered with buffered saline and further protected by a cover slip.

An eyepiece micrometer was used to measure vessel diameters. Surgical denervation of the wing vessels was accomplished by first locally anesthetizing the nerve trunk which enters the wing by placing a drop of procaine (1%) on the exposed nerve and then sectioning the nerve with a scalpel.

A detailed description of the bat wing technique was recently published (12).

**Results**

**Diameter changes in arterial vessels as a result of surgical denervation.**—The first procedure was to record diameter changes in the major arterial vessel and its subsequent branches down to the capillary network before and after surgical denervation. The major artery entering the wing gives off branches designated here as first-order vessels. Branches of the first-order vessels are called small arteries or second-order vessels and in turn give rise to arterioles or third-

**TABLE 1**

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Unchanged</th>
<th>Increased</th>
<th>Decreased</th>
<th>Control avg. diam. ((\mu m \pm SD))</th>
<th>Avg. change after denervation ((\mu m \pm SD))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major arteries</td>
<td>0</td>
<td>17</td>
<td>0</td>
<td>63.2 ± 11.5</td>
<td>+24.8 ± 2.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>1st order</td>
<td>6</td>
<td>11</td>
<td>0</td>
<td>36.5 ± 9.8</td>
<td>+5.4 ± 1.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2nd order</td>
<td>8</td>
<td>9</td>
<td>0</td>
<td>17.2 ± 5.0</td>
<td>+3.4 ± 1.4</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>3rd order</td>
<td>24</td>
<td>7</td>
<td>12</td>
<td>8.2 ± 2.9</td>
<td>-0.69 ± 0.3</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>4th order</td>
<td>30</td>
<td>7</td>
<td>28</td>
<td>4.3 ± 2.0</td>
<td>-0.0 ± 0.2</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
order vessels. The final vessel before the capillary net is called the terminal arteriole or fourth-order vessel.

Following nerve section, all major arteries increased in size, while some first- and second-order vessels increased and others showed no change. Some third- and fourth-order vessels showed no change, some increased in diameter, and others decreased. (See Table 1.)

Two important factors regarding these subjective measurements should be taken into account. First, the diameter recorded for an actively contracting vessel was its size during the relaxation phase which, in the final compilation, gave no indication of an increase in frequency of contraction or the duration of each contraction. The enhanced contractile activity frequently seen following denervation would, of course, alter blood flow into the capillary nets. Secondly, only one small vascular section could be measured in the wing for any single experiment, and it is possible that the area under observation was not the most active or responsive following denervation. For example, there are, on the average, 12.3 second-order vessels branching from each first-order vessel. Branching from successive vessels results in a total of 120 fourth-order vessels dependent on one first-order vessel for their blood supply. It is not to be expected that all of these more distally located vessels would behave in the same way. If such were the case, the changes in peripheral resistance would be enormous. This would account for the instances in which no diameter changes were seen in successive branches of the major artery. The activity and change in diameter size of a distal vessel would also be determined in part by the extent to which denervation affected its parent vessel. The direction of the diameter changes of all the vessels as a result of surgical denervation is shown in Figure 1.

It was also noted that the third- and fourth-order vessels contracting spontaneously before denervation continued to contract after nerve section. Vessels that had been inactive during the control period became active. It was seen that the major artery and the first- and second-order vessels showed no change in diameter when the animal was agitated and struggling against the restraining wing clips. In the intact animal, such movement results in alterations in vessel diameters varying from intense constriction to moderate reduction with increased vasomotion.

According to these observations of diameter changes and spontaneous contractile activity following denervation of arterial vessels, the major artery invariably increases in diameter and loses its ability to contract spontaneously, while the first- and second-order vessels may show an increase in diameter or no change in size. They also lose ability to contract spontaneously. Third- and fourth-order vessels decrease in diameter or are unchanged, and a very few increase in size. However, in these, cessation of contractile activity does not occur and may be initiated in previously quiescent vessels.

Diameter changes in denervated arterial vessels following intravenous injection of methacholine (Mecholyl).—To determine whether further relaxation of large arterial vessels could be elicited after nerve section, a supramaximal dose of Mecholyl (0.125 mg/kg)
was injected intravenously through the tail vein of the bat. If a basal vascular tone remained in arterial vessels after surgical denervation, then a substance such as methacholine, a vasodilator, should produce further relaxation. However, it was seen during microscopic observation of the vessels following an injection of methacholine that the diameters of the major artery and the first- and second-order vessels did not increase beyond that resulting from surgical denervation under such parasympathetic pharmacological influence. On the other hand, the spontaneously active and constricted third- and fourth-order vessels relaxed and became quiescent when treated with methacholine. The entire vascular bed was thus maximally dilated.

**Diameter changes in arterial vessels following intravenous injection of an adrenergic receptor-blocking drug.**—The next experimental procedure was to compare the results of pharmacological denervation with surgical denervation. Phenoxybenzamine (Dibenzyline), in a dose of 5 mg/kg, was injected into the tail vein, and diameter changes and spontaneous contractile activity of the arterial vessels were recorded. In general, the results were in the same direction as those seen with surgical denervation, but they were not as consistent. Phenoxybenzamine was not as effective as surgical denervation in enlarging diameters of the large arterial vessels. When surgical denervation was performed in animals pretreated with phenoxybenzamine, further relaxation of these large vessels occurred. Also, this adrenergic receptor-blocking agent, even in such a large dose, was not effective in curtailing spontaneous contractile activity of arterial vessels of any order, and often, in third- and fourth-order vessels, contractile activity began in vessels that were previously inactive.

The intravenous injection of dibenzyline resulted in an increase in diameter in all major arteries but caused little change in first-order vessels. Some second-order vessels increased, while others showed no change. Third- and fourth-order vessels either showed no change or a slight increase, and a few decreased in diameter. These results are presented in Table 2.

**Diameter changes in arterial vessels following intravenous injection of a ganglionic blocking agent.**—A second procedure for pharmacological denervation was the use of pentolinium (Ansolysen) to produce ganglionic

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Unchanged</th>
<th>Increased</th>
<th>Decreased</th>
<th>Control avg. diam. (μ ± SD)</th>
<th>Avg. change after pentolinium (μ ± SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major arteries</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>66.6 ± 11.8</td>
<td>4.7 ± 6.1</td>
<td>N.S.</td>
</tr>
<tr>
<td>1st order</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>41.6 ± 7.8</td>
<td>2.8 ± 1.5</td>
<td>N.S.</td>
</tr>
<tr>
<td>2nd order</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>15.4 ± 5.9</td>
<td>1.6 ± 1.0</td>
<td>N.S.</td>
</tr>
<tr>
<td>3rd order</td>
<td>11</td>
<td>3</td>
<td>3</td>
<td>8.8 ± 2.9</td>
<td>0.29 ± 0.54</td>
<td>N.S.</td>
</tr>
<tr>
<td>4th order</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>5.5 ± 2.3</td>
<td>0.48 ± 0.44</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
BLOOD FLOW THROUGH TERMINAL ARTERIAL VESSELS

A comparison between the diameter changes, expressed as percent of change of average diameter, after surgical denervation, adrenergic blockade, and ganglionic blockade.

blockade. After an intravenous injection of pentolinium (40 mg/kg), diameter changes and spontaneous contractile activity were recorded. Pentolinium was less effective than either surgical denervation or adrenergic receptor blockade in altering diameters of spontaneous contractile activity.

The responses of the vessels to intravenous injection of pentolinium were similar to those seen after the administration of the adrenergic receptor-blocking drug, although the changes were not statistically significant (Table 3). Also there was no evidence of any inhibition of spontaneous contractile activity.

These data support the suggestion that the alteration in size and spontaneous contractile activity of the third- and fourth-order vessels is dependent on the degree of diameter change in their parent vessels. In the absence of marked relaxation of the large arterial vessels (upstream vasodilation), there is no adequate distending stimulus for changes in the behavior of the more distally located arteriolar vessels. A comparison between the diameter change, expressed as percent of change of average diameter, after surgical denervation, adrenergic blockade, and ganglionic blockade, is shown in Figure 2.

Discussion

Surgical section of the nerve entering the wing of the bat results in relaxation of large arterial vessels and abolishes their ability to contract spontaneously, and terminal arteriolar vessels show a decrease in resting diameter and enhanced spontaneous contractile activity. To further emphasize these changes it was noted that all of the major arterial vessels were dilated after surgical denervation. In contrast, most of the first- and second-order vessels showed increases in diameter. These observations suggest removal of tonic vasoconstrictor impulses to the major artery and the first- and second-order vessels. At the same time, the more distally located vessels showed changes in diameter and behavior, but in the opposite direction. About 1/3 of the third-order vessels observed in a small vascular area of the entire wing and almost 3% of the fourth-order vessels were smaller in diameter and showed enhanced contractile activity. These responses of the third- and fourth-order vessels were very similar to those seen when intraluminal pressure was raised artificially (11). The decrease in diameter and the increase in contractile activity of these precapillary vessels after surgical denervation of the wing could be attributed to an elevation of intraluminal pressure in them caused by upstream vasodilation. In the absence of nervous control of parent vessels, the regulation of flow through capillary nets could be taken over by local control at the level of the terminal vessels. Baez et al. (13) have suggested this same mechanism to explain observations made on mesenteric vessels following alterations in intraluminal pressure. Johansson and Bohr (14) describe an increase in the frequency of rhythmical contractions of strips from small subcutaneous arteries from the dog's paw following stretch, and they suggest that this myogenic mechanism occurs in vivo to increase active tone in response to distension by intravascular pressure.

It is tempting to say that the resting diameter and contractile activity of terminal arterioles and precapillary sphincters are entirely dependent on local factors in the intact animal, especially since there is no convincing evidence in reports regarding the distribution...
of sympathetic nerves to these vessels. Recently Rhodin (2) gave a detailed presentation of the ultrastructure of arterioles and precapillary sphincters in which he described numerous nerve axons in the areas of arterioles, terminal arterioles, and precapillary sphincters. He based identification of vessels on their size as well as on the number of smooth muscle cell layers, and the site of the precapillary sphincter is that used by Chambers and Zweifach (15) in 1944. A precapillary sphincter has since been defined as the final smooth muscle cell preceding a capillary vessel (16). Accurate classification of vessels should be based on their function and position in the vascular bed. Using Rhodin's method of identification, the vessel reported here as a second-order vessel would correspond to a terminal arteriole in his terminology. Rhodin expresses the belief that the control of the smooth muscle cells of terminal arterioles and precapillary sphincters is not only through myoneural junctions but also through numerous myoendothelial junctions which he finds in these vessels. The myoendothelial junctions serve as "conduction devices" for humoral transmitter substances.

An intravenous injection of a supramaximal dose of methacholine into an animal whose vessels were denervated demonstrated that the larger arterial vessels were maximally dilated after separation from central nervous control. A residual vascular tone after denervation has been demonstrated in experiments in which blood flow to a limb was shown to increase following section of vasoconstrictor nerves; blood flow could be further increased with a chemical dilator (17, 18). The observations reported here that only the spontaneously active and constricted third- and fourth-order vessels were further dilated by methacholine indicates that basal vascular tone may be the result of the enhanced activity of the third- and fourth-order vessels because the larger arterial vessels are maximally dilated after removal of the influence of vasoconstrictor nerves. The residual tone is not equally distributed among all of the various-sized arterial vessels in the vascular bed, but it resides in the small vessels that are not predominantly under nervous control.

The responses of the microvasculature to adrenergic and ganglionic blockade were similar to those resulting from surgical denervation although they were not as marked. It seems apparent from these observations that while adrenergic and ganglionic blocking drugs may succeed in lowering systemic blood pressure through relaxation of large arterial vessels, they do not assure increased perfusion of capillary networks. The absence of relaxation of terminal arteriolar vessels following the intravenous injection of phenoxybenzamine is not in agreement with Altura and Zweifach (19) who reported dilation of all microvessels in the rat mesentery. However, Johansson and Bohr (14) were unable to inhibit spontaneous rhythmic activity of small subcutaneous arterial strips taken from dogs pretreated with phenoxybenzamine. According to Baez et al. (13), relaxation of precapillary vessels did not occur following the administration of subcutaneous pentolinium although systemic blood pressure was markedly reduced.

In summary, the behavior of the terminal microvessels in a bat's wing following denervation strongly supports the concept that the control of blood flow through these vessels to the capillaries beyond is predominantly through local factors. The contractile activity of the terminal arterioles and precapillary sphincters is independent of sympathetic vasoconstrictor impulses and is based primarily on myogenic factors.

Acknowledgment

The author expresses grateful appreciation to Silvia Rofé for expert technical assistance.

References

BLOOD FLOW THROUGH TERMINAL ARTERIAL VESSELS


Blood Flow through Terminal Arterial Vessels after Denervation of the Bat Wing
MARY P. WIEDEMAN

doi: 10.1161/01.RES.22.1.83
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1968 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/22/1/83

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/