Contractile Activity of Arterioles in the Bat Wing during Intraluminal Pressure Changes

By Mary P. Wiedeman, Ph.D.

ABSTRACT

Spontaneous contractile activity in the smooth muscle cells that invest arteriolar vessels is widely variable. Arteriolar branches originating from the same parent vessel contract independently of one another with no similarity in frequency or duration of individual contractions.

Direct microscopic observations were made of these vessels in the wing of unanesthetized bats before and after surgical denervation or nerve block by procaine. A major artery of the wing was cannulated and buffered saline was perfused in retrograde fashion to produce periods of increased intra-arterial pressure. Elevation of intraluminal pressure led to enhanced contractile activity both before and after denervation of the vessels. It is concluded that these vessels respond by contraction to increased pressure and that the response is not dependent on nervous activity.

ADDITIONAL KEY WORDS spontaneous arteriolar contractions intraluminal pressure denervation myogenic response autoregulation capillary blood flow

The fact that vascular smooth muscle has the inherent property of automaticity and that its contractile activity can be enhanced by stretching occupies an important place in present day discussions of autoregulation of blood flow.1-3 The contraction of arterial vessels in response to distention is a basis for the myogenic theory of autoregulation. According to Folkow,2 flow resistance can be increased to a considerable extent over a period of time by increasing the frequency and duration of contraction of precapillary sphincters when intraluminal pressure is elevated.

It seemed possible to determine directly whether precapillary vessels constrict more frequently and the contractions continue for a greater period of time when intraluminal pressure is increased within physiological limits by utilizing a technique of microscopic observation of the small blood vessels while varying intraluminal pressure. If they do, this would strengthen Folkow's contention that basal vascular tone depends on this myogenic property and that it can produce autoregulation.1, 2

Microscopic observation of vessels in the wing of the bat seemed ideal for such a study because neither anesthesia nor surgery are necessary and observations can be made at high powers of magnification without disturbing the normal circulation. Thus, interference which might obliterate or reduce autoregulation of blood flow is minimal. Also, direct observation of the vessels makes it possible to ascertain when the perfusion pressure reaches a level that exceeds physiological limits and overdistends the vessel.

Method

Common brown bats (Myotis) were used. An unanesthetized bat was prepared for observation by placing it in a suitable holder and extending one wing over a glass plate. The wing was held in place by spring clips and a drop or two of mineral oil was put between the wing and the glass for visual clarity. To further increase clarity, a small area of the wing was stripped of its epidermis. The epidermis can be removed with fine forceps without causing injury to the underlying structures, and the denuded area is then protected with buffered saline solution. A chamber was arranged over the denuded area by plac-
ing a cover slip on top of two small slivers of glass. Saline was introduced under the cover slip with a dropper. Observations of the vessels were made at a magnification of 1200 X. In this vascular bed, the average diameter of the major artery entering the wing is 70 \( \mu \) arterioles which branch from it have a diameter averaging 19 \( \mu \) arterioles and the terminal arterioles which are the vessels whose contractile activity was followed in this study have an average diameter of 7 \( \mu \).

Elevation of intraluminal pressure was accomplished by cannulation of the major artery of the wing at a distal portion. In this way a retrograde perfusion of buffered saline could be made. The cannula was attached by polyvinyl tubing to a T-tube; one arm was connected to a 1-ml syringe or a perfusion pump and the other arm was connected to a Statham pressure transducer (Fig. 1). The transducer was in turn connected to an Electronics for Medicine Recorder. Thus the amount of pressure produced as a result of the infusion of buffered saline could be recorded. It was not possible for technical reasons to determine the actual pressure change in the cannulated artery; the pressure recorded in the perfusion system served as an index of change in intravascular pressure, as did direct observation (see below). Duration of contraction of the vessel under observation was marked on the record by depressing a foot pedal attached to the recorder.

After anesthetizing the area locally with procaine, denervation of the wing vessels was accomplished by sectioning the nerve trunk which originates in the brachial plexus and enters the wing at the shoulder. Abolition of central nervous control was recognized by relaxation of the arterial vessels and their inability to respond in a normal way when the animal was made to struggle. Terminal arteriolar vessels were not affected by denervation, evidenced by the fact that their diameters did not change and their spontaneous vasomotion continued.

**Results**

1. **SPONTANEOUS ACTIVITY IN TERMINAL ARTERIOLES IN INTACT ANIMALS**

The number of spontaneous contractions of smooth muscle cells encircling the terminal arterioles were widely variable both in frequency of occurrence and duration with no direct relationship between frequency and duration. The contractions varied in intensity and at times caused blood flow to stop. Terminal arterioles branching from the same arteriole showed no similarity in spontaneous activity; some were quiescent and others were extremely active. In instances where several terminal arterioles were active, there was a closer correlation between the frequency with which they contracted than between the duration of contractions.

It is not possible to give any meaningful average values for the frequency and duration of contractions of a spontaneously active ter-

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**FIGURE 1**

Diagrammatic representation of method used to produce changes in intraluminal pressure in terminal arterioles while observing their responses microscopically.
CONTRACTILE ACTIVITY OF ARTERIOLES

Terminal arteriole because of uncontrolled variables in the living animal, but it can be stated that these small vessels undergo contractile activity of varying frequency and duration, independently of one another.

2. CONTRACTILE ACTIVITY IN TERMINAL ARTERIOLES DURING CONTINUOUS ELEVATION OF PERFUSION PRESSURE

A modified Ringer's solution (each in mm per liter: CaCl₂, 0.05; KCl, 6.20; NaCl, 112.9; MgCl₂, 1.0; NaH₂PO₄, 1.0, and NaHCO₃, 5.0) at room temperature was perfused into the major artery of the bat's wing at a constant known rate using a Harvard Apparatus Co. infusion pump. The rates most frequently used were 0.002 ml per min and 0.005 ml per min. During the infusion periods, the average total amount of buffered saline injected into the animal was 0.06 ml. This amount of buffered saline was considered to be too small to evoke any changes in contractile activity of the arterioles, either through dilution of blood or by changes in blood volume. In earlier experiments, intravenous injections of buffered saline in much larger amounts (0.2 ml) did not affect contractile activity in arteriolar vessels, so presumably the injected saline was not responsible for the enhanced activity.

Microscopic observation of the cannulated artery from the cannula tip to the area selected for recording activity in a terminal arteriole showed that the infusion of buffered saline caused the oncoming blood to be forced away from the cannula tip, and as pressure continued to rise in the perfusion system, the column of blood was pushed back upstream by the column of saline. The forward flow of arterial blood was diverted into arterial branches proximal to the cannula. Infusion of saline was never carried to the point where saline flowed into the arterial branch that was the parent vessel for the area under study.

The results of these studies, which included 15 trials in 9 animals, showed that as pressure in the system increased so did the length of time during which an arteriolar vessel was contracted. The average number of seconds during a 2-min period that the terminal arteriole was contracted when the infusion pump was not running was 25. After the pump was turned on and the pressure recorded by the strain gauge began to rise, the average length of contraction in a 2-min period was 44 sec with a range from 11 to 82 sec; at a higher pressure, contraction endured for 60 sec, ranging between 25 and 82 sec. As pressure continued to rise, so did the length of time the small vessels were contracted, and at a relatively high pressure, contraction time was 95 sec with a range from 73 to 114 sec.

3. CONTRACTILE ACTIVITY IN TERMINAL ARTERIOLES DURING A SLIGHT ELEVATION IN PERFUSION PRESSURE

A series of trials was run in a group of 14 animals in which pressure was elevated manually to a preselected level with a 1-ml syringe and maintained at this pressure for a 2-min period. A selection was made for a pressure which was not great enough to clear the cannulated artery of blood for any great distance beyond the tip of the cannula. The actual pressure at the tip of the cannula and the rate at which buffered saline was introduced into the animal was unknown, but it was assumed that a small increase in intraluminal pressure was produced. Although the results in this series were not so consistent as in the first group where the infusion rate was known, the response of the vessels was in the same direction; enhanced contractility of the terminal arterioles during the 2-min elevation period caused them to be contracted 53% of the time (average), which represented an increase of 17% over the average time for the control period.

4. CONTRACTILE ACTIVITY IN TERMINAL ARTERIOLES DURING A MODERATE ELEVATION IN PERFUSION PRESSURE

When the preselected level of infusion pressure was doubled over the value considered to be a slight elevation, almost 50% of the responses showed a stepwise increment in contractile activity between a slight elevation and the further increase. A typical record of the contractile activity in response to a slight and then moderate elevation of perfusion pressure is shown in Figure 2. In 22 trials, all of the observed vessels were contracted for a longer period with either a slight or moderate elevation than during their control period.
Representative experiment showing contractile activity of a terminal arteriole during a control period and during stepwise increments in perfusion pressure.

5. CONTRACTILE ACTIVITY IN TERMINAL ARTERIOLES FOLLOWING DENERVATION

To ascertain to what extent, if any, nervous control influenced contractile activity of the terminal arterioles in the conditions described above, some of the experimental procedures were repeated in bats following section of the major nerve entering the wing. Their diameters were unchanged and spontaneous contractions continued to occur.

In 17 observations in 15 animals that had surgical denervation, the spontaneous contractions in a 2-min period rose by 33.4 sec (average); in the 12 instances in which there was an increase over the control value, there was a 27% increase. It can be postulated that the apparent increase in blood flow through the arterial system after denervation acts as a stimulus (via increased intraluminal pressure) to the vascular smooth muscle of the arterioles which are not dilated after nerve section. In any event, the contractile activity of the smallest arterioles is enhanced when their parent vessels, devoid of central nervous control, are dilated.

It is possible that all nerves to the observed area were not interrupted by surgical section of the main trunk. To test the possibility that vasoconstrictor nerves might escape division if they were carried in vascular fascia or approached from another direction, chemical denervation with procaine was carried out. A narrow strip of epidermis in the form of a circle was removed from the wing and a ligature soaked in 1% procaine was placed in the "moat." In this way all vessels and nerve fibers leading into the observed area were covered with procaine. Arterial vessels within the circle were then observed before and after nerve section and the application of 1% procaine. There were no differences in diameter changes or vasomotor activity of the vessels when the two methods were used separately or combined.

Manual elevation of perfusion pressure to the level considered as slight for 2 min and then to moderate for 2 min in the denervated wing resulted in enhanced contractile activity of terminal arterioles and they were con-
TRACTED 60% OF A 2-MIN PERIOD AT SLIGHT ELEVATION AND 67% WHEN THE ELEVATION WAS MODERATE.

These results suggest that the increased activity of vascular smooth muscle cells which occurs during elevation of intraluminal pressure is not dependent on nervous activity.

Discussion

Microscopic observation of augmented contractile activity of vascular smooth muscle induced by variations in intraluminal pressure is not new; it has been reported by other investigators and from this laboratory, in respect to greater frequencies of venous vasmotion during distention of the veins.

Nicol and Webb described a powerful contraction of small arterioles or venules resulting from a sudden increase in internal pressure. Nicol observed constriction of arteriolar vessels at their origin during resumption of blood flow after flow had been artificially blocked. Johnson saw arteriolar diameters become smaller at increased arterial pressure. Folkow has applied this myogenic response to an explanation for autoregulation with a very convincing set of arguments.

The results reported here of responses seen confirm the belief that an increase in intraluminal pressure will augment contractile activity of the precapillary vessels. This means of regulation of blood flow through capillary nets would act in conjunction with other known activators of smooth muscle such as local changes in metabolites or circulating neurohumors. It might be expected to contribute to control of flow during changes in intraluminal pressure of the same magnitude as those produced by normal shifts in flow and pressure in the larger parent vessels.

Direct microscopic observation also made it apparent that the precapillary vessels will respond to very slight pressure changes which might be too small to be noticeable in measurements of systemic pressure.

Denervation of the blood vessels of the bat's wing resulted in a predictable response. The loss of central nervous control of the larger arterial vessels, which allowed them to assume a bigger resting diameter, increased the contractile activity of the smooth muscle cells of the precapillary arterioles.

The heightened responsiveness of the terminal vessels to increased intraluminal pressure after nerve section demonstrates the lack of dependence of this portion of the vascular bed on nerves for control of blood flow.

It is concluded that precapillary vessels will constrict, thereby altering blood flow into capillary nets, when the smooth muscle which invests them is stimulated by an increase in intraluminal pressure. The achievement of regulation of flow by this means results in "fingertip" control by these delicate vessels which react independently of the central nervous system.

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References

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MARY P. WIEDEMAN

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