Pressure Releases a Transferable Endothelial Contractile Factor in Cat Cerebral Arteries

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When exposed to an increasing transmural pressure, middle cerebral arteries of the cat exhibit reduction of internal diameter which is mediated by vascular muscle cell depolarization. This laboratory has recently demonstrated that this "pressure-induced" activation is dependent upon the presence of an intact endothelium. The present studies were undertaken to determine if this phenomenon is due to inhibition of tonically released endothelium-derived relaxing factors (EDRF) or release of a contractile substance. When cerebral arterial segments were pressurized to between 40 and 160 mm Hg there was 13.2% reduction in internal diameter accompanied by significant muscle cell depolarization from \(-53 \pm 2.7\) to \(-22 \pm 1.4\) mV. There was a significant positive correlation between the \(\Delta E_m\) and step increases in transmural pressure. These excitatory responses were lost and vessels dilated to pressure when the endothelium was removed. Upon exposing the denuded vessel to a pressurized intact donor, the denuded vessel recovered its ability to contract and depolarize suggesting that a contractile substance might be released from the vascular endothelium upon pressurization. The EDRF antagonist oxyhemoglobin did not alter the excitatory response to pressure in these isolated cerebral arteries further suggesting that the reduction in diameter and muscle cell depolarization results from the release of a contractile substance from the vascular endothelium and not inhibition of EDRF. (Circulation Research 1989;65:193–198)

Recent reports have demonstrated that the vascular endothelium of various arterial beds may release factors that are contractile in nature.1–7 In the cerebral circulation, this apparent endothelium-dependent contraction is observed on elevation of transmural pressure and has been speculated to be partially responsible for blood flow autoregulation.4,8

One of the primary problems in defining a contractile factor thought to be released from the vascular endothelium is the ubiquitous presence of endothelium-derived relaxing factor(s) (EDRF).9–12 It is possible that the contractile response observed upon various maneuvers (e.g., hypoxia, pressure, and stretch) may be the result of inhibition of tonic release of EDRF.13

The present studies were designed to determine whether elevation of transmural pressure triggers endothelium-dependent contraction of cat cerebral arteries by effecting the synthesis and release of diffusible endothelial contractile factor(s) or, alternatively, by depressing the release of EDRF. The experiments were carried out using a novel bioassay system in which segments of intact and denuded cerebral arteries are pressurized and perfused in series.14 The results suggest that elevation of transmural pressure induces the release of a transferable contractile factor released from the endothelium that acts via muscle cell membrane depolarization.

Materials and Methods

General

Adult mongrel cats were anesthetized with sodium pentobarbital (60 mg/kg) and their brains removed. Middle cerebral arteries were dissected free of arachnoid and placed in 4° C bicarbonate-buffered physiological salt solutions. The middle cerebral arteries were then cut into 7–10 mm long segments and placed in an organ chamber maintained at 37° C. The arterial segments were then cannulated at both ends with PE 10 polyethylene cannulas and perfused and suffused with physiological saline solution containing (mM) \(\text{Na}^+ 141, \text{Cl}^- 125, \text{Ca}^{2+} 2.5, \text{K}^+\)
Double-Vessel Perfusion System

Endothelium removal was accomplished as previously described.4,15 Briefly, vessels were perfused with a dilute collagenase solution (1.0 μg/ml collagenase in physiological saline solution) at a transmural pressure of 100 mm Hg. Note that NE contracts (negative values) cat middle cerebral arteries. Addition of acetylcholine dilated the NE-precontracted vessels (before collagenase perfusion). After collagenase perfusion, NE still contracts these vessels but subsequent addition of acetylcholine resulted in further contraction rather than the previously observed dilation. This was our functional test for endothelial removal. Vertical lines through each bar represent the SEM of seven vessels. All values, except that depicted as PSS were significantly different from zero at p < 0.05.

**Electrical and mechanical responses to increases in transmural pressure were measured in both vessels. The denuded arterial segment (recipient) was always studied in isolation before the intact donor segment was placed in series. Flow through these vessels was 1.2 ml/min. Vessels were connected by a PE 10 polyethylene tube. The control set up is pictorially depicted in another manuscript appearing in this journal.14**

In all cases, it was determined beforehand that the recipient segment either maintained or reduced its diameter upon raising transmural pressure and that the response was lost (i.e., diameter increased on increasing perfusion pressure) when the endothelium was disrupted by collagenase perfusion. Electrical and mechanical measurements were done before and after oxyhemoglobin (10⁻⁷ M) perfusion to block EDRF. Oxyhemoglobin was prepared according to previously published techniques.16 The efficiency of oxyhemoglobin (10⁻⁷ M) to block EDRF in cat cerebral arteries was tested by its effect on the ACh (5×10⁻⁶ M) dilation on NE-precontracted arteries.

**Electrical Measurements**

Electrical measurements were made according to techniques previously described.4,8,15,17 Briefly, glass microelectrodes were used that had been filled with 3 M KCl, had tip resistance of 50–80 MΩ, and typically had tip potentials of <4 mV. The primary criteria for successful impalement included a sharp drop in voltage from baseline upon entry of the microelectrode tip into the cell and a sharp return to zero upon exit. Flow was usually stopped for 3–10 minutes to allow for successful cell impalement, but occasionally, a 20-minute period was necessary. It should be noted, however, that even when flow was stopped there were usually small leaks in the vessels coming from the tied side branches, which necessitated some small flow compensation to maintain pressure; therefore, mixing of solutions between vessels always occurred. Multiple impalements were made at each pressure. In this report, ‘n’ refers to the number of cells impaled, not the number of vessels.

**Vessel Diameter**

Vessel diameters were measured with the aid of a high-resolution stereomicroscope (Zeiss) on which a video camera (Hitachi) was mounted. The image was displayed on a TV monitor, and vessel diameters were measured using a Colorado Video image analyzer. Magnification at the screen was ×75, and the system was calibrated with a filar micrometer to an accuracy of ±2.0 μm. Vessel dimensions included internal diameter, external diameter, and wall thickness; however, our primary measurement was internal diameter. Again, flow through the system was usually halted during the time precise measurements were taken to eliminate possible movement artifact. During the time flow was halted (1–8 minutes), both
membrane potential and diameter response were stable at the particular pressure studied.

**Statistical Analysis**

Data were compared and significance tested by paired and nonpaired Students t tests and analysis of variance. Slopes were tested for significance and linearity via regression analysis. Significance was defined by a value of */p* < 0.05.

**Results**

Internal arterial diameter and intracellular membrane potentials were recorded in isolated, pressurized cat middle cerebral arteries before and after endothelium removal and on receiving flow from a pressurized intact donor. Endothelial cell removal was accomplished by perfusing vessels with a collagenase solution as described previously. The efficacy of endothelial cell removal was determined in the present preparation by recording the dilatory response to 5x10⁻⁶ M ACh in NE-precontracted vessels. As can be seen on Figure 1, before collagenase perfusion, 5x10⁻⁶ M ACh significantly dilated the 5x10⁻⁶ M NE-precontracted vessels (*/p* < 0.05). However, after collagenase perfusion, ACh resulted in further contraction (reduction in diameter) of precontracted vessels (n=7). Note that after collagenase perfusion, the washout of NE with physiological saline solution perfusion for 15–20 minutes did not affect diameter. Only those vessels that did not dilate after enzyme perfusion were used as “deendothelialized vessels.”

The data relating internal arterial diameter to step increases in transmural pressure are given in Figure 2. In this figure, each vessel is plotted separately due to the large variability in size of the middle cerebral arteries from different cats. Five arterial segments decreased in diameter and two maintained diameter when the transmural pressure was varied between 80 and 160 mm Hg. There was a mean reduction in diameter of 13.2% between 80 and 160 mm Hg. These changes in diameter were always observed within 20 seconds of changing pressure, and the diameter increased, often past control, when the pressure was reversed. On removal of the endothelium in these arteries, there was always an increase in diameter (by a mean of 13.9%) when pressure was increased from 80 to 160 mm Hg. All data are paired, and numbers next to each line refer to the same vessel in each frame (Figure 2).

The data summarizing the membrane potential (E_m) measurements under the above experimental conditions, that is, with and without endothelium, are depicted in Figure 3. There was a progressive depolarization at each step change in transmural pressure (from -53.03±2.7 to -22.6±1.4 mV at 40 and 160 mm Hg, respectively), when the endothelium was intact. The slope of 0.18 with a correlation coefficient of 0.77 was significantly different from zero at */p* < 0.01. When the endothelium was disrupted by collagenase perfusion, the positive relationship between reduction in E_m and increase in transmural pressure was lost, and the slope was not significantly different from zero (Figure 3).

When these same vessels received flow from an intact donor, they again depolarized and contracted as transmural pressure was elevated (both vessels connected in series) (Figure 4). There was an 8% reduction in diameter when pressure was increased from 40 to 160 mm Hg, and there was recovery of a significant (*/p* < 0.01) positive slope relating E_m as a function of pressure with a correlation coefficient of 0.68. These changes in diameter and E_m occurred within 60 seconds of raising pressure and returned toward control when pressure was reduced.

Removal of the endothelium did not result in a significant reduction in diameter or membrane depolarization at each transmural pressure (Figures 2 and
Figure 3. Summary of membrane potentials obtained in muscle cells of cat middle cerebral arteries after elevations in transmural pressure. Left panel represents the control state in which the vascular endothelium is intact and demonstrates progressive membrane depolarization as transmural pressure is elevated. Right panel represents data obtained when the endothelium is removed and transmural pressure is elevated. When the endothelium is removed, elevation in transmural pressure no longer results in membrane depolarization. Each point represents a single impalement at that pressure. The numbers over each group of data represent the number of cells (numerator) and the number of vessels (denominator).

This point is important in that it suggests that EDRF is not tonically released in this preparation.

In some experiments, we used oxyhemoglobin to block the potential action of EDRF on our bioassay. Oxyhemoglobin (10^{-7} M) did not block the contractile response of a deendothelialized recipient vessel receiving flow from a pressurized intact donor segment (Figure 5). There was still a significant positive relation between reductions in E_m and elevations in transmural pressure with a statistically significant slope and a change in E_m of 20 mV between 40 and 160 mm Hg (not shown). These data again suggest that there is not a tonic release of EDRF which pressure might inhibit in that even in

Figure 4. Summary of dimensional (diameter) and electrical data obtained in denuded (endothelium removed) cat middle cerebral arteries which received flow from a pressurized intact donor (segment A in the insert) middle cerebral artery from the same animal. All data were obtained from vessel segment B (i.e., without endothelium in the insert). Left panel depicts the internal diameter of the same denuded vessels as in Figure 2 (right panel) but in series with an intact, pressurized donor segment. Right panel represents the membrane potential response in the same vessels as Figure 3 (right panel) but in series with an intact donor. Note that when exposed to a pressurized donor segment, the denuded vessels recover their ability to contract and depolarize. Only one vessel (left panel, #6) tended to increase in diameter as pressure was elevated. The numbers over each group of data represent the number of cells (numerator) and vessels (denominator).
the presence of oxyhemoglobin step elevations in transmural pressure still resulted in membrane depolarization and reduction in diameter.

We conducted a separate set of experiments in which ACh (5 x 10^-6 M) dilated norepinephrine (5 x 10^-4 M) precontracted vessels. In these experiments (n=3), incubation with oxyhemoglobin (10^-7 M) blocked the ACh-induced dilation when the endothelium was intact (data not shown).

Discussion

These data demonstrate that pressurization of cat middle cerebral arteries induces the release of a transferable contractile substance from the vascular endothelium. This conclusion is based on the recovery of pressure-dependent contraction and the return of a positive relation between E_m and pressure when a denuded arterial segment was in series with an intact donor. It is not likely that the observed responses result from inhibition of tonically released EDRF in that 1) the denuded arterial segment did not depolarize or contract on endothelium removal, suggestive of tonic EDRF release; 2) the excitatory effect of elevation in transmural pressure could be transferred from an intact vessel to one without endothelium and the intact vessel did not exert a tonic inhibitory action (i.e., hyperpolarization or dilation) on the denuded one; and 3) the excitatory effect of elevations of transmural pressure was not blocked by oxyhemoglobin, a potential EDRF antagonist. While these findings do indeed suggest the presence of an endothelial contractile factor, they do not completely rule out a role for EDRF. The absolute evidence for an endothelial contractile factor important in myogenic activation of arteries would be the isolation of a biologically active substance from arterial perfusate upon elevation of pressure.

Bioassay techniques have been used in the past to understand the nature of EDRF. The system described in this study is novel in that it uses segments of the same artery and allows exposure of both the intact and denuded segment to the same transmural pressure and other environmental conditions. The use of intact pressurized arterial segments also maintains normal physiological geometry, which may be important if factors such as wall stress and shear forces are found to be important.

These studies are important because there is a potential physiological significance for a contractile substance released from the vascular endothelium that appears to be transferable. The fact that the response is contractile, is dependent on the level of transmural pressure, and is reversed quickly upon reduction of pressure suggests a potential role in blood flow autoregulation. It appears transferable, and therefore, it may be possible to determine its identity, which is essential to absolutely rule out a role for EDRF. Since the responses were stable during the 1–8 minute stop-flow experimental periods, it would appear that this putative factor is stable for that period. However, it is possible that mixing, which could occur through small leaks in the recipient vessel, is still occurring when flow is halted. Thus, any statement at this point regarding stability is speculative.

A recent report by Yanagisawa et al identified and sequenced a peptide, which is contractile in nature, from the cultured vascular endothelium of porcine aorta. The effect of this peptide, termed endothelin, was not easily reversible. In our preparation, the contractile response to step increases in pressure was rapid, occurring within 20 seconds, and was reversible in that the vessel diameter began to return toward control (i.e., low pressure state) upon reduction in pressure. Thus, there are some dissimilarities between the substance released upon pressurization in our preparation and that of endothelin. However, it is possible that under the experimental conditions of our preparation there may be release of a peptide that closely resembles endothelin but is easily reversible, or that under the conditions of high transmural pressure the same peptide may exert a different action.

Whatever the nature of this putative substance, in our preparation it appears to work via a mechanism of electromechanical coupling. The range of membrane depolarization to elevations in transmural pressure is similar to that observed in single isolated cerebral arteries. Previous work has suggested that the depolarization in pressurized arteries is due to an increase in Ca^2+ conductance. Thus, it will be important to test the effect of any potential endothelium-dependent vasomotive agent on ion conductance systems.

It is possible that the findings reported here may have implications regarding certain cerebrovascular pathologies, namely, propagated vasospasm. Under conditions of severe hypoxia and subarachnoid...
hemorrhage\textsuperscript{18–20} cerebral vasospasm occurs, which often appears to propagate beyond the area of original insult. Our finding that a contractile factor is released from the endothelium of one vessel segment and then exerts its action on a downstream segment provides a mechanism for a propagated vasoactive event. In certain arterial preparations, it has been demonstrated that severe hypoxia can result in contraction that is endothelium-dependent.\textsuperscript{2} If an endothelium-dependent contractile substance is released from a specific area of insult, it is possible that the vasoactive event might be propagated downstream.

References

15. Harder DR, Madden JA: Electrical stimulation of the endothelial surface of pressurized cat middle cerebral artery results in TTX-sensitive vasoconstriction. \textit{Circ Res} 1987;60;831–836

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