Pressure-Dependent Membrane Depolarization in Cat Middle Cerebral Artery

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SUMMARY. This study was undertaken to examine the effect of increasing transmural pressure on membrane electrical properties of cat middle cerebral arterial muscle. Middle cerebral arteries were removed from the cat brain, cannulated, and prepared so that transmural pressure within a segment could be manipulated. Intracellular membrane potential was recorded with glass micro-electrodes at various transmural pressures. There was a positive slope relating changes in intracellular membrane potential as a function of transmural pressure with a correlation coefficient of 0.79. Blockade of nerve excitation with tetrodotoxin and inhibition of α-adrenergic receptors with phentolamine not only did not block the pressure-induced depolarization, but increased the slope of the intracellular membrane potential vs. pressure relationship. This slope was increased upon elevation of extracellular calcium concentration from 2.5 to 4.0 mM and was significantly reduced upon reduction of extracellular calcium concentration to 0.5 mM. When arterial preparations were equilibrated at 0 mm Hg prior to pressurization, action potentials were recorded only when pressure was initially elevated, while a sustained depolarization was recorded during the pressure plateau. However, when arteries were equilibrated at a transmural pressure of 100 mm Hg for 90 minutes, spontaneous action potentials were recorded which increased in frequency as a function of pressure until they were inactivated when intracellular membrane potential approached —30 mV at high transmural pressures. Photomicrographs demonstrated that these vessels either maintained or decreased diameter upon pressurization. These findings provide a cellular mechanism for myogenic regulation of cerebral arterial diameter. (Circ Res 55: 197–202, 1984)

THE myogenic response in blood vessels refers to contraction of vascular muscle cells in response to an application of force (Bayliss, 1902). In microvascular beds studied in vivo, contraction is elicited by increasing transmural pressure; similarly, muscle strips from certain arteries contract when stretched (see review, Johnson, 1980). The mechanism by which a force applied to vascular muscle initiates an active response is not well understood.

Stretching portal vein leads to increased electrical spike activity (Johansson and Mellander, 1975). There is also increased spike activity when gut smooth muscle is stretched, an action which has been postulated to be due to an increase in Na+ permeability (Bulbring and Kuriyama, 1963). Halpern et al. (1984) have shown that pressurization of small rat cerebral arteries in vitro results in muscle activation and reduction of diameter, an action that is blocked by removal of Ca++ and addition of EGTA. Thus, it is possible that stretching arterial muscle cells results in activation by alteration of membrane permeabilities to ions.

Cerebral blood flow exhibits autoregulation to changing blood pressure. It is plausible that part of this autoregulation may be maintained by myogenic responses of cerebral arterial muscle. Pial arteries on the surface of cat brain constrict as arterial blood pressure rises (Wahl and Kuschinsky, 1979; Hernandez et al., 1978). Similarly, in man, retinal blood vessels constrict as arterial blood pressure is elevated (Tachibana et al., 1982).

It is the purpose of this paper to examine some of the cellular mechanisms of the myogenic response in cat middle cerebral arteries by recording intracellular electrical activity of arterial muscle cells upon elevation of transmural pressure.

Methods

Pressurization of Cerebral Arteries

Mongrel cats (3–5 kg) were anesthetized with sodium pentobarbital (30 mg/kg, ip), decapitated, and the brain removed. Middle cerebral arteries were removed and placed in cold (4°C) Krebs solution. Great care was taken not to stretch or damage the arteries during dissection. Arachnoid covering the vessels was cut with small iris scissors so that lifting the vessel from the brain did not cause undue stretching. Vessels were placed in an organ bath for mounting onto a glass pipette. One end of a 5- to 8-mm segment of artery was carefully pulled onto a 150-μm o.d. glass pipette and tied in place with a 30-μm silk suture under a binocular microscope. All major side branches, as well as the opposite end, were tied off with similar suture material, leaving a blind sack with only very small side branches of the artery remaining patent. The uncannulated end of the vessel was secured in plastic jaws.
and the length adjusted to that which had previously been measured while in the brain. The pipette was attached to a volume reservoir which could be maintained at any desired pressure. Since the volume inside the vessel was insignificant compared to the volume source, the reservoir was considered infinite so that the pressure at which it was maintained was the pressure of the entire system, including the inside of the vessel. Pressure was regulated by connecting the bulb portion of a standard sphygmomanometer to the reservoir. When the reservoir was pumped up to the desired pressure, the sphygmomanometer was clamped. The entire system was calibrated with a mercury monometer. A pressure transducer (Statham) was in line between the reservoir and the pipette so that transmural pressure could be continually monitored. The preparation was continually suffused with a physiological salt solution containing (in mM): 141 Na⁺; 4.7 K⁺; 2.5 Ca²⁺; 0.72 Mg²⁺; 124 Cl⁻; 1.7 H₂PO₄⁻; 25 HCO₃⁻; 11.0 glucose; and 3 Heps. Solutions were aerated with 95% O₂/5% CO₂, maintaining a pH of 7.35-7.4 and Pco₂ of 37-40 torr. Pco₂ was measured with a blood gas analyzer (Radiometer). Identical solutions were used to pressurize arterial preparations. The Pco₂ and PO₂ within the reservoir were maintained by filling the pressurizing bulb with calibrated gases. Both the pressurization and suffusion solutions were maintained at 37°C. Calcium concentrations were manipulated without osmotic compensation. Tetrodotoxin (Sigma) and phentolamine (Cal Biochem) were added directly to the suffusion solutions at a final concentration of 10⁻⁶ M.

Electrical Measurements
For measurements of intracellular activity, glass microelectrodes were used according to techniques previously described (Harder and Sperelakis, 1978). They were filled with 3 M KCl, had tip resistances of 60-80 MΩ and tip potentials less than 3 mV. The main criteria for a successful impalement included a sharp drop in voltage from baseline on entry of the microelectrode into the cell and a sharp return to zero upon exit. Identical criteria were applied throughout the range of pressure steps (i.e., low values of Eₘ were not discarded when transmural pressure was at 0 mm Hg). The recording amplifier used was Dagan (model 8100) intracellular probe system, which possessed capacitance neutralization and an internal bridge circuit. The microelectrodes used were of the floating type. A 25-μm silver wire was inserted and wedged into the microelectrode tip. The electrode tip then was broken and the wire extended through the electrode shank, leaving an extremely small tip on a very fine wire providing minimal inertia for movement. With this arrangement, it was sometimes possible for the electrode tip to remain inside an arterial muscle cell during vessel pressurization. Cell impalements were made with the aid of a sliding micromanipulator (Aus Jena).

Optical Measurements
Relative measurements of the internal diameter of cerebral arteries in response to changing transmural pressures were made with the aid of a high resolution binocular microscope (Zeiss). Microelectrode placement was made at 32X magnification. After the electrode tip was brought adjacent to the adventitial surface of the vessel, magnification was increased to 100X. Photographs could be taken of selected preparations to observe arterial diameter during pressure manipulation with a 35-mm camera mounted on a trinocular tube.

Due to the relatively large size of the arteries (300-700 μm o.d.) and the fact that a wide working area was needed to position the floating microelectrodes, magnifications higher than 100X could not be obtained, making it difficult to measure small changes in diameter accurately. This situation was compounded by the fact that these large vessels do not always maintain a perfect cylindrical shape. Therefore, we feel that we can only estimate diameter changes, stating that there was an increase, decrease, or no observable changes in diameter in response to increasing or decreasing pressure.

Only those vessels which could be observed to either decrease or show little increase in diameter to elevation of pressure beyond 60 mm Hg were used for study. Although all vessels increased in diameter when initially pressurized from 0 to 50 or 60 mm Hg, approximately 25% of the preparations were rejected after this initial pressurization, since they continued to increase in diameter beyond 60 mm Hg, acting like a balloon. It is realized that rejecting this population of vessels may be selecting a bias population; however, if arteries which previously maintained diameter upon pressure are purposely damaged by pinching them at one end with a forceps or subjecting them to undue stretch, they indeed do act like a balloon by increasing in diameter when pressurized to the same level. The care required in mounting these vessels cannot be overstated. Damage to any portion of the arterial segment used for study resulted in a preparation which had to be rejected.

Results
Pressure-induced Depolarization of Cat Middle Cerebral Artery: Effect of TTX and Phentolamine
Resting membrane potential (Eₘ) was measured from cat middle cerebral arteries with glass microelectrodes. The Eₘ (± se) obtained from 25 cells within 15 cat middle cerebral arteries was —68 ± 1.6 mV when the transmural pressure was 0 mm Hg. Upon elevation of transmural pressure from 0 to 150 mm Hg, the arterial muscle cells progressively depolarized. As can be seen in Figure 1, there is a positive slope with a correlation coefficient of 0.79 when Eₘ is plotted as a function of pressure, even though there is considerable dispersion of Eₘ's at the various pressures. Individual cell impalements were made after each pressure step so that mechanical artifact which could be caused by pressurization was eliminated. Statistical analyses, including slopes and correlation coefficients obtained under various conditions, are given in Table I.

To examine the possibility that the membrane depolarization observed upon vessel pressurization may be due to stimulation of periartrial nerves and release of excitatory neurotransmitters, we measured Eₘ in pressurized vessels after blocking nerve excitation with tetrodotoxin (TTX, 10⁻⁶ M) and blocking excitatory α-adrenergic receptors with phentolamine (10⁻⁶ M). As can be seen in Figure 2, inhibition of neurotransmission did not prevent the "pressure-
TABLE 1
Em vs. Transmural Pressure: Data Summary

<table>
<thead>
<tr>
<th></th>
<th>Control 2.5 mM Ca++</th>
<th>0.5 mM Ca++</th>
<th>4.0 mM Ca++</th>
<th>TTX, phentolamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>15</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>No. of cells</td>
<td>82</td>
<td>43</td>
<td>57</td>
<td>41</td>
</tr>
<tr>
<td>Slope Em/P</td>
<td>0.145</td>
<td>0.066*</td>
<td>0.210†</td>
<td>0.189†</td>
</tr>
<tr>
<td>se slope</td>
<td>0.010</td>
<td>0.008</td>
<td>0.016</td>
<td>0.009</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.79</td>
<td>0.48</td>
<td>0.86</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Note: at each [Ca]o concentration, the slope is the pooled estimate. The comparison of slopes between concentration results is a t-test with t > 3 and P < 0.01.
* Slope significantly less than control at P < 0.01.
† Slope significantly greater than control at P < 0.01.

induced depolarization but, rather, tended to increase the slope and improved the correlation coefficient (Table 1). Such findings demonstrate that release of norepinephrine from adrenergic nerves is not responsible for the Em response to pressure, and may even suggest that transmitters from TTX-sensitive nerve endings inhibit "pressure-induced" depolarization.

Effect of Varying [Ca]o on Pressure-induced Muscle Membrane Depolarization

When the membrane response to increasing transmural pressure was studied in middle cerebral arteries bathed in solution where [Ca]o was reduced to 0.5 mM, significant reduction in the slope and correlation coefficient of the Em vs. pressure relationship was observed (Table 1). Conversely, elevation of [Ca]o from 2.5 to 4.0 mM significantly increased the relationship between elevation of transmural pressure and arterial muscle cell depolarization (Table 1). However, when [Ca]o was elevated beyond 5.0 mM, the arterial muscle cells began to hyperpolarize, and "pressure-induced" changes in Em became less apparent (not shown). This action of very high [Ca]o (i.e., >5.0 mM) is probably due to membrane stabilization. Em recorded in low [Ca]o solutions was −57 ± 2.0 mV.

Pressure-induced Electrical Spike Generation

When these studies were initially carried out, we allowed the arterial preparations to equilibrate for 90 minutes at 0 mm Hg before increasing transmural pressure. On several occasions, it was possible to maintain a cell impalement during pressure steps by raising pressure in very small increments. Under these conditions, regenerative electrical spike activity could be recorded only when transmural pressure was initially elevated, while Em decreased but remained quiescent when pressure was maintained (Fig. 3).

However, when the arterial preparations were allowed to equilibrate at a transmural pressure of 100 mm Hg for 90 minutes prior to study, surprisingly different results were obtained. In six out of seven middle cerebral arteries studied in this fashion, spontaneous "pacemaker-like" action potentials could be recorded (Fig. 4). These action potentials appeared to occur primarily in areas of the vessel that exhibited a narrowed lumen with respect to the vessel as a whole, with more dilated areas often exhibiting a quiescent membrane. Obviously, this...
last point needs to be studied in greater detail and is beyond the scope of the present study.

When vessels that had been equilibrated at 100 mm Hg were studied throughout the range of transmural pressures, action potentials typically occurred at between 80 and 100 mm Hg, which increased in frequency as pressure was elevated further, and voltage inactivated when the $E_m$ approached $-30$ mV at high transmural pressures (Fig. 5). (Similar data were obtained in five experiments.) Such spontaneous activity appears to be myogenic, since it also occurred in the presence of TTX and phentolamine. Although not shown, these action potentials were not observed when $[Ca]_o$ was lowered to 0.5 mm and were blocked by the Ca$^{2+}$ channel blocker, verapamil ($10^{-6}$ M). The action potential characteristics are summarized in Table 2.

Pressure-induced Changes in Diameter

As explained in Methods, it was difficult to measure, accurately, small changes in diameter to increases in transmural pressure. However, photomicrographs were taken in six arterial preparations which were translucent enough to afford a clear image of internal diameter. Noticeable decreases in diameter with elevation of transmural pressure could be observed in four preparations, one of which is shown in Figure 6. When the pressure was rapidly (i.e., within 2–5 seconds) elevated and maintained at from 20 to 90 mm Hg, a decrease in internal diameter is observed, accompanied by an increase in wall thickness (Fig. 6, A and B). When the transmural pressure was rapidly reduced from 140 to 60 mm Hg, a phasic increase in diameter can easily be seen (Fig. 6, C and D; this photo was taken 10 seconds after pressure was reduced). The maximum degree of reduction in internal diameter ranged between 10 and 30 $\mu$m. However, given the difficulties described in Methods, it was very difficult to quantify such changes accurately. The $E_m$ values recorded

TABLE 2
Summary of Action Potential Characteristics in Middle Cerebral Artery Equilibrated at 100 mm Hg

<table>
<thead>
<tr>
<th>Trans. Press.</th>
<th>Frequency (A.P./sec)</th>
<th>Amplitude (mV)</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mm Hg</td>
<td>0.457 ± 0.084</td>
<td>21 ± 2.34</td>
<td>6</td>
</tr>
<tr>
<td>140 mm Hg</td>
<td>1.1 ± 0.129*</td>
<td>17 ± 4.64</td>
<td>4</td>
</tr>
<tr>
<td>170 mm Hg</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>100 mm Hg</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

*Significantly greater than at 100 mm Hg ($P < 0.01$).
The results of this study demonstrate that elevation of transmural pressure in cat middle cerebral arteries results in muscle cell membrane depolarization. Pressure-mediated muscle cell depolarization occurring in the presence of neural blockade and inhibition of excitatory \( \alpha \)-adrenergic receptors suggests that this response is myogenic in nature. However, given the amount of recent literature demonstrating endothelial factors controlling arterial diameter (Furchgott, 1983), further work needs to be done to demonstrate a purely myogenic mechanism. An increase in the slope of the \( E_m \) vs. pressure relationship after neural blockade may even suggest that stretching of adventitial nerves upon vessel pressurization may release inhibitory neurotransmitters. Extensive dilatory innervation has been demonstrated in cat cerebral arteries (Lee et al., 1978). While studying guinea pig mesenteric arteries at 0 and 60 mm Hg, Keef and Neild (1982) found that excitatory junction potential amplitude is elevated at 60 mm Hg, consistent with the notion that transmitter release is augmented under conditions of elevated pressure.

The increase in slope of the \( E_m \) vs. pressure relationship upon elevation of \([Ca]_o\), and its reduction upon lowering of \([Ca]_o\), suggest that the observed membrane depolarization is \( Ca^{++} \) dependent. The \( Ca^{++} \) equilibrium potential in most excitable cells is around 130 mV (Sperelakis, 1979); therefore, an increase in permeability would be expected to drive the membrane in a positive direction (i.e., depolarization). However, it cannot be determined from these studies whether other ions, namely, \( Na^+ \), are also involved.

Spontaneous action potential generation observed when middle cerebral arteries were equilibrated for 90 minutes under high transmural pressure suggests that ionic permeabilities change as a function of time under pressure, since similar activity is not observed when vessels are equilibrated for the same period of time under no pressure and then are exposed to high transmural pressure. The observed voltage inactivation at high transmural pressures confirms the degree of membrane depolarization and is consistent with previous data regarding voltage inactivation of inward current in arterial muscle (Harder and Sperelakis, 1979). Of interest is the finding that spike frequency increases during intermediate increases in transmural pressure (i.e., from 100 to 140 mm Hg). If such activity occurs in vivo, then cerebral vessels exposed to higher pressures (e.g., as in hypertension) may have a greater frequency of spike discharge and elevated arterial tone. Inhibition of spike activity with verapamil suggests that at least part of the inward current is carried by \( Ca^{++} \) and may contribute directly to muscle cell activation. It is difficult to explain why action potentials occurring during initial pressurization of vessels equilibrated at 0 mm Hg did not show similar voltage inactivation characteristics, except that they may be mediated by ionic channels with different inactivation kinetics or may be neurally mediated, since they were recorded in the absence of neural blockade with TTX. Spontaneous electrical activity has also been observed in rabbit cerebral arteries exposed to high pressures (Lusamvuku et al., 1979).

We have observed (Harder et al., 1983) that when cerebral arteries are loaded by stretching a segment between wires, the membrane is not depolarized compared to the resting state. We have no satisfying explanation why, when arterial segments are stretched over wires, their muscle cells do not depolarize, except that cellular damage may occur when small segments of arteries are cut and threaded by wires, not allowing us to observe this phenomenon. Whereas classic methods of measuring tension from helical strips or ring segments result in excellent responses to vasoactive agents, markedly different results would be obtained from pressurized arteries which are depolarized, since contraction in cerebral arterial muscle is closely linked to the level of \( E_m \) (Harder, 1981; Harder and Waters, 1983; Hermensmeyer et al., 1982). It must be remembered, however, that the present study deals with
an artificial in vitro preparation, and still may not reflect the true in vivo situation.

Whereas it is easy from studies such as these to link myogenic activity to cerebral blood flow autoregulation, it must be remembered that cerebral arterial muscle is very sensitive to a wide array of metabolic influences, including adenosine, pH, PCO₂, and a variety of known and putative neurotransmitters (Kuschinsky et al., 1972; Betz, 1972; Winn et al., 1979; Lee et al., 1976; Harder et al., 1981; Harder, 1982). Furthermore, there is evidence suggesting that, when arterial pressure increases, sympathetic regulation of cerebral blood flow may increase (Busija et al., 1980; Heistad et al., 1978). Even though it is obvious that there are many factors contributing to the regulation of cerebral blood flow, a myogenic mechanism is intriguing, due to its simplicity. Under control conditions, pial (Wahl and Kuschinsky, 1978) and retinal (Tachibana et al., 1982) arteries reduce their diameter when blood pressure is elevated.

If the myogenic response in cerebral arteries is, indeed, a function of Eₘ, then environmental factors which tend to change Eₘ, such as elevated cerebral spinal fluid K⁺ or serotonin, would markedly effect any myogenic regulation. Similarly, in certain hypertensive or metabolic disease states where ionic permeabilities are altered, the membrane response to pressure would be expected to differ relative to normal, thereby markedly changing any regulation component of blood flow.

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