Evidence that Intraluminal Pressure Affects High Potassium- and Serotonin-Induced Contractions Differently in the Bovine Middle Cerebral Artery: An in Vitro Study

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The effects of changing intraluminal pressure on contractions induced by 70 mM potassium (K+), 10⁻⁷, 10⁻⁴, and 10⁻³ M serotonin (5-HT) were studied in vitro in bovine middle cerebral arteries. Changes in vessel outside diameter in whole-mounted cylindrical sections of artery were detected with a photoelectric infrared device. High K⁺- or 5-HT (10⁻³ M)-induced contractions peaked at 25 mm Hg and were significantly correlated with increasing intraluminal pressure between 25 and 175 mm Hg. Contractions induced with lower concentrations of 5-HT (10⁻⁷, 10⁻⁸ M), norepinephrine, and histamine peaked at 75 mm Hg but were not significantly correlated with rising pressure. Phenolamine (2 × 10⁻⁶ M) added to the extraluminal bath had negligible influence on pressure's ability to affect K⁺- and 5-HT-induced contractions differently. Reducing bath temperature to 27°C reduced the K⁺ response at each pressure, but similar temperature changes had little effect on the 5-HT-induced contractions. The K⁺ response became less sensitive to increasing pressure at low temperatures. Nifedipine (10⁻⁷ M) almost totally eliminated K⁺-induced contractions, while significantly reducing the responses to all concentrations of 5-HT. The 5-HT responses appeared more sensitive to increasing intraluminal pressure in the presence of nifedipine. Maximum Ca⁺⁺-induced contractions in the presence of 10⁻³ M 5-HT and high K⁺ occurred at 25 mm Hg, while Ca⁺⁺-induced contractions and Ca⁺⁺-induced contractions in the presence of 10⁻⁷ 5-HT or K⁺ plus 5-HT were maximum at 75 mm Hg. This study reveals significant differences in the ability of increasing intraluminal pressure to affect a electromechanical-induced contraction compared to a pharmacological-induced contraction. The results suggest that pressure has selective effects on the mechanisms that control cytoplasmic calcium in the smooth muscle cell. (Stroke 1987;18:92-100)

ELEVATED pressure due to hypertension has been linked to an increase in vascular resistance that could be due to structural changes in the vascular wall, an increase in neurogenic or humoral activity, or a change in the wall mechanics of the smooth muscle itself. In some studies, it is difficult to determine whether the reported change in smooth muscle activity is the result of, or the cause of, hypertension; i.e., are these changes considered pathological? For instance, a relation has been described between developing hypertension and decreasing membrane potential in the tail arteries from young rats. In another study using cat middle cerebral arteries, such a relation between increasing transmural pressure and changes in membrane potential is described as a normal smooth muscle response. Thus, it appears important to study vascular properties throughout the pressure range in normotensive as well as hypertensive animals. Such studies should provide insight into whether the reported changes associated with hypertension are to be regarded as extensions of normal responses, or if specific abnormalities are developed only in the hypertensive state.

The following investigation was designed to study the effects of intraluminal pressure on the vasoactivity of potassium and serotonin in the middle cerebral artery of young calves in vitro. If a relation exists between intraluminal pressure and resting membrane potential, then pressure may affect a potassium-induced contraction differently than a pharmacologically induced contraction. We chose high concentrations of potassium and serotonin as our contractile agents because these agonists are believed to induce contraction by different contractile mechanisms. Potassium induces a contraction in smooth muscle by depolarizing the membrane (electromechanical coupling), making it more permeable to Ca⁺⁺, while serotonin, besides changing the membrane potential, can induce a contraction through pharmacomechanical coupling. A photoelectric infrared device was used that detected changes in vessel outside diameter in whole-mounted cylindrical sections of artery.

Materials and Methods

Middle cerebral arteries (MCA) were collected from calves within 15 minutes of death. Calves were between 2 and 6 months old. Sections of white matter were removed along with each vessel to maintain intact...
branching. The vessels were threaded onto a glass rod in a petri dish containing a standard Krebs solution (n-Krebs) maintained at 37°C (reduced, in some experiments, to 27°C) and gassed with 12% oxygen, 5% carbon dioxide, and 83% nitrogen. This concentration of oxygen was chosen for its approximation of PO₂ found in vivo. Twelve percent oxygen produced a bath PO₂ of 95 mm Hg. After brain tissue was gently teased away from the artery, branches were tied off with 10-0 suture. The absence of leaks was determined by the ability of the vessel to maintain constant pressure.

**Model**

Arteries 1 cm in length were cannulated between glass tubes and stretched longitudinally to their approximate intact length as measured before removal from the brain. At 75 mm Hg intraluminal pressure, outside diameter ranged from 1.4 to 1.8 mm. The arteries were perfused intraluminally with n-Krebs by means of a peristaltic pump (Harvard Apparatus), while the pressure was monitored with Statham pressure transducers. Pressure changes were introduced into the system by an inverted reservoir with an air pressure bulb. The tissue bath was placed in a photoelectric diameter gauge, which produced an infrared light beam emitted by a gallium arsenide diode, amplified (DC), and recorded on a Grass polygraph. The resolving power of this device was less than 0.05 μm.

**Solutions and drugs**

All vessels were equilibrated for 30 minutes at an intraluminal flow rate of 4.1 ml/min in n-Krebs solution of the following millimolar composition: NaCl 118.8, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, CaCl₂ 1.2, NaHCO₃ 14.9, and dextrose 11.2. Osmolarity of the gassed solution was 276 mOsm, and pH was 7.4. Serotonin creatine sulfamate (5-HT), l-norepinephrine bitartrate (NE), and histamine dihydrochloride (Sigma) were dissolved in deionized water before being added to the extraluminal surface of the artery. Nifedipine (NF) (Sigma) was dissolved in 50% ethanol and 15% polyethylene glycol and kept in the dark until used. NF was added to the extraluminal bath 10 minutes before any contractions were induced. High potassium (K⁺) solutions (70 and 125 mM) were made by increasing KCl and decreasing NaCl concentrations, thus maintaining osmolarity. In 15 experiments phentolamine mesylate (CIBA) was added to the n-Krebs and high potassium solutions. Drug amounts are reported as final extraluminal bath concentrations.

Twenty-eight arteries were washed with calcium-free Krebs solution plus 2.0 mM EGTA for 30 minutes, then washed with calcium-free Krebs solution without EGTA for 15 minutes both intraluminally and extraluminally. CaCl₂ was then added to the extraluminal bath containing calcium-free 70 mM K⁺ solution, 5-HT, or both so that the bath accumulated the following Ca²⁺ concentrations: 0.1 mM, 0.6 mM, 1.6 mM, and 2.6 mM. The above washing procedure was repeated before each pressure change. Intraluminal pressure was changed in 50 mm Hg increments. Arteries were allowed to equilibrate for 10 minutes between pressure changes.

**Statistical Analysis**

Data were analyzed using least squares linear regression of outside diameter against intraluminal pressure and correlation coefficients (r). Data were also analyzed using Student's t test for paired and unpaired data. The 0.05 level of probability was accepted as significant for both analyses. Results are expressed as means ± SEM. Contractions are reported as the difference between outside diameter (in millimeters) at peak response and baseline outside diameter before adding the agonist. Baseline outside diameter was relatively constant between 25 and 175 mm Hg.

**Results**

From previous experiments we were aware that between 25 and 175 mm Hg MCA can maintain a relatively constant outside diameter in vitro due to myogenic autoregulation. Thus, the experiments were conducted between these pressures.

We initially selected concentrations of potassium (70 mM) and serotonin (10^-6 M) that produced similar contractile responses in vitro at what we estimated would be the mean intraluminal pressure (75 mm Hg) in bovine MCA in vivo. Decreasing intraluminal pressure from 75 to 25 mm Hg caused the 5-HT response to decrease slightly and the K⁺ responses to increase (Figure 1). Increasing the pressure above 75 mm Hg reduced K⁺-induced contractions but did not significantly affect the magnitude of 5-HT-induced contractions. After each 50 mm Hg increase in pressure (beginning at 25 mm Hg) the K⁺ responses were reduced significantly (p<0.05), while the 5-HT responses were not significantly affected by any pressure change. The contractions induced by K⁺ at 175 mm Hg were reduced 56 ± 4.5% compared with contractions induced at 25 mm Hg. The contractions induced by 5-HT (10^-6 M) were reduced by only 18 ± 4.9% between the same pressure changes. The correlation coefficients

![Figure 1. Effects of intraluminal pressure on K⁺- and 5-HT-induced contractions in MCA. Each point is the mean ± SEM of 10 arteries. After each 50 mm Hg increase in pressure, the K⁺ response is significantly (p<0.05) depressed compared with the previous response (*), while the 5-HT responses appear little affected by similar pressure changes.](image-url)
Table 1. Slope for the Relation between Pressure and the Amount of K+ and 5-HT Response

<table>
<thead>
<tr>
<th>Arteries (##)</th>
<th>70 mM K+</th>
<th>10(^{-5}) M 5-HT</th>
<th>10(^{-6}) M 5-HT</th>
<th>10(^{-7}) M 5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>15</td>
<td>12</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Var.</td>
<td>1.5 \times 10^{-3}</td>
<td>5.0 \times 10^{-4}</td>
<td>3.4 \times 10^{-4}</td>
<td>2.0 \times 10^{-4}</td>
</tr>
<tr>
<td>r</td>
<td>-0.99*</td>
<td>-0.95*</td>
<td>-0.65</td>
<td>-0.35</td>
</tr>
</tbody>
</table>

*Significant correlation with changing pressure.

indicate that the amount of contraction induced by 70 mM K\(^+\) was significantly correlated with increasing pressure \((r = -0.99)\), while 10\(^{-6}\) M 5-HT was not \((r = -0.65)\) (Table 1). Thus, we felt the difference between the 5-HT and K\(^+\) responses in relation to changing pressure was real and warranted further investigation.

We first wanted to ascertain that the different effects of pressure on K\(^+\) and 5-HT were not concentration-specific. The experiment was repeated with other concentrations of K\(^+\) and 5-HT. The inhibiting effect of pressure on the K\(^+\) response persisted after complete depolarization of the artery with 125 mM K\(^+\), which produced a maximum contraction (Figure 2). Increasing the concentration of 5-HT to 10\(^{-5}\) M did not significantly change the amount of contraction induced at any pressure compared to the amount induced by 10\(^{-6}\) M, but the maximum response with this dose of 5-HT occurred at 25 mm Hg instead of 75 mm Hg (Figure 3). Decreasing the concentration of 5-HT to 10\(^{-7}\) M decreased the response at all pressures, and the maximum response was shifted slightly to the right (125 mm Hg). An examination of the slopes (Table 1) for these 3 concentrations of 5-HT revealed that the slope increased as the 5-HT concentration increased. The linear relation between the amount of contraction produced by 10\(^{-5}\) M 5-HT and intraluminal pressure was significant \((r = -0.95)\), but the relation was not significant for 10\(^{-6}\) and 10\(^{-7}\) M 5-HT.

We also looked at the influences of changing pressure on other drugs as well. Besides 5-HT, histamine and NE also produced a significant contraction in this preparation. The effects of pressure on histamine \((3 \times 10^{-6} \text{ M})\) and NE \((10^{-3} \text{ M})\) were similar to the effects of pressure on the lower concentrations of 5-HT (Figure 4). Maximum responses occurred in the middle pressure range (75 mm Hg). There was, however, a significant \((p<0.05)\) decrease in the histamine response on increasing the pressure from 125 to 175 mm Hg.

Some studies have suggested that a relationship exists between some types of experimental hypertension and an increased activity of the sympathetic nervous system.\(^{13}\) To determine if the initial difference in sensitivity to changing intraluminal pressure noted between 70 mM K\(^+\)- and 10\(^{-6}\) M 5-HT-induced contractions was due to the release of \(\alpha\)-adrenergic substances, these experiments were repeated with arteries treated with phentolamine \((2 \times 10^{-6} \text{ M})\). Phentolamine was added to the extraluminal bath 10 minutes before any contractions were induced and was left in the bath and wash solution for the duration of the experiment. This concentration totally eliminated the contractile response to NE \((10^{-3} \text{ M})\) at all pressures. A comparison of the effects of pressure on K\(^+\)- and 5-HT-induced contractions in the presence and absence of phentolamine indicated that the inhibition of \(\alpha\)-adrenergic receptors with phentolamine did not significantly change the ability of pressure to moderate high K\(^+\)- and 5-HT-induced contractions differently (Figure 5). However, the amount of contraction induced by 5-HT was significantly \((p<0.05)\) reduced (approximately 50% compared to that of control responses) at each pressure in the presence of phentolamine. The K\(^+\) responses were not significantly altered by phentolamine at any pressure.

Changes in temperature are often employed to differentiate contractile processes that involve active transport across smooth muscle membrane from pro-
Vinall and Simeone In Vitro Effects of Intraluminal Pressure in Bovine MCA

Effects of Intraluminal Pressure in Bovine MCA. Each point is the mean ± SEM of 4-10 arteries. *Significantly (p < 0.05) less response compared with that at the previous pressure.

Processes that proceed along established electrochemical gradients. Thus, we decided to investigate the effects of reduced bath temperature (27°C) on the relation between pressure and K⁺ (70 mM)- and 5-HT (10⁻⁶ M)-induced contractions. The potassium (70 mM) responses were significantly (p < 0.05) reduced at all pressures when the bath temperature was lowered (Figure 6A). The slope (1 × 10⁻³) of the K⁺ response at 27°C, though still significantly correlated with pressure, was reduced by 50% compared with the slope (2 × 10⁻³) of the K⁺ response at 37°C. The 5-HT (10⁻⁶ M)-induced contractions, though slightly increased at 75 and 125 mm Hg, were not significantly changed when the bath temperature was lowered (Figure 6B).

Nifedipine is a more selective inhibitor of calcium influx associated with electromechanical coupling compared to pharmacomechanical coupling, particularly in the cerebral artery. We reasoned that with the former mechanism inhibited by NF, 5-HT-induced contractions should be more dependent on pharmacomechanical mechanisms, and the effects of pressure, if any, on 5-HT-induced contractions associated with the latter contractile process should be revealed. NF (10⁻⁷ M) was added to the extraluminal bath 10 minutes before contractions were induced and was left in the bath and wash solutions for the duration of the experiment. The K⁺ response was almost completely inhibited by this dose of NF at all pressures (Figure 7A). Contractions induced by 10⁻⁷, 10⁻⁶, and 10⁻⁵ M 5-HT were significantly reduced by NF at each pressure compared to control responses. The amount of NF inhibition of the 5-HT response increased as intraluminal pressure increased: 47% at 25 mm Hg, 77% at 75 mm Hg, 92% at 125 mm Hg, and 100% at 175 mm Hg (Figure 7B). All concentrations of 5-HT in the presence of NF produced a maximum contraction at 25 mm Hg.
From the above results it appeared that NF was affecting more than just the movement of calcium triggered by depolarization of the membrane, and another approach to isolating the different effects of pressure was needed. Contractile responses of isolated smooth muscle to high concentrations of K+ and 5-HT in Ca-depleted Krebs on the readdition of Ca ++ results from increased influx of Ca ++ into the smooth muscle cell from the extracellular medium. Using this approach, we hoped to isolate the effects of pressure on calcium movement through electrical and pharmacomechanical channels. Arteries were first washed with calcium-free Krebs plus 2.0 mM EGTA for 30 minutes intraluminally and extraluminally, then washed with calcium-free Krebs without EGTA for 15 minutes. We first examined the effects of pressure on just Ca ++ readdition to the extraluminal bath. Ca ++ was added in accumulating doses at each pressure. The maximum response to all concentrations of Ca ++ occurred at 75 mm Hg (Figure 8A), and there was a significant (p < 0.05) decrease in the response induced by 2.6, 1.6, and 0.6 mM Ca ++ at 175 mm Hg when compared to the response at 75 mm Hg. If 10⁻⁷ M 5-HT were first added to the extraluminal bath, Ca ++ responses again peaked at 75 mm Hg for all Ca ++ doses (Figure 8B). A significant (p < 0.05) decrease in the response induced by 0.6 and 1.6 mM Ca ++ occurred as pressure was raised above 75 mm Hg to 175 mm Hg. Serotonin added to a calcium-free bath induced only a small phasic contraction that quickly relaxed to baseline. When Ca ++ was added to the bath in the presence of 10⁻⁵ M 5-HT, maximum contractions occurred at 25 mm Hg for all Ca ++ concentrations (Figure 9A). Contractions induced at 125 and 175 mm Hg by all concentrations of Ca ++ were significantly (p < 0.05) reduced compared to the peak responses seen at 25 mm Hg (Table 2). If Ca ++ were added to a Ca-free 70 mM K+ bath, the resulting contractions again were maximum at 25 mm Hg for all Ca ++ doses (Figure 9B). Like the Ca ++ /5-HT responses, contractions induced by all concentrations of Ca ++ in depolarized arteries were reduced by increasing the pressure to above 25 mm Hg. Reduction was significant at 125 and 175 mm Hg (Table 2). Calcium-free K+ solution never caused a contraction in this preparation. Some Ca-free treated arteries were first exposed to 70 mM K+ (Ca-free) solution, then 10⁻⁵ M 5-HT, before Ca ++ dose-response curves were plotted. The use of high concentrations of 5-HT in depolarizing solutions supposedly enhances the stimulation of pharmacomechanical mechanisms. When Ca ++ was added to this environment, contractions induced by 1.6 and 2.6 mM Ca ++ peaked at 75 mm Hg (Figure 9A and these responses were significantly (p < 0.05) greater than corresponding contractions in the presence of 10⁻⁵ M 5-HT or high K+ alone (Table 2). This maximum response to Ca ++ at 75 mm Hg emerged as bath Ca ++ concentration increased. Ca ++ -induced contractions in the presence of K+ plus 5-HT were also severely depressed at high pressures (175 mm Hg) compared with the maximum responses at 25 and 75 mm Hg for all concentrations of Ca ++ added to the bath.

**Discussion**

This study describes a significant difference in the ability of changing intraluminal pressure to affect an electromechanically induced contraction compared to a pharmacologically induced contraction in vitro in MCA. Contractions induced with high concentrations of K+ or 5-HT and associated with electromechanical coupling were maximum at the low end of the pressure curve and were significantly correlated with rising pressure. Contractions induced with lower concentrations of 5-HT and other contractile agents associated with pharmacomechanical coupling were maximum in the middle pressure range and did not indicate as strong a correlation with changing intraluminal pressure. This difference did not appear to depend on the release of α-adrenergic substances since phentolamine failed to alter pressure’s ability to affect K+ and 5-HT responses differently. Reduced temperature altered the

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**Figure 8.** Effects of pressure on A) Ca ++ -induced contractions and B) Ca ++ -induced contractions in the presence of 5-HT. Each point is the mean ± SEM of 4–6 arteries. The numbers to the right of each curve indicate the total Ca ++ (mM) concentration in the bath. *Significant (p<0.05) difference between amount of Ca ++ response induced at 75 and 175 mm Hg.

**Figure 9.** Effects of pressure on Ca ++ -induced contractions in the presence of A) 5-HT and B) K+. Each point is the mean ± SEM of 6 arteries. The numbers to the right of each curve indicate the total Ca ++ (mM) concentration in the bath.
Table 2. Effect of Pressure on Ca++-Induced Contractions

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Pressure (mm Hg)</th>
<th>25</th>
<th>75</th>
<th>125</th>
<th>175</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^{-5} M 5-HT</td>
<td>0.1</td>
<td>0.20 ±0.04</td>
<td>0.12 ±0.02*</td>
<td>0.07 ±0.02*</td>
<td>0.04 ±0.01*</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.47 ±0.04</td>
<td>0.38 ±0.06</td>
<td>0.24 ±0.05*</td>
<td>0.10 ±0.02*</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>0.51 ±0.05</td>
<td>0.47 ±0.07</td>
<td>0.34 ±0.05*</td>
<td>0.16 ±0.02*</td>
</tr>
<tr>
<td></td>
<td>2.6</td>
<td>0.51 ±0.05</td>
<td>0.49 ±0.06</td>
<td>0.40 ±0.06*</td>
<td>0.20 ±0.02*</td>
</tr>
<tr>
<td>70 mM K+</td>
<td>0.1</td>
<td>0.14 ±0.04</td>
<td>0.11 ±0.03</td>
<td>0.08 ±0.01*</td>
<td>0.03 ±0.01*</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.39 ±0.04</td>
<td>0.28 ±0.04</td>
<td>0.19 ±0.04*</td>
<td>0.09 ±0.02*</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>0.48 ±0.05</td>
<td>0.40 ±0.04</td>
<td>0.32 ±0.05*</td>
<td>0.14 ±0.04*</td>
</tr>
<tr>
<td></td>
<td>2.6</td>
<td>0.52 ±0.05</td>
<td>0.48 ±0.04</td>
<td>0.41 ±0.05*</td>
<td>0.22 ±0.06*</td>
</tr>
<tr>
<td>70 mM K+ plus 10^{-5} M 5-HT</td>
<td>0.1</td>
<td>0.35 ±0.04</td>
<td>0.07 ±0.03*</td>
<td>0.01*</td>
<td>0.005*</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.52 ±0.03</td>
<td>0.49 ±0.04*</td>
<td>0.06 ±0.02*</td>
<td>0.03*</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>0.56 ±0.04</td>
<td>0.63 ±0.04*</td>
<td>0.18 ±0.06*</td>
<td>0.05 ±0.01*</td>
</tr>
<tr>
<td></td>
<td>2.6</td>
<td>0.59 ±0.04*</td>
<td>0.70 ±0.052</td>
<td>0.37 ±0.09*</td>
<td>0.11 ±0.04*</td>
</tr>
</tbody>
</table>

Mean decreases in outside diameter are expressed as millimeters of change ± SEM.
*Significantly (p < 0.05) less than peak response.
†Significantly (p < 0.05) greater than corresponding K+ response.
‡Significantly (p < 0.05) greater than corresponding K+ or 5-HT (10^{-5} M) response.

effects of changing pressure on K+-induced contractions, but not 5-HT-induced contractions. Ca++-induced contractions in the presence of high concentrations of K+ or 5-HT were maximum at the low end of the pressure curve. Peak responses to Ca++ occurred at 75 mm Hg when Ca++ contractions were induced in the presence of 10^{-7} M 5-HT or K+ plus 5-HT, or in the absence of any agonist. Though we did not measure Ca++ influx, these results suggest that pressure has selective effects on the membrane mechanisms that control calcium movement into the smooth muscle cell.

Some studies have suggested that the stretching of vessel walls by pressure partially depolarizes the cell membrane, thus producing contraction and autoregulation of blood flow. Harder has shown a direct relation between intraluminal pressure and membrane potential in vitro in cat cerebral arteries that was not neurogenically mediated but was sensitive to changes in bath calcium. Thus, it is possible that the resulting change in resting membrane potential due to stretching of the vessel wall by increasing intraluminal pressure could limit the amount of depolarization produced by K+ or 5-HT, hence the amount of contraction. Similar pressure increases did not significantly change the contractions associated with pharmacomechanical coupling. Resting membrane potential (Erest) measurements in cat MCA reveal that high concentrations of 5-HT, 70 mM K+, and 125 mM K+ depolarize the vessel to -35 mV, -19 mV, and -8 mV respectively. In our preparation the contractions associated with this increasing state of depolarization were inhibited by increasing intraluminal pressure.

Depolarization of the membrane by potassium alone does not appear to explain the different relations between changing pressure and amount of contraction induced by K+ and 5-HT. Ca++-induced contractions and Ca++-induced contractions in the presence of 10^{-7} M 5-HT or 70 mM K+ plus 10^{-5} M 5-HT peaked at 75 mm Hg, while Ca++ contractions induced in the presence of 70 mM K+ or 10^{-5} M 5-HT were maximum at 25 mm Hg. The sustained contractions resulting from the addition of Ca++ to arteries bathed in Ca-free Krebs, in the presence of an agonist, are felt to result from the influx of calcium across a cell membrane made more permeable to calcium by the agonist. In contrast, the fast phasic part of an agonist-induced
contraction sometimes seen in the absence of extracellular calcium is felt to be due to the release of bound calcium from sequestered pools \(^\text{26,15}\) or the sarcoplasmic reticulum. \(^\text{27}\) We noticed that after washing with Ca-free Krebs, all 5-HT-induced contractions in this preparation were small and phasic. These contractions were significantly reduced by increasing pressure (unpublished observation). This residual phasic response may be due to release of sequestered calcium, or it may reflect an influx of calcium derived from less readily depleted membrane calcium stores. \(^\text{28}\) We can only speculate as to which of these mechanisms are occurring. Studies \(^\text{29,30}\) indicate that the intercellular calcium pool appears much smaller in cerebrovascular smooth muscle than in peripheral vessels. Consequently, some feel that cerebrovascular contractions are more dependent on free extracellular calcium ions. Vascular contractions associated with electromechanical coupling (high K\(^+\), \(10^{-5}\) M 5-HT) and more dependent on the influx of extracellular Ca\(^{++}\) were linearly related to increasing intraluminal pressure. Those responses associated with pharmacomechanical coupling (lower drug concentrations, 5-HT in depolarizing solution) were less linearly related to increasing pressure, while peak responses were shifted to the right. The use of high concentrations of 5-HT in depolarizing solution uncouples the electrical from the mechanical event. \(^\text{24}\) The resulting additional contraction is felt to result from the stimulation of pharmacological receptors. \(^\text{31}\) We consistently noted peak responses to the latter type of stimulation in the middle (75-125 mm Hg) pressure range. The emergence of this peak response (see Figure 10) appeared to be related to the concentration of extracellular Ca\(^{++}\). Thus, the intraluminal pressure may affect how Ca\(^{++}\) enters smooth muscle cells, not the release of sequestered Ca\(^{++}\).

Hogestatt and Andersson \(^\text{28}\) have described two separate agonist-activated calcium channel mechanisms, i.e., an initial fast followed by a slow, with different kinetic properties. Cooling produced a gradual dissociation of the two contractile components and depressed their maxima. We tried to isolate these two calcium mechanisms by reducing the bath temperature, hoping to study the effects of pressure on each component. However, such a dissociation of the K\(^+\) or 5-HT contractile responses was not noted at low temperatures in this artery. There was, however, a significant depression of overall K\(^+\)-induced contraction at all pressures at 27°C, while \(10^{-5}\) M 5-HT-induced contractions were not affected by the same temperature reductions. The slope (the degree of negative correlation with increasing pressure) of the K\(^+\) response also appeared depressed at low temperatures. Changes in temperature may affect both an electrogenic ion pump (for instance the Na\(^+\)/K\(^+\) pump) and the permeability of smooth muscle membranes to calcium. \(^\text{32}\) Though the direct relation between the Na\(^+\)/K\(^+\) pump and the contractile state is debatable, the contribution of the pump to the maintenance of the membrane potential is well accepted. It has also been suggested that high systemic pressure due to hypertension involves alterations in the Na\(^+\)/K\(^+\) pump. \(^\text{33,34}\) However, we cannot be certain that the above results reflect a reduction in Na\(^+\)/K\(^+\) pump activity; other pump mechanisms such as the Na\(^+\)/Ca\(^{++}\) pump may be involved. Besides affecting ion pumps, cooling may also affect the affinity of drug receptors. \(^\text{35}\) Superficial vessels, like the saphenous vein, exposed to moderate cooling demonstrate normal or even augmented responses to drugs, but high K\(^+\)-induced contractions are reduced. \(^\text{36}\) In deeper arteries, stepwise cooling from 37° to 5°C causes a progressive depression of contractile responses to both high K\(^+\) solution and pharmacological agents. \(^\text{37}\) It is interesting to note that augmented responses to NE or 5-HT after moderate cooling usually occur in superficial vessels only. \(^\text{38,39}\) This vascular characteristic is normally associated with blood vessels that play a role in thermoregulatory control. \(^\text{40,41}\)

Nifedipine, which can inhibit electromechanically dependent Ca\(^{++}\) influx in response to high potassium, electrical stimulation, or pharmacologically induced depolarization, \(^\text{17,42,43}\) as expected, almost totally eliminated the K\(^+\) response at all pressures. However, it was surprising that NF at the concentration used above reduced responses to 5-HT as much as it did — and at all pressures. If NF inhibits only the contractile mechanisms associated with membrane depolarization (studies \(^\text{42,43}\) have indicated that NF has no effect on the calcium influx that occurs in the absence of depolarization), little inhibition of those contractions associated with pharmacomechanical coupling should occur. However, the contractions induced by \(10^{-7}\) M 5-HT, a concentration of 5-HT that produces little depolarization, \(^\text{24}\) were significantly reduced by NF, supporting the contention that NF can inhibit the entry of calcium through the receptor-activated mechanisms as well as the electromechanical ones. \(^\text{45}\) Nifedipine may also be affecting the release of Ca\(^{++}\) from superfically bound calcium stores \(^\text{46}\) crucial to pharmacomechanical activation. Calcium from this superficial pool could be responsible for more stable 5-HT responses in n-Krebs in the face of increasing pressure. This calcium source may not be available in high concentrations of potassium. Whatever the explanation, it seems apparent that NF becomes more effective as an antagonist as pressure increases.

The use of whole-mounted cylindrical sections of artery vs. a helical or ring mount preparation may explain why results of this nature have not been described until recently. When ring segments of cerebral arteries are loaded by stretching between wires, the membrane is not depolarized compared to the resting state. \(^\text{47}\) When segments of MCA are cannulated and prepared so that transmural pressure can be manipulated, a relation between pressure and \(E_c\) is noted. \(^\text{9}\)

In conclusion, we feel that the different effects of changing intraluminal pressure on K\(^+\)- and 5-HT-induced contractions in vitro in the cerebral artery deserve further attention. Exactly what part of the excitation-contraction system is being affected is still debatable. Na\(^+\)/K\(^+\) pump, Ca\(^+\) release from storage sites, and Ca\(^{++}\) influx associated with electromechani-
cal vs. pharmacomechanical coupling are all probable points of impact. It is our opinion that a basic understanding of the normal physiology of the vascular system is needed before such abnormal pathological states as hypertension can be understood and treated. Some studies report that hypertension involves breakdowns in the basic mechanisms of the excitation–contraction system, such as calcium uptake and sequestration,8,9,49 or the Na+/K+ pump.50,51 This study suggests that changing intraluminal pressure may influence selective parts of the Ca++-dependent excitation–contraction coupling sequence in normal animals as well.

References

6. Friedman SM: An ion-exchange approach to the problem of intracellular sodium in the hypertensive process. Physiol Rev 1979;59:1605
43. Kanmura Y, Itoh T, Suzuki H, Ito Y, Kuriyama H: Effect of
nifedipine on smooth muscle cells of the rabbit mesenteric artery. *J Pharmacol Exp Ther* 1983;226:238-248


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