Pressure-Induced Activation of Membrane K⁺ Current in Rat Saphenous Artery

Viktor Berczi, William J. Stekiel, Stephen J. Contney, and Nancy J. Rusch

Pressurization of isolated arteries may result in Ca²⁺-dependent contraction and membrane depolarization. Because the open state probability of some vascular muscle K⁺ channels is augmented by rises in cytosolic Ca²⁺ and membrane depolarization, we investigated the possibility that increases in intraluminal pressure activate K⁺ channels in isolated, perfused rat saphenous arteries. Stepwise increases in intraluminal pressure from 5 to 205 mm Hg resulted in increasing, active arterial contraction, measured as smaller diameters in physiological salt solution than in Ca²⁺-free solution. Addition of 10 mM tetraethylammonium to the physiological salt solution to block arterial muscle K⁺ channels caused progressively greater diameter reductions at pressures above 25 mm Hg. Microelectrode measurements of membrane potential showed that tetraethylammonium depolarized arterial muscle more at 105 mm Hg (16±1 mV) than at 25 mm Hg (10±1 mV). The sensitivity of K⁺ current to tetraethylammonium was also demonstrated in patch-clamped vascular muscle cells from the same arteries. Peak whole-cell K⁺ current was suppressed 47% and 79% by 1 and 10 mM tetraethylammonium, respectively. This same current was enhanced 3.6-fold by the Ca²⁺ ionophore A23187 (10 μM), suggesting a Ca²⁺ dependence. We conclude that increases in intraluminal pressure progressively activate tetraethylammonium-sensitive K⁺ channels in the arterial muscle membrane. This can serve as a negative feedback mechanism to limit pressure-induced arterial constriction.

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KEY WORDS • vascular smooth muscle • tetraethylammonium compounds • blood pressure • calcium • potassium channels

Pressure-induced contraction of arterial muscle minimizes arterial diameter changes during local blood pressure alterations. Many studies have focused on the mechanisms for Ca²⁺ activation of arterial muscle in this response, as Ca²⁺ influx appears to be required for myogenic activation. However, the final level of arterial muscle contraction in response to changes in intraluminal pressure (IP) likely reflects the integrated reaction of multiple membrane ionic mechanisms. Of these, the function of arterial membrane K⁺ channels has received scant attention, despite their recognized role as key modulators of arterial contraction and excitability. In view of this, the purpose of this study was to test the hypothesis that arterial membrane K⁺ channels buffer the pressure-induced contraction of arterial muscle segments. Using tetraethylammonium (TEA) to block vascular muscle K⁺ channels in perfused rat saphenous arteries, we estimated the contribution of K⁺ current to arterial diameter and membrane potential (Eₘ) at IP values from 5 to 205 mm Hg. We used patch-clamp methods to document K⁺ current sensitivity to TEA-induced block and to increases of cytosolic Ca²⁺ in isolated arterial cells. The results suggest that activation of membrane K⁺ channels may be a mechanism for attenuation of acute pressure-induced constriction in small arteries.

Methods

Sprague-Dawley rats (360-430 g) were anesthetized with 40 mg/kg ketamine-HCl followed by 20-30 mg/kg sodium pentobarbital. The left or right saphenous artery was exposed, and 8-11-mm segments were carefully dissected free of connective tissue. These segments were used in perfusion studies for recording of arterial diameter and Eₘ responses as described below. In other studies, single arterial cells were enzymatically isolated from similar arterial segments for patch-clamp measurement of whole-cell K⁺ current.

Measurement of External Diameter in Perfused Arterial Segments

Segments of saphenous artery were placed in a perfusion chamber containing physiological salt solution (PSS) at 37°C that was aerated with 15% O₂-7% CO₂ and 78% N₂. The composition of the PSS was (mM) NaCl 119, KCl 4.7, MgSO₄ 1.17, CaCl₂ 1.6, NaHCO₃ 24.0, NaH₂PO₄ 1.18, EDTA 0.026, and glucose 5.5 (pH 7.35-7.45). Segments were cannulated and stretched longitudinally to their in vivo length. Arteries were both perfused (10 ml/hr) and superfused (5 ml/min) with PSS, and the external diameter was monitored by a continuous, on-line video technique described in detail elsewhere. Inflow and outflow pressures were measured continuously by pressure transducers. The input
Intraluminal Pressure (mmHg)

Patch-Clamp Measurements

Small segments (1 mm) from two saphenous arteries were placed in a 2-ml vial of enzyme solution containing (mM) NaCl 119, KCl 4.7, MgCl2 1.0, HEPES 10, CaCl2 0.5, and glucose 5.0, as well as 300 units/ml collagenase (type II, Worthington Biochemical Corp., Freehold, N.J.) and 30 units/ml elastase (Sigma). The vial was placed in a water-jacketed beaker on a microstirrer, and the arterial pieces were stirred slowly at 2 rpm for 20 minutes at 37°C. Dispersed, elongated cells were obtained at 5–10-minute intervals by gentle pipetting, and the cell suspension was placed in a test tube on ice for immediate use. Measurement of whole-cell K+ current was performed with a patch-clamp station previously described.9 Outward currents were elicited by 14 progressive voltage steps (10-mV increments, 200 msec) from a constant holding potential of -60 mV. External solution was (mM) NaCl 135, KCl 4.7, MgCl2 1.0, CaCl2 2.0, HEPES 5.0, and glucose 10 (pH 7.4). Pipette solution was (mM) K+-glutamate 130, MgCl2 1.0, EGTA 0.1, and HEPES 10 (pH 7.2).

Statistics

Data are expressed as mean±SEM. Statistical comparisons were carried out by analysis of variance with repeated measures. Significant differences between specific means were determined by Duncan’s new multiple range test. A value of p<0.05 was considered significant.

Results

Figure 1A shows traces of diameter measurements at 5, 85, and 165 mm Hg in PSS and after addition of 10 mM TEA. TEA reduced arterial diameter and induced phasic contractions at higher IP. Because the amplitude of oscillation was fairly constant, average midpoint was used for the determination of external diameter. Figure 1B compares the mean pressure–diameter relation of rat saphenous arteries in PSS, PSS+10 mM TEA, and Ca2+-free PSS. Compared with Ca2+-free PSS, external diameters in PSS (with 1.6 mM CaCl2) were smaller at

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IP values between 25 and 205 mm Hg, indicating an increasing pressure-induced contraction of arterial muscle in the presence of external calcium. In addition, superfusion of PSS containing 10 mM TEA produced progressive, significantly smaller diameters compared with normal PSS at IP values from 25 to 205 mm Hg (n=7). In Figure 1C, the average TEA-induced diameter reductions are plotted as percent change from PSS values. The results show that in the presence of TEA, diameter reductions were progressively augmented as IP increased from 5 to 85 mm Hg and then remained stable at higher pressures.

Compared with untreated vessels (n=4), endothelium removal (n=3) did not affect the magnitude of diameter reductions induced by 10 mM TEA relative to PSS at any pressure. Arterial diameter responses to increases in IP in PSS and Ca^{2+}-free PSS also were not altered by chemical sympathectomy. Thus, for each condition, we pooled diameter responses in untreated and sympathetomized vessels to obtain the mean data illustrated in Figure 1. Active diameter responses to pressure, however, were reduced after endothelium removal. For instance, diameter differences between PSS and Ca^{2+}-free PSS at 105 mm Hg were 5.2±1.2% in untreated and 1.7±0.3% in denuded arteries (p<0.05).

In further experiments, we used intracellular microelectrodes to assess the contribution of K+ channel current to E_m at a low (25 mm Hg) and higher (105 mm Hg) pressure. Figure 2A shows traces of actual E_m recordings in a single artery in PSS (left) and in PSS containing 3 mM TEA (right). The recordings show that changing IP in PSS from 25 to 105 mm Hg reduced E_m magnitude. Addition of 3 mM TEA caused a further depolarization at both pressures, with a greater effect at 105 mm Hg. At the latter pressure, spike potentials often were observed during E_m recordings, as shown in Figure 2B. Figure 2C shows the mean E_m values for six arteries exposed to 3 mM TEA. Values corresponding to the bars are (from left to right): 44±1, 34±1, 39±1, and 23±1 mV. Thus, an increase in IP from 25 to 105 mm Hg reduced E_m in PSS. Referenced to these values, TEA enhanced depolarization more at the higher than at the lower IP (16±1 and 10±1 mV, respectively).

Patch-clamp experiments confirmed that TEA significantly reduced K+ current amplitude in saphenous arterial muscle cells. Figure 3A illustrates the effect of 1 and 10 mM external TEA on K+ current elicited in a single cell (inset) and on the current–voltage relation for K+ current activation in six cells. The latter shows that the peak amplitudes of elicited K+ current in control recording solution were dose-dependently reduced by TEA, averaging 47% (1 mM) and 79% (10 mM) suppression at the most positive potential. In other cells, we added 10 μM A23187 to the external solution to evaluate the effect of elevated cytosolic Ca^{2+} on K+ current amplitude. The recording in Figure 3B (inset) shows that 10 μM A23187 increased peak K+ current amplitude in single cells relative to that in control solution. The corresponding current–voltage relation in six cells showed that A23187 increased K+ current at more positive potentials, showing a 3.6-fold enhancement of maximum K+ current amplitude.

**Discussion**

Our findings confirm that stepwise increases in IP activate arterial muscle in isolated rat saphenous artery. Local chemical sympathectomy did not affect this response, but it was blunted by endothelium removal as described by some but not by other laboratories. Subsequent Ca^{2+} entry appears to be involved in the pressure-induced activation of arterial muscle, as evidenced by the upward shift of the pressure–diameter relation in Ca^{2+}-free solution. Although the membrane pathways for this Ca^{2+} influx are unclear, they may include "stretch-activated" Ca^{2+}-permeant channels or dihydropyridine-sensitive Ca^{2+} channels. Opening of the latter is associated with pressure-induced membrane depolarization, which we measured directly using microelectrodes in this study.

The purpose of this investigation, however, was not to define the initiating mechanisms for pressure-induced contraction but to determine if this response was modulated by arterial muscle membrane K+ channels. We conclude that pressure-induced contraction of arterial muscle is attenuated by progressive activation of TEA-
sensitive K⁺ channels. The evidence for this is the following: 1) Addition of 10 mM TEA to PSS superfusate resulted in progressively greater diameter reductions at stepwise pressure increases above 25 mm Hg, suggesting incremental activation of K⁺ channels during pressure-induced contraction; 2) arterial muscle depolarization produced by 3 mM TEA was greater at 105 mm Hg than at 25 mm Hg, suggesting that K⁺ current contributes more to Eₜ than at lower pressures; and 3) macroscopic K⁺ current in patch-clamped arterial cells was suppressed by TEA in the concentration range (1–10 mM) used in the perfused vessel. This suggested that the electromechanical responses to TEA were consistent with its pharmacological block of arterial muscle K⁺ channels.¹²

The precise mechanism underlying pressure-induced K⁺ channel activation in arterial muscle is not apparent from our study. It appears, however, to be intrinsic to the arterial muscle cell rather than other vascular wall components, because sympathetic denervation and endothelium removal did not affect the mechanical responses to TEA. Likely candidates for K⁺ channel activation are the membrane depolarization and the rise in cytosolic Ca²⁺ associated with pressure-induced contraction. A steep, direct relation between K⁺ channel opening versus membrane depolarization and cytosolic Ca²⁺ has been demonstrated in arterial muscle cells.¹³,¹⁴ Also, our patch-clamp experiments revealed a predominant voltage- and Ca²⁺-dependent K⁺ current in rat saphenous arterial membranes. However, the nature of the TEA-sensitive K⁺ channel remains to be identified by single-channel studies combined with the use of more selective K⁺ channel blockers, because TEA at concentrations greater than or equal to 1 mM may block multiple K⁺ channel types.¹² Furthermore, other membrane ionic mechanisms, such as “stretch-induced” increases in K⁺ conductance or regulation of K⁺ channel opening by other cytosolic factors, also may be involved.⁵,¹⁵

These data provide initial information about the role of arterial muscle K⁺ channels during acute changes in IP. Information is also needed to determine if pressure-induced K⁺ channel activation is altered in arteries during chronic hypertension. It is known that basal and stimulated K⁺ efflux is elevated in arterial muscle from genetic and renal models of rat hypertension.¹⁶,¹⁷ Therefore, it is possible that the response of arterial muscle K⁺ channels to pressure is modified after chronic exposure to high blood pressure.

During pressure-induced contraction of rat saphenous arterial muscle, the hyperpolarization afforded by enhanced K⁺ channel current reduced, but did not prevent, Ca²⁺ influx from causing vascular muscle contraction. As such, K⁺ channel activation may act as a negative feedback mechanism to limit, rather than to control, the level of pressure-induced arterial contraction. It is possible that in the intact system during acute hypertension, pressure-induced K⁺ channel activation may function to buffer further rises in systemic arterial pressure.

**References**


**FIGURE 3.** Panel A: Graph showing effect of 1 and 10 mM tetraethylammonium (TEA) on whole-cell K⁺ currents in rat saphenous arterial cells. Currents were elicited from a constant holding potential of −60 mV to potentials indicated on the abscissa of the current–voltage relation. Inset: Traces showing effect of TEA on K⁺ current elicited by voltage steps from a holding potential of −60 mV to +60 mV. *TEA<control (Co), n=6. Panel B: Traces showing effect of 10 μM A23187 on whole-cell K⁺ current (inset) and graph showing current–voltage relation elicited by the same pulse protocols as in panel A. *Control (Co)<A23187, n=6.
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