Brief Communications

Stretch-Dependent Calcium Uptake Associated With Myogenic Tone in Rabbit Facial Vein

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Many blood vessels possess stretch-dependent myogenic tone. This tone contributes to the active resistance in small arteries, which regulates blood flow in the body. In this report we describe results indicating that stretch-dependent myogenic tone in the rabbit facial vein, a vascular preparation with useful experimental properties, is accompanied by an increased uptake of $^{45}\text{Ca}^{2+}$. This influx may not occur through voltage-gated $\text{Ca}^{2+}$ channels, since neither the extent of myogenic tone nor $^{45}\text{Ca}^{2+}$ influx is affected by a concentration of 1,4-dihydropyridine (PN 200-110, 10 nM) that reduces tone and $^{45}\text{Ca}^{2+}$ influx due to the depolarization by $K^+$. These results indicate that in myogenically active vessels a stretch-activated influx of $\text{Ca}^{2+}$ may occur through distinct $\text{Ca}^{2+}$-entry pathways. (Circulation Research 1988;63:669-772)

A number of vascular beds maintain a relatively constant blood flow despite changing perfusion pressure. Several explanations have been proposed for this autoregulation of flow. It seems likely that the interaction of metabolic dilation and myogenic constriction are important, the relative contribution of each varying with the vascular bed and the circumstances. The role of flow-dependent dilation, which undoubtedly plays a part, has yet to be assessed. The myogenic response refers to the intrinsic ability of the blood vessel wall to contract in response to increases in transmural pressure. It seems likely that myogenic vasoconstriction provides the basal tone of the vessel that is necessary for vasodilator influences to be effective. Although myogenic tone was described more than 80 years ago, its demonstration in isolated blood vessel segments is unusual because it is, on the whole, a feature of the smaller arteries. The mechanism whereby an increase in intramural pressure leading to stretch of the vascular wall produces tone is not known.

A number of observations from our laboratory have indicated that stretch of myogenically active vessels promotes the entry of extracellular $\text{Ca}^{2+}$ through channels distinct from those influenced by membrane depolarization or receptor activation. For instance, in the rabbit ear resistance artery, concentrations of nimodipine, a $\text{Ca}^{2+}$-entry inhibitor, which completely inhibit contractions to membrane depolarization by $K^+$, have no effect on stretch-activated myogenic tone. In the rabbit basilar artery, diltiazem is effective in inhibiting tone due to norepinephrine and $K^+$ but not tone due to stretch. In both the ear and basilar artery, all types of tone are highly dependent on the presence of extracellular $\text{Ca}^{2+}$.

In this study we report the first demonstration of a direct cellular influx of $^{45}\text{Ca}^{2+}$ when a myogenically active vessel is stretched. We used the buccal segment of the rabbit facial vein because it exhibits maintained myogenic tone when stretched and is of a size suitable for experimentation. Stretch-dependent tone in this vessel only occurs at temperatures greater than 32° C and is resistant to 1,4-dihydropyridine $\text{Ca}^{2+}$-entry blockers such as PN 200-110, whereas the contraction due to $K^+$ is not temperature-dependent; it is similar at 30° C and 42° C and is sensitive to $\text{Ca}^{2+}$-entry inhibitors. These features allow $\text{Ca}^{2+}$-entry mechanisms in the rabbit facial vein to be separated experimentally. (Circulation Research 1988;63:669-772)

Materials and Methods

Rabbits were anesthetized by intravenous administration of sodium pentobarbital (40 mg/kg). The animals were then bled and facial veins removed according to the method described by Pegram et al. Buccal segments of facial vein were dissected free and placed in physiological salt solution (PSS) of the following millimolar composition: NaCl 140, KCl 4.6, MgCl$_2$ 1.0, CaCl$_2$ 1.6, dextrose 10.0, and HEPES 5.0. The PSS was adjusted to pH 7.4 and gassed with oxygen (100%).

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Supported by United States Public Health Service grant HL-32985.

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Received August 10, 1987; accepted March 21, 1988.
Facial vein segments were dissected free of connective tissue and prepared for isolated tissue bath studies using methods described by Bevan and Osher. Following an hour equilibration period at 30°C, vessel segments were stretched to a preload of 0.5 g, a value shown to be optimal for this preparation.

Temperature-Dependent Tone

After several changes of the PSS, tissues were exposed to a maximally effective concentration of papaverine (10μM) for 3 minutes before the PSS was replaced again. To induce the temperature sensitive myogenic response in this vessel, the temperature indicator on the circulator bath was increased from 30°C to 42°C. The magnitude of the response was determined by the loss of tone that occurred when papaverine (10μM) was added. Papaverine (10μM) was used to determine the extent of active tone due to stretch activation; quantitatively similar results were obtained using isoproterenol (10μM) or PSS with no added Ca2+. Concurrently, measurements of contraction and Ca2+ influx were made with and without preloads (i.e., with and without stretch) at both 30°C and 42°C. In the case where myogenic tone developed in veins under stretch at 42°C, these measurements were also made after a 20-minute pretreatment with PN 200-110 (10nM) or its vehicle, ethanol (0.1%).

Responses to K+

For measurements of K+(80mM)-induced tone, experiments were made at 30°C. K+-rich PSS was prepared by equimolar substitution for Na+. Tissues were pretreated with prazosin (0.1μM) and propranolol (1μM) before responses to K+ (80mM) were measured. Tissues were then treated with either PN 200-110 (10nM) or ethanol (0.1%), and the response to K+ (80mM) was determined again. Simultaneous measurements of Ca2+ influx were made during the exposure of tissues to K+-rich PSS containing 45Ca2+ (1μCi/ml) in the presence of PN 200-110 (10nM) or ethanol (0.1%). Based on preliminary experiments, we determined that PN 200-110 (10nM) caused a 50% loss of K+ tone (I. Laher and J.A. Bevan, unpublished observation).

45Ca2+-Influx Measurements

Measurements of 45Ca2+ influx and vascular tone were made simultaneously in vessel segments prepared as described above. Ca2+ influx due to stretch activation or K+(80mM) activation was studied by exposing tissues to 45Ca2+-PSS (1μCi/ml) for 1 minute. The amount of 45Ca2+ entering during brief periods of exposure to label is assumed to be primarily due to influx from extracellular sources. The validity of this experimental protocol in measuring unidirectional influx of 45Ca2+ has been confirmed. The 45Ca2+-PSS used for pulse labeling of tissues was identical in composition (e.g., K+, PN 200-110, or ethanol concentrations) and temperature (30°C or 42°C) as that used during measurement of tone. At the end of the exposure to 45Ca2+ tissues were placed in 250 ml of bubbled ice-cold PSS to remove extracellularly bound 45Ca2+. Based on preliminary results we determined that the Ca2+ content of the tissue 30 minutes after placing in excess (250 ml) ice-cold PSS represents tightly bound, presumably intracellular, 45Ca2+. The tissues were removed from the ice-cold PSS, blotted dry, and weighed before they were soaked in EDTA (5 mM) overnight. Scintillation fluid was then added and radioactivity of the labeling solution and tissue samples counted (model LS8000, Beckman, Fullerton, California). The counts of radioactivity were converted to 45Ca2+ influx and expressed as micromoles per kilogram tissue per minute.

Statistics

Results were subject to analysis of variance using the Newmans-Keuls test of significance (p<0.05). The results are reported as the mean±SEM of at least 15 observations per group.

Drugs Used

Prazosin hydrochloride was a gift from Pfizer, New York, and PN 200-110 was a gift from Sandoz, Basel, Switzerland. PN 200-110 was dissolved in ethanol. 45CaCl2 (specific activity, 19.56 mc/mg) was purchased from New England Nuclear, Boston, Massachusetts.

Results

Resting influx of 45Ca2+ was similar in unstretched segments studied at either 30°C or 42°C (Figure 1A and 1B). Stretching facial vein segments at 42°C not only produced myogenic tone but also increased 45Ca2+ influx (p<0.01; Figure 1B). Neither change took place when vessels were studied at 30°C. This stretch-activated 45Ca2+ influx and contraction were not altered by a concentration of the Ca2+-entry inhibitor PN 200-110, which significantly reduced (p<0.01) the 45Ca2+ influx and contraction caused by 80 mM K+ at 30°C (Figure 1C). The K+-induced tone was associated with an increase in tone of similar magnitude to that caused by stretch at 42°C.

Discussion

In this study we report the first demonstration of an increased uptake of 45Ca2+ when a myogenically active vessel is stretched. We used the buccal segment of the rabbit facial vein because it exhibits maintained myogenic tone when stretched and is of a size suitable for experimentation. Stretch-dependent tone in this vessel only occurs at temperatures greater than 32°C and is resistant to 1,4-dihydropyridine Ca2+-entry blockers such as PN 200-110. Tone due to K+ is not temperature-dependent; it is similar at 30°C and 42°C and is sensitive to Ca2+-entry inhibitors. These features allow voltage-gated and stretch-activated Ca2+-entry mechanisms to be separated experimentally. Measuring 45Ca2+ influx in whole tissue segments

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FIGURE 1. Segments of rabbit facial vein were stretched to a 0.5 g preload and allowed to equilibrate at 30°C (A). A maximally effective concentration of papaverine (PPV, 10 μM) was added for 3 minutes and then washed out (see "Materials and Methods"). Thereafter, unidirectional 45Ca2+ influx (μmol/kg/min) measurements were made and compared to values obtained in unstretched segments. Similar experiments were made at 42°C (B) where some tissues were pretreated with PN 200-110 (10 nM). Changes in tone and 45Ca2+ influx due to K+ (84 mM) were measured at 30°C (C). a: Significantly greater than control (p<0.01) b: Significantly greater than treated (PN 200-110, 10 nM) (p<0.01).

has the advantage of allowing simultaneous measurements of vascular tone and stimulated entry of extracellular Ca2+. This method has proved useful in the separation of receptor- and potential-operated Ca2+ entry pathways in rabbit aorta.14

The central finding of this study is that the genesis of stretch-induced myogenic tone in the rabbit facial vein was accompanied by an increased influx of 45Ca2+ (Figure 1B). This increase in 45Ca2+ influx, as well as the stretch-activated myogenic tone, may occur through a 1,4-dihydropyridine (e.g., PN 200-110) insensitive pathway (Figure 1B). On the other hand, the K+-induced tone increase was accompanied by 45Ca2+ influx, which was attenuated by PN
200-110 (10 nM; Figure 1C). Only the "Ca" influx associated with stretch was temperature dependent.

The cellular mechanisms of maintained intrinsic tone are not established. A recent study in canine cerebral arteries suggests that the vascular endothelium releases an indomethacin-sensitive vasoconstrictor substance during stretch-induced tone.\(^\text{17}\) Stretch-induced tone in our study using vessel segments with intact endothelium was not altered by preincubation with indomethacin (10 \(\mu\)M; not shown). Harder has proposed that in some vessels pressure-dependent myogenic tone is due to Ca\(^{2+}\) entry leading to membrane depolarization.\(^\text{18}\) Although there is no doubt that myogenic tone is associated at least in some instances with changes in membrane potential, our results show that it can occur after the contraction of potassium is prevented by calcium entry inhibitors.\(^\text{6}\) Moreover, the extent of 1,4-dihydropyridine resistant, Ca\(^{2+}\)-dependent intrinsic tone is unrelated to the changes of membrane depolarization caused by extracellular K\(^+\).\(^\text{19}\) Thus, the resistance of stretch-induced tone to PN 200-110 is unlikely to be due to membrane potential regulation of the affinity of Ca\(^{2+}\)-entry inhibitors.\(^\text{20}\) It is clear that 1,4-dihydropyridines do not inhibit all Ca\(^{2+}\) entry due to voltage-dependent mechanisms. Such 1,4-dihydropyridine resistant Ca\(^{2+}\) channels have been described in striated and smooth muscle; these channels conduct calcium and barium equally and are inhibited by nickel.\(^\text{21}\) The stretch-activated, Ca\(^{2+}\)-dependent tone we report herein is not inhibited selectively by nickel, while barium only poorly substitutes for calcium in maintenance of myogenic tone in the facial vein (I. Laher and J.A. Bevan, unpublished observations).

The mechanical distension of a vessel causes the influx of sufficient Ca\(^{2+}\) to cause contraction through pathways distinct from those activated by membrane depolarization.

References

Key Words • calcium influx • myogenic tone • calcium entry inhibitors • vascular smooth muscle
Stretch-dependent calcium uptake associated with myogenic tone in rabbit facial vein.
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Circ Res. 1988;63:669-672
doi: 10.1161/01.RES.63.3.669

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