Acta Physiologica Hungarica, Volume 88 (3–4), pp. 197–206 (2001)

Axial stretching of extremity artery induces reversible hyperpolarization of smooth muscle cell membrane *in vivo*

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Received: February 5, 2002
Accepted: March 20, 2002

Circumferential stretch due to increases in pressure induces vascular smooth muscle cell depolarization and contraction known as the myogenic response. The aim of this study was to determine the *in vivo* effects of axial-longitudinal stretch of the rat saphenous artery (SA) on smooth muscle membrane potential (Em) and on external diameter. Consecutive elongations of the SA were carried out from resting length (L_0) in 10% increments up to 140% L_0 while changes in membrane potential and diameter were determined in intact and de-endothelized vessels. Axial stretching resulted in a small initial depolarization at 120% of L_0 followed by a progressive 20 to 33% hyperpolarizaion of vascular smooth muscle between 130% and 140% of L_0. At 140%, an average maximal 10.6 mV reversible hyperpolarization was measured compared to –41.2± 0.49 mV Em at 100% L_0. De-endothelialization completely eliminated the hyperpolarization to axial stretching and augmented the reduction of diameter beyond 120% L_0. These results indicate that arteries have a mechanism to protect them from vasospasm that could otherwise occur with movements of the extremities.

Keywords: arterial strain, endothelium, smooth muscle, vascular myogenic response

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Tangential (circumferential) stretch induced by transmural pressure in arteries (3–5, 13, 20) and veins (1, 12, 15, 18) elicits depolarization of the smooth muscle (VSM) cell membrane potential (Em) that is accompanied by an intrinsic myogenic increase in vascular tone. This response is endothelium independent and can be significantly augmented in response to long-term orthostatic pressure load in the limb veins (1, 16, 21). Similar augmentation occurs in arteries and veins in the early phase of arterial hypertension (14, 20) and in response to a noradrenergic stimulus (6, 18).

However, certain blood vessels, such as those in the heart (8) and in the extremities, are physiologically exposed not only to tangential stretch but also to axial (i.e. longitudinal) elongations. We have observed that extension of a limb from a full flexion position can alter the length of the saphenous vessels by substantially more than 20% in the rat (unpublished observation). In a previous study, we found that axial stretch of the *in situ* rat saphenous vein beyond its resting length caused endothelium-dependent hyperpolarization of the VSM Em and a concomitant suppression of the active tangential vascular tone (17). We proposed that this mechanism may serve to protect vessels from spasm when exposed to extreme physiological stretches. Since such responses have not been studied before in arteries, the present experiments were carried out to determine if a similar mechanism is operating in arteries.

Thus, the purpose of the present study was to determine whether incremental increases of axial stretch would hyperpolarize the VSM Em of the rat saphenous artery, and alter the active intrinsic myogenic tone. Further objectives were to study whether such responses depend on the presence of intact endothelium and to determine if they are reversible.

Materials and Methods

Experimental animals

Studies were performed in 7 male Sprague-Dawley rats (347±10.9 g body wt) anesthetized with a combination of ketamine HCl (30 mg/kg; Aveco, Fort Dodge, IA) and pentobarbital sodium (25 mg/kg; Anthony Products, Arcadia, CA). Anesthesia was maintained with occasional administration of supplemental doses appr. 10% of the initial dose.

Preparation of the saphenous artery

Vessel preparation and measurements were performed similar to that developed in an earlier study (17). In brief, anesthetized rats were placed on a temperature

controlled double-walled platform constructed of polystyrene with minimal water and chemical absorption properties. The platform was mounted on a microscope stage that could be controlled in two dimensions. A 2–2.5 cm long segment of the saphenous artery (SA) was surgically exposed through a skin incision and superfused with 37 °C physiological salt solution (PSS). The PSS was maintained at pH 7.3–7.4, pCO_2 35–40 mm Hg, and pO_2 100–120 mm Hg. The composition of the PSS in mM was: 119 NaCl, 4.7 KCl, 1.6 $CaCl_2$, 1.17 $MgSO_4$, 24.0 $NaHCO_3$, 1.18 NaH_2PO_4, 0.026 EDTA and 5.5 glucose. Special care was taken to minimize damage to the periadventitial connective tissue of the artery during the dissection process.

A specially constructed pair of miniature three-tined metal forks, the separation of which could be accurately controlled by a micromanipulator, was mounted on the animal platform (Fig. 1). The center tine of each fork was placed between the isolated adjacent saphenous vein (SV) and the SA segment, and the outer tines on the outer sides of each vessel. With this arrangement, the periadventitial connective tissue, and consequently the vessel segment, could be streched biaxially without causing axially asymmetric transverse distortions of the vessel wall. Either well-visualized side-branches or two strands from a silk suture glued to the vessel wall 8–10 mm apart, served as markers to measure the step increments of axial stretch. Baseline *in situ* vessel length (L_0) was defined as the shortest distance between the markers with no axial stretch or vessel folding. In order to maintain vessel length constant at a particular level of axial stretch during the time interval required for determination of the average Em value, the metal forks were carefully readjusted with the micromanipulator to compensate for an occasional tissue creep. The position of the extremity was maintained halfway between full extension and full flexion. A high resolution microscope eyepiece micrometer was used to measure L when stretching axially in 10% increments between 0 and 40%.

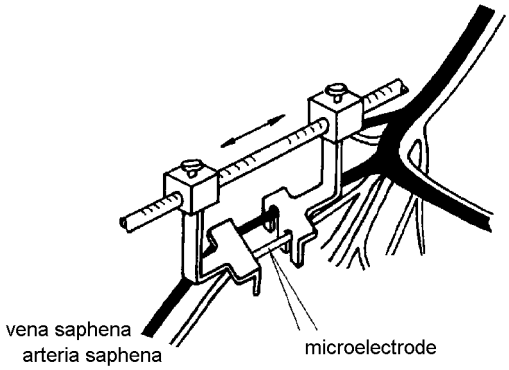

Fig. 1. Schematic drawing of the experimental arrangement. Three-tined metal forks mounted on a micromanipulator were applied to separate and axially stretch saphenous artery and vein segments *in situ*. Microelectrode was used to measure smooth muscle cell membrane potential

Arterial pressure was monitored from the contralateral femoral artery in each experimental animal using a Gould-Statham P23 ID pressure transducer and a Grass polygraph.

Measurement of VSM Em in situ

A miniature silicon rubber-coated metal plate, rigidly fixed to the frame of the animal platform, was inserted under the artery. In order to minimize pulsation-elicited artifacts during measurements of *Em* values, one side of the artery wall was stabilized by insertion of a row of miniature tungsten pins adjacent to the vessel into the coat of silicon rubber on the metal plate. Thus, the wall of the SA, which rested against the pins, could move longitudinally but not laterally. A conventional short-tapered glass micropipette filled with 3 M KCl having appr. 1 μm tip diameter and 60–100 MΩ impedance was mounted on a silver-silver chloride active lead connected to a capacity-compensated biological amplifier (Mentor Instrument; Minneapolis, MN). Output signals were recorded in parallel on a Tektronix storage oscilloscope and a the Grass polygraph. One average *Em* value was calculated at each selected vessel length, using the individual *Em* measurement data of five successful impalements of VSM cells made randomly from the adventitial side of the vessel. For a series of such successful impalements, max. 15–20 min period of time was required.

Measurement of SA outer diameter (De)

The SA De was measured optically by using a high-resolution microscope eyepiece micrometer with an accuracy of 5 μm.

Experimental protocol

To avoid neural influences, the saphenous vessels were locally sympathectomized by superfusing with PSS containing 10^{-6} M phentolamine for 5 min followed with PSS containing 6-hydroxydopamine (300 μg/ml) for 20 min (20). *Em* and De measurements were made one-hour following washout using normal PSS (1 vessel/animal). *Em* and De were measured first in the denervated endothelium-intact artery segment at L_0 and four consecutive 10% increases in vessel length, then again at L_0 in order to check reversibility of the responses. The measurements were then repeated one hour after de-endothelialization using air embolization. An air bolus was carefully introduced into the exposed SA segment via a 30 gauge needle inserted obliquely into its proximal end.

Upon filling of about 1 cm length of the artery with the air bolus, the distal end of the vessel was quickly occluded and maintained in this stop-flow condition for 6 min. At the end of this time, the air bolus was withdrawn from the proximal end via the needle, which was then removed. Normal blood flow was re-established by removal of the occluder. In separate vessel preparations, electron-microscopic examinations revealed that this techique completely removed the endothelial cell layer without damaging the VSM.

Statistics

Under both experimental conditions, data are shown as means ±SE of the measured variable. Stats Plus computer software package was applied using analysis of variance with repeated measures to estimate the significance of differences between means. A $P<0.05$ was regarded as significant.

Results

Initial values of *arterial blood pressure* (123±5.0 mm Hg) and *heart rate* (395± 10.9 bpm) of the animals did not change significantly during the experiments.

Effect of longitudinal stretch on Em *of VSM cell in endothelium-intact SA*

As illustrated in Figure 2, at zero axial stretch (L_0) *Em* averaged –41.2±0.49 mV. The *Em* response to 10% step increases of axial length was biphasic, with a small but significant depolarization at L_{20} (*Em* = –39.0±1.35 mV) relative to L_{10} (*Em* = –42.3± 0.55 mV), followed by a substantial 23% and a 33% hyperpolarization at L_{30} and L_{40} (*Em* = –48.1±0.95 mV and –51.8±0.67 mV), respectively. After restoring the vessel length to L_0, the *Em* returned back close to its initial value (–39.3±0.35 mV).

Effect of longitudinal stretch on Em *of VSM cell in de-endothelialized SA*

Compared to intact vessels, VSM cell membrane was significantly less polarized at all levels of axial stretch, except L_{20}, in de-endothelialized SA, even at the L_0 length. (*Em* = –39.3±0.35 mV). There was even a tendency toward further depolarization at lengths beyond L_{20} rather than the hyperpolarization, that was observed in endothelium-intact arteries (Fig. 2).

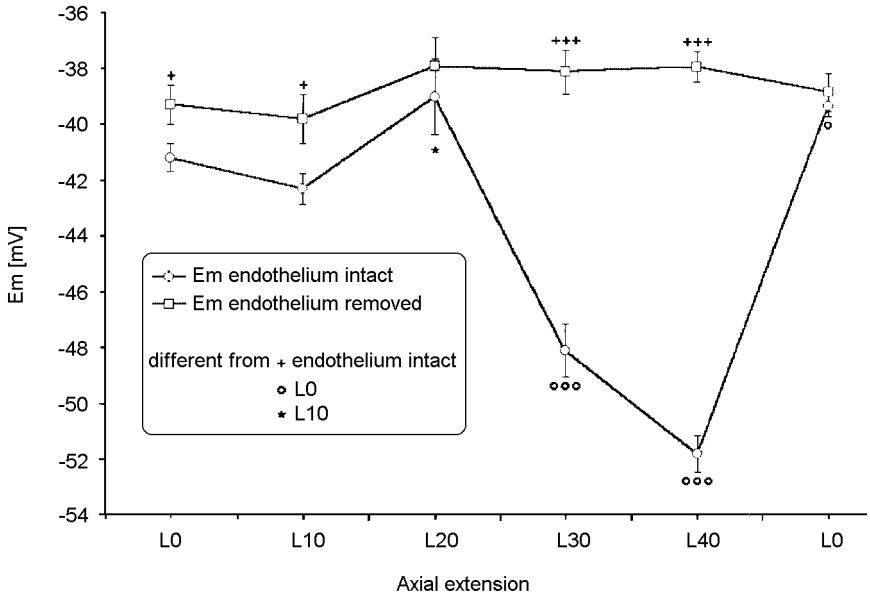

Fig. 2. Axial stretch evoked transmembrane potential (Em) responses of saphenous artery smooth muscle to stepwise elongations (10% increments from L_0 to L_{40} and returning to L_0) on intact (0) and endothelium denuded (?) segments measured *in situ*. Note that on intact artery segments the changes in *Em* were reversible when returning the vessel length to L_0 from L_{40}. Data is expressed as mean±SE

Changes in external diameter (De) of intact and de-endothelialized SA during axial stretching

De of SA averaged 683±33.2 μm at L_0 in intact vessels. The initial 10% elongation of the vessel resulted in an average 51±8 μm diameter reduction. Further elongations of the vessel by 10% (L_{10}–L_{20}, L_{20}–L_{30} and L_{30}–L_{40}) resulted in additional reductions of diameter 56±14.1, 72±6.4 and 59±4.9 μm, respectively as seen in Figure 3. De returned to initial values when the vessel length was set back to L_0 (680□}31.9 μm).

Stretch induced decreases in De were similar in de-endothelialized vessels with axial extensions between 110% to 120% of L_0 (37.3±8.2 and 59±9.5 μm, respectively) but were significantly enhanced with further extensions to 130 and 140% of L_0 (91.1±14.5 and 80.3±14 μm, respectively) compared to the diameter change at 110% L_0 (Fig. 3).

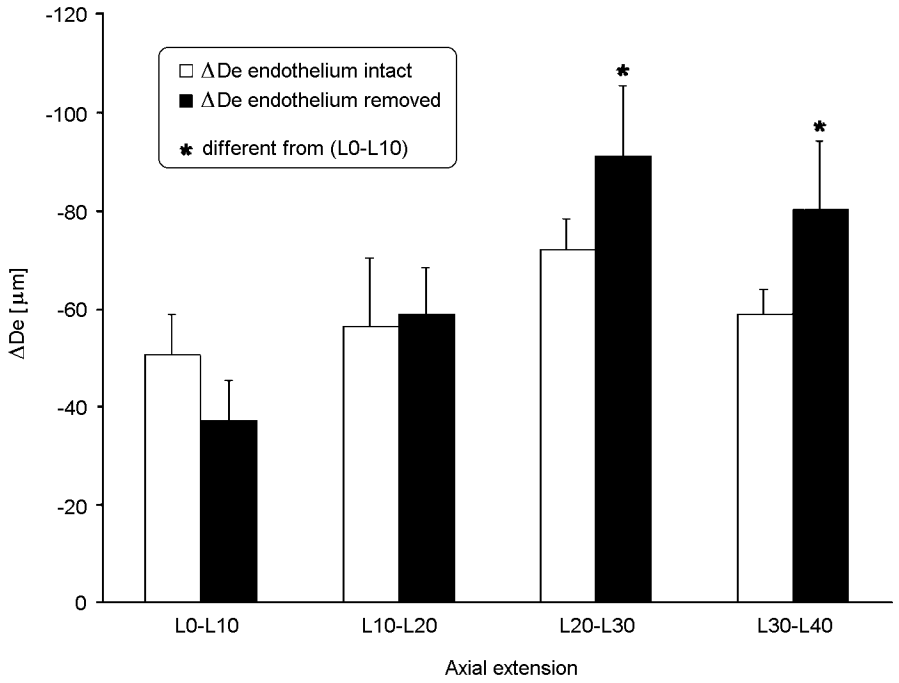

Fig. 3. Changes in external diameter (ΔDe) of intact and endothelium removed segments of saphenous artery at different levels of stepwise axial elongations (10% increments from L_0 to L_{40}) measured *in situ*. When endothelium was removed ΔDe was significantly larger between L_{20}–L_{30} and L_{30}–L_{40} compared to L_0–L_{10}. Data is expressed as mean±SE

Discussion

The major observation in this study was that increasing the length of the saphenous artery above the estimated resting level *in vivo* resulted in an endothelium dependent VSM hyperpolarization that attenuated the stretch induced reductions of diameter. This vascular response was reversible and did not depend on innervation.

These changes in bioelectrical and biomechanical properties of the artery related to axial stretch conform with responses of rat saphenous veins that we reported in an earlier study (17). Thus, this mechanism is present in both arteries and veins and may serve a meaningful role in protecting against vessel spasms during situations of large axial stretches. These responses are reversible, with the extended artery returning to its initial, fully relaxed length, and membrane potential.

The estimated *in vivo* "resting" axial length of the large saphenous vessels is by about 20% above the fully relaxed, somewhat compressed length. This extension ratio further increases by about 10% when the extremity is stretched during usual locomotor activity of the animal, and approaches 40% total extension in extreme situations (unpublished observation by E. Monos and S. J. Contney).

We found in earlier studies that local chemical denervation of blood vessels, such as the saphenous artery and vein of the rat, resulted in a moderate but statistically significant hyperpolarization of the VSM Em, the magnitude of which represents the sympathetic component of *Em* (16). Although it was not expected that local chemical denervation would cause substantial changes in Em-responses of the saphenous artery to axial stretch (17), denervation was carried out in this study to prevent any unexpected neural influence on the SA responses. The results of the present study provide evidence that the stretch induced active *Em* and De responses were intrinsic to the vasculature. Such intrinsic response could be initiated for instance by ATP released from perivascular nerves resulting in hyperpolarization of arterial VSM cells via endothelium dependent mechanism (22).

The cause of VSM *Em* hyperpolarization in SA remains to be determined. It is possible that with narrowing the vessel caliber due to the axial stretch, an elevated pressure head occurs along the narrow extended section, as seen in a Piezometer (7). This would increase the linear blood flow velocity gradient and consequently enhances shear rate on the endothelium surface. There is strong evidence that such shear forces, as natural stimuli, stimulate the endothelium to release nitric oxide which hyperpolarizes and relaxes the VSM (2, 11, 19). Preliminary studies in our laboratory using laser-doppler flowmetry (Periflux, PF3), together with direct volume flow measurements have suggested, that 30–40% local axial extension of SA results in a substantial elevation of the linear blood flow velocity in the elongated vessel segment while volume flow velocity decreases only slightly (unpublished obsterv ations by R. Roman and E. Monos).

It is noteworthy that even the largest axial stretch applied in this study did not cause significant VSM *Em* depolarization in the de-endothelized arteries. This was not seen in venous segments where depolarization did occur at this level of stretch (17). We presume, that sustained elongation of the artery induces substantial rearrangement of the anisotropic connective tissue network structure of the vascular wall (10) together with the VSM cells in a way that they are not stretched significantly, at least not in the external (i.e. adventitial) VSM cell layers of the SA media where probably the most *Em* measurements were made in our experiments. This assumption is consistent with the observations of Hill et al. (9) who found that sustained longitudinal stretching of rat cremaster arterioles in vitro resulted in only a short transient increase of intracellular Ca-ion concentration.

In conclusion, this study provides evidence that the *in vivo* longitudinal stretching of an extremity artery segment well beyond the resting length may induce hyperpolarization of its VSM cell *Em* when the endothelium is present, and a tendency to decrease in the active tone of vessel. This response is intrinsic to the intact vascular wall. Taking into account that maximal extension and flexion of an extremity is accompanied with a substantial lengthening of its vessels running in axial direction, these changes in electrical and biomechanical properties of the artery may reflect an intrinsic mechanism of the vascular wall that protects the vessels from spasm when they are axially overstreched.

Acknowledgements

This work was supported by National Science Foundation – MTA Grant Int-890894 (Hungary), and by OTKA T-30245 and ETT 149/2000 Hungarian Grants, and by PO1-HL29587.

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