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Excitability of Vascular Smooth Muscle

Alexandra V. Ulyanova

Abstract

Regulation of pressure and local blood flow occurs at the level of resistance arteries and arterioles. Under physiological conditions, these small vessels exist in a state of partial constriction, termed myogenic tone. Myogenic tone is considered to be an intrinsic property of arteriolar smooth muscle cells, which membranes depolarize in response to increase in the intraluminal pressure. Oscillations of membrane potential in smooth muscles are mediated by the activity of voltage-gated L-type Ca^{2+} channels, which provide an influx of Ca^{2+} to activate various voltage-gated and Ca^{2+} -sensitive channels of smooth muscle cells and to initiate endothelial Ca^{2+} signaling needed for vasodilation. Although a relationship between change in membrane potential and myogenic response is considered to be universal throughout various smooth muscle tissues, it may be regulated differently based on autoregulatory responses and channels expression. Here we review electrophysiological signature of arteriolar smooth muscle in various tissues, with an emphasis and specific examples of the excitability of 4th order arterioles isolated from skeletal muscle.

Keywords: vasculature, arteriolar smooth muscle, excitability, electrophysiology, voltage-gated Ca^{2+} and Na^{+} channels

1. Introduction

Regulation of pressure and local blood flow occurs at the level of resistance arteries and arterioles. Once blood exits the heart, it first flows into large elastic arteries, followed by smaller distributing arteries, which branch further into small resistance arteries and, finally, arterioles. The branching and reduction in vessel diameter actually results in the increase in total cross-sectional area of circulation. Because of their small diameter, resistance arteries and arterioles are the place of the largest pressure drop.

2. Myogenic tone

Resistance arteries and arterioles typically exhibit a state of partial constriction termed myogenic tone [1]. Myogenic tone is related to the level of the intraluminal pressure and provides a level of tone that vasodilators can act upon [2]. When the intraluminal pressure increases, resistance arteries and arterioles first dilate due to their elastic properties and then constrict to the new steady-state level. Myogenic tone is an intrinsic property of arteriolar smooth muscle cells and does not require endothelium [3, 4]. Resistance to blood flow is actively controlled by contraction

or relaxation of arteriolar smooth muscle cells wrapped around the vessel so that their tone regulates the vessel diameter [2]. Arterial walls are made up of three layers: the tunica intima, tunica media and tunica adventitia. While the tunica intima contains endothelial cells and a thin layer of connective tissue, the tunica media supplies mechanical strength and contractile power. It is composed of several layers of spindle-shaped smooth muscle cells arranged helically in a matrix of elastin and collagen fibers. In some places, endothelial cells make contacts with smooth muscle cells to transmit signals between the intima and media. The tunica adventitia is mostly a connective tissue sheath with no distinct outer border. Its role is to tether the vessel loosely to the surrounding tissue [5]. Arterioles, the smallest resistance arteries placed right before capillaries, have a single layer of spindle-shaped smooth muscle cells [6]. Endothelial cells frame the lumen of arterioles. The shape and orientation within the vessels help to distinguish between these two distinct cells types (**Figure 1**).

Development of the tone is associated with depolarization of smooth muscle cells. While the mechanisms underlying depolarization are not completely understood, it is known that development of myogenic tone depends on extracellular Ca^{2+} . At 0 Ca^{2+} , pressurized resistance arteries and arterioles are fully dilated. Adding Ca^{2+} up to ≈ 2 mM to the extracellular space causes maximal tone [7]. In addition to the development of basal tone, the myogenic mechanism is believed to underlie the response to acute changes in pressure and contribute to spontaneous vasomotor activity [7].

Myogenic tone is controlled by intrinsic as well as extrinsic mechanisms. Intrinsic mechanisms include constriction of arterioles in response to pressure increase (Bayliss effect), endothelial secretions (nitric oxide, EDHF, prostacyclin, endothelin), vasoactive metabolites (e.g. adenosine in exercising muscle), autacoids (local vasoactive paracrine secretions such as histamine), and temperature. Important physiological responses mediated entirely by intrinsic regulation include the autoregulation of flow, and functional and reactive hyperemia. Intrinsic regulation also contributes to pathological responses such as inflammation and arterial vasospasm. Extrinsic regulation is brought about by factors originating outside the organ, namely the vasomotor nerves (sympathetic, parasympathetic and others) and circulating hormones such as adrenaline, vasopressin and insulin (for review, see [8]).

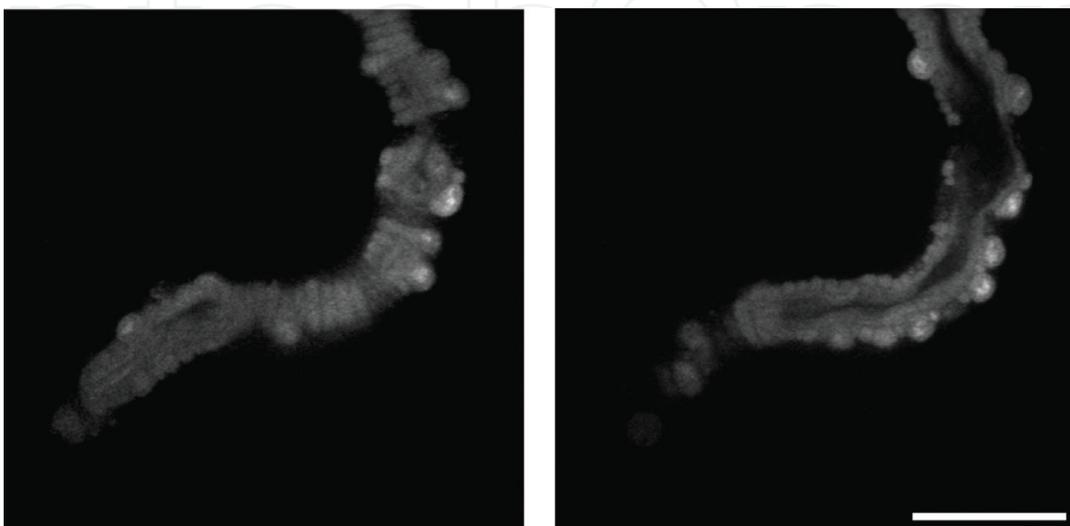


Figure 1. *4th order skeletal muscle arteriole loaded with 10 mM Fluo-4. A single layer of spindle-like vascular smooth muscle cells (VSMC) runs perpendicular to the vessel's length (left panel). Long endothelial cells (EC) frame the lumen of skeletal muscle arteriole (right panel, adapted from [6]). Scale bar = 50 μm .*

3. Skeletal muscle vasculature

Skeletal muscle is the largest organ in the body that receives about 20% of cardiac output at rest and up to 80% during exercise. In skeletal muscle, the local blood flow is regulated over a 20× range to meet the demands of exercise. Therefore, vascular resistance of skeletal muscle is a critical determinant of total peripheral resistance and blood pressure. Arterioles of skeletal muscle have a high myogenic tone at rest as they are subject to major hyperemia (increased blood flow) during exercise [8]. In case of orthostatic hypotension, a potential contributor to cardiovascular adaptation to prolonged periods of bed rest or microgravity, skeletal muscle arterioles may develop functional or structural adaptations. Despite the physiological importance of skeletal muscle arterioles, little is known about ionic mechanisms underlying their excitability. Even within the same skeletal muscle, arterioles might respond differently to pressure changes. For instance, the first-order arterioles isolated from fast-twitch (e.g., white gastrocnemius) skeletal muscle fiber demonstrated both functional and structural changes such as reduced myogenic tone, decreased contractile responsiveness, and reduced wall thickness with no change in luminal diameter [9, 10]. In contrast, arterioles isolated from slow-twitch (e.g., soleus) fibers show no difference in myogenic tone or contractile responsiveness but rather a structural remodeling resulting in a decreased arteriole diameter [11].

4. Excitability of vascular smooth muscle

After development of isolated vessel techniques, the electrophysiology of smooth muscle cells was extensively studied in small arteries isolated from various vascular beds [12].

4.1 Membrane potential of vascular smooth muscle

Both slow changes (myogenic tone) and fast spikes in the membrane potential have been observed in arteriolar smooth muscle cells. Arteriolar smooth muscle cells can generate rhythmical contractions (vasomotion) over an extended period of time. Vasomotion occurs spontaneously or in response to vasoactive stimulation with a frequency of 3–20 per minute. The exact physiological role of vasomotion is not clear. In cases of ischemia and hypertension, it serves as a protective mechanism. Vasomotion is not a consequence of heartbeat, respiration, or neuronal input. It is generated within arteriolar smooth muscle cells by an endogenous pacemaker mechanism driven by a cytosolic Ca^{2+} oscillator. The cytosolic Ca^{2+} oscillator depends on Ca^{2+} entry and is regulated by transmitters and hormones, which increase the formation of InsP_3 and diacylglycerol (DAG) and promote oscillatory activity (for review, see [13]).

From the early studies, it has been appreciated that an increase in intraluminal pressure leads to depolarization and consequent contraction of vascular smooth muscle cell (electromechanical coupling). Mechanisms of depolarization are still not well understood. Depolarization increases Ca^{2+} concentration inside the cell, which leads to activation of myosin light chain kinase by Ca^{2+} /calmodulin and consequent contraction. Propagation of depolarization is achieved through gap junctions between neighboring smooth muscle cells. The signaling between endothelial and smooth muscle cells via gap junctions is essential for normal vascular function. Gap junctions in arteriolar smooth muscle cells and endothelial cells are formed by connexins Cx37, Cx40, and Cx43. Coupling between arteriolar smooth muscle cells appears to be essential for the maintenance of oscillations in membrane potential, the intracellular $[\text{Ca}^{2+}]$, and vasomotion.

The exact signaling mechanisms that underlie detection of the mechanical stimulus and membrane depolarization are not completely understood (for review, see [7, 14]). Depolarization of vascular smooth muscle's membrane activates various voltage-gated ion channels, pumps and exchangers, including voltage-gated Ca^{2+} and K^+ channels, Ca^{2+} -activated BK_{Ca} and Cl_{Ca} channels, $\text{Na}^+/\text{Ca}^{2+}$ exchanger, Ca^{2+} -ATP pump and N^+/K^+ -ATPase, ATP-sensitive P2X receptor, and transient receptor potential (TRP) ion channels. Voltage-gated L-type Ca^{2+} channels are activated upon depolarization and increase Ca^{2+} concentration inside the cell. BK_{Ca} K^+ channels hyperpolarize smooth muscle cell back to its resting potential [15, 16]. Some vascular myocytes, particularly in large vessels, contract via pathways that appear to be unrelated to significant changes in the membrane potential (pharmacomechanical coupling).

Membrane potential of arteriolar smooth muscle cells is difficult to measure because of the changes in the intraluminal pressure and active vasomotor responses. Resting membrane potentials range from approximately -60 to -35 mV for physiological pressures (for review, see [17, 18]). Resting membrane potentials between -80 to -60 mV were previously reported for un-pressurized mice mesenteric artery [19], submucosal arterioles [20], and cerebral arterioles [21, 22]. A range of membrane potentials was reported for un-pressurized mice skeletal muscle arterioles (**Figure 2**). The average resting potential was reported to be around -68 mV (**Figure 2**, adapted from [6]). However, it's significantly more negative than -55 mV previously reported for un-pressurized arterioles in rat cremaster muscle [23]. Resting membrane potential in vascular smooth muscle cells is determined to a large extent by K^+ conductance [18]. Intracellular $[\text{Cl}^-]$ in vascular smooth muscle cells is around 55 mM, which is unusually high and mediated probably through $\text{Cl}^- \text{---} \text{HCO}_3^-$ exchanger. A small influx of Ca^{2+} and efflux of Cl^- ions at rest reduce the membrane potential from the Nernst equilibrium for K^+ [24].

4.2 Excitability of vascular smooth muscle

Most of the arteriolar smooth muscle cells are quiescent. In some cases, a drop in the intraluminal pressure (during trauma to a vessel) that leads to hyperpolarization and dilation generates membrane action potentials. Physiological role of membrane action potentials in arteriolar smooth muscle is unclear, as relatively small changes in the membrane potential (between -55 and -35 mV) are sufficient to control Ca^{2+} entry and to initiate contraction in response to stimulation by mechanical stress of the blood flow [14]. Spontaneous action potentials could be associated either with rhythmic vasomotor activity [23]; follow K^+ -induced hyperpolarization and dilation [25], or precede injury-induced constriction [26].

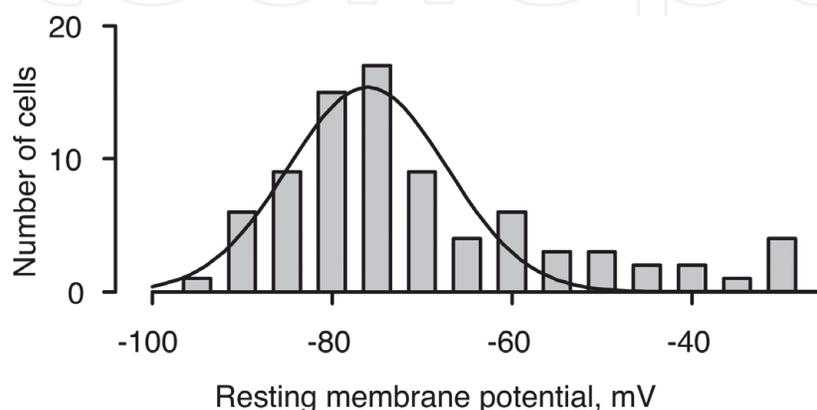


Figure 2. Resting membrane potential of un-pressurized 4th order arterioles isolated from mice skeletal muscle. Distribution of the resting membrane potential values was fitted by a single Gaussian function peaking at -77 ± 2 mV ($n = 81$, smooth line). The average resting potential was $V_{\text{rest}} = -68 \pm 2$ mV ($n = 81$). Adapted from [6].



Figure 3. Spontaneous action potentials were observed in un-pressurized skeletal muscle arterioles (2 mM Ca bath solution). Smooth muscle cells were current-clamped using gramicidin-perforated configuration, with intracellular solution containing 150 KCl. Adapted from [6].

Only a few recordings of action potentials in skeletal muscle arterioles were made so far [6, 23, 25, 27]. Spontaneous action potential spikes could be observed in pressurized small arteries [23, 25], as well as in un-pressurized arterioles as shown in **Figure 3** [6, 21].

4.3 Voltage-gated channels of arterial smooth muscle

Unlike in many other excitable tissues, action potentials in smooth muscle cells of arteries [28] and arterioles [21] are thought to be independent of voltage-gated Na⁺ channels, as they could not be blocked by the application of voltage-gated Na⁺ channel blocker tetrodotoxin (TTX). Depolarization of smooth muscle cells induced by the change in intraluminal pressure and/or tissue-stretch produces an increase in the intracellular [Ca²⁺], consequent myosin light chain phosphorylation and contraction (for review, see [14]). Since specific blockers of L-type voltage-gated Ca²⁺ channels suppress both the upstroke and the after-depolarizing components of action potentials, these channels are thought to be the main pathway for the depolarizing current. Nevertheless, several groups have demonstrated that voltage-gated Na⁺ channels are present in arterial beds and are activated upon membrane depolarization [6, 29].

4.3.1 Voltage-gated Ca²⁺ channels

Voltage-gated Ca²⁺ channels mediate influx of Ca²⁺ ions in response to membrane depolarization and regulate intracellular processes such as contraction, secretion, neurotransmission, and gene expression in many different cell types. Their activity is essential to couple electrical signals in the cell surface to physiological events in cells. Voltage-gated Ca²⁺ channels are comprised of the pore-forming α_1 subunit in complex with auxiliary subunits. A transmembrane disulfide-linked $\alpha_2\delta$, an intracellular β , and a γ subunit are components of most types of calcium channels. The α_1 subunit contains four repeats of a domain with six transmembrane segments, the fourth of which is the voltage-sensing S4 segment. The pore loop between transmembrane segments S5 and S6 in each domain determines ion conductance and selectivity (for review, see [30]). Ten distinct genes encode mammalian α_1 subunits of three subfamilies of voltage-gated Ca²⁺ channels. The amino acid sequences of these α_1 subunits are more than 70% identical within a subfamily but less than 40% identical among subfamilies. Voltage-gated Ca²⁺ channels are named using the chemical symbol of the principal permeating ion with the principal physiological regulator (voltage) indicated as a subscript according to the nomenclature developed for voltage-gated K⁺ channels [31]. The Ca_v1 subfamily (Ca_v1.1–Ca_v1.4) represents high-voltage activated L-type Ca²⁺ channels, the Ca_v2 subfamily (Ca_v2.1–Ca_v2.3) represents neuronal N-, P/Q-, and R-types Ca²⁺ channels, and Ca_v3 subfamily (Ca_v3.1–Ca_v3.3) represents low-voltage activated T-type Ca²⁺ channels.

The pharmacology and biophysics of Ca^{2+} channels subfamilies are quite distinct. L-type Ca^{2+} calcium channels typically require a strong depolarization for activation and do not inactivate at positive potentials. They are the main pathway for Ca^{2+} currents recorded in muscle cells, where they initiate contraction and secretion. The Ca_v1 subfamily is the molecular target of the organic Ca^{2+} channel blockers used widely in the therapy of cardiovascular diseases. L-type Ca^{2+} channels are blocked by the organic antagonists, including dihydropyridines (e.g., nifedipine), phenylalkylamines, and benzothiazepines. Dihydropyridines can be channel activators or inhibitors and therefore are thought to act allosterically to shift the channel toward the open or closed state rather than by occluding the pore. T-type Ca^{2+} channels are activated by weak depolarization and are transient at sustained depolarization. They are expressed in a wide variety of cell types, where they are involved in shaping the action potential and controlling patterns of repetitive firing. The Ca_v3 subfamily of voltage-gated Ca^{2+} channels is insensitive to both the dihydropyridines that block Ca_v1 channels and the spider and cone snail toxins that block the Ca_v2 channels. There are no widely useful pharmacological agents that block T-type Ca^{2+} currents specifically. Mibefradil blocks both T-type Ca^{2+} channels and with less potency L-type Ca^{2+} channels. Currents through expressed $\text{Ca}_v3.2$ channels could be selectively blocked by application of 40 μM of Ni^{2+} [32].

Currents through dihydropyridine-sensitive Ca^{2+} channels were recorded in arteries of various vascular beds [21, 33–36], including from smooth muscle cells of skeletal muscle arterioles (**Figure 4**, left panel). $\text{Ca}_v1.2$ considered to be the principal sub-type of voltage-gated Ca^{2+} channels involved in excitation-contraction coupling of vascular smooth muscle cells [37]. Since L-type Ca^{2+} blocker nifedipine did not eliminate basal tone in skeletal muscle arterioles, other Ca^{2+} entry mechanisms are believed to contribute to myogenic tone along with L-type Ca^{2+} channels [38]. In addition to L-type, vascular smooth muscle cells also express T-type Ca^{2+} channels (for review, see [30, 39]). Currents through T-type Ca^{2+} channels have been found in smooth muscle cells of arteries and arterioles of cerebral [21], mesenteric [40], renal [41], coronary [35], and skeletal [6] vascular beds as shown in **Figure 4** (right panel). Although the messenger RNAs for both, $\text{Ca}_v3.1$ and $\text{Ca}_v3.2$ T-type Ca^{2+} channels were found in rat cremaster arterioles [42], only L-type Ca^{2+} currents were recorded in single smooth muscle cells isolated from resistance arteries of hamster cremaster muscle [43]. The pressure/stretch stimulus initiates a depolarization that

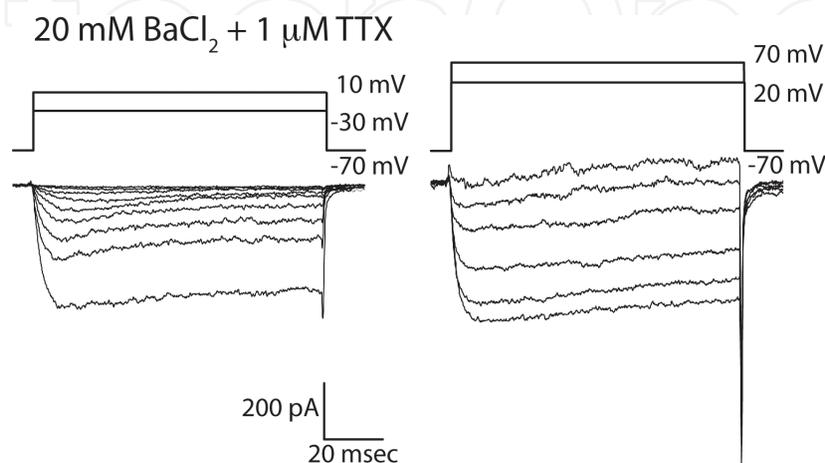


Figure 4.

Voltage-gated T-type and L-type Ca^{2+} channels are present in arteriolar smooth muscle. Whole-cell Ba^{2+} currents were recorded in the presence of 1 μM tetrodotoxin (TTX), voltage-gated Na^+ channel blocker as described in more detail in [6]. Two kinetically different components were observed: while voltage steps from -30 up to -10 mV produced fast inactivating T-type Ca^{2+} current (left panel), further depolarization activated slowly inactivating L-type Ca^{2+} current (right panel). Adapted from [6].

causes Ca^{2+} influx through voltage-gated L-type Ca^{2+} channels and initiates Ca^{2+} sparklets [44–46]. Both, Ca^{2+} sparklets and Ca^{2+} sparks (Ca^{2+} release from intracellular stores) signaling events, activate negative feedback mechanisms via Ca^{2+} -dependent K^+ currents thus preventing unrestrained depolarization [47–49]. While Ca^{2+} influx through L-type Ca^{2+} channels are believed to be involved in contraction of vascular smooth muscle, T-type Ca^{2+} channels, particularly $\text{Ca}_v3.2$ appear to be mostly involved in relaxation of coronary arteries, acting through activation of BK_{Ca} channels [40–43, 50, 51].

4.3.2 Voltage-gated Na^+ channels

In addition to voltage-gated Ca^{2+} channels, significant TTX-sensitive Na^+ currents were found in smooth muscle cells from rabbit main pulmonary artery [52], human aorta [53], murine mesenteric arteries [29], and skeletal muscle arterioles [6] as shown in **Figure 5**. The above observations suggest that more complex mechanisms are likely to generate action potential in vascular smooth muscle cells. When voltage-gated Ca^{2+} and Na^+ channels coexist in the same cell, Na^+ conductance is thought to be responsible for generation and propagation of action potential in excitable cells such as neurons, striated muscle, and neuroendocrine cells. It has lower threshold and faster rise time than Ca^{2+} conductance. After Na^+ channels rapidly inactivate, L-type voltage-gated Ca^{2+} channels open and further depolarize the cell, thus prolonging the plateau of an action potential. In contrast, T-type voltage-gated Ca^{2+} channels may open at resting potential and produce depolarizing current that brings the cell to threshold for Na^+ spike [54, 55].

Voltage-gated Na^+ channels consist of the subunit associated with auxiliary subunits. The pore-forming subunit is sufficient for functional expression, but the kinetics and voltage dependence of channel gating are modified by the subunits. These auxiliary subunits are involved in channel localization and interaction with cell adhesion molecules, extracellular matrix, and intracellular cytoskeleton. Similar to the voltage-gated Ca^{2+} channels, the subunits of Na^+ channels are organized in four homologous domains, each composed of six transmembrane segments. S4 segment is a voltage sensor, and a pore loop located between the S5 and S6 segments determines ion conductance and selectivity (for review, see [56]).

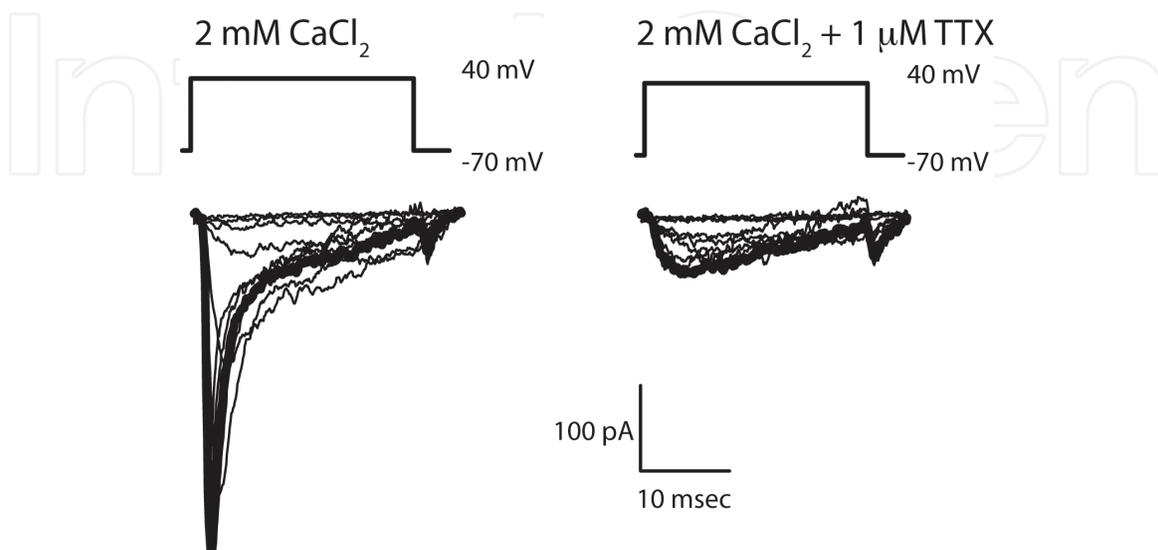


Figure 5. TTX-sensitive voltage-gated Na^+ channels are present in arteriolar smooth muscle. In arteriolar smooth muscle of skeletal muscle arterioles, voltage steps produced inward currents with at least two kinetically distinct components in 2 mM Ca solution (left panel). The fast component was through voltage-gated Na^+ channels as it was blocked by application of 1 μM TTX (right panel). Adapted from [6].

According to the nomenclature, the Na_v1 superfamily (Na_v1.1–Na_v1.9) represents voltage-gated Na⁺ channels. Unlike the different classes of voltage-gated Ca²⁺ channels, the functional properties of Na⁺ channels are relatively similar. Amino acid sequences of the nine mammalian Na⁺ channel isoforms are more than 50% identical to each other, but separate families are difficult to define. By this criterion, nine isoforms are considered to be members of one Na_v1 gene subfamily. Some voltage-gated Na⁺ channels (Na_v1.1, Na_v1.2, Na_v1.3, Na_v1.4, Na_v1.6, and Na_v1.7) could be blocked by tetrodotoxin that binds to the extracellular side of the pore.

Several isoforms of TTX-sensitive voltage-gated channels have been found previously smooth muscle cells of in vasa recta (Na_v1.3), portal vein (Na_v1.6 and Na_v1.8), and vas deference (Na_v1.6) [57–59]. The activity of these voltage-gated Na⁺ channels may be tightly controlled by elevations of intracellular Ca²⁺, potentially suppressing activity of Na⁺ channels [6]. For instance, Ca²⁺/CaM binding was shown to down-regulates skeletal muscle isoform Na_v1.4 by shifting its steady-state inactivation curve in the hyperpolarizing direction [60]. Na_v1.3 channels in descending vasa recta are suppressed by calmodulin inhibitors, while elevation of the intracellular [Ca²⁺] shifts the voltage-dependence of their activation to more positive voltages [57]. In addition, Ca²⁺-dependent down-regulation of Na⁺ channels can also occur via the PKC pathway since activation of PKC decreases peak sodium currents through brain Na_v1.2 and skeletal muscle Na_v1.4 channels by up to 80% [61, 62].

5. Conclusion

L-type voltage-gated Ca²⁺ channels are involved in excitation-contraction coupling of vascular smooth muscle cells. In addition, T-type Ca²⁺ channels were detected in arteriolar smooth muscle cells. These two types of voltage-gated Ca²⁺ channels together play an important role in the constriction/relaxation of smooth muscle cells by regulating Ca²⁺ signaling during myogenic tone. However, there are indications that other type of voltage-gated channels, specifically Na⁺ channels are present in various vascular beds. While the role of voltage-gated Ca²⁺ channels is well established, contribution of voltage-gated Na⁺ channel remains to be determined.

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Conflict of interest

The author declares no conflict of interest.

Abbreviations

DAG	diacylglycerol
EC	endothelial cell
EDHF	endothelium-derived hyperpolarizing factor
InsP ₃	inositol 1,4,5-triphosphate
TTX	tetrodotoxin
VSMC	vascular smooth muscle cell

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