Coupig a change in intraluminal pressure to vascular smooth muscle depolarization: still stretching for an explanation

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PRESURE-INDUCED (MYOGENIC) CONSTRICION of arterioles is believed to be a fundamental contributor to the local regulation of microvascular blood flow and pressure. Myogenic constriction provides a level of tone upon which vasodilators can act to lower resistance and match blood flow to metabolic requirements. The physiological importance of this regulatory mechanism, together with a growing interest in mechanotransduction per se, has provided the impetus to understand the underlying cellular mechanisms. In this issue of the American Journal of Physiology-Heart and Circulatory Physiology, Earley et al. (8) present new evidence linking the transient receptor potential (TRP) family cation channel TRPM4 to the initial events in myogenic constriction, and they offer new insights into how myogenic tone may be modulated by Ca\(^{2+}\) and protein kinase C (PKC). Importantly, increasing emphasis is being placed on TRP channels as potential sites for targeting pharmacological intervention in cardiovascular disease (16, 31).

Myogenic constriction is proposed to be initiated by the following sequence of events: 1) stretch-induced vascular smooth muscle cell (VSMC) depolarization, 2) the opening of voltage-gated Ca\(^{2+}\) channels (VOCCs), 3) a global increase in intracellular Ca\(^{2+}\), and 4) myosin light chain phosphorylation (4, 5, 20, 21). A growing literature suggests that those events are also accompanied by, or linked to, extracellular matrix-integrin interactions, cytoskeletal rearrangements, and changes in myofilament Ca\(^{2+}\) sensitivity (10, 12, 22, 38). A key question remains: at a molecular level, how is a change in intraluminal pressure coupled to a change in VSMC membrane potential? Candidate ionic mechanisms include the activation of nonselective cation channels or chloride channels and/or inhibition of constitutively active K\(^+\) channels (5); however, current evidence points most strongly to the activation of one or more nonselective cation channels (4, 19, 35, 39, 43). But what is the molecular identity of the VSMC cation channel? Is the channel intrinsically mechanoinsensitive? Or is it only activated secondary to stretch-induced generation of second messengers (1, 11)?

In recent studies, Brayden and colleagues (9, 44) have provided evidence for the involvement of members of the TRP-family of nonselective cation channels in the myogenic response. Those studies, as in their present work, combined electrophysiological approaches together with antisense manipulation of specific TRP protein expression in rat cerebral small arteries. This approach was necessary due to the lack of selective pharmacological inhibitors for the various TRP isoforms (3, 6). Collectively, the results point to critical roles for both TRPC6 and TRPM4 in pressure-induced VSMC depolarization and vasoconstriction. TRPC6 is stretch sensitive and Ca\(^{2+}\) permeable (3, 41), whereas TRPM4 is stretch insensitive, activated by intracellular Ca\(^{2+}\) and selective for monovalent cations (23). Although other TRP channels, notably TRPC1 (25) and TRPV2 (28), can also be mechanically activated, their functional roles in VSMCs are unknown. The roles of TRPC6 and TRPM4 in smooth muscle stretch activation are likely to be different from that of osmotically regulated TRP channels. Why both TRPC6 and TRPM4 should be required for pressure-induced depolarization in VSMCs has not been resolved, but one possibility is that they form a heteromultimeric channel. In that case, the knockdown of any one component (9, 44) could disable the entire channel complex. However, to date, heteromultimeric channel assembly has only been demonstrated within, but not across, TRP families (3, 6). Another possibility is that sequential activation of TRPC6/TRPM4 is needed for substantial membrane depolarization in response to a limited stretch stimulus. Specifically, the gating of TRPC6 channels by stretch might trigger Ca\(^{2+}\) influx, thereby increasing the open probability of TRPM4 to conduct the majority of inward Na\(^{+}\) current (9). Functional coupling of the two channels would probably require their colocalization, which has not yet been demonstrated. Regardless, the concerted activity of TRPC6 and TRPM4 could explain many of the characteristics of whole cell, stretch-activated current described in the smooth muscle (4, 19, 35, 39, 43), including a measurable Ca\(^{2+}\) influx independent of the downstream activation of VOCCs (43, 45, 47).

The use of knockout or transgenic models is an alternate and complementary approach to antisense oligonucleotides. At apparent odds with a critical role for TRPC6 is the finding that TRPC6\(^{-/-}\) mice show enhanced basal VSMC depolarization, a lower threshold for myogenic constriction, and elevated blood pressure (7). In part, compensation for TRPC6 deficiency occurs by upregulation of TRPC3, a closely related TRP protein regulated by diacylglycerol (DAG), which apparently accounts for the observed increase in a constitutively active cation current (see below) in TRPC6\(^{-/-}\) VSMCs. The mechanism underlying the increase in TRPC3 activity is attributed to the loss of negative regulation by TRPC6 rather than substitution of one isoform for another (7). In apparent contradiction, however, Reading et al. (37) found that TRPC3 knockdown (~50%) had no effect on the myogenic responsiveness of rat cerebral arteries.

The involvement of nonselective cation channels in pressure-induced depolarization is consistent with a wider role for such channels in VSMC signaling. Albert and Large (1) have described a number of cation conductances in VSMCs and...
suggest four channel classifications: stretch-activated, receptor-operated, constitutively active, and store-operated. These functions are not necessarily mutually exclusive; for example, TRPC1 is a putative store-operated channel (34) that is also stretch sensitive (25). Likewise, TRP6, when heterologously expressed, can be activated by patch pipette suction, by agonist-mediated signaling through phospholipase C (PLC), or by exogenous application of DAG analogs (41). Native VSMC cation channels share many of the same characteristics of TRPC6 (40) and TRPC3 (2), although it is not clear whether any of the same channels are directly activated by stretch. However, a common denominator between these procontraction mechanisms may be stretch activation of an upstream G protein, PLC, or phospholipase D (13, 33), resulting in the production of DAG, which subsequently becomes incorporated into the inner leaflet of the cell membrane and alters the lipid environment of the TRP channel(s) (41). The idea that a stretch-activated cation channel is also gated by DAG produced as a result of phospholipase activation fits with the recent observations of Park et al. (35) and Spassova et al. (41), except that DAG, itself, does not activate the cation conductance in the absence of membrane stretch (35). Related studies of patch-clamped VSMCs point to DAG as a critical modulator of a number of VSMC cation channels, with DAG exerting multiple and complex effects on cation conductances: both stimulatory effects and PKC-mediated inhibitory actions (1, 17). Many of these conductances are likely to involve TRP channels (2). To complicate the picture further, TRP channels are also known to be regulated by other phosphorylation mechanisms, including CamKII, protein kinase G, and nonreceptor tyrosine kinases (36). Thus it seems likely that a number of stimuli converge on VSMC cation channels through DAG and/or PKC. Can this convergence explain how norepinephrine, angiotensin II, and other agonists enhance myogenic gain or lower the threshold for myogenic constriction (27)? Possibly, as studies in intact blood vessels show DAG and inositol 1,4,5-trisphosphate to be produced following physiological stretch and that PLC inhibition attenuates myogenic responsiveness (29, 33, 35). A remaining challenge is to resolve the specific ionic conductances and selectivities of the native VSMC cation channels, along with the stimuli that activate them, and compare them with the properties of heterologously expressed TRP channels. However, it is unlikely that any known TRP isoform can explain all of the properties of the stretch-activated cation current associated with the myogenic response and that multiple TRP isoforms must act in concert (9) or form heteromultimers with unique properties (24).

Another contribution of the work by Earley et al. (8) is to provide links between TRP channel activation and other established players in myogenic signaling: namely changes in intracellular Ca\(^{2+}\) and PKC (15, 32). These authors show that PKC activation increases the Ca\(^{2+}\) sensitivity of TRPM4 in VSMC, thereby pointing to a mechanism by which PKC activation could enhance myogenic constriction (8). Indeed, multiple in vitro and in vivo studies on arteries had previously suggested a critical role for PKC in myogenic responsiveness (15, 32), possibly at the exclusion of stretch-induced membrane depolarization (22, 26). Whereas PKC activation is known to enhance Ca\(^{2+}\) entry through phosphorylation of VOCCs (18) and contribute to Ca\(^{2+}\) sensitization of the contractile proteins (14), it is now appreciated that at least part of the PKC effect may be explained by direct modulation of the upstream depolarizing mechanism (8).

Finally, myogenic vasoconstriction typically depends on Ca\(^{2+}\) entry from the extracellular space via VOCCs leading to an increase in global or cytosolic Ca\(^{2+}\). However, mounting evidence suggests an involvement of Ca\(^{2+}\) signaling events that operate in restricted domains. For example, the focal release of Ca\(^{2+}\) from intracellular stores (Ca\(^{2+}\) sparks) can produce Ca\(^{2+}\) concentrations approximating 10 \(\mu\)M (46) in a restricted space formed by close apposition of the sarcoplasmic reticulum and plasma membranes (42). This spatially restricted Ca\(^{2+}\) increase has been shown to activate large conductance Ca\(^{2+}\)-activated K\(^+\) channels causing membrane hyperpolarization (30) to counterbalance contractile stimuli. The present paper by Earley and colleagues (8) suggests that a similar scenario may exist for the Ca\(^{2+}\)-dependent activation of TRPM4, which is normally activated by Ca\(^{2+}\) in the 1–10 \(\mu\)M (or even higher) range but is activated in the submicromolar range after PKC activation. Regardless of PKC-dependent Ca\(^{2+}\) sensitization, TRPM4 appears to require Ca\(^{2+}\) levels considerably greater than those reported for pressure-induced changes in global intracellular Ca\(^{2+}\). Thus a physical location that allows TRPM4 to be exposed to high [Ca\(^{2+}\)] (e.g., colocalization with Ca\(^{2+}\)-permeable, mechanosensitive TRPs or by second-messengers produced by mechanosensitive enzymes) is an attractive hypothesis but is yet to be conclusively demonstrated.

The characterization of TRP channels in arterial smooth muscle, and the demonstration that several family members are either directly, or indirectly, activated by mechanical stimuli have provided an opportunity to integrate a number of cellular events implicated in myogenic signaling. In particular, links between stretch/pressure activation and changes in intracellular levels of Ca\(^{2+}\), PKC, and DAG are becoming evident. Furthermore, and importantly, these cellular events provide a plausible mechanism by which an increase in intraluminal pressure affects membrane depolarization and subsequent Ca\(^{2+}\) entry through VOCCs. Future challenges continue to involve identifying whether a single mechanosensor exists or whether an action of pressure is to alter, for example, the mobility/activity of several ion channel proteins. In addition, a more precise understanding of the spatiotemporal relationships between pressure-activated signaling molecules is required.

REFERENCES


