Ion channel switching and activation in smooth-muscle cells of occlusive vascular diseases

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Abstract

Blood vessels are essential for animal life, allowing flow of oxygen and nutrients to tissues and removal of waste products. Consequently, inappropriate remodelling of blood vessels, resulting in occlusion, can lead to disabling or catastrophic events: heart attacks, strokes and claudication. An important cell type of remodelling is the VSMC (vascular smooth-muscle cell), a fascinating cell that contributes significantly to occlusive vascular diseases by virtue of its ability to 'modulate' to a cell that no longer contracts and arranges radially in the medial layer of the vessel wall but migrates, invades, proliferates and adopts phenotypes of other cells. An intriguing aspect of modulation is switching to different ion transport systems. Initial events include loss of the $Ca_V1.2$ (L-type voltage-gated calcium) channel and gain of the $K_{Ca}3.1$ (IKCa) potassium channel, which putatively occur to enable membrane hyperpolarization that increases rather than decreases a type of calcium entry coupled with cell cycle activity, cell proliferation and cell migration. This type of calcium entry is related to store- and receptor-operated calcium entry phenomena, which, in VSMCs, are contributed to by TRPC [TRP (transient receptor potential) canonical] channel subunits. Instead of being voltage-gated, these channels are chemically gated – importantly, by key phospholipid factors of vascular development and disease. This brief review focuses on the hypothesis that the transition to a modulated cell may require a switch from predominantly voltage- to predominantly lipid-sensing ion channels.

Introduction

VSMCs (vascular smooth-muscle cells) are best known as the contractile cells of arteries and veins, regulating vessel calibre and thus determining tissue perfusion and venous drainage throughout the body. Not only must the cells perform this function but also they must continuously have the capacity to adapt as each tissue changes its demands or responds to injury. Because of this need, the cells retain remarkable plasticity throughout life, enabling reversible 'modulation' to an activated, non-contractile, migratory and proliferating cell phenotype [1]. In some situations, the cells may even differentiate into other cell types from the same lineage, including adipocytes and chondrocytes [2]. Often modulation is controlled and physiological, but it can also contribute significantly (positively or negatively) to major vascular abnormalities including atherosclerosis, neointimal hyperplasia, in-stent restenosis and allograft vasculopathy [3–5], abnormalities that underlie many deaths, disabilities and economic problems. Atherosclerosis, for example, causes most of the cases of angina, heart attack and stroke, leading to widespread disability, approx. 165 000 deaths per year (almost 14 times as many as caused by breast cancer, for example) and economic burden approaching £8 billion per year in the United Kingdom alone (BHF 2006 Coronary Heart Disease Stats, British Heart Foundation, http://www.bhf.org.uk).

The molecular machinery governing modulation of VSMCs is starting to emerge [1] but much remains to be elucidated. One feature is a dramatic switch in ion transport mechanisms, which are almost certainly a fundamental systems requirement of modulation and modulation-like phenomena, not only in VSMCs but also other cell types including lymphocytes and cancer cells [7–11]. Knowledge of these changes is necessary for understanding modulation but it also promises to reveal molecular foundations for accessible extracellular drug-binding sites that are dominant in modulating cells, and thus dominant in disease. The present paper focuses on selected findings arising in this area of research with a view to developing testable hypotheses on how the systems operate in VSMCs.

Loss of two dominant voltage-gated ion channels of the contractile cell

A feature of all contractile VSMCs is abundant expression of $K_{Ca}1.1$ (also referred to as BK_{Ca} or Maxi-K) channels: large-conductance Ca^{2+}-activated K^+ channels that are voltage-dependent, increasing in activity in response to membrane depolarization [12]. A function of the $K_{Ca}1.1$ channels is to provide negative feedback against depolarization, limiting Ca^{2+} influx through $Ca_V1.2$ (L-type voltage-gated Ca^{2+}) channels: channels that are also a striking feature of the contractile cells and a high-affinity site of action of the antihypertensive Ca^{2+} antagonists. Intriguingly, the switch

Key words: calcium channel, occlusive vascular disease, phospholipid, potassium channel, transcription factor, vascular smooth-muscle cell (VSMC).

Abbreviations used: ERK, extracellular-signal-regulated kinase; LDL, low-density lipoprotein; LPC, lysophosphatidylcholine; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; REST, repressor element 1-silencing transcription factor; TRP, transient receptor potential; TRPC, TRP canonical; TRPV, TRP vanilloid; VSMC, vascular smooth-muscle cell.

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to the proliferating phenotype is associated with loss or suppression of both of these ion channels [13–18]. It should not be excluded, however, that these channels could have roles in very early events of responses to injury [19] or return once the modulated cells have ceased their activity and begun a more quiescent existence. That is, timing is a crucial factor. It would be wrong to over-simplify the situation, assuming the cells are either in a pure contractile phenotype or constantly in a proliferating phenotype. Although there may be parallels with cancer cells, smooth-muscle cell remodelling is a physiological injury response and developmental process that shows the consistent property of stabilizing within reasonable boundaries. It is not, therefore, surprising to see expression of $Ca_V1.2$ subunits reappear [20] or be present in atherosclerotic plaques [21], which go through cycles of advancement and stabilization over months to years.

Shift of emphasis to TRPC family members: lipid-sensing channels

When $Ca_V1.2$ channels are lost, there is not concomitant loss of Ca^{2+} entry. Ca^{2+} entry is instead enabled by other ion channels, which are often resistant to therapeutic concentrations of Ca^{2+} antagonists and permeable to Na^+ and K^+ as well as Ca^{2+}. These channels may be the same as, or closely related to, the so-called store-operated channels, which were shown to be linked to VSMC proliferation [22]. There has been considerable effort to identify the molecular components of these channels, leading to the conclusion that they are partially accounted for by members of the TRP (transient receptor potential) channel family. Importantly, although TRP channels [23] are structurally related to voltage-gated ion channels, they do not require depolarization in order to be active. They are instead polymodal channels that are activated by several different endogenous chemical substances.

Attention focused firstly on the TRPC (TRP canonical) 1 channel, which was the first cloned human homologue of *Drosophila melanogaster* TRP and a candidate store-operated channel [24]. Early studies of VSMCs identified it as a contributor to native store-operated Ca^{2+} entry that fails to couple with contraction [25,26]. It is also a component of store-operated calcium channels of proliferating VSMCs [27–30]. There is up-regulation *in vivo* in response to vascular injury and stimulation by cholesterol [29,31,32]. Inhibiting TRPC1 inhibits VSMC proliferation [27,29]. Recently, links to angiotensin II-induced hypertrophy and the cell cycle were shown [29,30]. Through a strategy for developing isoform-specific channel blockers [33], we were able to make the important observation that antibody targeted to TRPC1 inhibits human neointimal hyperplasia [29], an adaptation that leads to failure in coronary bypass grafts and is due almost exclusively to smooth-muscle cell invasion, migration and proliferation. Therefore TRPC1 would seem to be an important voltage-independent Ca^{2+} channel subunit during modulation.

Focus on TRPC1 alone was, however, frustrating because we and others found TRPC1 to have weak (or no) function when expressed on its own in cell lines [34–36]. A way forward was indicated by the work of Strubing et al. [35] who first showed that TRPC1 has robust function in a heteromultimer with TRPC5. Although the mRNA species encoding TRPC5 has relatively low abundance, TRPC5 mRNA and protein are detected in several types of VSMC [30,36–41], TRPC5 is functional [33,36,41,42], and endogenous TRPC1 and TRPC5 co-localize and co-immunoprecipitate [36]. In VSMC types where mRNA encoding TRPC5 has been undetectable [32,43], studies have been of contractile cells, or TRPC5's roles may be taken by TRPC4, which is functionally similar to TRPC5 [44]. Variation in composition should not be surprising given that VSMCs have diversity in function, phenotype and embryonic origin. We have, however, detected mRNA encoding TRPC1, TRPC4 and TRPC5 in several blood vessels and suggest that the native channels of VSMCs commonly comprise heteromultimeric assemblies of all three TRPC proteins with various stoichiometries.

The general concept of TRP channels as direct chemical sensors, and particularly lipid sensors, has been an especially interesting aspect of the TRP field as a whole. TRPV (TRP vanilloid) 1, for example, is activated by anandamide, TRPV4 and related channels are activated by arachidonic acid metabolites, and TRPC3/6/7 is activated by diacylglycerol [23]. Because atherosclerotic progression and vascular development are influenced by lipid factors [3,45], we began to wonder whether there is lipid-sensing in TRPC1/5 channels. Using TRPC5 as a starting point, we searched for previously unrecognized activators based on lipid factors of atherosclerotic plaques or oxidized LDLs (low-density lipoproteins), which are drivers in the progression of atherosclerosis. Through this approach, we discovered that a highly effective activator is LPC (lysophosphatidylcholine) [40], which is approx. 40% of the oxidized LDL complex. LPC is not the only lipid activator: the widely studied signalling molecule sphingosine-1-phosphate, which is suggested to have pivotal roles in both mural cell recruitment during vascular development [45] and atherosclerosis [46], is a novel bipolar activator of the TRPC1/5 heteromultimeric channel [36]. Furthermore, sphingosine-1-phosphate-evoked migration of VSMCs was inhibited by specific TRPC5-blocking antibody or dominant-negative ion pore mutant of TRPC5 [36]. Activations by LPC and sphingosine-1-phosphate represent two examples of an expanding picture of polymodality in TRPC1/5 channels: the channels also show sensitivity to reactive oxygen species and reactive nitrogen species [47], which are also implicated in progression of vascular disease.

TRPC family members are not unique to the proliferative cell phenotype. However, up-regulation of *TRPC1* gene expression in the proliferating cells [27,29] will increase the influence of this gene. In addition, functional dominance will arise for other reasons: loss of $Ca_V1.2$ channels will prevent increases in Ca^{2+} entry in response to depolarization such that Ca^{2+} entry will instead increase with hyperpolarization, driven, for example, by $K_{Ca}3.1$ channel activity (see below). Furthermore, proliferating VSMCs are likely to be exposed to elevated concentrations of the phospholipid factors (e.g.

sphingosine-1-phosphate) that stimulate TRPC1/5 channel activity and inhibit $Ca_V1.2$ [36].

Gain of $K_{Ca}3.1$ channel

Seminal work from Neylon et al. [15] showed *de novo* expression of $K_{Ca}3.1$ (IKCa), an intermediate-conductance Ca^{2+}-activated K^+ channel encoded by the *KCNN4* gene [12]. It was later shown that inhibiting $K_{Ca}3.1$ channels suppressed smooth-muscle cell growth and, after angioplasty in the rat *in vivo*, reduced occlusion [48]. Blockade of $K_{Ca}3.1$ channels also reduced neointimal growth in the human saphenous vein, suggesting that appearance of this ion channel has general importance in the proliferative performance of smooth-muscle cells, and relevance to human disease [49].

Why would the cells change from expressing a large-conductance Ca^{2+}-activated K^+ channel ($K_{Ca}1.1$) to an intermediate-conductance Ca^{2+}-activated K^+ channel ($K_{Ca}3.1$)? The difference in conductance is probably unimportant because it can be compensated by more channels at the membrane. An important difference may be voltage dependence: $K_{Ca}1.1$ channels require a depolarizing stimulus to activate, whereas $K_{Ca}3.1$ channels do not. Therefore $K_{Ca}3.1$ channels can, theoretically, hyperpolarize the membrane potential all the way to the K^+ equilibrium potential (approx. -80 mV). Conceivably, extra negative membrane potential is necessary to provide sufficient electrical driving force on Ca^{2+} entry through Ca^{2+} channels that remain open at hyperpolarized membrane potentials, such as TRPC1/5 channels. We suggest that this is an important factor but recognize that it seems at odds with reports suggesting that $Ca_V3.1$ (depolarization-activated T-type Ca^{2+} channel) is also a feature of proliferating VSMCs (see below). A similar paradox appears in T-lymphocyte activation and may be explained by temporal aspects of different activation mechanisms [9]. To understand such issues, it will be important to explore the timing of different events in relation to the initial stimulus and have knowledge of the electrical activity of proliferating VSMCs *in situ*. It is, nevertheless, also conceivable that the voltage independence of $K_{Ca}3.1$ channels is not especially important: Instead, the channel may have other key properties, for example lipid-sensing [50].

Transcriptional control by REST (repressor element 1-silencing transcription factor) and other factors

There have been relatively few studies of the mechanisms controlling switches in ion channel expression during modulation but underlying principles and molecular components of the mechanisms are starting to emerge. One general observation is that changes in the channel proteins and their functions are often accompanied by changes in abundance of the mRNA species encoding the channels. Notably, expression of mRNA encoding $K_{Ca}3.1$ channels has been observed rapidly following vascular injury [51]. Therefore important control mechanisms are likely to lie at the level of transcription.

Two transcriptional control mechanisms have been identified for *KCNN4*. Both seem important in enabling expression. One is c-Jun of the AP-1 (activator protein 1) complex, which is a transcriptional activator and immediate early gene commonly induced as cells respond to insult. Physical association of c-Jun with *KCNN4* in VSMCs has been suggested [51]. This mechanism is counter-balanced by binding of REST, which is a transcriptional repressor that binds upstream of the *KCNN4* promoter and suppresses expression [49]. Critically, REST is down-regulated in proliferating VSMCs and neointimal hyperplasia, allowing expression of *KCNN4* [49]. Pharmacological studies suggest the Raf/MEK [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase)]- and ERK-signalling pathways are essential for PDGF (platelet-derived growth factor)-evoked expression of *KCNN4* [18].

The mechanisms underlying down-regulation of $K_{Ca}1.1$ and $Ca_V1.2$ channel expression have received limited attention, but there are important clues. Loss of function of the $K_{Ca}1.1$ channel may involve loss of its β-subunit, which is down-regulated by the NFAT (nuclear factor of activated T-cells) (c3) transcription factor [52]. MAPK inhibition reduces the loss of $Ca_V1.2$ [17]. Another pathway that may be involved is TNFα (tumour necrosis factor α) acting via NF-κB (nuclear factor κB) receptors because this pathway suppresses $Ca_V1.2$ expression in intestinal smooth-muscle cells [53] and has recognized roles in inflammation, which contributes to vascular diseases including atherosclerosis. Notably, up-regulation of *TRPC1* gene expression also occurs via this pathway in VSMCs and endothelial cells [30,54]. Therefore it is tempting to speculate that the NF-κB system is intimately involved both in the loss of $Ca_V1.2$ and increase in *TRPC1* gene expression. Although *TRPC1* gene has been suggested to be regulated by REST [55], we have not identified a REST-binding site in or near the *TRPC1* gene and do not find effects of manipulating REST on *TRPC1* gene expression (A. Cheong, I.C. Wood and D.J. Beech, unpublished work).

Changes in other aspects of ion transport

The above ion transport mechanisms are not the only ones associated with proliferating VSMCs. Others include voltage-gated Na^+ channel, T-type Ca^{2+} channel, ClC-3 chloride channel, TRPC6 channel, inward rectifier K^+ channel, $K_V3.4$ channel and Na^+–Ca^{2+} exchange [20,56–63]; conversely, loss of specific ion channels (e.g. $K_V1.5$) can also contribute [64]. Furthermore, changes in intracellular Ca^{2+} signalling are observed and roles of InsP_3 receptor-channel have been suggested. The control, importance, integration, timing and vessel-specific nature of many of these ion transport changes require further investigation.

Conclusions

There is ample evidence of profound change in the types of ion channel that are expressed or functionally important

when VSMCs make the transition from their contractile to proliferating phenotype. A critical event seems to be replacement of $Ca_V2.1$ with $K_{Ca}3.1$, which we suggest is important because it confers functional dominance on lipid-sensing channels such as those comprising TRPC1 and TRPC5 subunits. Further exploration of this hypothesis and the accompanying changes in other aspects of ion transport is important because the mechanisms are potentially of fundamental relevance to vascular development, physiology and disease. Components of the system are also promising and realistic targets for new therapeutic agents that could be useful to either increase or decrease VSMC proliferation depending on the context and timing of intervention.

This study was funded by the British Heart Foundation and the Wellcome Trust.

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Received 18 July 2007
doi:10.1042/BST0350890