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INVITED REVIEW

Regulation of vascular tone and arterial blood pressure: role of chloride transport in vascular smooth muscle

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Abstract Recent studies suggest that primary changes in vascular resistance can cause sustained changes in arterial blood pressure. In this review, we summarize current knowledge about Cl^- homeostasis in vascular smooth muscle cells. Within vascular smooth muscle cells, Cl^- is accumulated above the electrochemical equilibrium, causing Cl^- efflux, membrane depolarization, and increased contractile force when Cl^- channels are opened. At least two different transport mechanisms contribute to raise $[Cl^-]_i$ in vascular smooth muscle cells, anion exchange, and cation-chloride cotransport. Recent work suggests that TMEM16A-associated Ca^{2+}-activated Cl^- currents mediate Cl^- efflux in vascular smooth muscle cells leading to vasoconstriction. Additional proteins associated with Cl^- flux in vascular smooth muscle are bestrophins, which modulate vasomotion, the volume-activated LRRC8, and the cystic fibrosis transmembrane conductance regulator (CFTR). Cl^- transporters and Cl^- channels in vascular smooth muscle cells (VSMCs) significantly contribute to the physiological regulation of vascular tone and arterial blood pressure.

Keywords Blood pressure · Chloride channel · Hypertension · Kidney · Vascular resistance · Vasoconstriction

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Introduction

Adequate blood pressure is essential for proper organ function. While too low blood pressure (hypotension) can cause organ failure, elevated blood pressure (hypertension) is a major risk factor for cardiovascular disease, stroke, and chronic renal failure, which affects more than 25 % of the adult population worldwide [58]. Arterial blood pressure is determined by the volume of blood pumped by the heart (cardiac output) and the peripheral resistance of the vasculature. By Starling's Law, an increase in ventricular end-diastolic volume, which critically depends on the extracellular fluid volume, will increase stroke volume and hence cardiac output. A major hypothesis originally developed by Arthur Guyton in the early 1970s proposes that the kidneys control the level of long-term blood pressure by regulating extracellular fluid volume via the pressure natriuresis mechanism [43, 44]. According to this concept, any impaired capacity of the kidneys to excrete Na^+ will cause fluid retention, an increased cardiac output, and ultimately an increased blood pressure. The importance of renal Na^+ handling for blood pressure regulation and the pathogenesis of hypertension is strongly supported by genetic, experimental, and clinical evidence [22, 61, 69]. However, several more recent observations have challenged an exclusive role of the kidney for long-term blood pressure homeostasis [68, 76]. For example, selective genetic inactivation of the mineralocorticoid receptor in vascular smooth muscle cells (VSMCs) reduced vascular myogenic

tone, diminished the aging-related increase of blood pressure, and prevented arterial stiffening induced by aldosterone and high Na^+ intake in mice [35, 66]. These effects occurred without measurable changes in renal Na^+ handling, supporting that vascular mechanisms also impact on long-term blood pressure control. As the resistance to flow is inversely proportional to the fourth power of the radius of the blood vessel, small arteries and the arteriolar network are the main determinants of peripheral resistance. Their diameter is determined by contraction of the contractile cells within the wall, the VSMCs. In these cells, the cytosolic Cl^- concentration ($[Cl^-]_i$) is normally above the electrochemical equilibrium because of active Cl^- accumulation [20]. Hence, opening of Cl^- channels in the plasma membrane of VSMCs causes a depolarizing Cl^- efflux, which can activate voltage-dependent Ca^{2+} channels (Fig. 1). The ensuing rise in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) enhances force development by activating myosin light chain kinase via an increased formation of Ca^{2+}-calmodulin. Accordingly, alterations of $[Cl^-]_i$ in VSMCs should also modulate vascular tone. In this review, we give an update about the role of Cl^- for the regulation of peripheral resistance and arterial blood pressure.

Chloride transporters in vascular smooth muscle cells

In contrast to ions like Na^+ and K^+, in many cell types, the electrochemical gradient of Cl^- across the plasma membrane is not very far from the electrochemical equilibrium, which is expected from a passive distribution across the plasma membrane. Depending on the expression and activity of the various proteins that mediate Cl^- transport, $[Cl^-]_i$ can be either below or above the electrochemical equilibrium. In smooth muscle cells including VSMCs, the Cl^- equilibrium potential is normally above the resting membrane potential (E_m) (Table 1). At least two different mechanisms contribute to raise $[Cl^-]_i$ in VSMCs [20]: Cl^-/HCO_3^- exchange and Na^+-K^+-$2Cl^-$ cotransport. There is some evidence for a third mechanism, which has been referred to as "pump III" [20], but its molecular identity and driving force have not been identified so far. The Cl^-/H^+ antiporter ClC-3 is also expressed in VSMCs (like in other mammalian tissues) [55] and has been proposed to be involved in myogenic tone regulation [32]; however, ClC-3 mainly localizes intracellularly to endosomes [95].

Anion exchanger

Cl^- accumulation via anion exchange was initially shown for guinea pig ureter and vas deferens smooth muscle cells [2, 3]. In these cells, HCO_3^- is not at equilibrium due to the action of Na^+/H^+ exchange, which holds the intracellular pH at a more alkaline level than it would be if H^+ ions were in equilibrium with E_m [101]. The outward gradient for HCO_3^- thus provides the driving force for Cl^- accumulation via anion exchange. This mechanism has also been confirmed for smooth muscle

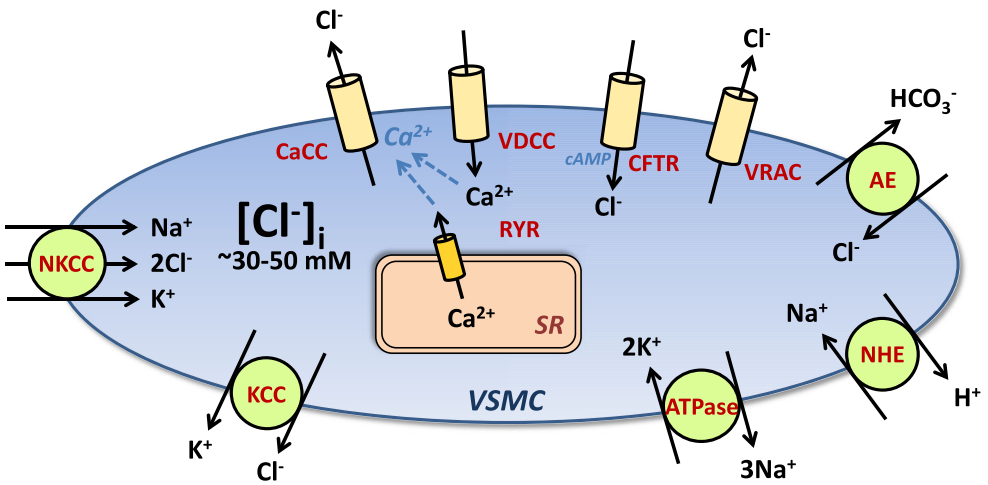

Fig. 1 Chloride homeostasis in vascular smooth muscle cells. NKCC and AE (in concert with NHE) accumulate chloride into vascular smooth muscle cells (*VSMCs*), while KCC cotransporters, which lower the intracellular Cl^- concentration ($[Cl^-]_i$), obviously play a minor role for steady state $[Cl^-]_i$. As a consequence, $[Cl^-]_i$ in VSMCs is normally above the electrochemical equilibrium. Hence, opening of plasma membrane Cl^- channels causes a depolarizing Cl^- efflux. Cl^- channels identified in VSMCs include Ca^{2+}-activated Cl^- channels (*CaCC*), volume-regulated Cl^- channels (*VRAC*), and the cAMP-regulated cystic fibrosis transmembrane conductance regulator (*CFTR*). Depolarization results in the activation of voltage-dependent Ca^{2+} channels (*VDCC*). The ensuing rise in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) enhances force development by activating myosin light chain kinase via an increased formation of Ca^{2+}-calmodulin. CaCC, which can be activated through Ca^{2+} release via ryanodine receptors (*RYR*) in the sarcoplasmic reticulum (*SR*) or Ca^{2+} release via IP_3 receptors activated by agonist stimulation of G protein-coupled receptors, boost responses to vasoconstrictive signaling. Activation of CFTR appears to occur predominantly at membrane potentials positive to the equilibrium potential for Cl^-, hence causing Cl^- influx and hyperpolarization. These mechanisms do not take place in all types of VSMCs, because the set of transport proteins expressed in VSMCs varies considerably along the vascular tree.

Table 1 Estimates for intracellular chloride concentration in smooth muscle cells. Different techniques have been used to determine $[Cl^-]_i$ in smooth muscle cells including double-barreled microelectrodes, ^{36}Cl content, using the fluorescent Cl^- probes, or X-ray microprobe analysis (adapted from [20])

	Technique used to determine $[Cl^-]_i$	$[Cl^-]_i$ (mM)	E_m (mV)	E_{Cl^-} (mV)	$[Cl^-]_{eq.}$ (mM)
Guinea pig vas deferens [3]	Cl^--sensitive microelectrode	41.2±6.7	67.6±7.8	24.5	8.0
GP ureter [2]	Cl^--sensitive microelectrode	51.1±4.0	48.7±5.4	18.6	16.6
Rabbit aorta [39]	^{36}Cl content	32.6	60.1±2.9	38.0	14.3
Rat aorta, cultured [53]	Fluorescent Cl^- probe (MQAE)	31.6±3.2	31.1		
Rat femoral artery [26]	Cl^--sensitive microelectrode	43.8±3.5	61.5±1.3	33.4	15.3
Human umbilical artery [29]	Cl^--sensitive microelectrode	33.8±0.9	57.0±1.0	35.6	15.2
Human placental artery [29]	Cl^--sensitive microelectrode	34.0±0.8	55.0±0.8	35.5	16.4
Mouse superficial brain arteries [87]	X-ray microprobe analysis	28.1±1.1			
Mouse saphenous arteries [87]	X-ray microprobe analysis	33.1±1.1			

cells cultured from embryonic rat aorta [54]. Of the three known Na^+-independent anion exchangers of the SLC4A family of bicarbonate transporters, only AE2 and AE3 were shown to be expressed in native arteries and in VSMCs [13]. AE2 was among seven genes, for which considerable association with increased blood pressure has been reported, but this association could not be confirmed in replication samples [94]. Conversely, a loss of AE2 or AE3 transport activity may cause a decrease of $[Cl^-]_i$ in VSMC leading to a diminished vascular tone and low blood pressure. Unfortunately, in vivo studies on AE2 knockout mice have been hampered because of the severe phenotype with growth retardation and early lethality around the time of weaning [38]. By contrast, AE3-knockout mice develop normally and show normal survival, although they are more susceptible to seizures [46] and suffer from impaired vision [4]. Cardiac performance and mean arterial pressure were not altered in anesthetized AE3-knockout mice [80].

Cation-chloride cotransporter

The first compelling evidence for Na^+-K^+-$2Cl^-$ cotransport in VSMCs was the finding of an ouabain-insensitive K^+ influx, which is sensitive to the NKCC1 inhibitor bumetanide and the presence of both extracellular Na^+ and Cl^- [75]. Inhibition of Na^+-K^+-$2Cl^-$ cotransport with bumetanide decreased $[Cl^-]_i$, hyperpolarized VSMCs, and attenuated the activation of L-type Ca^{2+} channels [5, 30]. Bumetanide also suppressed the constriction of mouse mesenteric arteries and rat afferent arterioles in response to angiotensin II [107].

Two different molecules mediate Na^+-K^+-$2Cl^-$ cotransport, NKCC1 and NKCC2, which belong to the family of cation-chloride cotransporters [36]. While the expression of NKCC2, which is mutated in Bartter syndrome type 1 [93], is restricted to epithelial cells of the thick ascending limb and the macula densa [36], where it mediates apical NaCl uptake, NKCC1 is expressed broadly including both epithelial and non-epithelial cells including VSMCs [47, 49]. Supporting a functional role for vascular tone of NKCC1 expression in VSMCs, the inhibitory action of bumetanide on contractions of mesenteric arteries was completely absent in NKCC1-knockout mice [52]. The role of NKCC1 for vascular contractility may differ along the vascular tree: When stimulated with phenylephrine, aortic smooth muscle rings from NKCC1-knockout mice exhibited no significant differences in maximum contractility and only moderate dose-response shifts, while a sharp reduction in mechanical force was noted in the portal vein [70]. Consistent with a role of NKCC1 in setting baseline vascular tone, bumetanide caused a decrease in blood pressure only in wild type but not in NKCC1-knockout mice [37]. Measurements of baseline blood pressure in NKCC1-knockout mice, however, yielded conflicting results: While one study demonstrated a lower systolic blood pressure [70], another study found even higher blood pressures in NKCC1-knockout mice compared with their wild-type controls, when they were fed a high salt diet [50]. The reason for this discrepancy remains unclear and may be related to the different blood pressure recording techniques used (tail cuff vs. telemetry), genetic backgrounds, and housing conditions. Furthermore, disruption of NKCC1 causes an increased expression of renal Na^+ transporters and reduced plasma ANP concentration [106] and a disinhibition of basal renin release through a direct effect on juxtaglomerular cells [19]. Thus, different levels of compensation may add additional variation between different studies and further complicate interpretation of the results.

Evidence for an elevated Na^+-K^+-$2Cl^-$ cotransport in VSMCs has also been obtained in several experimental models of hypertension in the rat [74]. A co-segregation of elevated Na^+-K^+-$2Cl^-$ cotransport in erythrocytes and blood pressure was observed for F2 hybrids of Milan normotensive and hypertensive rats suggesting a genetic association of anomalous NKCC1 function and hypertension [10]. More recently, also an epigenetic upregulation of NKCC1 activity has been detected in the aorta from spontaneously hypertensive

rats [21]. These findings support the notion that activation of NKCC1 is a general phenomenon of hypertension.

While NKCCs use the Na^+ gradient to accumulate Cl^- into the cell, K^+-Cl^- cotransporters (KCCs) use the K^+ gradient to lower $[Cl^-]_i$. There are some reports that K^+-Cl^- cotransport takes place in smooth muscle cells [1, 88]. Whether it is active under baseline conditions is so far unclear. KCC1 and KCC3, but not the neuronal specific KCC2, are expressed in VSMCs [87, 96]. KCC3-knockout mice, which are a model for the rare human genetic disorder Andermann syndrome [84], are hypertensive [11]. As expected from a KCC knockout, $[Cl^-]_i$ in VSMCs of the saphenous artery is increased [87], but the response of isolated saphenous arteries to changes in intravascular pressure, stimulation of alpha1-adrenoceptors, exogenous nitric oxide, or pharmacological blockade of Ca^{2+}-activated Cl^- channels with niflumic acid is unchanged [87]. These results argue against a major vascular intrinsic component of the increased arterial blood pressure in KCC3-knockout mice. Obviously, the local control of vascular smooth muscle tone does not require KCC3. Normalization of blood pressure by pharmacological autonomic blockade and increased urinary excretion rates of epinephrine and norepinephrine in KCC3-knockout mice rather point to a neurogenic origin of this phenotype [87].

Chloride channels in vascular smooth muscle cells

A contribution of Cl^- efflux to membrane depolarization induced by norepinephrine in VSMCs was detected already in the 1970s in the rat portal vein [104, 105] and rabbit pulmonary artery [18] by radioactive tracer flux experiments. Ca^{2+}-activated Cl^- currents in VSMCs were first reported in cells isolated from the pulmonary artery [15] and portal vein [16]. Since a rise of the cytosolic Ca^{2+} concentration is an early event after agonist stimulation of VSMCs, it was proposed that the opening of Ca^{2+}-activated Cl^- channels may be a key step in the electromechanical coupling in VSMCs [57] (Fig. 1). Studies using pharmacological blockers and Cl^--selective electrodes suggested also important roles of Cl^- currents for vasomotion [77], the myogenic response [31, 73, 110], and in VSMC proliferation [59]. However, for several reasons, the precise nature of the Cl^- transport proteins involved in these functions remained elusive until recently. Cl^- channels display largely similar low anion selectivities [33]; their expression varies greatly between different vascular beds [56, 63], different Cl^- channels coexist in the same VSMC [63], and classical Cl^- channel blockers like niflumic acid, DIDS, and flufenamic acid are unselective and have undefined modes of action [41, 102]. The molecular identification of Cl^- transport proteins is further complicated by the fact that all cellular expression systems express endogenous Cl^- channels.

Ca^{2+}-activated Cl^- channels

More than 25 years after the first characterization of Ca^{2+}-activated Cl^- currents in salamander photoreceptors [6] and Xenopus oocytes [7, 71], TMEM16A (anoctamin 1 (ANO1)) was independently identified by three groups as a component of Ca^{2+}-activated Cl^- channels [17, 90, 111]. It is a member of the TMEM16 family of transmembrane proteins, which has ten members in humans also known as anoctamins. Heterologous expression of TMEM16A in HEK293 cells and salamander oocytes induced Cl^- currents that recapitulated the biophysical and pharmacological properties of native Ca^{2+}-activated Cl^- channels [17, 90, 111]. Similar results were found for TMEM16B [90]. Treatment with silencing small interfering TMEM16A RNAs reduced Ca^{2+}-activated Cl^- channels in pancreatic and bronchial epithelial cell lines [17] and suppressed salivary production, which depends on functional Ca^{2+}-activated Cl^- channels in wild-type mice [111]. The reconstitution of purified human TMEM16A protein in proteoliposomes produced Ca^{2+}- and voltage-gated Cl^- currents with a submicromolar Ca^{2+} sensitivity, thus directly demonstrating that TMEM16A forms the pore of Ca^{2+}-activated Cl^- channels [99].

Expression of TMEM16A was identified in various VSMC types from rodent and human arteries [25, 27, 28, 62, 100, 108], while expression of TMEM16B appeared to be very low [27]. Inhibition of TMEM16A by small interfering RNAs or TMEM16A-specific antibodies [14, 25, 27, 28, 62, 100, 108] suppressed Ca^{2+}-activated Cl^- currents in VSMCs from mouse aorta, rat cerebral artery, and rat and rabbit pulmonary arteries. A systematic analysis of TMEM16A expression in mice by combined Western blotting and immunohistochemistry revealed high levels of expression in the large conduit arteries as well as in the second order and smaller arterioles in the brain, skeletal muscle, and retina, whereas TMEM16A expression in primary arterioles and small arteries was small or absent [45]. Throughout the arterial tree, expression densities of Ca^{2+}-activated Cl^- currents paralleled those of TMEM16A (Fig. 2), and targeted disruption of TMEM16A in VSMCs entirely eliminated Ca^{2+}-activated Cl^- currents as well as TMEM16A immunoreactivity in all vascular segments investigated, suggesting that in the mouse, Ca^{2+}-activated Cl^- currents in arterial VSMCs are largely generated by TMEM16A. In contrast to the mouse, TMEM16A was detected in rat and human small arteries [25, 27, 28, 62, 100, 108]. Thus some species differences appear to exist. Moreover, $T16A_{inh}$-A01 [28], an aminophenylthiazole that had been identified as a low μM inhibitor of TMEM16A-mediated currents by a cell-based high throughput screen [72], induced only a partial block of Ca^{2+}-activated Cl^- currents in rabbit pulmonary artery VSMCs even when administered at high concentrations [28].

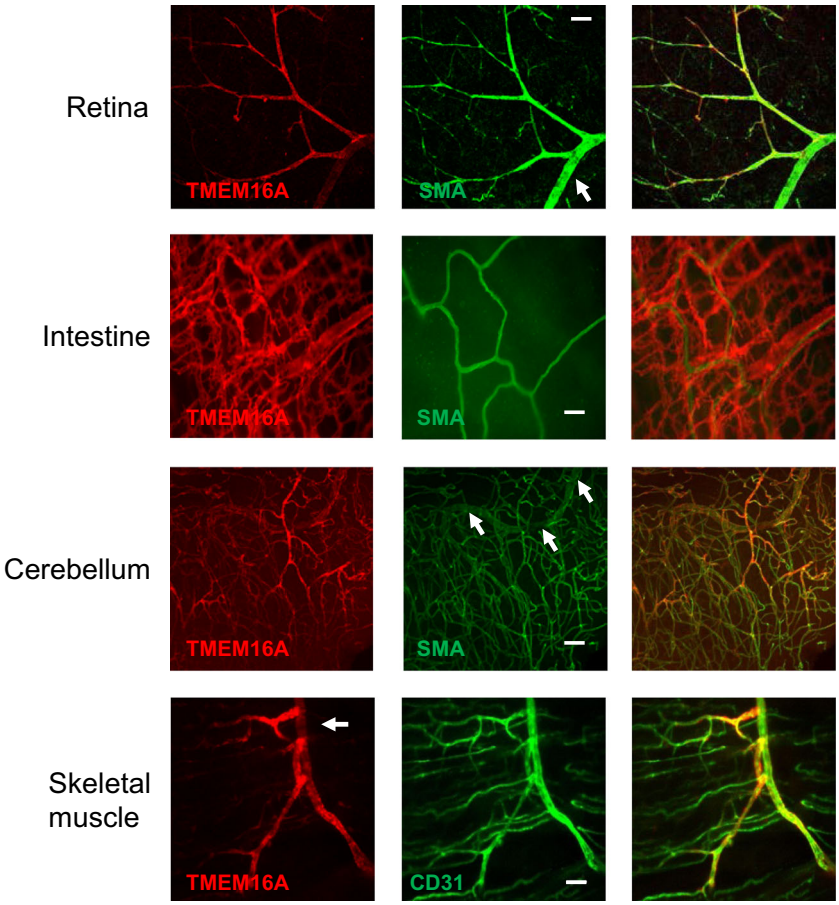

Fig. 2 Expression of TMEM16A in murine small blood vessels of various tissues. Double immunostaining for TMEM16A (*left*), smooth muscle actin (*SMA*), or endothelial marker CD31 (*middle*), and overlay (*right*) of various whole mount preparations. The *first row* is retina. Whereas first order arterioles do not show strong TMEM16A staining (*arrow*), smaller arterioles are more intensely stained. *Scale bar* indicates 30 μm. The *second row* is small intestine. While no arterioles are present in the projection images of the confocal stack and the small arteries do not express TMEM16A, an intense staining is found in the so-called intestinal cells of Cajal (*ICC*) [40], where TMEM16A is required for pacemaker activity [48]. *Scale bar* indicates 50 μm. The *third row* is a cerebellar slice. Significant TMEM16A expression is only found at the branching points of the largest vessel (*three arrows*), whereas smaller arterioles show stronger expression. *Scale bar* indicates 50 μm. The *fourth row* is a skeletal muscle slice. A *small artery running from the top to the bottom* (*arrow*) shows increasing TMEM16A expression with decreasing diameter caused by multiple branching events. Scale bar indicates 30 μm.

Downregulation of TMEM16A-associated Ca^{2+}-activated Cl^- currents through siRNA significantly diminished the vasoconstrictor response to noradrenaline and vasopressin in rat mesenteric arteries [25, 27, 62, 100, 108] and to intravascular pressure elevation in rat cerebral arteries [14]. Mouse mesenteric arteries and human visceral adipose tissue arteries preconstricted with the thromboxane mimetic U46619 were also relaxed by pharmacological inhibition of TMEM16A with $T16A_{inh}$-A01 [28]. In mice with an inducible VSMC-specific knockout of TMEM16A, the vasoconstrictor effects of U46619 were attenuated in retinal arterioles, but not in small mesenteric arteries, which have a very weak expression of TMEM16A [45]. These mice displayed reduced response to U46619 in isolated perfused hind limbs, were hypotensive, had smaller arterial pressure amplitudes, and developed less severe hypertension in response to chronic infusion of angiotensin II [45]. Collectively, these findings indicate that TMEM16A has a role for the Windkessel function of large conduit arteries and participates in the physiological regulation of basal vascular tone and vascular reactivity of resistance vessels to agonist-induced vasoconstriction in vivo.

Gating of TMEM16A is both voltage- and Ca^{2+} dependent. At $[Ca^{2+}]_i$ in the nM range, the current displays outward rectification which is lost when $[Ca^{2+}]_i$ is increased. The activation velocity is also strongly Ca^{2+} dependent: while the channel opens slowly at nM $[Ca^{2+}]_i$, activation is accelerated with increasing $[Ca^{2+}]_i$. These biophysical properties are well known from earlier studies on Ca^{2+}-activated Cl^- currents [57] and may serve presently unknown functions in VSMC. Recent observations suggest that Ca^{2+} exerts its modulatory influence on TMEM16A by directly binding to the channel protein. The Ca^{2+}-activated Cl^- current produced by reconstituted TMEM16A was not affected by calmodulin [99], and calmodulin did not alter activation or anion

permeability of heterologously expressed mouse TMEM16A [112, 113]. In addition to $[Ca^{2+}]_i$ and membrane voltage, PI(4,5)P_2 has been shown to act as a very potent inhibitor of TMEM16A at concentrations as low as 1 μM [81]. Since cytosolic PI(4,5)P_2 has been estimated to be in a range of 10–210 μM, relieve from tonic PI(4,5)P_2 inhibition may constitute a gating mechanism.

Activation of TMEM16A by localized increase in $[Ca^{2+}]_i$ also likely underlies the elusive stretch-regulated Cl^- current in the myogenic response [14]. The myogenic response helps to maintain blood flow constant in the face of fluctuations of perfusion pressure and depends on the intrinsic capacity of VSMCs in resistance vessels to increase their tone in response to increases in transmural pressure. Key events contributing to the myogenic response are a membrane depolarization and opening of L-type Ca^{2+} channels leading to an elevation of $[Ca^{2+}]_i$ [51]. Several mechanisms have been proposed to mediate the membrane depolarization in the myogenic response, including opening of mechanosensitive ion channels, activation of sphingosine-1-phosphate signaling, and activation of angiotensin AT1 receptors [89, 109, 114, 115]. The idea that increases in transmural pressure also activates Cl^- channels to depolarize and constrict cerebral resistance vessels originated from the observation that Cl^- channel blockers induced hyperpolarization and dilatation and that reductions in the extracellular Cl^- concentration augmented the myogenic tone [73]. Furthermore, direct measurements of Cl^- flux using a Cl^--selective electrode demonstrated a pressure-dependent increase in Cl^- efflux in intact cerebral resistance arteries [31]. Cell swelling induced by hypotonicity activated Cl^- currents with a comparable pharmacology also in pulmonary and renal arteries [110]. Initially, volume-regulated anion channels (VRAC) have been suggested to underlie this current [73]. However, specific inhibition by small interfering RNA demonstrated that a knockdown of TMEM16A causes a reduction of the myogenic response in cerebral resistance arteries by ~50 % [14]. Moreover, the Cl^- current evoked by cell swelling could be blocked by TMEM16A downregulation using short interfering RNAs, TMEM16A antibodies, or the fast Ca^{2+} chelating agent BAPTA and displayed similar biophysical properties as TMEM16A. Further studies will be required to assess whether activation of TMEM16A by localized increases in $[Ca^{2+}]_i$ also underlies other volume-regulated Cl^- currents in the cardiovascular system. In addition, it will be important to evaluate possible functions of the LRRC8 gene family, which recently has been shown to encode components of the volume-regulated anion channel VRAC [82, 103] that has been suggested to be important for VSMC proliferation [92].

Several pathologies are associated with an altered expression of TMEM16A. Pulmonary arterial VSMC expresses a prominent TMEM16A current [62]. Chronic hypoxia for 3–4 weeks in rats strongly upregulated TMEM16A protein expression and Ca^{2+}-activated Cl^- currents, which likely enhanced pulmonary reactivity to serotonin, a well-established mediator of chronic hypoxic pulmonary hypertension [97]. Very similar observations were made in rats treated with monocrotaline, an experimental drug for the induction of pulmonary hypertension [34]. In 2-kidney, 2-clip hypertensive rats, TMEM16A expression and Ca^{2+}-activated Cl^- currents were decreased in the basilar artery in an inverse relation to blood pressure [108]. Interestingly, knockdown of TMEM16A expression in cultured basilar VSMCs promoted angiotensin II effects on cell cycle, whereas TMEM16A overexpression suppressed the action of angiotensin II.

Besides the Ca^{2+}-activated Cl^- current encoded by TMEM16A, a cGMP-dependent Ca^{2+}-activated Cl^- current is co-expressed in many VSMC types. This current differs in several biophysical aspects from the classic Ca^{2+}-activated Cl^- current; in particular, it is voltage independent over the entire Ca^{2+} range. Downregulation of bestrophin-3 gene expression with small interfering RNAs suppressed the cGMP-dependent Ca^{2+}-activated Cl^- current in isolated cells as well as in vivo [64]. The amplitude of vasomotion was significantly dampened after downregulation of bestrophin-3, whereas vascular contractility was unaffected [12]. This suggests that bestrophins are involved in the regulation of tissue perfusion by synchronizing VSMC function. Bestrophin-3 belongs to the bestrophin family, which comprises four family members; each member forms a homotetrameric channel [9]. Interestingly, TMEM16A knockdown also inhibited the cGMP-dependent Ca^{2+}-activated Cl^- current, probably secondary to a suppression of bestrophin-3 [25]. The functional significance of this interaction is not clear. It has been suggested that bestrophin acts as a regulatory subunit of channels formed by TMEM16A to modify its biophysical properties [24].

Cl^- also plays a role for sequestration of Ca^{2+} into the sarcoplasmic reticulum. Regulation of Ca^{2+} by the sarcoplasmic reticulum is essential for normal contractile function of muscle cells. In skeletal muscle, the sarcoplasmic reticulum maintains a large Ca^{2+} gradient, but there is no electrical potential between its lumen and the cytoplasm [79]. Its membrane is permeable to Cl^-, and the entry of Cl^- into sarcoplasmic reticulum along with Ca^{2+} is thought to prevent the development of an electrical potential opposing further Ca^{2+} accumulation. Currently, the molecular pathway for Cl^- entry into the sarcoplasmic reticulum is not known, but bestrophin-1 has been suggested to form a Ca^{2+}-dependent counter-anion channel to balance the transient membrane potential associated with Ca^{2+} release and store refill [8].

Cystic fibrosis transmembrane conductance regulator

The cystic fibrosis transmembrane conductance regulator (CFTR) is a cyclic AMP-regulated Cl^- channel. Loss-of-function mutations in CFTR cause cystic fibrosis [91]. Human

patients with cystic fibrosis have decreased blood pressures [60, 98], an observation also made in piglets and mice carrying the most common CFTR mutation, CFTR-F508del [42, 78]. CFTR protein has been detected by Western blotting and immunofluorescence in the mouse aorta and intrapulmonary arteries [85, 86]. Cultured aortic VSMCs from wild type but not CFTR-knockout mice responded with an increased iodide efflux to cAMP agonists as well as to the $beta_{1/2}$ adrenoceptor agonist isoproterenol [85]. This effect was suppressed by the specific CFTR antagonist $CFTR_{inh}$-172. Correspondingly, aortic rings from CFTR-knockout mice showed a much stronger, endothelium-independent vasoconstrictor response to elevated K^+ than aortic rings from wild-type animals [85]. In addition, CFTR-knockout mice had a higher myogenic tone in pressurized isolated cerebral and mesenteric arteries [67]. Most of the inhibitory effects of CFTR activation on VSMC contraction were observed under depolarizing experimental conditions. Accordingly, activation of CFTR may predominantly operate at membrane potentials above the Cl^- equilibrium potential to repolarize the VSMC explaining the surprisingly relaxing effects of CFTR activation (Fig. 1). Since CFTR currents have not been demonstrated yet in VSMCs, the hyperpolarizing effect may also be secondary to a regulatory effect of CFTR on other membrane channels.

Conclusion

The findings presented in this review strongly suggest that Cl^- transporters and Cl^- channels in VSMCs significantly contribute to the physiological regulation of vascular tone. The observation that mice with an inducible VSMC-specific knockout of TMEM16A are hypotensive without showing any measurable changes in renal function [45] further supports the concept that primary changes in vascular function can cause sustained changes in arterial blood pressure [68, 76]. However, one should bear in mind that renal hemodynamics affects water and electrolyte homeostasis. Long-term blood pressure is particularly sensitive to alterations in the renal medullary circulation, and it has been demonstrated that a selective increase in vascular resistance in this vascular bed is sufficient to cause a sustained increase of arterial blood pressure [23, 65]. Accordingly, a decreased vascular contractility caused by vascular disruption of TMEM16A may also operate through an altered regulation of the extracellular fluid balance to reduce blood pressure.

The role of Cl^- for VSMC function is probably much more complex than the simple model that $[Cl^-]_i$ above equilibrium just sets the driving force to boost contraction via Cl^- channels. Cell-specific knockout mouse models will help to further advance the understanding of the role of Cl^- for the regulation of arterial blood pressure. However, as VSMCs are highly heterogeneous and have markedly different functional properties in different organs [83], it is a difficult task if nearly impossible to predict the effects of knockout strategies on systemic blood pressure.

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