Ion Channels in Endothelial Responses to Fluid Shear Stress

Fluid shear stress is an important environmental cue that governs vascular physiology and pathology, but the molecular mechanisms that mediate endothelial responses to flow are only partially understood. Gating of ion channels by flow is one mechanism that may underlie many of the known responses. Here, we review the literature on endothelial ion channels whose activity is modulated by flow with an eye toward identifying important questions for future research.

Fluid shear stress, the frictional drag force caused by blood flow over the endothelial luminal surface, is one of the environmental cues sensed by the endothelial cells that line the vasculature. Endothelial cell (ECs) experience a range of shear stress profiles in vivo that differ in magnitude, direction, and temporal characteristics, depending on location within the vasculature. These shear profiles are sensed by the endothelial cells and modulate cell behavior. Long-term unidirectional flow within the physiological range (typically 10–40 dynes/cm² for human arteries) promotes EC quiescence, with low proliferation and low inflammatory gene expression. Conversely, low flow or flow with changes in direction (multidirectional or oscillatory flow) promotes EC proliferation and turnover, and activates inflammatory pathways (22). In vivo, these flow patterns confer sensitivity to other risk factors such as hyperlipidemia, smoking, and diabetes, resulting in development of atherosclerotic plaques. On short time scales, decreases or increases of shear stress in resistance arteries trigger vasoconstriction or dilation, respectively. In the longer term, sustained changes in shear initiate inward or outward vessel remodeling (40, 67, 96). Fluid shear stress is also an important factor in controlling vascular development and the maturation of blood vessels (69).

Although our understanding of mechanotransduction is still fragmentary, several primary receptors of shear stress have been proposed, including G-protein-coupled receptors (GPCRs), junctional proteins, primary cilia, membrane lipids, and the apical glycocalyx (5). Mechanosensitive channels have also been proposed as primary detectors of shear stress, with many research groups reporting effects of shear stress on plasma membrane permeability and ionic flow. ECs are not excitable, so they do not express high levels of voltage-gated channels and do not show a propagated response to depolarization or hyperpolarization like neurons or muscle cells. However, the ionic conductances regulated by shear stress could influence a broad range of EC and vascular functions. The electrical coupling between ECs and smooth muscle cells (SMCs) also means that EC channels could directly control vascular tone (33). This review will discuss the changes in ion permeability observed in endothelial cells exposed to fluid shear stress and the roles various ionic fluxes may play in shear stress responses (Table 1).

Calcium

Calcium is a critically important cellular signal. It acts locally at the cell membrane, affecting ion channel and receptor activity, and globally throughout the cell, controlling transcription factors and functioning as a cofactor for enzymes (24). It is thus unsurprising that calcium is postulated to play a role in endothelial signaling in general and flow sensing in particular. The best-defined role for shear stress-activated calcium signaling in ECs is flow-induced vasodilation (FIV). Increased shear stress in arteries results in elevated EC cytosolic calcium, leading to activation of endothelial nitric oxide synthase (eNOS) and production of nitric oxide (NO). NO diffuses from the endothelium and triggers relaxation of smooth muscle cells, causing dilation of the vessels and thereby reducing wall shear stress to maintain homeostasis (35). In the FIV response, increased cytosolic calcium also triggers opening of calcium-activated potassium channels, which hyperpolarize the endothelium (82). The adjacent smooth muscle cells also hyperpolarize, likely through direct electrical coupling via gap junctions between the two cell types, increasing smooth muscle cell relaxation (46). Calcium signaling can also modulate several processes that are affected by shear stress. In addition to controlling NO production, calcium regulates endothelial prostacyclin synthesis, release of endothelin-1, and junctional permeability and contractility through myosin light chain
phosphorylation (17, 41, 57). However, whether changes in calcium are required for the effects of shear stress on these processes is unknown.

There have been many reports that show that onset of shear stress increases intracellular calcium in a magnitude-dependent manner (3, 4, 31, 44, 52, 66, 80, 84, 88, 105–107, 109, 123). Yet there is little agreement among different studies about the characteristics of this response, with large differences reported in threshold and half-maximal activation, latency, persistence of the calcium rise during application of shear stress, decay after stopping shear stress, and synchrony of the calcium response within a population of cells. This variability, which can presumably be attributed to differences in cell type, culture conditions, shear apparatus, and measurement techniques, suggests that calcium is regulated by multiple shear-sensitive components and multiple mechanisms.

### Calcium Sources, Stores vs. Plasma Membrane Channels

Cytosolic calcium levels can be increased due to influx of extracellular calcium through the plasma membrane and through release from intracellular stores. Influx in response to shear stress could be controlled by either direct mechanical gating of calcium permeable membrane channels or indirect activation of these channels through other pathways (3, 106). Release from stores is generally controlled through activation of phospholipase C (PLC), which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to produce diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). The latter gates calcium-permeable IP3R channels on the endoplasmic reticulum (ER), releasing calcium into the cytosol. Both RTKs and G proteins can stimulate PLC and are activated by shear stress (8, 18, 28, 59, 70). Activation of PLC and increased IP3 and DAG have also been observed within minutes after the application of shear (12, 89, 98). Thus elevation of cytosolic calcium via release from intracellular stores is expected.

Removing calcium from the extracellular solution blocks influx through the plasma membrane but does not affect release from stores; stores can be specifically depleted using thapsagargin. A number of studies have used these techniques to show that shear-stimulated increases in cytosolic calcium were wholly or partially dependent on release from stores, consistent with activation of the PLC pathway (3, 31, 44, 56, 76, 109). However, in some conditions, calcium increases were reported to be entirely due to influx from the medium (66, 86, 106, 107). There is little information about how calcium stores affect longer-term behavior of cells in vitro and virtually nothing about the role for this type of calcium signaling in endothelial cells in vivo.
**ATP and Its Receptors**

Shear stress can activate membrane receptors via both ligand-dependent and -independent mechanisms. There is evidence for ligand-independent activation of both VEGF receptors and GPCRs by flow; however, calcium signaling downstream of these receptors has not been reported (18, 59, 61). The best studied ligand-dependent shear-sensitive pathway involves adenosine triphosphate (ATP) receptors whose response to ATP is potentiated by shear stress. Several reports have demonstrated a rise in cytosolic calcium in response to onset of shear stress that requires extracellular ATP (31, 58, 118, 123–125). At low extracellular ATP concentrations, this rise is due to an influx of calcium through P2X4, an ATP-sensitive cation channel (123, 124). However, purinergic GPCRs like P2Y2 also contribute to calcium responses at higher ATP concentrations, or higher shear stresses, through activation of PLC and release of calcium from stores (118, 123). Strikingly, loss of P2X4 in ECs in vitro also decreases shear-induced transcription of Krüppel-like factor 2 (Klf2), an important flow-dependent transcription factor (102). In animal models, loss of P2X4 leads to a defect in both FIV and nitric oxide production, and defects in high shear stress-induced outward remodeling (125). However, since this is a global knockout, non-endothelial responses or developmental defects in the vasculature could contribute to this phenotype. When floxed P2Y2 was inducibly deleted in endothelial cells in adult mice, FIV decreased and mean arterial blood pressure increased, strongly suggesting that this receptor regulates shear stress responses (118). Whether the effect of P2Y2 loss is dependent on loss of calcium signaling was not investigated.

The mechanism by which shear stress potentiates purinergic receptor activity is unclear. Both P2Y2- and P2X4-dependent shear responses are blocked by apyrase, an enzyme that degrades extracellular ATP, indicating that these responses are ATP-dependent (118, 126). Consistent with this result, P2X4 is not directly gated by shear when expressed in heterologous systems (63). ATP is released by endothelial cells in response to shear stress and can act locally on time scales of a few seconds (122, 126). Coupled with local hydrolysis of ATP at the membrane and increased agonist delivery due to an increase in fluid flow rate, this release can partially explain the ATP-dependent calcium responses (31). However, because the mechanism of ATP release by shear stress is unknown, it has not been possible to determine whether the responses of P2X4 and P2Y2 are wholly due to increased concentration of extracellular ATP or whether there are additional signaling components that alter receptor sensitivity or change calcium dynamics. P2X4 does appear to display ATP release-independent responses to shear. In heterologous systems, P2X4 shows reduced inactivation and higher current density in response to ATP under shear stress compared with static conditions, which suggests that shear (directly or indirectly) stabilizes the open conformation of the channel (63). There are several possible mechanisms by which shear stress could alter P2X4 activity. For example, phosphoinositides inhibit inactivation and internalization of P2X4; thus shear stress-induced changes in phosphoinositide metabolism could influence channel activity (10).

**Force-Gated Channels, TRPV4, and Piezo1**

The simplest mechanism by which shear stress could induce calcium influx would be through direct activation of mechanically gated calcium permeable channels. Such channels could either be sensitive to membrane tension and/or curvature, or can respond to cellular deformation through tethering to the cytoskeleton and/or the extracellular matrix. Putative mechanosensitive calcium permeable channels have been reported in endothelial cells, and activities with similar pharmacological and/or physiological properties have been observed in response to shear stress (60, 68, 86, 106, 107, 110). It has therefore been hypothesized that such a channel may be partly or wholly responsible for calcium transients observed at the onset of shear in ECs. The identity of this channel or channels was not known at the time of these experiments; however, further studies have identified two mechanosensitive calcium permeable channels that may contribute to shear-evoked calcium responses in ECs. The first is transient receptor potential cation channel V4 (TRPV4), a member of the polymodal TRP channel family. TRPV4 is activated by cell swelling in several cell types, suggesting activation by membrane tension; it can also be activated by shear stress in ECs when expressed heterologously, suggesting direct mechanical activation (73, 80, 111). However, osmotic swelling-induced activation of TRPV4 has been reported to be indirect, mediated by accumulation of arachidonic acid, a lipid mediator known to activate TRPV4 (117, 119). Additionally, rapid translocation of TRPV4 to the cell surface in response to shear stress has been reported in heterologous systems, which could contribute to shear stress-activated TRPV4 currents (7). Interestingly, TRPV4 is one of the few TRP channels that has been shown to form heteromers with other TRP channel family members. It has been suggested that TRPV4 forms heterodimers with TRPC1 as well as a heterotrimeric complex of TRPV4, TRPC1, and Polycystin-2 (Pkd2), and that these heteromers conduct the
majority of the TRPV4-dependent shear-sensitive calcium response (30, 77). Although it is unclear whether TRPC1 or Pkd2 alter the shear sensitivity of TRPV4 channels, TRPC1 appears to alter inactivation of the heteromer and may play a role in the adaptation of the channel to prolonged shear stimuli (77).

Significant work has been done to address the role of TRPV4 in vivo in the vasculature. Genetic deletion of TRPV4 reduced both FIV in mice and calcium responses to onset of flow in ECs in vitro (48, 80). However, interpretation of this result is complicated by the effects of TRPV4 in other cell types, such as smooth muscle cells, and other systemic effects that might influence vascular function, such as osmoregulation, which could cause differences in blood pressure and serum ion concentrations (32, 74, 83). Pharmacological tools have been used to address the issue of systemic effects. Activation of TRPV4 with the phorbol ester 4-phorbol-12,13-didecanoate (4PDD) in isolated arteries induces vasodilation, whereas blockade of TRPV4 with the non-specific TRP channel blocker ruthenium red (RR) inhibited FIV (65). Pharmacological activation of TRPV4 with 4PDD also increased, whereas inhibition with RR reduced flow-induced cerebral arteriogenesis (104), providing some suggestive evidence for its involvement in flow-dependent remodeling in vivo. However, these pharmacological tools come with many caveats, especially RR, which has many off-target effects.

The other candidate is Piezo1 (Fam38a), which with Piezo2 forms a family of atypical channels directly activated by mechanical force and implicated in several mechanosensory processes (26, 95, 101, 120, 128). Like TRPV4, Piezo1 can be activated by shear stress when expressed heterologously in HEK cells, although the threshold for shear-induced currents seems to be above the threshold for calcium entry in ECs (100). Deletion of Piezo1 in ECs reduced calcium influx in response to the onset of shear stress and blocked their alignment in flow, a physiologically important flow response that has not been reported for other channels. Unlike loss of TRPV4 and P2X4, genetic deletion of Piezo1 in mice causes severe defects in vascular development, leading to embryonic lethality even with endothelial specific knockout (71, 100). Loss of Piezo1 also reduced the activation of the key flow-dependent transcription factor Klf2. The severity of the Piezo1 knockout phenotype suggests that this channel has a role beyond contributing to calcium entry in the short-term FIV response. Interestingly, loss or inhibition of Piezo1 in red blood cells reduced shear-stimulated release of ATP (23). If applicable to ECs, loss of ATP release could affect shear-induced activation of P2Y2 and P2X4, which could contribute to the Piezo1−/− phenotype. However, other downstream effects are likely, and the role of Piezo1 in sensing cellular stretch, independent of shear stress, may also contribute.

**Primary Cilia and Calcium Regulation**

In several cell types, flow-induced calcium signaling is controlled by a mechanosensory complex localized to primary cilia, a signaling complex that includes the TRP channel Pkd1 and its regulator Pkd2 (87). Bending of the cilia is an attractive mechanism for sensing very low flows (97). Embryonic endothelial cells exhibit primary cilia and show flow-induced calcium responses that are blocked by knockdown or genetic deletion of proteins involved in cilia formation, or of Pkd1 or Pkd2 (1, 88). Cilia appear to be required for flow-induced calcium responses in early zebrafish embryos, and loss of key cilia genes causes defects in embryonic angiogenesis (45, 62). In mice, loss of Pkd1 also affects vascular function, and knockout mice die in utero with pronounced hemorrhaging and moderate edema, whereas Pkd2-null mice show pronounced edema and focal hemorrhages (64, 121). However, global knockout mice also show profound defects in both vascular smooth muscle behavior and lymphatic development, which complicates the interpretation of the vascular phenotype (49, 93, 99). In adults, cilia are confined to regions of the vasculature that are under low flow, which correlates well with disruption of EC primary cilia by higher shear (54) and suggest that ciliary signaling is likely most important in early embryogenesis or in specific low-flow regions of adult vessels. However, recent work blocking cilia formation in endothelial and hematopoietic lineage cells through mutation of Ifitm8 produced no defect in retinal angiogenesis, suggesting that cilia per se may not be important for vascular development in mice (29). The difference between this phenotype and loss of Pkd2 or Pkd1 could be due to cilia-independent mechanosensory defects, since Pkd1 and 2 are not restricted to cilia (95, 108). Pkd2 in particular may interact with both TRPV4 and Piezo1 to affect mechanosensitivity through both of these channels (30, 95). Interestingly, mutation of Ifitm8 exacerbates atherosclerosis in apolipoprotein E-null mice, suggesting that ciliary signaling may mitigate inflammatory signaling in areas of low flow (29). However, Ifitm8 also has cilia-independent functions, so this phenotype may also involve other mechanisms (13, 14).

**Potassium**

In addition to calcium influx, onset of shear stress triggers rapid EC hyperpolarization. The characteristics of this hyperpolarization are consistent with
the activation of potassium channels and efflux of cytoplasmic potassium (92). Under static conditions, control of potassium flux is important for establishing resting membrane potential. In cells that express voltage-gated channels, this affects excitability and action potential dynamics; in ECs, which are nonexcitable, the role of potassium is less well understood. Inhibition of potassium channel activity in endothelial cells has been linked to lower shear stress-induced transforming growth factor β1 (TGFβ1) transcription and activity; it is also associated with production of cyclic guanosine monophosphate (cGMP) and, in isolated arteries ex vivo, with flow-induced vasoconstriction (25, 90, 91). However, these studies rely on broad spectrum potassium channel blockers and do not distinguish between subtypes that may have very different roles. Blocking potassium channels can also modulate other ionic fluxes, including calcium. Hyperpolarization due to potassium efflux increases the driving force for calcium influx, increasing calcium entry through calcium channels. Conversely, calcium-sensitive potassium channels can be activated in response to an influx of calcium. This cross talk between the two ionic fluxes can make it difficult to separate their roles. Although flow-induced changes in potassium flux were first reported in the 1980s (92), the effects of this response on endothelial cell behavior are still not well understood.

**$K_{\text{r}2.1}$ and $K_{\text{r}2.3}$**

The potassium current activated by the onset of shear stress is inwardly rectifying and blocked by barium, both characteristics of the potassium voltage-gated channel subfamily J (KCNJ) channels (55, 85, 92). This family contains 18 members, with KCNJ2 ($K_{\text{r}2.1}$) being the predominant member in ECs (37). $K_{\text{r}2.1}$ can be activated by shear in heterologous systems, making it a good candidate for the fast hyperpolarizing, flow-activated channel (53). $K_{\text{r}2.1}$ is also linked to a number of other flow-sensitive pathways. $K_{\text{r}2.1}$ activity and cell surface expression are regulated by GPCR signaling, 5′ AMP-activated protein kinase (AMPK), protein kinase A (PKA), and protein kinase C (PKC), all of which are affected by shear stress (2, 34, 129). Surprisingly, although the role of potassium efflux has been examined in ECs using the potassium channel blocker barium, $K_{\text{r}2.1}$ has not been targeted specifically, in part due to a lack of pharmacological tools. It is therefore unclear whether $K_{\text{r}2.1}$ is required for flow-induced activation of potassium efflux in ECs and whether activation of this channel interacts with other shear stress-sensitive kinases such as Akt or extracellular signal-regulated kinases (ERK1 and ERK2). Interestingly, $K_{\text{r}}$-like currents are also reduced by VLDL, which blocks flow-induced activation of $K_{\text{r}}$. This is consistent with reduced FIV in animal models of hypercholesterolemia, suggesting that these channels may play a role in pathological responses to shear in athero-prone model systems (36).

Genetic ablation of $K_{\text{r}2.1}$ inhibits extracellular potassium-evoked dilation in cerebral arteries, although this has been primarily attributed to the effect of $K_{\text{r}2.1}$ in smooth muscle cells (127). Loss of $K_{\text{r}2.1}$ also causes perinatal lethality, due at least in part to a cleft palate in these mice, making this mouse non-ideal for specific analysis of the role of $K_{\text{r}2.1}$ in endothelial cells (127). However, recent work using $K_{\text{r}2.1}$ heterozygotes, which show greatly reduced $K_{\text{r}}$ currents in isolated endothelial cells, suggests that this channel directly affects FIV (Levitan I, personal communication). Previous work linked potassium channel activity, specifically, the activation of small conductance calcium-activated potassium channels ($K_{\text{r}2.3}$, KCNN3), to NO-independent FIV (15). Unlike $K_{\text{r}2.3}$, the effects of $K_{\text{r}2.1}$ are NO-dependent (Levitan I, personal communication). This result suggests that $K_{\text{r}2.1}$ activation potentiates eNOS activity; increased calcium signaling is a likely mechanism, but this remains to be demonstrated. By contrast, $K_{\text{r}2.3}$ activation appears to be downstream of flow-induced calcium influx and contributes to FIV by increasing release of endothelial hyperpolarization factor or through direct electrically coupling with smooth muscle cells (15).

**Potassium and Cessation of Flow**

Cessation of flow has been studied as an aspect of ischemia, although fast responses to flow cessation occur without hypoxia or other metabolic sequelae. Several studies have shown that flow-adapted endothelial cells depolarize upon cessation of flow in vitro (19–21). This response is blocked by cromakalim, an activator of ATP-sensitive potassium channels, suggesting that depolarization is due to closing of these potassium channels (19). This would suggest that ATP-sensitive potassium channels in endothelial cells, like $K_{\text{r}}$ channels, are activated in response to shear stress. Long-term shear upregulates a number of potassium channels, including the ATP-sensitive channel $K_{\text{r}6.2}$ (KCNJ11) and the calcium-sensitive channel $K_{\text{r}3.1}$ (KCNN4), which may explain why this depolarization is not observed in cells under shear for short times (19, 113). $K_{\text{r}6.2}$-dependent depolarization of ECs after cessation of flow in mouse lungs ex vivo has also been reported (20). Although this response likely contributes to the effect of ischemia in vivo, it is unclear whether depolarization is a general response to decreasing shear stress and whether it plays a role in vasocon-
striction or in long-term adaption to decreased shear stress.

**Chloride**

After the fast, potassium-based hyperpolarization, shear stress induces a subsequent large depolarization in ECs in vitro. Surprisingly, this depolarization is reduced by chloride channel blockers, suggesting a role of chloride efflux in shear stress signaling (6, 43, 75). Although opening of chloride channels is most frequently associated with influx of chloride and therefore hyperpolarization, some cell types show high levels of cytosolic chloride, flipping the driving force and causing chloride efflux in response to channel opening. Since this current has a fairly slow latency, it is unlikely that chloride efflux is due to mechanically gated chloride channels. Rather, it could be downstream of flow-induced calcium influx, since inhibitors of the chloride current primarily target calcium-activated chloride channels (CaCCs), which are activated by cytosolic calcium. Interestingly, blocking CaCCs reduces shear-induced activation of Akt (43), which is downstream of the PECAM-mediated junctional signaling and upstream of eNOS activation (among other pathways) (116). Membrane depolarization also seems to affect Akt signaling in the flow cessation/ischemia model, suggesting that ECs may respond to all depolarizing signals by increasing phosphoinositide 3-kinase (PI3K) and Akt activity (20). Chloride channel activation shows a low shear threshold, similar to potassium channel activation, but saturates well below 10 dynes and is suppressed by higher shear stresses; by contrast, the potassium signal saturates ~12–15 dynes and does not appear to be inhibited by high shear stress (43, 92). These findings suggest that chloride signaling may play a role in the response to low shear stress and thus may be part of an inflammatory response. However, without knowing the identity of the channel(s) involved, the importance of this flow-activated current is difficult to ascertain due to the paucity of specific pharmacological tools for chloride channels.

**Sodium**

Sodium, along with potassium, is a major regulator of membrane potential. Dietary intake of high salt is correlated with hypertension in humans and reduces FIV in animal models (78). However, since sodium influx is important for smooth muscle contraction, it is difficult to determine to what extent endothelial cells mediate these effects. In lieu of a method to directly alter endothelial sodium influx without altering smooth muscle cells, a few groups have examined the role of sodium channels in endothelial shear stress responses in vitro. Although all non-specific cation channels conduct sodium ions to some degree, there are only two families of sodium-specific cation channels: the voltage-gated sodium channels (NaV) and epithelial sodium channels (ENaC). Although there is debate over whether either of these types of channels are expressed in ECs, and, if they are expressed, what their function might be, the role of both types of channels in shear stress signaling has been examined. One group reported that both loss of extracellular sodium and blocking NaV channels with tetrodotoxin increased ERK phosphorylation in response to shear stress (114). Although some NaV channels are directly sensitive to mechanical forces, the shear stress-induced depolarization of endothelial cells would likely be sufficient to activate these channels, potentiating depolarization (6, 11). This could suggest that the shear activation of the ERK pathway is in some way sensitive to membrane potential, much like the PI3K-Akt pathway. Another group observed ENaC-like channel activation in endothelial cells by flow and hydrostatic pressure (47). Since ENaC channels are activated by flow in heterologous systems, it is possible that these channels contribute directly to the mechanical response to flow (103). However, significant work remains to prove that sodium channels are important for endothelial responses to shear in vitro and in vivo. Information on different channels is summarized in **FIGURE 1**.

**Channel Activity and Shear Profiles**

Flow magnitude, pulsatility, direction, and temporal variations have major effects on EC phenotype. However, few studies have addressed how differing shear profiles affect channel activity. Early work suggested that calcium influx in ECs can be stimulated by steady or pulsatile flow but not oscillatory flow, suggesting that calcium influx is related to the anti-inflammatory adaptive response to laminar shear (51). This group also reported that pulsatile flow increased the frequency of EC calcium oscillations more strongly than laminar flow of the same average magnitude (52). Pulsatile flow, typical of arteries, activates some anti-inflammatory, vessel-stabilization pathways more strongly than steady flow; it will be interesting to test whether calcium oscillations contribute to these effects. However, work in animal models have suggested that TRPV4, which contributes to calcium responses to flow, is important for responses to oscillatory flow in zebrafish endocardium during valve development (50). Additionally, ciliary calcium signaling in adult mice, which theoretically contributes to shear sensing in regions of disturbed flow, modulates development of atherosclerosis
These studies suggest that calcium may also respond to oscillatory flow and be functionally important.

In terms of other ionic fluxes, laminar but not oscillatory shear was found to induce CaCC-mediated depolarization; by contrast, fast hyperpolarization, presumably due to $K_{\text{ir}}$ activation, was seen under all flow conditions (75). This result suggests that the slow depolarization of endothelial cells may be specific to laminar shear stress and could play a role in the anti-inflammatory adaptive response to shear stress, whereas the fast hyperpolarization may play a role in the inflammatory long-term response to disturbed shear. However, without more detailed information about the mechanisms of channel activation and their downstream consequences, it is difficult to determine how or whether channel activity is related to endothelial cell behavior in vivo.

**Concluding Remarks**

Given the amount of evidence for shear stress-activated (or -inhibited) conductances in endothelial cells, it is somewhat surprising that there is so little consensus about their function in shear stress signaling. In some cases, the specific channels responsible for changes in endothelial cell permeability have yet to be identified, but even where candidate channels are known, their role in shear stress signaling remains poorly understood. Although it is clear that FIV involves shear stress-induced activation of endothelial calcium and potassium channels, only a handful of reports address the effects of EC channels on other long-term physiological responses to shear stress, from development to remodeling to atherosclerosis. Similarly, few papers have addressed the role of channel activity in modulation of established shear stress EC responses, such as activation of kinases like Akt or ERK, changes in gene expression, and changes in cytoskeletal organization and EC alignment. Overall, although channels are clearly activated directly and indirectly by shear stress, functional roles remain to be explored in depth.

As targets for pharmaceutical intervention, channels are a mixed bag. Opening or closing a channel through pharmacological intervention can have profound effects on cell function or even viability, independent of more fine-tuned signaling responses. For example, chronic reduction of intracellular potassium activates inflammasomes, whereas chronically elevated calcium can trigger apoptosis (79, 94). However, if mechanosensitivity could be targeted independently of basal activity (e.g., shifting the threshold of activation by force), pharmacological tools could alter shear responses in a potentially useful way. This would be of particular relevance to channels like Kir2.1, which show altered flow responses in pathological condi-

![Diagram](image_url)

**FIGURE 1.** Diagram of putative shear stress-activated channel subtypes in endothelial cells and their interactions

Specific candidate genes are listed below their subtype. Flow activates non-specific cation channels, which depolarize the cell and increase cytoplasmic calcium. Flow also activates voltage-gated and epithelial sodium channels ($N_{\text{v}}$ and ENaC), which increase sodium influx, and calcium-sensitive chloride channels (CaCC), which increase chloride efflux. Activation of both of these channels leads to depolarization. Conversely, flow activates calcium-activated and inwardly rectifying potassium channels ($K_{\text{ir}}$, and $K_{\text{nc}}$), leading to hyperpolarization. Calcium influx directly activates both CaCCs and $K_{\text{ir}}$ channels. In contrast, hyperpolarization potentiates calcium influx through open channels by increasing the driving force, which propels calcium into the cell, whereas depolarization decreases this driving force. Additionally, depolarization directly activates voltage-gated channels like $N_{\text{v}}$, whereas hyperpolarization inhibits these channels.
tions (36). Although there are currently few drugs that target mechanosensitive channels, recent work identifying the Piezo1 agonist Yoda1 offers a proof of principal for a drug that modulates the mechanosensitivity of a channel (112). However, without better understanding the role of specific channels in the short- and long-term responses to shear stress, it is difficult to propose specific targets for drug development.

Despite these limitations, available evidence supports the importance of regulated channel activity in shear stress signaling. One aspect of potential interest concerns the ability of ECs to distinguish fine temporal features of shear stress profiles, such that subsecond dynamics influence signaling outputs and EC phenotype (38). Sensing changes in flow on this time scale would require pathways that are both activated and inactivated with suitably fast kinetics. Although G-protein signaling can be activated in seconds, rates of inactivation are slower and thus seem incompatible with sensing flow pulsatility. Channels, however, often have on and off rates on these time scales, particularly Piezo channels, which show rapid inactivation as well as activation (26). Elucidating the functions of specific channels in distinct EC flow responses therefore offers considerable promise for solving important problems in vascular biology.

We thank Dr. Irena Levitan for helpful comments, recommendations, and critical reading of this manuscript.

This work was supported by National Heart, Lung, and Blood Institute Grant PO1 HL-107235 to M.A.S.

No conflicts of interest, financial or otherwise, are declared by the author(s).

Author contributions: K.A.G. prepared figures; K.A.G. and M.A.S. drafted, revised, and edited the manuscript; K.A.G. and M.A.S. approved the final version of the manuscript.

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