American Heart Association | American Stroke Association

Regulation of Kir2.1 Function Under Shear Stress and Cholesterol Loading

Nhat-Tu Le, PhD; Jun-ichi Abe, MD, PhD

Lining the innermost layer of blood vessels, the endothelium is in direct contact with circulating blood elements and, consequently, is affected by mechanical forces generated by blood flow. Among these forces, fluid shear stress site-specifically regulates endothelial cells (ECs): In regions where the flowing blood is multidirectional (low shear stress or disturbed flow), ECs are activated and atherosclerotic plaque is detected; in regions where the flowing blood is unidirectional (high shear stress or laminar flow), ECs are normal and atherosclerotic plaque is not detected. These 2 flow patterns differently trigger changes, not only morphologies and functions but also biochemical and biological events of ECs.[1] Responding to high shear stress, ECs release vasoactive substances including prostacyclin, EC-derived hyperpolarizing factor, and nitric oxide (NO)—important factors that mediate EC-dependent vasodilation. NO increases the production of 3′, 5′-cGMP, subsequently activates cGMP-dependent protein kinase in vascular smooth muscle cells. Consequently, ECs orchestrate vascular tone and vascular homeostasis.[2] Accordingly, EC dysfunction contributes to a number of diseases, including atherosclerosis.[3]

Mechanosensory Pathways and Ion Channels

Several mechanosensory pathways such as PECAM-1 (platelet endothelial cell adhesion molecule 1)/vascular endothelial cadherin/VEGFR2 (vascular endothelial growth factor receptor 2)/VEGFR3 (vascular endothelial growth factor receptor 3) have been proposed in ECs. One of the important mechanosensory pathways is formed by groups of ion channels including P2X receptors, TRPV4 (transient receptor potential cation channel subfamily V member 4), the epithelial Na^+ channel, and Piezo1 (piezo-type mechanosensitive ion channel component 1)/Piezo2 (piezo-type mechanosensitive ion channel component 2). These channels have a crucial role in regulating early Ca^{2+} sparklets (localized Ca^{2+} influx) and subsequently increase NO by upregulating Akt (protein kinase B) and PKA (protein kinase A). In addition, voltage-insensitive small- and intermediate-conductance Ca^{2+}-activated K^+ channels are also activated by Ca^{2+} channels with outward-rectifying (with outward current) Cl^- channels, leading to membrane hyperpolarization. Interestingly, Piezo1 regulates ATP secretion from ECs and upregulates NO synthesis by activating the Gq/G11-coupled purinergic receptor of P2Y2.[4]

Cholesterol Loading, Kir2.1, and EC Dysfunction

In addition to Ca influx, the contribution of K^+ channels in regulating shear stress–induced EC hyperpolarization has been reported. The Kir2.1 isoform is the major K^+ channel in the endothelium of mesenteric resistance arteries.[5,6] Kir2.1 plays a crucial role in shear stress–induced vasodilation through an NO-dependent mechanism. Shear stress–induced Kir2.1 activation upregulates Akt kinase activity and subsequently induces vasodilation via endothelial NO synthase activation.[5] EC-specific Kir2.1 knockdown mice exhibit reduced TRPV4-dependent vasodilatation.[6] The elevation of lipid levels in blood, namely, hyperlipidemia or hypercholesterolemia, is a major risk factor for EC dysfunction and atherosclerosis.[7,8] Elevated levels of lipid in blood decrease NO bioavailability, impair EC-dependent vasodilation, and activate mechanisms that cause EC dysfunction and subsequent atherosclerotic plaque formation. Of note, it has been reported that cholesterol loading inhibits EC K^+ channels[9]; however, the actual role of Kir2.1 in regulating cholesterol loading-induced EC K^+ channel activation

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

From the Department of Cardiovascular Sciences, Houston Methodist Research Institute, Houston, TX (N.-T.L.); Department of Cardiology, The University of Texas MD Anderson Cancer Center, Houston, TX (J.-i.A.).

Correspondence to: Jun-ichi Abe, MD, PhD, Department of Cardiology, University of Texas MD Anderson Cancer Center, 2121 W, Holcombe Blvd., IBT8 803e, Houston, TX 77030. E-mail: jabe@mdanderson.org

J Am Heart Assoc. 2018;7:e008749. DOI: 10.1161/JAHA.118.008749.

and subsequent EC dysfunction and atherosclerosis formation remains to be examined.

In this issue of *Journal of the American Heart Association (JAHA)*, Fancher et al have assessed the functional role of cholesterol-mediated Kir2.1 channel suppression in a model of hypercholesterolemia by performing patch clamp analyses on isolated mouse ECs.[10] The authors presented experimental evidence demonstrating that Kir2.1 suppression is a contributing factor in the impairment of shear stress–mediated NO bioavailability and EC-dependent vasodilation induced by hypercholesterolemia. When loading ECs isolated from the resistance arteries with cholesterol using modified low-density lipoproteins, the shear stress–mediated increase of Kir2.1 current was inhibited. In microvascular ECs treated with acetylated low-density lipoprotein or ECs isolated from hypercholesterolemic apolipoprotein E–deficient ($ApoE^{-/-}$) mice, shear stress–induced Kir2.1 currents were significantly suppressed while the Kir2.1 protein expression level remained intact. In both scenarios, the suppression of Kir2.1 currents was rescued when cholesterol was removed by using methyl-β-cyclodextrin, confirming the role of cholesterol in suppressing Kir2.1 currents. In addition, the authors found that the shear stress–induced NO production and vasodilation of mesenteric arteries were impaired by cholesterol in $Kir2.1^{+/-}/ApoE^{-/-}$ but not $Kir2.1^{+/+}/ApoE^{-/-}$ mice. This impaired Kir2.1 current observed in $Kir2.1^{+/-}/ApoE^{-/-}$ mice was irreversible even if cholesterol was removed. Importantly, in this setting, the small- and intermediate-conductance Ca^{2+}-activated K^+ channel function appears largely intact. With these findings, the authors suggest that the reduction of Kir2.1 current is a key event in hypercholesterolemia-induced reduction of NO production and vasodilation under shear stress. Last, the authors found that reduced Kir2.1 expression in the hypercholesterolemic setting ($Kir2.1^{+/-}/ApoE^{-/-}$) led to an increase in atherosclerotic lesion formation, particularly in the atheroresistant area in the descending aorta. These data suggest the key role of Kir2.1 in hypercholesterolemia-induced EC dysfunction and atherosclerosis formation.

Posttranslational Modification of Kir2.1 and Lipidation

Because the authors could not find any change of Kir2.1 expression by shear stress and cholesterol loading, it is most likely that posttranslational modification of Kir2.1 may have some contribution for this activity change. The authors' group showed the possible role of direct binding of cholesterol with Kir2.1 in regulating its function and sensitivity (Figure 1).[11] But especially under the condition of shear stress, posttranslational modification of Kir2.1 may have a significant role in regulating Kir2.1 function. The Swiss-Prot knowledge database (https://www.nextprot.org/entry/NX_P63252/sequence) and the PhosphoSitePlus database (https://www.phosphosite.org/proteinAction.action?id=18795&showAllSites=true) list the following 5 co- or posttranslational modification sites in human Kir2.1 (KCNJ2 [potassium voltage-gated channel subfamily J member 2]): (1) N-myristoyl glycine (MG^2SVRTNR), (2) phosphoserine (S10), (3) phosphotyrosine (Y242), (4) phosphoserine (S425), and (5) S-nitrocyteine (TTC^{76}VDIRW; Figure 1).

Lipidation

Mixtures of lipid and proteins are the major components of biological cell membranes. The proteins can be directed to the cell membrane by co- or posttranslational modifications with specific lipids, which is called *lipidation*. Several lipidation processes have been reported on the basis of the identity of the attached lipids such as N-myristoylation, S-palmitoylation (S-acylation), S-prenylation, and the glycosylphosphatidylinositol anchor.[12] N-myristoylation is an important lipid modification of proteins containing a saturated acyl group of 14 carbons attached to the NH2-terminal glycine via an amide bond after cleavage of the initiating methionine. This process is catalyzed by N-myristoyl transferase, which recognizes a general consensus myristoylation sequence of glycine-X-X-X-(serine/threonine/cysteine) containing the NH_2-terminal glycine. This modification is generally regarded as cotransnlational and as a constitutive process, but it can occur posttranslationally. Caspase activation during apoptosis, for example, cleaves an internal glycine residue of the proapoptotic protein Bid and increases its N-myristoylation posttranslationally, leading to Bid mitochondrial translocation and subsequent apoptosis. It has been reported that cholesterol loading itself did not show any effect on EC apoptosis, but

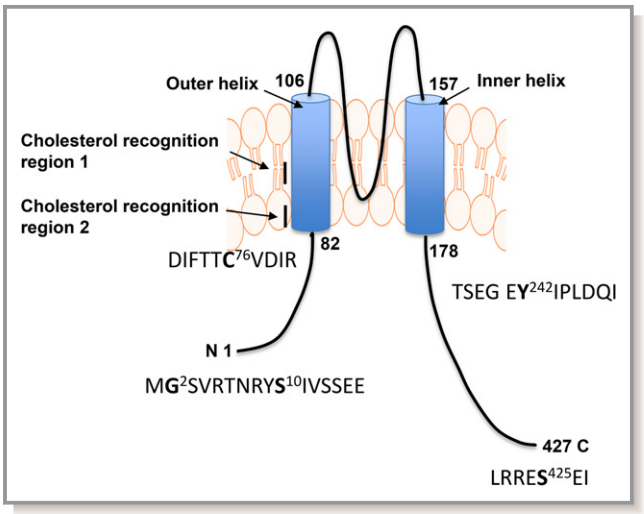

Figure 1. Scheme of Kir2.1 with posttranslation modification sites and cholesterol recognition regions.

cholesterol oxides (oxysterols) can increase EC apoptosis.[13] Because flow can increase $ONOO^-$, it is possible that cholesterol loading under flow increases oxysterols and induces EC apoptosis, which increases caspase activity and subsequently upregulates N-myristoylation of Kir2.1 and changes Kir2.1 function.

Phosphorylation (Y242 and S425)

Hinard et al[14] and Zhang et al[15] reported that protein tyrosine kinase inhibitors inhibit Kir2.1 currents, and the mutation of Y242 downregulates this inhibition. Vega et al have reported that PKA inhibits outward Kir currents, which were eliminated by the Ser425 mutation.[16] The increases in activation of various tyrosine kinases and cAMP/PKA signaling after shear stress have been reported. Consequently, the contribution of Y242 and S425 phosphorylation by shear stress to Kir2.1 activation remains unclear.

Phosphorylation (S10)

This site was detected by mass spectometry as the substrate of AMPK.[17] Although the functional consequence of Kir 2.1 S10 phosphorylation has not been reported, it is well known that shear stress can upregulate AMPK activity and inhibit EC activation; therefore, it is possible that AMPK activation induced by shear stress phosphorylates Kir2.1 S10 and activates its activity. In addition, it is well known that the activation of AMPK restores cellular energy balance by switching on catabolic pathways and switching off anabolic pathways and inhibits cholesterol content in the cellular membrane. Consequently, the interplay between shear stress–induced AMPK activation and cholesterol loading may be important to determine the activity of Kir2.1.

S-Nitrosocyteine ($TTC^{76}VDIRW$)

NO can selectively modify catalytically active protein cysteine residues to form S-nitrosocysteine, and this cysteine S-nitrosylation can regulate protein function, localization, and stability induced by the formation of S-nitrosoproteins (protein S-nitrosothiols). S-nitosylation of Drp1, for example, causes hyperactivation and excessive mitochondrial fission, and the nitrosylation mutant of Drp1 (C644A) significantly downregulates amyloid β–mediated mitochondrial fission and apoptosis. In the current study, Fancher et al found that shear stress can increase Kir2.1 activation, and it is well known that shear stress increases NO production; therefore, it is possible that Kir2.1 C76 S-nitrosylation is upregulated by shear stress. In fact, Hoffmann et al[18] and Huang et al[19] reported that shear stress increases S-nitrosylation in various molecules such as caspase 3, p21ras, and thioredoxin.

Cholesterol-Rich Lipid Raft and Myristoylated Akt

In the previous section we discussed the possible role of Kir2.1 co- or posttranslational modification in regulating its function. In the current study, the negative role of cholesterol loading in Kir2.1 function was suggested, but the molecular mechanism of this inhibition remains unclear. Cholesterol is not only a critical component of biological membranes but also an important component of the detergent-resistant microdomain of the plasma membrane called *lipid rafts*. Caveolae, which have been suggested as playing a mechanosensing role, are lipid raft structures that are specialized by containing caveolin proteins. Adam et al reported the functional role of lipid rafts in regulating Akt activation (Figure 2).[20] They showed that myristoylated Akt1,

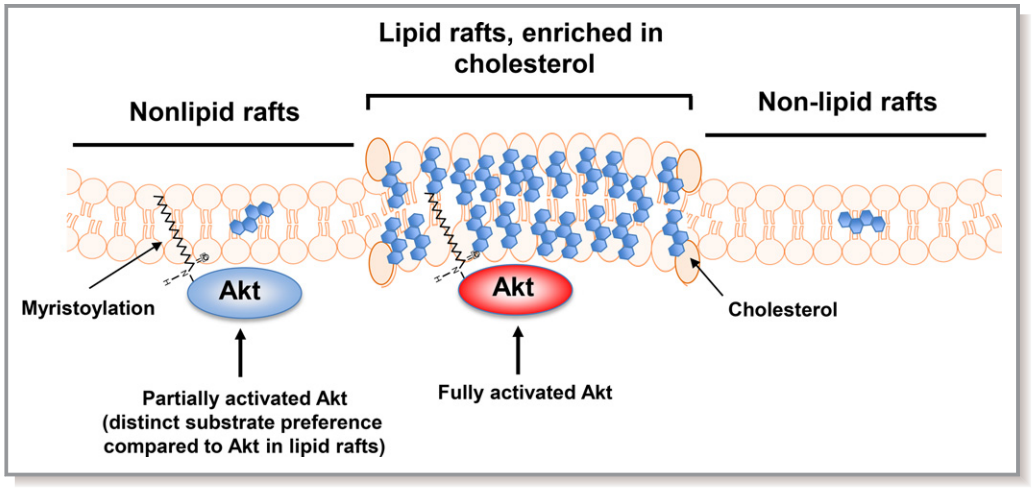

Figure 2. Scheme showing Akt (protein kinase B) activation in nonlipid and lipid rafts.[20] Blue indicates cholesterol.

which is the constitutively active form of Akt, is expressed mainly in the lipid raft fraction compared with wild-type Akt; the activated Akt in the lipid raft is attenuated by membrane cholesterol depletion, and cholesterol repletion restored this Akt activation. Interestingly, the authors proposed that the translocation of Akt to the cholesterol-rich lipid raft from the nonraft compartment is the key event for Akt to be fully activated. It is well known that myristoylation makes Akt localized to the cell membrane, leading to full activation by constitutive phosphorylation of threonine 308 and serine 473 of Akt. Adam et al proposed that not only the process of myristoylation but also the localization of Akt in the cholesterol-rich lipid raft is crucial for fully activating Akt. Because Kir2.1 activation was also regulated by cholesterol loading and depletion, as shown in the current study, it is possible that both the posttranslational modifications and the cholesterol-rich lipid raft localization of Kir2.1 may affect Kir2.1 activation. Further investigation will be necessary.

Is Activation of Kir2.1 a Good Therapeutic Target for Inhibiting Coronary Events?

Because overexpression of Kir2.1 can inhibit cholesterol loading–induced EC dysfunction, it is reasonable to assume that the activation of Kir2.1 can be a good target for inhibiting coronary events; however, Kir2.1 V93I mutation on chromosome 17q24.3, which is a gain-of-function mutant, was reported in familial ATFB9 (atrial fibrillation 9).[21] Consequently, systemic Kir2.1 activation may not be a good strategy. It will be necessary to consider endothelial-specific approaches to activate Kir2.1. In particular, the regulatory mechanism of cholesterol loading–mediated inhibition of Kir2.1 function remains unclear. Because attenuating the cholesterol loading–mediated inhibition of Kir2.1 function may not overactivate Kir2.1 function, even systemically, this may be a good strategy to inhibit atherosclerosis. This strategy simply impairs the hypercholesterolemia-induced attenuation of Kir2.1 function. These issues support further investigation to determine the functional role of posttranslational modifications of Kir2.1 under hypercholesterolemia and shear stress.

EC dysfunction and atherosclerosis are major risk factors for cardiovascular diseases. The contribution of the impairment of the K^+ channel in hypercholesterolemia-induced EC dysfunction has been suggested, but the exact mechanism remains unclear. Fancher et al have nicely shown the crucial role of Kir2.1 in cholesterol loading–induced EC dysfunction and atherosclerosis formation both in vitro and in vivo.[10] The role of ion channels as part of mechanosensory pathways has attracted substantial attention.[22] These findings provide us with a novel mechanism of hypercholesterolemia-induced vascular dysfunction and support the importance of endothelial Kir2.1 channels as a novel target for combating this disease. The overactivation of Kir2.1 may cause atrial fibrillation. Consequently, it will be crucial to determine how Kir2.1 function is inhibited by cholesterol loading to further explore Kir2.1 as a therapeutic target for suppressing coronary disease.

Acknowledgments

We thank Dr Keigi Fujiwara for discussions and critical reading of this article.

Sources of Funding

The research activities of the authors are supported by grants from the National Institutes of Health to Drs Le (HL-134740) and Abe (HL-130193, HL-123346, and HL-118462).

Disclosures

None.

References

1. Lu D, Kassab GS. Role of shear stress and stretch in vascular mechanobiology. *J R Soc Interface*. 2011;8:1379–1385.
2. Berk BC, Abe JI, Min W, Surapisitchat J, Yan C. Endothelial atheroprotective and anti-inflammatory mechanisms. *Ann N Y Acad Sci*. 2001;947:93–109; discussion 109–111
3. Abe J, Berk BC. Novel mechanisms of endothelial mechanotransduction. *Arterioscler Thromb Vasc Biol*. 2014;34:2378–2386.
4. Baratchi S, Khoshmanesh K, Woodman OL, Potocnik S, Peter K, McIntyre P. Molecular sensors of blood flow in endothelial cells. *Trends Mol Med*. 2017;23:850–868.
5. Ahn SJ, Fancher IS, Bian JT, Zhang CX, Schwab S, Gaffin R, Phillips SA, Levitan I. Inwardly rectifying K(+) channels are major contributors to flow-induced vasodilatation in resistance arteries. *J Physiol*. 2017;595:2339–2364.
6. Sonkusare SK, Dalsgaard T, Bonev AD, Nelson MT. Inward rectifier potassium (Kir2.1) channels as end-stage boosters of endothelium-dependent vasodilators. *J Physiol*. 2016;594:3271–3285.
7. Nelson RH. Hyperlipidemia as a risk factor for cardiovascular disease. *Prim Care*. 2013;40:195–211.
8. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res*. 2000;87:840–844.
9. Romanenko VG, Rothblat GH, Levitan I. Modulation of endothelial inward-rectifier K+ current by optical isomers of cholesterol. *Biophys J*. 2002;83:3211–3222.
10. Fancher IS, Ahn SJ, Adamos C, Osborn C, Oh M, Fang Y, Reardon CA, Getz GS, Philips SA, Levitan I. Hypercholesterolemia-induced loss of flow-induced vasodilation and lesion formation in Apolipoprotein E–Deficient Mice Critically Depend on Inwardly Rectifying K^+ Channels. *J Am Heart Assoc*. 2018;7:e007430. DOI: 10.1161/JAHA.117.007430.
11. Levitan I, Singh DK, Rosenhouse-Dantsker A. Cholesterol binding to ion channels. *Front Physiol*. 2014;5:65.
12. Casey PJ. Protein lipidation in cell signaling. *Science*. 1995;268:221–225.
13. Luchetti F, Crinelli R, Cesarini E, Canonico B, Guidi L, Zerbinati C, Di Sario G, Zamai L, Magnani M, Papa S, Iuliano L. Endothelial cells, endoplasmic reticulum stress and oxysterols. *Redox Biol*. 2017;13:581–587.
14. Hinard V, Belin D, Konig S, Bader CR, Bernheim L. Initiation of human myoblast differentiation via dephosphorylation of Kir2.1 K+ channels at tyrosine 242. *Development*. 2008;135:859–867.
15. Zhang DY, Wu W, Deng XL, Lau CP, Li GR. Genistein and tyrphostin AG556 inhibit inwardly-rectifying Kir2.1 channels expressed in HEK 293 cells via protein tyrosine kinase inhibition. *Biochim Biophys Acta*. 2011;1808:1993–1999.

16. Vega AL, Tester DJ, Ackerman MJ, Makielski JC. Protein kinase A-dependent biophysical phenotype for V227F-KCNJ2 mutation in catecholaminergic polymorphic ventricular tachycardia. *Circ Arrhythm Electrophysiol*. 2009;2:540–547.

17. Hoffman NJ, Parker BL, Chaudhuri R, Fisher-Wellman KH, Kleinert M, Humphrey SJ, Yang P, Holliday M, Trefely S, Fazakerley DJ, Stockli J, Burchfield JG, Jensen TE, Jothi R, Kiens B, Wojtaszewski JF, Richter EA, James DE. Global phosphoproteomic analysis of human skeletal muscle reveals a network of exercise-regulated kinases and AMPK substrates. *Cell Metab*. 2015;22:922–935.

18. Hoffmann J, Dimmeler S, Haendeler J. Shear stress increases the amount of S-nitrosylated molecules in endothelial cells: important role for signal transduction. *FEBS Lett*. 2003;551:153–158.

19. Huang B, Chen SC, Wang DL. Shear flow increases S-nitrosylation of proteins in endothelial cells. *Cardiovasc Res*. 2009;83:536–546.

20. Adam RM, Mukhopadhyay NK, Kim J, Di Vizio D, Cinar B, Boucher K, Solomon KR, Freeman MR. Cholesterol sensitivity of endogenous and myristoylated Akt. *Cancer Res*. 2007;67:6238–6246.

21. Xia M, Jin Q, Bendahhou S, He Y, Larroque MM, Chen Y, Zhou Q, Yang Y, Liu Y, Liu B, Zhu Q, Zhou Y, Lin J, Liang B, Li L, Dong X, Pan Z, Wang R, Wan H, Qiu W, Xu W, Eurlings P, Barhanin J, Chen Y. A Kir2.1 gain-of-function mutation underlies familial atrial fibrillation. *Biochem Biophys Res Commun*. 2005;332:1012–1019.

22. Nilius B, Droogmans G. Ion channels and their functional role in vascular endothelium. *Physiol Rev*. 2001;81:1415–1459.

Key Words: Editorials • posttranscriptional regulation • potassium channels • signal transduction