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Cholesterol and Kir channels

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Abstract

To date, most of the major types of Kir channels, Kir2s, Kir3s, Kir4s and Kir6s, have been found to partition into cholesterol-rich membrane domains and/or to be regulated by changes in the level of membrane cholesterol. Surprisingly, however, in spite of the structural similarities between different Kirs, effects of cholesterol on different types of Kir channels vary from cholesterol-induced decrease in the current density (Kir2 channels) to the loss of channel activity by cholesterol depletion (Kir4 channels) and loss of channel coupling by different mediators (Kir3 and Kir6 channels). Recently, we have gained initial insights into the mechanisms responsible for cholesterol-induced suppression Kir2 channels, but mechanisms underlying cholesterol sensitivity of other Kir channels are mostly unknown. The goal of this review is to present a summary of the current knowledge of the distinct effects of cholesterol on different types of Kir channels *in vitro* and *in vivo*.

Introduction

Cholesterol is one of the major lipid components of the plasma membrane in all mammalian cells and is essential for cell function and growth. An excess of cholesterol above the physiological level, however, is cytotoxic (34,93,94) and is associated with the development of cardiovascular disease (38,78,83). The basis for both cholesterol requirement and for its cytotoxicity is its ability to alter the function of integral membrane proteins. Indeed, a growing number of studies have demonstrated that changes in the level of membrane cholesterol affect the function of multiple membrane receptors and ion channels including different types of K⁺ channels (7,27,51,53,75,76), Ca²⁺ channels (1,8,43,46,87), Na⁺ channels (47,90) and Cl[−] channels (42,77). Multiple types of ion channels were also shown to be associated with cholesterol-rich membrane domains (reviewed by (48)). However, the mechanisms underlying cholesterol regulation of membrane proteins in general and of ion channels in particular are still not understood. Furthermore, the impact of cholesterol on different types of ion channels is highly heterogeneous. The most common effect is cholesterol-induced decrease in channel activity (decrease in open probability, unitary conductance and/or the number of active channels). This effect was observed for Ca²⁺ sensitive (7) and certain types of inwardly-rectifying K⁺ channels (75), voltage-gated Na⁺ channels (90), N-type Ca²⁺ channels (87) and volume-sensitive Cl[−] channels (42). In contrast, epithelial Na⁺ channels (eNaC) (81) and TrpC channels (43) are inhibited by cholesterol depletion, suggesting that multiple mechanisms are involved in the sensitivity of ion channels to cholesterol. In general, three mechanisms have been proposed to account for the sensitivity of ion channels to cholesterol: (1) direct interaction between the channels and cholesterol that involves binding of a cholesterol molecule to the channel protein (3,50) and (2) an increase in the energetic cost of channel transitions between closed and open states due to an increase in the stiffness of lipid membrane bilayer (2,46,47) and (3) association of

the channels with other regulatory proteins or lipids within the scaffolds generated by cholesterol-rich membrane domains (48,52). However, the mechanistic details and the structural basis for the sensitivity of ion channels to cholesterol have not been established yet.

This review focuses on inwardly rectifying K⁺ channels (Kir), a major class of K⁺ channels which play critical roles in the maintenance of membrane potential and K⁺ homeostasis regulating multiple cellular functions including membrane excitability, heart rate and vascular tone (5,39,62). Kir channels are classified into seven sub-families (Kir1–7) identified by distinct biophysical properties, such as the degree of current rectification and unitary conductance, and by their sensitivities to different mediators (reviewed by (39,62)). Several types of Kir channels belonging to the different sub-families were shown to be sensitive to the levels of membrane cholesterol and/or to be associated with cholesterol-rich membrane domains (lipid rafts) (Figure 1). In most cases, cholesterol-induced effects were observed on the level of whole-cell currents or changes in membrane potential. Surprisingly, the ways cholesterol affects different types of Kir channels are significantly different. Specifically, an increase in membrane cholesterol resulted in a significant decrease in Kir2 whole cell currents but, as discussed in more detail below, had no effect on the open probability, the unitary conductance or the rectification properties of the channels indicating that cholesterol-induced suppression of Kir2 channels is due to a decrease in the number of active channels. In contrast, Kir4 channels were shown to be inhibited by cholesterol depletion. The impact of cholesterol on Kir6 channels is still controversial. The goal of this review is to summarize what has been discovered so far about cholesterol sensitivities of different Kir channels and about the mechanisms underlying this sensitivity.

Kir2 channels

Kir2 channels are ubiquitously expressed in a variety of cell types, Kir 2.1–2.3 are expressed in the heart (30,55,89), all Kir2s (2.1–2.4) are expressed in neurons (61,73,86), and Kir2.1 and Kir2.2 are expressed in vascular smooth muscle (9,33,95) and endothelial cells (19,22,32). It is also known that Kir2 channels are critically involved in the regulation of the excitability and contraction of cardiac (56,68) and smooth muscle cells (95). In endothelial cells, Kir2 channels set the membrane potential under resting conditions and are suggested to be one of the primary flow sensors (18,64). In addition, Kir2 channels directly regulate neurovascular coupling in cerebral arterioles (20). Indeed, downregulation of Kir channels in ventricular myocytes is associated with heart failure in human patients (4). Furthermore, mutations in Kir2.1 are known to cause Andersen's syndrome, an autosomal disorder characterized by cardiac arrhythmias and periodic paralysis (71,88). It was also shown that targeted disruption of Kir2.1 gene in mice is lethal; the animals die within 12 hours after birth (95). Suppression or downregulation of Kir2 channels, therefore, is expected to have a major impact on the cardiovascular system.

Cholesterol-induced suppression of Kir2 activity *in vitro* and *in vivo*

Our studies have shown that Kir2 currents are strongly suppressed by an increase in membrane cholesterol both *in vitro* and *in vivo* (17,59,75,76). This effect was first observed in aortic endothelial cells, in which resting membrane potential is dominated by Kir2 channels (19). Endothelial Kir current is significantly suppressed by exposing the cells to methyl- β -cyclodextrin (M β CD) saturated with cholesterol, a well-known cholesterol donor, and facilitated by exposing the cells to M β CD not saturated with cholesterol that acts as a cholesterol acceptor (Figure 2A, B,(76)). More recently, we have shown (17) that endothelial Kir currents are also suppressed by low density lipoproteins (LDLs), acetylated LDL a modification of naturally occurring LDL that is commonly used in lipoprotein research to load cells with cholesterol (e.g. (23,74)) and by β -VLDL, a naturally occurring

lipoprotein that was also shown to load endothelial cells with cholesterol (35). The degree of Kir suppression correlated with the degree of cholesterol loading and was fully reversible upon the removal of cholesterol excess.

To test the effect of cholesterol on endothelial Kir currents *in vivo*, we compared the currents in endothelial cells freshly-isolated from aortas of Yorkshire pigs fed atherogenic high-cholesterol diet with the currents recorded in cells isolated from healthy animals (17). The pig model was chosen because porcine lipoprotein profiles are similar to those in humans (84) and because hypercholesterolemic pigs develop atherosclerotic lesions within a few months after the initiation of high cholesterol diet (13). As expected, feeding the pigs high-cholesterol diet resulted in significant elevation of plasma cholesterol levels with the plasma LDL increasing from 33 ± 13 mg/dL to 244 ± 143 mg/dL after one month on the diet. The level of endothelial cholesterol increased approximately two fold. Furthermore, endothelial cells isolated from hypercholesterolemic animals had significantly lower Kir currents than cells isolated from healthy animals. Hypercholesterolemia-induced Kir suppression was also fully reversible by decreasing membrane cholesterol *ex vivo* (Figure 2C). Furthermore, cholesterol-induced suppression of Kir was accompanied with a shift in endothelial membrane potential to a more depolarized value and, most importantly, with suppression of shear stress sensitivity of endothelial Kir channels virtually abolishing flow-induced hyperpolarization (2D). We have also shown that suppression of endothelial Kir correlated with a decrease in flow-induced vasodilatation of femoral arteries measured *in vivo* in the same animals. Suppression of endothelial Kir channels, therefore, may be one of the major mechanisms of endothelial dysfunction in the early stages of atherosclerosis (17,31). Furthermore, suppression of Kir was also observed in bone marrow-derived endothelial progenitor cells isolated from hypercholesterolemic animals suggesting that a loss of Kir activity may also contribute to dysfunction of endothelial progenitor cells (59).

Finally, comparative analysis of the four members of Kir2 family (Kir2.1–2.4) heterologously expressed in Chinese Hamster Ovary cells (CHO) showed that all Kir2 channels are suppressed by cholesterol but the degree of the suppression is not the same for different Kir2 sub-types: Kir2.1 and Kir2.2 channels are most sensitive to cholesterol (~70% inhibition), whereas Kir2.3 is significantly less sensitive (~30% inhibition) and Kir2.4 has intermediate cholesterol sensitivity (~50%) (Figure 3, (75)).

Sensitivity of Kir2 channels to the chiral structure of cholesterol

One of the key questions in elucidating the mechanisms underlying cholesterol effects on membrane proteins is whether cholesterol alters the function of membrane proteins by changing the physical properties of the membrane lipid bilayer or by specific cholesterol-protein interactions. We have addressed this question by partially substituting endogenous cholesterol in aortic endothelial cells by its optical isomer epicholesterol (76). The two stereoisomers, cholesterol (3 β -hydroxy-5-cholestene) and epicholesterol (3 α -hydroxy-5-cholestene) differ only in the rotational angle of the hydroxyl group at position 3 and are known to have similar effects on membrane ordering: both sterols induce a strong condensation effect on the area of the phospholipid molecules, a direct measure of membrane ordering (15), have similar effects on water permeability of the liposomes (6) and equally promote the formation of sterol-rich ordered membrane domains (91). Cholesterol-epicholesterol substitution, therefore, provides a powerful tool to discriminate between specific and non-specific effects of cholesterol. Using this approach, we have shown that substituting ~50% of endogenous cholesterol with epicholesterol has a profound and completely unexpected effect on endothelial Kir current increasing the current amplitude more than 2-fold (Figure 4, (76)). Changes in membrane ordering, therefore, cannot account for cholesterol-induced suppression of endothelial Kir. Instead, we proposed that specific cholesterol-protein interactions are important in the regulation of Kir2 channels and

suggested that epicholesterol-induced increase in Kir activity can be explained by a competition between the two sterols. It is important to point out, however, that sensitivity of the channels to the chiral structure of cholesterol does not necessarily mean that cholesterol interacts directly with the channels. It is equally possible that cholesterol interacts with another membrane protein, which in turn regulates the activity of Kir 2 channels.

“Silent channel” hypothesis

Surprisingly, in spite of the more than two fold changes in whole cell Kir2 currents, no or little effect was observed in the single channel properties of the channels: unitary conductance was not affected at all and the open probability was decreased less than 10% (75,76). Epicholesterol also had no effect on the open probability or unitary conductance of the channels. Rectification properties of the current have not been affected by either of these conditions as well. The simplest interpretation of these observations is that cholesterol-induced decrease in Kir2 whole cell current should be attributed to a decrease in the number of the channels rather than changes in the single channel properties. However, we have also shown that changes in membrane cholesterol have no effect either on Kir2.1 expression, as estimated by Western blot analysis and by immunostaining, or on its plasma membrane level, as estimated by tagging the extracellular domains of the channels (75), as was described earlier (96). Consistent with these observations, inhibition of protein synthesis had no effect on the increase in Kir current upon cholesterol depletion. Taken together, these observations led to a hypothesis that Kir2 channels exist on the plasma membrane in two modes: “active channels” that flicker between the closed and the open states with high open probability and “silent channels” that are stabilized in their closed state. Substitution of cholesterol by epicholesterol, in this case, would interfere with cholesterol-channel interactions and prevent the stabilization of the channels in the “silent” conformation state. We proposed further that Kir2 channels may be silent when they partition into a lipid raft domain and active outside of these domains.

Association of Kir2 channels with lipid rafts

Sensitivity of membrane proteins to cholesterol is frequently associated with their partitioning to cholesterol-rich membrane domains called lipid or membrane rafts. While the exact nature and composition of these domains remains controversial, they are generally defined as “small (10–200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes” (70). These domains are typically isolated either by their insolubility in cold detergents or by separating the membrane on sucrose density gradients or by a combination of the two methods. Using both approaches, we have shown that Kir2 channels partition into detergent-insoluble low-density membrane fractions confirming their association with the cholesterol-rich membrane domains (85). However, Kir2 channels do not partition exclusively to the raft domains but show clear double-peak distributions between low-density (cholesterol-rich) and high density (cholesterol-poor) membrane fractions (85). This observation is consistent with our hypothesis that Kir2 channels may exist in two functional modes. It is also noteworthy, that Kir2.1 which is more sensitive to cholesterol preferentially partitions into the raft domains whereas Kir2.3 that is less sensitive to cholesterol partitions preferentially into the non-raft domains (Figure 5, (47)). These observations support the hypothesis that partitioning into raft domains is important for the sensitivity of the channels to cholesterol. However, it is unlikely that changes in membrane cholesterol regulate Kir activity simply by changing their association with the raft domains because both cholesterol depletion and cholesterol enrichment result only in relatively mild shifts in the distributions of the channels between the raft and the non-raft membrane fractions (10–20%). Also, the fact that cholesterol and epicholesterol that showed a similar propensity to form lipid-ordered domains (91), have opposite effects on Kir2 function, suggests that partitioning in lipid rafts *per se* is unlikely to

account for the sensitivity of the channels to cholesterol. We suggest, therefore, that it is not the association of Kir2 channels with the raft *per se* but the interactions of the channels with other components within the raft that regulate channel activity (85). It is not clear yet, however, whether it is the lipid environment of a raft or protein-protein interactions forming within the raft scaffold that regulate Kir2 function. Possible candidates to regulate Kir2 function within the raft domains are caveolin, a regulatory phospholipid PIP2, or cholesterol itself. Studies are currently in progress to discriminate between these possibilities.

Kir3 channels

Kir3 channels constitute a family of G-protein gated channels that are activated by G protein $\beta\gamma$ subunits and play a major role in the inhibitory regulation of neuronal excitability (Kir3.2 and Kir 3.1/3.2 heteromers) and in the regulation of the heart rate (Kir3.4, Kir3.1/3.4 heteromers) (5,39,45,49,92). Loss of Kir3 channels leads to hyperexcitability in the brain, hyperactivity and seizures, as well as cardiac abnormalities. Furthermore, a mutation in the pore-forming region of Kir3.2 channels results in Weaver syndrome in mice that presents as multiple abnormalities in the neuronal activities (37,65,67). As described below, however, very little is known about the impact of cholesterol on Kir3 channels.

Association with lipid rafts and modulation by other proteins

Kir3.1/3.2 fusion proteins were shown to partition into low-density membrane fractions (lipid rafts) when expressed in CHO cells but their activity was not affected by blocking cholesterol synthesis with lovastatin (14). Interestingly, however, regulation of Kir3.1/3.2 channels by Neural Cell Adhesion Molecule (NCAM) was compromised by lovastatin treatment suggesting that integrity of lipid rafts are important for the interactions of Kir3 channels with NCAM (14). Furthermore, since multiple studies have shown that G-proteins are associated with the raft domains, as was reviewed recently (66), it is possible that $\beta\gamma$ regulation of the channels may also depend on the integrity of lipid rafts. Finally, Haas et al (26) have shown recently that deletion of a number of genes that have been shown to alter the lipid composition of yeast membrane significantly affect functional expression of Kir3.2 channels in the yeast membranes suggesting that membrane lipids may play an important role in the regulation of Kir3.2 trafficking and/or function. Clearly, more studies are needed to determine the role of cholesterol and cholesterol-rich domains in Kir3 function.

Kir4 channels

To date two members have been identified in Kir4 family

Kir4.1 expressed in multiple cell types, including glial and kidney epithelial cells, and implicated in the control of K^+ buffering and homeostasis, and Kir4.2 also found in multiple organs but with its physiological functions are not well established yet (39). Recently, Hibino and Kurachi (2007) (28) have shown that Kir4.1 channels are cholesterol sensitive but in contrast to Kir2 channels, Kir4.1 channels are inhibited by cholesterol depletion.

Cholesterol depletion results in loss of function of Kir4.1

Similarly to Kir2 and Kir3 channels, Kir4.1 was also found to be associated with both raft and non-raft fractions of brain membrane, which in this case were isolated using detergent extraction followed with sucrose density separation (28). Furthermore, Kir5.1 that co-assembles with Kir4.1 was also found in these fractions. However, depleting membrane cholesterol with M β CD, the same method that was used to test cholesterol sensitivity of Kir2 channels (76), resulted in strong inhibition of Kir4.1 activity (28). The activity of Kir4.1 channels was fully restored by repleting the cells with cholesterol. Hibino and Kurachi (2007) (28) suggested that the loss of Kir4.1 activity may be due to the dissociation of the

channels from a regulatory phospholipid PI(4,5)P₂ that is known to be required for the activation of multiple ion channels including Kir channels (29,44) and that also resides in cholesterol-rich membrane domains (69). This interpretation, however, does not explain the dramatic difference between Kir4.1 and Kir2 channels in terms of their sensitivity to cholesterol because Kir2 also require PI(4,5)P₂ for their function. Two observations may shed some light on possible explanations for this difference. One, Hibino and Kurachi (2007) (28) have shown that in contrast to Kir2 channels that partially colocalizes with caveolin (85), Kir4.1 channels showed very little co-localization with caveolin leading to a conclusion that they exist in noncaveolae raft domains. Two, while cholesterol depletion resulted only in a relatively small shift in the distribution of Kir2 channels between raft and non-raft membrane fractions (85), the same treatment resulted in virtual disappearance of Kir4.1 from the raft domains (28). These observations suggest that Kir2 and Kir4 channels may partition into distinct raft domains and that association of Kir2 channels with cholesterol-rich domains is much more stable than that of Kir4 channels. It is possible that these differences underlie the opposite effects of cholesterol on Kir2 and Kir4 function. Alternatively, it is also possible that the structural differences between Kir2 and Kir4 channels result in reverse sensitivities of these channels to cholesterol. However, since the structural determinants of the sensitivity of Kir channels to cholesterol are still unknown, it is impossible to make specific predictions about the possible structural basis for the different sensitivities of Kir2 and Kir4 to cholesterol.

Kir6 (K_{ATP}) channels

K_{ATP} channels are a family of heteromultimeric Kir channels that function as unique channel-enzyme complexes of Kir channels and ATP-binding cassette (ABC) sulfonylurea receptors (SUR) proteins (reviewed by (63,97)). A pore of the channel is formed by a tetramer of Kir6 subunits and each Kir 6 subunit is associated with one SUR protein (11,82). A fundamental feature of K_{ATP} channels is that they are sensitive to the level of cellular ATP thus providing a link between cellular metabolic state and membrane potential (39,58,63,97). More specifically, K_{ATP} channels are inhibited by the non-hydrolytic binding of ATP that interacts with the Kir6 subunits and activated by MgADP that interact with SUR subunits (63,97). SUR subunits are also critical for the pharmacological regulation of K_{ATP} channels. The channels are blocked by sulphonylureas, such as glibenclamide, glimepiride and repaglinide, and opened by a diverse group of agents including pinacidil, cromakalim, and nicorandil that stimulate K_{ATP} activity in a SUR-dependent way (e.g. (25,40,60)). Two members of Kir6 channels have been identified: Kir6.1 channels expressed in vascular smooth muscle cells and Kir6.2 channels expressed in pancreatic β -cells, heart, and brain (39,63,97). Kir6.1 channels are important in regulation of vascular tone and disruption of Kir6.1 gene in mice results in hypercontractility of coronary arteries that resembles Prinzmetal angina in humans (12,57,58). Kir6.2 are well known to play major roles in insulin secretion, cardioprotection and oxygen sensing in the brain, as well as cytoprotection during brain ischemia (21,58,63,97). Kir6.2 channels were also shown to be one of shear stress sensors in endothelial cells (10). Several studies focused on cholesterol sensitivity of K_{ATP} channels expressed in vascular tissues and in the heart but the findings and the interpretations are still controversial.

K_{ATP} channels in hypercholesterolemia

Mathew and Lerman (2001) (54) have shown that pinacidil, a K_{ATP} channel opener, was more effective in increasing coronary blood flow in hypercholesterolemic pigs than in normolipidemic animals. Glibenclamide, a K_{ATP} channel blocker, also had an enhanced effect in hypercholesterolemic animals. Mathew and Lerman (2001) (54) suggested, therefore, that K_{ATP} channels are actually enhanced under these conditions. In contrast, Genda et al (2002) (24) showed that blocking K_{ATP} channels in rabbit hearts has a similar

effect to that of hypercholesterolemia on the no-reflow phenomenon during ischemia-reperfusion injury. Conversely, pharmacological opening of K_{ATP} channels significantly diminished hypercholesterolemia-induced the no-reflow zone. Genda et al (2002) (24) suggested, therefore, that hypercholesterolemia-induced suppression of K_{ATP} channels is a major mechanism underlying microvascular injury under these conditions. Hyperlipidemia-induced suppression of K_{ATP} channels was also implicated in the development of left ventricular hypertrophy in hypercholesterolemic rabbits (41). One possibility to account for these differences in apparent K_{ATP} activity is the difference in the lipid profiles between hypercholesterolemic rabbits and pigs.

Coupling of Kir6.1 channels with PKA and PKC

It is known that in vascular smooth muscle cells, K_{ATP} channels are under control of protein kinases A (PKA) and C (PKC) with PKA activating the channels and PKC having an inhibitory effect (16). Pongo et al (2001) (72) showed that high-cholesterol diet inhibited PKC- K_{ATP} coupling in rabbit coronary arteries. Surprisingly, the same uncoupling effect was observed in response to supplementing the diet with lovastatin, an inhibitor of cholesterol synthesis. In both cases, the coupling was restored by farnesol. It is unlikely, therefore, that these effects can be attributed to changes of cholesterol levels *per se*. Instead, Pongo et al (2001) (72) suggested that PKC- K_{ATP} coupling depends on non-cholesterol mavalonate products. These observations also highlight a possible complication in interpreting experiments using lovastatin to test the effect of cholesterol. More recently, Sampson et al. (79,80) showed that Kir6.1 channels partition into caveolae and co-immunoprecipitate with caveolin and that their coupling with PKA is disrupted by cholesterol depletion. They also suggested that caveolae constitute the platform for PKC-induced inhibition of Kir6.1 channels. It is still not exactly clear how to reconcile the observations that both cholesterol-rich diet and cholesterol depletion lead to similar effects on PKC- K_{ATP} coupling. One possibility is that diet-induced hypercholesterolemia may have complex effects on the level of cellular cholesterol and while it may increase the total level of membrane cholesterol (17), at the same time it may decrease the level of cholesterol in caveolae (36). More studies are needed to clarify these discrepancies.

Concluding Remarks and Future Directions

Sensitivity to cholesterol and association with cholesterol-rich membrane domains appears to be a common feature for multiple types of Kir channels but there is a strong diversity in the effects of cholesterol on channel function, and while some Kir channels, specifically Kir2s, are suppressed by cholesterol, other Kirs, specifically Kir4.1, require cholesterol for their function. The basis for this diversity, however, is completely unknown. Our studies provided the first insights into the mechanisms responsible for cholesterol sensitivity of Kir2 channels suggesting that these channels are regulated by specific lipid-protein interactions but molecular details of these interactions are still not known (75,76,85). Very little is known about the mechanisms of cholesterol sensitivity of the other Kir channels. The only thing that is known about Kir3 channels is that they partition into lipid rafts (14) and it is proposed that partitioning into lipid rafts may affect their regulation by the G-proteins. Similarly, the only thing that is known about Kir4 channels is that they also partition into lipid rafts and that disruption of the rafts results in loss of Kir4 function. It is proposed that this inhibition is due to the dissociation of the channels from PI(4,5)P₂ (28). Finally, the role of cholesterol in regulation of Kir6 channels is quite controversial with some studies suggesting that Kir6 channels are enhanced by plasma hypercholesterolemia (54) while other studies suggest that hypercholesterolemia blocks Kir6 channels (24). The role of lipid rafts in coupling Kir6 channels to PKA and PKC is also somewhat controversial. To resolve these multiple controversies, it is clearly necessary to elucidate the mechanism(s) responsible for the sensitivity of Kir channels to cholesterol. The major question is what the

structural basis of the sensitivity of Kir channels to cholesterol is and whether different cholesterol-sensitive domains can be identified in different Kir channels. Another key question is whether cholesterol interacts with the channels directly or whether it regulates Kir activity through other signaling molecules, such as PI(4,5)P₂, caveolin or regulatory kinases. Addressing these two questions will lead to basic understanding of how different Kir channels are regulated by cholesterol and what is the molecular basis for the diversity of cholesterol effects.

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Figure 1. Phylogenetic tree of Kir channels presenting their sensitivity to cholesterol and/or association with raft and caveolae. Phylogenetic tree is adopted from (39).

Figure 2. Suppression of endothelial Kir channels *in vitro* and *in vivo*

A: Typical current traces recorded from bovine aortic endothelial cells under control and cholesterol-enriched conditions. B: The functional dependence of normalized Kir current on the level of membrane cholesterol. A and B are adopted from (76). C: Suppression of endothelial Kir by hypercholesterolemia in vivo: Representative recordings of Kir currents in freshly-isolated porcine aortas endothelial cells from control and hypercholesterolemic pigs, as well as from cells isolated from hypercholesterolemic pigs and treated with M β CD *ex vivo* to remove the surplus of cholesterol. The bar graphs show peak currents for the three cell populations. D: Membrane depolarization and impairment of flow-induced hyperpolarization by VLDL: Typical voltage traces from a control and a VLDL-treated cells exposed to 2 dyne/cm² shear stress. C and D are adopted from (17).

Figure 3. The differential sensitivities of heterologous Kir2.x channels to cholesterol enrichment

A: Typical current traces from control and cholesterol-enriched cells expressing individual Kir2.x channels. B: Mean current densities in cholesterol-enriched cells normalized to respective currents in control cells. From (75).

Figure 4. Distinct effects of cholesterol structural analogues on endothelial Kir channels
 A: Structure of cholesterol and epicholesterol. Cholesterol: R1=H, R2=OH; epicholesterol: R1=OH, R2=H. B: Chromatographic profiles of cholesterol and epicholesterol: The two isomers can be separated and quantified using GLC: peak 1 – CME (internal standard), peak 2 – epicholesterol, peak 3 – cholesterol. The three chromatographic profiles are: a – lipid extracts of the cells treated with MβCD-epicholesterol, b – mixture of CME and cholesterol, c – mixture of CME and epicholesterol. C: Typical current traces recorded from a cell exposed to MβCD-epicholesterol and from a control cell. D: Functional dependence of Kir current density on cholesterol and epicholesterol. Adopted from (76)

Figure 5. Distribution of Kir2 channels between raft and non-raft domains

Top: Typical immunoblots for Kir2.1, Kir2.3 and caveolin. Vertical lanes represent samples prepared from the membrane fractions separated by sucrose gradient using non-detergent method. Bottom: Densitometric analysis normalized to total intensity of all fractions. From: (85).