Comparative analysis of cholesterol sensitivity of Kir channels
Role of the CD loop

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Kir channels are important in setting the resting membrane potential and modulating membrane excitability. A common feature of Kir2 channels and several other ion channels that has emerged in recent years is that they are regulated by cholesterol, a major lipid component of the plasma membrane whose excess is associated with multiple pathological conditions. Yet, the mechanism by which cholesterol affects channel function is not clear. We have recently shown that the sensitivity of Kir2 channels to cholesterol depends on residues in the CD loop of the cytosolic domain of the channels with one of the mutations, L222I, abrogating cholesterol sensitivity of the channels completely. Here we show that in addition to Kir2 channels, members of other Kir subfamilies are also regulated by cholesterol. Interestingly, while similarly to Kir2 channels, several Kir channels, Kir1.1, Kir4.1 and Kir6.2∆36 were suppressed by an increase in membrane cholesterol, the function of Kir3.4* and Kir7.1 was enhanced following cholesterol enrichment. Furthermore, we show that independent of the impact of cholesterol on channel function, mutating residues in the corresponding positions of the CD loop in Kir2.1 and Kir3.4* inhibits cholesterol sensitivity of Kir channels, thus extending the critical role of the CD loop beyond Kir2 channels.

Cholesterol is one of the major lipid components of the plasma membrane in mammalian cells and it is essential for cell function and growth. The excess of cholesterol, however, is associated with multiple pathological conditions including the development of cardiovascular disease. Multiple studies have shown that an increase in membrane cholesterol regulates the function of a variety of ion channels, including different types of K+ channels, Ca2+ channels, Na+ channels and Cl− channels. Multiple types of ion channels were also shown to be associated with cholesterol-rich membrane domains. It is also known that the impact of cholesterol on different types of ion channels is highly heterogeneous. In most cases an increase in cholesterol levels leads to a decrease in channel function while in others, it leads to an increase in channel activity. For example, cholesterol enrichment resulted in decreased activity of Ca2+ sensitive channels, voltage-gated Na+ channels, N-type Ca2+ channels, volume-sensitive Cl− channels and the inwardly rectifying K+ channel, Kir2.1. On the other hand, epithelial Na+ channels (eNaC) and TrpC channels were inhibited by cholesterol depletion. However, little is still known about the mechanisms by which cholesterol regulates the function of ion channels. Our recent study provided the first structural insights into cholesterol sensitivity of ion channels by demonstrating that cholesterol sensitivity of Kir2 channels, a subfamily of inwardly rectifying K+ channels, critically depends on a specific loop in the C-terminus domain. Here we extend our studies to test the effect of cholesterol on representatives of all subfamilies of Kir channels. Our new observations show that effects of cholesterol are highly heterogeneous even within the same family of ion channels but that...
is quite controversial as well. While some studies have suggested that Kir6 channels are enhanced by plasma hypercholesterolemia, other studies suggest that hypercholesterolemia blocks Kir6 channels. In this study, we first examine the effect of cholesterol enrichment on a representative homomeric member of each of the functional Kir subfamilies. Since Kir5.1 is not functional as a homomer, we concentrate on the remaining six subfamilies and include homomers of Kir1.1, Kir2.1, Kir3.4*, Kir4.1, Kir6.2∆36 and Kir7.1. Kir3.4* refers to the homomerically active Kir3.4 pore mutant S143T, and Kir6.2∆36 refers to the C-terminal truncation mutant that renders these channels active as homomers in the absence of the SUR subunits. We used the Xenopus oocyte heterologous expression system to test the effect of cholesterol on different Kir subfamily members.
cholesterol (Kir1.1, Kir2.1, Kir3.4* and Kir6.2\(\Delta_{36}\)) than others (Kir4.1, Kir7.1).

Furthermore, the most striking observation is that while the function of most Kir channels was suppressed by elevation of cholesterol levels (Kir1.1, Kir2.1, Kir4.1 and Kir6.2\(\Delta_{36}\)), the function of two members of the Kir family, Kir3.4* and Kir7.1 was enhanced under the same conditions.

One possibility to explain the striking difference in cholesterol sensitivity between Kir2.1 and Kir3.4* is to suggest that cholesterol interacts with the two channels in completely different ways. An alternative possibility, however, is that the also affected by cholesterol but to a lesser extent. While these two channels were less sensitive to cholesterol, the effect of cholesterol was systematic and consistent in different batches of oocytes. Specifically, comparison of four different batches of oocytes in each of these cases shows that Kir4.1 function is always suppressed by cholesterol enrichment while Kir7.1 currents are always enhanced by elevation of membrane cholesterol (Fig. 1B).

These results demonstrate that even within the Kir family of channels itself, both the sensitivity and the impact of cholesterol on channel function vary. First, some Kir channels were more sensitive to cholesterol (Kir1.1, Kir2.1, Kir3.4* and Kir6.2\(\Delta_{36}\)) than others (Kir4.1, Kir7.1). Furthermore, the most striking observation is that while the function of most Kir channels was suppressed by elevation of cholesterol levels (Kir1.1, Kir2.1, Kir4.1 and Kir6.2\(\Delta_{36}\)), the function of two members of the Kir family, Kir3.4* and Kir7.1 was enhanced under the same conditions.

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Figure 1 depicts the effect of cholesterol enrichment on the activity of the representative Kir channels listed above. In agreement with data obtained for Kir2.1 expressed in endothelial cells,\(^{11,26}\) and in CHO cells,\(^{11,24}\) the function of Kir2.1 channels expressed in Xenopus oocytes was also significantly suppressed by cholesterol enrichment. As can be seen in Figure 1A, similarly to Kir2.1, Kir1.1 and Kir6.2\(\Delta_{36}\) showed a significant and comparable decrease in currents following cholesterol enrichment. In contrast, Kir3.4* currents were significantly enhanced by an increase in membrane cholesterol. The remaining two channels, Kir4.1 and Kir7.1, were also affected by cholesterol but to a lesser extent. While these two channels were less sensitive to cholesterol, the effect of cholesterol was systematic and consistent in different batches of oocytes. Specifically, comparison of four different batches of oocytes in each of these cases shows that Kir4.1 function is always suppressed by cholesterol enrichment while Kir7.1 currents are always enhanced by elevation of membrane cholesterol (Fig. 1B).

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![Figure 2](image-url)
same structural domains of the two channels are responsible for their cholesterol sensitivity even though the functional effects are opposite. Comparison of the crystallographic structures of the cytosolic domains of Kir2.1, Kir3.1, and Kir3.2 shows that they share high structural similarity. This is evident, for example, in the structural alignment of the crystallized cytosolic domains of Kir2.1 and Kir3.1 depicted in Figure 2A, which suggests that the topology of the cytosolic domain of Kir channels is conserved. It is expected, therefore, that Kir3.4 channels share the same structure of the cytosolic domain. To discriminate between the two possibilities described above, we examined whether cholesterol sensitivities of Kir2.1 and Kir3.4 depend on the same domain in the C-termini of the channels. Thus, as we have recently shown, mutations of residues in the CD loop (see Fig. 2A–C) of the cytosolic domain of Kir2.1 significantly decrease the sensitivity of Kir2.1 to cholesterol, we examined whether a similar effect would be observed for Kir3.4 channels. More specifically, since in Kir2.1, the L222I mutation resulted in the largest effect on cholesterol sensitivity in CHO cells, we examined the equivalent position in Kir3.4 (Fig. 2B and C). Here we show that similarly to our previous study in CHO cells, L222I mutation significantly decreased the sensitivity of Kir2.1 channels in oocytes. Most importantly, the equivalent mutation in Kir3.4 channels, L229I, had the same effect on cholesterol sensitivity of Kir3.4 channels (Fig. 2D).

Thus, although the impact of cholesterol enrichment on Kir2.1 and Kir3.4 function is opposite, cholesterol sensitivity in both channels is affected in a similar manner following mutations at the corresponding position in the CD loop of the cytosolic domain of the channel. This implies that the role of the CD loop in affecting cholesterol sensitivity extends beyond Kir2 channels to other Kir subfamilies, irrespective of the impact of cholesterol on the channel. Furthermore, these observations demonstrate that opposite effects of cholesterol on different ion channels may still be mediated by a common mechanism.

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