Optimal level of membrane cholesterol guarantees the proper functioning of Kv channels

Cholesterol is the most abundant lipid of the mammalian plasma membrane. It is well known that cholesterol influences membrane biophysical properties and regulates the activity of multiple types of ion channels, including K⁺ channels. The reported mechanisms for regulation of K⁺ channels by cholesterol are diverse. For example, direct cholesterol-channel interaction inhibits Kir2.1, but stimulates Kir3.4 and Kir3.1/Kir3.4 channels. The voltage-activated Kv1.3, Kv1.5 and Kv2.1 channels are all associated with cholesterol rich membrane domains (rafts) and modulated by cholesterol. Membrane cholesterol depletion also regulates these channels by modifying their voltage-dependent steady-state activation and inactivation kinetics.

![Diagram of cholesterol effect on Kv channels](image)

Fig. 1. Inhibition of Kv7.2/Kv7.3 channels is induced either by depletion or enrichment of plasma membrane cholesterol.

Kv7.2/Kv7.3 channels are preferentially expressed in neurons and generate the M-current that stabilizes the membrane potential and controls neuronal excitability. Both native M-current and heterologous expressed Kv7.2/Kv7.3 channels are modulated by different lipids. Opening of Kv7.X channels, for example, requires the presence of phosphatidylinositol 4, 5-bisphosphate (PIP₂). Polyunsaturated fatty acids (PUFAs) also promote the opening of native Kv7/M-channels by shifting the voltage-dependence of activation to more negative potentials. We report now that either an increase or a decrease in the basal level of cholesterol within the plasma membrane decreases the activity of Kv7.2/Kv7.3 channels expressed in HEK-293 cells. Interestingly, cholesterol depletion modifies the kinetics and voltage-dependence of Kv7.2/Kv7.3 channels, whereas cholesterol enrichment does not. Our results reveal that an optimum level of cholesterol guarantees the proper functioning of these channels. In contrast to the commonly accepted idea that ion channels which are inhibited by membrane cholesterol depletion are otherwise stimulated by cholesterol loading or vice versa, our data indicate that Kv7.2/Kv7.3 channels are significantly inhibited either by depletion or enrichment of plasma membrane cholesterol. Importantly, such optimum level refers to the available fraction (free/bound ratio) of plasma membrane cholesterol since our results show that Filipin III, a compound that sequesters but not depletes the plasma membrane cholesterol, also inhibits Kv7.2/Kv7.3 channels.
In general, two mechanisms are proposed to explain the functional effects of cholesterol on membrane ion channels: a direct interaction cholesterol-channel and changes in the physical properties of the plasma membrane. Our present data, obtained from three distinct experimental approaches to manipulate the free/bound ratio of membrane cholesterol (depletion by methyl-beta-cyclodextrin, complex by Filipin III, and oxidation of membrane cholesterol by COase), suggest that under basal conditions cholesterol stimulates Kv7.2/Kv7.3 channels via a direct interaction. As it is known COase does not modify the physical properties of the plasma membrane, however, it inhibits Kv7.2/Kv7.3 channels. Thus, the produced cholestenone molecule was unable to bind to or modulate Kv7.2/Kv7.3 channels, as cholesterol does. We conclude that a decrease in plasma membrane cholesterol inhibits Kv7.2/Kv7.3 channels because the direct interaction between cholesterol and these channels is lost.

On the other hand, we report that at a high membrane cholesterol level the Kv7.2/Kv7.3 channels were also functionally inhibited. Here we envisage two possibilities to explain this phenomenon. The first one involves a direct binding of one or more cholesterol molecule(s) to lower affinity sites on Kv7.2/Kv7.3 channels, in addition to those bound at basal cholesterol levels. Such additional binding of cholesterol molecule(s) would result in an inhibitory effect on the channels, in contrast to the first stimulatory cholesterol binding site(s). As a second possibility, since cholesterol enrichment changes the physical properties of the plasma membrane; it could indirectly alter the functioning of Kv7.2/Kv7.3 channels. A graphic representation of the modulation of Kv7.2/Kv7.3 by cholesterol is shown in Figure 1. To the best of our knowledge, this is the first report to reveal that a voltage-dependent ion channel requires an optimum level of cholesterol in the plasma membrane to maintain its proper functioning.

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