Cholesterol increases kinetic, energetic, and mechanical stability of the human β2-adrenergic receptor

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AUTHOR SUMMARY

The human β2-adrenergic receptor (β2AR), one of the best-characterized G protein-coupled receptors (GPCRs), belongs to class A GPCRs and is expressed in pulmonary and cardiac myocyte tissue. Together with its close relative β1AR, β2AR senses adrenalin in bronchial and cardiac muscle. The implication in a broad spectrum of diseases, such as asthma or heart failure, makes β2AR a potential therapeutic target. Several crystal structures of β2AR have been determined during the last years (1), providing unique insights into the structure–function relationship of GPCRs. Here, we quantify the structural properties of the human β2AR changing in the presence of cholesterol and highlight the extent to which these changes may influence the structure and function relationship of the GPCR.

Cellular membranes modulate the function of a large number of membrane proteins, such as GPCRs. This modulation is facilitated by chemical and physical interactions between the membrane proteins and lipids, sphingolipids, cholesterol, and other components of the cell membrane. Because the molecular composition of cellular membranes is heterogeneous and changes dynamically, the activity of GPCRs depends on their location in the cell membrane and the state of the cell. An essential component of eukaryotic membranes is the steroid cholesterol, which modulates chemical and physical properties of cellular membranes and plays a role in the dynamic formation of sphingolipid-enriched assemblies of lipids and membrane proteins (2). These metastable assemblies, known as lipid rafts, can regulate membrane proteins by different mechanisms. Indirect regulation of membrane proteins can be observed through the ability of cholesterol to modulate the biophysical properties of a lipid bilayer. Moreover, cholesterol directly regulates membrane proteins through specific interactions. Such interactions functionally regulate several GPCRs. Although understanding these processes is of cell biological and pharmacological importance (2, 3), how cells control the distribution of cholesterol and how cholesterol regulates the activities of GPCRs remain to be determined.

Cholesterol modulates the physiological function of the β2AR, and it also modulates the physiological function of other GPCRs. Furthermore, cholesterol and the more water-soluble cholesterol analog cholesteryl hemisuccinate enhance the thermal stability of β2AR. Moreover, cholesterol facilitates interactions between GPCRs and is helpful in crystallizing β2AR. A recently published X-ray crystallographic model provided fascinating insights into the specific cholesterol binding sites of the human β2AR and showed cholesterol to fit into a shallow surface groove formed by the transmembrane α-helices H1, H2, H3, and H4 (4). However, complete understanding of which interactions with cholesterol change kinetic, energetic, and mechanical properties of stable structural segments of the receptor requires experimentation.

Single-molecule force spectroscopy (SMFS) permits the quantification of inter- and intramolecular interactions that stabilize native transmembrane proteins embedded in lipid membranes and the allocation of these interactions to individual secondary structure elements, such as α-helices, β-sheets, or polypeptide loops (5). Dynamic SMFS (DFS) probes these interactions at different force-loading rates and approaches the mechanical, kinetic, and energetic properties of every stable structural segment stabilizing a membrane protein. Both SMFS and DFS have been applied to characterize these properties of various membrane proteins and follow how they change when the functional state or physiological environment of the membrane protein is altered. We used both approaches here to quantify the mechanical, kinetic, and energetic properties of...
stable structural segments of the human \( \beta_2 \)AR reconstituted into lipid membranes (Fig. P1). We characterized the effects of the cholesterol analog cholesteryl hemisuccinate on \( \beta_2 \)AR to better understand how cholesterol influences its properties.

Our results show that cholesterol significantly increases the strength of interactions that stabilize every stable structural segment of \( \beta_2 \)AR. Furthermore, cholesterol increased the kinetic and energetic stabilities of every structural segment, except the structural core segment of \( \beta_2 \)AR. We could not distinguish the extent to which these changes were caused directly by the binding of cholesterol to the receptor or indirectly through the ability of cholesterol to modulate the properties of the lipid bilayer. The stable structural segments of the \( \beta_2 \)AR that do not expose cholesterol binding sites must have changed properties by indirect interactions mediated through cholesterol.

In summary, the properties that changed in the presence of cholesterol are of sufficient magnitude to alter the structure and function relationship of \( \beta_2 \)AR. The fact that cholesterol increases the stability of the receptor might also support the hypothesis that cholesterol is an essential component in the crystallization of \( \beta_2 \)AR. However, the only stable structural segment that did not change properties in the presence of cholesterol was the core segment of \( \beta_2 \)AR, which is involved in ligand binding. This finding indicates that cholesterol may not necessarily influence the binding of a ligand to the core segment. Taken together, the findings indicate that the unchanged core segment containing multiple ligand binding sites and the changed properties of all other stable structural segments may represent a mechanism of how cholesterol modulates \( \beta_2 \)AR. Unraveling the mechanism of how cholesterol changes the structural properties of GPCRs will guide the understanding of how GPCRs being exposed to different assemblies in cellular membranes are functionally modulated. Such insight provides a more detailed understanding of how drugs can induce different functional responses of GPCRs.