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Understanding the mechanisms underlying functioning of proteins is one of the major challenges of the contemporary biology. Proteins are able to play their unique roles by changing their structural and functional properties in response to respective stimuli which is referred to as conformational transitions. While occurrence of conformational transitions is nowadays well established, the underlying molecular mechanisms are far from being fully understood. Ionic channels are a fascinating group of proteins which permeate ionic fluxes and thereby control the membrane voltage of cells. The currents flowing through single channels can be precisely measured using the patch-clamp technique. Interestingly, the time course of currents mediated by ionic channels literally reflect the conformational transitions, as they show all or nothing pattern of activity (reflecting thus open and shut conformations). Importantly, at the open state, the channel conductance typically remains at a constant value. It is worth emphasizing that a high precision of the patch-clamp recordings enables us to obtain a wealth of information on the kinetics of conformational transitions of studied channels. Kinetic description of single-channel events is nowadays considered a classic neurophysiological method but it needs to be pointed out that the molecular mechanisms underlying these conformational transitions are far from being fully described. Understanding these mechanisms is of key importance as it not only offers a better insight into protein functioning but also it is expected to be helpful in designing and synthesis of clinically relevant drugs acting on these receptors. The aim of this project is to further explore the mechanisms of conformational transitions for the ionotropic $GABA_AR$ which, in the adult brain, plays a key role in neuronal inhibition. Activation of this receptor is a consequence of binding of typically two agonist molecules (GABA) to the binding sites which are located at the interface between the principal and complementary subunits. Interestingly, agonist binding sites are located very distantly (5 nm) from the channel gate within the transmembrane region where pore opening or closing is eventually taking place. This implies that mechanical changes induced by agonist binding, before inducing pore opening, must concern vast parts of the macromolecule, making this process very complex. To accomplish the ambitious goal of describing these mechanisms for the $GABA_A$ receptor we propose an interdisciplinary approach based on advanced electrophysiological recordings (both macroscopic and single-channel), on molecular biology techniques to engineer mutants (mutagenesis) and on structural modeling based on homology modeling and molecular dynamics. We will induce a mutation (or series of mutations) in a precise localization and test, using patch-clamp technique, the impact of this structural change on kinetics of conformational transitions. Molecular dynamics simulations will enable us to indicate the most probable scenarios of molecular rearrangements accompanying the receptor activation in various localization within the macromolecule. Our recent study based on analogous methodology enabled us to demonstrate that between the closed and open states there is an intermediate – called preactivation (Szczot et al. 2014, Journal of Neuroscience). This discovery allowed to better understand some aspects of $GABA_AR$ functioning including its modulation by various pharmacological agents. This project consists of several specific goals which, however, converge to verification of a central hypothesis that interaction of agonist molecules with specific structural elements at the binding sites lead to mechanical movements of some rigid elements (showing beta sheet structure) within the extracellular region of the protein leading to a concerted movement both in the principal and complementary subunits. This, in turn, leads to movements of transmembrane helices due to interactions between structures of the extracellular domain and loops originating from the transmembrane region, eventually giving rise to the pore opening. We postulate that mechanical movement initiated at a binding site then divides into two “streams” in the principal and complementary subunits. Because of structural asymmetry between these subunits, molecular changes accompanying these “streams” re expected to show differences. On the other hand, mentioned above interactions mediated by long and rigid beta sheet structures are likely to make the two “streams” strongly functionally interdependent. In order to extract information regarding the sequential movement of respective elements within the channel macromolecule we will use so called REFER method (which technically requires high quality single-channel analysis) which has been already applied for this purpose to study a different ionotropic receptor (AChR). We expect that results obtained in the frame of this project wil significantly enrich our knowledge about molecular mechanisms of $GABA_AR$ activation. This knowledge will be helpful to describe the mechanisms of modulation of this receptor by endogenous and exogenous drugs (including mentioned above clinically relevant agents).