between the protein subunits. Our results demonstrate the large-amplitude collective motions that span across the different protein subunits and indicate the broad range of dynamics present in the dark state that prelude the allosteric changes which take place during the activation of the complex. We suggest that the intracellular domains and cytosolic extensions of the transmembrane α-helices in rhodopsin participate in correlated motions that influence their interaction with key structural elements of transducin, such as the N- and C-terminal α-helices. Despite the very transient nature of the interaction interface, persistent interdomain interactions involving hydrophobic clusters and charged groups are crucial in the stability of the complex. We propose the general structural features of the interaction interface and relate our results with atomic site distance measurements from electronic paramagnetic resonance (EPR) experiments. Our results further suggest novel mutagenesis experiments that can be used to investigate the stability and correlated dynamics of this model receptor system. This work is funded by the NSF, IBM and RPI.

1895-Plat

Differential Voltage-sensitivities Of Agonists At The Dopamine D_{2S} Receptor

Kristoffer Sahlholm, Daniel Marcellino, Johanna Nilsson, Kjell Fuxe, Peter Århem.
Karolinska Institutet, Stockholm, Sweden.

Voltage-sensitivity has recently been demonstrated for agonist potency and affinity at certain G protein-coupled receptors. Several of those studies employed electrophysiology assays coexpressing receptors with G protein-coupled potassium channels (GIRK) in *Xenopus* oocytes. Using this assay, we have previously shown that the potency of dopamine in activating GIRK via the dopamine D_{2S} receptor is reduced by depolarization from -80 to 0 mV. We have now investigated the voltage-sensitivities of a range of structurally related dopaminergic agonists at the D_{2S} receptor. We found β-phenethylamine, *p*- and *m*-tyramine to be voltage-insensitive, while both non-hydroxylated and monohydroxylated *N*,*N*-dipropyl-2-aminotetralin (DPAT) compounds were voltage-sensitive.

There were no differences in the fractional responses to β-phenethylamine, *p*- and *m*-tyramine, relative to the response to a saturating concentration of dopamine, between -80 and 0 mV. Whereas β-phenethylamine, *p*- and *m*-tyramine are all partial agonists at the D_2 receptor, *m*-tyramine behaved as a full agonist, indicating amplifaction in the signal transduction from receptor to channel. The differential voltage-sensitivity did not depend on signalling via distinct G-proteins, since both voltage-sensitive and -insensitive behaviour was observed when signalling occurred exclusively via a PTX-insensitive $G_{\alpha o1}$ subunit. The level of G-protein activation or extent of receptor reserve was not responsible, since voltage-sensitivity of dopamine persisted in the absence of a receptor reserve, and since *m*-tyramine was voltage-insensitive while behaving as a full agonist at GIRK activation. Instead, we speculate that these differences might relate to the structurally constrained binding orientations of DPAT ligands and dopamine, as suggested by mutational binding studies by others, as compared to β-phenethylamine, *p*- and *m*-tyramine, which have been suggested to bind in a more flexible manner. These findings suggest a means of investigating the physiological roles of receptor voltage-sensing and might hint at a mechanism of receptor voltage-sensitivity.

1896-Plat

"cAMP Sponge": A New Tool For Probing cAMP Microdomains In Living Cells

Konstantinos Lefkimmiatis[1,2], Meera Srikanthan[1,2], Silvana Curci[1,2], Aldebaran M. Hofer[1,2].
[1]Harvard Medical School, West Roxbury, MA, USA, [2]VA Boston Healthcare System, West Roxbury, MA, USA.

Specific buffers for second messengers (e.g. BAPTA for Ca2+ and ''InsP3 sponge'' for InsP3) have proven invaluable for studying complex signaling pathways. A buffer for cAMP would also be highly desirable, but no such tool currently exists. Here we present the generation and validation of a novel genetically encoded cAMP buffer, ''cAMP sponge'', based on the PKA regulatory subunit type 1, subtype beta (RIbeta). In order to avoid possible biological effects of RIbeta, we have engineered a recombinant form consisting of the two C-terminal cAMP binding domains, while the biologically active NH2 terminus was eliminated. In order to quantitatively assess the expression of our ''cAMP sponge'' at the single cell level, we labeled it with the fluorescent protein, mCherry. This allows quantitative and qualitative assessment of our chimera expression (based on fluorescence intensity measured at 610nm emission) on the microscope stage. Evaluation of the cAMP buffering power of our sponge in cell lines stably expressing a FRET-based sensor for cAMP (EPAC H30) showed significant attenuation of the response to PGE2, VIP and FSK. Sponge constructs targeted to cytoplasm, plasma membrane, and nucleus showed significant local attenuation of the response to cAMP-generating agonists. In control experiments, over-expression of mCherry alone or a double mutant of our sponge in which the two cAMP binding sites were inactivated showed no significant attenuation of cAMP signals in PGE2-, VIP- or FSK-challenged cells. Interestingly we found that the extra cAMP buffering power provided by the cAMP sponge can be compensated by second messenger derived from connected neighboring cells that do not express the exogenous buffer, revealing new aspects of gap junction mediated communication. This new tool should also prove valuable for assessing the importance of cAMP microdomains in secretion, migration and transcriptional regulation.

Platform AE: Voltage-gated K Channels - Gating: Structure & Function

1897-Plat

Towards The Chemical Synthesis Of A Voltage Gated K+ Channel

Francis Valiyaveetil.
Oregon Health And Science University, Portland, OR, USA.

Understanding the relationship between the atomic structure of a protein and the biological functions requires the ability to perturb the protein structure in a precise manner. This is generally accomplished by means of traditional site-directed mutagenesis. However, the modifications that can be introduced in this manner are limited by the set of naturally occurring amino acids. Chemical synthesis on the other hand facilitates the incorporation of a wide variety of side chain and peptide backbone modifications that enables precise modifications of the structural and electronic properties of the protein. Similar modifications are not possible using conventional mutagenesis, thus making chemical synthesis an important asset in investigations of protein structure and function. The size of the protein has been a major factor limiting the use of chemical synthesis to investigate proteins. In the field of membrane proteins, chemical synthesis has so far been accomplished only for relatively small (< 150 amino acids) proteins. Proteins of interest such as voltage gated ion channels are much bigger and are presently not amenable to chemical synthesis. To overcome this limitation, methods that can be used for the chemical synthesis of large (> 150 amino acid) membrane proteins are required. We are presently developing methodology for the chemical synthesis of the voltage gated K+ channel KvAP. Voltage gated ion channels are responsible for the generation of electrical impulses by excitable cells. They have been the subject of intense research, however, a number of key questions remain about the mechanisms of ionic selectivity and gating in these channels. We believe that using chemical synthesis for protein modification in combination with functional and structural studies will enable us to provide new information on these physiologically important processes.

1898-Plat

Investigating the Electromechanical Coupling in voltage-gated K^+ channels

Zarah Batulan, Georges A. Haddad, Mireille Marsolais, Rikard Blunck.
Université de Montréal, Montréal, QC, Canada.

While the gating of the voltage sensor as well as the opening of the ion conducting pore in voltage-gated ion channels has been investigated extensively, the electromechanical coupling between the peripheral voltage sensors and the central ion conducting pore needs to be further elucidated. Based on previous work (*Lu et al., 2002*) and the crystal structure of Kv1.2 (*Long et al, 2005*), it has been suggested that the C-terminal S6 is coupled to the S4-S5 linker region, and that this coupling leads to opening of the pore. Here, we set out to investigate the role of this interaction in the coupling mechanism in voltage-gated Shaker K^+ channels. Possible interaction sites were chosen and altered by point mutation. The Shaker channels were expressed in Xenopus oocytes and ionic and gating currents as well as fluorescence changes monitoring the S4 movement were determined using voltage-clamp fluorometry in a cut-open voltage-clamp setup.

We identified two spatially distinct interactions between the S6 and the S4-S5 linker that have a significant influence on the gating properties of Shaker. One interaction acts upon the closed and open state and its disturbance accelerates voltage sensor movement while slowing ionic currents. A second interaction only acts in the open state. Exchange of the responsible residue slows closing of the channel by trapping the voltage sensor in the open state. We are interpreting the interactions in order to form a molecular model for the electromechanical coupling process.

(supported by CIHR MOP-81351 and CRC 202965).