Monitoring of chloride and chloride-selective ion channels activity using genetically encoded fluorescent sensors.

Bregestovski P.

Mediterranean Institute of Neurobiology,13273, Marseille, France
Tel: 33 4 91 82 81 19, e-mail: pbreges@inmed.univ-mrs.fr

Chloride (Cl) is the most abundant physiological anion. It is present in every cell and participates in a variety of physiological functions. Despite the importance of Cl for cellular functions, there is little knowledge available on the regulation of Cl in healthy conditions and at diseases. This lack of information is primarily due to technical difficulties in the monitoring of Cl in biological organisms.

Quinoline-based fluorescent dyes sensitive to Cl have low biological toxicity, relatively good sensitivity and selectivity to Cl and rapid response to changes in Cl. The major disadvantage of all quinoline-based indicator dyes is, that they are prone to strong bleaching. This restricts the duration of the measurements and allows only a very low data acquisition rate.

Yellow fluorescent protein (YFP) is a weak sensor of Cl. Fusion YFP with the Cl-insensitive cyan fluorescent protein (CFP) allows FRET-based ratiometric measurements of Cl in neurons. However, the sensitivity of this protein to Cl is very low (EC50 > 160 mM), which is relatively far from physiological range of Cl in mammalian nervous system (5-30 mM). Thus, development of molecules with sensitivities closer to this physiological range would provide a useful tool for monitoring Cl in biological preparations.

Investigation of brain functioning requires also methods allowing dynamic analysis of network activity combined with determination of the single-cell properties. This strategy was developed at monitoring of calcium transients using rapid two photon microscopy. However, analysis of networks formed by neuronal circuits for specific synapses (glutamatergic, GABAergic or glycinergic) is humped by lack of adequate techniques. This problem could be solved at genetic incorporation of molecules capable to change fluorescence at activation of specific synapses. The best candidates for these molecules would be fluorescently modified postsynaptic receptor-operated channels. Development of these molecules is highly a challenging task.

Recently we have developed two novel Cl^- sensors that allow the monitoring of intracellular Cl^- concentrations in biological preparations. These sensors contain a CFP-YFP fusion peptide that has two principal novelties: (i) three mutations in YFP confer high sensitivity to Cl^- allowing this peptide, which we call "Cl-Sensor," to provide spectroscopic monitoring of Cl^- concentrations at physiological conditions; (ii) when introduced into the cytoplasmic domain of the human glycine receptor (GlyR) Cl-Sensor (called "BioSensor-GlyR") allows spectroscopic monitoring of GlyR channel activity. This invention provides the background for developing new techniques for non-invasive monitoring of Cl^--selective channel activity in living cells and transgenic animals.