Abstract

Purinergic P2X receptors are non-selective cationic channels gated by extracellular ATP. Up to now, seven mammalian subunits, termed P2X1-X7, have been cloned and characterized. These receptors comprise a new membrane channel family with distinct structural and functional features. P2X receptors take part in a signalling network called "purinergic signalling" which is widely exploited in both somatic and neuronal tissues. In the central nervous system, they are highly expressed in the hypothalamus and hypophysis, where they participate in the regulation of homeostatic and reproductional functions.

The main focus of my Thesis is on the expression and functional role of P2X receptors in supraoptic nuclei of the rat hypothalamus. These nuclei contain two populations of magnocellular neurons which produce either oxytocin or arginine-vasopressin. Delivery of the hormones into the systemic blood relies on the electrical activity of supraoptic neurons, which is in turn governed by the incomming synaptic inputs. It has been recently shown, that the process of hormone release from supraoptic neurons is regulated by extracellular ATP. However, purinergic signals that regulate hormone secretion are not well understood. The aim of my study was to identify subtypes of P2X receptors expressed in the supraoptic nuclei and to elucidate their impact on electrical activity and synaptic transmission of supraoptic neurones.

The second part of the Thesis comprises three studies focused on structure-function relationship of several P2X subtypes. Crystallographic data for closed channel showed that P2X receptor is composed of three subunits, each having two transmembrane domains. The channel pore is formed by second transmembrane regions, but the role of the first transmembrane domain is still unknown, as well as the binding sites for ATP and allosteric modulators. We used site-directed mutagenesis coupled with cell electrophysiology to solve the following questions: (1) where is the binding site for ivermectin, a positive allosteric modulator of P2X4 receptors, (2) what is the functional role of conserved aromatic residues in the upper-half of the first transmembrane region in P2X1, P2X2, P2X3, P2X4 and P2X7 receptors, and (3) what is the role of five conserved ectodomain cysteine-pairs in P2X4 receptor function.

My Thesis summarizes answers to these questions based on our results that have been published in four peer-reviewed articles and one review.