Summary

Purinergic P2X receptors represent a novel structural type of ligand-gated ion channels activated by extracellular ATP. So far, seven P2X receptor subunits have been found in excitable as well as non-excitable tissues. In the past ten years, the number of studies on P2X receptors has dramatically increased as investigators have begun to determine the physiological roles played by extracellular ATP and specific P2X receptor subtypes. It is already known that purinergic signaling is a key mechanism in pain sensation, brain injury, and immune processes. Little is known about their structure, mechanism of channel opening, localization and termination of ATP action by ectonucleotidases. Detailed knowledge about these events and the structure of purinergic receptor proteins evoke hope that new drugs will be developed that could prevent chronic pain and would be effective in protection against many diseases.

The aim of this work is to summarize recent investigations and describe our contribution to elucidating the structure of P2X receptors. We examined the structure of transmembrane domains of the P2X4 receptor subtype, the main purinergic receptor-channel in the central nervous system, the mechanism of channel opening and closing and its sensitivity to agonists and allosteric modulator ivermectin. To identify residues that might play a role in channel functioning, we performed scanning mutagenesis of both transmembrane domains of the P2X4 receptor. All 42 amino acids in both transmembrane domains were substituted, one by one, for cysteine or alanine, the mutated receptors expressed in HEK293 cells and their function examined using electrophysiological patch clamp technique. We found that both transmembrane domains of P2X4 receptor adopt helical structure and identified the most important residues for receptor function. We also examined the role of conserved tyrosine in the first transmembrane domain across five members of P2X receptor family, in P2X1, P2X2, P2X3, P2X4 and P2X7 receptors, and found that this aromatic residue plays an important role in 3D-structure of receptor channels and modulates specifically binding of agonist and/or channel gating.