The P2X4 receptor is ATP-gated cation channel. It is the only mamrnalian purinergic receptor which is modulated by extracellularly applied ivennectin (IVM). rVM is an allosteric
modulator that has several effects on receptor [unction: it increases sensitivity to agonists, potentiates maximum current amplitude and prolongs the deactivation kinetics of the channel after agonist washout. The aim of this study was to localize IVM binding site and using its positive allosteric effect to get new inforrnatioll about the structure and function of P2X. receptor. Initially we focused on identification of regions and residues responsible for IVM effect on channel function. We used several chimeras of P2X2 and P2X. receptors and P2X. receptors with single point mutatioll. Experiments with chimeric receptors revealed that extracellular sequence V49-V61 but not tbe sequ nce V64-Y315 is important for the effects af IVM on channel deactivation. Receptor-specific alanine mutations placed in transmembrane domains 029-V61 and N338-L358 showed the importance of residues
W50, V61 and V357 for TVM effect Oll channel deactivation. We tested further the irnportance of aH residues in transmembrane domains. Cysteine scanning mutagenesis supported the relevance of previously identified W50 residue and showed the importance ofresidues 029, R33, Q36, L40, V43, V47, N338, 0342, L346, A349 and 1356 for the binding oflVM molecule to the P2X. receptor. We used IVM also as a pharmacological tool to evaluate the importance of several ectodomain residues presumably involved in tbe ATP binding and/or channel gating
mechanism. We created alanine and rescue mutations of K67, F185, K190, F230, R278, D280, F294, R295 and K313 residues. The majority of these mutant receptors were lowresponsive or non-responsive to ATP, however, the modulatory effect of IVM was preserved.