Abstract

This work explores properties of nicotinic acetylcholine receptors. Nicotinic receptors are pentameric transmembrane proteins which open their cationic channel when their binding sites are occupied by molecules of agonists. Binding sites appear at the interface between subunit α and a neighbouring subunit if their order is appropriate. Both of the subunits interact with ligand, subunit α by it's loops A, B, C, the neighbouring subunit by loops D, E and possibly F. Ligand binding leads to far-reaching changes of receptor structure in many levels, reported are the movements of loop C but probably also of loop F and a quaternary twist. Receptor, when activated, can switch to a non-conducting state called desensitized. Probabilities of existence of different receptor states can be influenced by substances which bind either to the same site as agonist (competitive effect) or they act from a distinct site (noncompetitive, allosteric effect).

By mutating we replaced negatively charged aminoacids in F-loops of α and β subunits of receptor α3β4 by uncharged aminoacids. Single mutations αD192N, αE195Q, βE198Q and βD200N did not have significant effect while βD191N and βD192N or their combination affected the function of receptor. Activation curves of receptors mutated in these positions were shifted to higer concentrations which is demonstrated on agonists nicotine ane epibatidine. Receptor with double mutation could be activated only by epibatidine with its EC_{50} value approximately 18x higher than in wildtype receptor. Mutations, nevertheless, did not affected affinity of receptors to a competitive antagonist (+)-tubocurarine which suggests that binding properties of receptor were not directly impaired by mutation. Some agonists (acetylcholine, nicotine, carbachole) did really bind to the double mutated receptor but instead of

activating them they acted as inhibitors of epibatidine responses. Also desensitization of receptors in different time-scales was influenced by mutations. Mutated receptors desensitized slowlier and faster recovered from desensitization induced by application of epibatidine. These findings are discussed in context of linear model of activation KNF. We concluded that negatively charged positions 191 and 192 of β subunit are necessary for proper receptor gating, not for affinity of their binding sites and this function is not present in similar negatively charged aminoacids of subunit α.

On receptors α3β4 and αβεδ we tested effects of a new selective inhibitor of acetylcholine esterases C-547. This substance did not activate receptors but it caused their inhibition. Inhibition was result of faster and deeper desensitization of receptors. Binding site of C-547 on receptors was not identified, however, based on competition- and voltage- experiments it can be stated that the binding site is not located in ion channel nor it overlaps with binding site for agonists. Action of C-547 on nicotinic receptors appeared with micromolar concentrations which is three orders of magnitude higher than the concentration in which it acts on choline esterases.