Activation and Desensitization of $\alpha 3\beta 4$ Rat Neuronal Nicotinic Receptors: Role of Negatively Charged Amino Acids in $\beta 4$ F-Loop

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The nAChR-subunit topology is common for all other pentameric neurotransmitter receptor channels. These ionic channels constitute the Cys-loop receptor superfamily whose members share a conserved disulfide bridge in their extracellular ligand-binding domain.

The binding pockets for ACh are formed by the interface between each α -subunit and the adjacent subunit. The α -subunit contributes to the ACh-binding pocket the so-called principal component, which consists of a series of loops carrying highly conserved ACh-binding residues, mainly aromatic but also a cysteine pair. The neighboring subunit contributes a series of complementary ACh-binding residues. The role of negatively charged amino acids in the F-loop of the $\beta 4$ subunit in channel activation and desensitization was studied using the patch-clamp technique. The selected amino acids were changed to their neutral analogs via point mutations. Whole-cell currents were recorded in COS cells transiently transfected with the $\alpha 3\beta 4$ nico-

tinic acetylcholine receptor. The application of acetylcholine (ACh), nicotine (Nic), cytisine (Cyt), carbamylcholine (CCh), and epibatidine (Epi) to cells clamped at -40 mV produced inward currents which displayed biphasic desensitization. The EC₅₀ of Epi and Nic were increased by a factor of 3 to 6 due to single mutations D191N or D192N. Only Epi remained an agonist in the double-mutated receptors with EC₅₀ increased 17-fold. The interaction of the receptors with the competitive antagonist (+)tubocurarine (TC) was weakened almost 3-fold in the double-mutated receptors. The mutations increased the proportion of the slower desensitization component and increased the response plateau, resulting in decreased receptor desensitization. The double mutation substantially accelerated the return from long-term desensitization induced by Epi.

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