From the Interaction of Neurotoxic Peptides and Proteins with the Cys-loop Receptors to Novel Drugs

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Abstract

The talk is devoted to research on neurotoxic proteins and peptides carried out at our Institute in collaboration with leading Russian and foreign laboratories. It focuses on toxins interacting with nicotinic acetylcholine receptors (nAChRs) and other Cys-loop receptors : glycine receptors (GlyR), ionotropiic γ-aminobutyric acid and serotonin GABA-A and 5HT-3 receptors. Due to high homology, identification of functionally active individual subtypes cannot be reliably performed with antibodies and neurotoxins are more preferable because many have a considerable selectivity to a particular receptor subtype which can even be increased by design of novel analogs and derivatives. X-ray analysis of the neurotoxin complexes with Cys-loop receptors or receptor models, such as the acetylcholine-binding proteins (AChBPs) or receptor ligand-binding domains (LBD), provided information about the receptor binding sites opening new ways to drug design. These lines are illustrated by our recent work: using a novel computer program, α-conotoxin PnIA analogs were designed, synthesized, tested by radioligand analysis, electrophysiology and Ca2+ imaging and shown to have extremely high affinity for neuronal α7 nAChR which plays an important role in the neuroimmune axis and is a well-known target in drug design. Another example is a linear peptide azemiopsin isolated from the viper venom: contrary to all earlier known peptides and proteins from animal venoms which inhibit nAChRs and contain from 1 to 5 disulfides, azemiopsin has no disulfide bridges (thus greatly simplifying its synthesis) but selectively blocks the muscle nAChRs; its preclinical studies as a promising myorelaxant have been recently published. New ways to drug design can emerge from the discovered interactions of the human endogenous proteins with the Cys-loop receptors: it was recently shown that human SLURP-1 (protein of the Ly6 family having the same threefinger folding as snake venom α-neurotoxins) allosterically inhibits α9 nAChR, a target for novel analgesics. Neurocypres consortium members are devoted to study structural and functional aspects of Cys-loop receptors, a superfamily of ligand-gated ion channels that is crucial to the function of the peripheral and central nervous system. Cys-loop receptors (CLRs) share a generic protein architecture consisting of five subunits with an integral ion-channel that can open and close depending on ligand binding. CLRs comprise of nicotinic acetylcholine-, GABAA-, 5HT3-, and Glycine receptors. Dysfunction of CLRs is linked to muscle disorders (e.g. myasthenic syndromes), hyperexcitabiliy of the brain (e.g. epilepsy) and spinal cord (e.g. hyperekplexia / stiff baby syndrome) as well as nicotine addiction, while CLR subunit genes serve as candidates for frequent psychiatric diseases (e.g. schizophrenia). CLRs are molecular targets for clinically important drugs. Novel drugs are on the horizon, mediating highly selective therapeutic effects and providing new avenues for therapeutic use. Today, the quality of crystallographic data and the general knowledge of structure-function relationships have progressed to the extent that the rational design of ligands has become a feasible objective. Coordinated approaches, such as Neurocypres, are required to increase our knowledge of ligand-gated ion channel structure at high-resolution.

In 2008, Neurocypres brought together 20 groups of researchers in a large-scale integrated effort to capture high-resolution structural information encompassing either the entire structure of selected receptors, or of specific functional domains in these receptors. Since then, new discovery projects in the fields of nAChRs, GABAA and 5HT3 receptors have been established. Various projects were dedicated to understand the fundamentals of receptor structure, e.g structural data of the acetylcholine binding proteins (AChBPs), co-crystalized with various ligands, was obtained. For various different CLR ligands, in-depth knowledge was gathered on ligand binding to the AChBPs, e.g. various toxins, as well as small molecules important for drug design. Compounds relevant for medicinal use, for instance the anti-smoking compound varenicline, were studied. In addition AChBPs were engineered that can act as new tools for drug development for CLR receptors, for instance of the 5HT3R class. Another important area of the Neurocypres program was to advance new technologies for drug design and screening. New technologies were established with promising results on small molecules targeting the different members of the LGIC family. These included high-throughput electrophysiological analyses of receptor expression in oocytes, fluorescence based assays, and affinity measurements using surface plasmon resonance. Also the use of CLRs to probe neuronal function, as biosensors, has progressed to studies in neurons. Moreover, efforts to produce whole