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MEETING REPORT

NEUROTOXINS IN NEUROBIOLOGY: FROM BASIC RESEARCH TO CLINICAL APPLICATION

FRANCESCO CLEMENTI,[1] OLIVER J. DOLLY,[2] PIERLUIGI ONALI[3] and GIACINTO BAGETTA[4]*

[1]Department of Pharmacology, University of Milan, Milan, Italy, [2]Department of Biochemistry, Imperial College, University of London, London, U.K., [3]Department of Neuroscience B. Brodie, University of Cagliari, Cagliari, Italy; and [4]University of Calabria, 87030 Arcavacata di Rende (CS), Italy

The cholinergic system is extremely important in organism life since it controls and fine-tunes important functions of the central and peripheral nervous system, mostly by pre-synaptically controlling the release of a number of neurotransmitters including GABA, dopamine, glutamate and acetylcholine. It is, thus, not surprising that animals have developed a large variety of toxins targeted to key cholinergic transmission namely pre- and post-synaptic nicotinic and muscarinic receptors, acetylcholine esterase (AchE), molecules involved in neurotransmitter storage and release. These toxins are extremely powerful tools for understanding the functions of the targeted molecules and as reference components on which to model new and more selective drugs for intervening on the cholinergic system (see, for example, new drugs for Alzheimer disease treatment or against smoking addiction, but also more selective insecticides). These concepts formed the interest of the introductory session of the VIth Symposium of an International series on Neurotoxins in Neurobiology†.

The first part of the Symposium, in fact, dealt with the contribution of toxins to the understanding of neuronal nicotinic receptors. Although the structure of nicotinic receptors has been accurately described by Unwin (1993, 1995) at high resolution, both in the open and closed state, its definition at atomic level is not possible due to the impossible task of obtaining a crystallised structure of this complex molecule.

Prof. G. G. Lunt (University of Bath) circumvented the problem by looking for a heat labile enterotoxin of known crystallographic structure superimposable to the 4 transmembrane regions of the ligand gated ion channels. The model of the channel that he has then constructed allowed to hypothesize a series of structures that could be tested by molecular biology technique. This model is extremely interesting and will open a new way to look at the structured ligand gated ion channels but has some limitations

* Author to whom correspondence should be addressed.

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(for example, the enterotoxin does not form channels and a gated ion channel is much more complex than a pore).

The heterogeneity of neuronal nicotinic receptors was well illustrated by Prof. F. Clementi (University of Milan) and Dr S Wonnacott (University of Bath). Clementi showed the differences in structure, pharmacology and biophysics between the subtypes of the α-bungarotoxin binding receptors, the α7 and the α8, he also showed that the native receptor α4β2 can include or not an α5 subunit, and this inclusion greatly decreases the open probability of the channel after ligand binding without affecting the ligand affinity of the two subtypes. He stressed that for a real knowledge of the properties of the neuronal nicotinic receptors it is relevant to look at the properties of native receptors and not to rely only on the heterologously expressed receptors that can give misleading results.

S. Wonnacott presented a series of experiments on the selectivity of epibatidine and anatoxin-A and of a number of their derivatives on the α4β2 subtypes. These compounds were active not only as ligands but also stimulated *in vitro* dopamine release. A useful and novel compound for investigating the function of α3β2 receptor is α-conotoxin M II that is an antagonist at this receptor subtype.

Dr I. S. Blagbrough (University of Bath) presented a series of derivatives of norditerpenoid alkaloids from *Aconitum* and *Delphinium* plants that have selective α7 receptors. Dr W. R. Kem (University of Florida) presented another series of molecules derived from the toxin anabaseine selective toward α7 receptor that have a mild positive effect on learning behaviour.

Prof. A. Ménez (CEA, Gif-sur-Yvette) was able to understand why the long α-neurotoxins, as α-bungarotoxin, are able to block both muscular and central receptors, while short neurotoxins are able to block only the muscular receptors. The difference consists of a small loop delimited by a disulfide bond that is absent from the short toxin. All these studies open perspectives for novel selective compounds that will be extremely useful in understanding the relevance of nicotinic receptor subtypes in brain functions.

The last part of the Symposium on "cholinergic toxins" dealt with the molecules interacting with AChE. In this field, a great contribution to the understanding of the function of AChE comes from fasciculin (Dr F. Dajas, IIBCE, Montevideo), a toxin that binds AChE at two sites: one at the mouth of the pore, where ACh enters into the catalytic tunnel of the enzyme, and the other at the end of the pore where the hydrolysis of ACh takes place.

Dr C. Bon (Pasteur Institute, Paris) stressed also the relevance for the control of AChE function of the distribution of the electric charge at the periphery of the molecules that control the flow of ACh into the pore and that can be modified in range of ms by the membrane potential. Bon also reported the gene structure of *Bungarus* AChE that is soluble and present in high concentration into the snake venom. In comparing snake and non-snake genes he was able to localise a new exon that is present in the soluble molecule. A large discussion arose from the presentation of the data of Dajas and, in part, of Bon, on the role of AChE in non-cholinergic areas. A new role of AChE in development and in neuronal survival could be foreseen, at least in some tissue like retina.

Presentations from Prof. O. J. Dolly (University of London) and Prof. C. Montecucco (University of Padova) illustrated how deciphering the fundamental process of exocytotic release of transmitters has been aided by the use of *botulinum* (BoNT; types A to G) and tetanus (TeTX) neurotoxins. These are di-chain proteins produced by *Clostridium botulinum* and *tetani*, respectively, that exhibit fascinating properties,

being the most poisonous substances known due to unique abilities to irreversibly inhibit transmitter release in very specific fashions via a sophisticated mechanism. This entails neuronal targeting by binding to acceptors located exclusively on susceptible nerve endings, subsequent endocytosis and translocation to the cytosol where the toxins' light chain proteolytically cleaves vesicle associated membrane protein (VAMP), SNAP-25 or syntaxin —three proteins that are essential for regulated exocytosis. Cesare Montecucco described novel molecular features of the substrates that govern their exclusive susceptibility to the respective toxins (VAMP: TeTX, BoNT B, D, F and G; SNAP-25, BoNT A, C and E; syntaxin, C1). Oliver Dolly highlighted the dramatic success being achieved worldwide in exploiting the unique ability of BoNT to cause semi-permanent neuromuscular paralysis of the clinical treatment of a group of movement disorders, called dystonias and dysphonias. By injecting a minute quantity of BoNT into the affected muscles, the abnormal spasms can be diminished in these patients. Dolly reported *in vivo* imaging of the nerve sprouting that results from BoNT-induced neuromuscular paralysis. By using the endocytotic dye FM 1-43, the sprouts were shown to form functional synapses which contribute to the recovery of neurotransmission during a period when the original endplates remained poisoned. In contrast to the local action of BoNT at motor endings, after internalisation TeTX is retrogradely transported to the spinal cord and, eventually gets inside nerve terminals of inter-neurons where it blocks the release of inhibitory transmitters. Montecucco and colleagues have shown that TeTX can be endocytosed into synaptic vesicles and utilises the acidity acquired by the latter during recycling for translocation to the cytosol, its site of action.

Dr A. Grasso (CNR, Rome) described how α-latrotoxin increases transmitter release by Ca^{2+}-dependent and -independent mechanisms via its G-protein coupled receptor, latrophilin. Activation of protein kinase C results from the toxin's action. The group of Dr E. Sher (University of Milan) reported the usefulness of peptide toxins (e.g. ω-conotoxin, ω-agatoxin) in identifying the subtypes of voltage operated Ca^{2+} channels in neuroendocrine cells. L, N, P/O types were demonstrated in small-cell lung carcinoma and certain pancreatic β-cells where they control exocytosis. The venom of certain wasps contains polyamines which are potent antagonists of ionotropic glutamate receptors. A basis for this specificity of philanthotoxin was described by Prof. P. Usherwood (University of Nottingham) from elegant electrophysiological measurements on recombinant forms of glutamate receptors. Dendrotoxins from mamba snakes that selectively inhibit certain voltage-activated K^+ channels were described by Dr L. Smith (Fort Detrick). Site-directed mutagenesis of expressed dendrotoxin K has identified two domains (β-turn and 3_{10} helix) in this protein that are essential for its activity. The blockade of K^+ channels by dendrotoxins causes neuronal hyper-excitability and convulsive activity in rat brain, leading to neurodegeneration. Prof. G. Bagetta (University of Calabria at Cosenza) has exploited these toxins as tools to demonstrate the involvement of K^+ channel sub-types and to ascertain if glutamate receptors (NMDA and non-NMDA varieties) contribute to the perturbation of the neuronal circuits that result in convulsions.

Dr M. Stocker (Germany) examined the interaction of the peptide toxins, charybdotoxin and K-conotoxin PVIIA, and the vestibule of the shaker K^+ channel. By using point mutagenesis of both charybdotoxin and the channel, it was possible to calculate the electrostatic interaction energy between chosen pairs of residues, and to estimate their physical distances, thus allowing the representation of a possible model of toxin–channel interaction.

The last session of the Symposium was dedicated to the characterization of the activity of natural neurotoxins that can either act on signal transduction proteins, such as ion channels and G-protein coupled receptors, or on the metabolic pathways of the brain. Prof. L. Béress, (University of Kiel) described a multi-step purification procedure adapted to isolate five crab paralysing polypeptide toxins from the sea anemone *Anthopleura elegantissima*. The toxins have been characterized in terms of their amino acid sequence and their effects on the function of Na^+ channels. Prof. G. Escalona de Motta (University of Puerto Rico) examined the effect of maitotoxin, a polyether toxin known to increase intracellular Ca^{2+}. In muscle strips of the frog stomach, picomolar concentrations of maitotoxin isolated from the marine dinoflagellate *Gambierdiscus toxicus* were found to increase the force of contractions in a Ca^{2+}-dependent fashion; in contrast, higher concentrations of the toxin decreased the frequency of contractions, raising the possibility that the toxin acts on different Ca^{2+} channels located on different functional structures of the tissue preparation. The selective binding of the green mamba *(Dendroaspis angusticeps)* toxin was illustrated by E. Karlsson (Huddige Hospital, Sweden). Two of these toxins, MT-1 and MT-3 were iodinated and used to map the distribution of m1 and m4 receptors, respectively, in the rat brain. The use of these toxins has also allowed the demonstration of a selective loss of m4 receptors in the hippocampus of Alzheimer patients. The pharmacological properties of MT-3 were examined by Prof. P. Onali (University of Cagliari). MT-3 was found to be a potent and selective antagonist of the cloned and native m4 receptor and to be useful in the functional study of the m4 receptor in the rat brain. Prof. A. Vaccari (University of Cagliari) described some neurochemical changes that occur in the rat brain following the administration of the alcohol-aversive drug disulfiram. This drug was found to reduce the striatal binding of [^{3}H]tyramine and the vesicular uptake of [^{3}H]dopamine, to stimulate glutamate and dopamine release from the *striatum* of freely moving rats. The excessive accumulation of extracellular glutamate may lead to excitotoxicity and may be responsible for some neurological defects produced by disulfiram. Prof. P. Jenner (King's College, London) discussed the possible role of cytochrome P-450 isoforms in the pathogenesis of Parkinson's disease. This, on the basis of the ability of P-450 isoforms to metabolise the selective nigral toxin MPTP and to generate endogenous neurotoxin. The distribution in the brain of different P-450 isoforms was characterized by using a set of specific antipeptide antisera. Many P-450 isoforms were found to be localised in dopaminergic neurones of the *substantia nigra*, thus providing an immunobiological evidence for the possible involvement of P-450 in the neurodegenerative processes leading to Parkinson's disease.

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