

IONIC MECHANISMS INVOLVED IN THE NODAL SWELLING OF MYELINATED AXONS CAUSED BY MARINE TOXINS

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Abstract: This review describes the ionic mechanisms involved in the nodal swelling of frog myelinated axons caused by specific marine neurotoxins (ciguatoxins, brevetoxins, *Conus consors* toxin and equinatoxin-II), analysed using confocal laser scanning microscopy. We have focussed on toxins that either target neuronal voltage-dependent Na⁺ channels, or that form cation-selective pores and indirectly affect the functioning of the Na⁺-Ca⁺⁺ exchanger.

Key Words: Ciguatoxins, Brevetoxins, *Conus consors* Toxin, Equinatoxin-II, Nodal Swelling of Myelinated Axons, Confocal Laser Scanning Microscopy, Sodium Channels, Sodium-Calcium Exchanger

INTRODUCTION

The nodes of Ranvier of the myelinated axons play a key role in the conduction of nerve impulses, and changes in their protein structure and morphological characteristics have important physiopathological implications. In this article, we review studies performed *in vitro* in our laboratory using frog myelinated axons and specific marine neurotoxins in order to understand the ionic mechanisms involved in the swelling they produce at the nodes of Ranvier. Among the large number of neurotoxins that target ion channels, we have studied the effects of ciguatoxins (CTXs), brevetoxins (PbTx) and *Conus consors* toxin (CcTx), which modulate Na⁺ channel activity, and equinatoxin-II, which forms cation-selective pores in cell membranes.

SODIUM CHANNEL-ACTIVATING TOXINS

CTXs are a family of lipid-soluble, highly oxygenated, heat stable, cyclic polyether compounds which are responsible for ciguatera fish poisoning, a human seafood intoxication linked to the benthic dinoflagellate *Gambierdiscus toxicus* and acquired by eating certain contaminated species of fish (for a review,

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see [1]). PbTx are another family of potent, lipid-soluble, polyether compounds purified from the unarmoured dinoflagellate *Gymnodinium breve* (formerly known as *Ptychodiscus brevis*), which has been implicated in episodes of massive fish kills and in a severe form of microalgae-derived poisoning known as neurotoxic shellfish poisoning (for a review, see [2]). CTXs and PbTx bind to a common receptor site on the neuronal voltage-dependent Na⁺ channel-protein [2, 3]. As a consequence of their interactions, these toxins activate Na⁺ channels, notably at the resting membrane potential at which these channels are normally closed, and cause a membrane depolarization leading either to repetitive action potentials in response to a single electrical stimulation or to spontaneous action potentials [2, 4-6]. A similar increase in membrane excitability occurs in the presence of CcTx, a peptide of 30 amino acid residues isolated and purified from the crude venom of the piscivorous *Conus consors*. Hence, this conotoxin, through its ability to specifically activate neuronal voltage-dependent Na⁺ channels at the resting membrane potential, (i) depolarizes the nodal membrane of myelinated axons, inducing either spontaneous action potentials or repetitive action potentials in response to a single electrical stimulation, and (ii) causes trains of repetitive endplate potentials, which trigger muscle action potentials, in response to a single nerve stimulation applied to isolated nerve-muscle preparations [7].

The effects of various CTXs (P-CTX-1B, P-CTX-4B, P-CTX-3C, C-CTX-1), and PbTx (PbTx-1, PbTx-3), extracted and highly purified from either *G. toxicus* or various poisonous fish and *G. breve*, respectively, as well as CcTx, were studied on the morphology of isolated myelinated axons (from frogs) stained with the fluorescent dye FM1-43, using confocal laser scanning microscopy [4, 7-10]. During the action of all the toxins (10-600 nM), a time-dependent swelling of the nodes of Ranvier was observed. This effect occurred without any significant change in the internodal parts covered by the myelin sheath. In all cases, the effect appeared within 15-60 min of toxin addition to the standard medium, and was well maintained for an additional 60 min of toxin exposure. At that time, the nodal volume was increased by 80-100% with respect to the controls. The toxin-induced swelling was reversed within 20-60 min and prevented by external solutions in which the osmolality was increased by about 50% with D-mannitol (100 mM), sucrose (100 mM) or tetramethylammonium chloride (50 mM). Moreover, blocking the voltage-dependent Na⁺ channels with tetrodotoxin (TTX) prevented the nodal swelling. Hence, when myelinated axons were exposed to the toxins in the continuous presence of 1 μM TTX, no significant change in the nodal volume was detected for up to 120 min of exposure.

It is likely that CTXs, PbTx and CcTx, by enhancing membrane excitability, produce a continuous Na⁺ entry into axons (i) through toxin-modified Na⁺ channels permanently activated at the resting membrane potential, and (ii) through unmodified Na⁺ channels which open during the toxin-induced spontaneous and repetitive action potentials. From an electric point of view, such

a continuous entry of Na^+ ions is expected to be counterbalanced by an efflux of K^+ ions through voltage-dependent K^+ channels which open during spontaneous and repetitive action potentials. However, the gain of intra-axonal Na^+ ions during an action potential exceeds the loss of K^+ ions [11], resulting in a net entry of positive charges into the axons. Therefore, it is proposed that the toxin-induced continuous entry of Na^+ ions into the axons leads to an increased intra-axonal Na^+ concentration that raises the internal osmolality enough to directly or indirectly disturb the osmotic equilibrium between intra- and extra-axonal media. As a consequence, a water influx occurs to restore both the osmotic equilibrium and the intra-axonal Na^+ concentration to the initial levels, causing nodal swelling of the myelinated axons. Although no volume-regulatory mechanisms were detected during the nodal swelling induced by CTXs, PbTx and CcTx in the time-domain investigated, further studies are required to accurately determine the other ionic species and transporters involved in such a nodal swelling.

A CATION-SELECTIVE PORE-FORMING PROTEIN

The nodal volume of frog myelinated axons was also consistently increased by equinatoxin-II (EqTx-II), a cation-selective pore-forming protein isolated from the sea anemone *Actinia equina* [12-14], provided Ca^{++} was present in the external medium. The substitution of external Cl^- by methylsulfonate did not affect the toxin-induced increase in nodal volume. By contrast, the replacement of external NaCl by either sucrose or LiCl prevented the toxin from inducing nodal swelling. Interestingly, an increased nodal volume was detected in the presence of both EqTx-II and TTX, indicating that voltage-dependent Na^+ channels are not involved in the action of EqTx-II.

Previous microspectrofluorometric studies performed on neuroblastoma (NG108-15) cells preloaded with the Ca^{++} indicator fura-2/AM showed that EqTx-II markedly increased the intracellular Ca^{++} concentration, provided Ca^{++} was present in the extracellular medium. Such an increase, which was not dependent on extracellular Na^+ , was related to Ca^{++} influx occurring through pores formed by the toxin in the plasma membrane of neuroblastoma cells [15]. Considering that extracellular Ca^{++} is not essential for pore formation induced by EqTx-II [13, 14] and no nodal swelling was observed when Ca^{++} was withdrawn from the external medium containing Na^+ , it seems unlikely that Na^+ is directly involved in the nodal swelling induced by the toxin. Therefore, it is proposed that, when Ca^{++} is present in the extracellular medium, the entry of Ca^{++} into axons, through the pores formed by EqTx-II, increases intra-axonal Ca^{++} concentration and thereby activates the Na^+ - Ca^{++} exchanger in the normal mode, triggering an influx of Na^+ to extrude Ca^{++} . As stated above, such Na^+ entry into axons will lead to an increased nodal volume. This is supported by the fact that the EqTx-II-induced nodal swelling occurred provided both Ca^{++} and Na^+ were

present in the external medium, and was prevented by Li^+ , known to block the $\text{Na}^+-\text{Ca}^{++}$ exchanger.

CONCLUSION

In conclusion, marine toxins may be useful tools for dissecting the ionic channels and exchangers involved in the nodal swelling of myelinated axons. It is worth noting that nodal swelling, i.e. an increased membrane area of the nodes of Ranvier, is expected to have functional consequences for nerve conduction since the electric capacitance of the nodal membrane should be greatly increased. Hence, an increased nodal membrane capacitance will reduce the conduction velocity of myelinated axons.

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