available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Effects of ApC, a sea anemone toxin, on sodium currents of mammalian neurons

Emilio Salceda[a,*], *Anoland Garateix*[b], *Abel Aneiros*[b], *Héctor Salazar*[a], *Omar López*[a], *Enrique Soto*[a]

[a]*Instituto de Fisiología, Universidad Autónoma de Puebla, Apartado Postal 406, Puebla, Pue., CP 72001, Mexico*
[b]*Centro de Bioproductos Marinos, CITMA, La Habana, Cuba*

ARTICLE INFO

Article history:
Accepted 27 June 2006
Available online 15 August 2006

Keywords:
Anthopleura elegantissima toxin
Dorsal root ganglia neuron
Fast inactivation
Site-3 toxin
Voltage-gated sodium channel

Abbreviations:
4-AP, 4-aminopyridine
D50, duration of the action potential measured at 50% of its amplitude,
DRG, dorsal-root-ganglion
IC_{50}, half-maximum inhibitory concentration
L15, Leibovitz L15 medium
TEA-Cl, tetraethylamonium chloride
TTX, tetrodotoxin
TTX-S, tetrodotoxin-sensitive sodium current
TTX-R, tetrodotoxin-resistant sodium current
τ_h, inactivation time constant
$V_{1/2\ act}$, half-maximum voltage of activation
$V_{1/2\ inact}$, half-maximum voltage of inactivation

ABSTRACT

We have characterized the actions of ApC, a sea anemone polypeptide toxin isolated from *Anthopleura elegantissima*, on neuronal sodium currents (I_{Na}) using current and voltage-clamp techniques. Neurons of the dorsal root ganglia of Wistar rats (P5–9) in primary culture were used for this study. These cells express tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) I_{Na}. In current-clamp experiments, application of ApC increased the average duration of the action potential. Under voltage-clamp conditions, the main effect of ApC was a concentration-dependent increase in the TTX-S I_{Na} inactivation time course. No significant effects were observed on the activation time course or on the current peak-amplitude. ApC also produced a hyperpolarizing shift in the voltage at which 50% of the channels are inactivated and caused a significant decrease in the voltage dependence of Na^+ channel inactivation. No effects were observed on TTX-R I_{Na}. Our results suggest that ApC slows the conformational changes required for fast inactivation of the mammalian Na^+ channels in a form similar to other site-3 toxins, although with a greater potency than ATX-II, a highly homologous anemone toxin.

* *Corresponding author.*
E-mail address: esalceda@siu.buap.mx (E. Salceda).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.06.113

1. Introduction

Voltage-gated sodium channels (Na^+ channels) are integral membrane proteins responsible for the generation and propagation of action potentials in most excitable tissues. These channels are specific targets for a variety of neurotoxins that bind to various sites on the channel protein (Caterall, 2000) and therefore have been used as pharmacological tools to investigate the structure and function of Na^+ channels (Gordon et al., 1996; Caterall, 2000; Chen et al., 2002). To date, six neurotoxin-receptor sites have been identified by direct radiolabeled toxin studies (Strichartz et al., 1987; Fainzilber et al., 1994; Gilles et al., 2002). Typically, sea anemone toxins and scorpion α-toxins bind to the so-called neurotoxin receptor site-3 which has been located in the short extracellular loop IVS3-S4 of the Na^+ channel α-subunit with an important participation of the glutamic acid in position 1613 (rat brain Na^+ channel) (Rogers et al., 1996) or the aspartic acid in position 1612 (cardiac Na^+ channel) (Benzinger et al., 1998). Mutation of these acidic residues from the transmembrane segment IVS3 reduces the binding of sea anemone toxins and scorpion α-toxins. Both the IVS3-S4 loop and the amino acid residues noted could also be involved in the coupling of channel activation to fast inactivation. Beside, several other regions of the α-subunit may contribute to the receptor site, particularly the IS5-S6 and IVS5-S6 extracellular loops.

Site-3 neurotoxins comprise a structurally diverse group of peptide toxins isolated from scorpions (Meves et al., 1986; Possani et al., 1999), sea anemones (Norton, 1991), spiders (Fletcher et al., 1997; Little et al., 1998), and wasps (Sahara et al., 2000; Kinoshita et al., 2001). These compounds increase the action potential duration and often produce spontaneous firing.

The site-3 neurotoxins modify the inactivation process, although they act from the extracellular side, making them interesting for use as probes to study the inactivation mechanism of Na^+ channels. Also, the analysis of peptide sequences of the toxins may allow the identification of the amino acid residues of the channel that are essential for binding of the toxin. Moreover, the electrophysiological effects of site-3 toxins are analogous to those displayed by a number of naturally occurring mutant channel forms observed in periodic pathologies, such as paramyotonia congenita and hyperkalemic periodic paralysis (Cannon and Corey, 1993; Lehmann-Horn and Jurkat-Rott, 1999; Jurkat-Rott et al., 2000; Blumenthal and Seibert, 2003), constituting for this reason a potential tool for the development of animal models of these pathologies. Finally, because some site-3 toxins are highly efficacious and potent insect-selective toxins (Bosmans et al., 2002), these compounds could constitute a prototype for the design of new insecticides.

In this work, we have characterized the electrophysiological effects of APC, a peptide toxin obtained from the sea anemone *Anthopleura elegantissima*, on mammalian neurons.

2. Results

A total of 92 neurons were successfully current- or voltage-clamped for a sufficient time to allow the study of ApC actions. The capacitances of the DRG cells formed a unimodal histogram that was well fitted ($r^2=0.97$) by a Gaussian function with the mean = 51 ± 13 pF, corresponding to medium size DRG cells with a diameter of about 40 μm.

The average duration of the action potentials measured at 50% of its amplitude (D_{50}) was 2.1 ± 0.5 ms ($n=7$). Application of 3 μM ApC increased D_{50} to 34.7 ± 7.8 ms (Fig. 1). The maximum effect was always achieved within the first minute after perfusion with ApC. Washout (5 min) removed the toxin effects in 95 ± 2%. The mean membrane potential in these experiments was −59 ± 0.4 mV. ApC 3 μM produced a significant ($P<0.05$, Student's t test) depolarization in the cell membrane potential of 4.8 ± 0.4 mV. Washout (5 min) repolarized the cell membrane to −58 ± 0.5 mV.

In voltage-clamp experiments, the percentage of change in peak amplitude and activation and inactivation time constants were calculated for ionic currents from both TTX-S and TTX-R Na^+ currents before and about 2 min after toxin perfusion. Concentration–response curves for these variables were built using 0.1, 0.3, 1, 3, and 10 μM ApC.

The main effect of ApC was a concentration-dependent increase in the TTX-S I_{Na} inactivation time course (Fig. 2A) with no significant effects ($P>0.05$, Student's t test) on the activation time course or peak current amplitude. In the presence of 1 μM ApC, the inactivation time constant (τ_h) increased 35.7 ± 4.6% ($n=4$), whereas perfusion with 3 μM ApC produced 94 ± 20% ($n=12$) increase in τ_h. The maximum effect of ApC on τ_h occurred within the first minute after perfusion with toxin. Washout (5 min) removed 91 ± 9% of the effect of 3 μM ApC. The IC_{50} value derived from concentration–response curve (Fig. 2B) was 1.25 ± 0.9 μM. The ratio I_{5ms}/I_{peak} in control conditions at −30 mV had a value of 0.1 ± 0.02. Use of 3 μM ApC ($n=12$) significantly increased I_{5ms}/I_{peak} to 0.34 ± 0.02, whereas 10 μM ApC increased the ratio to 0.39 ± 0.04. A concentration of 3 μM was chosen for the remaining experiments in this study because its effect was close to the maximum, and because supplies of ApC were limited.

Current density versus voltage curves were obtained from current–voltage relationships by dividing peak ionic-current amplitudes by the corresponding cell-membrane capacity.

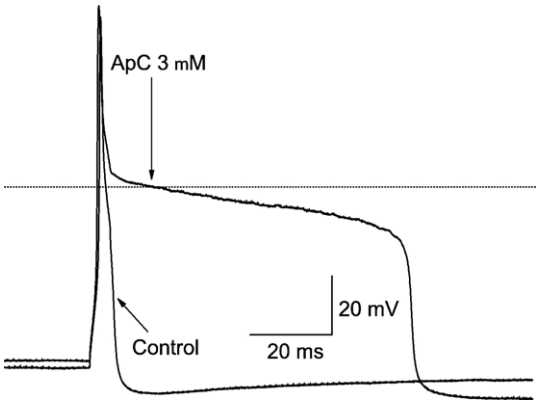

Fig. 1 – Effects of ApC on the action potential of DRG cells. Action potentials elicited by 100 pA, 2.5 ms stimuli were recorded under control conditions and after 3 μM ApC application. ApC increased the duration of action potential 1670% ($n=7$). The dotted line indicates the zero voltage level.