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Effect of sea anemone toxin anthopleurin-Q on sodium current in guinea pig ventricular myocytes

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KEY WORDS sea anemone; toxins; patch-clamp techniques; myocardium; sodium current

ABSTRACT

AIM: To investigate the effects of a sea anemone toxin anthopleurin-Q (AP-Q) isolated from *Anthopleura xanthogrammica* on sodium current (I_{Na}) in isolated guinea pig ventricular myocytes. **METHODS**: Single myocytes were dissociated by enzymatic dissociation method. I_{Na} was recorded using whole-cell patch-clamp technique. **RESULTS**: AP-Q (3 – 300 nmol/L) increased I_{Na} in a concentration-dependent manner. The EC_{50} value for increasing I_{Na} was 104 nmol/L (95 % confidence range: 78 – 130 nmol/L). AP-Q 300 nmol/L shifted the *I-V* curve to the leftward, changed the membrane potential of half maximal activation to more negative potential from (-36.3 ± 2.3) mV to (-43 ± 3) mV ($n = 6$, $P < 0.01$) and changed the membrane potential of half maximal inactivation to more positive potential from (-75 ± 6) mV to (-59 ± 5) mV ($n = 6$, $P < 0.01$). AP-Q 300 nmol/L shortened the half-recovery time of I_{Na} from (114 ± 36) ms to (17 ± 2) ms ($n = 6$, $P < 0.01$). The fast inactivation time constant (τ_f) of I_{Na} was markedly increased by AP-Q 300 nmol/L. **CONCLUSION**: AP-Q has a stimulating effect on I_{Na} with slowing the inactivation course of I_{Na}.

INTRODUCTION

Sea anemone toxins are polypeptide toxins extracted from marine invertebrates-sea anemones. There are many kinds of sea anemone toxins, including anemone sulcata toxins (ATX) extracted from *Anemona sulcata*, actinia equina toxins (EqT) from *Actinia equina*, anthopleurin toxins from *Anthopleura xanthogrammica* and so on. The toxins were first isolated as cardiac stimulators and neurotoxins[1] and these two activities remain the primary focus of attention. Most toxins are known to bind selectively to sodium channels in neuronal cells, cardiac myocytes, and skeletal muscles, and prolong the time course of sodium current (I_{Na}) by slowing channel inactivation with little or no effect on activation[2~6]. Binding studies and electrophysiological experiments have suggested a higher affinity of sea anemone toxins for tetrodotoxin (TTX)-insensitive than for TTX-sensitive sodium channels[3-5,7,8], implying a different channel structure in the reactive region. Otherwise, some report suggested that sea anemone toxins could increase the susceptibility of the sodium channel to TTX and lignocaine[9]. In mammalian cardiac muscle preparations, the delay in sodium current inactivation can induce prolongation of the action potential duration (APD)[5,8], which can lead to voltage oscillations on the plateau and may be associated with malignant tachyarrhythmias in the intact heart. Increased sodium entry may cause a positive inotropic effect via Na^+-Ca^{2+} exchange[10-12].

Anthopleurin-Q (AP-Q) is one of the anthopleurin toxins extracted from *Anthopleurin xanthogrammica*. Its electrophysiological characters are unknown. In our previous study, AP-Q 30 – 300 nmol/L showed concentration-dependent positive inotropic effect could not be blocked by verapamil in guinea pig left atrium, and AP-Q 300 nmol/L markedly prolonged the APD of isolated guinea pig papillary muscles (inner data). So we postulated that it might act through modulating sodium channel. In the present study, the effect of AP-Q on I_{Na} in guinea pig ventricular myocytes was investigated using whole-cell patch clamp technique.

MATERIALS AND METHODS

Drugs and animals AP-Q was supplied by

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Qingdao Marine Biology Research Institute. It is a stable and basic polypeptide of 40 amino acid residues, M_r = 4840, purity > 99 %. It was easily dissolved in distilled water.

Collagenase I, protease E, bovine serum albumin (BSA), Na_2ATP, HEPES, CsCl, $CdCl_2$, and CsOH were products of Sigma Co. Egtazic acid was purchased from Fluka Biochemica. Tetrodotoxin (TTX) was purchased from Hebei Aquatic Product Research Institute. Other analytical reagents were products of Shanghai Chemical Reagent Plant. Male or female guinea pigs weighing (300 ± 40) g were supplied by the Medical Experimental Ainimal Center of Tongji Medical College of Huazhong University of Science and Technology, Grade II, Certificate № 19-023.

Preparation of single ventricular myocytes Single ventricular myocytes were isolated using enzymatic dissociation method similar to that previously described[13].

I_{Na} recording technique I_{Na} was recorded using whole-cell patch-clamp technique with a patch-clamp amplifier (PC-II, Huazhong University of Science ane Technology). Pclamp 6.0 software (P-6, Huazhong University of Science and Technology) was used to produce the signal, collect and process the data. The resistance of the glass electrodes used were 2 – 3 MΩ when they were filled with the pipette solution and immersed in the extracellular solution. The pipette solution for I_{Na} measurments contained (mmol/L): CsCl 120, $MgCl_2$ 5.0, $CaCl_2$ 1.0, Na_2ATP 5.0, egtazic acid 11, HEPES 10, Glucose 11, the pH was adjusted to 7.3 with CsOH. The extracellular solution for I_{Na} measurements contained (mmol/L): NaCl 40, choline-Cl 100, KCl 5.4, $CaCl_2$ 1.0, $MgCl_2$ 1.0, NaH_2PO_4 0.33, HEPES 10, Glucose 10, the pH was adjusted to 7.4 with NaOH. In the extracellular solution, we added $CdCl_2$ 100 μmol/L to block $I_{Ca,L}$ and $NiCl_2$ 40 μmol/L to block $I_{Ca,T}$. The experiments were performed at room temperature (19 – 20 ℃).

Data analysis The data were analyzed by using Sigmaplot (Jandel Scientific) software, and were presented as $\bar{x} \pm s$. Concentration-effect relationship was calculated with the Hill equation and statistical significances were analyzed by t-test. A value of $P < 0.05$ was considered significant.

RESULTS

Effect of AP-Q on I_{Na} When the holding potential was – 90 mV, and the cells were depolarized to – 30 mV for 25 ms at a frequency of 0.1 Hz, an inward current was invoked, which could be blocked by TTX 30 μmol/L, therefore referred to as I_{Na}. AP-Q 300 nmol/L increased the amplitude of peak I_{Na} from (110 ± 14) pA/pF to (176 ± 24) pA/pF ($n = 6$, $P < 0.01$), it also slowed the decay course of I_{Na} apparently. Before treatment with AP-Q, the transient inward I_{Na} inactivated rapidly after the depolarization, while after perfusion with AP-Q 300 nmol/L, the inward current was decayed slowly. The onset of action was at 1 – 2 min after addition of the agent, and about 5 min were required for reaching the steady effects, which could sustained for more than 20 min. The effect could only be partially reversed after washing the cells with drug-free solution for 10 min, the amplitude of peak I_{Na} after washing out was (151 ± 17) pA/pF. However, I_{Na} recorded after AP-Q 300 nmol/L could be blocked completely by TTX 60 μmol/L (Fig 1).

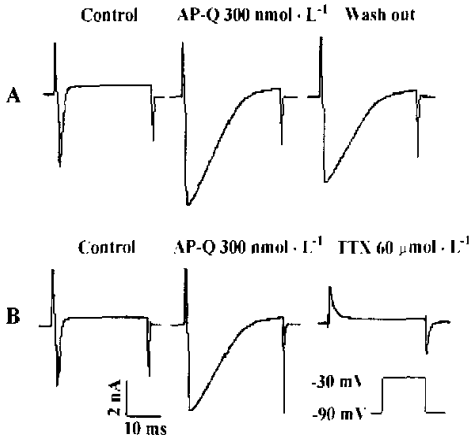

Fig 1. Sodium current (I_{Na}) elicited by depolarization to – 30 mV at the holding potential of – 90 mV in guinea pig ventricular myocytes. A) The increase in I_{Na} by AP-Q 300 nmol/L could be partially reversed after washing out. B) I_{Na} recorded after AP-Q 300 nmol/L could be blocked completely by TTX 60 μmol/L.

AP-Q 3 – 300 nmol/L produced a concentration-dependent increase of peak I_{Na}. The EC_{50} value for increasing of peak I_{Na} was 104 nmol/L (95 % confidence range: 78 – 130 nmol/L) with the maximal increase of peak I_{Na} up to 57 % ± 12 % at AP-Q 300 nmol/L (Fig 2). The maximal increase in peak I_{Na} did not further enhance with the concentration raised to 1 μmol/L.

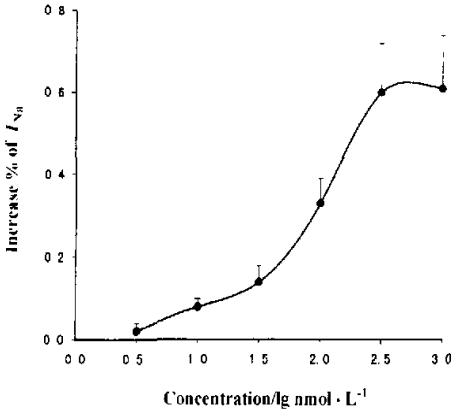

Fig 2. Concentration-dependent increase in I_{Na} by AP-Q. I_{Na} were elicited by depolarization to − 30 mV from the holding potential of − 90 mV. n = 6 cells. $\bar{x} \pm s$.

Effect of AP-Q on current-voltage relationship of I_{Na} Current-voltage relationship (I-V) curves were generated by applying a series depolarizing pulses from a holding potential of − 90 mV to different membrane potentials (− 60 − + 60 mV) with a 10 mV increment. The maximal activation of I_{Na} occurred at − 30 mV. After a 5-min exposure of the cell to AP-Q 300 nmol/L, peak I_{Na} was increased at the membrane potentials of − 50 − 0 mV ($P < 0.05$ or 0.01), but the increasing effect was not significant at positive membrane potentials (+ 10 − + 60 mV), so the I-V curve shifted to leftward and downward. But the average maximal amplitude of I_{Na} still occurred at V_m = − 30 mV (Fig 3).

Effects of AP-Q on steady-state activation and inactivation of I_{Na} On the basis of data obtained from current-voltage relationship, activation curves were obtained (Fig 4), they were fitted by the Boltzmann function: $G_{Na}/G_{Namax} = 1/[1 - \exp(V - V_{1/2})/k]$, where G_{Na} was membrane conductance transferred from membrane current, G_{Namax} was the maximal membrane conductance, $V_{1/2}$ was the membrane potential of half maximal activation and k was slope factor. The values of $V_{1/2}$ in control and in AP-Q 300 nmol/L were (− 36.3 ± 2.3) mV and (− 43 ± 3) mV (n = 6, $P < 0.01$), respectively, with a slope factor k of (3.6 ± 1.5) mV and (3.5 ± 1.1) mV (n = 6, $P > 0.05$), respectively. AP-Q shifted activation curve towards more negative potential without change the slope factor (Fig 4).

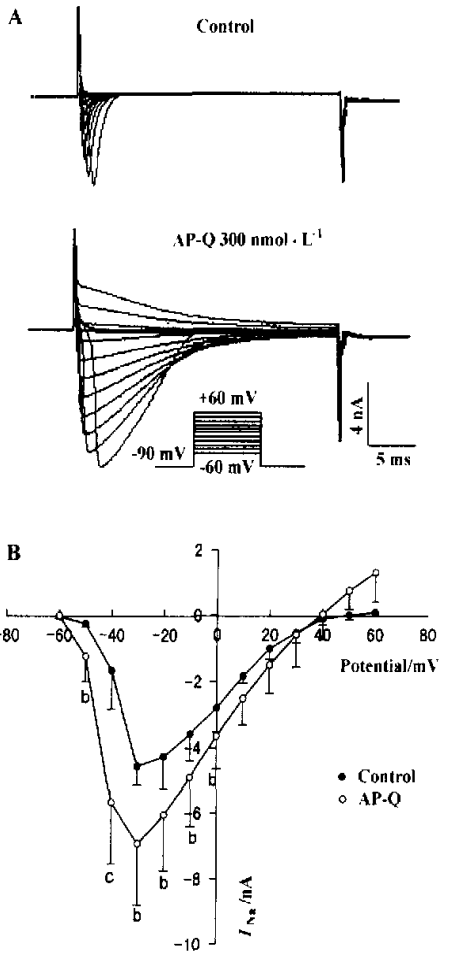

Fig 3. Voltage-dependent effect of AP-Q in I_{Na}. A) I_{Na} traces before and after AP-Q 300 nmol/L. B) Current-voltage relationship (I-V) curves of I_{Na}. I_{Na} were evoked by the step pulses from − 60 mV to + 60 mV at the holding potential of − 90 mV. n = 6 cells. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

The steady-state inactivation of I_{Na} was evaluated using a conventional double pulse protocol. The inactivating prepulse was stepped from a holding potential of − 90 mV to membrane potentials of − 120 − 0 mV in 10 mV steps for 1 s. The test pulse was stepped to − 30 mV for 25 ms. The current elicited by the test pulse (I_{Na}) was normalized as a fraction of the maximal current (I_{Namax}) obtained when the prepulse was − 120 mV and plotted as a function of the prepulse membrane potential (Fig 4). The inactivation curves were fitted by the Boltzmann function: $I_{Na}/I_{Namax} = 1/[1 + \exp(V - V_{1/2})/k]$, where $V_{1/2}$ was the membrane potential of

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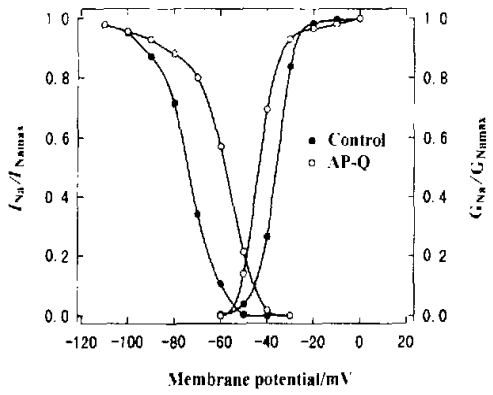

Fig 4. Effects of AP-Q 300 nmol/L on steady state activation and inactivation curves of I_{Na}. $n = 6$ cells.

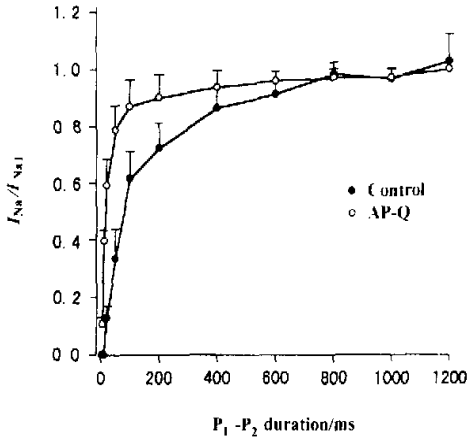

Fig 5. Effect of AP-Q 300 nmol/L on recovery time from inactivation of sodium channel. The recovery of I_{Na} from inactivation was studied by using paired pulses (P_1 and P_2) to −30 mV from a holding potential of −90 mV, P_1 and P_2 separated by an interval varying from 5 −1200 ms. I_{Na1} was I_{Na} elicited by P_1, and I_{Na2} was I_{Na} elicited by P_2. $n = 6$ cells. $\bar{x} \pm s$.

half maximal inactivation and k was slope factor. $V_{1/2}$ in control and in AP-Q 300 nmol/L were (-75 ± 6) mV and (-59 ± 5) mV ($n = 6$, $P < 0.01$), respectively, with a slope factor k of (5.7 ± 2.4) mV and (7.1 ± 2.3) mV ($n = 6$, $P > 0.05$), respectively. AP-Q shifted inactivation curve towards more positive potential, it had a tendency to increase the slope factor, but the increase was not significant ($P > 0.05$) (Fig 4).

Effect of AP-Q on recovery of I_{Na} from inactivation The recovery of I_{Na} from inactivation was studied by using paired pulses delivered at 0.1 Hz. Two pulses (P_1 500 ms and P_2 50 ms) to −30 mV from a holding potential of −90 mV were separated by an interval varying from 5 − 1200 ms. I_{Na} elicited by P_2 (I_{Na2}) was normalized by I_{Na} elicited by P_1 (I_{Na1}) and plotted as a function of the P_1-P_2 interval. AP-Q 300 nmol/L shortened half-recovery time of I_{Na} from (114 ± 36) ms to (17 ± 2) ms ($n = 6$, $P < 0.01$) (Fig 5).

Effect of AP-Q on inactivation kinetics of I_{Na} Under control conditions and in the presence of AP-Q 300 nmol/L, the inactivation course of I_{Na} at membrane potentials of −40 − +10 mV can be fitted by biexponential: the fast inactivation course, which constitutes approximately 85 % of the total inactivation, had a time constant (τ_f) much longer in the presence of AP-Q than in control under all membrane potentials ($n = 6$, $P < 0.01$) (Fig 6). The slower time constant typically comprised 10 % − 15 % of the total inactivation was similar in control and toxin-treated cells.

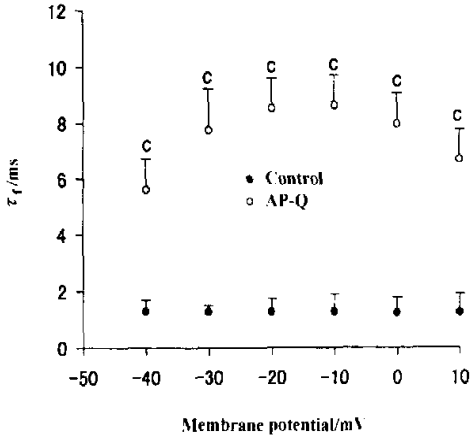

Fig 6. Effects of AP-Q 300 nmol/L on the time constant of the fast inactivation course (τ_f) of sodium channel. $n = 6$ cells. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

DISCUSSION

The results showed that AP-Q exerted an increasing effect on I_{Na} in a concentration-dependent and partially reversible manner. I_{Na} was not further increased when the concentration of AP-Q raised to 1 μmol/L, which suggested that the binding of AP-Q to the sodium channel got saturable at high concentrations. AP-Q 300 nmol/L increased I_{Na} at all tested potentials and shifted the I-V curve of I_{Na} to the left, which suggested that the stimulating in I_{Na} of AP-Q was voltage-dependent and its binding affinity to sodium channels decreased under

membrane depolarization. This was consistent with the effects of ATX on sodium channels in crayfish giant axons[2]. AP-Q 300 nmol/L shifted the activation curve leftwardly, and the half activation potential was changed from (− 36.3 ± 2.3) mV to (− 43 ± 3) mV, that is, made the sodium channel opened at more negative potentials, this also suggested that AP-Q could stimulate sodium channel activation in guinea pig ventricular myocytes. This result was different from other report on ATX which showed that ATX slowed the Na^+ current inactivation without affecting the activation process[2]. AP-Q 300 nmol/L shifted the steady inactivation curve to the right, and the half inactivation potential was changed to more positive potential from (− 75 ± 6) mV to (− 59 ± 5) mV, which suggested that AP-Q could increase the availability of sodium channels for activation in guinea-pig ventricular myocytes. AP-Q 300 nmol/L markedly shortened sodium channel recovery time from inactivation which meant that the sodium channel spending less time to recover, thus the duration of inactivation was decreased. This character should contribute to its stimulating effect on sodium channel.

In the absence of toxin, typical sodium currents decayed rapidly to zero level in several milliseconds after the depolarization, however in the presence of toxin, the currents decayed very slowly and the time constants of the inactivation course at AP-Q 300 nmol/L was significantly prolonged, we postulated that the toxin might modify the channels gating kinetics and inhibited the channel converting to close state from activation. This may be another reason for the increasing of the sodium current. However, the sodium channel could eventually inactivate completely, and the steady state inactivation curve could reach zero, this was different from some reports on other sea anemone toxins, which induced a noninactivating current, as reflected by the failure of steady state inactivation relations to reach zero, even at positive potential[2-5].

The results of the present study indicated that AP-Q increased I_{Na} with change of activation, inactivation and recovery characters of sodium channel at 300 nmol/L and the effect could be reversed by TTX, these effects on sodium channels may make it a potentially valuable tool in physiological and pharmacological research. Otherwise, AP-Q slowed the inactivation kinetics of sodium channel markedly, ie, sodium channels might decay slowly, sometimes exhibit repetitive reopenings, and the repolarization of action potential was delayed. So it could be implicated in imitating some pathophysiological models in the investigation of a variety of genetic diseases that target sodium channel inactivation in skeletal muscle (eg myotonias and periodic paralysis) and heart (long QT syndrome).

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海葵毒素 anthopleurin-Q 对豚鼠心室肌细胞钠电流的作用

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关键词 海葵；毒素类；膜片箝技术；心肌；钠电流

目的：研究从海葵（*Anthopleura xanthogrammica*）提取的毒素 anthopleurin-Q（AP-Q）对豚鼠心室肌钠电流（I_{Na}）的作用. **方法**：用酶消化法分离豚鼠单个心室肌细胞，用全细胞膜片箝技术记录心室肌细胞钠电流. **结果**：AP-Q 3 - 30 nmol/L 浓度依赖性地增大 I_{Na}，EC_{50}为 104 nmol/L（95 %可信范围：78 - 130 nmol/L）. AP-Q 300 nmol/L 使 *I-V* 曲线左移，使半数激活电压从（- 36.3 ± 2.3）mV 变为（- 43 ± 3）mV（$n = 6$，$P < 0.01$），半数失活电压从（- 75 ± 6）mV 变为（- 59 ± 5）mV（$n = 6$，$P < 0.01$）. AP-Q 300 nmol/L 使 I_{Na}半数恢复时间从（114 ± 36）ms 缩短为（17 ± 2）ms（$n = 6$，$P < 0.01$），并明显减慢 I_{Na}的快速失活时间常数（τ_f）. **结论**：AP-Q 对 I_{Na}有促进作用并减慢其失活过程.

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