**Laboratory Research**

**Effects of sea anemone toxin anthopleurin-Q on potassium currents in rats and guinea pig ventricular myocytes**

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**Objective** To investigate the effect of sea anemone toxin anthopleurin-Q (AP-Q) on potassium currents in isolated rats and guinea pig ventricular myocytes. **Methods** The ventricular cells of guinea pigs and SD rats were obtained by enzymatic dissociation method. Whole cell patch clamp technique was used to record potassium currents (I<sub>K</sub>, I<sub>A1</sub>, and I<sub>A2</sub>). **Results** AP-Q 3-100 nmol/L increased I<sub>K</sub> in a concentration-dependent manner, with an EC<sub>50</sub> value of 12.7 nmol/L. At a potential of +50mV, AP-Q 10 nmol/L increased I<sub>K</sub> from (13.3±3.4) pA pF<sup>-1</sup> to (19.46±4.3) pA pF<sup>-1</sup>. AP-Q 0.1-100 nmol/L increased I<sub>A1</sub> and I<sub>A2</sub> tail in a concentration-dependent manner with EC<sub>50</sub> values of 4.7 nmol/L and 5.0 nmol/L, respectively. AP-Q 1 pmol/L-100 nmol/L increased I<sub>A2</sub> in dose-dependent manner, with an EC<sub>50</sub> of 0.2 nmol/L. **Conclusions** The effect of AP-Q on I<sub>K</sub>, I<sub>A1</sub> and I<sub>A2</sub> may partly explain its mechanism in shortening APD and increasing RP. (J Geriatr Cardiol 2008; 5:243-247)

**Key words** sea anemone toxin AP-Q; ventricular myocytes; potassium current

Sea anemone toxins are poly peptides extracted from sea coelenterate anemones. These toxins specifically bind to ion channels in cardiac myocytes, neuronal cells and skeletal muscles. Anthopleurin-Q (AP-Q) is a 40 amino acid toxin extracted from yellow sea anemone Anthopleura xanthogrammica. Its effects have mainly been associated to ion channels in cardiac myocytes, neuronal cells and skeletal muscles. Anthopleurin-Q (AP-Q) is a 40 amino acid toxin extracted from yellow sea anemone Anthopleura xanthogrammica. Its effects have mainly been associated to ion channels in cardiac myocytes, neuronal cells and skeletal muscles. Anthopleurin-Q (AP-Q) is a 40 amino acid toxin extracted from yellow sea anemone Anthopleura xanthogrammica. Its effects have only been recently reported.

**Materials and methods**

**Isolation of ventricular myocytes**

Ventricular myocytes were dissociated from guinea pig and rat hearts by enzymatic dissociation method. All procedures were approved by the Animal Research Committee of the Graduate School of Medicine, Xinjiang Medical University. The animals were deeply anesthetized by pentobarbital sodium (>0.1 mg/g, ip). For the guinea pig hearts, thoracotomy was performed under artificial respiration and the aorta was cannulated to start retrograde perfusion of the heart. The heart was quickly excised, mounted on a horizontal Langendorff apparatus and perfused with Ca<sup>2+</sup>-free Tyrode’s solution containing 0.4 mg/ml collagenase (type I; Sigma) and protease (type E, 0.0125 mg/ml; Sigma) for 5 min at 37°C. For male Sprague-Dawley (SD) rats, the hearts were perfused with a cell isolation solution containing 0.4 mg/ml collagenase (type I; Sigma) and protease (type E, 0.0125 mg/ml; Sigma) for 20 min. Isolated cells were placed into 4-mL aliquots enzyme-free Tyrode’s solution containing bovine serum albumin (BSA, 0.025%; Sigma) and 200 μg/L ampicillin. The cells were stored in this enzyme-free solution at 22°C. When required, a drop of cell suspension was placed in a small chamber (volume 1 ml) on the stage of an inverted microscope. After 10-15 min, when the cells had settled onto the glass bottom of the chamber, the chamber was perfused at ~2.5 ml/min at room temperature (22°C). The cells were allowed to stabilize in the bath for at least 30 minutes before the experiment.

**Solutions and chemicals**

The control Tyrode solution contained: mmol/L, NaCl 140, KCl 5.4, CaCl<sub>2</sub> 1.8, MgCl<sub>2</sub> 0.5, NaH<sub>2</sub>PO<sub>4</sub> 0.3, glucose 5.5, and HEPES 5.0 (pH 7.4, adjusted with NaOH). This solution was aerated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>, pH 7.4. Mg-ATP, HEPES, BaCl<sub>2</sub>, TTX and 4-AP were products of Sigma Chemical Co.. EGTA purchased from Fluka Biochemika and doxetilide was obtained from Pfizer. The solutions were made with distilled water. AP-Q was supplied by Qindao Institute...
of Marine Biology. A stock solution of 4-AP (3mmol/L), TTX (30µmol/L), BaCl₂ (0.2 mmol/L), CdCl₂ and dofetilide (10 µmol/L) was made with distilled water, and the pH was adjusted to 7.4 using 2N HCl.

The internal solution: mmol/L, KCl 45, K-aspartate 85, Na-pyruvate 5, MgATP 5.0, EGTA 10, HEPES 10, glucose 11 (pH 7.3, adjusted with KOH). For Iᵥ, total measurements, 200 µmol/L CdCl₂ and 30 µmol/L TTX were added in the bath Tyrode’s solution to inhibit Iᵥ, L and Iᵥ,K, respectively. For Iᵥ, measurements, 200 µmol/L CdCl₂ was added to block Iᵥ. For Iᵥ,K, measurements, 200 µmol/L CdCl₂ was added in the bath Tyrode’s solution to block Iᵥ and 0.2 mmol/L BaCl₂ added to block Iᵥ,K.

Electrophysiology

The myocytes were voltage-clamped using the whole cell voltage-clamp method with Axopatch 1D (Axon Instruments) patch-clamp amplifier. 2.5-3.5MΩ 1 mm² Glass capillaries were pulled using a two-stage vertical pipette puller and then fire-polished. Voltage clamp protocols used in these experiments are computer-generated ramps, pulses, and voltage step changes. The liquid junction potential and gigaohm seal formation were corrected. Electrical access was usually obtained within 5-10 min, followed by an additional 25 min before the experiment. Pipette capacitance was electronically compensated, and membrane current was filtered at 1 kHz (low-pass Bessel filter). The capacitance of the patch was calculated using the relation Cm = I (dV / dt-1), where Cm was the cell membrane capacitance per surface unit. I was the net membrane current and dV / dt was the value of maximal voltage slope. Values of I were recorded in a cell-attached configuration and the rate of change in membrane potential was measured from intracellular action potentials recorded after breaking into the cell.

Membrane currents were analyzed from both depolarization and hyperpolarization protocols. Using voltage clamp technique, membrane potassium currents were evoked during voltage-ramp protocols. A holding potential of -40 mV was used to induce total potassium currents, given from -100 to +50 mV constant speed ramp stimulate (30 mV/s). Reversal potential (Erev) for potassium was -75mV.

Using voltage clamp technique, given from -40 mV to +50 mV, 300ms depolarizing pulses (holding potential of -40 mV), transient outward currents (Iₒ) were studied.

Using voltage clamp technique, given +50 mV, 7s depolarizing pulses (holding potential of -40 mV), delayed rectified outward potassium currents (Iᵥ) the tail currents were measured.

Using voltage clamp technique, given from -100 to +40 mV, 300 ms in 10-mV step pulses (holding potential of -40 mV), inward rectified currents (Iᵥ,K) were measured.

Statistical Analysis

The data were expressed as mean±SD. The differences before and after administrating AP-Q were analyzed using paired t-test. P value <0.05 was considered statistically significant.

Results

Effects of AP-Q on total potassium current in guinea pig ventricular myocytes

The sea anemone element 1 and 100nmol/L markedly increased the total inward potassium current and slightly increased the outward current. This result suggests that the sea anemone may have effects on different types of potassium electric current (Figure 1).

![Figure 1](image.png)

**Fig.1** Effect of AP-Q on total potassium current elicited by a ramp voltage protocol. The voltage ramp is from -100 mV to +50 mV in 5 s.

**Effects of AP-Q on Iₒ in rat ventricular myocytes**

When the cells were depolarized to +50 mV, AP-Q 10 nmol/L decreased Iₒ from 13.3 ± 3.4 pA pF⁻¹ (pre-administration) to 19.46 ± 4.3 pA pF⁻¹ (P<0.01, n = 12). The effect was partially restored after washing the cells with drug-free solution for 5 minutes. Iₒ could be blocked completely by 4-AP 3 mmol/L. AP-Q 3 - 100 nmol/L blocked Iₒ in a dose-dependent manner with an EC50 of 10.5 nmol/L (95% confidence interval, 8.1 to 21.6 nmol/L) (Figure 2 A).

**Effects of AP-Q on Iᵥ-curves of Iₒ in rat ventricular myocytes**

AP-Q 10 nmol/L reduced the amplitudes of different Iₒ membrane potentials, up-shift IV curves, but the decreasing effect was not significant. The results suggest that all has weak voltage-dependent inhibition of Iₒ (Figure 2 B).

**Effect of AP-Q on Iᵥ,K in guinea pig ventricular myocytes**

AP-Q 10 nmol/L increased Iᵥ,K and Iᵥ,K tail, from pre-administration 13.4 ± 2.9 pA pF⁻¹ and 4.5 ± 0.9 pA pF⁻¹ to 17.0 ±
4.1 pA pF-1 or 6.2 ± 1.3 pA pF-1, respectively (P < 0.01, n = 12). The increase in $I_{K}$ and $I_{K}^{t}$ tail was partially restored after washing with drug-free buffer. The percentage increase in $I_{K}$ and $I_{K}^{t}$ tail was 31±9% and 35±7%, respectively (Figure 3A). AP-Q 0.1-100 nmol/L increased $I_{K}$ and $I_{K}^{t}$ tail in a dose-dependent manner with an EC$_{50}$ of 4.7 nmol/L (95% confidence interval: 2.4 - 7.0 nmol/L) and 5.0 nmol/L (95% confidence interval: 3.8 - 6.2 nmol/L), respectively (Figure 3B).

Effect of AP-Q on $I_{K1}$ in guinea pig ventricular myocytes
AP-Q 1 pmol/L-100 nmol/L increased $I_{K1}$ in a dose-dependent manner at the membrane potential -100 mV with an EC$_{50}$ of 200 pmol/L (95% confidence interval 88 pmol/L - 237 pmol/L). AP-Q 100 nmol/L increased $I_{K1}$ from -35.12 ± 4.7 pA pF-1 to 50.1 ± 6.6 pA pF-1, a percentage increase of 43 ± 10% (P < 0.01, n = 11). After washing, the membrane potential was partially restored and decreased to -45.9 ± 6.4 pA pF-1. AP-Q 0.1 and 10 nmol L-1 increased all membrane potential of $I_{K1}$, especially the inward current of negative membrane potential (Figure 4).

Discussion
The results showed that AP-Q had a dose-dependent and partially reversible effect on guinea pigs ventricular myocytes $I_{K}$ and rat ventricular myocytes $I_{K}$. The effect of AP-Q on potassium currents is opposite to the majority of other types of sea anemone toxins, which block voltage dependent K$^{+}$ channels. For example, BgK toxin from Caribbean Sea sea anemone Bunodosoma granulifera, ShK toxin from sea anemone Stichodactyla helianthus, and the kaliclude and kalisepines in blood depressing substance (BDS-I and BDS-II) from sea anemone Sulcata, effectively block voltage dependent K$^{+}$ channels.5-8 The effect of AP-Q on $I_{K1}$ is similar to Bainh toxin from granulifera. Bainh can significantly increase $I_{K1}$ in single ventricle myocyte of rats or the dogs. AP-Q concentration which is greater than 100 nmol/L, can significantly slow down sodium channel deactivation function, and disrupt $I_{K}$ and $I_{K1}$ recording, hence no need to continuously increase the concentration.9
I_{K}, I_{K1}, and I_{K2} are the main ion currents involved in the myocardial action potential repolarization process. The low concentration of sea anemone element (0.1-100 nmol/L) increases I_{K} in a dose-dependent manner, which accelerates AP repolarization process and shorten APD. This effect might also reduce the elongated QT interval in long Q-T syndrome by shortening cardiac repolarization. I_{K1} plays an important role in the maintenance of resting membrane potential and the late AP repolarization in myocardial cells. I_{K1} is also conducive to the maintenance of platform period due to inward rectifying current. The main reason for maintaining myocyte resting potential in sea anemone is due to the enhancement of I_{K1} value. The absolute value of resting potential is increased, membrane hyperpolarized, the distance between membrane potential and threshold potential increased, and myocardial self-discipline decreased. All these effects favor canceling partial depolarizations and stabilizing myocardial trend, thus reducing the occurrence of arrhythmias.

The current anti-arrhythmic drugs are not ideal due to different reasons. The yellow sea anemone not only has an extremely rich marine resource, but also has a strong effect with little amount. Marine research is a broad research area and may become a new source of raw materials in the drug industry. Therefore, further study of the sea anemone toxin is still required to shed more light in its use as an anti-arrhythmic drug.

In conclusion, the effect of AP-Q on I_{K}, I_{K1} and I_{K2} may partly explain its mechanism in shortening APD and increasing RP.
References


