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Effect of acetylstrophanthidin on action potential duration and relation with extracellular potassium in sheep isolated Purkinje fibers[1]

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KEY WORDS acetylstrophanthidin; potassium; action potentials; Purkinje fibers

AIM: To study the relation between the effect of acetylstrophanthidin on action potential duration (APD) and the extracellular potassium concentration. **METHODS**: Effect of acetylstrophanthidin (AS 0.15 mmol·L^{-1}) on APD at different extracellular potassium concentrations was studied at the stimulation cycle lengths of 990 and 690 ms in sheep isolated cardiac Purkinje fibers using the standard microelectrode technique. **RESULTS**: At $[K^+]_o$ 4.0 mmol·L^{-1}, the biphasic effect of AS on APD appeared obviously. Both APD_{50} and APD_{90} were lengthened within the first 10 min of drug exposure. After 10 min, they were shortened at all pacing cycle lengths. On the other hand, at $[K^+]_o$ 5.4 mmol·L^{-1}, AS only shortened APD markedly without lengthening effect on it. The biphasic and monophasic effects of AS on APD were found at $[K^+]_o$ 4.0 mmol·L^{-1} and 5.4 mmol·L^{-1}, respectively. **CONCLUSION**: The effect of AS on APD was related to the concentration of $[K^+]_o$.

There were changes on action potential duration (APD) before afterdepolarization and triggered arrhythmias induced by digitalis[1-9]. Ito *et al*[10] studied the effects of 6 cardiac glycosides on the transmembrane potential and contractile characteristics of the right ventricle of guinea pigs. They divided 6 glycosides into 2 groups: group Ⅰ, consisting of digitoxin and proscillaridin, showed a dose-related prolongation of APD and time to peak tension; group Ⅱ, including ouabain and convallatoxol, primarily shortened the plateau of the action potential, whereas digoxin and dihydro-ouabain were essentially without dose-related effects on the APD. In our previous experiment[11], a biphasic effect of digoxin on APD and Q-T interval was found at lower concentrations of extracellular $[K^+]_o$ (4.0 mmol·L^{-1}). Therefore, there was a discrepancy between the data on the effect of digitalis on APD. The present study was to detect the effect of acetylstrophanthidin (AS) (0.15 mmol·L^{-1}) on APD and the relation with the concentrations of $[K^+]_o$ in adult sheep cardiac Purkinje fibers.

MATERIALS AND METHODS

Preparations Sheep hearts were obtained at a local abattoir. The hearts were, placed in cooled Tyrode's solution (near 4 ℃), and delivered to the laboratory where Purkinje fiber preparations were dissected[5]. The single free-running Purkinje fibers of left ventricle were used. The length of fibers was 14.7 ± 0.7 mm and width 0.10 ± 0.08 mm.

Solutions Tyrode's solution containing (in mmol·L^{-1}) NaCl 127, $CaCl_2$ 1.8, NaH_2PO_4 2.4, $MgCl_2$ 1.05, and glucose 5.5, was gassed with 95 % O_2 + 5 % CO_2 at 37 ± 0.5 ℃. To compare the effect of different concentrations of extracellular $[K^+]$ on APD during superfusing with AS, two concentrations of KCl (4.0 and 5.4 mmol·L^{-1}) were applied. AS (Sigma) was dissolved in distilled water to form a stock solution which was diluted with Tyrode's solution to a final concentration of 0.15 μmol·L^{-1} when used.

Intracellular recordings The intracellular action potential was measured with glass microelectrode (WPI, 1B150F-4 glass) filled

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with KCl 3 mol·L^{-1} and having resistances of 10 – 20 MΩ and small tip potentials. Single free-running Purkinje fibers were placed in a 2-mL tissue bath and superfused at 1 mL·min^{-1}, with normal Tyrode's solution. The glass microelectrode was impaled mid-length of a Purkinje fiber as the intracellular voltage-recording electrode and was connected via an Ag/AgCl half-cell to the input of a high-impedance amplifier. The preparation was stimulated by bipolar extracellular electrodes placed close to one end of the preparation (World Prement). Stimulating current pulses were 2 ms in duration with amplitudes 2 times of the threshold.

Experimental protocol Each Purkinje fiber was equilibrated at basic cycle length of 990 ms stimulation for a minimum of 1 h. To compare changing of APD, each fiber exhibited a normal action potential in the intracellular recordings, and was free of both spontaneous activity and afterdepolarizations. To study the biphasic and monophasic effects of AS on APD, the following protocol was used. At the end of the equilibration period, control measurements were made of the following parameters: APD at 50 % and 90 % repolarization (APD_{50} and APD_{90}); action potential amplitude; resting potential. The preparation was superfused with Tyrode's solution containing AS for 20 and/or 30 min and then washout for 60 min. During superfusing AS, transmembrane action potentials were recorded every 5 min.

The measuring method of all data was described in detail in our previous report[12,13].

Data analysis Data were expressed as $\bar{x} \pm s$ and analyzed by a paired t test.

RESULTS

Biphasic effect of AS on APD Biphasic effect of AS (0.15 μmol·L^{-1}) on APD_{50} and APD_{90} were observed ($n = 10$), at the concentration of $[K^+]_o$ 4.0 mmol·L^{-1} at stimulation cycle length of 990 and 690 ms. APD were prolonged initially by AS within the first 10 min (Fig 1).

APD_{90} was lengthened from 378 ± 14 ms at the control condition to 415 ± 19 ms at exposure to AS for 10 min ($P < 0.05$). Similar results on APD_{50} were seen. However, continued AS exposure resulted in a decrease on APD_{50} and APD_{90} at both cycle lengths of 990 and 690 ms. APD_{90} was shortened to 305 ± 10 ms ($P < 0.05$) at exposure to AS for 20 min. This initial prolongation and then shortening of APD was called biphasic effect (Fig 2). Increasing and decreasing in APD_{50} and APD_{90} were proportional to the pacing cycle lengths in all cases.

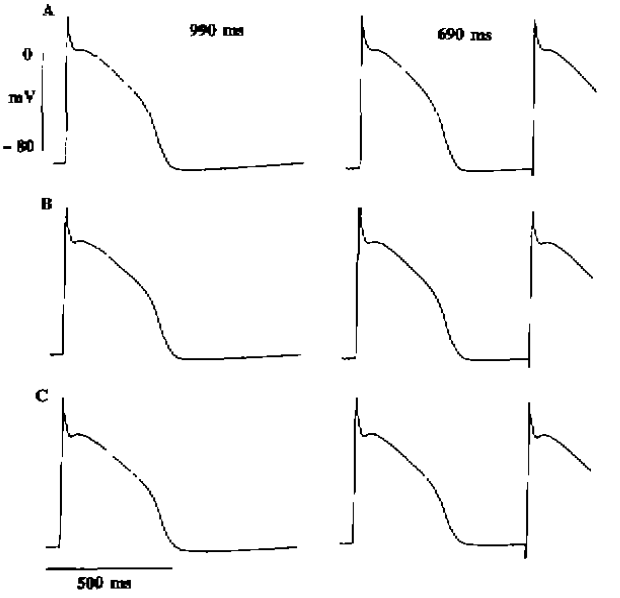

Fig 1. Initial lengthening effect of AS (0.15 μmol·L^{-1}) on APD at the pacing cycle lengths of 990 and 690 ms in sheep cardiac Purkinje fibers. (A) control. (B) and (C) after 5 and 10 min of AS exposure.

Monophasic effect of AS on APD Fig 3 showed a typical monophasic effect of AS (0.15 μmol·L^{-1}) on APD at the higher concentration of $[K^+]_o$ (5.4 mmol·L^{-1}, $n = 10$). AS shortened APD_{50} (upper panel) and APD_{90} (lower panel) from beginning of exposure to the drug to end at the pacing cycle lengths of 990 and 690 ms. At the control condition, APD_{90} was 324 ± 49 ms. After exposure to AS for 10, 20, and 30 min, APD_{90} shortened to 314 ± 40, 282 ± 30, and 229 ± 29 ms, respectively ($P < 0.05$). The similar results were obtained in APD_{50} and at cycle length of 690 ms. The decreasing in APD_{50} and APD_{90} were proportional to the pacing cycle lengths in all cases.

DISCUSSION

The major findings of the present study were that AS (0.15 μmol·L^{-1}) possessed a biphasic

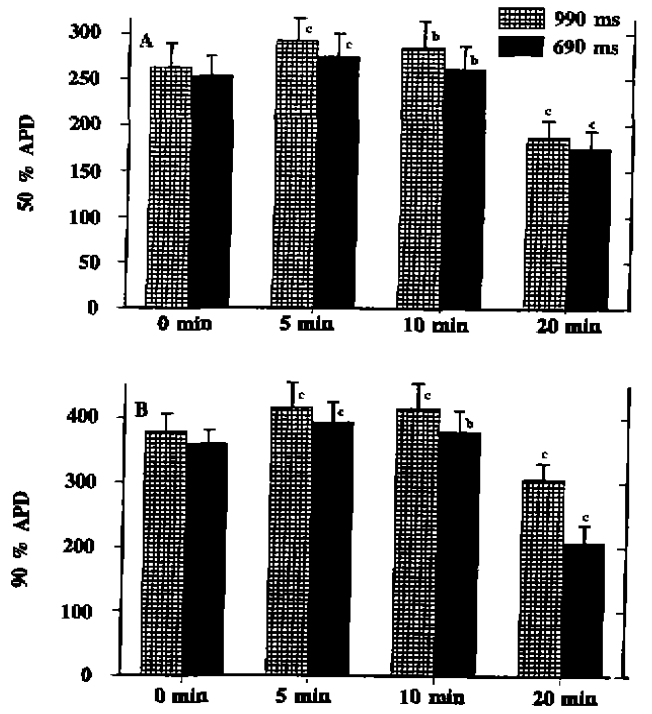

Fig 2. Biphasic effect of AS 0.15 μmol·L^{-1} on APD at [K$^+$]$_o$ 4.0 mmol·L^{-1}. $n = 10$, $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

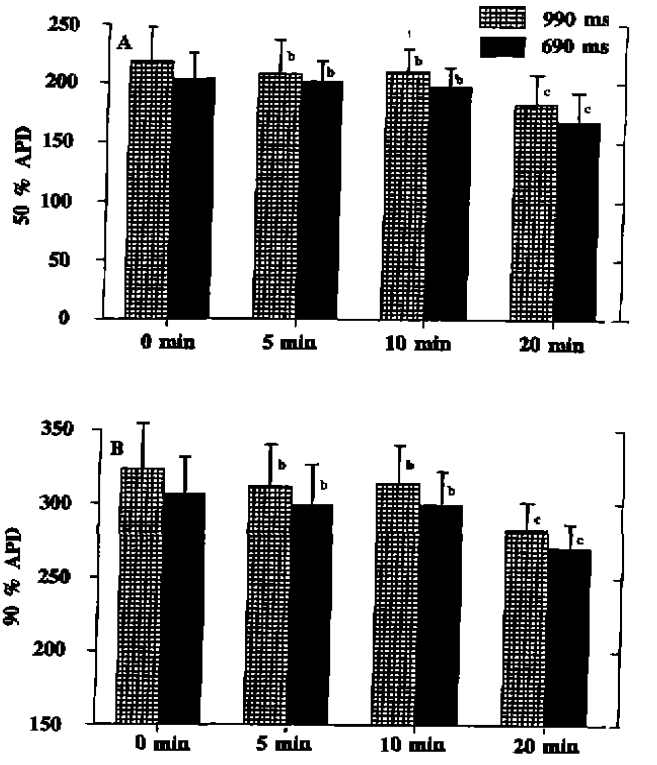

Fig 3. Monophasic effect of AS 0.15 μmol·L^{-1} on APD at [K$^+$]$_o$ 5.4 mmol·L^{-1}. $n = 10$, $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

effect on APD, including APD_{50} and APD_{90} at [K$^+$]$_o$ 4.0 mmol·L^{-1} at stimulation cycle lengths of 990 and 690 ms in sheep cardiac Purkinje fibers. This result was similar with the effect of digoxin on APD observed at [K$^+$]$_o$ 4.0 mmol·L^{-1}[11]. However, at higher concentration of [K$^+$]$_o$ (5.4 mmol·L^{-1}), only monophasic effect, shortening APD appeared in the same cases. Thus, the concentration of [K$^+$]$_o$ should be one of the important factors on the effect of AS on APD. There was a dose-dependent relationship between the effect of AS on APD and the concentration of [K$^+$]$_o$.

The mechanism of biphasic effect of AS on APD may relate with the concentration of [K$^+$]$_o$. At the lower concentration of [K$^+$]$_o$, since the Na$^+$/K$^+$ exchange pump produces repolarizing current by the net movement of monovalent cations, the initial application of AS could produce the prolongation of APD by suppressing repolarizing pump current. The subsequent shortening of APD may result from increase of [K$^+$]$_o$ in extracellular clefts. This accelerates the activation of a voltage-activated K$^+$ current[11]. However, because of the higher concentration of [K$^+$]$_o$ (5.4 mmol·L^{-1}), repolarizing current by the Na$^+$/K$^+$ exchange pump should be decreased and APD was shortened by AS from beginning to the end. This phenomenon was called monophasic effect of drug on APD.

In the present experiments, only one concentration of AS (0.15 μmol·L^{-1}) was used. However, we have got different effects of AS on APD at different stimulation cycle lengths. We did not find any relation between the concentration of AS and stimulation frequency.

It should be pointed out that delayed afterdepolarization and triggered arrhythmias induced by AS did not appear during prolongation of APD. These triggered activities always occurred during shorting of APD by the drug.

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酯毒毛花苷元对羊离体浦肯野纤维动作电位时程的影响及与细胞外钾离子的关系[1]

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关键词 酯毒毛花苷元; 钾; 动作电位; 浦肯野纤维

目的: 研究酯毒毛花苷元(AS)对羊心肌浦肯野纤维动作电位时程(APD)的影响, 以及与细胞外钾离子浓度的关系. **方法**: 标准玻璃微电极方法. **结果**: 胞外[K^+]浓度较低时(4.0 mmol·L^{-1}), AS 对 APD 的作用呈现双相性作用, 即在给 AS 的前 10 min, APD 延长, 而后缩短. 而在胞外[K^+]浓度较高时(5.4 mmol·L^{-1}), AS 只引起 APD 的缩短, 没有任何延长作用. **结论**: AS 对 APD 的影响与胞外[K^+]浓度有关.