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## 培养的大鼠颈上神经节交感神经元烟碱受体的动力学特性[1]

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郑建全, 何湘平, 杨爱珍, 刘传绩
(军事医学科学院毒物药物研究所, 北京 100850, 中国)

关键词 烟碱; 烟碱受体; 交感神经节; 药物动力学; 结合位点; 膜片箝技术

目的: 研究培养的新生大鼠颈上神经节交感神经元烟碱受体的动力学特性. 方法: 膜片箝技术的全细胞记录方法, 记录不同浓度烟碱诱发的电流, 使用 Clark 方程对烟碱作用的量效曲线进行拟合. 结果: 10, 20, 40, 80 和 160 μmol·L$^{-1}$烟碱诱发电流的幅度分别为: 0.91 ± 0.08, 1.56 ± 0.14, 2.53 ± 0.27, 3.93 ± 0.46 和 4.57 ± 0.55 nA ($n$ = 15), 经 Clark 方程拟合, 得到 $H$ = 1.097, $E_{max}$ = 5.958 nA, $K$ = 73.061 μmol·L$^{-1}$, 将 $H$ = 1 时拟合得到的 $E_{max}$(6.513 nA)和 $K$ 值(61.457 μmol·L$^{-1}$)代入 Clark 方程, 所计算出的理论值与相应浓度烟碱诱发电流的实测值基本相符. 结论: 烟碱与交感神经元烟碱受体作用的动力学特性符合一个作用位点的反应模型.

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# Electrophysiologic effect of enalapril on guinea pig papillary muscles *in vitro*

LIU Bo, Hu Da-Yi, GAO Yuan, LIU Xiu-Lan (*Heart Center, Beijing Red Cross Chaoyang Hospital affiliated to Capital University of Medical Sciences, Beijing 100020, China*)

**KEY WORDS** enalapril; action potentials; papillary muscles; ouabain; electrophysiology

**AIM**: To study the direct effect of enalapril on cellular electrophysiology of myocardium.
**METHODS**: Conventional microelectrodes technique was used to record the action potentials (AP) of guinea pig papillary muscles. **RESULTS**: Enalapril caused an increase of the AP amplitude (APA) and the resting potential (RP) in a concentration-dependent manner without any significant change of AP duration, $V_{max}$ and overshoot of AP. Superfusion of ouabain 0.5 μmol·L$^{-1}$ reduced APA and RP, induced stable delayed after-depolarizations (DAD) at different basic cycle lengths (BCL) in a frequency-dependent manner. At BCL 200 ms, the amplitude of DAD was large enough to induce nonsustained triggered activity (TA). In additional presence of enalapril 10 μmol·L$^{-1}$, the DAD amplitude at 500, 400, 300, and 200 ms were decreased from 5.3 ± 2.3, 5.9 ± 2.8, 7.4 ± 2.1, and 8.9 ± 1.3 to 2.6 ± 0.7, 3.1 ± 1.0, 3.7 ± 1.5, and 5.3 ± 1.1 (mV) respectively, all $P < 0.01$. The compensation intervals were increased in a similar frequency-dependent manner. The number of TA induced at BCL 200 ms was decreased from 3.6 ± 0.7 to 0.8 ± 0.2 ($P < 0.05$). **CONCLUSION**: Enalapril directly inhibits DAD and TA induced by ouabain through increasing RP and APA, which may contribute to its anti-arrhythmic effect.

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In patients with congestive heart failure, the incidence and complexity of ventricular arrhythmias were considerably lower in patients taking enalapril than taking placebo[1]. The possible anti-arrhythmic action of enalapril so far discussed was mainly due to relieving cardiac load by an increased fluid excretion and a diminution of peripheral vascular resistance. However, the direct effect of enalapril on cardiac cellular electrophysiologic properties, especially on arrhythmias caused by ouabain, was largely unknown. The purpose of this study was to elucidate the direct effect of enalapril on dalayed afterdepolarizations (DAD) and triggered activity (TA) induced by ouabain.

## MATERIALS AND METHODS

**Electric measurements** Twenty guinea pigs of either sex weighing 350 – 400 g, provided by Animal Center of Beijing Medical University, were killed by cervical dislocation. The right ventricular papillary muscle was placed in Tyrode's solution equilibrated with 95 % $O_2$ + 5 % $CO_2$ at 37 ± 1 ℃. The muscle was superfused with Tyrode's solution 8 – 10 mL·min$^{-1}$ with pH 7.4 ± 0.5. A pair of stainless electrodes were placed under the papillary muscles to stimulate the muscle at 1 Hz, 2 ms, and twice the diastolic threshold. After 1-h equilibration, transmembrane action potentials (AP) were measured with a glass microelectrode filled with KCl 3 mol·L$^{-1}$ connected to microelectrode amplifier (MEZ-8300, Nihon Kohden, Japan). AP and the miximal rate of membrane depolarization ($V_{max}$) continuously displayed on the oscilloscope showed on the Acquisition and Analysis of Medical Signals (NSA-Ⅲ, Nanjing Longhai Technical Co) by use of an IBM compatible computer. AP amplitude (APA), overshoot (OS) of AP and AP duration (APD) at 20 %, 50 %, and 90 % repolarization were measured.

**Experimental protocol** The effects of enalapril on AP were studied in a non-cumulative manner. Enalapril was washed out after a stable maximal effect was achieved, usually about 30 min. Enalapril 1, 10, 30, 50 μmol·L$^{-1}$ were added 10 min after AP returned to control condition. In a seperate experiment, the muscles were superfused with Tyrode's solution containing ouabain 0.5 μmol·L$^{-1}$ and DAD at the end of AP were monitored. When the DAD amplitude (DAD-Amp) reached a toxic value of ≥5 mV, enalapril 10 μmol·L$^{-1}$ was added to test the effect of enalapril on the toxic effect of ouabain. DAD-Amp and compensation interval (DAD-C, meassured from the beginning of AP to the plateau of DAD) of DAD were meassured at the end of 20 stimuli of various basic cycle lengths (BCL, 200, 300, 400, 500, 600, 800, and 1000 ms) at intervals of 20 s.

**Solutions and drugs** The Tyrode's solution contained (mmol·L$^{-1}$): NaCl 137, KCl 5.4, $NaHCO_3$ 11.9, $NaH_2PO_4$ 0.42, $MgCl_2$ 1.05, $CaCl_2$ 1.8, glucose 5.5, pH 7.4 ± 0.5. Enalapril maleate was purchased from Sigma Co and dissolved in $Me_2SO$ to give a stock solution of 100 mmol·L$^{-1}$. The same concentration of $Me_2SO$ was added to Tyrode's solution for control purposes. Ouabain was provided by Sigma Co, and dissolved in Tyrode's solution to 0.5 μmol·L$^{-1}$.

**Statistics** The differences among groups in Tab 1 were analysed by ANOVA and $q$ test. In Tab 2 and 3 the differences among groups were analysed by paried $t$ test through self-control design.

## RESULTS

***Effect of enalapril on AP*** Enalapril 1, 10, 30, and 50 μmol·L$^{-1}$ caused concentration-dependent increase of APA and RP. APA increased by 3.3 % ($P>0.05$), 6.7 % ($P<0.05$), 12.6 % ($P<0.01$), and 19.3 % ($P<0.01$) respectively. RP increased by 6.9 % ($P>0.01$), 9.2 % ($P<0.01$), 11.5 % ($P<0.01$), and 13.8 % ($P<0.01$), respectively, compared with control group. However, enalapril had no significant effect on $V_{max}$, OS, and APD (Tab 1).

**Tab 1. Effect of enalapril on AP of guinea pig papillary muscles. BCL = 1000 ms, $n$ = 7 guinea pig, $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.**

| Enalapril/μmol·L$^{-1}$ | 0 | 1 | 10 | 30 | 50 |
|---|---|---|---|---|---|
| APA/mV | 119 ± 8 | 123 ± 10$^a$ | 127 ± 4$^b$ | 134 ± 9$^c$ | 142 ± 13$^c$ |
| RP/mV | −87 ± 4 | −93 ± 2$^c$ | −95 ± 4$^c$ | −97 ± 4$^c$ | −99 ± 5$^c$ |
| $V_{max}$/V·s$^{-1}$ | 260 ± 46 | 270 ± 53$^a$ | 280 ± 30$^a$ | 290 ± 40$^a$ | 297 ± 20$^a$ |
| OS/mV | 32 ± 6 | 30 ± 11$^a$ | 37 ± 9$^a$ | 32 ± 3$^a$ | 39 ± 5$^a$ |
| $APD_{50}$/ms | 180 ± 40 | 173 ± 3$^a$ | 174 ± 13$^a$ | 171 ± 29$^a$ | 181 ± 15$^a$ |
| $APD_{90}$/ms | 215 ± 26 | 214 ± 21$^a$ | 190 ± 43$^a$ | 217 ± 25$^a$ | 221 ± 17$^a$ |

**Effects of enalapril on DAD and TA induced by ouabain** Ouabain caused a decrease of APA, RP, $V_{max}$ and OS by 11.6 % ($P<0.01$), 15.5 % ($P<0.01$), 33.1 % ($P<0.05$), and 39.5 % ($P<0.05$), respectively, compared with control group. In the presence of enalapril 10 $\mu mol \cdot L^{-1}$, APA, RP, $V_{max}$, and OS increased by 13.3 % ($P<0.01$), 17.1 % ($P<0.01$), 16.6 % ($P<0.01$), and 8.5 % ($P>0.05$) (Tab 2), respectively, compared with ouabain alone.

**Tab 2. Effect of enalapril (Ena) on AP under ouabain (Oub). BCL = 500 ms, $n$ = 7 guinea pigs, $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control; $^dP>0.05$, $^fP<0.01$ *vs* Ouabain.**

| | Control | Enalapril/ 10 $\mu mol \cdot L^{-1}$ | Ouabain/ 0.5 $\mu mol \cdot L^{-1}$ | Ena + Oub |
|---|---|---|---|---|
| APA/mV | 116±6 | 127±4$^b$ | 98±6$^c$ | 111±8$^f$ |
| RP/mV | −86±3 | −95±4$^c$ | −76±7$^c$ | −89±6$^f$ |
| $V_{max}/V \cdot s^{-1}$ | 260±52 | 280±30$^a$ | 176±28$^b$ | 191±17$^d$ |
| OS/mV | 38±2 | 37±9$^a$ | 23±6$^b$ | 29±5$^d$ |
| $APD_{50}$/ms | 170±35 | 174±13$^a$ | 180±35$^a$ | 171±29$^d$ |
| $APD_{90}$/ms | 213±18 | 190±43$^a$ | 227±25$^a$ | 222±27$^d$ |

Superfusion of Tyrode's solution containing ouabain 0.5 $\mu mol \cdot L^{-1}$ for 0.5 h caused stable DAD, the amplitude of which was increased with higher BCL of driving forces (Tab 3).

At BCL 200 ms, large DAD were induced followed by non-sustained TA (Fig 1). In the presence of enalapril 10 $\mu mol \cdot L^{-1}$ after toxic effect of ouabain was achieved, DAD-Amp was decreased and DAD-C was increased at all 7 BCL (Tab 3). At 50 $\mu mol \cdot L^{-1}$, DAD were nearly abolished. In the medial concentration of enalapril 10 $\mu mol \cdot L^{-1}$ the number of TA induced by ouabain at BCL 200 ms was decreased from 3.6±0.7 to 0.8±0.2 ($P<0.05$) (Fig 1).

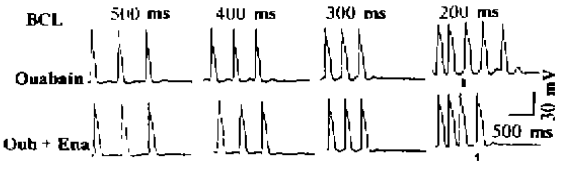


**Fig 1. Effect of enalapril 10 $\mu mol \cdot L^{-1}$ on DAD and TA induced by ouabain 0.5 $\mu mol \cdot L^{-1}$ in guinea pig papillary muscle.**

## DISCUSSION

Previous studies showed that ACE inhibitors reduced ventricular arrhythmia in animal models[2] and human trials[1]. In the present study we demonstrated that enalapril caused increases in RP and APA, which further increased electric synchronization and cardiac refractory periods[3], is also an important factor in mediating the inhibitory effect of enalapril on DAD and TA induced by ouabain in guinea pig papillary muscles.

DAD are oscillations occurring after full repolarization. Three different mechanisms were proposed for their initiation: a transient inward currents ($I_{ti}$) carried by $Na^+$; a current carried by $Na^+/Ca^{2+}$ exchanger; a catecholamine-induced $Cl^-$ currents[4]. The ACE inhibitor, captopril, may exert inhibitory effect on ouabain-induced DAD[5]. Another ACE inhibitor, perindoprilat, decreased $I_{ti}$ and prevented the action of norepinephrine on the $I_{ti}$, which explained why ACE inhibitors reduced ouabain-induced or reperfusion arrhythmias[5]. However, fosinoprilate caused a prolongation of the action potential, reduction of delayed rectifier $K^+$ currents and enhancement of L-type $Ca^{2+}$ currents in guinea pig ventricular myocytes and at 100 to 300 $\mu mol \cdot L^{-1}$, it may cause early after-depolarizations[6].

**Tab 3. Effect of enalapril (Ena, 10 $\mu mol \cdot L^{-1}$) on amplitude and compensation intervals of DAD induced by ouabain (Oub, 0.5 $\mu mol \cdot L^{-1}$). $n$ = 6 guinea pigs, $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* Oub.**

| BCL/ms | | 200 | 300 | 400 | 500 | 600 | 800 | 1000 |
|---|---|---|---|---|---|---|---|---|
| DAD-Amp/mV | Oub | 8.9±1.3 | 7.4±2.1 | 5.9±2.8 | 5.3±2.3 | 4.6±0.9 | 3.3±0.8 | 2.1±1.2 |
| | Oub + Ena | 5.3±1.1$^c$ | 3.7±1.5$^c$ | 3.1±1.0$^c$ | 2.6±0.7$^c$ | 2.2±0.5$^c$ | 1.5±0.4$^b$ | 1.4±0.3$^a$ |
| DAD-C/ms | Oub | 232±56 | 262±33 | 306±22 | 334±21 | 363±31 | 435±52 | 571±54 |
| | Oub + Ena | 365±28$^c$ | 360±53$^c$ | 359±33$^c$ | 389±31$^c$ | 412±24$^b$ | 525±68$^b$ | 672±64$^b$ |

In the present study, enalapril greatly inhibited DAD and TA caused by ouabain in frequency-dependent manner by an increment of APA and RP, which may in turn decrease the heart excitability induced by ouabain, ischemia, or ischemia-reperfusion. Captopril increased the Na/K pump in rabbit heart cells[7]. The activation of electrogenic $Na^+$ pump and the increment of $K^+$ currents to accelerate repolarization of myocardium might explain the effect of enalapril on RP and APA. However, it had no effect on APD, $V_{max}$ and OS, suggesting that it had no effect on $Na^+$ currents or $Ca^{2+}$ currents.

In conclusion, enalapril inhibited DAD and TA induced by ouabain in a frequency-dependent manner through decreasing heart excitability which may contribute to its anti-arrhythmic effect.

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## 依那普利对离体豚鼠乳头状肌的电生理作用

刘 勃，胡大一，高 渊，刘秀兰 (首都医科大学附属北京红十字朝阳医院心脏中心，北京 100020，中国)

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关键词 依那普利；动作电位；乳头状肌；哇巴因；电生理学

目的：研究依那普利(Ena)对豚鼠乳头状肌电生理特性，哇巴因诱发的延迟后除极(DAD)和触发电活动(TA)的直接作用. 方法与结果：采用标准玻璃微电极技术记录豚鼠乳头状肌动作电位. Ena 呈浓度依赖性增加静息膜电位(RP)和动作电位幅度(APA)，而对 0 期最大除极，超射，和动作电位时程无明显影响. Ena 10 μmol·L$^{-1}$则可明显抑制哇巴因 0.5 μmol·L$^{-1}$诱发的 DAD 和 TA，DAD 幅度分别由 5.3±2.3，5.9±2.8，7.4±2.1 和 8.9±1.3 降至 2.6±0.7，3.1±1.0，3.7±1.5 和 5.3±1.1 (mV) ($P$ 均<0.01)，刺激周长为 200 ms 时 TA 数目由 3.6±0.7 降至 0.8±0.2 ($P$<0.05). 结论：Ena 通过增加心肌细胞 RP 和 APA 抑制哇巴因诱发豚鼠乳头状肌 DAD 和 TA.