375—378
BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1994 Jul; 15 (4): 375—378

# 卡托普利对培养大鼠心肌细胞缺氧和复氧损伤的保护作用[1]

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**Protective effect of captopril on cultured rat myocardial cells with anoxia and reoxygenation injury**[1]

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**ABSTRACT** The effect of captopril (Cap) on electric activity of cultured rat myocardial cells under anoxia and reoxygenation was studied with standard microelectrode techinques. Results showed that anoxic solution caused lowerings of MDP, APA, and $V_{max}$, and a shortening of $APD_{50}$. All myocytes revealed multiform arrhythmias, and most cells stopped beating within 30 min, while only 40 % of the cells exhibited arrhythmias but none stopped beating in the presence of 40 mg·L$^{-1}$ under the same condition. During reoxygenation, most cells resumed beating in 10 min but some of these cells stopped beating again. The electric activities in rebeating cells during reoxygenation for 30 min were lower than those in normoxic cells. Cap (40 mg·L$^{-1}$)-treated cells rebeat quickly after reoxygenation and no cell stopped beating any more, with parameters higher than those in untreated cells. These results demonstrate that Cap yields some beneficial effects on preventing anoxia and reoxygenation injury in cultured rat myocardial cells.

**KEY WORDS** action potentials; anoxia; myocardial reperfusion injury; captopril; cultured cells

**摘要** 应用细胞内微电极技术观察到卡托普利(captopril，Cap)在40 mg·L$^{-1}$浓度下能延长培养大鼠心肌细胞在缺氧环境中的搏动时间，减少搏动节律失常的发生，部分纠正由复氧所致异常心肌细胞电活动参数，缓解心肌细胞复极后再次停搏的发生。

**关键词** 动作电位；缺氧；心肌再灌注损伤；卡托普利；培养的细胞

卡托普利(Cap)是含巯基(-SH)血管紧张素转换酶抑制剂，具有降压作用[1]。新近研究发现它还有自由基清除作用[2]，促进离体心脏缺血再灌注心肌内ATP的恢复[3]，抗再灌注心律失常作用[4,5]。本文以培养搏动大鼠心肌细胞为实验材料，从细胞动作电位(AP)水平观察Cap对心肌细胞缺氧和复氧损伤所致电活动变化的影响。

## MATERIALS AND METHODS

**心肌细胞制备与培养** 心肌细胞制备与培养按常规方法[6]进行。1—4 d龄Sprague-Dawley大鼠(中国科学院上海实验动物中心提供)心室肌，用0.1 %胰蛋白酶(1∶250 Difco Certified，USA)溶液分次消化细胞，含20 %小牛血清的Minimum Essential Medium Eagle (MEM Eagle)液，分装在3.8 cm×2.3 cm×6 cm的分

Received 1992-05-08 Accepted 1993-11-17
[1] Project supported by the Fund of Chinese National Educational Commission (Grant № 90—35) and the Foundation of Hypoxia Public Laboratory of Shanghai Institute of Physiology, Chinese Academy of Sciences.

隔细胞培养瓶内，瓶中置盖玻片供细胞贴附生长，细胞密度为1 $10^6$ $ml^{-1}$置于95 % $O_2$ + 5 % $CO_2$中，37 ℃恒温箱内，每隔2 d更换生长液，培养1—10 d的心肌细胞可用于实验。

**电位记录、缺氧和复氧** 通过循环灌流装置对培养搏动大鼠心肌细胞进行持续灌流，心肌细胞在正常灌流液中搏动频率及节律稳定后用微电极插入细胞，通过电位记录系统记录心肌细胞电位。灌流液为1 % 小牛血清 Eagle's 液持续充以95 % $O_2$ + 5 % $CO_2$，$pO_2$在80 kPa (600 mm Hg)以上，pH 7.0—7.1；缺氧灌流液为无小牛血清、无糖 Eagle's 液，持续充以95 % $N_2$ + 5 % $CO_2$，$pO_2$低于5.33 kPa (40 mm Hg)，pH 6.6—7.7。照相记录正常心肌细胞电位后将正常灌流液改为缺氧灌流液，分别记录缺氧即刻及以后每5 min的电位，直至细胞停搏。细胞停搏后迅速恢复正常灌流环境，停搏细胞复跳后每5 min记录电位一次，至细胞复跳后30 min整个过程中微电极维持在同一细胞内。

**Cap的作用** Cap (由中美合资上海施贵宝制药有限公司惠赠，批号：80061)加双蒸水配成4 $g \cdot L^{-1}$溶液，每次取该溶液0.5 ml加入19.5 ml的灌流液中，灌流液Cap浓度为10 $mg \cdot L^{-1}$。实验中分别于缺氧或复氧开始时加Cap观察Cap对培养搏动大鼠心肌细胞缺氧及缺氧后复氧所致电活动变化的影响。另外在正常培养搏动大鼠心肌细胞灌流液中加以Cap以观察Cap对正常心肌细胞电活动的影响。

## RESULTS

**Cap对缺氧所致心肌细胞电活动异常的影响** 培养搏动大鼠心肌细胞在缺氧后1 min开始出现持续快速节律(200—300 bpm)、持续缓慢节律(10—15 bpm)伴以短暂停搏、快慢节律交替及早搏等。在异常节律出现的同时可观察到早期后除极(EAD)、延迟后除极(DAD)、最大舒张期电位(MDP)波动及动作电位振幅(APA)高低交替的电交替现象。上述异常电活动发生率在对照组为100 %，用药组为40 % ($P<0.01$)；用Cap的细胞在缺氧30 min内无一细胞停搏，对照组有9/11细胞停止搏动($P<0.01$)，见Fig 1.

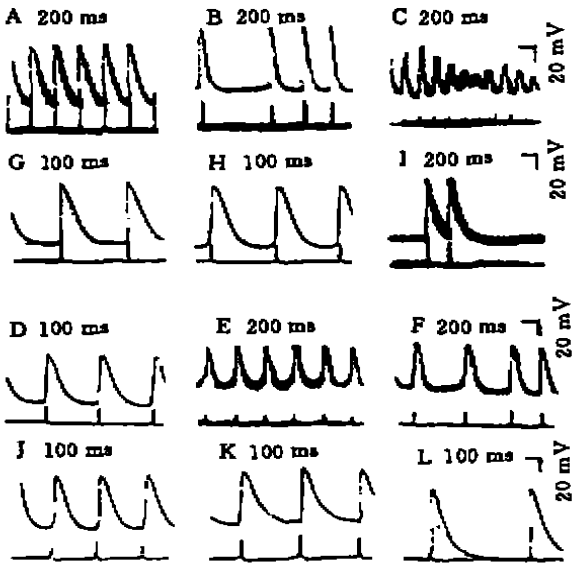


**Fig 1. Action potential (upper tracing) and $V_{max}$ (lower tracing, calibrated lines are 10 $V \cdot s^{-1}$) of cultured rat myocardial cells during anoxia for 5 min (A, G), 10 min (B, H), 20 min (C, I), and after reoxeating for 1 min (D, J), 5 min (E, K), and 10 min (F, L), respectively. A-F are control groups, while G-L are captopril treated groups.**

缺氧对照组在缺氧后心肌细胞的MDP下降，APA以及动作电位零相最大除极速率($V_{max}$)降低，动作电位时程(APD)缩短，随时间延长变化程度加重，直至细胞停搏。Cap组与对照组比较这种细胞电活动参数的改变未见明显改善($P>0.05$)，见Tab 1.

**Cap对复氧所致心肌细胞电活动异常的影响** 因缺氧而停搏的心肌细胞在恢复正常灌流环境后能逐渐复跳，在复跳即刻多数细胞(8/11)表现为短阵快节律(200 bpm以上)历时数分钟后恢复正常节律搏动，部分细胞(2/11)为缓慢节律(10—20 bpm)间以二联律、三联律、短暂停搏，1个细胞持续快节律(240 bpm)上述细胞中有4个细胞在复苏5—10 min再次停搏，再次停搏的细胞中有3个很快再次复跳，另一细胞未能再次复跳。在复氧后心肌细胞复跳即刻心肌细胞电活动参数有进一步下降趋势，

**Tab 1. Protective effect of captopril on electric activity of cultured rat myocardial cells under anoxia and reoxygenation.** $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

| | Time/min | Group | Cells | MDP/−mV | APA/mV | $V_{max}$/V·s$^{-1}$ | $APD_{50}$/ms |
|---|---|---|---|---|---|---|---|
| Anoxia | 0 | Control | 11 | 68±9 | 79±9 | 26±8 | 58±4 |
| | | Captopril | 10 | 68±7$^a$ | 78±6$^a$ | 26±7$^a$ | 60±5$^a$ |
| | 1 | Control | 11 | 67±10 | 79±10 | 20±8 | 53±7 |
| | | Captopril | 10 | 65±9$^a$ | 75±7$^a$ | 19±4$^a$ | 53±4$^a$ |
| | 5 | Control | 11 | 60±9 | 69±10 | 21±9 | 53±6 |
| | | Captopril | 10 | 58±6$^a$ | 67±10$^a$ | 19±4$^a$ | 52±7$^a$ |
| | 10 | Control | 10 | 60±11 | 62±9 | 16±5 | 52±6 |
| | | Captopril | 10 | 59±6$^a$ | 62±12$^a$ | 18±4$^a$ | 52±6$^a$ |
| | 15 | Control | 7 | 58±11 | 63±10 | 17±5 | 52±5 |
| | | Captopril | 10 | 59±11$^a$ | 62±14$^a$ | 16±4$^a$ | 51±6$^a$ |
| | 20 | Control | 5 | 60±9 | 62±12 | 16±6 | 52±5 |
| | | Captopril | 10 | 59±6$^a$ | 62±13$^a$ | 15±6$^a$ | 51±4$^a$ |
| | 25 | Control | 3 | 57±9 | 62±11 | 16±6 | 52±5 |
| | | Captopril | 10 | 59±7$^a$ | 65±13$^a$ | 18±6$^a$ | 52±4$^a$ |
| | 30 | Control | 2 | 51±8 | 61±13 | 12±5 | 50±4 |
| | | Captopril | 10 | 55±10$^a$ | 60±15$^a$ | 14±5$^a$ | 52±5$^a$ |
| Reoxygenation | 1 | Control | 11 | 48±7 | 51±8 | 13±9 | 49±5 |
| | | Captopril | 11 | 59±3$^b$ | 63±9$^b$ | 20±5$^b$ | 53±8$^a$ |
| | 5 | Control | 10 | 62±10 | 64±10 | 14±7 | 52±6 |
| | | Captopril | 11 | 59±5$^a$ | 66±9$^b$ | 21±4$^b$ | 52±6$^a$ |
| | 10 | Control | 8 | 61±8 | 64±10 | 18±5 | 53±6 |
| | | Captopril | 10 | 62±6$^a$ | 70±10$^a$ | 26±4$^c$ | 53±8$^a$ |
| | 15 | Control | 8 | 60±12 | 62±12 | 17±5 | 53±5 |
| | | Captopril | 10 | 62±10$^a$ | 71±8$^a$ | 24±3$^a$ | 52±5$^a$ |
| | 20 | Control | 7 | 61±9 | 63±9 | 18±7 | 54±4 |
| | | Captopril | 10 | 63±4$^a$ | 69±9$^a$ | 21±5$^a$ | 52±5$^a$ |
| | 25 | Control | 7 | 62±6 | 66±8 | 17±6 | 56±4 |
| | | Captopril | 10 | 63±4$^a$ | 72±5$^b$ | 23±3$^b$ | 53±5$^a$ |
| | 30 | Control | 7 | 64±6 | 65±8 | 18±5 | 57±5 |
| | | Captopril | 9 | 67±5$^a$ | 74±6$^b$ | 24±3$^b$ | 54±5$^a$ |

以后逐渐恢复. 在缺氧时未加 Cap 而复氧过程中加用 Cap (40 mg·L$^{-1}$)的所有细胞在10 min 内复跳，未出现再次停搏现象($P<0.05$)，多数细胞(8/11)复苏即刻表现为100—150 bpm 的节律搏动伴以偶发早搏，2/11表现为慢节律，1/11则为短阵快节律，见 Fig 1；用药组心

肌细胞电活动参数在缺氧过程中与对照组无显著差异，但在复氧过程中 MDP，APA，$V_{max}$则较对照组增大($P<0.05$或0.01)见 Tab 1.

**Cap 对正常心肌细胞电活动的影响** 正常节律搏动的培养大鼠心肌细胞在含 Cap (40 mg·L$^{-1}$)灌流液中持续灌流15 min，搏动频率及节律未出现明显改变，各项细胞电活动参数亦未见任何有意义的改变.

## DISCUSSION

本实验中未见 Cap 有改善因缺氧而引起的心肌细胞电活动参数改变，我们曾将 Cap 浓度增至80 mg·L$^{-1}$仍未发现 Cap 对心肌细胞电活动参数有任何影响，此与 Hemsworth[7]报道相符，并发现 Cap 对正常搏动的培养大鼠心肌细胞电活动参数无直接影响，然而 Cap 确能提高心肌细胞耐缺氧的能力，使心肌细胞在缺氧过程中节律搏动失常发生减轻. 复氧早期心肌细胞出现明显节律搏动异常，有细胞复跳后再次停搏出现，电活动参数亦现进一步异常变化，提示复氧损伤存在. 复氧时加用 Cap 后，复跳时间缩短，再次停搏发生减少，电活动参数较对照组有一定程度改善，支持 Cap 对复氧损伤细胞有保护作用. 然而增加 Cap 浓度至80 mg·L$^{-1}$时未见有更显著的保护作用产生. 本文结果提示 Cap 对缺氧和缺氧后复氧心肌细胞有保护作用，从而为 Cap 用于急性心肌梗塞早期溶栓疗法，紧急 PTCA 术中对再灌注心律失常、心力衰竭及猝死的防治提供了参考.

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