

## 吗啡后处理对大鼠心肌细胞 ATP 敏感性钾通道的影响 \*

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**摘要** 在氰化钠(NaCN)处理模拟细胞缺血的单个大鼠心肌细胞上, 应用膜片钳电压钳制全细胞记录模式, 研究吗啡后处理对缺血心肌细胞膜 ATP 敏感性钾通道的影响, 并探讨吗啡后处理可能涉及的阿片受体类型. 吗啡后处理可使 ATP 敏感性钾通道电流( $I_{K_{ATP}}$ )增加( $61.4 \pm 13.6\%$ ), 促进  $K_{ATP}$  通道开放. 特异阻断  $\kappa$ -阿片受体不能阻止  $I_{K_{ATP}}$  增加, 而非特异性阻断阿片受体或特异阻断  $\delta$ -阿片受体均可阻止  $I_{K_{ATP}}$  增加. 结果表明, 吗啡后处理促进  $K_{ATP}$  通道开放与  $\delta$ -阿片受体的激活有关.

**关键词** 膜片钳, 心肌细胞, 模拟缺血, 吗啡后处理, ATP 敏感性钾通道

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缺血后处理是指心肌缺血后、再灌注之前进行反复短暂的再灌注/缺血干预. 研究表明, 缺血后处理可减轻再灌注损伤, 具有强大心肌保护作用<sup>[1]</sup>. 根据其发生作用的机制, 于再灌注开始时给予腺苷等药物也可产生后处理样心肌保护作用, 称为药物后处理<sup>[2,3]</sup>. 后处理尤其是药物后处理是在心肌缺血后实施的干预措施, 因此具有临床应用前景. 我们前期研究是采用离体大鼠心脏灌注模型, 于再灌注开始即刻给予吗啡进行灌注即吗啡后处理, 研究发现, 吗啡后处理可减少肌酸激酶同工酶(CK-MB)的释放和心肌梗死面积, 具有心肌保护作用, 当给予非选择性阿片受体阻断剂纳洛酮和选择性  $\kappa$ -阿片受体阻断剂 nor-binaltorphimine 时, 前者可以完全逆转而后者可部分逆转吗啡(morphine)的保护作用. 当于再灌注即刻给予选择性线粒体 ATP 敏感性钾通道(mito $K_{ATP}$ )阻断剂 5-羟基癸酸甘油酯(5-hydroxydecanoate, 5-HD)时, 也可部分阻断吗啡后处理心肌保护作用, 因此认为, 吗啡后处理心肌保护作用可能与  $\kappa$ -阿片受体的激活以及 mito $K_{ATP}$  通道的开放有关<sup>[4]</sup>.

研究表明, 大鼠心肌有  $\kappa$ -和  $\delta$ -阿片受体, 无  $\mu$ -阿片受体<sup>[5]</sup>.  $K_{ATP}$  通道既存在于心肌细胞膜即肌膜  $K_{ATP}$ (sarc $K_{ATP}$ )通道, 也存在于线粒体即 mito $K_{ATP}$  通道, 而有关 sarc $K_{ATP}$  通道在吗啡后处理中作用的

研究则尚未见报道. 本研究利用膜片钳技术记录  $K_{ATP}$  通道电流( $I_{K_{ATP}}$ ), 观察吗啡后处理对 sarc $K_{ATP}$  通道的影响, 旨在探讨吗啡后处理心肌保护作用的离子通道机制. 由于选择性  $\kappa$ -阿片受体阻断剂仅可部分逆转吗啡保护作用, 因此本研究也将探讨吗啡后处理可能涉及的其他阿片受体类型.

### 1 材料与方法

#### 1.1 实验溶液与试剂

无钙台式液(mmol/L): NaCl, 137; KCl, 5.4; MgCl<sub>2</sub>, 1.0; HEPES, 10; 葡萄糖, 10. 用 NaOH 调节 pH 值至 7.4. Kraft-Brühe(KB)液(mmol/L): L-谷氨酸, 50; KCl, 40; KH<sub>2</sub>PO<sub>4</sub>, 20; 牛磺酸, 20; MgCl<sub>2</sub>, 3.0; KOH, 70; EGTA, 0.5; HEPES, 10; 葡萄糖, 10. 用 KOH 调节 pH 值至 7.4. 电极内液(mmol/L): L-aspartic acid potassium(L-天冬氨酸钾), 140; MgCl<sub>2</sub>, 1.0; EGTA, 0.5; HEPES, 10; ATP, 0.5. 用 KOH 调节 pH 值至 7.4. 细胞外液即浴液(mmol/L): N-甲基-D-谷氨酸, 137; KCl, 5.4; CaCl<sub>2</sub>, 1.8; MgCl<sub>2</sub>, 1.0; HEPES, 10; nifedipine(硝

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本地平), 10  $\mu\text{mol/L}$ . 用 HCl 调节 pH 值至 7.4. MI(metabolic inhibition)台式液(mmol/L): N- 甲基 -D-谷氨酸, 137; KCl, 5.4;  $\text{CaCl}_2$ , 1.8;  $\text{MgCl}_2$ , 1.0; HEPES, 10; 硝苯地平, 10  $\mu\text{mol/L}$ , NaCN, 2.0 (实验时向细胞外液加入 NaCN, 使其终浓度为 2 mmol/L).  $\text{K}_{\text{ATP}}$  通道阻断剂格列苯脲(Glibenclamide, Glib, Sigma 公司)用二甲基亚砜(dimethyl sulfoxide, DMSO)溶解, 配成浓度为 1 mmol/L 的母液. 钙通道阻断剂硝苯地平(Nifedipine, Sigma 公司)用 DMSO 溶解, 配成浓度为 100 mmol/L 的母液, 溶液中 DMSO 终浓度不超过 0.1%. 特异性  $\kappa$ -阿片受体阻断剂 nor-binaltorphimine(nor-BNI, 终浓度 5  $\mu\text{mol/L}$ , Sigma 公司)用乙醇(ethanol)溶解, 特异性  $\delta$ -阿片受体阻断剂 naltrindole(NTD, 终浓度 5  $\mu\text{mol/L}$ , Sigma 公司)用甲醇(methanol)溶解, 吗啡(morphine, 终浓度 3  $\mu\text{mol/L}$ )、纳洛酮(naloxone, NAL, 终浓度 10  $\mu\text{mol/L}$ )、氰化钠(NaCN, 终浓度 2 mmol/L)均以高纯水配制, 以上试剂均先配成母液, 实验时以 Bolus 方式加入溶液中, 达到所需终浓度. 实验中所采用的试剂浓度均参照以往研究资料<sup>[6-8]</sup>.

## 1.2 大鼠心室肌细胞的急性分离

健康成年 Sprague-Dawley 大鼠, 200~230 g, 雌性, 由北京维通利华实验动物技术有限公司提供. 腹腔注射 1%戊巴比妥钠 50 mg/kg 实施麻醉, 注射 5 U/g 肝素防止血液凝固, 麻醉后打开胸腔, 迅速取出心脏, 置于 4 $^{\circ}\text{C}$  无钙台式液中, 游离主动脉根部, 将心脏悬挂于 Langendorff 灌流装置上, 经主动脉逆行灌流, 先以无钙台式液灌流, 灌流速度 8~10 ml/min, 5 min 后改用含 0.5 g/L 胶原酶 II (Worthington 公司), 1 g/L 牛血清白蛋白(BSA, Sigma 公司)的酶液灌流心脏, 灌流时间(20 $\pm$ 5) min, 循环水浴使灌流液始终保温于 37 $^{\circ}\text{C}$ , 灌流过程中始终以 95%  $\text{O}_2$ +5%  $\text{CO}_2$  混合气饱和. 完毕后剪下心室肌, 置于 KB 液中, 用小剪刀将其剪碎, 轻轻吹打, 200 目尼龙网过滤. 室温下 KB 液孵育 1~2 h, 然后置于 4 $^{\circ}\text{C}$  冰箱保存备用. 实验在室温(20~25 $^{\circ}\text{C}$ )下进行.

## 1.3 全细胞电流的记录与分析

在全细胞记录模式下, ATP 敏感性钾通道电流( $\text{I}_{\text{KATP}}$ )的记录方案为: 钳制电位-40 mV, 测试电位从-40 mV 至 0 mV, 持续时间 100 ms, 每 15 s 一次, 取测试电位结束时的电流强度做分析.  $\text{K}_{\text{ATP}}$  电流由膜片钳放大器(Axopatch 200B Intergrating Patch Clamp, Axon Instruments, USA)记录, 采样频

率为 10 kHz, 1 kHz 低频滤波器降低高频率噪声. 数据采集软件为 pclamp 9.2, 数据分析软件为 clampfit 9.2(Axon Instruments, USA)和 ORIGIN 7.5 (OriginLab Corporation, USA). 玻璃微电极由毛细玻璃管(BF150-110-107052)用拉制仪(Narishige Model PB-7, Japan)分两步拉制而成, 电极电阻控制在 2~3 M $\Omega$ .

## 1.4 实验方案

实验分组: a. 对照组. 在全细胞记录模式下, 向溶液加入 NaCN 模拟心肌细胞缺血, 观察电流出现, 随后加入  $\text{K}_{\text{ATP}}$  通道特异性阻断剂 glibenclamide(Glib) 以验证电流是否为  $\text{I}_{\text{KATP}}$ , Glib 终浓度为 0.5  $\mu\text{mol/L}$ . b. 缺血对照组. 在全细胞记录模式下, 向溶液加入 NaCN 模拟缺血, 观察电流出现, 直至电流消失, 观察 NaCN 引起的电流变化过程. c. 正常心肌细胞 morphine 组. 在全细胞记录模式下, 先以含吗啡的溶液灌流 10 min, 吗啡溶液浓度为 3  $\mu\text{mol/L}$ , 再加入 NaCN 模拟缺血, 观察吗啡对正常心肌细胞是否有影响. d. 缺血心肌细胞 Morphine 组. e. naloxone(NAL)+ morphine 组. f. naltrindole(NTD) + morphine 组. g. nor-binaltorphimine(nor-BNI) + morphine 组. 以上各组均为在全细胞记录模式下, 向溶液加入 NaCN 模拟缺血, 待电流出现并稳定后再加入相应试剂, 观察电流变化, 最后均加入 0.5  $\mu\text{mol/L}$  Glib 以确认  $\text{I}_{\text{KATP}}$ . 其中 morphine 溶液浓度为 3  $\mu\text{mol/L}$ , NAL 为 10  $\mu\text{mol/L}$ , NTD 为 5  $\mu\text{mol/L}$ , nor-BNI 为 5  $\mu\text{mol/L}$ .

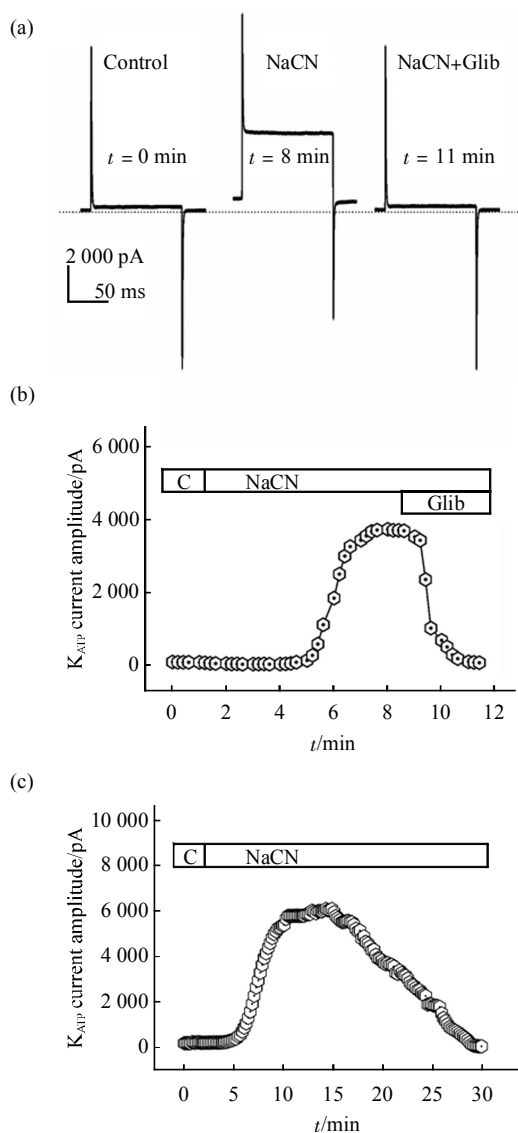
## 1.5 统计学处理

所有数据均以均数  $\pm$  标准误差表示( $\bar{x} \pm s$ ), 以 SPSS11.5 统计软件包进行统计学分析, 采用配对  $t$  检验,  $P < 0.05$  为有统计学差异.

## 2 结 果

### 2.1 NaCN 模拟心肌细胞缺血

NaCN 可阻断氧化磷酸化, 使细胞内 ATP 耗竭, 故用来模拟心肌细胞缺血<sup>[9]</sup>,  $\text{K}_{\text{ATP}}$  通道在细胞缺血 ATP 浓度降低时开放. 本实验对照组全细胞记录模式下 NaCN 对心肌细胞钾电流的影响如图 1a, b 所示, 向溶液加入 NaCN 模拟缺血, 可见外向电流出现, 经 Glib (0.5  $\mu\text{mol/L}$ ) 鉴定为  $\text{I}_{\text{KATP}}$  ( $n = 8$ ). 缺血对照组是向溶液加入 NaCN 模拟缺血, 心肌细胞平均(5  $\pm$  2) min 开始出现电流, 渐至最大值, 然后缓慢下降直至基础值, 期间未见电流回升现象(图 1c,  $n = 8$ ).

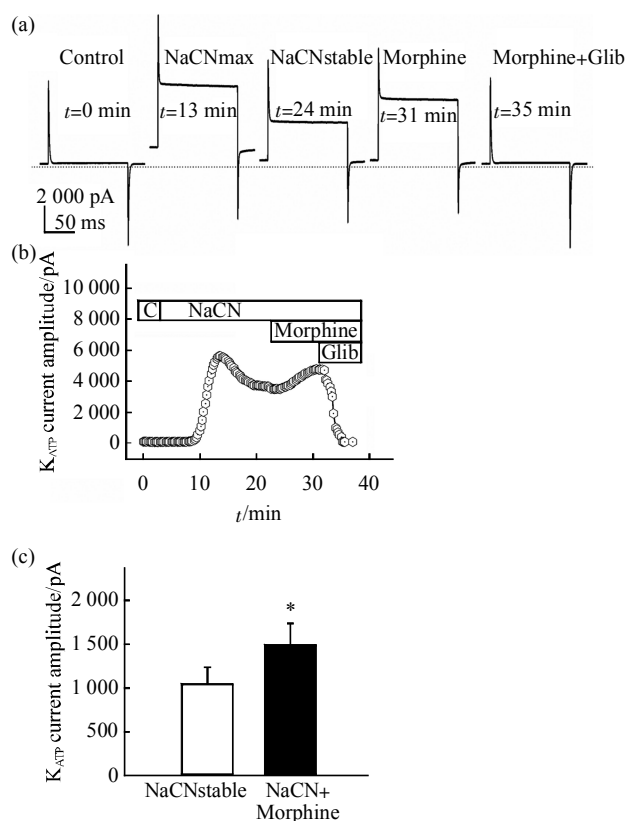


**Fig. 1** Activation of sarcolemmal adenosine triphosphate-sensitive potassium current ( $I_{KATP}$ ) by mimic ischemia with NaCN

(a) Traces of  $I_{KATP}$  elicited by a 100 ms voltage pulse to 0 mV from a holding potential of  $-40$  mV in control, in the presence of NaCN, and in the presence of NaCN and  $0.5 \mu\text{mol/L}$  glibenclamide. The  $I_{KATP}$  elicited by mimic ischemia with NaCN was sensitive to glibenclamide. (b) Corresponding time course of  $I_{KATP}$  activation by mimic ischemia with NaCN. Current was monitored every 15 s using voltage protocol described in (a). Current amplitude was plotted against time. Following the administration of glibenclamide, the time course of  $I_{KATP}$  was down abruptly. (c) The time course of  $I_{KATP}$  elicited by mimic ischemia with NaCN.  $I_{KATP}$  was down slowly without administration of glibenclamide. Abbreviations in Figure 1~4 are expressed as: C, control; G or Glib, glibenclamide; NaCNmax, the maximal amplitude of  $I_{KATP}$ ; NaCNstable, the steady state of  $I_{KATP}$ ; NTD, naltrindole; M, morphine; nor-BNI, nor-binaltorphimine.

## 2.2 吗啡后处理对模拟缺血心肌细胞 $I_{KATP}$ 的影响

缺血心肌细胞 morphine 组, 在全细胞记录模式下向浴液加入 NaCN 模拟缺血, 可见  $I_{KATP}$  出现并渐至最大值, 待  $I_{KATP}$  稳定后加入 morphine ( $3 \mu\text{mol/L}$ ), 观察发现  $I_{KATP}$  增大, 由  $I_{KATP}$  稳定值 ( $1034.4 \pm 201.8$ ) pA 增加到 ( $1474.5 \pm 267.6$ ) pA, 增加 ( $61.4 \pm 13.6\%$ ) ( $P < 0.01, n = 6$ ), 如图 2a, b, c 所示。



**Fig. 2** Effect of morphine postconditioning on activation of adenosine triphosphate-sensitive potassium current ( $I_{KATP}$ ) by mimic ischemia with NaCN

(a) Traces of  $I_{KATP}$  elicited by mimic ischemia with NaCN in control, in the presence of  $2 \text{ mmol/L}$  NaCN, and in the presence of NaCN and  $3 \mu\text{mol/L}$  morphine. The voltage protocol was as described in Figure 1.  $I_{KATP}$  was identified by  $0.5 \mu\text{mol/L}$  glibenclamide. (b) Corresponding time course of  $I_{KATP}$  activation by mimic ischemia with NaCN. Current was monitored every 15 s using voltage protocol described in Figure 1. Current amplitude was plotted against time. (c) Effect of morphine postconditioning on activation of  $I_{KATP}$  by mimic ischemia with NaCN. The voltage protocol was as described in Figure 1. Current amplitude was measured at the end of the 100 ms test pulse. Morphine postconditioning increased the amplitude of mimic ischemia-activated  $I_{KATP}$  by ( $61.4 \pm 13.6\%$ ) ( $*P < 0.01, \text{NaCN} + \text{Morphine} \text{ vs. } \text{NaCN alone}; n = 6$ ).

### 2.3 吗啡对正常心肌细胞 $I_{KATP}$ 的影响

正常心肌细胞 morphine 组, 在细胞记录模式下向细胞外液中加入 morphine, 浓度为  $3 \mu\text{mol/L}$ , 记录时间至少 10 min, 其间未见任何电流出现, 吗啡对正常心肌细胞  $I_{KATP}$  无作用. 将吗啡洗脱后加入 NaCN 模拟缺血, 可见有外向电流出现, 经 Glib ( $0.5 \mu\text{mol/L}$ ) 鉴定为  $I_{KATP}$ , 说明心肌细胞膜存在功能正常的  $K_{ATP}$  通道, 如图 3a 所示 ( $n=8$ ).

### 2.4 非选择性阻断阿片受体后吗啡后处理对缺血心肌细胞 $I_{KATP}$ 的影响

NAL+ morphine 组在细胞记录模式下, 加入 NaCN 模拟缺血, 可见  $I_{KATP}$  出现并渐至最大值, 待  $I_{KATP}$  稳定后加入非选择性阿片受体阻断剂 naloxone ( $10 \mu\text{mol/L}$ ), 观察 5 min, 未见电流增加, 随后加入 morphine ( $3 \mu\text{mol/L}$ ), 继续观察至少 10 min, 仍未见电流增加, 最后用 Glib ( $0.5 \mu\text{mol/L}$ ) 确认所记录电流为  $I_{KATP}$  ( $n=6$ ), 图 3b 所示.

### 2.5 选择性阻断 $\delta$ -阿片受体后吗啡后处理对缺血心肌细胞 $I_{KATP}$ 的影响

NTD + morphine 组在细胞记录模式下, 加入 NaCN 模拟缺血, 可见  $I_{KATP}$  出现并渐至最大值, 待  $I_{KATP}$  稳定后加入选择性  $\delta$ -阿片受体阻断剂 naltrindole ( $5 \mu\text{mol/L}$ ), 观察 5 min, 未见电流增加, 随后加入 morphine ( $3 \mu\text{mol/L}$ ), 继续观察至少 10 min, 仍未见电流增加, 最后用 Glib ( $0.5 \mu\text{mol/L}$ ) 确认所记录电流为  $I_{KATP}$  ( $n=6$ ), 图 3c 所示.

### 2.6 选择性阻断 $\kappa$ -阿片受体后吗啡后处理对缺血心肌细胞 $I_{KATP}$ 的影响

nor-BNI+Morphine 组在全细胞记录模式下, 加入 NaCN 模拟缺血, 可见  $I_{KATP}$  出现并渐至最大值, 待  $I_{KATP}$  稳定后加入选择性  $\kappa$ -阿片受体阻断剂 nor-binaltorphimine ( $5 \mu\text{mol/L}$ ), 观察 5 min, 未见电流增加, 随后加入 morphine ( $3 \mu\text{mol/L}$ ), 可见  $I_{KATP}$  回升. 在  $I_{KATP}$  稳定值基础上增加 ( $86.0 \pm 10.5\%$ ), 由  $I_{KATP}$  稳定值 ( $715.2 \pm 74.9$ ) pA 增加到 ( $1322.2 \pm 139.3$ ) pA ( $P < 0.01, n=6$ ), 如图 4a, b, c 所示. 最后用 Glib ( $0.5 \mu\text{mol/L}$ ) 确认所记录电流为  $I_{KATP}$ .

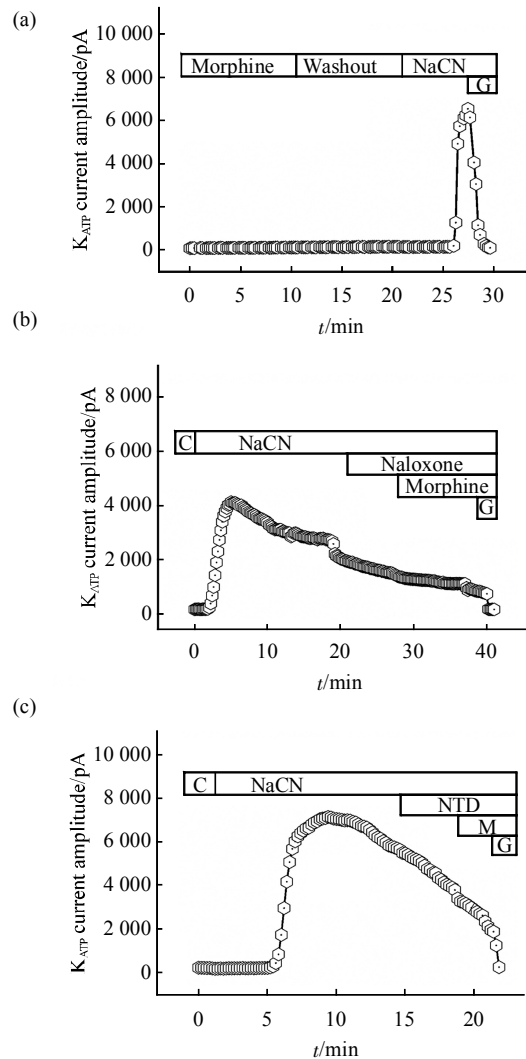
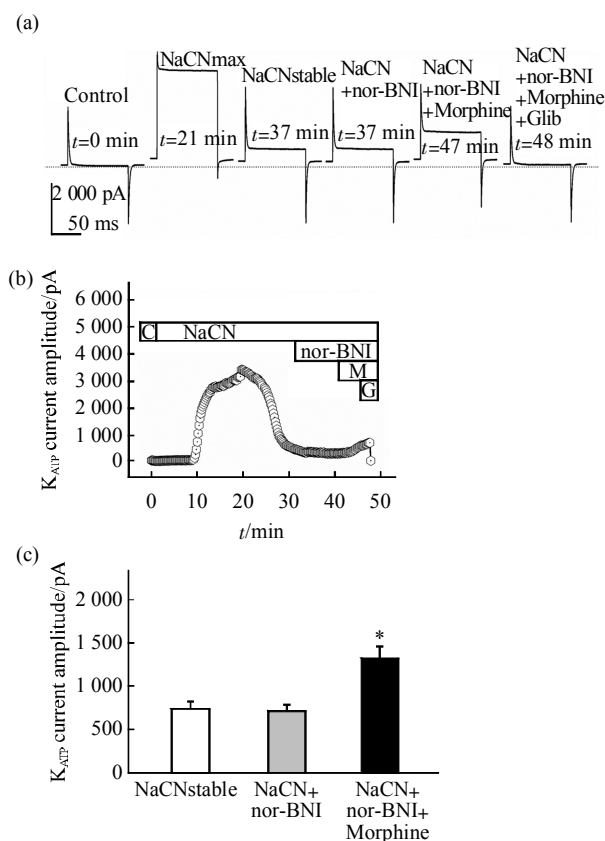


Fig. 3 Time courses of  $I_{KATP}$

(a) No  $I_{KATP}$  was elicited during the 10 min infusion of Morphine solution. Morphine alone does not activate  $I_{KATP}$ .  $K_{ATP}$  current was monitored every 15 s during the test pulse described in Figure 1. Current amplitude was measured at the end of the 100 ms test pulse. Subsequent application of 2 mmol/L NaCN activated  $I_{KATP}$  that was confirmed by  $0.5 \mu\text{mol/L}$  glibenclamide. (b), (c) Time courses of  $I_{KATP}$  elicited by mimic ischemia with NaCN in control, in the presence of 2 mmol/L NaCN, in the presence of NaCN and  $10 \mu\text{mol/L}$  naloxone (a nonselective opioid receptor antagonist, b) or  $5 \mu\text{mol/L}$  naltrindole (a selective  $\delta$ -opioid receptor antagonist, c), and in the presence of NaCN, naloxone (b) or naltrindole (c) and  $3 \mu\text{mol/L}$  morphine. The voltage protocol was as described in Figure 1. Current amplitude was measured at the end of the 100 ms test pulse. In these two groups, morphine postconditioning can not increase the amplitude of mimic ischemia-activated  $I_{KATP}$ .  $I_{KATP}$  was identified by  $0.5 \mu\text{mol/L}$  glibenclamide.



**Fig. 4 Effect of morphine postconditioning on  $I_{K_{ATP}}$  elicited by mimic ischemia with NaCN after specific blockade of  $\kappa$ -opioid receptor**

(a) Traces of  $I_{K_{ATP}}$  elicited by mimic ischemia with NaCN in control, in the presence of 2 mmol/L NaCN, in the presence of NaCN and 5  $\mu$ mol/L Nor-binaltorphimine (a selective  $\kappa$ -opioid receptor antagonist), and in the presence of NaCN, Nor-binaltorphimine and 3  $\mu$ mol/L morphine. The voltage protocol was as described in Figure 1.  $I_{K_{ATP}}$  was identified by 0.5  $\mu$ mol/L Glibenclamide. (b) Corresponding time course of  $I_{K_{ATP}}$  activation by mimic ischemia with NaCN. Current was monitored every 15 s using voltage protocol described in Figure 1. Current amplitude was plotted against time.  $I_{K_{ATP}}$  was identified by 0.5  $\mu$ mol/L Glibenclamide. (c) Effect of Morphine postconditioning on activation of  $I_{K_{ATP}}$  by mimic ischemia with NaCN. The voltage protocol was as described in Figure 1. Current amplitude was measured at the end of the 100 ms test pulse. Morphine postconditioning after specific blockade of  $\kappa$ -opioid receptor increased the amplitude of mimic ischemia-activated  $I_{K_{ATP}}$  by  $(86.0 \pm 10.5)\%$  ( $*P < 0.01$ , NaCN + nor-BNI + Morphine vs. NaCN + nor-BNI or NaCNstable;  $n = 6$ ).

### 3 讨 论

研究证明,  $mitoK_{ATP}$  通道参与后处理心肌保护<sup>[10~13]</sup>. 我们前期研究发现, 吗啡后处理心肌保护作用也与  $mitoK_{ATP}$  通道开放有关<sup>[4]</sup>, 而  $sarcK_{ATP}$  通道在吗啡后处理中的作用如何尚未见报道. 本研究

运用膜片钳技术在全细胞记录模式下研究吗啡后处理对  $sarcK_{ATP}$  通道电流( $I_{K_{ATP}}$ )的影响, 以 NaCN 代谢抑制模拟心肌细胞缺血, 耗竭细胞内 ATP, 使  $K_{ATP}$  通道开放. 实验时先向浴液加入 NaCN 模拟心肌细胞缺血, 同时观察  $I_{K_{ATP}}$  变化, 当  $I_{K_{ATP}}$  出现并至某一稳定水平后加用吗啡, 可见  $I_{K_{ATP}}$  回升, 表明吗啡后处理可使缺血心肌细胞  $I_{K_{ATP}}$  增加, 促进  $K_{ATP}$  通道开放. 本研究发现吗啡对正常心肌细胞  $I_{K_{ATP}}$  无影响, 即单用吗啡并不能激活  $K_{ATP}$  通道, 说明吗啡可促进已被 NaCN 模拟缺血激活的  $K_{ATP}$  通道开放, 对正常心肌细胞尚未激活的  $K_{ATP}$  通道并无作用, 即吗啡在心肌细胞缺血通道被激活后应用方可产生促进通道开放作用. 该研究也是目前有关  $sarcK_{ATP}$  通道参与吗啡后处理的首次研究.  $K_{ATP}$  通道开放对心肌保护有利, 已为许多研究所证实<sup>[10~13]</sup>. Noma<sup>[14]</sup>认为, 缺血缺氧引起  $K_{ATP}$  通道开放, 可使心肌细胞动作电位时程缩短, 减少钙内流, 减轻心肌缺血和早期再灌注时的钙超载, 从而产生心肌保护作用, 提高细胞生存能力. 因此, 促进缺血心肌细胞  $K_{ATP}$  通道开放可能是吗啡后处理心肌保护作用的离子通道机制之一. 本研究运用膜片钳技术记录全细胞  $K_{ATP}$  通道电流从细胞的离子通道水平为吗啡后处理促进  $K_{ATP}$  通道开放提供了直接证据. 由于  $sarcK_{ATP}$  通道和  $mitoK_{ATP}$  通道之间存在相互关联<sup>[15]</sup>, 当用 NaCN 代谢抑制模拟心肌细胞缺血时,  $sarcK_{ATP}$  通道开放的同时,  $mitoK_{ATP}$  通道也可能开放, 吗啡后处理促进  $sarcK_{ATP}$  通道开放的同时, 也可能促进  $mitoK_{ATP}$  通道开放. 因此  $sarcK_{ATP}$  通道和  $mitoK_{ATP}$  通道在吗啡后处理心肌保护中均可能发挥作用.

以往研究证明, 成年大鼠心肌细胞存在  $\kappa$ - 和  $\delta$ - 阿片受体而缺乏  $\mu$ - 阿片受体<sup>[15, 16]</sup>, 因此以急性分离的心肌细胞进行膜片钳实验就可排除  $\mu$ - 阿片受体的作用. 实验时先以 NaCN 代谢抑制模拟心肌细胞缺血, 待  $I_{K_{ATP}}$  稳定后依次加入非选择性阿片受体阻断剂 naloxone 与 morphine (NAL + morphine 组), 选择性  $\delta$ - 阿片受体阻断剂 naltrindole 与 morphine (NTD + morphine 组), 选择性  $\kappa$ - 阿片受体阻断剂 nor-binaltorphimine 与 morphine (nor-BNI + morphine 组). 结果发现, NAL + morphine 组和 NTD + morphine 组两组均未见  $I_{K_{ATP}}$  增大, 说明吗啡后处理促进  $K_{ATP}$  通道开放的作用可被非选择性阿片受体阻断剂 naloxone 以及选择性  $\delta$ - 阿片受体阻断剂 naltrindole 所阻断. 而在 nor-BNI + morphine

组  $I_{K_{ATP}}$  增大, 说明选择性  $\kappa$ -阿片受体阻断剂 *nor-binaltorphimine* 不能阻断吗啡后处理促进  $K_{ATP}$  通道开放的作用。以上研究证明, 吗啡后处理心肌保护作用是通过激动  $\delta$ -阿片受体所致, 与参与吗啡预处理心肌保护的阿片受体类型相同<sup>[17, 18]</sup>。而对缺血后处理的研究表明,  $\delta$ -、 $\kappa$ -阿片受体或单独<sup>[19, 20]</sup>或共同<sup>[21]</sup>参与缺血后处理心肌保护作用。我们前期在离体大鼠心脏进行的研究发现, 吗啡后处理心肌保护作用则与  $\kappa$ -阿片受体有关<sup>[4]</sup>, 这可能与两研究所采用的实验模型不同有关, 实际上在我们前期研究中当特异性阻断  $\kappa$ -阿片受体时仅可部分逆转吗啡后处理心肌保护作用, 说明吗啡后处理心肌保护作用并非完全归于  $\kappa$ -阿片受体的激活。

综上所述, 本研究运用膜片钳技术研究吗啡后处理这一心肌保护策略, 并对 *sarcK<sub>ATP</sub>* 通道参与吗啡后处理心肌保护进行了初步探讨, 研究表明, 吗啡后处理可促进缺血心肌细胞 *sarcK<sub>ATP</sub>* 通道开放, 该作用与激活  $\delta$ -阿片受体有关。

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## Effect of Morphine Postconditioning on Rat Cardiac Sarcolemmal $K_{ATP}$ Channels\*

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**Abstract** The  $K_{ATP}$  channel current ( $I_{K_{ATP}}$ ) was monitored using the whole cell configuration of the patch clamp technique in single ventricular cardiac myocytes enzymatically isolated from rat hearts. Sodium cyanide, a metabolic inhibitor, was used to mimic cell ischemia. Morphine postconditioning facilitated the further opening of the  $K_{ATP}$  channels. Following initial activation of  $I_{K_{ATP}}$  by mimic ischemia, morphine further increased current amplitude by  $(61.4 \pm 13.6)\%$ .  $K_{ATP}$  channels in normal cardiac myocytes were not affected by extracellular applications of morphine alone. Nor-binaltorphimine, a specific  $\kappa$ -opioid receptor antagonist, was unable to abolish the facilitation of morphine postconditioning when administered 5 minutes before morphine. However, naloxone, a non-specific opioid receptor antagonist, and naltrindole, a specific  $\delta$ -opioid receptor antagonist, can abolish the facilitation of morphine postconditioning. These results indicated that morphine postconditioning facilitate the further opening of the cardiac sarcolemmal  $K_{ATP}$  channels following the channel activation by mimic ischemia. The  $\delta$ -opioid receptor may be involved in morphine postconditioning.

**Key words** patch clamp, cardiac myocytes, mimic ischemia, morphine postconditioning, sarcolemmal  $K_{ATP}$  channel

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