

# Nicorandil, a Potent Cardioprotective Agent, Acts by Opening Mitochondrial ATP-Dependent Potassium Channels

Toshiaki Sato, MD, PhD, Norihito Sasaki, MD, PhD, Brian O'Rourke, PhD, Eduardo Marbán, MD, PhD, FACC

Baltimore, Maryland

- 
- OBJECTIVES** To determine the mechanism of cardioprotection afforded by nicorandil, an orally efficacious antianginal drug, we examined its effects on ATP-dependent potassium ( $K_{ATP}$ ) channels.
- BACKGROUND** Nicorandil can mimic ischemic preconditioning, while mitochondrial  $K_{ATP}$  (mito $K_{ATP}$ ) channels rather than sarcolemmal  $K_{ATP}$  (surface $K_{ATP}$ ) channels have emerged as the likely effectors.
- METHODS** Flavoprotein fluorescence and membrane current in intact rabbit ventricular myocytes were measured simultaneously to assay mito $K_{ATP}$  channel and surface  $K_{ATP}$  channel activities, respectively. In a cell-pelleting model of ischemia, cells permeable to trypan blue were counted as killed by 60 and 120 min of ischemia.
- RESULTS** Nicorandil (100  $\mu$ mol/liter) increased flavoprotein oxidation but not membrane current; a 10-fold higher concentration recruits both mito $K_{ATP}$  and surface $K_{ATP}$  channels. Pooled dose-response data confirm that nicorandil concentrations as low as 10  $\mu$ mol/liter turn on mito $K_{ATP}$  channels, while surface $K_{ATP}$  current requires exposure to millimolar concentrations. Nicorandil blunted the rate of cell death in a pelleting model of ischemia; this cardioprotective effect was prevented by the mito $K_{ATP}$  channel blocker 5-hydroxydecanoate but was unaffected by the surface $K_{ATP}$  channel blocker HMR1098.
- CONCLUSIONS** Nicorandil exerts a direct cardioprotective effect on heart muscle cells, an effect mediated by selective activation of mito $K_{ATP}$  channels. (J Am Coll Cardiol 2000;35:514-8) © 2000 by the American College of Cardiology
- 

Nicorandil, a hybrid ATP-dependent potassium ( $K_{ATP}$ ) channel opener and nitrate compound (1), is used clinically for the treatment of angina pectoris (2). The cardioprotective effects of nicorandil in ischemic hearts have received much attention: nicorandil can improve the recovery of postischemic contractile dysfunction and can reduce infarct size in several animal models (3-5) and in humans (6-9). The initial hypothesis to explain these observations invoked sarcolemmal  $K_{ATP}$  (surface $K_{ATP}$ ) channels: opening of surface $K_{ATP}$  channels would abbreviate excitability such that calcium overload and energy consumption would be attenuated (10). However, recent studies provide evidence that mitochondrial  $K_{ATP}$  (mito $K_{ATP}$ ) channels rather than surface $K_{ATP}$  channels are the dominant players (11,12).

The selective mito $K_{ATP}$  channel inhibitor 5-hydroxydecanoate (5HD) (13) abolishes the infarct size-limiting effect of nicorandil (5). Furthermore, it has been reported that nicorandil given orally to rats is preferentially distributed into heart mitochondria (14). Therefore, we hypothesized that nicorandil targets mito $K_{ATP}$  channels and the nicorandil-induced cardioprotection is mediated by opening of mito $K_{ATP}$  channels. To test this hypothesis, we simultaneously assayed the activity of surface $K_{ATP}$  channels and mito $K_{ATP}$  channels by measuring membrane current and flavoprotein fluorescence in rabbit ventricular myocytes (12).

## METHODS

**Preparation of rabbit myocytes.** The investigation conforms with *The Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985). Isolated ventricular myocytes were obtained from New Zealand white rabbits (weighing 1 to 2 kg) by conventional enzymatic dissociation methods (15). Cells were then filtered through

---

From the Institute of Molecular Cardiobiology, Johns Hopkins University, Baltimore, Maryland. Dr. Toshiaki Sato's present address: Department of Physiology, Oita Medical University, 1-1 Idaigaoka, Hasama, Oita 879-5503, Japan. This study was supported by NIH R37HL36957 (to Dr. Marbán), Banyu Fellowship in Lipid Metabolism and Atherosclerosis (to Dr. Sato), and an unrestricted gift from Chugai Pharmaceutical Co.

Manuscript received May 24, 1999; revised manuscript received September 10, 1999, accepted October 21, 1999.

#### Abbreviations and Acronyms

5HD	= 5-hydroxydecanoate
CONT	= control group
DNP	= 2,4-dinitrophenol
$I_{K,ATP}$	= surface $K_{ATP}$ current
IPC	= ischemic preconditioning
$K_{ATP}$	= ATP-dependent potassium
mito $K_{ATP}$	= mitochondrial $K_{ATP}$
NICO	= nicorandil-treated group
surface $K_{ATP}$	= sarcolemmal $K_{ATP}$

nylon mesh and washed several times with  $Ca^{2+}$ -free solution. The calcium concentration was gradually brought back to 1 mmol/liter.

**Flavoprotein fluorescence and electrophysiologic recording.** After isolation, cells were cultured on laminin-coated coverslips in M199 with 5% fetal bovine serum at 37°C and experiments were performed over the next day. Cells were mounted in a recording chamber and were superfused with external solution containing (in mmol/liter): NaCl, 140; KCl, 5;  $MgCl_2$ , 1;  $CaCl_2$ , 1; and HEPES, 10 (pH 7.4 with NaOH) at room temperature (approximately 22°C). Whole-cell current and flavoprotein fluorescence were recorded simultaneously. The internal pipette solution contained (in mmol/liter) the following: potassium glutamate, 120; KCl, 25;  $MgCl_2$ , 0.5; potassium EGTA, 10; HEPES, 10; and  $MgATP$ , 1 (pH 7.2 with KOH). Whole-cell currents were elicited every 6 s from a holding potential of -80 mV by two consecutive steps to -40 (for 100 ms) and 0 mV (for 380 ms). Currents at 0 mV were measured 200 ms into the pulse. Endogenous flavoprotein fluorescence was excited with a xenon arc lamp with a bandpass filter centered at 480 nm, but only during the 100-ms step to -40 mV to minimize photobleaching. Emitted fluorescence was recorded at 530 nm by a photomultiplier tube and digitized. By focusing on individual myocytes with a  $\times 40$  objective, whole-cell current and fluorescence were monitored simultaneously from one cell at a time. The redox signal was averaged during the excitation window and calibrated with the values after exposure to 2,4-dinitrophenol (DNP), which uncouples respiration from ATP synthesis, collapses the mitochondrial potential and induces maximal oxidation. Therefore, the values of flavoprotein fluorescence were expressed as a percentage of the DNP-induced fluorescence.

**Cell pelleting model of ischemia.** The cell pelleting model of ischemia modified from Vander Heide et al. (16) was used to quantify cell injury. In brief, cells were washed with incubation buffer (in mmol/liter): NaCl, 119;  $NaHCO_3$ , 25;  $KH_2PO_4$ , 1.2; KCl, 4.8;  $MgSO_4$ , 1.2;  $CaCl_2$ , 1; HEPES, 10; glucose, 11; creatine, 24.9; taurine, 58.5; and supplemented with 1% BME amino acids and 1% MEM nonessential amino acids (pH 7.4 with NaOH). An aliquot

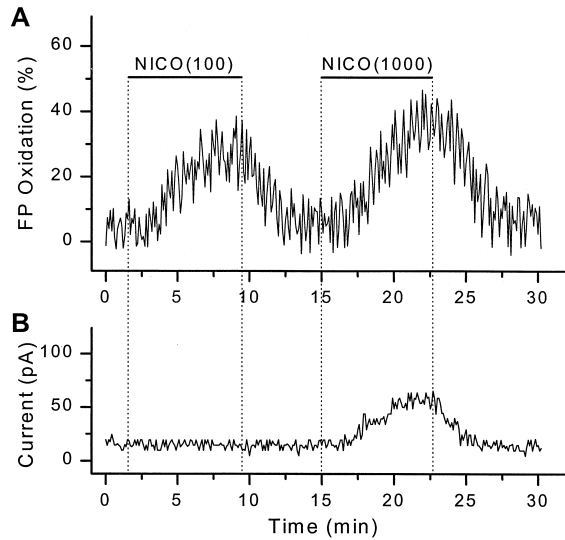
of each cell suspension (0.5 ml) was placed into a microcentrifuge tube and centrifuged for 15 s into a pellet. Approximately 0.25 ml of excess supernatant was removed to leave a thin fluid layer above the pellet, and 0.2 ml of mineral oil was layered on the top of the pellet to prevent gaseous diffusion. After 60 and 120 min of pelleting, 5  $\mu$ l of cell pellet was sampled through the oil layer and mixed with 75  $\mu$ l of 85 mosm/liter hypotonic staining solution (in mmol/liter):  $NaHCO_3$ , 11.9;  $KH_2PO_4$ , 0.4; KCl, 2.7;  $MgSO_4$ , 0.8;  $CaCl_2$ , 1 with 0.5% glutaraldehyde and 0.5% trypan blue. Microscopic examination was performed 2 to 5 min after mixing to determine the permeability of the cells to trypan blue. Cells permeable to trypan blue were counted as killed and expressed as a percentage of the total cells counted (>200 for each sample). Four groups of experiments were performed. In the control group (CONT), cells were pelleted and sampled at 60 and 120 min. For the nicorandil-treated group (NICO), nicorandil at a concentration of 100  $\mu$ mol/liter was added to the solution 15 min before the pelleting. Cells treated with nicorandil in the presence of 500  $\mu$ mol/liter of 5HD (NICO+5HD) or in the presence of 30  $\mu$ mol/liter HMR1098 (NICO+HMR1098) were likewise pelleted and sampled. Once applied, drugs were not washed out and thus were present throughout the period of simulated ischemia. Experiments were performed at 37°C. Individual experiments in each group were performed on cells isolated from different hearts.

**Chemicals.** DNP was obtained from the manufacturer (Sigma Chemical; St. Louis, Missouri), as was sodium 5HD (Research Biochemicals International; Natick, Massachusetts). Nicorandil was a gift (Chugai Pharmaceutical Co., Ltd; Tokyo, Japan), as was HMR1098 (Hoechst Marion Roussel Chemical Research; Frankfurt, Germany).

**Data analysis.** Data are presented as mean  $\pm$  SEM, and the number of cells or experiments is shown as *n*. Analysis of variance combined with Fisher post-hoc test was used to test for significance among groups. A value of *p* < 0.05 was considered significant.

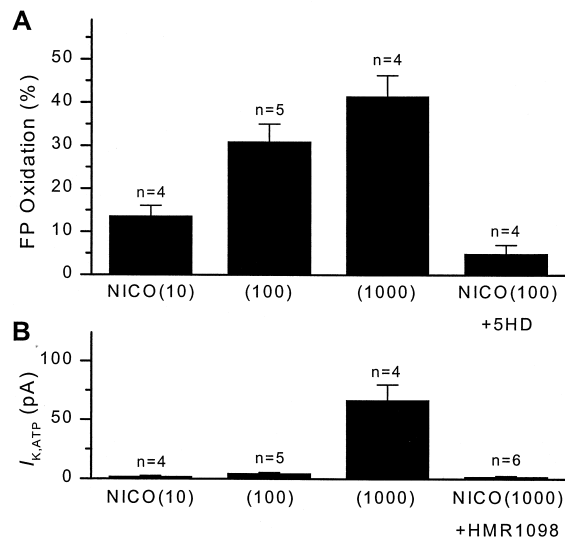
## RESULTS

Figure 1 shows representative results from simultaneous measurements of flavoprotein fluorescence and surface  $K_{ATP}$  current ( $I_{K,ATP}$ ) in a single ventricular myocyte. Nicorandil at a concentration of 100  $\mu$ mol/liter reversibly oxidized the flavoproteins but did not activate  $I_{K,ATP}$ . A second exposure to nicorandil at a concentration of 1 mmol/liter increased both flavoprotein fluorescence and  $I_{K,ATP}$ . As summarized in Figure 2, nicorandil increased flavoprotein fluorescence in a concentration-dependent manner. Nicorandil at concentrations of 10 and 100  $\mu$ mol/liter reversibly increased flavoprotein oxidation to  $14 \pm 2\%$  (*n* = 4) and  $31 \pm 4\%$  (*n* = 5) of the DNP value, respectively, without affecting  $I_{K,ATP}$ . However, a very high concentration (1 mmol/liter)

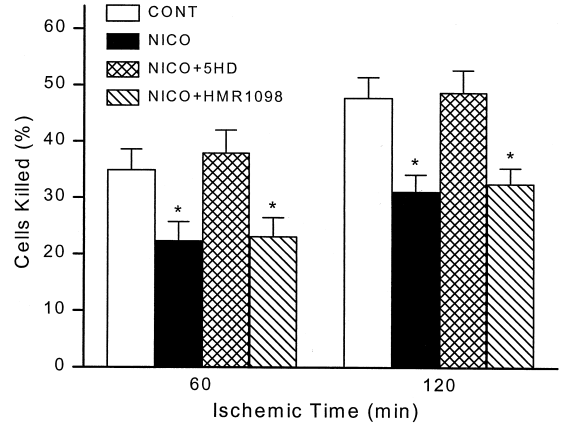


**Figure 1.** Relative potency of nitorandil in opening surface  $K_{ATP}$  and mito  $K_{ATP}$  channels. Records of simultaneous flavoprotein fluorescence (A) and membrane currents (B) in a single rabbit ventricular myocyte. The flavoprotein fluorescence was calibrated by exposing the cells to DNP (100  $\mu\text{mol/liter}$ ) at the end of experiments. Bar indicates periods when the cells were exposed to nitorandil (NICO), 100  $\mu\text{mol/liter}$  or 1,000  $\mu\text{mol/liter}$  as noted.

of nitorandil was required to increase not only flavoprotein oxidation but also  $I_{K_{ATP}}$ . The selective mito  $K_{ATP}$  channel blocker 5HD (500  $\mu\text{mol/liter}$ ) (13) virtually abolished the nitorandil (100  $\mu\text{mol/liter}$ )-induced flavoprotein oxidation. However, nitorandil (1 mmol/liter)-induced  $I_{K_{ATP}}$  was completely inhibited by 30  $\mu\text{mol/liter}$  HMR1098, a selec-



**Figure 2.** Summarized dose-response data for mitochondrial (A) and surface (B) effects. Nitorandil (100  $\mu\text{mol/liter}$ )-induced flavoprotein oxidation was prevented by 500  $\mu\text{mol/liter}$  5-hydroxydecanoate (NICO [100]+5HD), whereas nitorandil (1 mmol/liter)-induced  $I_{K_{ATP}}$  was inhibited by 30  $\mu\text{mol/liter}$  HMR1098 (NICO[1,000]+HMR1098).



**Figure 3.** Summarized data for cardioprotective effects of nitorandil. Cells killed by 60 or 120 min of simulated ischemia were plotted as a percentage of the total viable cells before ischemia. CONT = control group; NICO = nitorandil (100  $\mu\text{mol/liter}$ )-treated group; NICO+5HD = nitorandil and 5HD (500  $\mu\text{mol/liter}$ )-treated group; NICO+HMR1098 = nitorandil and HMR1098 (30  $\mu\text{mol/liter}$ )-treated group. \* $p < 0.01$  vs. CONT.

tive surface  $K_{ATP}$  channel blocker (17). These results suggest that nitorandil primarily activates mito  $K_{ATP}$  rather than surface  $K_{ATP}$  channels in rabbit ventricular cells.

In the next series of experiments, we tested the idea that mito  $K_{ATP}$  rather than surface  $K_{ATP}$  channels act as the effectors for cardioprotection afforded by nitorandil, using a cell-pelleting model of ischemia. Figure 3 plots the fraction of cells killed by 60 and 120 min of simulated ischemia as a percentage of the total number of viable cells before ischemia. Pelleting for 60 and 120 min killed  $35 \pm 4\%$  ( $n = 4$ ) and  $48 \pm 4\%$  ( $n = 4$ ) of cells, respectively (CONT). Inclusion of nitorandil (100  $\mu\text{mol/liter}$ ) significantly decreased the percentage of cells killed during ischemia to  $22 \pm 3\%$  ( $n = 4$ ) after 60 min and  $31 \pm 3\%$  ( $n = 4$ ) after 120 min ischemia (NICO,  $p < 0.01$  vs. CONT). The cardioprotective effects of nitorandil were abolished by 500  $\mu\text{mol/liter}$  5HD ( $38 \pm 4\%$  after 60 min and  $49 \pm 4\%$  after 120 min ischemia, respectively). In contrast, the selective surface  $K_{ATP}$  channel inhibitor HMR1098 (30  $\mu\text{mol/liter}$ ) did not abolish the cardioprotection by nitorandil (NICO+HMR1098,  $p < 0.01$  vs. CONT). These results indicate that nitorandil-induced cardioprotection against ischemic damage is mediated by opening of mito  $K_{ATP}$  channels but not surface  $K_{ATP}$  channels.

## DISCUSSION

**Nitorandil targets mito  $K_{ATP}$  channels.** Previous studies in our laboratory have demonstrated that the mito  $K_{ATP}$  channel opener diazoxide oxidizes flavoproteins without affecting  $I_{K_{ATP}}$  in rabbit ventricular myocytes (12). Using the same experimental design, our present results demonstrate that nitorandil reversibly oxidized the mitochondrial matrix in a concentration-dependent manner, while milli-

molar concentrations are required to elicit  $I_{K_{ATP}}$ . The oxidative effects of nicorandil actually reflect the opening of mito $K_{ATP}$  channels, because the mito $K_{ATP}$  channel blocker 5HD completely abolished the nicorandil-induced flavoprotein oxidation. These results indicate that nicorandil primarily activates mito $K_{ATP}$  channels in intact rabbit ventricular cells.

**Mito $K_{ATP}$  channels serve as effectors of cardioprotection.**  $K_{ATP}$  channel openers may shorten the action potential duration, thereby reducing cellular calcium overload and preserving viability in ischemic myocardium: this was initially proposed as the mechanism for protection of ischemic myocardium. Nevertheless, this hypothesis cannot account for the mechanism of cardioprotection, because abbreviation of action potentials is not necessary for protection (18-20). Alternatively, recent pharmacologic evidence hints that mito $K_{ATP}$  channels are the dominant players. The mito $K_{ATP}$  channel opener diazoxide protects rabbit ventricular myocytes in a cell pelleting model of ischemia (12) and improves functional recovery after ischemia in isolated rat and rabbit hearts (11); this diazoxide-induced protection is prevented by 5HD (11,12). In the present study, the cardioprotective effect of nicorandil was examined in a cellular ischemia model. Previous studies have shown that simulated ischemia preconditions myocytes in this model and that the underlying mechanisms for the protection are similar to those in intact hearts (21,22). Critz et al. (23) reported that nicorandil caused neither surface $K_{ATP}$  channel opening nor cardioprotection in rabbit myocytes. However, we found that nicorandil protects against cell death to the same degree as does genuine ischemic preconditioning. Although the reason for this discrepancy is unknown, the level of nucleotide diphosphates or intracellular pH during ischemia may affect the nicorandil-induced cardioprotection. To probe the final effector for cardioprotection, we used selective blockers of either mito $K_{ATP}$  or surface $K_{ATP}$  channels. HMR1098, a potent surface $K_{ATP}$  channel blocker (17), completely inhibited the nicorandil-induced  $I_{K_{ATP}}$  (Fig. 2B). However, the cardioprotective effects of nicorandil were not blocked by HMR1098. In contrast, the mito $K_{ATP}$  channel blocker 5HD completely abolished the nicorandil-induced cardioprotection. These results indicate that mito $K_{ATP}$  rather than surface $K_{ATP}$  channels are involved in the cardioprotection afforded by nicorandil. In separate experiments not shown herein, we determined that nitric oxide donors only weakly favor the opening of mito $K_{ATP}$  channels (24). Thus, it seems unlikely that the protective effect of nicorandil is solely conferred by its nitrate moiety.

Lethal injury to the heart can be dramatically blunted by brief periods of prior ischemia (25). Such ischemic preconditioning (IPC) exists in most species, including human (26-28). Diazoxide mimics IPC and reduces infarct size in rabbit hearts (29). However, 5HD abolishes genuine IPC (30,31). These results implicate mito $K_{ATP}$  channels as

effectors of IPC. Nicorandil can mimic IPC by reducing infarct size in rabbit hearts, and this cardioprotection is abolished by 5HD (5). Interestingly, Sakai et al. (14) reported subcellular localization of nicorandil in myocardial mitochondria. Therefore, taken together, it is reasonable to consider that nicorandil targets mito $K_{ATP}$  channels and that cardioprotective effects of nicorandil are mediated by opening of mito $K_{ATP}$  channels.

**Clinical implications.** Despite their favorable cardioprotective property, enthusiasm for  $K_{ATP}$  channel openers has been tempered by the fear that they may promote the development of ventricular arrhythmias (32). This potential drawback limits the clinical utility of surface $K_{ATP}$  channel openers. In contrast, the selective mito $K_{ATP}$  channel opener diazoxide protects the myocytes from ischemia (11, 12, 29), suggesting that mito $K_{ATP}$  channels might be useful targets for the ischemic cardioprotection. We found that nicorandil, a clinically available anti-ischemic agent, appears to be a fairly selective mito $K_{ATP}$  channel opener. It has been approved for human use (2) and has cardioprotective effects in humans (6-9). The clinical utility of nicorandil indicates drugs that target mito $K_{ATP}$  channels, without activating surface $K_{ATP}$  channels, may be safe and effective for the protection of ischemic myocardium.

## CONCLUSION

Our results indicated that nicorandil exerts a direct cardioprotective effect on heart muscle cells, an effect mediated by the selective activation of mito $K_{ATP}$  channels. It links, for the first time, the basic phenomenon of ischemic preconditioning with the existing pharmacopeia for ischemic syndromes. Our findings support the principle that mito $K_{ATP}$  channels are valuable new targets for anti-ischemic drug development.

---

**Reprint requests and correspondence:** Dr. Eduardo Marbán, Institute of Molecular Cardiobiology, Johns Hopkins University, Ross 844/720 Rutland Avenue, Baltimore, Maryland 21205. E-mail: marban@jhmi.edu.

---

## REFERENCES

1. Taira N. Nicorandil as a hybrid between nitrates and potassium channel activators. *Am J Cardiol* 1989;63:18J-24J.
2. Krumenacker M, Roland E. Clinical profile of nicorandil: an overview of its haemodynamic properties and therapeutic efficacy. *J Cardiovasc Pharmacol* 1992;20:S93-102.
3. Gross GJ. The cardioprotective effects of nicorandil. *Rev Contemp Pharmacother* 1993;4:199-206.
4. Mizumura T, Nithipatikom K, Gross GJ. Effects of nicorandil and glyceryl trinitrate on infarct size, adenosine release, and neutrophil infiltration in the dog. *Cardiovasc Res* 1995;29:482-9.
5. Imagawa J, Baxter GF, Yellon DM. Myocardial protection afforded by nicorandil and ischaemic preconditioning in a rabbit infarct model in vivo. *J Cardiovasc Pharmacol* 1998;31:74-9.
6. Saito S, Mizumura T, Takayama T, et al. Antiischemic effects of nicorandil during coronary angioplasty in humans. *Cardiovasc Drugs Ther* 1995;9:257-63.
7. Kobayashi Y, Goto Y, Daikoku S, et al. Cardioprotective effect of

- intravenous nicorandil in patients with successful reperfusion for acute myocardial infarction. *Jpn Circ J* 1998;62:183–9.
8. Ito H, Taniyama Y, Iwakura K, et al. Intravenous nicorandil can preserve microvascular integrity and myocardial viability in patients with reperfused anterior wall myocardial infarction. *J Am Coll Cardiol* 1999;33:654–60.
  9. Patel DJ, Purcell HJ, Fox KM. Cardioprotection by opening of the  $K_{ATP}$  channel in unstable angina: is this a clinical manifestation of myocardial preconditioning? Results of a randomized study with nicorandil. CESAR 2 investigation. Clinical European studies in angina and revascularization. *Eur Heart J* 1999;20:51–7.
  10. Nichols CG, Lederer WJ. Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. *Am J Physiol* 1991;261:H1675–86.
  11. Garlid KD, Paucek P, Yarov-Yarovsky V, et al. Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive  $K^+$  channels: possible mechanism of cardioprotection. *Circ Res* 1997;81:1072–82.
  12. Liu Y, Sato T, O'Rourke B, Marban E. Mitochondrial ATP-dependent potassium channels: novel effectors of cardioprotection? *Circulation* 1998;97:2463–9.
  13. Sato T, O'Rourke B, Marban E. Modulation of mitochondrial ATP-dependent  $K^+$  channels by protein kinase C. *Circ Res* 1998;83:110–4.
  14. Sakai K, Tsuchiya Y, Kitajima S, Hamada H. Myocardial distribution and biotransformation in vitro and in vivo of nicorandil in rats, with special reference to mitochondria. *J Cardiovasc Pharmacol* 1999;33:163–8.
  15. Liu Y, Gao WD, O'Rourke B, Marban E. Synergistic modulation of ATP-dependent  $K^+$  currents by protein kinase C and adenosine: implications for ischemic preconditioning. *Circ Res* 1996;78:443–54.
  16. Vander Heide RSV, Rim D, Hohl CM, Ganote CE. An in vitro model of myocardial ischemia utilizing isolated adult rat myocytes. *J Mol Cell Cardiol* 1990;22:165–81.
  17. Sato T, Sasaki N, Scharaseyov J, O'Rourke B, Marbán E. Selective pharmacological agents implicate mitochondrial, but not sarcolemmal,  $K_{ATP}$  channels in ischemic cardioprotection. *Circulation*. In Press.
  18. Yao Z, Gross GJ. Effects of the  $K_{ATP}$  channel opener bimakalim on coronary blood flow, monophasic action potential duration, and infarct size in dogs. *Circulation* 1994;89:1769–75.
  19. Grover GJ, Dalonzo AJ, Parham CS, Darbenzio RB. Cardioprotection with the  $K_{ATP}$  opener cromakalim is not correlated with ischemic myocardial action potential duration. *J Cardiovasc Pharmacol* 1995; 26:145–52.
  20. Grover GJ, Dalonzo AJ, Dzwonczyk S, Parham CS, Darbenzio RB. Preconditioning is not abolished by the delayed rectifier  $K^+$  blocker dofetilide. *Am J Physiol* 1996;40:H1207–14.
  21. Armstrong SC, Downey JM, Ganote CE. Preconditioning of isolated rabbit cardiomyocytes: induction by metabolic stress and blocked by the adenosine antagonist SPT and calphostin C, a protein kinase C inhibitor. *Cardiovasc Res* 1994;28:72–7.
  22. Liu Y, Gao WD, O'Rourke B, Marban E. Cell-type specificity of preconditioning in an in vitro model. *Basic Res Cardiol* 1996;91: 450–7.
  23. Critz SD, Liu GS, Chujo M, Downey JM. Pinacidil but not nicorandil opens ATP-sensitive  $K^+$  channels and protects against simulated ischemia in rabbit myocytes. *J Mol Cell Cardiol* 1997;29:1123–30.
  24. Sasaki N, Sato T, Ohler A, et al. Activation of mitochondrial ATP-dependent potassium channels by nitric oxide. *Circulation*. In Press.
  25. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986; 74:1124–36.
  26. Cohen MV, Downey JM. Ischemic preconditioning: can the protection be bottled? *Lancet* 1993;342:6.
  27. Yellon DM, Alkhulaifi AM, Pugsley WB. Preconditioning the human myocardium. *Lancet* 1993;342:276–7.
  28. Kloner RA, Yellon DM. Does ischemic preconditioning occur in patients? *J Am Coll Cardiol* 1994;24:1133–42.
  29. Baines CP, Liu GS, Birincioglu M, Critz SD, Cohen MV, Downey JM. Ischemic preconditioning depends on interaction between mitochondrial  $K_{ATP}$  channels and actin cytoskeleton. *Am J Physiol* 1999;276:H1361–8.
  30. Auchampach JA, Grover GJ, Gross GJ. Blockade of ischaemic preconditioning in dogs by the novel ATP dependent potassium channel antagonist sodium 5-hydroxydecanoate. *Cardiovasc Res* 1992; 26:1054–62.
  31. Hide EJ, Thiemermann C. Limitation of myocardial infarct size in the rabbit by ischaemic preconditioning is abolished by sodium 5-hydroxydecanoate. *Cardiovasc Res* 1996;31:941–6.
  32. Chi L, Uprichard AC, Lucchesi BR. Profibrillatory actions of pinacidil in a conscious canine model of sudden coronary death. *J Cardiovasc Pharmacol* 1990;15:452–64.