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Mechanisms of the Effects of Nicorandil in the Isolated Rat Heart During Ischemia and Reperfusion: A ^{31}P-Nuclear Magnetic Resonance Study

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ABSTRACT

Nicorandil (SG75) is a potent K^+-channel activator with an additional nitro moiety. In the present study we investigated the potential mechanisms (K^+-channel activation and nitric oxide [NO] release) for the effects of nicorandil on isolated perfused rat hearts during total global ischemia using ^{31}P-nuclear magnetic resonance. After a 10-min control perfusion, hearts were subjected to treatment with nicorandil-containing (100, 300, or 1000 μM) buffer for 10 min, 15 min of total global ischemia, and 30 min of reperfusion. At high dose (10^{-3} M), nicorandil reduced ATP depletion during ischemia by 26% compared with untreated hearts. Blockade of K^+ channels by glibenclamide prevented this protective effect. At all doses (10^{-4} to 10^{-3} M), nicorandil reduced the accumulation of protons during ischemia compared with untreated hearts (pH 6.22 ± 0.03 vs. 6.02 ± 0.05 in untreated hearts at the end of ischemia). This effect was preserved after blockade of K^+ channels by glibenclamide. Hearts treated with nitroglycerine before ischemia also showed reduced proton accumulation. Therefore, NO release accompanied by increased coronary flow before ischemia, which is caused by the nitro moiety of nicorandil and nitroglycerine treatment, results in reduced proton accumulation. During reperfusion, a pro-arrhythmic effect was observed in hearts treated with the nonpharmacologically high dose of nicorandil (1000 μM). Thus, we conclude that the effects of nicorandil are caused

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by the simultaneous action of both mechanisms K^+-channel activation and *NO release. The activation of K^+ channels prevents deterioration of ATP during ischemia, whereas NO release and increased coronary flow reduce the accumulation of protons—and thus the decrease in pH—during ischemia.*

Key Words: *Ischemia; K^+-channel activation; Magnetic resonance spectroscopy; Nicorandil; NO release; Reperfusion*

INTRODUCTION

Maintenance of myocardial ion homeostasis involves both numerous cation transport systems and a high energetic state of the cardiomyocytes. As long as the heart is in stable metabolic condition, the K^+ channels keep the balance of high intracellular and low extracellular K^+ concentrations. Ischemic stress leads to an increase of the ADP/ATP ratio, which results in closing of the K^+ channels, an increase of inorganic phosphate, and depolarization of the cardiomyocytes. Next, massive Ca^{2+} influx will occur and cause ischemic injury to the cell (1).

Beneficial effects of K^+-channel activators in the heart have been shown under ischemic condition. Activation of the K^+ channels causes reduced sensitivity to lowered ATP levels (2). Depolarization is attenuated or prevented in hearts treated with K^+-channel activators, which lowers Ca^{2+} influx (3,4) and the extent of ischemic injury.

A variety of K^+-channel activators has been studied to date, and all of them lower blood pressure in hypertensive patients. Nicorandil (*N*-(2-hydroxyethyl)nicotinamidenitrate, SG 75) is the most effective K^+-channel opener in comparison with other K^+-channel openers of the nicotinamide type (1). In addition, nicorandil may be beneficial for the treatment of unstable angina pectoris (5–11). The nicorandil molecule holds two moieties that might exert beneficial effects during ischemia. Nicorandil activates K^+ channels through its nicotinamide structure and acts as a potent nitrate by its nitro group. The aims of this study were to define the effects of nicorandil during control perfusion, ischemia, and reperfusion in the isolated rat heart over a wide dosage range beyond clinically used dosages and to elucidate whether the mechanism of the protective effect of nicorandil during ischemia are related to alterations in myocardial energetics and intracellular pH.

MATERIALS AND METHODS

Isolated Rat Heart Preparation

Male Wistar rats (340–360 g) were anesthetized by intraperitoneal injection of 40 mg sodium pentobarbital. A transverse laparotomy and a left and a right anterolateral thoracotomy were performed. The heart was rapidly excised and immersed in ice-cold buffer. The aorta was dissected free and mounted onto a cannula attached to a perfusion apparatus, as previously described (12). Retrograde perfusion of the heart with oxygenated Krebs-Henseleit buffer was started in the Langendorff mode at a constant temperature of 37°C and a coronary perfusion pressure of 100 mm Hg. The investigation conforms to the NIH guidelines for the care and use of laboratory animals and was approved by the local authorities (Regierung von Unterfranken).

Cardiac Performance Measurements

A small flexible polyethylene tube was pierced through the apex of the left ventricle to allow drainage of flow from Thebesian veins. A water-filled latex balloon was inserted through an incision in the left atrial appendage, via the mitral valve, and secured by a ligature. The volume of the balloon was adjusted to maintain an end-diastolic pressure of 8–11 mm Hg. The balloon was connected to a Statham pressure transducer (Gould Instruments, Glen Burnie, MD) via a small-bore polyethylene tube for continuous measurement of left ventricular pressures and heart rate on a four-channel graphic recorder (Watanabe, Tokyo, Japan). Performance was estimated as rate-pressure product (mm Hg/min), that is, the product of heart rate (min^{-1}), and left ventricular developed pressure (mm Hg), that is, the difference of systolic and diastolic pressure. Coronary flow was measured by an ultrasound flowmeter (Transonic Systems Inc., Ithaca, NY). The time for onset of ischemic contracture was measured at an increase of the end-diastolic pressure by 5 mm Hg.

Nuclear Magnetic Resonance Measurements

The perfused hearts were placed in a 20-mm outer diameter nuclear magnetic resonance (NMR) sample tube (Wilmad, Buena, NJ) and inserted into a ^{31}P- and ^{23}Na-NMR probe head (Paul Morris Inc., Gloucester, Canada)

that was seated in the bore of a superconducting super-wide-bore (150 mm) 7.05-T magnet (Oxford Instruments, Oxford). The probe head held two replaceable coils with 20-mm inner diameter for ^{31}P at 121.44 MHz and 30-mm inner diameter for ^{23}Na at 79.353 MHz.

A Bruker AM 300 NMR console (Bruker, Rheinstetten, Germany) was used for acquisition of NMR spectra. Each radiofrequency coil was tuned and matched before each experiment. Before the ^{31}P-NMR measurement, the magnet was shimmed with a 14-channel Bruker Shim Supply to maximum area of the free induction decay of the ^{23}Na signal, which resulted in a linewidth of 8 to 11 Hz for a single-pulse ^{23}Na-NMR spectrum.

For ^{31}P-NMR measurements, 152 scans were averaged with a pulse length of 37 μsec (pulse angle of 45 degrees) and an interpulse delay of 1.93 sec, yielding one spectrum in 5 min. Free induction decays were exponentially multiplied with a line broadening of 20 Hz, Fourier transformed, and the phase was corrected interactively. For each experiment, 13 consecutive ^{31}P-NMR spectra were recorded.

Intracellular pH of all spectra was calculated from the chemical shift difference between inorganic phosphate and phosphocreatine, as previously described (13). The resonance areas corresponding to ATP, phosphocreatine, inorganic phosphate, and phosphate monoesters were integrated by Bruker DISNMR89 integration routine. In each ^{31}P-NMR experiment, the area of the γ-P-ATP resonance of the first spectrum was set arbitrarily to 100% and used as a reference value for all resonances in the set of ^{31}P-NMR spectra obtained in a given protocol. Areas were corrected for partial saturation (1.12 for phosphocreatine, 1.08 for inorganic phosphate), and concentrations were calculated assuming 11.2 mM ATP content in the heart (14).

Pharmaceutical Agents and Buffer Solutions

Hearts were perfused with modified Krebs-Henseleit solution containing NaCl (118.0 mM), KCl (4.7 mM), $MgSO_4$ (1.2 mM), $CaCl_2$ (1.75 mM), EDTA (0.5 mM), $NaHCO_3$ (25.0 mM), and glucose (11.0 mM) dissolved in demineralized water (Fresenius, Bad Homburg, Germany). All ingredients were purchased from Sigma Chemie (Deisenhofen, Germany) and used without further purification. The perfusion solution was continuously equilibrated with 95% O_2 and 5% CO_2 to maintain a pH of 7.4. Agents were purchased from or kindly provided by the following: glibenclamide (Sigma Chemie), nicorandil and propanediol (Merck, Darmstadt, Germany), and nitroglycerine (G. Pohl-Boskamp, Hohenlockstedt, Germany).

A total of 21.1, 63.5, or 211.2 mg of nicorandil per liter Krebs-Henseleit buffer was dissolved to yield buffer concentrations of 100, 300, and 1000 μM nicorandil, respectively. A stock solution of glibenclamide, a selective blocker of ATP-dependent K^+ channels (15–17), dissolved in propanediol, was added to Krebs-Henseleit buffer (1 ml stock solution to 1 l Krebs-Henseleit buffer) to yield a concentration of 1 μM glibenclamide. Nitroglycerine was added as purchased to a modified Krebs-

Table 1

Perfusion Scheme for the Various Groups

Group	*n*	Control Period, 10 min	Treatment Period, 10 min	Gobal Ischemia, 15 min	Reperfusion, 30 min
Untreated	9	Krebs-Henseleit	Krebs-Henseleit	No flow	Krebs-Henseleit
Nico 100	10	Krebs-Henseleit	100 μM nicorandil	No flow	100 μM nicorandil
Nico 300	11	Krebs-Henseleit	300 μM nicorandil	No flow	300 μM nicorandil
Nico 1000	10	Krebs-Henseleit	1000 μM nicorandil	No flow	1000 μM nicorandil
Gli 0	9	Krebs-Henseleit	1 μM glibenclamide	No flow	1 μM glibenclamide
Gli 100	9	Krebs-Henseleit	100 μM nicorandil + 1 μM glibenclamide	No flow	100 μM nicorandil + 1 μM glibenclamide
Gli 300	9	Krebs-Henseleit	300 μM nicorandil + 1 μM glibenclamide	No flow	300 μM nicorandil + 1 μM glibenclamide
Gli 1000	9	Krebs-Henseleit	1000 μM nicorandil + 1 μM glibenclamide	No flow	1000 μM nicorandil + 1 μM glibenclamide
Nitro 100	9	Krebs-Henseleit	100 μM nitroglycerine	No flow	100 μM nitroglycerin

Henseleit buffer (NaCl 114.6 mM, glucose 4.8 mM) to result in the same concentrations for NaCl and glucose as the Krebs-Henseleit buffer listed above.

Protocol

The protocols for perfusion of each group are listed in Table 1. Hearts underwent 10 min of perfusion with Krebs-Henseleit buffer as the control period, followed by 10 min of perfusion with drug-containing buffer (or Krebs-Henseleit buffer for untreated group), before 15 min of total global normothermic ischemia and 30 min of reperfusion with drug-containing buffer. Four groups of hearts were studied:

1. Untreated hearts: perfused with Krebs-Henseleit buffer (Untreated);
2. Nicorandil-perfused hearts: three subgroups, perfused with buffer containing 100 μM (Nico 100), 300 μM (Nico 300), or 1000 μM nicorandil (Nico 1000);
3. Glibenclamide-perfused hearts: four subgroups, perfused with buffer containing 1 μM glibenclamide (Gli 0), or 100 μM nicorandil + 1 μM glibenclamide (Gli 100), or 300 μM nicorandil + 1 μM glibenclamide (Gli 300), or 1000 μM nicorandil + 1 μM glibenclamide (Gli 1000);
4. Nitroglycerine-perfused hearts: perfused with buffer containing 100 μM nitroglycerine (Nitro 100).

An additional group of hearts was treated with Krebs-Henseleit buffer plus propanediol to exclude effects of propanediol. Data not shown.

Statistical Analysis

The values of the individual hearts were averaged for each parameter to obtain mean values for each group. Data from the various groups were compared for each parameter by factorial analysis of variance followed by Scheffe's F-test (18), unless otherwise stated. Calculations were performed with the StatView SE+Graphics, Statistic Utility (Abacus Concepts, Berkeley, CA). Data are presented as means ± SE.

RESULTS

Control Period

At all concentrations, nicorandil caused an increase of coronary flow (Fig. 2a) by 73 ± 7, 55 ± 13, and 45 ± 9% ($p < 0.05$ vs. Untreated) in hearts perfused with 100, 300, and 1000 μM nicorandil, respectively. Glibenclamide did not alter coronary flow significantly. The combination of nicorandil and glibenclamide increased coronary flow (32 ± 4,* 61 ± 8,* and 71 ± 11% for 100, 300, and 1000 μM nicorandil + 1 μM glibenclamide, respectively; *$p < 0.05$ vs. 1 μM glibenclamide). Perfusion of hearts with buffer containing 100 μM nitroglycerine increased coronary flow from 20.7 ± 0.8 to 24.2 ± 0.8* ml/min (*$p < 0.05$), that is, by 27 ± 4%.

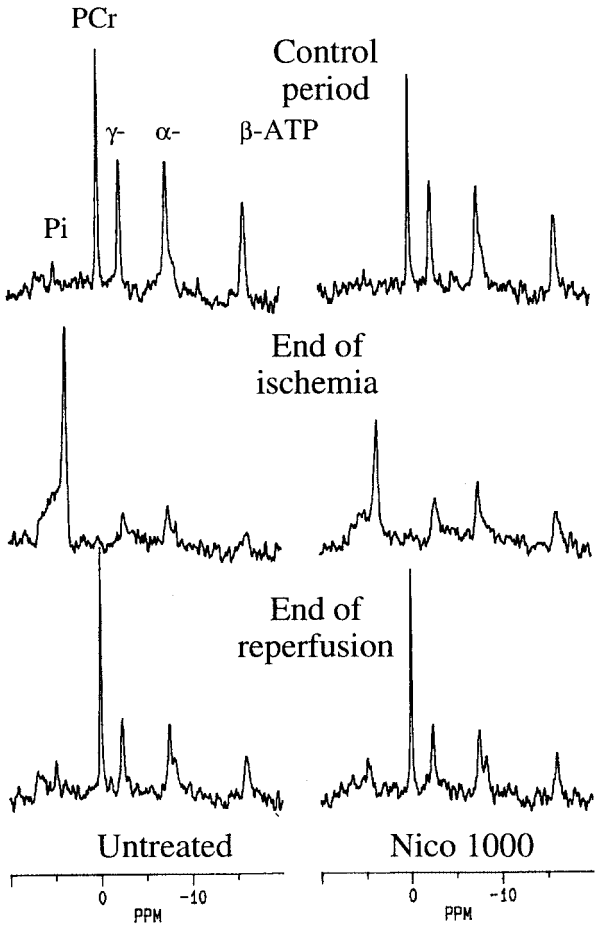

Figure 1. ^{31}P-NMR spectra of untreated (left column) and 1000 μM nicorandil-treated (right column) hearts during control period, at the end of ischemia, and at the end of reperfusion. Pi, inorganic phosphate; PCr, phosphocreatine.

As summarized in Table 2, lower concentrations of nicorandil (100 and 300 μM) led to an increase of the rate-pressure product due to increased left ventricular developed pressure, which presumably is due to the turgor effect. The turgor, or ''garden hose,'' effect describes the phenomenon that the myocardial wall is stretched in pro-

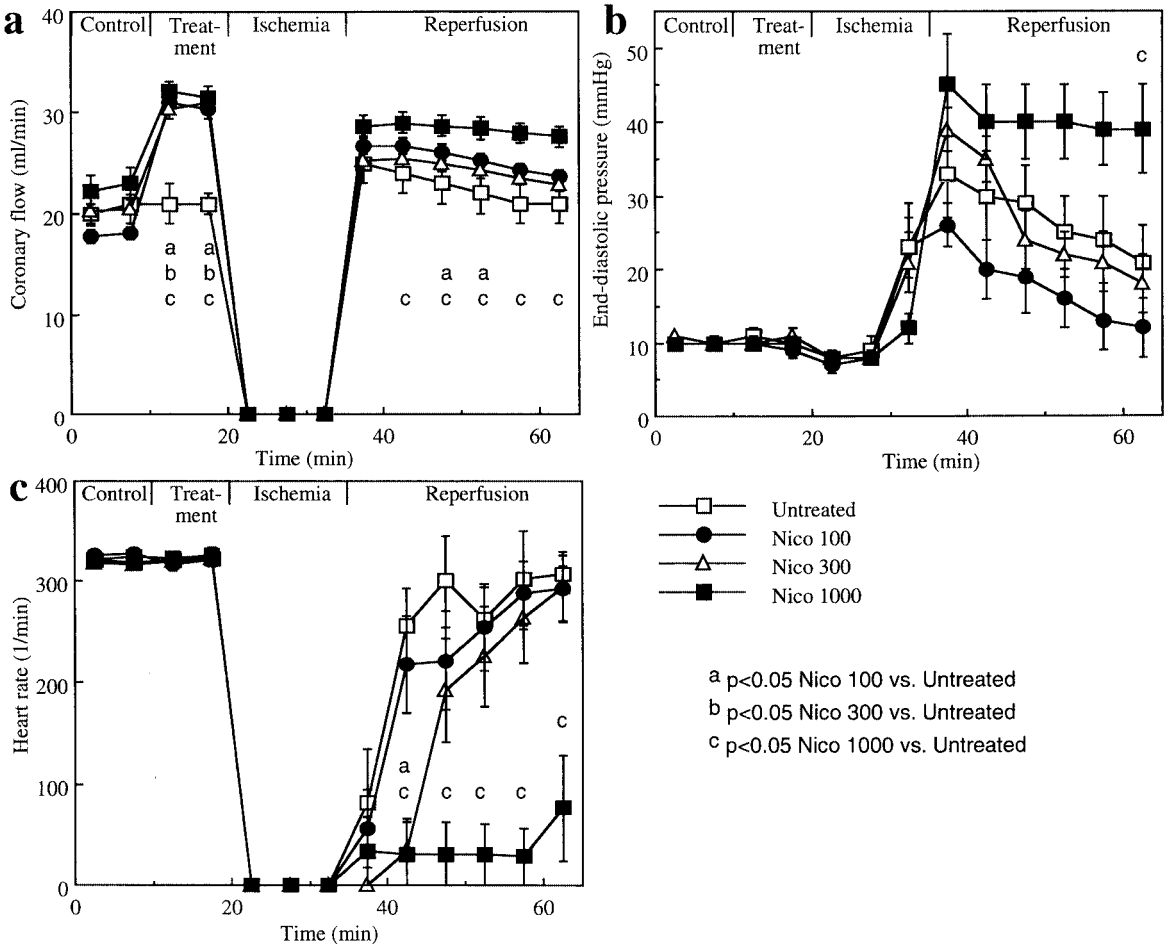

Figure 2. Parameters of perfusion and mechanical performance in untreated hearts and hearts treated with 100, 300, or 1000 μM nicorandil during control perfusion, treatment, ischemia, and reperfusion. (a) Coronary flow (ml/min), (b) end-diastolic pressure (mm Hg), and (c) heart rate (min^{-1}).

portion to coronary artery perfusion pressure. In the isolated heart setting, therefore, as coronary flow (and perfusion pressure) increases, end-diastolic pressure increases in parallel. Because of the Starling mechanism, this then leads to increased left ventricular developed pressure due to increased preload. For high-dose nicorandil (1000 μM), a negative inotropic effect offsets the effect of increased flow on performance. Therefore, no net effect on the rate-pressure product occurred. The rate-pressure product also increased in hearts perfused with nicorandil + glibenclamide. However, the negative inotropic effect of high nicorandil was not seen in these hearts. Hearts perfused with 100 μM nitroglycerine similarly had increased rate-pressure products. The heart rate was not affected by perfusion with either nicorandil or nicorandil + glibenclamide nor by perfusion with nitroglycerine. Therefore, all changes in rate-pressure product directly reflect changes in left ventricular developed pressure.

During the control period, end-diastolic pressure did not change before and during treatment with nicorandil. Additional treatment with glibenclamide or treatment with nitroglycerine alone had no effect on heart rate, end-diastolic and left ventricular developed pressure, and thus the rate-pressure product. During the control period, nicorandil, glibenclamide, and nitroglycerine had no effect on intracellular pH or concentrations of ^{31}P metabolites. Typical spectra of untreated hearts and hearts treated with 1000 μM nicorandil are given in Fig. 1.

Table 2

Mechanical Function of Hearts Expressed as Heart Rate (HR, min^{-1}), Left Ventricular Developed Pressure (LVDP, ml/min), and Rate-Pressure Product (RPP, 10^3 mm Hg/min) During Control Perfusion Before and During Treatment

	HR		LVDP		RPP	
	Control	Treatment	Control	Treatment	Control	Treatment
Untreated	321 ± 7	323 ± 6	97 ± 5	92 ± 4*	31.0 ± 1.9	29.9 ± 1.5
Nico 100	326 ± 5	321 ± 6	95 ± 4	109 ± 4*	30.9 ± 1.0	35.3 ± 1.5*
Nico 300	319 ± 5	324 ± 7	105 ± 5	114 ± 5	32.7 ± 1.7	35.8 ± 1.4*
Nico 1000	321 ± 8	327 ± 7	98 ± 2	92 ± 3*	31.6 ± 1.2	30.1 ± 1.1
Gli 0	317 ± 7	321 ± 10	87 ± 5	78 ± 6*	27.5 ± 1.8	24.8 ± 1.8*
Gli 100	333 ± 8	345 ± 6	91 ± 3	100 ± 5	30.6 ± 1.6	34.3 ± 1.5*
Gli 300	319 ± 7	325 ± 4	94 ± 5	112 ± 6*	29.7 ± 1.5	36.3 ± 1.7*
Gli 1000	322 ± 10	332 ± 8	91 ± 7	116 ± 7*	29.6 ± 3.1	39.6 ± 3.3*
Nitro 100	323 ± 8	311 ± 9	97 ± 5	109 ± 6*	31.2 ± 1.6	33.8 ± 1.5*

*$p < 0.05$ Control vs. Treatment.

Ischemia

During total global ischemia, all hearts were arrested within 2.5 min. Therefore, heart rate, left ventricular developed pressure, and rate-pressure product were all zero. During ischemia, end-diastolic pressure gradually increased in all groups (Table 3). The increase of end-diastolic pressure was slower in high-dose nicorandil compared with untreated hearts. Therefore, the time for onset of ischemic contracture was significantly delayed in high-dose nicorandil (12.1 ± 0.6, 12.0 ± 0.8, 11.6 ± 0.9, and 14.8 ± 0.8* min for Untreated and 100, 300, and 1000 μM nicorandil; *$p < 0.05$). Although there was no effect of 1 μM glibenclamide on the time for onset of ischemic contracture, hearts perfused with nicorandil + glibenclamide showed a significantly faster increase of end-diastolic pressure (12.9 ± 1.5, 7.0 ± 0.9,* 8.3 ± 0.7,* and 8.6 ± 1.1* min for 1 μM glibenclamide and 100, 300, and 1000 μM nicorandil + 1 μM glibenclamide, respectively; *$p < 0.05$). Nitroglycerine-treated hearts showed a faster increase of end-diastolic pressure than untreated or 100 μM nicorandil-treated hearts (time for onset of ischemic contracture 8.8 ± 0.8 min, $p < 0.05$ vs. Untreated, 100 μM nicorandil).

During ischemia, phosphocreatine declined rapidly in all groups. Within 5 min, phosphocreatine levels de-

Table 3

End-Diastolic Pressure (EDP), Time to Ischemic Contracture (TIC), ATP, Inorganic Phosphate (Pi), and Intracellular pH at the End of Ischemia

	EDP (mm Hg)	TIC (min)	ATP (mM)	Pi (mM)	pH
Untreated	23 ± 6	12.1 ± 0.6	3.92 ± 0.34	28.34 ± 1.90	6.02 ± 0.05
Nico 100	23 ± 4	12.0 ± 0.8	3.92 ± 0.56	28.56 ± 1.12	6.28 ± 0.12*
Nico 300	21 ± 4	11.6 ± 0.9	4.26 ± 0.56	30.69 ± 1.79	6.18 ± 0.12*
Nico 1000	12 ± 2	14.8 ± 0.8*	5.71 ± 0.56*	23.63 ± 1.12*	6.22 ± 0.03*
Gli 0	23 ± 5	12.9 ± 1.5	4.37 ± 0.56	30.58 ± 0.78	6.03 ± 0.03
Gli 100	45 ± 5†	7.0 ± 0.9*†	3.81 ± 0.34	35.06 ± 1.12	6.23 ± 0.04*†
Gli 300	43 ± 3†	8.3 ± 0.7*†	3.47 ± 0.45	31.36 ± 1.23	6.23 ± 0.05*†
Gli 1000	43 ± 6†	8.6 ± 1.1*†	3.14 ± 0.45	36.40 ± 1.34	6.20 ± 0.05*†
Nitro 100	31 ± 4	8.8 ± 0.8*	2.69 ± 0.45*	32.48 ± 1.46	6.15 ± 0.04*

*$p < 0.05$ vs. Untreated.
†$p < 0.05$ vs. Gli 0.

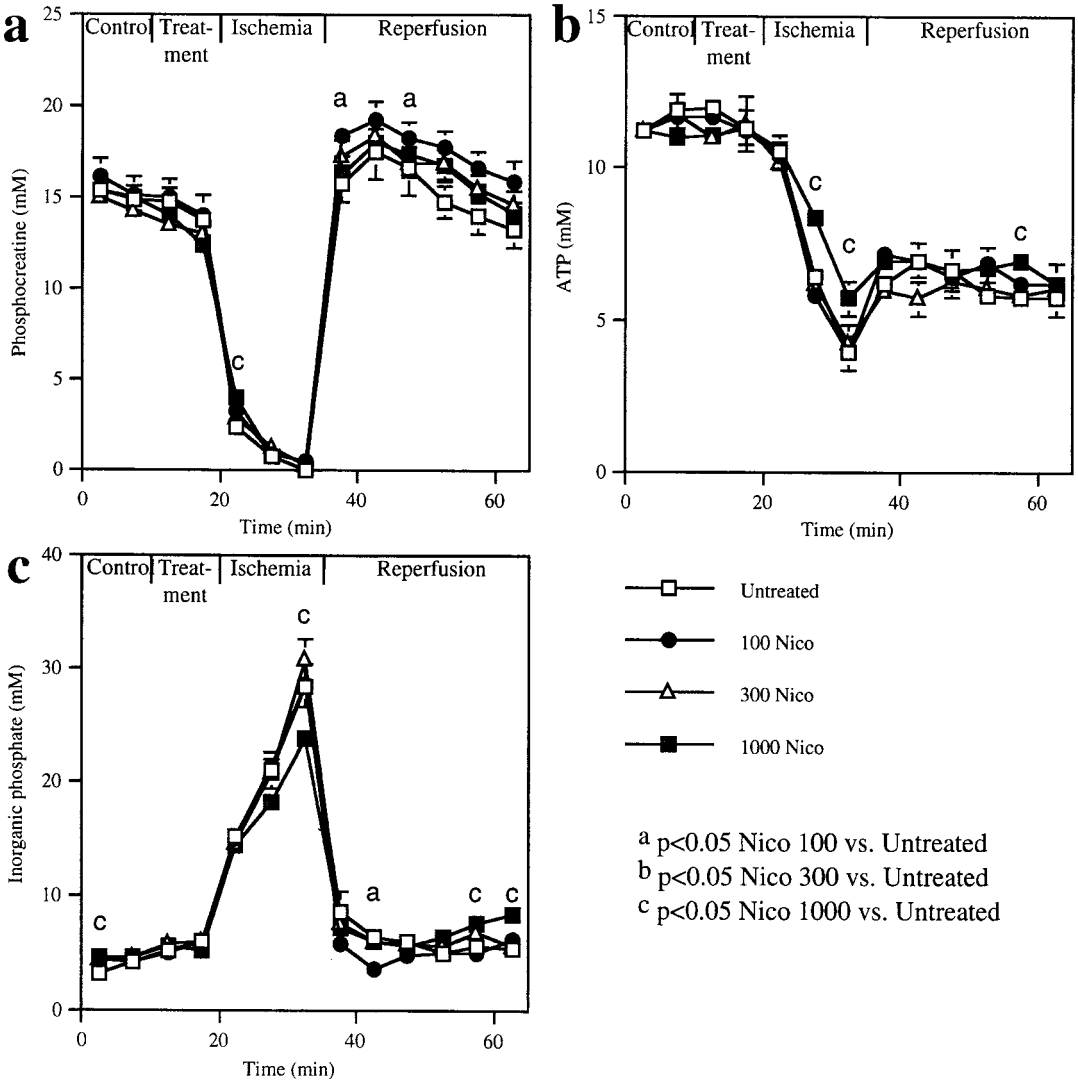

Figure 3. Parameters of ^{31}P metabolites in untreated hearts and hearts treated with 100, 300, or 1000 μM nicorandil during control perfusion, treatment, ischemia, and reperfusion. (a) Phosphocreatine, (b) ATP, and (c) inorganic phosphate. (All values are given as mM.)

creased to less than 20% of control perfusion and fell to zero until the end of ischemia (Fig. 3a). During ischemia, ATP declined in all groups. Depletion of ATP was reduced only in hearts treated with 1000 μM nicorandil ($p < 0.05$ Nico 1000 vs. Untreated). Here, ATP at the end of ischemia was significantly higher (51 ± 5% of preischemia, $*p < 0.05$ vs. Untreated) than in untreated hearts (35 ± 3%) or hearts treated with low-dose nicorandil (35 ± 5% in 100 μM, 38 ± 5% in 300 μM nicorandil; Fig. 3b). Hearts treated with nicorandil + glibenclamide showed depletion of ATP as in the untreated group. Inorganic phosphate increased rapidly during early ischemia, with a more gradual rise there after (Fig. 3c). Reduced ATP depletion of hearts treated with 1000 μM nicorandil was reflected by lower inorganic phosphate levels at the end of ischemia (Table 3). Treatment of hearts with nitroglycerine did not influence high-energy phosphate depletion during ischemia.

In all groups intracellular pH fell rapidly during ischemia (Fig. 4). In hearts treated with nicorandil, intracellu-

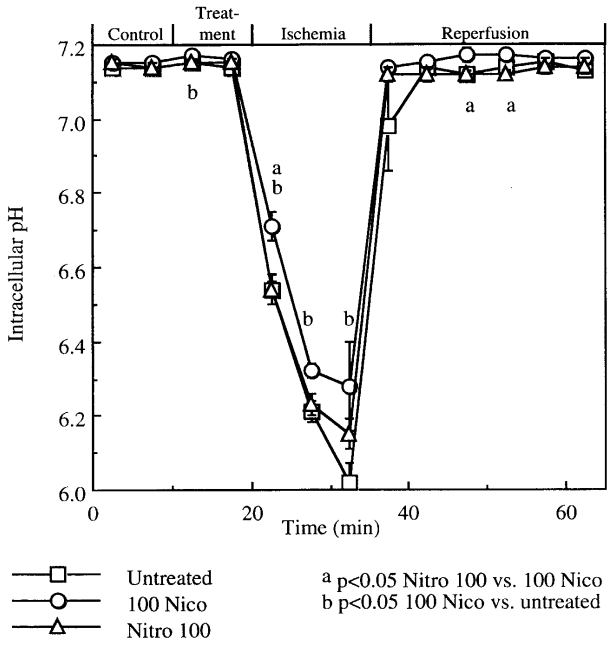

Figure 4. Intracellular pH value from untreated hearts and hearts treated with 100 μM nicorandil or 100 μM nitroglycerine during control, treatment, ischemia, and reperfusion.

lar acidosis was attenuated at all dosages (6.02 ± 0.05, 6.28 ± 0.12,* 6.18 ± 0.02,* and 6.22 ± 0.03* for Untreated and 100, 300, and 1000 μM nicorandil, respectively; *$p < 0.05$ vs. Untreated). This was also observed in hearts treated with nicorandil + glibenclamide (6.23 ± 0.04, 6.23 ± 0.05, and 6.20 ± 0.05 for 100, 300, and 1000 μM nicorandil + 1 μM glibenclamide; $p < 0.05$ vs. Gli 0). In hearts treated with nitroglycerine, proton accumulation was also attenuated (6.15 ± 0.04; $p < 0.05$ vs. Untreated).

Reperfusion

During reperfusion, coronary flow returned to control values in the untreated, glibenclamide-treated, and low-dose nicorandil groups. Hearts treated with 1000 μM nicorandil had a significantly higher coronary flow (Fig. 2a) during reperfusion. Untreated hearts and hearts perfused with low-dose nicorandil (100 and 300 μM) resumed mechanical function within the first 5 min of reperfusion. End-diastolic pressure declined during reperfusion but did not fully recover (Table 4). In these groups, left ventricular developed pressure returned to approximately 80% of control values at the end of reperfusion. Because the heart rate was not affected in the groups mentioned above, rate-pressure product returned to 80 ± 8, 76 ± 9, and 67 ± 11% of untreated, treated, 100 and 300 μM nicorandil-treated hearts, respectively (Table 4).

At 1000 μM nicorandil, 9 of 10 hearts fibrillated during reperfusion. Here, the end-diastolic pressure remained elevated at extremely high levels, whereas heart rate, left ventricular developed pressure, and rate-pressure product did not recover.

Hearts treated with nitroglycerine showed a tendency for reduced recovery of left ventricular developed pres-

Table 4

End-Diastolic Pressure (EDP), Left Ventricular Developed Pressure (LVDP), and Rate-Pressure Product (RPP) at the End of Reperfusion

	EDP (mm Hg)	LVDP (mm Hg)	LVDP (% of control)	RPP (10^3 mm Hg/min)	pH
Untreated	23 ± 6	76 ± 7	72 ± 11	23.2 ± 2.3	7.13 ± 0.02
Nico 100	23 ± 4	82 ± 9	83 ± 8	26.5 ± 3.2	7.16 ± 0.00
Nico 300	21 ± 4	92 ± 6	88 ± 5	24.5 ± 4.3	7.14 ± 0.02
Nico 1000	12 ± 2	12 ± 11	13 ± 12*	3.7 ± 3.5*	7.15 ± 0.01
Gli 0	23 ± 5	49 ± 13	52 ± 13	15.1 ± 4.4	7.1 ± 0.03
Gli 100	45 ± 5	56 ± 14	58 ± 12	17.6 ± 4.8	7.13 ± 0.01
Gli 300	43 ± 3	29 ± 8	32 ± 10*	9.6 ± 2.8	7.09 ± 0.02
Gli 1000	43 ± 6	31 ± 10	37 ± 13*	9.8 ± 3.9	7.08 ± 0.03
Nitro 100	31 ± 4	58 ± 14	57 ± 14	14.2 ± 3.7	7.14 ± 0.02

*$p < 0.05$ vs. Untreated.

sure (57 ± 14% of control) than the untreated hearts (72 ± 11% of control) or 100 μM nicorandil-treated hearts (83 ± 8% of control). However, recovery of left ventricular developed pressure in 100 μM nicorandil + 1 μM glibenclamide-treated hearts (58 ± 12% of control) was similar to nitroglycerine-treated hearts. In hearts treated with 300 or 1000 μM glibenclamide, recovery of left ventricular developed pressure was reduced in comparison with untreated hearts (Table 4).

During reperfusion, phosphocreatine recovered quickly in all groups (Fig. 3a). In nicorandil-treated hearts there was an initial overshoot above control values leveling off at control concentrations, whereas glibenclamide-treated and nitroglycerine-treated hearts recovered to control values without initial overshoot. ATP recovered to ~50% of control value in all groups. Inorganic phosphate levels remained elevated above control levels (Fig. 3c). During reperfusion, the pH recovered in all groups to a mean value of 7.11 ± 0.01 with no differences among the groups (Table 4).

DISCUSSION

Effects of Nicorandil and Nitroglycerine on Coronary Flow

In all treated groups, an increase of coronary flow was observed as reported previously (19–23). This is due to relaxation of the vascular smooth muscle by nicorandil. To differentiate whether this effect was caused by the nitric oxide (NO) donor effect or the K^+ channel activation, we compared two sets of experiments with nitroglycerine or glibenclamide. Glibenclamide (1 μM) is an effective blocker of ATP-dependent K^+ channels (24) and is reported to be effective in experiments with cromakalim and pinacidil on smooth muscle cells (25–27). Our results (Fig. 2a) suggest two independent mechanisms for the vasodilatory effect of nicorandil. Stimulation of the guanylate cyclase by NO causes an increase of cGMP levels and hyperpolarization of the smooth muscle cell membrane (28). However, the nitro moiety causes stronger effects than the K^+-channel activation. As reported by Holzmann (6) and Kukovetz et al. (28), cGMP levels and relaxation of the vascular smooth muscle was enhanced by nicorandil compared with other nitro compounds. This suggests an additional relaxing effect of nicorandil, which is independent of the production of cGMP, as supported by the work of Shono et al. (29). A direct inhibition of the Ca^{2+} influx in the cell by nicorandil was discussed by Taira et al. (30), because an increased level of cytosolic cGMP (31) causes a decrease of cytosolic Ca^{2+} levels in smooth muscle (32,33). However, the effect of nicorandil on heart and vascular muscle (9,30), as well as the relaxation of rabbit coronary arteries (34), is different from primary Ca^{2+} channel antagonists. Kukovetz et al. (28) used methylene blue for the nonspecific inhibition of guanylate cyclase and cGMP formation by nitro compounds and antagonized the effects of nicorandil on cGMP up to a concentration of 47 μM. The relaxing effects of nicorandil were still 55% above control, which was shown to be induced by hyperpolarization.

In contrast to nitroglycerine, nicorandil causes dilation of large and small coronary vessels (20,35,36). Whereas nitroglycerine has high potential for dilatation of large vessels, its effect on small coronary resistance vessels is minimal (37). Thus, Hashimoto et al. (36) and Berdeaux et al. (20) classified the action of nicorandil on small vessels as response to hyperpolarization of the membranes, whereas large vessels are dilated by NO release.

Effects of Nicorandil with and without K^+-Channel Blockade by Glibenclamide and Nitroglycerine on Mechanical Function

Conflicting reports on positive inotropic effects of nicorandil in high concentrations in anesthetized dogs (38) and negative inotropic effect (39,40) due to shortening of the action potential are reported in the literature. Our data show an increase of mechanical performance during low (100 μM) and medium (300 μM) dose nicorandil treatment. Because in the constant pressure model mechanical performance is directly linked to coronary flow, we suggest that these changes are caused by a turgor effect. High dose (1000 μM) nicorandil, however, causes depression of mechanical performance (39) besides an increased coronary flow as reported previously. Therefore, reduced mechanical performance might be a result of the mild negative inotropic effect of nicorandil due to shortening of the action potential (22,41). A similar dose dependence was found for low versus high doses of cromakalim (42). Blockade of K^+ channels with glibenclamide under nicorandil treatment had no effect on the turgor effect. However, the depression of mechanical performance (left ventricular developed pressure and rate-pressure product) due to a negative inotropic effect of high dose nicorandil was prevented. During reperfusion, flow was comparable in nitroglycerine- and nicorandil- (100 μM) treated hearts. However, rate-pressure product was higher in nicorandil-treated hearts. This finding supports our suggestion that the action potential is shortened,

whereas the nitro effect does not change hyperpolarization in cardiac muscle. Kukovetz et al. (28) were able to show the same two effects of nicorandil, hyperpolarization, and cGMP stimulation in bovine coronary vascular smooth muscle. In our study, we found no effect of nicorandil on heart rate and end-diastolic pressure.

Effects of Nicorandil on ^{31}P Metabolism and Proton Accumulation

Concentrations of ^{31}P-NMR visible metabolites were equal in all groups during the control period. There was no change in ^{31}P metabolites when the buffer was switched from Krebs-Henseleit buffer to nicorandil or nicorandil + glibenclamide containing buffer. During ischemia, reduced depletion of ATP was found in hearts treated with 10^{-3} M Nico. In the nitroglycerine-treated hearts, there was no evidence for a reduced ATP depletion during ischemia. Thus, this effect cannot be caused by increased coronary flow before ischemia. It is most likely caused by K^+-channel activation. This mechanism is supported by the fact that ATP degradation was not reduced in hearts with blocked K^+ channels, for example, when treated with 10^{-3} M nicorandil + glibenclamide.

Low-dose nicorandil had no effect on ATP depletion. Our finding is in contrast to the work of Gross et al. (43), who reported that the loss of total adenine nucleotides was prevented by 7 μM nicorandil at 30-min reperfusion after 20 min of low-flow ischemia. Recently published work (44,45) in isolated cells suggests that low concentrations (10 or 100 μM) of nicorandil activate only the mitochondrial K^+ channels, whereas high concentrations (1000 μM) activate both mitochondrial and sarcolemmal K^+ channels. In contrast to isolated cells, in our model of total global ischemia the effects on mitochondrial or sarcolemmal K^+ channels cannot be distinguished. Thus, the reasons for these differences remain unclear at present.

Hearts treated with nicorandil at any concentration showed reduced proton accumulation during ischemia. Because this effect was not influenced by K^+-channel blockade with glibenclamide, we conclude that the reduction of H^+ accumulation is an effect of the nitro moiety of nicorandil.

Adverse Effects of Nicorandil During Ischemia-Reperfusion Injury

In hearts treated with high-dose nicorandil (10^{-3} M) ventricular fibrillation occurred during reperfusion. Because K^+-channel activators cause a shortening of the action potential *and* attenuate membrane depolarization (42,46), we speculate that the latter effect causes ventricular fibrillation. However, the concentration of nicorandil used in our study was four orders in magnitude higher than serum levels of nicorandil in patients. Therefore, the direct clinical relevance of this finding remains uncertain.

Mechanisms of Nicorandil During Ischemia-Reperfusion Injury

We conclude that the protective mechanisms of nicorandil during ischemia are related to the simultaneous action of both properties of the molecule. First, K^+-channel activation during ischemia prevents ATP depletion in hearts treated with high-dose (1000 μM) nicorandil. Second, NO release and increased pre-ischemic coronary flow might weaken the accumulation of protons—and thus the decrease of pH—during ischemia.

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