Salutary action of nicorandil, a new antianginal drug, on myocardial metabolism during ischemia and on postischemic function in a canine preparation of brief, repetitive coronary artery occlusions: comparison with isosorbide dinitrate

GALEN M. PIEPER, PH.D., AND GARRETT J. GROSS, PH.D.

ABSTRACT The effects of two antianginal drugs, nicorandil and isosorbide dinitrate (ISDN), on metabolism and function of the ischemic myocardium were studied in a preparation of multiple coronary occlusions in barbital-anesthetized dogs. The preparation consisted of three 5 min occlusions of the left anterior descending coronary artery interspersed by 30 min of reperfusion. An equihypotensive dose of nicorandil (7.5 μg/kg/min) or ISDN (12.5 μg/kg/min) was infused 15 min before and during the second occlusion period. Hemodynamics, myocardial segment shortening (%SS), tissue blood flow, and myocardial oxygen consumption were determined throughout. Uptake of free fatty acids (FFA), glucose, and lactate were determined during control and ischemic periods. At the end of the final 30 min reperfusion period, biopsy samples of transmural tissue were taken for analysis of phosphocreatine, adenine nucleotides, and total tissue water content. No major hemodynamic changes were produced by either drug except for a 5 to 10 mm Hg decrease in mean aortic pressure. Compared with untreated and ISDN-treated hearts, hearts of dogs treated with nicorandil exhibited reversal of a significant increase in FFA uptake during recurrent ischemia. This was accompanied by an attenuation of the increase in oxygen extraction and CO₂ production in the ischemic zone by nicorandil, but not by ISDN. Nicorandil, but not ISDN, improved %SS during reperfusion. Endocardial ATP and total adenine nucleotides were preserved in both nicorandil- and ISDN-treated hearts. Tissue edema was also attenuated by both compounds. Thus, nicorandil improved both function and metabolism during recurrent myocardial ischemia independent of a hemodynamic effect, whereas ISDN only attenuated the loss of adenine nucleotides and increase in tissue water.


REPETITIVE, brief episodes of myocardial ischemia are common during angina pectoris in patients with coronary artery disease. Intermittent cross-clamping or repeated, brief periods of coronary artery occlusion are also used during cardiac surgery. However, it is not clear from experimental studies whether recurrent ischemic episodes of brief duration have a cumulative effect on the development of regional postischemic myocardial dysfunction.¹–⁴

Nicorandil [SG-75; 2-nicotinamidoethyl-nitrate (es-

ter)] has been developed as a new antianginal drug with potent vasodilating properties. It has recently been shown to improve postischemic segment dysfunction in anesthetized⁵ and conscious⁶ dogs after a single 10 or 15 min coronary artery occlusion. The improvement in postischemic myocardial function after nicorandil may be partially related to a reduction in afterload, but the improved functional recovery in the presence of nicorandil is significantly greater than that after an equihypotensive dose of nifedipine.⁷ Thus suggests that the beneficial effects of nicorandil on the ischemic myocardium may be independent of peripheral hemodynamics and that other mechanisms must be involved in its action.

The purpose of this study was to determine whether there is a metabolic component of the action of nicorandil independent of its hemodynamic effect that
might explain its salient effect on postischemic myocardial function. Thus, we purposely chose a low dose of
nicorandil that would not significantly alter the rate-pressure product or myocardial oxygen consumption to
determine whether the recovery of contractile function was enhanced after reperfusion. The experimental
preparation of repeated, short periods of coronary artery occlusion was chosen for these studies to mimic
the recurrent ischemia of anginal attacks observed clinically. Isosorbide dinitrate was chosen for purposes of
comparison because this compound has a similar hemodynamic profile and plasma half-life to those of
nicorandil.

Methods

Surgical preparation and functional analysis. Twenty-four adult mongrel dogs (15 to 25 kg) of both sexes were used in this
study. Animals were anesthetized with an intravenous combination of 15 mg/kg sodium pentobarbital and 200 mg/kg barbitral sodium. Dogs were ventilated with a Harvard respirator (tidal volume of 15 ml/kg and 10 to 15 respirations per min) and supplemented with 100% oxygen. Atelectasis was prevented by
maintaining a trap with an end-expiratory pressure of 5 to 7 cm of water. Body temperature was maintained at 38°C with a
heating pad. Blood gases were monitored (ABL-2) at various times throughout the experiment and kept within the normal
physiologic range.

Mean arterial and left ventricular pressures were monitored by
inserting a double pressure transducer–tipped catheter (Millar
PC 771) into the aorta and left ventricle via the left carotid artery.
Left ventricular dP/dt was determined by electronic differentiation
of the left ventricular pressure pulse. The right femoral vein
was cannulated for the administration of drugs.

A left thoracotomy was performed at the fifth intercostal
space, the pericardium was incised, and the heart was suspended in
a cradle. A portion of the left anterior descending coronary
artery (LAD) was isolated and an electromagnetic flow probe (Statham SP 7515) was placed around the vessel. LAD coronary blood flow was measured with a flowmeter (Statham 2202).
Distal to the flow probe, a micrometer-driven mechanical
occluder was placed to produce a total occlusion of the LAD.
The electrocardiogram was monitored using limb lead II and a
tachograph was used for a continuous measurement of heart rate.
All measurements were monitored on a Grass Model 7 poly
graph. A cannula was positioned via the left external jugular vein
in the local anterior intraventricular coronary vein, which
drained the ischemic LAD area to obtain blood samples (figure 1).
The cannula tip always lay just distal to the coronary occluder to
ensure sampling primarily from the ischemic-reperfused area.
Roberts et al.8 and others9 have previously shown that blood
sampled by this technique is almost exclusively (95%) draining
the LAD perfusion bed. In three separate dogs, an epicardial
venous catheter was placed directly into the area draining the
ischemic-reperfused region and duplicate blood gas samples
were obtained before and during coronary artery occlusion.
Blood gas values and myocardial oxygen extraction (%O2E) and
oxygen consumption (MVO2) were not different when obtained
by the epicardial stick method or by the coronary venous cannula
(preocclusion %O2E = 57.3 ± 3.7 vs 54.0 ± 3.0 and MVO2 =
10.2 ± 0.7 vs 10.0 ± 1.3 ml/min/g, respectively; occlusion
%O2E = 69.5 ± 9.7 vs 64.0 ± 2.7 and MVO2 = 2.8 ± 0.7
vs 2.6 ± 0.7 ml/min/g, respectively). Both aortic and local
coronary venous blood samples were withdrawn for substrate
analysis and determination of blood gases and oxygen content.
Blood gases, % oxygen saturation (%O2sat) and hemoglobin
(Hb) values were measured with a Radiometer ABL 2 blood gas
analyzer. Oxygen content (vol%) in arterial and coronary venous
blood samples was determined by multiplying 1.34 × Hb × %O2sat + 0.003 × PO2. %O2E was calculated as:

\[
%O_2E = \frac{AO_2 - CVO_2}{AO_2} \times 100
\]

and MVO2 (in ml/min/100 g) as: \(AO_2 - CVO_2\) × coronary
blood flow (ml/min/100 g), where \(AO_2\) is arterial oxygen
content and \(CVO_2\) is coronary venous \(O_2\).

Myocardial segment function (% segment shortening, %SS)
was measured in the regions perfused by the left anterior
descending and left circumflex coronary arteries with the use of
two sets of piezoelectric crystals. The crystals were inserted into
the myocardium (approximately 10 to 15 mm apart and 7 to 9
mm deep) parallel to subendocardial fiber orientation.7 The leads
of each crystal were connected to an ultrasonic amplifier that
transformed the sound pulse transmitted between the two crystals
into an electrical signal proportional to the distance between the
crystals. The crystals were recalibrated and the tracings
were monitored with an oscilloscope (Soltec 520). By recording
changes in transmission time, the distance between the two
crystals was measured. With left ventricular dP/dt, systolic
length (SL) was determined at maximal negative dP/dt and
diastolic length (DL) was determined just before the onset of systole. %SS was calculated as:

\[
%SS = \frac{DL - SL}{DL} \times 100
\]

At the completion of each experiment, the depth of each crystal
was verified. The %SS data were normalized by use of a value of
10.0 for the control diastolic length.

Myocardial blood flow. Myocardial blood flow was determined
by the radioactive microsphere technique. The left atrial
appendage was cannulated for the administration of micro-

FIGURE 1. Diagram of experimental preparation. Blood samples were
taken from the local coronary vein for the measurement of blood gases,
free fatty acids, glucose, and lactate. Biopsy samples of transmural tissue
were obtained at the end of the third 30-min reperfusion period for the
determination of phosphocreatine, total adenine nucleotides, and tissue
water content. Radioactive microspheres were used to measure coronary
collateral blood flow and piezoelectric crystals to determine %SS by
sonomicrometry. Note that the coronary vein catheter is actually advanced
to a position well within the shaded, ischemic region.

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spheres while the right femoral artery was cannulated for the withdrawal of a reference blood flow sample. Carbonized plastic microspheres (15 ± 3 μm diameter) labeled with 141Ce, 51Cr, 105Ru, or 85Sr were suspended in isotonic saline with 0.01% Tween 80 added to prevent aggregation. The radioactive microspheres were ultrasonicated for 5 min followed by 5 min of vortexing. Approximately 2 to 4 × 10^6 spheres were injected into the left atrium followed by a 0.1 ml saline wash. Just before sphere administration, a reference blood flow sample was withdrawn from the femoral artery at a constant rate of 6.5 ml/min for 3 min.

At the completion of each experiment, India Ink was injected into the LAD to delineate the perfusion area. The heart was removed and stored overnight in 10% formalin. The heart was sectioned into subepicardium, midmyocardium, and subendocardium of the normal (three pieces) and ischemic regions (five pieces) and the tissue samples were weighed. All samples were counted (Searle Analytic 1195) to determine the activity of each isotope in each sample. The activity of each isotope was also determined in the reference blood flow samples. Myocardial blood flow was calculated by a preprogrammed computer (Apple IIe) to obtain the true activity of each isotope in individual samples and tissue blood flow was determined with the equation:

\[ Q_m = Q_r \cdot C_m/C_r \]

where \( Q_m \) = myocardial blood flow (ml/min/g tissue); \( Q_r \) = rate of withdrawal of the reference blood flow sample (6.5 ml/min); \( C_r \) = activity of reference blood flow sample (cpm); \( C_m \) = activity of tissue sample (cpm/g). Transmural blood flow was calculated as the weighted average of the three layers in each region. Myocardial blood flow distribution (endocardial-epicardial ratio, endo/epi) was calculated by dividing the average subendocardial flow of each region by the average subepicardial flow.

**Blood chemical analysis.** Arterial and local coronary venous blood samples were collected into ice-chilled tubes containing sodium fluoride (to prevent glucose metabolism in vitro) and potassium oxalate (anticoagulant). A 0.5 ml aliquot of the collected blood was immediately added to 1.0 ml of ice-cold, 6% perchloric acid for deproteination. Samples were then centrifuged and enzymatically analyzed within 3 hr for blood lactate (Sigma Diagnostics) with the use of a Gilford 250 recording spectrophotometer.

The remaining blood was centrifuged to obtain plasma for both glucose and free fatty acid (FFA) determinations. Twenty microliter aliquots of plasma were taken and analyzed enzymatically for glucose (Sigma Diagnostics) within 3 hr after termination of the experiment. Plasma FFA was extracted by adding 100 μl of plasma to 6.0 ml of chloroform/heptane/methanol (200/150/7 volumes) in tightly capped tubes containing 100 mesh-activated silicic acid. Plasma FFA was extracted by the method of Laurell and Tibbling10 and stored at −20°C for analysis at a later date. FFA was analyzed in triplicate by the analysis outlined by Hron and Menahan.11 Plasma FFA and glucose concentrations were corrected to whole blood and multiplied by regional myocardial blood flow. The arterial-local coronary venous concentration difference was multiplied by the regional ischemic myocardial blood flow in milliliters per minute per 100 g to determine substrate uptake in the ischemic-reperfused zone. Since it was not possible to obtain tissue blood flow measurements at all periods, the uptakes in control periods 2, 3, and 4 were estimated by the proportional relationship of LAD blood flow determined by the electromagnetic flow probe to that assessed by the microsphere method during the control period 1.

**Experimental design.** The experimental design consisted of an initial control period followed by three consecutive 5 min coronary artery occlusions with 30 min of reperfusion allowed after each occlusion period. Hemodynamics, %SS, MVO, and the uptake of various substrates were measured 5 min before each occlusion (control 1, control 2, and control 3), during each occlusion (occlusion 1, occlusion 2, and occlusion 3), and 25 min after occlusion 3 (control 4). Regional myocardial blood flow (radioactive microspheres) was measured 5 min before occlusion 1 and during each occlusion period. Saline (control series), nicorandil (7.5 μg/kg/min), or isosorbide dinitrate (ISDN) (12.5 μg/kg/min) was infused via a cannula in the right femoral vein for 15 min before and during the second 5 min coronary occlusion (total infusion time = 20 min). These doses of nicorandil and ISDN produced only 5 to 10 mm Hg decreases in systemic arterial blood pressure. The plasma half-life of nicorandil12 and ISDN13 in dogs is 42 and 39 min, respectively.

**Tissue biopsy and analysis.** At 30 min after release of the third coronary occlusion, simultaneous transmural drill biopsy samples were obtained from reperfused and nonischemic zones of the left ventricle and frozen between liquid nitrogen-chilled aluminum blocks.14 Frozen tissue biopsy samples were cleaned of blood residue and subdivided into epicardial, midmyocardial, and endocardial layers. Neutralized, perchloric acid extracts of tissues were analyzed for ATP, ADP, AMP, and phosphocreatine on a Gilford 250 spectrophotometer with the use of coupled enzymatic assays.15-17

The energy charge potential of the adenylate pool was calculated with the formula:

\[ \frac{\text{ATP} + 0.5 \text{ADP}}{\text{ATP} + \text{ADP} + \text{AMP}} \]

Additional portions of frozen tissue were weighed and dried overnight at 95°C to determine tissue water content and to convert tissue metabolite concentrations to units of micromoles per gram dry weight. All values are given as the mean ± SE (n = 8, each group). Differences between group means were compared by a two-way analysis of variance and Fisher’s least significant difference. Values are compared with the preocclusion control by one-way analysis of variance for repeated measures or Dunnett’s t test. Paired and unpaired t tests were used to compare two means within the same animal and between animals, respectively. Means were considered significantly different if p < .05.

**Results**

**Blood gases and MVO.** During each of the three occlusion periods in untreated hearts, MVO and coronary venous pH and oxygen content decreased while coronary venous PCO and %O2E increased significantly (p < .05) compared with the preocclusion control (table 1). %O2E was higher during the second occlusion period as compared with the first occlusion period. The calculated net changes in these variables for all experimental groups are listed in figure 2. The administration of nicorandil, but not ISDN, significantly attenuated or abolished the decrease in coronary venous pH and O2 content and the increase in %O2E and venous PCO during occlusion 2 (figure 2).

**Hemodynamics and blood flow.** There were no major changes in heart rate, mean aortic pressure, left ventricular systolic and end-diastolic pressure, or left ventricular maximum dP/dt between the three experimen-
TABLE 1
Oxygen extraction and coronary venous blood gas indexes in untreated hearts

<table>
<thead>
<tr>
<th></th>
<th>CVpH (units)</th>
<th>CVPCO₂ (mm Hg)</th>
<th>CVO₂ (vol %)</th>
<th>( \frac{O₂ \text{ extraction}}{(A-CV) × 100} )</th>
<th>MVO₂ (ml/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control₁</td>
<td>7.37 ± 0.02</td>
<td>40 ± 2</td>
<td>9.5 ± 0.7</td>
<td>54.0 ± 3.2</td>
<td>10.0 ± 1.3</td>
</tr>
<tr>
<td>Occlusion₁</td>
<td>7.30 ± 0.03</td>
<td>45 ± 3</td>
<td>8.6 ± 0.6</td>
<td>57.7 ± 2.9(^a)</td>
<td>2.0 ± 0.7(^a)</td>
</tr>
<tr>
<td>Control₂</td>
<td>7.35 ± 0.02</td>
<td>40 ± 2</td>
<td>9.4 ± 0.6</td>
<td>55.3 ± 3.7</td>
<td>11.7 ± 2.6</td>
</tr>
<tr>
<td>Occlusion₂</td>
<td>7.31 ± 0.03</td>
<td>44 ± 3</td>
<td>7.5 ± 0.4</td>
<td>64.0 ± 2.7(^a)</td>
<td>2.6 ± 0.7(^a)</td>
</tr>
<tr>
<td>Control₃</td>
<td>7.33 ± 0.02</td>
<td>40 ± 2</td>
<td>8.4 ± 0.7</td>
<td>58.7 ± 4.1</td>
<td>11.4 ± 1.0</td>
</tr>
<tr>
<td>Occlusion₃</td>
<td>7.30 ± 0.03</td>
<td>43 ± 3</td>
<td>7.5 ± 0.6</td>
<td>64.0 ± 3.5(^a)</td>
<td>2.0 ± 0.4(^a)</td>
</tr>
<tr>
<td>Control₄</td>
<td>7.33 ± 0.02</td>
<td>40 ± 2</td>
<td>9.2 ± 0.7</td>
<td>56.8 ± 3.2</td>
<td>10.1 ± 1.2</td>
</tr>
</tbody>
</table>

All values are mean ± SEM (n = 8).
A = arterial. CV = local coronary venous.
\(^a\)Significantly different from respective preocclusion control value (p < .05). All control measurements were taken 5 min before the respective occlusion period. Control measurements 2, 3, and 4 were taken 25 min after reperfusion.

tal groups as a result of coronary artery occlusion. Both nicorandil and ISDN produced modest decreases in mean aortic pressure that were not significantly different from the baseline values for each group (table 2). Mean aortic pressures in the two drug-treated groups were slightly, but significantly (p < .05), lower than those observed in control animals. The doses of nitrates chosen did not produce any changes in left ventricular pressures, dP/dt, heart rate, rate-pressure product, or MVO₂ (table 2).

There were no significant changes in mean coronary artery (LAD) blood flow in the hearts of control or nicorandil-treated dogs during the control period. In contrast, coronary blood flow decreased in hearts of dogs treated with ISDN between control 1 and 2 (31 ± 4 to 23 ± 2 ml/min, p < .05).

During each occlusion period, the rate-pressure product was equivalent and did not vary between groups of animals (figure 3). MVO₂ tended to be higher during the second occlusion period in control animals compared with that in drug-treated animals, but this difference was not statistically significant. There was

FIGURE 2. Changes in local coronary venous pH, PCO₂, O₂ content, %O₂E between control period 2 and occlusion 2 in control (C), nicorandil (NC), and ISDN-treated dogs. All values are the mean ± SEM (n = 8, each group). Nicorandil attenuated the decrease in coronary venous pH and O₂ content and the increase in PCO₂ and %O₂E produced by coronary occlusion.
TABLE 2

<table>
<thead>
<tr>
<th>Hemodynamic variable before occlusion period 1 or before occlusion period 2 in the presence of nicorandil or ISDN</th>
<th>Untreated</th>
<th>Nicorandil</th>
<th>ISDN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
<td>96 ± 7</td>
<td>95 ± 4</td>
<td>96 ± 6</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>109 ± 7</td>
<td>107 ± 5</td>
<td>106 ± 6</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Heart rate (min⁻¹)</td>
<td>140 ± 8</td>
<td>142 ± 6</td>
<td>155 ± 6</td>
</tr>
<tr>
<td>Rate pressure product (mm Hg min⁻¹ × 10⁴)</td>
<td>1.57 ± 0.17</td>
<td>1.52 ± 0.11</td>
<td>1.65 ± 0.14</td>
</tr>
<tr>
<td>Maximum dP/dt (mm Hg sec⁻¹)</td>
<td>2400 ± 200</td>
<td>2200 ± 180</td>
<td>2460 ± 215</td>
</tr>
<tr>
<td>MVO₂ (ml/min⁻¹/100 g⁻¹)</td>
<td>10.0 ± 1.3</td>
<td>8.1 ± 0.8</td>
<td>7.5 ± 0.8</td>
</tr>
<tr>
<td>CBF (ml/min⁻¹)</td>
<td>31 ± 6</td>
<td>31 ± 5</td>
<td>31 ± 4</td>
</tr>
</tbody>
</table>

All values are the mean ± SEM of eight dogs in each group.

LVSP and LVEDP = left ventricular systolic and end-diastolic pressures; CBF = left anterior descending coronary blood flow.

*Significantly different from the untreated group (p < .05).

no change in MVO₂ during subsequent occlusion periods in drug-treated animals. In addition, collateral blood flow did not change in subsequent occlusion periods and was not altered by the presence of drug.

Regional myocardial blood flow. Tissue blood flow data during preocclusion (control) and during each 5 min occlusion period (occlusions 1, 2, and 3) were determined by the microsphere technique. No significant changes were observed in the nonischemic (left circumflex–perfused) region throughout the experimental period in control or nicorandil-treated hearts (data not shown). However, in the ISDN group, transmural myocardial blood flow in the nonischemic area decreased from a baseline of 0.93 ± 0.08 ml/min/g in the first occlusion period to 0.68 ± 0.06 and 0.73 ± 0.08 ml/min/g in the second and third occlusion periods. The endo/epi blood flow ratio was 1.28 ± 0.4 and 1.11 ± 0.08, respectively. In the ischemic region, blood flow decreased markedly and to a similar extent during all three occlusion periods in the three groups (figure 3).

![Figure 3](http://circ.ahajournals.org/)
of ischemia. These occlusions resulted in figures and during the three 30 min occlusion periods (figure 4).

During the first 30 min reperfusion period in the absence of drugs, the recovery of %SS in the three groups was similar (figure 4). Immediately after drug treatment during the second 30 min reperfusion period, %SS in nicorandil-treated dogs recovered 100% as compared with the initial control 1 value, whereas %SS in the saline and ISDN-treated dogs was significantly decreased (p < .05) compared with their respective control 1 values (figure 4). When the %SS values during the second 30 min reperfusion period were compared with their respective control 2 values, %SS in nicorandil-treated dogs recovered to a greater extent than those in the saline and ISDN-treated animals and %SS in the ISDN group was significantly increased with that in the control group (figure 5). At the end of the third 30 min reperfusion period, functional recovery in all three groups was depressed and %SS was approximately 30% to 40% less than the original control 1.

In addition, the left ventricular weights, ischemic area weights, and the percent area at risk for ischemia were similar in all groups (left ventricular weights: 100.8 ± 9.8, 96.5 ± 6.8, and 96.1 ± 5.8 g; ischemic area weights: 31.0 ± 5.7, 30.3 ± 3.4, and 27.4 ± 1.5 g; % area at risk: 29.8 ± 3.2%, 31.2 ± 2.4%, and 29.2 ± 2.2% for control, nicorandil, and ISDN groups, respectively). These data indicate that during coronary occlusion the three groups were subjected to similar degrees of ischemia.

Myocardial segment function. %SS data at 5 min before each occlusion period (controls 1, 2, and 3), at the end of 30 min of reperfusion after occlusion 3 (control 4), and during occlusion in the various groups are shown in figures 4 and 5. No significant changes in %SS occurred in the nonischemic (left circumflex-perfused) area throughout the experiment in any of the three groups (data not shown). In the ischemic-reperfused (LAD-perfused) region, each coronary occlusion resulted in a reduction in %SS to negative values in all groups, which is indicative of passive systolic lengthening. There were no significant differences between groups during the occlusion periods (figure 4).

FIGURE 4. %SS (expressed as a percent of control period 1 = 100%) during the three 5 min occlusion periods and at various times after reperfusion in the control, nicorandil, and ISDN-treated dogs. All values are the mean ± SEM (n = 8, each group).

FIGURE 5. %SS (expressed at a percent of control period 2) in control, nicorandil, and ISDN-treated dogs. All values are the mean ± SEM (n = 8, each group).
TABLE 3
Effects of repeated ischemic-reperfusion episodes on plasma glucose extraction by the myocardium

<table>
<thead>
<tr>
<th></th>
<th>Control₁</th>
<th>Control₂</th>
<th>Control₃</th>
<th>Control₄</th>
<th>Control₁</th>
<th>Control₂</th>
<th>Control₃</th>
<th>Control₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated series</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-CV [glucose]</td>
<td>0.18 ± 0.06</td>
<td>0.18 ± 0.06</td>
<td>0.42 ± 0.11</td>
<td>0.43 ± 0.13</td>
<td>0.35 ± 0.07</td>
<td>0.24 ± 0.06</td>
<td>0.28 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Glucose uptake</td>
<td>5.88 ± 2.84</td>
<td>1.57 ± 0.96</td>
<td>14.65 ± 5.21</td>
<td>2.63 ± 0.73</td>
<td>15.54 ± 4.56</td>
<td>0.88 ± 0.67</td>
<td>14.42 ± 5.60</td>
<td></td>
</tr>
<tr>
<td>Nicorandil series</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-CV [glucose]</td>
<td>0.16 ± 0.06</td>
<td>0.28 ± 0.11</td>
<td>0.37 ± 0.07</td>
<td>0.34 ± 0.07</td>
<td>0.28 ± 0.06</td>
<td>0.33 ± 0.09</td>
<td>0.30 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Glucose uptake</td>
<td>7.32 ± 2.25</td>
<td>3.68 ± 1.87</td>
<td>28.17 ± 11.08</td>
<td>3.11 ± 1.17</td>
<td>12.86 ± 2.26</td>
<td>3.66 ± 1.50</td>
<td>13.90 ± 4.22</td>
<td></td>
</tr>
<tr>
<td>ISDN series</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-CV [glucose]</td>
<td>0.20 ± 0.08</td>
<td>0.39 ± 0.10</td>
<td>0.35 ± 0.07</td>
<td>0.37 ± 0.12</td>
<td>0.42 ± 0.11</td>
<td>0.43 ± 0.15</td>
<td>0.62 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>Glucose uptake</td>
<td>11.09 ± 3.67</td>
<td>3.74 ± 1.19</td>
<td>13.41 ± 1.97</td>
<td>3.92 ± 1.33</td>
<td>18.72 ± 5.24</td>
<td>4.62 ± 1.78</td>
<td>26.27 ± 7.17</td>
<td></td>
</tr>
</tbody>
</table>

Each point represents the mean of eight animals ± SEM. Glucose concentrations were corrected to whole blood for determination of glucose uptake. Since microspheres were not used for tissue blood flow determinations at control₁, control₂, and control₄, uptake at these times was calculated with tissue blood flow estimates based on LAD blood flow measurements by the electromagnetic flowmeter.

Abbreviations are as in table 1.

a Differences compared with control₁ and occlusion₁.

b Differences between control and occlusion values.

%SS in the nicorandil-treated dogs was somewhat greater than in the saline and ISDN group, but this difference was not significant (p < .1).

Myocardial glucose extraction. Arterial and coronary venous glucose concentrations were unaltered by the repetitive ischemic-reperfusion protocol (not shown). In contrast, the arterial–coronary venous) glucose concentration difference and glucose uptake (table 3) was stimulated after the first ischemic and reperfusion episode (control 2). The alteration in the pattern of glucose extraction was attenuated in the presence of nicorandil or ISDN.

Myocardial free fatty acid (FFA) extraction. Arterial and coronary venous FFA concentrations were unaltered by multiple ischemic-reperfusion episodes (data not shown). The arterial–coronary venous FFA concentrations and the FFA uptake during each period are shown in table 4. Baseline FFA uptake values were not significantly different between experimental groups before occlusion period 1 based on analysis of variance (F ratio = 1.96, df = 2 and 19, p > .1). It was observed that there was a progressive increase in FFA arterial–coronary venous differences and FFA uptake in subsequent control periods in untreated dogs (FFA uptake: 6.65 ± 1.85, 13.99 ± 3.02, 14.24 ± 3.59, and 18.55 ± 5.04 μmoles/min/100 g, respectively). In contrast, nicorandil produced a progressive decrease in FFA uptake during control periods (13.35 ± 5.02, 10.26 ± 1.94, 6.64 ± 1.23, and 7.77 ± 0.84 μmoles/min/100 g, respectively). This pattern was significantly different than that observed in untreated hearts. Changes in FFA uptake during control periods in animals treated with ISDN were similar to those observed in untreated animals (5.43 ± 1.08, 7.19 ± 1.62, 11.02 ± 4.14, and 14.84 ± 5.67 μmoles/min/100 g, respectively). Thus, nicorandil, unlike ISDN, attenuated or reversed the progressive increase in FFA uptake after recurrent ischemia.

While the uptake of FFA was reduced during each period of ischemia in all three groups compared with the uptake in the respective control period, that in untreated hearts during both the second control and second ischemic period was increased relative to that during the first control and ischemic period. During ischemic periods in untreated hearts, the uptake of FFA was twice as high in the second as in the first ischemic period (figure 6). Nicorandil, but not ISDN, reversed this increase. When the net change in FFA uptake was calculated, there was an increase in FFA uptake in both the ischemic periods (2 and 3) that followed in untreated animals. This was not observed in the ISDN-treated group. In contrast, a net decrease in FFA uptake was observed in both ischemic periods 2 and 3 in the nicorandil-treated group (figure 6).

Myocardial lactate extraction. Uptake of lactate was reduced in all experimental groups during all three ischemic periods (table 5). There was no major alterations in the arterial and coronary venous concentra-
TABLE 4
Effects of repeated ischemic-reperfusion episodes on plasma FFA extraction by the myocardium

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Occlusion</th>
<th>Control</th>
<th>Occlusion</th>
<th>Control</th>
<th>Occlusion</th>
<th>Control</th>
<th>Occlusion</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated series</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>A-CV [FFA] (nmol·ml⁻¹ plasma)</td>
<td>137 ± 16</td>
<td>141 ± 44</td>
<td>281 ± 84</td>
<td>235 ± 32</td>
<td>255 ± 54</td>
<td>166 ± 54</td>
<td>149 ± 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFA uptake (μmol·min⁻¹·100 g⁻¹)</td>
<td>6.65 ± 1.85</td>
<td>1.03 ± 0.32</td>
<td>13.99 ± 3.02</td>
<td>2.26 ± 59</td>
<td>14.24 ± 3.59</td>
<td>1.41 ± 0.71</td>
<td>18.55 ± 5.04</td>
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<td></td>
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<tr>
<td>Nicorandil series</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-CV [FFA] (nmol·ml⁻¹ plasma)</td>
<td>203 ± 78</td>
<td>150 ± 44</td>
<td>148 ± 30</td>
<td>132 ± 45</td>
<td>138 ± 14</td>
<td>167 ± 18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFA uptake (μmol·min⁻¹·100 g⁻¹)</td>
<td>13.35 ± 5.02</td>
<td>2.14 ± 0.85</td>
<td>10.26 ± 1.94</td>
<td>1.40 ± 0.63</td>
<td>6.64 ± 1.23</td>
<td>1.53 ± 0.47</td>
<td>7.77 ± 0.84</td>
<td></td>
<td></td>
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<tr>
<td>ISDN series</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>A-CV [FFA] (nmol·ml⁻¹)</td>
<td>95 ± 20</td>
<td>134 ± 29</td>
<td>151 ± 19</td>
<td>179 ± 26</td>
<td>207 ± 58</td>
<td>279 ± 57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFA uptake (μmol·min⁻¹·100 g⁻¹)</td>
<td>5.43 ± 1.08</td>
<td>1.59 ± 0.55</td>
<td>7.19 ± 1.62</td>
<td>1.92 ± 0.74</td>
<td>2.17 ± 0.90</td>
<td>14.84 ± 5.67</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each point represents the mean of eight animals ± SEM. FFA concentrations were corrected to whole blood for determination of FFA uptake. For other details see table 3.

Abbreviations are as in table 1.

* Differences compared with control, and occlusion₁.

⁺ Differences between control and occlusion values.

Tensions of lactate in any of the groups. Generally, there was an increase in lactate concentration in coronary venous samples during the ischemic periods (baseline, 1.19 ± 0.26; occlusion 1, 1.70 ± 0.44; occlusion 2, 1.63 ± 0.38; occlusion 3, 1.75 ± 0.39 mmol/liter). Neither nicorandil nor ISDN had any significant effect on lactate metabolism, as determined by comparison with the control group.

Myocardial tissue energy metabolism and water content. Repetitive, short periods of myocardial ischemia resulted in a rebound in myocardial phosphocreatine concentration (table 6). There was no statistically sig-
TABLE 5
Effects of repeated ischemic-reperfusion episodes on blood lactate extraction by the myocardium

<table>
<thead>
<tr>
<th></th>
<th>Untreated series</th>
<th>Nicorandil series</th>
<th>ISDN series</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Occlusion</td>
<td>Control</td>
</tr>
<tr>
<td>A-CV [lactate] (mmoles/liter)</td>
<td>0.30 ± 0.27</td>
<td>0.14 ± 0.43</td>
<td>0.63 ± 0.20</td>
</tr>
<tr>
<td>Lactate uptake (µmole·min⁻¹·100 g⁻¹)</td>
<td>59.06 ± 13.98</td>
<td>5.06 ± 5.68A</td>
<td>59.96 ± 17.72</td>
</tr>
</tbody>
</table>

Each point represents the mean ± SEM. For other details, see table 3.
Abbreviations are as in table 1.
 Differences between control and occlusion values.

significant reduction in ATP or the various adenine nucleotide fractions. However, the absolute concentrations of both ATP and total adenine nucleotides tended to be lower in the endocardial layer of the reperfused left ventricle, especially when normalized to the nonischemic region within the same heart (figure 7). This reduction in endocardial ATP and total adenine nucleotides was prevented by the administration of either nicorandil or ISDN. There was no significant difference in the energy (adenylate) charge potential between layers of nonischemic and reperfused areas. The values in the endocardial layers of the reperfused area were 0.911 ± 0.006 (untreated), 0.910 ± 0.003 (nicorandil), and 0.906 ± 0.005 (ISDN).

An increase in tissue water was also evident in the reperfused region of untreated hearts (figure 8). Both nicorandil and ISDN attenuated the tissue edema in the reperfused region after repetitive ischemic-reperfusion episodes.

Discussion
The observations in this study suggest that nicorandil may be an effective therapeutic agent in the treatment of recurrent myocardial ischemia. The salient effect of

TABLE 6
Effect of multiple, short ischemic-reperfusion episodes on transmural myocardial energy metabolism

<table>
<thead>
<tr>
<th></th>
<th>Nonischemic zone</th>
<th>Reperfused zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epi</td>
<td>Mid</td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>40.77±6.71</td>
<td>39.25±5.15</td>
</tr>
<tr>
<td>ATP</td>
<td>24.67±1.32</td>
<td>26.14±1.07</td>
</tr>
<tr>
<td>Total adenine nucleotides</td>
<td>29.08±1.57</td>
<td>30.88±1.55</td>
</tr>
<tr>
<td>Nicorandil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>35.72±3.79</td>
<td>33.86±3.48</td>
</tr>
<tr>
<td>ATP</td>
<td>23.65±0.92</td>
<td>25.25±1.05</td>
</tr>
<tr>
<td>Total adenine nucleotides</td>
<td>28.43±1.17</td>
<td>30.26±1.38</td>
</tr>
<tr>
<td>ISDN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>36.57±3.13</td>
<td>34.87±3.90</td>
</tr>
<tr>
<td>ATP</td>
<td>23.04±1.36</td>
<td>23.31±1.24</td>
</tr>
<tr>
<td>Total adenine nucleotides</td>
<td>27.67±1.46</td>
<td>28.37±1.19</td>
</tr>
</tbody>
</table>

Each point represents the mean ± SEM of seven or eight hearts expressed in units of µmole per g dry weight.
 Significantly different from corresponding layer in nonischemic zone.
Nicorandil has been of ischemic postischemic on reperfusion period, postischemic in previously adenine to be related, unlike the effects this is more physiological significance since the concentration of nicorandil (2 mM) was much higher than that expected in our preparation. The mechanism for functional improvement by nicorandil is believed to be related to a metabolic component of its action. Nicorandil, but not ISDN, prevented or attenuated the increase in myocardial FFA uptake and %O₂E within the ischemic zone during a repeated episode of ischemia.

**Substrate utilization in the ischemic zone.** It is commonly believed that enhanced FFA uptake is detrimental during ischemia and agents that reduce FFA uptake or oxidation during ischemia are beneficial. The reduction in FFA uptake in ischemic regions of nicorandil-treated hearts is assumed to result in a concomitant reduction in oxidation of FFA. Although the latter was not directly measured, the observation that nicorandil attenuated the increase in %O₂E and coronary venous CO₂ production would be consistent with a reduction in FFA oxidation. The corresponding absence of attenuation of the changes in FFA uptake, %O₂E, and CO₂ production by ISDN could explain why this agent was not as effective as nicorandil in preventing postischemic dysfunction. Since increased FFA oxidation contributes to a relative
“oxygen wasting” phenomenon, it is possible that this could also explain why nicorandil prevented the decline in adenine nucleotides after multiple ischemic episodes. However, this does not appear to explain why ISDN preserved adenine nucleotides. It is possible that the preservation of adenine nucleotides by these two agents may be either unrelated to changes in substrate uptake during ischemia or arise from different mechanisms than those for improved functional recovery.

**Relationship to previous studies.** In an isolated report on a preparation of recurrent ischemia, Korb et al. reported that nicorandil might have a detrimental effect during myocardial ischemia. They showed that nicorandil increased FFA metabolism and oxygen debt during a 3 min occlusion interval. The present study does not confirm these findings. Instead, our study suggests the opposite conclusion. There are some probable reasons for these discrepancies.

Korb et al. used coronary sinus blood for venous substrate and blood gas concentrations as well as for blood flow determinations. In contrast, we collected blood for venous substrates and blood gases from the local vein draining the ischemic bed. The local vein samples should be more representative of the ischemic area and not greatly contaminated from adjacent nonischemic sources, which may dilute blood samples obtained from the coronary sinus. Furthermore, we made our blood flow measurements in the ischemic area by the radioactive microsphere method. Again, this would be a more precise measure of ischemic myocardial blood flow than that obtained from measurements of coronary sinus blood flow. The flow value obtained for the determination of coronary sinus blood flow may have been artificially high due to the contribution of blood flow from adjacent nonischemic areas, which may contribute significantly to total coronary sinus blood flow. Thus, any calculations of substrate uptake based on measures of coronary sinus blood flow compound the error in measurement of coronary sinus substrate concentration and lead to erroneous conclusions. Finally, in their preparation of repetitive occlusion Korb et al. used ischemia of different durations (3 min vs 5 min). It is not known whether this time differential is of significance. However, it is likely that Korb et al. could not differentiate a priming effect of repetitive ischemia because they combined their data from two to three control occlusions or two to three drug-treated occlusions within the same dog. The averaging of substrate utilization from multiple periods of occlusion or reperfusion within the same animal may not be justified based on our results.

**Limitations and uniqueness of the preparation.** The preparation of recurrent ischemia chosen for these studies was created by ischemic episodes of short duration. While most experimental protocols consist of ischemic durations of 10 min or longer, shorter periods may be relevant to certain clinical situations. The brevity of this ischemic interval may produce events that are not entirely characteristic of prolonged ischemia. It is likely that metabolic events arising during early myocardial ischemia (less than 5 min) are not similar to those occurring during ischemia of longer duration (more than 5 min). Thus, there is a progression of metabolic events that evolve from the transition from the nonischemic to the overtly ischemic myocardium.

It should be emphasized that our preparation is more representative of the initial transition phase of acute myocardial ischemia. One manifestation of this phase is that net lactate production by the heart has not yet commenced, although coronary venous blood lactate concentration may be increased. Since we collected local coronary venous blood samples for substrates between the third and fourth minute of ischemia, it is not surprising that not all hearts showed net lactate production during the ischemic interval. This observation is consistent with other investigators who observed only marginal lactate production after 5 min or significant lactate production after 10 min of ischemia. Alternatively, others have shown net lactate production only on reperfusion after a 2 min occlusion period. Unfortunately, we did not measure substrate utilization during early reperfusion. Such determinations would be complicated by the variability of the hyperemic blood flow and may not be an accurate index of lactate production.

Instead of focusing on the reperfusion phase for substrate utilization, we chose as one of our primary foci a comparison of events occurring during each ischemic interval since this might influence the functional recovery of the succeeding reperfusion period. Between the first and second occlusion cycles, we observed a priming effect on substrate utilization before and during the ischemic period. We know of no other study that has detected such a priming phenomenon. This is most likely because investigators who use a preparation of recurrent ischemia measure functional indexes rather than metabolic variables. Thus, our studies are unique in that regional myocardial blood flow, regional contractile function, regional $\text{MVO}_2$, transmyocardial substrate uptake, and regional myocardial energy metabolism were all measured within the same hearts. It is not yet known whether the substrate priming after the first occlusion cycle is a compensatory mechanism to regenerate chemical energy stores and to
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protect the myocardium from further ischemic insult. Alternatively, when the substrate priming (i.e., enhanced FFA extraction) carries over into the second occlusion cycle, it may have deleterious consequences, as indicated by the observation of posts ischemic dysfunction.

Adenine nucleotides in recurrent ischemia. Repetitive ischemic episodes of short duration do not apparently produce a cumulative effect on adenine nucleotide loss from myocardial tissue. It has been suggested that the largest decline in myocardial ATP occurs after the first occlusion (10 or 15 min duration), with a slower rate of depletion in subsequent episodes. It is not yet known whether this difference in ATP hydrolysis between first and subsequent ischemic episodes occurs in preparations of shorter duration such as that used in our study. We assume that the reduction in myocardial adenine nucleotides should be the same in all groups after the first occlusion period since collateral blood flow and MVO2 in the ischemic zone was similar in all groups in the absence of drug infusions. Despite this, both nicorandil and ISDN were effective in preserving myocardial adenine nucleotides when given before and during the second occlusion period. This suggests that while a single 5 min episode of myocardial ischemia may cause hydrolysis of ATP stores, there may not be a concomitant efflux of nucleosides and bases. In such a case, rephosphorylation to normal ATP levels would still be possible on reperfusion. However, in the absence of pharmacologic intervention between the first and second occlusion periods, a progressive loss in tissue energy metabolites probably occurs. Therefore, the largest loss of myocardial adenine nucleotides may well occur after the second ischemic episode in our preparation of repetitive ischemia, in contrast to the preparation of Reimer et al.

It was not ascertained in either the preparation of Reimer et al. or in our preparation whether a reduced rate of ATP depletion in subsequent ischemic cycles is related to the degree of functional impairment during the reperfusion period immediately after a previous ischemic interval. Thus, it could be argued that posts ischemic hypofunction may result in decreased demand for ATP utilization. However, such a scenario would predict that since nicorandil improves contractile function it should result in a greater utilization of myocardial ATP. However, both nicorandil and ISDN attenuated the loss of myocardial adenine nucleotides, despite different levels of posts ischemic function. This suggests that the preservation of myocardial adenine nucleotides by these drugs proceeds by mechanisms other than preservation of contractile function.

Postischemic contractile function after recurrent ischemia. Some controversy also exists as to whether repetitive occlusions produce a progressive decrease in regional contractile function. A number of studies, including the present one, suggest that %SS progressively decreases after each succeeding ischemic episode. In contrast, Lange et al. reported an initial decrease in contractile function that was not decreased further after a second or third occlusion. Some of this controversy may be related to the variation in experimental protocol including: the duration of the occlusion period, the duration of reperfusion, and the number of occlusion periods. It is also difficult to compare various studies on contractile function since collateral blood flow and area at risk were not provided in all cases. While the occlusion and reperfusion periods in the study by Lange et al. are similar to those of the present study, they did not find a progressive deterioration in myocardial contractile function with repetitive ischemic episodes. This may be related to the higher and more variable blood flow values reported by Lange et al. compared with those observed in the present study during ischemia. Higher collateral blood flow during ischemia would be conducive to better recovery of function during reperfusion. While we did show a progressive deterioration in contractile function during subsequent ischemic episodes in untreated hearts, we were also able to demonstrate that our preparation was still sensitive to therapeutic improvement in wall function, despite an initial decline in %SS.

Implications of the study. Our preparation of repeated, brief coronary artery occlusions was used to test the efficacy of nicorandil and ISDN during myocardial ischemia and reperfusion. Our observations may have potential clinical applications for drug testing. In this regard, the choice of cycle for drug intervention may influence the results obtained. The preparation chosen for these studies appears to be ideal since drug intervention occurred at the point of the substrate priming effect. Therefore, interventions at this point may be useful in determining whether a drug will have a beneficial effect on postischemic myocardial dysfunction.

Finally, the low doses of nitrates chosen for this study were well within the range of those that would be used clinically. The results of the study indicate that nicorandil significantly improves postischemic contractile dysfunction independent of hemodynamic changes. The metabolic data suggest that nicorandil may prove to be a more desirable clinical agent than ISDN in the treatment of angina pectoris.

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Salutary action of nicorandil, a new antianginal drug, on myocardial metabolism during ischemia and on postischemic function in a canine preparation of brief, repetitive coronary artery occlusions: comparison with isosorbide dinitrate.
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