Effects of Calcium Channel Blocker on Responses of Blood Flow, Function, Arrhythmias, and Extent of Infarction Following Reperfusion in Conscious Baboons

Stephen F. Vatner, Thomas A. Patrick, Delvin R. Knight, W. Thomas Manders, and John T. Fallon

Two groups of chronically instrumented, conscious baboons were studied. The effects of coronary artery occlusion for 3 hours and reperfusion for 1 week were examined on measurements of left ventricular function, ischemic-zone wall thickness, regional myocardial blood flow, arrhythmias, and extent of necrosis. The experimental group of animals (n = 7) was treated with the calcium channel blocker nisoldipine (0.1 μg/kg/min) from 1 hour after coronary occlusion to 3 hours after coronary reperfusion. The control group (n = 6) received the vehicle (n = 4) or saline (n = 2). The effects of coronary artery occlusion and reperfusion on arterial pressure, left ventricular systolic pressure, heart rate, and left ventricular dP/dt were similar in both groups. Systolic wall thickening was reversed to paradoxical wall thinning during occlusion in both groups, and there was no recovery to systolic wall thickening over the 1-week period in either group. There were differences in regional blood flow; during coronary artery occlusion, nisoldipine increased blood flow significantly in the endocardium and epicardium of nonischemic and ischemic zones. There was a major difference in the number of arrhythmic beats per minute on reperfusion; during reperfusion, the number of arrhythmias rose markedly in the vehicle-treated group but actually fell in the nisoldipine-treated group. The size of areas at risk, infarcts, infarcts related to the area at risk, and amount of total creatine kinase (CK) and MB-CK appearing in blood were not significantly different in the two groups. Thus, in the conscious baboon, nisoldipine administered 1 hour after coronary artery occlusion exerted a marked effect in diminishing reperfusion-induced arrhythmias and improved blood flow to the ischemic zone during occlusion but did not salvage ischemic tissue. (Circulation Research 1988;62:105-115)

Coronary artery reperfusion, if initiated soon enough after coronary artery occlusion, can ameliorate the effects of myocardial ischemia. If full reperfusion occurs within 20 minutes of coronary occlusion, no permanent damage occurs. However, after 3-6 hours of coronary artery occlusion, reperfusion does little to salvage myocardial tissue or regional myocardial function. Since this window of opportunity for major beneficial effects by reperfusion is relatively narrow, it is important to determine if this critical period can be extended by additional therapeutic intervention. Calcium channel blockers, administered prior to or soon after coronary artery occlusion, have been shown to reduce infarct size, improve blood flow to the ischemic zone, exert a beneficial effect on mechanical function, and reduce ischemia-induced arrhythmias. These beneficial effects of calcium channel blockers have been demonstrated in the presence of permanent coronary occlusion and reperfusion. It is important to note that these prior studies demonstrating beneficial effects of calcium channel blockers have been conducted primarily in nonprimate models. Since major differences between primates and other mammals exist in the regulation of the coronary circulation under normal conditions and in the presence of myocardial ischemia, it is not clear that results from lower species can be extrapolated to primates and eventually to humans. In fact, in contrast to studies in nonprimate models, prior studies in primates and humans have not consistently demonstrated beneficial effects of calcium channel blockers in the setting of either coronary artery occlusion or reperfusion.

The goal of the present investigation was to examine the mechanism of the apparent difference in species response by determining whether a calcium channel blocker would improve blood flow in ischemic tissue during coronary artery occlusion and reperfusion or would induce a "steal" due to the vasodilating properties in the nonischemic zone. Nisoldipine, a dihydropyridine derivative with potent vascular dilating action, was used. Additional goals of this investigation were to determine if the calcium channel blocker exerted a beneficial effect on mechanical function, induction of arrhythmias, and ultimate extent of myocardial necrosis. Potentially, since prior
differences could be due to species or open-chest versus unanesthetized experiments, these studies were conducted in conscious, chronically instrumented baboons.

Materials and Methods

Animal Preparation

Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and with those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHHS Publication No. [NIH] 85-23, revised 1985). Fourteen male baboons (Papio anubis, n = 13; Papio cynocephalus, n = 1) underwent operation for instrumentation allowing hemodynamic monitoring during subsequent experiments. After premedication with ketamine hydrochloride (6–8 mg/kg) and general anesthesia with halothane (0.5–1.0 vol%), the chest was opened through a midline sternotomy, the pericardium incised, and the heart exposed. The midleft anterior descending coronary artery was carefully dissected, a hydraulic occluder was implanted, and the potential ischemic area was delineated by occluding the artery for less than 30 seconds. A pair of ultrasonic crystals for measurement of wall thickness was implanted across the left ventricular (LV) free wall in the center of the area intended to become ischemic. A miniature solid-state pressure gauge (Konigsberg Instruments, Pasadena, California) was implanted in the LV cavity. Heparin-filled Tygon catheters were implanted in the ascending aorta and left atrium. Transducer wires and catheters were tunneled to the back of the animal and buried in subcutaneous pouches.

Measurements

LV pressure was measured with the implanted miniature pressure gauge, which was calibrated in vitro as well as in vivo during the experiments by systolic arterial pressure and measured with Statham P23ID strain gauge manometers (Statham Instruments, Oxford, California). In 6 animals, regional myocardial function was measured with an ultrasonic transit time dimension gauge previously described. The instrument measures the transit time of acoustic signals traveling at a sonic velocity of 1.58 × 10^6 mm/sec between two intramyocardial crystals. The drift of this instrument, although minimal (less than 0.01 mm/6 hr), was effectively eliminated by repeated calibrations throughout the experiment.

The radioactive microsphere technique as previously described by Domenech et al was used in this laboratory to measure regional myocardial blood flow. In this study, 15 ± 1 μm microspheres labeled with 85Ce, 95Nb, 89Sr, 67Sc, 103Ru, 113Sn, or 118In were used (New England Nuclear, Boston, Massachusetts). Four of these isotopes were administered to each animal. Since these experiments included a 1-week follow-up after coronary artery occlusion, a correction factor for blood flow in the ischemic myocardium was used to minimize error due to a potential "microsphere loss." To correct for microsphere loss, individual values for blood flow in the ischemic tissue were multiplied by the ratio of average blood flow in the nonischemic myocardium to blood flow in the ischemic myocardium observed before coronary artery occlusion.

Protocol

Experiments were performed 3–4 weeks after operation, when the animals had recovered from surgery and no signs of infection were present. On the day of the experiments, the animals were sedated with ketamine hydrochloride (6–8 mg/kg i.m.) and placed in a chair after catheters and instrumentation had been exteriorized, with lidocaine used for local anesthesia. When the effects of ketamine had worn off and the animal was conscious in the chair, control recordings of LV pressure, rate of change of LV pressure (dP/dt), heart rate, phasic and mean arterial pressure, ischemic zone wall thickness, and electrocardiographic lead II were obtained. Prior experiments indicated that no detectable effects of ketamine on hemodynamics persisted 4 hours after sedation. After control recordings of hemodynamics, a first (control) injection of microspheres was performed.

Coronary artery occlusion was then accomplished by inflating the hydraulic occluder. Complete coronary artery occlusion was confirmed in 6 of these animals during the experiment by complete loss of systolic function in the ischemic zone and was later verified by left ventricular angiography in the remaining 8 animals. In 1 of the 14 animals, complete occlusion could not be verified by either flow or function measurement, and accordingly, this animal was not included in the data. All animals developing arrhythmias were treated with 2% lidocaine i.v. during just the first hour of coronary artery occlusion. Hemodynamic effects of lidocaine persisted for less than 5 minutes. Microspheres were injected 1 and 3 hours after coronary artery occlusion for a second and third determination of regional myocardial blood flow. The occlusion was released after 3 hours in all animals. Two minutes before release of occlusion, 2 ml 2% lidocaine i.v. was administered. The coronary artery occluder was released slowly over 2–3 minutes. At 3–6 minutes after reperfusion, a fourth injection of microspheres was performed.

One group of baboons (n = 7) was treated with nisoldipine, 0.1 μg/kg/min, beginning immediately after injection of microspheres at 1 hour after coronary artery occlusion. An additional bolus of nisoldipine, 1.0 μg/kg, was administered immediately after coronary artery reperfusion to ensure sufficient blood levels and to prevent reperfusion damage if it occurred and was attributable to a calcium-related mechanism. Nisoldipine was administered continuously in a 20% solution of polyethylene glycol in saline at a rate of 0.38 ml/min for a total of 5 hours. The infusion of nisoldipine was discontinued 3 hours after coronary artery reperfusion. The other group of baboons re-
received either saline (n = 2) or vehicle (n = 4), which was administered in identical amounts and manner as the nisoldipine. Myocardial function was monitored continuously during coronary artery occlusion and for the first 6 hours after reperfusion. Ketamine, 0.4 mg/kg, was administered if the animals became restless or showed evidence of discomfort. Serial samples of blood (5 ml) were withdrawn from the animals over a 24-hour period. The samples were taken every hour for 8 hours, every 2 hours for the next 8 hours, and every 4 hours for the final 8 hours. The samples were collected in tubes containing EGTA and centrifuged. The plasma was decanted and frozen immediately at -70° C. Creatine kinase (CK) in plasma was assayed spectrophotometrically as described by Rosalki. 57 CK-MB isoenzyme was measured using a modified microbatch filtration method with anion exchange glass beads as described by Henry et al. 58 The animals were returned to their cages 24 hours after reperfusion. Hemodynamic parameters were measured again at 2 and 4 days and 1 week after reperfusion.

After 1 week, the baboons were killed with a lethal dose of sodium pentobarbital. Autopsy was performed and the heart removed. The heart was placed in a perfusion apparatus for dual perfusion of the coronary circulation. The ascending aorta was perfused retrogradely with Evans blue dye, 1 mg/100 ml saline, while the left anterior descending artery was cannulated at the site of occlusion and perfused at the same pressure with saline alone. Patency of the occluded coronary artery was verified, and the heart was placed in 5% buffered formalin for 2 days. The ventricles were sliced into multiple 5-10 mm thick transverse rings. Individual rings were weighed, and the basilar side was photographed. One ring from each heart containing the infarct was processed in its entirety for light microscopy. The infarct size was calculated by planimetry of epicardium, ventricular cavities, and infarct borders from photographs of individual ventricular rings at 2.5× magnification

**Table 1. Hemodynamics**

<table>
<thead>
<tr>
<th></th>
<th>Coronary artery occlusion</th>
<th>Coronary artery reperfusion</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control 1 hour 3 hours</td>
<td>5 minutes 1 hour 3 hours</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>V 99±5.4 99±2.3 98±5.2</td>
<td>106±5.8 94±4.0 94±5.9</td>
</tr>
<tr>
<td></td>
<td>N 111±1.9 109±4.7 101±4.9</td>
<td>100±4.4 95±4.1† 96±6.0†</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>V 112±6.4 117±6.1 123±2.3</td>
<td>127±6.9 133±5.3† 133±6.1†</td>
</tr>
<tr>
<td></td>
<td>N 116±6.0 122±4.3 141±5.9†</td>
<td>141±5.8† 140±6.7† 149±8.1†</td>
</tr>
<tr>
<td>LV pressure (mm Hg)</td>
<td>V 123±5.8 116±4.4 121±7.5</td>
<td>127±7.9 115±3.7 117±7.1</td>
</tr>
<tr>
<td></td>
<td>N 134±2.6 127±5.2 122±6.2</td>
<td>125±7.1 123±4.4 119±8.7</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>V 9.2±1.4 11.6±1.5 15.0±0.6†</td>
<td>17.0±1.0† 14.2±1.2† 12.6±0.8</td>
</tr>
<tr>
<td></td>
<td>N 9.3±0.6 11.7±0.8 12.3±1.5</td>
<td>12.2±1.0 12.0±1.2 12.3±1.6</td>
</tr>
<tr>
<td>LV dP/dt (mm Hg)</td>
<td>V 2,783±107 2,393±96 2,529±168</td>
<td>2,537±172 2,062±183† 2,304±277</td>
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<tr>
<td></td>
<td>N 3,088±206 2,583±266 2,885±282</td>
<td>2,855±364 2,549±171 2,515±292</td>
</tr>
<tr>
<td>Ischemic-zone wall thickening (mm)</td>
<td>V 1.47±0.17 -0.64±0.32† -0.54±0.28†</td>
<td>-1.49±0.50† -1.40±0.38† -1.33±0.31†</td>
</tr>
<tr>
<td></td>
<td>N 1.64±0.12 -0.92±0.46† -1.00±0.51†</td>
<td>-1.05±0.58† -1.09±0.60† -1.53±0.22†</td>
</tr>
<tr>
<td>Arrhythmic beats (beats/min)</td>
<td>V 0.5±0.5 0.4±0.1 0.6±2.5</td>
<td>14.5±2.5 76.5±22† 41.5±21.5</td>
</tr>
<tr>
<td></td>
<td>N 0.2±0.1 6.4±5.3 15.3±6.8</td>
<td>3.1±1.4* 12.8±8.4* 3.1±1.4</td>
</tr>
</tbody>
</table>

V, vehicle-treated; N, nisoldipine-treated.
* p<0.05 difference between groups.
† p<0.05 difference from control.
without knowledge of the treatment group. The area at risk was determined by two techniques. One technique involved planimetry of the zone, which did not stain blue following Evans blue dye infusion. This technique was used to provide area at risk for the histologically processed slice and for the entire heart in 1 animal in which the blood flow measurement was inaccurate. The area at risk was also determined using the blood flow technique. This technique was used in 12 of the 13 animals studied and involved calculation of area at risk by measurement of blood flow in all pieces of myocardium. Reductions of blood flow greater than 85% were assumed to indicate myocardium totally at risk. Blood flow reductions of 25-85% were considered to be proportionately less at risk. The two techniques for assessing area at risk, blood flow and dye, correlated well (Figure 1). For blood flow measurements, the myocardial samples were divided into three layers (endocardium, midwall, and epicardium), weighed, and counted for 10 minutes in a multichannel gamma well counter using a germanium detector with appropriately selected energy windows to obtain values for regional myocardial blood flow.

**Data Analysis**

All signals were recorded on magnetic tape during experiments with a 14-channel tape recorder and played back on two multichannel direct-writing oscillographs displaying 16 simultaneous channels of data. Arrhythmias, as reflected in lead II of the ECG, were quantitated by counting all beats for 15 minutes at each data point. Mean values and standard errors were calculated. Data were stored on magnetic tape and on an IBM PC/AT computer. Significant changes between nisoldipine- and vehicle-treated groups were evaluated by analysis of variance and Scheffe’s test for multiple comparisons. 59

**Results**

**Left Ventricular Function (Table 1)**

During control and at 1 hour of coronary artery occlusion, there were no differences between the two groups. Coronary artery occlusion did not affect LV systolic pressure, mean arterial pressure, or LV dP/dt significantly but increased heart rate and LV end-diastolic pressure significantly. Wall thickening in the ischemic zone was reduced by more than 100%; i.e., paradoxical wall thinning was observed in both groups after coronary artery occlusion. Also, at 3 hours of coronary artery occlusion, there were no significant differences between the two groups. However, in the nisoldipine group, mean arterial and LV systolic pressures tended to fall from values obtained at 1 hour after coronary artery occlusion.

During the initial period after coronary artery reperfusion, there were no differences between the two groups with the exception that LV end-diastolic pressure rose slightly and paradoxical wall thinning was intensified in the vehicle-treated group; this was not observed in the nisoldipine-treated group. From control levels prior to coronary artery occlusion to 1 week after coronary artery reperfusion, there were no statistically significant differences between the two groups for hemodynamics (Figures 2-4).

**Regional Myocardial Blood Flow (Table 2)**

The uncorrected blood flow values are included in Table 2, as well as ischemic zone samples corrected for microsphere loss. Prior to coronary artery occlusion, endocardial blood flow was higher than epicardial blood flow, and no differences for corrected blood flows were observed between the two groups. Prior to coronary artery occlusion, the uncorrected blood flows were lower in the ischemic zone than in the nonischemic zone. Blood flows in the nonischemic zone rose similarly in both groups at 1 hour after coronary artery occlusion.
FIGURE 4.  Effects of coronary artery occlusion (CAO) for 3 hours and reperfusion (CAR) for 1 week are compared for absolute values of wall thickening in ischemic zone in vehicle-treated group (Solid, n = 3) and nisoldipine-treated group (Dashed, n = 3). Time of treatment is shown by bar at top. Wall thickening was replaced by paradoxical systolic wall thinning during coronary artery occlusion. There was no significant return of systolic wall thickening over 1-week period.

In the nisoldipine-treated group, blood flow rose further at 3 hours after coronary artery occlusion. Blood flow in the nonischemic zone did not rise further at 3 hours after occlusion in the vehicle-treated group and was significantly lower (p<0.01) than blood flow in the nonischemic zone in the nisoldipine-treated group. During the initial reperfusion period, blood flow in the nonischemic zone remained significantly higher in the treated group compared with that in the non-treated group.

In the ischemic zone, blood flow was reduced to nearly zero in both the endocardium and epicardium in both groups of animals at 1 hour after coronary artery occlusion. At 3 hours after coronary artery occlusion, blood flow was slightly but significantly (p<0.05) higher in both the endocardium and epicardium in the nisoldipine-treated group than in the vehicle-treated group. During the initial reperfusion period, blood flow returned to both endocardium and epicardium with no differences observed in corrected blood flows between the two groups. Uncorrected blood flows were lower in the nisoldipine-treated group than in the vehicle-treated group upon reperfusion.

Arrhythmias (Table 1)

There were no differences in number of arrhythmic beats per minute in the two groups during control or at 1 hour after coronary artery occlusion. At 3 hours after occlusion, the number of arrhythmic beats tended to be greater in the nisoldipine-treated group, but the difference was not statistically significant. Ventricular fibrillation with rapid defibrillation occurred in 1 animal in each group at 35–40 minutes after occlusion.

During the initial reperfusion period, the number of arrhythmic beats increased significantly in the vehicle-

Table 2. Regional Blood Flow (ml/min/g)

<table>
<thead>
<tr>
<th></th>
<th>Coronary artery occlusion</th>
<th>Coronary artery reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1 hour</td>
</tr>
<tr>
<td>Nonischemic zone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocardial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (n = 6)</td>
<td>1.10±0.09</td>
<td>1.32±0.11</td>
</tr>
<tr>
<td>Nisoldipine (n = 6)</td>
<td>1.30±0.18</td>
<td>1.38±0.19</td>
</tr>
<tr>
<td>Epicardial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (n = 6)</td>
<td>0.81±0.10</td>
<td>0.98±0.10</td>
</tr>
<tr>
<td>Nisoldipine (n = 6)</td>
<td>0.83±0.17</td>
<td>0.99±0.14</td>
</tr>
<tr>
<td>Ischemic zone (uncorrected)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocardial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (n = 6)</td>
<td>1.09±0.11</td>
<td>0.01±0.02</td>
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<tr>
<td>Nisoldipine (n = 6)</td>
<td>0.96±0.19</td>
<td>0.03±0.01</td>
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<tr>
<td>Epicardial</td>
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<td>Vehicle (n = 6)</td>
<td>0.77±0.06</td>
<td>0.03±0.01</td>
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<td>Nisoldipine (n = 6)</td>
<td>0.54±0.11</td>
<td>0.04±0.02</td>
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<td>Ischemic zone (corrected)</td>
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<tr>
<td>Endocardial</td>
<td></td>
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<tr>
<td>Epicardial</td>
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<tr>
<td>Vehicle (n = 6)</td>
<td>0.81±0.10</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Nisoldipine (n = 6)</td>
<td>0.83±0.17</td>
<td>0.06±0.03</td>
</tr>
</tbody>
</table>

*p<0.05 difference between groups.
FIGURE 5. Effects of coronary artery occlusion (CAO) for 3 hours and reperfusion (CAR) for 1 week are shown on number of arrhythmic beats per minute in vehicle-treated group (■——■, n = 6) and nisoldipine-treated group (▲——▲, n = 7). Time of treatment is shown by bar at top. Significantly greater numbers of arrhythmic beats per minute were observed on CAR and for 1 hour after CAR in vehicle-treated group but actually fell in the nisoldipine-treated group (Figure 5). These differences were significant (p<0.01). Ventricular fibrillation occurred with rapid defibrillation in 1 animal at 45 minutes after reperfusion in the vehicle-treated group. At 1 and 3 hours after coronary artery reperfusion, there were significantly more arrhythmias in the vehicle-treated group, but these differences were only statistically significant at 1 hour after reperfusion. Between 6 hours and 1 week after reperfusion, there were no differences between the two groups (Figure 5). The majority of arrhythmias were ventricular in origin. When only ventricular arrhythmias were analyzed, statistically significant differences were still observed between the two groups during the initial period of reperfusion and at 1 hour after reperfusion.

Pathology (Table 3)

The areas at risk and the size of infarcts were similar in the vehicle-treated and the nisoldipine-treated groups. The amount of infarct was also correlated with the area at risk in the two groups. These relations

\[
\begin{array}{|c|c|c|c|c|c|c|}
\hline
\text{Baboon} & \text{Hemorrhage*} & \text{Body weight (kg)} & \text{LV + RV weight (g)} & \text{Infarct weight (INF) (g)} & \text{Area at risk (AAR) (g)} & \% \text{INF:AAR} \\
\hline
\text{Vehicle or saline-treated} & & & & & & \\
1 & 3 & 23.0 & 87.2 & 2.8 & 8.9 & 31.3 \\
2 & 4 & 22.4 & 94.7 & 7.3 & 13.6 & 53.8 \\
3 & 2 & 22.0 & 106.1 & 4.2 & 6.3 & 66.9 \\
4 & 2 & 23.5 & 91.0 & 1.1 & 7.2 & 15.2 \\
5 & 0 & 23.5 & 78.9 & 5.5 & 19.7 & 27.9 \\
6 & 4 & 34.0 & 100.6 & 11.7 & 18.9 & 61.8 \\
\text{Mean ± SEM} & 2.5 ± 0.6 & 24.7 ± 1.9 & 93.1 ± 4.0 & 5.4 ± 1.5 & 12.5 ± 2.4 & 42.8 ± 8.5 \\
\hline
\text{Nisoldipine-treated} & & & & & & \\
1 & 2 & 21.0 & 59.5 & 4.3 & 7.0 & 61.4 \\
2 & 3 & 25.0 & 127.1 & 7.9 & 21.3 & 37.1 \\
3 & 1 & 20.0 & 92.5 & 2.4 & 4.1 & 59.1 \\
4 & 2 & 23.5 & 93.4 & 7.1 & 7.9 & 89.8 \\
5 & 2 & 27.0 & 89.6 & 3.7 & 7.6 & 48.8 \\
6 & 4 & 28.0 & 115.3 & 13.9 & 19.9 & 69.9 \\
7 & 3 & 26.0 & 110.3 & 3.7 & 5.2 & 71.2 \\
\text{Mean ± SEM} & 2.4 ± 0.4 & 24.4 ± 1.1 & 98.2 ± 8.3 & 6.1 ± 1.5 & 10.4 ± 2.7 & 62.5 ± 6.4 \\
\hline
\end{array}
\]

* LV, left ventricular; RV, right ventricular.

*0, none; 1, focal on histology; 2, grossly evident, less than 25% of infarct; 3, grossly evident, 25—50% of infarct; 4, grossly evident, >50% of infarct.

† By Evan's blue dye.
(Figure 6) were similar in the two groups. All infarcts were characterized as anterior-septal, transmural, and healing. Histologically, infarcts were similar for both groups. There was a prominent rim of both endocardial and epicardial sparing, usually 4–10 myocytes thick. The lateral borders were irregular with "islands" of viable myocytes. Myocytes along the infarct borders often were enlarged and vacuolated and contained large bizarre single nuclei or, in some instances, numerous small nuclei. Centrally, infarct zones contained hyper-eosinophilic mummified muscle with accentuated cross striations and absent nuclei. Foci of hemorrhage were present in this central necrotic zone in all but one heart studied. The amount of hemorrhage, quantified on a scale of 0–4, was not significantly different for the two groups (Table 3). Between the borders and the necrotic
centers, granulation tissue containing variable numbers and types of mononuclear inflammatory cells was present. Within this healing zone, the nisoldipine-treated hearts often had a loose, edematous-like appearance in contrast to the vehicle-treated hearts in which this zone of tissue repair was densely populated with cells and connective tissue matrix (Figure 7).

Creatine Kinase

The curves for appearance of MB-CK and total CK in blood were similar for the two groups (Figures 8 and 9). Both curves showed abrupt increases in CK after reperfusion, reaching a peak at 2–5 hours after coronary artery reperfusion. There were no significant differences between the two groups for appearance of MB-CK or total CK in blood.

Discussion

The major limitations to the successful use of coronary artery reperfusion in patients are the critical time period after which reperfusion can no longer exert beneficial effects and the potential deleterious effects of reperfusion injury. To minimize these limitations, several therapeutic strategies have been devised in experimental preparations. One intervention, which has been demonstrated to be useful in nonprimate experimental models, is the administration of a calcium channel blocker. These agents dilate vascular smooth muscle and can depress electrical and mechanical function to varying degrees. While there has been some discussion in the literature as to potential differences among calcium channel blockers in the presence of myocardial ischemia, there is sufficient evidence that all types of calcium channel blockers, e.g., benzeneacetonitriles, benzothiazepines, and dihydropyridines, exert beneficial effects in nonprimate models. Therefore, distinctions due to classes of calcium channel blockers will not be discussed further.

Because the calcium channel blockers relax vascular smooth muscle, there is some concern that vasodilation of resistance vessels in the nonischemic zone might induce a steal and actually reduce blood flow in the ischemic zone, as occurs with isoproterenol. This was not observed in the present investigation. Blood flow in the nonischemic zone rose significantly in the treated group between 1 and 3 hours after coronary artery occlusion, i.e., after nisoldipine infusion was initiated. Despite the vasodilation in the nonischemic zone, blood flow did not fall and actually rose significantly in the ischemic zone during this period in the nisoldipine-treated group. The effects of calcium channel blockers on regional myocardial blood flow during coronary artery occlusion and reperfusion have not been assessed previously in primates. However, prior studies in other species have been equally distributed in showing blood flow in the central ischemic zone to rise or not change in response to calcium channel blockers.

Even though the changes in blood flow in the ischemic zone were statistically significant, they were trivial in terms of absolute levels of myocardial blood flow and most likely physiologically not significant enough to allow for myocardial salvage. However, it is possible to conclude from these data that the calcium channel blocker did not induce a coronary steal. This was most likely attributable to the fact that these agents also induce considerable dilation of large coronary arteries and, under the conditions of the present experiments, did not reduce arterial driving pressure.

Blood flows in the ischemic zone were examined for "microsphere loss," a phenomenon that is probably attributable to the swelling and edema following myocardial ischemia and reperfusion. While the correction factor may play an important role in interpretation of blood flow values in the ischemic zone prior to coronary artery occlusion and after reperfusion, the
correction factor did not have a major effect on the data during coronary artery occlusion because myocardial blood flow was less than 0.1 ml/min/g. The corrected and uncorrected blood flow values in the ischemic zone were similar in the two groups during coronary artery occlusion. However, the uncorrected values during control and reperfusion tended to be lower in the nisoldipine group. These differences between the treated and nontreated groups suggest that either there was more microsphere loss in the nisoldipine group or that there was more edema and swelling in that group. In support of the latter possibility, it is interesting to note that in the nisoldipine-treated group, the infarcts tended to appear more edematous. It is conceivable that the calcium channel blocker, potentially through its vasodilating action, predisposes to edema formation in the infarcted region.

In contrast to the majority of prior studies on the effects of calcium channel blockers, the present investigation shows no apparent effect on myocardial tissue salvage. The difference between the present results and other studies may be related to species, because the lack of preformed collateral channels in the primate 46 would prevent a major improvement in blood flow to the ischemic zone. Indeed, as discussed above, while blood flow rose in the ischemic zone in the nisoldipine-treated group, it did not rise to levels that would be considered compatible with salvage of ischemic myocardium. In support of this concept, while prior studies in primates and humans with myocardial ischemia have been controversial, 21,41-45,62 most of these studies have failed to demonstrate salutary effects of calcium channel blockers. 5,14-40

It is also clear that administration of any therapeutic intervention during ischemia must occur soon enough, i.e., prior to development of complete necrosis. 20,38,52,63 We introduced the calcium channel blocker at 1 hour after coronary artery occlusion, which is clearly prior to development of complete necrosis 13-18 and might be considered the earliest time to exert a beneficial effect in the clinical setting. Furthermore, prior studies have demonstrated that treatment with a calcium channel blocker at this point following coronary artery occlusion but prior to reperfusion would have beneficial effects. 5,12,13,16,30,37,38

Another possibility that must be considered is that upon reperfusion, further injury occurs. 54,65 Mechanisms that have been considered include oxygen free radicals, 46 tissue edema, 67 calcium, 68 and hemorrhagic infarction. 69 The last mechanism was observed in both groups of animals and has been shown to be the result of, rather than the cause of, tissue injury. 70 The results of the present study make it unlikely that a mechanism involving the calcium channel is responsible for reperfusion injury. It is interesting that others have already speculated that reperfusion injury is not responsible for a major amount of cellular necrosis following reperfusion. 71

Prior studies found that calcium channel blockers exerted a protective effect on arrhythmias during coronary artery occlusion. 21-25 This was not observed in the present investigation. However, the major beneficial action of the calcium channel blocker was the striking reduction in reperfusion arrhythmias observed in the nisoldipine-treated group. These results suggest that changes in calcium flux may be responsible for the reperfusion arrhythmias in the baboon because there were no major differences in hemodynamics, area at risk, extent of infarction, or corrected ischemic zone blood flow during reperfusion in the two groups. The calcium channel blocker may have preferentially affected viable cells, responsible for the reperfusion arrhythmias, without affecting the cells already necrotic or destined to become necrotic. An interesting possibility, suggested in a preliminary report by Thandroyen et al, 72 is that regeneration of ATP in the face of enhanced calcium as occurs during reoxygenation may facilitate ventricular fibrillation.

In summary, administration of a calcium channel blocker at 1 hour after coronary artery occlusion to conscious baboons did not induce a coronary steal and did not reduce the ultimate extent of myocardial infarction. However, the calcium channel blocker did induce a marked reduction in reperfusion arrhythmias. While these arrhythmias are observed only in some patients undergoing coronary artery reperfusion with thrombolytic therapy, 73,74 the frequency of these arrhythmias may be greater and of more significance if earlier reperfusion is instituted.

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