



Feature Article

Nisoldipine Attenuates Myocardial Stunning Induced by Multiple Coronary Occlusions in Conscious Pigs and this Effect is Independent of Changes in Hemodynamics or Coronary Blood Flow

Seong-Wook Park*, Xian-Liang Tang*†, Yumin Qiu*†, Jian-Zhong Sun* and Roberto Bolli*†

*Experimental Research Laboratory, Section of Cardiology, Department of Medicine, Baylor College of Medicine, Houston, TX 77030 and †the Experimental Research Laboratory, Division of Cardiology, University of Louisville, Louisville, KY 40292, USA

(Received 21 August 1995, accepted in revised form 16 October 1995)

S.-W. PARK, X.-L. TANG, Y. QIU, J.-Z. SUN AND R. BOLLI. Nisoldipine Attenuates Myocardial Stunning Induced by Multiple Coronary Occlusions in Conscious Pigs and this Effect is Independent of Changes in Hemodynamics or Coronary Blood Flow. *Journal of Molecular and Cellular Cardiology* (1996) **28**, 655–666. Recent studies suggest that calcium channel blockers attenuate reversible post-ischemic myocardial dysfunction (myocardial “stunning”) *in vivo*. This beneficial effect, however, has been shown either in open-chest preparations, which are subject to the confounding influence of many unphysiological conditions, or in models in which treatment caused significant hemodynamic alterations. Furthermore, all of the studies have been conducted in the dog, and almost all of them have examined the effect of calcium antagonists after a single ischemic episode. The goal of the present investigation was to assess the effect of nisoldipine in a conscious pig model of repetitive ischemia, and to determine whether the drug exerts direct cardioprotection independent of hemodynamic changes. A total of 33 conscious pigs were used. Pigs underwent a sequence of 10 2-min coronary occlusions, each separated by 2 min of reperfusion, and were randomly assigned to a treated group ($n=11$), in which nisoldipine was infused at a rate of $0.5 \mu\text{g}/\text{kg}/\text{min}$ from 15 min before the first coronary occlusion till 30 min after the last reperfusion, and a control group ($n=12$), which received vehicle. Results showed that there were no significant differences between the two groups with respect to ischemic bed size or hemodynamic variables throughout the experiment. Collateral blood flow to the ischemic regions was virtually nil in both groups. During the sequence of coronary occlusions, systolic thickening fraction in the ischemic region decreased similarly in the two groups. After the 10th reperfusion, however, the recovery of wall thickening was markedly enhanced in treated compared to control pigs, with the differences being statistically significant at 5, 15, and 30 min and 1, 3, 4 and 5 h. The total deficit of wall thickening after the 10th reperfusion (an integrative assessment of post-ischemic dysfunction) was 51% less in the treated compared with the control group ($P<0.001$). This study demonstrates that nisoldipine markedly attenuates myocardial stunning after multiple ischemic episodes in conscious pigs, the improvement is evident immediately after the end of the ischemic episodes and is sustained throughout the recovery phase. This beneficial effect is independent of any favourable hemodynamic changes, and therefore indicates a direct cardioprotective action of nisoldipine. © 1996 Academic Press Limited

KEY WORDS: Nisoldipine; Calcium channel blockers; Repetitive ischemia; Myocardial stunning; Post-ischemic dysfunction; Pig.

Please address all correspondence to: Roberto Bolli, Division of Cardiology, University of Louisville, Ambulatory Care Building, Louisville, KY 40292, USA.

Introduction

Post-ischemic myocardial dysfunction, or myocardial "stunning" (Braunwald and Kloner, 1982), is the protracted depression of contractility that is observed after a reversible ischemic insult (Bolli, 1990). Because calcium channel blockers attenuate the severity of ischemic injury (Naylor *et al.*, 1990) and because the severity of myocardial stunning is related to the severity of the ischemic insult (Bolli, 1990), calcium channel blockers have been used in experimental models of myocardial stunning and several investigators have reported beneficial effects (Lamping and Gross, 1985; Przyklenk and Kloner, 1988; Warltier 1988; Przyklenk *et al.*, 1989; Taylor *et al.*, 1990; Ehring *et al.*, 1992; Gross and Pieper, 1992). The direct effects of calcium channel blockers on myocardial stunning *in vivo*, however, are still unclear because these results (Lamping and Gross, 1985; Przyklenk and Kloner, 1988; Warltier, 1988; Przyklenk *et al.*, 1989; Taylor *et al.*, 1990; Ehring *et al.*, 1992; Gross and Pieper, 1992) were obtained either in anesthetized, open-chest preparations, or in conditions in which the drugs caused significant changes in hemodynamic variables. Several investigators (Vatner *et al.*, 1971a, b; Cobb *et al.*, 1974; Vatner and Braunwald, 1975; Templeton *et al.*, 1975; Manders and Vatner, 1976; Jugdutt, 1985; Bolli *et al.*, 1986; Laxson *et al.*, 1989; Ning *et al.*, 1990) have pointed out that observations in open-chest models may be confounded by the effects of anesthesia, surgical trauma, abnormal hemodynamic conditions, excessive level of circulating catecholamines, and other factors. Certain conclusions derived from open-chest preparations have subsequently been found not to be applicable to conscious animals (Vatner *et al.*, 1971a, b; Jugdutt, 1985; Bolli *et al.*, 1986; Ning *et al.*, 1990). Recently, it has been shown that, in the 15-min coronary occlusion model of myocardial stunning, both the severity of post-ischemic dysfunction (Triana *et al.*, 1991) and the magnitude of the associated free radical generation (Li *et al.*, 1993) are greatly exaggerated in open-chest as compared with conscious animals preparations, raising the possibility that observations made in the former may not necessarily apply to the latter. Furthermore, the stunned myocardium is extremely sensitive to a number of hemodynamic variables, so that favorable modifications of afterload, preload, heart rate and regional myocardial blood flow could increase its systolic shortening independently of changes in intrinsic contractile properties (Stahl *et al.*, 1986; Przyklenk and Kloner, 1988).

This study was undertaken to determine whether nisoldipine, a dihydropyridine-derivative calcium channel blocker, attenuates myocardial stunning in conscious pigs in the absence of significant alterations of hemodynamic variables. Nisoldipine was chosen because it exerts no negative inotropic and chronotropic effects at therapeutic doses (van der Giessen *et al.*, 1989). The infusion rate of nisoldipine used in this study was selected because it has been found to be the highest that does not cause significant change in systemic hemodynamic variables in conscious pigs (Duncker *et al.*, 1988). Because all of the previous studies of calcium channel blockers in stunned myocardium (Lamping and Gross, 1985; Fuster *et al.*, 1988; Przyklenk and Kloner, 1988; Warltier, 1988; Przyklenk *et al.*, 1989; Taylor *et al.*, 1990; Ehring *et al.*, 1992; Gross and Pieper, 1992) have been performed in dogs, we elected to use a swine model in order to assess the effect of nisoldipine in a species that resembles humans with respect to the lack of native coronary collateral vessels and myocardial xanthine oxidase activity (Eckstein, 1954; White and Bloor, 1981; Bloor *et al.*, 1986; White *et al.*, 1986; Muxfeldt and Schaper, 1987; Podzuweit *et al.*, 1987; Roth *et al.*, 1987; Schaper *et al.*, 1988; Grum *et al.*, 1989). A protocol of repetitive coronary occlusions was chosen because most of the previous studies (Lamping and Gross, 1985; Kloner, 1988; Warltier, 1988; Przyklenk *et al.*, 1989; Taylor *et al.*, 1990; Ehring *et al.*, 1992) have used a single coronary occlusion protocol and because repetitive ischemia occurs frequently in patients with coronary artery disease (Deanfield *et al.*, 1983; Cohn, 1985).

Materials and Methods

A total of 33 pigs were used for this investigation. The experimental model and techniques have been described previously (Sun *et al.*, 1995). The study was performed in accordance with the guidelines of the Committee on Animals of Baylor College of Medicine and with the *Guide for the Care and Use of Laboratory Animals* (Department of Health and Human Services, Publication No. [NIH] 86-23).

Experimental preparation

Domestic pigs of either sex (27.7 ± 1.3 kg) were premedicated with ketamine hydrochloride (20 mg/kg, i.m.) and atropine (0.04 mg/kg, i.m.). Sixty minutes later, anesthesia was induced with methohexital sodium (4–8 mg/kg, i.v.), following

which the animals were intubated and anesthesia was maintained with 0.5–1.0% methoxyflurane. A left thoracotomy was performed under sterile conditions at the level of the fifth intercostal space. Tygon catheters were placed in the left atrium and right ventricle and an additional catheter was introduced into the femoral artery and advanced to the thoracic aorta. A hydraulic occluder and a Doppler flow velocity probe were implanted around the mid left anterior descending coronary artery (LAD). Two insulated copper wires were sutured to the right ventricle to record the electrocardiogram. To measure left ventricular (LV) wall thickening, 10-MHz pulsed Doppler ultrasonic crystals (Zhu *et al.*, 1986) were sutured to the epicardial surface, three in the center of the region to be rendered ischemic and another in an area remote from it (posterior LV wall); each probe was sutured with four 6-0 prolene stitches penetrating 0.5–1.0 mm into the myocardium, thus producing minimal trauma. To avoid the “tethering” effect of non-ischemic myocardium on adjacent ischemic-reperfused myocardium, the crystals were placed at least 1.0 cm inside the boundaries of the ischemic region, which were identified by occluding the LAD for 30 s. All wires and catheters were tunneled under the skin and exteriorized through small incisions on the back. The chest was closed in layers and a small tube was left in the thorax to evacuate air and fluid post-operatively. Antibiotics were administered i.v. before surgery and daily thereafter (cefazolin 30 mg/kg b.i.d. and gentamicin 0.7 mg/kg b.i.d.). Arterial blood gases, hematocrit, rectal temperature, and heart rate were measured daily after instrumentation.

Experimental protocol

Throughout the experiment, pigs were studied while lying quietly in a specially designed cage. Aortic and left atrial pressures were measured with Statham P23 Db pressure transducers. All measured variables (aortic pressure, left atrial pressure, LAD blood flow velocity, wall thickening, and the electrocardiogram) were recorded simultaneously on an eight-channel, direct writing oscillograph (Gould Brush System 200). Pigs underwent a sequence of 10 2-min coronary occlusions, each separated by 2 min of reperfusion, followed by several hours of observation. Hemodynamic variables and thickening fraction were measured at baseline, before occlusion, 1 min after each reperfusion, and then 5, 15, 30 min and 1, 2, 3, 4, and 5 h after the last reperfusion or until thickening fraction recovered.

To measure regional myocardial blood flow, radioactive microspheres were injected at 30–60 s into the fifth LAD occlusions as previously described (Bolli *et al.*, 1988b). The animals were randomly assigned to a treated or a control group. In the treated group, nisoldipine was infused intravenously at the rate of 0.5 $\mu\text{g}/\text{kg}/\text{min}$ (1.2 ml/min) from 15 min before the first coronary occlusion till 30 min after the 10th reperfusion (total dose: 41.5 $\mu\text{g}/\text{kg}$). The nisoldipine solution was prepared from a stock solution (0.5 mg/ml, dissolved in polyethylene glycol 400). The stock solution was diluted with 0.9% NaCl immediately before infusion and administered after filtering through a 0.22- μm Millipore filter. Because of the photosensitive nature of nisoldipine, all solutions, syringes and cannulas were protected from light. Control animals underwent the same protocol described above except that vehicle was infused instead of nisoldipine.

Measurement of regional myocardial function

Regional myocardial function was assessed as systolic thickening using a pulsed Doppler probe, as previously described (Zhu *et al.*, 1986; Bolli *et al.*, 1988a, b; Triana *et al.*, 1991; Li *et al.*, 1993; Sekili *et al.*, 1993; Sun *et al.*, 1995). The beginning of systole was determined from the peak of the QRS complex on the right ventricular electrogram and the end of systole from the onset of the rapid rise in LAD blood flow velocity after systole (Sun *et al.*, 1995). Percentage systolic thickening fraction was calculated as the ratio of net systolic thickening to end-diastolic wall thickness, multiplied by 100 (Bolli *et al.*, 1988b). The total deficit of wall thickening after reperfusion (an integrative assessment of the severity of post-ischemic dysfunction) was calculated by measuring the area comprised between the wall thickening-*v*-time line and the baseline (100% line) during the recovery phase (Triana *et al.*, 1991; Li *et al.*, 1993; Sekili *et al.*, 1993; Sun *et al.*, 1995) (Fig. 1); the recovery phase was defined as the interval between the 10th reperfusion and the time when thickening fraction returned to values >90% of baseline values (Sun *et al.*, 1995). In all animals, measurements from at least 10 beats were averaged at baseline, and from at least five beats at all subsequent time points. As indicated above, three thickening Doppler probes were implanted in the potentially ischemic region. The measurements used for this study are those derived from the probe that gave the lowest values of wall thickening (i.e., the most severe degree of myocardial stunning) after reperfusion.

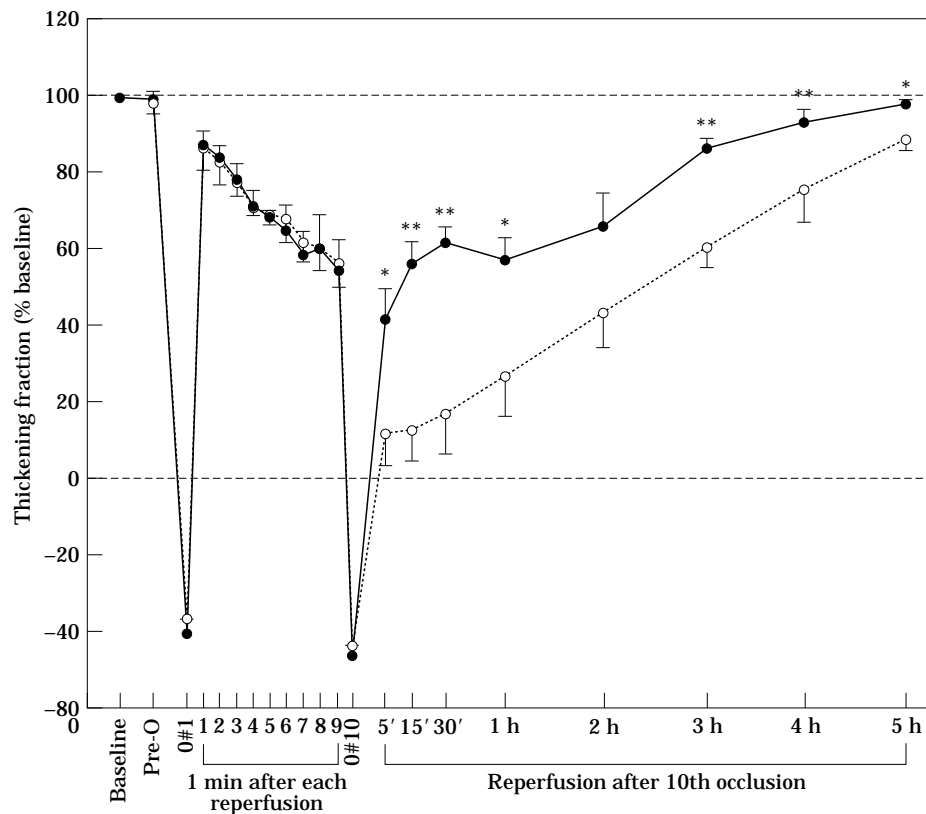


Figure 1 Systolic thickening fraction in the ischemic-reperfused region. Shown are the measurement of thickening fraction obtained before administration of nisoldipine or vehicle (Baseline), 10 min after the infusion of the nisoldipine or vehicle solution [preocclusion (Pre-O)], 1 min into the first LAD occlusion (O#1), 1 min into each of the first nine reperfusion (1-9), 1 min into the 10th occlusion (O#10), and at selected times during the 5-h reperfusion interval following the 10th coronary occlusion in the control and nisoldipine-treated groups. Measurements taken in the control group are represented by the dashed line with open circles ($n=12$), measurements taken in the treated group are represented by the continuous line with solid circles ($n=11$). Thickening fraction is expressed as a percentage of baseline values. Data are mean \pm S.E.M. * $P<0.05$ v control; ** $P<0.01$ v control.

Post mortem tissue analysis

At the end of the study, the pigs were given heparin (5000 U, i.v.), after which they were anesthetized with pentobarbital sodium (35 mg/kg, i.v.) and killed with a bolus of KCl. The hearts were excised and the size of the occluded coronary vascular bed was determined by tying the LAD at the site of the previous occlusion and by perfusing the aortic root for 2 min with a 0.5% solution of Monastral blue dye in 6% dextran 70 in normal saline at a pressure of 100 mmHg (Sun *et al.*, 1995). The rationale for using dextran in the perfusate was to prevent myocardial edema, which may hinder perfusion and cause underestimation of myocardial blood flow by the microsphere technique. The heart was then cut into 1.0-cm thick transverse slices, which were incubated for 20 min at 38°C in a 1% solution of triphenyltetrazolium chloride to verify the absence of infarction. The portion of the left ventricle supplied by the previously occluded coronary artery

(occluded bed) was identified by the absence of blue dye and separated from the rest of the left ventricle. Both components were weighed to determine occluded bed size as a percentage of total LV weight. Four transmural specimens (1-2 g) were then obtained from both the ischemic and the non-ischemic regions (to avoid admixture of ischemic and non-ischemic tissue, ischemic specimens were obtained at least 0.5 cm inside the boundaries of the occluded bed). Each specimen was divided into endocardial and epicardial halves, weighed, and placed in scintillation vials containing 10% neutral buffered formalin. Regional myocardial blood flow was calculated by standard methods (Bolli *et al.*, 1988b).

Statistical analysis

Data were reported as mean \pm S.E.M. Hemodynamic variables and wall thickening fraction were analysed by a two-way repeated measures ANOVA

(time and group) to determine whether there was a main effect of time, a main effect of group, or a time-group interaction. If the global tests showed a significant main effect or interaction, *post hoc* contrasts between different time-points in the same group or between different groups at the same time point were performed with paired or unpaired Student's *t*-tests, as appropriate, with the Bonferroni correction (Wallenstein *et al.*, 1980). The total deficits of wall thickening were analysed by an unpaired Student's *t*-test. All statistical analyses were performed using the SAS software system (SAS Institute, 1988). Two-way ANOVA was performed using the procedure GLM (General Linear Models) (SAS Institute, 1988).

Results

Exclusions

Of the 33 pigs instrumented, 10 (30%) were excluded for the following reasons: intraoperative death (one pig), ventricular fibrillation on perfusion (four pigs; two in each group), unexplained death 3 h after reperfusion (one control pig), lack of dyskinesia during ischemia (one treated pig), spontaneous post-operative LAD occlusion (one pig), and failure of the balloon occluder during occlusion (two pigs). The remaining 23 pigs (12 controls and 11 treated) form the basis of this report.

Post mortem tissue analysis

Post mortem tissue analysis was performed in all pigs. Tetrazolium staining demonstrated absence of infarction in every pig, confirming that the injury associated with the 10 cycles of 2-min occlusion/2-min reperfusion was completely reversible (Sun *et al.*, 1995). In all animals, post mortem perfusion confirmed that the Doppler ultrasonic crystals were at least 1 cm away from the boundaries of the ischemic region.

Arterial blood gases, hematocrit, and temperature

As shown in Table 1, arterial pH, P_{O_2} , hemacrit, and rectal temperature were within physiological limits, and were comparable between control and treated groups (Table 1).

Hemodynamic variables

The infusion of nisoldipine produced no significant hemodynamic changes (Table 2). All measured variables (heart rate, systolic arterial pressure, diastolic arterial pressure, rate-pressure product, left atrial pressure, and LAD blood flow) were comparable between the two groups throughout the protocol (Table 2). These results indicate that the effect of nisoldipine on myocardial contractility was assessed in the absence of any indirect influence of the hemodynamic determinants of post-ischemic dysfunction.

Occluded bed size and regional myocardial blood flow

The LV weight averaged 114.5 ± 8.9 g and 114.0 ± 7.6 g in control and treated pigs, respectively; the mean size of the occluded/reperfused vascular bed was 20.4 ± 2.2 g ($17.9 \pm 1.1\%$ of LV weight) and 23.6 ± 3.6 g ($20.3 \pm 2.2\%$ of LV weight), respectively.

Regional myocardial blood flow to the ischemic region (measured during the fifth LAD occlusion) was virtually zero in both the subepicardial and the subendocardial layers of the LV wall in the two groups (Table 3). There were no statistically significant differences between the two groups with respect to epicardial, endocardial, or mean transmural flow to the non-ischemic zone (Table 3), indicating that the dose of nisoldipine used in this study had no coronary vasodilator effects.

Regional myocardial function

Systolic thickening fraction in the non-ischemic region remained stable during the sequence of LAD occlusions and the subsequent 5 h of reperfusion (Table 2). In addition, thickening fraction in the non-ischemic zone did not differ significantly between the groups (Table 2). Thus, the infusion of nisoldipine did not affect myocardial contractility in the non-ischemic zone.

Baseline systolic thickening fraction in the region to be rendered ischemic was $27.2 \pm 2.0\%$ and $26.7 \pm 1.6\%$ in the control and treated group, respectively. Figure 1 illustrates the serial measurements of thickening fraction expressed as a percentage of baseline measurements. After administration of either nisoldipine or vehicle (pre-occlusion measurements), the values of thickening fraction were virtually unchanged: $26.6 \pm 1.8\%$ and

Table 1 Basic physiological variables

Group	Body weight (kg)	pH	Po ₂ (mmHg)	Hematocrit (%)	Temperature (°C)
Control	28.3 ± 1.8	7.48 ± 0.01	86.3 ± 2.1	38.6 ± 1.4	39.3 ± 0.2
Treated	27.1 ± 2.0	7.49 ± 0.01	85.0 ± 2.2	39.8 ± 1.6	39.2 ± 0.1

Data are mean ± S.E.M. The control group ($n=12$) received vehicle; the treated group ($n=11$) received nisoldipine 0.5 µg/kg/min from 15 min before the first occlusion till 30 min after the last reperfusion.

27.9 ± 1.8% in control and treated pigs, respectively. The extent of paradoxical systolic thinning during ischemia did not change significantly with subsequent occlusions, so that during the 10th occlusion it was similar to that measured during the first occlusion (Fig. 1). The extent of systolic thinning during the first or 10th occlusion was similar in the two groups.

During the ten occlusion-reperfusion cycles there was a significant deterioration of wall motion with repeated ischemia, but no significant differences between the groups were noted (Fig. 1). After the ninth reperfusion, thickening fraction was 56.6 ± 6.0% and 55.1 ± 8.2% of baseline values in the control and treated groups, respectively. After the 10th reperfusion, a delayed recovery of contractile function was observed in both groups, indicating myocardial stunning. Analysis of the recovery by two-way ANOVA showed a main effect of group as well as a main effect of time, but no interaction between group and time. In control pigs, wall thickening was significantly decreased compared to baseline values for 4 h after the 10th reperfusion. In treated pigs, however, the rate of recovery was significantly faster throughout the reperfusion phase, with thickening fraction averaging 57.2 ± 5.3% of baseline value at 15 min in treated v 13.8 ± 8.7% in control pigs ($P<0.01$), 62.8 ± 3.7% v 18.0 ± 11.1% at 30 min ($P<0.01$), 57.6 ± 5.9% v 27.3 ± 10.9% at 1 h ($P<0.05$), 66.8 ± 8.3% v 44.1 ± 9.5% at 2 h (n.s.), 86.7 ± 2.6% v 61.3 ± 5.5% at 3 h ($P<0.01$), 93.6 ± 3.2% v 76.5 ± 3.9% at 4 h ($P<0.01$), and 97.9 ± 1.7% v 88.9 ± 2.9% at 5 h ($P<0.05$). The total deficit of wall thickening after the 10th reperfusion [an integrative assessment of post-ischemic dysfunction (Sun *et al.*, 1995)] was 51% less in treated pigs compared with controls ($P<0.001$) (Fig. 2).

Discussion

The present study demonstrates that nisoldipine markedly attenuates myocardial stunning in conscious pigs subjected to multiple recurrent ischemic

episodes. The drug prevented approximately half of the total post-ischemic dysfunction present in this model. This beneficial effect was rapid, being evident as early as 5 min after the last ischemic insult, and was sustained, being present throughout the 5-h recovery phase. The attenuation of myocardial stunning occurred independently of any significant change in hemodynamic variables, indicating a direct cardioprotective effect of the drug.

Methodological considerations

A pig model was chosen for several reasons. First, the paucity of collateral vessels in the porcine heart (Eckstein, 1954; White and Bloor, 1981; White *et al.*, 1986; Bloor *et al.*, 1986; Roth *et al.*, 1987; Schaper *et al.*, 1988) eliminates the variability in collateral flow that is inherent in canine models. Since collateral flow is the major determinant of the severity of myocardial stunning (Bolli *et al.*, 1988b) the elimination of this variable results in more reproducible post-ischemic dysfunction among different animals. Second, there are no published data regarding the effect of calcium channel blockers on post-ischemic myocardial contractile function *in vivo* in species other than the dog. Since the canine heart has a native coronary collateral circulation, it is unknown whether calcium channel blockers attenuate myocardial stunning *in vivo* in conditions of zero-flow ischemia. Third, like the human heart, the porcine heart lacks appreciable preformed collateral anastomoses (Eckstein, 1954; White and Bloor, 1981; White *et al.*, 1986; Bloor *et al.*, 1986; Roth *et al.*, 1987; Schaper *et al.*, 1988) and has minimal xanthine oxidase activity (Muxfeldt and Schaper, 1987; Podzuweit *et al.*, 1987; Grum *et al.*, 1989).

The rationale for using 10 2-min coronary occlusions was two-fold. First, almost all of the previous studies of calcium channel blockers in models of stunning have used a single coronary occlusion (Lamping and Gross, 1985; Przyklenk and Kloner, 1988; Warltier, 1988; Przyklenk *et al.*, 1989; Taylor *et al.*, 1990; Ehring *et al.*, 1992). The pathophysiology of myocardial stunning after repetitive

Table 2 Hemodynamic variables

Group	Baseline	Pre-occlusion	O ₁	R _d	Reperfusion					
					5 min	30 min	1 h	2 h	3 h	4 h
HR (beats/min)										
Control	106±5	102±3	104±5	103±5	107±4	109±4	106±3	110±4	113±5	119±2
Treated	112±6	114±8	119±7	113±8	125±9	116±7	116±7	115±6	120±6	118±4
SAP (mmHg)										
Control	123±4	124±4	122±5	123±5	120±4	120±4	120±3	115±3	114±2	108±2
Treated	121±4	121±5	116±3	116±3	112±3	113±3	117±3	116±2	113±3	112±3
DAP (mmHg)										
Control	78±4	78±3	83±5	83±4	75±4	76±4	74±4	73±4	72±3	70±2
Treated	79±3	77±5	73±3	72±3	71±3	70±3	73±3	72±2	73±2	72±2
RPP										
Control	12.9±0.6	12.5±0.4	12.6±0.5	12.6±0.6	12.8±0.5	13.1±0.6	12.6±0.4	12.6±0.4	12.9±0.6	12.9±0.4
Treated	13.5±0.9	13.8±1.1	13.3±0.9	12.9±0.9	14.0±1.0	13.0±0.7	13.5±0.7	13.4±0.7	13.6±0.7	13.3±0.6
LAP (mmHg)										
Control	7.1±0.7	6.8±0.6	8.0±1.0	—	4.7±0.7	6.0±0.5	5.2±0.4	4.7±0.4	4.9±0.3	4.8±0.5
Treated	7.3±1.0	7.0±0.9	9.0±0.9	—	6.7±0.8	6.0±0.5	5.8±0.6	5.3±0.6	5.4±0.6	5.8±0.6
LAD Flow (ml/min)										
Control	16.2±2.4	17.0±2.4	0	—	24.3±3.7	17.6±2.7	17.1±2.4	16.8±2.2	16.6±2.2	16.3±2.2
Treated	18.2±2.8	18.8±2.6	0	—	29.6±4.4	23.9±3.3	19.8±2.6	17.7±1.9	18.6±2.6	20.3±4.0
% ThF (NIZ)										
Control	26.1±1.7	26.6±1.7	26.9±1.9	—	24.4±2.1	25.4±1.9	24.1±1.7	25.4±1.9	24.0±2.0	23.6±2.3
Treated	25.8±2.9	26.2±2.9	26.7±3.2	—	27.1±2.7	26.6±2.7	26.9±3.0	27.8±2.9	26.1±3.1	25.9±2.9

Data are mean ± S.E.M. HR, heart rate; SAP, systolic arterial pressure; RPP, rate-pressure product (heart rate × systolic arterial pressure/1000); LAP, mean left atrial pressure; LAD Flow, blood flow in the left anterior descending coronary artery; % ThF (NIZ), % thickening fraction in the non-ischemic zone. Baseline measurements were taken before administration of nisoldipine or vehicle; pre-occlusion measurements were taken just before the first coronary occlusion. The control group received vehicle (n = 12); the treated group received nisoldipine 0.5 μg/kg/min from 15 min before the first occlusion till 30 min after the last reperfusion (n = 11).

Table 3 Regional myocardial blood flow

Group	Ischemic zone flow (ml/min/g)			Non-ischemic zone flow (ml/min/g)			IZF/NZF × 100%
	Epi	Endo	Mean	Epi	Endo	Mean	
Control	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	1.22 ± 0.09	1.44 ± 0.14	1.32 ± 0.11	3.11 ± 0.65
Treated	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	1.16 ± 0.05	1.43 ± 0.11	1.29 ± 0.08	3.18 ± 0.68

Data are mean ± S.E.M. Epi, epicardial flow; Endo, endocardial flow; Mean, mean transmural flow; IZF, ischemic zone flow; NZF, non-ischemic zone flow. IZF/NZF, ratio of transmural ischemic zone flow to simultaneous transmural non-ischemic zone flow.

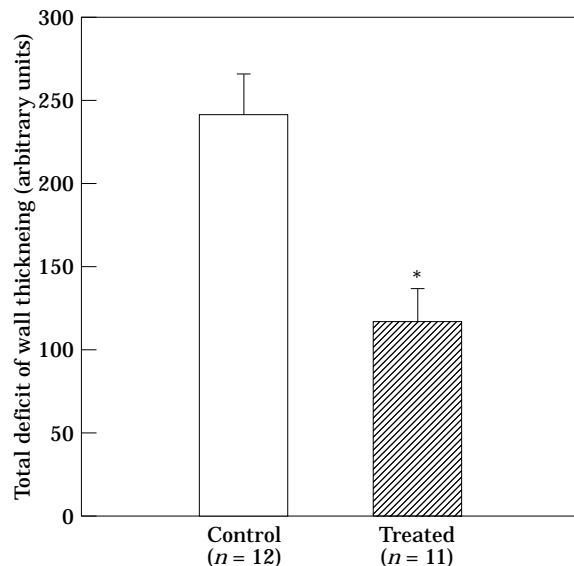


Figure 2 Total deficit of wall thickening after the 10th reperfusion in the control and nisoldipine-treated groups. The total deficit of wall thickening was measured in arbitrary units. Data are mean ± S.E.M. * $P < 0.001$ v control.

ischemia differs from that after a single ischemic episode in many respects (Bolli, 1990). For example, the dysfunction develops more gradually, is unrelated to collateral flow (Cohen and Downey, 1990; Bolli *et al.*, 1995), and the total ischemic burden is much greater. Also, the initial ischemic episodes "precondition" the heart against the stunning caused by the subsequent episodes (Cohen and Downey, 1990; Bolli *et al.*, 1995). Consequently, conclusions derived from models of single coronary occlusion may not apply to models of repetitive occlusions.

Second, the pathophysiology of myocardial stunning after repetitive ischemia is a clinically relevant problem, because many patients with coronary artery disease experience multiple recurrent episodes of ischemia (painful or silent) in the same territory as a result of recurrent spasm and/or thrombosis (Deanfield *et al.*, 1983; Cohn 1985; Fuster *et al.*, 1988). This is the main reason why several previous investigators have used models of myocardial stunning induced by multiple brief ischemic episodes

(Becker *et al.*, 1986; Hoffmeister *et al.*, 1986; Cohen and Downey, 1990; Bunch *et al.*, 1991; Yao *et al.*, 1993).

Previous studies

Calcium antagonists have been found to enhance recovery of ventricular function in isolated heart models (Naylor *et al.*, 1990), but it is often difficult to discern how much of the dysfunction seen in these models is due to stunning v infarction. Furthermore, results obtained in buffer-perfused, non-working, globally ischemic hearts may not necessarily be extrapolated to regional ischemia in blood-perfused hearts in whole animals. Nisoldipine has been reported to reduce myocardial infarct size in conscious dogs (Crottogini *et al.*, 1985), but no data are available regarding effect of this drug on myocardial stunning in conscious animal models. Several calcium channel blockers, including verapamil, diltiazem, nifedipine, nitrendipine, and amlodipine, have shown to improve recovery of function in regionally stunned myocardium in intact animals (Lamping and Gross, 1985; Przyklenk and Kloner, 1988; Warltier, 1988; Przyklenk *et al.*, 1989; Taylor *et al.*, 1990; Ehring *et al.*, 1992; Gross and Pieper, 1992). However, in most of these studies it is unclear whether the beneficial effects reflected a direct protective action or were mediated by favourable modifications of afterload, preload, heart rate, and regional myocardial blood flow. A direct protective action was reported in an open-chest dog model by Przyklenk *et al.* (1989). In this study, intracoronary administration of nifedipine was found to enhance the functional recovery of the stunned myocardium independently of any effect on systemic hemodynamics or regional myocardial blood flow. However, interpretation of these results is difficult because the beneficial effects of nifedipine were noted even when treatment was started 30 min after reperfusion, whereas myocardial stunning has been suggested to result from a transient calcium overload immediately after reflow (Kusuoka *et al.*, 1987), and the increased cytosolic

calcium levels have been demonstrated to return to normal within a few minutes after reperfusion (Steenbergen *et al.*, 1987; Marban *et al.*, 1987). In a recent elegant study, Ehring *et al.* (1992) demonstrated a direct protective effect of nisoldipine in enflurane-anesthetized dogs; in this study, the mean aortic blood pressure was kept constant and the regional myocardial blood flow to the ischemic area did not change. The protective effect, however, was observed only when nisoldipine was given before ischemia, not when it was given at reperfusion, in direct contradiction to the nifedipine study (Przyklenk *et al.*, 1989). Our results are consistent with the previously reported beneficial effects of calcium channel blockers and expand them by demonstrating that nisoldipine alleviates myocardial stunning in a conscious porcine model of repetitive ischemia independently of hemodynamic effects. We have recently reproduced the present results using a different series of animals (Sun *et al.*, 1996).

Mechanism of the protection of nisoldipine against stunning

Considerable evidence suggests that a transient calcium overload develops during early reperfusion and contributes to the pathogenesis of myocardial stunning after global ischemia *in vitro* (Kusuoka *et al.*, 1987; Steenbergen *et al.*, 1987; Marban *et al.*, 1987). However, this rise in intracellular free calcium during early reperfusion appears to be mediated by increased sodium/calcium exchange rather than via the L-type calcium channels (Bolli, 1990). Consequently, it seems unlikely that the primary mechanism of action of nisoldipine in the present study was blockade of calcium channels during early reperfusion. The failure of nisoldipine to attenuate stunning when given at the time of reperfusion (Ehring *et al.*, 1992) is also consistent with this interpretation. Previous studies have demonstrated that both the severity of myocardial stunning and the magnitude of the concomitant free radical generation upon reperfusion are determined primarily by the severity of the injury incurred during ischemia (Bolli *et al.*, 1988a, b; Li *et al.*, 1993). Consequently, any intervention that attenuates the severity of ischemic injury would be expected to attenuate the severity of the subsequent reperfusion injury. Calcium channel blockers attenuate the severity of ischemic injury by decreasing the influx of calcium during ischemic phase, which results in decreased ATP consumption (Nayler *et al.*, 1990). We therefore propose that the major

mechanism of action of nisoldipine in the present study was a decrease in calcium influx during ischemia, which resulted in attenuation of ischemic injury and, as a secondary effect, an attenuation in reperfusion injury.

It is important to stress that the beneficial effects of nisoldipine observed herein are not in conflict with the concept that oxygen-derived free radicals play a major role in myocardial stunning (Bolli, 1990). As elaborated above, generation of oxygen radicals upon reperfusion is closely related to the severity of the antecedent ischemic injury (Bolli *et al.*, 1988b; Bolli, 1990; Li *et al.*, 1993). Consequently, manipulations which alleviate the severity of ischemic injury would be expected to alleviate indirectly oxyradical-mediated damage upon reperfusion.

Another possible mechanism by which nisoldipine might produce a beneficial effect is by virtue of an antioxidant action. Calcium channel blockers, such as nisoldipine, have been suggested to lessen free radical-induced injury (Herbaczynska-Cedro and Gordon-Majszak, 1990; Nayler, 1992), and there is evidence that they can inhibit oxyradical-mediated lipid peroxidation of myocardial membranes (Herbaczynska-Cedro and Gordon-Majszak, 1990; Weglicki *et al.*, 1990; Sugawara *et al.*, 1994). However, in these studies (Herbaczynska-Cedro and Gordon-Majszak, 1990; Weglicki *et al.*, 1990; Sugawara *et al.*, 1994) calcium channel blockers were used in concentrations that were well above their pharmacologically active values. Furthermore, the inability of nisoldipine to alleviate myocardial stunning when given at reperfusion (Ehring *et al.*, 1992) argues against an effect on oxyradicals. Thus, it seems unlikely that the beneficial effect of nisoldipine might be due to its antioxidant action.

Conclusions

We have demonstrated a direct beneficial effect of nisoldipine against myocardial stunning in a conscious pig model of repetitive ischemia. Approximately half of the total post-ischemic dysfunction was eliminated by the administration of nisoldipine, and this effect was independent of hemodynamic changes. The present results corroborate previous studies in which protective effects of calcium channel blockers were shown in anesthetized open-chest preparations or in conditions in which significant changes of hemodynamics developed. The present results also expand previous

observations obtained in models of stunning caused by a single coronary occlusion.

With regard to the clinical implications of our data, there are many situations in which myocardial stunning may play an important role in precipitating left ventricular failure with its attendant morbidity and mortality (Bolli, 1992). Repetitive ischemia occurs commonly in patients with angina pectoris and other manifestations of coronary artery disease. The results of the present study imply that nisoldipine might be effectively administered to attenuate post-ischemic dysfunction in such clinical situations. Indeed, recent studies (Scognamiglio et al., 1991; Sheiban et al., 1993) suggest a beneficial effect of calcium channel antagonists in patients with post-ischemic myocardial stunning.

Acknowledgements

We thank Dr David Wood for helpful discussion and support of this study and Miles Laboratories, Inc., for supplying nisoldipine. We gratefully acknowledge Jennifer S. Pocius, and Alejandro Tumang, for expert technical assistance.

This study was supported in part by NIH Grant HL-43151 and SCOR Grant HL-42267 (R.B), and a grant from Miles Laboratories, Inc.

References

- BECKER LC, LEVINE JH, DiPAULA AF, GUARNIERI T, AVERSA-ANO TR, 1986. Reversal of dysfunction in postischemic stunned myocardium by epinephrine and post-extrasystolic potentiation. *J Am Coll Cardiol* 7: 580-589.
- BLOOR CM, WHITE FC, LAMMERS RJ, 1986. Cardiac ischemia and coronary blood flow in swine. In: Stanton HC, Mersmann HJ, eds, *Swine in Cardiovascular Research*. Boca Raton, Florida. CRC Press, 87-97.
- BOLLI R, 1990. Mechanism of myocardial "stunning". *Circulation* 82: 723-738.
- BOLLI R, 1992. Myocardial "stunning" in man. *Circulation* 86: 1671-1691.
- BOLLI R, MYERS ML, ZHU WX, ROBERTS R, 1986. Disparity of reperfusion arrhythmias after reversible myocardial ischemia in open chest and conscious dogs. *J Am Coll Cardiol* 5: 1047-1056.
- BOLLI R, PATEL BS, JEROUDI MO, LAI EK, McCAY PB, 1988a. Demonstration of free radical generation in "stunned" myocardium of intact dogs with the use of the spin trap alpha-phenyl *N-tert*-butyl nitron. *J Clin Invest* 82: 476-485.
- BOLLI R, ZHU WX, THORNBY JI, O'NEILL PG, ROBERTS R, 1988b. Time-course and determinants of recovery of function after reversible ischemia in conscious dogs. *Am J Physiol* 254: H102-H114.
- BOLLI R, ZUGHAIB M, LI X-Y, TANG X-L, SUN J-Z, TRIANA JF, McCAY PB, 1995. Recurrent ischemia in the canine heart causes recurrent bursts of free radical production that have a cumulative effect on contractile function: A pathophysiological basis for chronic myocardial "stunning". *J Clin Invest* 96: 1066-1084.
- BRAUNWALD E, KLONER RA, 1982. The stunned myocardium: Prolonged, postischemic ventricular dysfunction. *Circulation* 66: 1146-1149.
- BUNCH FT, THORNTON J, DOWNEY JM, 1991. Adenosine, an endogenous protectant against staccato ischemia. (Abstract) *Circulation* 84: II-306.
- COBB FR, BACH RJ, GREENFIEL JC, 1974. Regional myocardial blood flow in awake dogs. *J Clin Invest* 53: 1618-1625.
- COHEN MV, DOWNEY JM, 1990. Myocardial stunning in dogs: Preconditioning effect and influence of coronary collateral flow. *Am Heart J* 120: 282-291.
- COHN PF, 1985. Silent myocardial ischemia: classification, prevalence, and prognosis. *Am J Med* 79 [Suppl. 3A]: 2-12.
- CROTTIGINI AJ, DEPAOLI JR, BARRA JG, FISCHER EC, CHATRUC MR, PICHEL RH, DE LA FUENTE L, 1985. The effect of the new calcium antagonist nisoldipine (BAY k-5552) on myocardial infarct size limitation in conscious dogs. *Am Heart J* 110: 753-760.
- DEANFIELD JE, SELWYN AP, CHIERCHIA S, MASERI A, RIBEIRO P, KRIKLER S, MORGAN M, 1983. Myocardial ischemia during daily life in patients with stable angina: its relation to symptoms and heart rate changes. *Lancet* 2: 753-758.
- DUNCKER DJ, SAXENA PR, VERDOUW PD, 1988. Systemic haemodynamics of dihydropyridine derivatives in conscious pigs with or without propranolol. *Eur J Pharmacol* 157: 401-409.
- ECKSTEIN RW, 1954. Coronary interarterial anastomoses in young pigs and mongrel dogs. *Circ Res* 2: 460-465.
- EHRING T, BOEHM M, HEUSCH G, 1992. The calcium antagonist nisoldipine improves the functional recovery of reperfused myocardium only when given before ischemia. *J Cardiovasc Pharmacol* 20: 63-74.
- FUSTER V, BADIMON L, COHEN M, AMBROSE JA, BADIMON JJ, CHESEBRO J, 1988. Insights into the pathogenesis of acute ischemic syndromes. *Circulation* 77: 1213-1220.
- GROSS GJ, PIEPER GM, 1992. Beneficial actions of am- lodipine in the multiple-stunned canine myocardium. *Cardiovasc Drugs Ther* 6: 67-75.
- GRUM CM, GALLAGHER KP, KIRSH MM, SCHLAFAER M, 1989. Absence of detectable xanthine oxidase in human myocardium. *J Mol Cell Cardiol* 21: 263-267.
- HERBACZYNSKA-CEDRO K, GORDON-MAJSZAK W, 1990. Nisoldipine inhibits lipid peroxidation induced by coronary occlusion in pig myocardium. *Cardiovasc Res* 24: 683-687.
- HOFFMEISTER HM, MAUSER M, SCHAPER W, 1986. Repeated episodes of regional myocardial ischemia: Effect on local function and high energy phosphate levels. *Basic Res Cardiol* 81: 361-372.
- JUGDUTT BI, 1985. Different relations between infarct size and occluded bed size in barbiturate-anesthetized versus conscious dogs. *J Am Coll Cardiol* 6: 1035-1046.
- KUSUOKA H, PORTERFIELD JK, WEISMAN HF, WEISFELDT ML, MARBAN E, 1987. Pathophysiology and pathogenesis of stunned myocardium: Depressed Ca²⁺ activation of contraction as a consequence of reperfusion-induced

- cellular calcium overload in ferret hearts. *J Clin Invest* **79**: 950-961.
- LAMPING KA, GROSS GJ, 1985. Improved recovery of myocardial segment function following a short coronary occlusion in dogs in nicorandil, a potential new antianginal agent, and nifedipine. *J Cardiovasc Pharmacol* **7**: 158-166.
- LAXSON DD, HOMANS DC, DAI X-Z, SUBLETT E, BACHE RJ, 1989. Oxygen consumption and coronary reactivity in postischemic myocardium. *Circ Res* **64**: 9-20.
- LI X-Y, MCCAY PB, ZUGHAI B M, JEROUDI MO, TRIANA JF, BOLLI R, 1993. Demonstration of free radical generation in the "stunned" myocardium in the conscious dog and identification of major differences between conscious and open-chest dogs. *J Clin Invest* **92**: 1025-1041.
- MANDERS WT, VATNER SF, 1976. Effects of sodium pentobarbital anesthesia on left ventricular function and distribution of cardiac output in dogs, with particular reference to the mechanism for tachycardia. *Circ Res* **39**: 512-517.
- MARBAN E, KITAKAZE M, KUSUOKA H, PORTERFIELD JK, YUE DT, CHACKO VP, 1987. Intracellular free calcium concentration measured with ¹⁹F NMR spectroscopy in intact ferret hearts. *Proc Natl Acad Sci USA* **84**: 6005-6009.
- MUXFELDT M, SCHAPER W, 1987. The activity of xanthine oxidase in hearts of pigs, guinea pigs, rats, and humans. *Basic Res Cardiol* **82**: 486-492.
- NAYLER WJ, BUCKLEY DJ, LEONG J, 1990. Calcium antagonists and the "stunned" myocardium. *Cardioscience* **1**: 61-64.
- NAYLER WG, 1992. The role of oxygen radicals during reperfusion. *J Cardiovasc Pharmacol* **20** [Suppl. 5]: S14-S17.
- NING X-H, ZWENG TN, GALLAGHER KP, 1990. Ejection- and isovolumic contraction-phase wall thickening in nonischemic myocardium during coronary occlusion. *Am J Physiol* **258**: H490-H499.
- PODZUWEIT T, BRAUN W, MULLER A, SCHAPER W, 1987. Arrhythmias and infarction in the ischemic pig heart are not mediated by xanthine oxidase-derived free oxygen radicals. *Basic Res Cardiol* **82**: 493-505.
- PRZYKLENK K, GHAFARI GB, EITZMAN DT, KLONER RA, 1989. Nifedipine administered after reperfusion ablates systolic contractile dysfunction of postischemic "stunned" myocardium. *J Am Coll Cardiol* **13**: 1176-1183.
- PRZYKLENK K, KLONER RA, 1988. Effect of verapamil on postischemic "stunned" myocardium: Importance of the timing of treatment. *J Am Coll Cardiol* **11**: 614-623.
- ROTH DM, MARUOKA Y, ROGERS J, WHITE FC, LONGHURST JC, BLOOR CM, 1987. Development of coronary collateral circulation in left circumflex ameroid-occluded swine myocardium. *Am J Physiol* **253**: H1279-H1288.
- SAS INSTITUTE, 1988. *SAS/STAT User's Guide*, Release of 6.03 Edition. Cary, NC. SAS Institute, 675-712.
- SCHAPER W, GORGE G, WINKLER B, SCHAPER J, 1988. The collateral circulation of the heart. *Prog Cardiovasc Dis* **31**: 57-77.
- SCOGNAMIGLIO R, PONCHIA A, FASOLI G, MIRAGLIA G, DALLA-VOLTA S, 1991. Exercise-induced left ventricular dysfunction in coronary heart disease. A model for studying the stunned myocardium in man. *Eur Heart J* **12** [Suppl. G]: 16-19.
- SEKILI S, MCCAY PB, LI X-Y, ZUGHAI B M, SUN J-Z, TANG X-L, THORNBY JI, BOLLI R, 1993. Direct evidence that the hydroxyl radical plays a pathogenetic role in myocardial "stunning" in the conscious dog and that stunning can be markedly attenuated without subsequent adverse effect. *Circ Res* **73**: 705-723.
- SHEIBAN I, TONNI S, BENUSSI P, MARINI A, TREVI GP, 1993. Left ventricular dysfunction following transient ischemia induced by transluminal coronary angioplasty. Beneficial effects of calcium antagonists against post-ischemic myocardial stunning. *Eur Heart J* **14** [Suppl. A]: 14-21.
- STAHL LD, AVERSANO TR, BECKER LC, 1986. Selective enhancement of function of stunned myocardium by increased flow. *Circulation* **74**: 843-851.
- STEENBERGEN C, MURPHY E, LEVY L, LONDON RE, 1987. Elevation in cytosolic free calcium concentration early in myocardial ischemia in perfused rat hearts. *Circ Res* **60**: 700-707.
- SUGAWARA H, TOBISE K, ONODERA S, 1994. Absence of antioxidant effects of nifedipine and diltiazem on myocardial membrane lipid peroxidation in contrast with those of nisoldipine and propranolol. *Biochem Pharmacol* **47**: 887-892.
- SUN J-Z, TANG X-L, KNOWLTON AA, PARK S-W, QIU Y, BOLLI R, 1995. Late preconditioning against myocardial stunning: An endogenous protective mechanism that confers resistance to postischemic dysfunction 24 hours after brief ischemia in conscious pigs. *J Clin Invest* **95**: 388-403.
- SUN J-Z, TANG X-L, PARK S-W, QIU Y, TURRENS JF, BOLLI R, 1996. Evidence for an essential role of oxygen-derived free radicals in the genesis of late preconditioning against myocardial stunning in conscious pigs. *J Clin Invest* **97**: 562-576.
- TAYLOR AL, GOLINO P, ECKELS R, PASTOR P, BUJA LM, WILLERSON JT, 1990. Differential enhancement of post-ischemic segmental systolic thickening by diltiazem. *J Am Coll Cardiol* **15**: 737-747.
- TEMPLETON GH, WILDENTHAL K, WILLERSON JT, MITCHELL JH, 1975. Influence of acute myocardial depression on left ventricular stiffness and its elastic and viscous components. *J Clin Invest* **56**: 278-285.
- TRIANA JF, LI XY, JAMALUDDIN U, THORNBY JI, BOLLI R, 1991. Postischemic myocardial "stunning": Identification of major differences between the open-chest and the conscious dog and evaluation of the oxygen radical hypothesis in the conscious pig. *Circ Res* **69**: 731-747.
- VATNER SF, BRAUNWALD E, 1975. Cardiovascular control mechanisms in the conscious state. *N Engl J Med* **29**: 970-976.
- VATNER SF, FRANKLIN D, BRAUNWALD E, 1971a. Effects of anesthesia and sleep on circulatory response to carotid nerve stimulation. *Am J Physiol* **220**: 1249-1255.
- VATNER SF, HIGGINS CB, PATRICK T, FRANKLIN D, BRAUNWALD E, 1971b. Effects of cardiac depression and of anesthesia on the myocardial action of a cardiac glycoside. *J Clin Invest* **50**: 2585-2595.
- VAN DER GIESSEN WJ, VAN WOERKENS LJ, DUNCKER DJ, ROELANDT JRTC, VERDOUW PD, 1989. Acute hemodynamic effects of nisoldipine and pimobendan in conscious pigs with chronic heart failure. *J Cardiovasc Pharmacol* **14**: 653-658.
- WALLENSTEIN S, ZUCKER CL, FLEISS JL, 1980. Some statistical methods useful in circulation research. *Circ Res* **47**: 1-9.

- WARLTIER DC, GROSS GJ, BROOKS HL, PREUSS KC, 1988. Improvement of postischemic, contractile function by the calcium channel blocking agent nitrendipine in conscious pigs. *J Cardiovasc Pharmacol* **12** [Suppl. 4]: S120-S124.
- WEGLIICKI WB, MAK IT, SIMIC MG, 1990. Mechanisms of cardiovascular drugs as antioxidants. *J Mol Cell Cardiol* **22**: 1199-1208.
- WHITE FC, BLOOR CM, 1981. Coronary collateral circulation in the pig: Correlation of collateral flow with coronary bed size. *Basic Res Cardiol* **76**: 189-196.
- WHITE FC, ROTH DM, BLOOR CM, 1986. The pig as a model for myocardial ischemia and exercise. *Lab Animal Sci* **36**: 351-356.
- YAO Z, CAVERO I, GROSS GJ, 1993. Activation of cardiac KATP channels: an endogenous protective mechanism during repetitive ischemia. *Am J Physiol* **264**: H495-H504.
- ZHU WX, MYERS ML, HARTLEY CJ, ROBERTS R, BOLLI R, 1986. Validation of a single crystal for measurement of transmural and epicardial thickening. *Am J Physiol* **251**: H1045-H1055.