

Cardioprotective Effects of Amlodipine on Ischemia and Reperfusion in Two Experimental Models

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The cardioprotective effect of amlodipine, a long-acting dihydropyridine derivative, was studied in 2 experimental models of ischemia and reperfusion.

Isolated and blood-perfused feline hearts were made globally ischemic for 60 minutes and then reperfused for 60 minutes. Alterations of left ventricular developed pressure and compliance were monitored in both amlodipine-treated hearts and saline-treated control animals. Changes in perfusion pressure indicated that amlodipine significantly reduced myocardial oxygen consumption and coronary vascular resistance. Furthermore, a progressive increase in resting left ventricular diastolic pressure indicated that amlodipine, administered before the onset of global ischemia, attenuated the development of ischemic contracture. Return of contractile function 60 minutes after reperfusion and maintenance of tissue concentrations of electrolytes were significantly better in the amlodipine-treated group than in the control animals.

In intact canine hearts, regional myocardial ischemia was induced for 90 minutes, followed by 6 hours of reperfusion. Although the hemodynamic variables and the size of the region of risk did not differ significantly between treated animals and control animals, the infarct size was significantly smaller in the amlodipine-treated group than in the control animals, and a gradual reduction in coronary blood flow was observed in the control group that was prevented in the amlodipine group. A comparison of these findings with those observed with oxygen radical scavengers also is discussed.

A detailed report of these studies was published in *The American Journal of Cardiology* (1989;64:1011-1161). This review is included here to maintain continuity of the symposium for the convenience of the reader.

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Amlodipine is a dihydropyridine calcium antagonist shown to have a gradual onset and a sustained duration of action.¹⁻³ Because of the selectivity of the dihydropyridines for vascular smooth muscle and their lack of significant cardiac side effects,^{4,5} they may have the potential to limit myocardial injury during ischemia and reperfusion.⁶⁻⁸

Myocardial ischemia is associated with impaired calcium homeostasis.⁹⁻¹¹ Ischemic tissue injury causes an increase¹²⁻¹⁴ in free intracellular calcium that leads to diminished recovery of myocardial contractile function, compromised membrane integrity and decreasing reserves of cellular adenosine triphosphate. Inasmuch as the short-acting calcium antagonists diltiazem and nifedipine have been shown to protect the myocardium from irreversible tissue injury after global or regional ischemia followed by reperfusion,¹⁵⁻¹⁷ we assessed the potential for additional cardioprotective effects of a calcium antagonist with a prolonged duration of action, amlodipine, in 2 experimental models. The isolated and blood-perfused feline heart provided an experimental model for the induction of global myocardial ischemia¹⁸ and was used to ascertain the effects of amlodipine on mechanical and biochemical function after global ischemia and reperfusion. The intact canine heart provided a model for regional myocardial ischemia followed by reperfusion.^{19,20} In this review, we summarize our previously reported experimental work with these 2 models,²¹ followed by a discussion of the potential usefulness of amlodipine and its mechanisms of action in myocardial ischemia and reperfusion syndromes. We also compared these findings with those observed with oxygen radical scavenging agents.

EXPERIMENTS IN ISOLATED AND BLOOD-PERFUSED FELINE HEARTS

In an experimental model of isolated and blood-perfused feline hearts made globally ischemic for 60 minutes and followed by reperfusion for 60 minutes, infusion of amlodipine before the induction of global ischemia significantly ($p < 0.05$) curtailed the development of ischemic contracture. There was minimal or no increase in left ventricular end-diastolic pressure in the amlodipine-treated hearts, compared with a significant progressive increase in left ventricular end-diastolic pressure in saline-treated control animals.

Although no ventricular fibrillation occurred during the ischemic period, all hearts fibrillated immediately when reperfused. A better spontaneous recovery of sinoatrial rhythm (85.7%) was observed in the amlodipine-

treated hearts than in the saline-treated hearts (54.5%), but the difference was not statistically significant.

Significant ($p < 0.05$) decreases in left ventricular compliance were observed in the isolated hearts of both groups. However, the shift to the left in pressure-volume curves of the amlodipine-treated group was significantly ($p < 0.05$) less than that in the placebo group.

Pretreatment with amlodipine attenuated the declines in left ventricular developed pressure (Fig. 1) and left ventricular $+dP/dt$ (Fig. 2) observed in all hearts after ischemia and reperfusion. Thus, amlodipine protected the reperfused ischemic heart from depression of myocardial contractility. Coronary blood flow increased 30% ($p = 0.021$) in the saline-treated hearts and 127% ($p = 0.002$) in the amlodipine-treated hearts (Fig. 3). Despite the obviously greater increase in the amlodipine group, the differences between treatments were short of statistical significance.

Amlodipine significantly reduced the loss of intracellular potassium and the accumulation of calcium during reperfusion (Fig. 4). These results support the conclusion that this agent preserves both systolic and diastolic functions of the isolated ischemic and reperfused heart.

EXPERIMENTS IN INTACT CANINE HEARTS

Is amlodipine capable of actually reducing anatomic myocardial infarct size? To answer this question, anesthetized dogs were subjected to 90 minutes of coronary artery occlusion followed by 6 hours of reperfusion through a critical stenosis. The dogs were assigned randomly to treatment with either amlodipine (150 $\mu\text{g}/\text{kg}$ dissolved in 20 ml saline) or with saline alone. Treatment was administered 75 minutes after the start of regional

myocardial ischemia and 15 minutes before the onset of reperfusion.

The overall area at risk (measured by the Evans-blue dye technique) for both amlodipine-treated and control dogs was $40.2 \pm 4.9\%$. Amlodipine achieved a 24.8% reduction ($p \leq 0.025$) in ultimate infarct size (measured by the triphenyltetrazolium chloride technique), determined as a percentage of the area at risk (Fig. 5). A significant difference ($p < 0.05$) was also observed when the infarct size was expressed as a percent of the left ventricle: $18.6 \pm 1.5\%$ for the saline group and $13.6 \pm 1.6\%$ for the amlodipine group. There were no significant differences in hemodynamics between the 2 groups.

The calcium concentration of tissue within the area of risk was greater than that of myocardial tissue from the nonischemic region (Fig. 6). Amlodipine significantly decreased the elevation of calcium in the noninfarcted tissue within the area at risk and somewhat diminished calcium in the center of the infarcted zone.

DISCUSSION

These experiments in isolated and blood-perfused feline hearts show that amlodipine given before the onset of global ischemia preserves myocardial function. Similar cardioprotection has been demonstrated with other calcium antagonists.²²⁻²⁴ In addition, we demonstrated in the intact canine heart that amlodipine given after the onset of ischemia 15 minutes before reperfusion reduces the extent of myocardial necrosis after 90 minutes of regional ischemia. Calcium antagonists,^{6,15,17} administered before the onset of ischemia, have been shown to reduce infarct size in models of permanent occlusion and ischemia-reperfusion.

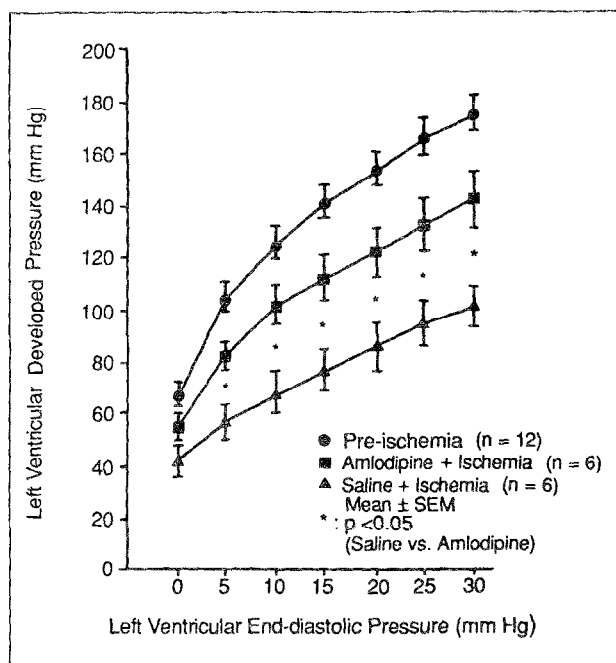


FIGURE 1. Ventricular function curves of the isolated and blood-perfused feline heart. SEM = standard error of the mean. (Redrawn, with permission, from Hoff et al.²¹)

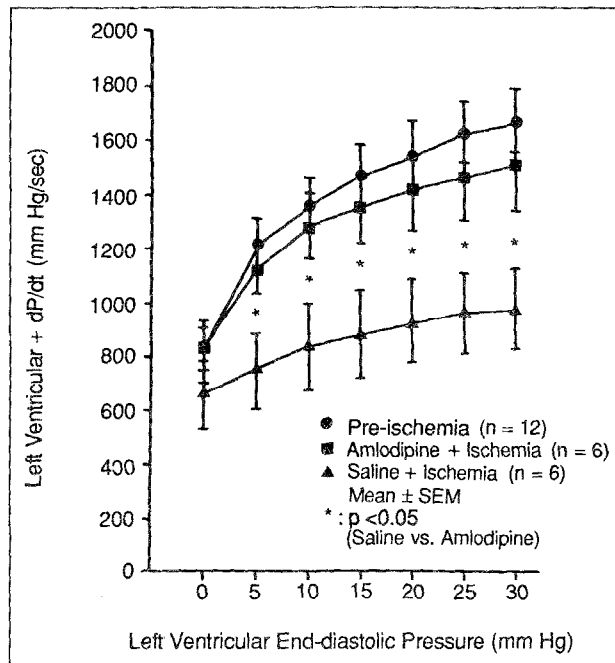


FIGURE 2. Left ventricular $+dP/dt$ of the cat blood-perfused, isolated heart. SEM = standard error of the mean. (Redrawn, with permission, from Hoff et al.²¹)

The isolated and blood-perfused feline heart allowed an assessment of the effects of amlodipine on myocardial performance and electrolyte balance independent of hemodynamic and central nervous system variations. In addition, this model had the advantage of assuring global ischemia and eliminating the possible mechanism of coronary steal.²⁵

Experiments in isolated myocytes have shown that pretreatment with the calcium antagonists nifedipine and diltiazem did not prevent hypercontracture, but pretreatment with the nonspecific agents lidoflazine and flunarazine did afford protection.²⁶ These findings suggest a possible intracellular action of calcium regulation. Spere-

lakis²⁷ suggested that the increase in calcium during ischemia might be a consequence of failure of the metabolic processes to extract free calcium from the cell. Dhalla et al.²⁸ demonstrated an inhibition of sarcoplasmic reticular calcium adenosine triphosphatase uptake and sodium-calcium exchange mechanisms during anoxia and ischemia. It is likely that calcium has more than 1 route of entry during reperfusion. The dihydropyridines are believed to bind selectively to sites directly within the ion channel,²⁹ which may impart some undetermined action independent of ion flux.³⁰ This action may provide an explanation for the observation that specific calcium antagonists can postpone, but not abolish, the reperfusion-induced accumulation of calcium that leads to cell death.

Our data from the isolated cardiac preparation suggest that amlodipine limits the alterations in electrolyte homeostasis, namely, the increases in total tissue calcium and sodium and the decrease in potassium associated with ischemia and reperfusion. The increase we observed in tissue calcium is compatible with the rapid accumulation of calcium associated with reperfusion. This cannot occur solely through activation of the slow channels.³¹ Various mechanisms have been proposed for the gradual deterioration of the membrane during ischemia. These include activation of phospholipases³² or accumulation of lipids³³ or oxygen free radicals,³⁴ which remove the physical barriers allowing the free diffusion of calcium down its concentration gradient at reperfusion. The decline in total calcium after pretreatment with amlodipine may result from an inhibition of the events that trigger membrane damage during ischemia; the metabolic and structural changes that normally provide the substrate for reperfusion injury (calcium overload) may be eliminated.

Amlodipine inhibited the increase in left ventricular end-diastolic pressure throughout the 60 minutes of ischemia. By suppressing the induction of myocyte hypercon-

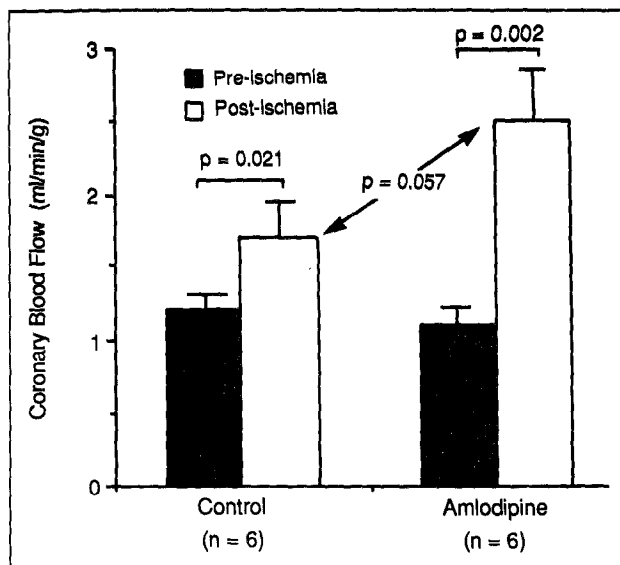


FIGURE 3. Coronary blood flow before and after 60 minutes of ischemia plus reperfusion. (Redrawn, with permission, from Hoff et al.²¹)

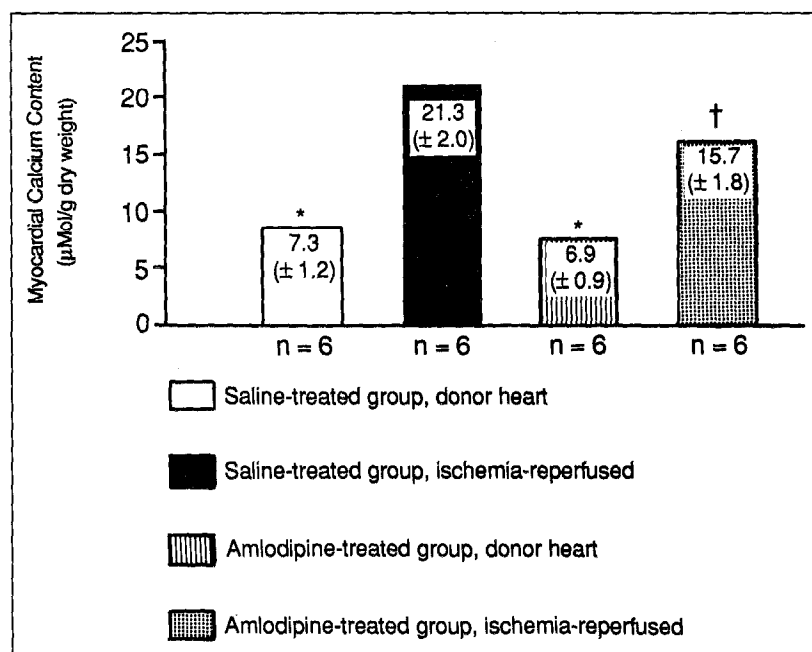


FIGURE 4. Myocardial calcium content (μmol/g, dry weight). *p ≤ 0.05: donor heart vs ischemia-reperfused heart. †p ≤ 0.05: saline-treated group vs amlodipine-treated group. (Data from Hoff et al.²¹)

tracture, amlodipine may obviate additional mechanical disruption and chemical degradation of the sarcolemmal membrane and render the cells less susceptible to calcium overload during reperfusion.

The intact canine heart model provided the opportunity to assess drug responses under more physiologic conditions. The ability of amlodipine to reduce the extent of myocardial necrosis that occurs during the late stage of ischemia and reperfusion was assessed in this model. We previously demonstrated that reproducible regional myocardial necrosis can be induced in the canine heart after temporary coronary occlusion followed by reperfusion.^{15,19,20} With physiologic hemodynamic and central nervous system responses maintained, it was possible to monitor drug-related changes in systemic arterial blood pressure, heart rate, coronary blood flow and myocardial oxygen consumption.

First-generation calcium antagonists administered before the onset of coronary occlusion in intact animal hearts have been shown to reduce infarct size and im-

prove cardiac function.^{15,35} By directly inhibiting neutrophil activation, these agents may help to reduce the ultimate extent of damage.^{36,37} However, they have not been shown to be effective if administered at the time of perfusion.³⁰ Solutions containing low concentrations of calcium or acidic reperfusate cannot prevent cellular damage, although they can delay its onset.³¹

Much of the damage associated with reperfusion is thought to occur within minutes of the resumption of flow.³⁸ Total cellular calcium concentration remains constant during ischemia and increases precipitously during reperfusion.³⁹ The rapid loss of sarcolemmal membrane integrity associated with restoration of flow and derangement of homeostatic mechanisms most likely prevent calcium antagonists from improving cardiac function and reducing infarct size when administered during reperfusion only. Ischemia primes the cells for destruction through activation of proteases, oxygen free radicals and calcium adenosine triphosphatase.^{11,28} During reperfusion, the sarcolemma is disrupted, a phenomenon that is

FIGURE 5. Region of tissue representing the area at risk as a percentage of the left ventricle and myocardial infarct size taken as a fraction of the area at risk. NS = not significant. (Redrawn, with permission, from Hoff et al.²¹)

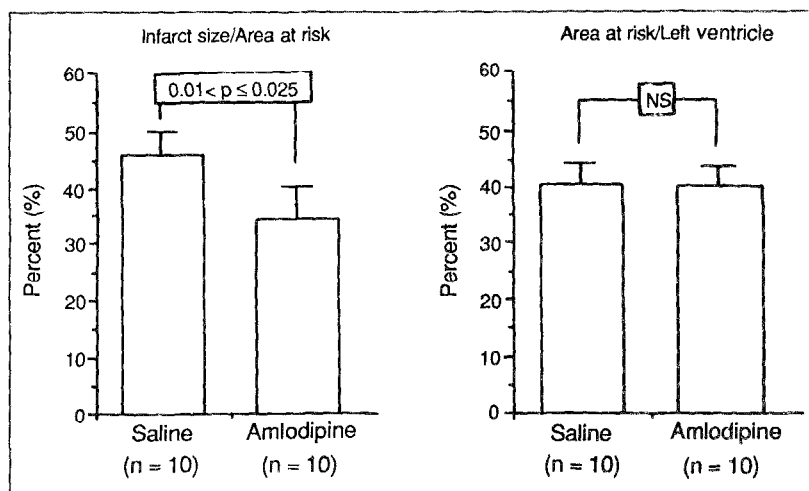
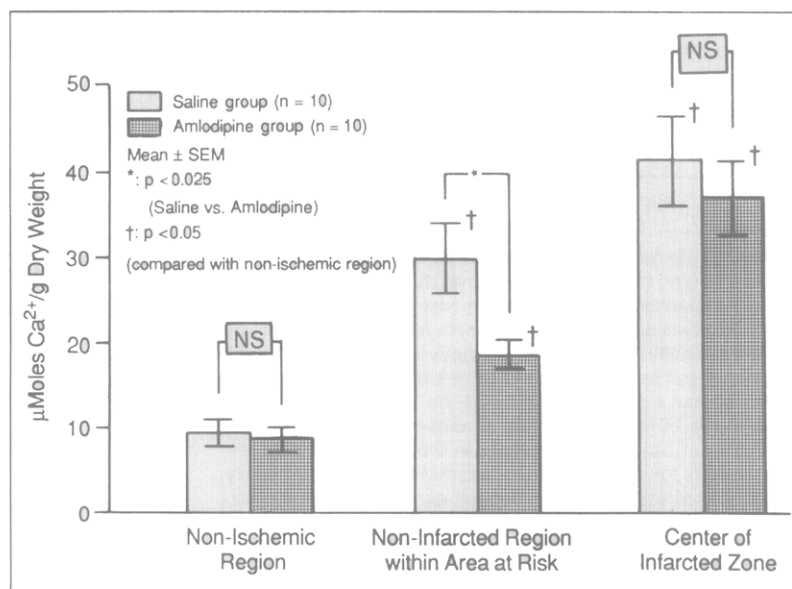


FIGURE 6. Tissue calcium (Ca^{2+}) concentration in the canine myocardium after 90 minutes of occlusion and 6 hours of reperfusion. NS = not significant; SEM = standard error of the mean. (Redrawn, with permission, from Hoff et al.²¹)



not manifest during ischemia alone, although the cell membrane has altered permeability.^{31,40,41} Hence, calcium can move freely into the cytoplasmic space during reperfusion. Calcium influx may be one of the final common pathways leading to myocardial dysfunction and necrosis.

Amlodipine administered 15 minutes before the beginning of reperfusion reduced infarct size and intracellular calcium concentrations in the noninfarcted tissue in the region at risk. Neither verapamil nor diltiazem administered at the time of reperfusion exhibited a cardioprotective effect, although nifedipine and the calmodulin antagonist, W-7, were effective when administered at reperfusion or before ischemia.³⁰ Thus, calcium antagonists may have different effects on the sarcolemmal membrane, with the second-generation calcium antagonist, amlodipine, showing a greater ability to prevent sarcolemmal disruption on reperfusion. Additionally, nifedipine may provide an intracellular action similar to that of W-7 on calmodulin.⁴²

The results of our experiments suggest that the reduction in myocardial infarct size was independent of altered hemodynamics, which did not show differences between treated and control dogs. These results agree with our findings in the isolated hearts and suggest that amlodipine's mechanism of action is not due simply to altering heart rate or blood pressure.

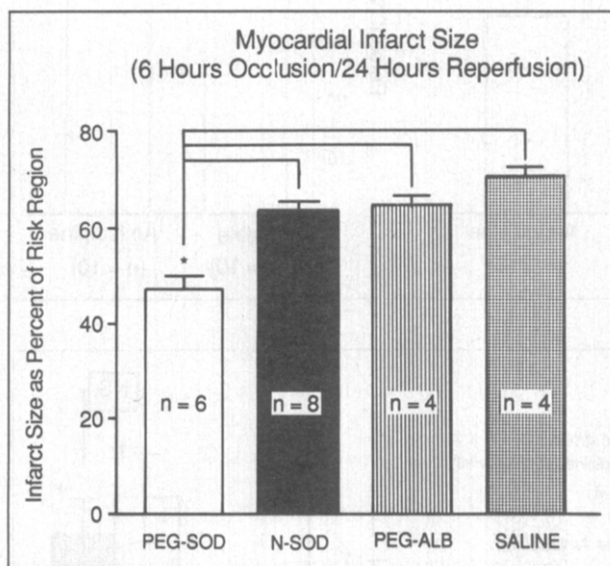


FIGURE 7. Reduction in myocardial infarct size in the canine heart subjected to 6 hours of regional ischemia followed by 24 hours of reperfusion. Salvage of myocardial tissue is achieved with the polyethylene conjugated form of superoxide dismutase (PEG-SOD) in contrast to the native enzyme (SOD), which does not result in a reduction in irreversible myocardial injury. PEG-SOD has a pharmacologic half-life >30 hours as compared to the 10-minute half-life of native SOD. The continued generation of oxygen free radicals in the postischemic heart may account for the progressive extension of myocyte injury. An agent that will provide protection against the damage associated with reperfusion must possess a pharmacokinetic profile that will extend into the period during which irreversible cell injury can occur. PEG-ALB = polyethylene conjugated form of albumin.

Excessive influx of calcium occurs almost exclusively during reperfusion and cannot be due entirely to increased conductance through the calcium channel.^{31,43} The administration of amlodipine 15 minutes before reperfusion achieved a reduction of infarct size typical of hearts treated before ischemia, but distinct from those treated only during reperfusion. Amlodipine administered prophylactically during late ischemia may provide enough protection to sustain the sarcolemma and calcium homeostasis mechanisms during reperfusion. Lowered accumulation of tissue calcium compared with control animals indicates that amlodipine may directly protect the myocardium during ischemia, and the effect may persist into the reperfusion phase either through membrane stabilization or intracellular mechanisms. Moreover, amlodipine may inhibit activation of leukocytes that would curtail enzymatic and oxidative damage consequent to ischemia and reperfusion.^{36,37}

The evidence for generation of oxygen free radicals during myocardial reperfusion would suggest that superoxide dismutase (SOD) and other free radical scavengers might alter the extent of myocardial injury. A number of studies from our laboratory as well as those of others have supported the hypothesis that free radical scavengers have a protective action against the extension of myocardial injury related to reperfusion.⁴⁴ Chi et al⁴⁵ have presented evidence for the sustained presence of oxygen radical scavenger activity as being essential for the prevention of infarct extension rather than delay of necrosis after reperfusion. Figure 7 presents a summary of the results of a comparison made between the native form of SOD (with a half-life of 10 minutes) and the polyethylene conjugated form of the enzyme (PEG-SOD), which has a half-life in excess of 30 hours. Only the PEG-SOD was effective in reducing ultimate infarct size when assessed 24 hours after reperfusion. The interpretation of the results conforms to that cited earlier⁴⁶ in which there was a direct demonstration of sustained oxyradical generation in the postischemic heart. Additional studies are needed to determine whether there is a link between the reduction in infarct size as achieved with calcium antagonists and that obtained with oxygen radical scavengers. Do oxyradicals impair the removal of calcium from the myocardial cell? Do calcium antagonists provide their beneficial effect by interfering with ion movement in the slow calcium channel? Do calcium antagonists affect cell-to-cell interactions that are known to participate in reperfusion injury such as the events involving neutrophil-mediated cell injury? Substantial investigative efforts must now be directed toward resolving some of the questions regarding the manner in which several diverse pharmacologic agents can achieve a similar outcome with respect to reducing myocardial reperfusion injury.

The pharmacokinetic profile of amlodipine may prove valuable in protection of the postischemic heart. A single intravenous dose of 10 mg resulted in an absolute bioavailability of 64% and a calculated half-life of 34 hours. Similar values were obtained after oral administration of the drug. The pharmacokinetic data with amlodipine contrast with the results of similar studies using most

other dihydropyridine and nondihydropyridine calcium antagonists. Most dihydropyridine calcium antagonists have an elimination half-life of about 3 to 10 hours. The nondihydropyridine calcium antagonists show an elimination half-life of 3 to 6 hours. The prolonged generation of oxyradicals in the postischemic heart may adversely affect calcium homeostasis. It is intriguing to suspect, therefore, that amlodipine may serve to modulate the otherwise unfavorable change in intracellular calcium and thereby reduce the extent of irreversible cell injury associated with reperfusion. The prolonged pharmacologic action of amlodipine would provide the extended duration of protection necessary to reduce the extent of the cellular damage in the postischemic heart.

There is a growing body of evidence indicating the participation of reactive oxygen metabolites (superoxide anion, hydrogen peroxide, hydroxyl anion) as contributors to the tissue damage associated with reperfusion.^{44,47} The exact mechanism by which reactive oxygen species produce alterations in cardiac function and myocyte viability is not known. Recent studies have suggested that the sarcolemmal membrane is altered by exposure to superoxide anion, and this may result in depressing the calcium-pump mechanism that facilitates efflux of the divalent ion from the myocardial cell.^{48,49} The inhibition of sarcolemmal calcium-pump activities by superoxide anion was decreased by the addition of dithiothreitol or cysteine in a dose-dependent manner.⁴⁹ Heart sarcolemmal sulfhydryl groups were depressed by superoxide anion, hydrogen peroxide and hydroxyl anion. SOD, catalase and D-mannitol showed protective effects on the sulfhydryl group depression by the several reactive oxygen metabolites. The increase in intracellular calcium observed to occur in myocardial ischemia/reperfusion injury may be a manifestation of an oxygen free radical-mediated depression of the mechanism(s) responsible for removal of cytosolic free calcium. Spin trapping techniques have been used to demonstrate the formation of free radicals during regional myocardial ischemia. Evidence has been provided to indicate the presence of oxygen- and carbon-centered radical adducts in the coronary venous blood draining from the ischemic bed on reperfusion of the ischemic heart.⁵⁰ Studies in the canine heart subjected to 90 minutes of regional ischemia followed by reperfusion have demonstrated the sustained generation of oxyradicals 1 to 3 hours after the restoration of blood flow. There was an associated progressive increase in infarct size supporting the view that a chain reaction of oxyradicals contributes to the propagation of myocardial cell damage in the postischemic heart.⁴⁶

In conclusion, experimental data demonstrate that amlodipine, a second-generation long-acting dihydropyridine with somewhat unique binding properties, is effective in preserving myocardial contractile function and attenuating calcium accumulation after 60 minutes of ischemia and reperfusion when administered before the onset of ischemia. It also limits ultimate infarct size when administered 15 minutes before the beginning of reperfusion and after 75 minutes of regional ischemia. The protective effect was associated with reduced accumulation

of calcium in noninfarcted tissue in the area at risk. Our data suggest that amlodipine may have utility as a cardio-protective agent to preserve cardiac function and cell viability during myocardial ischemia and reperfusion.

Comparable data with respect to the reduction in infarct size and the preservation of contractile function after brief periods of global or regional ischemia have been obtained with agents known to scavenge oxygen free radicals. There is evidence to suggest that oxyradicals may influence the mechanisms that control calcium accumulation in the myocardial cell and thereby lead to irreversible cell injury or an alteration in contractile function. It is intriguing to speculate that a common mechanism that subjects the myocyte to permanent damage may be operative. Amlodipine, with its interesting pharmacologic and pharmacokinetic profiles, may provide a means of modulating the myocardial injury in the postischemic heart.

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