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Na-H Exchange Inhibition with Cariporide Limits Functional Impairment Caused by Repetitive Ischemia

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Abstract

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Summary:

Intracellular calcium ([Ca]i) overload on reperfusion may be one of the mechanisms responsible for ischemia-induced regional myocardial dysfunction. Because inhibiting the Na-H exchanger (NHE) limits intracellular sodium ([Na]i) and subsequent [Ca]i accumulation, we hypothesized that NHE inhibition would attenuate regional dysfunction in response to 25 cycles of ischemia (I, 2-min) and reperfusion (R, 8-min) of the left circumflex coronary artery (LCx) in conscious swine. Six animals were instrumented to measure arterial pressure, regional myocardial blood flow (colored microspheres), systolic wall thickening (WTh) in the normally perfused (left anterior descending, LAD) and LCx regions (sonomicrometry), LCx blood flow velocity (Doppler), and to reversibly occlude the LCx (hydraulic occluder). Each animal completed three protocols separated by 7 days: ISC, 25 I/R cycles; CAR, 25 I/R cycles + NHE inhibition (cariporide); and VEH, vehicle administration for 4.2 h. Regional myocardial blood flow was measured during LCx occlusion in the first protocol and 10 min after I/R 25 in all protocols. Systemic hemodynamics were similar among and within each protocol. Blood flow measured during LCx occlusion confirmed that perfusion was reduced ($p < 0.05$) to this compared with the LAD region. During ISC, LCx WTh was reduced ($p < 0.05$) after five I/R cycles, and a stable reduction ([almost equal to]55% of baseline; $p < 0.05$) was present after 20 I/R cycles. During CAR, LCx systolic WTh was reduced ($p < 0.05$) only after 15 and 25 I/R cycles ([almost equal to]80 and 72%, respectively). The decrease in LCx WTh was greater in ISC than in CAR ($p < 0.05$). LCx WTh was not altered during VEH, while LAD WTh was similar within and among all protocols. Regional blood flow measured after 25 I/R cycles was not different among protocols. Our results indicate that NHE inhibition delays the onset and limits the degree of regional dysfunction in response to repeated bouts of ischemia and reperfusion.

Patients with coronary artery disease may exhibit chronic left-ventricular dysfunction that improves with revascularization (1). Although compromised contractile function may occur from exposure to

persistent sublethal ischemia (2,3), persistent regional dysfunction may be caused by recurrent bouts of ischemia (4). In this paradigm, dysfunctional yet viable myocardium is perfused antegrade from a significantly stenosed artery or retrograde by collateral vessels or both. In either instance, imbalances between myocardial oxygen supply and demand occur when the metabolic requirement for oxygen increases (e.g., exercise).

Although the precise mechanisms responsible for postischemic myocardial dysfunction are not clear, evidence exists that both calcium overload on reperfusion (5,6) and free radical generation are involved (7). Data implicating the former possibility are that calcium transport is abnormal in postischemic myocardium (5,8) and that ischemia-induced myocardial dysfunction is attenuated when intracellular calcium ([Ca]i) entry is limited (9).

Whereas direct inhibition of [Ca]i entry may be effective in limiting myocardial dysfunction, results indicate that reducing the stimulus for [Ca]i entry on reperfusion also may be protective. A primary stimulus for [Ca]i entry on reperfusion is the accumulation of intracellular sodium ([Na]i) during ischemia and early reperfusion (6), resulting in the subsequent exchange of sodium for calcium via the Na-Ca exchanger (10). Therefore, interventions that limit [Na]i entry can reduce [Ca]i overload, and thus, myocardial dysfunction.

Studies concerned with limiting [Na]i entry during ischemia and reperfusion have focused on the Na-H exchanger (NHE; 11-13). In the presence of intracellular acidosis, this ubiquitous membrane protein exchanges one proton for one sodium, thereby limiting acidosis at the expense of increased [Na]i (14). As with direct limitation of [Ca]i, NHE inhibition lessens ischemia-induced dysfunction resulting from one or two bouts of myocardial ischemia in anesthetized animal models (11,13,15,16).

Because of the effect of NHE inhibition on [Na]i and [Ca]i, we hypothesized that NHE inhibition using cariporide would attenuate the impairment of regional function induced by repeated bouts of ischemia and reperfusion in a conscious animal model of myocardial stunning. Stable regional dysfunction was produced by 25 cycles of left circumflex coronary artery occlusion (2 min) and reperfusion (8 min) in conscious swine. When this protocol was performed in the same animals in the presence of NHE inhibition using cariporide, the onset of regional dysfunction was delayed, and the degree of impairment was lessened throughout the protocol.

MATERIALS AND METHODS

Surgical procedures

Surgical and experimental protocols used in this study were approved by the Animal Use and Care Committee at the University of California, Davis. One day before surgery, pigs were given 400 mg sulfamethoxazole and 80 mg trimethoprim orally (Tribrisson) and then were fasted overnight. Immediately before surgery, animals were sedated with ketamine (25 mg/kg, i.m.) and atropine (0.05 mg/kg, i.m.), intubated after relaxation by mask induction with 1-3% halothane, and then were given 1 g of cefazolin sodium (Kefzol, i.v.). Anesthesia was maintained during the aseptic procedure with 1-3% isoflurane. With the animal in a dorsal recumbent position, an incision was made in the neck, the carotid artery was isolated, and a polymeric silicone (Silastic) catheter was inserted and advanced to the descending aorta. Next, the pig was repositioned so that a left lateral thoracotomy could be performed through the fifth intercostal space, a Silastic catheter inserted into the left atrial appendage, and four recording electrodes sewn to the surface of the myocardium. A 1- to 2-cm segment of the proximal left circumflex coronary artery (LCx) then was dissected from adherent tissue, and a hydraulic occluder (In Vivo Metric, Healdsburg, CA, U.S.A.) and Doppler flow probe (Triton Technology, San Diego, CA, U.S.A.) placed around the vessel. Two pairs of sonomicrometer dimension gauges (5 MHz, 2.5 mm in diameter; J.W. Inc, San Diego, CA, U.S.A.) were placed across the left ventricular free wall for measuring systolic wall thickening (WTh; Triton Technology). One set of gauges was placed distal to the hydraulic occluder in the area perfused by the LCx (i.e., the region to be made ischemic), whereas the second pair was positioned approximately midway between the apex and base of the heart in a region supplied by the left anterior descending coronary artery (LAD; the normally perfused region). Accurate placement of the gauges was confirmed by decreased systolic WTh or frank wall thinning in the LCx region, with no change or increased systolic WTh in the LAD perfusion territory in response to a brief (20- to 30-s) inflation of the occluder. Dimension gauges were repositioned if placement criteria were not met. Throughout the surgical procedure, arterial blood gases were monitored so that pH could be maintained between 7.35 and 7.45. All catheters and wires were exteriorized on the pig's back. After surgery, each animal was fitted with a custom-made jacket (Alice King Chatham, Los Angeles, CA, or Fabric Expressions, Seattle, WA, U.S.A.) to protect catheters and their exit sites.

The health status of the animals was monitored 7 days per week. Kefzol (1 g, i.a.) was administered daily for 7 days, followed by oral Tribrisson each day thereafter. Catheters were flushed and exit sites cleaned every second day. The pigs were housed individually in pens and fed once per day. We have used these techniques previously (17,18).

Hemodynamic and blood-flow measurements

Systolic, diastolic, and mean arterial blood pressures, heart rate (HR), ECG, LCx blood-flow velocity, and signals obtained from the sonomicrometer dimension gauges were monitored and recorded on a Gould TA 4000 (Cleveland, OH, U.S.A.). The heart rate times systolic blood pressure product (RPP) was used to estimate myocardial oxygen demand.

Systolic WTh was measured over the ejection phase of systole. The timing of systole was determined by using the peak of the S wave and the end of the T wave of the ECG (19). This method of timing the ejection phase was compared with more traditional methods (e.g., dP/dt) and found to be similar both at rest and during demand-induced regional ischemia in conscious pigs (18,19). Regional myocardial function data are expressed as percentage systolic

WTh, calculated as end-systolic WTh minus end-diastolic WTh divided by end-diastolic WTh times 100. All hemodynamic and regional function calculations were determined over five to 10 consecutive cardiac cycles.

Regional blood flow was measured by injecting [almost equal to]3 × 10^6 colored microspheres (e.g., blue, yellow, red, or white; 15 μm, Triton Technology) into the left atrium 15 s after initiating withdrawal of the reference sample from the carotid artery. The withdrawal was continued for a total of 2 min (7 ml/min), and myocardial blood flow was calculated by using techniques that have been described (20). Renal blood flow also was measured so that adequate mixing of the microspheres could be documented (i.e., <15% difference in blood flow between right and left kidney).

Experimental protocols

At least 7 days after surgical instrumentation, baseline arterial blood gases and rectal temperatures were obtained while the animals stood quietly in the transport cage. Next, pigs were suspended in a large animal sling and mild sedation attained by using propofol ([almost equal to]0.2 mg/kg/min, i.a., a gift from Zeneca Pharmaceuticals, Wilmington, DE, U.S.A.). For the duration of each experimental protocol, supplemental oxygen (0.25-0.50 L/min; 100% O_2) was administered using a nasal cannula. After hemodynamic variables and blood gases were stable (e.g., for 20-30 min), animals began a 60-min baseline period in one of three experimental protocols, each separated by 7 days.

Twenty-five ischemia and reperfusion (I/R) cycles + vehicle; ISC. The purpose of the ISC protocol was to document the time course of development and the extent of regional dysfunction in response to 25 I/R cycles of the LCx. Vehicle (saline; [almost equal to]12-ml bolus) was administered into the left atrium at the beginning of the 60-min baseline period, and a constant infusion (0.04 ml/min) was started at the end of this period and continued throughout the 25 I/R cycles. Each I/R cycle consisted of inflating the hydraulic occluder for 2 min followed by reperfusion for 8 min. The diameter of the occluder was decreased by using an indeflator to an extent whereby phasic variations (both audible and visual) in LCx blood-flow velocity were eliminated. Subsequent inflations during both protocols requiring LCx occlusion were guided by the same parameters, so that similar reductions in LCx blood-flow velocity were achieved. Although hemodynamic variables were monitored throughout the entire protocol, MAP, HR, RPP, LCx and LAD systolic WTh, and LCx blood-flow velocity were calculated immediately before occlusion and at 2 min of occlusion for I/R 1, 5, 10, 15, 20, and 25. Arterial blood gases and rectal temperatures were taken before I/R 1, 5, 10, 15, 20, and 25. In addition, to document whether the I/R regimen elicited indices of myocyte damage, arterial blood samples were obtained for later analysis of plasma creatine kinase (21).

Blood flow was determined after ~1.5-min during the last 2-min LCx occlusion. Because of the limited number of colors available whose wavelengths could be separated accurately, occlusion blood flow was assessed only during this protocol. Regional blood flow was measured [almost equal to]10 min after I/R 25 (or sham I/R 25) in all three protocols. Whereas the blood flow after VEH was used to determine baseline perfusion, transmural flow after ISC and CAR was used to assess whether regional perfusion was altered in the presence of regional dysfunction.

Twenty-five I/R cycles + NHE inhibition; CAR. The purpose of this protocol was to examine whether NHE inhibition attenuates the time course and/or the magnitude of regional dysfunction in response to 25 I/R cycles of the LCx. Experimental procedures were identical to those described in ISC, except that the NHE inhibitor [cariporide (formerly known as HOE 642), 3 mg/kg bolus, 12 ml total, i.a.) was administered into the left atrium at the beginning of the baseline period, followed 60 min later by a constant infusion of cariporide (0.08 mg/kg/min, i.a.) that continued for the duration of the 25 I/R cycles. The dose and method of administration of cariporide was chosen based on preliminary data from three pigs indicating that this regimen resulted in adequate plasma concentrations (e.g., 1.0-1.5 μM). After I/R 25, regional blood flow was measured, and arterial blood samples were obtained for measuring plasma creatine kinase.

Time/vehicle control; VEH. The purpose of this protocol was to verify that alterations in regional function in ISC and CAR were due to ischemia per se rather than a result of cumulative time and/or prolonged sedation. Vehicle (saline; 12 ml, i.a.) was administered before, and a constant infusion started (0.04 ml/min, i.a.) at the end of the 60-min baseline period that continued for [almost equal to]4.2 h (i.e., 25 cycles × 10 min). Although no occlusions were performed in this protocol, hemodynamic variables were calculated and blood gases and rectal temperatures obtained after the time equivalent of cycles 1, 5, 10, 15, 20, and 25. Regional blood flow was measured as described.

Adenosine infusion

To document vascular (i.e., LCx blood-flow velocity) and myofibrillar (i.e., regional systolic WTh) responsiveness after each experimental protocol, hemodynamic variables were assessed before and during adenosine infusion. Adenosine (5 mg/ml) was infused at incremental rates (0.5, 1.0, and 1.5 ml/min; i.a.) for 2-3 min at each rate. This procedure was performed ~10 min after the last occlusion (i.e., ISC or CAR) or VEH.

Euthanization and postmortem procedures

Within 2 h of the last protocol, animals were intubated, anesthetized, and the heart excised after its arrest by using saturated KCl. Next, an incision was made on the surface of the myocardium from base to apex at the level of the hydraulic occluder to estimate the demarcation between normally perfused and ischemic myocardium. The myocardium then was cut into two transverse rings ([almost equal to]1.5 cm thick) along the hoop axis from base to apex, starting ~0.5 cm below the plane of the LCx. Each ring then was sectioned (1- to 2-g segments) progressively from the LAD, through the LCx, to the RCA perfusion territories. The tissue was divided further into epicardial and endocardial layers, and sample blocks were placed into homogenizing vials and processed by using the standard procedures (20). Confirmation of the LCx-perfusion territory was provided by the lower microsphere blood-flow measurements during ischemia in tissue samples at the site of the LCx sonomicrometer dimension gauges.

Statistical analysis

Data are expressed as group mean ± SEM. A two-way analysis of variance with repeated measures over time was used to evaluate hemodynamic and hematologic variables. Post hoc tests (Tukey) were performed when significant treatment (i.e., ISC, CAR, VEH) or time (i.e., baseline-I/R 25) effects or both were obtained (i.e., $p < 0.05$). A one-way analysis of variance was used to assess whether differences existed in transmural myocardial blood flow and plasma creatine kinase.

RESULTS

Exclusions

Of 20 pigs originally instrumented for study, six were excluded because of ventricular fibrillation that occurred either during an occlusion or on reperfusion (n = 4, ISC protocol; n = 2, CAR protocol), and seven were excluded because of instrumentation problems that occurred before each animal completed all three protocols. One animal was used to confirm a previous study (22) that similar reductions in regional myocardial function can be reproduced when I/R cycles are separated by >=6 days (data not shown). Therefore, statistical analyses were performed by using data from six animals (37.7 ± 2.9 kg at time of surgery) that completed all three experimental protocols.

General results

Although arterial pH, PCO_2, and PO_2 were similar in pigs among conditions, propofol administration concomitant with supplemental oxygen altered blood gases from baseline within each protocol (Table 1). Supplemental oxygen increased arterial PO_2, whereas propofol suppressed respiratory frequency, resulting in increased PCO_2 concentrations. However, arterial pH remained within physiological limits (e.g., 7.40-7.45), thus precluding any influence(s) of acid/base status on the results. The dose of propofol used, as well as each animal's rectal temperature, also did not vary either between pigs, or within animals on separate study days (data not shown). Moreover, plasma creatine kinase was similar before initiating the I/R regimen (505 ± 134 and 325 ± 72 U/L) and after I/R 25 (295 ± 81 and 294 ± 132 U/L) in ISC and CAR, respectively.

TABLE 1. Arterial blood gases

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Hemodynamic variables

Mean arterial pressure, HR, RPP (Fig. 1), LAD systolic WTh (Fig. 2; Table 2), and LCx blood-flow velocity (Table 3) did not differ in pigs within or among protocols before or during LCx occlusion. Moreover, LCx systolic WTh was not different during occlusion within or between the ISC and CAR protocols (Table 2).

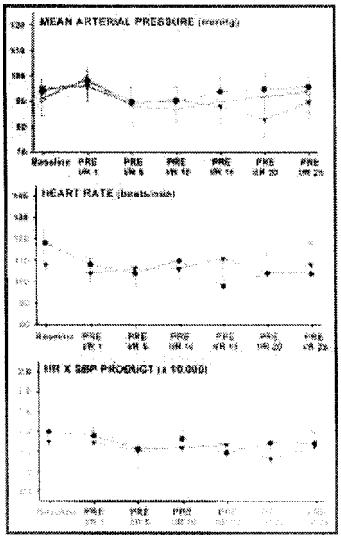

FIG. 1. Mean arterial pressure, heart rate (HR), and HR times systolic blood pressure (SBP) product responses in pigs at baseline and during 25 ischemia (I) and reperfusion (I/R) cycles (I); 25 I/R cycles + cariporide ([black down pointing small triangle]-[black down pointing small triangle]); and time/vehicle control conditions ([black circle, horizontal bar, black circle]). Values expressed as mean ± SEM. Baseline measures are a combination of those taken immediately and 30 min after sedation with propofol. Values before the first I/R cycle were obtained 60 min after sedation. Successive measurements were taken at [almost equal to]50-min intervals (i.e., five I/R cycles, 10 min per cycle).

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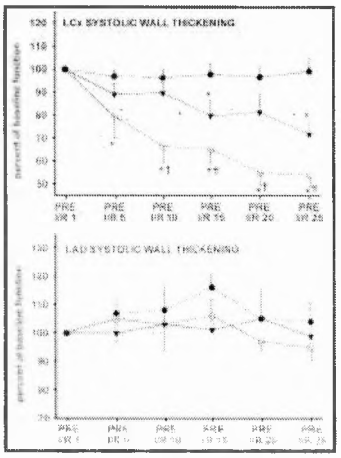

FIG. 2. Left circumflex (LCx) and left anterior descending (LAD) coronary artery regional systolic wall thickening in pigs at baseline and during 25 ischemia (I) and reperfusion (I/R) cycles ([white circle, horizontal bar, white circle]); 25 I/R cycles + cariporide ([black down pointing small triangle]-[black down pointing small triangle]); and time/vehicle control conditions ([black circle, horizontal bar, black circle]). Values expressed as mean ± SEM. Pre-I/R 1 measures are a combination of those taken 30 and 60 min after sedation with propofol. Successive measurements were taken at [almost equal to]50-min intervals (i.e., five I/R cycles, 10 min per cycle) and are expressed as a percentage of baseline (i.e., pre-I/R 1) systolic wall thickening. *$p < 0.05$ versus respective baseline condition; †$p < 0.05$ versus CAR and VEH.

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TABLE 2. Regional systolic wall thickening and left circumflex blood-flow velocity

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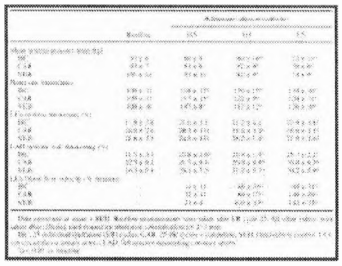

TABLE 3. Hemodynamic variables during adenosine (5 mg/ml) infusion

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Absolute LCx systolic WTh was similar among animals in each condition at baseline and before I/R 1. Over time, however, LCx systolic WTh was reduced compared with baseline at I/R 5, 10, 15, and 25 in the ISC protocol, and at I/R 25 during the CAR condition. Similar results were obtained when LCx systolic WTh was expressed as a percentage of baseline and compared within each condition over time (Fig. 2). Specifically, the percentage of baseline function was reduced at I/R 5, 10, 15, 20, and 25 in ISC, and at I/R 15 and 25 in CAR. When results were compared among groups, the percentage of baseline function was depressed in ISC, compared with both CAR and VEH, at I/R 10, 15, 20, and 25. LCx systolic WTh was not altered over time in VEH when expressed either in absolute terms or as a percentage of baseline. A representative tracing of regional function before I/R 1, during LCx occlusion, and before I/R 25, for the ISC and CAR protocols, is shown in Fig. 3.

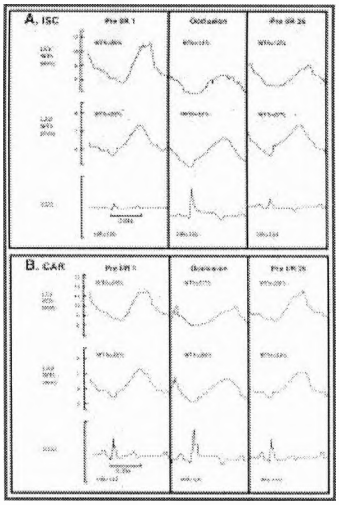

FIG. 3. A tracing from one animal showing systolic wall thickening (WTh) in the left circumflex (LCx) and left anterior descending (LAD) perfusion territories before ischemia and reperfusion (I/R) cycle 1, during an LCx occlusion, and before I/R cycle 25. **A:** ISC, 25 I/R cycles. **B:** CAR, 25 I/R cycles + cariporide. Gain of the electrocardiogram (ECG) was not constant.

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Because no differences existed in transmural blood flow between rings 1 and 2, the data were combined. Regional myocardial blood-flow measurements confirmed that inflation of the hydraulic occluder reduced transmural blood flow to the LCx-perfusion territory. Moreover, transmural blood flow in both the LCx and LAD regions was similar when compared among protocols after I/R 25 (Fig. 4). Adequate mixing of the microspheres was documented because <15% difference existed in blood flow between the right and left kidneys.

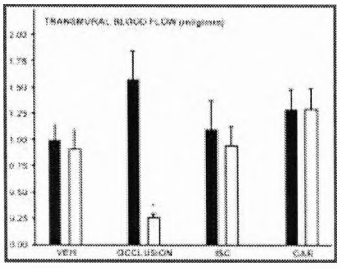

FIG. 4. Transmural blood flow in regions perfused by the left circumflex (LCx, ([white square]) and left anterior descending (LAD, [black small square]) coronary arteries. Values expressed as mean ± SEM. Blood flow was measured after 25 ischemia and reperfusion (I/R) cycles (ISC), 25 I/R cycles + cariporide (CAR); 25 mock I/R cycles (VEH), and during LCx occlusion (Occlusion). *p < 0.05 versus all other measurements.

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Adenosine infusion

All hemodynamic variables were similar among protocols before adenosine administration (Table 3). There were no differences in the hemodynamic responses to adenosine infusion (MAP, HR, LCx blood-flow velocity) at all three infusion rates (i.e., 0.5, 1.0, 1.5 ml/min). However, in the LCx region, adenosine infusion at a rate of 1.0 ml/min increased systolic WTh in the CAR and VEH, but not ISC, protocols. Adenosine infusion at a rate of 1.5 ml/min increased systolic WTh in all protocols.

DISCUSSION

This study used a long-term porcine model of repetitive myocardial ischemia and reperfusion (I/R) to produce stable regional dysfunction. We demonstrated that NHE inhibition with cariporide delayed the onset and limited the degree of regional dysfunction in response to 25 I (2-min)/R (8-min) cycles. In light of previous work (11,13,15,16), we can postulate the mechanisms for this effect and the potential for therapy in patients with chronic ischemic dysfunction.

Mechanisms of regional dysfunction

A single episode of ischemia followed by timely reperfusion results in persistent regional dysfunction (23). Although there probably are numerous mechanisms responsible for postischemic myocardial dysfunction [e.g., generation of free radicals (7)], data indicate that calcium overload on reperfusion likely is an important mechanism (5,6,8). In this regard, a single episode of brief ischemia and reperfusion has been shown to impair calcium homeostasis, illustrated by reduced transport in the sarcoplasmic reticulum (5), prolongation of the calcium transient, and elevation of diastolic calcium (8). In addition, findings suggest that the severity of ischemic dysfunction after brief ischemia and reperfusion is limited by interventions that reduce calcium flux at the onset of reperfusion (9). Taken together, these results strongly suggest the importance of reperfusion calcium overload as a potential mechanism for regional dysfunction after ischemia.

Calcium overload after brief ischemia may result from several intra- and extracellular pathways, including SR calcium channels, sarcolemmal calcium

channels, and the Na-Ca exchanger (10). One mechanism stimulating entry of calcium through the Na-Ca exchanger is the increase in intracellular sodium [Na]i during ischemia and early reperfusion as a consequence of acidosis and resultant Na-H exchange (24). Data supporting this mechanism indicate that NHE inhibition before and during an ischemic episode reduces [Na]i and [Ca]i, as well as the impairment of ischemia-induced myocardial dysfunction (11,15,16). Although several NHE inhibitors have been used to demonstrate these beneficial effects (11,15), results indicate that some (e.g., amiloride, ethylisopropyl amiloride) may directly inhibit the Na-Ca exchanger (25). In contrast, the NHE inhibitor used in our study, cariporide, is a specific inhibitor of the NHE subtype 1 with no known effect on the Na-Ca exchanger at its IC_{50} (~0.1-0.3 μM; 15).

NHE inhibition in repetitive ischemia

The efficacy of NHE inhibition has not been examined previously in a chronic model of repetitive ischemia that is capable of inducing stable reductions in regional myocardial function. Our data show that cariporide administered systemically to provide plasma concentrations of 1-1.5 μM delays the onset and attenuates the magnitude of ischemia-induced LCx regional dysfunction (Fig. 1). These protective effects were observed in the absence of any effect of cariporide on systemic hemodynamics, arterial blood gases, LAD systolic WTh, and transmural myocardial blood flow. These results are consistent with the findings of Sack et al. (13), who demonstrated less postischemic dysfunction after two 10-min periods of coronary occlusion in animals pretreated with the NHE inhibitor HOE 694. Our experiments add to these findings by (a) demonstrating a beneficial effect in a long-term conscious animal preparation with repetitive brief ischemic episodes, (b) providing concurrent measurements of myocardial blood flow with and without NRE inhibition, and (c) demonstrating a beneficial effect of NHE inhibition on the vasodilator response to adenosine. Our observations of chronically reduced function despite normal blood flow also are consistent with previous animal models using repetitive ischemia (22) or progressive coronary artery stenosis (26). Differences in these models should be noted, because we observed stable reductions in LCx regional function after ~3 h (i.e., I/R20), whereas Shen and Vatner (26) noted these changes by using ameroid-induced LCx constriction after 20 days.

NHE inhibition and vascular function

In addition to delaying the onset and reducing the extent of ischemia-induced regional dysfunction, NHE inhibition also improved the vasodilator response to adenosine after 25 I/R cycles. For example, a greater amount of adenosine was required (i.e., 1.5 ml/min) to increase LCx systolic WTh after ISC compared with the CAR and VEH protocols (i.e., 1.0 ml/min; Table 3). Although no statistical difference existed among protocols in the LCx flow-velocity increase in response to maximal adenosine infusion (i.e., 1.5 ml/min), increases observed during the VEH (142%) and CAR (140%) protocols were higher than ISC (98%; p = NS). This [almost equal to]40% increase in blood-flow velocity may reflect some degree of preservation of endothelium-dependent function by NHE inhibition.

Repetitive ischemia as a model for hibernation

Chronic regional dysfunction in patients with coronary artery disease is often termed "hibernating myocardium" (1,2). Hibernating myocardium can develop from chronic sublethal ischemia (27-31) or repeated brief ischemic episodes or both, resulting in chronic myocardial dysfunction (4).

The applicability of the paradigm of stable, reduced flow as a mechanism of chronic dysfunction in humans may be questioned because data from other studies (animal and human) indicate that regional blood flow is maintained under conditions of hibernating myocardium (4,26). If this is correct, then transient rather than chronic reductions in blood flow may be responsible for regional dysfunction. This paradigm is supported by the study of Shen and Vatner (26), who showed that regional myocardial blood flow was similar 20 days after placement of an ameroid constrictor, despite reductions in regional WTh. This study further supported the model of repetitive ischemia causing hibernation, because persistent reductions in regional myocardial function occurred in response to spontaneous excitement (e.g., feeding; 26).

Clinical evidence for this conclusion was provided by measurement of regional blood flow and function in patients with chronic collateral-dependent dysfunction (4). In that study, mild reductions of flow were present at rest in regions of impaired function. However, flow reserve in response to dipyridamole was impaired markedly, and the degree of functional impairment was related significantly to flow reserve rather than baseline flow. As in the animal study noted earlier, these investigators concluded that "impaired function probably results from repetitive episodes of ischemia with persistent stunning ..." (4). These results, together with those from other studies (32-34), suggest that in many cases hibernation is the result of repetitive stunning rather than, or in addition to, chronic ischemia.

Given these findings, our protocol, consisting of 25 cycles of ischemia (2 min) and reperfusion (8 min), resulting in stable reductions in regional systolic function, may be considered a model of acute myocardial hibernation (35). However, because our protocol did not last weeks/months/years (as in human myocardial hibernation), the applicability of these findings to patients is not yet established.

Limitations

Concern may be raised that the CAR protocol was biased favorably to a late effect of ischemic preconditioning (36). This bias is unlikely because (a) a previous study using a similar protocol showed that the late protective effect of repetitive ischemia was absent within 6 days of the last ischemic episode (22), (b) a pilot experiment showed no change in the response to 25 I/R cycles separated by 7 days, and (c) baseline systolic WTh was similar at the onset of each protocol (i.e., there was no persistent regional dysfunction). Second, although the mechanism of protection of cariporide may be extrapolated from this and other studies (11,13,15,16), we performed no measures of $[Na]_i$ and $[Ca]_i$. Thus it is possible that other nonspecific factors associated with cariporide could account for the protective effects in the absence of reductions in either [Na] and $[Ca]_i$. Third, cariporide was administered before the onset of repetitive ischemia, and it is unknown whether NHE inhibition during the development of regional dysfunction, or even after it is established, would be similarly effective. Fourth, concern may be raised that total occlusion of the LCx was not achieved because transmural blood flow ([almost equal to]0.25 ml/g/min) and systolic WTh (<5%) in this region were not zero. The most likely explanation for this finding is that even though the visual and audible indices of phasic flow were abolished during each occlusion, some flow still may have passed through the occluder and perfused the LCx region. However, any perfusion that did exist during LCx occlusion likely was similar within and between protocols for each animal because the indeflator was pressurized identically, and occlusion-induced reductions in LCx blood-flow velocity and systolic WTh were similar within and between protocols. Finally, the possibility exists that collateral vessels were formed by our occlusion protocol. However, because the induction of collaterals by repetitive occlusion

requires that multiple occlusions be performed over several days (37,38), stimulation of this vasculature by our protocol is unlikely. In any case, absolute LCx WTh was not different during occlusion either between the ISC and CAR protocols or over time within each condition, thus allowing comparison between these two protocols.

Implications

This study demonstrated that NHE inhibition with cariporide delays the onset and limits the extent of ischemia-induced regional dysfunction in a long-term porcine model of repetitive ischemia. Whereas extrapolation of these data from our experimental animal model should be done cautiously, our findings suggest that NHE inhibition in patients with chronic ischemic dysfunction may limit the degree of functional impairment without surgical intervention. Because left ventricular dysfunction is an important determinant of survival, such treatment also may decrease mortality in these patients.

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