Mechanisms of Intracellular pH Regulation During Postischemic Reperfusion of Diabetic Rat Hearts

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A marked decrease in the activity of the amiloride-sensitive Na^+/H^+ exchanger has been demonstrated in hearts from streptozotocin (STZ)-induced diabetic rats. The aim of this study was to investigate the contribution of other specific sarcolemmal transport mechanisms to intracellular pH (pH_i) recovery upon reperfusion in STZ-induced diabetic rat hearts and their relation to recovery of ventricular function. Isovolumic rat hearts were submitted to a zero-flow ischemic period of 28 min at 37°C and then reperfused for 28 min. The time course of pH_i decline during ischemia and of recovery on reperfusion was followed by means of ^{31}P-labeled NMR. The perfusion buffers used were either HEPES or CO_2/HCO_3^-. An HCO_3^--dependent (amiloride-insensitive) mechanism contributed to pH_i recovery after ischemia in the diabetic rat hearts. Even when the Na^+/H^+ exchanger was blocked by amiloride in nominally HCO_3^--free solution, a rapid rise in pH_i occurred during the first 3 min of reperfusion. The early rise in pH_i was reduced by external lactate and inhibited by α-cyano-4-hydroxycinnamate. This suggested that a coupled H^+-lactate efflux may be a major mechanism for acid extrusion in the initial stage of reperfusion. The observation of a higher functional recovery on reperfusion in diabetic hearts is in accordance with previous studies using HCO_3^- buffer. However, this study shows that a good recovery of function occurred even more rapidly in diabetic hearts receiving HEPES-buffered solution than in those receiving HCO_3^--buffered solution. This suggests that the HCO_3^--dependent mechanism of regulation may be depressed in diabetic rat hearts. *Diabetes* 44:196–202, 1995

Intracellular pH (pH_i) regulation and the consequences of this regulation may play an important role during ischemia and especially on reperfusion after an ischemic episode (1). In nuclear magnetic resonance (NMR) experiments designed to examine pH_i in isolated hearts, we have previously shown (2) that the recovery of myocardial pH_i from ischemia is slowed down with a pharmacological block of the sarcolemmal Na^+/H^+ exchanger in normal rat hearts. This also occurred in diabetic rat hearts in which the activity of the Na^+/H^+ exchange process has been shown to be decreased (3). These data, together with those of studies showing that inhibition of the antiporter could reduce Na^+ accumulation during reperfusion (4,5), have evidenced the critical role of the Na^+/H^+ exchanger in the recovery of pH_i during this period. In addition, inhibition of the function of this transporter, either in diabetic hearts (2,6) or by amiloride and its analogues in normal hearts (2,4,5,7,8), improved the recovery of cardiac contractility after reperfusion. As yet, no specific sarcolemmal transport mechanisms other than the Na^+/H^+ exchanger have been reported to contribute to pH_i regulation in diabetic hearts.

Indeed, recent work performed on mammalian ventricular myocytes has demonstrated that an Na^+/HCO_3^--dependent carrier mechanism contributes substantially to pH_i recovery from an ammonium-induced intracellular acid load (9). Moreover, such a system has been shown to operate in intact isolated ferret heart that has been submitted to a short period of global ischemia with a moderate decrease in pH_i (10). In addition to the two previously mentioned mechanisms (the Na^+/H^+ exchanger and the Na^+-dependent HCO_3^- transporter), removal of ischemic metabolic products, such as lactate, during ischemia/reperfusion, via a lactate-H^+ cotransport, may also participate in pH_i recovery after ischemia (10,11). Diabetes may influence the activity of these systems as a consequence of metabolic changes (12) and/or changes in composition of the cardiac sarcolemmal membrane (13).

Our study was designed to investigate and compare the relative contribution of specific sarcolemmal transport mechanisms of pH_i recovery in hearts from normal rats and hearts from streptozotocin (STZ)-induced diabetic rats upon reperfusion following a 28-min period of ischemia at 37°C. The time course of change in pH_i during ischemia and upon reperfusion was followed by means of phosphorus NMR spectroscopy. The results were then considered in relation to recovery of ventricular function after reperfusion.

RESEARCH DESIGN AND METHODS

Experimental model of diabetes. All procedures were in accordance with the regulations laid down by the Ministère de l'Agriculture et de la Forêt, France, for the care and use of laboratory animals.

Male Wistar rats weighing between 250 and 300 g were fasted overnight and made diabetic by a single injection of STZ (Sigma; 40 mg/kg) into the femoral vein. STZ-induced diabetic and age-matched control animals were maintained on the same diet until they were used 4 weeks later. Development of diabetes and its persistence were followed by serial quantitative measurements of glucose in the urine with reagent strips. On the day of the experiment, the diabetic state was assessed by measuring nonfasting glucose concentrations in blood samples collected at the time of heart excision.

Heart perfusion. Hearts were quickly removed from normal or STZ-induced diabetic rats anesthetized with thiopentone (5 mg/100 g body wt i.p.) and immersed in ice-cold buffer. The aorta was dissected free and then mounted onto a cannula attached to a perfusion apparatus.

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pH_i, intracellular pH; NMR, nuclear magnetic resonance; STZ, streptozotocin; FID, free induction decay; CHC, α-cyano-4-hydroxycinnamate.

Retrograde perfusion of the heart was started in the Langendorff mode at a constant coronary pressure of 80 cm H_2O and a constant temperature of 37°C. A small vent made of polyethylene tubing was pierced through the apex of the left ventricle for drainage of flow from Thebesian veins. The perfusate was either a Krebs-Henseleit HCO_3^--buffered solution gassed with 95% O_2–5% CO_2 (pH 7.4) or a nominally HCO_3^--free 20 mmol/l HEPES-buffered solution equilibrated with 100% O_2 and pH-adjusted to 7.4 at 37°C with NaOH. Both solutions were phosphate-free, KH_2PO_4 being replaced by an equivalent concentration of KCl (14). They were supplemented with 11 mmol/l glucose and contained 1.25 mmol/l free Ca^{2+}. Perfusion pressure was monitored with a Statham 23 ID pressure transducer connected by polyethylene tubing to a side arm on the aortic cannula. Isovolumic left ventricular–developed pressure was monitored using a fluid-filled latex balloon inserted into the left ventricular cavity via the left atrium. The initial left ventricular end diastolic pressure was adjusted to ~10 mmHg. Developed pressure was calculated by subtracting end diastolic pressure from systolic pressure. Pressure signals were monitored on a Gould 2200 recorder. For all experiments, hearts were allowed to contract spontaneously.

NMR spectroscopy. Hearts perfused in the Langendorff mode as described above were inserted into a 20-mm diameter glass NMR tube and placed into a ^{31}P-labeled NMR probe that was seated in the bore of a superconducting 4.7 Tesla magnet (Oxford). Temperature was maintained at 37°C during the course of the experiment by blowing warm air over the NMR sample tube. The coronary effluent was evacuated from the NMR tube using a vacuum pump. ^{31}P-NMR spectra were generated at 80.9 MHz using a Bruker-Nicolet WP-200 spectrometer. Field homogeneity was adjusted using the water ^1H signal. Generally, ^{31}P-NMR spectra were accumulated for 4 min, averaging data obtained from 342 free induction decays (FIDs), by using a pulse angle of 45° and a recycle time of 0.7 s, with a spectral width of 6,000 Hz and 2,048 data points. To study changes in pH during the first 4 min of ischemia and the first 4 min of reflow with greater time resolution, 1-min time-resolved spectra were acquired by averaging 240 FID with a pulse of 15° and 256 ms recycle time. The spectral width was 8,000 Hz, and 2,048 data points were collected. Before Fourier transformation, the FID was multiplied by an exponential function that generated a 20-Hz line broadening. pH_i was measured from the chemical shift of the pH-dependent peak of P_i signal relative to the phosphocreatine peak using a standard titration curve.

Experimental groups and protocol. Groups were distinguished as follows for normal and diabetic hearts.

Control rats (group I). Control hearts were perfused with $HEPES^-$ or HCO_3^--buffered solution for 24 min, followed by 28 min of zero-flow global ischemia (aortic inflow line clamped) and then 28 min of reperfusion.

HEPES/HCO_3^- plus amiloride (group II). At the end of a 20-min stabilization period in HEPES-buffered perfused hearts, 1 mmol/l amiloride was added to the perfusate 4 min before inducing ischemia. After 2 min of reperfusion, perfusate was switched from a HEPES to an HCO_3^--buffered solution. Amiloride was present throughout ischemia and reperfusion.

HEPES plus amiloride (group III). At the end of a 20-min stabilization period in HEPES-buffered perfused hearts, 1 mmol/l amiloride was added to the perfusate 4 min before inducing ischemia and was present during both ischemia and reperfusion.

HEPES plus lactate (group IV). The 24-min stabilization period in HEPES-buffered perfused hearts was followed by 28 min of ischemia. Hearts were then reperfused with HEPES-buffered solution containing 30 mmol/l sodium lactate. In these experiments, NaCl was reduced by an equimolar concentration.

HEPES plus α-cyano-4-hydroxycinnamate (CHC) (group V). At the end of a 22-min stabilization period in HEPES-buffered perfused hearts, 4 mmol/l CHC was added to the perfusate 2 min before inducing ischemia and was present during both ischemia and reperfusion.

CHC was dissolved in absolute ethanol before being added to the perfusate. The maximum ethanol concentration was 0.005%, which was without effect on any parameter when added by itself in control experiments. Amiloride, CHC, and sodium lactate were from Sigma.

Lactate determinations. Tissue lactate levels after ischemia were determined by standard enzymatic procedure (15) using neutralized perchloric acid extracts of frozen ventricles. The same enzymatic procedure was used to determine lactate in myocardial effluent samples collected before ischemia and after 30, 60, 90, 120, 150, and 180 s of reperfusion.

TABLE 1
Characteristics of normal and diabetic rats

	Normal rats	Diabetic rats
n	11	11
Body wt (g)	385.0 ± 8.4	280.8 ± 8.8*
Heart wt (mg)	1,207.5 ± 49.4	1,062.5 ± 50.6
Heart:body ratio (mg/g)	3.12 ± 0.07	3.78 ± 0.10*
Blood glucose (mmol/l)	9.22 ± 0.6	40.6 ± 1.5*

* $P < 0.05$ vs. normal.

Statistical analysis. The data were analyzed using either Student's t test for unpaired data or analysis of variance followed by Student-Newman-Keuls test to locate differences between groups. Differences were considered significant at the level of $P < 0.05$.

RESULTS

Characteristics of the animals from both groups are presented in Table 1. Only STZ-injected rats with blood glucose levels exceeding 20 mmol/l were considered diabetic. Heart weights tended to be lower in diabetic rats compared with normal rats, although the difference was not significant. The 4-week period of diabetes was chosen because previous studies have characterized cardiac alterations during this period. In particular, the decrease in the activity of the amiloride-sensitive Na^+/H^+ exchange was demonstrated (3) along with changes in pH_i sensitivity of contractile force (16).

Effects of diabetes on the pH_i during ischemia and reperfusion

Control hearts (group I). Figure 1*A* shows time courses of decline in pH_i during ischemia and of recovery on reperfusion in HEPES-buffered perfused hearts. Similar time courses were obtained in HCO_3^--buffered perfused hearts (not shown). Preischemic pH_i values of diabetic hearts were identical to those of normal hearts (7.14 ± 0.05 vs. 7.14 ± 0.03 U with HEPES-buffered solution [n = 5] and 7.14 ± 0.03 vs. 7.17 ± 0.03 [n = 5] in HCO_3^--buffered solution, respectively) and in close agreement with those previously obtained using ion-sensitive microelectrodes (3) or the ^{31}P-NMR technique (2). A somewhat slower fall in pH_i during ischemia was observed in diabetic hearts, but the mean pH_i values reached at each time did not differ significantly from those of normal hearts. Moreover, values reached at the end of ischemia were not significantly different between the two groups. However, upon reperfusion, different kinetics of pH_i recovery were observed between normal and diabetic rat hearts. Indeed, restoration of pH_i to near preischemic values occurred rapidly in normal hearts (7.08 ± 0.04 within ~4 min). By contrast, pH_i recovery was significantly slower in the diabetic rat hearts (6.88 ± 0.09 within 4 min) and hardly reached preischemic values after 28 min of reperfusion. This confirmed our previous observation in hearts perfused with HCO_3^--buffered solution (2).

HEPES/HCO_3^- plus amiloride (group II). In this group, amiloride was given to the hearts before ischemia and during reperfusion (Fig. 1*B*). Hearts received HEPES-buffered solution until the beginning of reperfusion. We then switched to an HCO_3^-/CO_2-buffered solution. In the presence of amiloride, Na^+/H^+ could not operate. Nevertheless, upon switching to HCO_3^- in normal hearts, although pH_i recovery was not complete, it did recover up to ~6.9 within ~4 min and then reached a plateau. In diabetic hearts, pH_i went up within 4 min to a lower value than in normal hearts (pH_i ~6.6) and

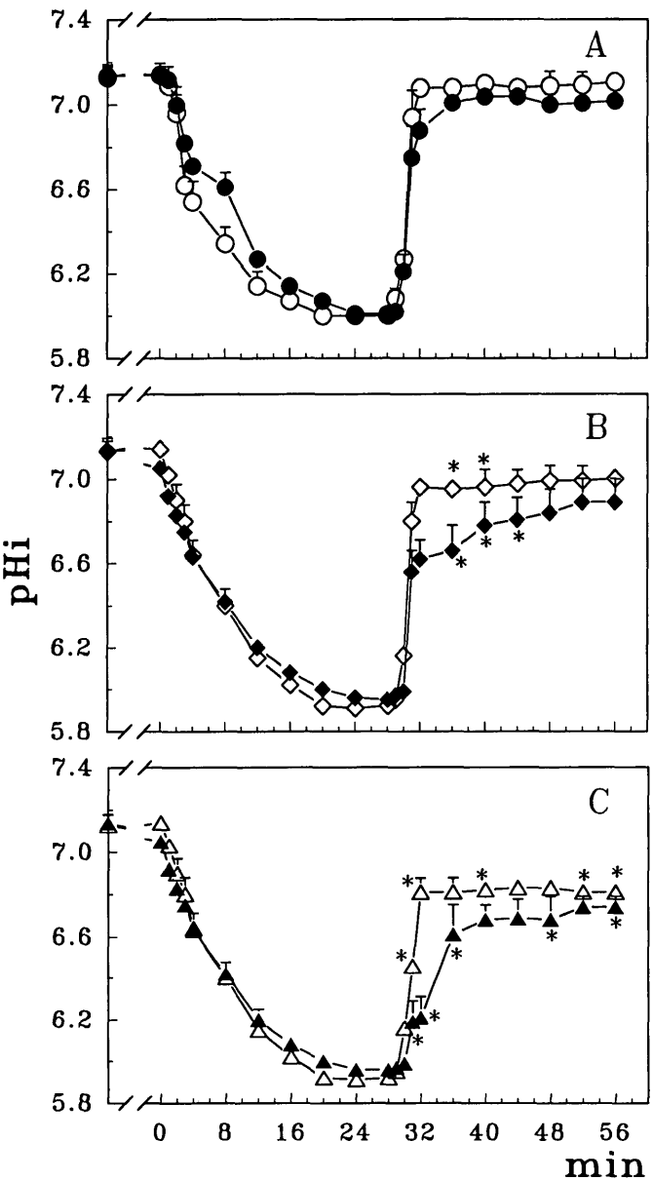

FIG. 1. Time courses of changes in pH_i during zero-flow ischemia and reperfusion of normal (white symbols) and diabetic (black symbols) hearts. Ischemia was induced at zero time. *A*: group I hearts were perfused with a HEPES-buffered solution. *B*: group II hearts were perfused in the presence of amiloride and received HEPES-buffered solution until switching to HCO_3^-/CO_2 buffer at the beginning of reperfusion. *C*: group III hearts were perfused with HEPES-buffered solution in the presence of amiloride. n = 5 hearts in each group. *$P < 0.05$ vs. the corresponding normal or diabetic hearts of group I.

then slowly increased to reach a plateau, below preischemic values, as in normal hearts.

HEPES plus amiloride (group III). Amiloride was given to hearts before ischemia and reperfusion as above, but these hearts received HEPES-buffered solution throughout (Fig. 1*C*). Under these conditions, some pH_i recovery during reperfusion was still observed in both normal and diabetic hearts, although it was slower than in the previous group (i.e., group II) and pH_i remained at a lower value after 28 min. The slowing and lowering of pH_i recovery were more marked at this point for the group of diabetic hearts.

HEPES plus lactate and HEPES plus CHC (groups IV and V). It has previously been shown that ~85% of the very high levels of tissue lactate at the end of ischemia may be washed out of the tissue within ~2 min of reperfusion (17). To reduce transmembrane lactate gradient, we added 30 mmol/l lactate to the perfusate at the time of reperfusion. In the presence of exogenous lactate (Fig. 2*B*), pH_i recovery at 2 and 3 min of reperfusion was markedly decreased in normal hearts compared with control conditions. The effect was less pronounced in diabetic hearts (with a significant difference only at 3 min of reperfusion). We also studied the effects of 4 mmol/l of CHC, which is a lactate carrier inhibitor (18). In the presence of CHC (Fig. 2*C*), the initial recovery of pH_i at reperfusion was markedly inhibited in both diabetic and normal hearts. pH_i remained at low, near-end ischemic values for the first 3 min and then increased very slowly.

Recovery of left ventricular function. Preischemic values of left ventricular developed pressure were not significantly different between normal and diabetic hearts receiving either HEPES (108 ± 18.2 and 104 ± 11.8 mmHg, respectively, n = 6 in both groups) or HCO_3^- buffer (111.7 ± 13.8 and 109.4 ± 10.0 mmHg, respectively, n = 8 in both groups). As previously observed (2), heart rate was slightly decreased after 4 weeks of STZ-induced diabetes (252 ± 10.3 vs. 270 ± 7.7 beats/min in normal hearts). Figure 3 shows the percentage of recovery of ventricular function (expressed as the product of heart rate and developed pressure) during reperfusion of control hearts from normal and diabetic rats. Diabetic hearts showed a better function recovery than normal hearts whichever perfusion buffer was used. However, the difference between the recoveries of both groups (i.e., normal and diabetic) was more marked in hearts that received HCO_3^- buffer compared with those receiving HEPES buffer.

Switching from HEPES to an HCO_3^--buffered solution at the beginning of reperfusion (in the presence of amiloride, group II) (Fig. 4*A*) resulted in a rather good recovery of ventricular function of diabetic hearts over the first 15 min of reperfusion. Then recovery of function decreased and plateaued at ~40% during the last 10 min of reperfusion. In normal hearts, after an initial recovery of ~50% after 10 min of reperfusion, ventricular function progressively deteriorated. When hearts received HEPES buffer plus amiloride throughout ischemia and reperfusion (group III) (Fig. 4*B*), the recovery of ventricular function after 15 min of reperfusion in diabetic hearts was slightly lower than in group II at the same time, then it slowly declined. In normal hearts, the percentage of function recovery after 10 min of reperfusion was not different from that of diabetic hearts. Then the recovery slowly declined.

The percentage of recovery of ventricular function in hearts receiving HEPES buffer in which we supplied external lactate during reperfusion (group IV) (Fig. 4*C*) was initially higher in the diabetic group (for example, after 10 min). Then ventricular function declined and remained at the same level in both groups of hearts.

The effects of CHC (group V) on recovery of ventricular function were not determined because of the known inhibition of mitochondrial pyruvate transport and oxidation (19).

Lactate in myocardial effluent. Preischemic lactate level in the myocardial effluent of diabetic hearts was very low and about half that in the myocardial effluent of normal hearts (group I) (Fig. 5). After 30 s of reperfusion after ischemia, lactate level was markedly increased in both groups, the level being much higher in the myocardial effluent of normal hearts. Then it decreased in both groups and was no longer significantly different from its preischemic level after 2 min of reperfusion.

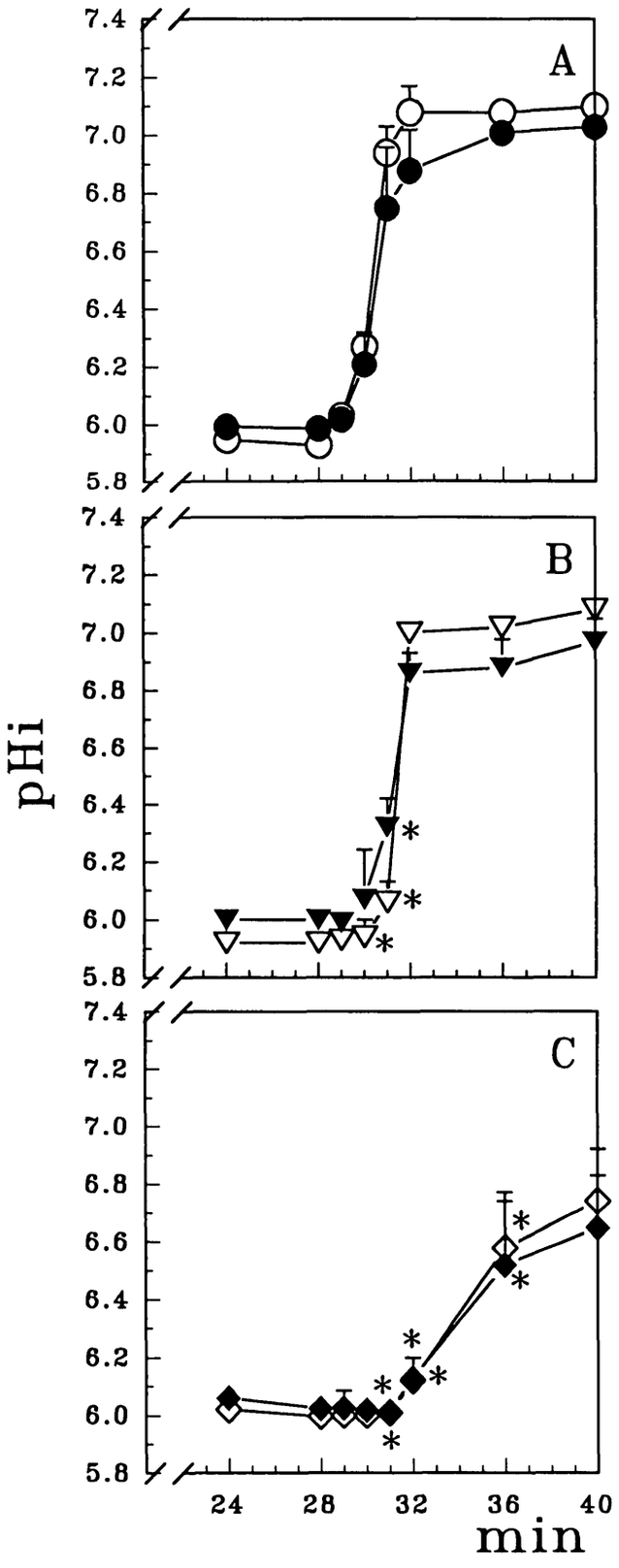

FIG. 2. Time courses of changes in pH_i during the last 4 min of ischemia (end ischemia at t = 28 min) and the first 12 min of reperfusion of normal (white symbols) and diabetic (black symbols) hearts. *A*: group I hearts. *B*: group IV hearts (in the presence of external lactate). *C*: group V hearts (in the presence of CHC). n = 5 hearts in each group. *$P < 0.05$ vs. without external lactate or without CHC.

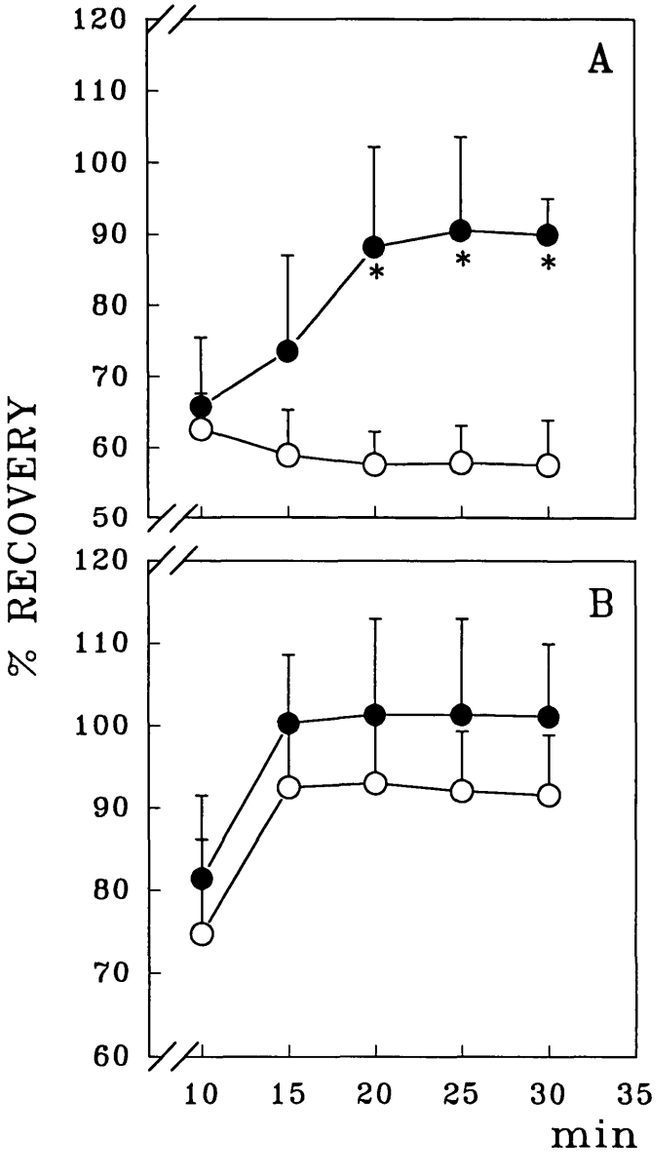

FIG. 3. Time courses of functional recovery with reperfusion of normal (○) and diabetic (●) hearts. The percentage of recovery of ventricular function was calculated from the products of heart rate and developed pressure obtained before and after ischemia. Hearts were perfused with either an HCO_3^--buffered solution (*A*) or a HEPES-buffered solution (*B*). n = 7 hearts in each group. *$P < 0.05$ vs. normal hearts.

DISCUSSION

This study shows that an HCO_3^--dependent (amiloride-insensitive) mechanism contributes to pH_i recovery after ischemia in hearts from diabetic rats, as well as in hearts from normal rats. In addition, when the Na^+/H^+ exchanger was blocked by amiloride in nominally HCO_3^--free solution, a rapid rise in pH_i still occurred at the very beginning of reperfusion (during the first 2–3 min); this was, however, less abrupt in diabetic hearts. This early rise in pH_i, which could be reduced by supplying external lactate and inhibited by CHC in the two groups of hearts, suggests that a coupled H^+-lactate efflux may be a major mechanism for acid extrusion in the initial stage of reperfusion.

Our study did not demonstrate that HCO_3^--dependent pH_i recovery required sodium, because we could not remove external Na^+ in our perfused heart model. However, in light of the recent demonstration of an efficient contribution of an

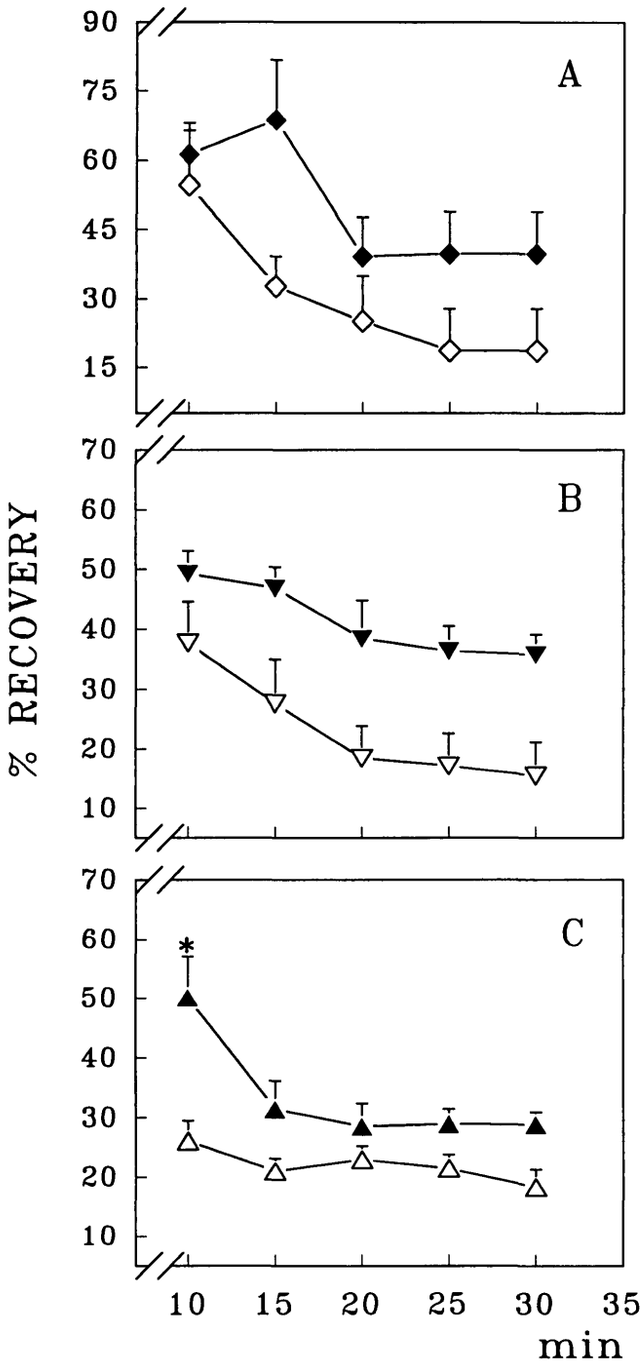

FIG. 4. Time courses of functional recovery with reperfusion of normal (white symbols) and diabetic (black symbols) hearts. *A*: group II hearts; *B*: group III hearts; *C*: group IV hearts. n = 4–6 hearts in each group. *P < 0.05 vs. normal hearts.

Na^+-HCO_3^- symport to acid equivalent extrusion in the guinea pig ventricular myocyte (9), and since such a symport was also shown to contribute to recovery from intracellular acidosis in ferret cardiac tissue (10), it may be inferred that HCO_3^--dependent recovery in our experiments likely occurred via an Na^+/HCO_3^- cotransport. The comparison of the kinetics of pH_i recovery in control hearts (group I; both normal and diabetic hearts) and in hearts in which we switched from HEPES to HCO_3^-/CO_2 buffer in the presence of amiloride (group II; both normal and diabetic hearts) would seem to indicate that the HCO_3^--dependent process was slowed down in diabetic hearts.

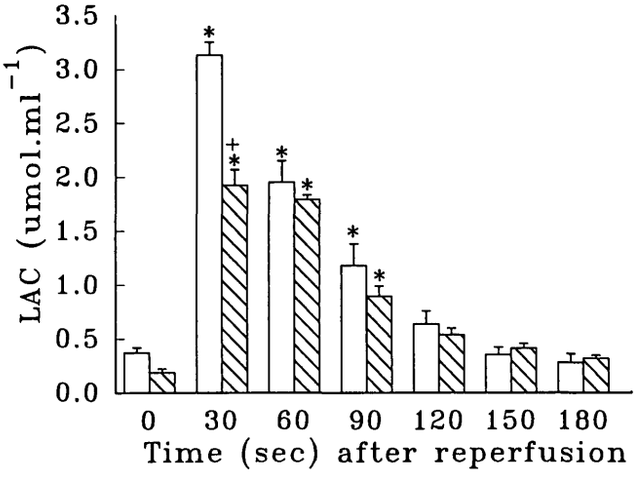

FIG. 5. Myocardial effluent lactate during the first 3 min of reperfusion of normal (□) and diabetic (▨) hearts. n = 6 hearts in each group. *P < 0.05 vs. the preischemic value at zero time; +P < 0.05 vs. normal hearts at the same time.

In the group of hearts that received HEPES buffer plus amiloride throughout ischemia and reperfusion (group III; both normal and diabetic hearts), we may assume that the contribution of Na^+/H^+ exchange and of an HCO_3^--dependent mechanism was substantially less than that in group I hearts. Indeed, even in nominally HCO_3^--free solution, the possible contribution of HCO_3^--dependent pH_i regulation induced by the presence of trace amounts of HCO_3^- cannot be set aside (20). The possibility that the Na^+/H^+ exchanger was not inhibited completely or specifically by 1 mmol/l amiloride could also be considered. This has been discussed previously (2,4). In particular, 1 mmol/l amiloride decreased the rise in Na^+_i that occurred in nontreated ischemic rat hearts during the first few minutes of reperfusion (4). This concentration of amiloride has also been shown to inhibit the recovery of pH_i from an intracellular acid load (3,16). Yet, under the experimental conditions of both normal and diabetic hearts of group III, some pH_i recovery was observed upon reperfusion, up to ~6.8 and ~6.7, respectively. This suggests that other systems whose activities are decreased by diabetes may contribute to extrude excess acid from myocardial cells. One such system may be an H^+-lactate coefflux. Indeed, lactate production increases very rapidly in ischemic myocardium of normal rats. In this study, the tissue lactate accumulated at the end of ischemia was significantly less in diabetic hearts than in normal hearts (76.74 ± 11.67 vs. 39.33 ± 3.07 μmol/g dry wt in group I hearts). This may account for a less important H^+-coupled lactate efflux, which is consistent with the lower lactate level in the myocardial effluent of diabetic hearts at the very beginning of reperfusion (Fig. 5). To examine the contribution of such a coupled efflux, we used two approaches, i.e., either a high concentration of external lactate or a monocarboxylate analogue (18). Both manipulations resulted in a slowing down, or even absence, of pH_i increase during the first few minutes of reperfusion. This was particularly obvious during the first 3 min, especially in the presence of CHC. The smaller effect of external lactate may be due to the experimental procedure. CHC was indeed present during ischemia and reperfusion, whereas lactate was added to the perfusate only at the time of reperfusion. It could be argued that there is no evidence here for a complete inhibition of the H^+-lactate cotransport

by CHC. However, this cyano-cinnamate derivative has been shown to be an efficient inhibitor of lactate efflux in cardiac myocytes (K_i ~0.04 mmol/l in cardiac myocytes from guinea pig) (18). In addition, both of the monocarboxylate carriers with distinct kinetic properties that have been demonstrated in rat heart cells are inhibited by CHC (11). Finally, our determinations of lactate in the effluent of normal hearts that received CHC (data not shown) clearly showed no increase in lactate level upon reperfusion when compared with preischemic level. The slow pH_i increase, which then developed after 3 min of reperfusion in the presence of CHC would likely occur via another contributing mechanism, also involving washout of metabolites, possibly CO_2 efflux (10).

The observation of a higher functional recovery on reperfusion in diabetic hearts is in accordance with previous studies (2,21,22). However, all these studies compared ventricular function of diabetic and normal hearts that received HCO_3^- buffer, that is to say under conditions where not only the Na^+/H^+ exchange but also an HCO_3^--dependent mechanism was likely to be involved in pH_i regulation. In this study, it is clear from Fig. 3 (group I hearts) that a good recovery of function occurred even more rapidly in diabetic hearts perfused with HEPES buffer than in those that received HCO_3^- buffer. In addition, Fig. 3 also shows that recovery of function in normal hearts was significantly improved when those hearts were perfused with HEPES buffer rather than HCO_3^- buffer. This indicates that the more rapid recovery of function of diabetic hearts and the marked improvement in function recovery of normal hearts when both received HEPES buffer may be related to a lesser contribution of HCO_3^--dependent pH_i regulation. Indeed, as discussed above, the HCO_3^--dependent mechanism is likely the Na^+-HCO_3^- symport (9,10). Activation of this system will promote Na^+ influx into the cell, as will activation of the Na^+/H^+ exchange process. In normal hearts in the presence of HCO_3^-, full activation of both the Na^+/H^+ exchanger and the Na^+-HCO_3^- symport would thus favor Na^+ overloading. In contrast, in the presence of HEPES, Na^+ overloading would be reduced since it results essentially from one of the two mechanisms operating for Na^+ influx (i.e., Na^+/H^+ exchange). Moreover, Na^+ influx would be further reduced in HEPES-buffered perfused diabetic hearts in relation to the depressed Na^+/H^+ exchange (3,6). Furthermore, if diabetic hearts had a reduced activity of the (likely) Na^+-dependent HCO_3^- transport, this might also account for their greater recovery of function when receiving HCO_3^- buffer compared with that of normal hearts under similar conditions. Indeed, it has been shown that an increase in intracellular Na^+ causes excessive Ca^{2+} uptake (4), at least in part by Na^+-Ca^{2+} exchange (23), and depressed recovery of cellular function with reperfusion (4). A reduced activity of the Na^+-Ca^{2+} exchange in diabetic hearts (24) might also contribute to their protection against reperfusion damage. However, this is probably not the primary mechanism in view of the marked differences observed in function recovery between HEPES-buffered and HCO_3^--buffered perfused hearts. In diabetic hearts of group II (Fig. 4*A*), the recovery of function during the first 15 min of reperfusion was of the same order of magnitude as in diabetic hearts of group I perfused with HCO_3^- buffer. Function declined at that time point instead of improving. Function declined even earlier in normal hearts. This earlier decline in normal hearts may again be related to a more important Na^+ influx via the HCO_3^--dependent system. The decline in function in group II hearts as well as in group III hearts after the first 10 or 15 min of reperfusion is likely to be related to a lower pH_i recovery than in group I and its consequences on contractile function (25). In addition, in group II hearts, although not apparent from our results (i.e., Fig. 1*B*), a small acidosis (CO_2 entry and hydration) may have occurred upon switching to a CO_2/HCO_3^--buffered solution. This has been observed by others (9,10). The possibility still cannot be excluded that differences in the sensitivity of contractile proteins to decrease in pH_i might exist and account for the observed differences in function recovery of the two groups of hearts. For example, hearts from diabetic animals have been shown to have decreased sensitivity to external Ca^{2+} (26), whereas in skinned cardiac fibers, a slight but significant increase in Ca^{2+} sensitivity was observed (27). However, in group III hearts, in which the mechanisms of pH_i regulation that may drive Na^+ into the cell were probably not operating, or only slightly, the recovery of function was only slightly better in diabetic hearts than in normal hearts. This, again, would seem to indicate a reduction in the activity of the HCO_3^--dependent mechanism for pH_i regulation in diabetic hearts. Further research is needed in isolated ventricular myocytes from diabetic rat hearts to confirm the Na^+-dependency of the HCO_3^--dependent process and to elucidate whether or not it is depressed in diabetes.

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