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Metabolic inhibition in the perfused rat heart: evidence for glycolytic requirement for normal sodium homeostasis

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JOHN WHANG,[1] PAUL CANNON,[1] AND JOSÉ KATZ[1,2]
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Dizon, José, Daniel Burkhoff, Joseph Tauskela, John Whang, Paul Cannon, and José Katz. Metabolic inhibition in the perfused rat heart: evidence for glycolytic requirement for normal sodium homeostasis. *Am. J. Physiol.* 274 (*Heart Circ. Physiol.* 43): H1082–H1089, 1998.—Subcellular compartmentalization of energy stores to support different myocardial processes has been exemplified by the glycolytic control of the ATP-sensitive K^+ channel. Recent data suggest that the control of intracellular sodium (Na_i) may also rely on glycolytically derived ATP; however, the degree of this dependence is unclear. To examine this question, isolated, perfused rat hearts were exposed to hypoxia, to selectively inhibit oxidative metabolism, or iodoacetate (IAA, 100 µmol/l), to selectively inhibit glycolysis. Na_i and myocardial high-energy phosphate levels were monitored using triple-quantum-filtered (TQF) ^{23}Na and ^{31}P magnetic resonance spectroscopy, respectively. The effects of ion exchange mechanisms (Na^+/Ca^{2+}, Na^+/H^+) on Na_i were examined by pharmacological manipulation of these channels. Na_i, as monitored by shift reagent-aided TQF ^{23}Na spectral amplitudes, increased by ~220% relative to baseline after 45 min of perfusion with IAA, with or without rapid pacing. During hypoxia, Na_i increased by ~200% during rapid pacing but did not increase in unpaced hearts or when the Na^+/H^+ exchange blocker ethylisopropylamiloride (EIPA, 10 µmol/l) was used. Neither EIPA nor a low-Ca^{2+} perfusate (50 µmol/l) could prevent the rise in Na_i during perfusion with IAA. Myocardial function and high-energy phosphate stores were preserved during inhibition of glycolysis with IAA and continued oxidative metabolism. These results suggest that glycolysis is required for normal Na^+ homeostasis in the perfused rat heart, possibly because of preferential fueling of Na-K-adenosinetriphosphatase by glycolytically derived ATP.

magnetic resonance spectroscopy

REGULATION OF INTRACELLULAR SODIUM (Na_i) content is a critical function of cardiac myocytes, because sodium ions participate in many myocardial processes. In pathological states such as hypoxia or ischemia, increased Na_i has been associated with arrhythmias (35), impaired contractile function (9), and cell injury caused by the resultant increase in intracellular calcium (7, 30, 51). Elucidation of the mechanisms controlling Na_i content is thus of fundamental importance.

The large extracellular sodium (Na_o)-to-Na_i gradient is actively maintained by Na-K-adenosinetriphosphatase (ATPase), the primary mechanism of Na^+ extrusion from within the cell. Entry of Na^+ into the cell occurs via a variety of ion channels, exchangers, and cotransporters, including the voltage-gated Na^+ channel, Na^+/Ca^{2+} exchanger, Na^+/H^+ exchanger, Na^+-K^+-$2Cl^-$ cotransporter, and background Na^+ leak channels. Increased myocardial Na_i content during pathologi-

cal states is a reflection of the altered balance between Na^+ influx down the transsarcolemmal gradient and active efflux via Na-K-ATPase. Although prior studies have implicated Na^+/H^+ exchange (33, 34, 41, 47), Na^+/Ca^{2+} exchange (32), and voltage-gated Na^+ channels (6) as mechanisms that may contribute to increased Na_i during interventions causing metabolic inhibition, decreased Na^+ efflux caused by inhibition of Na-K-ATPase may also be a major factor (9, 16, 57).

Results of prior studies have led to the concept of a functional subcellular compartmentalization of energy stores whereby energy (ATP) derived from oxidative phosphorylation and glycolysis may preferentially fuel different cellular processes (54). For example, it has been shown that the gating properties of the ATP-sensitive potassium channel are dependent on ATP derived from glycolysis (55, 56). Similarly, results of several studies suggest that there is also a close relationship between Na-K-ATPase activity and glycolysis (9, 15, 38, 39, 47). One recent study demonstrated the ability of a continued supply of glucose to maintain normal Na_i levels and Na-K-ATPase activity during low-flow ischemia, as well as in preserving myocardial function during reperfusion (9). These data suggest that ATP derived from glycolysis is sufficient to maintain normal Na_i content, presumably by maintaining Na-K-ATPase activity. However, whether maintenance of normal Na_i requires glycolysis is unknown, particularly for the intact heart.

We thus examined this question in isolated, perfused rat hearts using interventions that selectively inhibit production of oxidative or glycolytically derived ATP. Triple-quantum-filtered (TQF) ^{23}Na magnetic resonance spectroscopy (MRS) was used to monitor Na_i, and ^{31}P MRS was used to monitor intracellular high-energy phosphate levels. Pharmacological inhibition of Na^+/Ca^{2+} and Na^+/H^+ exchange was used to examine the relative contributions of these putative mechanisms to increasing Na_i during metabolic inhibition.

METHODS

Heart perfusion. Nonfasting male Wistar rats weighing ~400 g were anesthetized with ketamine (60 mg/kg) and xylazine (20 mg/kg). Bilateral sternotomy was performed, the pericardium was removed, and the heart was excised above the great vessels. The aorta was then cannulated, and the heart was perfused in a retrograde manner at a constant perfusion pressure of ~90 mmHg. The heart was submerged in perfusate effluent within a 20-mm glass nuclear magnetic resonance (NMR) tube. Left ventricular developed pressure (LVDP) was monitored via a balloon placed within the left ventricle, with the balloon volume adjusted to an end-diastolic pressure of ~10 mmHg and not changed for the

duration of the experiment. Coronary perfusion pressure was monitored via a fluid-filled line attached proximal to the aortic cannula, and coronary effluent was collected for metabolite measurements. In most experiments, the atrioventricular node was damaged, and agar wicks containing concentrated KCl and copper electrodes were used to pace the hearts at 200 beats/min. Physiological data including LVDP, left ventricular end-diastolic pressure (LVEDP), and perfusion pressure were monitored continuously on a Gould recorder. The entire perfusion line was enclosed within a heated, water-jacketed system, allowing good temperature control despite the length of the perfusion system ($\sim$8 ft).

Perfusate and reagents. The primary perfusate used was a modified Tyrode solution containing (in mmol/l) 144 NaCl, 5 KCl, 0.9 $MgCl_2$, 6 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 1.5 $CaCl_2$, and 15 dextrose. The perfusate was adjusted to pH 7.40, heated to 37°C, and bubbled with 95% O_2-5% N_2. Hearts were allowed to stabilize for $\sim$30 min before measurements and interventions. Previous data have suggested that the buildup of glycolytic intermediates (sugar phosphates) following maneuvers that inhibit glycolysis can adversely affect high-energy phosphate levels and cardiac function (23, 43, 48). Thus, in experiments in which glycolysis was inhibited, dextrose was omitted, the hearts were exposed to 15 min of substrate-free perfusion with 2 mg/l glucagon to deplete myocardial glycogen, and acetate (5 mmol/l) was then used as substrate for oxidative metabolism. A similar protocol has been demonstrated to prevent the accumulation of sugar phosphates and to allow the preservation of high-energy phosphate levels and myocardial function after glycolytic inhibition (23). The effectiveness of glucagon perfusion was determined in parallel experiments in which freeze-clamped samples from control and glucagon-treated hearts were assayed for glycogen content using a spectrophotometric method (45). Hypoxia was induced by boiling the perfusate for 10 min, followed by bubbling with 100% N_2 for at least 60 min before use. This resulted in a drop in Po_2 from 540 mmHg in the oxygenated perfusate to 38 mmHg in the hypoxic perfusate, as measured from a blood gas analyzer (Nova Biomedical). Iodoacetate (IAA, an inhibitor of glyceraldehyde-3-phosphate dehydrogenase; Ref. 53), at a concentration of 100 µmol/l, was used as a selective inhibitor of glycolysis and ethylisopropylamiloride (EIPA, 10 µmol/l) was used as an inhibitor of Na^+/H^+ exchange. For most ^{23}Na MRS experiments, the thulium(III) complex of 1,4,7,10-tetraazacyclododecane-N, N', N', N''-tetra(methylene-phosphonate) ($TmDOTP^{5-}$) was used as a paramagnetic shift reagent to resolve intra- from extracellular Na spectra (4.5 mmol/l). Because of chelation of divalent cations by this shift reagent, the concentration of Ca^{2+} in the perfusate in experiments using $TmDOTP^{5-}$ was increased by 3.0 mmol/l to maintain a free Ca^{2+} level of $\sim$1 mmol/l, which was confirmed by a Ca^{2+}-sensitive electrode (Orion).

NMR methods. All NMR experiments were performed on a Bruker WB-AM 300 spectrometer, using 20-mm ^{23}Na and ^{31}P probes. TQF ^{23}Na MRS was used to monitor Na_i; this was preferred over standard single-quantum techniques because previous data from our laboratory have demonstrated the ability of TQF Na MRS to monitor relative changes in Na_i in the absence of a paramagnetic shift reagent during constant-perfusion interventions (10). This is a particular advantage in physiological studies in light of the known effects of shift reagents on the electrolyte milieu and cardiac function (4, 5, 42). Results obtained using shift reagent could thus be confirmed in other experiments in the absence of shift reagent. TQF ^{23}Na spectra were acquired at 79.4 MHz using 4,000 data points, a sweep width of 4 kHz, and 384 transients

($\sim$2.5 min). An established pulse sequence to detect TQF coherences was used (40). Relative changes in Na_i during interventions were monitored by following changes in amplitudes of TQF Na_i spectra, which were measured from baseline to peak and normalized to control values. ^{31}P NMR spectra were obtained at 121.5 MHz with 240–480 transients ($\sim$4–8 min), a sweep width of 8 kHz, 4,000 data points, pulse width of 9 µs, acquisition time of 0.125 s, and a recycle time of 1.05 s. Relative changes in high-energy phosphate levels during interventions were determined by following changes in amplitudes of ^{31}P peaks, which were measured from baseline to peak and normalized to control values. Intracellular pH was determined by measuring the chemical shift difference between inorganic phosphate (P_i) and phosphocreatine (PCr) and applying a previously determined relation (13).

Protocols. Our hypothesis is that glycolytically derived ATP is required for normal sodium homeostasis. To test this hypothesis, the experimental protocols were designed to determine whether selective inhibition of glycolytic as opposed to oxidative mechanisms causes a differential response in Na_i. Changes in Na_i were then examined under conditions in which mechanisms involved in the regulation of Na_i content were manipulated to investigate the mechanism by which glycolysis may control Na_i. Table 1 summarizes the experimental protocols. *Protocols 1* and *2* compare the effect on Na_i of selective inhibition of oxidative phosphorylation with hypoxia to the selective inhibition of glycolysis with IAA. The efficacy of IAA in inhibiting glycolysis was documented by analyzing lactate content in the coronary effluent of hearts exposed to IAA using a spectrophotometric method (36). To examine the possible role of Na^+/H^+ exchange in the increase in Na_i during metabolic inhibition, EIPA, a potent inhibitor of this exchange mechanism (41), was used simultaneously with hypoxia or IAA (*protocols 3* and *4*, respectively). To examine whether intracellular acidosis associated with continued ventricular pacing during hypoxia may have contributed to increased Na_i, one group of hypoxic hearts was not paced and was allowed to beat at their spontaneous rate (*protocol 5*). For comparison, hearts exposed to IAA were also not paced (*protocol 6*). In *protocol 7*, the potential contribution of the Na^+/Ca^{2+} exchange mechanism to increased Na_i during inhibition of glycolysis was examined indirectly by perfusion with a low-Ca^{2+} perfusate ($\sim$50 µmol/l) to lower intracellular Ca^{2+} before the use of IAA (14).

In view of the fact that paramagnetic shift reagents chelate divalent cations and may have effects on membrane properties, the effects of IAA on Na_i were also examined using TQF ^{23}Na spectroscopy in the absence of shift reagent with both normal and low extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$; 1.5 mmol/l and 50 µmol/l, respectively). In the absence of shift reagent, the ^{23}Na TQF spectrum has a single peak whose amplitude is the sum of a contribution from Na_o (which is constant so long as Na_o content and coronary perfusion flow

Table 1. *Experimental protocols*

Protocol	Treatment	Glycolysis	OxPhos	Na^+/H^+	Na^+/Ca^{2+}
1	Hypoxia-pace	+	−	+	+
2	IAA-pace	−	+	+	+
3	Hypoxia-EIPA	+	−	−	+
4	IAA-EIPA	−	+	−	+
5	Hypoxia	+	−	+	+
6	IAA	−	+	+	+
7	IAA-low Ca^{2+}	−	+	+	decreased

For each protocol, $n = 3$. OxPhos, oxidative phosphorylation; IAA, iodoacetate; EIPA, ethylisopropylamiloride; +, mechanism is active; −, mechanism has been inhibited.

are constant; Ref. 10) and a contribution from Na_i (which will vary during interventions that affect Na_i content). Under normal conditions, Na_o contributes ~75% of the total TQF spectral amplitude, whereas Na_i contributes ~25% (10). Accordingly, TQF [23]Na spectroscopy without shift reagent will be less sensitive to changes in Na_i than TQF spectroscopy in the presence of a shift reagent.

Finally, for the basic comparison of the effect of selectively inhibiting oxidative phosphorylation versus glycolysis on Na_i content (*protocols 1* and *2*), ^{31}P MRS was performed in a separate group of hearts undergoing identical protocols to examine the effect on high-energy phosphate levels.

Statistical methods. Data are reported as means ± SD. Differences between two groups were assessed using Student's *t*-tests. For TQF Na_i data, normalized spectral amplitudes were plotted versus time of intervention, and data were analyzed by repeated-measures analysis of variance. Post hoc comparisons were made using Dunnett's test.

RESULTS

Control experiments revealed no significant changes in Na_i or myocardial function in hearts perfused with either dextrose or acetate as substrate for 1 h ($n = 3$ for each, see Table 2 for physiological data). There was a small (~15%) decrease in high-energy phosphates in both control groups during this time (Table 3). This suggests that the isolated rat hearts perfused with either acetate or dextrose were otherwise stable for the duration of the planned interventions. Hearts perfused with glucagon demonstrated myocardial glycogen content that was ~10% that of control hearts ($n = 3$ for each, Table 4). As opposed to control hearts, lactate efflux was essentially undetectable after perfusion with IAA, demonstrating effective inhibition of glycolysis ($n = 3$ for each, Table 4).

Figure 1 shows a typical series of TQF spectra acquired from a perfused rat heart exposed to 45 min of IAA. The intra- and extracellular sodium resonances are resolved by use of TmDOTP^{5-} (Na_i is represented by the *right peak* in each pair of spectra). As shown, the Na_i peak increases, whereas the extracellular peak is relatively constant during the 45-min period. Figures 2–5 are plots of the TQF Na_i spectral amplitude versus time of exposure for each intervention ($n = 3$ hearts for each, all spectra normalized to baseline). Na_i, as monitored by the TQF [23]Na spectral amplitude, increased to ~220% of baseline after 45 min of perfusion with IAA (Fig. 2*A*) and increased by a similar amount after 45 min of hypoxia (Fig. 2*B*) with hearts paced at 200 beats/min.

During inhibition of Na^+/H^+ exchange with EIPA, Na_i did not change significantly during hypoxia, whereas

EIPA did not affect the rise in Na_i during perfusion with IAA (Fig. 3, *B* and *A*, respectively). This suggests that Na^+/H^+ exchange plays a prominent role in the rise in Na_i during hypoxia, possibly from intracellular acidosis associated with continued pacing at 200 beats/min during inhibition of oxidative metabolism. To test this hypothesis, hypoxia was induced in the absence of pacing. Spontaneous heart rates decreased markedly during hypoxia (baseline, 230 ± 28 beats/min, range 40–70 beats/min after 45 min of hypoxia), and Na_i did not rise significantly under this condition (Fig. 4*B*). In contrast, Na_i increased in unpaced hearts exposed to IAA despite the fact that heart rate also fell in this group to comparable levels as in the hypoxia group (baseline, 240 ± 25 beats/min, range 30–80 beats/min after 45 min, Fig. 4*A*).

During perfusion with a low-Ca^{2+} perfusate (50 μmol/l), LVDP and LVEDP decreased markedly. When glycolysis was then inhibited with IAA, Na_i still increased about twofold after 45 min (Fig. 5). Because, as detailed in METHODS, paramagnetic shift reagents may interfere with membrane function and divalent cation concentrations, IAA experiments with normal and low $[Ca^{2+}]_o$ were repeated in the absence of TmDOTP^{5-} ($[Ca^{2+}]_o$ values of 1.5 mmol/l and 50 μmol/l, respectively). The TQF spectral amplitude in the absence of shift reagent increased ~150% for both experiments, a magnitude consistent with a significant rise in Na_i during a constant perfusion intervention (Ref. 10; data not shown). Thus the changes in Na_i noted above were not caused by an effect of the shift reagent during metabolic inhibition.

The functional effects of the main interventions, IAA versus hypoxia (paced), are summarized in Table 2. As opposed to during hypoxia, LVDP was well preserved at the end of IAA infusion. LVEDP was similar at the end of both interventions. The effects of hypoxia or IAA exposure on myocardial high-energy phosphate levels are summarized in Table 3 for PCr, β-ATP, and P_i. PCr and β-ATP levels were similar to control levels after IAA exposure. In contrast, there was a significant

Table 3. *High-energy phosphates during metabolic inhibition*

	Control*	Hypoxia	IAA
PCr, % baseline	83 ± 5.3	43 ± 14.0†	84 ± 9.5
β-ATP, % baseline	87 ± 3.1	68 ± 13.9‡	83 ± 15.5

Values are means ± SD; $n = 3$ hearts/group. PCr, phosphocreatine. *Results after 1-h perfusion with either dextrose or acetate (pooled data); †$P < 0.05$ hypoxia vs. control; ‡$P < 0.10$ hypoxia vs. control.

Table 2. *Myocardial function during metabolic inhibition*

	Control*	Hypoxia	IAA
LVDP, % baseline	90 ± 5.6	44 ± 9.6†	85 ± 2.6
LVEDP, mmHg	9.5 ± 2.1	10 ± 5.4	8.3 ± 3.4

Values are means ± SD; $n = 3$ hearts/group. LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure. *Results after 1-h perfusion with either dextrose or acetate (pooled data); †$P < 0.05$, hypoxia vs. control.

Table 4. *Myocardial glycogen content and lactate efflux*

	Control	Postintervention
Myocardial glycogen content*	14.5 ± 3.9	1.5 ± 0.1
Lactate efflux†	0.83 ± 0.23	0

Values are means ± SD; $n = 3$ hearts/group. *Expressed as μmol glucosyl units/gram wet wt, intervention = perfusion with 2 mg/l glucagon; †expressed as μmol·min^{-1}·gram wet wt^{-1}, intervention = 45-min perfusion with 100 μmol/l IAA.
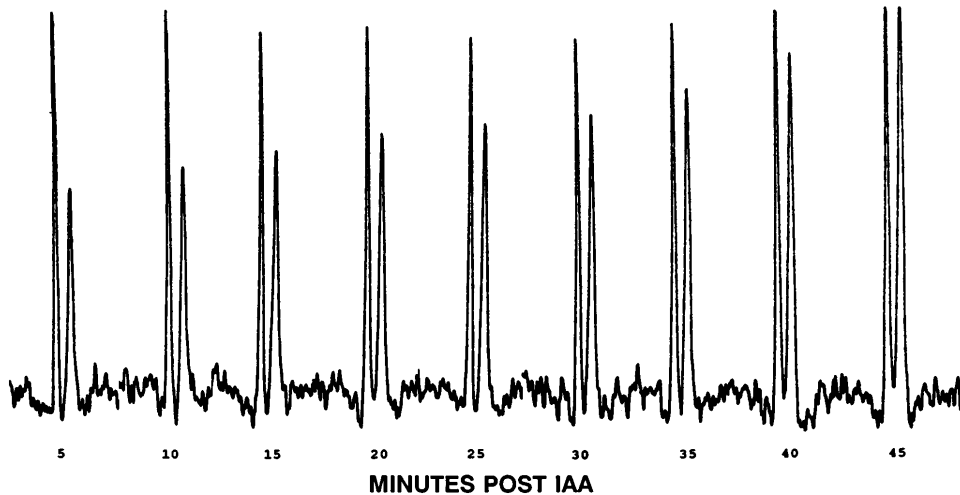
Fig. 1. Series of triple-quantum-filtered (TQF) ^{23}Na spectra acquired in a perfused rat heart exposed to 45 min of iodoacetate (IAA). Intracellular sodium (Na$_i$) is represented by *right peak* in each pair of spectra. Note progressive rise in the TQF Na$_i$ spectral amplitude and relative constancy of extracellular sodium (*left peak*) spectral amplitude.

MINUTES POST IAA

depression in PCr as well as decreased β-ATP levels after hypoxia. There was no significant change in the level of P$_i$ after IAA exposure, nor was there the appearance of a sugar phosphate resonance indicating accumulated glycolytic intermediates. In contrast, P$_i$ levels increased approximately threefold after hypoxia. Baseline intracellular pH values averaged 7.19 ± 0.08. There was no significant change in intracellular pH during exposure to IAA. In contrast, during hypoxia intracellular pH fell significantly (mean 6.75 ± 0.21, $P < 0.05$).

DISCUSSION

Weiss and Hiltbrand (54) suggested a general principle concerning functional compartmentalization of energy in myocytes: energy derived from oxidative metabolism is used to support the contractile function of the myocardium, whereas energy from glycolysis supports membrane functions. Although previous data suggest that this principle may apply for glycolysis and the control of myocardial Na$_i$ (9, 15, 37, 47), we sought to validate this concept by directly comparing the effect of selective suppression of glycolytic versus oxidative metabolism on changes in Na$_i$ in an intact heart model. We then attempted to delineate the mechanism by which glycolysis may control Na$_i$.

The suppression of oxidative metabolism by hypoxia was evident in the decrease in myocardial high-energy phosphate levels and mechanical function that we observed, which is consistent with previous data (27,
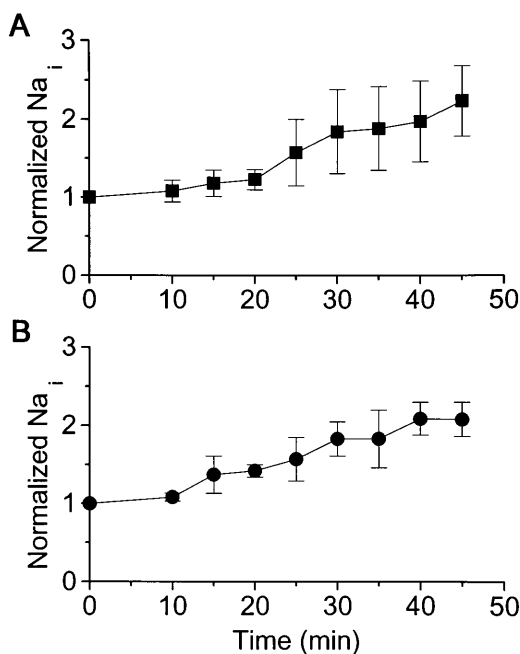

Fig. 2. *A*: plot of normalized TQF Na$_i$ spectral amplitude vs. time of exposure to IAA (100 μmol/l). *B*: similar plot for hypoxia. Points are means ± SD; $n = 3$ hearts/group. Spectral amplitudes increased ~2-fold for both interventions and became significantly greater than baseline after 35 min of perfusion for IAA and 25 min for hypoxia ($P < 0.05$).
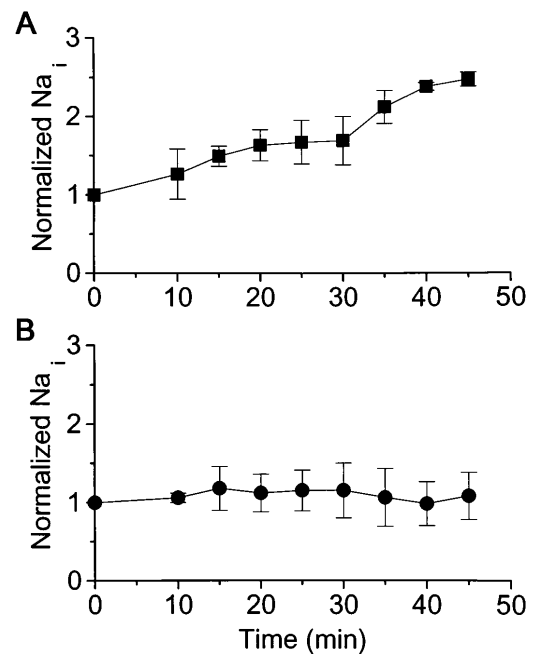

Fig. 3. *A*: plot of normalized TQF Na$_i$ spectral amplitude vs. time of exposure to IAA + ethylisopropylamiloride (EIPA, 10 μmol/l). *B*: similar plot for hypoxia + EIPA. Points are means ± SD; $n = 3$ hearts/group. EIPA prevented rise in Na$_i$ for hypoxia but not for IAA. Spectral amplitudes became significantly greater than baseline after 15 min for IAA + EIPA ($P < 0.05$).
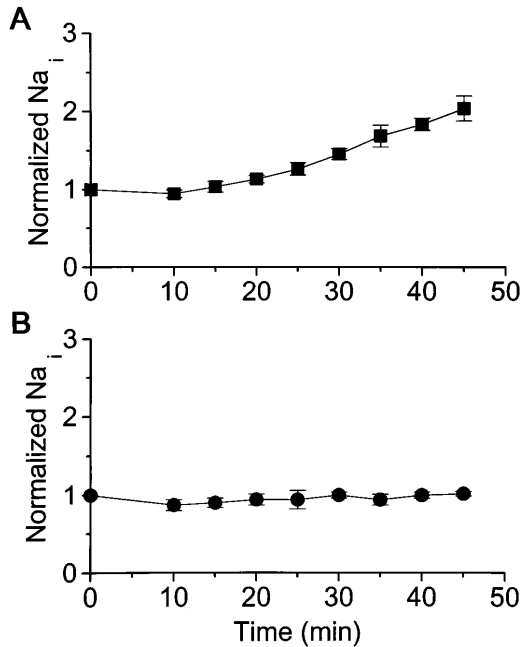
Fig. 4. *A*: plot of normalized TQF Na_i spectral amplitude vs. time of exposure to IAA in absence of pacing. *B*: similar plot for hypoxia in absence of pacing. Points are means ± SD; *n* = 3 hearts/group. Na_i did not increase during hypoxia when hearts were not paced. Absence of continued pacing did not prevent rise in Na_i during IAA exposure. Spectral amplitudes became significantly greater than baseline after 25 min for IAA ($P < 0.05$).

31). IAA, at the dose that we used (100 µmol/l), has been established as a potent, relatively specific inhibitor of glycolysis (43, 53). The suppression of lactate efflux that we observed during perfusion with IAA is consistent with the effective inhibition of glycolysis. The preservation of high-energy phosphate levels and myocardial function that we observed during use of IAA with acetate present is consistent with sparing of oxidative mechanisms and agrees with the results of prior studies of glycolytic inhibition in perfused hearts
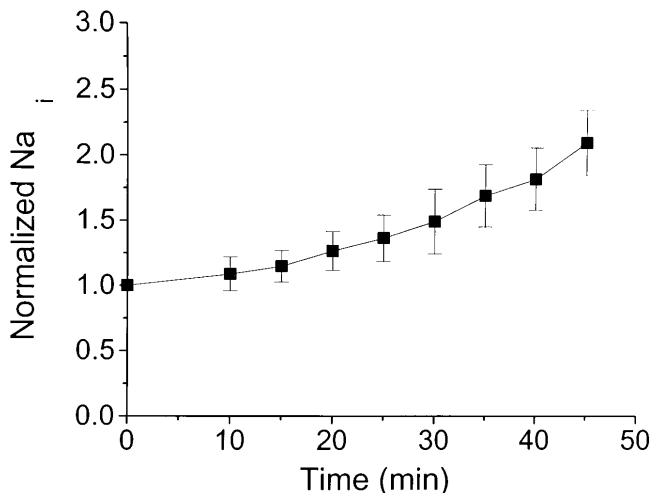

Fig. 5. Plot of normalized TQF Na_i spectral amplitude vs. time of exposure to IAA in presence of 50 µmol/l extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) (*n* = 3 hearts). Spectral amplitudes became significantly greater than baseline after 25 min of perfusion ($P < 0.05$). Inhibition of Na^+/Ca^{2+} exchange with low $[Ca^{2+}]_o$ did not prevent increase in Na_i during exposure to IAA.

(1, 20, 43). The preserved mechanical and metabolic functions that we observed after IAA exposure following depletion of myocardial glycogen with glucagon support the use of this maneuver to prevent the accumulation of toxic glycolytic intermediates (23).

Na_i content, as assessed using TQF ^{23}Na spectral amplitudes, increased when either glycolysis or oxidative metabolism was selectively inhibited in the presence of constant pacing. However, when heart rate was allowed to fall spontaneously or when Na^+/H^+ exchange was inhibited by EIPA during hypoxia, Na_i remained at baseline levels. These results suggest that the rise in Na_i during hypoxia with glycolysis intact is primarily caused by influx of Na^+ in exchange for H^+ generated from intracellular acidosis (12). In contrast, the increase in Na_i caused by inhibition of glycolysis was not affected by inhibition of Na^+/H^+ exchange. This suggests that Na^+/H^+ exchange was not a primary mechanism involved in the increase in Na_i during inhibition of glycolysis; intracellular acidosis would also not be expected to be great in the absence of glycolysis and lack of lactate production (32, 50), consistent with the lack of an observed change in intracellular pH during exposure to IAA in the current study.

Previous data suggest that cellular control of calcium may also be supported by glycolysis (32, 58). If this were the primary effect of glycolytic inhibition, then Na^+/Ca^{2+} exchange could theoretically result in increased Na_i, offering an explanation for our observations after use of IAA. Decreasing extracellular Ca^{2+} to low levels would be expected to significantly decrease influx of Ca^{2+} through the Na^+/Ca^{2+} exchanger, as previously demonstrated in rat synaptosome preparations (14). As shown in Fig. 5, perfusion with a low-Ca^{2+} solution followed by IAA exposure failed to prevent the rise in Na_i. The experiments performed in the absence of $TmDOTP^{5-}$ confirmed that the use of shift reagent did not in itself cause the rise in Na_i during glycolytic inhibition by altering extracellular Ca^{2+} levels.

Thus, although glycolysis may play a role in the control of various cellular ions, neither exchange of Na^+ for Ca^{2+} nor for H^+ appears to explain the increase in Na_i during glycolytic inhibition. Previous data also suggest that voltage-gated Na^+ channels are not a primary mechanism of increased Na_i during metabolic inhibition (29, 47). We did not specifically examine the possible contribution of the Na^+-K^+-$2Cl^-$ cotransporter to Na^+ influx during metabolic inhibition, whose contribution during control conditions has been estimated to be minor (22) but may increase during ischemic conditions (44, 46). Thus, although net Na_i is a balance between Na^+ influx and efflux, the cumulative data presented here suggest that a major component of the increase in Na_i during glycolytic inhibition may be caused by decreased active extrusion of Na_i by Na-K-ATPase. Because selective inhibition of oxidative phosphorylation by hypoxia did not cause increased Na_i independent of Na^+/H^+ exchange, but increased Na_i did occur with inhibition of glycolysis in every case, this suggests a significant dependence of Na-K-ATPase on glycolytic ATP. Such an inference is consistent with previous results that suggest a close relationship be-

tween glycolysis and Na-K-ATPase, including the results of Cross et al. (9) and others (15, 38, 39, 47), who through rubidium MRS were able to directly demonstrate the efficacy of continued glycolytic metabolism to preserve Na-K-ATPase activity in rat hearts exposed to low-flow ischemia. Our observation of normal Na_i during hypoxia (in the absence of rapid pacing or during use of EIPA) but elevated Na_i during glycolytic inhibition (despite preserved energy levels and myocardial function) provides additional support for the hypothesis of functional compartmentalization of myocardial energy stores as proposed by Weiss and Hiltbrand (54).

In their experiments on isolated perfused rat hearts, Cross et al. (9) demonstrated the ability of continued glycolytic activity to preserve Na_i levels and myocardial function during low-flow ischemia followed by reperfusion. More recently, this same group has shown that the degree of glycolytic activity, rather than the level of preischemic myocardial glycogen stores, correlates with the functional recovery of perfused rat hearts after ischemia (8). Several other studies have also demonstrated the critical effect of continued glycolytic metabolism in improving myocardial preservation after ischemia (3, 21, 26, 27, 52). Collectively, these studies suggest that despite the relatively minor glycolytic contribution to total myocardial energy production, maintenance of glucose metabolism is important for maintenance of global myocardial function in ischemic states. Although a recent study suggests that glucose flux through aerobic mechanisms may underlie its benefit during ischemia (28), the paradoxically important role of glucose may be more readily explained by the critical role that glycolysis plays in Na^+ homeostasis and, perhaps more generally, its importance for membrane pumps, as hypothesized here and suggested by others.

The results we obtained with EIPA during hypoxia and rapid pacing are similar to results previously obtained with the use of EIPA during ischemia. Pike et al. (41) demonstrated the ability of EIPA to prevent the increase in Na_i during short periods of ischemia in perfused rat hearts, thus implicating Na^+/H^+ exchange as a primary mechanism of Na_i accumulation during ischemia (theoretically, long periods of ischemia could result in glycogen depletion, and hence, increased Na_i via inhibition of glycolysis as well). The fact that EIPA could negate the increase in Na_i otherwise seen during hypoxia with rapid pacing in our study suggests that Na^+/H^+ exchange plays an important role in cellular Na^+ accumulation during this intervention as well, which is consistent with the results of prior studies in perfused rabbit hearts by Anderson et al. (2). This is not surprising, because both ischemia and hypoxia cause an increased myocardial reliance on glycolysis and hence an increased production of lactate and resultant intracellular acidosis. However, in the absence of significant metabolic stress such as occurred in our unpaced hearts exposed to hypoxia, the activity of Na-K-ATPase is presumably able to compensate for Na^+ gained from Na^+/H^+ exchange. This observation is also consistent with the results of Anderson et al. (2).

This study used TQF ^{23}Na MRS to qualitatively monitor Na_i content during interventions. Although we did not confirm changes in Na_i content by an independent method, multiple prior studies have demonstrated the ability of multiple-quantum ^{23}Na MRS to follow changes in Na_i (10, 11, 19, 24, 25, 42, 49, 51a). Although theoretically changes in NMR relaxation times during interventions can affect TQF spectral amplitudes, previous work in our laboratory has shown that relaxation effects have a negligible impact on TQF ^{23}Na spectral amplitudes for interventions such as hypoxia or use of IAA. Although we cannot rule out the possibility that IAA had effects on the heart beyond selective inhibition of glycolysis, the preservation of high-energy phosphate levels and myocardial function that we observed supports specific glycolytic inhibition by IAA. Prior investigators have suggested the specificity of the dose that we used (43), and others have used IAA at even higher concentrations with maintenance of high-energy phosphate levels and myocardial function when alternative substrates for oxidative metabolism were present (20, 23). IAA could also theoretically directly inhibit the Na^+ pump by its effects on sulfhydryl groups (17, 18), and thus our observations could be accounted for without having to invoke glycolytic inhibition. However, increases in Na_i and depression in Na^+ pump activity have been noted in studies using other means to inhibit glycolysis (9, 15, 47). Our data support the hypothesis of preferential fueling of Na-K-ATPase by glycolytic ATP and, although inferential, are consistent with the results from Cross et al. (9), who were able to correlate the activities of Na-K-ATPase and glycolysis during low-flow ischemia in perfused rat hearts.

In conclusion, the results of this study suggest that glycolysis is required for normal Na^+ homeostasis in a perfused rat heart model. Ion exchange mechanisms may play a secondary role in the increase in Na_i during glycolytic inhibition, although Na^+/H^+ exchange is a major mechanism underlying the increased Na_i with hypoxia and, as shown previously, during short periods of ischemia. These results, combined with previously available data, lead us to infer that Na-K-ATPase may be preferentially fueled by glycolysis. These results have important clinical implications in that they further support the notion that glucose metabolism plays a more fundamental role in myocardial physiology and pathophysiology than was believed in the past, despite the relatively small amount of ATP derived from glycolysis compared with oxidative phosphorylation. These data may have relevance in explaining why certain groups of patients such as diabetics more often have a worse outcome during myocardial ischemic processes and why interventions that enhance glucose utilization uniformly improve myocardial preservation during ischemia.

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