A reevaluation of the roles of hexokinase I and II in the heart

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Southworth R, Davey KA, Warley A, Garlick PB. A reevaluation of the roles of hexokinase I and II in the heart. Am J Physiol Heart Circ Physiol 292: H378–H386, 2007. First published September 1, 2006. doi:10.1152/ajpheart.00664.2006.—Hexokinase is responsible for glucose phosphorylation, a process fundamental to regulating glucose uptake. In some tissues, hexokinase translocates to the mitochondria, thereby increasing its efficiency and decreasing its susceptibility to product inhibition. It may also decrease free radical formation in the mitochondria and prevent apoptosis. Whether hexokinase translocation occurs in the heart is controversial; here, using immunogold labeling for the first time, we provide evidence for this process. Rat hearts (6 groups, n = 6/group), perfused with either glucose- or glucose + oleate (0.4 mmol/l)-containing buffer, were exposed to 30-min insulin stimulation, ischemia, or control perfusion. Hexokinase I (HK I) and hexokinase II (HK II) distributions were then determined. In glucose-perfused hearts, HK I-mitochondrial binding increased from 0.41 ± 0.04 golds/mm in control hearts to 0.71 ± 0.10 golds/mm after insulin and to 1.54 ± 0.38 golds/mm after ischemia (P < 0.05). Similarly, HK II-mitochondrial binding increased from 0.16 ± 0.02 to 0.53 ± 0.08 golds/mm with insulin and 0.44 ± 0.07 golds/mm after ischemia (P < 0.05). Under basal conditions, the fraction of HK I that was mitochondrial bound was five times greater than for HK II; insulin and ischemia caused a fourfold increase in HK II binding but only a doubling in HK I binding. Oleate decreased hexokinase-mitochondrial binding and abolished insulin-mediated translocation of HK I. Our data show that mitochondrial-hexokinase binding increases under insulin or ischemic stimulation and that this translocation is modified by oleate. These events are isoform specific, suggesting that HK I and HK II are independently regulated and implying that they perform different roles in cardiac glucose regulation.

glucose metabolism; insulin; ischemia; immunogold electron microscopy

MYOCARDIAL GLUCOSE UPTAKE is primarily governed by the glucose transporters GLUT1 and GLUT4 (48). Once inside the myocyte, glucose is phosphorylated to glucose-6-phosphate (G-6-P) by hexokinase, which traps it within the cell. The phosphorylation of glucose by hexokinase maintains intracellular free glucose concentrations at a low level, providing the concentration gradient necessary for continued glucose flux into myocytes. Hexokinase is therefore essential for the regulation and maintenance of myocardial glucose uptake (45).

Inulin-responsive tissues express two hexokinase isoforms, hexokinase I (HK I) and hexokinase II (HK II) (2, 7, 11, 32). Under physiological conditions, HK II is predominantly located in the cytosol (2, 11, 24, 32), whereas HK I is predominantly bound to the mitochondria (24, 32, 44, 45). Binding of hexokinase to the mitochondria is thought to have several biochemical advantages: making hexokinase less susceptible to product inhibition (10), providing hexokinase with preferential access to mitochondrially produced ATP (2, 10, 44), and providing the mitochondria with ADP for oxidative phosphorylation (3, 19). The interaction of hexokinase with the mitochondria has also been proposed to have beneficial nonenzymatic roles in cellular protection by decreasing mitochondrial superoxide leakage (9) and preventing apoptosis (3, 27, 31).

In skeletal muscle, insulin has been shown to induce translocation of HK II from the cytosol to mitochondrial membranes (40). Thus insulin’s effect of increasing glucose uptake by externalizing GLUT4 is complemented by an increased capacity to phosphorylate glucose by insulin-mediated hexokinase translocation to the mitochondria. This dual response means that the threshold at which hexokinase becomes a rate-limiting step to glucose accumulation is raised (15). Whether cardiac hexokinase also responds to insulin in this manner is currently controversial, with the most recent study concluding that cardiac hexokinase translocation does not occur (12, 34). Although GLUT4 is known to translocate to cardiac membranes during ischemia, no studies to date have investigated whether hexokinase also translocates to the mitochondria to support cardiac glucose accumulation.

Characterizing hexokinase behavior in tissue is difficult because its activity is directly linked to its intracellular location and chemical microenvironment. Assessing hexokinase activity in tissue homogenates, as has been done in the past (24, 32), is likely to have limited relevance to hexokinase activity in situ, since hexokinase-mitochondrial interaction is likely to be disrupted or absent. Estimation of the extent of mitochondrial binding by cellular fractionation has also been shown to be of limited value. Although the extent of hexokinase-mitochondrial binding in astrocytes by fractionation has been measured as 15–40%, parallel studies in an intact cell population by confocal microscopy showed that ~70% of hexokinase was mitochondrial bound (26); tissue homogenization would thus appear to lead to a dissociation of hexokinase from the mitochondria and erroneous results. Heat lability, used in the past to distinguish between HK I and HK II isofoms, has also been discredited. Because hexokinase lability is highly influenced by a number of factors, including solvent conditions, the presence of other proteins, and in particular mitochondrial binding, the use of this technique to distinguish the isofoms is flawed (32, 45). Here, we describe an immunogold technique that we have developed that allows the localization of both HK I and HK II isofoms in cardiac tissue in situ. Using this technique, combined with established stereological quantification methods, we are able to assess the extent of hexokinase association with the mitochondria.

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Although the predominant energy substrate for the heart during normal metabolism is fatty acids, glucose metabolism becomes more prevalent during insulin stimulation (47) and ischemia (46). The interplay between glucose and fatty acid metabolism is well defined (36, 42), but in isolated heart studies that investigate the behavior of cardiac glucose transporters and hexokinases, the effect of competing energy substrates is rarely examined.

In this study, therefore, we use immunogold labeling and stereological analyses to 1) visualize and quantify HK I and HK II distribution in cardiac tissue, 2) determine whether insulin stimulation and ischemia induce translocation of either or both hexokinase isoforms to the mitochondria, and 3) determine whether these changes are modified by competing energy substrates.

MATERIALS AND METHODS

Reagents

Perfusion media were supplied by BDH (Poole, Dorset, UK). Monoclonal anti-mouse HK I and affinity-purified anti-mouse HK II were supplied by Chemicon Europe (Hampshire, UK), and goat anti-mouse 10-nm colloidal gold antibodies were supplied by BB International (Cardiff, UK).

Animals

Male Wistar rats (250–300 g; n = 6/group) were anesthetized with pentobarbital sodium (120 mg/kg ip) and heparinized via the femoral vein (200 IU). Hearts were excised and immediately immersed in ice-cold Krebs-Henseleit buffer. Hearts were cannulated and perfused under a constant pressure of 100 cmH2O. The basic perfusion fluid was a glucose and having the following ionic composition (in mmol/l): 144.0 Na+/H11001, 6.0 K+/H11005, 2.5 Ca2+/H11001, 130.0 Cl−/H11001, 1.2 SO42−/H11002, 25.0 HCO3−/H11002, and 0.5 Na2EDTA. To investigate the effects of competing substrates on myocardial glucose handling, extra groups of hearts were perfused with 11 mmol/l glucose and 0.4 mmol/l oleate (bound to 0.2 mmol/l myocardial glucose handling, extra groups of hearts were perfused for a baseline period of 60 min before being exposed to 30 min of insulin stimulation (100 IU/l insulin in Krebs buffer at 10% of perfused) and with glucose and oleate in combination. Hearts were insulin treated, ischemic, or control, with either glucose as sole energy substrate or with glucose and oleate in combination. Hearts were perfused fixed for 5 min with 2% formaldehyde + 0.2% glutaraldehyde in Krebs-Henseleit buffer (pH 7.4). They were then incubated for 1 h in fixative before longitudinal left ventricular sections were cut, after which they were placed in 2.3 mol/l sucrose overnight at 4°C. Midmyocardial longitudinal sections (1–2 mm2) were then cut from these samples, mounted on specimen pins, cryofixed by plunging into liquid nitrogen, and stored in liquid nitrogen before cryosectioning.

Sections (70 nm thick) were cut with glass knives at −80°C by a RMC MTXl ultramicrotome with a RMC CRX cryoadaptation and mounted on 3-mm nickel grids coated on one side with 0.3% Pioloform film. The grids were then floated on standard buffer consisting of PBS + 0.1% BSA-c (Aurion) + 0.1% sodium azide (pH 8.2) until all required sections had been cut. Ten grids per sample were used (3 each for HK I and HK II analyses and 2 in each control group). The grids were then transferred from the standard buffer onto a droplet of PBS + 0.05 mol/l glycine for 20 min and then washed (3 × 5 min) with standard buffer. Each grid was then incubated on an individual 25-μl droplet of primary antibody for 45 min (diluted in standard buffer as follows: anti-HK I at 1:200 and anti-HK II at 1:800). Controls were incubated on standard buffer only.

Grids were then thoroughly washed (6 × 5 min) in the standard buffer before incubation on 25-μl droplets of the gold-conjugated secondary antibody for 30 min with antibodies diluted in standard buffer as follows: HK I goat anti-mouse 10-nm colloidal gold antibody, 1:100; and HK II goat anti-rabbit 10-nm colloidal gold antibody, 1:100. Again, optimal concentrations of optimal secondary antibodies had been previously determined (data not shown).

Grids were washed as before in standard buffer before a final wash in PBS and postfixation using PBS + 1% glutaraldehyde (10 min). The grids were then washed in distilled water before they were embedded in nine parts 2% methyl cellulose and one part 3% uranyl acetate on ice and blotted dry before analysis.

Transmission electron microscopy of cryosections. Sections were examined and micrographs were obtained with the use of a JEOL JEM-1200 EXII transmission electron microscope at an accelerating voltage of 80 kV (magnification of ×15,000 to ×20,000).

Relative labeling index calculations. To calculate the relative labeling indexes (RLI) for cellular membranes, test lines were superimposed on the micrographs. For each membrane present, the number of gold particles associated with it was counted to give the observed labeling (no). The length of the membrane with which these gold particles were associated was then assessed by counting the number of intersections the test lines made with the membrane, to give the observed proportion (to/tI) of the total number of test lines (tI).

To calculate RLI for cellular compartments, test grids were applied to the micrographs. Gold particles were counted for each cellular compartment present to give nC. Compartment size was then estimated by counting the number of times a compartment intersected the joint of two grid lines to give the observed number of points (P). The nC was then calculated as follows: nC = (tI/tP) × P (29). RLI was then calculated as follows: RLI = nC/nC.

RLIs were evaluated to assess the distribution of gold labeling across tissue sections. In randomly labeled membranes or compartments, RLI was ≤ 1, whereas in preferentially labeled membranes or compartments RLI was > 1 (29).

Generation of a “mitochondrial binding ratio.” Because the labeling efficiencies of the anti-HK I and anti-HK II antibodies for their
respective targets could not be assumed to be the same, direct comparisons of absolute amounts of these proteins were not possible. Similarly, labeling densities of membranes (e.g., mitochondrial-bound hexokinase) and intracellular compartments (e.g., intracellular vesicle hexokinase) could not be directly compared because the former are quantified per unit length and the latter per unit area. By generating an artificial “mitochondrial binding ratio,” defined below, we obtained an index of the percentage of each hexokinase isoform bound to the mitochondria in each of our experimental groups, which enabled these comparisons to be made: mitochondrial binding ratio = (membrane labeling density × 1,000/vesicle labeling density).

**Statistical Analysis**

All data are expressed as means ± SE. Data were analyzed by ANOVA, followed by *t*-test for unpaired data with Bonferroni correction. A *P* value of <0.05 was considered statistically significant.

**RESULTS**

**Preliminary Data Establishing Labeling Specificity**

To determine the amount of labeling resulting from the nonspecific binding of the secondary antibodies, controls, which consisted of labeling of sections with the gold-conjugated secondary antibodies in the absence of the specific primary antibodies, were included in each incubation. Micrographs from these control sections are shown in Fig. 1; no gold labeling was observed, confirming secondary antibody specificity.

**HK I Labeling Specificity**

Specific HK I labeling was observed in the mitochondrial membranes (RLI = 1.2). In the remaining cellular membranes, HK I labeling was randomly distributed (RLI of sarcolemmal, T tubules, and all remaining membranes = 0). Specific HK I labeling was identified in intracellular vesicles (RLI = 24.0), whereas all other compartments had little or no gold labeling present (for mitochondria, myofibril, and remaining compartments, RLI = 0). Representative micrographs of HK I labeling are shown in Fig. 2.

**HK II Labeling Specificity**

Specific HK II labeling was identified in the mitochondrial membranes (RLI = 1.2); labeling in all remaining membranes was randomly distributed (RLI of sarcolemmal and residual membranes = 0; for T tubule, RLI = 0.1). HK II labeling was also identified in intracellular vesicles (RLI = 21.9), whereas the remaining compartments were labeled randomly (mitochondria RLI = 0.3; myofibril RLI = 0, and residual RLI = 0.1). Representative micrographs of HK II labeling are shown in Fig. 3.

**Effect of Insulin on Cardiac Hexokinase Distribution**

Insulin caused increased binding of HK I to mitochondrial membranes in hearts perfused with glucose as sole substrate (mitochondrial membrane labeling increased from 0.42 ± 0.4 to 0.71 ± 0.10 golds/mm, *P* < 0.05; Fig. 4, A and B). Oleate had no effect on the extent of mitochondrial HK I labeling observed under control conditions; however, oleate abolished the insulin-stimulated translocation of HK I to the mitochondria. Insulin induced significant translocation of HK II from intracellular pools to the mitochondrial membrane. In glucose-only perfused hearts, mitochondrial HK II labeling more than trebled from 0.16 ± 0.02 to 0.53 ± 0.08 golds/mm (Fig. 4, C and D; *P* < 0.05). Insulin also induced HK II translocation to the mitochondria in the presence of oleate, although the effect was less dramatic (from 0.13 ± 0.01 to 0.33 ± 0.04 golds/mm; *P* < 0.05). Decreased hexokinase labeling in the intracellular vesicles indicates that the increased labeling at the mitochondria was due to translocation from the intracellular vesicles.

**Effect of Ischemia on Cardiac Hexokinase Distribution**

Ischemia induced significant increases in mitochondrial-HK I binding, irrespective of buffer substrate composition (binding increased from 0.41 ± 0.04 to 1.54 ± 0.38 golds/mm in glucose-only-perfused hearts, *P* < 0.05; Fig. 5A). This increased HK I binding occurred with a concomitant increase in nonmitochondrially bound HK I, irrespective of buffer substrate composition (intracellular vesicle HK I labeling increased from 21.8 ± 4.9 to 40.4 ± 7.3 golds/mm in glucose-only-perfused hearts, *P* < 0.05; Fig. 5B), indicating that this was not a translocation of HK I from one pool to another. Ischemia also induced translocation of HK II from the cytosol to the mitochondria (from 0.16 ± 0.2 to 0.44 ± 0.07 golds/mm in glucose-only-perfused hearts, *P* < 0.05; Fig. 6), irrespective of buffer substrate composition. Decreased labeling of HK II in the intracellular vesicles indicated its translocation to mitochondria.

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*Fig. 1. Representative control electron micrographs of gold-conjugated secondary antibody labeling in the absence of primary antibody for hexokinase I (HK I; A) and hexokinase II (HK II; B). Gold labeling is absent on both micrographs, indicating specificity of secondary antibody labeling. T, T tubules; M, mitochondria; Z, Z-line of sarcomere.*
Mitochondrial Binding Ratios

The mitochondrial binding ratios show that, under aerobic perfusion conditions, the proportion of total HK I bound to the mitochondria was five times greater than that of HK II (19 vs. 3.5; Fig. 7). However, under insulin stimulation and ischemia, although HK I-mitochondrial binding increased twofold (from 19 to 35), HK II-mitochondrial binding increased fourfold (from 3.5 to 14). Under all conditions, oleate caused dissociation of both HK I and HK II from the mitochondria and dramatically diminished the stimulation of hexokinase-mitochondrial binding caused by either ischemia or insulin.

Fig. 2. Representative electron micrographs of cardiac HK I and HK II distribution. Under control conditions, both HK I and HK II were distributed between the mitochondrial membranes (arrowheads) and intracellular vesicles (arrows). A: HK I labeling in control sections of a heart perfused with glucose. B: HK I labeling in control sections of a heart perfused with glucose + oleate. C: HK II labeling in control sections of a heart perfused with glucose. D: HK II labeling in control sections of a heart perfused with glucose + oleate.

Fig. 3. Representative electron micrographs of cardiac HK I and HK II distribution between the mitochondrial membranes (arrowheads) and intracellular vesicles (arrows) after insulin stimulation. A: HK I labeling after insulin stimulation in heart perfused with glucose. B: HK I labeling after insulin stimulation in heart perfused with glucose + oleate. C: HK II labeling after insulin stimulation in heart perfused with glucose + oleate. D: HK II labeling after insulin stimulation in heart perfused with glucose + oleate.
Effect of Insulin, Ischemia, and Substrate on Myocardial Energetics

$^{31}$P-NMR analysis showed no significant change in myocardial energetics due to insulin stimulation (data not shown).

Phosphocreatine, ATP, $P_i$, and intracellular pH remained at control levels throughout the perfusion protocol, with no significant difference being observed between hearts perfused with glucose alone or those perfused with glucose plus oleate.

Fig. 4. Bar charts showing changes in HK I (A and B) and HK II (C and D) distribution between mitochondrial membranes and intracellular vesicles after insulin stimulation in hearts perfused with glucose and hearts perfused with glucose + oleate.

Fig. 5. Representative electron micrographs of cardiac HK I and HK II distribution between the mitochondrial membranes (arrowheads) and intracellular vesicles (arrows) after ischemia. A: HK I labeling after ischemia in heart perfused with glucose. B: HK I labeling after ischemia in heart perfused with glucose + oleate. C: HK II labeling after ischemia in heart perfused with glucose. D: HK II labeling after ischemia in heart perfused with glucose + oleate.
When hearts were made ischemic, intracellular phosphocreatine levels fell rapidly within 10 min, reaching zero by 20 min. Intracellular ATP concentrations fell more slowly, reaching ~10% of control values after 30 min of ischemia, with Pi levels rising accordingly. Intracellular pH fell from 7.0 during control perfusion to 6.3 after 30 min of ischemia. The presence of oleate in the buffer before ischemia had no effect on any of these parameters.

**DISCUSSION**

In this study, we demonstrate that both HK I and HK II translocate to the mitochondria during both ischemia and insulin stimulation and that this translocation is heavily modified by the presence of an alternate energy substrate such as oleate. Although, initially, these findings conflict with the most recent study of the response of hexokinase to insulin in cardiac tissue (12), they are, however, consistent with the known behavior of hexokinase in other insulin-responsive tissues. Our data represent the first direct study of hexokinase-mitochondrial interaction in ischemic cardiac tissue. We also demonstrate significant differences between the responses of HK I and HK II to insulin and ischemia, suggesting that translocation of the two isoforms is independently regulated and that they perform different roles in the heart.

**Methodological Considerations**

As we outline in our introduction, immunogold labeling has distinct advantages in terms of hexokinase characterization; however, it is not without its own limitations. Low labeling densities can lead to skewed results, and higher labeling densities can obscure the fine details of the translocation process. It is crucial to carefully control these parameters to ensure accurate and reproducible results.
densities occur, especially for proteins that are not highly expressed in relation to other cellular components (22). In immunoelectron microscopy, nonspecific labeling is frequently a problem (35). We have accounted for this by using RLI to confirm the specificity of our antibodies. We have also included control tissue sections for each of our samples, in which the primary antibody was omitted to ensure that nonspecific binding of gold-conjugated secondary antibodies had not occurred. In terms of labeling quantification, it must be noted that mitochondrial membrane labeling is quantified per unit length, whereas labeling in cytosol is quantified per unit area. For this reason, increased labeling density at the mitochondria will not result in an equal decrease in the cytosol, even if there is no overall change in tissue hexokinase content. In general, the labeling of hexokinase at mitochondrial membranes (per unit membrane length) must be considered the more sensitive index of hexokinase translocation. Because the antibodies for HK I and HK II have different binding efficiencies for their targets, the labeling densities for the two isoforms cannot be compared directly. The mitochondrial binding ratio that we calculate, however, provides an index of these relative labeling densities.

Another potential limitation of this study is the use of the Langendorff mode rather than a working heart mode perfusion. Although the latter technique is routinely used for studies of cardiac energetics in isolated perfused hearts, it is more complicated to perform than the Langendorff mode perfusion, particularly in the enclosed space of an NMR probe. We therefore decided that Langendorff mode perfusion was sufficient for this study; however, it must be noted that, in a working heart preparation, substrate uptake and oxidation may be higher because of higher cardiac workload, and hexokinase distribution may differ accordingly.

Response of Cardiac Hexokinase to Insulin Stimulation

We demonstrate that insulin stimulation induces a significant increase in the translocation of both HK I and HK II to the mitochondria (Fig. 4). This has been demonstrated indirectly in other tissue types by shifts in hexokinase activity from cell fractions containing cytosol to those containing mitochondria (34, 41, 49).

There has been a long-running controversy as to whether hexokinase translocates to the mitochondria in cardiac tissue under insulin stimulation. In 1992, Russell et al. (34) demonstrated insulin-mediated binding of hexokinase to mitochondria in isolated working rat hearts. The same group later contradicted their findings, concluding that hexokinase did not translocate to the mitochondria in response to insulin, citing differences in their enzyme assay protocols as the explanation for the discrepancy (12). Examination of their methodology, however, reveals that, in the former study, hearts were perfused with buffer containing 10 mmol/l glucose as sole substrate, whereas, in the latter study, the perfusion buffer contained 5 mmol/l glucose + 0.4 mmol/l oleate. Our data resolve this confusion and, in doing so, confirm the findings of both of their studies. We demonstrate that insulin inducible hexokinase translocation to the mitochondria in hearts perfused with glucose as the sole substrate; in the presence of oleate, however, there is no translocation of HK I and greatly diminished translocation of HK II (Fig. 4). Even under supraphysiological concentrations of insulin, fatty acids remain a dominant modulator of glucose metabolism. This highlights the importance of accounting for buffer substrate composition when investigating cardiac glucose metabolism.

The physical binding of hexokinase to the mitochondria, although not changing the affinity of hexokinase for glucose (39), decreases its $K_m$ for ATP and relieves it from allosteric inhibition by both G-6-P (25) and glucose-1,6-bisphosphate (4). Thus the binding of hexokinase to the mitochondria increases its capacity to phosphorylate glucose. It has been proposed that the redistribution of HK II during insulin stimulation may be causally linked to the increased intracellular glucose concentrations resulting from GLUT4 translocation to the cell surface (1, 38). In addition to inducing HK II binding to the mitochondria, insulin is also thought to stimulate HK II activity directly (1). Under insulin stimulation, where GLUT4 is externalized to maximize glucose uptake, glucose phosphorylation rather than glucose uptake has been shown to be the rate-limiting step in myocardial glucose accumulation (16). We demonstrate that insulin promotes hexokinase mitochondrial interaction and is likely to be a significant mechanism through which insulin increases cardiac glucose uptake.

Response of Hexokinase to Ischemia

We have demonstrated increased HK I labeling after ischemia at both mitochondrial membranes and cytosol, indicating not just translocation from one site to the other but an increase in total myocyte HK I content (Fig. 6). Although ischemia has been previously shown to increase cardiac hexokinase mRNA expression in pig hearts (14), de novo HK I synthesis is unlikely to become significant within the 30–min time frame used in our study. It is more likely that our data reflect increased amounts of “mature” HK I following posttranslational modification of a pro-HK I pool not recognized by the primary antibody; such posttranslational modifications to HK I have been previously suggested to modify hexokinase activity in cancer cells (30). Our data also show that more HK I is present in postischemic myocardium and that a greater proportion of it is mitochondrially bound, as indicated by a doubling in the mitochondrial binding ratio (Fig. 7). Although increased mitochondrial binding has been demonstrated in the ischemic piglet brain (21) and recently in the heart after a 5-min preconditioning period of ischemia (49), both of these studies use data showing changes in hexokinase activity in subcellular fractions to infer that hexokinase translocation has occurred. We have directly demonstrated ischemia-mediated hexokinase translocation to the mitochondria in cardiac tissue and subdivided the hexokinase isoform distributions for the first time.

We have also demonstrated a significant increase in HK II mitochondrial binding in response to ischemia. Because this occurred in the face of diminishing intracellular glucose, as opposed to increasing intracellular glucose during insulin stimulation, it is possible that insulin- and ischemia-stimulated translocations of HK II occur via independent mechanisms. Translocation of GLUT4 to the cell surface is independently controlled by two mechanisms; insulin induces GLUT4 translocation via phosphatidylinositol 3-kinase (6, 13), and ischemia acts via AMP kinase (23, 33). It is possible that these mechanisms control HK I and HK II translocation in a similar
manner. Although comparative studies have yet to be performed to investigate this, activation of AMP kinase has been shown to increase HK II transcription in skeletal muscle (37). The binding of hexokinase to the mitochondria during ischemia is likely to perform two functions. First, as we propose with insulin stimulation, such binding acts synergistically with GLUT4 externalization to promote maximal glucose extraction from the cardiac interstitial space. Second, there is increasing evidence that hexokinase-mitochondrial interaction may have alternate nonenzymatic roles in cardiac protection. It has been proposed that the binding of hexokinase to the mitochondrial voltage-dependent anion channel-adenine nucleotide translocator complex provides it with “preferential access” to intramitochondrial ATP. This links the rate of glucose phosphorylation (and thus glucose uptake) to the rate of mitochondrial oxidative phosphorylation (8), thereby preventing lactate accumulation [for review, see Wilson (45)]. It has also recently been shown that mitochondrial-bound hexokinase returns ADP through the adenine nucleotide translocator to the mitochondrial matrix, setting up an ATP/ADP cycle, which lowers mitochondrial membrane potential and decreases free radical production (11). We were not reliably able to quantify sugar phosphorylation and limit their suppression of hexokinase-mitochondrial interaction. We were not reliably able to quantify sugar phosphorylation and limit their suppression of hexokinase-mitochondrial interaction. We were not reliably able to quantify sugar phosphorylation and limit their suppression of hexokinase-mitochondrial interaction. We were not reliably able to quantify sugar phosphorylation and limit their suppression of hexokinase-mitochondrial interaction.

Relative Roles of HK I and HK II

HK I and HK II differ in their affinities for glucose and ATP (32), relative tissue amounts (32), cellular distribution, and allosteric regulation [P, antagonizes product inhibition by G-6-P on HK I, whereas it exaggerates G-6-P inhibition on HK II (43)]. This has led to the suggestion that HK I and HK II may play different roles in regulating glucose metabolism; HK I may be involved in catabolism, whereas HK II may be involved in anabolism (45). Our data support this theory; both the greater amount and the greater mitochondrial binding of HK I denote significant capacity for glycolytic flux through this isoform, suggesting a major catabolic role. When oleate is present (i.e., under more physiological conditions), HK I remains relatively insensitive to insulin, suggesting that cardiac glucose catabolism does not increase with increased blood glucose concentrations. Although proportionately less HK II is mitochondrial bound than HK I under control conditions, insulin stimulation induces a four- to fivefold increase in HK II binding, consistent with a specialized anabolic role in increasing G-6-P availability for glycogen synthesis. During ischemia, where glucose entry into the cell is paramount for cell survival, both HK I and HK II are recruited to the mitochondria to maximize glucose metabolism. Although some of these responses occur by translocation of existing HK I and HK II to the mitochondria, we also demonstrate a rapid increase in tissue HK I content. Again, this would be consistent with the two proposed roles of HK I: to maximize G-6-P production in a time of extreme need and to occupy mitochondrial binding sites that would otherwise bind Bax and Bak and initiate apoptosis during and after an ischemic insult.

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