Myocardial metabolism of exogenous FDP is consistent with transport by a dicarboxylate transporter

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Myocardial metabolism of exogenous FDP is consistent with transport by a dicarboxylate transporter. Am J Physiol Heart Circ Physiol 281: H2654–H2660, 2001.—The extent to and the mechanism by which fructose-1,6-bisphosphate (FDP) crosses cell membranes are unknown. We hypothesized that its transport is either via band 3 or a dicarboxylate transporter. The question was addressed in isolated Langendorff rat hearts perfused under normoxic conditions. Groups of hearts received the following metabolic substrates (in mM): 5 FDP; 5 FDP + either 5, 10, or 20 fumarate; 10 FDP and either 5, 10, or 20 fumarate; or 5 FDP + 2,4,4’-dinitrostilbene-2,2’-disulfonate (DNDS), a band 3 inhibitor. FDP uptake and metabolism were measured as production of [13C]lactate from [13C]FDP or [14C]lactate and 14CO2 and [14C]lactate from uniformly labeled [14C]FDP in sample perfusates. During 30 min of perfusion, FDP metabolism was 12.4 ± 2.6 and 31.2 ± 3.0 μmol for 5 and 10 mM FDP, respectively. Addition of 20 mM fumarate reduced FDP metabolism over a 30-min perfusion period to 3.1 ± 0.6 and 6.3 ± 0.5 μmol for 5 and 10 mM FDP groups, respectively. DNDS did not affect FDP utilization. These data are consistent with transport of FDP by a dicarboxylate transporter system.

glycolysis; energetics; dicarboxylic acid metabolism; band 3 transporter; biological transport

DURING MYOCARDIAL HYPOXIA or mild ischemia, mitochondrial metabolism becomes impaired, and glycolytic flux increases to partially meet cellular ATP demands. Glycolytic ATP production, which often does not fully compensate for loss of mitochondrial energy production due to flux limitations primarily at the level of phosphofructokinase, can result in damage to the myocardium. One strategy, used experimentally to augment glycolytic ATP production during conditions of metabolic stress, is to provide exogenous fructose-1,6-bisphosphate (FDP). This compound is a naturally occurring glycolytic intermediate that enters glycolysis after the rate-limiting step of phosphofructokinase. A number of reports indicate FDP effectively protects and enhances recovery of tissue functions from oxygen-deficient conditions. For example, FDP provided to hypoxic or ischemic myocardium improved recovery of mechanical function after 20-min low-flow ischemia (9). Indeed, FDP administration to a wide variety of tissues during metabolic energy production deficiencies usually modestly improved tissue function and/or energetic state (1, 5, 9–11, 17, 18, 23, 25). That only modest effects were observed has been assumed to be due to low FDP utilization because of a limitation of its transport into the tissues (5).

Despite FDP’s promise as a therapeutic agent, little has been done to assess its mechanism(s) of action. The first studies of how FDP might exert its salutary influence focused on its metabolic actions. It was shown that FDP reduced myocardial glycolytic rate, oxygen consumption, and glucose oxidation (22, 25). It also was found that FDP caused the myocardium to become reduced as indicated by increased adenine dinucleotide fluorescence (6). This is consistent with enhanced flux through glycolysis. However, these changes may have been a consequence of FDP depression of mechanical function via altered Ca2+ metabolism. Definitive results showing that FDP directly influences myocardial metabolism were obtained by Tavazzi et al. (25) who found that perfused rat hearts converted [14C]FDP to [14C]lactate and 14CO2. Evidence that FDP entered cells rather than being converted to fructose was provided by data for metabolism of exogenous fructose by hearts, which was only about 10% that found with FDP (25), and fructose metabolism was undetectable in vascular smooth muscle (4).

FDP is a highly charged molecule at physiological pH, is not expected to readily cross cell membranes,
and the mechanism of its transport is not known. One recent report showed FDP is capable of crossing artificial lipid bilayers in a dose-dependent manner (2). However, a membrane transporter has not been identified for FDP. Therefore, the purposes of this study were to verify that FDP is transported and metabolized in Langendorff-perfused rat hearts, test the hypothesis that FDP enters cardiomyocytes by a dicarboxylate transport system, and attempt to assess FDP maximum utilization rates. We found that perfused rat hearts were able to metabolize exogenous FDP and exogenous fumarate, a dicarboxylate, indicating that both were capable of entering cardiomyocytes. Furthermore, we discovered that fumarate inhibited FDP utilization by isolated perfused hearts consistent with translocation via a dicarboxylate transporter. We conclude that the keto (linear) form of FDP utilizes a dicarboxylate or analogous transporter to enter cells based on the structural similarity of this form of FDP and dicarboxylates. This is the first evidence in support of a specific transport mechanism for FDP into cells. We were able to increase FDP utilization rates to about three times that for glucose observed under physiological substrate conditions, but this rate was considered to be less than maximal. These observations may lead to insights on strategies to further increase FDP uptake and metabolism by metabolically stressed tissues and eventually FDP may become a useful intervention during ischemic or hypoxic conditions to maintain tissue-energetic state and enhance survivability.

MATERIALS AND METHODS

General approach. We employed two methods to address the nature of FDP transport and metabolism by isolated perfused rat hearts. We first used [13C]-nuclear magnetic resonance (NMR) to assess the ability of these hearts to metabolize [13C]FDP and [13C]fumarate as a representative dicarboxylate and then used [14C]FDP to quantify FDP metabolism and assess competition between it and fumarate.

Perfused hearts. Hearts were rapidly removed from pentobarbital anesthetized (30 mg/kg body wt ip), heparin injected (1000 units intra-vena cava), 300–500 g male Wistar (Morini, S. Polo D’Enza; Rome, Italy) or Sprague-Dawley (Harlan, Indianapolis, IN) rats maintained on standard laboratory rat chow and ad libitum drinking water. Each heart was placed in ice-cold perfusion buffer solution and the aorta was cleaned of excess tissue and cannulated with a stainless-steel cannula connected to a perfusion buffer solution reservoir set to provide 80 cmH2O perfusion pressure. Perfusion was begun as soon as the aorta cannula was patent. The entire procedure typically required <1 min after the thoracotomy was begun. The perfusate from the initial 10-min perfusion period was not recirculated. The perfusion buffer solution was then switched to containing FDP, fumarate, or FDP and fumarate (as described below) and the perfusates were recirculated. For the [13C]FDP NMR studies, the total perfusion buffer volume was 12 to 14 ml and for the [14C] studies the total recirculated volume was 50 (perfusions for 30 min or less) or 100 ml (perfusions for >30 min). Each heart was perfused with only one solution in addition to the basic one used in the initial perfusion. The basic perfusion buffer solution was modified Krebs-Henseleit-Ringer bicarbonate, pH 7.4 containing 12 IU insulin/l (and in mM) NaCl, 2.5 CaCl2, 4.8 KCl, 1.2 MgSO4, 0.6 KH2PO4, 25 NaHCO3, and 10 glucose. The perfusion solutions were equilibrated with 95% O2–5% CO2 at 37°C by a constant flow of gas through a bubbling stone ([14C]FDP studies) or falling film oxygenator (13) ([13C]FDP studies).

We perfused hearts with buffer solutions to which FDP or FDP and fumarate were added to several concentrations. The solutions were adjusted to maintain ionic strength and free Ca2+ concentration equal to that of perfusion solution to which 5 mM FDP was added. Free calcium was measured using an Orion (Boston, MA) ion selective calcium electrode and an Orion (model 920A) ion meter. FDP-Na3 contributed ~2.5 mosmol/mM and its addition to 5 mM increased ionic strength from 310 to 320 mosmol and reduced the Ca2+ concentration from 2.08 to 1.34 mM. To maintain the perfusion buffer solution, ionic strength, and free Ca2+ concentrations at these values at any other FDP concentration tested, we omitted NaCl and added CaCl2. Addition of fumarate to FDP-containing buffers did not change free Ca2+, and NaCl equivalent to the fumarate added was omitted.

[123FDP synthesis and purification. The [1-13C]FDP was synthesized from [U-13C]Fructose as previously described (5) and further purified by anion exchange column chromatography. Briefly, [1-13C]FDP was synthesized enzymatically from [1-13C]fructose using hexokinase and phosphofructokinase with the ATP supplied by an ATP-regenerating system consisting of phosphocreatine and creatine kinase. Neutralized products were applied to a 2.5-×50-cm column of Dowex 1 × 8–200 at a flow rate of ~2 ml/min and eluted with a gradient of NH4HCO3 (0.75–1.5 M). Fractions, collected every 2 min, were evaporated in a Speed Vac (Savant Instruments; Holbrook, NY) until residual water and NH4HCO3 in the form of NH3 and CO2 were removed. Each fraction was assayed for ATP and FDP and fractions with a FDP/ATP of >50 were used for heart perfusions. The [1-13C]FDP was used at a concentration of ~2 mM in the heart perfusion buffer.

13C NMR Spectroscopy. Frozen perfusate samples (2.5 ml each) were lyophilized in a Speed Vac (Savant Instruments). The powder was suspended in 1 ml 99% D2O with 25 mM 3-(trimethylsilyl)1-propane-sulfonic acid (TMSPS) as a chemical shift reference. A 0.65-ml sample was transferred to a 5-mm NMR tube. 13C NMR spectroscopy was performed on a Bruker DRX 500 spectrometer with the following acquisition parameters: 300 scans with 16 dummy scans, 30 pulse angle at 125.77 MHz, 33,333-Hz sweep width, and a 1-s predelay. 32 K points were acquired and processed with 1-Hz line broadening before Fourier transform. All spectra were broadband proton decoupled and referenced with TMS at 0 ppm. NMR data were processed using Bruker software for peak amplitude determination.

Metabolic rate measurements. FDP metabolism was assessed by measuring [14C]-lactate and [14CO2 production from [U-14C]FDP. The [1-14C]FDP (2.5 to 10 μCi) to give radioscopic activities of 4 × 109 to 4 × 109 dpm per ml/min of perfusion medium was added to the perfusion buffer just before beginning perfusion of hearts with the FDP-containing perfusion solution. Separation of [14C]-lactate from [14C]FDP was carried out using ion-pairing HPLC. For this purpose, perfusate samples were collected at the desired times from the reservoir and immediately deproteinized by adding ice-cold 70% HClO4 (1:10; vol/vol) and centrifugation. After neutralization with 5 M K2CO3 and subsequent centrifugation for 10 min at 4°C to remove precipitated potassium perchlorate, perfusate samples were filtered through a 0.45-μm Millipore filter and then loaded (200 μl) onto a Kromasil C-18, 5-μm particle size, 4.6 × 250-mm HPLC column. The column was
previously equilibrated with buffer having the following composition: 10 mM tetrabutylammonium hydroxide (as the pairing reagent), 10 mM KH$_2$PO$_4$, 0.25% methanol, pH 7.00. Lactate (both cold and [1$^{14}$C]) was isocratically eluted with a retention time of 6.3 min (capacity factor, $k'$ = 4.22, which is defined as $(V_i-V_o)/V_o$, where $V_i$ is the retention volume of peak i, and $V_o$ is the void volume of the column) at a flow rate of 1.2 ml/min and a constant column temperature of 23°C. Because FDP was retained by the stationary phase during lactate elution, before any new sample injection, the column was cleaned with methanol/water (40:60, vol/vol) for 15–20 min at a flow rate of 1.2 ml/min. HPLC apparatus consisted of a Constametric 3500 dual pump system (Thermoquest, Rodano; Milan, Italy) connected to a SpectraSystem UV6000LP spectrophotometric diode array detector (Thermoquest) set up between 200 and 300 nm wavelengths for chromatogram acquisition. The detector sensitivity was increased with a 5-cm light path flow cell. Acquisition and analysis of chromatographic data were performed on a personal computer using ChromQuest software supplied by the HPLC manufacturers. Lactate peak recognition in perfusates was affected at 210 nm by comparing the retention times of known quantities of standard lactate. After verifying enzymatically that FDP was not present, and hence no [1$^{14}$C]FDP was present, 2 ml of the collected lactate peak of perfusate samples were then added to 8 ml of liquid scintillation cocktail (Aquasol-2, DuPont NEN; Boston, MA) and radioactivities were determined by liquid scintillation spectroscopy. Production of $^{14}$CO$_2$ was determined by bubbling the gas from the recirculating heart perfusate through 20 ml of 10 M NaOH. The radioactivities in 2-ml samples of the NaOH removed during and at the end of perfusion were measured by liquid scintillation spectroscopy. Perfusate samples (2 ml each) were taken at the same times as the samples of the CO$_2$ trap, placed in a closed vial, and acidified with 1 ml of 12 N H$_2$SO$_4$. The CO$_2$ released from each vial during a 2-h incubation period with gentle shaking was trapped in 1 ml of 10 M NaOH. The $^{14}$CO$_2$ in the NaOH trap was determined by liquid scintillation spectroscopy. By analyzing the recirculated perfusate, we obtained an average conversion of FDP to its metabolic products rather than minute-to-minute estimates that would be achieved by analyses of coronary effluents, but we increased the sensitivity of the measurements, particularly of lactate mass and of $^{14}$CO$_2$, at low FDP radio-specific activities.

Radioactivities in the lactate and CO$_2$ traps (corrected for dilutions and sample sizes) were divided by the radiospecific activities of the perfusate [1$^{14}$C]FDP to obtain the moles FDP metabolized to these products.

Statistics. Statistical significances were determined using a two-tailed paired Student's $t$-test assuming unequal variances. A $P$ value $<$0.05 was accepted as significant.

RESULTS

FDP and dicarboxylates are highly charged at physiological pH, and it was of interest to assess that these molecules cross cardiac sarcolemma. We examined the fates of [1-1$^{13}$C]FDP and [U-1$^{13}$C]fumarate included in the perfusates of isolated Langendorff-perfused rat hearts. One advantage of 1$^{13}$C NMR spectroscopy is the ability to identify all metabolic products from the metabolism of a 1$^{13}$C-labeled substrate. Shown in Fig. 1 are results from perfusing hearts with [1$^{13}$C]FDP. The perfusate contained [3-1$^{13}$C]lactate as the primary metabolite as would be expected if FDP crossed the cell membrane and acted as a substrate for glycolysis. It is important to note that no new resonances (between 66 and 66.5 ppm) corresponding to [1-1$^{13}$C]fructose appear. Therefore, no detectable conversion of FDP to fructose appears to occur outside the cell, and thus the phosphorylated form (FDP) appears to be the form transported and metabolized.

On the basis of the similarities in planar structures, we hypothesized that the linear form of FDP (the keto form) utilizes a dicarboxylate transporter to cross the plasma membrane. To study whether dicarboxylates are transported into myocardial cells, we included 2 mM of [U-1$^{13}$C]fumarate in the perfusion solution for hearts. One advantage of 1$^{13}$C NMR spectrum of the perfusate after 20 min of perfusion is shown in Fig. 2. The only metabolic product of [U-1$^{13}$C]fumarate we are able to detect is [U-1$^{13}$C]malate. Because fumarate is...
converted to malate and malate appears in the perfusate, we conclude that the fumarate that enters cardiac myocytes is catabolized by fumarase in the mitochondria, and the product, malate, is transported out of the myocytes. Therefore, fumarate and malate can both cross the plasma membrane of cardiac myocytes, indicating the presence of a dicarboxylate transport system in heart sarcolemma.

We then ascertained whether FDP is transported by the same mechanism as dicarboxylates by determining whether metabolism is inhibited by the dicarboxylic acid fumarate. Because dicarboxylate transporters typically are characterized by the ability of other dicarboxylates to compete for transport and by having an apparent Michaelis-Menton constant ($K_m$) for transport in the millimolar range, we examined the uptake and oxidation of 5 mM [U-14C]FDP in the presence and absence of 10 mM unlabeled fumarate. Shown in Fig. 3 is the total 14CO2 production (from gas trapping and dissolved in perfusate) from [U-14C]FDP at the indicated FDP concentrations and with fumarate. Included in this figure are data from experiments in which we included 10 µM 4,4’-dinitrostilbene-2,2’-disulfonate (DNDS) a band 3 inhibitor. Because the mechanism for FDP transport into cells was unknown, we also hypothesized that the band 3 anion transporter may be a transport mechanism for FDP to enter myocardial cells. First, there is a concentration-dependent oxidation of FDP the rate at 20 mM being about six times the rate at 5 mM. Second, uptake and oxidation of FDP are significantly inhibited about 70% by 10 mM of fumarate, indicating possible competition of transport between FDP and fumarate and suggesting a common transport system. Third, DNDS does not significantly affect 14CO2 production from [U-14C]FDP, indicating FDP uptake is not via the band 3 anion exchanger.

Because myocardial FDP metabolism is expected to primarily yield both 14CO2 and [14C]lactate, we separated [14C]lactate from [14C]FDP (as described in MATERIALS AND METHODS). FDP conversion to lactate accounted for 32% of total lactate produced by the heart at 5 and 10 mM FDP and for 60% at 20 mM FDP. The sums of [14C]lactate and 14CO2 production from [14C]FDP are shown in Fig. 4. As with 14CO2 production, DNDS does not and fumarate does inhibit lactate production from FDP. The relationships between FDP metabolism and its inhibition by fumarate at 10 and 20 mM FDP and 5 to 20 mM fumarate are shown in Fig. 5. At a ratio of fumarate to FDP of 4:1, FDP utilization is nearly completely inhibited (85%). Fumarate causes proportional decreases in both lactate and CO2 production from FDP compared with when fumarate is absent with an apparent IC50 of 8 mM at 5 mM FDP. The fraction of total FDP metabolized to lactate remains constant in hearts perfused with 5 mM FDP in the presence or absence of either fumarate or DNDS (Fig. 5).
6). Therefore, fumarate inhibits FDP transport rather than metabolism because inhibition of metabolism would change the ratio of lactate/CO₂ production from FDP (see DISCUSSION). However, at 20 mM FDP, the fraction of FDP converted to lactate increases significantly. The high total glycolytic rate observed with 20 mM FDP (Fig. 4) appears to result in a pyruvate production rate in excess of the capacity of pyruvate dehydrogenase to convert it to acetyl CoA under the conditions of the experiment. The rate of FDP transport/metabolism does not approach a maximum at 20 mM FDP.

DISCUSSION

These studies clearly show that FDP is transported and metabolized to CO₂ and lactate. The flux of FDP through the glycolytic pathway is 3 μmol·g⁻¹·min⁻¹ at 20 mM FDP, which is about 3.5 times the flux of glucose with 11 mM glucose as the only exogenous substrate (20). Because this flux rate is approximately sixfold higher than the rate observed with 5 mM FDP, the maximum flux with FDP is not achieved at 5 mM FDP. We suspect the increase in flux is due to a proportional increase in keto-FDP and that at 20 mM the ~0.4 mM keto-FDP is less than the \( K_m \) for transport. It is likely that if the maximum transport rate \( V_{\text{max}} \) were to be achieved, FDP metabolic flux would simply mirror transport, because glycolytic enzymes are relatively unregulated from FDP on, except during ischemia, and under strongly reducing conditions (20).

For several decades, the glycolytic intermediate FDP has been administered in attempts to improve cellular energetic state during periods of ischemia or hypoxia. The rationale for the use of FDP has been that because oxidative metabolism is inhibited, glycolytic ATP production will become substantially more important in meeting cellular ATP demands. The ATP yield of the glycolytic oxidation of FDP is four molecules of ATP per molecule of FDP compared with two molecules of ATP per molecule of glucose. However, a key rate-limiting step in glycolysis is phosphofructokinase, and during ischemia, its activity becomes more restricted at low pH as does that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (20). Therefore, if FDP could enter the cell, glycolysis would no longer be regulated by phosphofructokinase activity, more substrate would be available for the remainder of the pathway, and the rate of glycolytic ATP production could be increased. Even under severely ischemic conditions, FDP likely would increase flux through GAPDH simply by mass action as the activity of this enzyme is influenced by substrate concentration, H⁺, lactate, and NADH. However, the ultimate limitation to flux through GAPDH would be NAD⁺ availability.

FDP provided during periods of ischemia or hypoxia has resulted in modest improvement of tissue function in a wide variety of tissues studied. Possible limitations to the usefulness of FDP during ischemia may include limited transport of FDP into cells and inade-
quate lactate removal may limit FDP metabolism when the cell is very reduced. In Langendorff-perfused rat hearts, FDP was shown to preserve high-energy metabolites during anoxia, restore myocardial metabolism and contractility during reperfusion (9), and improve myocardial efficiency under well-perfused and well-oxygenated conditions (22). Improvement in tissue metabolic state during metabolic stress has been reported in a number of other tissue types, including kidney (1), liver (19), brain (26), erythrocytes (8), smooth muscle (5), and intestine (24). Many of these effects were likely due to FDP entering cells, but also due to the known calcium chelating activity of FDP (see Ref. 6). It has been demonstrated that FDP is converted to lactate by heart and vascular smooth muscle, and thus some of the effect of FDP in improving tissue energetic state must have resulted from glycolysis of FDP (5, 25). However, the magnitude of the improvement has typically been modest. Because FDP is a highly charged molecule at physiological pH, it has generally been presumed that the transport of FDP has limited its utilization and hence its effectiveness. However, the mechanism of FDP transport has remained unknown.

In the current study, we have shown that FDP can be metabolized to lactate in normal Langendorff-perfused rat heart indicating transport into myocytes and subsequent metabolism of FDP by glycolysis. We also demonstrated that the dicarboxylic acid fumarate can be converted to malate, indicating transport of both malate and fumarate across the plasmalemma of the myocyte. We hypothesized that keto-FDP shares enough structural similarities to dicarboxylic acids that the keto-FDP could be transported using dicarboxylate transporters.

Dicarboxylate transport activity in the plasma membrane has been extensively examined in kidney cells (14, 15, 23), intestinal cells (27, 28), and liver cells (29). However, it has long been known that substrates such as succinate and fumarate can enter cardiovascular cells (3, 7, 16, 17). Therefore, dicarboxylate transport activity likely exists in a wide variety of cell types. Typically, the $K_m$ for transport of dicarboxylic acids has been reported to be in the range of ~0.4 mM to 34 mM (14, 27, 29) with the sodium-dependent type having a lower $K_m$ than the sodium-independent type. From our data, neither the $K_m$ nor the sodium dependence of dicarboxylate transport can be determined (see discussion below). However, if keto-FDP [2% of total (12)] is the transported form, then concentrations of keto-FDP that were used would be 0.1, 0.4, and 1 mM and thus likely would be below or near the $K_m$ for the transporter. This is consistent with our data in Figs. 3 and 4, showing that transport and metabolism of FDP increased at least linearly as total FDP was increased from 5 to 20 mM.

FDP transport/metabolism is inhibited by fumarate, indicating competition between the two. Fumarate is transported into the cell and subsequently into the mitochondria where it is converted to malate from where it is transported into the sarcoplasm and into the extracellular space. It is likely this efflux is to achieve transsarcolemmal equilibrium, but the extracellular fluid contains no malate, and equilibrium is not obtained because of the large "extracellular volume" of the perfused heart. Alternatively, malate efflux is in exchange for extracellular fumarate. The observed inhibition of myocardial FDP utilization by fumarate might occur at either transport or metabolism. The fact that fumarate does not change the ratio of FDP-derived CO$_2$ and lactate indicates fumarate does not compete with FDP for metabolism. If it did, the $^{14}$CO$_2$/[14C]lactate ratio would decrease with fumarate as substrate. At 5 mM FDP, it does not, but it does at higher concentrations of FDP, regardless of whether or not fumarate is present. The latter findings indicate flux through pyruvate decarboxylase (pyruvate dehydrogenase complex) is restricted relative to pyruvate conversion to lactate at FDP concentrations above 5 mM. Flux of FDP-derived pyruvate to acetyl CoA likely would be higher without insulin as a stimulator of glucose transport and without glucose as additional exogenous substrate but we have not determined this.

The mechanism for inhibition of FDP transport by fumarate, like the kinetics of FDP transport, cannot be accurately discerned from these data, because at FDP concentrations are much lower than $K_m$. The plot of rate versus FDP for all data is linear. We attempted several approximations including fit of the data to $v = V_{max}S/(K_m + K_i + K_iS/K_m)$, where $v$ is the rate, $S$ is the substrate concentration, $I$ is the inhibitor concentration, and $K_i$ is the inhibition constant. A plot of 1/slope of this line versus $I$ gives a $K_m/V_{max} = 0.2$ and an estimate of $K_i = 1.5$. This analysis does not provide insight into the nature of the inhibition by fumarate. Fumarate certainly changes the slope of v versus S indicating fumarate is not uncompetitive, but competitive and noncompetitive inhibition both predict changes in $V_{max}/K_m$. Based solely on structural similarities between FDP and fumarate, we would expect inhibition to be competitive.

It is possible that rather than a metabolic action, FDP protects against the untoward effects of hypoxia and ischemia in several tissues by chelating extracellular Ca$^{2+}$. This would be particularly germane to the heart, because reduced Ca$^{2+}$ decreases cardiac mechanical performance and oxygen consumption, and the ischemic insult is lessened because of a more favorable oxygen supply/demand ratio. Even if the extracellular Ca$^{2+}$ concentration is adjusted to compensate for added FDP, it is possible FDP protects via chelation of intracellular Ca$^{2+}$. In order for this to happen, free FDP would need to accumulate inside cells. This is unlikely, because FDP transported into cells is metabolically active and all enzymatic steps beyond phosphofructokinase are near equilibrium, which ensures a relatively high flux from aldolase on with little change in pathway substrate concentrations. Also, aldolase concentration is about 800 $\mu$M (21), and the calculated total FDP concentrations in aerobic, and ischemic hearts are 90 and 250 $\mu$M, respectively (17) indicating that the free FDP concentration is very low. In view of
these considerations and based on the results of the present studies, it is more plausible that when extra-
cellular Ca\(^{2+}\) concentrations are controlled, FDP con-
fers protection to oxygen-deficient tissues via a meta-
bolic action.

The present studies determine the extent to which the heart metabolizes FDP and test the possibilities
that FDP is transported either via the band 3 transport system or via a dicarboxylate transporter. The data are
consistent with transport of the keto form of FDP,
which probably enters cells via the same transporter
transport is inhibited by the dicarboxylate fumarate,
the highest concentration we can effectively test, and its
consistent with transport of the keto form of FDP,
cell or via a dicarboxylate transporter. The data are
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REFERENCES

1. Didlake R, Kirchner KA, Lewin J, Bower JD, and Markov
AK. Attenuation of ischemic renal injury with fructose 1,6-
2. Ehringer WD, Niu W, Chiang B, Wang OL, Gordon L, and
Chien S. Membrane permeability of fructose-1,6-diphosphate in
lipid vesicles and endothelial cells. *Mol Cell Biochem* 210: 35–45,
2000.
3. Finder DR and Hardin CD. Transport and metabolism of
exogenous fumarate and 3-phosphoglycerate in vascular smooth
4. Hardin CD and Roberts TM. Compartmentation of glucose and fructose 1,6-bisphosphate metabolism in vascular smooth
5. Hardin CD and Roberts TM. Metabolism of exogenously ap-
nous fructose 1,6-bisphosphate on myocardial energy metabo-
7. HOHL C, OESTREICH R, ROSEN P, WIESSER R, and GRIESHABER
M. Evidence for succinate production by reduction of fumarate
during hypoxia in isolated adult rat heart cells. *Arch Biochem
8. Lazzarino G, CATTANI L, COSTRINI R, MULIERI L, CANDIANI A,
and GALZIGNA L. Increase of intraerythrocytic fructose-1,6-
diphosphate after incubation of whole human blood with fruc-
9. Lazzarino G, NUUTINEN ME, TAVAZZI B, CERRONI L, DI PEIERRO D, and GIARDINA B. Preserving effect of fructose-1,6-
diphosphate on high-energy phosphate compounds during an-
oxia and reperfusion in isolated Langendorff-perfused rat hearts.
10. MARKOV AK. Hemodynamics and metabolic effects of fructose-1,6-
diphosphate in ischemia and shock. Experimental and clinical
11. MARKOV AK, OGLETHORPE NC, BLAKE TM, LEHAN PH, and
HELLEMS HK. Hemodynamic, electrocardiographic, and meta-

bolic effects of fructose diphosphate on acute myocardial ische-

12. MIDFORT CF, GUPTA RK, and ROSE IA. Fructose 1,6-bisphos-
phate: isomeric composition, kinetics, and substrate specificity
13. Morgan HE, Chua BH, Fuller EO, and Siehl D. Regulation of
protein synthesis and degradation during in vitro cardiac work.
14. Pajor AM. Sequence and functional characterization of a renal
15. Pajor AM and VALMONTE HG. Expression of the renal Na’/
dicarboxylate cotransporter, NaDC-1, in COS-7 cells. *Pflügers
16. PEARL JM, HIRAMOTO J, LAKS H, DRINKWATER DC JR, and
17. PENNEY DG and CASECARANO J. Anaerobic rat heart. Effects of
glucose and tricarboxylic acid-cycle metabolites on metabolism
18. Planas ME, Sanchez S, Gonzalez P, Rodriguez de Oliveira
J, and Bartrons R. Protective effect of fructose 1,6-bisphos-
phate against carrageenan-induced inflammation. *Eur J Phar-
19. ROIG T, DE OLIVEIRA JR, BARTRONS R, and BERMUDEZ J.
Fructose 1,6-bisphosphate protects against l-galactosamine tox-
icity in isolated rat hepatocytes. *Am J Physiol Cell Physiol* 266:
20. Rovetto MJ, Lamberton WF, and Neely JR. Mechanisms of
glycolytic inhibition in ischemic rat hearts. *Circ Res* 37: 742–
751, 1975.
21. Srivastava DK and Bernhard SA. Biophysical chemistry of
metabolic reaction sequences in concentrated enzyme solution
and in the cell. *Annu Rev Biophys Biophys Chem* 16: 175–204,
2007.
22. Starnes JW, Seiler KS, Bowles DK, Giardina B, and Lazz-
arino G. Fructose-1,6-bisphosphate improves efficiency of work
in isolated perfused rat hearts. *Am J Physiol Heart Circ Physiol*
23. Sullivan LP and Grantham JJ. Specificity of basolateral organ-
ic anion exchanger in proximal tubule for cellular and extra-
24. Sun JX, Farias LA, and Markov AK. Fructose 1,6-diphos-
phate prevents intestinal ischemic reperfusion injury and death in
25. Tavazzi B, Starnes JW, Lazzarino G, Di Pierro D, Nuuti-
nen EM, and Giardina B. Exogenous fructose-1,6-bisphos-
phate is a metabolizable substrate for the isolated normoxic rat
26. Trimarchi GR, ARECADA FA, De Luca R, Imperatore C, San-
toro G, Trimarchi F, and Costa G. Neuroprotective activity of
fructose-1,6-bisphosphate following transient forebrain ischemia
27. Wolffram S, BADERTSCHER M, and Scharrer E. Carrier-medi-
ated transport is involved in mucosal sucinate uptake by rat
28. Wolffram S, HAGEMANN C, GRENACHER B, and Scharrer E.
Characterization of the transport of tri- and dicarboxylates by pig
intestinal brush-border membrane vesicles. *Comp Biochem