The Effects of Inhibition of Oxidative Phosphorylation and Glycolysis on Contractility and High-Energy Phosphate Content in Cultured Chick Heart Cells

Andrew J. Doorey and William H. Barry
From the Cardiovascular Division, Brigham and Women's Hospital and the Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, Massachusetts

SUMMARY. Cultured embryonic chick ventricular cells were exposed to various concentrations of cyanide ion to determine the relationships between graded inhibition of oxidative phosphorylation, contractile performance, and high energy phosphate contents. Exposure to cyanide produced a decline in contractile amplitude (CA) of cell motion of the spontaneously beating myocytes within 30-40 seconds. The threshold cyanide concentration for this effect was $10^{-5}$ M, and progressive increments in cyanide concentration produced further declines in contractile amplitude to 18% of control at a cyanide concentration of $1.5 \times 10^{-4}$ M. Control values for adenosine triphosphate and phosphocreatine were 28.9 ± 0.08 and 17.9 ± 0.05 nmol/mg protein, respectively, and exposure of cultured cells to cyanide produced a decline in adenosine triphosphate concentration within 5-10 seconds. At low concentrations of cyanide, the decrement in high energy phosphate content paralleled the decline in contractile function after 10 minutes of cyanide exposure. However, as the concentration of cyanide was increased to above $10^{-4}$ M, high energy phosphate content did not decline below 50-60% of control values, despite further decrements in amplitude of contraction. Exposure of cultured cells to $10^{-6}$ M verapamil, which abolishes mechanical activity, resulted in preservation of high energy phosphate contents in cells exposed to cyanide at concentrations less than $10^{-4}$ M. However, there was no effect of verapamil on high energy phosphate contents in the presence of higher concentrations of cyanide. Exposure of these cells to inhibitors of glycolysis, 2-deoxyglucose (20 mM), or iodoacetate ($10^{-4}$ M) resulted in a decline in contractile amplitude (55% of control after 10 minutes of exposure to 2-deoxyglucose) that was prevented by provision of the Krebs cycle substrates, acetate or pyruvate. However, glycolytic blockade with 2-deoxyglucose, in the presence of cyanide ($10^{-4}$ M) and acetate, did produce further depression of contractile amplitude and high energy phosphate contents. These findings suggest that there is a quantitative relationship between the degree of depression of contractility and adenosine triphosphate and phosphocreatine levels only during mild-to-moderate degrees of inhibition of oxidative phosphorylation, and that negative inotropy during severe impairment of oxidative phosphorylation may preserve high energy phosphate. Energy derived from glycolysis, although not essential for maintenance of contractile function when oxidative phosphorylation is unimpaired and Krebs cycle substrate is provided, can contribute importantly to maintenance of high energy phosphate content and contractility during inhibition of oxidative phosphorylation. (Circ Res 53:192–201, 1983)

DIMINUTION of contractile force in myocardium during hypoxia is generally believed to be due to depressed mitochondrial oxidative synthesis of high energy phosphates (HEP). Initial studies suggested that negative inotropic effects develop before substantial changes in HEP content occur in cardiac muscle made acutely hypoxic (Parvez et al. 1957; Pool et al., 1966). More recently, others have found that acute changes in contractile function associated with the onset of myocardial hypoxia are preceded by and in general parallel significant changes in HEP (Scheuer and Stezoski, 1968; Dhalla et al., 1972; Hearse, 1979). A linear correlation between HEP content and contractile function has been reported in several cardiac preparations after more prolonged cellular hypoxia (Scheuer and Stezoski, 1968; Jarmakani et al., Reibel and Rovetto, 1978). These studies suggest that both during the onset of hypoxia and at a steady state level of hypoxia, depression of contractile function is quantitatively related to depression of HEP. However, Feinstein et al. (1968) did not observe a good correlation between contractile function and HEP content during recovery from myocardial hypoxia, and hypothesized that steady state levels of myocardial high energy phosphate compounds are not as important in determining mechanical capacity as are their rates of synthesis. Furthermore, there may be subcellular
compartmentation of HEP (Gudbjarnason et al., 1971), and the mechanical effects induced by hypoxia may alter HEP content (Sperelesson and Schnei
der, 1977; Hearse, 1979). Thus the exact relationships between the negative inotropic effects of myocardial hypoxia and cellular HEP content or HEP synthetic rates are unresolved.

In addition, energy derived from the glycolytic pathway can influence the effects of hypoxia. Diminished glycolysis induced by a glucose-free medium has been shown to have deleterious effects on the speed of onset and degree of recovery from a hypoxic insult in intact cardiac tissue (Weissler et al., 1968; Hearse and Chain, 1972; Burton et al., 1980), and contractile performance during hypoxia can be favorably influenced by increasing glucose concentrations in the tissue perfusate (Apstein et al., 1976). It has been documented that glycolytic pathway blockade with iodoacetate eliminates residual contractility during severe hypoxia in cultured chick heart cells (Barry et al., 1980), and similar results have been found in isolated rat hearts (Apstein et al., 1978), and rat papillary muscles (Pirzada et al., 1975; Bing and Fishbein, 1979).

Difficulties in producing homogenous, graded inhibition of energy production in myocardial tissue during which alterations in contractility and metabolic changes may be assessed during steady state conditions contribute to these uncertainties relating cardiac function to oxidative metabolism (Allison and Holsinger, 1977; Paradise et al., 1981). Cultured cardiac cells offer several advantages for studying these issues, as diffusion gradients and tissue inhomogeneity are minimized. Several investigators have used cultured or isolated heart cells to study the effects of interruption of oxidative metabolism (Harr
y and Slater, 1965; Seraydarian et al., 1968; Che
eval et al., 1972; Lacuara and Lacuara, 1973; Acosta et al., 1978; Raji et al., 1980; Higgins et al., 1981). Changes in beating rate, ATP content, and cellular enzyme release have been assessed, but contractile performance has not been quantified and correlated with metabolic interventions.

In the experiments to be described, we have studied cultured chick embryo ventricular cells in which contractility can be assessed by measurement of amplitude of cell motion (Barry et al., 1975, 1978; Biedert et al., 1979), and varying degrees of inhibition of metabolic energy production induced by exposure to inhibitors of mitochondrial electron transport (hypoxia, cyanide) and inhibitors of glycolysis (2-deoxyglucose, iodoacetate). Utilizing this preparation, we have sought to determine the relationships between graded degrees of reduction in HEP synthesis by mitochondrial oxidative phosphorylation and changes in myocardial contractility and HEP content. In addition, we have investigated the importance of glycolytically derived HEP in maintaining total HEP content and contractile function in the presence and absence of inhibition of oxidative phosphorylation, and the influence of ab-

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<td><strong>Tissue Culture</strong></td>
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Cultures of beating chick embryo ventricular cells were prepared as described previously (Barry et al., 1980). Briefly, 10-day-old chick embryo hearts were removed under sterile conditions, and the ventricles were cut into 0.5 mm fragments and placed in Ca- and Mg-free Hanks' solution. The ventricular fragments were gently agitated in 10 ml 0.025% (wt/vol) trypsin in Ca- and Mg-free Hanks' solution at 37°C for four cycles of 7 minutes each. The supernatant suspensions containing cells dissociated by these cycles were placed in 20 ml of cold trypsin inhibitor medium. This suspension was centrifuged at 2000 rpm for 10 minutes, the supernatant phase was discarded, and the cells were resuspended in culture medium consisting of 6% heat-inactivated fetal calf serum, 40% medium 199 (Grand Island Biological Co.), 0.1% penicillin streptomycin antibiotic solution, and 54% low potassium salt solution containing (mm): NaCl, 116; NaH2PO4, 1.0; MgSO4, 0.8; KCl, 1.18; and NaHCO3, 26.2. Final concentrations (mm) were: Na, 144; K, 4.1; HCO3, 17; Ca, 0.97; Cl, 131; Mg, 1.0; and glucose, 5. The suspension of cells was diluted to 4 x 10^5 cells/ml and placed in plastic tissue culture petri dishes containing 25-mm circular coverslips (VWR-VanLab thickness No. 2). Cultures were incubated in a humidified 5% CO2-95% air atmosphere at 37°C. Confluent layers two to three cells thick in which 70–80% of the cells were synchronously contracting developing by 2–3 days. Contractility studies were done on day 3 of culture. Plastic microspheres 2–3 μm in diameter (3M Co.) were added to the cultures on day 2 of the culture. The plastic microspheres became attached to cell surfaces and were moved by contraction of individual cells in the layers. This provided an improved image for contraction recording and measurement.

| Measurement of Contractility |

A glass coverslip with attached cultured heart cells was placed in a chamber provided with inlet and exit ports for culture perfusion. The chamber was placed on the stage of an inverted phase contrast microscope (Leitz Diavert) enclosed in a lucte box with controlled temperature (37°C). The inlet to the perfusion chamber was connected by polyethylene tubing to several syringe pumps so that the culture could be sequentially perfused with medium containing various substrates or metabolic inhibitors. During continuous perfusion, medium bathing a cell in the center of a coverslip exchanged with a time constant of 15 seconds at a flow rate of 0.96 ml/min. More abrupt medium changes could be produced by completely removing control medium from the chamber and then switching to a test perfusion solution, noting the instant the meniscus of test solution fluid covered the cell. The optical apparatus was supported by an air table to damp building vibrations and the cells were magnified using a 40X objective. The image was monitored by a low-light-level TV camera (Dage 650 SSX) attached to the microscope observation tube using a X2 coupler. The TV camera had an interlace defeat so that the image was composed of 262 raster lines. The motion detector moni-
were recovered with respective efficiencies of 96 ± 2% and 95 ± 2%. Cellular protein was measured using the method of Lowry et al. (1952).

Production of Hypoxia for Contractility and HEP Studies
Marked hypoxia is required to effect significant depression of contractility in these cultured cardiac cells, and therefore an oxygen-extraction apparatus is necessary to produce a sufficient degree of O₂-deficiency (Barry et al., 1980). We used an O₂ extraction system similar to that described by Meites and Meites (1948). Commercial 100% nitrogen gas was sequentially bubbled through 4 wash flasks. Flasks 1 and 2 contained 100 g of amalgamated zinc added to 100 ml of 0.1 m vanadium sulfate activated with H₂SO₄. Flask 3 contained a few crystals of Na₂SO₃ in 10 mm Na borate, and flask 4 contained distilled H₂O₂, through which the gas was bubbled to remove any traces of vanadium. The gas then was directed through copper tubing to a glass flask containing the perfusion solution. The space above the perfusion solution was continuously gassed and the solution stirred with a magnetic stirrer to promote rapid equilibration of gas tension. Equilibrated fluids then were warmed up in a glass syringe and delivered to the perfusion chamber via the inner lumen of a specially constructed double lumen tube, the outer lumen of which is gassed with hypoxic gas. The PO₂ of hypoxic perfusate was not increased when infused into the perfusion chamber in this manner.

HEP studies were done as before, with certain modifications. Several large glass syringes were filled with hypoxic medium and placed in ice slush. Within 30 minutes they cooled to 1.2 ± 0.5°C without significant change in the PO₂ (1.9 torr before, 2.2 torr after cooling). The medium of a closed incubation chamber was then rapidly exchanged repeatedly with warm hypoxic medium (37°C) via a long needle inserted into a small hole in the top of the chamber. Equilibrium at the desired hypoxic PO₂ was achieved after two or three exchanges. After 10 minutes of incubation, the entire chamber was immersed in ice slush and the medium repeatedly exchanged with the cold hypoxic medium. Equilibrium of chamber contents at 1°C and PO₂ < 2 came after three exchanges. The coverslips were then rapidly immersed in liquid N₂ within one-half second. The coverslips were then sequentially removed, covered with cold HClO₄, and analyzed as above.

Reagents
All chemicals and reagents were obtained from Sigma Chemicals, with the following exceptions: KCN (Aldrich Chemicals), verapamil (Knoll Pharmaceuticals), IAA (Eastman Kodak), and Na acetate (Fisher Scientific).

Statistics
The Mann-Whitney test was used to compare groups nonparametrically.

Results
Effects of Cyanide (CN) on Contractility and HEP Content
We first sought to determine whether a quantitative relationship exists between the degree of inhibition of oxidative phosphorylation and myocardial cell contractile performance. Perfusion of spontaneously beating cells with CN-containing medium

Perfusion Solutions
The ionic composition of the perfusion medium has been described. Solutions of Na acetate, 2-deoxyglucose (2-DG), iodoacetate (IAA), and 2,4-dinitrophenol (2,4-DNP) were made by adding the substances directly to completed culture medium. The perfusion medium was similar to the medium used for culture except that the concentration of Ca was 0.6 mm and of glucose 3 mm. A stock solution of cyanide (CN) was made daily by dissolving 130 mg of KCN in 20 ml of culture medium, resulting in a 0.1 M solution. Appropriate amounts of this solution were added to 50–150 ml of culture medium to achieve the desired final concentration of CN. The pH of all solutions was adjusted to 7.30–7.35.

Measurement of ATP and Phosphocreatine (PC)
For the high energy phosphate (HEP) content studies, 10 coverslips were placed vertically in a lucite basket and immersed in a 100-ml lucite chamber filled with the desired medium. Continuous gassing was carried out via a port in the top of the chamber. The chamber was maintained at 37°C by immersion in a heated water bath, which gently agitated the chambers to minimize unstirred layers. At the completion of an experimental intervention, the basket holding the coverslips was immediately transferred to a chamber containing culture medium chilled to 1–2°C, within 1.5 seconds. Individual coverslips were then rapidly immersed in cold HCLO₄, and analyzed as above.
produced a decline in contractile amplitude of cell motion (Fig. 1). After abrupt exposure to CN, the onset of this decline occurred within 20–30 seconds, and reached >90% of its ultimate magnitude by 2 minutes (Fig. 2A). A steady state of contractile amplitude depression was reached by 10 minutes and remained unchanged between 10 and 30 minutes of exposure. The reversibility of the CN-induced changes in contractile amplitude was assessed by washout of the CN solution after a 10-minute exposure period. The contractile amplitude rapidly recovered and returned to control by 45 minutes of washout (Fig. 2B). Thus, CN produced graded, reversible negative inotropic effects in cultured chick embryo myocardial cells.

The effects of this same concentration range of CN on ATP content are also shown in Figure 2A. A decrease in ATP content began 10 seconds after exposure to 1.3 x 10^-4 M CN, clearly preceding the decline in contractility by 20 to 30 sec. Little further decline in ATP or contractile amplitude occurred between 2 and 10 minutes, indicating that a quasi steady state of [ATP] and contractility was reached after 10 minutes of exposure to CN. On washout (Fig. 2B), [ATP] and contractile amplitude gradually returned to control levels by 45 minutes.

To determine steady state effects of graded inhibition of oxidative phosphorylation on HEP contents and contractility, we exposed the cells to various concentrations of CN for 10 minutes. The results are shown in Figure 3. A steep dose-response relationship existed for effects of CN on contractility. The threshold CN concentration for mechanical effects was 0.1 x 10^-4 M, whereas near-maximal negative inotropic effects (depression of contractile amplitude to 18% of control) were reached at a [CN] of 1.5 x 10^-4 M. Further increases in [CN] produced little further mechanical suppression, 10^-3 M CN decreasing contractile amplitude to 9 ± 2% of control.

Control HEP contents were ATP = 28.9 ± 0.8 nmol/mg prot (n = 146) and PC = 17.9 ± 0.5 nmol/mg prot (n = 106). Exposure to increasing CN concentrations produced a progressive decline in ATP content that paralleled the decline in contractile amplitude at low [CN] (Fig. 3A). However, ATP content reached a plateau of 50–60% of control as [CN] was increased to concentrations >10^-4 M, despite further falls in contractile amplitude. It was not possible to produce a further decline in HEP with higher [CN]. Thus, falls in contractile amplitude and ATP occurred in parallel during mild to moderate
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Effects of Oxidative Phosphorylation Inhibition on Contractile Amplitude of Cell Motion (CA) and Cellular ATP Content

<table>
<thead>
<tr>
<th>PO₂</th>
<th>CA [m]</th>
<th>ATP [%]</th>
<th>CN [m]</th>
<th>CA [m]</th>
<th>ATP [%]</th>
<th>2,4-DNP [m]</th>
<th>CA [m]</th>
<th>ATP [%]</th>
</tr>
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<tbody>
<tr>
<td>9</td>
<td>86 ± 4</td>
<td>74 ± 6</td>
<td>0.1 × 10⁻⁴</td>
<td>92 ± 2</td>
<td>81 ± 9</td>
<td>10⁻⁴</td>
<td>83.0 ± 4</td>
<td>57 ± 5</td>
</tr>
<tr>
<td>1</td>
<td>27 ± 3</td>
<td>52 ± 6</td>
<td>1.3 × 10⁻⁴</td>
<td>35 ± 3</td>
<td>61 ± 3.5</td>
<td>1.5 × 10⁻⁴</td>
<td>36 ± 6</td>
<td>56 ± 5</td>
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Groups of cultured myocardial cells were exposed to specific concentrations of oxygen, CN or 2,4-DNP. Measurements of contractile amplitude and ATP content were made after 10 minutes of exposure and are expressed as percent control. (n = 11-24; means ± SEM).

Role of Energy Produced by Glycolysis in Maintenance of Contractile Activity

The role that the glycolytic pathway might play in supplying energy for contractile function has not been fully delineated. We sought to answer this question by studying the effect of inhibitors of glycolysis on contractile amplitude in the presence and absence of impaired oxidative phosphorylation. Exposure of cultured cells to glycolytic blockade alone resulted in a decline in contractile amplitude. Ten and 20 mM 2-deoxyglucose (2-DG) depressed contractile amplitude to 76 ± 10% and 63 ± 4% of control, respectively, after 10 minutes. ATP content declined to 68 ± 4% of control and PC to 56 ± 6% of control 10 minutes after exposure to 20 mM 2-DG (Table 2). Na acetate (5 mM) had no effect on contractile amplitude under control conditions, yet prevented the decline in contractile amplitude resulting from exposure to 2-DG. ATP content remained at 83 ± 4% of control, while PC declined to 57 ± 5% of control.
from 2-DG. Pyruvate (5 mM) provided a similar protective effect. Thus, the negative inotropic effects of glycolytic blockade in this system likely resulted from inadequate delivery of Krebs cycle substrates, usually provided by the glycolytic pathway in the form of pyruvate. Interestingly, although provision of Krebs cycle substrate prevented the decline in contractile amplitude during exposure to 2-DG, it did not prevent the decline in HEP (Table 2). This suggests that there is some compartmentalization of HEP within the cell, in a pool being supplied by glycolysis but not required for maintenance of contractility.

We next examined the role of glycolytically derived ATP in maintaining contractility during impaired production of ATP by oxidative phosphorylation. As discussed above, this can only be specifically assessed when adequate Krebs cycle substrate is made available. Preliminary studies revealed that Na acetate (5 mM) shifted the CN-contractile amplitude concentration-effect-response curve. CN $10^{-4}$ M and Na acetate (5 mM) depressed contractile amplitude to 62 ± 3% of control (n = 12) vs. a contractile amplitude of 50 ± 2% after exposure to CN $10^{-4}$ M alone (n = 16, P < 0.05). Therefore, cells treated with CN and Na acetate were used as controls for determination of the effects of CN, Na acetate, and 2-DG. There is probably near-complete inhibition of glycolysis with 20 mM 2-DG, since 10 mM 2-DG causes 90% inhibition of glycolysis when added to cultured rat heart cells with impaired oxidative phosphorylation (Higgins et al., 1981). The results are shown in Table 3. When cells treated with CN and acetate were exposed to 2-DG, a further decline in contractile amplitude and ATP was observed, the difference being significant at the P < 0.01 and P < 0.02 levels, respectively. At least one experiment in each group was performed using iodoacetate (IAA) ($10^{-4}$ M) as a glycolytic inhibitor. The results were indistinguishable from the results seen with 2-DG.

Thus, even when care is taken to exclude changes secondary to Krebs cycle substrate availability, in the setting of partial inhibition of oxidative phosphorylation, glycolysis contributes energy that can be used for maintenance of contractile activity. In contrast, glycolytic blockade had no deleterious effects on contractile amplitude under these experimental conditions when oxidative phosphorylation was not partially inhibited.

### HEP Content Protection with Verapamil

The plateau of HEP content levels at high [CN], when contraction was severely depressed, was of interest. This suggested that the initial falls in HEP content were due to continued HEP utilization by contraction in the face of impaired energy synthesis. However, when contractile amplitude was markedly decreased by higher [CN], energy utilization was decreased, resulting in a stable HEP content in spite of further decreases in the rate of HEP production. That decreased cellular energy utilization due to negative inotropy may exert a 'protective' effect on cellular HEP has been suggested (Sperelakis and Schneider, 1977; Hearse, 1979; Watts et al., 1980). To test this hypothesis, we studied the effects of verapamil on the relationship between [CN] and HEP contents. Verapamil ($10^{-6}$ M) produces electro-mechanical uncoupling within 40–90 seconds in these cultured myocardial cells (Barry and Smith, 1982). Therefore, cultures were incubated in verapamil alone for 3 minutes before exposure to the experimental solution of $10^{-6}$ M verapamil and varying concentrations of CN. As shown in Figure 4, at low and intermediate [CN], the HEP levels were significantly higher in the cells treated with verapamil, probably reflecting the benefits of decreased mechanical energy utilization during impaired energy.

### Table 2

<table>
<thead>
<tr>
<th>2-Deoxyglucose (20 mM)</th>
<th>Contractile amplitude</th>
<th>ATP</th>
<th>Phosphocreatine</th>
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<tr>
<td></td>
<td>72 ± 5</td>
<td>68 ± 4</td>
<td>56 ± 6</td>
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One group of cultured myocardial cells were exposed to 2-deoxyglucose (20 mM) alone, and another group was exposed to 2-deoxyglucose plus Na acetate (5 mM) as well. Measurements were made after 10 minutes of exposure and are expressed as percent of control values. (n = 8–18, means ± SEM). The values for contractile amplitude are significantly different (P < 0.01) between groups, but those for ATP and phosphocreatine content are not.

### Table 3

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<tr>
<th>Cyanide (10$^{-4}$ M)</th>
<th>Contractile amplitude</th>
<th>ATP</th>
<th>Phosphocreatine</th>
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<tr>
<td>Na acetate (5 mM), 2-deoxyglucose (20 mM)</td>
<td>62 ± 3</td>
<td>68 ± 5</td>
<td>36 ± 3</td>
</tr>
</tbody>
</table>

Cyanide (10$^{-4}$ M) and Na acetate (5 mM) depressed contractile amplitude to 62 ± 3% of control (n = 12) vs. a contractile amplitude of 50 ± 2% after exposure to CN $10^{-4}$ M alone (n = 16, P < 0.05). Therefore, cells treated with CN and Na acetate were used as controls for determination of the effects of CN, Na acetate, and 2-DG. There is probably near-complete inhibition of glycolysis with 20 mM 2-DG, since 10 mM 2-DG causes 90% inhibition of glycolysis when added to cultured rat heart cells with impaired oxidative phosphorylation (Higgins et al., 1981). The results are shown in Table 3. When cells treated with CN and acetate were exposed to 2-DG, a further decline in contractile amplitude and ATP was observed, the difference being significant at the P < 0.01 and P < 0.02 levels, respectively. At least one experiment in each group was performed using iodoacetate (IAA) (10$^{-4}$ M) as a glycolytic inhibitor. The results were indistinguishable from the results seen with 2-DG.
FIGURE 4. Effects of exposure to CN on ATP (panel A) and phosphocreatine (panel B) contents in the presence and absence of verapamil (10^-6 M). Abolition of contractile activity was produced by exposure to verapamil before cells were exposed to CN. Assays were performed after 10 minutes of CN exposure. Results are expressed as means ± SEM (n = 8-31). Differences between the group exposed to CN alone and the group exposed to CN and verapamil are significant at CN = 0.1 × 10^-4 M and 10^-3 M (P < 0.01).

Discussion

The individual contributions of glycolytic and oxidative energy sources to myocardial cell function have been difficult to elucidate. The use of cultured cardiac cells reduces the problems of tissue diffusion barriers and inhomogeneities of substrate concentrations. We have previously measured contractile amplitude in spontaneously contracting cultured heart cells to define the relationship between hypoxia, glycolysis, and cardiac cellular mechanical function (Barry et al., 1980). The present study was designed to determine the relationships between inhibition of oxidative phosphorylation, cultured cell mechanical function, and HEP contents.

Cellular hypoxia of a degree required to influence the mechanical function of cultured myocardial cells is difficult to produce. In most of these experiments, therefore, we used cyanide to induce graded degrees of inhibition of oxidative phosphorylation. Cyanide inhibits mitochondrial electron transport and oxidative phosphorylation in a manner similar to cellular hypoxia. In the concentration range used in our experiments, CN has been found to depress oxidative metabolism in cultured rat heart cells (Higgins et al., 1981) and to decrease mechanical tension dependent on respiration in vascular smooth muscle (Coburn et al., 1979). In addition, CN produces mechanical dysfunction in both rat and canine myocardial tissue that is indistinguishable from that induced by hypoxia (Pirzada et al., 1975). Whereas CN can cause nonspecific effects on other cellular enzyme systems, these occur at CN concentrations several orders of magnitude higher than those required for effects on enzymes in the respiratory chain (Dixon and Webb, 1978).

Progressive increases in [CN] over a narrow range produced a profound and reproducible decrement in steady state contractility. This indicates that cardiac mechanical function is closely related to mitochondrial oxidative energy synthesis, as has been previously suggested (Neeley et al., 1967; Graham et al., 1968; Hearse, 1979). Indeed, CN in this concentration range produced a decline in HEP contents of the cultured heart cells. After abrupt exposure to CN, a decline in [ATP] preceded a decline in contractile function, a finding consistent with the results of Dhalla et al. (1972) and Hearse (1979) in intact heart preparations. With prolonged exposure to CN over a concentration range of 10^-6 to 10^-4 M, cellular ATP content declined, and contractile amplitude decreased roughly in parallel. However, further increments in [CN] > 10^-4 M failed to cause further significant decrements in ATP content despite a further depression of mechanical function over this [CN] range. A plateau of [ATP] during severe hypoxia despite progressive and profound depression of mechanical function has also been found in the hypoxic isolated rat heart models (Scheuer, 1968; Hearse, 1979). The reason for a plateau in ATP content at more severe degrees of inhibition of oxidative phosphorylation is not clear, but it may be due in part to decreased mechanical energy consumption secondary to falls in rate and amplitude of contraction.

Reversibility of the mechanical dysfunction induced by CN has been reported in cultured heart cells (Hyde et al., 1972), in intact canine hearts (Pirzada et al., 1975), and in frog atria (Nargeot et al., 1978), and is consistent with the reversible inhibition of cytochrome oxidase by CN (Dixon and Webb, 1964). In our experiments, a coincident re-
covery of contractile amplitude and [ATP] was observed after washout of CN and was complete within 45 minutes. It is important to note that the time course of recovery of ATP content and contractility on washout of CN was much slower than the rate of recovery of contractility after reoxygenation of hypoxic cells (Barry et al., 1980). This probably is due to a relatively slow rate of dissociation of CN from binding sites within the cell. However, at the CN concentrations used, tissue levels of CN in these cells were too low to permit accurate measurement of the rate course of CN washout. Other factors, such as depletion of cellular purines during CN exposure, could possibly play a role as well (Braunwald and Kloner, 1982).

There was a failure of PC levels to return to 100% control values (65 ± 5% of control after 45 minutes of washout). Complete mechanical recovery after a transient period of oxidative phosphorylation inhibition has been described (Scheuer et al., 1968), and failure of PC content to return to control values despite normalization of ATP content and mechanical function after such an insult has been previously reported as well (Hearse and Chain, 1972).

The protective effect of verapamil on HEP content during inhibition of cellular respiration is of interest. Our findings are consistent with results reported in several experimental models of hypoxia (Nayler et al., 1976, 1978; Higgins et al., 1980). It is important to note that, in our studies, direct myocardial cellular effects of this calcium channel blocker are of primary importance, as changes in cardiac afterload or myocardial blood flow, confounding factors in determining the mechanism of action in intact heart or in vivo systems, are not relevant in tissue-cultured cells. Exposure of cultured heart cells to verapamil during moderate impairment of oxidative phosphorylation appeared to result in preservation of HEP contents, possibly due to diminished contractile energy use. That no protection was seen at [CN] ≥ 10−4 M, where contractility was severely reduced in cells not exposed to verapamil, lends further support to this theory. Thus, decrements in contractile amplitude may preserve cellular HEP despite impaired oxidative energy synthesis, resulting in a failure of HEP content to relate linearly to contractile activity. The lack of protective effects of verapamil with more marked inhibition of oxidative phosphorylation is suggestive of significant noncontractile energy use in these quiescent, uncoupled cells. These findings also suggest that verapamil probably has no direct effect on energy production or utilization, aside from its negative isotropic effects.

Although 20–30% of cultured cells used in these experiments are non-muscle cells, the relative homogeneity of this preparation allows other uncertainties regarding the relationship of HEP content and contractility to be addressed. Several investigators have reported correlations between contractile function and tissue HEP content during ischemia or hypoxia (Dhalla et al., 1972; Jarmakani et al., 1978; Hearse, 1979); however, the existence of such a correlation between HEP content and contractile function on a cellular level has not been established. It is theoretically possible that individual cells retain full contractile activity as oxidative phosphorylation is progressively inhibited until a threshold concentration of HEP is reached and contraction suddenly ceases (Reibel and Rovetto, 1978). If there were tissue heterogeneity in the degree of inhibition of oxidative phosphorylation, total tissue contractility and tissue HEP content values might then appear to gradually decline in parallel. However, our results demonstrate that individual cells had graded decrements in contractile amplitude during progressive metabolic inhibition, rather than an all-or-none response.

The upward shift of the CN contractility dose-response curve by acetate is of interest. Acetate has been found to have protective effects on cardiac HEP contents during ischemia (Vary et al., 1979) and acidosis (Schaeffer et al., 1978). This effect is mediated, in part at least, by increased Krebs cycle flux and the provision of reducing equivalents for mitochondrial oxidative phosphorylation (Schaeffer et al., 1978). It is possible that a similar mechanism existed in our cells exposed to CN. Acetate may induce an increase in mitochondrial reducing equivalents during steady state mitochondrial electron transport inhibition which could increase mitochondrial electron transport and, hence, oxidative phosphorylation and contractility.

Role of Glycolysis

A preferential role for glycolytically derived energy in cardiac membrane function has been suggested by a number of studies (MacLeod and Daniel, 1965; Girardier, 1971; McDonald et al., 1971; Higgins et al., 1981), including preliminary results from our own laboratory (Hasin et al., 1980). Contractile function has not always been found to vary concomitantly with glycolytically dependent membrane function, however. In the present study, we have demonstrated that glycolytically derived energy can support a low level of mechanical work during steady state impairment of oxidative energy synthesis. Maintenance of HEP content during impaired oxidative phosphorylation by glycolysis has also been reported in guinea pig papillary muscles (McDonald et al., 1971) and in cultured fetal mouse hearts (Ingwall et al., 1975). Interestingly, however, several studies have shown that glycolytic blockade does not alter mechanical performance during normal Krebs cycle activity (Apstein et al., 1976; Frezza et al., 1976; Bing and Fishbein, 1979). During normal myocardial function, glycolysis provides about 4% of total ATP produced (Neely et al., 1975). During severe cellular hypoxia, >80% of ATP utilized is derived from glycolysis (Jennings et al., 1981). However, glycolysis alone during hypoxia (Opie, 1968; Neely et al., 1975) or ischemia (Apstein et al., 1978) can provide only 8–33% of normal myocardial energy needs. Thus, whereas glycolytically derived
energy apparently is relatively unimportant for mechanical function when oxidative phosphorylation is unimpeded, it can, in part, support contractile performance when oxidative phosphorylation is compromised.

Our finding of a decreased HEP content despite normal contractile amplitude when glycolysis alone is blocked in the presence of Krebs cycle substrate suggests HEP compartmentation in these cells, and is consistent with the decline in ATP content after 2-DG/pyruvate exposure reported in cultured heart cells by Girardier (1971). Under these conditions, oxidative phosphorylation is not limited, and contractile amplitude is unchanged; yet HEP is significantly decreased, likely in a pool largely dependent on glycolysis. In our experiments, phosphorylation of 2-DG to a nonmetabolizable analog (Kipnis and Cori, 1959; Hyde, 1972) may also have contributed to HEP depletion in cells exposed to this substance. In addition, detection of HEP deficiency in this "glycolytically dependent" pool may be facilitated in these cells because of the relative dependence of embryonic cells on glycolysis (Vleugels et al., 1976; Hoeter and Opie, 1978). It is known that patterns of substrate utilization and cellular enzyme activities change during embryonic development (Wildenthal, 1973; Ingwall et al., 1980). The reported progressive development of the creatine kinase system during fetal growth may, for instance, explain the low phosphocreatine levels found in these embryonic ventricular cells, where this system may not yet be fully developed. However, there have been no reports suggesting that these developmental changes effect the fundamental relationships between high energy phosphate synthesis and utilization and contractile function, the primary focus of this work.

The lack of correlation between changes in HEP contents and contractility during glycolytic inhibition in the presence of Krebs substrate, in addition to the "plateau" of HEP contents occurring at more severe degrees of inhibition of oxidation phosphorylation, make it likely that HEP contents in cellular compartments and/or rates of production and utilization of HEP have a more important effect on contractile function than net cellular HEP content. Thus, the relationships between oxidative phosphorylation, HEP content, and contractility in cultured heart cells appear complex. However, we can conclude that contractile strength appears largely dependent on energy derived from oxidative phosphorylation and, after abrupt inhibition of oxidative phosphorylation, [ATP] declines prior to a decrease in contractility. At a steady state of inhibition of oxidative phosphorylation, there appears to be a quantitative relationship between the decline in [ATP] and depression of contraction only for mild-to-moderate degrees of inhibition of oxidative phosphorylation. Diminished mechanical energy consumption caused by the negative inotropic effects may serve to preserve cellular HEP during more severe inhibition of oxidative phosphorylation.

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Address for reprints: William H. Barry, M.D., Cardiovascular Division, Brigham and Women’s Hospital, 75 Francis Street, Boston, Massachusetts 02115.

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A J Doorey and W H Barry

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