The effects of the exogenous provision of lactate and the endogenous production of lactate on protein synthesis in the heart

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We have investigated the effects of exogenous addition of lactate and of the stimulation of endogenous production of lactate on protein synthesis in the anterogradely perfused rat heart. In the absence of exogenous lactate, hearts release lactate into the perfusate. At lactate concentrations of 0.2 mM and greater, the heart takes up lactate. The best fit for lactate uptake plotted against exogenous lactate concentration is a rectangular hyperbola with a maximal rate of 220 $\mu\text{mol}/2\text{ h}$ per heart (wet wt. about 1 g). Uptake is half-maximal at about 1.3 mM-lactate. The stimulation of protein synthesis also exhibits a rectangular-hyperbolic dependence on exogenous lactate concentration, with maximal stimulation being about 38%. Half-maximal stimulation occurs at about 0.9 mM-lactate. We stimulated endogenous lactate production by perfusion with 2-cyanocinnamate (an inhibitor of mitochondrial pyruvate transport) at concentrations up to 70 μM . Cardiac outputs, intracellular pH and the concentrations of phosphocreatine and the adenine nucleotides were not altered. Atrial protein-synthesis rates were unchanged, but ventricular rates were decreased. We conclude that endogenous lactate production is unlikely to stimulate protein synthesis and that the stimulation of protein synthesis by exogenous lactate is related to its uptake.

INTRODUCTION

The provision of exogenous lactate (or other non-carbohydrate fuel substrates) stimulates protein synthesis in the perfused heart [1–4] by an unknown mechanism. In hearts taken from fasted rats, a correlation between protein-synthesis rates and PCr/creatine concentration ratios was observed in perfusions with various fuels (glucose, lactate, pyruvate, acetoacetate or 3-hydroxybutyrate) [3]. However, the correlation was not seen when hearts from fed rats were used [3]. With lactate plus acetate as fuels, protein-synthesis rates were stimulated [4], but pH_i fell [5], thus excluding mediation by intracellular alkalization [6]. Interventions that stimulate cardiac protein synthesis also often stimulate cardiac lactate output (see Table 1). If lactate were to mediate the effects of some of these interventions, at least three mechanisms could be suggested. Protein synthesis could be stimulated by (i) a direct action of increased extracellular lactate, (ii) increased intracellular production of lactate, or (ii) re-uptake of endogenously produced lactate from the extracellular medium. In this paper, we have examined the effects of exogenously supplied lactate on cardiac protein synthesis and lactate uptake. We have also examined whether an increase in endogenous lactate production induced by perfusion with 2-cyanocinnamate (an inhibitor of mitochondrial pyruvate transport at low concentrations [7,8]) can stimulate cardiac protein synthesis.

EXPERIMENTAL

Materials and animals

2-Cyanocinnamic acid was obtained from Lancaster Syntheses, Morecambe, Lancs., U.K. A 2 mM solution was freshly prepared in 154 mM- NaHCO_3 with warming or, preferably, a 35 mM solution was freshly prepared in dimethyl sulphoxide. Other chemicals and biochemicals were obtained from Merck, Dagenham, Essex, U.K., Sigma, Poole, Dorset, U.K., or Boehringer-Mannheim, Lewes, East Sussex, U.K. Radiochemicals and NCS

were from Amersham International, Amersham, Bucks., U.K. All solutions were prepared with double-distilled deionized water. Male Sprague–Dawley rats, from Harlan–Olac, Bicester, Oxon., U.K., were kept in the animal house at 22 °C for at least 3 days before use. During this time, they had free access to food and water. Lighting was set for a 12 h-light/12 h-dark cycle, with the light phase beginning at 08:00 h.

Anterograde heart perfusions

Hearts from fed rats of 250–300 g body wt. were removed and perfused anterogradely with 100 ml of perfusate as described previously [6,9,10]. To assist interconversion of results, the heart-wet-wt./body-wt. ratio is 0.0032 and the dry-wt./wet-wt. ratio is 0.2. Four types of perfusions were performed: (i) perfusions in which pH_i and pH_o were varied [6], (ii) perfusions in which various exogenous concentrations of sodium lactate were present, (iii) perfusions with 2-cyanocinnamate, and (iv) perfusions in which hearts were ‘volume-overloaded’ [10–12]. Perfusions in which pH_i and pH_o were varied were carried out precisely as described in [6], and previously unpublished results from that series of experiments are quoted.

In all other perfusions, the perfusate was Krebs & Henseleit [13] 25 mM- NaHCO_3 -buffered saline (measured pH at 37 °C = 7.62) equilibrated with O_2/CO_2 (19:1). For the perfusions in which the exogenous concentrations of sodium lactate (prepared as in [14]) were varied, all perfusates contained 5 mM-glucose. When present, sodium lactate was included only in the anterograde phase of the perfusions. To accommodate the extra Na^+ concentration, the NaCl concentration was decreased as necessary. The filling pressure was 0.5 kPa and the aortic pressure was 7 kPa. To measure protein synthesis, the perfusate for the anterograde perfusion contained additionally 0.4 mM-[U- ^{14}C]-phenylalanine (sp. radioactivity about 0.04 Ci/mol) and the remaining amino acids necessary to support protein synthesis each at a concentration of 0.2 mM. Hearts were perfused anterogradely for 2 h, during which time the cardiac output was

Abbreviations used: DMO, 5,5'-dimethylloxazolidine-2,4-dione; PCr, phosphocreatine; the subscript ‘i’ refers to an intracellular value; the subscript ‘o’ refers to an extracellular value.

Table 1. Effects of various interventions on protein synthesis, lactate output and ATP concentration in the anterogradely perfused rat heart

Intervention	Effect on protein synthesis	Effect on lactate output	Effect on ATP concn.	Reference
Hypertensive aortic pressures	Stimulated (+20%)	None	Decreased (–20%)	[31]; M. A. Bogoyevitch & P. H. Sugden (unpublished work)
'Volume-overload'	None	Increased (5–10-fold)	Decreased (–25%)	[10–12]; the present work
Insulin	Stimulated (+65%)	Increased (5–10-fold)	None (may be an increase in ATP/ADP ratio)	[10,16]
Adrenaline	Stimulated (+15 to 32%)	Increased (doubled)	Decreased	[6,17]; M. A. Bogoyevitch & P. H. Sugden (unpublished work)
Isoprenaline	None	Increased (about 5-fold)	Decreased (–25%)	[15]
Hypoxia (50% O ₂)	None	Increased (> 10-fold)	Not known (but unchanged in presence of insulin)	[15]
Acetate (10–20 mM)	Stimulated (+55%)	No effect in absence of glucose	None (10 mM-lactate also present)	[4,15]; the present work
Increased pH _o	Stimulated (+65 to 70%)	Initial increase then re-uptake	None	[5,16]

monitored [14]. Samples of perfusate were removed periodically during the perfusion and were treated as described previously [14]. Perfusate volumes were estimated by the dilution of [U-¹⁴C]phenylalanine radioactivity. At the end of the perfusion period, hearts were either removed from the cannulae, blotted and frozen, or were 'freeze-clamped' with tongs cooled in liquid N₂. Protein was prepared for the measurement of [U-¹⁴C]-phenylalanine incorporation as described previously [15].

Low concentrations (70 μM or less) of 2-cyanocinnamate predominantly inhibit mitochondrial uptake of pyruvate [7,8]. In experiments with 2-cyanocinnamate, the fuel provided was usually 20 mM-glucose, although in some experiments 20 mM-sodium acetate (prepared as described in [14]) was substituted and the NaCl concentration was decreased accordingly. 2-Cyanocinnamate was present in both the retrograde pre-perfusion and the anterograde perfusion. In the latter, the filling pressure was 0.5 kPa and the aortic pressure was 7 kPa. Cardiac output was monitored during these perfusions. Protein-synthesis rates were measured as above. Perfusion volumes were estimated as above or as described previously [14].

In some experiments with 2-cyanocinnamate, steady-state pH_i was determined by the partition of [2-¹⁴C]DMO as described previously [6,16]. Hearts were perfused anterogradely for 75 min. [2-¹⁴C]DMO was added after 15 min. Either ³H₂O or [³H]sucrose was added at 55 min or 65 min, respectively, in order to measure the extracellular and intracellular spaces (0.40 and 0.45 ml/g wet wt. respectively). The sizes of the extracellular and intracellular spaces were unaffected by any of the perfusion conditions.

In the final set of perfusions, hearts were subjected to a 'volume overload' as described previously [10–12]. Here the filling pressure for the anterograde perfusion was 2 kPa and the aortic pressure was 7 kPa. The perfusate glucose concentration was 20 mM. Protein synthesis was measured as above.

Other methods

Perfusate glucose, lactate and pyruvate concentrations, specific radioactivities of [U-¹⁴C]phenylalanine and the cardiac concentrations of PCr, creatine, ATP, ADP and AMP were measured as described previously [6,10,15,17] by standard techniques [18,19]. Data were analysed by using Graphpad (ISI, San Diego, CA, U.S.A.) or Microstat II (Ecosoft, Indianapolis, IN, U.S.A.) software. Results are expressed as means ± S.E.M. Statistical significance was assessed by using a two-tailed Student's *t* test, with *P* values of < 0.02 being taken as establishing a significant difference.

RESULTS AND DISCUSSION

Effects of increasing pH_o on lactate release from perfused hearts

As shown in earlier experiments (Fig. 2 in [6]) and by others [20], an increase in pH_o initially increased lactate output by the perfused heart. The maximum perfusate lactate concentration was about 1 mM and occurred at 30 min of perfusion at a pH_o of 8.21. Thereafter, there was re-uptake of lactate at the higher pH_o values [6]. By using polynomial ($y = a + bx + cx^2 + dx^3 + ex^4$) regression analysis, the correlations between protein-synthesis rates over the 30–120 min period and perfusate lactate concentrations at 60 min or 120 min were relatively poor (*P* = 0.011 or

Fig. 1. Correlation between protein-synthesis rates and lactate uptake in hearts perfused at various values of pH_o.

Data were taken from the experiments described in [6] in which protein-synthesis rates were shown to be stimulated by increasing perfusate pH_o over the range 7.18–8.08 (pH_o values given are those after 120 min of perfusion; see in Table 1 [6]). Protein-synthesis rates were measured over the 30–120 min period of the anterograde perfusion. Lactate uptake was measured by subtraction of the lactate present in the perfusate at 120 min from that at 60 min. A negative value indicates a net output of lactate. The equation for the polynomial regression line shown is $y = 1.766 + 0.0277x - (0.918 \times 10^{-4})x^2 - (1.280 \times 10^{-5})x^3 - (0.196 \times 10^{-6})x^4$ ($r^2 = 0.573$, $P = 2.72 \times 10^{-6}$, 23 degrees of freedom). Goodness-of-fit was assessed by using intermediate weighting.

Fig. 2. Dependence of lactate uptake by the perfused heart on perfusate lactate concentration

Hearts were perfused as described in the Experimental section. Net lactate uptake was measured over the 120 min of antegrade heart perfusion. Time-averaged perfusate lactate concentrations were calculated. A negative value indicates a net output of lactate. For the purposes of curve-fitting to a rectangular hyperbola, the minimum value of lactate uptake ($-49.7 \mu\text{mol}/2 \text{ h per heart}$) was subtracted from all values. The equation for the rectangular hyperbola shown is $(y + 49.7) = 269x / (0.911 + x)$ ($r^2 = 0.771$, $P < 10^{-16}$, 67 degrees of freedom). Goodness-of-fit was assessed by using actual distances.

Fig. 3. Dependence of protein-synthesis rates in the perfused heart on perfusate lactate concentration

Hearts were perfused as described in the Experimental section. Protein-synthesis rates were measured over the 120 min of antegrade heart perfusion and plotted against time-averaged perfusate lactate concentrations. For the purposes of curve fitting to a rectangular hyperbola, the minimum value of protein-synthesis rate obtained in the perfusions ($1.18 \text{ nmol of phenylalanine incorporated}/2 \text{ h per mg of protein}$) was subtracted from all values. The equation for the rectangular hyperbola shown is $(y - 1.18) = 0.454x / (0.939 + x)$ ($r^2 = 0.628$, $P = 8.44 \times 10^{-9}$, 34 degrees of freedom). Goodness-of-fit was assessed by using actual distances.

0.073 respectively). However, the correlation (Fig. 1) between protein-synthesis rates and lactate re-uptake over the 60–120 min period was better ($P = 1.2 \times 10^{-5}$). These experiments suggest the possibility that re-uptake of endogenously produced lactate may be involved in the stimulation of cardiac protein synthesis by

Fig. 4. Dependence of protein-synthesis rates on lactate uptake in perfused hearts

Hearts were perfused as described in the Experimental section. Protein-synthesis rates and net lactate uptake were measured over the 120 min of antegrade heart perfusion. A negative value of lactate uptake indicates a net output of lactate. In (a), the equation for the polynomial regression line shown is $y = 1.331 + (1.142 \times 10^{-3})x - (0.123 \times 10^{-6})x^2 + (0.172 \times 10^{-7})x^3 - (0.714 \times 10^{-10})x^4$ ($r^2 = 0.635$, $P = 2.88 \times 10^{-8}$, 31 degrees of freedom). Goodness-of-fit was assessed by using actual distances. In (b), the relative percentage stimulation of protein synthesis is plotted against lactate uptake. The basal protein-synthesis rate in the absence of exogenous lactate was $1.274 \pm 0.026 \text{ nmol}/2 \text{ h per mg of protein}$ (6 observations). The maximum rate of protein synthesis in the presence of nominally 15 mM exogenous lactate was $1.623 \pm 0.018 \text{ nmol}/2 \text{ h per mg of protein}$ (6 observations). The relative percentage stimulation was thus calculated as $100(\text{protein-synthesis rate} - 1.274) / (1.623 - 1.274)$. The nominal initial lactate concentrations were 0, 1 mM, 2 mM, 3 mM, 7.5 mM and 15 mM. Expression of the results in this manner assists in the calculation of the proportion of the stimulation by a given intervention that can be attributed to (re-)uptake of lactate. The equation (values $\pm \text{S.E.M.}$) for the linear-regression line drawn is $y = 7.27(\pm 10.75) + 0.422(\pm 0.081)x$ ($r^2 = 0.870$, $P = 10^{-4}$, 4 degrees of freedom).

increased pH_o/pH_i [6]. The reasons for the increase in lactate output with increasing pH_o/pH_i are not clear. They may include a mismatch between O_2 supply and demand when the pressure-volume work done is increased by the rise in pH_o/pH_i [6,16]. Alternatively, an increase in cytoplasmic pH may diminish the pH gradient across the mitochondrial inner membrane, thereby decreasing the efficiency of oxidative phosphorylation and consequently increasing the dependence of the heart on anaerobic glycolysis, or it may increase glycolytic flux by activation of phosphofructokinase [21,22].

Table 2. Cardiac outputs of hearts perfused with 2-cyanocinnamate

Hearts (6 in each group) were perfused as described in the Experimental section. The stability of the cardiac output over the perfusion period was assessed from the ratio of cardiac outputs at 110 and 10 min.

2-Cyanocinnamate concn. (μM)	Cardiac output at 70 min (ml/min per g wet wt.)	Cardiac output at 110 min cardiac output at 10 min (%)
0	61 $\pm$ 3	108 $\pm$ 6
20	64 $\pm$ 3	104 $\pm$ 7
40	52 $\pm$ 3	105 $\pm$ 6
70	50 $\pm$ 3	96 $\pm$ 4

Effects of perfusate lactate concentration on cardiac lactate uptake

In these experiments, the perfusate lactate concentrations were monitored every 10 or 20 min over the 2 h perfusion period. In the absence of exogenous lactate, hearts tended to release lactate initially. This was sometimes followed by a subsequent re-uptake. At higher initial exogenous lactate concentrations (nominally 7.5–15 mM), lactate uptake was linear with time. At intermediate concentrations, lactate uptake rates were not linear with time over the entire 2 h perfusion period, but lactate was never entirely removed from the perfusate. From these data, the time-averaged perfusate lactate concentration was calculated for each perfusion. Net lactate uptake or release was calculated either from linear regression analysis of lactate uptake at exogenous lactate concentrations of 7.5–15 mM or from initial and final perfusate lactate concentrations when lactate release was not linear with time. The lactate uptake values are thus not necessarily initial rates. We did not attempt to maintain the lactate concentrations in the perfusates by infusing lactate over the course of the experiment. Apart from the difficulty we have in rapidly monitoring lactate concentrations, such experiments are not practicable when hearts are net producers of lactate.

Lactate uptake values were plotted against the time-averaged perfusate lactate concentrations, and the data were fitted to a rectangular hyperbola (Fig. 2). In the absence of exogenous lactate, perfused hearts are net producers of lactate. At about 0.2 mM-lactate, the heart switches to net uptake of lactate, which increases with increasing perfusate lactate concentrations to a

maximum of about 220 $\mu\text{mol/heart}$ per 2 h of perfusion. This corresponds to a rate of lactate uptake of 4–5 nmol/ μl of intracellular water per min, and is less than the V_{max} for lactate transport calculated for guinea-pig cardiomyocytes (28 nmol/ μl of intracellular water per min [23]). However, our maximal rate of lactate uptake is not a kinetic V_{max} , as such (see [23] for further discussion). The time-averaged lactate concentration at which lactate uptake is half-maximal (i.e. 110 $\mu\text{mol/heart}$ per 2 h) is about 1.3 mM. This value is close to the K_m for lactate uptake (2.3 mM) obtained by Poole *et al.* [23] for guinea-pig cardiomyocytes at 11 °C.

Glucose uptake is inhibited by lactate uptake. In the absence of exogenous lactate, the rate of glucose uptake is 140 $\pm$ 9 $\mu\text{mol/heart}$ per 2 h (3 observations). At an initial lactate concentration of 10 mM, the rate of glucose uptake is 26 $\pm$ 9 $\mu\text{mol/heart}$ per 2 h of perfusion (3 observations, $P < 0.001$). The rates of utilization of glucose equivalents (i.e. glucose + $\frac{1}{2}$ lactate) in each condition are thus similar.

Effects of perfusate lactate concentration and cardiac lactate uptake on protein synthesis

Rates of protein synthesis exhibited a rectangular-hyperbolic dependence on time-averaged lactate concentration (Fig. 3). Protein synthesis was half-maximally stimulated at 0.94 mM-lactate, which is very similar to the value for lactate uptake. Since plasma lactate concentrations are in this range, a physiological role for lactate is implied with respect to both cardiac energy provision and maintenance of cardiac protein-synthesis rates. The maximal stimulation of protein synthesis as predicted by Fig. 3 is 38 %. We also correlated protein-synthesis rates from individual perfusions with individual values of lactate uptake (Fig. 4a). As expected, a strong correlation was observed, which appeared to approach a maximum at the highest lactate uptake rates. The linear-regression line with means of data for the stimulation of protein synthesis expressed as a percentage of the maximal stimulation (at a nominal exogenous lactate concentration of 15 mM) is shown in Fig. 4(b). From this plot, we assessed the maximum proportion of the stimulation of protein synthesis by increased pH_i [6,16] that could be attributed to lactate re-uptake over the period of measurement of protein synthesis. Protein synthesis is maximally stimulated at pH_i values of about 7.65–7.70 or greater [6,16], when lactate re-uptake was calculated to be about 60–65 $\mu\text{mol/heart}$ over 2 h of perfusion [6]. This is only sufficient to account for about 35 % of the maximum stimulation of protein synthesis. In addition, we have observed stimulation of protein synthesis (albeit proportion-

Table 3. Glucose uptake, and lactate and pyruvate output by hearts perfused with 2-cyanocinnamate

Hearts (4–6 in each group) were perfused as described in the Experimental section. In Expt. 1, glucose uptake and lactate output were determined over the 10–110 min antegrade-perfusion period. In Expt. 2, glucose uptake, and lactate and pyruvate output were determined over the 0–120 min antegrade perfusion period. The proportions of glucose converted into lactate or pyruvate that is released into the perfusate are shown in parentheses. N.D., not determined. Statistical significance versus perfusions in the absence of 2-cyanocinnamate: ^a $P < 0.02$, ^b $P < 0.01$, ^c $P < 0.001$.

2-Cyanocinnamate concn. (μM)	Glucose uptake	Glucose converted into lactate	Glucose converted into pyruvate
Expt. 1. Results expressed in units of $\mu\text{mol}/100$ min per g dry wt. of heart			
0	333 $\pm$ 28	67 $\pm$ 25 (21 %)	N.D.
20	573 $\pm$ 43 ^c	208 $\pm$ 50 (34 %)	N.D.
40	1336 $\pm$ 218 ^b	655 $\pm$ 166 (47 %)	N.D.
70	1561 $\pm$ 20 ^c	823 $\pm$ 62 ^c (53 %)	N.D.
Expt. 2. Results expressed in units of $\mu\text{mol}/120$ min per heart			
0	116 $\pm$ 23	14 $\pm$ 5 (12 %)	4 $\pm$ 2 (2 %)
70	483 $\pm$ 41 ^c	337 $\pm$ 32 ^c (70 %)	93 $\pm$ 10 ^c (20 %)

Fig. 5. Lactate and pyruvate outputs from hearts perfused with 2-cyanocinnamate

Hearts were perfused as described in the Experimental section. There were 4–6 observations at each concentration of 2-cyanocinnamate. The fuel was 20 mM-glucose. In (a), the 2-cyanocinnamate concentrations used were: none (●), 20 μM (▲), 40 μM (■) and 70 μM (□). A full statistical analysis was not undertaken, but the lactate released after 110 min of perfusion at all 2-cyanocinnamate concentrations was significantly different ($P < 0.001$) from that for perfusions in the absence of 2-cyanocinnamate. In (b), pyruvate output (▲) was monitored in addition to lactate output (■). Here the 2-cyanocinnamate concentration was 70 μM. Pyruvate output in the absence of 2-cyanocinnamate was very low (4 ± 2 μmol/heart over the 2 h perfusion period).

ally less than in the perfused heart) by increased pH_o/pH_i in isolated cardiomyocytes [24]. Here, lactate production is undetectable, presumably because the cells are quiescent and metabolizing glucose only at a basal rate.

In all perfusions, cardiac metabolite concentrations were measured. In the 36 perfusions from which data were used for Figs. 3 and 4, there were no detectable changes in ATP concentrations (mean $\pm$ S.E.M. for all 36 perfusions: 22.1 ± 0.3 nmol/mg of protein), ADP (5.11 ± 0.07 nmol/mg of protein), AMP (1.00 ± 0.03 nmol/mg of protein), ATP+ADP+AMP (28.2 ± 0.4 nmol/mg of protein), creatine (13.5 ± 0.4 nmol/mg of protein) or PCr+creatine (38.9 ± 0.7 nmol/mg of protein). At the highest two exogenous lactate concentrations (nominally 7.5 and 15 mM), PCr concentrations were 29.5 ± 0.7 and 29.5 ± 0.3 nmol/mg of protein respectively (6 observations). These were significantly greater ($P < 0.001$) than the PCr concentration in the absence of exogenous lactate (24.0 ± 0.6 nmol/mg of protein, 6 observations). We attempted to correlate protein-synthesis rates with PCr concentrations, as we have done successfully previously [5,6,16]. However, the polynomial ($y =$

$a + bx + cx^2 + dx^3 + ex^4$) regression line showed three points of inflexion over the range of measured PCr values, and was thus not considered to be significant [6]. Thus, in this instance, it cannot be suggested that protein-synthesis rates are regulated by PCr, creatine or PCr/creatine concentrations.

Characteristics of hearts perfused with 2-cyanocinnamate

Perfusion of hearts with 2-cyanocinnamate up to 70 μM did not significantly diminish cardiac performance (Table 2). As expected from [8], lactate output, pyruvate output and glucose uptake were all significantly stimulated by 2-cyanocinnamate (Fig. 5 and Table 3). There were small decreases in pH_o in perfusions with 2-cyanocinnamate (Table 4). These were presumably caused by the release of lactic acid and pyruvic acid into the perfusate. The rates of lactate output in the presence of 70 μM-2-cyanocinnamate were greater than those seen with high concentrations of insulin [10] or with hypoxia produced with perfusates gassed with $O_2/N_2/CO_2$ (10:9:1) [11,15], but were similar to those seen in the initial 30 min when hearts were perfused at high pH_o values [6]. (In [6], the dry weights of the perfused hearts were about 0.2 g.) The perfusate lactate concentrations after 110–120 min of perfusion in the presence of 70 μM-2-cyanocinnamate were 3–7 mM (Fig. 5 and Table 3). The perfusate concentration of pyruvate (which also stimulates cardiac protein synthesis [2,3]) reached about 2 mM after 120 min (Fig. 5b). The proportion of glucose taken up that was released as lactate+pyruvate was also significantly increased by 2-cyanocinnamate, being about 90% at 70 μM-2-cyanocinnamate (Table 3). The reason that cardiac output is maintained even under this condition is presumably because glycolytically produced NADH that is not utilized for reduction of pyruvate can still be oxidized by the mitochondria. Furthermore, because mitochondrial oxidation of NADH is unaffected by 2-cyanocinnamate, release of pyruvate should not lead to any sequestration of the cytoplasmic NAD^+ pool as NADH. It is difficult to deduce from our data whether there is any change in intracellular redox state in the perfusions with 2-cyanocinnamate. This is relevant, because it has been suggested that increased reduction of the $NAD^+/NADH$ couple may stimulate protein synthesis [25].

Protein synthesis in hearts perfused with 2-cyanocinnamate

Ventricular protein-synthesis rates were decreased significantly rather than increased by perfusion with 2-cyanocinnamate with 20 mM-glucose as fuel (Table 4, Expt. 1). Rates in atria were unaltered (Table 4). We tried to identify the cause of the inhibition of ventricular protein synthesis. A decrease in pH_i to hypophysiological values inhibits protein synthesis in the perfused heart [6]. Although lactate and pyruvate production was increased by 2-cyanocinnamate (Fig. 5), no decrease in cardiac pH_i was detectable (Table 4). This implies that the working heart can maintain a stable pH_i even in the face of increased lactate production, and hence that intracellular acidosis does not occur when coronary flow rates are high. It also implies that the transport of endogenous lactate out of the heart is rapid under these conditions. There were no significant decreases in ATP or PCr concentrations, nor were there any consistently significant increases in ADP or AMP concentrations in hearts perfused with 2-cyanocinnamate (Table 5). The inhibition of protein synthesis is not attributable to changes in the concentrations of these metabolites.

Because 2-cyanocinnamate inhibited protein synthesis in glucose-perfused hearts for an unknown reason, we investigated whether this compound also inhibited protein synthesis when hearts were perfused with acetate, which alone can satisfy the fuel requirements of the heart [4,9] and which does not give rise to pyruvate. No inhibition was observed, suggesting that 2-

Table 4. Protein-synthesis rates, pH_o and pH_i in hearts perfused with 2-cyanocinnamate

Hearts (4–6 in each group) were perfused as described in the Experimental section. The initial pH_o value was 7.62 at 37 °C. The values of pH_o and pH_i quoted were measured after 75 min of anterograde perfusion. N.D., not determined. Statistical significance versus perfusions in the absence of 2-cyanocinnamate: ^a $P < 0.02$, ^b $P < 0.01$, ^c $P < 0.001$.

2-Cyanocinnamate concn. (μM)	Protein-synthesis rate (nmol of phenylalanine incorporated/ 2 h per mg of protein)		pH_o	pH_i
	Ventricle	Atrium		
Expt. 1. Hearts perfused with 20 mM-glucose				
0	1.251 ± 0.027	2.290 ± 0.059	7.75 ± 0.01	7.46 ± 0.03
20	1.271 ± 0.071	2.638 ± 0.132	7.74 ± 0.01	7.48 ± 0.01
40	1.053 ± 0.060^a	2.518 ± 0.146	7.66 ± 0.01^b	7.48 ± 0.03
70	0.956 ± 0.050^c	2.363 ± 0.082	7.62 ± 0.02^b	7.48 ± 0.01
Expt. 2. Hearts perfused with 20 mM-sodium acetate				
0	1.579 ± 0.053	N.D.	N.D.	N.D.
70	1.648 ± 0.034	N.D.	N.D.	N.D.

Table 5. PCr and adenine nucleotide concentrations in hearts perfused with 2-cyanocinnamate

Hearts were perfused as described in the Experimental section. There were 4–6 observations in each group. Statistical significance versus perfusions in the absence of 2-cyanocinnamate: ^a $P < 0.02$, ^b $P < 0.01$.

2-Cyanocinnamate concn. (μM)	Metabolite concn. (nmol/mg of protein)			
	PCr	ATP	ADP	AMP
0	23.9 $\pm$ 1.5	19.3 $\pm$ 1.2	6.0 $\pm$ 0.3	1.3 $\pm$ 0.1
20	22.2 $\pm$ 1.0	25.0 $\pm$ 0.7 ^b	7.4 $\pm$ 0.3 ^a	1.0 $\pm$ 0.1
40	24.1 $\pm$ 1.3	21.1 $\pm$ 0.7	6.8 $\pm$ 0.1	1.3 $\pm$ 0.2
70	22.9 $\pm$ 1.0	22.1 $\pm$ 1.1	6.8 $\pm$ 0.2	1.4 $\pm$ 0.1

cyanocinnamate does not exert 'non-specific' inhibitory effects on protein synthesis (Table 4, Expt. 2).

Protein synthesis in other situations when lactate output is raised

We studied another condition (the 'volume-overloaded' heart) in which the lactate output is increased (Fig. 6). Here the left-atrial filling pressure is raised, and consequently the hydraulic work done by the heart is increased because of the increase in the volume component [10,11]. In these hearts, there is a mismatch between O_2 supply and demand because of insufficient coronary flow and the limited O_2 -carrying capacity of the perfusate. This leads to an increased reliance of the heart on anaerobic glycolysis [10]. There

was no evidence that protein synthesis is stimulated in 'volume-overloaded' hearts (Table 6). These experiments were not unequivocal, since 'volume-overload' significantly decreases ATP and total adenine nucleotide concentrations (by 20–25%) and the ATP/ADP concentration ratio (Table 6). It could be argued that the decrease in the ATP concentration could limit any stimulatory effects of an intervention (see also [15]). For this reason, we have tried to draw together data from our own work using the anterogradely perfused heart relating to this problem (Table 1). Stimulation of protein synthesis can be observed in situations where ATP concentrations are decreased by 20–25% (hypertensive aortic pressures, adrenaline). The 'volume-overload' model thus probably supports the conclusions from experiments with 2-cyanocinnamate. Data from experiments with hypoxia [15] are also supportive, but here there was a decrease in cardiac output. It thus seems that the 2-cyanocinnamate model is the only condition identified thus far in which there is no stimulation of protein synthesis when lactate output is increased, and adenine nucleotide and creatine metabolite concentrations or cardiac output are unchanged.

Possible mechanisms by which lactate may stimulate protein synthesis

Our conclusion from these experiments is that the stimulation of protein synthesis by lactate is dependent on either its extracellular concentration or its uptake. It seems unlikely that the former could directly affect protein-synthesis rates. However, uptake of lactate could cause a change in the concentration of some effector of protein synthesis. It is difficult to rationalize the difference between uptake of exogenous lactate and intracellular

Table 6. Protein synthesis and metabolite concentrations in 'volume-overloaded' perfused hearts

Hearts (6 in each group) were perfused as described in the Experimental section. The aortic pressure was 7 kPa. Statistical significance versus perfusions at 0.5 kPa filling pressure: ^a $P < 0.01$, ^b $P < 0.001$.

Filling pressure (kPa)	Protein-synthesis rate (nmol of phenylalanine incorporated/2 h per mg of protein)	Metabolite concn. (nmol/mg of protein)						
		PCr	Creatine	ATP	ADP	AMP	ATP+ADP + AMP	ATP/ADP
0.5	1.634 $\pm$ 0.045	27.2 $\pm$ 1.3	16.2 $\pm$ 1.1	23.2 $\pm$ 0.6	6.7 $\pm$ 0.4	1.0 $\pm$ 0.1	31.0 $\pm$ 1.0	3.48 $\pm$ 0.14
2.0	1.670 $\pm$ 0.080	27.0 $\pm$ 0.8	17.3 $\pm$ 0.7	17.5 $\pm$ 0.5 ^b	5.9 $\pm$ 0.2	1.1 $\pm$ 0.1	24.5 $\pm$ 0.7 ^b	2.95 $\pm$ 0.5 ^a

Fig. 6. Lactate output from 'volume-overloaded' hearts

Hearts were perfused as described in the Experimental section at left-atrial filling pressures of 0.5 (■) or 2.0 (▲) kPa. Each value of lactate output at 2.0 kPa was significantly different ($P < 0.01$) from that at the corresponding time at 0.5 kPa.

production of lactate with respect to the stimulation of protein synthesis. However, there does seem to be a difference, since the former causes an intracellular acidosis [5,26], whereas the latter does not, and instead decreases pH_o (Table 4). Lactate is transported (or diffuses at high concentrations) into and out of the heart across the sarcolemma as the free acid [23,27]. If the intracellular concentration were to be higher when lactate is supplied exogenously than when its endogenous production is stimulated by 2-cyanocinnamate, this could account for the lowering of pH_i by exogenous lactate, the relative constancy of pH_i with 2-cyanocinnamate and the stimulatory effect of exogenous lactate on protein synthesis. More complex interpretations are possible. In the heart, one of the major mechanisms in the recovery from intracellular acidosis is an increased activity of the Na^+/H^+ exchanger [28,29]. If transport of endogenous lactate out of the heart is sufficiently rapid, there may be no change in pH_i and hence no activation of Na^+/H^+ exchange. However, if lactate uptake decreases pH_i , Na^+/H^+ exchange may be activated. Increased membrane Na^+ flux into the cardiac cell may stimulate protein synthesis by an unknown mechanism [30]. Alternatively, it may be that the mitochondrial oxidation of pyruvate derived from lactate is the critical determinant for the stimulation of protein synthesis.

We conclude that endogenous lactate production is unlikely to be responsible for the stimulation of protein synthesis by insulin, adrenaline or acetate (which stimulates protein synthesis in the absence of glucose without stimulating lactate production [4,9]). However, we have not yet excluded the possibility that part of

the stimulation of protein synthesis by increased pH_o/pH_i could be caused by re-uptake of endogenous lactate by the heart.

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