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Effects of increasing extracellular pH on protein synthesis and protein degradation in the perfused working rat heart

Stephen J. FULLER, Catherine J. GAITANAKI* and Peter H. SUGDEN
Department of Cardiac Medicine, National Heart and Lung Institute, University of London, Dovehouse Street, London SW3 6LY, U.K.

Increasing the extracellular pH over the range pH 7.4–8.9 stimulated protein synthesis by about 60% in the rat heart preparation anterogradely perfused *in vitro*. Protein degradation was inhibited by this pH increase. The magnitudes of the effects at pH 8.9 on protein synthesis and degradation were similar to those of high concentrations of insulin. Cardiac outputs were increased, as were cardiac phosphocreatine contents, indicating that the alterations in extracellular pH did not adversely affect the physiological viability of the preparation. ATP contents were unaltered. The creatine kinase equilibrium was used to assess the magnitude of the change in intracellular pH induced by these treatments. The increase in intracellular pH was about 0.2 for a 1-unit increase in extracellular pH. Thus small changes in intracellular pH have dramatic effects on cardiac protein turnover.

INTRODUCTION

Recently, interest in the regulation of intracellular processes by intracellular pH (pH_i) has increased (for reviews, see [1–5]). pH_i affects metabolic activity, $[Ca^{2+}]_i$, cyclic AMP concentrations and the conductivity of some ion channels, and it may be involved in transmembrane signalling. Increases in pH_i can be induced by peptide hormones/growth factors and protein kinase C activators, and can be relatively large (as much as 0.1–0.3 pH unit). The major mechanism involved in this pH_i increase is thought to involve the Na^+/H^+ antiporter by which H^+_i is expelled in exchange for Na^+_o. In isolated sheep heart Purkinje fibres or cultured cardiac myocytes from chick embryos, pH_i can also be conveniently manipulated by altering pH_o [6,7]. It is important to realize that relatively large changes in pH_o *in vitro* induce only relatively small changes in pH_i. This is because of the operation of intracellular buffering mechanisms or other limiting factors (such as permeability barriers), and possibly because of the constitution of the media. The gradient of a plot of steady-state pH_i against pH_o is only about 0.20–0.23, as measured directly by using intracellular microelectrodes or pH-sensitive dyes [6,7]. Because of the involvement of pH_i in the regulation of intracellular processes, we decided to investigate whether there were any effects of increased pH_o on cardiac protein turnover. As a model, we used the working (anterogradely perfused) rat heart *in vitro*, because the cardiac output can be used to assess the physiological viability of the preparation. We show that increased pH_o has dramatic effects on cardiac protein turnover and suggest that this may be the result of an increase in pH_i. We discuss the possible relevance of these results with respect to other interventions known to affect cardiac protein turnover.

EXPERIMENTAL

Materials and animals

Materials and rats were from sources given previously [8].

Perfusion buffers

Most perfusions were carried out with modified Tyrode's solution equilibrated with O_2. When Hepes was buffer, a solution containing 10 mM-Hepes, 120 mM-NaCl, 6 mM-KCl, 1 mM-$MgCl_2$, 2 mM-$CaCl_2$ and 5 mM-glucose was adjusted at 22–25 °C to the desired pH by using 10 M-NaOH and a pH-meter calibrated between 7.00 and 9.00. Solid NaCl was then added to give a total Na^+ concentration of 140 mM. When Tris was buffer, a solution containing 10 mM-Tris base, 140 mM-NaCl, 6 mM-KCl, 1 mM-$MgCl_2$, 2 mM-$CaCl_2$ and 5 mM-glucose was adjusted at 22–25 °C to the desired pH with 12 M-HCl. Additionally, some perfusions were carried out with Krebs–Henseleit bicarbonate-buffered saline [9] (119 mM-NaCl, 25 mM-$NaHCO_3$, 4.7 mM-KCl, 2.5 mM-$CaCl_2$, 1.2 mM-$MgSO_4$, 1.2 mM-KH_2PO_4 and 5 mM-glucose) equilibrated with O_2:CO_2 (19:1) with a measured pH of 7.40 at 37 °C.

Heart perfusions

After a retrograde pre-perfusion during which cannulation was completed, hearts from 275–325 g fed male rats were perfused anterogradely essentially as described previously [10,11]. The buffer volume was 120 ml for protein-synthesis measurements and 100 ml for protein-degradation measurements. The filling pressure was 0.5 kPa and the aortic pressure was 7.0 kPa. Cardiac output (i.e. the sum of aortic and coronary flows) was monitored throughout. For protein-synthesis

Abbreviations used: the subscript 'i' refers to an intracellular value, the subscript 'o' refers to an extracellular value and the subscript 'c' refers to a cytoplasmic value; DMO, 5,5'-dimethyloxazolidine-2,4-dione.

* Permanent address: Laboratory of Animal Physiology, Department of Zoology, School of Science, Aristotelian University of Thessaloniki, Thessaloniki 54006, Greece.

measurements, pre-perfusion buffers did not contain amino acids but were otherwise identical with the perfusion buffers. For protein-degradation measurements, both buffers were the same. When required, insulin and/or cycloheximide were added to both the pre-perfusion and the perfusion buffers.

Ventricular protein synthesis was measured as described in detail in [12,13]. Amino acids were added after 10 min of anterograde perfusion. [U-^{14}C]Phenylalanine concentration was 0.4 mM (sp. radioactivity 0.04 Ci/mol). At this concentration, extracellular [U-^{14}C]phenylalanine specific radioactivity rapidly equilibrates with that of phenylalanyl-tRNA [14]. The remaining amino acids required for protein synthesis were each present at 0.2 mM. Under these conditions, protein synthesis is linear with time for 2 h [12]. Hearts were 'freeze-clamped' in aluminium tongs cooled in liquid N_2 at 2 h after addition of amino acids and were stored at −20 °C until processing. Protein degradation was measured by phenylalanine release in the presence of 20 μM-cycloheximide [15] during 90 min of anterograde perfusion by using an isotope-dilution method [15,16].

Measurement of cardiac pH_i by using [^{14}C]DMO

Cardiac pH_i was measured in perfusions with Krebs–Henseleit buffer in the presence and absence of insulin by using DMO [17]. Hearts were anterogradely perfused with 100 ml of medium. After 40 min, [2-^{14}C]DMO (0.5 ml of 100 mM, 0.1 Ci/mol) was added. To half of the perfusions, ^3H_2O (0.5 ml, containing 10 μCi) was added after 70 min. To the remainder, [^{3}H]inulin (0.05 ml, containing 10 μCi) was added after 90 min. Hearts were removed from the cannulae after 100 min, weighed and homogenized in 5 ml of water. Trichloroacetic acid (0.15 ml of 100%, w/v) was added, and protein was removed by bench centrifugation. Samples of perfusate were taken at the end of the perfusions. Heart supernatants and perfusates were counted for radioactivity by using a dual-isotope programme. Extracellular and intracellular spaces were calculated, and hence the distribution ratio ($[DMO]_i/[DMO]_o$) of [2-^{14}C]DMO was obtained. pH_i was calculated from the equation [18]:

$$pH_i = pK_a + \log\left\{\frac{[DMO]_i}{[DMO]_o}\cdot(1+10^{pH_o-pK_a})-1\right\}$$

pK_a was taken to be 6.28 [18] and pH_o was 7.40.

Other methods

Protein was determined as in [19], with bovine serum albumin as standard. For protein-degradation experiments, heart dry weights were measured [15] and were converted into protein equivalents by using a protein/dry-weight ratio of 0.8 [8]. Metabolites were measured in freeze-clamped hearts by standard techniques [20]. Glucose uptake and lactate output were measured as described elsewhere [10]. Results are presented as means ± S.E.M. Statistical significance was determined by an unpaired Student's t test, with values of $P < 0.05$ taken as being significant.

RESULTS

Effects of increasing pH_o on cardiac performance

Cardiac outputs in hearts perfused at pH 8.4 or pH 8.9 were greater than at pH 7.4 (Table 1) because of greater aortic flows. There were no significant differences in flows in hearts perfused at pH 7.4 with either Krebs–Henseleit buffer or with Hepes-buffered Tyrode's solution (results not shown).

Table 1. Effects of pH_o on aortic flow, coronary flow and cardiac output

Hearts were perfused anterogradely as described in the Experimental section. Measurements were made after 80 min of anterograde perfusion. Hepes was used to buffer at pH 7.4, Hepes or Tris was used at pH 8.4 and Tris was used at pH 8.9. Since insulin did not affect cardiac performance (results not shown; see also [23]), results in the absence and presence of insulin were combined. Statistical significance versus perfusions at pH 7.4: $^aP < 0.01$, $^bP < 0.001$.

		Flow or output (ml/min per heart)		
pH_o	n	Aortic flow	Coronary flow	Cardiac output
7.4	15	34 ± 2	15 ± 1	49 ± 2
8.4	19	55 ± 4^b	16 ± 1	72 ± 5^b
8.9	6	55 ± 6^b	16 ± 2	71 ± 8^a

Effects of increasing pH_o on protein synthesis in perfused hearts

For Tyrode's solutions, all pH values given in this paper refer to those at 22–25 °C. For Hepes, the pK_a/°C is −0.014. The pH of the Hepes solutions at 37 °C is thus approx. 0.2–0.3 pH unit less than at 22–25 °C. Thus, for the protein-synthesis experiments, perfusions with Krebs–Henseleit buffer and Hepes-buffered Tyrode solutions at pH 7.4 and 7.7 were used as controls. For Tris, the pH/°C is −0.028 [21]. The pH of Tris-buffered solutions at 37 °C is thus approx. 0.3–0.4 pH unit less than at 22–25 °C. The possibility that buffer constitution itself might be affecting protein synthesis was excluded by 'overlapping' their pH values.

As shown in Table 2, perfusion with Krebs–Henseleit solution, pH 7.4, and Hepes-buffered Tyrode's solution, pH 7.4 or 7.7, gave similar (within 10–15%) rates of protein synthesis (thus excluding artefacts arising from the temperature-dependence of buffer pH or buffer constitution). The same was true for Hepes-buffered Tyrode's solution, pH 8.4, and Tris-buffered Tyrode's solution, pH 8.4 (Table 2). Protein synthesis was progressively stimulated by increasing the pH_o above pH 7.4–7.7 (Table 2). At pH 8.9, the stimulation of protein synthesis was similar to that produced by maximally effective insulin concentrations. (In Krebs–Henseleit buffer at pH 7.4, protein synthesis is maximally stimulated by 0.1 munits of insulin/ml [22].) Stimulation by increased pH_o and by maximally effective concentrations of insulin was not additive, the maximum attainable being about 60%. It thus follows that the proportional stimulation of protein synthesis by insulin is pH-dependent.

Effects of increasing pH_o on protein degradation in perfused hearts

As in perfusions with Krebs–Henseleit buffer [15], protein degradation was linear with time in hearts perfused with Hepes at pH 7.4 (Fig. 1). Rates were

Table 2. Effects of pH_o and insulin on protein synthesis and glucose uptake

Hearts were perfused as described in the Experimental section. In Expt. 1 the insulin concentration was 10 munits/ml; in Expt. 2 it was 20 munits/ml. The extent of stimulation of protein synthesis compared with perfusions with Hepes, pH 7.4, is given in parentheses as a percentage. Statistical significance for protein synthesis: [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$, versus perfusions at pH 7.4 with Hepes buffer; [d]$P < 0.01$, [e]$P < 0.001$, versus perfusions with Krebs–Henseleit buffer at pH 7.4; [f]$P < 0.001$ versus Hepes at pH 7.7. Statistical significance for glucose uptake: [g]$P < 0.001$ versus perfusions in the absence of insulin at the same pH.

Perfusion buffer	Protein synthesis (nmol of phenylalanine incorporated/h per mg of protein)	Glucose uptake (μmol/h per heart)
Expt. 1		
Hepes (pH 7.4)	0.62±0.01	88±10
Krebs–Henseleit (pH 7.4)	0.68±0.02 (+10)[a]	105±4
Hepes (pH 7.4)+insulin	0.96±0.03 (+56)[c]	211±19[g]
Hepes (pH 7.7)	0.72±0.02 (+16)[b]	88±17
Hepes (pH 8.0)	0.77±0.01 (+25)[c,d]	63±8
Hepes (pH 8.0)+insulin	1.00±0.02 (+61)[c,e,f]	247±14[g]
Hepes (pH 8.4)	0.91±0.03 (+47)[c,e,f]	76±14
Expt. 2		
Hepes (pH 7.4)	0.54±0.03	–
Hepes (pH 8.4)	0.79±0.03 (+48)[c]	–
Hepes (pH 8.4)+insulin	0.87±0.04 (+62)[c]	–
Tris (pH 8.4)	0.76±0.02 (+42)[c]	–
Tris (pH 8.9)	0.90±0.02 (+68)[c]	–

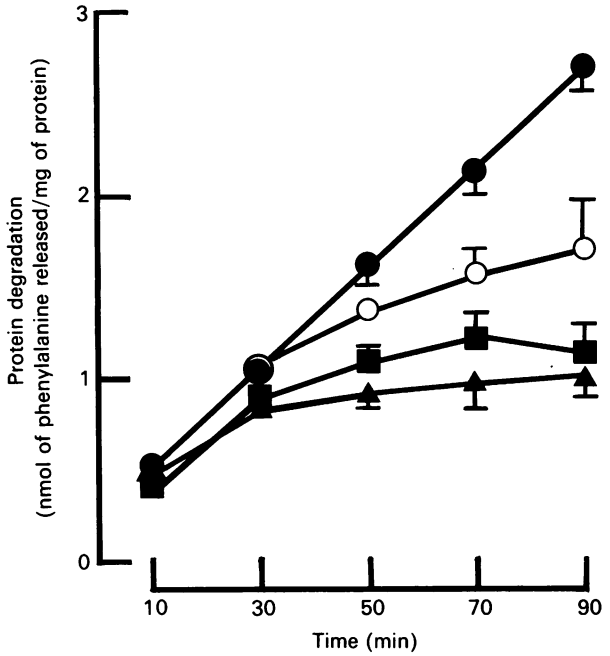

Fig. 1. Effects of pH_o on protein degradation

Hearts were perfused and protein degradation was measured as described in the Experimental section. Conditions of pH_o were as follows: ●, Hepes (pH 7.4); ○, Tris (pH 8.4); ■, Tris (pH 8.9); ▲, Hepes (pH 7.4)+25 munits of insulin/ml. Results are presented as means of six to eight independent observations. For simplicity, S.E.M. bars are shown only when there were significant differences from perfusions with Hepes, pH 7.4 (●). For perfusions with Tris, pH 8.4 (○), the values at 70 and 90 min were significantly different from those for Hepes, pH 7.4 (●), at $P < 0.01$. For perfusions with Tris, pH 8.9 (■), the values at 50, 70 and 90 min were significantly different from those for Hepes, pH 7.4 (●), at $P < 0.01$, $P < 0.001$ and $P < 0.001$ respectively. For perfusions with Hepes (pH 7.4)+insulin (▲), the values at 50, 70 and 90 min were significantly different from those for Hepes, pH 7.4 (●), at $P < 0.001$.

similar (about 1.3 μmol of phenylalanine released/h per g dry wt. in Fig. 1; see [15,23] for earlier results). Protein degradation was progressively inhibited by increasing pH_o above pH 7.4. The rates of protein degradation at pH 8.4 and pH 8.9 were not constant with time. If the inhibition were to require an increase in pH in some intracellular compartment such as the lysosomes, the non-linearity may represent the time taken to alter the pH in that compartment.

The effects of pH_o 8.9 or of insulin (25 munits/ml) at pH_o 7.4 on protein degradation were very similar (Fig. 1). (In Krebs–Henseleit buffer, pH 7.4, cardiac protein degradation was maximally inhibited by 0.1 munits of insulin/ml [15].) Unlike perfusions with insulin with Krebs–Henseleit buffer pH at 7.4 [15], the rate of protein degradation in the presence of insulin in Hepes at pH 7.4 was not linear. We do not have any explanation of this effect, unless it is related to buffer constitution.

Glucose uptake and lactate output of perfused hearts

Both glucose uptake (Table 2) and lactate output (results not shown; see [10] for typical behaviour) were significantly stimulated by insulin at pH_o 7.4 or 8.0. Increasing pH_o alone did not significantly increase glucose uptake (Table 2) or lactate output (results not shown).

Adenine nucleotide and phosphocreatine concentrations in perfused hearts

In order to detect any disturbances in energy metabolism, perfused hearts used in protein-synthesis experiments were assayed for adenine nucleotide and

Table 3. Adenine nucleotide and phosphocreatine contents in perfused hearts

Hearts were perfused anterogradely for a total of 130 min as described in the Experimental section. They were then freeze-clamped. When it was present, the insulin concentration was 10 munits/ml. Statistical significance versus perfusions with Hepes, pH 7.4: $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

Perfusion buffer	n	Content (nmol/mg of protein)				
		ATP	ADP	AMP	Phosphocreatine	ATP/ADP
Hepes (pH 7.4)	11	19.1±0.9	6.3±0.3	0.88±0.09	21.4±1.3	3.03±0.07
Hepes (pH 7.4)+insulin	4	16.8±0.9	3.9±0.3^{c}	0.63±0.03	22.1±1.4	3.87±0.34^{b}
Hepes (pH 8.0)	5	18.0±0.7	4.7±0.2^{b}	0.74±0.09	27.1±1.1^{a}	3.84±0.14^{c}
Hepes (pH 8.4)	9	18.8±0.6	5.3±0.6	0.87±0.13	32.8±1.3^{c}	3.48±0.27
Tris (pH 8.9)	5	17.4±0.9	5.3±0.2	0.93±0.09	36.4±0.6^{c}	3.30±0.09^{a}

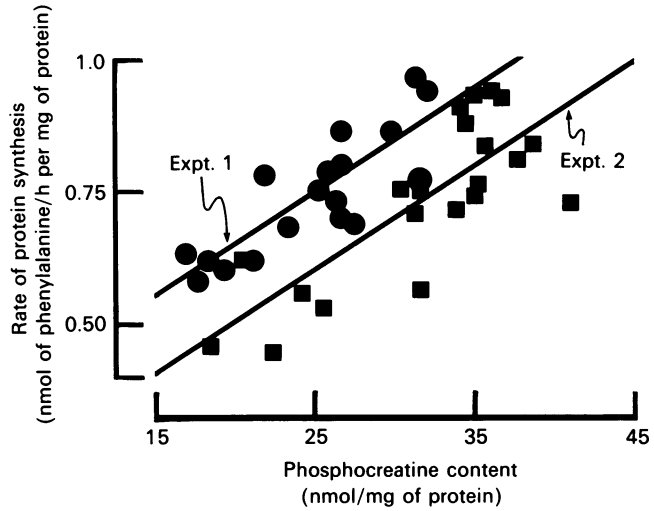

Fig. 2. Correlation between cardiac phosphocreatine contents and rates of protein synthesis

Values for individual perfusions in Hepes- or Tris-buffered Tyrode's solution from Expts. 1 (●) and 2 (■) (see Table 2) were plotted. For Expt. 1, $r = 0.83$, $n = 18$, $P < 0.001$. For Expt. 2, $r = 0.79$, $n = 21$, $P < 0.001$.

phosphocreatine contents (Table 3). Phosphocreatine contents were progressively increased by increasing pH_o. ATP was unaltered. Increasing pH_o caused some inconsistent decreases in ADP and increases in the ATP/ADP ratio. ATP content was not detectably altered by insulin, but ADP content was decreased, leading to an increase in the ATP/ADP ratio, as we have shown previously [13]. In these experiments in Hepes-buffered Tyrode's solution, we did not observe any increase in phosphocreatine content in the presence of insulin, although we have seen increases in perfusions with Krebs–Henseleit buffer [13].

Correlation between rates of protein synthesis and phosphocreatine contents

There was a linear correlation (Fig. 2) between rates of protein synthesis and terminal cardiac phosphocreatine contents in perfusions with Tyrode's solutions in the absence of insulin in both Expt. 1 and Expt. 2 (Table 2). There were no consistent correlations between rates of protein synthesis and ATP, ADP or AMP contents, or with ATP/ADP ratios. Although such plots are of interest, they should be interpreted with caution, since a correlation does not indicate a cause.

Variation of pH_c as a function of pH_o as assessed by the creatine kinase equilibrium

Details of the use of the creatine kinase equilibrium to calculate the variation of cytoplasmic pH_i as a function of pH_o are given in Fig. 3 legend. A plot of α ($= pH_c - pK_{eq.}$) against pH_o was linear ($r = 0.90$, $P < 0.001$, $n = 10$). The slope ($= \Delta pH_c/\Delta pH_o$) was 0.22, in good agreement with values obtained by others using more direct measurements [6,7]. If the change in log ($[ADP]_{total}/[ATP]_{total}$) is small compared with the change in log ([phosphocreatine]/[creatine]) (which is the case in our experiments), then a plot of log ([phosphocreatine]/[creatine]) versus pH_o gives $\Delta pH_c/\Delta pH_o$. This plot (not shown) has a slope of 0.19 ($r = 0.89$, $P < 0.001$, $n = 10$). Knowing that pH_i is 7.18 (see below) at a pH_o of 7.4, the value of pH_c at a given pH_o can be calculated from the equation $pH_c = 7.18 + 0.22\ (pH_o - 7.4)$.

Steady-state pH_i in the presence and absence of insulin

Extracellular space was 0.351±0.008 ml/g wet wt. ($n = 14$), and the sum of the extracellular and intracellular spaces was 0.801±0.008 ml/g wet wt. ($n = 14$). The DMO distribution ratio in the absence of insulin was 0.629±0.018 ($n = 12$), equivalent to a pH_i of 7.18. This agrees with more direct measurements of pH_i at a pH_o of 7.4 [6,7]. In the presence of 20 munits of insulin/ml the DMO distribution ratio was 0.655±0.015 ($n = 12$), equivalent to a pH_i of 7.20. Thus insulin did not cause any detectable change in overall cardiac pH_i.

DISCUSSION

Magnitude of changes in pH_i as a function of changes in pH_o

As we described in the Introduction, large changes in the pH_o of Tyrode's solutions induce relatively small changes in pH_i in cardiac tissue *in vitro* [6,7]. We do not contend that changes in pH_o of the magnitude used here (1.5 pH units) are encountered physiologically, but rather that small changes in pH_i may lead to large effects on protein turnover. With respect to the time course of pH_i change, steady-state pH_i is attained after about 30 min in sheep Purkinje fibres [6]. In hearts perfused through an intact coronary circulation, a steady-state

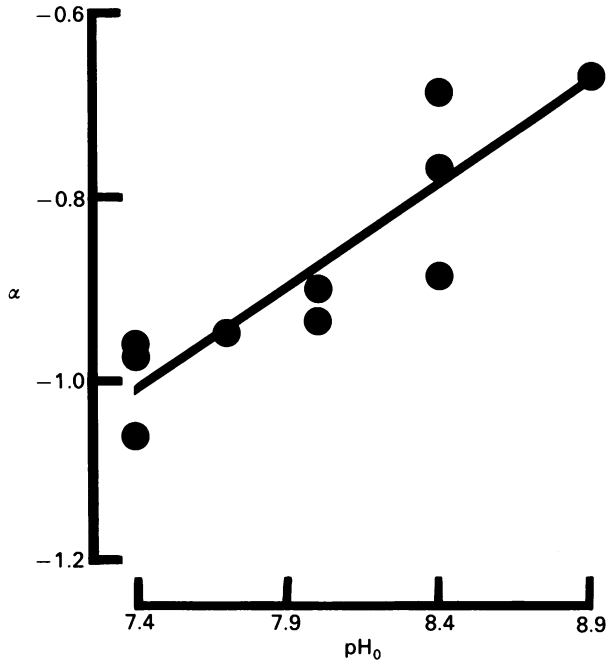

Fig. 3. Dependence of pH_i on pH_o as assessed by the creatine kinase equilibrium

Creatine kinase catalyses the following cytoplasmic reaction [52,53], which is close to equilibrium *in vivo*:

$$\text{Creatine} + \text{ATP} \rightleftharpoons \text{phosphocreatine} + \text{ADP} + \text{H}^+$$

Thus
$$K_{eq.} = \frac{[\text{phosphocreatine}]\cdot[\text{ADP}]_c\cdot[\text{H}^+]_c}{[\text{creatine}]\cdot[\text{ATP}]_c}$$

If the whole heart total [ADP]/[ATP] ratios reflect cytoplasmic [ADP]/[ATP] ratios ([ATP]/[ADP] ratios in respiring heart mitochondria are similar to the ratios given in Table 3 [54]), and if other factors (such as $[Mg^{2+}]_c$ and the proportion of adenine nucleotides bound to proteins) remain constant, then:

$$pH_c = pK_{eq.} + \log\left(\frac{[\text{phosphocreatine}]}{[\text{creatine}]}\right) + \log\left(\frac{[\text{ADP}]_{total}}{[\text{ATP}]_{total}}\right)$$

Let

$$\alpha = \log\left(\frac{[\text{phosphocreatine}]}{[\text{creatine}]}\right) + \log\left(\frac{[\text{ADP}]_{total}}{[\text{ATP}]_{total}}\right)$$

Thus

$$pH_c - pK_{eq.} = \alpha$$

α was calculated from results given in Table 3 and other results (not shown) by using a value for creatine content of 87.5 nmol/mg of protein [55] and was plotted against pH_o.

pH_i might be achieved more rapidly. We started measurements 20–25 min after exposure of the hearts to the various pH_o buffers; thus a steady state should have been attained.

The mechanism by which pH_o modulates pH_i *in vitro* should be considered. The Tyrode's solutions used here and elsewhere [6,7] were nominally HCO_3^-/CO_2-free, thus preventing the operation of HCO_3^--dependent pH_i-modulating systems [4,5]. A possible mechanism involves the operation of the Na^+/H^+ antiporter [6,24], with H^+_i leaving the cell in exchange for Na^+_o. *In vivo*, pH_i might reflect pH_o more closely when HCO_3^--dependent buffering systems will be operational. In acidosis induced by injection of HCl or breathing an atmosphere containing an increased partial pressure of CO_2, pH_i quite closely reflected pH_o [17].

We would have liked to have measured pH_i more directly during our perfusions. Unfortunately, microelectrode or indicator-dye methods are not suitable in the beating heart. pH_i can be measured by using DMO. However, theoretical considerations show that, on increasing pH_o from 7.4 to 8.4, this method would not discriminate between an unchanged normal pH_i of 7.18 and an expected pH_i of 7.40. (The differences in total cardiac DMO content would be less than 5%). This situation arises because pH_i is likely to be much less than 8.4, and hence DMO in the extracellular space constitutes a large proportion of the total heart DMO. The method would discriminate between a pH_i of 7.18 and 8.4, but we would expect hearts to fail if such a large increase were to occur. Wash-out experiments might be preferable, but we have not done these as yet.

Possible mechanisms by which pH_i and pH_o might affect protein turnover

The overall effects of increased pH_i or pH_o are to produce a more positive nitrogen balance in the perfused heart. For protein synthesis, it is probable that in our acute experiments translation (rather than transcription or RNA processing) is affected, since effects are observed so rapidly. In cell-free translation systems, a sharp pH optimum of 7.4 has been demonstrated [25]. If protein degradation is in part lysosomal, intracellular alkalinization may increase intralysosomal pH and hence inhibit the process. Equally, the pH optima of any hypothetical non-lysosomal proteases may be on the acidic side.

The effects that we have observed may not be the direct result of an increase in pH_o or pH_i, since several other cellular processes are pH-dependent (see the Introduction). Perhaps most importantly, there are complex relationships between intracellular and extracellular H^+, Na^+ and Ca^{2+} concentrations [7,26–28]. Changes in pH_i alter intracellular Ca^{2+} handling and changes in pH_o alter sarcolemmal Ca^{2+} handling [7]. These alterations, combined with an increased sensitivity of the myofibrillar ATPase to Ca^{2+}_i [29], probably cause the increase in cardiac output and contractility observed with increased pH_i and pH_o. It has been proposed that increased Ca^{2+}_i may stimulate protein synthesis. However, perfusion of hearts with media containing increased Ca^{2+}_o (which results in increased Ca^{2+}_i) did not alter rates of protein synthesis [30] or degradation [31], although, in certain isolated-cell systems, Ca^{2+} has been shown to stimulate synthesis [32].

The increase in heart volume work when pH_o was increased could affect protein turnover. However, an increase in cardiac output itself induced by increasing the filling pressure at a constant aortic pressure (presumably mediated by an increase in Ca^{2+}_i) did not alter rates of synthesis or degradation [23,33]. Furthermore, we have observed stimulation of protein synthesis by increased pH_o in quiescent cardiac myocytes and in K^+-arrested retrogradely perfused hearts (S. J. Fuller, C. J. Gaitanaki & P. H. Sugden, unpublished work).

In the perfused heart, protein synthesis is stimulated and protein degradation is inhibited by the following: insulin, non-carbohydrate fuels, increases in aortic pressure or leucine (for reviews, see [34,35]). (In our

hands, protein degradation is not inhibited by non-carbohydrate fuels in the working rat heart [17], although Chua *et al.* [36,37] have shown inhibition in the retrogradely perfused heart.) An attractive hypothesis is that these interventions might cause an increase in pH_i. Insulin increases pH_i in frog skeletal muscle (by 0.1–0.3 unit) [38–40], cultured skeletal muscle cells [40a] and rat adipocytes [41] by stimulation of Na^+/H^+ exchange (reviewed in [2,5]; see also [42]). Its stimulation of frog muscle glycolysis has been ascribed to this [43]. However, others have not detected insulin-induced increases in pH_i. As discussed in [40], no insulin-induced alkalinization could be detected in rat heart [44], fibroblasts [45] or mammalian skeletal muscle [46], although it did potentiate increases in pH_i induced by growth factors or thrombin in some cells [45,47]. We could not detect any insulin-induced change in pH_i in the perfused heart, although measurements of global pH_i might not detect any subcellular pH changes and would not detect transients. Insulin does, however, have some effect on membrane ion-transport systems, since it hyperpolarizes mammalian heart and skeletal muscle [48–51]. Even if an insulin-induced increase in pH_i were responsible for its effects on protein turnover, the hormone's effects cannot be solely explained by modulation of pH_i. Thus an increase in pH_o to pH 8.4 did not stimulate cardiac glucose transport or lactate output (processes which are insulin-sensitive), and insulin did not increase cardiac output [23] or cardiac phosphocreatine contents in this particular series of experiments (Table 3). Although our findings may not be of direct relevance to insulin action, we consider that, since the effects of pH_o on protein turnover are relatively large, their mechanism and physiological importance merit further investigation.

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