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Stimulation of protein synthesis by raised extracellular pH in cardiac myocytes and perfused hearts

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Protein synthesis was stimulated in freshly-isolated rat cardiac myocytes by increasing the extracellular pH of Hepes-buffered Tyrode's solutions over the range pH 7.4–8.4. The maximal stimulation was about 45%. Protein synthesis in anterogradely-perfused rat hearts was stimulated by 11% by increasing the pH of the bicarbonate-containing perfusion medium from pH 7.4 to 7.8. This manoeuvre increased intracellular pH by 0.12 units. A concomitant increase in phosphocreatine concentration was observed. These findings are consistent with the hypothesis that intracellular pH may exert profound effects on tissue protein synthesis rates.

Protein synthesis; Intracellular pH; Hepes buffer; Bicarbonate buffer; (Rat heart)

1. INTRODUCTION

We have recently shown that the protein synthesis rate in the perfused rat heart can be dramatically stimulated by increasing the pH of the perfusion buffer over the range pH 7.4 to pH 8.9 [1]. We attributed this stimulation to an increase in pH_i and a putative direct effect on translation. However, increasing the pH_o also caused an increase in cardiac output and we could not be certain that the increased contractility was not responsible for the stimulation of protein synthesis. Furthermore, the perfusion buffers used were nominally free of bicarbonate, an anion of considerable importance in physiological buffering. Experiments carried out in the nominal absence of bicarbonate have been criticized on the grounds of being of little relevance to the situation in vivo [2]. We therefore assessed the importance of contractility in the response of protein synthesis to increased pH_o by using quiescent isolated cardiac myocytes and also studied the effects of increasing pH_o in bicarbonate-containing buffers on perfused heart protein synthesis.

2. EXPERIMENTAL

Materials were from standard sources [3,4]. Cardiac myocytes were prepared as described previously [4] except that the dispersed myocytes from the pooled third and fourth collagenase digestions were washed 3 × at 37°C in oxygenated Tyrode's solution buffered with Hepes (Sigma, cell culture grade) and adjusted to the requisite pH [1] but in this case containing 50 μM added Ca, 10 mM glucose and 2% BSA. This procedure took about 15 min. This basic Tyrode's solution was also used for the incubations which were carried out under an atmosphere of O_2 at 37°C with gentle shaking every 10 min. Protein synthesis was measured over 60 min from the incorporation of [U-^{14}C]phenylalanine in the presence of the remaining necessary amino acids as described previously [4].

Anterograde heart perfusions were carried out at a filling pressure of 0.5 kPa and an aortic pressure of 7.0 kPa as described in detail previously [5,6]. The perfusion buffers were modified Krebs-Henseleit bicarbonate-buffered saline solutions [7]. They were equilibrated with 95% O_2/5% CO_2 and contained 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4 and either 129 mM NaCl plus 15 mM $NaHCO_3$ (measured at pH 7.39 and 37°C) or 109 mM NaCl plus 35 mM $NaHCO_3$ (measured pH at 7.79 and 37°C). The buffer used in the retrograde pre-perfusion was the name as that used in the anterograde perfusion. Protein synthesis was measured over 90 min from the incorporation of [U-^{14}C]phenylalanine in the presence of the remaining necessary amino acids as described previously [6]. Intracellular pH was measured using the [^{14}C]DMO method as described previously [1]. Metabolite concns. in neutralized perchloric acid-extracts of freeze-clamped hearts were measured by standard spectrophotometric techniques [8].

Cardiac myocyte protein was measured by the biuret method [9] in a sample of cells washed free of BSA. Results are presented as means ± SE. Statistical significance was assessed by a paired or unpaired 2-tailed Student's t-test with $P<0.05$ taken as indicating significance.

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Abbreviations: BSA, bovine serum albumin; DMO, 5,5′-dimethyloxazolidine-2,4-dione; subscript 'i', intracellular value; subscript 'o', extracellular value

3. RESULTS AND DISCUSSION

3.1. *Effects of the variation of pH_o on cardiac myocyte protein synthesis*

The steady-state pH_i of cardiac myocytes can be conveniently manipulated by exposure of the cells to buffers of varying pH [10]. In cardiac cells, the dependence of steady-state pH_i on pH_o is rather weak ($\Delta pH_i/\Delta pH_o$

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Table 1

Stimulation of protein synthesis in isolated cardiac myocytes by increased pH_o or insulin

Condition	Rate of protein synthesis (% control)
pH_o 7.7	137 ± 6[a]
pH_o 8.0	147 ± 1[c]
pH_o 8.4	143 ± 6[b]
pH_o 7.4 + insulin (6.7 nM)	195 ± 10[c]

Protein synthesis rates in a 1 h incubation are expressed as a percentage of control rate at pH_o = 7.4 (absolute value = 422 ± 28 pmol Phe incorporated/h per mg myocyte protein) and are the means ± SE for 3–4 separate myocyte preparations

[a] $P<0.05$, [b] $P<0.01$, [c] $P<0.001$ vs control incubation at pH_o = 7.4, paired t-test

Table 2

Stimulation of protein synthesis and increase in pH_i resulting from increased pH_o in the perfused heart

pH_o	Rate of protein synthesis (pmol Phe incorporated/h per mg protein)	pH_i
7.4	723 ± 28	7.36 ± 0.01
7.8	810 ± 21[a]	7.48 ± 0.01[b]

Protein synthesis rates were measured in a 1.5 h anterograde perfusion. There were 12–14 observations for protein synthesis and 6 for pH_i

[a] $P<0.02$, [b] $P<0.001$ vs incubation at pH_o = 7.4, unpaired t-test

= 0.2–0.4 [10–12]). Following a transition in pH_o, a new steady-state pH_i is achieved in 15–20 min [10]. This is approximately the length of time taken to wash the myocytes free of collagenase using the Tyrode's buffers. Hence, pH_i will have reached a new steady-state by the time measurement of protein synthesis was initiated. In Hepes-buffered Tyrode's solution at pH 7.4, protein synthesis was linear with time for at least 1 h (not shown). Absolute rates of protein synthesis were about the same as in Krebs-Henseleit medium (compare results in table 1 with those in [4]). Protein synthesis was stimulated by increased pH_o (table 1). Stimulation was maximal at a pH_o of 8.0–8.4, i.e. at lower values than in the perfused heart [1]. The maximal stimulation expressed as a percentage of the basal rate in cardiac myocytes was less than that in the perfused heart (147% and 168%, respectively). Furthermore, the maximal stimulation of protein synthesis by pH_o in cardiac myocytes was not as great as the stimulation by insulin, contrasting with the situation in the perfused heart. However, in spite of these quantitative differences, the observations in cardiac myocytes are not inconsistent with our hypothesis that increasing pH_i causes an increase in protein synthesis.

3.2. *Effects of variation of pH_o on protein synthesis in perfused hearts in bicarbonate-containing buffers*

A valid criticism [2] of our earlier work on the effects of pH_o on protein synthesis in perfused hearts [1] is that the perfusion buffers were nominally bicarbonate-free. Hepes- or Tris-buffered solutions containing $NaHCO_3$ which are gassed with O_2 do not maintain a stable pH. Unfortunately, the ability to increase the pH of Krebs-Henseleit bicarbonate-buffered saline by increasing the bicarbonate concentration is limited by the solubility of $CaCO_3$. We could therefore increase pH_o only from about 7.4–7.8. This small increase significantly stimulated protein synthesis by about 12% (table 2). We showed directly that increased pH_o raised pH_i by 0.29 units per change in pH_o (table 2). Adenine nucleotide concns. were not significantly changed by increased pH_o but phosphocreatine concns. and ATP/ADP ratios increased (table 3). We have argued [1] that since the creatine kinase reaction is close-to-equilibrium in the perfused heart [13] and involves the participation of a proton [14], increased phosphocreatine concns. may indicate an increase in pH_i. Using results in table 3 as described previously [1], the calculated change in pH_i between pH_o values of 7.4 and 7.8 was 0.10 units (cf. table 2).

3.3. *General conclusions*

Increased contractility is unlikely to be the cause of the increased protein synthesis rates seen in perfused hearts when pH_o is increased. Protein synthesis is increased when pH_o is increased even in buffers containing bicarbonate. It is possible that agents which increase pH_i (hormones, growth factors, etc., [15,16]) may increase protein synthesis through this mechanism. Even agents which stimulate protein synthesis in the absence

Table 3

Adenine nucleotide and phosphocreatine concentrations in the perfused heart

pH_o	Metabolite concentration (μmol/g protein)					ATP/ADP ratio
	ATP	ADP	AMP	ATP + ADP + AMP	Phospho-creatine	
7.4	23.1 ± 0.7	7.5 ± 0.3	1.0 ± 0.1	31.5 ± 0.9	16.2 ± 1.0	3.09 ± 0.10
7.8	23.7 ± 0.6	6.7 ± 0.3	1.2 ± 0.1	31.6 ± 1.0	21.6 ± 1.2[b]	3.54 ± 0.10[a]

Metabolite concns. were measured after 1.5 h of anterograde perfusion. There were 5 or 6 observations in each group

[a] $P<0.05$, [b] $P<0.001$ vs perfusions at pH 7.4, unpaired t-test

of any change in pH_i (e.g. insulin in rat heart [1,17]) could alter the pH optimum of the process (which, in cell-free translation systems, is on the alkaline side of normal pH_i [18]) to bring it closer to the normal intracellular value. We are well aware that pH_o and pH_i interact with the intracellular concns. of other ions and that one of these may be the causative agent of the increased protein synthesis rate.

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REFERENCES

[1] Fuller, S.J., Gaitanaki, C.J. and Sugden, P.H. (1989) Biochem. J. 259, 173–179.
[2] Thomas, R.C. (1989) Nature (London) 337, 601.
[3] Preedy, V.R., Smith, D.M., Kearney, N.F. and Sugden, P.H. (1984) Biochem. J. 222, 395–400.
[4] Fuller, S.J. and Sugden, P.H. (1989) FEBS Lett. 247, 209–212.
[5] Sugden, P.H. and Smith, D.M. (1982) Biochem. J. 206, 473–479.
[6] Fuller, S.J. and Sugden, P.H. (1988) Am. J. Physiol. 255, E537–E547.
[7] Krebs, H.A. and Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. 210, 33–66.
[8] Bergmeyer, H.U. (1974) Methods of Enzymatic Analysis, Academic Press, New York.
[9] Gornall, A.G., Bardawill, C.J. and David, M.M. (1949) J. Biol. Chem. 177, 751–766.
[10] Wallert, M.A. and Frohlich, O. (1989) Am. J. Physiol. 257, C207–C213.
[11] Deitmer, J. and Ellis, D. (1980) J. Physiol. (London) 304, 471–488.
[12] Kim, D. and Smith, T.W. (1987) Am. J. Physiol. 253, C137–C146.
[13] Bittl, J.A. and Ingwall, J.S. (1985) J. Biol. Chem. 260, 3512–3517.
[14] Lawson, J.W.R. and Veech, R.L. (1979) J. Biol. Chem. 254, 6528–6537.
[15] Busa, W.B. (1986) Annu. Rev. Physiol. 48, 389–402.
[16] Frelin, C., Vigne, P., Ladoux, A. and Lazdunski, M. (1988) Eur. J. Biochem. 174, 3–14.
[17] Bailey, I.A., Radda, G.K., Seymour, A.M. and Williams, S.R. (1982) Biochim. Biophys. Acta 720, 17–27.
[18] Winkler, M.M. (1982) in: Intracellular pH – Its Measurement, Regulation and Utilization in Cellular Functions (Nuccitelli, R. and Deamer, D.W., eds), pp. 325–340, Alan R. Liss, New York.