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Acute α_1-adrenergic stimulation of cardiac protein synthesis may involve increased intracellular pH and protein kinase activity

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In the presence of 5 μM-DL-propranolol and in HCO_3^--containing buffers, 1 μM-adrenaline acutely stimulated protein synthesis by about 25% in the anterogradely perfused rat heart. This stimulation was opposed by low (1–10 nM) concentrations of prazosin, but not by similar concentrations of yohimbine, suggesting involvement of the α_1-adrenoceptor. Under the same conditions, adrenaline raised intracellular pH (pH_i) by about 0.1 unit. The increase in pH_i induced by adrenaline was prevented by 5 nM-prazosin, but not by 5 nM-yohimbine, again suggesting involvement of the α_1-adrenoceptor. Since an increase in pH_i stimulates protein synthesis in the heart [Sugden & Fuller (1991) Biochem. J. 273, 339–346], the increase in pH_i induced by adrenaline may be involved in its stimulation of protein synthesis. Adrenaline also increased phosphocreatine concentrations. As discussed, the increase in pH_i induced by adrenaline may be responsible for this effect. Using second-order polynomial regression analysis, we showed that rates of protein synthesis were significantly correlated ($P < 0.0001$) with phosphocreatine concentrations. We discuss two possible reasons for this correlation: (i) increases in pH_i stimulate protein synthesis and separately raise phosphocreatine concentrations, or (ii) the increase in protein synthesis rates is a consequence of the raised phosphocreatine concentrations induced by the increase in pH_i. Rates of protein synthesis were not significantly correlated with ATP/ADP concentration ratios, nor with any of the following: ATP, ADP, AMP or total adenine nucleotide concentrations. In freshly isolated adult rat cardiomyocytes, the protein kinase inhibitor staurosporine (1 μM) prevented stimulation of protein synthesis by 0.3 μM-adrenaline (and by 1 μM-phorbol 12-myristate 13-acetate or 1 m-unit of insulin/ml). The results are discussed within a mechanistic framework initiated by stimulation of the hydrolysis of membrane phospholipids by α_1-adrenergic agonists.

INTRODUCTION

We have shown that adrenaline acutely stimulates protein synthesis in anterogradely perfused rat hearts in the presence of the β-adrenergic antagonist propranolol [1]. Additionally, adrenaline acutely stimulates protein synthesis in cardiomyocytes freshly isolated from adult rats [1]. The latter was classified as an α_1-adrenergic effect by the use of various adrenergic agonists and antagonists [1]. We have also shown that increasing pH_o during heart perfusions or in incubations of cardiomyocytes increases protein synthesis rates and PCr concentrations [2,3]. Further investigation of this effect demonstrated a correlation between protein synthesis, pH_i and the concentration of creatine metabolites [4]. On the basis of these results, we suggested that protein synthesis is acutely sensitive to variation in pH_i and that modulation of pH_i might be provide a mechanism through which protein synthesis rates could be regulated [2,4]. This could take the form either of a direct effect of pH_i on protein synthesis or of an indirect effect whereby pH_i influences the concentrations of creatine metabolites through the creatine kinase equilibrium or influences an unidentified co-variable. The creatine metabolites or other factor might then affect protein synthesis rates.

The plasma-membrane Na^+/H^+ exchanger is important in the regulation of pH_i in many cells, expelling intracellular protons in exchange for Na^+_o (reviewed in [5]). Numerous agents have been reported to increase Na^+/H^+ exchange and/or pH_i in certain cell lines (reviewed in [6,7]). These include polypeptide hormones and growth factors, tumour-promoting phorbol esters and α_1-adrenergic agonists. The observed increases in pH_i are about 0.1–0.3 unit. A possible mechanism involves stimulation of membrane phosphoinositidase activity. leading to hydrolysis of membrane phosphatidylinositols to produce the 'second messenger' molecules diacylglycerol and $Ins P_3$. Diacylglycerol then stimulates the activity of the Ca^{2+}/phospholipid-dependent protein kinase, PKC, which in turn catalyses the phosphorylation and activation of the Na^+/H^+ exchanger, leading to the expulsion of intracellular protons (reviewed in [7]). We have proposed that the stimulation of protein synthesis by adrenaline might involve such a mechanism [1]. In this paper, we present further evidence in support of this hypothesis.

EXPERIMENTAL

Materials and animals

Sources of materials and animals have been given previously [1,4]. In addition, staurosporine was from Boehringer-Mannheim U.K., Lewes, East Sussex, U.K., and a 10 mM stock solution was prepared in dimethyl sulphoxide. Insulin (Novo, human Actrapid; stock solution of 100 units/ml) was obtained from the Brompton Hospital pharmacy. Stock solutions of BSA, PMA, adrenaline and adrenergic antagonists were prepared as described previously [1,8].

Anterograde heart perfusions

Hearts from fed rats of 250–300 g body wt. were removed and perfused at a filling pressure of 0.5 kPa and an aortic pressure of 7 kPa as described previously [4,9,10]. The perfusate was Krebs

Abbreviations used: DMO, 5,5'-dimethyloxazolidine-2,4-dione; PCr, phosphocreatine; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; the subscripts 'i' or 'o' refer to an intracellular or an extracellular value, respectively.

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& Henseleit [11] HCO_3^--buffered saline (15 mM-$NaHCO_3$, pH 7.4 at 37 °C) equilibrated with O_2/CO_2 (19:1). The fuel provided was 5 mM-glucose. The initial volume of the perfusate was 84 ml. Zero time was taken as the time when the switch to anterograde perfusion was made. DL-Propranolol, prazosin or yohimbine solutions of the appropriate concentrations were added after 5 min. Adrenaline (20 μl of 5 mM) was added after 10 min. In preliminary experiments, we ensured that protein synthesis rates were not affected by the carry-over of dimethyl sulphoxide or L-ascorbic acid present in stock solutions.

Protein synthesis was measured over the 10–100 min perfusion period by the incorporation of 0.4 mM-[U-^{14}C]phenylalanine (sp. radioactivity 0.04 Ci/mol) in the presence of the remaining amino acids necessary for protein synthesis (each at a final concentration of 0.2 mM). Measurement of protein synthesis was initiated by the addition of 16 ml of a concentrated mixture of the amino acids in Krebs–Henseleit saline. After 100 min, hearts were freeze-clamped by using tongs cooled in liquid N_2 and were stored at −80 °C. The sp. radioactivity of the [U-^{14}C]phenylalanine in the perfusate was calculated from liquid-scintillation counting of perfusate samples and measurement of the phenylalanine concentration [12,13]. Frozen heart tissue was powdered and extracted in 0.56 M-$HClO_4$ (4 ml/g of heart). After centrifugation at 4 °C in a bench centrifuge, the precipitated protein was washed and prepared for liquid-scintillation counting as described previously for ventricular protein [4,14]. PCr, ATP, ADP and AMP were measured as described previously [1,4,14] by standard enzyme-coupled spectrophotometric assays [15].

Steady-state pH_i was determined by the partition of [2-^{14}C]DMO [2,4]. Hearts were perfused with 100 ml of medium and the appropriate additions (propranolol, adrenaline, prazosin and yohimbine) made as described above, except that amino acids were omitted. After 15 min of anterograde perfusion, [2-^{14}C]DMO (0.5 ml of 100 mM adjusted to pH 7.4 with NaOH, sp. radioactivity 0.1 Ci/mol) was added. To half of the perfusions, ^3H_2O (0.1 ml, containing 10 μCi) was added after 40 min. To the other half of the perfusions, [^{3}H]inulin (0.1 ml, containing 10 μCi) was added after 50 min. After 60 min, the hearts were removed from the cannulae, cut open and blotted lightly. The hearts were processed as described previously [2,4]. The pH_i was calculated as before [2,4] by using a pK_a value of 6.28 for DMO [16] and the empirically determined values for extracellular and intracellular spaces (0.36 and 0.44 ml/g respectively). The sizes of these spaces were unaffected by any of the perfusion conditions.

Measurement of [U-^{14}C]phenylalanine incorporation into protein, and of ATP concentrations in isolated cardiomyocytes

Cardiomyocytes were isolated from the hearts of 250–300 g fed rats by collagenase digestion as described previously [1]. They were resuspended at a myocyte protein concentration of 3.5–5.5 mg/ml (determined as described previously [1]) in collagenase-free Krebs–Henseleit saline containing 25 mM-$NaHCO_3$, 2% BSA, 10 mM-glucose and 50 μM added Ca^{2+} (incubation buffer). They were kept at 37 °C before use within 20 min of preparation. After isolation and resuspension, 75–95% of the myocytes were rod-shaped, and they were quiescent.

The methodology used to measure [U-^{14}C]phenylalanine incorporation into protein has been described and characterized previously [1]. Incubations were carried out in quadruplicate with the mean taken as one experimental observation. Resuspended myocytes (100 μl) were added to incubation medium (50 μl) which contained 4.8 μM-staurosporine in silicone-treated glass tubes. No staurosporine was present in the control incubations. The myocytes were preincubated at 37 °C for 20 min. Stock solutions of adrenaline, insulin or PMA were diluted in incubation medium, and 50 μl was then added to the myocytes. The preincubations were continued for a further 20 min. Measurement of protein synthesis was initiated by the addition of 40 μl of an amino acid mixture containing [U-^{14}C]phenylalanine as described previously [1]. Thus the final concentrations of staurosporine, adrenaline, insulin and PMA were 1 μM, 0.3 μM, 1 m-unit/ml and 1 μM respectively. Protein synthesis was terminated 60 min after the addition of amino acids by the addition of 1 ml of ice-cold 5% trichloroacetic acid, and the precipitated protein was prepared for liquid-scintillation counting [1]. Blank incubations in the absence of myocytes were performed simultaneously and were subtracted [1]. When stock solutions of agents had been prepared in dimethyl sulphoxide, we ensured that dimethyl sulphoxide at the concentrations present in the incubations did not affect protein synthesis rates. ATP was measured by the luciferase method [1,17].

Statistical methods

Second-order polynomial regression analysis with intermediate weighting was carried out with Graphpad software (ISI, San Diego, CA, U.S.A.), and statistically significant correlations were taken as being established at $P < 0.0001$. Other stastical analyses were undertaken with a Microstat-II statistics package (Ecosoft, Indianpolis, IN, U.S.A.). For data other than those analysed by non-linear regression, Student's t tests for paired for unpaired data (used as appropriate) were two-tailed, and P values of < 0.05 between groups were taken as indicating significant differences.

RESULTS

Effects of α-adrenergic antagonists on the stimulation of protein synthesis by adrenaline in perfused hearts

All perfusions were carried out in the presence of 5 μM-DL-propranolol to decrease the β-adrenergic effects of adrenaline. We have shown previously that propranolol did not affect basal rates of protein synthesis, but increased the stimulation of protein synthesis by adrenaline, since it at least partially prevents the decrease in ATP concentration that occurs on β-adrenergic stimulation [1]. Adrenaline (1 μM) stimulated protein synthesis by about 25% in this series of experiments (Table 1). This stimulation was halved by 1 nM-prazosin and was abolished by 10 nM-prazosin. At a concentration of 1 nM, yohimbine did not affect the stimulation, but 100 nM-yohimbine halved it and 1 μM-yohimbine decreased it by about 75%. In the absence of adrenaline, 1 μM-prazosin or 1 μM-yohimbine did not alter the rates of protein synthesis (results not shown). Thus the stimulation of protein synthesis by adrenaline was about 100 times more sensitive to inhibition by prazosin than to inhibition by yohimbine, and was hence probably an α_1-adrenergic effect. This agrees with our findings in cardiomyocytes, where the stimulation was about 100–1000 times more sensitive to inhibition by prazosin than to inhibition by yohimbine [1].

Adenine nucleotide and PCr concentrations in perfused hearts

Adenine nucleotide and PCr concentrations were assayed in all hearts in which protein synthesis rates were measured (Table 1). Adrenaline increased PCr concentrations. This increase was prevented by 10 nM-prazosin or 100 nM-yohimbine. Given certain assumptions, we have argued that increases in PCr concentrations may be indicative of increases in pH_i [2,4]. Even in the presence of propranolol, adrenaline decreased ATP, ADP and (ATP + ADP + AMP) concentrations (Table 1). These decreases were prevented by 10 nM-prazosin. The decrease in ADP concentration was partly prevented by 100 nM-yohimbine. There were no detectable changes in AMP concentrations, and only in one instance was there any alteration in the ATP/ADP ratio.

Table 1. Effects of α-adrenergic antagonists on the stimulation of protein synthesis by adrenaline and on PCr and adenine nucleotide concentrations in perfused hearts

Details of methods used are given in the Experimental section. Perfusions were carried out with Krebs–Henseleit saline. DL-Propranolol was added to all perfusions after 5 min of anterograde perfusion to a final concentration of 5 μM. When present, prazosin or yohimbine was added as appropriate at the same time as propranolol. When present, adrenaline was added to a final concentration of 1 μM after 10 min of anterograde perfusion. Perfusions were for a total of 100 min, after which time the hearts were freeze-clamped. Protein synthesis was measured over the 10–100 min interval. There were 5–14 separate observations in each group. Statistical analysis was by a two-tailed unpaired *t* test: $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ versus control perfusions (line 1); $^{d}P < 0.05$, $^{e}P < 0.02$, $^{f}P < 0.01$, $^{g}P < 0.001$ versus perfusions in the presence of 1 μM-adrenaline alone (line 2).

Perfusion conditions	Rate of protein synthesis (nmol of phenylalanine incorporated/90 min per mg of protein)	Metabolite concn. (nmol/mg of protein)					
		PCr	ATP	ADP	AMP	ATP+ADP +AMP	ATP/ADP
Control	1.310±0.018	19.3±0.9	25.9±0.8	7.38±0.22	1.03±0.11	34.3±0.9	3.53±0.11
+adrenaline	1.640±0.035[c]	27.8±1.7[c]	19.3±0.9[c]	5.38±0.22[c]	1.23±0.12	24.3±1.0[c]	3.42±0.24
+adrenaline+1 nM-prazosin	1.475±0.063[b,d]	26.4±1.0[c]	20.1±1.1[c]	4.93±0.47[c]	1.42±0.27	26.6±1.2[c]	4.27±0.48[a]
+adrenaline+10 nM-prazosin	1.226±0.041[g]	17.1±1.3[f]	24.4±1.4[e]	7.49±0.53[g]	0.94±0.05	32.9±1.9[g]	3.27±0.13
+adrenaline+1 nM-yohimbine	1.674±0.057[c]	27.9±1.5[c]	18.5±1.5[c]	5.54±0.46[c]	1.17±0.16	25.2±1.8[c]	3.38±0.23
+adrenaline+100 nM-yohimbine	1.493±0.063[b,d]	20.3±1.7[d]	20.5±0.6[b]	6.48±0.30[a,e]	0.89±0.11	27.9±0.5[c]	3.19±0.19
+adrenaline+1 μM-yohimbine	1.278±0.107[e]	23.5±2.5	19.2±1.6[a]	5.84±0.56[b]	1.41±0.32	24.4±1.0[c]	3.33±0.20

Hence, in the presence of adrenaline, adenine nucleotide concentrations were maintained only when both prazosin and propranolol were present together.

Correlations between protein synthesis rate and PCr or adenine nucleotide concentrations

Second-order polynomial regression analysis was performed with protein synthesis rate as the dependent variable and with PCr, ATP, ADP, AMP, (ATP+ADP+AMP) or ATP/ADP concentrations as the independent variable. Protein synthesis rates were significantly correlated ($P < 0.0001$) only with PCr concentrations (Fig. 1). It could be suggested that the regression line shown in Fig. 1 is biased by the two points where PCr concentration is greater than 35 nmol/mg of protein. However, if these points are excluded, the curve is similar and the correlation by second-order polynomial regression analysis is still significant, with a P value of 6.05×10^{-8}.

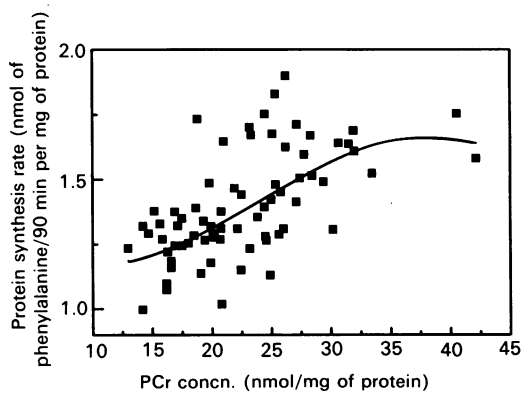

Fig. 1. Correlation between rates of protein synthesis and PCr concentrations in hearts perfused with adrenaline and α-adrenergic antagonists

Data from 71 heart perfusions were plotted. Hearts were perfused with Krebs–Henseleit saline in the presence of 5 μM-DL-propranolol to which the following additions were made: no addition, 1 μM-adrenaline, 1 μM-adrenaline+various concentrations (1 nM–1 μM) of prazosin, and 1 μM-adrenaline+various concentrations (1 nM–1 μM) of yohimbine. The following equation describes the second-order polynomial regression line: $y = 1.845 - 0.122x + (7.391 \times 10^{-3})x^2 - (1.487 \times 10^{-4})x^3 + (9.374 \times 10^{-7})x^4$ ($r^2 = 0.398$, $P = 8.09 \times 10^{-9}$).

Effects of adrenaline and α-adrenergic antagonists on pH_i in perfused hearts

These perfusions were also carried out in the presence of propranolol. Propranolol alone did not affect pH_i. In the absence of propranolol and in Krebs–Henseleit saline, pH_i was 7.323±0.025 (6 observations) compared with 7.314±0.009 (Table 2; 14 observations) in its presence. Perfusion of hearts with 1 μM-adrenaline in the presence of propranolol resulted in an increase in pH_i of about 0.1 unit (Table 2). From data in [4], this increase in pH_i should stimulate protein synthesis by about 21%. The measured increase was 25% (Table 1). Preliminary experiments have shown that adrenaline in the presence of

Table 2. Effect of adrenaline and α-adrenergic antagonism on pH_i in the perfused heart

Details of methods used are given in the Experimental section. DL-Propranolol was added to all perfusions at a final concentration of 5 μM after 5 min of anterograde perfusion. When present, prazosin or yohimbine was added to a final concentration of 5 μM after 5 min of anterograde perfusion. When present, adrenaline was added to a final concentration of 1 μM after 10 min of anterograde perfusion. All perfusions were for 60 min. They were 5–12 separate observations in each group. Statistical analysis was assessed by an unpaired two-tailed Student's *t*-test: $^{a}P < 0.01$, $^{b}P < 0.001$ versus control perfusions; $^{c}P < 0.02$ versus perfusions in the presence of adrenaline; $^{d}P < 0.02$ for perfusions in the presence of adrenaline and yohimbine versus those in the presence of adrenaline and prazosin.

Perfusion conditions	pH_i
Control (+propranolol)	7.314±0.010
+adrenaline	7.411±0.021[b]
+adrenaline+prazosin	7.317±0.010[c]
+adrenaline+yohimbine	7.376±0.015[a,d]

Table 3. Effects of staurosporine on the stimulation of protein synthesis by adrenaline, TPA or insulin in isolated cardiomyocytes

Cardiomyocytes were isolated and incubated in modified Krebs–Henseleit saline as described in the Experimental section. Protein synthesis rates were expressed relative to the control rate in the absence of staurosporine, which was 469 ± 30 pmol of phenylalanine incorporated/h per mg of myocyte protein (16 separate preparations of myocytes). The numbers of observations refer to separate preparations of cardiomyocytes used for each paired observation. Statistical significance was assessed by a paired *t* test: [a]$P < 0.001$ versus control incubations in the absence of staurosporine; [b]$P < 0.05$, [c]$P < 0.001$ versus the same incubation condition in the absence of staurosporine.

Incubation condition	No. of observations	Protein synthesis rate (% of control)	
		No staurosporine	+1 μM-staurosporine
Control	7	(100)	88 ± 4[b]
+0.3 μM-adrenaline	7	124 ± 3[a]	94 ± 5[c]
+1 μM-PMA	4	144 ± 4[a]	100 ± 4[c]
+1 m-unit of insulin/ml	6	172 ± 7[a]	97 ± 6[c]

propranolol also increased pH_i in hearts perfused with Hepes-buffered Tyrode's solution, pH about 7.7 at room temperature. The increase in pH_i induced by adrenaline was prevented by 5 nM-prazosin (Table 2). Values of pH_i in the presence of adrenaline and in the presence of adrenaline + 5 nM-yohimbine were not significantly different. The concentrations of α-antagonists were chosen on the basis of their effects on protein synthesis (Table 1). These data suggest that the adrenaline-induced increase in pH_i is an α_1-adrenergic effect.

Effects of staurosporine on the stimulation of isolated cardiomyocyte protein synthesis by adrenaline, PMA and insulin

Staurosporine is a general inhibitor of protein kinases, but probably displays a predilection for PKC (reviewed in [18]). It was not possible to use this compound in anterogradely or retrogradely perfused hearts, since it affected heart function. Here, therefore, we used isolated cardiomyocytes. We have shown elsewhere that adrenaline, PMA and insulin stimulate protein synthesis in this system [1,8]. This is confirmed in Table 3. Staurosporine (1 μM) alone slightly inhibited control rates of protein synthesis. However, staurosporine completely prevented the stimulation of protein synthesis by adrenaline, PMA or insulin, decreasing the rates to those seen in the presence of staurosporine alone. None of the interventions significantly altered myocyte ATP concentrations (results not shown). These results suggest that the stimulation of protein synthesis by adrenaline, PMA or insulin involves protein phosphorylation events which may or may not be mediated by PKC.

DISCUSSION

ATP depletion following adrenergic stimulation in the anterogradely perfused heart

All perfusions were carried out in the presence of propranolol. Propranolol does not affect basal rates of protein synthesis, but does increase the stimulation of protein synthesis by adrenaline [1]. The reason for this is probably related to the finding that propranolol partly prevents the decrease in adenine nucleotide concentrations (especially in ATP) resulting from the positive inotropic and chronotropic effects of β-adrenoceptor stimulation. In perfusions using buffers of limited O_2-carrying capacity, the increased rate and force of contraction may induce a mismatch between O_2 supply and demand, leading to ATP depletion and inhibition of protein synthesis under some conditions (discussed in [1]). However, as shown in Table 1, α_1-adrenergic drive may also contribute to the decrease in adenine nucleotide concentrations in perfused hearts. This presumably results from the positive inotropic and possibly chronotropic effects of α_1-adrenergic stimulation (reviewed in [19]). In the presence of adrenaline, adenine nucleotide concentrations were maintained only when both prazosin and propranolol were simultaneously present. It should be noted that adrenaline stimulated protein synthesis even in the face of a decrease in adenine nucleotide concentrations (Table 1), suggesting that their concentrations were of minor importance over the range encountered in these experiments. Similarly, there was a decrease in protein synthesis when α_1-adrenergic antagonists were added in the presence of adrenaline, although adenine nucleotide concentrations were increased (Table 1). The relative unimportance of adenine nucleotide concentrations in affecting the rates of protein synthesis in these experiments was confirmed by the regression analyses.

Mechanisms by which α_1-adrenoceptor stimulation might increase cardiac protein synthesis

Background. Our recent work has identified three interventions which stimulate cardiac protein synthesis. First, increases in pH_o stimulate protein synthesis in perfused hearts and isolated cardiomyocytes, and the protein synthesis rate correlates with pH_i and with the concentrations of creatine metabolites [2–4]. Secondly, PMA stimulates protein synthesis in isolated cardiomyocytes [8]. Since the Ca^{2+}/phospholipid-dependent protein kinase, PKC, is the only known cellular receptor for PMA, activation of this protein kinase can probably modify protein synthesis rates. (Care was taken to exclude membrane effects of PMA.) Thirdly, α_1-adrenergic stimulation increases protein synthesis rates in isolated myocytes [1]. In addition, adrenaline stimulates protein synthesis in perfused hearts, probably through an α-adrenergic effect [1]. The effects of adrenaline and PMA in myocytes are insensitive to actinomycin D, implying that they are exerted at the level of translation [1,8]. These effects are thus additional to the hypertrophic action of α_1-adrenergic stimulation in cultured neonatal-rat cardiomyocytes, since this is probably exerted at the level of transcription (reviewed in [20,21]). In the present paper we have shown that stimulation of protein synthesis in the perfused heart by adrenaline is an α_1-adrenergic effect. Stimulation by adrenaline concomitantly increases pH_i in the perfused heart, and this is also an α_1-adrenergic effect. In addition to increasing pH_i, adrenaline also increases PCr concentrations and, as elsewhere [2,4], protein synthesis rates are strongly correlated with PCr concentrations. The stimulation of protein synthesis by adrenaline in isolated cardiomyocytes is prevented by staurosporine, a protein kinase inhibitor.

Mechanisms of modulation of protein synthesis by α_1-adrenergic stimulation. There are at least three possible mechanisms which could account for our observations. First, adrenaline increases pH_i and this stimulates protein synthesis. Secondly, adrenaline changes the concentrations of creatine metabolites, possibly because the alterations in pH_i affect the concentrations of the substrates within the creatine kinase equilibrium [4]. The alterations in the concentrations of creatine metabolites may influence the rates of protein synthesis. Thirdly, adrenaline influences protein synthesis by some unidentified mechanism.

How might α_1-adrenergic stimulation increase pH_i? α_1-Adrenergic actions are thought to be mediated though the hydrolysis of membrane phosphatidylinositols (and possibly other phospholipids [22]) by activation of phospholipases and hence through the diacylglycerol and InsP_3 pathways (reviewed in [23]). An enhancement of the hydrolysis of membrane phosphatidylinositols by α_1-adrenergic stimulation has been demonstrated in the perfused heart [24]. An increase in diacylglycerol concentration should activate PKC, which will phosphorylate its protein substrates, one of which may include the Na^+/H^+ exchanger (reviewed in [7]). Stimulation of Na^+/H^+ exchange may cause an increase in pH_i, thereby stimulating protein synthesis [2].

If this hypothesis is correct, PMA should increase pH_i in cardiac cells. PMA does increase Na^+/H^+ exchange and pH_i in cultured chick heart myocytes [25]. In myocytes cultured from foetal mouse hearts, PMA increases pH_i when the Ca^{2+} ionophore A23187 is present [26]. The requirement for A23187 may stem from the necessity to raise $Ca^{2+}{}_i$ concentrations in order to allow stimulation of PKC. PMA increased the rate of recovery of contractile function following acid loading by NH_4Cl pre-pulsing in single isolated cardiomyocytes from adult rats, presumably by a PKC-catalysed phosphorylation and activation of the Na^+/H^+ exchanger [27]. There is thus reasonable evidence that PKC activation may, under some circumstances, increase pH_i. However, a problem is that Na^+/H^+ exchange is thought to be more important in the recovery from intracellular acidosis rather than promoting intracellular alkalinization (reviewed in [7]). Additionally, PMA may stimulate protein synthesis not by promoting Na^+/H^+ exchange, but rather by stimulating phosphorylation of components of the protein synthesis machinery [28].

It should be noted that adrenaline increased pH_i in HCO_3^--containing buffers (Table 2). Thomas [29] has suggested that some observed increases in pH_i induced by hormones or growth factors may not be physiological, since the buffers used are often nominally HCO_3^--free. In these circumstances, the HCO_3^--dependent ion exchangers which, in addition to Na^+/H^+ exchange, are also involved in regulation of pH_i (reviewed in [5]) would be inoperative. In the presence of HCO_3^-, pH_i may not be increased by hormones or growth factors (reviewed in [29]). Under these conditions, the net effect of growth factors and hormones on the various ion exchangers involved in the regulation of pH_i may be to enhance proton cycling across the plasma membrane and thereby increase the sensitivity of pH_i regulation [30].

Here and elsewhere [2,4], we have observed correlations between protein synthesis, pH_i and PCr concentrations. The possibility that concentrations of creatine metabolites might regulate protein synthesis must also be considered (see also [4]). We think that the correlation between PCr concentration and pH_i probably results from the involvement of a proton in the creatine kinase equilibrium, and we have suggested that more direct evidence of a change in pH_i should be sought if changes in PCr concentrations are detected [4]. Others have also shown correlations between protein synthesis and PCr concentrations in heart and skeletal muscle under a variety of conditions [31–34], although the relationship is not universal [31,32]. Protein synthesis involves the hydrolysis of ATP and GTP to ADP and GDP. GTP/GDP concentration ratios are in equilibrium with those of ATP/ADP. Undoubtedly, a decrease in ATP or in ATP/ADP below a certain level will inhibit protein synthesis. Thus PCr (or PCr/Cr) could increase protein synthesis by increasing 'energy status' through the creatine kinase equilibrium. However, we do not see any change in the ATP/ADP concentration ratios when PCr is increased by increasing pH_i or by adrenaline (Table 1; [2,4]). Furthermore, ATP concentrations are slightly decreased by adrenaline (Table 1), making the proposition unattractive. Thus, if PCr is to regulate protein synthesis, the protein synthesis machinery should itself be allosterically sensitive to PCr or Cr concentration or to the PCr/Cr concentration ratio. This cannot be excluded.

We are also well aware that any alteration in pH_i or pH_o may have considerable effects on the intracellular concentrations and the membrane fluxes of ions such as Na^+, K^+ and Ca^{2+} [35,36]. Activation of phosphoinositidase may lead to increased production of InsP_3, which could cause Ca^{2+} mobilization from intracellular stores and hence may raise $Ca^{2+}{}_i$ concentrations. As discussed in [4], it is possible that some of these phenomena may be relevant to the effects on protein synthesis that we have observed. Indeed, others have suggested that $Na^+{}_i$ or Na^+ flux may be important in the regulation of cardiac protein turnover [37]. Equally, however, concentrations or fluxes of Na^+ ions may affect pH_i. As discussed in [4], Ca^{2+} ions alone are not thought to regulate cardiac protein synthesis [38]. The possibility remains, however, that they may act synergistically with diacylglycerol to activate PKC. As discussed in ref. [4], a further factor could be lactate, the output of which by the perfused heart is increased by adrenergic stimulation [14].

Is modulation of pH_i a generalized mechanism for regulation of protein synthesis?

It is important to consider whether changes in cardiomyocyte pH_i occur *in vivo* and whether these may be involved in the regulation of protein synthesis. Under normal conditions, acidemia or alkalinaemia are relatively limited in extent and are acute. We do not see any role for pH_o in the regulation of cardiac protein synthesis under normal conditions. In pathological conditions, for example diabetic ketoacidosis, there may be considerable alterations in pH_o, the extremes compatible with life being 6.8 and 7.8 (reviewed in [39]). There may be shifts in pH_i under these conditions, and protein synthesis could be affected.

Are there other conditions under which pH_i is changed? In addition to α_1-adrenergic stimulation, numerous hormones, growth factors and other manipulations activate Na^+/H^+ exchange in cultured cell lines and can potentially increase pH_i (reviewed in [6,7]). Some of these interventions increase protein synthesis rates. We have examined whether three other interventions which are known to increase cardiac protein synthesis also increase cardiac pH_i. First, it is well known that insulin stimulates cardiac protein synthesis [40], and has been reported to increase pH_i in frog sartorius muscle [16] and L6 muscle-cell cultures [41]. However, in agreement with others [42], we could not detect any effect of insulin on pH_i in perfused hearts [2]. Secondly, non-carbohydrate fuels such as lactate or acetate stimulate cardiac protein synthesis as effectively as insulin [31,43]. Using hearts from fasted rats, a correlation between protein synthesis and PCr/Cr concentration ratios was seen on perfusion with pyruvate, lactate or ketone bodies [31], suggesting the possibility of a pH_i change. This correlation was not seen in hearts from fed rats, although protein synthesis rates were increased. In our hands, non-carbohydrate fuels decreased pH_i. In control perfusions with 5 mM-glucose as fuel, pH_i was 7.178 ± 0.016 (12 observations). In the presence of 10 mM-lactate and 10 mM-acetate, pH_i was 7.016 ± 0.021 (8 observations; $P < 0.001$ versus control) (S. J. Fuller, C. J. Gaitanaki & P. H. Sugden, unpublished work). Thirdly, protein synthesis in the anterogradely perfused heart is increased when the aortic pressure is raised to hypertensive levels [44]. In arrested-drained retrogradely perfused hearts (but not in beating hearts), stimulation of protein synthesis correlated with increased PCr/Cr concentration ratios [32]. However, we could not detect any

increase in cardiac pH_i at hypertensive aortic pressures in the anterogradely perfused heart. Using the hypotensive and hypertensive heart preparations described in [44], pH_i in the hypotensive preparation was 7.312 ± 0.014 (6 observations), whereas in the hypertensive preparation it was 7.325 ± 0.016 (6 observations) (S. J. Fuller & P. H. Sugden, unpublished work). Thus the only situation thus far identified where protein synthesis and pH_i are concomitantly increased is after α_1-adrenergic stimulation.

There may also be conditions *in vivo* or *in vitro* (such as ischaemia) where pH_i and PCr concentrations may decrease, rather than increase. In skeletal muscle, contractile activity *in vitro* or ischaemia *in vivo* decreased protein synthesis rates [33,34]. There was a correlation between PCr concentrations and protein synthesis [33,34]. In the perfused heart, ischaemia decreased pH_i [45] and also decreased protein synthesis rates and PCr concentrations, although no detailed correlations between protein synthesis and PCr were presented [46]. Again we suggest that the decreases in PCr concentrations may reflect decreases in pH_i, probably resulting from the observed intracellular lactoacidosis, and that the decreases in protein synthesis may result directly from the decreases in pH_i or alterations in the concentrations of creatine metabolites.

Involvement of the β-adrenoceptor in the modulation of the cardiac protein turnover

Morgan and colleagues have recently reported an acute stimulation of protein synthesis by agents which raise cyclic AMP concentrations [47] under conditions where there was little change in ATP concentrations. These effects are presumably exerted at the level of translation, and implicate the β-adrenoceptor in the regulation of protein synthesis. This group has also suggested that the stimulation of protein synthesis by raised aortic pressure might be mediated by cyclic AMP [47,48] (but note also a recent report which demonstrated that atrial or ventricular stretch stimulated hydrolysis of membrane phosphatidylinositols [49]). Clearly, the results of Morgan's group are different from our own. Of relevance to Morgan's findings is the observation that the β-adrenergic agonist isoprenaline causes cardiac hypertrophy *in vivo*. Recent evidence suggests that the hypertrophy results from the increased cardiac workload or from a direct action of β-adrenergic stimulation on the myocytes, presumably resulting in a stimulation of transcription and/or translation, or an inhibition of protein degradation [50]. Alternatively, β-adrenergic stimulation may act presynaptically by stimulating noradrenaline release from sympathetic nerves [51]. Any direct action of isoprenaline on the cardiomyocytes presumably must involve an increase in cyclic AMP concentrations. We failed to detect any acute stimulation of protein synthesis by isoprenaline in isolated cardiomyocytes [1]. Under these conditions, there was no decrease in myocyte ATP concentrations. Contraction amplitude and rate of shortening in our myocytes responded to isoprenaline in a similar manner to that described in [52], indicating that they possessed functional β-adrenoceptors (results not shown). Thus the relative importance of α_1- versus β-adrenoceptor stimulation of the regulation of cardiac protein synthesis remains controversial.

Protein kinases and the stimulation of protein synthesis by adrenaline

Results with the protein kinase inhibitor staurosporine (Table 3) imply participation of protein kinases in the stimulation of protein synthesis by adrenaline, PMA and insulin. We were unsuccessful in obtaining convincing dose–response curves for staurosporine in this system. It may be reasonable to suggest that staurosporine inhibits the effects of PMA on protein synthesis by inhibition of PKC. However, the finding that staurosporine inhibited the effects of adrenaline or insulin cannot be taken as definitely indicating an involvement of PKC. At least for insulin, we suspect that the result is more likely to be associated with the general effect of inhibition of a number of protein kinases by this compound.

General conclusion

In conclusion, the results here, together with our previous observations, suggest that the signal-transduction pathway involved in the stimulation of cardiac protein synthesis by adrenaline may include a rise in pH_i. Further work will be needed to establish whether the observed increase in pH_i is an obligatory event in the pathway leading to stimulation of protein synthesis, or whether it is merely coincidence.

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