

# Effects of catecholamines on protein synthesis in cardiac myocytes and perfused hearts isolated from adult rats

## Stimulation of translation is mediated through the $\alpha_1$ -adrenoceptor

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Protein-synthesis rates in freshly isolated cardiac myocytes from adult rats were acutely stimulated by 20–30% by 1  $\mu$ M-adrenaline, by 1  $\mu$ M-noradrenaline or by 1  $\mu$ M-phenylephrine, but were not stimulated by 1  $\mu$ M-isoprenaline. Stimulation by 1  $\mu$ M-adrenaline was completely prevented by 100 nM-prazosin. Yohimbine was much less effective in preventing stimulation, and 20  $\mu$ M-DL-propranolol was completely ineffective. The stimulation of protein synthesis by adrenaline was still observed after inhibition of transcription by actinomycin D. None of these manipulations affected myocyte ATP contents. In anterogradely perfused hearts, protein-synthesis rates were stimulated by 1–2  $\mu$ M-adrenaline in the presence of 10  $\mu$ M-DL-propranolol (to decrease the  $\beta$ -adrenergic effects of adrenaline). ATP contents were not altered, but phosphocreatine contents were increased. These observations lead us to conclude that cardiac protein synthesis can be stimulated acutely at the level of translation by  $\alpha_1$ -adrenergic stimulation. We discuss possible roles for protein kinase C and intracellular alkalization in the mediation of this effect.

## INTRODUCTION

*In vivo*, imposition of an increased workload on the heart results in an increase in its tissue mass. Myocytes enlarge and lay down extra sarcomeres. Non-myocytic cells divide [1,2]. This condition is recognized clinically as cardiac hypertrophy. There is still discussion as to whether, for myocytes, the phenomenon involves solely an increase in cell size in the absence of mitosis/cytokinesis (i.e. true hypertrophy) [3]. For convenience the term 'hypertrophy' will be used here to indicate an increase in cardiac protein mass. Administration of certain catecholamines *in vivo* causes the heart to hypertrophy (for a review, see [4]). It is unclear whether this hypertrophy results entirely from a direct action of catecholamines on the cardiac myocytes, or whether there is also an indirect cause resulting from, e.g., the pressor effects of the catecholamines. Experiments in perfused hearts have not been very helpful, since results have not always been consistent with known mechanisms of action of adrenergic agonists (see the Discussion section for an amplification of this point). In cultured myocytes isolated from neonatal-rat ventricles,  $\alpha_1$ -adrenergic agonists stimulated protein synthesis and caused true myocyte hypertrophy [5–7]. It has been suggested that this hypertrophy is primarily mediated through enhanced transcription [7,8]. There is evidence, however, that induction of cardiac hypertrophy *in vivo* involves enhancement of both transcription and translation [9]. In the present paper, we report the acute stimulation of protein synthesis by adrenergic agonists, using freshly isolated cardiac myocytes and perfused hearts from adult rats, and that these particular effects can be attributed to stimulation of translation.

## EXPERIMENTAL

### Materials and animals

Biochemicals were obtained from Sigma, Poole, Dorset, U.K., except when otherwise stated. Collagenase (Worthington type 1) was from Lorne Diagnostics, Twyford, Berks., U.K. [ $^{14}$ C]Phenylalanine and NCS tissue solubilizer were from Amersham International, Amersham, Bucks., U.K. General laboratory chemicals were from BDH, Dagenham, Essex, U.K. All solutions were prepared in double-distilled deionized water. Stock solutions of adrenergic agonists and propranolol were freshly prepared in the appropriate incubation or perfusion media containing 0.1 mM-ascorbate, and were diluted as required. Prazosin and yohimbine were dissolved in dimethyl sulphoxide before dilution with medium containing ascorbate. Actinomycin D was also dissolved in dimethyl sulphoxide and subsequently diluted with medium. Bovine serum albumin (fraction V) was extensively dialysed against water before use. Male Sprague-Dawley rats were from Harlan-Olac, Bicester, Oxon., U.K. They were kept as described previously [10] and had free access to food and water.

### Preparation of cardiac myocytes

Hearts from 250–300 g fed rats were perfused retrogradely at 10 kPa pressure and at 37 °C with a modified Krebs & Henseleit buffer [11] containing 10 mM-glucose, 0.1% BSA and a lowered concentration (10  $\mu$ M) of added  $\text{Ca}^{2+}$ . After 6 min, the added  $\text{Ca}^{2+}$  concentration was increased to 50  $\mu$ M, and collagenase was added at a concentration of 1 mg/ml. After a further 5 min, the softened heart was removed from the cannula, and

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myocytes were liberated by four consecutive incubations (with shaking) each of 5 min at 37 °C with low-Ca<sup>2+</sup> Krebs & Henseleit buffer containing 1 mg of collagenase/ml and 2% BSA. No Ca<sup>2+</sup> was added to the first incubation medium, and the media for the three subsequent incubations each contained 50 μM added Ca<sup>2+</sup>. The digests were filtered through nylon gauze. Myocytes in the filtrates from the third and fourth digestions were pooled and collected by sedimentation under gravity. They were washed three times at 37 °C in collagenase-free Krebs & Henseleit buffer containing 2% BSA and 50 μM added Ca<sup>2+</sup>, and collected at 37 °C by sedimentation under gravity. The myocytes were resuspended at a myocyte protein concentration of 3.5–5.5 mg/ml and were kept at 37 °C before use within 20 min of preparation. A sample (1 ml) was removed for determination of myocyte protein content. These cells were allowed to sediment under gravity and were washed twice with 5 ml of BSA-free medium (to remove BSA, which would otherwise interfere). Preliminary experiments showed that this washing procedure decreased the protein content of the supernatant to undetectable levels. After washing, protein was precipitated with 5% trichloroacetic acid and redissolved in 0.55 ml of 0.1 M-NaOH.

#### Measurement of rates of protein synthesis in cardiac myocytes

The resuspended myocytes freshly isolated from a single heart were swirled gently to ensure uniform distribution before pipetting samples (100 μl) into silicone-treated glass tubes. A wide-aperture pipette tip was used for this operation to prevent damage to the cells. The amount of myocyte protein added per tube (0.35–0.55 mg) differed between experiments, although it was obviously the same within each individual experiment. The myocytes were preincubated in quadruplicate at 37 °C with the various agents as described in the Tables and Figures in a total volume of 200 μl of low (50 μM)-added-Ca<sup>2+</sup> Krebs & Henseleit buffer supplemented with 10 mM-glucose and 2% BSA. 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. After the appropriate preincubation, measurement of protein synthesis was initiated by the addition of 40 μl of Krebs & Henseleit buffer (no Ca<sup>2+</sup> added) containing 2.5 mM-[U-<sup>14</sup>C]phenylalanine (sp. radioactivity 2 Ci/mol), and all the other amino acids necessary to support protein synthesis, each at a concentration of 1.25 mM. Incubations were performed at 37 °C under an atmosphere of O<sub>2</sub>/CO<sub>2</sub> (19:1), and myocytes were gently shaken at 10 min intervals. Except when time courses were examined, protein synthesis was terminated after 1 h by addition of 1 ml of ice-cold 5% trichloroacetic acid. The precipitated protein was collected by bench centrifugation at 4 °C. ATP was measured in the supernatant by the luciferase method [12]. Protein pellets were washed four times by resuspension in 2 ml of 5% trichloroacetic acid, and were dissolved in 1 ml of NCS tissue solubilizer after neutralization with 50 μl of 0.5 M-NaOH. Preliminary experiments showed that this washing protocol was sufficient to decrease radioactivity in the trichloroacetic acid supernatant to background levels. Radioactivity incorporated was measured in a toluene-based fluor. Except when

stated, the mean of the quadruplicate incubations was taken as one experimental observation. The consistency of [U-<sup>14</sup>C]phenylalanine incorporation between the quadruplicate incubations assured us that each tube contained a similar number of myocytes. In the absence of myocytes, [U-<sup>14</sup>C]phenylalanine was also linearly incorporated with time into trichloroacetic acid-precipitable material at a maximum of about 15% of the control rate. This blank was dependent on the presence of BSA, and increased with the time for which the stock solution of amino acids had been stored at -20 °C before use. It was not the result of incomplete washing. Although we thought that the likeliest explanation for this blank was that protein was being synthesized through microbiological contamination, the blank incorporation was not sensitive to inhibition by penicillin, chloramphenicol or puromycin. We do not understand the origin of the blank. However, blanks were carried out routinely and were subtracted from the radioactivity incorporated into protein in the presence of myocytes.

#### Measurement of rates of protein synthesis in anterogradely perfused hearts

Hearts from fed 250–300 g rats were perfused anterogradely as described in detail previously [13–15]. The perfusate was Krebs & Henseleit buffer (2.5 mM-Ca<sup>2+</sup>) equilibrated with O<sub>2</sub>/CO<sub>2</sub> (19:1) and supplemented with 5 mM-glucose. The left-atrial filling pressure was 0.5 kPa and the aortic pressure was 7 kPa. After switching from the retrograde pre-perfusion to anterograde perfusion, the perfusate (100 ml, recirculated) contained additionally 0.4 mM-[U-<sup>14</sup>C]phenylalanine, and the remaining amino acids necessary to support protein synthesis, each at 0.2 mM. When adrenaline was present, it was added to the anterograde perfusate after 10 min of anterograde perfusion to a concentration of 1 μM. A further similar addition of adrenaline was made after 70 min of anterograde perfusion (total adrenaline added per perfusion was hence 0.2 μmol). The anterograde perfusion was continued for a total of 130 min. When DL-propranolol was present, it was added to the anterograde perfusate to a concentration of 10 μM 5 min before addition of adrenaline. On completion of the perfusion periods, the hearts were freeze-clamped with aluminium tongs pre-cooled in liquid N<sub>2</sub>. The frozen hearts were powdered and extracted with 0.56 M-HClO<sub>4</sub>. The precipitated protein was collected by bench centrifugation at 4 °C and was washed free of unbound [U-<sup>14</sup>C]phenylalanine [15]. Incorporation of [U-<sup>14</sup>C]phenylalanine into protein was measured as described in ref. [15]. The supernatant from the HClO<sub>4</sub> extractions were neutralized to about pH 7 with 5 M-KOH/0.5 M-Tris/HCl by using Universal Indicator (BDH). ATP, ADP, AMP and phosphocreatine contents were measured by standard spectrophotometric techniques [16].

#### Other methods, and expression of results

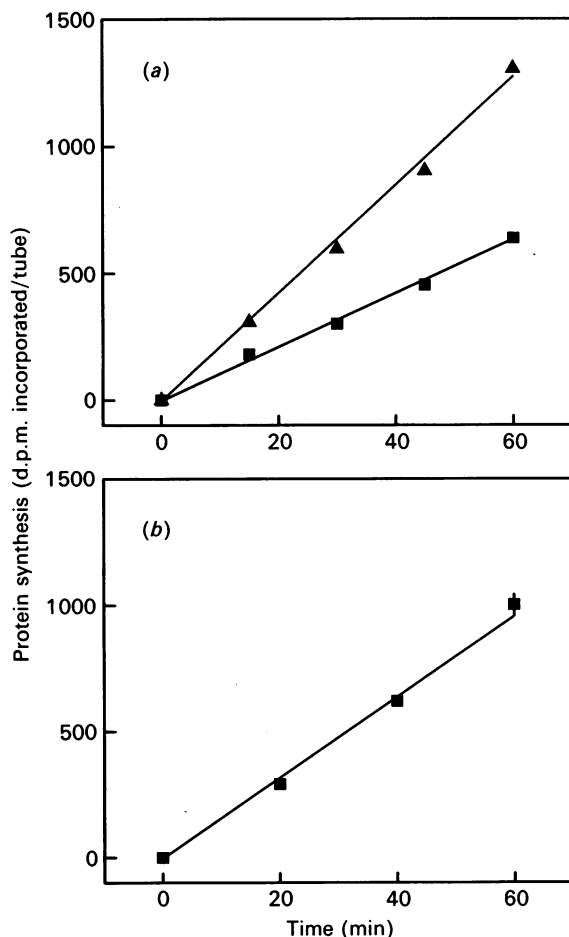
In the experiments in which cardiac myocyte protein synthesis was characterized (Figs. 1–3), phenylalanine incorporation into protein is presented in terms of d.p.m. When incorporation of [U-<sup>14</sup>C]phenylalanine was expressed in molar terms, the calculation was based on the specific radioactivity of the added amino acid (for a justification, see the Discussion section). All results are expressed as phenylalanine incorporation relative to cardiac myocyte or whole heart protein. Protein was

measured by the biuret method [17] (with BSA as standard) in a sample of myocytes washed free of BSA (see above for the protocol) or in NaOH digests of HClO<sub>4</sub>-washed heart protein. Results are presented as means  $\pm$  S.E.M. Statistical significance was assessed by a two-tailed unpaired or paired Student's *t* test as appropriate, with *P* values of  $< 0.05$  taken as being statistically significant.

## RESULTS

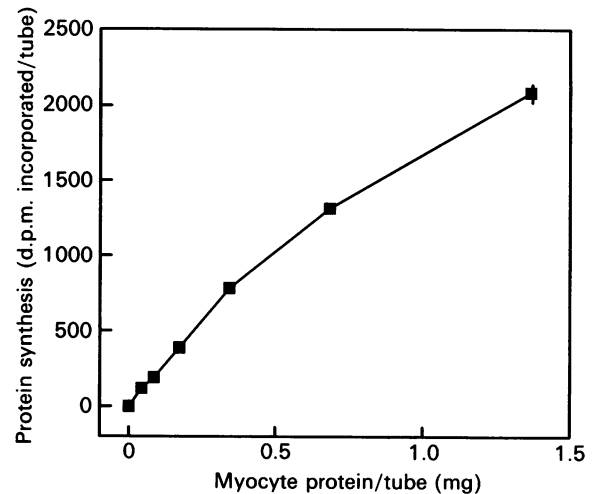
### Preliminary characterization of protein synthesis in isolated cardiac myocytes

We undertook a number of preliminary experiments to ensure that the cardiac myocytes, as prepared by ourselves, were suitable for the proposed investigation. The normal histological criteria of cardiac myocyte integrity are (i) rod-shaped appearance and (ii) lack of contractile activity. The myocytes used in the experiments reported



**Fig. 1.** Time course of protein synthesis in isolated cardiac myocytes

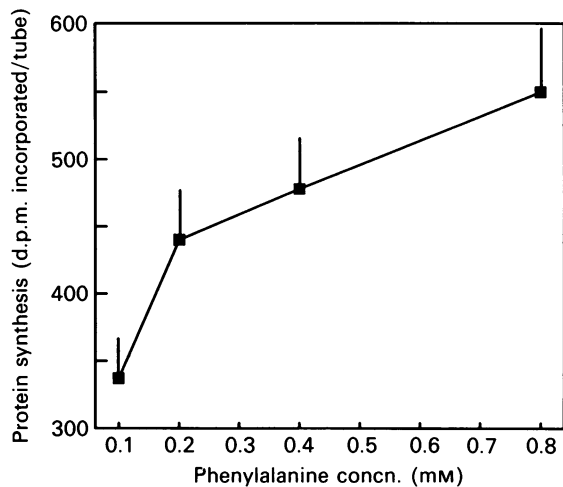
Cardiac myocytes were preincubated as appropriate for 10 min and the protein synthesis was measured for various times as described in the Experimental section: (a) shows a control experiment (■) and one in which 1 munit of insulin/ml was present (▲); (b) shows an experiment in which 1 μM-adrenaline was present. Experiments in (a) and (b) were carried out at different times with different myocyte preparations. In (a), the amount of myocyte protein present per tube was 0.4 mg; in (b) it was 0.5 mg.



**Fig. 2.** Dependence of protein synthesis on cardiac myocyte protein concentration

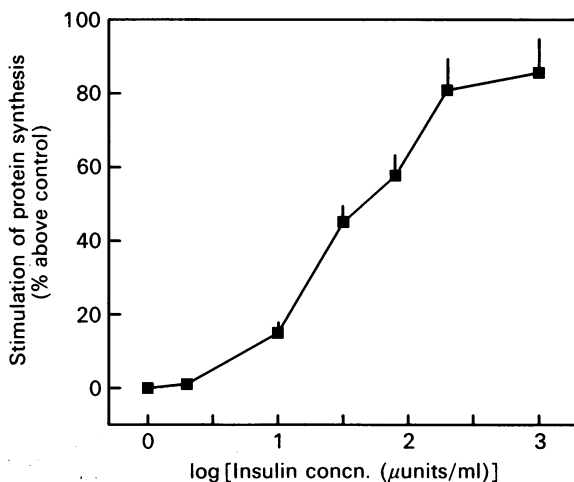
Various quantities of cardiac myocytes were incubated for 1 h as described in the Experimental section. Results of a single typical experiment are presented.

here were examined by phase-contrast microscopy before and after incubation. They were 75–95% rod-shaped and were quiescent. Protein synthesis in control incubations and in the presence of 1 μM-adrenaline or 1 munit of insulin/ml was linear with time for at least 1 h (Fig. 1). The absence of any significant lag in the time courses suggests that the length of pre-exposure (10 min) of myocytes to agonists was sufficient to elicit a maximal response and that the equilibration of specific radioactivity between extracellular [U-<sup>14</sup>C]phenylalanine and [U-<sup>14</sup>C]phenylalanyl-tRNA was rapid. Incubations were routinely carried out for 1 h. Protein synthesis was linear with cardiac myocyte protein added per tube up to about 0.35 mg of protein per tube, and thereafter declined slightly (Fig. 2). We had to balance the need for a relatively low number of cells per tube (in order to ensure linearity of protein synthesis with myocyte protein added) against the need for incorporation of as much radioactivity as possible (in order to ensure maximum sensitivity). We routinely carried out experiments over the range of 0.35–0.55 mg of myocyte protein added per tube. (Note that the amount of myocyte protein added per tube in each individual experiment was identical.) Protein-synthesis rates were not significantly different over a phenylalanine concentration range of 0.2–0.8 mM (Fig. 3). We chose to use routinely a phenylalanine concentration of 0.4 mM, mainly to allow direct comparison of rates of protein synthesis that we obtained in myocytes with those we obtained in the perfused hearts. We also assayed ATP contents routinely. ATP contents were unaffected by any of the treatments, including those in which adrenergic antagonists were present. Values (in μmol/g of protein for 12 observations) were as follows: control incubations,  $20.3 \pm 1.0$ ; +1 μM-adrenaline,  $21.6 \pm 1.2$ ; +1 munit of insulin/ml,  $21.8 \pm 1.3$ . The RNA concentration in cardiac myocytes washed free of BSA was  $8.33 \pm 0.73$  μg/mg of protein (three observations). This value was similar to those routinely observed in the ventricles of perfused hearts [18] and serve to confirm the likelihood that the myocytes used for protein determinations were not contaminated with the BSA present



**Fig. 3. Dependence of isolated cardiac myocyte protein synthesis on phenylalanine concentration**

Cardiac myocytes were incubated for 1 h with various concentrations of [ $U\text{-}^{14}\text{C}$ ]phenylalanine as described in the Experimental section. The amount of myocyte protein per tube was 0.5 mg. Results of a single typical experiment are presented.



**Fig. 4. Effect of insulin concentration on protein synthesis in isolated cardiac myocytes**

Cardiac myocytes were preincubated for 10 min with various concentrations of insulin, and protein synthesis was measured for 1 h as described in the Experimental section. Stimulation is expressed as the percentage increase over the control. Three separate preparations of myocytes were used in these experiments.

during their isolation. We also confirmed that protein synthesis in cardiac myocytes was responsive to low concentrations of insulin (Fig. 4). The sensitivity and the responsiveness were similar to those in ventricles of the anterogradely perfused heart [18]. Stimulation of myocyte protein-synthesis rates was half-maximal at about 30  $\mu\text{units/ml}$  and the maximal stimulation of protein synthesis attainable was about 80%. These results serve to confirm that protein synthesis in the isolated myocytes is characteristically insulin-responsive.

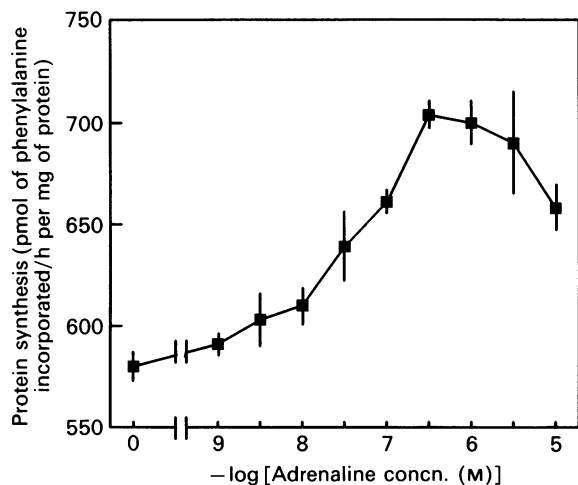
### Effects of adrenergic agonists on rates of protein synthesis in cardiac myocytes

Protein synthesis in cardiac myocytes was stimulated by 20–30% by 1  $\mu\text{M}$ -adrenaline, 1  $\mu\text{M}$ -noradrenaline or 1  $\mu\text{M}$ -phenylephrine (Table 1). Isoprenaline (1  $\mu\text{M}$ ) did not stimulate. This spectrum of response implies participation of the  $\alpha$ -adrenoceptor. The concentration of adrenaline necessary for half-maximal stimulation of protein synthesis was about 30 nM (Fig. 5). The magnitude of the stimulation by maximally effective concentrations (1  $\mu\text{M}$ ) of adrenaline was much less than for maximally effective concentrations [about 100–200  $\mu\text{units/ml}$  (Fig. 4)] of insulin (Table 1). The stimulation of protein synthesis by maximally effective concentrations of insulin and adrenaline was additive (Table 1). In contrast with the situation in the anterogradely perfused heart [15], isoprenaline did not affect protein synthesis in the presence of insulin (Table 1). Myocyte ATP contents were not affected by any of the preceding treatments (see above for values in the presence of insulin or adrenaline; remaining results not shown).

**Table 1. Effects of adrenergic agonists on rates of protein synthesis in cardiac myocytes in the absence or presence of insulin**

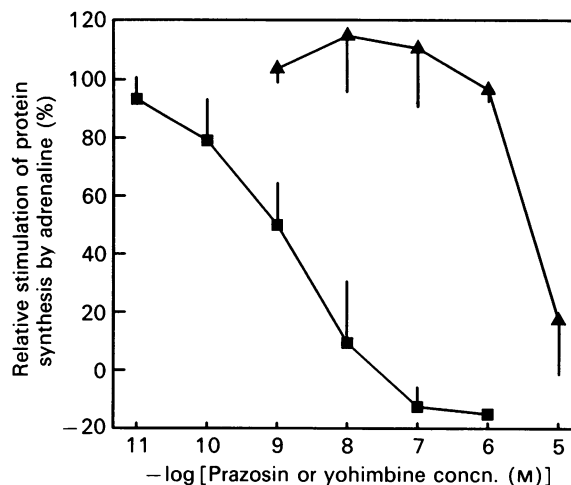
In Expt. 1, no insulin was added. In Expt. 2, insulin was present. Cardiac myocytes were incubated for 10 min with the various agents. When present, adrenergic agonists were added to a concentration of 1  $\mu\text{M}$ , and insulin was added to a concentration of 1 munit/ml. After the preincubation, measurement of the rate of protein synthesis was initiated by the addition of amino acids and was allowed to continue for 60 min. Further details are given in the Experimental section. Control experiments were performed in the absence of the various agents, and the rate of protein synthesis was taken as 100%. Five separate preparations of myocytes were used in both Expts. 1 and 2. The absolute rates of protein synthesis in Expts. 1 and 2 were  $426 \pm 53$  and  $391 \pm 34$  pmol of phenylalanine incorporated/h per mg of protein. Rates of protein synthesis in the presence of the various agents were expressed relative to the rates in the control experiments. There was no further stimulation of protein synthesis by insulin alone when its concentration was increased from 1 to 10 munits/ml. Here, the rate of protein synthesis relative to the control rate was  $192 \pm 11\%$ . Statistical significance was assessed by a paired *t* test. For Expt. 1,  $^*P < 0.05$ ,  $^bP < 0.01$ , versus control incubations (value set at 100%). For Expt. 2,  $^cP < 0.05$  versus incubations in the presence of 1 munit of insulin/ml. (Effects of insulin alone were not analysed statistically.)

Addition	Rate of protein synthesis (% of control value)
Expt. 1 (insulin absent)	
Adrenaline	$128 \pm 3^b$
Noradrenaline	$120 \pm 6^a$
Phenylephrine	$127 \pm 4^b$
Isoprenaline	$97 \pm 3$
Expt. 2 (insulin present)	
No further addition	$188 \pm 11$
Adrenaline	$210 \pm 10^c$
Isoprenaline	$194 \pm 11$



**Fig. 5. Dependence of the stimulation of protein synthesis in cardiac myocytes on adrenaline concentration**

Cardiac myocytes were preincubated with various concentrations of adrenaline at 37 °C for 10 min, and protein synthesis was measured for 1 h as described in the Experimental section. Three separate preparations of myocytes were used in these experiments.



**Fig. 6. Inhibition of the adrenaline-induced stimulation of protein synthesis in cardiac myocytes by  $\alpha$ -adrenergic antagonists**

Cardiac myocytes were incubated with various concentrations of prazosin (■) or yohimbine (▲) at 37 °C for 10 min. Adrenaline was then added to a concentration of 1  $\mu$ M, and the myocytes were incubated for a further 10 min, after which time protein synthesis was measured as described in the Experimental section. Three separate preparations of myocytes were used in these experiments. The relative stimulation of protein synthesis by adrenaline was calculated by subtracting the control (no addition) rate from the rate in the presence of adrenaline and antagonist. This value was then expressed as a percentage of the rate in the presence of adrenaline minus the control rate.

**Effect of adrenergic antagonists on the stimulation of protein synthesis by adrenaline in cardiac myocytes**

Further characterization of the stimulation of protein synthesis by adrenaline (1  $\mu$ M) showed it to be an  $\alpha_1$ -adrenergic effect, since it was completely prevented by the  $\alpha_1$ -adrenergic antagonist prazosin at a concentration of 100 nM (Fig. 6). The  $\alpha_2$ -antagonist yohimbine did not prevent stimulation at a concentration of 1  $\mu$ M but partially (80%) prevented stimulation at a concentration of 10  $\mu$ M. The  $\beta$ -antagonist DL-propranolol (20  $\mu$ M) did not prevent stimulation of protein synthesis by 1  $\mu$ M-adrenaline (results not shown). Control experiments showed that the rates of protein synthesis in the absence of adrenaline were not inhibited by 1  $\mu$ M-prazosin, 10  $\mu$ M-yohimbine or 20  $\mu$ M-DL-propranolol (results not shown).

**Effects of adrenaline on protein synthesis in anterogradely perfused hearts**

A problem with perfusing hearts with adrenergic agonists is that there is a tendency for ATP contents to decrease [15,19], presumably because of their positive inotropic and chronotropic effects. This complicates interpretation of their action on protein synthesis [15].

**Table 2. Effect of adrenaline on the rates of protein synthesis and adenine nucleotide and phosphocreatine contents in the anterogradely perfused heart**

Details of methods used are given in the Experimental section. DL-Propranolol was added to a final concentration of 10  $\mu$ M after 5 min of anterograde perfusion. One addition of adrenaline (to a concentration of 1  $\mu$ M) was made after 10 min of anterograde perfusion; a second similar addition was made at 70 min. Perfusions were for a total of 130 min. There were 5–10 observations in each group. Statistical significance was calculated by using a two-tailed unpaired *t* test: <sup>a</sup>*P* < 0.05, <sup>b</sup>*P* < 0.01, <sup>c</sup>*P* < 0.001 for perfusions in the presence of adrenaline versus control perfusions, or for perfusions in the presence of adrenaline + propranolol versus perfusions in the presence of propranolol.

Perfusion conditions	Rate of protein synthesis (pmol of phenylalanine incorporated/h per mg of protein)	Metabolite concn. ( $\mu$ mol/g of protein)					
		ATP	ADP	AMP	ATP + ADP + AMP	Phosphocreatine	ATP/ADP ratio
Control	646 ± 18	25.0 ± 0.8	6.2 ± 0.4	0.9 ± 0.1	32.1 ± 0.8	20.3 ± 1.6	4.3 ± 0.4
+ propranolol	625 ± 33	23.7 ± 2.5	6.3 ± 0.5	1.0 ± 0.1	31.1 ± 2.9	19.2 ± 1.7	3.5 ± 0.1
+ adrenaline	748 ± 44 <sup>a</sup>	18.7 ± 0.9 <sup>c</sup>	3.4 ± 0.1 <sup>c</sup>	0.6 ± 0.1	22.8 ± 0.9 <sup>c</sup>	33.8 ± 1.5 <sup>c</sup>	5.5 ± 0.5
+ adrenaline + propranolol	932 ± 22 <sup>c</sup>	21.3 ± 0.8	5.4 ± 0.3	0.8 ± 0.1	27.5 ± 1.0 <sup>a</sup>	27.5 ± 2.0 <sup>a</sup>	4.0 ± 0.1 <sup>b</sup>

**Table 3. Effects of actinomycin D on the stimulation of protein synthesis by adrenaline or insulin**

Cardiac myocytes were preincubated for 20 min with 5  $\mu\text{g}$  of actinomycin D/ml. Adrenaline (final concn. 1  $\mu\text{M}$ ) or insulin (final concn. 1 munit/ml) was then added and the incubations were continued for a further 10 min, after which time measurement of protein synthesis was initiated by addition of amino acids and was allowed to continue for 60 min. Three separate preparations of myocytes were used for these experiments. The values in parentheses indicate the rate of protein synthesis expressed relative to control values in the absence or presence of actinomycin D. The control rate in the presence of actinomycin D was  $84 \pm 3\%$  of that in its absence. The stimulation of protein synthesis was calculated by subtracting the appropriate control rate from the rate in the presence of adrenaline or insulin. Because of the nature of the experiment (i.e. we wished to test for the existence of stimulation of protein synthesis in the presence of actinomycin D), no statistical analysis of results was undertaken. Cardiac myocyte ATP contents were unaffected by actinomycin D and were as follows, in the absence and presence of actinomycin D respectively: control,  $20.6 \pm 1.8$  and  $22.6 \pm 3.5$ ; +1  $\mu\text{M}$ -adrenaline,  $22.5 \pm 2.8$  and  $23.4 \pm 3.8$ ; +1 munit of insulin/ml,  $22.8 \pm 2.7$  and  $23.8 \pm 3.6$   $\mu\text{mol/g}$  of protein. Further details of the incubations are given in the Experimental section.

Addition	Rate of protein synthesis (pmol of phenylalanine incorporated/h per mg of protein)			
	No actinomycin D		+ actinomycin D	
	Rate	Stimulation	Rate	Stimulation
Control	$438 \pm 56$ (100%)	–	$367 \pm 45$ (100%)	–
+ adrenaline	$541 \pm 68$ (124 $\pm$ 1%)	$103 \pm 12$	$471 \pm 50$ (129 $\pm$ 2%)	$104 \pm 5$
+ insulin	$751 \pm 72$ (173 $\pm$ 8%)	$313 \pm 22$	$674 \pm 59$ (186 $\pm$ 11%)	$307 \pm 23$

We examined the effects of adrenaline on protein synthesis in the absence or presence of the  $\beta$ -adrenergic antagonist propranolol. Protein synthesis was stimulated by adrenaline in the absence of propranolol, but the stimulation was only 16% (Table 2). ATP concentrations were decreased and phosphocreatine concentrations were increased. Addition of propranolol did not affect protein-synthesis rates or metabolite concentrations as compared with control perfusions. However, in the presence of propranolol, adrenaline stimulated protein synthesis by 49%. Under these conditions, adrenaline did not significantly decrease ATP concentrations, and still increased phosphocreatine concentrations. These findings suggest that (i) in order to observe effects of catecholamines on cardiac protein synthesis, care must be taken to maintain ATP concentrations, and (ii) the stimulation of protein synthesis in the perfused heart is probably an  $\alpha$ -adrenergic effect.

#### Effects of actinomycin D on the stimulation of protein synthesis by adrenaline in cardiac myocytes

Use of actinomycin D established that the acute stimulation of protein synthesis by adrenaline in these experiments was exerted at the level of translation. As a control, we also included incubations with insulin, which is known to exert its acute effects at the level of translation [20,21]. Actinomycin D decreased the rates of protein synthesis by the same absolute amount in all incubations (Table 3). Protein synthesis was still stimulated by the same absolute amount by adrenaline or insulin in the presence of actinomycin D as in its absence. This behaviour is similar to that which we have seen previously when cardiac myocyte protein synthesis is acutely stimulated by 12-tetradecanoylphorbol 13-acetate [22].

#### Lack of effect of angiotensin II

In this paper, we have categorized the stimulation of protein synthesis by adrenaline as an  $\alpha_1$ -adrenergic effect.  $\alpha_1$ -Adrenoceptor stimulation in cardiac myocytes increases membrane phosphatidylinositol hydrolysis by stimulation of phosphoinositidase activity [23]. Angiotensin II also stimulates phosphoinositidase activity in cardiac myocytes [24] where there are functional angiotensin II receptors [25]. It has been suggested that some of the effects of the hormone on cardiac myocyte contractility are attributable to stimulation of diacylglycerol production and protein kinase C activity [24]. In

**Table 4. Lack of effect of angiotensin II on cardiac myocyte protein synthesis at low and high  $\text{Ca}^{2+}$  concentrations**

Cardiac myocytes were preincubated for 10 min with the various concentrations of angiotensin II, after which time protein synthesis was measured as described in the Experimental section. The results from a single preparation of myocytes are shown, although results in two other separate experiments were similar.

Angiotensin II concn. (M)	Rate of protein synthesis (pmol of phenylalanine incorporated/h per mg of protein)	
	50 $\mu\text{M}$ added $\text{Ca}^{2+}$	1.5 mM added $\text{Ca}^{2+}$
0	$371 \pm 22$	$470 \pm 18$
$10^{-9}$	$396 \pm 12$	$471 \pm 9$
$10^{-7}$	$411 \pm 17$	$458 \pm 20$
$10^{-5}$	$415 \pm 12$	$458 \pm 9$

our earlier experiments, we showed that phorbol esters stimulated protein synthesis in cardiac myocytes [22]. Hence it might be predicted that angiotensin II would stimulate cardiac myocyte protein synthesis. We investigated the effects of angiotensin II on protein synthesis in isolated cardiac myocytes at angiotensin II concentrations of between 1 pM and 10  $\mu$ M at low and high added  $\text{Ca}^{2+}$  concentrations (Table 4). We could not detect any effect of angiotensin II on protein synthesis, although we did observe that protein synthesis was stimulated at the higher  $\text{Ca}^{2+}$  concentration by about 25%.

## DISCUSSION

### General comments on the use of isolated cardiac myocytes in protein-synthesis experiments

Cardiac myocytes freshly isolated from adult fed rats represent a suitable system for the study of cardiac protein synthesis. The control rates of protein synthesis are about 400 pmol of phenylalanine incorporated/h per mg of myocyte protein (Tables 1 and 3). These values are about 60% of the control rates observed in the anterogradely perfused heart (Table 2). We consider that these rates are satisfactory. It should be remembered that the myocytes are quiescent, and hence any stimulation of protein synthesis that might be attributable to either stretch [26,27] or contractility [28] will have been removed. Williams *et al.* [29] showed that the rate of protein synthesis in control anterogradely perfused hearts was 1909 pmol of phenylalanine incorporated/2 h per mg of protein. In cardiac myocytes prepared by these workers and subsequently incubated to allow synthesis of protein, the rate was 859–880 pmol/h per mg of protein [29]. When these rates were corrected for the different RNA concentrations and incubation times in the two experiments, the efficiencies of protein synthesis in the perfused hearts and isolated myocytes were 119 and 80 pmol of phenylalanine incorporated/h per  $\mu$ g of RNA respectively. Thus, under conditions that we can compare directly with our own, myocytes synthesized protein at about 65% of the rate in the anterogradely perfused heart. Our experiments were carried out at added  $\text{Ca}^{2+}$  concentrations of 50  $\mu$ M. When the added  $\text{Ca}^{2+}$  concentration was raised to 1.5 mM, protein-synthesis rates were increased by about 25% (Table 4).

Protein synthesis in the anterogradely perfused heart is sensitive to insulin [18]. This insulin stimulation is itself prevented by decreased cardiac 'energy status' [15], and so can serve as a criterion of the metabolic integrity of the cardiac myocytes. We have observed a stimulation of protein synthesis by insulin in isolated cardiac myocytes. The sensitivity and responsiveness are similar to those in the anterogradely perfused heart (Fig. 4 and [18]). Furthermore, ATP concentrations (at about 20  $\mu$ mol/mg of myocyte protein; see the Results section) were also similar to those in anterogradely perfused hearts (Table 2).

In all protein-synthesis experiments, there is always concern as to whether the radiolabelled precursor rapidly and completely equilibrates with the aminoacyl-tRNA pool. The findings that we do not see a significant lag in protein synthesis after the addition of [ $^{14}\text{C}$ ]phenylalanine under any condition (Fig. 1) suggest rapid equilibration of the extracellular [ $^{14}\text{C}$ ]phenylalanine and intracellular [ $^{14}\text{C}$ ]phenylalanyl-

tRNA pools. In perfused hearts, the specific radioactivities of the two pools of [ $^{14}\text{C}$ ]phenylalanine rapidly equalize [30]. Thus the finding that rates of protein synthesis in cardiac myocytes and perfused hearts compare reasonably well with one another leads us to conclude that equalization of the specific radioactivities is complete. Furthermore, no significant increases in protein-synthesis rates were observed on doubling the [ $^{14}\text{C}$ ]phenylalanine concentration in the myocyte incubations from 0.4 to 0.8 mM (Fig. 3). We should point out that in cardiac myocyte cultures from neonatal-rat or embryonic-chick hearts (which may not be comparable with our myocytes) as a source of cells, the specific radioactivity of the radiolabelled aminoacyl-tRNA pool usually only attained 50% of that in the medium over periods of up to 2 h [28,31], although this value of specific radioactivity was reached within 7.5 min and was then unchanged [31]. Increasing the extracellular concentration of radiolabelled amino acid did not increase the rate of protein synthesis in this system [32]. The phenomenon of unequal specific radioactivities may be related to preferential recycling of unlabelled amino acid derived from intracellular protein degradation back into protein. The inequality between the extracellular [ $^{14}\text{C}$ ]phenylalanine and [ $^{14}\text{C}$ ]phenylalanyl-tRNA specific radioactivities was probably not the result of the technical difficulties in measuring the specific radioactivity of the [ $^{14}\text{C}$ ]phenylalanyl-tRNA pool. Calculations of protein-synthesis rates either based on [ $^{14}\text{C}$ ]phenylalanyl-tRNA specific radioactivities in a pulse-labelling experiment or using the double-labelling protocol of Zak and colleagues [33,34] (which obviates the problem of equalization of the two amino acid pools) gave similar results [28]. The latter technique could not be used in our experiments, since it is only really suitable for cells in prolonged culture.

### Other work of relevance to the action of adrenergic agonists on cardiac protein synthesis

Although the effects of adrenergic agonists have been investigated in the perfused heart, this system has not been especially useful, since results have not always been consistent. Mallov [35] showed that pre-treatment with catecholamines *in vivo* enhanced protein synthesis in ventricular slices subsequently incubated *in vitro*, but that there was no acute effect of adrenaline on protein synthesis in slices *in vitro*. Källfelt *et al.* [36] showed a stimulation of protein synthesis by isoprenaline, adrenaline and noradrenaline in perfused hearts, but, surprisingly, an inhibition by dibutyryl cyclic AMP. Dibutyryl cyclic AMP or glucagon also inhibited protein synthesis in atrial strips or perfused hearts [37]. In a preliminary report, Morgan and his colleagues showed that, in perfused hearts, adrenaline stimulated protein synthesis in the absence of insulin, but inhibited it in the presence of insulin [19]. (Physiological concentrations of insulin stimulate cardiac protein synthesis; see, e.g. [18].) In these experiments, glucagon stimulated protein synthesis. We have detected an inhibition of protein synthesis by isoprenaline and other agents which raise intracellular cyclic AMP concentrations in the presence of insulin [15]. However, we were unable to demonstrate any effect of isoprenaline on basal protein synthesis in the absence of insulin [15]. Hearts perfused *in vitro* with catecholamines show decreases in ATP and total adenine nucleotide contents [15,19]. This is likely to result from insufficient

O<sub>2</sub> delivery by simple physiological saline solutions in the face of positive chronotropism and inotropism. That O<sub>2</sub> delivery may be inadequate under these conditions is supported by the finding that lactate output was dramatically increased by catecholamines in the perfused heart provided with glucose as a fuel [15]. Since protein synthesis is an endergonic process, decreased nucleoside triphosphate contents might explain a number of the above findings in perfused hearts (see [15] for a more detailed discussion). Morgan and colleagues avoided the problems of inadequate O<sub>2</sub> delivery by using tetrodotoxin-arrested hearts. Agents that raised intracellular cyclic AMP concentrations stimulated protein synthesis [38]. These findings implicated the  $\beta$ -adrenoceptor/adenylate cyclase system in the regulation of cardiac protein synthesis. The same group has also recently suggested a role for cyclic AMP in the stimulation of protein synthesis in the perfused heart by pressure-overload [39].

#### Translational stimulation of protein synthesis by $\alpha$ -adrenergic agonists

In contrast with the results of Morgan's group [38], we have shown here that  $\alpha_1$ -adrenergic agonists stimulate protein synthesis at the level of translation in the heart. The presence of  $\alpha_1$ -adrenoceptors on the sarcolemma of rat ventricular myocytes has been established by radioligand binding or autoradiography [40,41].  $\alpha_1$ -Adrenoceptor stimulation is thought to stimulate phosphoinositidase-mediated hydrolysis of membrane phosphatidylinositols, leading to stimulation of production of diacylglycerol and inositol trisphosphate (reviewed in [42]). This is known to occur in rat cardiac myocytes [23], although  $\alpha_1$ -adrenoceptor stimulation may also additionally decrease cyclic AMP concentrations [43]. Diacylglycerol stimulates protein kinase C to phosphorylate substrate proteins, and inositol trisphosphate increases cytoplasmic Ca<sup>2+</sup> concentration by causing its mobilization from intracellular stores (reviewed in [42]).

One way in which the problems of O<sub>2</sub> delivery encountered in the perfused heart in the face of adrenergic stimulation can be avoided is through the use of isolated myocytes. In this paper, we have demonstrated acute  $\alpha_1$ -adrenergic stimulation of protein-synthesis translation in freshly isolated cardiac myocytes (Tables 1 and 3). Although the magnitude of the maximal stimulation by adrenaline is relatively small (about 30%) compared with that of insulin (about 85%), it should be recognized that the development of cardiac hypertrophy probably requires only a relatively small enhancement of protein synthesis over protein degradation [8], and a large stimulation of protein synthesis *in vitro* should not necessarily be expected. It is also of interest that adrenaline stimulated protein synthesis even in the presence of maximally effective insulin concentrations (Table 1), which contrasts with the response to other stimulators of cardiac protein synthesis, such as non-carbohydrate fuels [44]. There is no suggestion that insulin causes cardiac hypertrophy, in spite of its effects on cardiac protein turnover [18,45]. We suggest that insulin may regulate the diurnal nutrient-related turnover of cardiac protein, upon which more specific controls are superimposed.

As discussed above, we did not measure the [<sup>14</sup>C]phenylalanyl-tRNA specific radioactivity in our isolated myocytes. Equalization of precursor specific radio-

activities is not a problem in the perfused heart, since it is so rapid [30]. Partly for these reasons, we also carried out some experiments in this system. We demonstrated stimulation of protein synthesis by adrenaline in the presence of propranolol, suggesting that it is an  $\alpha$ -adrenergic effect (Table 2). Thus the effect is presumably analogous to the effects of adrenaline in isolated myocytes (Table 1). The inclusion of propranolol prevented any decrease in ATP concentrations, probably by decreasing  $\beta$ -adrenergic drive. Phosphocreatine concentrations were increased by adrenaline (Table 2). A possible reason for this finding is discussed below.

#### Possible mechanisms responsible for the $\alpha$ -adrenergic stimulation of protein synthesis

Creatine kinase catalyses a reaction that is close to equilibrium in the perfused heart [46,47]. The synthesis of phosphocreatine from ATP and creatine involves production of a proton [48]. We have suggested that an increase in the phosphocreatine content in the heart may be indicative of an increase in cytoplasmic pH under some circumstances [49]. Calculations using data in Table 2 as described previously [49] suggest that (if our assumptions [49] are justified) adrenaline in the presence of propranolol may raise cytoplasmic pH by about 0.15 unit compared with perfusions with propranolol alone. This increase in cytoplasmic pH should be sufficient to increase the rate of protein synthesis by about 35–40% (see [49]). The observed increase was about 45–50% (Table 2). A plausible mechanistic hypothesis might involve  $\alpha_1$ -adrenergic stimulation of cardiac phosphoinositidase activity [23], diacylglycerol stimulation of protein kinase C activity (for reviews, see [42,50]) and hence activation of the Na<sup>+</sup>/H<sup>+</sup> antiporter [51–53] leading to proton efflux from the cell. It should be noted that the effects that we have observed were obtained in bicarbonate-containing media, thereby avoiding some of the objections raised by Thomas [54]. In the absence of propranolol, adrenaline was less effective in stimulating protein synthesis compared with the control perfusions, stimulating by about 15%. However, phosphocreatine concentrations were still increased. The calculated increase in cytoplasmic pH under these conditions was about 0.2 unit, which should theoretically be sufficient to stimulate protein synthesis by about 50% [49]. This lesser stimulation of protein synthesis in the face of this putative increase in cytoplasmic pH might be explained by a counteraction resulting from the decreased ATP concentration (Table 2).

We have recently shown that 12-tetradecanoylphorbol 13-acetate, which is thought to act as a long-lived diacylglycerol analogue [50], stimulates translation in cardiac myocytes.  $\alpha_1$ -Adrenergic stimulation might act analogously. There are two differences. First, the responses of protein synthesis to maximally effective concentrations of adrenaline and insulin are additive, whereas those to 12-tetradecanoylphorbol 13-acetate and insulin are not [22]. Secondly, the stimulations of protein synthesis induced by 12-tetradecanoylphorbol 13-acetate or adrenaline differ in magnitude, the former being the greater (Table 1 and [22]). However, these differences do not exclude the possibility that both phorbol esters and  $\alpha_1$ -adrenergic agonists may stimulate protein synthesis through a protein kinase C-dependent mechanism, and that subtle regulation occurs at other levels.



### Translational and pretranslational control of cardiac protein synthesis

Others have shown that chronic exposure (up to 4 days) of cells cultured from neonatal rat hearts to  $\alpha_1$ -adrenergic agonists induced hypertrophy and stimulated protein synthesis [5,6]. The point of action of the agonists was not defined. Simpson *et al.* [7] have more recently suggested that the induction of the hypertrophic response by  $\alpha_1$ -adrenergic stimulation was primarily transcriptional. There is no doubt that  $\alpha_1$ -adrenergic agonists acutely stimulate generalized transcription in cardiac myocyte cultures as measured by uridine or uridylate incorporation in intact cells or nuclei isolated from pre-treated intact cells [55]. There is enhanced transcription of proto-oncogenes [2,56], rRNA genes [7] and the  $\alpha$ -actin and myosin light chain-2 genes [7,55]. Some of these effects are also induced by 12-tetradecanoylphorbol 13-acetate [55,56]. It is thus likely that the development of cardiac hypertrophy involves enhancement of both transcription and translation, and supporting evidence for this hypothesis has been obtained *in vivo* [9]. It is possible that stimulation of translation is expressed as a rapidly observed enhancement of protein synthesis and that stimulation of transcription, because of the more complex processing involved, may lead to a longer-term enhancement and may regulate the specificity of proteins synthesized.

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