Biochem. J. (1992) 282, 85–90 (Printed in Great Britain)

Stimulation of cardiac protein synthesis by insulin-like growth factors

Stephen J. FULLER,* John R. MYNETT and Peter H. SUGDEN
Department of Cardiac Medicine, National Heart and Lung Institute (University of London), Dovehouse Street, London SW3 6LY, U.K.

The effects of the insulin-like growth factors (IGF)-1 and -2 on the rates of protein synthesis in freshly isolated cardiac myocytes from adult rats were compared with those of insulin. At concentrations of 50–100 nM, each agent stimulated protein synthesis by about 70%. There was no additional stimulation upon combination of insulin with IGF-1 or IGF-2 at these high concentrations. When compared over a range of concentrations, the relative response to each agent was insulin > IGF-1 ≥ IGF-2. Concentrations of 1 nM-IGF-1, 1 nM-IGF-2 or 0.2 nM-insulin enhanced the rates of protein synthesis by 36%, 30% or 34% respectively. A combination of 0.2 nM-insulin and 1 nM-IGF-1 or 1 nM-IGF-2 increased the stimulation of protein synthesis to 46%. In contrast, the effects of 1 nM-IGF-1 and 1 nM-IGF-2 were not additive. The possible mechanistic basis for this difference is discussed. At a concentration of 50 nM, epidermal growth factor (EGF), fibroblast growth factor and platelet-derived growth factor were each without effect on protein synthesis. In anterogradely perfused rat heart preparations, 2 nM-IGF-1 or 2.4 nM-IGF-2 increased protein synthesis and lactate production, but 9.2 nM-EGF did not. From a consideration of the plasma free concentrations of IGF-1 and IGF-2, we suggest that these factors may contribute to the maintenance of rate of cardiac protein synthesis *in vivo*.

INTRODUCTION

The insulin-like growth factors (IGFs)-1 and -2 are single-chain polypeptides which are structurally and functionally related to each other as well as to insulin (reviewed in [1–3]). In man the IGFs are expressed both during the growth period and in adult life. *In vitro*, IGF-1 and IGF-2 have insulin-like metabolic activity (such as stimulation of glucose uptake), but only at concentrations greater than their plasma concentration [1,3]. Their biologically relevant effects include stimulation of cell proliferation and differentiation, which occur *in vitro* at nanomolar concentrations [3,4]. The physiological roles of IGF-1 and IGF-2 have thus been proposed to involve promotion of growth, whereas acute metabolic actions are thought to be mediated by insulin itself [1].

Many factors influence the rate of muscle protein turnover *in vitro* and *in vivo* (for a review, see [5]). In a variety of skeletal-muscle cell cultures, IGF-1 and IGF-2 stimulated protein synthesis and inhibited protein degradation at concentrations that occur physiologically [6–8]. In those studies, insulin was less effective than the IGFs, and effects were only observed at hyperphysiological concentrations. In contrast, Airhart *et al.* [9] have demonstrated that physiological concentrations of insulin stimulated protein synthesis in skeletal-muscle cells cultured from chick embryos. However, hyperphysiological concentrations of insulin were required to stimulate protein synthesis in analogous cardiac-muscle cell cultures [9]. Our own studies have shown that insulin stimulates protein synthesis at physiological concentrations in perfused hearts and in freshly isolated cardiac myocytes from adult rats [10,11]. Furthermore, experiments *in vivo* have shown that protein synthesis in both skeletal and cardiac muscles in approx. 5-week-old rats is sensitive to physiological concentrations of insulin [12]. The reasons for the differing responses of muscle preparations to insulin and the IGFs may reflect the fact that cultured cells are more closely related to embryonic cells than to adult cells. As discussed in [5], IGFs may be generally more important than insulin in the regulation of protein turnover in embryonic cells. The aim of this study was therefore to compare the effects of IGF-1 and IGF-2 on protein synthesis with those of insulin, using cardiac preparations isolated from adult animals. In this way, we hoped to establish whether the IGFs are potentially important regulators of cardiac protein synthesis in adult animals.

EXPERIMENTAL

Materials and animals

Sources of materials and animals have been given previously [11,13]. In addition, human recombinant IGF-1, IGF-2 and epidermal growth factor (EGF), pig platelet-derived growth factor (PDGF) and bovine brain fibroblast growth factor (FGF) were from Boehringer–Mannheim U.K., Lewes, East Sussex, U.K. Insulin (Novo, human Actrapid; stock solution of 100 units/ml) was obtained from the Royal Brompton National Heart Hospital pharmacy. Stock solutions of growth factors were prepared as follows: IGF-1 and IGF-2 (50 μg/ml) in 10 mM-HCl; PDGF (50 μg/ml) and EGF (500 μg/ml) in water; FGF (50 μg/ml) as supplied in 0.7 M-NaCl/20 mM-Tris/HCl, pH 7.4. Solutions of growth factors were stored in separate batches at −80 °C until use. IGF-1 and IGF-2 were neutralized with 10 mM-NaOH before use.

Measurement of protein synthesis in isolated cardiac myocytes

Cardiac myocytes were isolated from the hearts of 250–300 g fed rats by collagenase digestion as described previously [11], except that the low-Ca^{2+} perfusion buffer did not contain albumin and the added Ca^{2+} concentration was decreased to 5 μM. Cells were resuspended at a myocyte protein concentration of 3.5–5.5 mg/ml in collagenase-free Krebs & Henseleit saline containing 25 mM-$NaHCO_3$, 2% (w/v) BSA, 10 mM-glucose and

Abbreviations used: EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF-1, insulin-like growth factor-1; IGF-2, insulin-like growth factor-2; PDGF, platelet-derived growth factor.

* To whom correspondence should be addressed.

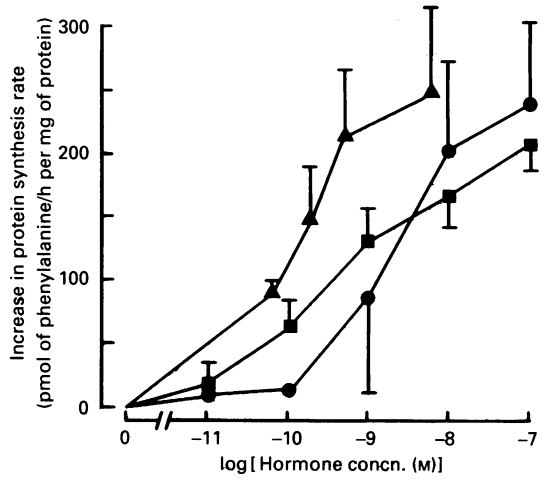

Fig. 1. Dependence of the stimulation of cardiac myocyte protein synthesis on IGF-1, IGF-2 and insulin concentrations

Myocytes were incubated with insulin (▲), IGF-1 (■) or IGF-2 (●) as described in the Experimental section. Stimulation of protein synthesis by each agent is expressed as the absolute increase in rate of protein synthesis above that in its absence. The data were obtained from 3–5 separate preparations of myocytes. Basal rates of protein synthesis were 403 ± 23, 363 ± 20 or 285 ± 53 pmol of phenylalanine incorporated/h per mg of myocyte protein for insulin, IGF-1 or IGF-2 respectively.

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 protein synthesis has been described and characterized previously [11]. The mean of quadruplicate incubations was taken as one experimental observation. Myocytes were preincubated in a volume of 200 μl at 37 °C for 20 min in the absence or presence of insulin and/or other growth factors. Measurement of protein synthesis was initiated by addition of 40 μl of an amino acid mixture containing 2.4 mM-[U-^{14}C]phenylalanine (sp. radioactivity 2.0 Ci/mol) and the remaining amino acids required for the support of protein synthesis each at 1.2 mM. Protein synthesis was terminated after 60 min by addition of 1 ml of ice-cold 5% (w/v) trichloroacetic acid, and the precipitated protein was washed and prepared for liquid-scintillation counting [11].

Measurement of protein synthesis and lactate production in anterogradely perfused hearts

Hearts were taken from fed or overnight-fasted 250–300 g rats and were perfused anterogradely as described in detail previously [14,15]. The perfusate for the retrograde pre-perfusion was Krebs & Henseleit buffer (2.5 mM-Ca^{2+}) equilibrated with O_2/CO_2 (19:1) and supplemented with 5 mM-glucose. After switching from the retrograde pre-perfusion to anterograde perfusion, the perfusate (100 ml, recirculated) contained additionally 0.4 mM-[U-^{14}C]phenylalanine (sp. radioactivity about 0.04 Ci/mol) and the remaining amino acids necessary to support protein synthesis each at 0.2 mM. The left-atrial filling pressure was 0.5 kPa and the aortic pressure was 7 kPa. The sp. radioactivity of the [U-^{14}C]phenylalanine was calculated by liquid-scintillation counting of the perfusate samples and measurement of the phenylalanine concentration [15,16]. Insulin and growth factors were present only during the anterograde perfusion.

After 2 h of the anterograde perfusion, the hearts were freeze-clamped with aluminium tongs pre-cooled in liquid N_2. The frozen hearts were powdered in liquid N_2 and extracted with 0.56 M-$HClO_4$. The precipitated protein was collected by bench centrifugation at 4 °C and was washed free of unbound [U-^{14}C]phenylalanine [15]. Incorporation of [U-^{14}C]phenylalanine into protein was measured as described previously [15]. The supernatant fractions from the initial $HClO_4$ extractions were neutralized with 5 M-KOH/0.5 M-Tris/HCl, by using Universal Indicator (BDH). ATP, ADP, AMP, phosphocreatine and creatine contents in these extracts were measured by standard spectrophotometric techniques [17].

For the determination of the rate of lactate release by the heart, samples (0.5 ml) of perfusate were taken at the times indicated, frozen in liquid N_2 and stored at −20 °C. Perfusate volumes were determined from the dilution of [U-^{14}C]phenylalanine, and lactate concentrations were assayed spectrophotometrically [17].

Other methods and expression of results

Protein was measured by the biuret method [18] (with BSA as standard) in a sample of myocytes washed free of BSA or in NaOH digests of $HClO_4$-washed heart protein [11]. Results are presented as means ± S.E.M. Statistical significance was assessed by a two-tailed unpaired or paired Student's t test as appropriate, with $P < 0.05$ taken as being statistically significant.

RESULTS

Effects of IGF-1, IGF-2 and insulin on rates of protein synthesis in cardiac myocytes

We have previously demonstrated that cardiac myocyte protein synthesis is stimulated by insulin, with a maximum effect at 1000 μunits/ml (6.7 nM) [11]. This was confirmed in the present series of experiments (Fig. 1). Half-maximal stimulation occurred at an insulin concentration of about 0.15 nM and served as a standard with which the effects of IGF-1 and IGF-2 could be compared. IGF-1 stimulated the rate of protein synthesis over the concentration range 0.01–100 nM, and IGF-2 stimulated the rate of protein synthesis over the concentration range 0.1–100 nM (Fig. 1). It was not possible to extend the dose–response curves for IGF-1 and IGF-2 beyond the upper concentration of 100 nM, because of the expense involved. The shapes of the curves for IGF-1 and IGF-2 made it difficult to determine whether maximum stimulation of protein synthesis had been achieved. However, indirect evidence presented below (Table 1) suggested that the responses to 100 nM-IGF-1 or -IGF-2 were close to maximum. Concentrations of IGF-1 and IGF-2 that gave half the stimulation of protein synthesis observed at 100 nM were about 0.4 nM and 2 nM respectively. Thus IGF-1 was about 3-fold and IGF-2 about 10-fold less potent in stimulating protein synthesis than was insulin.

The dose–response curves to IGF-1, IGF-2 and insulin (Fig. 1) were conducted in different preparations of myocytes. Direct comparisons between them with respect to absolute rates of protein synthesis are thus not strictly valid. Consequently, additional experiments were carried out in which the effects of IGF-1, IGF-2 and insulin were compared with respect to maximum response, sub-maximal response and additivity of response within the same preparations of myocytes (Table 1).

At concentrations of 50 nM-IGF-1, 50 nM-IGF-2 and 6.7 nM-insulin the stimulation of protein synthesis by each factor was similar and was not significantly different from that produced by 67 nM-insulin, suggesting that maximal stimulation of protein synthesis had been achieved (Table 1, Expt. 1). Also, there was

Table 1. Effects of combinations of IGF-1, IGF-2 and insulin on the rates of protein synthesis in cardiac myocytes

Protein synthesis rates were measured as described in the Experimental section for 3–5 separate preparations of myocytes in Expts. 1–3. The concentrations of IGF-1, IGF-2 and insulin represent the values in the incubations when protein synthesis rates were measured. Results are expressed relative to the rate of protein synthesis in control incubations. Absolute rates of protein synthesis (in pmol of phenylalanine incorporated/h per mg of myocyte protein) were: Expt. 1, 617±90; Expt. 2, 328±35; Expt. 3, 462±66. Statistical significance was determined by a paired Student's t test: $^{a}P < 0.05$, $^{b}P < 0.02$, $^{c}P < 0.01$ versus control incubations. Additionally, for Expt. 3, $^{d}P < 0.05$ versus IGF-2 or 0.2 nM-insulin alone, $^{e}P < 0.02$ versus IGF-1 alone, $^{f}P < 0.01$ versus 0.2 nM-insulin alone.

Expt. no.	Incubation conditions	Protein synthesis rate (% of control)
1	Control	(100)
	50 nM-IGF-1	163±9[b]
	50 nM-IGF-2	170±8[b]
	6.7 nM-insulin	166±6[c]
	50 nM-IGF-1 + 6.7 nM-insulin	176±8[b]
	50 nM-IGF-2 + 6.7 nM-insulin	179±9[b]
	67 nM-insulin	174±9[b]
2	Control	(100)
	1 nM-IGF-1	132±4[b]
	1 nM-IGF-2	124±7[a]
	1 nM-IGF-1 + 1 nM-IGF-2	135±5[b]
	10 nM-IGF-1	143±8[a]
	10 nM-IGF-2	153±7[b]
	10 nM-IGF-1 + 10 nM-IGF-2	155±8[a]
	67 nM-insulin	162±9[a]
3	Control	(100)
	1 nM-IGF-1	136±6[c]
	1 nM-IGF-2	130±5[c]
	0.2 nM-insulin	134±8[a]
	1 nM-IGF-1 + 0.2 nM-insulin	146±9[b,e,f]
	1 nM-IGF-2 + 0.2 nM-insulin	146±9[b,d]
	67 nM-insulin	158±12[b]

Table 2. Effects of various growth factors on the rate of protein synthesis in cardiac myocytes

Rates of protein synthesis were measured as described in the Experimental section for 3–4 separate preparations of cardiac myocytes. The absolute rate of protein synthesis was 617±56 pmol of phenylalanine incorporated/h per mg of myocyte protein. Statistical significance for differences from control incubations: $^{a}P < 0.05$, $^{b}P < 0.02$, $^{c}P < 0.01$.

Incubation conditions	Protein synthesis rate (% of control)
Control	(100)
50 nM-FGF	107±4
50 nM-PDGF	96±5
50 nM-EGF	108±3
50 nM-IGF-1	155±9[a]
50 nM-IGF-2	155±7[b]
6.7 nM-insulin	152±8[c]

no further stimulation of protein synthesis when 6.7 nM-insulin was present in addition to either 50 nM-IGF-1 or 50 nM-IGF-2 (Table 1, Expt. 1). These results thus provide indirect evidence that IGF-1 and IGF-2 at concentrations of 50–100 nM are sufficient to achieve a maximal or near-maximal response in this system.

In Expt. 2 of Table 1, the effects of 1 nM- or 10 nM-IGF-1 or -IGF-2 were compared alone and in combination. At these submaximally effective concentrations, the stimulation of protein synthesis was not additive, and the response was the same as that of the more effective of the two agents alone, i.e. IGF-1 at 1 nM and IGF-2 at 10 nM (Table 1, Expt. 2). In contrast, a combination of 0.2 nM-insulin with either 1 nM-IGF-1 or 1 nM-IGF-2 was able to enhance the stimulation of myocyte protein synthesis significantly above that by IGF-1, IGF-2 or insulin alone (Table 1, Expt. 3). The significance of these results is discussed below.

To ascertain whether the stimulation of myocyte protein synthesis by IGF-1 and IGF-2 is specific to these factors or whether it represents a feature of growth factors in general, myocytes were incubated with a variety of other growth factors. At a concentration of 50 nM, the rate of myocyte protein synthesis was unaffected by FGF, PDGF and EGF (Table 2).

Effects of IGF-1, IGF-2, EGF and insulin on protein synthesis, lactate release and adenine nucleotide and creatine metabolite concentrations in perfused hearts

The effects of IGF-1 and IGF-2 on cardiac protein synthesis were examined further with the anterogradely perfused rat heart preparation (Table 3). Cost dictated that the maximum concentrations of IGF-1 and IGF-2 that we could use were 2 nM and 2.4 nM respectively. In Expt. 1, 67 nM-insulin stimulated the rate of protein synthesis by 33%, whereas 2 nM-IGF-1 stimulated it by 19%. The relative magnitudes of these effects are consistent with those observed in cardiac myocytes at these concentrations (Fig. 1). The experiment with IGF-2 (Expt. 2) was conducted in rats fasted overnight, a manoeuvre known to enhance insulin-sensitivity [19]. Here, insulin stimulated protein synthesis by 40%. However, the stimulation of protein synthesis by IGF-2 was not significant, although, at 18% over control rates, the increase was as expected from experiments in isolated myocytes (Fig. 1). In contrast, EGF (9.2 nM) caused a slight but significant inhibition of protein synthesis (Table 3, Expt. 2).

The rate of lactate production in the perfused heart in the presence of 2 nM-IGF-1 (Fig. 2*a*) or 2.4 nM-IGF-2 (Fig. 2*b*) was significantly greater than in control hearts, but significantly lower than in the presence of 67 nM-insulin. This is consistent with the relative potencies of IGF-1, IGF-2 and insulin at these concentrations with respect to the stimulation of protein synthesis (Fig. 1). EGF (9.2 nM) had no effect on the rate of lactate production (results not shown).

There were no differences in the adenine nucleotide, phosphocreatine and creatine concentrations in the hearts perfused in the presence of IGF-1 or insulin (Table 3, Expt. 1). There were minor effects of IGF-2 on ADP concentration and EGF on phosphocreatine concentration (Table 3, Expt. 2). There was also an increase in phosphocreatine concentration in response to insulin. The reasons for the different responses of phosphocreatine concentrations to insulin between Expts. 1 and 2 in Table 3 are not clear, but such a variable response has been noted previously [15,20].

DISCUSSION

This paper has specifically aimed to examine the relative effects of IGF-1, IGF-2 and insulin on protein synthesis. As reviewed in [5,11], it is important that there is rapid isotopic equilibration of the immediate precursor for protein synthesis ([U-^{14}C]phenylalanyl-tRNA) with the extracellular [U-^{14}C]phenylalanine. Ideally, the specific radioactivity of the two pools should be

Table 3. Effects of IGF-1, IGF-2, EGF and insulin on the rate of protein synthesis and the concentrations of adenine nucleotides and creatine metabolites in perfused rat hearts

Hearts (4–6 in each group) were perfused anterogradely as described in the Experimental section. Fed rats were used in Expt. 1, and overnight-fasted rats were used in Expt. 2. Statistical significance versus control perfusions by an unpaired Student's t test: $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

Expt. no.	Perfusion conditions	Protein synthesis rate (pmol of phenylalanine/2 h per mg of protein)	Metabolite concn. (nmol/mg of protein)						
			ATP	ADP	AMP	ATP+ADP +AMP	Phosphocreatine	Creatine	Phosphocreatine +creatine
1	Control	1520±41	19.4±0.6	5.7±0.1	0.7±0.1	25.7±0.6	24.9±1.4	22.4±0.5	47.3±1.7
	2 nM-IGF-1	1802±80[a]	18.8±1.0	5.6±0.2	0.9±0.1	25.3±1.2	24.3±0.8	23.4±0.8	47.7±2.6
	67 nM-insulin	2023±57[c]	19.1±0.4	5.2±0.2	0.8±0.1	25.0±0.6	26.0±1.9	20.3±1.4	46.2±3.3
2	Control	1281±60	22.0±1.1	5.3±0.2	0.8±0.1	28.2±1.2	23.1±0.9	26.4±2.1	49.4±3.0
	2.4 nM-IGF-2	1510±119	21.3±1.2	4.7±0.1[a]	0.9±0.1	26.9±1.4	24.4±0.9	23.3±1.6	47.7±2.1
	9.2 nM-EGF	1114±41[a]	23.8±1.3	5.0±0.2	0.8±0.1	29.6±1.4	28.1±1.5[a]	25.9±0.6	54.0±1.9
	67 nM-insulin	1789±89[b]	24.3±1.0	4.7±0.2	0.9±0.1	29.9±1.1	32.0±1.7[b]	24.0±1.1	56.0±2.4

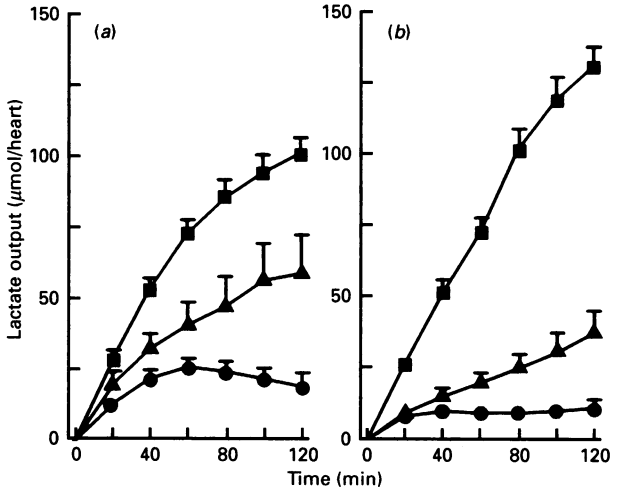

Fig. 2. Effect of IGF-1, IGF-2 and insulin on lactate output in perfused rat hearts

Hearts were perfused as described in the Experimental section with 2 nM-IGF-1 (*a*, ▲) or with 2.4 nM-IGF-2 (*b*, ▲). For comparison, perfusions under basal conditions (no IGF added, ●) or in the presence of 67 nM-insulin (■) are also shown. Statistical significance for perfusions in the presence of IGF-1 or IGF-2 versus those under basal conditions was determined by a Student's t test for unpaired data. In (*a*): $P < 0.05$ at 80 min and $P < 0.01$ at 100 and 120 min. In (*b*), $P < 0.05$ at 60, 80, 100 and 120 min.

identical, although it is still possible to obtain meaningful results if the specific radioactivity of [U-^{14}C]phenylalanyl-tRNA is constant for all interventions studied but is less than that of extracellular [U-^{14}C]phenylalanine. However, in the absence of measurements of the specific radioactivity of [U-^{14}C]phenylalanyl-tRNA, it is difficult to conclude unequivocally whether the effects of various agents are the result of stimulation of protein synthesis itself, whether they result from a decreased dilution of [U-^{14}C]phenylalanyl-tRNA specific radioactivity owing to an inhibition of protein degradation, or whether there may be effects of the agents at the level of amino acid transport. In the perfused heart, the [U-^{14}C]phenylalanyl-tRNA specific radioactivity rapidly reaches that of the extracellular [U-^{14}C]phenylalanine when the concentration of the latter is 0.4 mM [21]. It is very difficult to measure the specific radioactivity of [U-^{14}C]phenylalanyl-tRNA reliably in isolated cardiomyocytes, on account of the small amounts of [U-^{14}C]phenylalanyl-tRNA available and the problems with contamination of samples with unbound phenylalanine. We have discussed the validity of our measurements of protein-synthesis rates in cardiac myocytes in detail recently [11]. We concluded that the specific radioactivity of [U-^{14}C]phenylalanyl-tRNA rapidly attained that of the extracellular [U-^{14}C]phenylalanine. The reasons were as follows. First, measured rates of protein synthesis in isolated cardiac myocytes (where the stretch stimulus to protein synthesis [22,23] is absent) were about 60% of those in perfused hearts, where isotopic equilibration is not a problem. Secondly, although protein-synthesis rates increased progressively between 0.2 mM- and 0.8 mM-[U-^{14}C]phenylalanine, rates at 0.4 mM- and 0.8 mM-[U-^{14}C]phenylalanine were not statistically significantly different. Thirdly, no lag was detectable in the time course of protein synthesis. Fourthly, we always try to support protein-synthesis results obtained in isolated cardiac myocytes with results obtained in perfused hearts (as we have done in this study). We thus consider it unlikely that our results are attributable to variations in [U-^{14}C]phenylalanyl-tRNA specific radioactivity.

The results reported here show that both IGF-1 and IGF-2 stimulate cardiac myocyte protein synthesis to the same extent as insulin. The relative potencies are insulin > IGF-1 ≥ IGF-2 (Fig. 1). However, the relative physiological response to each of these factors will be determined by their plasma concentrations in relation to these curves. Plasma concentrations of insulin are normally 0.1–0.6 nM [12,24], which covers the range on the dose–response curve of about 25–75% of maximal stimulation (Fig. 2). Since the stimulation of myocyte protein synthesis by sub-maximally effective concentrations of insulin and the IGFs is partially additive (Table 1, Expt. 3), there is potential for the IGFs to contribute to stimulation.

Unlike insulin, most of the plasma IGF-1 and IGF-2 is bound to specific proteins [1–3,25] and this restricts their free concentrations to only 1–5% of their total concentrations [25,26]. In the rat, the serum concentrations of IGF-1 and IGF-2 are dependent on age. In foetal rat serum, total IGF-1 and IGF-2 concentrations are 34 nM and 220 nM respectively [27]. During the early growth phase, IGF-1 concentrations increase steadily [28], but IGF-2 concentrations decrease 20–100-fold within the first 25 days after birth [29]. In man, the total plasma concentration of IGF-1 is about 10 nM at birth, increases to about 50 nM at 20 years and declines steadily thereafter [2]. IGF-2 concentrations in man are low prenatally but increase during the first postnatal year [30], and remain about 4 times higher than for IGF-1 after the first

few years of life [31]. Despite the presence of the IGF-binding proteins, the free concentrations of IGF-1 and IGF-2 in man should still be sufficient to stimulate protein synthesis, assuming a similar sensitivity to that demonstrated here in rat cardiac myocytes (Fig. 1).

One function of the IGF-binding proteins is to protect the IGFs from degradation, and this, in combination with a slow but steady secretion of the IGFs by the liver, maintains stable plasma concentrations of these factors [2,3]. In contrast, plasma concentrations of insulin vary diurnally, depending on the nutritional status of the animal [3]. The relative contribution of insulin, IGF-1 and IGF-2 to the maintenance of rates of protein synthesis may therefore vary throughout the day, as well as with the age of the animal.

Like insulin, the effects of IGF-1 and IGF-2 are transmitted through specific receptors located in the plasma membrane of the target tissue [32]. However, there is considerable cross-reactivity between insulin, IGF-1 and IGF-2 and their respective receptors. Thus insulin can interact with the IGF-1 receptor, but not with the IGF-2 receptor, and IGF-1 and IGF-2 can interact strongly with each other's receptor and more weakly with the insulin receptor [1,2,32]. There is also recent evidence that hybrid receptors consisting of one half ($\alpha\beta$) of an insulin receptor coupled to one half ($\alpha\beta$) of an IGF-1 receptor may exist in normal human tissue [33]. These hybrid receptors are responsive to both insulin and IGF-1. These observations make the assignment of receptors responsible for individual insulin and IGF actions very difficult.

Although sub-maximally effective concentrations of insulin and either IGF-1 or IGF-2 are partially additive with respect to their stimulation of protein synthesis, the effects of sub-maximally effective concentrations of IGF-1 and IGF-2 are not (Table 1, Expts. 1 and 3). One interpretation of this finding is that, whereas the insulin response may occur through its own receptor, the effects of IGF-1 and IGF-2 may both be transmitted through a single IGF receptor. Since protein synthesis is more sensitive to IGF-1 (Fig. 1 and Table 1), this single receptor should be the IGF-1 receptor. Interaction of IGF-2 at the IGF-1 receptor could explain the lack of additivity of 1 nM-IGF-1 and 1 nM-IGF-2 (Table 1, Expt. 2), because they would bind to the same receptor population. The effect of adding 1 nM-IGF-2 would thus approximate to moving 0.3 (i.e. log2) unit along the dose–response curve from 1 nM-IGF-1, which would not enhance protein synthesis detectably (Fig. 1). In contrast, interaction of insulin with its own receptor would generate a separate signal to that produced at the IGF-1 receptor (in response to either IGF-1 or IGF-2) and would be partially additive to it (Table 1, Expt. 3). In support of this idea, many of the other biological effects of IGF-2 are known to be mediated through the IGF-1 receptor (reviewed in [34]). In addition, human IGF-1 receptors stably expressed in Chinese-hamster ovary cells bind IGF-2 with an affinity only 2-fold lower than that with which IGF-1 is bound [35]. This is similar to the approx. 3-fold difference in potency with which these factors stimulate protein synthesis (Fig. 1). The effects of insulin and IGF-1 require the protein tyrosine kinase activity of their receptors [32,34], an activity not present in the IGF-2 receptor. These pieces of evidence lend additional support to our contention that the stimulation of protein synthesis by IGF-2 may be mediated by the IGF-1 receptor.

In addition to their effects on protein synthesis, IGF-1 and IGF-2 stimulated lactate production in anterogradely perfused hearts (Fig. 2). This confirms and extends earlier studies in retrogradely perfused hearts in which an impure preparation of IGFs was shown to stimulate glucose uptake, lactate production and efflux of 3-*O*-methylglucose with a potency similar to that of insulin [36]. Addition of lactate to hearts perfused with glucose elevates the rate of protein synthesis [37]. It could be suggested that the IGFs and insulin stimulate protein synthesis by stimulating lactate production. We have examined this possibility in detail [37], and have concluded that such a possibility is unlikely, since stimulation of protein synthesis by lactate correlates with net uptake of lactate rather than net production. A more direct action of IGFs and insulin on protein synthesis is implied.

In conclusion, the experiments reported here show that both IGF-1 and IGF-2 are potentially as effective as insulin in stimulating cardiac protein synthesis. The stability of their concentrations in plasma may enable them to act as a buffer to prevent any large alterations in protein synthesis rates that might otherwise occur in response to variations in insulin concentration.

This work was supported by grants from the British Heart Foundation and the British Diabetic Association.

REFERENCES

1. Humbel, R. E. (1990) Eur. J. Biochem. **190**, 445–462
2. Sara, V. R. & Hall, K. (1990) Physiol. Rev. **70**, 591–614
3. Froesch, E. R., Schmid, C., Schwander, J. & Zapf, J. (1985) Annu. Rev. Physiol. **47**, 443–467
4. Van Wyk, J. J. (1984) in Hormonal Proteins and Peptides (Li, C. H., ed.), vol. 12, pp. 81–125, Academic Press, Orlando
5. Sugden, P. H. & Fuller, S. J. (1991) Biochem. J. **273**, 21–37
6. Gulve, E. A. & Dice, J. F. (1989) Biochem. J. **260**, 377–387
7. Ballard, F. J., Read, L. C., Francis, G. L., Bagley, C. J. & Wallace, J. C. (1986) Biochem. J. **233**, 223–230
8. Janeczko, R. A. & Etlinger, J. D. (1984) J. Biol. Chem. **259**, 6292–6297
9. Airhart, J., Arnold, J. A., Stirewalt, W. S. & Low, R. B. (1982) Am. J. Physiol. **243**, C81–C86
10. Fuller, S. J. & Sugden, P. H. (1986) FEBS Lett. **201**, 246–250
11. Fuller, S. J., Gaitanaki, C. J. & Sugden, P. H. (1990) Biochem. J. **266**, 727–736
12. Reeds, P. J., Hay, S. M., Glennie, R. T., Mackie, W. S. & Garlick, P. J. (1985) Biochem. J. **227**, 255–261
13. Sugden, P. H. & Fuller, S. J. (1991) Biochem. J. **273**, 339–346
14. Sugden, P. H. & Smith, D. M. (1982) Biochem. J. **206**, 473–479
15. Fuller, S. J. & Sugden, P. H. (1988) Am. J. Physiol. **255**, E537–E547
16. Rubin, I. B. & Goldstein, G. (1970) Anal. Biochem. **33**, 244–254
17. Bergmeyer, H. U. (1974) Methods of Enzymatic Analysis, Academic Press, New York
18. Gornall, A. G., Bardawill, C. J. & David, M. M. (1949) J. Biol. Chem. **177**, 751–766
19. Brady, L. J., Goodman, M. N., Kalish, F. N. & Ruderman, N. B. (1981) Am. J. Physiol. **240**, E184–E190
20. Fuller, S. J., Gaitanaki, C. J. & Sugden, P. H. (1989) Biochem. J. **259**, 173–179
21. McKee, E. E., Cheung, J. Y., Rannels, D. E. & Morgan, H. E. (1978) J. Biol. Chem. **253**, 1030–1040
22. Xenophontos, X. P., Gordon, E. E. & Morgan, H. E. (1986) Am. J. Physiol. **251**, C95–C98
23. Smith, D. M. & Sugden, P. H. (1987) Biochem. J. **243**, 473–479
24. Frayn, K. N. & Maycock, P. F. (1979) Biochem. J. **184**, 323–330
25. Zapf, J., Schoenle, E., Jagers, E., Sand, I. & Froesch, E. R. (1979) J. Clin. Invest. **63**, 1077–1084
26. Furlanetto, R. W., Underwood, L., Van Wyk, J. J. & D'Ercole, A. J. (1977) J. Clin. Invest. **60**, 648–657
27. Gluckman, P. D. & Butler, J. H. (1982) J. Endocrinol. **99**, 223–232
28. Sara, V. R., Hall, K., Lins, P. E. & Fryklund, L. (1980) Endocrinology (Baltimore) **107**, 622–625
29. Moses, A. C., Nissley, P. C., Short, P. A., Rechler, M. M., White, R. M., Knight, A. B. & Higa, O. Z. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 3649–3653
30. Hall, K. & Sara, V. R. (1984) Clin. Endocrinol. Metab. **13**, 91–112
31. Enberg, G. & Hall, K. (1984) Acta Endocrinol. (Copenhagen) **107**, 164–170
32. Rechler, M. M. & Nissley, S. P. (1985) Annu. Rev. Physiol. **47**, 425–442
33. Soos, M. A. & Siddle, K. (1989) Biochem. J. **263**, 553–563

34. Czech, M. P. (1989) Cell **59**, 235–238
35. Steele-Perkins, G., Turner, J., Edman, J. C., Hari, J., Pierce, S. B., Stover, C., Rutter, W. J. & Roth, R. A. (1988) J. Biol. Chem. **263**, 11486–11492
36. Meuli, C. & Froesch, E. R. (1975) Eur. J. Clin. Invest. 93–99
37. Fuller, S. J. & Sugden, P. H. (1991) Biochem. J. **281**, 121–127

Received 3 May 1991/18 September 1991; accepted 1 October 1991