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The effect of glucagon administration on protein synthesis in skeletal muscles, heart and liver *in vivo*

Victor R. PREEDY* and Peter J. GARLICK†
Clinical Nutrition and Metabolism Unit, London School of Hygiene and Tropical Medicine, Hospital for Tropical Diseases, 4 St. Pancras Way, London NW1 2PE, U.K.

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1. Infusion of glucagon (0.5mg/h per 100g body wt.) into fed rats for 6h inhibited protein synthesis in skeletal muscle, but not in heart. The order of sensitivity of three muscles was plantaris > gastrocnemius > soleus. Treatment with glucagon for periods of 1h or less had no effect. Liver protein synthesis was inhibited by glucagon treatment for 10min, but stimulated after 6h. 2. The effect of glucagon on muscle was not secondary to impaired food absorption or to depletion of amino acids by increased gluconeogenesis, since the inhibition of protein synthesis was observed in postabsorptive and amino acid-infused rats. 3. The failure of glucagon to inhibit muscle protein synthesis after 1h may have been caused by the increase in plasma insulin that occurred at this time, since an inhibition was detected in insulin-treated diabetic rats. 4. The lowest infusion rate that gave a significant decrease in muscle protein synthesis was 6μg/h per 100g body wt., despite a small increase in plasma insulin. This gave plasma glucagon concentrations in the high pathophysiological range, suggesting that glucagon may be significant in the pathogenesis of muscle wasting in metabolic stresses such as diabetes and starvation.

Elevated concentrations of circulating glucagon are characteristic of a number of catabolic states, such as starvation and diabetes (Unger *et al.*, 1963; Adibi *et al.*, 1976; Stearns & Benzo, 1978). In these states marked loss of muscle protein occurs as a result of decreased protein synthesis and increased proteolysis (Pain & Garlick, 1974; Pain *et al.*, 1983). The involvement of the low concentrations of circulating insulin in these changes has been extensively studied, both in isolated tissues (Jefferson *et al.*, 1977; Flaim *et al.*, 1980) and in the whole animal (Pain & Garlick, 1974; Pain *et al.*, 1983), but the role of glucagon has been relatively neglected. One of glucagon's most documented functions appears to be in the regulation of glucose production by the liver (Alford & Chisholm, 1979). Whether this is accompanied by effects in the peripheral tissues has been the subject of controversy for many years (Beatty *et al.*, 1963; Cherrington & Vranic, 1974; Pozefsky *et al.*, 1976; Daniel *et al.*, 1977; Schneider *et al.*, 1981). In a previous study (Preedy *et al.*, 1980) we showed that addition of glucagon to perfused rat hemicorpus preparations resulted in a lowering of rates of protein synthesis in plantaris and gastrocnemius muscles, but not in soleus. We now report experiments that show that this effect of glucagon on skeletal-muscle protein synthesis can also be observed in the whole animal.

* To whom reprint requests should be sent. Present address: Cardiothoracic Institute, 2 Beaumont Street, London W1N 2DX, U.K.

† Present address: Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, Scotland, U.K.

Methods

Materials

Biochemicals were obtained from Sigma (Poole, Dorset, U.K.) and L-[4-^{3}H]phenylalanine (25Ci/mmol) was purchased from Amersham International (Amersham, Bucks., U.K.). Monocomponent glucagon, its accompanying diluent, and monocomponent insulin protamine–zinc were obtained from Novo Laboratories (Basingstoke, Hants., U.K.), and Trasylol was from Bayer (U.K.) Ltd. (Haywards Heath, Sussex, U.K.). The amino acid mixture, Synthamin 17 without added electrolytes (Travenol Laboratories, Thetford, Norfolk, U.K.), contained 16.9g of N/litre, with amino acids in the following concentrations

(g/litre): alanine, 20.8; arginine, 10.4; glycine, 20.8; histidine, 4.4; isoleucine, 4.8; leucine, 6.2; lysine, 5.8; methionine, 5.8; phenylalanine, 6.2; proline, 4.2; threonine, 4.2; tryptophan, 1.8; tyrosine, 0.4; valine, 4.6.

Animals

Male Wistar rats were obtained from Charles River (Margate, Kent, U.K.) and caged singly in a humidified and temperature-controlled environment on a 12h-light/12h-dark cycle. They were fed *ad lib* until they weighed 120–140g. 'Postabsorptive' animals had their food removed at 23:00h the night before measurements were made. 'Insulin-treated diabetic' rats were given an intravenous injection of streptozotocin (13.3mg/100g body wt.) when they weighed 75–80g; 24h afterwards and for a further 5 days they were given subcutaneous injections of insulin (protamine–zinc; 2units/day per 100g body wt.) daily at 09:00h.

Experimental procedures

Experiments were performed between 09:00 and 13:00h (injections or 1h infusions) or between 09:00 and 17:00h (6h infusions). [4-^{3}H]Phenylalanine (150mM, 25μCi/ml; 1ml/100g body wt.) was given by tail-vein injection for measurement of the rate of protein synthesis (Garlick *et al.*, 1980). Rats were killed by decapitation after 2min (four rats per group) or 10min (six rats per group). Blood was collected in heparinized tubes and tissues were rapidly removed and frozen in liquid N_2 as described previously (Garlick *et al.*, 1980).

Chemical determinations

The specific radioactivity of free and protein-bound phenylalanine, the concentration of RNA and protein in tissues and the concentrations of insulin and glucose in plasma were measured as described previously (Garlick *et al.*, 1980, 1983). Glucagon, in plasma which had been stored at −20°C in the presence of Trasylol (200 K.I. units/ml of plasma), was measured by a double-antibody radioimmunoassay with a kit supplied by IRE-UK Ltd. (High Wycombe, Bucks., U.K.). Muscle amino acid concentrations were determined with an amino acid analyser (Locarte, London, U.K.) as described by Preedy (1981).

Calculation of rates of protein synthesis

Fractional rates of protein synthesis (k_s, i.e. the % of the tissue protein pool renewed daily by synthesis) were calculated from the specific radioactivity of phenylalanine in protein (S_B) 10min after injection of the label and the mean specific radioactivity of free phenylalanine in the tissue (S_A) in the period 0–10min (McNurlan *et al.*, 1979), i.e.

$$k_s = (S_B \times 100)/(S_A \times t)$$

where t is the period of incorporation (days). The value of S_A was calculated from the measured specific radioactivities at 2min and 10min (Garlick *et al.*, 1980) to obtain the data shown in Table 1. In subsequent experiments, no 2min animals were used. The value of S_A was taken to be the same as the measured value at 10min, since the experiment in Table 1 had shown that the specific radioactivity of free phenylalanine in muscle did not change appreciably between 2 and 10min, and that this was not affected by glucagon.

Statistics

Results are expressed as means ± S.E.M., with numbers of observations in parentheses. Differences between groups within experiments were assessed by analysis of variance followed by two-tailed t tests, the pooled estimates of variance being used when appropriate.

Results

Table 1 shows the effects of glucagon 10min after injection into fed rats. There was a significant ($P<0.005$) rise in the concentration of glucose in plasma [from 10.1 ± 0.2 (6) to 12.8 ± 0.5 (6) mM], accompanied by a large increase in insulin concentration [from 27.6 ± 2.7 (6) to 73.9 ± 16.7 (6) μunits/ml; $P<0.05$]. The hormone had no effect on the fractional rate of protein synthesis in skeletal muscle (plantaris, gastrocnemius or soleus) or cardiac muscle, but caused a small though statistically significant fall in protein synthesis in liver.

Infusion of glucagon for 6h into fed rats (Table 1) caused a decrease in the concentration of glucose in plasma [from 9.3 ± 0.2 (6) to 7.7 ± 0.2 (6) mM; $P<0.001$], accompanied by a small and statistically insignificant increase in insulin [from 14.3 ± 3.4 (6) to 22.0 ± 2.1 (6) μunits/ml]. Rates of protein synthesis in plantaris, gastrocnemius and soleus muscles were significantly decreased by glucagon infusion, but there was no significant effect in heart. The effect of the hormone in the skeletal muscles was greatest in plantaris (24%), less in gastrocnemius (18%) and least in soleus (15%). In liver the rate of protein synthesis in hormone treated rats was apparently higher than that in controls, which is the opposite effect to that seen with single injections of glucagon (Table 1). The rates of synthesis in fed rats after 6h infusion were in general lower than in fed rats that had not been infused (Table 1). This represents the progression from the fed to the fasted state, since food was not given during the 6h

Table 1. *Effect of single injection and 6h constant infusion of glucagon on fractional rates of protein synthesis in tissues of fed rats.*

Fed rats were given a single intraperitoneal injection of glucagon (0.2mg in 0.1ml of diluent/100g body wt.) or diluent alone, followed immediately by tail-vein injection of [4-^{3}H]phenylalanine, and killed 2 or 10 min later. A second set were immobilized by wrapping in a towel and were infused into a tail vein with glucagon (2mg/ml) or diluent alone at a rate of 0.3ml/h per rat for 6h. Shortly before the end, they were injected with [4-^{3}H]phenylalanine via the infusion cannula and the infusion was continued for another 2 or 10min. Results are means ± S.E.M.; $n = 6$ (or 5 when marked †). Differences between controls and glucagon-treated: $^*P<0.05$, $^{**}P<0.01$.

	Fractional synthesis rate (%/day)			
	Single injection		6h infusion	
Tissue	Control	+Glucagon	Control	+Glucagon
Gastrocnemius	16.34±0.41	16.51±1.01	11.14±0.39	9.19±0.35**
Plantaris	14.02±0.36	13.86±0.69	14.77±0.36	11.21±0.50**
Soleus	18.75±0.75	17.33±0.65	15.45±0.64	13.21±0.32*
Heart	19.90±0.54	18.49±0.28	17.48±0.65	18.01±0.36
Liver	82.61±1.59	73.47±1.58**	66.82±1.70†	81.91±2.63**

Table 2. *Effects of infusions of glucagon on protein synthesis in gastrocnemius muscle of normal, insulin-treated diabetic and amino acid infused rats*

Normal fed rats were infused with carrier for 30min or with glucagon for 30min or 60min as described in the legend to Table 1. A second set were made diabetic as described in the text and were infused as above. A third set of normal fed rats was infused for 6h with a mixture of amino acids, with or without glucagon. Values are means ± S.E.M. ($n = 6$). Differences between controls and glucagon-treated rats: $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Rats	Treatment	Fractional synthesis rate (%/day)	Plasma insulin (μunits/ml)	Plasma glucose (mM)
Normal	Control	15.20±0.39	23.1±3.5	9.4±0.2
	30min glucagon	15.91±0.36	51.3±4.4***	10.3±0.4
	60min glucagon	14.68±0.58	59.3±6.1***	10.3±0.6
Diabetic	Control	15.92±0.47	69.3±18.1	6.2±1.4
	30min glucagon	15.83±0.49	82.6±14.3	18.2±1.5***
	60min glucagon	13.90±0.44**	59.9±9.7	20.9±2.1***
Amino acid-infused	Control	15.39±1.03	32.3±3.3	9.5±0.2
	6h glucagon	12.30±0.35*	28.1±2.8	8.1±0.1***

infusion period. However, for the sake of convenience, rats that were fed until the start of the infusion will continue to be referred to as 'fed' to distinguish them from rats that were fasted overnight, and hence were 'postabsorptive' at the start of the infusion.

Table 2 shows the effects of glucagon infusion for 30min and 60min in fed rats. There was a small increase in plasma glucose and a doubling of plasma insulin, but rates of protein synthesis in gastrocnemius muscle were unaltered. Table 2 also shows the results obtained by the same protocol but in diabetic rats that were maintained by daily injections of insulin. Glucagon infusion caused a large rise in plasma glucose concentration at both times. The plasma insulin was higher than in non-diabetic rats, but as expected was unaltered by glucagon. Rates of protein synthesis in diabetic rats were not altered after 30min of infusion with glucagon, but after 60min they were lower than in controls ($P<0.01$).

The effects of glucagon infusion for 6h in fed rats that were infused simultaneously with an amino acid mixture are also shown in Table 2. The rate of infusion, 11.4mg of N/h per 100g body wt., was chosen to supply N at an hourly rate similar to the normal rate of intake of dietary N averaged over the day. In amino acid-infused rats glucagon decreased the plasma glucose concentration, but had no effect on plasma insulin at the end of 6h. The inhibitory effect of glucagon on muscle protein synthesis was, however, similar to that obtained without infusion of amino acids ($P<0.02$).

Experiments described thus far have used pharmacological doses of glucagon. Table 3 shows the dose–response relationship in which lower amounts were infused for 6h in postabsorptive rats. The mean glucagon concentration in controls

Table 3. *Effect of various doses of glucagon infused for 6 h on protein synthesis in gastrocnemius muscle of postabsorptive rats*

Postabsorptive rats were infused with carrier alone (controls) or glucagon in concentrations of 0.0002, 0.002, 0.02, 0.2 or 2 mg/ml at a rate of 0.3 ml/h per rat. At the end of 6 h, rates of protein synthesis and plasma glucagon, insulin and glucose were measured as described in the text. Values are means ± S.E.M.; $n = 6$ (or 5 when marked †). Significance of differences from controls: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Plasma glucagon (ng/ml)	Fractional synthesis rate (%/day)	Plasma insulin (μunits/ml)	Plasma glucose (mM)
0.75 ± 0.10	9.14 ± 0.51	11.7 ± 1.6	6.6 ± 0.3
0.82 ± 0.06	9.89 ± 0.31	12.6 ± 0.6	5.8 ± 0.3*
1.62 ± 0.18	8.71 ± 0.30	14.8 ± 1.6	7.4 ± 0.2*
11.50 ± 1.06	7.52 ± 0.41*	17.4 ± 2.8*	7.3 ± 0.2*
138.33 ± 9.97	7.62 ± 0.59*	27.2 ± 2.3***†	7.3 ± 0.2*
1315 ± 70†	6.32 ± 0.53***†	26.4 ± 2.3***†	7.6 ± 0.2**†

Table 4. *Effect of glucagon infusion for 6 h on free amino acids in gastrocnemius muscles from fed, amino acid-infused and postabsorptive rats*

Free amino acid concentrations were determined in gastrocnemius muscles taken after 6 h of infusion. Animals used in these studies were those described in Tables 1 and 2, and a postabsorptive group treated the same as the group with the highest dose of glucagon in Table 3. Values are means ± S.E.M.; $n = 6$. Significance of differences from control values: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

	Muscle amino acid concentrations (nmol/g wet wt.)					
	Fed		Fed, amino-acid infused		Postabsorptive	
Amino acid	Control	+Glucagon	Control	+Glucagon	Control	+Glucagon
Aspartic acid	625 ± 39	557 ± 78	641 ± 50	647 ± 23	558 ± 56	538 ± 45
Threonine	533 ± 39	296 ± 37**	881 ± 49	282 ± 11***	618 ± 17	270 ± 16***
Serine	1050 ± 33	605 ± 40***	1270 ± 70	510 ± 20***	818 ± 35	432 ± 25***
Glutamine	3460 ± 50	1850 ± 110***	2680 ± 120	1640 ± 90***	2490 ± 140	1710 ± 150**
Glutamic acid	1560 ± 220	765 ± 73**	2310 ± 170	1220 ± 110***	2070 ± 200	1420 ± 220
Proline	294 ± 21	129 ± 9***	451 ± 21	102 ± 20***	226 ± 17	228 ± 54
Glycine	3920 ± 120	3250 ± 260*	10400 ± 400	7540 ± 450***	5380 ± 290	4420 ± 200*
Alanine	2760 ± 91	2040 ± 170**	3130 ± 210	2640 ± 200	1990 ± 170	1690 ± 94
Cysteine	79 ± 10	78 ± 9	22 ± 3	18 ± 2	34 ± 4	24 ± 2*
Valine	138 ± 5	146 ± 14	251 ± 12	247 ± 6	185 ± 8	200 ± 15
Methionine	57 ± 12	16 ± 2*	155 ± 6	32 ± 2***	39 ± 5	24 ± 1*
Isoleucine	81 ± 17	72 ± 7	129 ± 5	119 ± 2	98 ± 5	109 ± 10
Leucine	101 ± 9	118 ± 9	149 ± 6	152 ± 4	139 ± 9	152 ± 13
Lysine	1040 ± 110	1060 ± 50	1780 ± 140	2210 ± 250	1570 ± 200	1230 ± 250
Histidine	226 ± 23	218 ± 20	423 ± 29	333 ± 22*	254 ± 28	247 ± 20
Arginine	314 ± 35	227 ± 23	103 ± 10	70 ± 5*	334 ± 43	315 ± 50

was 0.75 ng/ml. This value is higher than generally reported for the concentration of immunoreactive glucagon in rat plasma. We do not know the reason for this. The assay used was a commercial kit designed for use on human plasmas, which utilizes the 30K antibody. Our high values in rats may have resulted from differences in the treatment of rats. Values reported by others have generally been measured in venous blood from anaesthetized rats, whereas our measurements were on mainly arterial blood from unanaesthetized rats. In spite of the high control values, it is important to emphasize that the increments in concentration caused by infusion of glucagon should be correctly indicated by the assay.

The lowest rate of glucagon infusion, which raised the concentration of hormone in plasma by 0.07 ng/ml, caused a significant decrease in the plasma glucose concentration, but all other rates of infusion caused a small but significant rise. There was also a steady increase in plasma insulin concentration with increasing rates of infusion. This increase in insulin was statistically significant for glucagon concentrations of 11.5 ng/ml and above. The rate of protein synthesis in gastrocnemius muscle was not altered significantly by the two lowest rates of glucagon infusion, but was significantly decreased by the three highest rates (i.e. when the glucagon concentration exceeded 11.5 ng/ml).

Table 5. *Effect of glucagon infusion for 1 h on free amino acids in gastrocnemius muscles from normal and insulin-treated diabetic rats*

Free amino acids in gastrocnemius-muscle homogenates were determined in samples taken from those animals described in the legend to Table 2. Values are means ± S.E.M.; $n = 6$. Significance of differences from control values: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

	Muscle amino acid concentrations (nmol/g wet wt.)			
	Normal		Insulin-treated diabetic	
Amino acid	Control	+Glucagon	Control	+Glucagon
Aspartic acid	453±27	389±25	627±55	671±49
Threonine	765±34	668±66	532±51	368±18*
Serine	987±42	814±38**	976±87	820±58
Glutamine	3060±180	2540±120*	3020±160	2890±160
Glutamic acid	1660±240	2480±610	2470±250	2560±420
Glycine	5100±380	4960±360	6600±590	6630±790
Alanine	2690±120	2590±90	3290±320	2430±50
Cysteine	12±1	13±2	12±2	12±1
Valine	181±5	102±5***	163±8	119±3**
Methionine	42±7	24±4*	29±2	11±2***
Isoleucine	89±3	47±3***	71±5	51±1*
Leucine	107±4	51±4**	87±6	59±1**
Lysine	1500±170	1520±260	966±63	725±92
Histidine	331±16	302±23	266±28	212±30
Arginine	750±72	654±105	448±27	248±39**

Table 4 shows the concentrations of amino acids in gastrocnemius muscle of rats infused with glucagon for 6h, along with their respective controls. Aromatic amino acids are not included because of the large dose of [^{3}H]phenylalanine injected to measure rates of protein synthesis. In fed rats the concentrations of the branched-chain amino acids, aspartate, cysteine, lysine and histidine were not significantly altered by glucagon infusion, but the concentrations of most other amino acids were significantly lowered. The biggest decrease (72%) was observed for methionine. Infusion of amino acids increased the concentrations of most amino acids, but the pattern of change when glucagon was infused was similar. In particular, aspartate, cysteine, lysine and the branched-chain amino acids were unaltered by glucagon. Decreases occurred for most others, that of methionine again being the greatest (80%). In rats that were postabsorptive before infusion of glucagon, the pattern of changes resulting from the hormone was also similar to that in fed rats. Threonine exhibited the largest fall (67%), but that of methionine was much smaller than before (39%).

The changes in amino acid concentrations in muscle of rats infused with glucagon for 1 h are shown in Table 5. Similar changes occurred whether rats were fed or diabetic, but there were some differences from the pattern found in 6h infusions. In particular the branched-chain amino acid concentrations were significantly decreased in hormone-treated rats, whereas some of the amino acids that decreased in 6h infusions did not change significantly in 1h (e.g. glycine, glutamine, alanine). Methionine concentration was decreased during glucagon treatment by 42% in fed rats and by 62% in diabetic rats.

Discussion

There appear to have been only two previous studies in which direct measurements of the action of glucagon on protein synthesis in skeletal muscle were made. Beatty *et al.* (1963) showed that the rate of incorporation of labelled glycine into protein of incubated muscle was inhibited by glucagon, but did not attempt to measure the specific radioactivity of the free amino acid precursor. More recently, our own measurements of the label in free and protein-bound tyrosine in muscle of the perfused rat hemicorpus also indicated an inhibitory action of glucagon (Preedy *et al.*, 1980). The present study, which appears to have been the first attempt to make direct measurements of the effect of the hormone on muscle protein synthesis *in vivo*, has confirmed that the effects of glucagon observed in isolated tissues *in vitro* can be reproduced in the whole animal.

Our results also indicate that the effect of glucagon on muscle may be direct, rather than secondary to some other change. Glucagon is well known to affect gastrointestinal motility (Stunkard *et al.*, 1955). It was thus possible that a decreased

rate of absorption might have been responsible for the effect on muscle protein synthesis (cf. starvation: Garlick *et al.*, 1973, 1983). However, this possibility was ruled out, since glucagon infusion had an effect on muscle protein synthesis in rats that were postabsorptive before the infusion began (Table 3).

Daniel *et al.* (1977) also observed an effect of glucagon on muscle, but they concluded that it resulted from a depletion of glucogenic amino acids, particularly alanine, because liver gluconeogenesis was stimulated. Table 4 shows that in our experiments the alanine concentration was not consistently decreased when glucagon was infused for 6h, although some other amino acids were (i.e. threonine, serine, glutamate, glutamine and methionine).

The branched-chain amino acids have been shown to stimulate muscle protein synthesis *in vitro* (e.g. Fulks *et al.*, 1975; Buse & Reid, 1975; Li & Jefferson, 1978). However, they are clearly not implicated in the action of glucagon in muscle, since their concentrations were not altered by infusion of the hormone for 6h (Table 4) and fell when protein synthesis was not altered (Table 5). Only methionine and threonine showed a consistent fall in concentration in all groups with lowered rates of protein synthesis (Tables 4 and 5), and experiments with muscle *in vitro* have been unable to demonstrate any effect of these on protein synthesis. It therefore seems unlikely that changes in amino acid concentrations are responsible for the actions of glucagon on muscle.

The effect of glucagon in fed rats was only detectable at 6h of infusion, and not after 10, 30 or 60 min. However, in the 30 and 60min infusions there was also a substantial increase in the concentration of insulin (Table 2). This is a well-recognized sequel of glucagon administration, but after 6h infusion it was much less pronounced and was no longer statistically significant. Insulin has a stimulatory effect on muscle protein synthesis, which can act within as short a period as 10min (Garlick *et al.*, 1983). The rise in insulin may therefore have counteracted the effect of glucagon with 30 or 60min infusions, as has been suggested previously (Schneider *et al.*, 1981; Parrilla *et al.*, 1974; Marliss *et al.*, 1970). This hypothesis is supported by the results from the insulin-treated diabetic rats, which did show an effect of glucagon after 60min of infusion. Although the concentration of insulin in these rats was high, relative to that in normal fed rats, it was not influenced by glucagon administration.

The amount of glucagon used in earlier experiments (Tables 1 and 2) was very high, and constituted a pharmacological dose. We were therefore concerned to know whether the effects observed in skeletal muscle could be reproduced with lower doses. The dose–response experiment (Table 3) shows that the lowest dose to achieve a significant inhibition of protein synthesis relative to the control occurred when glucagon concentrations were elevated to about 11.5ng/ml. In pathological states, such as diabetic ketoacidosis, the glucagon concentration may rise as high as 1ng/ml (Muller *et al.*, 1973). Also, Katsilambros *et al.* (1970) have reported that in streptozotocin-induced diabetic rats, venous plasma concentrations of 4.71ng/ml are obtained, compared with 0.14ng/ml in controls. In separate experiments, but with the same glucagon assay as that used here, we have obtained concentrations in plasma of alloxan-diabetic and 3-day-starved rats of 1.5 ± 0.1 (6)ng/ml and 1.6 ± 0.5 (3)ng/ml respectively (V. R. Preedy & P. J. Garlick, unpublished work). When concentrations similar to these (i.e. in the pathophysiological range) were obtained in rats given infusions of glucagon (i.e. 1.62ng/ml; Table 3), the rate of protein synthesis was lower than in the controls. Although this difference in synthesis was not statistically significant, it should be pointed out that the difference between synthesis in this group and that with the lowest rate of glucagon infusion (producing a mean plasma concentration of 0.82ng/ml) did approach significance ($P = 0.07$), suggesting that there might indeed be an effect of the hormone at concentrations seen in intact animals in response to starvation, diabetes or other metabolic stresses. Furthermore, an important consideration is that the effects of glucagon may be greater than those indicated by the data in Table 3, since each increment in glucagon concentration was accompanied by an increment in insulin concentration (which is not observed in starvation or diabetes). These increases in insulin were within the range of those found in fed and fasted rats (Garlick *et al.*, 1983), and may have decreased the overall magnitude of glucagon's effects on muscle protein synthesis.

The response to glucagon in each individual tissue was different. We made measurements on three different skeletal muscles and showed that the plantaris and gastrocnemius were more sensitive than the soleus muscle. This order of sensitivity of different muscles to glucagon, i.e. plantaris > gastrocnemius > soleus, is the same as that observed in the perfused rat hemicorpus in response to glucagon (Preedy, 1981; Preedy *et al.*, 1980), and may be related to the muscle composition: plantaris and gastrocnemius contain mainly fast-twitch fibres, whereas soleus is predominantly slow. The same order of sensitivity has been noted for the action of insulin on protein synthesis (Preedy & Garlick, 1983) and for the decreases in protein synthesis induced by starvation (Preedy &

Garlick, 1983) and diabetes (Flaim *et al.*, 1980; Pain *et al.*, 1983).

Protein synthesis in cardiac muscle was not apparently influenced by the hormone. However, heart is known to contain a glucagon-stimulated adenylate cyclase (Sutherland & Robinson, 1969), so we must conclude that protein synthesis in this tissue may not be regulated by changes in cyclic AMP concentration. A similar conclusion was reached by Martin-Requero *et al.* (1981), who noted that the glucagon-stimulated decrease in protein synthesis in liver was unrelated to changes in cyclic AMP concentrations. Our own results in liver are similar to those of Martin-Requero *et al.* (1981) and Ayuso-Parrilla *et al.* (1976), who showed a rapid decrease in protein synthesis after glucagon administration. Studies in perfused liver have also shown this inhibitory effect of the hormone, but have also shown that it depends on the supply of amino acids (Woodside *et al.*, 1974). We are not able to explain why liver protein synthesis in the group infused for 6h with glucagon appears higher than that of controls. An important consideration, however, is that this effect was really a maintenance of synthesis by glucagon compared with control values that had declined, apparently as a result of the inability of the rats to feed during the 6h infusion.

Overall, these studies show that administration of a pharmacological dose of glucagon decreases protein synthesis in skeletal muscle, but that the hormone may also be active within the range of concentrations as seen in starvation and diabetes. The magnitude of the effect is not large, but even small perturbations in muscle protein synthesis may have an important influence on the availability of amino acids for gluconeogenesis, as skeletal muscle comprises a very large proportion (45%) of body mass.

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