The effect of insulin-like growth factor II on glucose uptake and metabolism in rat skeletal muscle *in vitro*

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The effect of insulin-like growth factor II (IGF II) on the rates of lactate formation, glycogen synthesis and glucose transport in the presence of a range of concentrations of insulin were investigated using an isolated preparation of rat skeletal muscle. IGF II, at a concentration of 65 ng/ml, caused a small but significant increase in the rates of these processes at a basal physiological insulin concentration (10 μ units/ml), but was without effect in the presence of 1, 100, 1000 or 10000 μ units of insulin/ml. Hence IGF II increased the insulin sensitivity of this tissue. This effect was removed if the incubation medium was supplemented with an equimolar concentration of IGF binding protein 1 (BP1). It is suggested that changes in the concentration of IGF II and/or BP1 may regulate glucose uptake and metabolism in skeletal muscle and have physiological significance in the control of blood glucose level.

INTRODUCTION

Insulin-like growth factor II (IGF II) is a single-chain polypeptide which shows significant sequence similarity with pro-insulin and insulin-like growth factor I (IGF I) [1]. It is the major insulin-like growth factor present in human plasma, in which the concentration is about 700 ng/ml. In contrast to IGF I, the plasma concentration of IGF II does not appear to be under stringent control by growth hormone [2] and, unlike insulin, the majority of IGF I and IGF II is present in the plasma as complexes with specific binding proteins, which probably serve to protect the IGFs from degradation and may, in addition, regulate the availability of both peptides to cells [3]. In particular, the plasma concentration of IGF binding protein 1 (BP1), which has a molecular mass of 28 kDa, appears to be regulated by insulin [4]: an increase in the level of insulin results in a decrease in the plasma concentration of BP1. Hence insulin, via changes in the level of this binding protein, may be able to regulate the availability of the IGFs within tissues.

It is an interesting question as to whether this particular effect of insulin could play an important role in the regulation of blood glucose levels since both IGF I and IGF II cause insulin-like effects [5]. An intravenous bolus injection of IGF II into rats lowers the blood glucose level and enhances the rate of disappearance of [14 C]glucose from serum [6]. However, although the effects of IGF II on some tissues have been studied [7–9], the quantitative importance of the target tissues in mediating the hypoglycaemic effect of IGF II has yet to be established.

Although IGF II stimulates the rates of glucose transport and lipogenesis in adipocytes [7], these effects are considered to be mediated through the insulin receptor [10,11]. In contrast, in heart and skeletal muscle, the metabolic effects of the IGFs appear to be mediated by receptors which possess high affinities for the IGFs and a low affinity for insulin [8,9,12]. Multiplication-stimulating activity (a partially purified extract of peptides from the media of the BRL 3A cell line, which contains predominantly IGF II), stimulates the rate of uptake of xylose and α -aminoisobutyrate into intact soleus muscle *in vitro* [13]. Similarly, IGF II stimulates the uptake of glucose and amino acids in L6 myoblasts [14,15]. Since human recombinant IGF II has now become available, it was considered important to study, sys-

tematically, the effects of this peptide on the rates of glucose transport, glycolysis and glycogen synthesis in skeletal muscle in the presence of various concentrations of insulin. In addition, studies have been carried out to assess the potential of BP1 to influence the effects of IGF II on glucose metabolism in muscle.

MATERIALS AND METHODS

Animals

Male Wistar rats (140–160 g) were fasted overnight for 12–14 h prior to each experiment and killed by cervical dislocation.

Chemicals and enzymes

All biochemicals and enzymes were obtained from sources given previously [16]. In addition, D-[U- 14 C]glucose and 3-O-[3 H]methylglucose were obtained from Dupont, Stevenage, Herts. U.K., and [U- 14 C]sucrose was from BDH Ltd., Poole, Dorset, U.K.

Human recombinant IGF II was a gift from Kabi Pharmacia, Stockholm, Sweden. The 28 kDa BP 1, isolated from amniotic fluid [17], was kindly provided by Dr J. M. P. Holly, Department of Endocrinology, St. Bartholomew's Hospital Medical School, London, U.K.

Experimental procedures

Soleus muscle strips were prepared as previously described [16] and preincubated for 30 min at 37 °C in 3.2 ml of Krebs–Henseleit bicarbonate-buffered medium containing defatted BSA (1.5%) and glucose (5.5 mM). For measurement of rates of lactate formation and glycogen synthesis, muscle strips were transferred to flasks containing 3 ml of incubation medium supplemented with insulin (1–10000 μ units/ml) and 0.4 μ Ci of [U- 14 C]glucose/1 ml, and incubated for 1 h with or without IGF II (65 ng/ml). In further experiments, conducted to investigate the interaction between insulin and IGF II, the response of the rate of lactate formation to the IGF II concentration was studied by incubating muscle strips in the presence of either 1 or 10 μ units of insulin/ml. The lactate concentration in the medium at the end of the incubation was determined by enzymic analysis [18]. The rate of radiochemical lactate formation, an index of the proportion of total lactate formed which is derived from exogenous glucose,

Fig. 1. Time course showing the uptake of 3-O-[³H]methylglucose into soleus muscle *in vitro* in the presence of a maximal concentration of insulin (1000 μ units/ml)

Values are means $\pm$ S.E.M. for eight separate observations.

was determined using ion-exchange chromatography to separate radiolabelled glucose and lactate in the incubation medium [19]. The rate of glycogen synthesis was measured from [¹⁴C]glucose incorporation into glycogen [20].

The rate of glucose transport was measured from the accumulation of 3-O-[³H]methylglucose in the soleus muscle: the incubation medium contained 3-O-[³H]methylglucose (1 μ Ci/ml), the extracellular marker [U-¹⁴C]sucrose (0.1 μ Ci/ml) and insulin. At the end of the incubation, the muscles were frozen in liquid nitrogen, then digested with 1 M-KOH at 70 °C for 1 h. The digest was neutralized by the addition of 0.5 ml of distilled water and 150 μ l of glacial acetic acid, then added to scintillation fluid for the measurement of radioactivity using a Liquid Scintillation Counter (Beckman Model LS1701).

In the presence of a supraphysiological (maximally stimulating) concentration of insulin (1000 μ units/ml) the rate of 3-O-[³H]methylglucose uptake into soleus muscle was linear for at least 10 min (Fig. 1). As the rate of glucose transport into soleus muscle in the presence of physiological concentrations of insulin (i.e. 10–100 μ units/ml) was less than that at 1000 μ units of insulin/ml, and since the stimulatory effect of IGF II on glucose metabolism was known to be apparent only at a concentration of 10 μ units of insulin/ml (see Figs. 2 and 3), it was considered that the linearity of the transport process in the presence of a lower concentration of insulin (10 μ units/ml) would be maintained up to 15 min. In addition, an incubation period of 15 min allowed sufficient time to complete the dissection procedure.

Statistical analysis

An unpaired Student's *t* test was used to analyse the significance of the difference between control and experimental groups.

RESULTS

As in previous work, insulin (10–1000 μ units/ml) stimulated the rates of glucose transport, glycogen synthesis and lactate formation in the incubated soleus muscle *in vitro*. The increased rate of lactate formation (i.e. glycolysis) in the presence of insulin is a consequence of a stimulation of the rate of glucose transport, since this process is the flux-generating step for lactate formation in this tissue (Fig. 2) [21].

The effect of IGF II on insulin-stimulated glucose metabolism was studied. IGF II at 65 ng/ml had no effect on the rates of glucose transport, glycogen synthesis or lactate formation in the presence of 1, 100, 1000 or 10000 μ units of insulin/ml. However, it caused a small but significant increase in the rates of all of these

processes at an insulin concentration of 10 μ units/ml (Figs. 2–4). A number of samples ($n = 4$ –8) from the studies shown in Figs. 2 and 3 were analysed for the concentration of radiochemical lactate. No statistically significant effect of IGF II on the rate of radiochemical lactate formation was apparent at an insulin concentration of 10 μ units/ml, or indeed at any of the other insulin concentrations studied (results not shown). However, a significant stimulation of the rate of radiochemical lactate formation in the presence of 10 μ units of insulin/ml was apparent in a second study in which a large number of samples were analysed ($n = 18$; Table 1). Hence the magnitude of this effect of IGF II is small, and consequently a large sample size is required in order to achieve statistical significance.

Therefore, IGF II increased the sensitivity of the rates of glucose transport and glycogen synthesis to insulin, where sensitivity is defined as the concentration of insulin which produces a half-maximal stimulation. Similarly, an increase in the insulin concentration in the incubation medium from 1 to 10 μ units/ml increased the sensitivity of lactate formation to IGF II in the muscle preparation (Fig. 5). There was insufficient IGF II available to establish if the maximal response of lactate formation from isolated soleus muscle was affected by the

Fig. 2. Effect of IGF II on the rate of lactate formation in incubated soleus muscle at various concentrations of insulin

Values are means $\pm$ S.E.M. for between 8 and 24 observations. ●, Control incubations; □, addition of IGF II (65 ng/ml). A statistically significant difference between control and experimental means is denoted by *** $P < 0.001$.

Fig. 3. Effect of IGF II on the rate of glycogen synthesis in incubated soleus muscle in the presence of various concentrations of insulin

Values are means $\pm$ S.E.M. for between 8 and 24 observations. ●, Control incubations; □, addition of IGF II (65 ng/ml). A statistically significant difference between control and experimental means is denoted by ** $P < 0.005$.

Fig. 4. Effect of IGF II on the rate of 3-O-methylglucose transport in incubated soleus muscle in the presence of various concentrations of insulin

Values are means ± S.E.M. for at least six separate incubations. ●, Control incubations; □, addition of IGF II (65 ng/ml). A statistically significant difference between control and experimental means is denoted by ***P* < 0.005.

Table 1. Effect of BP1 and IGF II on the rate of lactate formation and glycogen synthesis in soleus muscle *in vitro* in the presence of 10 μunits of insulin/ml

Values are mean ± S.E.M. for at least 18 separate incubations. The significance of the difference between control and experimental means is denoted by **P* < 0.02, ****P* < 0.001.

Incubation conditions		Rate (μmol/h per g wet weight)		
IGF II	BP1	Total lactate formation	Radiochemical lactate formation	Glycogen synthesis
—	—	10.75 ± 0.53	6.77 ± 0.68	1.89 ± 0.10
+	—	15.74 ± 1.00***	10.39 ± 1.20*	2.29 ± 0.17*
—	+	11.63 ± 0.58	7.12 ± 0.67	1.83 ± 0.08
+	+	11.96 ± 0.57	6.95 ± 0.71	2.05 ± 0.11

Fig. 5. Effect of insulin on the rate of lactate formation at several concentrations of IGF II in the incubated soleus muscle preparation

Values are means ± S.E.M. for at least five separate incubations. Muscles were incubated with IGF II (0–750 ng/ml) in the presence of either 1 μunit of insulin/ml (□) or 10 μunits of insulin/ml (●). A statistically significant difference between IGF II-treated and control means at either 1 or 10 μunits of insulin/ml is denoted by **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

presence of insulin in the incubation medium. However, the results provide evidence that insulin and IGF II behave synergistically with respect to their stimulatory effects on glucose metabolism in isolated incubated skeletal muscle *in vitro*.

BP1 had no effect on the rates of lactate formation or glycogen synthesis at 10 μunits of insulin/ml. However, it removed the stimulatory effect of IGF II on these processes in the presence of 10 μunits of insulin/ml (Table 1).

Seasonal variation accounts for the different baseline values for lactate formation in Fig. 2 and Table 1, since these two studies were conducted at different times of the year. This variability in the magnitude of basal and insulin-stimulated responses of carbohydrate metabolism has been observed previously in the isolated soleus muscle preparation [22]. Nevertheless, it is noteworthy that the magnitude of the IGF II-mediated increases in the rate of lactate formation in the presence of 10 μunits of insulin/ml were comparable in the two studies (38 % in Fig. 2 and 46 % in Table 1).

DISCUSSION

IGF II, at a concentration of 65 ng/ml (9 nM), increased the rates of glucose transport, glycolysis and glycogen synthesis in isolated incubated soleus muscle, but only at an insulin concentration of 10 μunits/ml (0.07 nM) (Figs. 2–4 and Table 1). Higher concentrations of IGF II (250 ng/ml and above) were required to produce an increase in the rate of glycolysis in the presence of 1 μunit of insulin/ml (Fig. 5). In adipocytes it has been shown that an increase in the insulin concentration in the incubation medium from 1 to 10 μunits/ml causes a translocation of IGF II receptors from an intracellular compartment to the plasma membrane [23]. Although there are no reports of translocation of IGF II receptors in muscle, the effect of a low concentration of IGF II at 10 μunits of insulin/ml, but not at 1 μunits/ml, is consistent with an insulin stimulation of receptor translocation. The result of the stimulation of the rates of these processes by IGF II at such a low concentration of insulin is an increase in the insulin-sensitivity of these processes in the skeletal muscle preparation. This could be due to, for example, the second messenger pathway, which is activated by the binding of IGF II to its receptor, converging with that of insulin. However, failure to demonstrate an effect of IGF II on the rates of lactate formation, glycogen synthesis and glucose transport at 100 μunits of insulin/ml is not consistent with IGF II increasing insulin-sensitivity. This may be explained by the difficulty of detecting statistically significant increases when the effect of insulin approaches its maximum.

It is suggested that the effects of IGF II are not caused by an interaction with the IGF I or insulin receptor, but are the result of an interaction with its own receptor, for the following reasons. First, IGF I stimulates glucose transport, glycolysis and glycogen synthesis in the isolated soleus muscle preparation independent of the concentration of insulin in the incubation medium; in relation to insulin, therefore, the effects of IGF II are quite distinct from those of IGF I [24]. Secondly, an affinity cross-linking study identified IGF II receptors in rat soleus muscle and suggested that, at concentrations similar to that used in the present study (i.e. approx. 10 nM), multiplication-stimulating activity (predominantly IGF II) would not interact significantly with either the insulin or the IGF I receptor in this tissue [13]. Thirdly, IGF II (10 nM) stimulated the rate of transport and phosphorylation of 2-deoxyglucose in L6 myoblasts, which paralleled IGF II binding to its receptor in these cells [14]. In addition, a similar concentration of IGF II stimulated glycogen synthesis in Hep G2 cells via its own receptor; polyclonal antibodies to the IGF II receptor mimicked the ability of IGF II to increase glycogenesis. Also, the IGF II response was not affected by the presence of a monoclonal antibody which inhibits cellular responses mediated via the IGF I receptor [25,26]. Thus data from the present study suggest that a low concentration of

IGF II (< 10 nM) may have specific effects on glucose utilization in skeletal muscle, but only at basal concentrations of insulin, which may be necessary for the expression of a significant number of IGF II receptors on the plasma membrane.

In contrast, other studies have suggested that the stimulation of glucose and amino acid transport by IGF II is mediated via the IGF I receptor. The IGF II-mediated stimulation of glucose and amino acid transport in L6 myoblasts appeared to be the result of an IGF I receptor interaction, since antibodies which block the IGF II receptor were without effect on the response to IGF II in these cells [15]. In the isolated soleus muscle, metabolic effects of multiplication-stimulating activity were only apparent at very high concentrations (> 100 nM), and a close relationship was observed between the stimulatory effects of the peptide on glucose and amino acid transport and its ability to displace ¹²⁵I-IGF I from the IGF I receptor. In contrast, ¹²⁵I-IGF I was displaced from the IGF II receptor by IGF II at concentrations which had no detectable effects on skeletal muscle metabolism [13]. However, insulin was omitted from the incubation medium in both of these studies. It is noteworthy that in the present study the concentration of IGF II required to stimulate glycolysis in the presence of a sub-physiological concentration of insulin (1 μ units/ml) was approx. 40 nM, i.e. 4-fold higher than when a basal physiological concentration of insulin (10 μ units/ml) was included in the incubation medium (Fig. 5). Thus these studies suggest that, in conditions of insulin insufficiency, the effect of a high concentration of IGF II is the result of an interaction with the IGF I receptor.

Although the present investigation provides evidence to suggest that the insulin-like effects of IGF II are mediated via the IGF II receptor, there is much controversy as to whether the IGF II receptor subclass fulfils any role in initiating the intracellular signalling which is associated with either the growth-promoting or metabolic effects of IGF II [27,28]. The IGF II receptor is structurally distinct from the IGF I/insulin receptor subclass, and it is not known how its small cytoplasmic domain (which lacks tyrosine kinase activity) could transduce ligand binding to metabolic and/or mitogenic effects within cells. The IGF II receptor appears to cycle continuously between the plasma membrane and an intracellular compartment [29]. Hence it has been suggested that the association of IGF II with its receptor may be a mechanism solely for ensuring degradation of the peptide; this hypothesis is supported by the observation that antibodies to the IGF II receptor inhibited the degradation of IGF II by > 90% [15]. However, the IGF II-receptor-mediated increase in Ca²⁺ influx in BALB/c3T3 cells was shown to be coupled to a pertussis-sensitive G-protein [30]. In conclusion, there is evidence supporting the ability of the IGF II receptor to couple ligand interaction to biological responses, although it is far from clear what role either IGF II or the IGF II receptor may play *in vivo* [1,27,28].

An interesting and potentially important observation in this study was that the presence of BP1 in the incubation medium inhibited the effect of IGF II on the rate of glycolysis and glycogen synthesis (Table 1). This effect is consistent with that found in other studies; e.g. the stimulation of sulphate incorporation into chondrocytes and glucose incorporation into fatty acids in adipocytes by crude IGF preparations was inhibited in the presence of BP1 [31]. BP1 has also been shown to inhibit the rate of *in vitro* sulphation in cartilage which was stimulated by IGF I [17]. Since the plasma concentration of BP1 is regulated by insulin [4], and since skeletal muscle has been shown to be quantitatively important in mediating glucose disposal after a meal [32], the results of the present work support the hypothesis that changes in the plasma concentration of BP1 may be of physiological importance in the control of blood glucose levels,

by modulating the availability of IGF II [3]. Thus the increase in the plasma insulin concentration and the subsequent decrease in the plasma concentration of BP1 which are associated with a glucose load would be expected, via an increase in the effectiveness of IGF II, to increase the rates of glucose uptake and glycogen synthesis in skeletal muscle.

The concentration of free IGF II, in either the plasma or the interstitial fluid, is not well established. Although the total plasma concentration in humans is high (700 ng/ml) [33], > 95% of this is complexed to binding proteins and is therefore unavailable to the tissues [3]. Hence the free concentration of IGF II in plasma may be approx. 30 ng/ml, which is similar to the concentration used in the present work. However, the effective concentration of IGF II in the interstitial fluid is difficult to assess, since both IGF I and II and the binding proteins can be produced locally in most tissues [1,34]. Furthermore, the binding proteins that are released from vascular endothelial cells may actually enhance, rather than inhibit, the effects of these peptides [35].

The effects of the peptide on isolated muscle reported in this paper suggest that the hypoglycaemic effect of IGF II may be caused by its ability to increase the sensitivity of glucose utilization and glycogen synthesis to insulin. However, the role of IGF II in the regulation of glucose metabolism may differ between species, since the high plasma concentration observed in adult humans is unusual. For most species, including the rat, high levels of IGF II are present only in the fetal state, and the concentration of the peptide in plasma declines rapidly after birth [36].

We acknowledge the financial support of the Medical Research Council and the Science and Engineering Research Council.

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Received 11 October 1991/4 March 1992; accepted 18 March 1992