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PARALLEL EFFECTS OF INSULIN-LIKE GROWTH FACTOR-II AND INSULIN ON GLUCOSE METABOLISM OF DEVELOPING MOUSE EMBRYONIC LIMB BUDS IN CULTURE

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SUMMARY. The present study was undertaken to evaluate the functional significance of the previously observed increase in the level of IGF-II receptors despite the loss of growth promoting activity of IGF-II in differentiated limb buds in organ culture. In the present study IGF-II and insulin at similar concentration (0.1-1 µg/ml) stimulated the glucose uptake by the tissue and incorporation into glycogen (~2 and 4 times control) and stimulated the activity of glycogen synthase (~2 times control) of the limb buds irrespective of the differentiation stage of the tissues. IGF-I had little or no effect. None of the hormones (IGF-I,II or insulin) had any effect on the CO_2 production by the limb buds. These results suggest a regulatory role of IGF-II, complementary or overlapping with insulin, in glucose metabolism of the developing limb buds. The absence of the effects of both IGF-II and insulin on CO_2 production by the tissue, however, indicate that the IGF-II and insulin regulation of glucose metabolism of the limb buds is predominantly on glycogen synthesis.

The classical definition of insulin-like growth factors (IGF)/somatomedin (SM) is growth factors with insulin-like activity (ILA) which promote the actions of growth hormone in skeletal tissue (1). On the basis of their isoelectric point, IGF are divided into two groups, basic or IGF-I and acid-neutral or IGF-II (2-5). The two groups of IGF have been shown to possess two distinctly different cell surface receptors (6-12). IGF-I receptor, similar to insulin receptor, is an oligomeric protein with higher affinity for IGF-I than IGF-II and very low affinity for insulin. IGF-II receptor, unlike insulin receptor, is a non-oligomeric protein with higher affinity for IGF-II than IGF-I and no affinity for insulin.

More recent studies have revealed that IGF growth promoting effects are not confined to cartilage but are also noted in a variety of other

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non-skeletal tissues (13-14). In some tissues IGF may have ILA by acting via their own receptors rather than through cross-reactivity with insulin receptors (13-15). The overall roles of IGF-I and II in growth and metabolism of specific tissues are not yet fully defined, however, results of various studies suggest that IGF-I and IGF-II are more important as adult and fetal growth factors respectively (16,17). A recent report from our laboratory, studying the in vitro effects of IGF-I and II on the growth and development of mouse embryonic limb buds from blastoma stage to well differentiated cartilage tissue, provides further support for this conclusion. IGF-II was shown to be growth promoting in non-differentiated, but not in differentiated, tissue whereas IGF-I was shown to be growth promoting in differentiated, but not in non-differentiated limb buds, (18). IGF-II receptors, however, despite the loss of growth promoting activity of IGF-II, were increased in the differentiated limb buds. This would suggest that IGF-II may have important tissue metabolic action, other than growth promotion, in the more differentiated limb buds. The present study explores this possibility by investigating the ILA of IGF in differentiating limb buds by measuring the effects of IGF, in parallel with insulin, on glucose metabolism of the limb buds, in organ culture, at different stages of their development.

MATERIALS AND METHODS

Hormones

IGF-I and II used in these studies were purified to homogeneity in our laboratory. IGF-I (B-SM) was purified as previously described (3) with the addition of reverse phase high pressure liquid chromatography (HPLC) in the final purification step (18,20). IGF-II was purified in a similar manner to IGF-I as previously described (18-20). Porcine insulin was a gift from Novo Laboratories (Toronto, Ontario).

Limb Bud Organ Cultures

Organ cultures of limb buds (LB) from 11 day old mouse embryos (Swiss white) were established by the modification (18) of the organ culture technique of Trowell (21). Fore limb buds were cultured in Bigger's medium containing a 25% salt solution, without any added serum. The designation of non-differentiated (at the commencement of the culture) and differentiated (on or after 3 days in culture) tissue in the text correspond to the morphological and biochemical evaluation of the tissues as previously described (18).

Glucose Metabolism

Glucose metabolism of the limb buds was studied by simultaneously measuring the utilization of D-[U ^{14}C]glucose in formation of CO_2 and

incorporation into tissue and glycogen. Optimal conditions for CO_2 production and glucose uptake by the tissue in the presence and absence of the hormones were established in preliminary experiments. Accordingly, tissues on appropriate days of culture were transferred in tightly stoppered tubes, fitted with a well containing filter paper, and incubated overnight at 37 C in the culture medium (0.5 ml) supplemented with [U-^{14}C]glucose (0.2 μCi/tube) and varying concentrations of IGF or insulin. At the end of the incubation $^{14}CO_2$ trapped in the filter paper (10) and ^{14}C incorporated into tissue and glycogen were measured as previously described (22).

Glycogen Synthase Activity

For measurement of glycogen synthase activity, tissues were pre-incubated overnight, with fresh culture medium containing varying concentrations of IGF or insulin. At the end of incubation, tissues were removed and lyophilyzed. The glycogen synthase activity of the lyophilyzed tissues, subsequent to homogenization in 0.25 M sucrose containing 1.7 mM EDTA and 10 mM NaF, was determined by previously described methods (23,24). Homogenates (30 μl) were added to 60 ul Tris maleate buffer (40 mM, pH 7.6), containing 1.7 mM EDTA, 10 mM NaF, 0.48% glycogen and 5 mM uridine diphospho-[U-^{14}C]glucose (UDPG) with and without the glucose-6-phosphate (32 mM) as substrate. The reaction mixture was then incubated for 15 min at 37 C. The incubation was terminated by addition of 2N NaOH followed by precipitation with 1 mg/ml of glycogen. Glycogen synthase activity is expressed as percent of active or independent form, I (measured in absence of the substrate), of the total, [I plus D (dependent or inactive form, measured in presence of the substrate)].

RESULTS

Effect of IGF on glucose uptake and CO_2 production by the limb buds at different stages of differentiation.

In initial experiments, based on our previous studies on the effects of IGF-I and II on thymidine and sulfate incorporation by the limb buds (18), a single dose (1μg/ml) of IGF-I and II was chosen, to study their effects on glucose uptake by the limb buds at different stages of differentiation (0-4 days in culture). As shown in Fig. 1, IGF-II significantly ($p < 0.05$) increased (approximately 3 times control) the ^{14}C uptake by the limb buds at all stages of differentiation. IGF-I had no significant effect. None of the IGF had any effect on CO_2 production by the tissues.

Comparison of the effects of IGF and insulin on CO_2 production and glucose uptake by non-differentiated and differentiated limb buds.

Since no developmental stage significant differences in the effects of IGF-I or II in stimulating the glucose uptake by the limb buds was noted, we compared the effects of IGF-I, II, and insulin on the glucose metabolism of the limb buds only at the commencement of the culture (non-differentiated

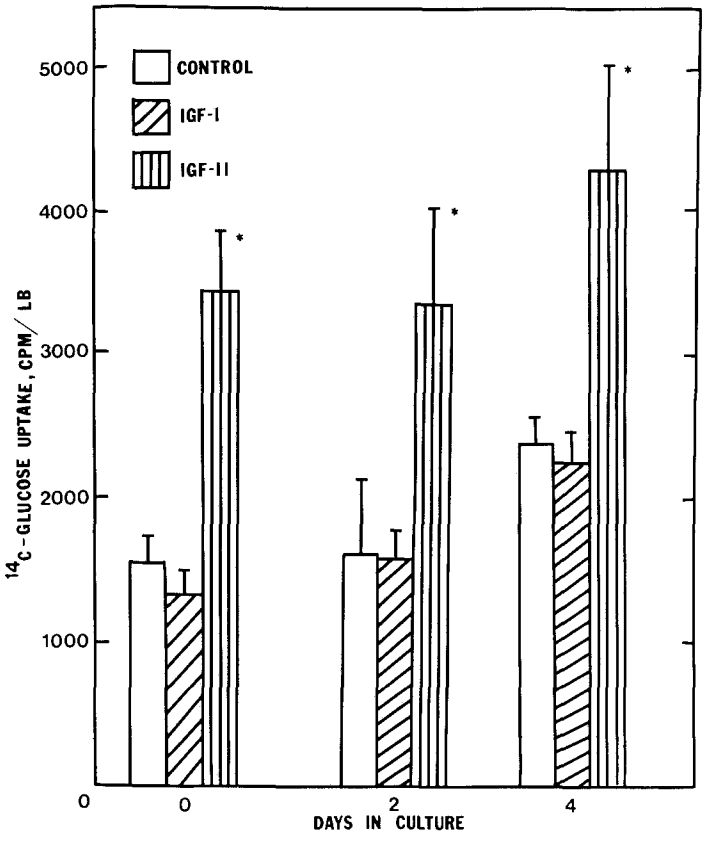

Figure 1 Effects of IGF-I and II on glucose uptake by the developing limb buds. On the indicated days of culture, tissues were transferred to tubes containing culture medium supplemented with [U-^{14}C]glucose in the presence and absence of (control) IGF-I or II (1ug/ml). After overnight incubation at 37 C the glucose uptake by the tissues was measured as described in the methods. Results represent mean $\pm$ SD (CPM/LB) of 4 plates per experimental condition from three separate cultures. *, $p < 0.05$ or better, compared with the mean of the control, determined by one-way analysis of variance followed by Duncan's multiple range test.

tissue) and on or after 3 days in culture (differentiated tissue). As shown in Fig. 2, IGF-II and insulin (1 μg/ml) stimulated glucose uptake with similar magnitude in non-differentiated and differentiated tissue. Insulin, similar to IGF-I and II and unlike its effect on most other tissues, had no effect on CO_2 production by the non-differentiated or differentiated tissue. The dose dependency of IGF-II and insulin on stimulation of the glucose uptake by the differentiated and non-differentiated tissue was studied. As shown in Fig 3, increasing concentrations, from 0.1 to 1.0 μg, of both IGF-II and insulin, resulted in significant ($p<0.05$) increase in glucose uptake in the differentiated tissues. The magnitude of stimulation by both IGF-II and

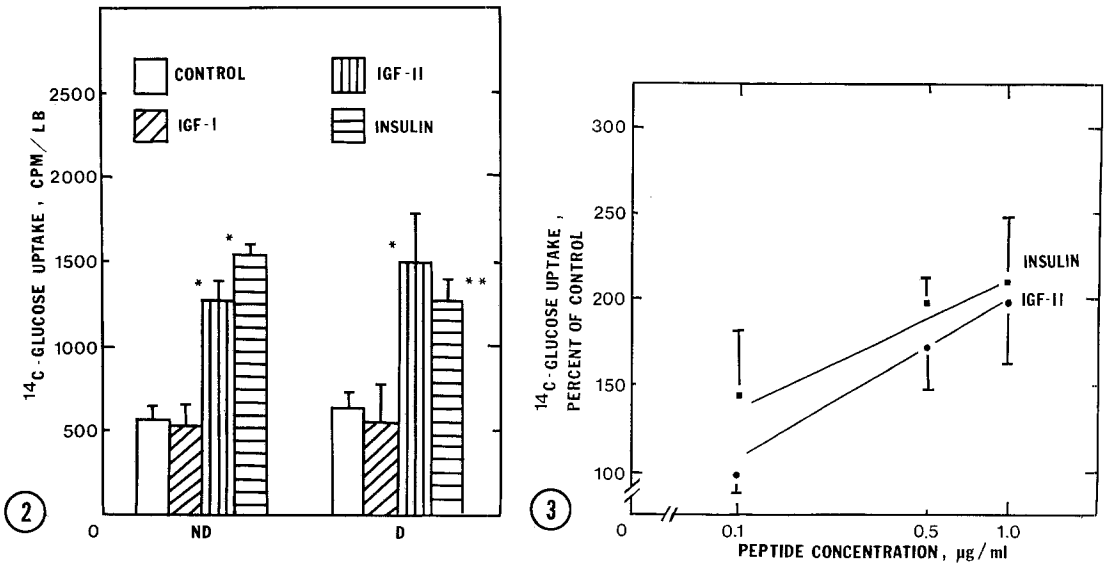

Figure 2 Comparison of the effects of IGF-I, II and insulin on glucose uptake by the differentiated and non-differentiated limb buds. Limb buds at the commencement (non-differentiated, ND) or after 3 days (differentiated, D) of culture were incubated with [U-^{14}C]glucose for measurement of glucose uptake by the tissues as described in the methods. Experimental condition and representation of the results are same as in Figure 1. * and **, $p<0.05$ and $p<0.01$, respectively.

Figure 3 Dose response curves for IGF-II and insulin stimulation of glucose uptake by differentiated limb buds after 3 days in culture. The experimental condition was the same as described in Figures 1 and 2. Results represent percent stimulation over control (in absence of IGF-II or insulin), mean $\pm$ SD of 4 plates per experimental condition from two separate experiments.

insulin was comparable. The results were similar in nondifferentiated tissue. The slopes of the dose response curves of IGF-II and and insulin were not different.

Effects of IGF and insulin on glycogen synthesis by the limb buds.

The effects of IGF and insulin on glycogen synthesis were measured by the glucose incorporation into glycogen as described in Methods. IGF-II and insulin (Fig. 4), but not IGF-I, stimulated the incorporation of labeled glucose to glycogen similar to their effects on the glucose uptake by the differentiated limb buds. The magnitude of stimulation of glycogen synthesis by both IGF-II and insulin was similar at similar concentrations (0.1 and 1.0 ug/ml). A similar observation was made in the non-differentiated tissue.

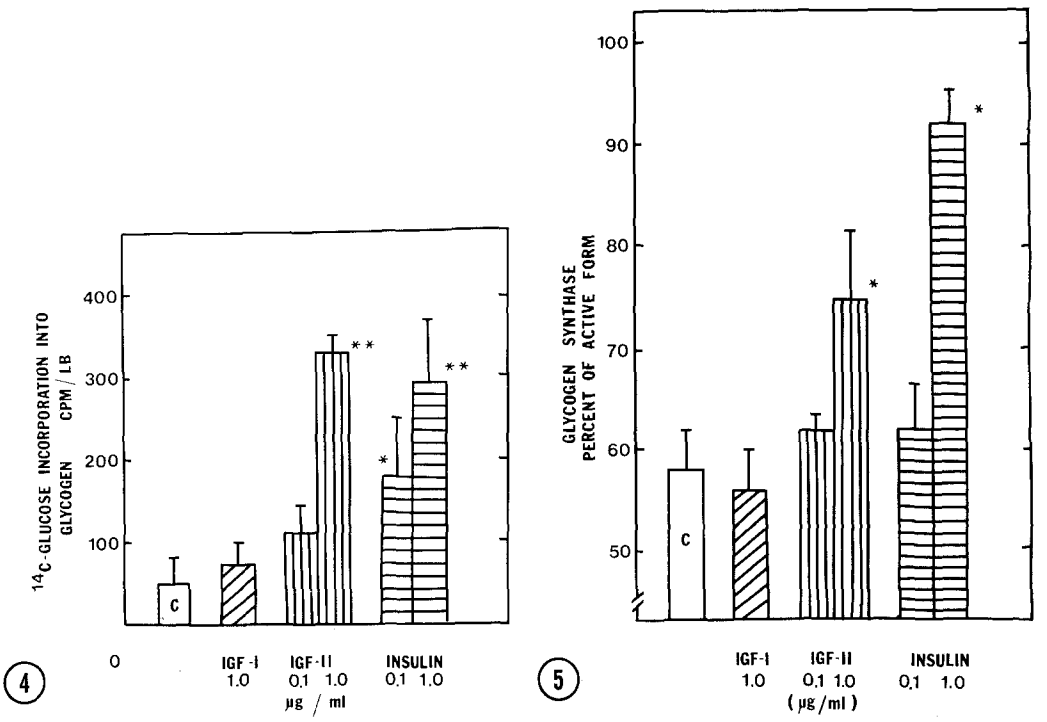

Figure 4 Effects of IGF-I, II and insulin on glycogen synthesis in the differentiated limb buds. Tissues after 3 days in culture were incubated with [U-^{14}C]glucose in absence (C) and presence of IGF-I, II or insulin under conditions similar to that described for Figures 1 and 2. ^{14}C-glucose incorporated in glycogen was determined after precipitation of glycogen as described in methods. Statistical representation of the results are same as in Figure 3.

Figure 5 Effects of IGF-I, II and insulin on glycogen synthase activity of differentiating limb buds. Tissues after 3 days in culture were pre-incubated with and without (C) IGF-I, II or insulin. The glycogen synthase activities of the pre-incubated tissues were determined and expressed by the methods described in text. The statistical presentation of the data are same as in Figures 1, 2 and 4.

Effects of IGF and insulin on glycogen synthase activity in limb buds.

Next we compared the effects of IGF and insulin on glycogen synthase activity of the limb buds. IGF-II but not IGF-I, stimulated the glycogen synthase activity, similar to the effects on glucose uptake in tissue and incorporation into glycogen, in the differentiated (Fig 5) and non-differentiated limb buds (results not shown). The potency and effectiveness of IGF-II and insulin in stimulation of glycogen synthase activity (~2 times control) were similar.

DISCUSSION

These studies show that insulin and IGF-II, but not IGF I, stimulated glucose incorporation into tissue and glycogen and activated glycogen synthase of the developing limb buds at the different stages of differentiation. The similar potency and effectiveness of IGF-II and insulin, in context of low affinity of IGF-II for insulin receptor and no affinity of insulin for IGF-II receptor, would suggest that each hormone is acting through its own receptor in affecting glucose metabolism by the limb buds. Since IGF-II and insulin did not affect tissue production of CO_2 from glucose, the major effects of insulin and IGF-II on glucose metabolism in limb buds would appear to be stimulation of glycogen synthesis.

The findings in these studies that IGF-I did not affect glucose metabolism, in contrast to IGF-II and insulin, are not readily explained in view of IGF-I cross-reactivity with IGF-II and insulin receptors. It is possible that this experimental model, using the limb buds in organ culture, derives in problems with hormone degradation or limitation of diffusion of the hormone into the tissues. This could result in tissue exposure to lower concentration of the hormone during incubation and the lower concentration of IGF-I would not derive in significant cross-reactivity with IGF-II or insulin receptors to effect biological action.

Our studies suggesting a similar regulatory role of both IGF-II and insulin on glucose metabolism of the limb buds is not unique. Others have reported that both IGF and insulin stimulate glucose metabolism by cultured hepatoma cells (15,25) and stimulate growth and differentiation of chick embryos (26). The actions of IGF and insulin have been assumed to be predominantly on growth promotion and glucose metabolism respectively. Our studies along with others (13-14,25-27), however, suggest that IGF and insulin may have similar actions in some tissues, particularly when non-differentiated or of fetal origin, and differing actions in other tissues. This control evolution may be important in normal growth and development of various tissues and organ. In view of the structural and functional similarities of IGF-I and

insulin receptors, both of which are protein kinases (28-31), a common regulatory pathway, involving autophosphorylation of their receptor as an early event, may be postulated for these two hormones. The IGF-II receptor is, however, structurally different from insulin receptor and lacks kinase activity (32-34). A possible explanation would be that there are multiple entry points into the regulatory pathway of growth and metabolism and IGF-II acts at a common post receptor step without prior phosphorylation of the receptor to elicit the same responses as IGF-I or insulin. Further definition of the mechanisms by which IGF-I,II and insulin ultimately regulate growth and metabolism may explain the teleological rationale for the predominant biological actions of IGF and insulin in various tissues at different stages of development.

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