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Acute actions of insulin-like growth factor II on glucose metabolism in adult rats

F. Stümpel and H. Hartmann

Department of Medicine, Division of Gastroenterology and Endocrinology, Georg-August-Universität, Göttingen, FRG

Summary. The metabolic potency of recombinant human insulin-like growth factor II was studied in anaesthetized adult rats by obtaining dose-response curves for the hypoglycaemic action and for the stimulation of glucose metabolism during euglycaemic clamping. Compared to insulin, about 50 times higher doses of insulin-like growth factor II were required to result in identical in vivo responses, with half-maximally effective serum concentrations for the stimulation of glucose disposal during clamp studies of about 0.8 and 50 pmol/ml, respectively. A similar difference in potency was observed for the dose-dependent stimulatory actions on glucose metabolism in individual target tissues. Half-maximally effective serum concentrations in the range of 0.8 to 3.0 pmol/ml for insulin and of 40 to 70 pmol/ml for insulin-like growth factor II were seen to be required for 2-deoxyglucose uptake, glycogen formation in skeletal muscle and lipogenesis in epididymal fat. Maximal responses were identical with both peptides. These data suggest that in vivo acute metabolic actions of insulin-like growth factor II on carbohydrate metabolism occurred through insulin receptors.

Key words: Insulin-like growth factor II, insulin action, euglycaemic clamping, glucose metabolism, lipogenesis, glycogenesis.

Insulin-like growth factor II (IGF-II) is a polypeptide hormone structurally related to proinsulin and insulin-like growth factor I (IGF-I) [1, 2]. Whereas IGF-I (also previously named somatomedin C) has been shown to mediate most of the action of growth hormone on target tissues [3], the physiological role of IGF-II, particularly in humans, remains less well understood. In the rat, IGF-II is regarded as a fetal growth factor, while post-natally, plasma IGF-II levels decrease and tissue IGF-II mRNA expression apparently ceases [4, 5]. Recently, direct evidence for a physiological role of IGF-II in rodent embryonic growth was provided by studies where the IGF-II gene was disrupted by gene targetting [6]. Conversely, in man low IGF-II concentrations have been reported for fetal blood compared to adult human plasma [7].

Acute metabolic actions – qualitatively resembling those of insulin – have been reported for IGF-II from in vivo and in vitro studies [8–10]. When injected into normal and hypophysectomized rats IGF-II was shown to lower blood glucose and to stimulate tissue glycogen and lipid synthesis in the presence of an anti-insulin serum [10]. In addition, a recent study performed in fasted lambs, suggested a slight stimulatory action of IGF-II on glucose clearance [11]. In man, indirect evidence for acute effects of IGF-II on glucose metabolism is provided by investigations reported from patients with non-islet-cell tumour-associated hypoglycaemia [12–15]. Furthermore, various in vitro studies, including work with human adipocytes, demonstrated insulin-like metabolic effects of IGF-II [16]. Several lines of evidence suggest that these metabolic actions of IGF-II were not mediated through IGF-II receptors but through insulin receptors, to which IGF-II can bind with low affinity [17–19].

For further characterization of acute actions of IGF-II on glucose metabolism dose-response curves were obtained in the present investigation to determine the hypoglycaemic potency following i.v. bolus injection and the stimulatory action on glucose disposal utilising the euglycaemic clamp technique in anaesthetized rats. In addition, while using labelled tracers such as [^{14}C]glucose and 2-[^{3}H]deoxyglucose during clamping, IGF-II action on individual tissues and on different metabolic pathways, e.g. glycogen synthesis and lipogenesis, was studied.

Materials and methods

Materials

Chemicals were reagent grade and obtained from commercial sources. Enzymes were purchased from Boehringer Mannheim (Mannheim, FRG) and porcine insulin was from Serva (Heidelberg, FRG);

Table 1. Time course of free and total serum insulin-like growth factor II concentrations during euglycaemic clamp studies

IGF-II infusion ($nmol \cdot kg^{-1} \cdot h^{-1}$)	IGF-II concentration (pmol/ml)			
	Time (min)	0	90	120
IGF-II (50)	Total	22.0 ± 3.4	53.8 ± 4.7	47.3 ± 8.2
	Free	1.9 ± 0.4	34.1 ± 9.6	24.8 ± 6.4
IGF-II (170)	Total	26.3 ± 2.9	179.3 ± 24.3	197.3 ± 36.8
	Free	1.85 ± 0.7	172.1 ± 42.4	167.0 ± 16.9

Data are means ± SD of at least seven experiments at each infusion rate

recombinant human IGF-II was a gift from Ciba Geigy (Basel, Switzerland). 2-Deoxy-(1-^{3}H)-D-glucose (specific activity of ~14 Ci/mmol) was obtained from Amersham-Buchler (Braunschweig, FRG) and (U-^{14}C)-D-glucose (specific activity ~4 mCi/mmol) from New England Nuclear (Dreieich, FRG). Insulin radioimmunoassay kit was purchased from Pharmacia (Ratingen, FRG). The anti-IGF-II serum for radioimmunological determination of IGF-II was a gift from Prof. J. Zapf, Zürich, Switzerland.

Animals

Male Wistar rats (220–290 g body weight) were obtained from Mus Rattus (Brunnthal, FRG). They were kept on a 12 h light/dark cycle with free access to a standard diet (Altromin 1324; Altromin, Lage, FRG).

Hypoglycaemic action

Experiments were started at 14.00 hours; food was removed 8 h before to assure absence of ingested nutrients in the intestine and to achieve a similar hepatic glycogen content. Anaesthesia was obtained by intraperitoneal injection of pentobarbital (60 mg/kg). Animals were kept on heated pads to maintain body temperature throughout the experiments. After preparation of the right jugular vein a polyvinylchloride catheter (external diameter ~0.8 mm), obtained from Mahrt und Hoerning (Göttingen, FRG), was inserted and positioned in the right atrium for injection of hormones and blood sampling. Following an equilibration period of 20 min with repeated three-fold determinations of blood glucose (samples of 50 µl at 10-min intervals), an i.v. bolus of insulin or IGF-II was administered in a volume of 250 µl – 0.5, 2.0, 4.0, 8.0 and 12.0 $nmol \cdot kg^{-1}$ of insulin or 25, 50, 100, 200 and 400 $nmol \cdot kg^{-1}$ body weight of IGF-II in 0.9% weight/volume (w/v) sodium chloride (NaCl) solution containing 0.3% (w/v) bovine serum albumin (BSA). For determination of blood glucose subsequent blood samples (50 µl) were collected at 10-min intervals, deproteinized in 500 µl perchloric acid (0.33 mol/l) and analysed for glucose content by the glucose-dehydrogenase method as described [20]. Hypoglycaemia was quantitated by calculating the area over the blood glucose concentration curve for individual experiments by the trapezoid method [21]. The basal line was given by the mean of the three values prior to hormone injection.

Euglycaemic clamp studies

Animals were fasted for 24 h, anaesthetized and kept during the experiment as described above. In addition to the venous catheter an arterial cannula was inserted into the right carotid artery. According to the method of DeFronzo et al. [22] euglycaemic clamp studies were performed by continous venous hormone infusion (1.2 $ml \cdot h^{-1}$, Unita 1 b; Braun, Melsungen, FRG) of insulin (0.5, 1.0, 2.0, 4.0, 8.0, 10.0 and 13.0 $nmol \cdot kg^{-1} \cdot h^{-1}$) or IGF-II (25, 50, 100, 170 and 300 $nmol \cdot kg^{-1} \cdot h^{-1}$) dissolved in 0.9% (w/v) NaCl solution containing 0.3% BSA. A priming IGF-II bolus (one-third of the respective dose per hour) was given. Euglycaemia was maintained by infusing a solution of exogenous glucose (0 to 1.5 $ml \cdot h^{-1}$, glucose concentration ranging from 50 to 220 mg/ml) using a variable infusion pump (Wissenschaftliche Werkstätten; Universität Göttingen, FRG). Repeated measurements of arterial blood glucose concentrations (samples of 10 µl serum obtained at 10- to 20-min intervals, Beckman Glucose Analyzer II; Beckman, München, FRG) were performed to guide the adjustment of the glucose infusion rate in accordance with short-term changes of glucose concentration. The glucose infusion rate during the last 30 min of the 2-h clamp experiment was used as a measure of insulin or IGF-II action on whole body glucose disposal. Blood samples (200 µl) for determination of insulin or IGF-II were taken at 0, 90 and 120 min.

Effects on individual tissues

Insulin and IGF-II action on glucose uptake in individual tissues during euglycaemic clamping were estimated by the use of labelled 2-deoxyglucose as described by Kraegen et al. [23]. In brief, animals received an i.v. bolus injection of 2-deoxy-D-(1-^{3}H)-glucose (50 µCi in a volume of 300 µl 0.9% [w/v] NaCl solution) at 60 min after the commencement of the glucose clamp followed by subsequent arterial blood sampling for determination of glucose and 2-deoxyglucose concentrations. At the completion of the clamp (at 120 min) tissue samples were taken from diaphragm, white and red parts of the gastrocnemius muscle, heart and epididymal fat to be analysed for tissue accumulation of 2-deoxyglucose. Analysis and calculation of glucose metabolic index Rg' were performed as described in detail elsewhere [24].

Incorporation of labelled glucose into lipids of epididymal fat pads and into tissue glycogen stimulated by insulin or IGF-II was determined in separate experiments following an i.v. bolus injection of (U-^{14}C)-glucose (in 250 µl 0.9% [w/v] NaCl solution) at 60 min during euglycaemic clamping with tissue sampling at 120 min as described by Zapf et al. [10]. Extraction of lipids from epididymal fat was performed according to Stansbie et al. [25]. Lipogenesis was estimated by dividing radioactivity incorporated into lipids by the area under the blood radioactivity time curve following bolus injection. Glycogen formation was studied by analysing individual tissues for radioactivity incorporated into glycogen as described [10]. Tissue glycogen content was determined enzymatically [26]. Glycogenesis was estimated by dividing radioactivity incorporated into tissue glycogen by tissue glycogen content with correction for serum radioactivity following bolus injection of labelled glucose.

Analytical methods

Radioactivity in aqueous samples was counted in 10 ml Hydroluma scintillant (Baker, Deventer, The Netherlands) in a beta-counter (Mark III; Searle, Des Plaines, Ill., USA); lipids were counted using 10 ml Instant Szint. Gel II (Canberra Packard, Zürich, Switzerland). Serum hormone samples obtained at steady-state conditions during clamp experiments were measured in duplicate. Insulin concentrations were determined by a commercial radioimmunoassay (radioactivity was counted with a Gamma-Master 1277; Ratingen, FRG). For the measurement of total IGF-II concentrations 1 ml of 0.5 mol/l HCl was added to 100 µl serum and applied to Sep-Pak C_{18}-cartridges (Waters Associates, Milford, Mass., USA). IGF-II was eluted with methanol, lyophilized and dissolved in 1 ml of assay buffer (phosphate-buffered saline pH 7.4 with 0.2% [w/v] human serum albumin). Free IGF-II was separated from protein bound on a Sephadex G 50 column (1 × 100 cm, flow rate 12 ml/h) in serum samples of 300 µl under neutral conditions (0.15 mol/l NH_4HCO_3 buffer, pH 7.7) as described in detail elsewhere [10], lyophilized and

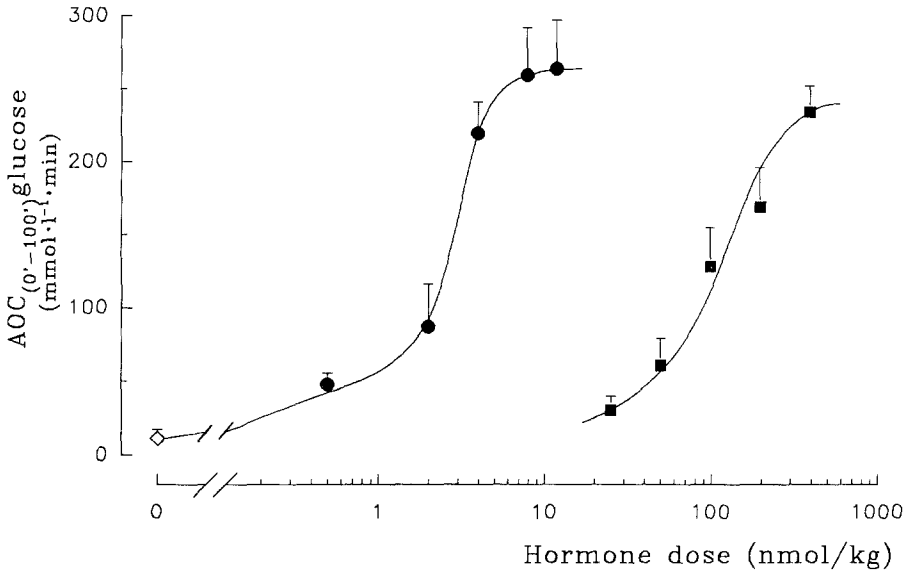

Fig. 1. Hypoglycaemic potency. Anaesthetized rats received i.v. bolus injections of insulin (●—●) or IGF-II (■—■). Hypoglycaemia was quantitated by integrating the area over the curve (AOC) of blood glucose decrease for 100 min following the hormone injection. Control animals (◇) received an identical volume of sodium chloride solution. Values are means ± SD of at least five experiments each at individual doses

dissolved in 1 ml of assay buffer. After appropriate dilution 100 µl each were assayed radioimmunologically using a specific rabbit antibody.

Results

Hypoglycaemic potency

Following an i.v. bolus a dose-dependent decrease of blood glucose concentration was observed with insulin and IGF-II. At the highest doses glucose nadirs in the range of 1.94–2.22 mmol/l were observed; death from hypoglycaemia did not occur. Figure 1 demonstrates the hypoglycaemic potency calculated by integrating the area over the curve (AOC) for blood glucose decrease. Compared to insulin the potency of IGF-II at a half-maximally effective dose was about 2%, e.g. ~2 nmol/kg for insulin and ~100 nmol/kg for IGF-II, whereas identical maximal hypoglycaemic effects were observed with both peptides. Saline-injected control animals did not show significant changes in blood glucose concentrations.

Euglycaemic clamp studies

Figure 2a depicts the stimulatory action of insulin and IGF-II on whole body glucose disposal. A dose-dependent increase was observed for both peptides with an identical maximal stimulation of ~19.0 $mg \cdot kg^{-1} \cdot h^{-1}$. However, an approximately 30-fold higher dose of IGF-II was required to achieve similar rates of glucose disposal with half-maximally effective doses of ~1.0 $nmol \cdot kg^{-1} \cdot h^{-1}$ for insulin and of ~50.0 $nmol \cdot kg^{-1} \cdot h^{-1}$ for IGF-II, respectively. Half-maximally effective serum concentrations of insulin and IGF-II (measured in samples obtained at steady-state conditions during clamping) were in the range of 0.8 pmol/l for insulin and 50 pmol/ml for IGF-II (Fig. 2b). The time course for serum concentrations of the free and total portion of IGF-II is given in Table 1. Sodium chloride infused control animals did not require exogeneous glucose.

Stimulation of 2-deoxyglucose uptake in individual tissues

For insulin- and IGF-II-treated rats maximal stimulation of 2-deoxyglucose uptake was identical in several target tissues. The uptake was dose-dependent as demonstrated in Figure 3 for skeletal muscle and diaphragm. Half-maximally effective serum concentrations however, were considerably higher in IGF-II-infused animals (see Fig. 3 and Table 2 for individual tissues). The respective serum concentrations were in the range of 0.8 to 0.3 pmol/ml for insulin and 40 to 70 pmol/ml for IGF-II.

Stimulation of lipogenesis in epididymal fat pads

Half-maximally effective serum concentrations of insulin and IGF-II for the stimulation of lipogenesis are given in Table 2. The effects of both peptides were dose-dependent with similar maximal stimulatory actions.

Stimulation of glycogenesis

Incorporation of labelled glucose into tissue glycogen was stimulated by insulin and IGF-II demonstrating a similar difference in potency as for the metabolic actions described above. At infusion rates of insulin and IGF-II that resulted in almost identical stimulation of total body glucose disposal during euglycaemic clamping (infusion rates of 2.0 and 100 $nmol \cdot kg^{-1} \cdot h^{-1}$) glycogenesis in red and white skeletal muscle was stimulated to a similar extent (Fig. 4).

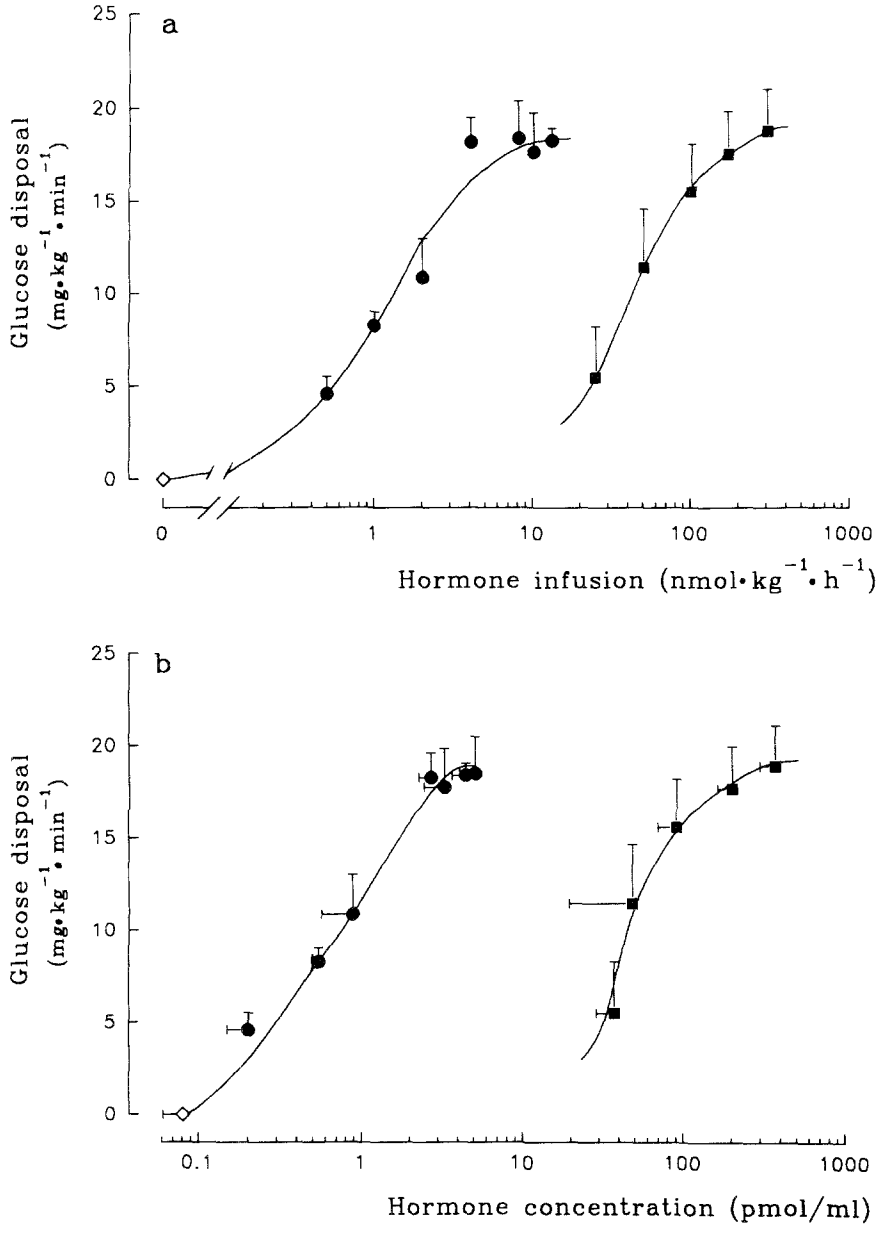

Fig. 2 a, b. Stimulation of whole body glucose disposal during euglycaemic clamping. Anaesthetized rats received i.v. infusions of insulin (●—●) or IGF-II (■—■). Exogenous glucose was infused to maintain euglycaemia. The amount of glucose infused at steady-state conditions (90–120 min after commencement) is equivalent to whole body glucose utilisation. **a** Hormone infusion rates. **b** Arterial hormone concentrations at steady-state. Control animals (◇) received identical volumes of sodium chloride solution. Values are means ± SD of at least five experiments each at individual doses

Discussion

In the present study acute actions of IGF-II were observed in vivo, qualitatively resembling those of insulin but with a considerably lower potency for IGF-II of approximately 2% that of insulin. IGF-II-induced hypoglycaemia has been described previously in experimental animals; however, an estimation of its potency compared to insulin, e.g. by obtaining respective dose-response curves, was hampered by the limited availability of serum-derived IGF-II [8, 10]. Furthermore, these early observations suggested that only free IGF-II, but not the IGF carrier protein complex, was available to insulin target tissues [10]. The present results obtained with recombinant human IGF-II using the euglycaemic clamp provide further evidence that free IGF-II would be required for acute metabolic effects, since a stimulatory action of IGF-II on total body glucose disposal and on glucose metabolism in individual tissues was observed only when serum concentrations of free IGF-II were raised substantially. Since the observed half-maximally effective serum IGF-II concentrations exceeded the reported affinity of IGF-II for the IGF-II receptor several times [18], the present data are in line with the assumption that acute metabolic actions of IGF-II were mediated through insulin receptors [17]. Thus, the present data obtained in vivo support conclusions derived from previous in vitro studies [19]. It is of interest to note that in a recent investigation comparing the acute effects of recombinant human IGF-I and IGF-II on glucose metabolism in fasted lambs, a lower potency of IGF-II was reported, i.e. an identical infusion of both peptides resulted in a considerably smaller increase of glucose clearance in IGF-II treated animals [11]. These latter data do not agree with previous results obtained with IGF-I in anaesthetized rats suggesting a potency of IGF-I on glucose metabolism almost identical to that observed in the present

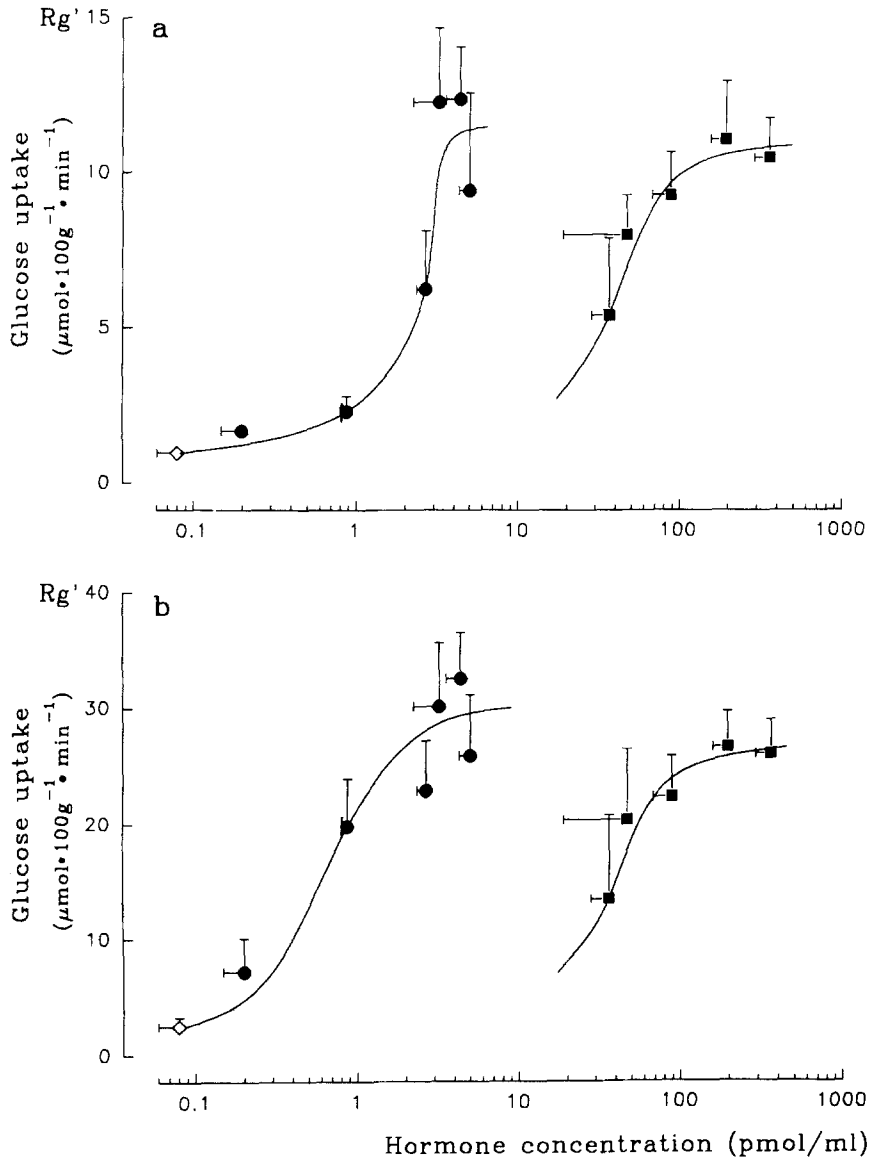

Fig. 3a, b. Stimulation of 2-deoxyglucose uptake in red parts of gastrocnemic muscle (**a**) and diaphragm (**b**) by insulin and IGF-II. Anaesthetized rats received i. v. infusions of insulin (●—●) or IGF-II (■—■) as depicted in Figure 2. At 60 min after commencement of the euglycaemic clamp an i. v. bolus of labelled 2-deoxyglucose was given followed by tissue sampling at 120 min. Measurement of radioactivity and calculation of glucose metabolic index (Rg') were performed as described in Methods. Hormone concentrations were measured in arterial blood samples obtained at steady-state conditions during clamping (means of values measured at 90 and 120 min after commencement of hormone infusion). Control animals (◇) received sodium chloride infusions. Values are means ± SD of at least five experiments each at individual doses

study with IGF-II [27]. Since neither IGF-II serum concentrations nor dose-response curves were reported for the studies in lambs, there is little evidence for a different stimulatory potency of IGF-II compared to IGF-I on glucose metabolism. However, since experimental animals were fasted for a prolonged period, fasting-related changes of IGF binding proteins resulting in a reduced potency of IGF-II remain possible. As to the role of IGF-I and IGF-II in short-term regulation of protein metabolism, little information regarding IGF-II is available at present. For IGF-I, a more sensitive regulatory response of protein metabolism compared to carbohydrate metabolism has been claimed [11].

Using isotopic tracer methodology the effects of IGF-II on individual tissues were studied in the present work. Tissue glucose uptake, assessed with 2-deoxyglucose, as well as the stimulation of lipogenesis and glycogenesis were affected in a variety of tissues with a similar difference in potency for IGF-II and insulin as that observed for the respective effects on total body glucose metabolism. Thus, a preferential action of IGF-II on glucose metabolism of an individual tissue appears unlikely.

The data derived from the present study do not suggest a physiological role of IGF-II in the regulation of carbohydrate metabolism in adults. Given the rather weak "somatomedin-like" activity of IGF-II compared to IGF-I [28] and the apparent non-dependence of circulating IGF-II concentrations from growth hormone [29], the physiologi-

Table 2. Stimulation of 2-deoxyglucose uptake and lipogenesis in epididymal fat pads by insulin and insulin-like growth factor II

	Half-maximally effective serum concentration (pmol/ml)[a]	
	Insulin	IGF-II
2-deoxyglucose uptake	1.5	50
Lipogenesis	2.0	70

[a] Data are derived graphically from individual dose-response curves with at least four experiments at each individual dose

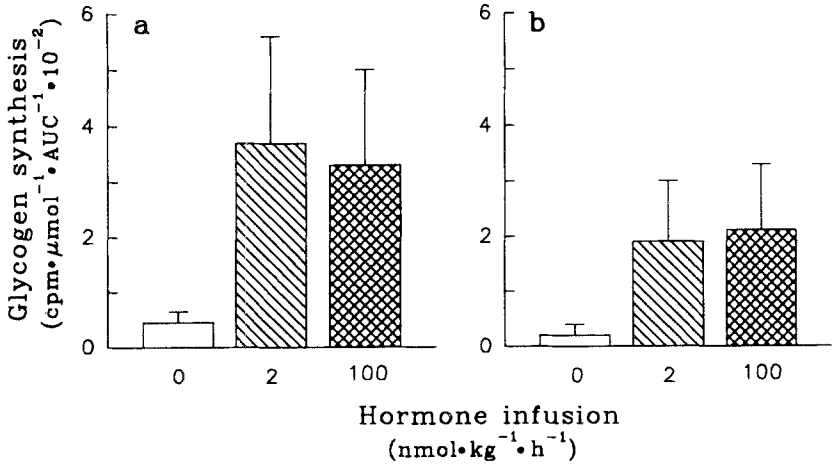

Fig. 4a, b. Stimulation of glycogen formation in red (**a**) and white (**b**) parts of gastrocnemic muscle during euglycaemic clamping. Animals received i.v. infusions of 2.0 nmol·kg^{-1}·h^{-1} insulin (▨) and 100 nmol·kg^{-1}·h^{-1} IGF-II (▩). Glycogen synthesis was measured with (U-^{14}C)-glucose as described in Methods. Control animals (□) received sodium chloride solution. Values are means ± SD of at least four experiments at each dose

cal function of circulating IGF-II in adulthood can hardly be explained by current knowledge. However, caution should be taken in extrapolating the present data for IGF-II obtained in adult rat towards human physiology in view of the different developmental pattern, i.e. very low serum IGF-II levels in adult rats [30] compared to rather high levels in adult humans [7]. Furthermore, species differences in IGF binding proteins have been noted that could influence biological actions of IGF-II [31].

Recent in vitro studies have reported an IGF-II-dependent modulation of the endocytosis of mannose 6-phosphate containing ligands, e.g. lysosomal enzymes, thereby potentially regulating their extracellular concentrations [19]. However, the recognition of the biological relevance of IGF-II in human adults will depend on future studies.

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Dr. H. Hartmann
Medizinische Universitätsklinik
Robert-Koch-Strasse 40
W-3400 Goettingen
FRG