The effect of epidermal growth factor and IGF-I infusion on hepatic and renal expression of the IGF-system in adult female rats

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Abstract

Systemic administration of epidermal growth factor (EGF) in neonatal rats results in reduced body weight gain and decreased circulating levels of IGF-I, suggesting its involvement in EGF-induced growth retardation.

We investigated the effect of EGF and/or IGF-I administration for 7 days on circulating IGF-I and IGFBP levels and hepatic and renal IGF-system mRNA expression profiles in adult female rats. EGF administration (30 μg/rat/day) did not influence body weight, liver or kidney weight. In contrast, IGF-I (400 μg/rat/day) and EGF/IGF-I administration increased both body weight and kidney weight. Also, serum IGF-I and the 30 kDa IGFBPs (IGFBP-1 and -2) were significantly increased in these groups. Serum IGFBP-3 levels increased in the IGF-I group along with increased hepatic IGFBP-1 and -3 mRNA levels. In contrast, in the EGF administration group serum IGFBP-3 levels were significantly decreased; however, the mRNA levels remained unchanged. In the EGF/IGF-I administration group, serum IGF-I and IGFBP-3 levels were significantly lowered when compared with the IGF-I administration group. This was in contrast to the effect on kidney weight increase that was identical for the IGF-I and EGF/IGF-I groups. The decrease in serum IGFBP-3 was not reflected at the hepatic IGFBP-3 mRNA level. IGFBP-3 expression might be regulated at a post-transcriptional level although EGF induced IGFBP-3 proteolysis could not be demonstrated *in vitro*.

We conclude that EGF administration reduced serum IGFBP-3 whereas IGF-I administration increased the level of IGFBP-3 and IGF-I and resulted in an increased body and kidney weight in adult female rats.

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Introduction

Insulin-like growth factor I (IGF-I) is a multifunctional growth factor produced in a variety of body fluids and tissues including liver and kidney. IGF-I is a member of the IGF-system that also consists of IGF-II, two types of IGF receptor and six different IGF binding proteins (IGFBPs) (Kelley *et al.* 1996). Furthermore, four IGFBP related proteins have been described (Baxter *et al.* 1998). The IGFBPs are abundantly present in the circulation, organs and tissues and act as major modulators of IGF action by transporting the IGFs to their sites of action and controlling the bioavailability of the IGFs to their surface receptors (Kelley *et al.* 1996). However, little is known about the *in vivo* regulation of their production.

The epidermal growth factor (EGF) belongs to a family of growth factors that regulate cell proliferation, migration and differentiation through binding to receptor tyrosine kinases on target cells (Miettinen 1997). EGF has been shown to act as a mitogen as well as a differentiation factor for many cell types (Bonassar & Trippel 1997). It is present in the circulation at very low concentrations that, nevertheless, also elicit cellular responses *in vitro* (Bujia *et al.* 1993, Murakami *et al.* 1994). In addition, the kidney is thought to be the major site of EGF synthesis in mammals (Fisher *et al.* 1989).

Given the large number of hormones, cytokines and growth factors present in the circulation, organs and tissues, interactions between growth factor families are a field of growing interest. Several studies have focused on the interaction between the EGF and IGF systems *in vitro*. EGF addition to isolated rat renal collecting duct cells increased IGF-I mRNA levels 3-fold (Rogers *et al.* 1991). EGF addition to human keratinocytes in primary culture reduced IGFBP-3 mRNA levels to 50% of control values whereas IGF-I addition had no effect (Edmondson *et al.* 1999). Furthermore, addition of EGF to rat hepatocytes in primary culture had a dose-dependent stimulatory effect

on IGF-I and IGFBP-1 production (Barreca *et al.* 1992). Accordingly, IGF-I modulates the EGF receptor action in keratinocyte proliferation (Krane *et al.* 1991), whereas EGF stimulates IGF-I biosynthesis in the liver and in the kidneys. However, only a few studies have examined these interactions during systemic administration *in vivo* (Frystyk *et al.* 1996, Vinter-Jensen *et al.* 1996).

In the present paper we investigated the effects of short term (7 days) EGF and/or IGF-I administration on the expression of the circulating, hepatic and renal IGF-system genes in young adult female rats.

Materials and Methods

Animals

Female Wistar rats (mean body weight 190 g) obtained from our own breeders (Department of Pathology, Aalborg Hospital, Denmark) were used in the study. The animals were housed individually in cages on white special spanwall bedding. They were fed a standard laboratory diet (No. 1310, Lage, Germany), had free access to water and were kept at constant temperature (21 °C), humidity (55 ± 5%) and at a ratio of 12-h light: 12-h darkness cycle (0600–1800 h light). The study complied with Danish regulations for care and use of laboratory animals.

Study design

The rats were randomly allocated into four groups of eight animals:

(1) Placebo control: animals infused with 0·1 M acetic acid.

(2) EGF group: animals infused with 30 μg/rat/day human recombinant (hr) EGF.

(3) IGF-I group: animals infused with 400 μg/rat/day hrIGF-I.

(4) EGF/IGF-I group: animals infused with 30 μg/rat/day hrEGF and 400 μg/rat/day hrIGF-I.

The growth factors were dissolved in 0·1 M acetic acid and delivered by osmotic minipumps (Model 2002, Alzet, Alza, Palo Alto, CA, USA) subcutaneously implanted on the back of each rat under light ether anesthesia. The infusion rate was 1 μl/h for 7 days.

Treatment was initiated at day 0. The animals were weighed at days 0, 2, 4, and 7. At day 7 the animals were anesthetized with pentobarbital (50 mg/kg i.p.). Non-fasting blood samples were collected from the retrobulbar plexus through heparinized capillary tubes under light ether anesthesia. On day 7 the animals were decapitated, the serum samples were kept at − 80 °C for later analysis. The left kidney and liver were removed, weighed and snap frozen in liquid nitrogen.

Serum IGF-I

Serum IGF-I was extracted using acid ethanol (30 μl serum and 750 (μl acid ethanol) in order to determine total levels of circulating IGF-I as described previously (Daughaday *et al.* 1980). Serum extracts were diluted 1:200 in assay buffer (40 mmol/l phosphate pH 8·0, 0·6 mmol/l sodium merthiolate and 5% (w/v) human serum albumin). IGF-I concentrations were analyzed by an in-house IGF-I RIA using a polyclonal rabbit antibody (Nichols Institute Diagnostics, San Capistrano, CA, USA) and rhIGF-I as standard (Amgen Biologicals, CA, USA, purchased from Amersham International, Amersham, Bucks, UK). Mono-iodinated rhIGF-I (^{125}I-(Tyr^{31}) IGF-I) was obtained from Novo Nordisk A/S Bagsvaerd, Denmark. The intra-assay and inter-assay coefficients of variation were 5% and 10% respectively.

Serum IGFBPs

SDS-PAGE and Western ligand blotting (WLB) analyses were executed according to the method of Hossenlopp (Hossenlopp *et al.* 1986) as described previously (Flyvbjerg *et al.* 1992).

Gene-expression of IGF system genes (mRNA) in tissues

Gene-expression of IGFBP 1–4 and -5 (renal) was measured by Northern blot analysis. Total RNA was extracted from kidney and liver samples by the guanidinium thiocyanate method (Chomczynski & Sacchi 1987). Glyoxilated-RNA samples were electrophoresed in 1% agarose gels submerged in 10 mM sodium phosphate (pH 7·2) and transferred to nylon membranes (Hybond N$^+$, Amersham, Hertogenbosch, The Netherlands). Filters were hybridized with 1–2 × 10^6 c.p.m. per ml of ^{32}P-labeled cDNA fragments encoding for each of the six mouse IGFBPs (Schuller *et al.* 1994) and 18S rRNA at 65°C, according to the method of Church & Gilbert (1984).

Gene expression of IGFBP-5 (hepatic), IGFBP-6, IGF-I, -II, IGF-I receptor, and the IGF-II/mannose-6-phosphate (Man-6-P) receptor was below Northern blot detection levels and was measured by a semi-quantitative reverse transcriptase (RT) PCR approach using 18S internal standards (Ambion, Austin, TX, USA). One microgram of total RNA was reverse transcribed with 200 units of Moloney murine leukaemia virus (M-MLV) reverse transcriptase (Gibco BRL, Breda, The Netherlands) and primed with 3′-primers specific for the IGF-system genes. The RT reaction was carried out in 20 μl (total volume) cDNA buffer (75 mM KCl, 3 mM $MgCl_2$, 50 mM Tris–HCl, pH 8·3), 10 mM DTT, 1 mM of each deoxy-NTP, and 20 U of RNAse guard (Pharmacia, Roosendaal, The Netherlands). The reaction mix was incubated for 1 h at 37 °C, heated to 95 °C for 5 min, and then diluted to 100 μl. The PCR reaction was carried out with 2 μl of diluted RT mixture in PCR buffer (50 mM KCl, 1·5 mM $MgCl_2$, and 20 mM Tris–HCl, pH 8·3), 25 pmol forward and reverse primers and 1U of

Table 1 (a) Body weight at the start (day 0) and end (day 7) of the study, liver weight at day 7 and kidney weight at day 7 (all mean ± S.E.M., $n=8$) in adult female rats treated with placebo, EGF, IGF-I or EGF+IGF-I. (b) Liver and kidney weights, expressed as percentage of total body weight and related to placebo control animals

(a)

	Placebo control	EGF	IGF-I	EGF+IGF-I
Body weight (g), day 0	190·6 ± 0·4	190·5 ± 0·4	190·5 ± 0·4	190·3 ± 0·3
Body weight (g), day 7	191·7 ± 2·1	191·0 ± 2·3	196·6 ± 2·7*	202·6 ± 2·2**#
Liver weight (mg), day 7	3970 ± 347	4528 ± 591	4225 ± 302	4236 ± 386
Kidneys weight (mg), day 7	1177 ± 19	1213 ± 16	1456 ± 15***	1519 ± 23***

(b)

	Placebo control	EGF	IGF-I	EGF+IGF
Liver	100	114	104	101
Kidneys	100	104	121***	123***

*P=0·04, **P=0·002, ***P=<0·001, when compared with placebo controls.
#P=0·002, when compared with the IGF-I infusion group.

Hi-Taq DNA Polymerase (Bioprobe Systems, France). PCR reactions were performed separately and the products analyzed on 1% agarose gel and transferred to nylon membranes (Hybond N^+, Amersham). Filters were hybridized with $1–2 \times 10^6$ c.p.m. per ml ^{32}P-labeled cDNA fragments encoding for the corresponding IGF-system genes and 18S rRNA. Oligonucleotide primers designed for the amplification were purchased from Pharmacia Biotech and were as follows: IGFBP-5: sense primer, 5′-TTGCCTCAACGAAAAGAGC-3′; antisense primer, 5′-AGAATCCTTTGCGGTCACA (size of corresponding PCR fragment: 377 bp). IGFBP-6: sense primer, 5′-GTCTACAGCCCTAAGTGCGCCCCAG-3′; antisense primer, 5′-CACAGTTTGG-CACATAGA GCCCAC (size of corresponding PCR fragment: 345 bp). IGF-I: sense primer 5′-AAAATGACCGCACCTCC A-AC-3′; antisense primer, 5′-AGATCACAGCTCC GGAAGCA-3′ (size of corresponding PCR fragment: 299 bp). IGF-II: sense primer 5′-GCCCCGGAGAGAC TCTGTGCG-3′; antisense primer, 5′-GCCCACGGGG TATCTGGGG-AA-3′ (size of corresponding PCR fragment: 253 bp). IGF-I receptor: sense primer, 5′-GAG TACAACTACCGCTGCTGGAC-3′; antisense primer, 5′-AGGGGCCTTCACAGGGGA-TACA-3′ (size of corresponding PCR fragment: 380 bp). IGF-II/Man-6-P receptor: sense primer, 5′-CCAGTTTCTTCTGCCAG CAAGGG-3′; antisense primer, 5′-GCTCCTCTCC AAG-AGCCTCTAC-3′ (size of corresponding PCR fragment: 330 bp).

Quantification

Autoradiographs of ligand blots were scanned using a laser densitometer (Shimadzu model CS 90001 PC, Shimadzu Europe GmbH, Duisburg, Germany) and the relative densities of the bands expressed in arbitrary absorbency units per square millimeter (AU/mm^2). Northern blots were scanned on a Phosphor Imager (Molecular Dynamics, Sunnyvale, CA, USA) and quantified using ImageQuant software (Molecular Dynamics, Sunnyvale, CA, USA). All measured IGF-system results were expressed relative to 18S rRNA levels, as mean ± S.E.M., with n indicating the number of animals studied.

Statistical analysis

Data were examined for distribution, variance homogeneity (F-test) and analyzed using Student's unpaired t-test if appropriate. Otherwise, the significance of difference was compared by one-way analysis of variance (ANOVA) followed by pair-wise comparisons with the least significant difference (LSD)-method. The interaction between EGF and IGF-I administration was compared by 2-way ANOVA followed by a regression model to assess the significance of the contribution of EGF and IGF-I. All data are expressed as mean ± S.E.M., with n indicating the number of rats studied and P-values less than 5% are considered significant.

Results

Body weight

In Table 1A the body weights of the different study groups at the beginning (day 0) and end (day 7) of the experimental period are shown. The mean body weights on day 0 were between 190 and 191 g with no differences between the groups. At day 7, body weights of control animals and rats treated with EGF were identical. Rats treated with IGF-I or EGF/IGF-I significantly increased weight to 197 ± 3 g (P=0·04 day 0 versus day 7) and 203 ± 2 g (P=0·002 day 0 versus day 7) respectively. In addition, the EGF/IGF-I treated group demonstrated a significantly increased weight gain when compared with the group that received EGF alone (EGF versus EGF/IGF-I, P=0·002).

Table 2 Serum IGF-I and serum IGFBP levels (all mean ± S.E.M., $n=8$) in adult female rats after 7 days of placebo, EGF, IGF-I or EGF+IGF-I treatment

	Placebo control	EGF	IGF-I	EGF+IGF-I
IGF-I (μ/l)	438 ± 15	396 ± 25	1537 ± 92***	964 ± 94*#
IGFBP-3 (AU/mm^2)	180·4 ± 12·9	78·8 ± 8·6***	325·8 ± 17·0***	213·9 ± 32·1
IGFBP-1 and -2 (AU/mm^2)	36·7 ± 5·8	40·6 ± 5·8	90·7 ± 10·7**	112·1 ± 20·0**
IGFBP-4 (AU/mm^2)	22·4 ± 1·7	36·9 ± 3·4*	19·2 ± 2·2	40·7 ± 5·0**

*$P \leq 0{\cdot}02$, **$P \leq 0{\cdot}008$, ***$P \leq 0{\cdot}001$, when compared with placebo controls.
#$P=0{\cdot}01$, when compared with the IGF-I infusion group.

Liver and kidney weights

At the end of the treatment the weights of the liver and kidneys were determined (Table 1A).

When compared with placebo control, infusion regimens did not demonstrate a significant effect on liver weight. In kidneys, EGF infusion alone did not result in an organ-specific weight increase while IGF-I and EGF/IGF-I treatments significantly increased the weight to 121% ($P<0{\cdot}001$) and 123% ($P<0{\cdot}001$) of the control weight, respectively (Table 1B).

Serum IGF-I

By the end of the infusion period serum IGF-I levels were determined in all infusion groups (Table 2). In the EGF-treated group serum IGF-I levels were unchanged (396 ± 25 μg/l). In the IGF-I treated group the IGF-I levels were significantly increased to 1537 ± 92 μg/l ($P<0{\cdot}001$). In the group treated with EGF/IGF-I IGF-I levels significantly increased to 964 ± 94 μg/l ($P=0{\cdot}01$) However, compared with the IGF-I infusion group the increase in IGF-I was significantly lower in the EGF/IGF-I group ($P=0{\cdot}01$).

Serum IGFBPs

Using WLB four distinct bands were obtained. A double band at 42 and 38 kDa represents IGFBP-3, a single band at 30 kDa represents IGFBP-1 and -2 and a 24 kDa band represents IGFBP-4 (as described previously) (Flyvbjerg *et al.* 1992).

In the EGF-treated group IGFBP-3 levels were significantly lowered to 44% of control values ($P<0{\cdot}001$) whereas in the IGF-I treated group IGFBP-3 levels were significantly increased to 181% of control values ($P<0{\cdot}001$). In the EGF/IGF-I-treated group the circulatory IGFBP-3 values remained unchanged (Table 2). Circulating 30 kDa IGFBPs (IGFBP-1 and -2) were unchanged in the EGF treatment group. However, in the IGF-I and EGF/IGF-I groups the 30 kDa IGFBPs (IGFBP-1 and -2) were significantly increased to 247% and 305% of control values (both $P=0{\cdot}007$). IGF-I infusion did not significantly modify IGFBP-4 levels, whereas EGF and EGF/IGF-I infusion demonstrated significantly increased IGFBP-4 levels (165% and 182% of control values; $P=0{\cdot}02$ and $P=0{\cdot}008$ respectively) (Table 2).

Hepatic IGF system expression

Compared with the mRNA expression in control rats, EGF infusion did not significantly modify hepatic IGF system mRNA levels (Fig. 1).

IGF-I-infused animals demonstrated significantly increased hepatic IGFBP-1 ($P=0{\cdot}03$) and IGFBP-3 ($P=0{\cdot}001$) mRNA levels when compared with placebo infused rats (Fig. 1A), whereas IGFBP-5 mRNA levels were significantly decreased in the IGF-I infusion group ($P=0{\cdot}05$) (Fig. 1B). IGFBP-5 mRNA levels in the EGF/IGF-I infusion group were the result of a significant interaction between EGF and IGF-I that, however, could not be significantly attributed to EGF and/or IGF-I.

In the EGF/IGF-I infusion group, hepatic IGFBP-1, -2 and -3 mRNA levels were significantly increased ($P=0{\cdot}03$, $P=0{\cdot}01$ and $P=0{\cdot}001$ respectively) (Fig. 1). IGFBP-2 mRNA levels in the EGF/IGF-I infusion group were the result of a significant interaction between EGF and IGF-I that could be significantly attributed EGF ($P<0{\cdot}001$) and IGF-I ($P<0{\cdot}02$).

Hepatic IGF-II and IGF-I receptor mRNA could not be reliably quantified in any of the groups.

Renal IGF system expression

Compared with the expression levels in control rats, EGF infusion did not change renal mRNA levels of most of the IGF system genes. Only IGFBP-4 demonstrated significantly increased ($P=0{\cdot}03$) whereas IGFBP-6 demonstrated significantly decreased ($P=0{\cdot}03$) renal mRNA levels (Fig. 2A and B). IGF-I-infused animals demonstrated significantly decreased renal IGFBP-6 mRNA levels ($P=0{\cdot}001$) (Fig. 2B).

In the EGF/IGF-I infusion group, the renal IGFBP-4 mRNA levels were significantly increased ($P=0{\cdot}05$) (Fig. 2A), whereas renal IGFBP-6 and IGF-II/Man-6-P receptor mRNA levels were significantly decreased ($P=0{\cdot}02$ and $P=0{\cdot}01$ respectively) (Fig. 2B). IGFBP-6 mRNA

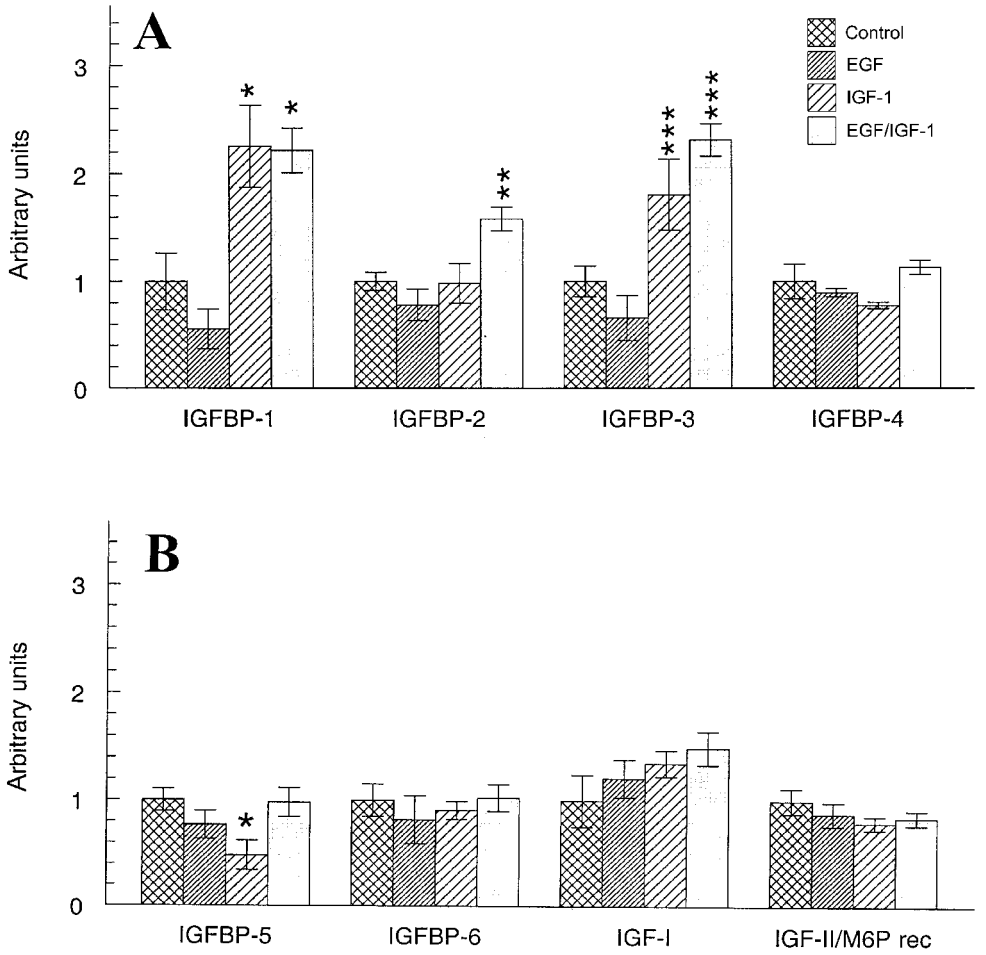

Figure 1 (A) Hepatic IGFBP-1, -2, -3 and -4 mRNA levels in young adult female rats, infused for 7 days with acetic acid (placebo control), 30 μg/kg/day EGF, 400 μg/kg/day IGF-I and 30 μg/kg/day EGF+400 μg/kg/day IGF-I. Values are based on quantitations of Northern blots where each lane contained 20 μg of total RNA and was compensated for RNA loading differences. Values are represented as means ± S.E.M. ($n=8$) and expressed relative to placebo control. *$P=0{\cdot}03$, **$P=0{\cdot}01$, ***$P=0{\cdot}001$, statistical significance level between the indicated group and the placebo control. (B) Hepatic IGFBP-5, -6, IGF-I and IGF-II/Man-6-P receptor mRNA levels in young adult female rats, infused for 7 days with acetic acid (placebo control), 30 μg/kg/day EGF, 400 μg/kg/day IGF-I and 30 μg/kg/day EGF+400 μg/kg/day IGF-I. Values represent the quantified outcome of RT-PCR reactions, compensated for differences in efficiency and the amount of starting material. Values are represented as means ± S.E.M. ($n=8$) and expressed relative to placebo control. *$P=0{\cdot}05$, statistical significance level between the indicated group and the placebo control.

levels in the EGF/IGF-I infusion group were the result of a significant interaction between EGF and IGF-I that could be significantly attributed to EGF ($P=0{\cdot}04$) and IGF-I ($P=0{\cdot}006$).

Renal IGF-I and IGF-II mRNA could not be reliably quantified in any of the groups.

Discussion

In the present study we investigated the effects of short-term systemic treatment with EGF and/or IGF-I on growth parameters and hepatic and renal expression of the IGF system genes in young adult female rats.

EGF infusion did not show any effect on body weight gain. This is in agreement with our earlier EGF infusion study in young adult male rats where EGF administration for 4 weeks (400 μg/kg/day), did not significantly change body weight gain (Frystyk *et al.* 1996). In newborn animals, EGF treatment (doses 50–1000 μg/kg/day) results in retarded organ and body growth (Hoath *et al.* 1988). This growth retardation was most prominent when EGF was applied directly after birth and persisted until weaning (Hoath 1986). EGF is normally present in very

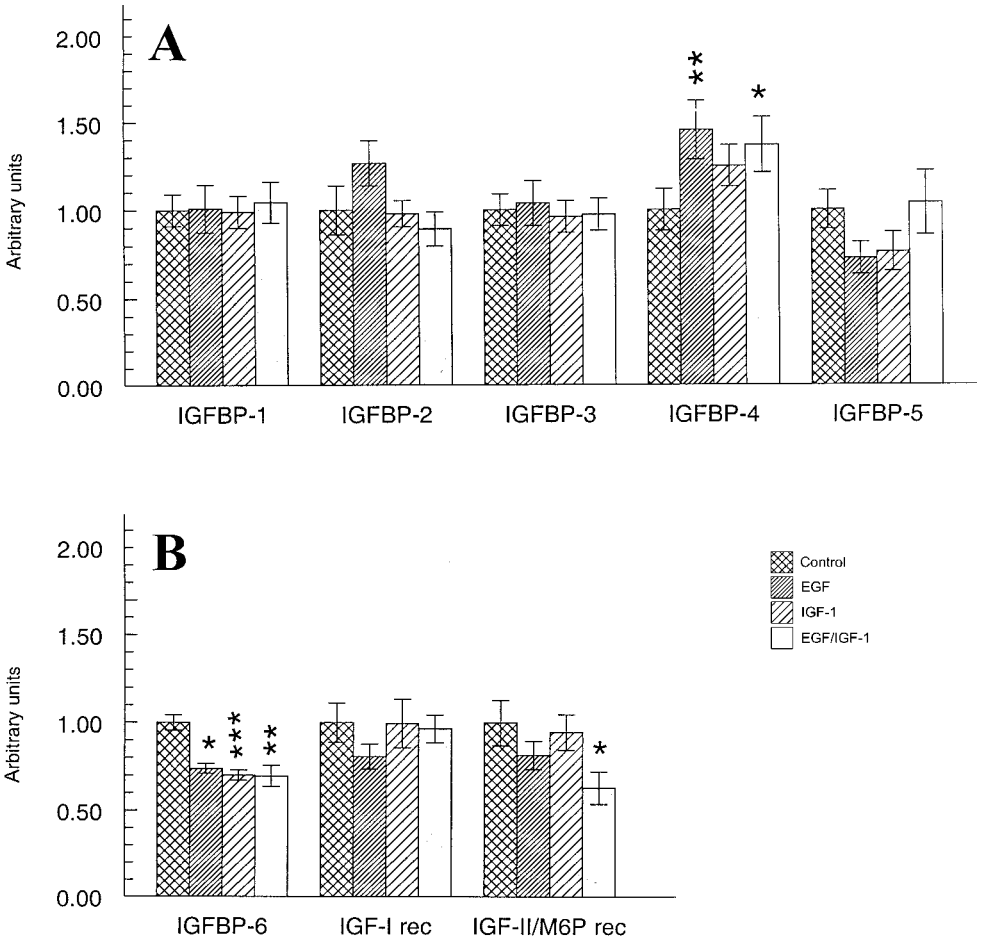

Figure 2 (A) Kidney IGFBP-1, -2, -3, -4 and -5 mRNA levels in young adult female rats, infused for 7 days with acetic acid (placebo control), 30 μg/kg/day EGF, 400 μg/kg/day IGF-I and 30 μg/kg/day EGF+ 400 μg/kg/day IGF-I. Values are based on quantitations of Northern blots where each lane contained 20 μg of total RNA and was compensated for RNA loading differences. Values are represented as means ± S.E.M. (n=8) and expressed relative to placebo control. *P=0·05, **P=0·03, statistical significance level between the indicated group and the placebo control. (B) Kidney IGFBP-6, IGF-I receptor and IGF-II/Man-6-P receptor mRNA levels in young adult female rats, infused for 7 days with acetic acid (placebo control), 30 μg/kg/day EGF, 400 μg/kg/day IGF-I and 30 μg/kg/day EGF+400 μg/kg/day IGF-I. Values represent the quantitated outcome of RT-PCR reactions, compensated for differences in efficiency and the amount of starting material. Values are represented as means ± S.E.M. (n=8) and expressed relative to placebo control. *P=0·02, **P=0·01, ***P=0·005, statistical significance level between the indicated group and the placebo control.

low (0·1<2 μg/l) quantities in circulation (Nexø *et al.* 1992). Systemic administration at the doses used in these studies, therefore, represents a pharmacological situation.

In contrast to EGF infusion, IGF-I infusion resulted in a significantly increased body weight gain. This is in agreement with an earlier study where 240 μg/rat/day IGF-I was infused for 14 days in 6-week-old female rats (van Neck *et al.* 1997). EGF/IGF-I infusion also resulted in a significantly increased body weight gain. However, weight gain in this group did not significantly differ from the IGF-I infusion group.

Liver weight was not increased in any of the growth factor treatment regimens. This is in agreement with earlier studies (Frystyk *et al.* 1996, van Neck *et al.* 1997). In contrast, kidney weight was significantly increased in the IGF-I and in the EGF/IGF-I treatment groups but not by EGF treatment alone. The observed effect of IGF-I infusion on kidney weight is consistent with earlier findings (Gruaz *et al.* 1997, van Neck *et al.* 1997), as is the ineffectiveness of EGF on kidney growth (Frystyk *et al.* 1996). This is in contrast to the findings of Breider *et al.* (1996) who infused male and female animals with

100 μg/kg/day EGF for 4 weeks and observed both liver and kidney enlargement. Interestingly, in this study, kidney enlargement only was observed in female animals.

The effects of systemic EGF administration have been extensively studied in neonatal rats. In newborn animals EGF administration reduced the circulatory IGF-I levels but increased the mRNA and protein expression of IGFBP-1 (Murray *et al.* 1993). Older animals demonstrated strongly diminished responses to EGF infusion. In our study, EGF infusion did not affect the 30 kDa IGFBPs (IGFBP-1 and -2) and the hepatic mRNA expression did not change. In contrast, treatment with EGF/IGF-I increased the 30 kDa protein band and the hepatic IGFBP-1 and -2 mRNA expression. IGFBP-4 was increased in both the EGF and EGF/IGF-I infusion groups whereas the corresponding hepatic mRNA levels were unchanged. EGF infusion reduced IGFBP-3 levels. This was also observed in previous studies (Frystyk *et al.* 1996, Vinter-Jensen *et al.* 1996). In addition, the EGF/IGF-I infusion group also revealed reduced IGFBP-3 levels when compared with the IGF-I infusion group, however, with unchanged hepatic IGFBP-3 mRNA levels. This is in contrast to *in vitro* studies where a 5-fold reduction in IGFBP-3 mRNA was demonstrated in cultured keratinocytes by treatment with EGF (Wraight & Werther 1995, Edmondson *et al.* 1999). In our *in vivo* experiment, a post-transcriptional event probably underlies the observed decrease in IGFBP-3, as hepatic IGFBP-3 mRNA was not significantly decreased in the EGF and EGF/IGF-I infusion groups. To test the effect of EGF on IGFBP-3 stability, ^{125}I-IGFBP-3 was incubated with different doses of EGF ranging from 0·001 to 500 μg/ml. EGF had no effect on IGFBP-3 proteolysis *in vitro* excluding a direct IGFBP-3 proteolytic effect (data not shown). EGF infusion did not influence the hepatic mRNA levels of the other members of the IGF-system. Barreca described a dose-dependent stimulation of IGF-I secretion in cultured liver cells, already detectable at a concentration of 30 μg/l (Barreca *et al.* 1992). In our rat *in vivo* experiments, however, the EGF infusion group did not show a stimulatory effect on circulatory IGF-I levels, nor did EGF infusion significantly increase hepatic IGF-I mRNA expression.

In the IGF-I infusion group, circulatory levels of the 30 kDa IGFBPs (IGFBP-1 and -2) and IGFBP-3 were significantly increased. These changes were reflected by corresponding changes in the hepatic mRNA profiles of IGFBP-1 and -3. In a previous study, IGF-I infusion for 14 days also upregulated hepatic IGFBP-3 mRNA levels (van Neck *et al.* 1997). Compared with the IGF-I infusion group, IGFBP-3 levels in the EGF/IGF-I infusion group were significantly decreased. Decreased IGFBP-3 levels may result in a release of free, biodegradable IGF-I which may explain the decreased circulating pool of IGF-I as observed in the EGF/IGF-I group.

In the IGF-I infusion group, circulatory IGF-I levels were increased 3-fold, achieving levels similar to those measured in an earlier IGF-I infusion study (van Neck *et al.* 1997). In the EGF/IGF-I infusion group, circulatory IGF-I levels were significantly reduced. As identical amounts of IGF-I were infused in both the EGF/IGF-I and IGF-I group, EGF, therefore, seemed to decrease the amount of circulatory IGF-I without affecting its mRNA level.

In kidney, only IGFBP-6 mRNA levels were lower in the IGF-I treatment group. IGF-I infusion did not influence the local expression of the other IGF-system members despite its pronounced effects on kidney growth.

To our knowledge the present study is the first to investigate the very complex interaction or cross-talk between the EGF and the IGF-system *in vivo*. IGF-I and EGF/IGF-I infusions significantly increased circulatory IGF-I but had no effect on hepatic IGF-I levels. Kidney weight was also significantly increased in these groups. EGF infusion lowered circulatory IGFBP-3 without an effect on hepatic IGFBP-3 mRNA levels.

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