Acute effects of insulin-like growth factor I on inter-organ amino acid flux in protein-catabolic dogs

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The effects of acute administration of human recombinant insulin-like growth factor-I (rhIGF-I) on amino acid (AA) flux between hindlimbs, liver and gut were investigated in anaesthetized post-operative dogs. rhIGF-I produced about a 10-fold increase in plasma IGF-I concentrations above baseline values ($P < 0.001$), increased the plasma levels of glucagon and adrenaline ($P < 0.05$), and evoked a fall in plasma glucose ($-55 \pm 8\%$; $P < 0.001$) and plasma total AA levels ($-23 \pm 8\%$; $P < 0.05$). AA flux in post-absorptive dogs under NaCl infusions was characterized by an efflux of AA from the hindlimbs (as a result of the protein-catabolic situation), an equal AA balance across the gut and an AA uptake by the liver. The administration of rhIGF-I increased hepatic AA uptake in the NaCl group from 3.51 ± 0.8 to 7.5 ± 0.4 μmol/min per kg ($P < 0.01$) and in the AA-infused group from 16.8 ± 0.6 to 22.4 ± 1.5 μmol/min per kg ($P < 0.05$), but did not influence the AA balance across hindlimbs and gut. Glucose infusions normalized the plasma concentrations of counter-regulatory hormones without influencing the inter-organ AA balances. We conclude that hypoaminoacidaemia caused by rhIGF-I infusions is the result of a stimulated AA uptake by the liver, but is unrelated to alterations of AA exchange across the hindlimbs.

INTRODUCTION

Insulin-like growth factor-I (IGF-I) is a polypeptide with M_r 7600 that is structurally related to insulin [1]. IGF-I produces a variety of metabolic effects. At supraphysiological doses, IGF-I lowers blood glucose in non-diabetic animals and humans, with a hypoglycaemic potency of 1.5–7% compared with that of insulin on a molar basis [2]. IGF-I stimulates growth in several pathophysiological conditions associated with low IGF-I levels, e.g. hypophysectomized rats [3], diabetic rats [4] and Snell dwarf mice [5].

In mediating these effects, IGF-I not only stimulates cell growth but also increases protein accretion. It was shown that IGF-I accelerates amino acid (AA) uptake from hepatocytes [6], increases net protein synthesis in cartilage [7], enhances tyrosine incorporation in isolated muscles of lean mice [8] and lowers circulating AAs by decreasing protein breakdown in normal rats [9]. Prolonged infusions of IGF-I increase body weight in thermally injured rats [10], decrease the rate of loss of protein in lambs during infusion of recombinant tumour necrosis factor [11] and reverse diet-induced catabolism in humans [12], but are ineffective in improving nitrogen balance in normal human subjects [13].

The mechanisms involved in these changes of AA metabolism are not yet clear. Therefore the purpose of this study was to investigate the influence of recombinant human (rh) IGF-I on the inter-organ transfer of AAs in dogs. We performed our study in a catabolic situation where amino nitrogen is delivered from skeletal muscle to the liver.

METHODS

Animal preparation

Male Beagle dogs aged about 2 years and weighing 10–15 kg were used. The dogs were fasted overnight (12 h). On the morning of the study all dogs were subjected to general anaesthesia using N_2O/O_2 (1:1) plus 0.5% halothane. Laparotomy was performed through a midline abdominal incision for placement of silicone rubber catheters (1 mm inner diameter, 2.2 mm outer diameter; Dow Corning, Midland, MI, U.S.A.). The catheters were implanted into the femoral artery, portal vein, hepatic vein and femoral vein as described previously [14,15]. Another catheter was placed in the internal jugular vein and was used for infusion of the substrates: saline (0.9% NaCl), AAs, glucose and rhIGF-I. Flow probes of appropriate inner and outer diameters (Heilige G.m.b.H., Freiburg, Germany) were placed around the vena cava and the portal vein. The operation and the implantation of the catheters took about 1 h. The experiment was started 30 min after completion of these procedures.

Experimental design

We compared two groups of experimental animals (six dogs in each group) with two groups of control animals (five dogs per group). Each animal of the experimental groups received a primed (40 μg/kg) continuous (1.5 μg/min per kg) infusion of rhIGF-I (Kabi Biopharma, Stockholm, Sweden) for 180 min and either a saline or an AA infusion (2.2 mg of AA/min per kg, solution of 10% Vamin, from Kabi Nutrition, Stockholm, Sweden). The dosage was chosen according to a pilot experiment in which this dose had been shown to lower the plasma glucose level by about 40%. All dogs in the two experimental groups received glucose solutions starting 90 min after the start of rhIGF-I administration. Euglycaemic conditions with glucose levels as found in the pre-period were held between 60 and 90 min after the start of the glucose load. The control animals received either NaCl or AAs (same dosage as the experimental group) for 180 min, but without infusion of glucose.

For determination of arterial plasma concentrations of IGF-I, glucose, AAs, insulin and glucagon, blood was drawn 10 min

Abbreviations used: rhIGF-I, recombinant human insulin-like growth factor-I; AA(s), amino acid(s).

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before and at the start of rhIGF-I administration, at 10 min intervals thereafter up to 90 min, and at 150, 165 and 180 min. After the start of the glucose infusions, arterial glucose levels were determined every 5 min. Organ balances and plasma levels of cortisol and catecholamines were analysed 60, 75, 90, 150, 165 and 180 min after the start of the infusion programme. At these time points, blood-flow measurements were performed and blood was drawn simultaneously from the femoral artery and the hepatic, portal and femoral veins.

Analytical methods

Plasma glucose and lactate were determined enzymically (Boehringer Mannheim Biochemicals, Indianapolis, IN, U.S.A.) Total plasma IGF-I was determined after acid-ethanol extraction and measured with a double-antibody radioimmunoassay method (IRMA-mat IGF-I test kit; Byk-Sangtec, Dietzenbach, Germany). Glucagon, insulin (Serono, Milano, Italy) and cortisol (Incstar, Stillwater, MN, U.S.A.) were estimated by commercial radioimmunoassay methods.

Adrenaline and noradrenaline were analysed by h.p.l.c. with electrochemical detection [16].

Blood samples for determination of plasma AAs (2 ml) were drawn in heparinized syringes. For determination of AAs, 1 ml of plasma was deproteinized with 0.1 ml of 3% sulphosalicylic acid containing β-thienylalanine (1 nM) as the internal standard. After centrifugation, 1 part of the sample was diluted with 99 parts of water containing 2 mmol of NaN_3. The AAs were analysed by automatic pre-column derivative formation with *o*-phthalaldehyde and h.p.l.c. separation [17], with some modifications as described previously [14].

Blood flow in the artery and portal vein was measured electromagnetically and was corrected for the corresponding packed cell volume to obtain plasma flow values. The hindlimb was assumed to contain 25% of the total skeletal muscle; therefore, plasma (blood) flow in the hindlimb was multiplied by 4 to calculate plasma (blood) flow of the total skeletal muscle [18]. The hepatic and muscle plasma flows determined in the present experiment were similar to those that we and others had reported previously [14,15,18].

Calculation and statistics

Net release or removal of AAs was calculated by multiplying the arteriovenous plasma concentration differences by the rate of plasma flow. In the liver there is a dual supply of substrates, i.e. via the portal vein and the hepatic artery. It was assumed that 72% of the hepatic blood flow was via the portal vein and 28% via the hepatic artery [19,20]. These plasma flow values were multiplied by the respective concentrations in the portal vein and artery to calculate the total amount of substrate inflow into the liver. Balances across the hindlimbs were assumed to be representative of balances across skeletal muscle. We chose to refer to the substrate balance across the extrahepatic splanchnic tissue as that of 'gut'. A positive balance indicates net substrate uptake (disappearance), and a negative balance indicates net substrate release. Neutral balance is defined as not being different from zero. Organ balances were determined at 60, 75 and 90 min ('hypoglycaemic state') and at 150, 165 and 180 min ('normoglycaemic state') after the start of rhIGF-I administration. Since there was no significant difference among the balances done either at 60, 75 and 90 min or at 150, 165 and 180 min, they were averaged.

Analysis of variance with repeated measures was used to determine whether there was a difference between the values obtained for the experimental and control groups respectively. Analysis of statistical differences were performed by using Wilcoxon's matched-pairs signed rank test, and (exact permutational) *P* values were derived in view of the small sample size. All data are expressed as means ± S.E.M.

RESULTS

Anaesthesia and operation procedures (abdominal incision, catheter insertion) increased the pre-operative plasma levels of glucose from 5.8 ± 0.16 to 7.0 ± 0.27 mM ($P < 0.01$), of urea from 4.3 ± 0.2 to 8.3 ± 0.4 mM ($P < 0.001$) and of glucagon from 120 ± 12 to 231 ± 21 pg/ml ($P < 0.01$). The plasma levels of total AA (before, 2850 ± 170, after, 2703 ± 97 μM) and insulin (before, 10.6 ± 0.9, after, 11.4 ± 0.76 μ-units/ml) remained unchanged by the operation procedure. The post-operative values were measured 30 min after the end of the operation, immediately before the onset of the experimental procedures.

During the basal period, the average plasma flow rates in the hindlimbs, liver and gut of all dogs were 6.2 ± 0.2, 18.6 ± 1.2 and 13.8 ± 1.0 ml/min per kg. rhIGF-I infusions did not alter the blood flow in any of the tissue beds examined.

Effect of rhIGF-I on insulin, glucagon, adrenaline, noradrenaline and cortisol levels

A 90 min infusion of rhIGF-I produced an 8–10-fold increase in plasma IGF-I concentrations above baseline, from 81 ± 15 to

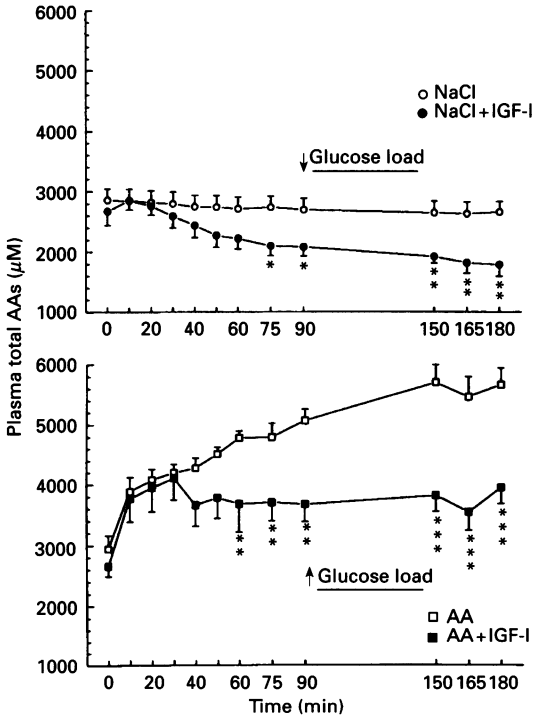

Figure 1 Arterial total AA levels

Arterial total AA levels were measured during saline (NaCl) or AA (2.2 mg of AA/min per kg) administration or during infusions of IGF-I (40 μg/kg + 1.5 μg/min per kg) together with either saline (NaCl + IGF-I) or AA (AA + IGF-I). The two IGF-I groups received a glucose load at 90 min after the start of IGF-I infusions, to obtain normoglycaemic conditions. Results are expressed as means ± S.E.M. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus the respective control group.

Table 1 Arterial concentration of AAs after infusion of NaCl (control), NaCl + IGF-I and AA (control), or AA + IGF-I, measured 90 min after start of administration

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, versus corresponding control group.

	Concn. (μM)			
	NaCl infusion		AA infusion	
	Control	IGF-I	Control	IGF-I
Glutamate	18.8±1.9	13.4±1.3*	69±3.8	48±5.9*
Alanine	672±120	587±101	1213±65	858±47**
Glutamine	401±28	301±25*	721±41	563±48*
Asparagine	51±6.6	37±5.4	51±3.1	43±4.3
Serine	118±10.6	96±9.8	264±33	168±21*
Histidine	80±6.7	62±4.7*	167±16.1	124±14.7
Glycine	168±5.1	141±3.8**	432±17.4	280±25***
Threonine	235±26	183±23	434±68	274±34
Citrulline	64±8.7	55±6.3	59±6.2	68±8.0
Taurine	98±15.2	81±16.1	83±9.8	76±9.4
Arginine	138±17.0	89±10.3*	316±21	213±36
Tyrosine	32±1.8	18±2.6**	34±1.2	25±4.7
Valine	187±17.4	138±13.9*	332±20	263±32
Isoleucine	81±10.7	49±6.2*	204±14.3	118±16.6**
Leucine	147±12.5	95±6.0**	255±19	187±28
Methionine	50±4.2	37±5.1	125±7.7	87±14.8*
Tryptophan	66±5.4	52±4.2	60±4.9	54±3.3
Phenylalanine	58±9.4	43±6.8	111±4.5	119±13.0
Total AAs	2710±211	2128±167*	4923±133	3633±276**

876±89 ng/ml in the NaCl group and from 93±12 to 794±66 ng/ml in the AA group ($P < 0.001$). At 150 min after the start of the rhIGF-I infusions, while maintaining plasma glucose at baseline (coefficient of variance < 5%), plasma IGF-I levels were 959±100 ng/ml in the NaCl group and 923±95 ng/ml in the AA group. A 90 min infusion of rhIGF-I did not alter plasma insulin levels in the NaCl group (12.3±0.8 versus 11.2±1.0 μ-units/ml), but evoked a significant decrease in plasma insulin in the AA group (13.8±1.4 versus 8.9±0.6 μ-units/ml; $P < 0.05$). During combined infusions of glucose and rhIGF-I, plasma insulin levels rose to 14.3±2.1 μ-units/ml in the NaCl group and to 14.4±2.8 μ-units/ml in the AA group (not significant). Insulin levels in the two control groups remained unchanged. In the hypoglycaemic state, 60–90 min after onset of the rhIGF-I infusions, plasma glucagon and plasma adrenaline levels were significantly higher ($P < 0.05$) in both experimental groups. Glucagon increased to 497±82 pg/ml in the saline group ($P < 0.01$) and to 281±104 pg/ml ($P < 0.05$) in the AA group (controls 164±26 and 173±21 pg/ml, respectively). Adrenaline rose to 630±189 pg/ml in the saline and to 605±131 pg/ml in the AA group (controls 235±96 and 284±85 pg/ml respectively; $P < 0.05$). During the euglycaemic study period, plasma glucagon and adrenaline values decreased to pre-period concentrations. Plasma cortisol and noradrenaline values were unaffected by the rhIGF-I infusions.

Effect of rhIGF-I on plasma glucose concentrations

Plasma glucose fell rapidly and remained 50–60% below baseline values between 60 and 90 min after the start of rhIGF-I infusions in both experimental groups. The mean glucose levels during the glucose load were 7.2±0.4 mM in the NaCl + rhIGF-I group and 7.8±0.46 mM in the AA + rhIGF-I group.

Effect of rhIGF-I on AA metabolism

Arterial AA concentrations remained constant during NaCl infusions, but increased considerably during AA infusions (+71% after 90 min). In both experimental conditions the addition of rhIGF-I significantly lowered the plasma [−24% ($P < 0.05$) in the NaCl group and −29% ($P < 0.01$) in the AA group]. Glucose loading did not alter plasma AA levels (Figure 1). Table 1 shows the arterial concentrations of individual AAs after 90 min of rhIGF-I infusion. The AA-lowering effect of rhIGF-I was found for virtually all AAs measured.

Table 2 AA balances across skeletal muscle and liver

Values are means±S.E.M. *$P < 0.05$, **$P < 0.01$, versus corresponding control group.

		Balance (μmol/min per kg)			
		NaCl infusion		AA infusion	
Tissue		Control	IGF-I	Control	IGF-I
Skeletal muscle	Alanine	−2.14±0.39	−3.64±0.62*	−1.12±0.15	−2.93±0.60*
	Glutamine	−2.23±0.61	−3.19±0.47	−2.04±0.27	−2.83±0.72
	Glutamate	−0.01±0.11	−0.02±0.02	+0.24±0.02	−0.31±0.06
	Valine	+0.08±0.06	−0.11±0.06*	+0.38±0.08	+0.65±0.07*
	Isoleucine	+0.04±0.04	−0.06±0.07	+0.33±0.06	+0.41±0.06
	Leucine	+0.03±0.06	−0.16±0.04*	+0.31±0.10	+0.52±0.08
	Total AA	−7.28±1.46	−10.31±2.24	−2.63±0.72	−3.64±1.46
Liver	Alanine	+2.40±0.23	+4.30±0.21**	+5.32±0.18	+8.33±1.07*
	Glutamine	+0.20±0.07	+0.61±0.10	+4.31±0.20	+3.92±0.43
	Glutamate	+0.08±0.02	+0.23±0.05*	+0.34±0.11	+0.62±0.14
	Valine	−0.25±0.03	−0.11±0.03	+0.01±0.04	+0.06±0.02
	Isoleucine	−0.18±0.02	−0.06±0.04*	−0.01±0.05	+0.01±0.05
	Leucine	−0.28±0.07	−0.07±0.02*	+0.06±0.05	+0.12±0.04
	Total AA	+3.51±0.80	+7.54±0.40**	+16.81±0.60	+22.43±1.52*

During the saline infusion there was AA release from skeletal muscle and AA uptake by the liver and gut. Infusion of AAs decreased AA release from skeletal muscle ($P < 0.05$) and increased AA uptake by the liver ($P < 0.001$) and gut (0.53 ± 0.31 versus 4.03 ± 1.23; $P < 0.05$) (Table 2). rhIGF-I caused a moderate increase of peripheral release of AAs (not significant) and an increase of hepatic AA uptake ($P < 0.01$ in the NaCl group and $P < 0.05$ in the AA group). Gut AA balances became neutral under rhIGF-I and co-infusion of NaCl or glucose, and remained positive (uptake) under AA infusions. The glucose load had no additional influence on the AA balances.

Of the individual AAs, only alanine, glutamate and the branched-chain AAs valine, leucine and isoleucine were significantly affected (Table 2). The infusion of rhIGF-I increased alanine [+76% ($P < 0.05$) with NaCl and +140% ($P < 0.05$) with AA] release from skeletal muscle, and stimulated alanine uptake from the liver [NaCl infusion +80% ($P < 0.01$); AA infusion +60% ($P < 0.05$)], but did not influence the alanine or glutamine balances across the gut (results not shown). Of the other AAs, IGF-I infusions evoked release of valine and leucine from the hindlimbs during NaCl infusion, and stimulated peripheral uptake of valine during AA infusion. In the liver, IGF-I evoked a higher extraction of glutamate (+82%) and decreased the release of leucine and isoleucine during NaCl infusion.

The administration of rhIGF-I together with glucose to obtain normoglycaemic conditions had no additional effect on the inter-organ balances of the measured AAs.

DISCUSSION

The infusion of rhGF-I in the first period resulted in significant hypoglycaemia, and this was associated with a rise in the plasma levels of the counter-regulatory hormones glucagon and adrenaline. During this period an associated fall in the plasma levels of all measured AAs occurred. Since we have not used radioisotopes during this study, we were unable to determine whether these changes are secondary to decreased protein breakdown, or are due to improved protein synthesis. The influence of rhIGF-I on protein turnover was measured in conscious fasted rats. In this study rhIGF-I evoked a decrease in leucine flux and a decline in the incorporation of [1-^{14}C]leucine into muscle and liver protein, indicating an inhibition of tissue protein synthesis [9]. Similarly, rhIGF-I administration in lambs made catabolic by infusion of recombinant tumour necrosis factor resulted in a 15% decrease in rate of net protein loss [11].

At 90 min after start of rhIGF-I infusion, the plasma levels decreased by about 25%. A similar effect of rhIGF-I on total plasma AA levels was found in conscious fasted rats [9]. In that study it was proved that this effect of rhIGF-I was independent of insulin, which is also known to be potent in decreasing plasma AA. This has been confirmed in our study, since mean plasma insulin values were not affected by the rhIGF-I infusion in the saline group or were even lowered in the dogs receiving AA.

Our study allowed us for the first time to differentiate which of the organs was most affected by rhIGF-I administration. The data clearly indicate that the fall in plasma AAs must have been due to an increased uptake of AAs by the liver. The total plasma AA levels during the administration of rhIGF-I in the two groups varied between 600 and 1300 μM. Taking 1000 μM as an average and dividing this amount by the sum of extracellular water plus plasma water [mean body weight of 12.5 kg $\times$ 0.22 (22% of the total body fluids)] and dividing again by 90 min (the time it took for the change to occur), we obtain a value of about 4 μmol/min per kg. This value corresponds to the increased AA uptake of the liver under IGF-I.

From our data we cannot deduce the intra-hepatic utilization of the AAs taken up at a higher rate. Because of the lowered plasma glucose levels, it is unlikely that rhIGF-I increased the rate of gluconeogenesis. Therefore the increased hepatic uptake of AAs must have been due either to enhanced protein synthesis by the liver for resident proteins or secretory proteins, or to increased use of AAs for oxidation.

Unexpected was the finding that the net release of AAs across the hindlimbs was not decreased by rhIGF-I during both NaCl and AA infusions. In our experimental model, nitrogen is delivered from the hindlimbs to the gut and liver. This is a characteristic metabolic situation in the protein-catabolic state, where muscular proteins are degraded to AAs. Therefore, in this situation AA efflux is stimulated as compared with the conscious non-fasting state [14,18,21]. The reason for the lack of efficiency of rhIGF-I in reducing AA liberation from the skeletal muscle may be related to the hypoglycaemia with increased plasma levels of glucagon and adrenaline. Previous investigations have shown that hypoglycaemia is associated with the stimulation of protein breakdown [22]. Since the levels of both hormones returned to pre-infusion values during the euglycaemic state, and the effects of rhIGF-I on muscular AA release and on hepatic AA uptake were independent of whether the dogs were in hypoglycaemia or euglycaemia, a significant contribution of glucagon or adrenaline to the metabolic changes appears unlikely. Data from muscle preparations indicate that AAs, and particularly the branched-chain AAs valine, isoleucine and leucine, are important in preventing protein breakdown [23]. On the other hand, studies by Abumrad et al. [24] have indicated that the uptake of AAs is dependent on the increase in AAs in the artery. Recently it was shown that a low dose of rhIGF-I failed to effect protein anabolism in normal volunteers [25]; in that study, no alterations in plasma glucose concentration were observed. Rates of proteolysis, protein synthesis and leucine oxidation were unaffected by rhIGF-I administration. This failure of rhIGF-I to induce protein anabolism may be due to the lack of induction of pivotal growth-hormone-dependent serum cofactor(s) necessary for this effect, such as IGF-I binding protein-3.

In conclusion, we were able to demonstrate for the first time that the hypoaminoacidaemia occurring during rhIGF-I infusion results from a stimulated amino acid uptake by the liver. Even in the absence of AA infusions, rhIGF-I increased AA uptake across the liver. However, the maximal effect of rhIGF-I on AA uptake by the liver was achieved in the presence of abundant AA. In contrast, there was hardly any effect of rhIGF-I on the AA metabolism across the skeletal muscle or gut, in either the presence or the absence of AA infusion. This finding provides evidence that rhIGF-I was ineffective in reversing stimulated AA efflux from skeletal muscle in a catabolic state. However, a mediator which stimulates hepatic AA uptake in catabolic situations could be a valuable clinical tool for the treatment of catabolic patients, because impaired liver metabolism is a bad prognostic sign for these types of patients [26].

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