Factors regulating the rat insulin-like growth factor-binding protein-1 response to octreotide

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Abstract

Insulin-like growth factor-binding protein-1 (IGFBP-1) production is increased by somatostatin and its analogues. In order to determine the time course and identify possible mechanisms of this increase *in vivo* we administered octreotide to rats and determined IGFBP-1 concentrations by RIA. After 60 min of anaesthesia, the mean baseline IGFBP-1 concentrations were 166 (95% confidence interval 123 to 225) ng/ml and increased in saline-infused animals to 729 (488 to 1086) ng/ml after 180 min. IGFBP-1 was stimulated transiently in response to octreotide, with circulating IGFBP-1 concentrations peaking at 1605 (1220 to 2111) ng/ml at 105 min during a continuous infusion of octreotide (100 µg/kg per h). In conscious chronically cannulated rats, baseline IGFBP-1 concentrations were 22 (18 to 28) ng/ml, 8-fold less than in the anaesthetised state, and were stimulated in the short term

after administration of an octreotide bolus (100 µg/kg s.c.) to reach 88 (62 to 126) ng/ml at 60 min. A similar response was seen after i.v. administration to conscious rats. Intravenous bolus of octreotide (100 µg/kg) in rats anaesthetised for 3 h resulted in an increase in IGFBP-1 to peak at 1556 (1268 to 1910) ng/ml at 60 min. The IGFBP-1 response to octreotide was diminished in high-fat fed hyperinsulinaemic rats. The pattern of disappearance of iodinated IGFBP-1 from the circulation was not influenced by octreotide. The changes in GH, insulin and glucose concentrations alone did not sufficiently account for the patterns of response observed. We conclude that, in rats, octreotide stimulates IGFBP-1 acutely and this response is potentiated by factors related to anaesthesia.

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Introduction

Circulating concentrations of insulin-like growth factor-binding protein-1 (IGFBP-1) are dynamically regulated in a manner that suggests that it has an acute physiological role (Lee *et al.* 1993, Lewitt 1994). Since IGFBP-1 blocks the availability of insulin-like growth factors (IGF) for insulin-like actions *in vivo* and can increase blood glucose levels (Lewitt *et al.* 1991, 1993), we have proposed that this role is to counter-regulate IGF availability for glucose homeostasis (Lewitt 1994). Although in many circumstances circulating concentrations of IGFBP-1 and insulin are closely inversely correlated (Brismar *et al.* 1988, Holly *et al.* 1988, Suikkari *et al.* 1988), there are exceptions to this relationship (Rutanen *et al.* 1993, Hopkins *et al.* 1994, Hilding *et al.* 1995). It is now recognised that multiple regulatory pathways other than insulin determine IGFBP-1 production *in vivo*, including stimulation by other counter-regulatory hormones, such as glucocorticoids (Conover *et al.* 1993), glucagon (Hilding *et al.* 1993) and catecholamines (Hooper *et al.* 1994).

Somatostatin has been implicated in the regulation of IGFBP-1 (Ørskov *et al.* 1994). Octreotide is a long-acting somatostatin analogue, which has been used therapeutically to attenuate secretion of polypeptide hormones, including growth hormone (GH) and insulin (Cai *et al.* 1986). In humans it stimulates IGFBP-1 regardless of GH status and changes in insulin levels (Ezzat *et al.* 1991, 1992). The stimulatory effect on IGFBP-1 of lanreotide, another somatostatin octapeptide analogue, also appears to be independent of changes in circulating insulin concentrations (Wolthers *et al.* 1994). However, one study has shown that the stimulatory effect of a somatostatin infusion is abolished by a euglycaemic-hyperinsulinaemic clamp (Ørskov *et al.* 1994). In support of an independent effect on IGFBP-1, octreotide has been shown to stimulate IGFBP-1 mRNA expression and protein production by the human hepatoma cell line HepG2 (Ren *et al.* 1992).

Rat IGFBP-1 mRNA expression is increased 1 h after administration of octreotide *in vivo*, apparently independently of glucose and insulin concentrations (Flyvbjerg *et al.* 1995). We have established the rat as a model for

studies of the regulatory mechanisms of IGFBP-1 (Lewitt *et al.* 1992, 1994) and have developed a sensitive RIA (Lewitt *et al.* 1994). The aim of this study was to determine the effect of octreotide on circulating IGFBP-1 levels and to determine the time course and possible mechanisms of the response *in vivo*.

Materials and Methods

Materials

Octreotide was kindly donated by Sandoz Australia (North Ryde, NSW, Australia) and was diluted in 0.15 mol/l NaCl for i.v. infusion or s.c. administration. For the pharmacokinetic studies, human (h) IGFBP-1, purified from amniotic fluid, was iodinated by the chloramine T method as previously described (Baxter *et al.* 1987) and detected by immunoprecipitation with antibody, A2 (Baxter *et al.* 1987).

Animals

Male Wistar rats were used for experiments which were conducted with the approval of the Animal Experimentation Ethics Committees, Royal Prince Alfred Hospital and the University of Sydney. Insulin resistance was induced by feeding a diet in which fat (Safflower oil) comprised 59% of the calories for 4 weeks before experiments. The diet was isocaloric with normal rat chow, in which carbohydrate comprised 69% of calories. High-fat fed animals weighed 341 ± 8 g ($n=26$), significantly more than littermates fed on a diet high in carbohydrate (304 ± 8 g; $n=32$; $P<0.001$).

Experiments were conducted from 0800 h, 16 h after removal of food. In experiments carried out under anaesthesia, pentobarbital (30 mg/kg) and ketamine (50 mg/kg) were administered i.p. and the animals were kept on warming pads to maintain body temperature at 37 °C. Groups of three or four animals were anaesthetised, cannulated via one jugular vein for infusion of 0.15 mol/l NaCl or octreotide at a rate of 1 ml/h and via the contralateral carotid artery for blood sampling. The time from induction of anaesthesia until the start of octreotide or saline administration was approximately 1 h. In preliminary experiments, xylazine (10 mg/kg i.p.) and ketamine (100 mg/kg i.p.) were used for anaesthesia, and similar IGFBP-1 responses were observed. In experiments carried out in the conscious state, a cannula was inserted into the jugular vein under anaesthesia and exteriorised through the skin at the base of the skull. Animals were allowed to recover and experiments were conducted 72 h later in unrestrained animals. Octreotide was given by s.c. or i.v. bolus. In each series of experiments, a baseline sample was taken, and blood samples (250 µl) drawn at the times indicated. The cells were separated and resuspended to a

final volume of 250 µl in 0.15 mol/l NaCl and replaced after the subsequent sample to prevent volume depletion. Cannulae were kept patent with 50 U/ml sodium heparin. Plasma was stored at -20 °C for later assay.

Assays

Rat IGFBP-1 concentrations were measured by specific RIA with a sensitivity of 1 ng/ml as previously described (Lewitt *et al.* 1994). In brief, a specific antiserum, designated B3 was used at a final dilution of 1:10 000. The between-assay imprecision was 4.5% measured at 0.82 ng/assay tube. The within-assay imprecision was 11.8% at 0.16 ng, 3.7% at 0.82 ng and 5.4% at 7.6 ng/assay tube. Plasma insulin was measured by RIA using a guinea-pig anti-rat insulin antibody against a rat standard (Linco Res Inc, St Louis, MO, USA). The sensitivity was 35 pmol/l. The between-assay imprecision was 12% and the within-assay imprecision was 8.5% measured at 165 pmol/l. Plasma glucose concentrations were measured by a glucose oxidase/peroxidase method with between-assay and within-assay imprecisions of <5%. Rat GH concentrations were measured by a commercially available RIA (Amersham International, Amersham, Bucks, UK) with a sensitivity of 3.2 ng/ml. The between-assay imprecision was 8.3% and the within-assay imprecision was 3.0% measured at 1.3 ng/assay tube. For each measurement all of the samples from a single animal were run in the same assay.

Pharmacokinetic studies

Studies of IGFBP-1 disposal were carried out in anaesthetised rats. Two hours after the induction of anaesthesia, radioiodinated hIGFBP-1 (10×10^6 c.p.m.) was injected via the jugular venous cannula. Blood samples (250 µl) were drawn at the times indicated and the cells separated, resuspended and returned as described above. Duplicate aliquots of 25 or 50 µl were precipitated with 5 volumes of 10% trichloroacetic acid (TCA) or with rabbit anti-hIGFBP-1 antibody (A2) at a final dilution of 1:1000. After an overnight incubation at 4 °C, immunoprecipitated counts were separated with goat anti-rabbit serum and polyethylene glycol as previously described (Baxter *et al.* 1987). Total, TCA-precipitated and immunoprecipitated counts were determined in a γ -counter. Pharmacokinetic analyses were performed using standard equations (Gibaldi 1982).

Statistics

In each experiment, animals were randomised to each treatment group. The number of animals per group appears in the figure legends. IGFBP-1, insulin and GH concentrations were log-transformed to achieve a normal distribution before data analysis. IGFBP-1, insulin and GH

Figure 1 Effect of a continuous infusion of octreotide on IGFBP-1, insulin and glucose levels in fasting anaesthetised rats. IGFBP-1 (A), insulin (B) and glucose (C) concentrations were measured in eight animals given octreotide 100 µg/kg per h (■) and compared with those in nine animals infused with saline alone (□). Octreotide stimulated IGFBP-1 concentrations transiently ($P<0.001$; ANOVA, corrected for repeated measures). Insulin concentrations were lower in the animals given octreotide ($P<0.05$). Values represent the mean $\pm$ S.E.

results are expressed in the text as the mean and 95% confidence interval, constructed from the transformed data and then back-transformed to the original scale of measurement. The results are expressed in the figures as the mean $\pm$ S.E. The changes in IGFBP-1, insulin and glucose concentrations and between-group effects were compared by ANOVA for repeated measures (Super-ANOVA, Abacus Concepts, Berkeley, CA, USA). GH levels were compared by ANOVA. Significant differences were identified using the Fisher's PLSD post-hoc test. Statistical significance was set at $P<0.05$.

Results

Experiment 1

In this experiment rats were randomised to a normal-fat diet or a high-fat diet and then further randomised to receive saline or octreotide infusion. Figure 1A shows the effect of a continuous infusion of octreotide 100 µg/kg per h on circulating IGFBP-1 concentrations in anaesthetised rats fed on a normal-fat diet. One hour of anaesthesia was required for cannulating the carotid and jugular vessels in groups of three or four rats before commencement of the infusions. Baseline IGFBP-1 concentrations were 166 (95% confidence interval 123 to 225) ng/ml and increased in the saline-infused animals to a plateau at 120 min. At 180 min, IGFBP-1 concentrations were 729 (488 to 1086) ng/ml. The increase in IGFBP-1 was greater in the octreotide-infused rats but was transient, reaching peak levels of 1605 (1220 to 2111) ng/ml 105 min after commencement of the infusion. The changes in IGFBP-1 concentration were analysed by ANOVA corrected for

repeated measures. Overall, the increase in IGFBP-1 levels with time was significant ($P<0.001$), and the increase in concentration was significantly greater in octreotide-infused animals ($P<0.001$). We measured circulating insulin concentrations in these animals and these are shown in Fig. 1B. There appeared to be an initial fall in insulin concentration followed by an increase. This change in insulin concentration with time was statistically significant ($P<0.001$) and there was a significant difference in the insulin profiles between saline- and octreotide-infused animals ($P<0.05$). Glucose concentration (Fig. 1C) appeared to increase with time in both groups. This change was statistically significant, but there was no significant difference in the glucose values between the saline and octreotide infusions.

In order to determine the importance of insulin in the IGFBP-1 response to octreotide, we used rats fed on a diet high in fat. Figure 2A demonstrates the IGFBP-1 responses in these animals to continuous infusions of saline or octreotide. Baseline IGFBP-1 concentrations, measured 1 h after the induction of anaesthesia, were 46 (25 to 83) ng/ml, approximately 4-fold lower than the levels in normal-fat fed rats. IGFBP-1 concentrations increased in the saline-infused animals to 780 (503 to 1208) ng/ml at 180 min. The increase in IGFBP-1 was greater in the octreotide-infused rats, and this difference appeared to be transient, reaching peak levels of 1120 (792 to 1586) ng/ml 135 min after commencement of the infusion. The change in IGFBP-1 levels with time was significant ($P<0.001$), and the increase in concentration was greater in octreotide-infused animals ($P<0.05$). When compared with the littermates fed on a normal-fat diet, the IGFBP-1 levels were lower in the high-fat fed animals ($P<0.001$) and the effect of octreotide was less ($P<0.02$). Insulin

Figure 2 Effect of hyperinsulinaemia on the IGFBP-1, insulin and glucose responses to a continuous infusion of octreotide in fasting anaesthetised rats. IGFBP-1 (A), insulin (B) and glucose (C) concentrations were measured in 12 animals in which hyperinsulinaemia had been induced with a high-fat diet. Animals were infused with octreotide 100 µg/kg per h (■; $n=8$) or saline (□; $n=8$). Octreotide stimulated IGFBP-1 concentrations transiently ($P<0.05$; ANOVA, corrected for repeated measures). Values represent the mean $\pm$ S.E.

concentrations were higher in high-fat fed animals than in normal-fat fed animals ($P<0.001$), and there was no difference in insulin response between saline and octreotide infusions (Fig. 2B). In the high-fat fed animals there was no significant difference in glucose levels between saline and octreotide infusions (Fig. 2C).

GH concentrations were measured in samples taken at 0, 15 and 180 min ($n=6$ in each group). Baseline GH concentrations showed considerable interindividual variation, but overall did not vary significantly between groups and fell in all animals during anaesthesia. In the animals given octreotide, a greater early decline in GH concentration was observed, so that in normal-fat fed rats at 15 min concentrations were 25.5 (17.0 to 39.0) ng/ml in saline-treated normal rats and 12.9 (9.4 to 16.6) ng/ml after octreotide ($P<0.05$). In the high-fat fed animals, GH concentrations were 38.8 (25.1 to 71.3) and 14.6 (7.2 to 24.2) ng/ml 15 min after commencement of saline and octreotide infusions respectively ($P<0.02$).

Experiment 2

IGFBP-1 increases during anaesthesia in the rat by complex mechanisms (Lewitt *et al.* 1992), which may have influenced the response to octreotide. We therefore repeated the experiments in the conscious chronically cannulated state. IGFBP-1 levels, shown in Fig. 3, were at least 8-fold lower in the conscious animals than in the anaesthetised rats. IGFBP-1 levels increased during the 3 h sampling period in all four groups ($P<0.001$). The greatest increase was seen in the normal-fat fed conscious rats given octreotide ($P<0.02$), and in this group IGFBP-1 concentrations rose from 22 (18 to 28) to 88 (62 to 126) ng/ml

within 60 min. In the high-fat fed animals, basal IGFBP-1 concentrations were 7.4 (5.0 to 10.8) ng/ml and were significantly less than in conscious normal-fat fed rats throughout the sampling period ($P<0.01$). In the high-fat fed animals, there was also a greater increase in IGFBP-1 in response to s.c. administration of octreotide compared with saline ($P<0.05$), but this increase was less than that in the normal-fat fed animals ($P<0.05$).

Figure 4A shows insulin concentrations in the conscious normal-fat fed rats. Initial concentrations were 96 (76 to 122) pM, approximately half the values in anaesthetised animals. Overall there was a significant change in insulin concentration with time during the study ($P<0.001$), with no statistically significant difference between the saline and octreotide treatment groups. Although there was a statistically significant variation in glucose concentration with time ($P<0.01$; Fig. 4B), there was no significant difference in response to the saline and octreotide infusions. In the conscious high-fat fed rats, insulin concentrations were 364 (272 to 486) pM. In the high-fat fed animals there was no significant difference in insulin or glucose levels between animals given octreotide or saline (data not shown).

Experiment 3

We examined whether the pattern of IGFBP-1 response related to the mode of delivery of octreotide. In a group of conscious normal-fat fed animals, a similar IGFBP-1 response to 100 µg/kg s.c. was seen in four rats given an i.v. bolus of 100 µg/kg octreotide and in four animals given 200 µg/kg s.c. (data not shown). The effect of a 100 µg/kg i.v. bolus of octreotide was determined. If the

Figure 3 IGFBP-1 response to a subcutaneous bolus of octreotide in conscious chronically cannulated rats. IGFBP-1 concentrations were measured in normal rats given octreotide 100 µg/kg (■; $n=8$) or saline (□; $n=7$) and compared with those in rats with hyperinsulinaemia induced with a diet high in fat and given octreotide (●; $n=7$) or saline (○; $n=7$). Octreotide stimulated IGFBP-1 concentrations in the normal-fat fed rats ($P<0.02$; ANOVA, corrected for repeated measures) and in high-fat fed animals ($P<0.05$). Compared with values in high-fat feeding rats, IGFBP-1 levels were higher in normal-fat fed animals during both saline and octreotide administration ($P<0.01$). Octreotide stimulated IGFBP-1 concentrations in the high-fat fed animals ($P<0.01$). Values represent the mean $\pm$ S.E.

bolus was administered at time 0 after 1 h of anaesthesia, no significant change in IGFBP-1 concentration was observed (data not shown). However, if the bolus was administered after a further 2 h of sampling under anaesthesia (Fig. 5), IGFBP-1 concentrations promptly increased in response to octreotide to reach peak levels of 1556 (1268 to 1910) ng/ml, values similar to those in animals given a continuous infusion of octreotide. When analysed by ANOVA corrected for repeated measures, there was a significant increase with time ($P<0.001$) and a significant difference between octreotide- and saline-treated animals ($P<0.05$). Insulin and glucose concentrations tended to fall after this late octreotide bolus, but these changes did not reach statistical significance.

Experiment 4

IGFBP-1 concentrations increased most rapidly after octreotide was administered by bolus (Experiments 2 and 3). We evaluated the potential role of altered clearance of IGFBP-1 by determining the disappearance of radioiodinated IGFBP-1. Radioiodinated hIGFBP-1 with or without 100 µg/kg octreotide was injected into normal

rats via a jugular cannula 2 h after the induction of anaesthesia, and blood samples were taken for 2 h. The disappearance of total radioactivity, TCA-precipitable counts and immunoprecipitable counts is shown in Fig. 6. There were no significant differences in the disappearance curves for saline- and octreotide-infused animals. Calculated from the immunoprecipitable counts and using the area under the mean curve extrapolated to infinity, the clearance rate of hIGFBP-1 was 3.19 ml/min per kg during the saline infusion and 2.78 ml/min per kg during octreotide infusion. In the first sample, taken 2 min after tracer injection alone, $85.6 \pm 0.9\%$ of hIGFBP-1 was in an immunoprecipitable form, which is not different from $84.0 \pm 1.9\%$ in the samples taken 2 min after octreotide co-infusion. The percentage immunoprecipitable by A2 diminished with time so that, 120 min after injection, 22.3 ± 3.5 and $16.9 \pm 4.8\%$ of counts were immunoprecipitated in samples without and with octreotide injection respectively (difference not significant). Using a three-compartment model for the disappearance curves of each animal, the intermediate half-life for radioiodinated hIGFBP-1 injected during anaesthesia was 11.7 ± 1.1 min, which is not significantly different from the value of 9.0 ± 1.2 min obtained when the hIGFBP-1 was injected during an octreotide infusion.

Discussion

In this study a continuous infusion of octreotide resulted in a transient stimulation of rat IGFBP-1 in anaesthetised rats. A bolus of octreotide (100 µg/kg) administered to conscious rats or anaesthetised animals resulted in a rapid increase in IGFBP-1 concentration, to reach maximum values within 60 min. During continuous infusion (100 µg/kg per h), the peak response occurred later, at approximately 105 min. Although the time course of the response may be related to the mode of administration, its transient nature may not be entirely attributable to the pharmacokinetics of the drug. Increasing circulating insulin concentrations, for example, may have had an inhibitory action in the anaesthetised rats. In the conscious rats, the effect of octreotide over the increment during a saline infusion was lost with time. Here the effect of increasing 'stress-related' stimulatory factors during the 3 h sampling period may have exceeded the earlier difference between the octreotide and saline infusions.

Although the stimulation of IGFBP-1 in response to octreotide in humans appears to be related to suppression of GH (Ezzat *et al.* 1991), the observation that stimulation occurs even in GH-deficient subjects supports the hypothesis that octreotide modulates IGFBP-1, at least in part, independently of GH (Ezzat *et al.* 1992). Octreotide is expected to influence prevailing GH concentrations in the rat (Cai *et al.* 1986), and this in turn may influence IGFBP-1 transcription (Seneviratne *et al.* 1990). In our

Figure 4 Effect of an s.c. bolus of octreotide on insulin (A) and glucose (B) in conscious chronically cannulated rats. Insulin and glucose concentrations were measured in eight rats after octreotide 100 µg/kg (■) and were compared with values in seven control animals (□). There was a significant difference in the insulin levels between octreotide- and saline-treated animals ($P<0.05$; ANOVA, corrected for repeated measures). Values represent the mean $\pm$ S.E.

Figure 5 Effect of an intravenous bolus of octreotide on IGFBP-1 levels in anaesthetised rats. Octreotide stimulated IGFBP-1 concentrations in three animals given octreotide 100 µg/kg per h at 120 min (■) compared with three animals infused with saline alone (□) ($P<0.05$; ANOVA, corrected for repeated measures). Values represent the mean $\pm$ S.E.

study, changes in GH concentration in response to octreotide did not appear to account for the IGFBP-1 responses observed. GH concentrations were suppressed by octreotide to a similar extent in normal and hyper-

Figure 6 Disappearance of an i.v. bolus of radioiodinated hIGFBP-1 (hIGFBP-1*) from the circulation of anaesthetised rats. Two hours after the induction of anaesthesia, hIGFBP-1 tracer (10×10^6 c.p.m.) was injected alone (A; $n=3$) or with 100 µg/kg octreotide (B; $n=3$) via a jugular cannula, and blood samples were taken for 2 h. The disappearance curves for total radioactivity (■), TCA-precipitable counts (□) and radioactivity immunoprecipitable with antibody to hIGFBP-1 (●) are shown.

insulinaemic rats, but the IGFBP-1 response was clearly greater in the normal animals. We cannot exclude, however, the possibility that GH suppression might be a prerequisite for a response to other factors. Low GH concentrations may also have contributed to the increasing IGFBP-1 concentrations seen in rats infused with saline

alone under anaesthesia. Circulating insulin concentration is an important determinant of circulating IGFBP-1 levels in the rat (Unterman *et al.* 1990, Lewitt *et al.* 1994). In this study, high-fat feeding resulted in some weight gain and an insulin-resistant state characterised by hyperinsulinaemia and normal fasting glucose values. Insulin sensitivity was preserved with respect to basal IGFBP-1 production and the increase in IGFBP-1 in response to octreotide was impaired in animals with hyperinsulinaemia. Hyperinsulinaemia has also been observed to abolish the IGFBP-1 response to somatostatin infusion in humans (Ørskov *et al.* 1994). Although these observations support a role for insulin in determining the IGFBP-1 response to octreotide, the prevailing insulin concentration is not likely to be solely responsible for the observed effects. An IGFBP-1 response to octreotide was observed in the hyperinsulinaemic animals, even though the changes in insulin levels were not significantly different from the saline infusion. Furthermore, the effect of octreotide was most marked during anaesthesia in the face of rising insulin concentrations during the sampling period and higher insulin concentrations than in the conscious rat. In support of an insulin-independent stimulation of IGFBP-1, one study in normal awake humans showed dose-dependent increases in IGFBP-1 in response to lanreotide, despite identical initial suppression of serum insulin at each dose (Wolthers *et al.* 1994).

The most notable difference between the experimental groups was the marked elevation of plasma IGFBP-1 concentrations in the anaesthetised rats compared with those in conscious animals. Anaesthesia is associated with reduced basal and insulin-suppressed hepatic glucose output in the rat (Clark *et al.* 1990). It is therefore possible that the high IGFBP-1 concentration during anaesthesia is related to hepatic resistance with respect to the inhibition of IGFBP-1 by insulin. In keeping with this concept, it has been observed that IGFBP-1 responses to insulin diminish with age (Rutanen *et al.* 1993). However, in humans with insulin resistance associated with obesity (Conover *et al.* 1992) and fasting (Cotterill *et al.* 1993), insulin sensitivity with regard to IGFBP-1 production is preserved.

It has been suggested that the stimulatory effect of octreotide is a direct hepatic effect. It can stimulate IGFBP-1 expression in HepG2 human hepatoma cells (Ren *et al.* 1992), but the direct stimulatory effect required incubation for at least 12 h. Flyvbjerg *et al.* (1995) showed that IGFBP-1 hepatic mRNA expression was stimulated 1 h after octreotide administration to rats, with no concomitant changes in glucose or insulin. However, in their study the possibility of an early transient effect on insulin secretion, as suggested in our experiments, could not be excluded. In view of the relatively rapid response of IGFBP-1 to a bolus of octreotide, we considered and excluded the possibility that octreotide had an effect on IGFBP-1 clearance in addition to stimulating specific mRNA levels (Flyvbjerg *et al.* 1995).

The increase in IGFBP-1 caused by octreotide was greater during anaesthesia than in the conscious state. This could be explained by the presence of factors that are able to potentiate IGFBP-1 stimulation by octreotide and which increase during anaesthesia. There are several possible mediators of this effect. Glucocorticoids increase circulating IGFBP-1 concentrations in the rat (Luo *et al.* 1990), but we have previously demonstrated that the increases in IGFBP-1 during the stresses of anaesthesia and insulin-induced hypoglycaemia are not reflected by changes in corticosterone levels alone (Lewitt *et al.* 1992). Glucagon stimulates hIGFBP-1 *in vivo* (Hilding *et al.* 1993) and *in vitro* (Lewitt & Baxter 1989). We did not measure glucagon concentrations in our study, but these are more likely to have been inhibited by octreotide (Cai *et al.* 1986). Catecholamines are also candidates, since they increase during anaesthesia and stimulate IGFBP-1 in sheep (Hooper *et al.* 1994). Catecholamine concentrations were not measured in these experiments.

In summary, we have shown in the rat that octreotide administration leads to a rapid increase in circulating IGFBP-1 concentrations. The data support a contributory role of insulin inhibition in determining this response, but neither the changes in insulin nor the changes in GH concentration could entirely account for the changes in circulating IGFBP-1 levels. Furthermore, there was a marked increase in circulating IGFBP-1 concentrations during anaesthesia and a greater IGFBP-1 response to octreotide in this state. These experiments indicate the complexity of the response, and further studies are under way to further define the multiple factors responsible for stimulating IGFBP-1.

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