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EFFECTS OF IGF-I OR LR^{3}IGF-I INFUSION ON COMPONENTS OF THE GH/IGF-I AXIS IN PIGS

by

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ABSTRACT

Insulin-like growth factor-I (IGF-I) promotes protein and DNA synthesis, inhibits protein breakdown and enhances growth in normal rats. A variant of IGF-I, Long R^3IGF-I (LR^3IGF-I), that binds poorly to IGF binding proteins is 10-fold more potent than IGF-I *in vitro*. LR^3IGF-I is even more anabolic in rats than IGF-I. In pigs, LR^3IGF-I inhibits growth, while IGF-I has no effect on growth performance.

The aim of this project was to determine why LR^3IGF-I has such divergent effects in two different species. One hypothesis is that LR^3IGF-I may have different effects on the GH/IGF-I axis depending on the species. I therefore investigated the endocrine regulation of IGF-I and IGF binding protein-3 (IGFBP-3) in the pig and determined the effects of IGF-I and LR^3IGF-I treatment on porcine IGF-I and IGFBP-3 expression at the gene and protein level.

In the first study, the effects of continuous 4 day infusion of IGF-I or LR^3IGF-I alone and in combination with porcine growth hormone (pGH) on growth performance and plasma hormone levels was investigated. This study examined whether the poor growth response that had been previously observed was due to hypoglycaemia induced by the IGF peptides and whether exogenous administration of IGF-I or LR^3IGF-I affected components of the GH/IGF-I axis. This was determined by measuring plasma IGF-I, IGFBP-3 and GH levels. LR^3IGF-I significantly decreased average daily gain when compared to animals receiving IGF-I, and decreased feed intake. Although co-administration with pGH did return growth performance of LR^3IGF-I treated pigs to normal, there were no synergistic actions on growth performance between the two peptides. Neither IGF-I or LR^3IGF-I when administered as a chronic infusion affected plasma glucose levels, indicating that the poor growth response observed in these animals was not due to hypoglycaemia. Plasma insulin levels were reduced by IGF-I and LR^3IGF-I, consistent with similar studies in man and sheep where IGF-I treatment suppressed plasma insulin levels. IGF-I and LR^3IGF-I treatment suppressed average plasma GH levels, suggesting that the peptides were acting on the GH/IGF-I axis to inhibit GH production or secretion from the pituitary. LR^3IGF-I also blunted the magnitude of the pulsatile expression of GH by decreasing the area under the GH peaks. Similar effects of IGF-I on GH secretion have also been reported in man and sheep. In the present study, the decrease in plasma GH was associated with a decrease in plasma IGF-I and IGFBP-3 in LR^3IGF-I treated animals. Co-administration with pGH was not able to return plasma levels of IGF-I and IGFBP-3 to normal. In the rat, LR^3IGF-I treatment has no effect on plasma IGF-I levels, and plasma IGFBP-3 levels are increased in these animals. These results suggest that in the pig, LR^3IGF-I inhibits

components of the GH/IGF-I axis and this is responsible for the poor growth response seen in pigs.

Following on from these findings, I postulated that the decrease in plasma IGF-I and IGFBP-3 were due to a suppression in gene transcription. In order to determine this, it was first necessary to establish in which porcine tissues IGF-I and IGFBP-3 gene expression was regulated by GH. Female pigs of the same age as used in the previous study were treated with 70 μg/kg/day porcine GH for 5 days, sacrificed and samples of liver, kidney, muscle, stomach and small intestine were snap frozen in liquid nitrogen and stored at -80^{0}C. Total RNA was extracted and expression of IGFBP-3 mRNA was measured by RNase protection assay using a previously constructed porcine IGFBP-3 probe. For IGF-I analysis, expression of IGF-I *class 1* and *class 2* transcripts was quantitated in a similar manner using porcine specific probes. In the liver, pGH treatment significantly increased IGF-I *class 1*, *class 2* and IGFBP-3 gene expression. In the kidney, there were no changes in IGF-I *class 1* gene expression and IGF-I *class 2* mRNA could not be detected. Kidney IGFBP-3 gene expression was significantly increased. None of the other tissues examined showed changes in IGF-I or IGFBP-3 gene expression in response to pGH treatment. This is the first study to investigate GH regulated gene expression of IGFBP-3 in different porcine tissues. GH dependent expression of the two different IGF-I transcripts in pigs was only reported in August 1996 and was confined to mRNA analysis in liver, adipose tissue and longissimus dorsi and semitendinosus muscle. IGF-I *class* 1 expression has been shown to increase with GH treatment in liver, adipose tissue, semitendinosus but not longissimus dorsi muscle. From the present study it was concluded that in the liver, IGF-I *class 1, class 2* and IGFBP-3 gene expression are increased with pGH treatment, and IGFBP-3 mRNA is increased in the kidneys of pGH treated animals.

In the next part of the project, pigs were intravenously infused with IGF-I or LR^{3}IGF-I for 5 days to determine their effect on endogenous IGF-I and IGFBP-3 gene expression in liver and kidney. In agreement with the earlier study, LR^{3}IGF-I treatment decreased plasma IGF-I and IGFBP-3 concentrations. Both IGF-I and LR^{3}IGF-I treatment decreased liver IGF-I *class 2* but not IGF-I *class 1* gene expression. Liver IGFBP-3 and kidney IGF-I and IGFBP-3 gene expression were not affected by either IGF-I or LR^{3}IGF-I treatment. It is most likely that IGF-I *class 2* mRNA expression is more sensitive to changes in plasma GH status than IGF-I *class 1* mRNA, since only expression of *class 2* transcripts was affected by IGF-I or LR^{3}IGF-I treatment. Expression of liver IGF-I *class 2* mRNA has been shown to be closely correlated to plasma IGF-I levels. The reduction in liver IGF-I *class 2* mRNA is therefore likely to be a major contributor to the reduction in plasma IGF-I levels. Although plasma IGFBP-3 levels were significantly reduced in LR^{3}IGF-I treated animals, liver and kidney IGFBP-3 mRNA expression

was not affected. The decrease in plasma IGFBP-3 may therefore be a result of post translational modifications such as mRNA instability or IGFBP-3 protease activity. The observed increases in kidney IGFBP-3 expression during pGH treatment could be a result of increased IGF-I in the circulation binding to kidney IGF type 1 receptors and increasing IGFBP-3 expression.

The final study was designed to investigate if combination treatment of IGF-I or LR^3IGF-I with pGH for 14 days was able to restore liver IGF-I gene expression. Pigs receiving LR^3IGF-I treatment alone or in combination with pGH lost their appetite and refused to eat. Two pigs (one from the LR^3IGF-I group, the other from the LR^3IGF-I + pGH group) had to be euthanised after 7 and 9 days of treatment. Another LR^3IGF-I treated pig was found dead in its pen on day 10 of treatment. This made data analysis for this group not possible. IGF-I treatment alone and in combination with pGH had similar effects on plasma IGF-I and IGFBP-3 levels as described in previous studies. In this study, pGH treatment alone significantly increased liver IGF-I *class 2* but not *class 1* mRNA. The dose administered in this trial was less than half that administered in the previous experiment, and confirms that IGF-I *class 2* mRNA expression is more sensitive to GH status than IGF-I *class 1* mRNA expression. IGF-I treatment for 14 days significantly decreased liver IGF-I *class 2* mRNA, which were restored to normal when pGH was administered in combination with IGF-I. There were no effects on liver and kidney IGF-I *class 1* or IGFBP-3 mRNA levels for any of the treatments. Co-administration of pGH with IGF-I can therefore compensate for some of the effects IGF-I treatment has on components of the GH/IGF-I axis. It is unlikely that pGH has the same effects on IGF-I mRNA expression in pigs treated with LR^3IGF-I, since plasma IGF-I and IGFBP-3 levels are reduced even when the two hormones are given in combination.

This project has shown that LR^3IGF-I treatment in pigs reduces average daily gain and feed intake. This is not due to the hypoglycaemic effects often associated with IGF treatment, but rather a result of reduced plasma GH levels. This reduction in plasma GH is achieved by either direct inhibition of GH mRNA expression or secretion from the pituitary and/or via a long-loop feedback inhibition at the hypothalamus by regulating somatostatin and/or growth hormone releasing hormone. The reduction in plasma GH affects components of the GH/IGF-I axis, which leads to reduction in IGF-I gene and protein expression as well as reduced plasma IGFBP-3 levels.

The different responses to LR^3IGF-I treatment between rats and pigs may be due to different levels of GH sensitivity between the species. Growth performance in the rat can be increased by GH at doses of 1 mg/kg/day, without altering plasma IGF-I levels, while in pigs doses as low as 30 μg/kg/day are sufficient to improve growth performance and elevate plasma

IGF-I levels. Since most of the growth promoting actions of GH are mediated by IGF-I, then this suggests that in the rat, IGF-I expression is not very sensitive to GH status. If IGF peptides do reduce plasma levels of GH in the rat as they do in the pig, the growth promoting actions of IGF-I are not impaired in rats, since IGF-I levels are not reduced with IGF-I treatment in this species.