Intracerebral administration of insulin-like growth factor I decreases circulating growth hormone levels in the fetal pig

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Abstract. Radioimmunoassayable IGF-I levels were measured in the cerebrospinal fluid and plasma of pig fetuses at 94 days gestational age. Mean plasma IGF-I levels were 128.5 ± 5.8 μg/l while the concentration in the cerebrospinal fluid was 25.8 ± 4.4 μg/l. The effect of intracerebroventricular administration of IGF-I on circulating GH levels was also studied in pig fetuses in utero. Eighteen pig fetuses were fitted with indwelling carotid artery and jugular vein catheters. Nine fetuses were given 1500 ng of pure IGF-I in 100 μl 0.9% saline by direct injection into a right lateral ventricle. Nine further fetuses (controls) were similarly given 100 μl of saline without IGF-I. GH levels in the control fetuses were ~200 μg/l and showed marked fluctuations with episodic intervals of about 40 min. By contrast, in the IGF-I-treated fetuses, GH levels were dramatically lowered by 20 min after IGF administration and remained low throughout the 4-h study. The episodic variations in GH were abolished and levels remained fairly constant at ca. 40 μg/l. From these results we surmise that the low levels of IGF-I in the fetus may contribute to their high GH levels. At this stage it is not possible to identify whether the IGF-I inhibition is a direct effect on the pituitary or is mediated by increased somatostatin, decreased GHRH or both.

Hypothalamic control over the release of growth hormone via somatostatin and growth hormone-releasing hormone is well documented. However, as with other pituitary hormones, such as gonadotropins, there is also a variety of feedback mechanisms at both the hypothalamic and pituitary level influencing secretion of GH. It has been shown, both in vitro and in vivo, that IGF-I can influence GH secretion through a negative feedback pathway (1-3). It has been suggested that such an action may be effected through a decrease in GHRH (4,5), increased SRIH (1) and a direct pituitary effect (1).

Much less is definitively known about the control of GH in the fetus. Ontogenic studies in fetuses show that SRIH and GHRH mechanisms are present in the late gestation fetus (6,7). Similarly, in the pig fetus SRIH has been shown to have an effect on GH secretion (8). However, these mechanisms may not be fully developed since GH levels are much higher in the fetus than in the post-natal pig (9-11). It is also known that the bioassayable somatomedin levels are lower in the fetal circulation than post-natally in the pig (11); however, bioassayable somatomedin levels may not reflect immunoassayable levels. We surmise that the high GH levels in the fetal pig may be due, at least in part, to the lack of inhibitory feedback occasioned by the low IGF-I levels.

In this study we have attempted to clarify this by measuring radioimmunoassayable IGF-I levels in plasma and cerebrospinal fluid (CSF) from the fetal pig and elevating IGF-I levels in the central nervous system (CNS) to see if this can reduce plasma GH levels in the fetal pig.

Materials and Methods
We studied 18 fetuses from four commercial crossbred sows at 98 days gestational age. The sows were sedated
with 1 mg/kg azaperone (Stressnil®, Janssen Pharmaceuticals) administered im, and then anesthetised with 2.5 mg/kg metomidate (Hypnodil®, Janssen Pharmaceutical) given iv. Anesthesia was maintained under oxygen/nitrous oxide inhalation with incremental metomidate as required.

Each fetus was exposed by laparotomy and hysterotomy, and vinyl catheters were inserted into the left carotid artery and external jugular vein and tied in place as described elsewhere (12). A 23-gauge needle was inserted into the head of the fetus such that the tip lay in the right lateral ventricle of the brain. Positioning of the needle in the ventricle was verified by the efflux of cerebrospinal fluid through the needle. Pure IGF-I, 1500 ng in 100 µl saline (13), was gently infused into the ventricle. The needle was slowly withdrawn and the skin and subcutaneous tissues covered the hole. The placenta and uterus were sutured and the fetus returned to the abdomen while the free ends of the catheters remained outside the sow.

Blood samples (1 ml) were taken at 20-min intervals for 4 h through the jugular vein catheter into heparinised syringes. The blood was transferred to tubes containing EDTA and stored on ice until rapidly centrifuged, plasma was separated and stored at −20°C until assayed for GH as previously described (10).

Cerebrospinal fluid was taken via a needle inserted into the cisterna magna of untreated fetuses and the levels of IGF-I were measured in these samples and in pretreatment fetal blood samples by an 'in house' limited reagent radioimmunoassay (14,15). Buffer for all reagents was 64 mmol/l phosphate buffer (pH 7.4) containing 0.25% bovine serum albumin, 0.02% sodium azide and 0.02% Triton X-100.

Before measurement in the radioimmunoassay, samples were extracted by acid/ethanol extraction (16), neutralised and lyophilised. Recombinant human IGF-I (courtesy of Dr A Skottner, KabiVitrum, Sweden) was used as standard and, following iodination using the Iodogen method (17), as radioligand. An antiserum specific for IGF-I was raised 'in house' by repeated sc injections of an IGF-I-egg albumin conjugate in rabbits and used at an initial dilution of 1:10 000. Assays were performed by incubation of standards (or re-constituted sample extracts) with tracer and antibody for 24 h at 4°C. Separation of bound and free antigen was achieved using a 30-min incubation with a magnetisable solid-phase second antibody consisting of goat anti-rabbit serum covalently linked to BioMag 4100 (Advanced Magnetics Inc, Cambridge, MA). The limit of sensitivity of the assay was 0.15 µg/l, the inter- and intra-assay variations were 10.2 and 3.2%, respectively. The assay showed <1% cross-reactivity with IGF-II and <0.1% with insulin.

Statistical analysis was made using a two-way analysis of variance comparing the mean pretreatment level for each fetus with each separate posttreatment levels for that animal.

Results

Mean IGF-I levels in the pretreatment samples of fetal pig plasma were measured at 128.5 ± 5.6 µg/l.

Fig. 1.

Temporal changes in plasma concentration of porcine growth hormone (pGH) in a representative pig fetus following intracerebroventricular administration of A. 1500 ng IGF-I in 100 µl of saline or B. 100 µl of saline.
Table 1. Plasma concentrations of porcine GH in 98-day-old pig fetuses and the response to intracerebroventricular injection of 1500 ng of IGF-I or 100 µl saline (pGH µg/l; mean ± SEM). 

<table>
<thead>
<tr>
<th>mins</th>
<th>-10</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I (N)</td>
<td>145±13</td>
<td>141±13</td>
<td>90±14</td>
<td>94±10</td>
<td>63±13</td>
<td>47±8</td>
</tr>
<tr>
<td>Saline (N)</td>
<td>170±71</td>
<td>106±16</td>
<td>241±68</td>
<td>162±13</td>
<td>157±28</td>
<td>168±39</td>
</tr>
</tbody>
</table>

NS NS NS p<0.01 p<0.02 p<0.01

while the mean cerebrospinal fluid concentration of IGF-I was 25.8 ± 4.4 µg/l. Mean maternal plasma levels of IGF-I were 252 µg/l.

In the control fetuses given saline alone, there were clear, marked episodic spikes of GH at 40-60 min intervals (Fig. 1). In control fetuses, there was no significant difference (p>0.05) between the pretreatment baseline levels and the GH levels following saline administration into the cerebral ventricle.

In the IGF-I treated fetuses, the levels of GH in the plasma during the pretreatment period were not significantly different from the levels in the saline-treated fetuses (Table 1). However, following IGF-I administration, plasma GH fell quickly to much lower levels and were significantly different (p<0.01) from control fetuses by 10 min and significantly different (p<0.05) from pretreatment levels by 20 min. In addition, the episodic secretion of GH in the IGF-I treated fetuses was less evident because of the much dampened GH levels, and there was little evidence of a spiked secretory pattern of GH (Fig. 2). The GH levels in the treated fetuses remained significantly lower (p<0.01) than those in the controls throughout the period of study.

All fetal plasma samples had higher concentrations of pGH than those measured in maternal plasma (10.2 ± 3.6 µg/l).

Discussion

Intracerebroventricular administration of IGF-I to pig fetuses in utero resulted in a rapid and sustained decrease in circulating pGH levels, but the mechanism by which the IGF-I decreases circulating pGH levels remains unclear.

It is possible that there may have been an enhanced clearance of pGH from the plasma. Plasma GH in the fetal pig has been calculated as having a half-time of 18 min (18), which would fit with the decrease observed 20 min after the IGF administration. However, there may also have been an immediate effect on GH release. In vitro studies using the rat indicate that IGF-I may inhibit the secretion of hypothalamic GHRH (5), and also increase SRIH release (1). There is also reported to be an effect of IGF-I at the pituitary level where it can inhibit the effects of GHRH on GH secretion from pituitary cells in culture (4) and levels of

![Fig 2. The mean ± SEM plasma pGH levels in pig fetuses receiving either 100 µl saline (○) or 1500 ng IGF-I in 100 µl saline (●) by direct injection into a lateral ventricle.](image-url)
mRNA for IGF-I (19,20). However, the mechanism by which the decrease in GH levels is effected in the fetal pig is yet to be elucidated.

The immediate effect of IGF-I on plasma pGH levels was surprising. Studies in the post-natal rat have shown that GH levels were not depressed until 2 hours after IGF-I administration. There are a number of possible reasons for the difference between these two sets of results. The very high levels of GH in the fetal pig may make it easier to detect a decrease in circulating GH compared with the very different pattern of GH secretion in the adult rat.

In contrast to these findings, recent studies in the post-natal sheep (21) demonstrate that central levels of IGF-I have no effect on GH secretion. It has been suggested that the effects observed in the rat are a result of leakage of the administered substances from the CSF into the peripheral blood stream (21,22). Possibly the same is true in the pig fetus and the effects seen are a result of elevated plasma IGF levels. We did not have sufficient IGF-I to test this in the fetal pig, but it has been shown that acute intravenous administration of IGF-I to the fetal lamb can have an effect on GH secretion (23).

It should be noted that IGF-I appears to have an effect on GH levels at a time when circulating IGF-I itself is not closely regulated by GH. Although IGF-I has been shown to stimulate many fetal cell types and is present in a wide variety of fetal tissues (24). If IGF-I acts as a cell growth-stimulating factor then, in view of the very rapid growth and cell division in the fetus, high levels of IGF-I would be expected. Despite the high growth rate and GH levels in the fetus, IGF-I concentrations are low and there is no clearly defined role for IGF-I in the fetus. We have previously published data showing that bioassayable somatomedin activity in the pig fetus is about one quarter of the levels in post-natal blood (11); radioreceptor studies revealed a similar level (25). The results of the present study indicate that radioimmunoassayable IGF-I is also present at about 25% of normal post-natal pig blood levels since we have measured normal IGF-I levels at between 200 and 300 ng/l.

There are no previously published data on the levels of IGF-I in the cerebrospinal fluid of the pig fetus. Our finding that the concentration of IGF-I in the CSF is lower than in fetal peripheral blood concurs with the findings of low IGF-I levels in the CSF in sheep and humans (21,26). Assuming a CSF volume of 5 ml, administration of 1500 ng of IGF-I would increase the concentration of IGF-I in the CSF by 300 μg/l; a 30-fold increase. This is likely to result in an even greater relative increase in free (non-binding protein bound) IGF-I and it is possible that at this high concentration the IGF-I may also be exerting an effect through insulin or type 2 receptors.

These data show the presence of a pathway for IGF-I to influence GH release, but this does not necessarily indicate a physiological role for IGF-I in regulation of GH secretion. It has been shown in sheep (27), that although intravenously administered IGF-I appears rapidly in the lymph, it does not cross into the CSF (27). There is considerable expression of IGF-I mRNA in the fetal brain (28) and this seems likely to be the source of IGF-I in the CSF. Thus circulating levels of IGF-I may not alter CSF levels of IGF-I to which the hypothalamus is exposed.

In conclusion, the pig fetus seems to be sensitive to changes in cerebrospinal fluid IGF-I concentration and these results support the suggestion that the low level of IGF-I in the fetal circulation may be one of the factors associated with high plasma GH levels in the pig fetus. However, the pathway through which these changes are produced remains unclear.

Acknowledgments

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