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Original article

Decreased circulating growth hormone levels following centrally administered insulin-like growth factor-1 is not mediated by somatostatin in the pig fetus

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Summary — Twenty-six pig fetuses (at 94 days gestational age) were fitted with carotid artery catheters. Eight fetuses were given 1 500 ng of IGF-1 (in 100 μl) directly into a lateral cerebral ventricle; 7 further fetuses received the IGF-1 together with 150 μl of a potent specific anti-somatostatin serum into a ventricle, 5 other fetuses received the anti-somatostatin serum alone while 6 controls received normal sheep serum. Administration of IGF-1 caused a rapid decrease in circulating growth hormone (pGH) levels but there was no significant change in plasma levels of somatostatin immediately following the IGF-1 administration, suggesting that the decrease in pGH was not mediated by somatostatin secretion. Further evidence that somatostatin was not involved in this effect was provided by the lack of effect of concurrent antisomatostatin serum on the IGF-1-induced decrease in pGH. Thus the high circulating levels of GH in the fetus may result from a lack of feedback of IGF-1, but not through the somatostatin–pituitary axis.

pig / fetus / IGF-1 / somatostatin / growth hormone

Résumé — La diminution des niveaux d'hormone de croissance circulante après administration centrale d'IGF-1 (*insulin-like growth factor* 1) n'est pas régulée par la somatostatine chez les fœtus de porc. *Vingt-six fœtus de porc (à 94 j de la gestation) ont été équipés de catheters intracardotidiens. Huit d'entre eux ont reçu 1 500 ng d'IGF-1 pur (dans 100 μl), directement dans un ventricule cérébral latéral; 7 autres ont reçu l'IGF-1 mélangé à 150 μl d'un puissant antiserum spécifique de la somatostatine dans un ventricule, 5 autres encore ont reçu l'antisérum seul tandis que 6 témoins recevaient serum normale. L'administration de l'IGF-1 a provoqué une chute rapide de la concentration de pGH circulante sans que simultanément soit noté un changement significatif du niveau plasmatiques de somatostatine ce qui suggère que la chute de pGH n'était pas due à une sécrétion de somatostatine. Une autre preuve que la somatostatine n'intervient pas dans ce processus fut l'absence d'effet de l'antisérum anti-somatostatine sur la chute de pGH induite par l'IGF-1. Ainsi les niveaux élevés de GH circulante chez les fœtus pourraient provenir non pas d'une absence de retrocontrôle par IGF-1 mais d'une immaturité de l'axe somatostatine–hypophyse fœtale.*

porc / fœtus / IGF-1 / somatostatine / hormone de croissance

INTRODUCTION

Although growth hormone (GH) levels are much higher in the pig fetus than in the post-natal animal (Atinmo *et al,* 1976; Spencer et al, 1983a) the reverse is true of the levels of insulin-like growth factor 1 (IGF-1), which are lower in the pig fetus (Spencer *et al,* 1983a, 1989a). We have speculated that the high GH levels in fetal plasma are a result of reduced negative feedback on GH release because of the low levels of IGF-1, and have recently published results supporting this hypothesis (Spencer *et al,* 1989b, 1991a). However, in those studies it was not possible to determine the mechanism by which the intracerebroventricularly (icv) administered IGF-1 produced a decrease in circulating GH levels.

Both growth hormone-releasing factor (GRF) and somatostatin (SRIF) are present in the pig fetus from mid-gestation (Polkowska *et al,* 1985) and intravenous administration of SRIF can acutely decrease GH levels in the fetal pig (Spencer *et al,* 1985). However, full control over GH release may not be established *in utero* since in the pig large doses of SRIF are required to produce changes in plasma GH levels. Large doses of SRIF are also required to lower GH plasma levels in the sheep fetus (Gluckman *et al,* 1979). The present study combines central administration of IGF-1 and intracerebral SRIF immuno-neutralization in an attempt to investigate whether the decrease in GH levels brought about by icv IGF-1 administration is effected through the SRIF pathways.

MATERIALS AND METHODS

Six cross-bred sows at 104 days gestational age provided the 26 fetuses used in this study. The sows were sedated with 1 mg/kg azaperone (Stressnil, Janssen Pharmaceuticals) given im and anaesthetized with 2.5 mg/kg metomidate iv (Hypnodil, Janssen Pharmaceuticals). The sows were ventilated with O_2 and N_2O and anaesthesia maintained with incremental azaperone/metomidate as required. Fetuses were fitted with carotid artery catheters (Macdonald *et al,* 1981) and substances were administered into a lateral cerebral ventricle by direct injection while *in utero* as previously described (Spencer *et al,* 1989, 1991a). The uterus was closed and the fetuses returned to the abdomen.

IGF-1 was purified to homogeneity from ovine plasma (Moore *et al,* 1990) and showed equal potency and cross-reactivity to recombinant IGF in all systems so far investigated (Hodgkinson *et al,* 1989; Spencer *et al,* 1991b). Anti-somatostatin serum was raised in sheep to a somatostatin–human serum globulin conjugate (Spencer *et al,* 1983b) and concentrated by lyophilization. The equivalent of 0.8 ml of plasma was given in a volume of 150 μl.

Eight fetuses were given 1 500 ng of pure IGF-1 in 100 μl saline directly into a lateral ventricle. Seven further fetuses were given 1 500 ng of IGF-1 co-administered with 150 μl of the anti-somatostatin serum (capable of binding 94 μg 125I Tyr1-SRIF *in vitro*). Five other fetuses were given icv anti-somatostatin treatment alone, while 6 received 150 μl normal sheep serum. Different treatments were spread randomly over the different sows to remove any inter-litter effects. Blood samples (2 ml) were taken through the carotid cannula 10 min before, and immediately prior to, treatment and at intervals thereafter for at least 90 min.

Blood samples from the fetuses were collected into EDTA on ice for plasma GH measurement by specific radioimmunoassay (Spencer *et al,* 1983c). Aliquots of plasma were also assayed for somatostatin by radioimmunoassay as described below. Plasma (1 vol) was extracted with (2 vol) M HCl: absolute ethanol (5% : 95%) for 15 min at room temperature, and centrifuged. The supernatant was lyophylized and dissolved in half the original volume of assay buffer prior to immunoassay. Briefly, the assay protocol was: 100 μl extracted plasma, 200 μl specific anti-somatostatin serum (used at a final dilution of 1 : 20 000) and 100 μl (20 000 dpm) iodinated Tyr1-SRIF (New England Nuclear; Boston, MA,

USA) were incubated in a phosphate assay buffer containing 1 000 KIU/ml aprotinin (Trasylol, Sigma, St Louis, USA), and 50 mmol.l^{-1} EDTA at 4 °C for 24 h. Separation of free from bound label was effected using 150 µl dextran coated charcoal (0.5% activated charcoal and 0.05% dextran in phosphate buffer containing 1% bovine serum albumin) followed by a 15-min incubation at room temperature and centrifugation at 2 000 rpm for 15 min. The assay has a sensitivity of 3 pg/ml. The antiserum does not cross-react with: insulin, IGF-1, glucagon, TSH, ACTH, ADH, LHRH, oxytocin, porcine prolactin or GH, amongst other substances. All samples were measured in the same assay.

Where necessary, the GH values were log transformed to normalize the distribution and variation prior to statistical analysis. Statistical analysis of the data was made by comparing the change in GH concentrations from the mean pre-treatment value for each individual fetus using 2-way analysis of variance (paired *t*-test).

RESULTS

Plasma samples from fetuses receiving intracerebroventricular IGF-1 showed a rapid, sustained depression ($P < 0.05$) in plasma GH concentrations following administration of IGF-I (fig 1). In contrast to the change in GH, plasma levels of somatostatin did not show any significant alteration over the immediate post-treatment period (fig 2).

As a more direct indication of the part played by somatostatin in mediating the decrease in GH, IGF-1 was co-administered together with anti-somatostatin serum. This co-administration failed to attenuate the IGF-I induced drop in plasma GH (fig 1); the decrease in GH was similar in both magnitude and duration to that observed following IGF-1 administration alone.

As in earlier studies (Spencer *et al*, 1989b, 1991a), there was no significant change in GH levels following either saline or normal serum given in a similar volume into a lateral cerebral ventricle (fig 3), but a marked variation in GH secretion was seen in individual control animals. There was also no significant change in GH levels following icv administration of antisomatostatin serum alone, when changes within individual fetuses were analyzed (fig 3).

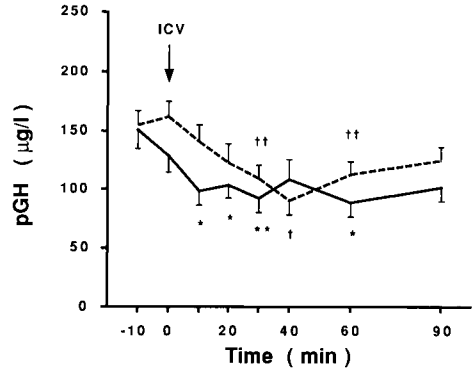

Fig 1. Change in the level of circulating pGH following intracerebroventricular administration of 1 500 ng IGF-1 or icv administration of 1 500 ng IGF-1 concurrently with icv anti-somatostatin serum to pig fetuses *in utero*. * $P < 0.05$, ** $P < 0.01$ compared with mean pre-treatment levels by paired *t*-test for IGF-1 alone. + $P < 0.05$, ++ $P < 0.01$ for IGF-1 + anti-somatostatin treated pigs. Values are means ± SEM of log-transformed data.

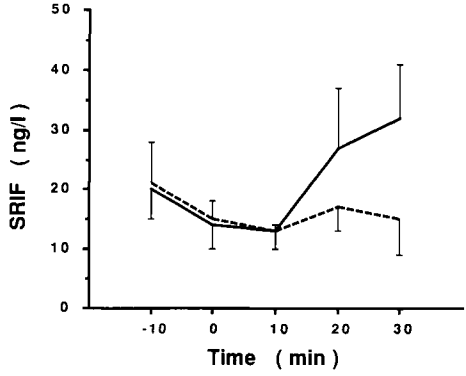

Fig 2. Plasma levels of somatostatin immediately before and after intracerebroventricular administration of : saline or 1 500 ng IGF-1 to pig fetuses *in utero*. Values are means ± SEM.

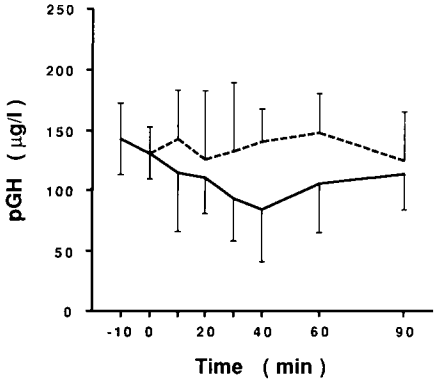

Fig 3. Change in the level of circulating pGH following intracerebroventricular administration of either normal sheep serum or anti-somatostatin serum to fetal pigs *in utero*. The data are shown as means ± SEM adjusted by covariate analysis to common pre-treatment means.

DISCUSSION

These data confirm the previously reported finding of a decrease in plasma GH concentrations following icv administration of IGF-1 to the fetal pig *in utero* (Spencer *et al*, 1989b, 1991a). Furthermore, the lack of change in measurable plasma somatostatin concentration suggests that this effect is not mediated *via* an immediate hypersecretion of hypothalamic somatostatin.

Although the sampling times may not be optimal for measurement of somatostatin, and although measurement of peripheral somatostatin may not adequately reflect hypothalamic somatostatin secretion as seen by the pituitary somatotrophes, the suggestion that somatostatin is not involved in mediating the IGF-1 effect on pGH release is given further support by the inability of anti-somatostatin serum to reverse the IGF-1 induced depression in GH levels. The effects seen are not likely to be a non-specific result of administration of substances into the brain since it has been shown that neither saline nor normal serum injections icv have an effect on circulating GH levels. As has been previously reported (Spencer *et al*, 1991a), the episodic pattern of plasma GH levels seen in the control fetuses was greatly attenuated in the animals given IGF-1 in these studies.

In the adult rat it has been shown that centrally administered IGF-1 can cause a lowering of circulating GH levels (Abe *et al*, 1983; Tannebaum *et al*, 1983) but it has not been possible to show a similar effect either *in vivo* (Spencer *et al*, 1991b), or *in vitro* (Blanchard *et al*, 1988) with post-natal sheep. However, using a monolayer culture of fetal ovine pituitary cells, it has been shown that IGF-1 can inhibit both basal and GRF-stimulated GH secretion (Blanchard *et al*, 1988), and late gestation lambs have also been reported to respond to intravenous IGF-1 administration with a decrease in circulating GH (de Zegher *et al*, 1988). These results suggest that IGF-1 may have an effect on GH release at the level of the pituitary.

In the rat, decreased GH levels following IGF-1 administration have been linked to stimulation of secretion of hypothalamic SRIF (Berelowitz *et al*, 1981) but it would appear that the IGF-1-related decrease in GH in the fetal pig is not related to SRIF secretion. These data suggest that although somatostatin is present in the pig fetus at this gestational age (Polkowska *et al*, 1985), endogenous SRIF levels are not involved in GH regulation at this stage of development. This conclusion is supported by the lack of effect of administration of anti-somatostatin serum alone on circulating GH levels. However, the fetal pituitary is apparently able to respond to SRIF since decreased GH levels have been found following intravenous SRIF adminis-

tration in the pig fetus (Spencer *et al*, 1985), although the doses used were pharmacological and the effect was variable.

It has been reported that following central administration of hormones to the rat, the administered substances leak rapidly into the peripheral circulation (Tannenbaum and Patel, 1986). It is possible that similar leakage from the cerebral space occurs in the pig fetus, although we have not been able to adequately examine this possibility in the fetal pig to date. In a recent study using post-natal sheep, icv administration of large doses of the N-Met IGF-1 variant did not result in any detectable leakage of the administered peptide into the general circulation and there was no effect on GH secretion (Spencer *et al*, 1991b). Direct evidence for leakage of IGF-1 from the cerebral compartment of the fetal pig in the present studies could not be obtained as the N-Met variant was not available. Measurement of natural sequence IGF-1 in plasma was not a practicable indicator since the calculated elevation in plasma IGF-1 concentration would be too small to accurately assess the amount of peptide entering the blood stream.

In conclusion, these data confirm the suppressive effect of intracerebral administration of IGF-1 on GH secretion in the fetal pig, and indicate that this decrease in GH is not mediated through hypothalamic somatostatin.

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