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Short Report

Modulation of the control of the hypothalamic growth hormone (GH)-releasing factor (GRF) on the GH secretion in fetal and neonatal pigs

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ABSTRACT An ontogenic aspect of the growth hormone (GH) secretion was studied in fetal and neonatal pigs. It is known that GH secretion is under the dual control of hypothalamic peptides: a GH-releasing factor (GRF) and somatostatin (SRIF), which exerts a major inhibitory influence (Frohman and Jansson, 1986). It has been also reported that chronic treatments (continuous or pulsatile) of pituitary cells to GRF caused a loss of responsiveness of somatotropes to the secretagogue (Hulse *etal.,* 1986). This phenomenon was explained by desensitization (Bilezikjian and Vale, 1983).Then, we have studied the role of hypothalamic GRF to maintain the responsiveness of somatotrope cells to the secretagogue during animal development. For this purpose, we compared the effects of 3-days pulsatile treatments of anterior pituitary (AP) tissue cultures from 95-days female fetuses, 110-days fetuses and 12-days neonatal pigs to GRF on GH response to this secretagogue, using a *in vitro* superfusion system. Every day, pulsatile treatments of GRF maintained GRF-estimulated GH secretion in neonatal female pigs with a similar response. However, a significant reduction in GH response was observed day to day in fetuses. In previous reports, when we exposured AP tissue to GRF resulted in a rapid GH release in fetuses and neonates. However, somatostatin and IGF-I given during a GRF pulse inhibited the GH response in neonates but not in fetuses. Therefore, the relative resistance of the fetal somatotropes to the inhibitory effects of somatostatin and IGF-I showed an immaturity of regulatory mechanism in fetuses which, at least in part, could be responsible for the desensitization effects of GRF in fetuses, but not in piglets. These data suggest a fundamental difference between the GH regulatory process of fetuses and piglet pituitaries. The ability of the somatotrope to maintain GH response to GRF is developmentally regulated, and the presence of the mature stimulatory (GRF)/ inhibitory(SRIF or IGF-I) mechanism might contribute to avoid the desensitization effects to the secretagogues.

Materials and Methods

We have used an *in vitro* superfusion system (Torronteras *et al.,* 1997). This system consists in small columns fashioned from 1-ml disposable plastic syringes, which are filled with AP tissue pieces. The AP pieces are superfused with complete MEM-medium at a flow rate of 0.1 ml/min (6 ml/h), using a peristaltic pump, and fractions samples are collected on ice at 10-min intervals. AP tissue was obtained from 3 stages of female German Landrace Pigs: fetuses of 95 days postcoitum, 110 days p.c. and piglets of 12 days postpartum. Each AP gland was cut separately into pieces of approximately $1mm^3$. Each perfusion chamber contained AP tissue derived from only 1 animal. In order to reduce variations, pGH secretion was expressed as nanograms of GH per 10 min per milligram of AP. In each perifusion experiment six chambers were control groups receiving medium alone and the other six chambers received GRF treatment. Data reported for each group (GRF and control) correspond to 6 replicates obtained in 3 (one per age) independent experiments. The pituitary tissue was perifused for 3 consecutive days (Fig. 1). Each day, three consecutive 10 min pulses of GRF were given at 1 h intervals. The dose of the first pulse correspond to 1 nM GRF and the two following pulses correspond to 10 nM GRF. We collected medium samples for 4 h (from 8:00 a.m. to 12:00 p.m.) at 10 min intervals. A competitive enzyme immunoassay (Serpek *et al.,* 1993) for pGH was performed. Integrated area under the curve (AUC) was calculated by substracting basal GH release data from GH data from GRF pulses. The net values for the collection period were summed up to obtain the AUC. The AUCs of GH profiles of each day and different ages were assessed for an 180-min perifusion period, expressed as nanograms of GH per 180 min per milligram of AP, calculated immediately after the first GRF pulse and are shown as means ± SEM (n=6; Fig. 2).

Results

The effects of prolonged exposure to GRF on GH secretion from fetal and neonatal pituitary tissue were evaluated for detecting the presence of desensitization response as a decrease in the AUC, assessed during the 180-min period after GRF treatment, and compared among the three days of treatment. Exposure to three 10-min pulses of GRF resulted in a rapid increment in GH secretion into the culture medium by both fetal and neonatal pituitary tissue. This increment of GH release was more relevant during the first day and was similar in the three ages (Fig. 1). However, it is of interest to note that the amount of stimulated GH was significantly greater in neonates (AUC was 184 ± 18,3 ng GH.180 $min^{-1}.mg^{-1}$ pituitary) than in both fetuses 95 and 110 days p.c. (103.4 ± 10.9 and 130 ± 12.5 ng GH.180 $min^{-1}.mg^{-1}$ pituitary, respectively). In neonates, GRF-induced GH release remained significantly elevated over second and third day of experiment (AUC was 207.7 ± 10 and 156.3 ± 12.3 ng GH.180 $min^{-1}.mg^{-1}$ pituitary, respectively). However, in fetuses the

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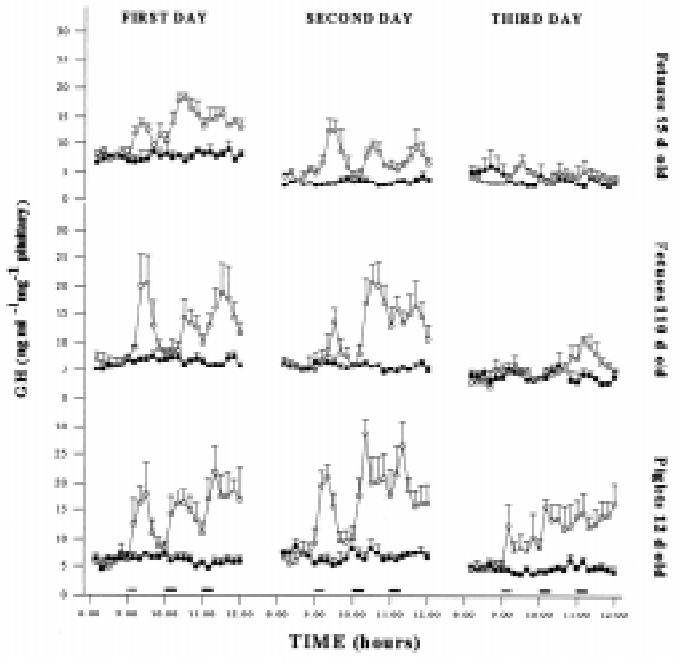

Fig. 1. Effect of three GRF pulses *(1, 10 and 10 nM, respectivey) during three consecutive days on GH release in vitro from anterior pituitary tissue of female fetuses or piglets (mean± SEM from 6 replicates).*

AUC of stimulated GH release was decreasing along second and third day. In the case of 95 days-fetuses, the decrease of GRF-induced GH release started in the second day (AUC, 55.1 ± 12 ng GH.180 $min^{-1}.mg^{-1}$ pituitary), whereas in 110 days-fetuses just occurred in the third day (AUC, 51.3 ± 8.9 ng GH.180 $min^{-1}.mg^{-1}$ pituitary).

Conclusions These data indicated that, compared with fetuses pituitaries, neonatal pituitaries were resistant to the suppressive effects of long GRF treatment. The GRF-induced GH release was markedly reduced in fetuses pituitaries after second or third day of GRF pulses, but was unchanged in neonatal pituitaries. These findings showed a important difference in GH regulation in the pituitary of the neonates compared to the fetuses, suggesting that the GRF signal is amplified with a pulsatil exposure to the secretagogue in the neonates, but not in the fetuses. This may represent resistance to the desensitizing effects of GRF in neonates and/or greater susceptibility of the immature pituitary to the desensitization effects of GRF. These observations are unlikely to be due to differential cell damage. Fetus somatotropes do not appear to be damaged by long treatment since they present no significant changes on basal GH secretion. In 110-days fetuses and neonates, within each day of treatment, induced GH secretion by second and third GRF pulse (10 nM) was similar or higher than GH response to the first pulse (1nM). However, in 95-days fetuses, where desensitization effect day to day is greater, the amount of GH release by 10nM GRF is less than that stimulated by first GRF pulse (1nM). These results could suggest that the dose of secretagogue does not cause desensitization effects of the GH-secretory responsiveness, but rather the frequency of pulses might be an important factor to provoke this phenomenon in fetuses. In rats (Kovacs *et al.,* 1994), it has also been demonstrated that the appearence of desensitization to repetitive pulses of a GRF analog is dependent on the frequency rather than on the dose of pulses. But, why does this affect fetuses more? Our findings suggest that the capacity of the somatotropes to attenuate GH release in response to GRF pulses could be developmentally regulated. In previous studies (Torronteras *et al.,* 1997), we have observed that endogenous inhibitor factors like somatostatin (SRIF) or insulin-like growth factor (IGF-I) given during a GRF pulse in a similar superfusion system were able to inhibit the stimulated GH response in neonates pituitaries tissue but not in fetuses. The relative resistance of the fetal somatotropes to the inhibitory effects of SRIF and IGF-I suggested an immaturity of fetal regulatory mechaninsm of GH secretion. The existence of this mature and complete stimulatory/inhibitory mechanism in neonates pigs could be responsible, at least in part, for remaining a releaseble GH pool in the somatotrope cells and/or to avoid a down-regulation of GRF receptors.

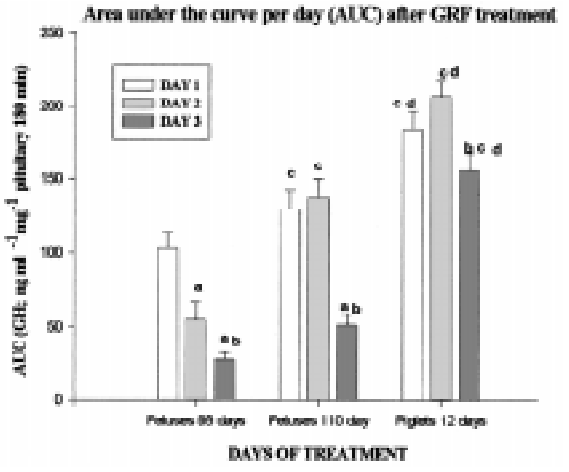

Fig. 2. Histograms of the effect of GRF on AUC and GH release from German Landrace 95-day fetuses, 110-day fetuses and 12-day piglets. *AUC of total GH released during a 180 min period after the first GRF pulse. Eac h histogram bar represents the AUC of total induced GH secretion during a day of treatment. Significant differences are: a, p<0.05 vs. 1st day within same age. b, p<0.05 vs. 2nd day within same age. c, p<0.05 vs. 95 day old within same day. d, p<0.05 vs. 110 day-old within same day.*

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