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Regulation of growth hormone secretion from human fetal pituitaries : interactions between growth hormone releasing factor and somatostatin (*)

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Summary.

Using an explant culture system, we have demonstrated that human somatotropes respond to growth hormone releasing factor (GRF) and somatostatin (SRIF) from as early as 9.5 weeks of fetal age. Responsiveness to GRF increases significantly as a function of age up to midgestation while SRIF inhibition of basal growth hormone (GH) release does not change. SRIF has little effect on GRF-stimulated GH secretion from early gestation pituitaries, but its ability to block GRF stimulation gradually increases with fetal age from 9.5 to 16 weeks. The response to GRF remains predominant throughout this developmental period : 100 times more SRIF than GRF must be added to the cultures in order to block the GRF stimulatory effect and maintain GH secretion at basal (control) levels. Finally, adding SRIF 30 min prior to the GRF does not increase the inhibitory activity of SRIF.

Our data suggest that the mechanisms that permit an interaction between GRF and SRIF are developing, but slowly, in the early to midgestation human fetal somatotrope and that GRF stimulatory pathways predominate. This may help to explain the very high levels of GH in fetal serum during the first half of gestation.

Introduction.

Serum growth hormone (GH) levels are detectable as early as 9-10 weeks of gestation in the human ; they rise rapidly, reaching a peak ($\simeq$ 130 ng/ml) at 20-24 weeks, then decrease gradually until birth (Kaplan and Grumbach, 1976). However, even at the end of gestation, circulating levels of GH are high : they are significantly elevated above what is seen in the normal adult and are often in the range found in acromegalics.

Using a pituitary culture system, we have been investigating the ontogeny of pituitary responsiveness to known regulators of somatotrope activity, to

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determine why serum GH levels are so high and fluctuate so dramatically during fetal life. Our previous studies have shown that fetal somatotropes can be stimulated *in vitro* by growth hormone releasing factor (GRF) and inhibited by somatostatin (SRIF) in a dose-related fashion (Goodyer *et al.*, 1984). Surprisingly, when GRF and SRIF were added together at the same concentrations, GRF blocked SRIF inhibition of GH release (Goodyer *et al.*, 1984), suggesting that stimulatory pathways predominate in the early gestation human fetal somatotrope. The present studies were undertaken to examine this hypothesis further.

Materials and methods.

Cultures. — Human fetal anterior pituitary glands (9.5-16 weeks fetal age) were obtained at the time of therapeutic abortion by D and C (Dept. of Obstetrics and Gynecology, Hôpital Notre-Dame, with approval from the local Institutional Ethics Committees). The tissues were minced under sterile conditions and 0.5 mm^3 explants placed on 40 mesh stainless steel grids in 1.5 ml of Ham's F-10 culture medium (Flow Laboratories, Mississauga, Ontario, Canada), supplemented with 10 % heat-inactivated fetal bovine serum (Hyclone Sterile Systems, Logan, Utah, USA) and antibiotics (penicillin, 200 IU/ml, Glaxo Labs, Montreal, Quebec, Canada ; gentamycin, 5 µg/ml, Schering, Pointe Claire, Quebec, Canada ; and amphotericin B, 40 µg/ml, Squibb Canada, Montreal, Quebec, Canada). The cultures were housed in a humidified hydrojac incubator at 37 °C with 5 % CO_2 in air and maintained for up to 20 weeks with daily changes of medium. Fetal age was determined by footlength according to Streeter (1920).

Test protocols. — Acute tests were begun after one week in culture, when the secretion profile for GH was stable (Goodyer *et al.*, unpublished), and were repeated at 2 to 3 day intervals until the end of the culture. Following a 1 hour preincubation, there were two consecutive 3 hour incubations, the first (CON) in control medium and the second (EXP) in medium containing test factors ; thus each grid acted as its own control. In addition, a series of incubations were performed without test factors during both 3 hour periods in order to determine the basal hormone secretion pattern and establish a separate control group of data. hGRF(1-44) and SRIF(1-14) were purchased from Peninsula Laboratories (San Carlos, California, USA). Responsiveness to GRF and SRIF did not change significantly as a function of culture age suggesting that there was no spontaneous maturation of their regulatory mechanisms under our culture conditions.

Hormone assays. — Culture media samples were collected in polypropylene vials and stored at − 20 °C until they could be processed for radioimmunoassay. hGH was measured using kit materials from the National Hormone and Pituitary Program (NIADDK, Bethesda, Maryland). Random samples were prescreened at several dilutions to determine parallelism to the standards ; all samples were then assayed in duplicate at the appropriate dilution. Intra-assay variation was 10 %,

inter-assay variation was 12 %. The minimal detectable quantity of hGH was 2.5 ng/ml. NIAMDD-hGH-RP-1(AFP-4793B) was used for standards.

Statistical analysis. — Data from the acute tests were initially expressed as ng of hGH produced per ml of culture medium during each 3 h incubation period. The measurements were then paired as a ratio for each grid (EXP/CON) in order to eliminate any differences caused by variable amounts of tissue per grid (*i.e.* each grid acted as its own control). The ratios were multiplied by 100 to create

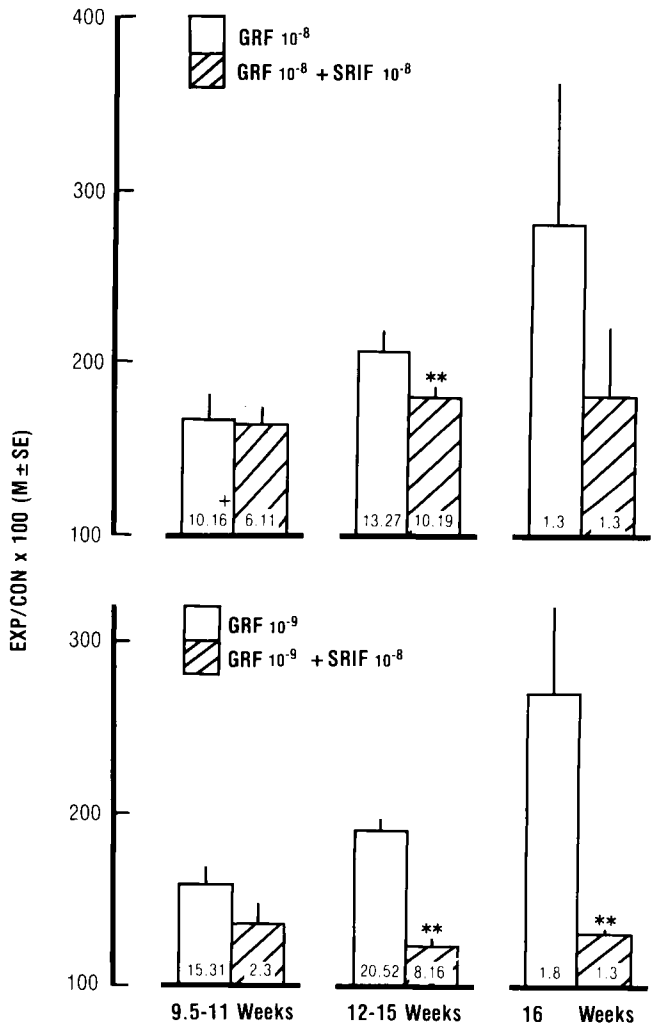

FIG. 1. — *Effects of GRF alone* (upper panel : 10^{-8} ; lower panel : 10^{-9} M) *and in combination with SRIF*(10^{-8} M) *on GH release from human fetal pituitaries as a function of fetal age* (9.5-16 weeks fetal age). Data are expressed as percent EXP/CON (mean +/− SE). + (n = no. of pituitaries, no. of observations). ** $p \leq 0.01$: as compared to GRF treatment alone by ANOVA and Duncan's multiple range test. Response to GRF 10^{-8} and 10^{-9} M increased significantly between 9.5-11.5 weeks and 12-15 weeks ($p \leq 0.01$) as well as between 9.5-11.5 weeks and 16 weeks ($p \leq 0.02$).

percentages and the data grouped according to the factor tested. Statistical differences between each group were analyzed using ANOVA for uneven numbers followed by Duncan's multiple range test.

Results.

GRF +/− SRIF as a function of fetal age. — Figure 1 shows the effects of GRF, alone and in combination with SRIF, as a function of fetal age. In the upper panel GRF and SRIF were tested at the same concentration (10^{-8} M), while in the lower panel SRIF(10^{-8} M) was 10 fold higher than GRF(10^{-9} M). We found that, along with the previously recognized age-related increase in responsiveness to GRF(10^{-8} or 10^{-9} M), there was an increase in the ability of SRIF(at 10^{-8} M) to inhibit GRF stimulation of GH release. This was especially apparent when SRIF was ten-fold higher in concentration than the GRF(lower panel). However, SRIF did not completely block the GRF effect in any test group.

Relative potencies of GRF and SRIF. — We examined the relative potencies of GRF and SRIF by holding each factor constant (at 10^{-8} M) and varying the other's concentration (SRIF from 10^{-7}-10^{-10} M ; GRF from 10^{-8}-10^{-10} M) (fig. 2). These experiments show that one must add 100 times more SRIF than GRF in order to observe a significant block of the GRF stimulatory effect : the amount of GH released is then similar to basal (control) levels.

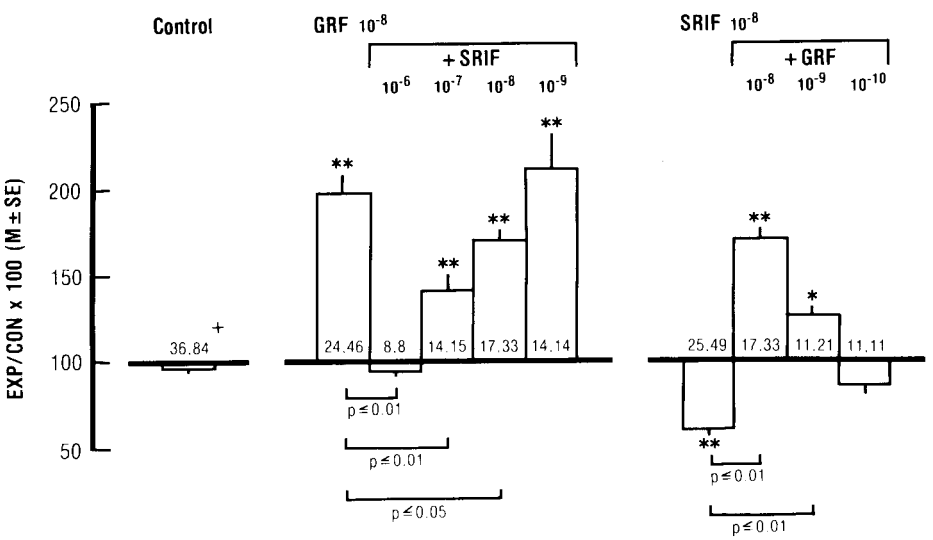

FIG. 2. — *Study of the relative potencies of GRF and SRIF in regulating GH release from human fetal pituitaries* (9.5-16 weeks). Data are expressed as percent EXP/CON (mean +/− SE). + (n = no. of pituitaries, no. of observations). * $p \leq 0.05$, ** $p \leq 0.01$: statistical significance when compared to control data. ⌊——⌋ : statistical significance for the two treatment groups indicated. Statistical analysis by ANOVA and Duncan's multiple range test.

When the data were analysed as a function of fetal age (table 1), we found that SRIF significantly inhibited basal GH secretion to a similar degree in all three age groups. However, it was only after 12 weeks that a significant dose-related inhibition of GRF-stimulated GH release could be observed with SRIF at 10^{-6}-10^{-9} M. It is possible that the pituitary cells from 9.5-11.5 week fetuses may respond in a dose-dependent manner as well but only with higher doses of SRIF (*eg.* $> 10^{-7}$ M).

TABLE 1

Relative potencies of GRF and SRIF as a function of fetal age.

	9.5-11.5 weeks	*12-15 weeks*	*16 weeks*
Control :	98 ± 2(14,36) +	100 ± 2(21,42)	103 ± 3(1,3)
GRF 10-8 :	167 ± 15(10,16)**	207 ± 12(13,27)**	280 ± 82(1,3)
+ *SRIF 10-6* :	111 (1, 1)	94 ± 4(7, 7) **	—
+ *SRIF 10-7* :	162 ± 32(3, 3)**	125 ± 10(10,11) *	209 ± (1,1)
+ *SRIF 10-8* :	164 ± 10(6,11)**	170 ± 6(10,19)**	181 ± 40(1,3)
+ *SRIF 10-9* :	182 ± 13(3, 3)**	203 ± 19(10,11)**	362 (1,1)
SRIF 10-8 :	54 ± 5(10,15)**	63 ± 4(14,32)**	54 ± 1(1,2)°
+ *GRF 10-8* :	164 ± 10(6,11)** **	170 ± 6(10,19)** **	181 ± 40(1,3)
+ *GRF 10-9* :	136 ± 12(2, 3)	124 ± 5(8,16)*	131 ± 5(1,3)
+ *GRF 10-10* :	105 ± 24(2, 2)°	80 ± 6(8, 8)	96 (1,1)
SRIF 10-8 30' pre-GRF 10-8 :	162 ± 13(3, 5)**	180 ± 14(6,11)**	199 (1,1)

+ Mean ± SE (n = no. of pituitaries, no. of observations) ; EXP/CON × 100.
° Mean ± SD (n = no. of pituitaries, no. of observations) ; EXP/CON × 100.
* $p \leq 0.05$, ** $p \leq 0.01$: statistical significance as compared to control data.
⌊——⌋ : statistical significance for the two treatment groups indicated :
* $p \leq 0.05$, ** $p \leq 0.01$.

Effect of pretreatment with SRIF. — In an attempt to increase the inhibitory activity of SRIF, we added it to cultures thirty minutes prior to the GRF. However, there was no significant change in somatotrope response : the GRF effect remained predominant (fig. 3). This was true whether the data were analysed as a function of fetal age (table 1) or as a single group (fig. 3 : 9.5-16 weeks fetal age).

Discussion.

In most mammalian species, plasma GH levels during fetal life are markedly elevated above those observed in the normal adult (Gluckman, 1983). Since these high GH concentrations do not appear to play a critical role in fetal growth or development, and because they gradually decrease during late gestation and the immediate postnatal period, it has been proposed that they are a consequence of

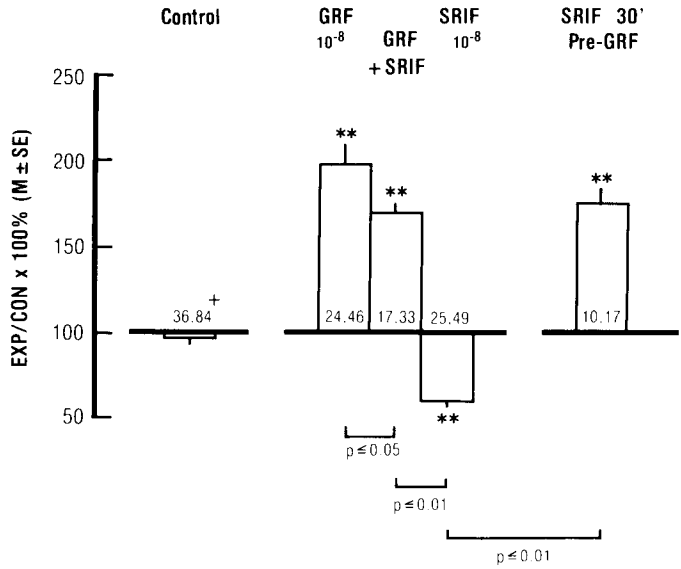

FIG. 3. — *Effect of adding SRIF* (10^{-8} M) *thirty minutes before GRF* (10^{-8} M) (9.5-16 weeks). Data are expressed as percent EXP/CON (mean +/− SE). + (n = no. of pituitaries, no. of observations). ** $p \leq 0.01$: statistical significance when compared to control data. ⌊——⌋ : statistical significance for the two treatment groups indicated. Statistical analysis by ANOVA and Duncan's multiple range test.

immature control mechanisms (Kaplan and Grumbach, 1976 ; Gluckman, 1983). Although there are many levels of the hypothalamic-pituitary axis that may be involved in this process, it is very difficult, if not impossible, to examine most of them in the human. One hypothesis that can be tested is whether certain mechanisms within the somatrope itself are immature such that it is more sensitive to stimulatory factors and/or relatively refractory to inhibitory factors.

In vivo and *in vitro* data support the concept that the human somatotrope is responsive to the hypothalamic hormones GRF and SRIF during both fetal and early neonatal life. Shimano *et al.* (1985) have reported that serum GH levels in normal newborns (3-28 days postnatal) are markedly stimulated by a 1 μg/kg dose of GRF. Roti *et al.* (1984) have administered SRIF to women in labour and found a significant decrease in cord levels of GH at the time of delivery. In addition, Delitala *et al.* (1978) have observed that, during the first day of life, newborns are able to respond to an *iv bolus* of somatostatin with a small but significant decrease in serum GH levels. Our own culture studies have shown that human fetal somatotropes respond to both GRF and SRIF in a dose-related fashion from as early as the ninth week of fetal life (Goodyer *et al.*, 1984). Responsiveness to GRF increases at each dose tested (10^{-8}-10^{-10} M) from the ninth week to midgestation (Goodyer *et al.* present data). In contrast, there is very little change in the inhibitory effect of SRIF(10^{-7}-10^{-10} M) on basal GH secretion during this same period of development (Goodyer *et al.*, unpublished).

In a preliminary study (Goodyer *et al.,* 1984), GRF and SRIF were added simultaneously, at the same concentration, to fetal pituitary cultures ; we were surprised to observe that SRIF had no effect at all on GRF-stimulated GH secretion. This finding is contrary to what has been reported for adult rat pituitary cultures, where adding equimolar GRF and SRIF results in a significant inhibition of GRF-stimulated GH release and often a decrease in GH secretion to below control levels (Law *et al.,* 1985 ; Sheppard *et al.,* 1985 ; Cuttler *et al.,* 1986). That is, when GRF and SRIF interact at the level of the adult rat somatotrope, SRIF appears to exert the dominant influence on GH release.

Since our initial experiments were done with fairly early gestation human pituitary glands (n = 7, 9.5-11.5 weeks ; n = 1, 13.5 weeks), it was decided to repeat the study using glands from older fetuses. The present results show clearly that, along with an increase in responsiveness to GRF as a function of fetal age, there is an age-related increase in the ability of SRIF to inhibit GRF-stimulated GH release. However, even with the oldest pituitaries, the level of inhibition is significantly less than what has been observed with adult rat pituitary cultures.

Another indication of the refractoriness of the fetal somatotrope to SRIF is that, in order to observe a marked inhibition of the GRF stimulatory effect, we had to add 10-100 times more SRIF than GRF. This was especially evident in the early gestation pituitary cultures. In addition, adding the somatostatin thirty minutes prior to the GRF did not appear to increase the efficacy of SRIF. These data support our previous hypothesis that the early to mid-gestation human fetal somatotrope is preferentially responsive to GRF and relatively insensitive to SRIF (Goodyer *et al.,* 1984).

In vivo and *in vitro* studies of the rat somatotrope suggest that, in this species as well, GRF rather than SRIF is the major factor regulating GH secretion in the late gestation fetus (Rieutort, 1981 ; Khorram *et al.,* 1983 ; Baird *et al.,* 1984). Following birth, there is a gradual decrease in responsiveness to GRF ; at the same time, SRIF slowly increases its influence on both basal and GRF-stimulated GH secretion until in the adult it appears to play a critical role in regulating GH release (Walker *et al.,* 1977 ; Rieutort, 1981 ; Bowers *et al.,* 1981 ; Oliver *et al.,* 1982 ; Khorram *et al.,* 1983 ; Cella *et al.,* 1985 ; Szabo and Cuttler, 1986 ; Cuttler *et al.,* 1986 ; Wehrenberg, 1986).

A comparison of the human and rat studies suggests that development of the hypothalamic-somatotrope axis may be occuring in a similar fashion in the two species although the timing in relation to parturition differs considerably. Unfortunately, we can only speculate as to what mechanisms are involved during these ontogenetic changes, since so few data are as yet available. Because human and rat fetal somatotropes respond to both GRF and SRIF, specific receptors for both peptides must be present on these cells. However, the difference in relative responsiveness suggests that, during early stages of development, either the GRF receptor exists in greater number or the GRF-receptor complex interacts more efficiently with its transducing systems than the SRIF-receptor complex. Studies of the ACTH receptor in ovine fetal adrenals (Durand *et al.,* 1985) and of the β-adrenergic receptor in rabbit fetal lung (Roberts *et al.,* 1984) have demonstrated age-related increases in both receptor

concentrations and receptor-transducer coupling activities. These changes correlate with increasing tissue sensitivity to their respective stimulatory factors, suggesting that both are important for maturation of the regulatory mechanisms.

Conclusion.

The present studies demonstrate that there are two distinctive features of the early to mid-gestation human fetal somatotrope : 1) preferential responsiveness to the stimulatory actions of GRF, and 2) relative resistance to the inhibitory effects of SRIF. Our data also suggest that, by the fourth month of fetal life, new regulatory mechanisms begin to develop that permit somatostatin to have a greater influence on GH release.

However, it is clear that there are still many gaps in our knowledge. Further studies are needed to define exactly which (GRF and SRIF) receptor-coupled transducing mechanisms are active during fetal life. We must also examine two related aspects of somatotrope activity, synthesis and storage of GH, to determine how these processes are regulated during development. The final task will be to assess to what degree changes in fetal serum GH concentrations are the result of, on the one hand, developmental changes within the somatotrope itself and, on the other, alterations in the circulating levels of hypothalamic and peripherally-derived factors.

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Résumé. *Régulation de la sécrétion de GH par les hypophyses fœtales humaines : interactions entre GRF et somatostatine.*

En utilisant un système de cultures d'explants d'hypophyses fœtales humaines, nous avons démontré que la sécrétion de l'hormone de croissance (GH) pouvait être modulée à la fois par le facteur de libération de la GH (GRF) et par la somatostatine (SRIF), dès la 9e semaine de gestation. La réponse au GRF augmente en fonction de l'âge du fœtus entre la 9e et la 16e semaine, tandis que l'effet de SRIF sur le taux basal de GH varie peu pendant la même période. En revanche, l'effet inhibiteur de la SRIF sur la sécrétion de la GH stimulée par GRF, qui reste faible entre la 9e et la 11e semaine, augmente progressivement entre la 12e et la 16e semaine de vie fœtale. Cependant, la réponse au GRF reste prédominante tout au long de cette période : on doit ajouter cent fois plus de somatostatine que de GRF pour maintenir la sécrétion de la GH au niveau basal. De plus, l'effet inhibiteur de la SRIF n'est pas augmenté si celle-ci est ajoutée trente minutes avant le GRF.

Nos données suggèrent que les mécanismes qui permettent l'interaction entre la somatostatine et le GRF dans la cellule somatotrope fœtale évoluent relativement lentement entre la 9e et la 16e semaine de gestation mais privilégient l'action stimulante du GRF. Ces résultats peuvent expliquer les niveaux élevés d'hormone de croissance observés chez le fœtus pendant la première moitié de la vie fœtale.

References

BAIRD A., WEHRENBERG W. B., LING N., 1984. Ontogeny of the response to growth hormone-releasing factor. *Regulatory Peptides,* **10,** 23-28.

BOWERS C. Y., REYNOLDS G. A., CHANG D., HONG A., MOMANY F., 1981. A study on the regulation of growth hormone release from the pituitaries of rats *in vitro. Endocrinology,* **108,** 1071-1080.

CELLA S. G., LOCATELLI V., DE GENNARO V., PUGGIONI R., PINTOR C., MULLER E. E., 1985. Human pancreatic growth hormone (GH)-releasing hormone stimulates GH synthesis and release in infant rats. An *in vivo* study. *Endocrinology,* **116,** 574-577.

CUTTLER L., WELSH J. B., SZABO M., 1986. The effect of age on somatostatin suppression of basal, growth hormone (GH)-releasing factor-stimulated, and dibutyryl adenosine 3′,5′-monophosphate-stimulated GH release from rat pituitary cells in monolayer culture. *Endocrinology,* **119,** 152-158.

DELITALA G., MELONI T., MASALA A., ALAGNA S., DEVILLA L., COSTA R., 1978. Action of somatostatin, levodopa and pyridoxine on growth hormone (GH) secretion in newborn infants. *Biomedicine,* **29,** 13-15.

DURAND P., CATHIARD A.-M., LOCATELLI A., SAEZ J. M., 1985. Biochemical modifications involved in the maturation of the ovine fetal adrenal gland in late gestation : their modalities and regulation. *Reprod. Nutr. Dév.,* **25,** 963-976.

GLUCKMAN P. D., 1983. The fetal neuroendocrine axis, 1-42. In MARTINI L., JAMES V. H. T., *Fetal endocrinology and metabolism,* Vol. **5,** Acad. Press, New York.

GOODYER C. G., HALL G., GUYDA H., ROBERT F., GIROUD C. J.-P., 1977. Human fetal pituitary in culture : hormone secretion and response to somatostatin, luteinizing hormone releasing factor, thyrotropin releasing factor and dibutyryl cyclic AMP. *J. clin. Endocrinol. Metab.,* **45,** 73-85.

GOODYER C. G., MARCOVITZ S., BEREZUIK M., DE STEPHANO L., LEFEBVRE Y., 1984. *In vitro* modulation of GH secretion from early gestation human fetal pituitaries, 209-212. In ELLENDORFF F., GLUCKMAN P. D., PARVIZI N., *Fetal neuroendocrinology,* Perinatology Press, New York.

KAPLAN S. L., GRUMBACH M. M., 1976. Development of hormonal secretion by the human fetal pituitary gland, 255-276. In MARTINI L., GANONG W. F., *Frontiers in neuroendocrinology,* Vol. **4,** Raven Press, New York.

KHORRAM O., DePALATIS L. R., McCANN S. M., 1983. Development of hypothalamic control of growth hormone secretion in the rat. *Endocrinology,* **113,** 720-728.

LAW G. J., RAY K. P., WALLIS M., 1985. Effects of growth hormone releasing factor and somatostatin on growth hormone secretion and cellular cyclic AMP levels. FEBS Letters, **179,** 12-16.

OLIVER C., GIRAUD P., LISSITZKY J. C., COTE J., BOUDOURESQUE F., GILLIOZ P., CONTE-DEVOLX B., 1982. Influence of endogenous somatostatin on growth hormone and thyrotropin secretion in neonatal rats. *Endocrinology,* **110,** 1018-1022.

RIEUTORT M., 1981. Ontogenetic development of the inhibition of growth hormone release by somatostatin in the rat : *in vivo* and *in vitro* (perifusion) study. *J. Endocrinol.,* **89,** 355-363.

ROBERTS J. M., McDONALD J. V., JACOBS M. M., BARNES P. J., BALLARD P. J., GONZALES L. W., CHENG J. B., 1984. Pulmonary adrenoreceptors : ontogeny, modulation and role in perinatal adaptation, 163-182. In JAFFE, R. B., DELL'ACQUA S., *The endocrine physiology of pregnancy and the peripartal period,* Raven Press, New York.

ROTI E., ROBUSCHI G., ALBONI A., EMANUELE R., D'AMATO L., GARDINI E., SALVI M., DALL'AGLIO E., GNUDI A., BRAVERMAN L. E., 1984. Inhibition of fetal growth hormone (GH) and thyrotrophin (TSH) secretion after maternal administration of somatostatin. *Acta endocrinol.,* **106,** 393-399.

SHEPPARD M. S., MOOR B. C., KRAICER J., 1985. Release of growth hormone (GH) from purified

somatotrophs : interaction of growth hormone-releasing factor and somatostatin and role of adenosine 3′,5′-monophosphate. *Endocrinology,* **117,** 2364-2370.

SHIMANO S., SUZUKI S., NAGASHIMA K., YAGI H., SAKAGUCHI M., KUROUME T., 1985. Growth hormone responses to growth hormone releasing factor in neonates. *Biol. Neonate,* **47,** 367-370.

STREETER J., 1920. *Contributions in embryology,* Vol. **XI,** Publ. 274.

SZABO M., CUTTLER L., 1986. Differential responsiveness of the somatotroph to growth hormone-releasing factor during early neonatal development in the rat. *Endocrinology,* **118,** 69-73.

WALKER P., DUSSAULT J. H., ALVARADO-URBINA G., DUPONT A., 1977. The development of the hypothalamo-pituitary axis in the neonatal rat : hypothalamic somatostatin and pituitary and serum growth hormone concentrations. *Endocrinology,* **101,** 782-787.

WEHRENBERG W. B., 1986. The role of growth hormone-releasing factor and somatostatin on somatic growth in rats. *Endocrinology,* **118,** 489-494.