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SOMATOMEDIN-C SECRETION BY SERTOLI CELLS IN VITRO : CHARACTERIZATION AND REGULATION

Somatomedin-C (Sm-C) was characterized in serum-free conditionned medium of porcine Sertoli cells cultured for 1 day in the presence of Transferrin, Vitamin E, Insulin and 3H-Leucine. Sm-C was purified by Sep-pak extraction followed by monoclonal anti-Sm-C antibody affinity chromatography and reverse phase C18 HPLC. Immunoreactive (IR) 3H-Leu-Sm-C co-eluted with pure 125-I-Sm-C on HPLC, gives a good parallelism to pure Sm-C in a specific radioimmunoassay. 48 hours IR-Sm-C secretion by Sertoli cells in serum-free conditionned medium (without insulin and 3H-Leu) is as follow :

Conditions	IR-Sm-C (ng/million cells)
Control	6.5 - 6.9
Testosterone 10^{-7}M	6.5 - 6.5
FSH 100 ng/ml	3.5 - 2.5
GH 2 µg/ml	7.0 - 7.2
FGF 100 ng/ml	9.0 - 10.8
FSH + Testosterone	1.2 - 5.0
FSH + Testosterone + GH	0.8 - 5.0
FSH + Testosterone + GH + FGF	7.0 - 5.4

Conclusions. Sertoli cells secrete Somatomedin-C, in quantity compatible with an autocrine-paracrine role in the testis. Fibroblast Growth Factor (FGF) but not GH stimulates this secretion, as reported with fibroblasts.

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SOMATOMEDIN C/INSULIN LIKE GROWTH FACTOR 1: AN INTRATESTICULAR DIFFERENTIATING FACTOR OF LEYDIG CELLS?

We have recently reported that, in the testis, porcine immature Sertoli cells cultured in a serum-free defined medium, release Somatomedin C / immunoreactive IGF 1 (irSmC) stimulated by FGF but not by GH (Ann. Endocrinol, 1985, 46, 287). In the present report, we show that SmC/IGF1 is a potent regulator of Leydig cell differentiation. The effect of SmC/IGF 1 on Leydig cell activity was studied by incubating porcine immature Leydig cells with a biosynthetic SmC. SmC/IGF1 exerted a dose and time dependent stimulating effect on Leydig cell function, with the maximal response obtained at 50 ng/ml after 48hrs treament. SmC/IGF1 increased both LH/hCG binding (x4-5), basal(x4) and hCG-stimulated (x15.5) testosterone secretion. The slight effects of SmC/IGF1 (100ng/ml for 48hrs) on Leydig cell number (x1.3) and DNA synthesis (x1.5) in the comparison with the high steroidogenic effect support the concept that SmC/IGF1 acts as a cytodifferenciative factor rather than a growth factor. The steroidogenic action of SmC/IGF1 was not suppressed by cytosine arabinoside C, a DNA synthesis inhibitor, supporting the concept of the cytodifferenciative role of this peptide. In conclusion, the production by Sertoli cells of irSmC/IGF1 associated with the stimulating effect of SmC/IGF1 on steroidogenesis, suggest that this peptide could be an intratesticular regulator of Leydig cell differentiation.

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SOMATOMEDIN-C (Sm-C) RECEPTORS IN CULTURED PIG LEYDIG CELLS (LC): CHARACTERIZATION AND REGULATION. ROLE OF Sm-C ON LC FUNCTIONS

Using affinity cross-linking we have shown the presence of Sm-C receptors (Cm-C-R) in cultured pig LC. The Sm-C-R were further characterized by competition binding (KD 1.10^{-9}M). When LC were cultured in a chemically defined medium without hormones, the number of Sm-C-R varied from 10000 to 15000 sites/cell. Treatment of LC with hCG induced a 2 to 3 fold increase in Sm-C-R. This effect was time-(maximum effect after 48 h) and dose-dependent (ED50 ≈ 10^{-10}M). Moreover LC treatment with Sm-C for 3 days induced an increase of hCG receptors (4-6 fold) and of hCG responsiveness (cAMP and testosterone production) (8-10 fold) with ED50 of about 2 ng/dl. In addition, Sm-C stimulated LC DNA synthesis (ED50 ≈ 10 ng/ml). These results show : 1) LC contain specific Sm-C-R which are positively regulated by hCG ; 2) Sm-C increased both hCG receptors number and hCG steroidogenic responsiveness of LC. Since Sertoli cells secrete Sm-C (see Chatelain et al) our data strongly suggest that Sm-C might play a role in the paracrine regulation of LC. Therefore Sm-C could play a key role on the delayed puberty observed in children with isolated growth hormone deficiency.

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SERUM LEVELS OF SOMATOMEDINS AND SOMATOMEDIN BINDING PROTEIN IN PREGNANT WOMEN AND THEIR INFANTS

Serum levels of IGF-I, IGF-II and the low molecular form of somatomedin binding protein (SMBP) were determined in pregnant women and their infants. Longitudinal studies during pregnancy were performed in healthy women, women with GH deficiency (n=3) and women with diabetes or gestational diabetes (n=44). IGF-I, IGF-II and SMBP were determined by radioimmunoassays using polyclonal antibodies.

The serum levels of IGF-I and SMBP, but not IGF-II, increased during pregnancy in both healthy women and women with diabetes. In GH deficient women the levels rose to normal pregnant levels. The birth weight percentile of the infants increased with the maternal IGF-I levels and decreased with the maternal SMBP levels. The ratio between maternal IGF-I and SMBP levels improved the correlation to the birth weight percentile. No correlation was found between the birth weight and the isolated values of IGF-I, IGF-II, SMBP and C-peptide in cord blood. The IGF-II levels in cord blood from infants of diabetic women were 50% higher than those of nondiabetic women.

The correlation between maternal levels of the birth weight of the infants indicates that maternal IGF-I levels may play a role in the transfer of nutrients to the fetus. The elevated IGF-II levels in cord blood of infants of diabetic women suggest that IGF-II could participate in the glucose homeostasis of the fetus.

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INSULIN-LIKE GROWTH FACTORS AND THEIR BINDING PROTEINS (IGF-BP) IN HUMAN FOETAL SERUM

IGF I and IGF II were measured in serum samples collected from the umbilical cord in healthy foetuses (in utero) and newborns. Results (mean ± SE) were as follows :

Gest. age	n	IGF I	IGF II	IGF II/IGF I
21 w.	14	55 ± 3.0	383 ± 12	7.2 ± 0.4
22 w.	16	52 ± 3.0	354 ± 15	7.2 ± 0.5
23 w.	20	52 ± 2.6	259 ± 17	5.0 ± 0.2
24 w.	9	58 ± 3.5	335 ± 15	5.9 ± 0.5
25-32 w.	9	54 ± 6.2	392 ± 17	7.6 ± 0.6
Neonate	31	143 ± 7.5	653 ± 27	4.9 ± 0.3
Adult	12	279 ± 11.4	1282 ± 64	4.7 ± 0.3
Hypopit.	12	51 ± 6.0	500 ± 65	9.8 ± 0.6

In foetuses, IGF I levels were similar to those in cases of total GH deficiency. IGF II levels were on average 6.5 times those of IGF I. No correlation was seen between IGF levels and growth parameters estimated by ultrasounds. Western blot analysis of IGF-BPs showed that foetal serum contained smaller quantities than normal adult serum of 42 and 39 K MW forms (which in the adult appear chiefly in the ~ 150 K [IGF-BP] complex) and large quantities of the 34 and 30 K forms, which have a selective affinity for IGF II and are minor forms in adults. Such a pattern occurs in GH deficiency. Conclusion : The profile of IGFs and IGF-BPs in foetal serum is similar to that seen after birth when GH control is absent.

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SEX HORMONE BINDING GLOBULIN (SHBG) IN MATERNAL AND FETAL BLOOD AND IN AMNIOTIC FLUID DURING THE PERINATAL PERIOD

To investigate the regulation and physiological significance of SHBG in fetal life, it's distribution in maternal and fetal blood and in amniotic fluid was measured. In spite of high circulating estrogens, the fetal SHBG-levels were 13.4-fold below the maternal blood-concentrations: 3.o ± 1.o (SD) ug/ml (n = 62) versus 4o.1 ± 9.3 (SD) ug/ml (n = 64). No significant difference was found regarding fetal sex in maternal and fetal blood: female 2.9 ± o.8 ug/ml (n = 29) and male 3.2 ± 1.o ug/ml (n = 33). In amniotic fluid the SHBG-concentration was 1.1 ± o.6 ug/ml in female and o.9 ± o.5 in male pregnancies. This difference is not significant. After the sharp fall of estrogens postnatally the maternal SHBG-concentration declined by 5o% during the first puerperal week. In contrast, the SHBG-levels increased by almost 1oo% during the first week in the newborns of both sexes (5.6 ± 1.8 ug/ml). These findings suggest, that fetal and maternal SHBG-levels are independently regulated. While maternal SHBG is strongly influenced by circulating estrogens, the regulation in fetuses and newborns seems to be controlled by mechanisms other than induction and suppression by estrogens and androgens. It may be speculated, that the low SHBG-levels during fetal life are related to the increased biological steroid activity during this period. In contrast to other studies the measurement of SHBG in amniotic fluid did not prove to help identify fetal sex.

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