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PROLACTIN(PRL) SECRETION OF PREMATURE AND FULL-TERM NEONATES IN NORMAL AND VARIOUS PATHOLOGICAL CONDITIONS. H. Takahashi, H. Kondoh and M. Takata, Department of Pediatrics, Kanazawa Medical University, Ishikawa-ken 920-02, JAPAN

To investigate the physiological role of PRL in early life, we have studied serum PRL concentrations longitudinally in large cohort of premature and full-term neonates in normal and pathological conditions.

We have studied serum PRL in full-term neonates(n=84), premature neonates(n=50), those of amniotic fluid(n=29) and umbilical cord specimen. We also studied serum PRL in children with dehydration(n=30) before and after fluid therapy. Serum PRL has been measured by immunoradiometric assay, using newly developed one-step solid phase PRL assay kit.

Full-term neonates revealed high PRL in male (156.4± 74.1ng /ml) and in female(178.4± 74.1ng/ml) and these high PRL values immediately after birth gradually fell over one to four months postnatally. Small for date(SFD) premature neonates at 31-36 weeks showed significantly higher PRL, compared to those of appropriate for date(AFD), but no significant difference was noted in SFD and AFD full-term neonates. Significantly higher PRL was found in asphyxia of full-term neonates, but not in those of premature infants. Increased PRL in children with dehydration revealed significant decrease after fluid replacement therapy.

We have extensively studied serum PRL in perinatal period in normal and various pathological conditions, because PRL has numerous and diverse actions. This study appeared to be the first report of ontogenesis of prolactin secretion in the fetus, neonates and infants in the largest series. The meaning of high PRL in the perinatal period remains to be elucidated.

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LACK OF 150kDa TERNARY COMPLEX FORMATION IN SERUM FROM THE HUMAN FETUS *IN UTERO*: INCREASED BIOAVAILABILITY OF CIRCULATING IGF-I

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IGF-I has been proposed to be an important endocrine growth factor in human fetal growth. We have determined IGF-I concentrations in acid chromatographed serum obtained *in utero* from 34 human fetuses of Rhesus Iso-immunized pregnancies. Furthermore, IGFBPs and their molecular weight forms in fetal serum, were investigated, to asses the bioavailability of circulating IGF-I. IGF-I concentrations increased significantly (r=0.52, p<0.005) from a mean of 40µg/l at 20 weeks of gestation to 75µg/l at 35 weeks and decreased (r=0.57, p<0.001) as the hemoglobin concentration decreased (range 30-140% of the median of normal fetuses). Western ligand blot analysis of fetal serum displayed decreased IGFBP-3 and increased IGFBP-1 and IGFBP-2 as compared to adult serum (this has been confirmed by specific RIAs). Proteolysis of ^{125}I IGFBP-3 was not increased in fetal serum as compared to non-pregnant adult serum. Neutral size cromatography of pools of fetal serum displayed the presence of excess ^{125}I IGF-I binding sites almost exclusively in the 50kDa complex. Western immunoblot analysis of the fractions displayed the following molecular weight distribution of immunoreactive IGFBP-3 (irIGFBP-3):

irIGFBP-3 in	fetus (w 19-25)	fetus (w 26-29)	fetus (w 30-35)	adult
150kDa complex	13%	14%	16%	62%
50kDa complex	87%	86%	84%	38%

In conclusion there is a lack of ternary ALS/IGFBP-3/IGF-I complex formation in human fetal serum and increased IGF-I availability due to its presence predominantly in the 50kDa complex or smaller forms. Furthermore, less IGF-I is bound by high affinity IGFBP-3. Finally we suggest that intra-uterine growth retardation in severe cases of Rhesus Iso-immunization could be attributed to decreased serum IGF-I concentrations.

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HIGH LEVELS OF GRANULOCYTE–COLONY STIMULATING FACTOR (G–CSF) IN CORD BLOOD AND AMNIOTIC FLUID.

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The hematopoietic growth factor G-CSF stimulates the production and function of granulocytes. Fetal and neonatal granulocytes have functional defects and therefore preterm born neonates tend to develop severe sepsis. The pathophysiology, intrauterine regulation, and production of G-CSF in utero and in fetus remain to be clarified. We analysed the serum of cord blood (n=20) from the 34th to the 42th week of gestation and amniotic fluid (n=20) from the 15th to the 22th week of gestation. The murine cell line NFS-60 was used in a proliferation assay to detect G-CSF. NFS-60 cells did not respond to cytokines like rh IL-1 to IL-3, IL-6, EPO, TNF and TGF-beta. The lower detection limit of G-CSF was 5 pg/ml. Cord blood levels ranged from 5 to 2.700 pg/ml (n=20) and amniotic fluid levels from 120 to 9000 pg/ml. In cord blood increasing activity of G-CSF was demonstrated towards the end of pregnancy (39th to 42th week of gestation). Twins had the same G-CSF levels. There is a close linkage between G-CSF levels and abnormal alpha fetoprotein. Based on these normative data we plan a prospective study to evaluate if intrauterine G-CSF levels can act as a predictive risk factor to develop postnatal sepsis.

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PROTEIN KINASE C ISOTYPE EXPRESSION DURING EARLY PITUITARY DEVELOPMENT. L. Cuttler, M. Axline, and A.P. Fields, Depts of Pediatrics/Pharmacology, Case Western Reserve Univ, Cleveland OH, USA

Protein kinase C (PKC) is a critical element for signal transduction in many cell types, and its activation mediates the secretion of pituitary hormones in developing and mature animals. Although it has recently been recognized that PKC consists of a family of functionally distinct isotypes with tissue-specific patterns of distribution, the expression of specific PKC isotypes during pituitary development is not known. In order to determine the ontogeny of pituitary PKC isotypes, we prepared extracts from pituitary homogenates of newborn (2-4 days [d]-old; n= 2 preparations), juvenile (12-d-old; n= 1 preparation), and adult male (3-4 months; n= 4 preparations) Sprague-Dawley rats. The soluble fractions of the pituitary homogenates from each age group were subjected to SDS-PAGE, and immunoblot analyses performed using affinity-purified, highly specific antibodies to PKC isotypes α, ßI, ßII, γ, δ, ϵ, and ζ (3-6 experiments/isotype). PKC isotypes α and ζ were expressed in high abundance in immature and mature pituitary extracts. However, the expression of PKC ßI, ßII, γ, and ϵ were markedly age-dependent; these isotypes were clearly expressed by adult pituitaries but not detected in neonatal and juvenile pituitaries. PKC γ was detected in very low quantities in all age groups. Conclusion: PKC expression in the pituitary is isotype-specific and is subject to strict developmental progression. The achievement of a mature pattern of pituitary PKC isotype expression appears to involve an expanded capacity to produce diverse and specific PKC isotypes.

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21-HYDROXYLASE DEFICIENCY SCREENING AND INCIDENCE IN ISRAEL 1986-1991. H. Front, M. Schreiber, J. Sack, Sheba Medical Center, Tel Hashomer, Israel.

From June 1987 until December 1991 we screened a countrywide random sample of 90,228 newborns for 21 OHD measuring 17 α - OH progesterone (17 OHP) from blood spotted on filter paper. 2 Arabs and 1 Jew, were detected with levels of 17 OHP between 135 - 315 ng/ml, suggesting very low incidence of 1 in 30,000 live births. In order to compare these findings to the incidence of 21 OHD in the total population born in Israel during the years 1986-1991, and if we missed infants who were among those who were screened, all the archives of the hospitals in the country and the pediatric endocrinologists collaborated. The incidence of 21 OHD nationwide was 1:19,000, 1:30,000 for the Jews and 1:8,000 for the Arabs. The incidence of 21 OHD among Arab newborns in the northern part of the country was even higher 1:5,000 (14:71130). The M/F ratio was 1:2.6 and the ratio of salt-wasting to simple virilizing was 5:1. Two male patients were diagnosed prenatally, 21 patients (17 F and 4 M) during the first month after birth and 6 others subsequently. Conclusions: The high ratio of F/M suggests that male patients were missed or died early due to salt-wasting. The high incidence of this disease in the northern part of the country and among the Arabs, suggests that screening in this part of the country is warranted.

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HORMONE-RECEPTOR INTERACTION OF HUMAN CHORIONIC GONADOTROPIN (hCG) AND ITS GLYCOSYLATION VARIANTS, AS STUDIED BY MONOCLONAL ANTIBODIES (MCA).

The hCG receptor has recently been cloned and revealed to be a member of the G protein-coupled receptor family (1). Receptor binding of hCG involves surface regions of both protein subunits of the α/β heterodimer hormone (2) and presumably several regions of the large extracellular domain (ECD)(3), which is the unique structure of all sequenced glycoprotein hormone receptors sequenced so far. How this complex binding leads to activation of a signal transduction cascade (G-protein activation and cAMP increase) is still unclear. The carbohydrate chains of hCG (two asparagine linked ones on each subunit) seem to play a critical role in signal transduction since deglycosylated hCG (degly-hCG) binds the receptor with high affinity but without intrinsic activity (2). To elucidate the functional role of the carbohydrate moieties in receptor interaction we chose a MCA based sandwich-type assay. Previously, we have described fourteen epitopes on the antigenic surface of hCG: 5 epitopes on the α subunit, 5 β subunit epitopes and 4 epitopes resulting from formation of the $\alpha\beta$ heterodimer (4). Having assessed that every epitope discovered on soluble hCG is also present on soluble degly-hCG (5) we compared the epitope accessibility of receptor bound hCG and degly-hCG to ^{125}I-labeled MCA. We found that receptor bound hCG exposed only two epitopes (β3 and β5), while all other 12 epitopes were not detectable. In contrast none of the 14 epitopes were accessible on receptor bound degly-hCG. This observation suggests that receptor binding of hCG and degly-hCG results in hormone receptor complexes in which the ECD of the receptor covers most of the respective ligands surface. In addition hCG and degly-hCG differ in distinct receptor bound orientations since degly-hCG exposed none of the 14 epitopes and thus seemed to be more buried by the ECD. If these two different orientations of receptor binding would be responsible for differences in signaltransduction competence (agonistic hCG, antagonistic degly-hCG) will be addressed in further investigations. This study shows that MCA against hCG can be used as tools to describe receptor interaction of hCG and hCG-variants. Furthermore, the sandwich assay approach can probably help to investigate receptor binding of hCG-variants of several patients such as children with the recently described carbohydrate-deficient glycoprotein syndrome (CDG) (6) or hCG secreting tumors.

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