
Marco A. Rivarola y Alicia Belgorosky
Servicio de Endocrinologia, Hospital de Pediatria Garrahan, Buenos Aires, Argentina

As in previous meetings of the Endocrine Society (ENDO 05 and ENDO 06) we will intend to review the present Annual Meeting, ENDO 07, the most relevant endocrine meeting of the year. The 89th Meeting took place in Toronto, Canada, at the Metro Toronto Convention Center, between saturday June 2 and Tuesday June 5, 2007.

However, the overall approach for ENDO this year will break with recent tradition by moving away from a central meeting theme and offering a broader array of topics for attendees. Among other innovative features of ENDO 07 are: “The Year In” presentations, which will review important articles published over the preceding 12 months; Endocrine Debates, which will offer two sides of compelling subjects with audience participation in the form of electronic voting; an expanded poster session program, adding a fourth day (totaling 2369 posters); conversations with basic researchers, which are designed to introduce basic science researchers to leaders in their field. These new features complement a strong slate of established programs, such as 16 plenary presentations, Out-of-the Box Lectures, New Technology Lectures, a greatly expanded Meet the Professor program (92 sessions), 77 symposia (3 speakers each) divided in basic, basic/clinical, translational and clinical, Oral Presentations (total 335) and other educational sessions. Moreover, the Exhibit Floor offered approximately 200 booths with diverse materials of interest for more than 8800 attendees. The web site of the Endocrine Society is "www.endo-society.org".

Obviously, it is impossible to summarize all the activity of this huge Meeting. Additional information can be gathered by reading the abstracts available in the web page of the Endocrine Society http://www.abstracts2view.com/endo/.

In the opening ceremony, Dr Leonard Wartofsky, President of the Endocrine Society gave the presidential address reflecting a year of successes and new horizons. Reaching new heights in attendance at the annual meeting, making unprecedented strides in policy initiatives and public outreach, providing greater resources for members through the release of several Clinical Practice Guidelines, creating strong financial and strategic planning goals, and building an enduring foundation for the new Clack T. Sawin Memorial Library and Resource Center were some of the multiple achievements announced.

As it is already a tradition, the Endocrine Nurses Society Symposium was part of this year activities.

Several laureate award winners were honoured for their work. Among them, the 2007 Distinguished Physician Award was presented to Bernardo I. Wajchenberg, M.D., from Hospital das Clinicas, Universidad Sao Paulo, in recognition of outstanding contributions to the practice of clinical endocrinology. The award includes a $3,000 honorarium. Moreover, other endocrinologists from Latino America were invited to participate either as speakers in a Symposium (Dr. Ana Latronico from Brasil) or in Meet the Professor sessions (Drs Berenice Mendonca from Brasil, Veronica Mericq from Chile, and Marco A. Rivarola from Argentina).
Implicit in the justification for the enormous increase in investment in bio-molecular research that has occurred over the past 20 years has been the promise that this would provide insights relevant to the understanding of human pathophysiology and the ultimate alleviation of suffering from human disease. To this end, model organisms are enormously attractive as they provide tractable and controllable systems in which precise data can be obtained. In contrast the study of complex human disease is fraught with difficulties related to the multifactorial nature of most human illness, and the challenges of controlling for the effects of largely immeasurable confounding factors, both genetic and environmental. While it is critical that research on complex human phenotypes and diseases continues an approach that focuses on extreme human phenotypes has established itself as a useful complementary strategy. Firstly, it is more likely that extreme human phenotypes are caused by tractable monogenic or oligogenic defects. Secondly, the consequences of such defects are not always identical to those seen in animal models. Thirdly, once a link between a major mutation particular gene and a human phenotype is established it is much more likely that subtle variation in those genes is involved in influencing susceptibility to common human diseases. Finally, discoveries in this area may lead to effective mechanism-based therapies which provide justification for the continuation of investment in basic biomedical research. I will discuss advances that have come from the studies of two cohorts of humans with extreme phenotypes, namely obesity and insulin resistance.

Several human phenotypes were commented by Dr O’Rahili:

Mutations of leptin receptor (LEPR). The prevalence of pathogenic LEPR mutations in a cohort of subjects with severe, early-onset obesity was 3%. Circulating levels of leptin were not disproportionately elevated, suggesting that serum leptin cannot be used as a marker for leptin-receptor deficiency. Congenital leptin-receptor deficiency should be considered in the differential diagnosis in any child with hyperphagia and severe obesity in the absence of developmental delay or dysmorphism. Humans congenitally lacking proopiomelanocortin (POMC) gene products. These patients all presented in early life with features of hypocortisolemia secondary to ACTH deficiency, leading to hypoglycemia, prolonged jaundice, susceptibility to the effects of infection and, in one case, neonatal death. The children responded well to physiological replacement with glucocorticoids but all subsequently developed marked obesity in association with hyperphagia. All children thus far reported have pale skin and red hair, features consistent with the known role of POMC-derived peptides in the determination of the phaeomelanin to eumelanin ratio in melanocytes. Thus, the cardinal features of congenital POMC deficiency are isolated ACTH deficiency, hyperphagia, and severe early-onset obesity. Although red hair may be an important diagnostic clue in patients of Caucasian origin, its absence in patients originating from other ethnic groups should not result in this diagnostic consideration being excluded. The prohormone convertases (PC1 and PC2) are expressed in neuroendocrine tissues and act upon a range of substrates including proinsulin, proglucagon, and POMC. PC1 is itself synthesized as an inactive precursor, then undergoes two autocatalytic events, first within the endoplasmic reticulum and then within the secretory vesicles of the regulated secretory pathway to generate a fully active 66-kDa isoform that is stored in mature secretory granules.
Reported phenotype has been an adult female with severe early-onset obesity, hypogonadotropic hypogonadism, postprandial hypoglycemia, hypocortisolemia, and evidence of impaired processing of POMC and proinsulin. This patient suffered from severe small intestinal absorptive dysfunction as well as the characteristic severe early-onset obesity, impaired prohormone processing, and hypocortisolemia. Heterozygous mutations in the MC4R in humans that were associated with dominantly inherited obesity. The clinical features of MC4R deficiency include hyperphagia, which invariably starts in the first year of life. Alongside the increase in fat mass, MC4R-deficient subjects also have an increase in lean mass and a marked increase in bone mineral density, thus they often appear “big-boned”. They exhibit accelerated linear growth in early childhood, which does not appear to be due to dysfunction of the GH axis and may be a consequence of the disproportionate early hyperinsulinemia seen in these patients. The accelerated linear growth and the disproportionate early hyperinsulinemia are consistent with observations in the MC4R knockout mouse. Brain-derived neurotrophic factor (BDNF) regulates the development, survival, and differentiation of neurons through its high-affinity receptor, tropomyosin-related kinase B (TrkB). Unlike other neurotrophins, BDNF is secreted in an activity-dependent manner that allows for highly controlled release. Recently, BDNF has been implicated in the regulation of body weight because its expression is reduced by fasting and BDNF administration causes weight loss in wild-type mice through a reduction in food intake. BDNF has also been implicated in memory and a range of behaviors using a number of conditional knockout models. They reported a child with severe obesity, impaired short-term memory, and developmental delay who had a de novo missense mutation impairing the function of TrkB, the tyrosine kinase receptor that mediates the effects of both BDNF and the neurotrophin, NT4/5. They have also identified a patient with severe hyperphagia and obesity and a complex neurobehavioral phenotype including impaired cognitive function and memory as well as distinctive hyperactive behaviour.


Steven W Lamberts, Dept of Med, Erasmus Med Ctr, Rotterdam, Netherlands

Chronic stress has been implicated in insulin resistance, obesity, atherosclerosis and cognitive loss. Cortisol action is mediated through glucocorticoid receptors (GR). We investigated whether variations in the GR gene are associated with changes in cortisol sensitivity, as well as with these disorders. Mutations in the GR gene are rare. In only 12 patients mutations, mainly in the ligand-binding domain of the GR gene were found. In the absence of symptoms of cortisol excess, these patients present with hypokalemia and hypertension (due to mineralocorticoid excess), and hirsutism and menstrual disorders in women (due to adrenal androgen excess).

We studied glucocorticoid sensitivity in 216 healthy individuals with two overnight suppression tests (1 and 0.25 mg dex). Serum cortisol concentrations showed a strong intraindividual stability. Subsequently we studied the associations with 4 GR gene polymorphisms (polym).

The N363S and BclI polym (allele freq. 3.7%, and 36.5%, resp) were associated with an increased sensitivity to dex, and increased BMI.

The ER22/23EK polym (allele freq. 3.4%) was associated with relative resistance to dex. ER22/23EK heterozygote young men were taller, stronger and had more muscle mass. Later in life this polym was associated with a healthier metabolic profile, and towards the end of life the risk of dementia was lower, while carriers lived longer. The fourth polym, lying in the ‘ATTTA’ motif of the 3’ untranslated region has an allele
frequency of 14.5%. Homozygote GR-9 carriers had increased CRP, increased risk of coronary heart disease and myocardial infarction, but were protected from persistent Staphylococcus aureus nasal carriage. Three polym were functional. In mononuclear leukocytes of homozygous carriers the N363S had increased and the ER22/23EK polym had decreased GR-mediated transactivation, while GR-9 polym carriers had a decreased transrepression by glucocorticoids. The ER22/23EK polym increases expression of the less active GR-A translational isoform, the GR-9 polym increases GR mRNA stability, and the N363S and (the intronic) BclI polym cause changes in gene expression for which the mechanisms have not yet been explained. Small variations in the GR gene cause measurable changes in glucocorticoid sensitivity. These observations might help to improve the choice of an optimal cortisol replacement dose and a potential “safe” dose of glucocorticoids in the treatment of immune diseases.

**Does Breast Cancer Start in the Womb?**
_Ana M Soto, Dept of Anat & Cell Biol, Tufts Univ Sch of Med, Boston, MA_

Recent data have suggested that perturbations in the fetal environment may predispose individuals to disease and/or organ dysfunction, which become apparent in adulthood. This new emphasis on the fetal origins of adult diseases has prompted scientists to hypothesize that fetal exposure to environmental estrogens may be an underlying cause of the increased incidence of uterine leiomyoma, testicular cancer and breast cancer observed in European and US populations over the last 50 years.

Humans are routinely exposed to bisphenol-A (BPA), an estrogenic compound that leaches from dental materials, food and beverage containers and other plastic consumer products. In mice, prenatal exposure to environmentally relevant levels of BPA accelerated the development of the fetal mammary gland and induced alterations of the mammary gland architecture which manifested during puberty and adulthood, long after the period of exposure has ended. In these mice, BPA increased the number of terminal end buds and terminal ends and increased lateral branching of the ducts. Just as significant, BPA exposed mice showed an enhanced sensitivity to estradiol. All these parameters are associated with an increased risk for developing breast cancer.

But...does prenatal exposure to BPA induce mammary gland neoplasia? To answer this question, we chose a rat model because it more closely mimics the human disease regarding hormone factors and histopathology than the available mouse models. Indeed, fetal exposure to BPA induces the development of ductal hyperplasias and carcinoma in situ which manifest during early adulthood. These highly proliferative lesions have an increased number of estrogen receptor alpha-positive cells. Thus, fetal bisphenol A exposure is sufficient to induce the development of preneoplastic and neoplastic lesions in the mammary gland in the absence of any additional treatment aimed at increasing tumor development.

Women prenatally exposed to diethylstilbestrol are now reaching the age at which breast cancer is commonly diagnosed. Emerging epidemiological data are revealing an increased incidence of breast cancer in this population. Hence, animal experiments and epidemiological data strengthen the hypothesis that exposure to xenoestrogens during early development may be an underlying cause of the increased incidence of breast cancer observed over the last 50 years.

**Advances in the Understanding of the Genetic Basis of Bone and Mineral Disorders.**
_Rajesh V Thakker, Nuffield Dept of Med; OCDEM, Univ of Oxford, Oxford, UK_
The parathyroids play a central role in calcium homeostasis, and disorders that result in altered parathyroid hormone (PTH) secretion lead to abnormalities of bone and mineral metabolism. Thus, PTH over-secretion due to parathyroid tumours, which affect 3:1,000 of the population, is a major cause of hypercalcaemia which may be associated with osteoporosis and kidney stones. PTH deficiency, which results in hypocalcaemia and occurs in 1:4,000 live births, may be associated with tetany, epilepsy, cataracts and skeletal malformations. Molecular genetic studies of these parathyroid disorders have increased our understanding of calcium homeostasis, as illustrated by investigations of diseases associated with hypoparathyroidism which have helped to elucidate pathways regulating PTH secretion and development of the parathyroids from the embryonic third and fourth branchial pouches. These studies have identified some of the genes (e.g. GATA3, Gdm2 and Hoxa3) involved in the parathyroid developmental pathway. For example, GATA3 haploinsufficiency has been shown to cause the hypoparathyroidism-deafness-renal dysplasia (HDR) syndrome, which is an autosomal dominant syndrome. GATA3 belongs to a family of dual zinc-finger transcription factors that are involved in vertebrate embryonic development. Indeed functional assessments, using electrophoretic mobility shift and yeast-two-hybrid assays, of the GATA3 abnormalities associated with HDR has revealed 3 classes of mutations: those that lead to a loss of DNA binding which represent over 90% of all mutations, and involve a loss of the carboxy-terminal zinc finger; those that result in a reduced DNA-binding affinity; and those that do not alter DNA binding or affinity but likely alter a conformational change. Moreover, the HDR phenotype is consistent with the expression pattern of GATA3 during human and mouse embryogenesis in the developing kidney, otic vesicle and parathyroids. Thus, mutant mice deleted for one copy of Gata3, i.e. heterozygotes (+/-), develop deafness due to degeneration of the outer hair cells in the cochlea, whilst Gata3 null mice, i.e. homozygotes (-/-), which are embryonically lethal, develop renal hypoplasia and parathyroid abnormalities. Thus, studies of hypoparathyroid patients and mouse models have helped to elucidate some of the genes involved in the embryological development of the parathyroids, which play a major role in bone and mineral metabolism.

ROY O GREEP AWARD LECTURE: Pituitary Development and Disease: Roles of Transcription Factors and Signaling Pathways.

Sally A Camper, Univ of Michigan Med Sch, Ann Arbor, MI

Pituitary hormone deficiency and pituitary adenomas are common problems. Studies in genetically engineered and mutant mice have advanced understanding of the mechanisms underlying these disease processes. In most cases, genes discovered in the mouse have led quickly to the discovery of lesions in human patients and have revealed the genetic hierarchy of control of pituitary cell specification and growth. During development signaling molecules expressed in the infundibulum and ventral mesenchyme, such as WNTs, BMPs, and FGFs, control spatial patterns of transcription factor expression, leading to specialized cell types that produce pituitary hormones. Expression of noggin, an antagonist of BMP signaling, and TCF4, an effector of WNT signaling, in the infundibulum are critical for maintaining the balance of signaling pathways necessary for normal pituitary growth and morphology.

Many transcription factors play important roles in pituitary development and hormone production. The early-acting genes are not pituitary specific, and lesions in these genes cause defects in development of other organs and/or structures. Some of these are homeobox genes with overlapping functions and multiple roles during ontogeny, i.e. Pitx1 and Pitx2, Lhx3 and Lhx4. Defects in some of these genes cause apoptosis, leading
to pituitary hypoplasia. The pituitary functions of genes with broad expression patterns, like Gata2, a downstream target of Pitx2, can be dissected by tissue specific disruption. Prop1 and Pit1 are examples of homeobox transcription factors specifically involved in pituitary development. While the ultimate characteristics of adult Prop1 and Pit1 mutants are similar, there are dramatic differences in their effects on fetal and neonatal pituitary development. Prop1 mutants have delayed pituitary vascularization and dysmorphology owing to failure of progenitors to migrate away from the proliferative zone and undergo differentiation. Prop1 expression is necessary for Notch signaling and activation of Pit1 expression. Defects in the notch signaling target Hes1 cause pituitary hypoplasia and altered cell fate. We have used comparative genomics, gene expression profiling, and bioinformatics to identify regulatory sequences in Prop1 and downstream targets of Prop1 and Pit1. This gene discovery approach has revealed an exciting new group of transcription factors that are candidates for regulating pituitary development and the basis of human hormone deficiency disease.

New Concepts and Applications of In Vitro Engineered Follicles.

*Teresa K Woodruff, Dept of Ob and Gyn, Northwestern Univ, Evanston, IL*

In vitro ovarian follicle culture provides a tool to investigate folliculogenesis and may one day provide women with new fertility management options. The application of tissue engineering principles to ovarian follicle maturation enables the creation of biomimetic environments that coordinate the growth of the multi-cellular compartments within the follicle. Additionally, three-dimensional culture systems preserve follicle architecture, thereby maintaining critical cell-cell and cell-matrix interactions lost in traditional two-dimensional attached follicle culture systems. Finally, the in vitro system permits study of the fundamental biological principles underlying follicle maturation. For example, a lower matrix stiffness enhances follicle growth and permits coordinated cell growth, antral cavity formation, theca cell differentiation and oocyte maturation. Less rigid and dense matrices secrete estrogen at levels exceeding progesterone and androgen accumulation, which is characteristic of normal ovarian function. A more rigid and dense environment favors high progesterone and androgen secretion, which is observed in some cases of infertility. These studies reveal, for the first time, a direct link between the biomechanical environment and follicle function, and suggest a novel non-hormonal connection between follicle function and the rigidity of the environment. These advances in ovarian follicle biology may provide insights and technology useful to restore fertility in cases where reproductive function is threatened.

New Perspectives in Endometriosis.

*Linda C Giudice, Dept of Ob/Gyn, Univ of California-San Francisco, San Francisco, CA*

Endometriosis is a common, estrogen-dependent gynecologic disorder associated with pelvic pain, infertility, and increased risk of ovarian cancer. It is characterized by ectopic lesions of endometrium on the ovaries, pelvic peritoneum, and bowel that cause a local inflammatory response contributing to the associated infertility and pelvic pain. Most lesions arrive at their destination by retrograde menstruation which most women experience, although 6-10% have the disorder that is manifested after puberty and regresses after menopause. Recent advances demonstrate that there is a genetic component and that endometriosis lesions synthesize estradiol that is amitotic and a survival factor for the lesions. Ectopic lesions and eutopic endometrium (within the uterus) of women with disease are resistant to the actions of progesterone, complicating
the therapeutic paradigm, but also providing opportunities for innovation. Diagnosis of endometriosis is by direct visualization and biopsy of suspicious lesions at the time of surgery under general anesthesia. Treatment focuses on lowering endogenous estradiol levels by GnRH agonists, combined steroid hormone contraceptives, progestins, and more recently, aromatase inhibitors coupled with progestins. Also, because of an inflammatory component, some therapies have focused on components of the immune system, without major success. Microarray analyses of eutopic and ectopic endometrium demonstrate that lesions on the ovary (endometriomas) are distinct from peritoneal and rectovaginal disease. In addition, gene ontology analysis and further bioinformatic analyses demonstrate a resistance to progesterone action in eutopic endometrium, especially in the early secretory phase of the menstrual cycle. Endometrial biopsy and subsequent candidate gene analysis offer a diagnostic approach that precludes surgery and general anesthesia. Pathway analysis reveals involvement of the MAPK and k-ras pathways active in endometrium from women with vs. without the disorder. Interestingly, a mouse model of over-expression of k-ras and conditional Pten deletion in ovarian surface epithelium results in endometriosis (and endometrioid ovarian carcinoma). The possibility remains that similar mutations are contributory to establishment of the disease and the accompanying risk for endometrioid ovarian carcinoma observed in women with this disorder.

Predicting Endocrine Autoimmunity.

Anthony Weetman, The Med Sch, Univ of Sheffield, Sheffield, UK

Autoimmune endocrine diseases frequently co-exist and are associated with other non-endocrine autoimmune conditions, such that the traditional dichotomy between organ-specific and non-organ-specific autoimmunity is no longer helpful. The most important reason for these overlaps is the influence of genetic factors. As well as the long-established associations between autoimmune diseases and HLA-DR alleles (and especially with the DR3 specificity in Caucasians), it is now clear that many autoimmune diseases share an association with two genes that are critical to determining the overall level of T cell function, namely CTLA-4 and PTPN22. In the case of autoimmune polyglandular syndrome (APS) type 1, there is a single gene defect, namely a mutation in the AIRE gene, which leads to widespread autoimmune disease through failure to delete autoreactive T cells during thymic tolerance induction and this provides the best example of failure of central tolerance as a cause of autoimmunity. Notably, autoimmune thyroid disease is rather uncommon in APS type 1 and this implies the presence of other mechanisms that control autoreactive T cell activity, including peripheral tolerance mechanisms. The effect of environmental factors on susceptibility is likely to be at the level of peripheral tolerance. Prediction of autoimmune endocrine disease through genotyping and estimation of the influence of environmental markers is unlikely to be sufficiently sensitive and specific for clinical utility, whereas the slow course of autoimmune endocrine disease allows serological prediction using autoantibodies in a number of situations when disease is still in a subclinical form. The availability of new serological markers will improve the ability to predict the onset of clinically relevant endocrinopathies and this is best illustrated by the wide diversity of autoantibodies that have been uncovered in patients with APS type 1, including those directed against the calcium sensing receptor in patients with hypoparathyroidism. Another level of prediction is possible based on accurate association studies which establish the statistical likelihood of development of any particular disease in the presence of another. To date little cost benefit analysis has been
performed to evaluate such associations and their impact on screening programmes although these will be important in justification of clinical follow-up strategies.

**Maternal Programming of Neuroendocrine Systems through DNA Methylation**

*Michael Meaney, Douglas Hosp Research Ctr, McGill Univ, Verdun QC, Canada*

Early life experiences shape an individual’s physical and mental health across the lifespan. Not surprisingly, an upbringing that is associated with adversity can produce detrimental effects on health. A central theme that arises from studies in human and nonhuman species is that the effects of adversity are mediated by the interactions between a mother and her young. In this review we describe some of the long-term effects of maternal care on the offspring and we focus on the impact of naturally occurring variations in the behavior of female rats. Of particular interest are mothers that engage in high or low amounts of licking/grooming (LG) and arched-back nursing (ABN) of their pups, but do so within the normal range for this species. Such variations in LG-ABN can alter the function of the hypothalamic-pituitary-adrenal (HPA) axis, and cognitive and emotional development by directly affecting the underlying neural mechanisms. At the heart of these mechanisms is gene expression. By studying the hippocampal glucocorticoid receptor gene, they have identified that maternal care regulates its expression by changing two processes: the acetylation of histones H3-K9, and the methylation of the NGFI-A consensus sequence on the exon 1 promoter.

They reported that increased pup licking and grooming (LG) and arched-back nursing (ABN) by rat mothers altered the offspring epigenome at a glucocorticoid receptor (GR) gene promoter in the hippocampus. Offspring of mothers that showed high levels of LG and ABN were found to have differences in DNA methylation, as compared to offspring of ‘low-LG-ABN’ mothers. These differences emerged over the first week of life, were reversed with cross-fostering, persisted into adulthood and were associated with altered histone acetylation and transcription factor (NGFI-A) binding to the GR promoter. Central infusion of a histone deacetylase inhibitor removed the group differences in histone acetylation, DNA methylation, NGFI-A binding, GR expression and hypothalamic-pituitary-adrenal (HPA) responses to stress, suggesting a causal relation among epigenomic state, GR expression and the maternal effect on stress responses in the offspring.

Thus, they showed that an epigenomic state of a gene can be established through behavioral programming, and it is potentially reversible. Sustained “maternal effects” appear elsewhere in biology, including plants, insects, and lizards, and may have evolved to program advantages in the environments that the offspring will likely face as adults. These findings demonstrate that the structural modifications of the DNA can be established through environmental programming and that, in spite of the inherent stability of this epigenomic marker, it is dynamic and potentially reversible. Given the importance of early life and parent-child interactions to later behavior, prevention and intervention programs should target this critical phase of development.

**Social Regulation of GnRH and Its Receptors.**

*Russell D Fernald, Dept of Biol Sci, Stanford Univ, Stanford, CA*

How do social encounters produce changes in the brain? The social environment influences physiological, cellular and molecular processes in the brain but how is social information transduced into cellular and molecular changes? To discover general principles of behavioral influences on the brain, we study the most important event in an animals life, reproduction which is controlled via the highly conserved brain-pituitary-
gonadal axis. We use a model system in which socially dominant animals can reproduce while non-dominant animals cannot. We used an African cichlid fish, *Astatotilapia burtoni*, with two distinct, reversible male phenotypes: dominant (territorial, T) males that are larger, more brightly colored, more aggressive, and reproductively competent and non-dominant males (non-territorial, NT) that are smaller, camouflage colored, and have regressed gonads. By manipulating the essential features of the natural life of this species, including the social system, we ask experimental questions across the spectrum from behavioral to molecular levels of analysis. When adult males become socially dominant, hypothalamic neurons containing gonadotropin releasing hormone enlarge while social descent reverses this change. The dendrites of these cells also change, altering their interactions. They traced the neural circuits controlling these changes using gene expression maps, as well as conventional tracers. Since GnRH neurons are phylogenetically ancient, these studies should provide insight about reproductive regulation across vertebrate phyla. Among fish, even in the pituitary, T males have significantly higher levels of pituitary GnRH-R1 mRNA than NT males. Using quantitative PCR, they also compared the expression of specific subtypes of androgen (AR) and estrogen (ER) receptor genes between dominant and subordinated males in 3 divisions of the brain, the pituitary, and the testes. They measured mRNA levels of AR-alpha, AR-beta, ER-alpha, ER-betaa, and ER-betab, gonadotropin-releasing hormone 1 (GnRH1), and GnRH receptor 1 (GnRH-R1) relative to 18S rRNA. In the anterior part of the brain, they found that dominant males had higher mRNA expression of AR-alpha, AR-beta, ER-betaa, and ER-betab, but not ER-alpha, compared to subordinate males. This effect of dominance was reflected in a positive correlation between testes size and AR-alpha, AR-beta, ER-betaa, and ER-betab in the anterior brain. In addition, mRNA levels of all ARs and ERs in the anterior brain were positively correlated with mRNA level of GnRH1. In the middle and posterior portions of the brain, as well as the testes, steroid receptor mRNA levels were similar among dominants and subordinates. In the pituitary, ER-alpha mRNA level was positively correlated with testes size and AR-alpha mRNA was positively correlated with GnRH-R1 mRNA level. These data suggest that dominant male brains could be more sensitive to sex steroids, which may contribute to the increased complexity of the behavioral repertoires of dominant males.

**SYMPOSIA**

**REGULACION DE LA PUBERTAD**

**The Impact of Endocrine-Disrupting Chemicals on Female Puberty.**

*Jean Pierre Bourguignon, Pediat, Univ of Liege, Liege, Belgium*

Fetal or early postnatal exposure to endocrine disrupting chemicals (EDCs) possibly results in a spectrum of disorders throughout life. Because androgens play a key-role in sexual differentiation while many EDCs have antiandrogenic properties, early effects of EDCs can be observed in the male. In the female, vaginal adenocarcinoma in daughters of women exposed to the potent synthetic estrogen DES was among the first evidences of EDC effects. Since then, there were few observations indicating early female effects of estrogenic EDCs. Such a hypothesis was considered after we and others reported early puberty on average and an unexpectedly high incidence of sexual precocity in internationally adopted girls. The ethnic and nutritional factors did not provide unequivocal explanations. In these patients, detectable serum levels of a persisting derivative of the insecticide DDT, an estrogenic EDC, raised the question of early DDT effects on female sexual maturation and the consequences of withdrawal from the DDT.
exposed environment after migration. The immature (5-15 day old) female rat was used as a model since hypothalamic explants obtained at these ages were sensitive to a stimulatory effect of estradiol on the frequency of pulsatile GnRH secretion. With a 1:1,000 potency ratio, DDT accounted for the same in vitro effects as estradiol and involved similarly the estrogen receptor and the AMPA/kainate subtype of glutamate receptors. After administration of estradiol or DDT in vivo between 5 and 15 days, sexual precocity (early vaginal opening and first estrus) was thought to involve both peripheral and central mechanisms including early acceleration of pulsatile GnRH secretion and temporary inhibition of pituitary LH secretion. Accordingly, sexual precocity in migrating girls formerly exposed to EDCs could share a common pathophysiological mechanism with other conditions (e.g. congenital adrenal hyperplasia) resulting initially in peripheral precocity and secondarily in central puberty after management of the primary disorder. The relevance of our findings for sexual development in general remains speculative in view of the variety of EDCs spread in the environment and the possible mixture effects. EDCs should be considered among other factors to account for the still changing distribution of age at onset of puberty in some countries while subsequent effects on reproductive capacity and hormone-dependent cancers in the female need to be further monitored.

Evolution of Puberty

**Evolution and Contemporary Onset of Puberty: Mismatched?**

*Mark A Hanson, DOHaD, Univ of Southampton/Princess Anne Hosp, Southampton, UK*

During their life history many animals make tradeoffs between growth, reproduction, fecundity and longevity, to maximize reproductive success and Darwinian fitness. The ability to make such ‘choices’ at key points in development has itself evolved, and uses the processes of developmental plasticity. Whilst in some species short life is associated with high fecundity, human child development necessitates long parental investment and thus a low fecundity - long longevity strategy.

The timing of key stages in the life-history can be influenced by environmental conditions, e.g. poor conditions may accelerate metamorphosis but at the risk of smaller body size. An alternative strategy, adopted by *H. sapiens*, is to delay onset of puberty in poor conditions which may be adaptive if conditions subsequently improve. However impaired prenatal environment is associated with earlier puberty. This applies especially to females, for which life history theory suggests that reproductive success is tightly linked to nutritional status before and during pregnancy and lactation, whereas for the male, body size is the key determinant of reproductive success. The combination of impaired prenatal environment followed by plentiful postnatal nutritional environment should produce the greatest advancement in the timing of puberty in girls, and recent data confirm this. This effect is particularly evident in populations undergoing rapid socio-economic transitions such as migrants.

The issue of inappropriately named ‘precocious puberty’ is an example of the consequences of mismatch between evolved human biology and our contemporary environments. In pre-agricultural hominids it seems probable that puberty occurred relatively young to allow a mother to nurture several offspring through childhood. This timing may have matched neural maturation needed for contemporary social requirements. With, agriculture, settlement and stratified society came infection, malnutrition and delayed puberty onset, but still matched to longer childhood needed for learning more complex tasks. But recent dramatic improvements in environmental conditions in developed societies have removed these constraints and menarche has
advanced to be close again to its evolved timing - yet the demands on today’s adolescents require more, not fewer, years. Developed, and still less developing, societies are ill-equipped to deal with this mismatch.

**Role of GPR54 Mutations in Pubertal Disorders.**

_Anna Claudia Latronico, Endocrinology and Metab, Hosp das Clinicas da Fac de Medicina da USP, Sao Paulo, Brazil_

The kisspeptin-GPR54 signaling complex has been proposed as a gatekeeper of pubertal activation of GnRH neurons and the reproductive axis. Several loss-of-function mutations of the GPR54, a G protein-coupled receptor, were identified as a new cause of familial and sporadic isolated hypogonadotropic hypogonadism. In addition, mice bearing homozygous targeted disruptions of _gpr54_ exhibited a similar failure of sexual maturation. The normal hypothalamic content of GnRH in these animals indicated that the pathways of GnRH neuronal migration and GnRH synthesis remain intact and suggested a defect in the processing or secretion of GnRH. Indeed, subsequent studies in rodent and primate models have demonstrated localization of _KiSS1_ mRNA in the hypothalamus, colocalization of GPR54 in GnRH neurons, GnRH-dependent activation of LH and FSH release by intracerebroventricular or peripheral administration of kisspeptin, and increased hypothalamic _KiSS-1_ and _GPR54_ mRNA levels at the onset of puberty. More recently, a novel autosomal dominant GPR54 mutation, R386P, was identified in a Brazilian girl with idiopathic gonadotropin-dependent precocious puberty. _In vitro_ studies demonstrated that this mutation leads to prolonged activation of intracellular signaling pathways in response to kisspeptin. This non-constitutively activating mutation of GPR54 represents the first recognized genetic cause of gonadotropin-dependent precocious puberty. These findings have confirmed the major role of the kisspeptin/GPR54 complex in neuroendocrine regulation of the gonadotropic axis and therefore sexual maturation.

**Gubernacular development: its central role in testicular descent and cryptorchidism.**

_John M Hutson, Royal Children’s Hosp Res Inst, Parkville, VIC, Australia_

The understanding of normal testicular descent has changed dramatically in recent years. The process is now known to occur in 2 steps with the gubernaculum central to both. The first step requires enlargement of the gubernaculum under control of insulin-like hormone 3 (Insl 3), while in the second step the gubernaculum migrates to the scrotum under androgenic control. The genitofemoral nerve provides chemotactic and trophic stimuli to guide this migration, which shows many of the growth characteristics of an embryonic limb bud. Cryptorchidism is caused mostly by breakdown of this migratory phase. Recognition of early postnatal germ cell male development has led to recommending orchidopexy at 6 months of age. Inguinal hernia closure is tightly linked to testicular descent and recent studies suggest that both are regulated by the genitofemoral nerve. Finally, recent studies suggest that cryptorchidism can often be acquired, secondary to anomalies in hernia closure.

**Developmental Origin of Human Testicular Cancer: Environmental & Genetic Aspects.**

_Ewa Rajpert-De Meyts, Growth & Reprod, Univ Hosp of Copenhagen, Copenhagen, Denmark_

Testicular germ cell tumors occur predominantly in young adults but they originate very early in life. The two main tumor types, classical seminoma and nenseminomas, despite
their histological heterogeneity are derived from a common precursor cell, carcinoma in situ (CIS) cell. Growing evidence suggests that CIS is a result of malignant transformation of immature germ cells, most probably primordial germ cells or gonocytes, which in analogy to CIS cells, but in contrast to mature spermatogonia, show high expression of embryonic pluripotency genes, e.g. NANOG, POU5F1 (OCT-3/4) and TFAP2C (AP2-gamma). Testicular neoplasia occurs predominantly in testes with signs of disturbed early development, as documented in numerous histological and epidemiological studies. The incidence of testicular cancer has been growing in recent decades in many countries in the world but with marked geographic and ethnic differences suggesting the predominant etiological role for environmental or lifestyle factors. At the same time, similar trends were observed in incidences of cryptorchidism and mild forms of hypospadias, suggesting the common etiology for at least a subset of these disorders, which have been grouped together with testicular cancer in the testicular dysgenesis syndrome. Genetic factors undoubtedly play a role in the most severe form of testicular dysgenesis, with gene polymorphism most probably explaining the ethnic differences.

**Preservation of Spermatogenesis.**

Kolja Kvist, Pediat Surg, Univ Hosp of Copenhagen, Copenhagen, Denmark

Two groups of boys are particularly prone to experience fertility problems later in life. One group being boys suffering a disease for which the treatment involves gonadotoxic treatment regimens and the other is boys in whom the testes have not descended properly - cryptorchidism. In the western world approximately 2.5% of boys undergo surgery for cryptorchidism. The disease carries an increased risk of infertility upon reaching adulthood, ranging from up to 20% in unilaterally affected boys and up to 70% in bilaterally affected. Numerically the group of boys receiving gonadotoxic treatment is smaller, but never the less it is estimated that by year 2010 1 in 250 will be a longtime survivor of a malignant disease contracted in childhood. Presently these groups of patients have no fertility preserving options available. However, over the past decade studies on animals -including primates - have shown that spermatogonial stem cells from different species tolerate freezing, and upon thawing are capable of re-initiating spermatogenesis when injected into sterile testes. Furthermore ectopic grafting within and between animal species of intact testicular tissue have proven a feasible method of storing and accelerating initiation of spermatogenesis. Intriguingly it appears that contrary to xenografts of immature or neonatal testis tissue, xenografts of adult testis tissue are unable to maintain spermatogenesis. Other studies have ascertained that immunomagnetic cell-sorting, either alone or combined with density gradient centrifugation, is an effective method for achieving enriched and purified suspensions of stem cells, and in the process discarding malignant cells. Animated by these studies we undertook to evaluate the effect of cryopreservation on intact testicular tissue from pre-pubertal, cryptorchid boys, and in the process develop a protocol for the cryopreservation of such tissue. Judged by the histological appearance and hormone production, the tissue tolerated cryopreservation quite well. In lieu hereof it seems possible, that within a foreseeable future we shall be able to treat...
infertility arisen in childhood, either as a consequence of a congenital disorder or due to gonadotoxic treatment of a malignant disease, through preservation of spermatogenesis.

**NOVEL DISORDERS OF WATER METABOLISM: THERE’S SOMETHING NEW IN THE WATER**

**Aquaporin-1 in the Human Choroids Plexus**  
**Soren Nielsen, Inst. of Anatomy, Aarhus Univ Hosp. Aarhus C, Denmark**

The choroid plexus epithelium secretes electrolytes and fluid in the brain ventricular lumen at high rates. Several channels and ion carriers have been identified as likely mediators of this transport in rodent choroid plexus. This study aimed to map several of these proteins to the human choroid plexus. Immunoperoxidase-histochemistry was employed to determine the cellular and subcellular localization of the proteins. The water channel, aquaporin (AQP) 1, was predominantly situated in the apical plasma membrane domain, although distinct basolateral and endothelial immunoreactivity was also observed. The Na(+)−K(+)−ATPase alpha(1)-subunit was exclusively localized apically in the human choroid plexus epithelial cells. Immunoreactivity for the Na(+)−K(+)−2Cl(−) cotransporter, NKCC1, was likewise confined to the apical plasma membrane domain of the epithelium. The Cl(−)/HCO(3)(−) exchanger, AE2, was localized basolaterally, as was the Na(+)−dependent Cl(−)/HCO(3)(−) exchanger, NCBE, and the electroneutral Na(+)−HCO(3)(−) cotransporter, NBCn1. No immunoreactivity was found toward the Na(+)−dependent acid/base transporters NHE1 or NBCe2. Hence, the human choroid plexus epithelium displays an almost identical distribution pattern of water channels and Na(+) transporters as the rat and mouse choroid plexus. This general cross species pattern suggests central roles for these transporters in choroid plexus functions such as cerebrospinal fluid production.

**Nephrogenic Syndrome of Inappropriate Antidiuresis: A Novel Disorder in Water Balance.**  
**Stephen Gitelman, Univ of California-San Francisco, San Francisco, CA**

Maintaining appropriate water balance is essential for life. Homeostasis occurs via an intact thirst mechanism, mediated by sensors in the hypothalamus, and regulation of water loss in the kidney, governed by arginine vasopressin (AVP). Hyponatremia is encountered frequently in clinical medicine, often resulting from the syndrome of inappropriate antidiuretic hormone secretion (SIADH). We recently evaluated 2 unrelated male infants who had persistent hyponatremia and were unable to excrete a maximally dilute urine. However, they did not seem to fit the usual pattern for SIADH: their problems developed in the first months of life and persisted; they did not have any conditions often associated with SIADH (such as a CNS or pulmonary lesion); and their AVP levels were consistently unmeasurable. We hypothesized that these infants had a novel gain of function defect in the AVP signaling pathway. DNA sequencing of each patient’s V2 vasopressin receptor gene identified missense mutations at codon 137 in each (R137C or R137L) in each. This change maps to the presumed second cytoplasmic loop of this G-protein coupled receptor, and mutations in this same codon (R137H) have been reported previously to cause nephrogenic diabetes insipidus. Further analysis showed that the mutant receptors induce 4 to 7.5-fold greater basal activity than the wild-type V2 vasopressin receptor. Thus, these mutations do appear to cause constitutive activation of the V2R, as we had originally hypothesized. These findings represent a previously unrecognized genetic disease, which has been designated as nephrogenic
syndrome of inappropriate antidiuresis. A number of questions have emerged from these initial findings and will be addressed during the presentation, including: 1) what is the natural history of this disorder; 2) what is the frequency; 3) are there non-renal manifestations; 4) are heterozygotes affected; 5) what is the optimal therapy; and 6) how do these mutations cause constitutive activation of the receptor?

**Vasopressin Antagonists.**

*Florence Wong, Dept of Med, Univ of Toronto, Toronto, ON, Canada*

There are several clinical scenarios where hyponatremia is a common occurrence. In patients with the syndrome of inappropriate antidiuretic hormone (SIADH) secretion, hyponatremia is primarily a result of excessive water retention, caused by either exogenous administration or excess endogenous production of AVP. In edematous states such as congestive heart failure or cirrhosis, there is excess non-osmotic release of AVP, causing excess renal water retention and hence hyponatremia.

Traditional treatment of chronic hyponatremia in the euvoletic and hypervolemic patient is to gradually correct the serum sodium by restricting water intake to <800ml per day. However, this is unpalatable, and patient compliance is poor. AVP V2 receptor antagonists are non-peptide agents, whose structure is very similar to that of AVP. They bind to the V2 receptor with high degree of affinity, thereby preventing the binding of AVP, without themselves triggering receptor activation. To date, there are 4 agents being developed for clinical use, and all have been named vaptan.

Conivaptan is the only AVP receptor antagonist that has both V1 and V2 activity. Lixivaptan, tolvaptan and satavaptan are pure V2 receptor antagonists. All have been studied in patients with SIADH, congestive heart failure and cirrhosis with ascites. The results have been correction of hyponatremia, increased urine output, decreased urinary osmolality. There were significant patient withdrawals in all studies so far, partly related to the advanced state of their disease. Thirst was the most significant side effect. Despite this, renal function did not deteriorate, and the plasma norepinephrine, renin and aldosterone where measured remained unchanged. Satavaptan has also been used as an adjunct therapy to diuretics in the management of ascites in cirrhosis without hyponatremia. Cirrhotic patients with ascites taking satavaptan for a 12 week period had significantly less frequent paracenteses.

Therefore, it is clear than the V2 receptor antagonists will become the mainstay of treatment for hyponatremia in euvoletic and hypervolemic patients. The indication for their use may be broadened to include adjunct therapy in addition to diuretics in the control of ascites in normo-natrempic patients. Although all the V2 receptor antagonists have proven to be relatively safe, they still need to be used judiciously, so that patients will not become excessively dehydrated, with over-correction of hyponatremia.