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**2010**

**ARNOLD E., RIVERA J.C., THEBAULT S., MORENO-PARAMO D., QUIROZ-MERCADO H., QUINTANAR-STEPHANO A., BINART N., MARTINEZ-DE LA ESCALERA G., CLAPP C.**

High levels of serum prolactin protect against diabetic retinopathy by increasing ocular vasoinhibins.

*Diabetes*, 59 (12), 3192-3197, 2010

(Services cités : U845 (NB))

**OBJECTIVE:** Increased retinal vasopermeability (RVP) occurs early in diabetes and is crucial for the development of sight-threatening proliferative diabetic retinopathy (DR). The hormone prolactin (PRL) is proteolytically processed to vasoinhibins, a family of peptides that inhibit the excessive RVP related to DR. Here, we investigate the circulating levels of PRL in association with DR in men and test whether increased circulating PRL, by serving as a source of ocular vasoinhibins, can reduce the pathological RVP in diabetes. **RESEARCH DESIGN AND METHODS:** Serum PRL was evaluated in 40 nondiabetic and 181 diabetic men at various stages of DR. Retinal vasoinhibins were measured in rats rendered hyperprolactinemic by placing two anterior pituitary grafts under the kidney capsule and in PRL receptor-null mice. RVP was determined in hyperprolactinemic rats subjected to the intraocular injection of vascular endothelial growth factor (VEGF) or made diabetic with streptozotocin. **RESULTS:** The circulating levels of PRL increased in diabetes and were higher in diabetic patients without retinopathy than in those with proliferative DR. In rodents, hyperprolactinemia led to vasoinhibin accumulation within the retina; genetic deletion of the PRL receptor prevented this effect, indicating receptor-mediated incorporation of systemic PRL into the eye. Hyperprolactinemia reduced both VEGF-induced and diabetes-induced increase of RVP. This reduction was blocked by bromocriptine, an inhibitor of pituitary PRL secretion, which lowers the levels of circulating PRL and retinal vasoinhibins. **CONCLUSIONS:** Circulating PRL influences the progression of DR after its intraocular conversion to vasoinhibins. Inducing hyperprolactinemia may represent a novel therapy against DR.

**BACHELOT A., BOUILLY J., LIU Y., REBOURCET D., LEUX C., KUTTENN F., TOURAINE P., BINART N.**

Sequence variation analysis of the prolactin receptor C-terminal region in women with premature ovarian failure.

*Fert. Steril.*, 94 (7), 2772-2775, 2010

(Services cités : U845 (NB), U845 (VG))

Using a mouse model expressing only the PRL receptor short isoform mimicking premature ovarian failure, signaling pathways induced by PRL were analyzed in mouse ovaries. Sequencing of the coding portion of exons 10 and 11, specific to the long and short receptor isoform, respectively, did not reveal any mutation in 101 women with premature ovarian failure.

**BINART N., BACHELOT A., BOUILLY J.**

Impact of prolactin receptor isoforms on reproduction.

*Trends Endocrinol. Metab.*, 21 (6), 362-368, 2010

(Services cités : U845 (NB))

Prolactin is a hormone involved in growth, development, reproduction, metabolism, water and electrolyte balance, brain and behavior, and immunoregulation. Its actions on reproductive processes represent the largest group of functions identified for this hormone. Besides the classic long form of the prolactin receptor, many short form receptors have been identified in rodents and human tissues. Mouse mutagenesis studies have offered insight into the biology of the prolactin family, providing compelling evidence that different isoforms have independent biological activity. The possibility that short forms mediate cell proliferation is important for a variety of tissues including mammary glands and ovarian follicles. This review summarizes the current knowledge about prolactin signaling and its role in reproduction through either long or short isoform receptors.

**SAUVAT F., SARNACKI S., BINART N.**

Fertility preservation before puberty: from mice to men.

*Ann. Endocrinol.*, 71 (3), 231-236, 2010

(Services cités : Chirurgie Viscérale Pédiatrique, U845 (NB))

Malignant tumors account for 1% of childhood cancers. The incidence is to the order of 122 cases per million children. The five-year survival after cancer before the age of 16 years has improved from 50 to 80% in 40 years. Assessment of potential for preservation of fertility should thus be a systematic element of care for children treated for a malignant tumor (high-dose chemotherapy with alkylating agents, radiation therapy including the gonads) or those receiving hematopoietic stem cell grafts for malignant or benign disease (sickle-cell anemia, immune deficit). Among the techniques proposed, cryopreservation of ovarian tissue appears to be the most promising, or perhaps the only one available before puberty with encouraging result. Nevertheless the uncertainties, or even risks, related to these treatments, should not be neglected.

**SCHRAENEN A., LEMAIRE K., de FAUDEUR G., HENDRICKX N., GRANVIK M., VAN LOMMEL L., MALLET J., VODJDANI G., GILON P., BINART N., IN'T VELD P., SCHUIT F.**

Placental lactogens induce serotonin biosynthesis in a subset of mouse beta cells during pregnancy.

*Diabetologia*, 53 (12), 2589-2599, 2010

(Services cités : U845 (NB))

**AIMS/HYPOTHESIS:** Upregulation of the functional beta cell mass is required to match the physiological demands of mother and fetus during pregnancy. This increase is dependent on placental lactogens (PLs) and prolactin receptors, but the mechanisms underlying these events are only partially understood. We studied the mRNA expression profile of mouse islets during pregnancy to gain a better insight into these changes. **METHODS:** RNA expression was measured ex vivo via microarrays and quantitative RT-PCR. In vivo observations were extended by in vitro models in which ovine PL was added to cultured mouse islets and MIN6 cells. **RESULTS:** mRNA encoding both isoforms of the rate-limiting enzyme of serotonin biosynthesis, tryptophan hydroxylase (TPH), i.e. Tph1 and Tph2, were strongly induced (fold change 25- to

200-fold) during pregnancy. This induction was mimicked by exposing islets or MIN6 cells to ovine PLs for 24 h and was dependent on janus kinase 2 and signal transducer and activator of transcription 5. Parallel to Tph1 mRNA and protein induction, islet serotonin content increased to a peak level that was 200-fold higher than basal. Interestingly, only a subpopulation of the beta cells was serotonin-positive in vitro and in vivo. The stored serotonin pool in pregnant islets and PL-treated MIN6 cells was rapidly released (turnover once every 2 h).

**CONCLUSIONS/INTERPRETATION:** A very strong lactogen-dependent upregulation of serotonin biosynthesis occurs in a subpopulation of mouse islet beta cells during pregnancy. Since the newly formed serotonin is rapidly released, this lactogen-induced beta cell function may serve local or endocrine tasks, the nature of which remains to be identified.

**2009**

**BACHELOT A., BEAUFARON J., SERVEL N., KEDZIA C., MONGET P., KELLY P.A., GIBORI G., BINART N.**

Prolactin independent rescue of mouse corpus luteum life span: identification of prolactin and luteinizing hormone target genes.

*Amer. J. Physiol. - Endocrinol. Met.*, 297 (3), E676-E684, 2009

(Services cités : U845 (NB))

The corpus luteum (CL) plays a central role in the maintenance of pregnancy in rodents, mainly by secreting progesterone. Female mice lacking prolactin (PRL) receptor (R) are sterile due to a failure of embryo implantation, which is a consequence of decreased luteinizing hormone (LH) receptor expression in the CL and inadequate levels of progesterone. We attempted to treat PRLR(-/-) females with human chorionic gonadotropin (hCG) and showed a de novo expression of LHR mRNA in the corpora lutea. Binding analysis confirmed that the LHR in hCG-treated PRLR(-/-) animals was functional. This was accompanied with increased expression of steroidogenic enzymes involved in progesterone synthesis. Despite these effects, no embryo implantation was observed because of high expression of 20 $\alpha$ -hydroxysteroid dehydrogenase. To better appreciate the molecular mechanisms underlying maintenance of the CL, a series of mRNA expression-profiling experiments was performed on isolated corpora lutea of PRLR(-/-) and hCG-treated PRLR(-/-) mice. This approach revealed several novel candidate genes with potentially pivotal roles in ovarian function, among them, p27, VE-cadherin, Pten, and sFRP-4, a member of the Wnt/frizzled family. This study showed the differential role of PRL and LH in CL function and identified new targets of these hormones in luteal cells.

**DEVI Y.S., SHEHU A., STOCCO C., HALPERIN J., LE J., SEIBOLD A.M., LAHAV M., BINART N., GIBORI G.**

Regulation of Transcription Factors and Repression of Sp1 by Prolactin Signaling Through the Short Isoform of Its Cognate Receptor.

*Endocrinology*, 150 (7), 3327-3335, 2009

(Services cités : U845 (NB))

Prolactin (PRL) affects the development and function of the reproductive system by binding to two types of receptors, which differ by the size of their intracellular domain in rodents. Whereas the signaling pathway through the long form of the receptor (PRL-RL) is well characterized, signaling through the short form (PRL-RS) remains obscure. In this investigation, we examined transcription factors regulated by PRL in the ovary and decidua of mice expressing only PRL-RS in a PRLR null background. These mice provide a powerful in vivo model to study the selective signaling mechanism of PRL through PRL-RS independent of PRL-RL. We also examined the regulation of transcription factors in ovarian and uterine cell lines stably transfected with PRL-RS or PRL-RL. We focused our investigation on transcription factors similarly regulated in both these tissues and clearly established that signaling through PRL-RS does not activate the Jak2/Stat in vivo, but leads to severe down regulation of Sp1 expression, DNA binding activity, and nuclear localization, events that appear to involve the CamK pathway. Our in vivo and in culture data demonstrate that the PRL-RS activates a signaling pathway distinct from that of the PRL-RL.

**SAUVAT F., BINART N., POIROT C., SARNACKI S.**

Preserving fertility in prepubertal children.

*Hormone Res.*, 71 ( Suppl.1), 82-86, 2009

(Services cités : Chirurgie Viscérale Pédiatrique, U845 (NB))

**BACKGROUND:** As a result of advances in treatment, almost 80% of children and adolescents who currently receive a diagnosis of cancer become long-term survivors. Potential adverse consequences of treatment include impaired puberty and fertility due to gonadal removal, genital tract injury or damage to germ cells from adjuvant therapy. In recent years, treatment of solid tumors and hematological malignancies has been modified in an attempt to minimize damage to the reproductive system. Simultaneously, advances in assisted reproductive technologies have led to new possibilities for the prevention and treatment of infertility. We review experimental data in animal models and clinical experience in adults and discuss strategies to preserve fertility in prepubertal children. **CONCLUSIONS:** Fertility preservation should now be considered in children facing cancer treatment that has a high risk of gonadal toxicity including high-dose chemotherapy and bilateral irradiation of the gonads at toxic doses.

**2008**

**BREZILLON N.M., DASILVA L., L'HOTE D., BERNEX F., PIQUET J., BINART N., MOROSAN S., KREMSDORF D.**

Rescue of fertility in homozygous mice for the urokinase plasminogen activator transgene by the transplantation of mouse hepatocytes.

*Cell Transplant.*, 17 (7), 803-812, 2008

(Services cités : U845 (DK))

Development of the urokinase plasminogen activator/SCID (uPA/SCID) transgenic mouse model has opened new perspectives for the study of different biological mechanisms such as liver regeneration, stem cell differentiation, and human hepatic pathogens. We observed that homozygous uPA/SCID mice (uPA<sup>+/+</sup>/SCID) had a small offspring, indicating a fertility defect. The goal of this study was thus to rescue the fertility of homozygous uPA mice. A deregulation of ovarian function with an absence of corpus luteum was observed in female uPA<sup>+/+</sup>/SCID mice. In male uPA<sup>+/+</sup>/SCID mice, a decrease of the weight of the testes, epididymis, seminal vesicle, and prostate was measured. This was associated with an absence of seminal and prostatic secretions and a reduction in testicular sperm production. We hypothesized that the infertility of mice was the consequence of uPA-induced liver injury. Thus, in order to rescue liver function, hepatocytes from mice negative for the uPA transgene were transplanted into uPA<sup>+/+</sup>/SCID mice. Thirty days after cell transplantation, the livers of transplanted uPA<sup>+/+</sup>/SCID mice were totally repopulated and presented a normal morphology. Furthermore, transplantation restored normal body weight, life span, and reproductive organ function. In conclusion, we demonstrated that the transplantation of uPA<sup>+/+</sup>/SCID mice with healthy hepatocytes was sufficient to rescue the reproductive capacity of female and male uPA homozygous animals, highlighting the importance of normal liver function to reproductive capability.

**HALPERIN J., DEVI S.Y., ELIZUR S., STOCCO C., SHEHU A., REBOURCET D., UNTERMAN T.G., LESLIE N.D., LE J., BINART N., GIBORI G.**

Prolactin Signaling Through the Short Form of Its Receptor Represses FOXO3 and its Target Gene Galt Causing a Severe Ovarian Defect.

*Mol. Endocrinol.*, 22 (2), 513-522, 2008

(Services cités : U845 (NB))

Prolactin is a hormone with over 300 biological activities. Although the signaling pathway downstream of the long form of its receptor (RL) has been well characterized, little is known

about PRL actions upon activation of the short form (RS). Here, we show that mice expressing only RS exhibit an ovarian phenotype of accelerated follicular recruitment followed by massive follicular death leading to premature ovarian failure (POF). Consequently, RS-expressing ovaries of young adults are depleted of functional follicles and formed mostly by interstitium. We also show that activation of RS represses the expression of the transcription factor FOXO3 and that of the enzyme galactose-1-phosphate uridylyltransferase (Galt), two proteins known to be essential for normal follicular development. Our finding that FOXO3 regulates the expression of Galt and enhances its transcriptional activity indicates that it is the repression of FOXO3 by PRL acting through RS that prevent Galt expression in the ovary and causes follicular death. Co-expression of RL with RS prevents PRL inhibition of Galt, and the ovarian defect is no longer seen in RS transgenic mice that co-express RL, suggesting that RL prevents RS-induced ovarian impairment. In summary, we show that prolactin signals through RS and causes, in absence of RL, a severe ovarian pathology by repressing the expression of FOXO3 and that of its target gene Galt. We also provide evidence of a link between the POF seen in mice expressing RS, in mice with FOXO3 gene deletion as well as in human with Galt mutation.

**SAUVAT F., CAPITO C., SARNACKI S., POIROT C., BACHELOT A., MEDURI G., DANDOLO L., BINART N.**

Immature Cryopreserved Ovary Restores Puberty and Fertility in Mice without Alteration of Epigenetic Marks.

*PLoS ONE*, 3 (4), e1972, 2008

(Services cités : Chirurgie Viscérale Pédiatrique, U845 (NB))

Background: Progress in oncology could improve survival rate in children, but would probably lead to impaired fertility and puberty. In pre-pubertal girls, the only therapeutic option is the cryopreservation of one ovary. Three births have been reported after reimplantation of cryopreserved mature ovary. Conversely, reimplantation of ovary preserved before puberty (defined as immature ovary) has never been performed in humans. Methodology/Principal Findings: In order to analyze ovarian function, we performed transplantation using fresh or cryopreserved immature grafts in pre-pubertal or adult mice. Puberty as well as cyclic hormonal activity was restored. All follicle populations were present although a significant reduction in follicle density was observed with or without cryopreservation. Although fertility was restored, the graft is of limited life span. Because ex vivo ovary manipulation and cryopreservation procedure, the status of genomic imprinting was investigated. Methylation status of the H19 and Lit1 Imprinting Control Regions in kidney, muscle and tongue of offsprings from grafted mice does not show significant alteration when compared to those of unoperated mice. Conclusions/Significance: These results demonstrate that immature ovarian grafting can restore spontaneous puberty and fertility. However, these data suggest that follicle depletion leads to premature ovarian failure. This study addresses the very important epigenetics issue, and provides valuable information to the study of ovarian transplantation suggesting that these procedures do not perturb normal epigenetics marks. These results are highly relevant to the reimplantation question of immature cortex in women.

**VIENGCHAREUN S., SERVEL N., FEVE B., FREEMARK M., LOMBES M., BINART N.**

Prolactin Receptor Signaling Is Essential for Perinatal Brown Adipocyte Function: A Role for Insulin-like Growth Factor-2.

*PLoS ONE*, 3 (2), e1535, 2008

(Services cités : U845 (NB))

**BACKGROUND:** The lactogenic hormones prolactin (PRL) and placental lactogens (PL) play central roles in reproduction and mammary development. Their actions are mediated via binding to PRL receptor (PRLR), highly expressed in brown adipose tissue (BAT), yet their impact on adipocyte function and metabolism remains unclear.

**METHODOLOGY/PRINCIPAL FINDINGS:** PRLR knockout (KO) newborn mice were phenotypically characterized in terms of thermoregulation and their BAT differentiation assayed for gene expression studies. Derived brown preadipocyte cell lines were established to evaluate the molecular mechanisms involved in PRL signaling on BAT function. Here, we report that newborn mice lacking PRLR have hypotrophic BAT depots that express low levels of adipocyte nuclear receptor PPARgamma2, its coactivator PGC-1alpha, uncoupling protein 1 (UCP1) and the beta3 adrenoceptor, reducing mouse viability during cold challenge.

Immortalized PRLR KO preadipocytes fail to undergo differentiation into mature adipocytes, a defect reversed by reintroduction of PRLR. That the effects of the lactogens in BAT are at least partly mediated by Insulin-like Growth Factor-2 (IGF-2) is supported by: i) a striking reduction in BAT IGF-2 expression in PRLR KO mice and in PRLR-deficient preadipocytes; ii) induction of cellular IGF-2 expression by PRL through JAK2/STAT5 pathway activation; and iii) reversal of defective differentiation in PRLR KO cells by exogenous IGF-2.

**CONCLUSIONS:** Our findings demonstrate that the lactogens act in concert with IGF-2 to control brown adipocyte differentiation and growth. Given the prominent role of brown adipose tissue during the perinatal period, our results identified prolactin receptor signaling as a major player and a potential therapeutic target in protecting newborn mammals against hypothermia.

**2007**

**BACHELOT A., BINART N.**

Reproductive role of prolactin.

*Reproduction*, 133 (2), 361-369, 2007

(Services cités : U845 (NB))

The biological actions of prolactin (PRL), a polypeptide hormone, are mostly related to lactation and reproduction. These actions have been clarified by studies of PRL and PRL-deficient receptor mice, which have a clear phenotype of reproductive failure at multiple sites. This review aims to summarize current knowledge about PRL and its receptor, role in reproductive axis and presents information of hyperprolactinemia in reproductive medicine. Our understanding of the physiology and transduction pathway of PRL has largely increased in the past 20 years with the cloning of PRL and its receptor gene.

**2006**

**FLINT D.J., BINART N., BOUMARD S., KOPCHICK J.J., KELLY P.A.**

Developmental aspects of adipose tissue in GH receptor and prolactin receptor gene disrupted mice: site-specific effects upon proliferation, differentiation and hormone sensitivity.

*J. Endocrinol.*, 191 (1), 101-111, 2006

(Services cités : U809)

Direct metabolic effects of GH on adipose tissue are well established, but effects of prolactin (PRL) have been more controversial. Recent studies have demonstrated PRL receptors on adipocytes and effects of PRL on adipose tissue in vitro. The role of GH in adipocyte proliferation and differentiation is also controversial, since GH stimulates adipocyte

differentiation in cell lines, whereas it stimulates proliferation but inhibits differentiation of adipocytes in primary cell culture. Using female gene disrupted (ko) mice, we showed that absence of PRL receptors (PRLRko) impaired development of both internal and s.c. adipose tissue, due to reduced numbers of adipocytes, an effect differing from that of reduced food intake, where cell volume is decreased. In contrast, GHRko mice exhibited major decreases in the number of internal adipocytes, whereas s.c. adipocyte numbers were increased, even though body weight was decreased by 40-50%. The changes in adipose tissue in PRLRko mice appeared to be entirely due to extrinsic factors since preadipocytes proliferated and differentiated in similar fashion to wild-type animals in vitro and their response to insulin and isoproterenol was similar to wild-type animals. This contrasted with GHRko mice, where s.c. adipocytes proliferated, differentiated, and responded to hormones in identical fashion to controls, whereas parametrial adipocytes exhibited markedly depressed proliferation and differentiation potential and failed to respond to insulin or noradrenaline. Our results provide in vivo evidence that both GH and PRL stimulate differentiation of adipocytes but that the effects of GH are site specific and induce intrinsic changes in the precursor population, which are retained in vitro.

**PIWNICA D., FERNANDEZ I., BINART N., TOURAINÉ P., KELLY P.A., GOFFIN V.**  
A New Mechanism for Prolactin Processing into 16K PRL by Secreted Cathepsin D.  
*Mol. Endocrinol.*, 20 (12), 3263-3278, 2006  
(Services cités : U808, U809)

Cathepsins are lysosomal enzymes that were shown to release the antiangiogenic fragments 16K prolactin (PRL), endostatin, and angiostatin by processing precursors at acidic pH in vitro. However, the physiological relevance of these findings is questionable because the neutral pH of physiological fluids is not compatible with the acidic conditions required for the proteolytic activity of these enzymes. Here we show that cathepsin D secreted from various tissues is able to process PRL into 16K PRL outside the cell. To specifically target extracellular proteolysis, we used tissues from PRL receptor-deficient mice, which are unable to internalize PRL. As assessed by the use of specific inhibitors of proton extruders, we show that the proteolytic activity of cathepsin D requires local acid secretion driven by Na(+)/H(+) exchangers and H(+)/ATPase. Although it is usually assumed that cathepsin-mediated generation of antiangiogenic peptides occurs in the moderately acidic pericellular milieu found in malignant tumors, we propose a new mechanism explaining the extracellular activity of this acidic protease under physiological pH. Our data support the concept that secreted lysosomal enzymes could be involved in the maintenance of angiogenesis dormancy via the generation of active antiangiogenic peptides in nonpathological contexts.

**SLOT K.A., KASTELIJN J., BACHELOT A., KELLY P.A., BINART N., TEERDS K.J.**  
Reduced recruitment and survival of primordial and growing follicles in GH receptor-deficient mice.  
*Reproduction*, 131 (3), 525-532, 2006  
(Services cités : U809)

GH influences female fertility. The goal of the present study was to obtain more insight into the effect of loss of GH signalling, as observed in humans suffering from Laron syndrome, on ovarian function. Therefore, serial paraffin sections of ovaries of untreated and IGF-I-treated female GH receptor knock-out (GHR/GHBP-KO) mice were examined to determine the follicular reserve and the percentage of follicular atresia in each ovary. Our observations demonstrate that the amount of primordial follicles was significantly elevated in GHR/GHBP-

KO mice, while the numbers of primary, preantral and antral follicles were lower compared with wild-type values. The reduced number of healthy growing follicles in GHR/GHBP-KO mice was accompanied by a significant increase in the percentage of atretic follicles. IGF-I treatment of GHR/GHBP-KO mice for 14 days resulted in a reduced number of primordial follicles, an increased number of healthy antral follicles, and a decreased percentage of atretic follicles. The results of the present study suggest that GH may play a role, either directly or indirectly, via for instance IGF-I, in the recruitment of primordial follicles into the growing pool. Furthermore, GH seems to protect antral follicles, directly or indirectly from undergoing atresia.