

**Publications de l'U 845 – Equipe Binart (Inserm)  
(intégrée à l'unité KELLY de 1999 à 2005)**

**2007**

**BACHELOT A., BINART N.**

Reproductive role of prolactin.

*Reproduction*, 133 (2), 361-369, 2007 ; (Facteur d'Impact 2006 : **2,958**)

(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.**

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 ; (Facteur d'Impact 2005 : **3,319**)

(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., TOURAINE 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 ; (Facteur d'Impact 2005 : **5,872**)

(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 ; (Facteur d'Impact 2005 : **2,447**)

(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.

**2005**

**BACHELOT A., BINART N.**

Corpus luteum development: lessons from genetic models in mice.

*Curr. Top. Dev. Biol.*, 68 (.), 49-84, 2005

(Services cités : U584)

The corpus luteum is a transient endocrine gland that produces essentially progesterone, a required product for the establishment and maintenance of early pregnancy. In the absence of pregnancy, the corpus luteum will cease to produce progesterone, and the structure itself will regress in size over time. The life span and function of the corpus luteum is regulated by complex interactions between stimulatory (luteotrophic) and inhibitory (luteolytic) mediators. Although the process of luteal formation and regression has been studied for several decades, many of the regulatory mechanisms involved in loss of function and involution of the structure are incompletely understood. In rodents, prolactin is the major luteotrophic hormone by maintaining the structural and functional integrity of the corpus luteum for several days after mating. Other factors involved in steroidogenesis, control of cell cycle, apoptosis, and tissue remodeling have been shown to play a role in corpus luteum development and maintenance. Especially, PGF2alpha seems to be the most potent luteolytic hormone. One of the most important advances in the study of mammalian genes has been the development of techniques to obtain defined mutations in mice. These tools enable us to target specific genes and to analyze the impact of their loss on cell fate and function. With these approaches, several receptors, transcription factors, enzymes, and other factors have been linked to corpus luteum development and maintenance. These models are helping to define mechanisms of reproductive function and to identify potential new contraceptive targets and genes involved in the pathophysiology of reproductive disorders.

**BESCOND M., RAHMANI Z.**

Dual-specificity tyrosine-phosphorylated and regulated kinase 1A (DYRK1A) interacts with the phytanoyl-CoA alpha-hydroxylase associated protein 1 (PAHX-API), a brain specific protein.

*Int. J. Biochem. Cell Biol.*, 37 (4), 775-783, 2005

(Services cités : U584)

Down syndrome (DS) is the most common genetic defect correlated with mental retardation and delayed development. The specific genes responsible for these phenotypic alterations have not yet been defined. Dyrk1A (dual-specificity tyrosine-phosphorylated and regulated kinase 1A), the human ortholog of the *Drosophila* minibrain gene (*mnb*), maps to the Down syndrome critical region of human chromosome 21 and is overexpressed in Down syndrome fetal brain. In *Drosophila*, minibrain is involved in postembryonic neurogenesis. In human, DYRK1A encodes a serine-threonine kinase but despite its potential involvement in the neurobiological alterations associated with Down syndrome, its physiological function has not yet been defined. To gain some insight into its biological function, we used the yeast two-hybrid approach to identify binding partners of DYRK1A. We found that the C-terminal region of DYRK1A interacts with a brain specific protein, phytanoyl-CoA alpha-hydroxylase-associated protein 1 (PAHX-API, also named PHYHIP) which was previously shown to interact with phytanoyl-CoA alpha-hydroxylase (PAHX, also named PHYH), a Refsum disease gene product. This interaction was confirmed by

co-immunoprecipitation of PC12 cells co-transfected with DYRK1A and PAHX-AP1. Furthermore, immunofluorescence analysis of PC12 cells co-transfected with both plasmids showed a re-distribution of DYRK1A from the nucleus to the cytoplasm where it co-localized with PAHX-AP1. Finally, in PC12 cells co-transfected with both plasmids, DYRK1A was no longer able to interact with the nuclear transcription factor CREB, thereby confirming that the intracellular localization of DYRK1A was changed from the nucleus to the cytoplasm in the presence of PAHX-AP1. Therefore, these data indicate that by inducing a re-localization of DYRK1A into the cytoplasm, PAHX-AP1 may contribute to new cellular functions of DYRK1A and suggest that PAHX-AP1 may be involved in the development of neurological abnormalities observed in Down syndrome patients.

**CASTANET M., SURA-TRUEBA S., CHAUTY A., CARRE A., de ROUX N., HEATH S., LEGER J., LYONNET S., CZERNICHOW P., POLAK M.**

Linkage and mutational analysis of familial thyroid dysgenesis demonstrate genetic heterogeneity implicating novel genes.

*Eur. J. Human Genet.*, 13 232-239, 2005

(Services cités : E 0363, Génétique Médicale Pédiatrique, Métabolisme-Neurologie, U584)

The pathophysiology of thyroid dysgenesis (TD) is not elucidated yet in the majority of cases. The unexpected familial clustering of congenital hypothyroidism due to TD suggests a genetically determined disorder. Four genes have been hitherto involved in thyroid development, including migration and growth. Three of these encode transcription factors (the thyroid transcription factors 1 and 2 (TTF1 or NKX2.1 and TTF2 or FOXE1) and PAX8) while the other encodes the thyrotropin hormone receptor (TSHR). Some mutations have been reported in patients affected by thyroid defects, which supports the relevance of these four genes in TD. However, their involvement in the general TD population remains questionable. Therefore, to document their involvement, we performed a linkage analysis followed by mutational analysis in 19 multiplex TD families. The LOD score results failed to prove linkage between any of the four genes and the TD phenotype, whatever the postulated mode of inheritance. Manual extended haplotypes showed allele sharing among affected individuals of at least one of these four genes in the majority of families. Nevertheless, mutational analysis did not identify mutations in these cases, arguing in favor of identity by descent and not identity by state. Furthermore, as a main result of the present study, extended haplotypes confirmed by mutational analysis showed that the four genes were excluded in five out of the 19 investigated families, demonstrating the relevance of other genes. In conclusion, the present study demonstrates genetic heterogeneity in the TD disorder and suggests the involvement of novel genes. *European Journal of Human Genetics* advance online publication, 17 November 2004; doi:10.1038/sj.ejhg.5201321.

**DE ROUX N.**

Isolated Gonadotropic Deficiency with and without Anosmia: A Developmental Defect or a Neuroendocrine Regulation Abnormality of the Gonadotropic Axis.

*Hormone Res.*, 64 (Suppl.2), 48-55, 2005

(Services cités : U584)

Hypogonadotropic hypogonadism has been described in several human genetic diseases. Congenital isolated hypogonadotropic hypogonadism is classified into two categories: one that is associated with anosmia (Kallmann syndrome) and one that is apparently isolated. Mutations and deletions of the KAL1 gene, which encodes for a protein involved in cell adhesion, have been observed in many cases of the X-linked form of Kallmann syndrome. Recently, loss-of-function

mutations of fibroblast growth factor receptor-1 (FGFR1) were associated with an autosomal dominant form of Kallmann syndrome. Genotype-phenotype correlations confirm the large spectrum of the phenotype due to FGFR1 mutations. Cases of isolated hypogonadotropic hypogonadism were considered to be idiopathic until the description of mutations of the gonadotropin releasing hormone receptor, luteinizing hormone and follicle stimulating hormone genes. However, defects in these genes only account for a small percentage of familial cases, which suggests that other proteins may be involved in regulation of the gonadotropic axis. We recently described GPR54 as one of these proteins by genome mapping in a very informative family. In vivo studies and genotype-phenotype correlations indicate that gonadotropic axis regulation by GPR54 occurs mainly at the level of the hypothalamus. Copyright (c) 2005 S. Karger AG, Basel.

#### **DE ROUX N.**

Les résistances à la GnRH et le gène GPR54.

*Rev. Prat., Spec. No 16-19, 2005*

(Services cités : [U584](#))

#### **FLEENOR D., ODEN J., KELLY P.A., MOHAN S., ALLIOUACHENE S., PENDE M., WENTZ S., KERR J., FREEMARK M.**

Roles of the lactogens and somatogens in perinatal and postnatal metabolism and growth: studies of a novel mouse model combining lactogen resistance and growth hormone deficiency.

*Endocrinology, 146 (1), 103-112, 2005*

(Services cités : [U584](#))

#### **FLINT D.J., TRAVERS M.T., BARBER M.C., BINART N., KELLY P.A.**

Diet-induced obesity impairs mammary development and lactogenesis in murine mammary gland.

*Amer. J. Physiol. - Endocrinol. Met., 288 (6), E1179-E1187, 2005*

(Services cités : [U584](#))

We have developed a mouse model of diet-induced obesity that shows numerous abnormalities relating to mammary gland function. Animals ate approximately 40% more calories when offered a high-fat diet and gained weight at three times the rate of controls. They exhibited reduced conception rates, increased peripartum pup mortality, and impaired lactogenesis. The impairment of lactogenesis involved lipid accumulation in the secretory epithelial cells indicative of an absence of copious milk secretion. Expression of mRNAs for beta-casein, whey acid protein, and alpha-lactalbumin were all decreased immediately postpartum but recovered as lactation was established over 2-3 days. Expression of acetyl-CoA carboxylase (ACC)-alpha mRNA was also decreased at parturition as was the total enzyme activity, although there was a compensatory increase in the proportion in the active state. By day 10 of lactation, the proportion of ACC in the active state was also decreased in obese animals, indicative of suppression of de novo fatty acid synthesis resulting from the supply of preformed fatty acids in the diet. Although obese animals consumed more calories in the nonpregnant and early pregnant states, they showed a marked depression in fat intake around day 9 of pregnancy before food intake recovered in later pregnancy. Food intake increased dramatically in both lean and obese animals during lactation although total calories consumed were identical in both groups. Thus, despite access to high-energy diets, the obese animals mobilized even more adipose tissue during lactation than their lean counterparts. Obese animals also exhibited marked abnormalities in alveolar development of

the mammary gland, which may partially explain the delay in differentiation evident during lactogenesis.

**GOFFIN V., BERNICHTEIN S., TOURAIN P., KELLY P.A.**

Development and potential clinical uses of human prolactin receptor antagonists.

*Endocrine Rev.*, 26 (3), 400-422, 2005

(Services cités : Endocrinologie & Médecine de la Reproduction, U584)

There is a large body of literature showing that prolactin (PRL) exerts growth-promoting activities in breast cancer, and possibly in prostate cancer and prostate hyperplasia. In addition, increasing evidence argues for the involvement of locally produced (autocrine) PRL, perhaps even more than pituitary-secreted (endocrine) PRL, in tumor growth. Because dopamine analogs are unable to inhibit PRL production in extrapituitary sites, alternative strategies need investigation. To that end, several PRL receptor antagonists have been developed by introducing various mutations into its natural ligands. For all but one of these analogs, the mechanism of action involves a competition with endogenous PRL for receptor binding. Such compounds are thus candidates to counteract the undesired actions of PRL, not only in tumors, but also in dopamine-resistant prolactinomas. In this review, we describe the different versions of antagonists that have been developed, with emphasis on the controversies regarding their characterization, and the limits for their potential development as a drug. The most recently developed antagonist, Delta1-9-G129R-hPRL, is the only one that is totally devoid of residual agonistic activity, meaning it acts as pure antagonist. We discuss to what extent this new molecule could be considered as a lead compound for inhibiting the actions of human PRL in the above-mentioned diseases. We also speculate on the multiple questions that could be addressed with respect to the therapeutic use of PRL receptor antagonists in patients.

**KARGES B., de ROUX N.**

Molecular genetics of isolated hypogonadotropic hypogonadism and kallmann syndrome.

*Endocr. Dev.*, 8 (.), 67-80, 2005

(Services cités : U584)

Isolated hypogonadotropic hypogonadism (IHH) is characterized by complete or partial failure of pubertal development due to impaired secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In the molecular pathogenesis of IHH, the gonadotropin-releasing hormone receptor (GnRH-R) and associated proteins have evolved as a central element. GnRH-R germline mutations were among the first genetic alterations identified in patients with IHH. These mutations are associated with impaired GnRH binding, ligand-induced signal transduction, or both, leading to various degrees of LH and FSH deficiency. As GnRH-R mutations explain several but not all cases of IHH, the search for new candidate genes continued in informative families. In 2003, mutations of the KiSS-1-derived peptide receptor GPR54 were identified in patients with IHH, opening a new pathway in the physiologic regulation of puberty and reproduction. GPR54 is putatively involved in the control of GnRH secretion. IHH associated with impaired olfactory function (Kallmann syndrome) may be caused by mutations of the X-chromosomal KAL1 (encoding anosmin) or the fibroblast growth factor receptor 1 genes (FGFR1), both leading to agenesis of olfactory and GnRH-secreting neurons. In addition to their clinical and diagnostic value, the identification of genetic and functional alterations in IHH helps to unravel the complex regulation of the gonadotropic axis.

**KARGES B., KRAUSE G., HOMOKI J., DEBATIN K.M., de ROUX N., KARGES W.**

TSH receptor mutation V509A causes familial hyperthyroidism by release of interhelical constraints between transmembrane helices TMH3 and TMH5.

*J. Endocrinol.*, 186 (2), 377-385, 2005

(Services cités : [U584](#))

Mutations of the human thyrotrophin receptor (TSH-R) are a cause of thyroid adenomas and hyperthyroidism. Here we study mechanisms of receptor activation in a genomic TSH-R variant V509A located in transmembrane helix (TMH) 3, which we identify in a family with congenital hyperthyroidism, multiple adenomas and follicular thyroid cancer. Using molecular modelling and dynamic simulation, we predicted the release of amino acid residue A593 (located opposite in domain TMH5) from a tight 'knob-and-hole' interaction with TMH3, physiologically constrained in the native receptor state by the bulky side chain of V509. To experimentally validate this concept, we generated mutant TSH-R expression constructs for functional in vitro studies. TSH-R mutant V509A showed a 2.8-fold increase in basal cAMP production, confirming constitutive TSH-R activation. The addition of a second site suppressor mutant A593V to TSH-R V509A resulted in the normalization of basal cAMP release, and the dose-responsiveness to TSH ligand was maintained. These data thus demonstrate that TSH-R V509A activation is caused by the release of TMH3-TMH5 interhelical constraints, while the native TSH-R conformation is re-stabilized by the introduction of a spacious valine residue at position 593. In conclusion, we delineate a novel mechanism of constitutive TSH-R activation, leading to thyroid hyperfunction and neoplasia.

**KARGES B., GIDENNE S., AUMAS C., HADDAD F., KELLY P.A., MILGROM E., de ROUX N.**

Zero-Length Cross-Linking Reveals that Tight Interactions between the Extracellular and Transmembrane Domains of the Luteinizing Hormone Receptor Persist during Receptor Activation.

*Mol. Endocrinol.*, 19 (8), 2086-2098, 2005

(Services cités : [U584](#))

Several molecular models of glycoprotein hormone receptor activation have been proposed. It has been suggested that ligand binding to the ectodomain (ECD) leads to major changes in intramolecular interactions between the ECD and the transmembrane domain. We studied these intramolecular modifications by generating a recombinant LH/CG receptor (LHR) bearing an intramolecular cleavage site. We did this by inserting a furin site at position 316 in the hinge region of the ECD (LHR\_Fur316). Affinity for human chorionic gonadotropin (hCG) and cAMP production upon hCG stimulation was identical to those of wild-type LHR. Western blot analysis showed that the LHR\_Fur316 receptor was cleaved into two subunits linked by disulfide bridges. Chemical shedding of the ECD from the transmembrane domain did not increase basal adenylate cyclase activity, indicating that the first 294 residues did not act as an inverse agonist. The truncated LHR\_316 was still activated by hCG but with an EC(50) higher than that for the wild-type receptor. Zero length cross-linking was used to study intramolecular interactions between the two domains of LHR\_Fur316. Cross-linking efficiency was similar for the basal and activated states, which indicated that the two domains interacted closely in the basal state, and this tight interaction persisted during activation. Our data suggest that activation of the LHR results from subtle modifications of intramolecular interactions between the two domains and low-affinity binding of hCG to the extracellular loops or residues preceding the first transmembrane segment.

**KEDZIA C., LACROIX L., AMEUR N., RAGOT T., KELLY P.A., CAILLOU B.,**

**BINART N.**

Medullary thyroid carcinoma arises in the absence of prolactin signaling.

*Cancer Res.*, 65 (18), 8497-8503, 2005

(Services cités : U584)

Prolactin, a pituitary hormone, exerts pleiotropic effects in various cells. These effects are mediated by a membrane receptor highly expressed in many tissues. To analyze prolactin effects on the thyroid gland, we first identified prolactin receptor (PRLR) mRNAs by in situ hybridization. To further evaluate the physiologic relevance of PRLR actions in the thyroid in vivo, we used PRLR knockout mice. Whereas the histologic structure of thyroid of PRLR-null mice was not disturbed, we show that T4 levels are lower in null animals (13.63 +/- 2.98 versus 10.78 +/- 2.25 pmol/L in null mice), confirming that prolactin participates in the control of thyroid metabolism. To further investigate thyroid effects in mice, we measured body temperature and thyroid-stimulating hormone in young and adult male and/or female PRLR-null mice and their normal siblings. Surprisingly, in null animals, we saw medullary thyroid carcinoma (MTC) arising from parafollicular C cells producing calcitonin. The incidence of these carcinomas attained 41% in PRLR-null mice, whereas this malignant tumor occurs sporadically or as a component of the familial cancer syndrome in humans. This finding suggests that PRLR-null mice could represent a valuable animal model for MTC, which could be compared with existing MTC models. These observations suggest a possible link between the appearance of this carcinoma and the absence of prolactin signaling.

**KELLY P.A., RAHMANI Z.**

DYRK1A Enhances the Mitogen-activated Protein Kinase Cascade in PC12 Cells by Forming a Complex with Ras, B-Raf, and MEK1.

*Mol. Biol. Cell*, 16 (8), 3562-3573, 2005

(Services cités : U584)

Dual-specificity tyrosine-phosphorylated and regulated kinase 1A (Dyrk1A) is the human homologue of the *Drosophila* *mnb* (minibrain) gene. In *Drosophila*, *mnb* is involved in postembryonic neurogenesis. In human, DYRK1A maps within the Down syndrome critical region of chromosome 21 and is overexpressed in Down syndrome embryonic brain. Despite its potential involvement in the neurobiological alterations observed in Down syndrome patients, the biological functions of the serine/threonine kinase DYRK1A have not been identified yet. Here, we report that DYRK1A overexpression potentiates nerve growth factor (NGF)-mediated PC12 neuronal differentiation by up-regulating the Ras/MAP kinase signaling pathway independently of its kinase activity. Furthermore, we show that DYRK1A prolongs the kinetics of ERK activation by interacting with Ras, B-Raf, and MEK1 to facilitate the formation of a Ras/B-Raf/MEK1 multiprotein complex. These data indicate that DYRK1A may play a critical role in Ras-dependent transducing signals that are required for promoting or maintaining neuronal differentiation and suggest that overexpression of DYRK1A may contribute to the neurological abnormalities observed in Down syndrome patients.

**LACROIX M.C., GUIBOURDENCHE J., FOURNIER T., LAURENDEAU I., IGOUT A., GOFFIN V., PANTEL J., TSATSARIS V., EVAIN-BRION D.**

Stimulation of Human Trophoblast Invasion by Placental Growth Hormone.

*Endocrinology*, 146 (5), 2434-2444, 2005

(Services cités : U584)

A critical step in establishment of human pregnancy is the invasion of the uterus wall by the

extravillous cytotrophoblast (EVCT), a process regulated by multiple autocrine and paracrine factors. Hormones belonging to the GH/prolactin family are expressed at the maternofetal interface. Because they are involved in cell motility in various models, we examined the possible regulatory role of human placental GH (hPGH) in EVCT invasiveness. By using an in vitro invasion model, we found that EVCT isolated from first-trimester chorionic villi and cultured on Matrigel secreted hPGH and expressed human GH receptor (hGHR). These data were confirmed by in situ immunohistochemistry. EVCT expressed the full-length and truncated forms of hGHR, and the Janus kinase-2/signal transducer and activator of transcription factor-5 signaling pathway was activated in EVCT by hPGH treatment. Strong hPGH and hGHR expression was observed when EVCT invaded Matrigel and moved through the pores of the filter on which they were cultured. hPGH stimulated EVCT invasiveness, and this effect was inhibited by a Janus kinase-2 inhibitor. Interestingly, hPGH was more efficient than pituitary GH in stimulating EVCT invasiveness. These results offer the first evidence for a placental role of hPGH and suggest an autocrine/paracrine role of hPGH in the regulation of trophoblast invasion.

**MA F.Y., GRATTAN D.R., GOFFIN V., BUNN S.J.**

Prolactin-regulated tyrosine hydroxylase activity and messenger ribonucleic Acid expression in mediobasal hypothalamic cultures: the differential role of specific protein kinases.

*Endocrinology*, 146 (1), 93-102, 2005

(Services cités : U584)

**MA F.Y., ANDERSON G.M., GUNN T.D., GOFFIN V., GRATTAN D.R., BUNN S.J.**

Prolactin Specifically Activates Signal Transducer and Activator of Transcription 5b in Neuroendocrine Dopaminergic Neurons.

*Endocrinology*, 146 (12), 5112-5119., 2005

(Services cités : U584)

The hypothalamic neuroendocrine dopaminergic (NEDA) neurons are crucial in regulating prolactin secretion from the anterior pituitary. Rising prolactin concentrations stimulate these neurons to secrete dopamine, which acts via the pituitary portal vasculature to inhibit additional prolactin release. Prolactin is known to activate Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathways in other cell types, including neurons. The possible role of JAK-STAT signaling in NEDA neurons has therefore been examined in this study using fetal rat mediobasal hypothalamic cell cultures and an adult rat in vivo preparation. Cultured cells expressing the dopamine synthesizing enzyme tyrosine hydroxylase (TH) responded to prolactin with a time-dependent increase in phospho-STAT5, but not phospho-STAT1 or phospho-STAT3, nuclear labeling. This response was inhibited by the prolactin receptor antagonist Delta1-9-G129R-human prolactin and the JAK inhibitor AG490, but was unaffected by selected serine/threonine kinase inhibitors (H89, KN-93, bisindolymaleimide, or PD98059). Antibodies selective for STAT5a or STAT5b indicated that the response was restricted to STAT5b, with the number of TH cells displaying STAT5b nuclear immunoreactivity rising from less than 10% under basal conditions to approximately 70% after prolactin stimulation. STAT5a nuclear labeling remained unchanged at 6-10% of TH-positive cells. STAT5b selectivity was confirmed in vivo, where the injection of prolactin into bromocriptine-treated rats stimulated a time-dependent increase in STAT5b, but not STAT5a, nuclear staining in the TH-expressing neurons in the arcuate nucleus. These results extend our previous findings with STAT5b-deficient mice and strongly suggest that in NEDA neurons, prolactin signaling via the JAK/STAT pathway is mediated exclusively by STAT5b.

**MANHES C., GOFFIN V., KELLY P.A., TOURAINE P.**

Autocrine prolactin as a promotor of mammary tumour growth.

*J. Dairy Res.*, 72 (S1), 58-65, 2005

(Services cités : U584)

**NILSSON L., BINART N., BOHLOOLY-Y M., BRAMNERT M., EGECIOGLU E.,  
KINDBLOM J., KELLY P.A., KOPCHICK J.J., ORMANDY C.J., LING C., BILLIG H.**

Prolactin and growth hormone regulate adiponectin secretion and receptor expression in adipose tissue.

*Biochem. Biophys. Res. Commun.*, 331 (4), 1120-1126, 2005

(Services cités : U584)

Adiponectin is a hormone secreted from adipose tissue, and serum levels are decreased with obesity and insulin resistance. Because prolactin (PRL) and growth hormone (GH) can affect insulin sensitivity, we investigated the effects of these hormones on the regulation of adiponectin in human adipose tissue in vitro and in rodents in vivo. Adiponectin secretion was significantly suppressed by PRL and GH in in vitro cultured human adipose tissue. Furthermore, PRL increased adiponectin receptor 1 (AdipoR1) mRNA expression and GH decreased AdipoR2 expression in the cultured human adipose tissue. In transgenic mice expressing GH, and female mice expressing PRL, serum levels of adiponectin were decreased. In contrast, GH receptor deficient mice had elevated adiponectin levels, while PRL receptor deficient mice were unaffected. In conclusion, we demonstrate gene expression of AdipoR1 and AdipoR2 in human adipose tissue for the first time, and show that these are differentially regulated by PRL and GH. Both PRL and GH reduced adiponectin secretion in human adipose tissue in vitro and in mice in vivo. Decreased serum adiponectin levels have been associated with insulin resistance, and our data in human tissue and in transgenic mice suggest a role for adiponectin in PRL and GH induced insulin resistance.

**OHANNA M., SOBERING A.K., LAPOINTE T., LORENZO L., PRAUD C.,  
PETROULAKIS E., SONENBERG N., KELLY P.A., SOTIROPOULOS A., PENDE M.**

Atrophy of S6K1(-/-) skeletal muscle cells reveals distinct mTOR effectors for cell cycle and size control.

*Nat. Cell Biol.*, 7 (3), 286-294, 2005

(Services cités : U584)

The mammalian target of rapamycin (mTOR) and Akt proteins regulate various steps of muscle development and growth, but the physiological relevance and the downstream effectors are under investigation. Here we show that S6 kinase 1 (S6K1), a protein kinase activated by nutrients and insulin-like growth factors (IGFs), is essential for the control of muscle cytoplasmic volume by Akt and mTOR. Deletion of S6K1 does not affect myoblast cell proliferation but reduces myoblast size to the same extent as that observed with mTOR inhibition by rapamycin. In the differentiated state, S6K1(-/-) myotubes have a normal number of nuclei but are smaller, and their hypertrophic response to IGF1, nutrients and membrane-targeted Akt is blunted. These growth defects reveal that mTOR requires distinct effectors for the control of muscle cell cycle and size, potentially opening new avenues of therapeutic intervention against neoplasia or muscle atrophy.

**SAVINO W., COTTA-de-ALMEIDA V., VAN BUUL-OFFERS S.C., KOSTER J.G.,**

**DARDENNE M.**

Abnormal Thymic Microenvironment in Insulin-Like Growth Factor-II Transgenic Mice.

*Neuroimmunomodulation*, 12 (2), 100-112, 2005

(Services cités : UMR 8147, U584)

Objectives: Intrathymic T cell differentiation is driven by the thymic microenvironment, a tridimensional network of cells and extracellular matrix (ECM). Previous data showed that lymphoid and microenvironmental compartments are under the control of hormones and growth factors. We then attempted to define if insulin-like growth factor-II (IGF-II) was also involved in such a control. Methods: We used IGF-II transgenic (Tg) mice and studied their thymic microenvironment by immunohistochemistry. Moreover, we evaluated thymocytes in terms of their ability to adhere to thymic epithelial cells and to migrate through epithelial cells and ECM. Results: Transgenic IGF-II expression results in abnormalities of the thymic epithelium. Terminal differentiation of thymic epithelial cells (TEC) is modified, with the appearance of large clusters of cells immunoreactive to the monoclonal antibody KL1, which specifically recognizes highly differentiated TEC. Accordingly, treatment of cultured TEC with exogenous IGF-II induces the appearance of KL1+ cells and increases TEC proliferation. IGF-II Tg animals exhibit increased serum levels of the TEC-derived hormone thymulin. These effects were seen even when the IGF-II transgene was inserted in dwarf mice. Moreover, deposition of fibronectin and laminin is also enhanced in IGF-II Tg mouse thymus and in IGF-II-treated TEC cultures. Furthermore, ECM-mediated interactions between thymocytes and TEC are affected by exogenous IGF-II, as exemplified by the enhancement of thymocyte adhesion to TEC monolayers and thymocyte migration in thymic nurse cell complexes. Conclusions: Our data indicate that IGF-II pleiotropically affects the thymic epithelium, both in vivo and in vitro, and that some of these changes may have consequences on thymocyte/TEC interactions. Copyright (c) 2005 S. Karger AG, Basel.

**SCHAFFLER A., BINART N., SCHOLMERICH J., BUCHLER C.**

Hypothesis paper Brain talks with fat--evidence for a hypothalamic-pituitary-adipose axis ?

*Neuropeptides*, 39 (4), 363-367, 2005

(Services cités : U584)

The adipose tissue signals to the brain via its secretory products. However, it is unknown whether the brain itself can directly contact the fat tissue. In order to test this hypothesis, the adipocytic expression of receptors for pituitary hormones and hypothalamic peptides was investigated. Besides FSH- and LH-receptors, adipocytes do express the specific receptors for ACTH, TSH, GH, prolactin, oxytocin and the three receptor subtypes for vasopressin. Thus, the adipose tissue might no longer be regarded as an inert and steady tissue but as a fast acting player downstream of and under the control of the brain. Based on this, the potential existence and clinical impact of a hypothalamic-pituitary-adipose axis should further be investigated.

**SMANIOTTO S., de MELLO-COELHO V., VILLA-VERDE D.M., PLEAU J.M., POSTEL-VINAY M.C., DARDENNE M., SAVINO W.**

Growth Hormone Modulates Thymocyte Development in Vivo through a Combined Action of Laminin and CXC Chemokine Ligand 12.

*Endocrinology*, 146 (7), 3005-3017, 2005

(Services cités : U584, UMR 8147)

Previous evidence indicates that GH modulates thymic cell migration. In this study we approached this issue in vivo, studying thymocyte migration in GH transgenic animals and in

normal mice treated intrathymically with GH. Extracellular matrix and chemokines are involved in thymocyte migration. In this respect, thymocyte adhesion to laminin was higher in GH-treated animals than controls, and the numbers of migrating cells in laminin-coated Transwells was higher in GH-transgenic and GH-injected mice. Additionally, CXC chemokine ligand 12 (CXCL12)-driven migration was higher in GH-Tg and GH-treated animals compared with controls. Interestingly, although CXCR4 expression on thymocytes did not change in GH-Tg mice, the CXCL12 intrathymic contents were higher. We found that CXCL12, in conjunction with laminin, would additionally enhance the migration of thymocytes previously exposed to high concentrations of GH in vivo. Lastly, there was an augmentation of recent thymic emigrants in lymph nodes from GH-Tg and GH-injected animals. In conclusion, enhanced thymocyte migration in GH transgenic mice as well as GH-injected mice results at least partially from a combined action of laminin and CXCL12. Considering that GH is presently being used as an adjuvant therapeutic agent in immunodeficiencies, including AIDS, the concepts defined herein provide important background knowledge for future GH-based immune interventions.

**TEILUM K., HOCH J.C., GOFFIN V., KINET S., MARTIAL J.A., KRAGELUND B.B.**

Solution structure of human prolactin.

*J. Mol. Biol.*, 351 (4), 810-823, 2005

(Services cités : U584)

We report the solution structure of human prolactin determined by NMR spectroscopy. Our result is a significant improvement over a previous structure in terms of number and distribution of distance restraints, regularity of secondary structure, and potential energy. More significantly, the structure is sufficiently different that it leads to different conclusions regarding the mechanism of receptor activation and initiation of signal transduction. Here, we compare the structure of unbound prolactin to structures of both the homologue ovine placental lactogen and growth hormone. The structures of unbound and receptor bound prolactin/placental lactogen are similar and no noteworthy structural changes occur upon receptor binding. The observation of enhanced binding at the second receptor site when the first site is occupied has been widely interpreted to indicate conformational change induced by binding the first receptor. However, our results indicate that this enhanced binding at the second site could be due to receptor-receptor interactions or some other free energy sources rather than conformational change in the hormone. Titration of human prolactin with the extracellular domain of the human prolactin receptor was followed by NMR, gel filtration and electrophoresis. Both binary and ternary hormone-receptor complexes are clearly detectable by gel filtration and electrophoresis. The binary complex is not observable by NMR, possibly due to a dynamic equilibrium in intermediate exchange within the complex. The ternary complex of one hormone molecule bound to two receptor molecules is on the contrary readily detectable by NMR. This is in stark contrast to the widely held view that the ternary prolactin-receptor complex is only transiently formed. Thus, our results lead to improved understanding of the prolactin-prolactin receptor interaction.

**TOURAINÉ P., YOUSSEF N., ALYANAKIAN M.A., LECHAT X., BALLEYGUIER C., DUFLOS C., DIB A., MAY A., CAREL J.C., LABORDE K., SIGAL-ZAFRANI B., GOFFIN V., EYMARD B., BOITARD C., BROUSSE N., KUTTENN F.**

Breast inflammatory giantomastia in a context of immune-mediated diseases.

*J. Clin. Endocrinol. Metabol.*, 90 (9), 5287-5294, 2005

(Services cités : Anatomo-Pathologie, Endocrinologie & Médecine de la Reproduction, Infectiologie, Radiologie Adulte, U584, Explorations Fonctionnelles)

Context: Localized breast lesions have been described in lupic or diabetic patients. However, the description of breast gigantomastia in women presenting with autoimmune diseases has not been reported. Setting: The study took place within the Department of Endocrinology and Reproductive Medicine, Necker Hospital, Paris, France. Patients: We describe eight patients with inflammatory gigantomastia, occurring in a context of immune-mediated diseases: myasthenia, chronic arthritis, or thyroiditis. Main Outcome Measures: Together with hormonal, immunological, and breast magnetic resonance imaging (MRI) evaluation, breast histology enabled us to perform immunocytochemical and indirect immunofluorescence studies. Control sera were obtained from patients with (n = 10) and without (n = 7) antinuclear antibodies. Results: Six of the eight patients developed gigantomastia either at puberty or during pregnancy. Neither a hormonal oversecretion nor a specific immunological pattern was observed. All patients except one presented antinuclear antibodies. Histological study revealed a diffuse, stromal hyperplasia and a severe atrophy of the lobules. A rarefaction of adipocytes was also noted, as previously suggested on MRI. There was a perilobular lymphocytic infiltrate made of CD3+ lymphocytes. Study of sera from five of six cases of gigantomastia showed a nuclear immunofluorescence pattern in normal mammary ductal and lobular glandular epithelium, as well as in kidney and intestine epithelial cells. In control sera, a nuclear signal was observed only when antinuclear antibodies were present. Conclusions: We suggest that breast tissue may be a target tissue in autoimmune diseases, this process being favored by the hormonal milieu. However, the precise mechanism of such association is not individualized. The fact that stromal hyperplasia is the main histological feature justifies the search for the involvement of growth factors in such a process.

**2004**

**DOS SANTOS C., ESSIUX L., TEINTURIER C., TAUBER M., GOFFIN V., BOUGNERES P.**

A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone.

*Nat. Genet.*, 36 (7), 720-724, 2004

(Services cités : U584)

Growth hormone is used to increase height in short children who are not deficient in growth hormone, but its efficacy varies largely across individuals. The genetic factors responsible for this variation are entirely unknown. In two cohorts of short children treated with growth hormone, we found that an isoform of the growth hormone receptor gene that lacks exon 3 (d3-GHR) was associated with 1.7 to 2 times more growth acceleration induced by growth hormone than the full-length isoform ( $P < 0.0001$ ). In transfection experiments, the transduction of growth hormone signaling through d3-GHR homo- or heterodimers was approximately 30% higher than through full-length GHR homodimers ( $P < 0.0001$ ). One-half of Europeans are hetero- or homozygous with respect to the allele encoding the d3-GHR isoform, which is dominant over the full-length isoform. These observations suggest that the polymorphism in exon 3 of GHR is important in growth hormone pharmacogenetics.

**GOFFIN V., TOURAINE P.**

Pegvisomant Pfizer/Sensus.

*Curr. Opin. Investig. Drugs*, 5 (4), 463-468, 2004

(Services cités : U584)

Pfizer (formerly Pharmacia), in collaboration with its wholly owned subsidiary Sensus, has

developed and launched pegvisomant, a pegylated, genetically modified human growth hormone (hGH), for the treatment of acromegaly. Pegvisomant, in contrast to classical somatostatin analogs which lower hGH synthesis, exerts its anti-hGH action by preventing GH receptor activation. This drug is now available in the US and Europe for the treatment of acromegaly.

**IOVANE A., AUMAS C., de ROUX N.**

New insights in the genetics of isolated hypogonadotropic hypogonadism.

*Eur. J. Endocrinol.*, 151 Suppl 3 U83-U88, 2004

(Services cités : U584)

Isolated gonadotropic deficiency or isolated hypogonadotropic hypogonadism is defined as a low sexual hormone secretion by the gonads associated with low LH and FSH plasma levels.

Kallmann syndrome is defined as a congenital isolated gonadotropic deficiency associated with anosmia whereas the phenotype of the idiopathic form is limited to the gonadotropic axis. For several years, it has been known that mutations of the KAL-1 gene or loss-of-function mutations of GnRH receptor did not explain all familial cases of isolated gonadotropic deficiency with or without anosmia. Thus the existence of other genes playing a major role in the physiology of the gonadotropic axis was highly suggested. In 2003, fibroblast growth factor receptor 1 (FGFR1) and GPR54 were shown to be two of these genes. FGFR1 loss-of-function mutations were reported in Kallmann syndrome whereas inactivating mutations of GPR54 were described in the idiopathic form of the gonadotropic deficiency. These genetic studies have opened up a new chapter in the physiology and the pharmacology of the gonadotropic axis.

**MASSIN N., GOUGEON A., MEDURI G., THIBAUD E., LABORDE K.,  
MATUCHANSKY C., CONSTANCIS E., VACHER-LAVENU M.C., PANIEL B., ZORN  
J.R., MISRAHI M., KUTTENN F., TOURAINE P.**

Significance of ovarian histology in the management of patients presenting a premature ovarian failure.

*Hum. Reprod.*, 19 (11), 2555-2560, 2004

(Services cités : Endocrinologie & Médecine de la Reproduction, Endocrinologie et Croissance, Explorations Fonctionnelles, U584)

**BACKGROUND:** Premature ovarian failure (POF) is a heterogeneous syndrome, possibly due to mutations of genes involved in the normal development of the ovary and/or follicles. Based essentially on animal models, these mutations are associated with various ovarian phenotypes, from a complete absence of follicles to a partial follicular maturation. The aim of the present study was to determine whether ovarian histology, compared to pelvic ultrasonography, would be helpful in identifying which patients display an impaired follicular reserve and/or growth, and in orientating the search for POF aetiology. **METHODS AND RESULTS:** We studied a cohort of 61 patients suffering from POF with a normal karyotype. Their median age (range) at diagnosis was 26 years (15-39). The FSH plasma level was high, 67.0 IU/l (13-155). Estradiol and inhibin B plasma levels were low: 18.5 pmol/l (18.5-555) and 5 pg/ml (5-105) respectively. Both pelvic ultrasonography and ovarian biopsies were performed in each patient. The presence of follicles suggested at ultrasonography was confirmed at histology in 56% of the patients. Ovarian histology led to the distinction of two phenotypes: (i) small-sized ovaries, deprived of follicles; and (ii) normal-sized ovaries with partial follicular maturation. To confirm the value of ovarian biopsies, samples from 20 normal women were studied. These demonstrated that ovarian biopsy at random enables reliable assessment of follicular presence, especially when their size is <2 mm. **CONCLUSION:** Ovarian histology appears to be a reliable tool in evaluating the follicular

reserve, and helpful and complementary to clinical and hormonal phenotyping in orienting the search for the various genetic causes of POF syndrome.

**PENDE M., UM S.H., MIEULET V., STICKER M., GOSS V.L., MESTAN J., MUELLER M., FUMAGALLI S., KOZMA S.C., THOMAS G.**

S6K1(-)/S6K2(-) mice exhibit perinatal lethality and rapamycin-sensitive 5'-terminal oligopyrimidine mRNA translation and reveal a mitogen-activated protein kinase-dependent S6 kinase pathway.

*Mol. Cell. Biol.*, 24 (8), 3112-3124, 2004

(Services cités : [U584](#))

Activation of 40S ribosomal protein S6 kinases (S6Ks) is mediated by anabolic signals triggered by hormones, growth factors, and nutrients. Stimulation by any of these agents is inhibited by the bacterial macrolide rapamycin, which binds to and inactivates the mammalian target of rapamycin, an S6K kinase. In mammals, two genes encoding homologous S6Ks, S6K1 and S6K2, have been identified. Here we show that mice deficient for S6K1 or S6K2 are born at the expected Mendelian ratio. Compared to wild-type mice, S6K1(-) mice are significantly smaller, whereas S6K2(-) mice tend to be slightly larger. However, mice lacking both genes showed a sharp reduction in viability due to perinatal lethality. Analysis of S6 phosphorylation in the cytoplasm and nucleoli of cells derived from the distinct S6K genotypes suggests that both kinases are required for full S6 phosphorylation but that S6K2 may be more prevalent in contributing to this response. Despite the impairment of S6 phosphorylation in cells from S6K1(-)/S6K2(-) mice, cell cycle progression and the translation of 5'-terminal oligopyrimidine mRNAs were still modulated by mitogens in a rapamycin-dependent manner. Thus, the absence of S6K1 and S6K2 profoundly impairs animal viability but does not seem to affect the proliferative responses of these cell types. Unexpectedly, in S6K1(-)/S6K2(-) cells, S6 phosphorylation persisted at serines 235 and 236, the first two sites phosphorylated in response to mitogens. In these cells, as well as in rapamycin-treated wild-type, S6K1(-), and S6K2(-) cells, this step was catalyzed by a mitogen-activated protein kinase (MAPK)-dependent kinase, most likely p90rsk. These data reveal a redundancy between the S6K and the MAPK pathways in mediating early S6 phosphorylation in response to mitogens.

**PIWNICA D., TOURAINE P., STRUMAN I., TABRUYN S., BOLBACH G., CLAPP C., MARTIAL J.A., KELLY P.A., GOFFIN V.**

Cathepsin D Processes Human Prolactin into Multiple 16K-Like N-Terminal Fragments: Study of Their Antiangiogenic Properties and Physiological Relevance.

*Mol. Endocrinol.*, 18 (10), 2522-2542, 2004

(Services cités : [U584](#))

16K prolactin (PRL) is the name given to the 16-kDa N-terminal fragment obtained by proteolysis of rat PRL by tissue extracts or cell lysates, in which cathepsin D was identified as the candidate protease. Based on its antiangiogenic activity, 16K PRL is potentially a physiological inhibitor of tumor growth. Full-length human PRL (hPRL) was reported to be resistant to cathepsin D, suggesting that antiangiogenic 16K PRL may be physiologically irrelevant in humans. In this study, we show that hPRL can be cleaved by cathepsin D or mammary cell extracts under the same conditions as described earlier for rat PRL, although with lower efficiency. In contrast to the rat hormone, hPRL proteolysis generates three 16K-like fragments, which were identified by N-terminal sequencing and mass spectrometry as corresponding to amino acids 1-132 (15 kDa), 1-147 (16.5 kDa), and 1-150 (17 kDa). Biochemical and

mutagenetic studies showed that the species-specific digestion pattern is due to subtle differences in primary and tertiary structures of rat and human hormones. The antiangiogenic activity of N-terminal hPRL fragments was assessed by the inhibition of growth factor-induced thymidine uptake and MAPK activation in bovine umbilical endothelial cells. Finally, an N-terminal hPRL fragment comigrating with the proteolytic 17-kDa fragment was identified in human pituitary adenomas, suggesting that the physiological relevance of antiangiogenic N-terminal hPRL fragments needs to be reevaluated in humans.

**TOURAINÉ P., GOFFIN V., KELLY P.A., MORANGE I., JACQUET P.**

La prolactine : physiologie et pathologie.

in: *Traité de Médecine (4<sup>e</sup> édition)*. (Godeau P. eds.)

Flammarion Médecine-Sciences (Paris), 2004, pp.1941-1946.

(Services cités : Endocrinologie & Médecine de la Reproduction, U584)

**VIENGCHAREUN S., BOUZINBA-SEGARD H., LAIGNEAU J.P., ZENNARO M.C., KELLY P.A., BADO A., LOMBES M., BINART N.**

Prolactin potentiates insulin-stimulated leptin expression and release from differentiated brown adipocytes.

*J. Mol. Endocrinol.*, 33 (3), 679-691, 2004

(Services cités : U584)

The pituitary hormone prolactin (PRL) exerts pleiotropic effects, which are mediated by a membrane receptor (PRLR) present in numerous cell types including adipocytes. Brown adipose tissue (BAT) expresses uncoupling proteins (UCPs), involved in thermogenesis, but also secretes leptin, a key hormone involved in the control of body weight. To investigate PRL effects on BAT, we used the T37i brown adipose cell line, and demonstrated that PRLRs are expressed as a function of cell differentiation. Addition of PRL leads to activation of the JAK/STAT and MAP kinase signaling pathways, demonstrating that PRLRs are functional in these cells. Basal and catecholamine-induced UCP1 expression were not affected by PRL. However, PRL combined with insulin significantly increases leptin expression and release, indicating that PRL potentiates the stimulatory effect of insulin as revealed by the recruitment of insulin receptor substrates and the activation of phosphatidylinositol 3-kinase. To explore the in vivo physiological relevance of PRL action in BAT, we showed that leptin content was significantly increased in BAT of PRLR-null mice compared with wild-type mice, highlighting the involvement of PRL in the leptin secretion process. This study provides the first evidence for a functional link between PRL and energy balance via a cross-talk between insulin and PRL signaling pathways in brown adipocytes.

**2003**

**BARAN N., KELLY P.A., BINART N.**

Decysin, a new member of the metalloproteinase family, is regulated by prolactin and steroids during mouse pregnancy.

*Biol. Reprod.*, 68 (5), 1787-1792, 2003

(Services cités : U584)

More than 300 separated actions have been attributed to prolactin (PRL), which could be correlated to the quasi-ubiquitous distribution of its receptor. Null mutation of the PRL receptor (PRLR) gene leads to female sterility caused by a failure of embryo implantation. Using the PRLR knockout mouse model and the mRNA differential display method, among 45 isolated

genes, we identified UA+4 as a PRL and steroids-target gene during the peri-implantation period that encodes the decysin. Hormonally regulated in the uterus during pregnancy, this new member of disintegrin metalloproteinase is present in the uterus at the site of blastocyst apposition in nondifferentiated stromal cells at the antimesometrial pole and, interestingly, is colocalized with the PRLR. At midpregnancy, decysin expression persists specifically at the foeto-maternal junction around vessels. Although it has been previously suggested that decysin expression is related to immune function, its function during pregnancy remains to be clearly established.

**BERNICHTTEIN S., JEAY S., VAUDRY R., KELLY P.A., GOFFIN V.**

New homologous bioassays for human lactogens show that agonism or antagonism of various analogs is a function of assay sensitivity.

*Endocrine*, 20 (1-2), 177-190, 2003

(Services cités : U584)

The reference bioassay for lactogens is the Nb2 cell proliferation assay, whose extreme sensitivity allows the detection of very low amounts of lactogenic activity in biologic fluids. The use of rat Nb2 cells raises the problem of species specificity when analyzing lactogens of other origin, including human lactogenic hormones for which no reference bioassay currently exists. In this article, we describe two new homologous bioassays for human lactogens. One is a transcriptional bioassay generated by stably transfecting 293 human embryonic kidney fibroblasts using two plasmids, encoding the human prolactin receptor (hPRLR) and the PRL-responsive lactogenic hormone response element luciferase reporter gene. The second is a proliferation assay obtained by stably transfecting Ba/F3 cells with a plasmid encoding the hPRLR. We provide characterization of the various clones or cell populations that were isolated, and we describe experiments that were performed to achieve optimized protocols for both bioassays. These new assays were compared with other cells types exhibiting well-recognized PRL-mediated responses (proliferation of Nb2 or of human breast tumor cell lines), using various lactogen analogs. This comparative analysis provides strong evidence that the intrinsic characteristics of each bioassay dramatically affect the biologic properties attributed to the lactogen of interest. Depending on the assay, a given analog can exhibit agonistic or antagonistic properties. We hypothesize that in addition to species specificity, assay sensitivity is the key parameter in directing the apparent bioactivity of lactogens. Of course, in the end, it will be necessary to confirm the agonistic or antagonistic properties of the tested analogs, in vivo.

**BERNICHTTEIN S., KAYSER C., DILLNER K., MOULIN S., KOPCHICK J.J., MARTIAL J.A., NORSTEDT G., ISAKSSON O., KELLY P.A., GOFFIN V.**

Development of pure prolactin receptor antagonists.

*J. Biol. Chem.*, 278 (38), 35988-35999, 2003

(Services cités : U584)

Prolactin (PRL) promotes tumor growth in various experimental models and leads to prostate hyperplasia and mammary neoplasia in PRL transgenic mice. Increasing experimental evidence argues for the involvement of autocrine PRL in this process. PRL receptor antagonists have been developed to counteract these undesired proliferative actions of PRL. However, all forms of PRL receptor antagonists obtained to date exhibit partial agonism, preventing their therapeutic use as full antagonists. In the present study, we describe the development of new human PRL antagonists devoid of agonistic properties and therefore able to act as pure antagonists. This was demonstrated using several in vitro bioassays, including highly sensitive assays able to detect extremely low levels of receptor activation. These new compounds also act as pure antagonists in

vivo, as assessed by analyzing their ability to competitively inhibit PRL-triggered signaling cascades in various target tissues (liver, mammary gland, and prostate). Finally, by using transgenic mice expressing PRL specifically in the prostate, which exhibit constitutively activated signaling cascades paralleling hyperplasia, we show that these new PRL analogs are able to completely revert PRL-activated events. These second generation human PRL antagonists are good candidates to be used as inhibitors of growth-promoting actions of PRL.

**BERNICHTEN S., JOMAIN J.B., KELLY P.A., GOFFIN V.**

The N-terminus of human prolactin modulates its biological properties.

*Mol. Cell. Endocrinol.*, 208 (1-2), 11-21, 2003

(Services cités : U584)

The N-terminus is the most divergent region within the prolactin (PRL)/placental lactogen (PL)/growth hormone (GH) family. Since all of these ligands are able to activate the lactogen receptor, it has been usually assumed that the N-terminus plays no major role in biological actions of any family member. In this study, we generated several analogs of human PRL in which the N-terminus was truncated by 9 and iteratively up to the 14 first residues. Truncation did not alter protein folding, and it even decreased the formation of PRL aggregates that appear during the purification of refolded protein. Removal of the entire N-terminal loop (14 residues) decreased the affinity for the receptor by two-three-fold, and reduced the ability of the hormone to activate the human lactogen receptor. In contrast, removal of 13 or less residues improves receptor activation since these analogs are able to produce supra-maximal activities in a transcriptional bioassay, or in proliferation assays exhibit dose-response curves that are less bell-shaped, which reflects enhanced stabilization of receptor dimers. Altogether, these data suggest that the N-terminus of PRL is actually slightly detrimental to bioactivity, but may be required for other properties of the hormone.

**BINART N., MELAINE N., PINEAU C., KERCRET H., TOUZALIN A.M., IMBERT-BOLLORE P., KELLY P.A., JEGOU B.**

Male reproductive function is not affected in prolactin receptor-deficient mice.

*Endocrinology*, 144 (9), 3779-3782, 2003

(Services cités : U584)

Mice with a targeted disruption of the prolactin (PRL) receptor gene were used to study the physiological role of PRL in the control of the male reproductive function. Fertility parameters as well as body and reproductive organ weights (epididymis and testes) were unaffected in PRL receptor knockout mice. Testicular histology and sperm reserves were also normal. Compared with wild-type animals, knockout mice had no significant difference in basal plasma LH, FSH, and testosterone levels, and the weight of seminal vesicles and prostate was unaffected. Moreover, no alteration was detected in human chorionic gonadotropin-induced testosterone levels. It is concluded that the absence of PRL signaling is not detrimental to male testicular function and to fertility in the mouse.

**BINART N., IMBERT-BOLLORE P., BARAN N., VIGLIETTA C., KELLY P.A.**

A short form of the prolactin (PRL) receptor is able to rescue mammopoiesis in heterozygous PRL receptor mice.

*Mol. Endocrinol.*, 17 (6), 1066-1074, 2003

(Services cités : U584)

The heterozygous prolactin (PRL) receptor (PRLR +/-) mouse fails to develop a fully functional

mammary gland at the end of the first pregnancy and shows markedly impaired lobuloalveolar development and milk secretion in young females. The PRLR is expressed ubiquitously, with various proportions of long and short isoforms in different tissues. Conflicting data have appeared on the putative role of the receptor short forms, with both agonist and antagonistic actions proposed. To assess whether the mouse PR-1 short isoform of the PRLR is potentially able to transduce a signal, we overexpressed it in heterozygous mice and investigated its effect on the rescue of mammary development. PRLR<sup>+/-</sup> mice were not able to develop a functional mammary gland, but restoration of mammary alveolar development and an increase in the expressions of casein and whey acidic protein genes were observed in transgenic PRLR<sup>+/-</sup> mice expressing the short form of the PRLR, leading to a complete rescue of mammary gland development and function in young females. These results demonstrate that PR-1, the short form of the PRLR, can improve mammary development in PRLR<sup>+/-</sup> mice, which compensates for the haploinsufficiency of the receptor long form; this effect is probably caused by accelerated proliferation and an activation of the PRLR signaling cascade, resulting in activation of target genes involved in mammary development and milk synthesis.

**DE ROUX N., GENIN E., CAREL J.C., MATSUDA F., CHAUSSAIN J.L., MILGROM E.**  
Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54.

*Proc. Nat. Acad. Sci. USA*, 100 (19), 10972-10976, 2003

(Services cités : U584)

Hypogonadotropic hypogonadism is defined as a deficiency of the pituitary secretion of follicle-stimulating hormone and luteinizing hormone, which results in the impairment of pubertal maturation and of reproductive function. In the absence of pituitary or hypothalamic anatomical lesions and of anosmia (Kallmann syndrome), hypogonadotropic hypogonadism is referred to as isolated hypogonadotropic hypogonadism (IHH). A limited number of IHH cases are due to loss-of-function mutations of the gonadotropin-releasing hormone receptor. To identify additional gene defects leading to IHH, a large consanguineous family with five affected siblings and with a normal gonadotropin-releasing hormone receptor coding sequence was studied. Homozygosity whole-genome mapping allowed the localization of a new locus within the short arm of chromosome 19 (19p13). Sequencing of several genes localized within this region showed that all affected siblings of the family carried a homozygous deletion of 155 nucleotides in the GPR54 gene. This deletion encompassed the splicing acceptor site of intron 4-exon 5 junction and part of exon 5. The deletion was absent or present on only one allele in unaffected family members. GPR54 has been initially identified as an orphan G protein-coupled receptor with 40% homology to galanin receptors. Recently, a 54-aa peptide derived from the KiSS1 protein was identified as a ligand of GPR54. The present study shows that loss of function of GPR54 is a cause of IHH, and it identifies GPR54 and possibly KiSS1 protein-derived peptide as playing a major and previously unsuspected role in the physiology of the gonadotropic axis.

**GOFFIN V.**

New Homologous Bioassays for Human Lactogens Show That Agonism or Antagonism of Various Analogs Is a Function of Assay Sensitivity.

*Endocrine*, 20 (1-2), 177-190, 2003

(Services cités : U584)

The reference bioassay for lactogens is the Nb2 cell proliferation assay, whose extreme sensitivity allows the detection of very low amounts of lactogenic activity in biologic fluids. The

use of rat Nb2 cells raises the problem of species specificity when analyzing lactogens of other origin, including human lactogenic hormones for which no reference bioassay currently exists. In this article, we describe two new homologous bioassays for human lactogens. One is a transcriptional bioassay generated by stably transfecting 293 human embryonic kidney fibroblasts using two plasmids, encoding the human prolactin receptor (hPRLR) and the PRL-responsive lactogenic hormone response element luciferase reporter gene. The second is a proliferation assay obtained by stably transfecting Ba/F3 cells with a plasmid encoding the hPRLR. We provide characterization of the various clones or cell populations that were isolated, and we describe experiments that were performed to achieve optimized protocols for both bioassays. These new assays were compared with other cell types exhibiting well-recognized PRL-mediated responses (proliferation of Nb2 or of human breast tumor cell lines), using various lactogen analogs. This comparative analysis provides strong evidence that the intrinsic characteristics of each bioassay dramatically affect the biologic properties attributed to the lactogen of interest. Depending on the assay, a given analog can exhibit agonistic or antagonistic properties. We hypothesize that in addition to species specificity, assay sensitivity is the key parameter in directing the apparent bioactivity of lactogens. Of course, in the end, it will be necessary to confirm the agonistic or antagonistic properties of the tested analogs, *in vivo*.

**GOFFIN V., BERNICHTEIN S., KAYSER C., KELLY P.A.**

Development of new prolactin analogs acting as pure prolactin receptor antagonists.

*Pituitary*, 6 (2), 89-95, 2003

(Services cités : [U584](#))

Prolactin (PRL) promotes tumor growth, as recently highlighted by the spontaneous appearance of prostate hyperplasia and mammary neoplasia in PRL transgenic mice. Increasing experimental evidence argues for the involvement of autocrine PRL in this process. Human (h)PRL receptor antagonists have been developed to counteract these undesired proliferative actions of PRL. However, all PRL receptor antagonists obtained to date exhibit partial agonism, limiting their therapeutic use as full antagonists. This is the case for the first generation antagonists (the prototype of which is G129R-hPRL) that we developed ten years ago, which display antagonistic activity in some, but not all *in vitro* bioassays, and fail to inhibit PRL activity in transgenic mice expressing this analog. We recently developed new human PRL antagonists devoid of agonistic properties, and therefore able to act as pure antagonists. This was demonstrated using several *in vitro* bioassays, including assays able to detect extremely low levels of receptor activation. These new compounds also act as pure antagonists *in vivo*, as demonstrated by their ability to competitively inhibit PRL-triggered signaling cascades in various target tissues (liver, mammary gland and prostate). Finally, using transgenic mice specifically expressing PRL in the prostate, which have constitutively activated signaling cascades and prostate hyperplasia, these new PRL analogs are able to completely revert PRL-activated events to basal levels. These second generation antagonists are good candidates to be used as inhibitors of the growth-promoting actions of hPRL.

**GROSDEMOUGE I., BACHELOT A., LUCAS A., BARAN N., KELLY P.A., BINART N.**

Effects of deletion of the prolactin receptor on ovarian gene expression.

*Reprod. Biol. Endocrinol.*, 1 (1), 12, 2003

(Services cités : [U584](#))

Prolactin (PRL) exerts pleiotropic physiological effects in various cells and tissues, and is mainly considered as a regulator of reproduction and cell growth. Null mutation of the PRL receptor (R)

gene leads to female sterility due to a complete failure of embryo implantation. Pre-implantatory egg development, implantation and decidualization in the mouse appear to be dependent on ovarian rather than uterine PRLR expression, since progesterone replacement permits the rescue of normal implantation and early pregnancy. To better understand PRL receptor deficiency, we analyzed in detail ovarian and corpora lutea development of PRLR<sup>-/-</sup> females. The present study demonstrates that the ovulation rate is not different between PRLR<sup>+/+</sup> and PRLR<sup>-/-</sup> mice. The corpus luteum is formed but an elevated level of apoptosis and extensive inhibition of angiogenesis occur during the luteal transition in the absence of prolactin signaling. These modifications lead to the decrease of LH receptor expression and consequently to a loss of the enzymatic cascades necessary to produce adequate levels of progesterone which are required for the maintenance of pregnancy.

**KARGES B., KARGES W., de ROUX N.**

Clinical and molecular genetics of the human GnRH receptor.

*Hum. Reprod. Update*, 9 (6), 523-530, 2003

(Services cités : U584)

A functional GnRH receptor (GnRH-R) in the anterior pituitary is critical for normal LH/FSH secretion, pubertal development and reproduction. Inactivating mutations of the GnRH-R have been identified in patients with idiopathic hypogonadotropic hypogonadism. In this article we summarize phenotypic characteristics of these patients and focus on specific functional alterations of the human GnRH-R. In-vitro studies using recombinant receptor constructs demonstrate that GnRH-R missense mutations result in impaired ligand binding and reduced signal transduction, causing gonadotrophin deficiency. A detailed molecular understanding of receptor inactivation may help to design new GnRH agonists to therapeutically modulate GnRH-R function.

**LEMPEREUR L., BRAMBILLA D., SCOTO G.M., D'ALCAMO M., GOFFIN V., CROSTA L., PALMUCCI T., RAMPELLO L., BERNARDINI R., CANTARELLA G.**

Growth hormone protects human lymphocytes from irradiation-induced cell death.

*Br. J. Pharmacol.*, 138 (8), 1411-1416, 2003

(Services cités : U584)

1 Undesired effects of cancer radiotherapy mainly affect the hematopoietic system. Growth hormone (GH) participates in both hematopoiesis and modulation of the immune response. We report both r-hGH cell death prevention and restoration of secretory capacities of irradiated human peripheral blood lymphocytes (PBL) in vitro. 2 r-hGH induced cell survival and increased proliferation of irradiated cells. Western blot analysis indicated that these effects of GH were paralleled by increased expression of the antiapoptotic protein Bcl-2. 3 r-hGH restored mitogen-stimulated release of IL-2 by PBL. Preincubation of irradiated lymphocytes with the growth hormone receptor (GHR) antagonists B2036 and G120 K abrogated r-hGH-dependent IL-2 release. 4 These results demonstrate that r-hGH protects irradiated PBL from death in a specific, receptor-mediated manner. Such effect of r-hGH on PBL involves activation of the antiapoptotic gene bcl-2 and prevention of cell death, associated with preserved functional cell capacity. Finally, potential use of GH as an immunopotentiating agent could be envisioned during radiation therapy of cancer. *British Journal of Pharmacology* (2003) 138, 1411-1416.  
doi:10.1038/sj.bjp.0705173

**LONG W., WAGNER K.U., LLOYD K.C., BINART N., SHILLINGFORD J.M.,**

**HENNIGHAUSEN L., JONES F.E.**

Impaired differentiation and lactational failure of *ErbB4*-deficient mammary glands identify ERBB4 as an obligate mediator of STAT5.

*Development*, 130 (21), 5257-5268, 2003

(Services cités : U584)

The ERBB family of type 1 receptor tyrosine kinases and their ligands have crucial functions during mammary development, but the signaling networks that ultimately regulate ERBB activity in the breast have remained elusive. Here, we show that mice with Cre-lox mediated deletions of both *ErbB4* alleles within the developing mammary gland (*ErbB4*(Flox/Flox)Wap-Cre) fail to accumulate lobuloalveoli or successfully engage lactation at parturition owing, in part, to impaired epithelial proliferation. Analysis of the mammary differentiation factor STAT5 by immunohistochemistry and western blot revealed a complete ablation of STAT5 activation in *ErbB4*(Flox/Flox)Wap-Cre mammary epithelium at parturition. Consistent with disrupted STAT5 function, *ErbB4*(Flox/Flox)Wap-Cre mammary glands at parturition failed to express the mammary epithelial differentiation marker NPT2B. Defects in epithelial functional differentiation at parturition were accompanied by a profound reduction in expression of the STAT5-regulated milk genes casein beta and whey acidic protein. We propose that ERBB4 functions as an essential mediator of STAT5 signaling, and that loss of STAT5 activity contributes to the impaired functional differentiation of mammary glands observed in mice containing conditional *ErbB4* deletions.

**MOULIN S., BOUZINBA-SEGARD H., KELLY P.A., FINIDORI J.**

Jak2 and proteasome activities control the availability of cell surface growth hormone receptors during ligand exposure.

*Cell. Signal.*, 15 (1), 47-55, 2003

(Services cités : U584)

Several mechanisms participate in the down-regulation of growth hormone receptor (GHR) signalling under ligand exposure. In CHO cells expressing GHR, we show that ligand stimulation induces degradation of the total cell GHR content. Experiments with <sup>125</sup>I-hGH indicate that ligand-bound internalized receptors are not immediately replaced. Using cell surface biotinylation, we demonstrate for the first time that, concomitantly with the degradation of cell surface receptors, GHRs from the intracellular compartments are also degraded. We thus suggest that under prolonged ligand exposure, some GHRs are targeted to the cell surface, while others are routed to degradation compartments. Inhibitors of Jak2 and of the proteasome partially inhibited degradation of cell surface receptors, while these compounds completely inhibit the degradation of intracellular GHRs, resulting in their accumulation. We therefore propose that Jak2 and proteasome activities control the amount of intracellular GHRs, and thus the availability of receptors at the cell surface, during ligand exposure.

**MOULIN S., BOUZINBA-SEGARD H., KELLY P.A., FINIDORI J.**

Subcellular Trafficking of Growth Hormone Receptor and Jak2 under Ligand Exposure.

*Hormone Metab. Res.*, 35 (7), 396-401, 2003

(Services cités : U584)

In CHO cells, growth hormone stimulation induces a rapid degradation of mature and precursor forms of its receptor, but does not affect Jak2 concentration. Confocal analysis of the receptor and of specific markers for subcellular localization shows that ligand exposure induced the disappearance of cell surface receptors, while some receptors seem to be sequestered in the

endoplasmic reticulum (ER) and in the Golgi apparatus. Using a tagged version of Jak2 (HA-Jak2) and double immuno-fluorescence analysis with anti-HA and anti-Stat5 antibodies, we demonstrate that ligand stimulation induces Stat5 nuclear accumulation while Jak2 remains localized in the cytoplasm. Immunoblots of nuclear extracts confirm the Jak2 nuclear exclusion.

**ORMANDY C.J., NAYLOR M., HARRIS J., ROBERTSON F., HORSEMAN N.D., LINDEMAN G.J., VISVADER J., KELLY P.A.**

Investigation of the transcriptional changes underlying functional defects in the mammary glands of prolactin receptor knockout mice.

*Rec. Progres. Horm. Res.*, 58 297-323, 2003

(Services cités : U584)

Knockout (KO) mice have been created that carry null mutations of genes encoding molecules essential for prolactin (PRL) release, PRL, the receptor for prolactin (PRLR), and various members of the receptor's signaling pathway. This allowed an *in vivo* genetic analysis of the role of PRL in target organ function. In PRLKO and PRLRKO mice, mammary ductal side branching was absent, terminal end bud (TEB)-like structures persisted at the ductal termini well into maturity, and no alveolar buds formed along the ductal tree. Transplants of recombined mammary glands formed from stromal and epithelial elements with and without PRLR showed normal development, while supplementation of progesterone levels in PRLKO animals restored ductal side branching. During pregnancy, PRLR heterozygous animals initially showed normal ductal and alveolar development. However, alveolar development stalled during late pregnancy, preventing successful lactation. This defect could be rescued by the loss of a single allele of the suppressor of cytokine signaling (SOCS) I gene. Transplants of recombined glands containing PRLRKO epithelium and wild-type (WT) stroma formed alveolar buds during pregnancy but showed no lobuloalveolar development. Recombinations of WT epithelium and PRLRKO stroma showed normal development, demonstrating that a direct action of the lactogenic hormones is confined to the epithelium, to promote lobuloalveolar development. Transcript profiling of epithelial transplants expressing or not expressing PRLR was used during early pregnancy to investigate the transcriptional response to lactogens underlying this defect. Such profiling has identified a number of genes with well-characterized roles in mammary development, in addition to a number of novel transcripts.

**ROBERTSON F.G., HARRIS J., NAYLOR M.J., OAKES S.R., KINDBLOM J., DILLNER K., WENNBO H., TORNELL J., KELLY P.A., GREEN J., ORMANDY C.J.**

Prostate development and carcinogenesis in prolactin receptor knockout mice.

*Endocrinology*, 144 (7), 3196-3205, 2003

(Services cités : U584)

Hyperprolactinemia results in prostatic hypertrophy and hyperplasia, but it is not known whether prolactin plays an essential role in these processes in the prostate. To address this question, we investigated prostate development, gene expression, and simian virus 40 (SV40)T-induced prostate carcinogenesis in prolactin receptor knockout mice. These animals showed a small increase in dorsolateral and ventral prostate weight but no change in the weight of the anterior prostate. The dorsal but not ventral or lateral lobes showed a 12% loss of epithelial cells; all other morphological parameters were normal. The area of SV40T-induced prostate intraepithelial neoplasia was reduced by 28% in the ventral lobe but not the dorsal lobe, and no tumors were seen in 20 prolactin receptor knockout animals, compared with 1 of 11 detected in wild-type and 4 of 21 found in heterozygous animals. Oligonucleotide microarrays were used to identify

essential transcriptional roles of prolactin and revealed a small set of genes with decreased expression involved in sperm/oocyte interaction and copulatory plug formation. Infertility or reduced fertility was apparent in these animals. These findings establish essential though subtle roles for prolactin in the regulation of prostate morphology, gene expression, SV40T-induced neoplasia, and reproductive function.

**SAUNIER E., DIF F., KELLY P.A., EDERY M.**

Targeted expression of the dominant-negative prolactin receptor in the mammary gland of transgenic mice results in impaired lactation.

*Endocrinology*, 144 (6), 2669-2675, 2003

(Services cités : U584)

The F3-short form of the rat PRL receptor (F3-SPRLR) form acts as a dominant negative inhibitor in vitro. We have developed a transgenic mouse model in which the rat F3-SPRLR was expressed in mammary epithelium under the control of the mouse mammary tumor virus promoter. Two lines of mice were characterized and shown to express the transgene in the mammary gland. No developmental abnormalities or differences from wild-type littermates were observed on the basis of size, activity, or fertility. Mice with a low level of transgene expression had a mammary phenotype similar to the wild type. However, mice overexpressing the transgene (levels much higher than those of the endogenous long PRLR transcript) had impaired mammary gland differentiation and lactation. In these mice, whole-mount and histological analyses demonstrated normal ductal development, but severely reduced lobuloalveolar outgrowth. signal transducer and activator of transcription-5 phosphorylation and expression of beta-casein and whey acidic protein gene were decreased. In vivo bromodeoxyuridine incorporation at midpregnancy showed that the reduction in mammary development was not due to an inhibition of ductal growth and side-branching. This model demonstrates for the first time in vivo a function of the SPRLR and a local and targeted effect of PRL on the mammary gland that are essential for its function, but not for its development.

**SEGARD H.B., MOULIN S., BOUMARD S., de CREMIERS C.A., KELLY P.A., FINIDORI J.**

Autocrine growth hormone production prevents apoptosis and inhibits differentiation in C2C12 myoblasts.

*Cell. Signal.*, 15 (6), 615-623, 2003

(Services cités : U584)

Although C2C12 myoblasts express low levels of growth hormone receptor (GHR), we failed to see any effect of exogenous growth hormone (GH) on cell proliferation or differentiation. C2C12 cells stably overexpressing (sixfold) more in GHR (C2C12(GHR)) grew faster than parental cells in media containing 2% serum, and proliferated while parental cells died, in the absence of serum. These effects were independent of exogenous GH but were inhibited by anti-GH and anti-insulin-like growth factor (anti-IGF-1) antibodies, consistent with a local production of GH, which we confirmed by RT-PCR and radioimmunoassay. In C2C12(GHR) cells, we observed an increased activation of the Janus kinase 2 (Jak2), signal transducers and activator of transcription 5 (Stat5), mitogen-activated protein kinase (MAPK) and protein kinase B (Akt) upon acute GH stimulation. GHR overexpression also inhibited the formation of myotubes and the expression of markers for myoblast differentiation. Taken together, our data suggest that GH acts as an autocrine factor in C2C12 cells, to enhance proliferation and to inhibit differentiation.

2002

**ALLAN G.J., TONNER E., BARBER M.C., TRAVERS M.T., SHAND J.H., VERNON R.G., KELLY P.A., BINART N., FLINT D.J.**

Growth Hormone, Acting in Part through the Insulin-Like Growth Factor Axis, Rescues Developmental, But Not Metabolic, Activity in the Mammary Gland of Mice Expressing a Single Allele of the Prolactin Receptor.

*Endocrinology*, 143 (11), 4310-4309, 2002

(Services cités : U344)

The heterozygous prolactin (PRL) receptor (PRLR(+/-)) mouse fails to develop a fully functional mammary gland at the end of the first pregnancy and shows markedly impaired lobuloalveolar development and milk secretion in young females. PRL and GH, acting through the IGF system, have interactive effects to enhance epithelial cell survival. Thus, we propose that a reduction in the expression of the PRLR may lead to increased IGFBP-5 expression (proapoptotic) and that GH may rescue mammary development by increasing IGF-I, an important mitogen and survival factor for the mammary epithelium. Mammary IGF-binding protein-5 (IGFBP-5) concentrations and plasmin activity in PRLR(+/-) mice were increased on d 2 postpartum, indicative of increased cell death and extracellular matrix remodeling. After GH treatment, a restoration of mammary alveolar development and a reduction in the activities of IGFBP-5 and plasmin were observed. Despite the severely impaired mammary development in PRLR(+/-) mice, both mRNA and protein expression for caseins and acetyl-coenzyme A (acetyl-CoA) carboxylase and acetyl-CoA carboxylase-alpha mRNA increased at parturition, although not to the extent in wild-type animals. Surprisingly, GH treatment actually led to a further decrease in milk protein and acetyl-CoA carboxylase-alpha expression when expressed per cell. This was confirmed by the smaller alveolar size, the relative paucity of milk in the mammary glands of GH-treated animals, and the inability of their pups to gain weight. In a subsequent study IGFBP-5 was administered to wild-type mice and produced a 45% decrease in mammary DNA content, a 30% decrease in parenchymal tissue, and impaired lactation. These results suggest that GH can improve mammary development in PRLR(+/-) mice, but that it fails to enhance metabolic activity. This may be due to the maintenance by GH/IGF-I of a proliferative, rather than a differentiative, phenotype.

**BACHELOT A., MONGET P., IMBERT-BOLLORE P., COSHIGANO K., KOPCHICK J.J., KELLY P.A., BINART N.**

Growth hormone is required for ovarian follicular growth.

*Endocrinology*, 143 (10), 4104-4112, 2002

(Services cités : U344)

To analyze the consequences of the absence of GH receptor (GHR) and GH-binding protein (GHBP) on female reproductive function, we used a mouse model in which the GHR/GHBP gene has been disrupted by homologous recombination. The major effect on reproductive function seen in GHR/GHBP knockout (KO) compared with wild-type animals is a dramatic decrease in litter size; this defect is due to a reduction of the ovulation rate. The ovulatory response to exogenous gonadotropin treatment is also 3-fold reduced in GHR/GHBP KO compared with the wild-type ovaries. These results establish that the reduced rate of ovulation is essentially due to an ovarian defect rather than a deficiency in pituitary gonadotropins. The number of follicles per ovary is markedly reduced, although all categories of follicles are represented. Interestingly, the number of healthy follicles from antral and preovulatory stages is dramatically decreased in GHR/GHBP KO in comparison with wild-type follicles. The capacity of follicles to bind LH, FSH, and IGF-I was not diminished. IGF-I treatment using micropumps is not able to rescue

either fertility or ovarian responsiveness to exogenous gonadotropins, suggesting that the effect of GH is independent of IGF-I. In conclusion, these results indicate that the reduction of litter size in GHR/GHBP KO mice is the consequence of an alteration of the growth of follicles and suggest that the effects of GH effects on follicular growth are independent of IGF-I.

**BARAN N., KELLY P.A., BINART N.**

Characterization of a prolactin-regulated gene in reproductive tissues using the prolactin receptor knockout mouse model.

*Biol. Reprod.*, 66 (4), 1210-1218, 2002

(Services cités : U344)

Prolactin (PRL) exerts pleiotropic physiological effects in various cells and tissues, although it is mainly considered as a regulator of reproduction and cell growth. Null mutation of the prolactin receptor (PRLR) gene leads to female sterility due to a failure of embryo implantation. Using this mouse model and the method of mRNA differential display, we identified PRL target genes that are regulated during the peri-implantation period. We characterized 1 among the 45 isolated genes, UA-3, which is regulated in the uterus as well as in the ovary during early pregnancy. This gene corresponds to a P311 mouse cDNA that was originally identified for its high expression in late-stage embryonic brain and adult cerebellum. We report here that UA-3 is present in numerous tissues as well as in ovary and uterus at the site of blastocyst apposition, and that its expression is hormonally regulated. Moreover, *in situ* hybridization reveals high expression in ovarian granulosa cells and in uterine epithelium. Recently, it has been suggested that P311 expression is tightly regulated at several levels by mechanisms that control cellular growth, transformation, motility, or a combination of these. Taken together, these results suggest that P311 could be involved in these processes during pregnancy, although its function remains to be clearly established.

**BEAULOYE V., WILLEMS B., de CONINCK V., FRANK S.J., EDERY M., THISEN J.P.**

Impairment of Liver GH Receptor Signaling by Fasting.

*Endocrinology*, 143 (3), 792-800, 2002

(Services cités : U344)

Fasting causes a state of GH resistance responsible for low circulating IGF-I levels. To investigate whether this resistance may result from alterations in the GH signaling pathway, we determined the effects of fasting on the GH transduction pathway in rat liver. Forty-eight-hour fasted or fed male rats were injected with recombinant rat GH via the portal vein. Liver was removed 0 and 15 min after injection. Although GH stimulated Janus kinase 2 (JAK2) phosphorylation in all animals, this was severely blunted in fasted animals. Similarly, the phosphorylation of the GH receptor, although observed in both fasted and fed rats after GH injection, was markedly reduced in fasted rats. A rapid signal transducer and activator of transcription 5 (STAT5) tyrosine phosphorylation was also induced in the liver of fed animals in response to GH. In contrast, in fasted rats only a slight phosphorylated STAT5 signal was observed. The inhibitory effect of fasting on these GH signaling molecules occurred without changes in their protein content. Furthermore, the impairment of the JAK-STAT pathway in fasted animals was associated with increased liver suppressor of cytokine signaling 3 mRNA levels. Although glucocorticoids, which are increased by fasting, may cause GH resistance, adrenalectomy failed to prevent alterations in the JAK-STAT pathway caused by fasting. In conclusion, the GH resistance induced by fasting is associated with impairment of the JAK-

STAT signaling pathway. This might contribute to the decrease in liver IGF-I production observed in fasting.

**CHALLIER C., COCAULT L., BERTHIER R., BINART N., DUSANTER-FOURT I., UZAN G., SOUYRI M.**

The cytoplasmic domain of Mpl receptor transduces exclusive signals in embryonic and fetal hematopoietic cells.

*Blood*, 100 (6), 2063-2070, 2002

(Services cités : U344)

The Mpl receptor plays an important role at the level of adult hematopoietic stem cells, but little is known of its function in embryonic and fetal hematopoiesis. We investigated the signals sent by the MPL cytoplasmic domain in fetal liver hematopoietic progenitors and during embryonic stem (ES) cell hematopoietic commitment. Mpl was found to be expressed only from day 6 of ES cell differentiation into embryoid bodies. Therefore, we expressed Mpl in undifferentiated ES cells or in fetal progenitors and studied the effects on hematopoietic differentiation. To avoid the inadvertent effect of thrombopoietin, we used a chimeric receptor, PM-R, composed of the extracellular domain of the prolactin receptor (PRL-R) and the transmembrane and cytoplasmic domains of Mpl. This allowed activation of the receptor with a hormone that is not involved in hematopoietic differentiation and assessment of the specificity of responses to Mpl by comparing PM-R with another PRL-R chimeric receptor that includes the cytoplasmic domain of the erythropoietin receptor (EPO-R) ([PE-R]). We have shown that the cytoplasmic domain of the Mpl receptor transduces exclusive signals in fetal liver hematopoietic progenitors as compared with that of EPO-R and that it promotes hematopoietic commitment of ES cells. Our findings demonstrate for the first time the specific role of Mpl in early embryonic or fetal hematopoietic progenitors and stem cells. (*Blood*. 2002;100:2063-2070)

**FREEMARK M., AVRIL I., FLEENOR D., DRISCOLL P., PETRO A., OPARA E., KENDALL W., ODEN J., BRIDGES S., BINART N., BREANT B., KELLY P.A.**

Targeted deletion of the PRL receptor: Effects on islet development, insulin production, and glucose tolerance.

*Endocrinology*, 143 (4), 1378-1385, 2002

(Services cités : U344)

PRL and placental lactogen (PL) stimulate beta-cell proliferation and insulin gene transcription in isolated islets and rat insulinoma cells, but the roles of the lactogenic hormones in islet development and insulin production in vivo remain unclear. To clarify the roles of the lactogens in pancreatic development and function, we measured islet density (number of islets/cm<sup>2</sup>) and mean islet size, beta-cell mass, pancreatic insulin mRNA levels, islet insulin content, and the insulin secretory response to glucose in an experimental model of lactogen resistance: the PRL receptor (PRLR) -deficient mouse. We then measured plasma glucose concentrations after ip injections of glucose or insulin. Compared with wild-type littermates, PRLR-deficient mice had 26-42% reductions ( $P < 0.01$ ) in islet density and  $\beta$ -cell mass. The reductions in islet density and  $\beta$ -cell mass were noted as early as 3 wk of age and persisted through 8 months of age and were observed in both male and female mice. Pancreatic islets of PRLR-deficient mice were smaller than those of wild-type mice at weaning but not in adulthood. Pancreatic insulin mRNA levels were 20-30% lower ( $P < 0.05$ ) in adult PRLR-deficient mice than in wild-type mice, and the insulin content of isolated islets was reduced by 16-25%. The insulin secretory response to ip glucose was blunted in PRLR-deficient males in vivo ( $P < 0.05$ ) and in isolated

islets of PRLR-deficient females and males in vitro ( $P < 0.01$ ). Fasting blood glucose concentrations in PRLR-deficient mice were normal, but glucose levels after an ip glucose load were 10-20% higher ( $P < 0.02$ ) than those in wild-type mice. On the other hand, the glucose response to ip insulin was normal. Our observations establish a physiologic role for lactogens in islet development and function.

**GOFFIN V., KELLY P.A.**

in: *Hormone signaling*. (Goffin V., Kelly P.A. eds.)  
Kluwer Academic (Boston, London), 2002, pp.1-314.  
(Services cités : [U344](#))

**GOFFIN V., BINART N., TOURAINE P., KELLY P.A.**

Prolactin: The New Biology of an Old Hormone.

*Annu. Rev. Physiol.*, 64 47-67, 2002

(Services cités : [U344](#))

Prolactin (PRL) is a paradoxical hormone. Historically known as the pituitary hormone of lactation, it has had attributed to it more than 300 separate actions, which can be correlated to the quasi-ubiquitous distribution of its receptor. Meanwhile, PRL-related knockout models have mainly highlighted its irreplaceable role in functions of lactation and reproduction, which suggests that most of its other reported target tissues are presumably modulated by, rather than strictly dependent on, PRL. The multiplicity of PRL actions in animals is in direct opposition to the paucity of arguments that suggest its involvement in human pathophysiology other than effects on reproduction. Although many experimental data argue for a role of PRL in the progression of some tumors, such as breast and prostate cancers, drugs lowering circulating PRL levels are ineffective. This observation opens new avenues for research into the understanding of whether local production of PRL is involved in tumor growth and, if so, how extrapituitary PRL synthesis is regulated. Finally, the physiological relevance of PRL variants, such as the antiangiogenic 16K-like PRL fragments, needs to be elucidated. This review is aimed at critically discussing how these recent findings have renewed the manner in which PRL should be considered as a multifunctional hormone.

**GOFFIN V., TOURAINE P.**

Pegvisomant. Pharmacia.

*Curr. Opin. Investig. Drugs*, 3 (5), 752-757, 2002

(Services cités : [U344](#))

Pegvisomant, a polyethylene glycol (PEG) derivative of human growth hormone (GH) that acts as a highly selective GH receptor antagonist, is under development by Pharmacia (formerly Sensus) as a potential treatment for acromegaly. By February 2001, Sensus had submitted an NDA for the treatment of acromegaly, and an approvable letter indicating outstanding issues had been received by July 2001. Pegvisomant was granted Orphan Drug status by the FDA and was designated for Priority Review. Pegvisomant also received Orphan Drug designation in the EU and Japan. In March 2001, additional regulatory filings were being planned for later in 2001. In October 2001, Pharmacia was preparing an NDA in Japan for the treatment of acromegaly. By September 1998, phase I trials of the treatment were underway for diabetic retinopathy, and were planned for diabetic nephropathy in 1999. By September 1997, a phase II trial to test the effects of pegvisomant on insulin sensitivity and secretion in type II diabetes patients was underway. However, no development has been reported for these indications since the dates given. By 1994,

Sensus had licensed technology for development of GH receptor antagonists from Genentech and Ohio University. Sensus was to pay Genentech, and Genentech was to receive equity in Sensus and royalties from the commercialization of any product resulting from the agreement. In April 2000, the company entered into a licensing agreement with Shearwater Polymers for the PEGylation of pegvisomant using Shearwater's proprietary technology, which is now used to produce the 20-kDa PEG-derivative of pegvisomant. In June 1999, Pharmacia Corp (formerly Pharmacia & Upjohn) signed an agreement to purchase 19.9% of Sensus and to potentially acquire the remainder of the company at a later date. In March 2001, Pharmacia completed its purchase of Sensus. Analysts at Merrill Lynch predicted in February 2002 that the product would be launched in 2003, with US revenues of \$20 million, rising to \$115 million in 2006.

**GOFFIN V., KELLY P.A.**

Growth-promoting actions of prolactin, the hormone of lactation.

*J. Pediatr. Endocrinol. Metab.*, 15 (6), 787-788, 2002

(Services cités : U344)

**JEAY S., SONENSHEIN G.E., POSTEL-VINAY M.C., KELLY P.A., BAIXERAS E.**

Growth hormone can act as a cytokine controlling survival and proliferation of immune cells: new insights into signaling pathways.

*Mol. Cell. Endocrinol.*, 188 (1-2), 1-7, 2002

(Services cités : U344)

While growth hormone (GH) is classically defined as a peptide hormone, recent evidence supports a role for GH acting as a cytokine in the immune system under conditions of stress, counteracting immunosuppression by glucocorticoids. Lymphoid cells express the GH receptor, which belongs to the cytokine receptor superfamily, and GH can be produced by immune tissues, suggesting an autocrine/paracrine mode of action of GH. GH can act as a cytokine, promoting cell cycle progression of lymphoid cells and preventing apoptosis. These effects of GH were shown to be mainly mediated by the PI-3 kinase/Akt pathway and the transcription factor NF-kappaB. Expression of several cell cycle mediators, as well as Bcl-2, c-Myc and cyclin proteins were found to be regulated by GH. Survival of immune cells under conditions of stress was promoted by NF-kappaB. Thus, GH acts not only as a hormone but also as a cytokine, playing a potentially important role in immune system cells. Lastly, in this mini-review, we will discuss whether the discovery of these molecules in GH signaling pathways offers new insights into additional mechanisms of action whereby GH regulates apoptosis, proliferation and neoplastic transformation of cells of the immune system.

**KELLY P.A., BACHELOT A., KEDZIA C., HENNIGHAUSEN L., ORMANDY C.J., KOPCHICK J.J., BINART N.**

The role of prolactin and growth hormone in mammary gland development.

*Mol. Cell. Endocrinol.*, 197 (1-2), 127-131, 2002

(Services cités : U344)

Development and differentiation of the mammary gland occur primarily during pregnancy. Females homozygous (-/-) for the null mutation of the PRL receptor (PRLR) gene are sterile due to a complete failure of blastocysts to implant. In progesterone-treated mice pregnancy is rescued but the mammary gland is severely underdeveloped. Interestingly, females hemizygous for the PRLR (+/-) in their first lactation show an almost complete failure to lactate. This phenotype disappears in the second and subsequent pregnancies in inbred 129/Sv mice but is maintained in

inbred C57BL/6 mice. In GH receptor (GHR) KO mice litter size is markedly decreased, probably due to an ovarian defect. To assess the relevance of the GH and PRLRs in the mammary gland development, GHR and PRLR null epithelia were transplanted into cleared fat pads of wild-type mice. Such studies show that epithelial GHR is not required for functional mammary development. In contrast, epithelial PRLRs are required for mammary development and milk protein gene expression during pregnancy. Since ductal development is impaired in GHR  $-/-$  mice, it appears that GH signals through the stromal compartment. In summary, it is now established that GH and PRL activate Stat5 in separate compartments, reflecting their specific roles in ductal and alveolar development and differentiation.

**KUHN E.R., VLEURICK L., EDERY M., DECUYPERE E., DARRAS V.M.**

Internalization of the chicken growth hormone receptor complex and its effect on biological functions.

*Comp. Biochem. Physiol. [B]*, 132 (1), 299-308, 2002

(Services cités : U344)

In the chicken, as in mammals, GH is a pleiotropic cytokine that plays a central role in growth differentiation and metabolism by altering gene expression in target cells. In the growing and adult chicken it stimulates gene expression of IGF-I and inhibits gene transcription of the type III deiodinating enzyme (D3) and by doing so also increases T-3 concentrations. GH binding to its receptor leads to internalization of the GH-GHR complex to the Golgi apparatus. This process is linked to the episodic release pattern of GH during growth. At the same time, a sharp decline of the expression of cGHR occurs at hatching. An in vitro study using a COS-7 cell line transfected with the cDNA of the chicken GHR, revealed that GHR immunofluorescence was found in the perinuclear region and on the plasma membrane. Following GH-induced internalization, GH and GHR were colocalized in endocytic and later in large lysosomal vesicles. Neither receptor nor ligand was transferred to the nucleus as confirmed by confocal laser microscopy. The JAK/STAT pathway however, as reported for mammalian GH receptors, mediated GH-induced gene transcription in chickens. (C) 2002 Elsevier Science Inc. All rights reserved.

**PATEL S., LOCHHEAD P.A., RENA G., FUMAGALLI S., PENDE M., KOZMA S.C., THOMAS G., SUTHERLAND C.**

Insulin regulation of insulin-like growth factor-binding protein-1 gene expression is dependent on the mammalian target of rapamycin, but independent of ribosomal S6 kinase activity.

*J. Biol. Chem.*, 277 (12), 9889-9895, 2002

(Services cités : U344)

Insulin inhibits the expression of the hepatic insulin-like growth factor-binding protein-1 (IGFBP-1) and glucose-6-phosphatase (G6Pase) genes. The signaling pathway that mediates these events requires the activation of phosphatidylinositol 3-kinase, whereas transfection studies have suggested an involvement of Akt (protein kinase 13) and FKHR, a transcription factor regulated by Akt. We now demonstrate that insulin repression of endogenous IGFBP-1 gene transcription was blocked by rapamycin or by amino acid starvation. Rapamycin inhibited the mammalian target of rapamycin (mTOR) and the subsequent activation of p70/p85 S6 protein kinase-1 (S6K1) by insulin, whereas amino acid depletion prevented insulin induction of these signaling molecules. Importantly, we demonstrate that insulin regulation of the thymine-rich insulin response element of the IGFBP-1 promoter was also inhibited by rapamycin. However, sustained activation of S6K1 did not repress this promoter. In addition, rapamycin did not affect insulin regulation of G6Pase expression or Akt activation.

We propose that these observations indicate that an mTOR-dependent, but S6K-independent mechanism regulates the suppression of IGFBP-1 (but not G6Pase) gene expression by insulin. Therefore, although the insulin-responsive sequence of the G6Pase gene promoter is related to that of the IGFBP-1 promoter, the signaling pathways that mediate suppression of these genes are distinct.

**RAYNAUD-SIMON A., PERIN L., MEAUME S., LESOURD B., MOULIAS R., POSTEL-VINAY M.C., LE BOUC Y.**

IGF-I, IGF-I-binding proteins and GH-binding protein in malnourished elderly patients with inflammation receiving refeeding therapy.

*Eur. J. Endocrinol.*, 146 (5), 657-665, 2002

(Services cités : U344)

**OBJECTIVE:** To investigate the mechanisms determining the success or failure of refeeding therapy in malnourished elderly patients with inflammation by studying changes in plasma IGF-I, GH-binding protein (GHBP) and IGF-binding protein (IGFBP) levels and IGFBP-3 proteolysis. **DESIGN AND METHODS:** We studied 15 severely malnourished hospitalized elderly patients. Weight, food intake, plasma albumin, transthyretin, C-reactive protein (CRP), orosomucoid, interleukin-6 (IL-6), IGF-I, intact and proteolytically degraded IGFBP-3 and GHBP levels were determined on admission and during refeeding therapy designed to increase food intake to 40 kcal/kg body weight per day (15% protein). **RESULTS:** Plasma IGF-I, IGFBP-3 and GHBP levels were significantly low for age on admission in all malnourished elderly patients. They increased in nine patients as nutritional status improved (albuminemia >30 g/l; transthyretinemia >200 mg/l or weight gain >5% of initial body weight) and levels of inflammation markers decreased (group 1). In contrast, plasma IGF-I, IGFBP-3 and GHBP levels remained low in six patients in whom nutritional status failed to improve and levels of inflammation markers increased (group 2). IGF-I showed greater variations than IGFBP-3 or GHBP with respect to nutritional status. High plasma CRP and IL-6 levels were associated with high levels of IGFBP-3 proteolysis. **CONCLUSION:** Efficient refeeding therapy was associated with a significant increase in IGF-I plasma levels. In patients with severe and persistent inflammation, high levels of proteolysis of IGFBP-3 may have contributed to the low plasma IGF-I levels, persistence of hypercatabolism and lack of improvement in nutritional status.

**SAVINO W., POSTEL-VINAY M., SMANIOTTO S., DARDENNE M.**

The Thymus Gland: a Target Organ for Growth Hormone.

*Scand. J. Immunol.*, 55 (5), 442-452, 2002

(Services cités : FRE 2444, U344)

Increasing evidence has placed hormones and neuropeptides among potent immunomodulators, in both health and disease. Herein, we focus on the effects of growth hormone (GH) upon the thymus. Exogenous GH enhances thymic microenvironmental cell-derived secretory products such as cytokines and thymic hormones. Moreover, GH increases thymic epithelial cell (TEC) proliferation in vitro, and exhibits a synergistic effect with anti-CD3 in stimulating thymocyte proliferation, which is in keeping with the data showing that transgenic mice overexpressing GH or GH-releasing hormone exhibit overgrowth of the thymus. GH also influences thymocyte traffic: it increases human T-cell progenitor engraftment into the thymus; augments TEC/thymocyte adhesion and the traffic of thymocytes in the lymphoepithelial complexes, the thymic nurse cells; modulates in vivo the homing of recent thymic emigrants, enhancing the numbers of fluorescein isothiocyanate (FITC)+ cells in the lymph nodes and diminishing them in

the spleen. In keeping with the effects of GH upon thymic cells is the detection of GH receptors in both TEC and thymocytes. Additionally, data indicate that insulin-like growth factor (IGF)-1 is involved in several effects of GH in the thymus, including the modulation of thymulin secretion, TEC proliferation as well as thymocyte/TEC adhesion. This is in keeping with the demonstration of IGF-1 production and expression of IGF-1 by TEC and thymocytes. Also, it should be envisioned as an intrathymic circuitry, involving not only IGF-1, but also GH itself, as intrathymic GH expression is seen both in TEC and in thymocytes, and that thymocyte-derived GH could enhance thymocyte proliferation. Finally, the possibility that GH improve thymic functions, including thymocyte proliferation and migration, places this molecule as a potential therapeutic adjuvant in immunodeficiency conditions associated with thymocyte decrease and loss of peripheral T cells.

**SCHUFF K.G., HENTGES S.T., KELLY M.A., BINART N., KELLY P.A., IUVONE P.M., ASA S.L., LOW M.J.**

Lack of prolactin receptor signaling in mice results in lactotroph proliferation and prolactinomas by dopamine-dependent and -independent mechanisms.

*J. Clin. Invest.*, 110 (7), 973-981, 2002

(Services cités : U344)

Hypothalamic dopamine inhibits pituitary prolactin secretion and proliferation of prolactin-producing lactotroph cells by activating lactotroph dopamine D2 receptors (D2Rs). Conversely, prolactin (PRL) stimulates hypothalamic dopamine neurons via PRL receptors (PRLRs) in a short-loop feedback circuit. We used *Drd2*(-/-) and *Prlr*(-/-) mutant mice to bypass this feedback and investigate possible dopamine-independent effects of PRL on lactotroph function. The absence of either receptor induced hyperprolactinemia and large prolactinomas in females. Small macroadenomas developed in aged *Prlr*(-/-) males, but only microscopic adenomas were found in *Drd2*(-/-) male mice. Pharmacologic studies in *Prlr*(-/-) mice with D2R agonists and antagonists demonstrated a significant loss of endogenous dopamine tone, i.e., constitutive inhibitory signaling by the D2R, in the pituitary. However, *Prlr*(-/-) mice exhibited more profound hyperprolactinemia and larger tumors than did age-matched *Drd2*(-/-) mice, and there were additive effects in compound homozygous mutant male mice. In vitro, PRL treatment markedly inhibited the proliferation of wild-type female and male *Drd2*(-/-) lactotrophs, but had no effect on female *Drd2*(-/-) lactotrophs, suggesting a downregulation or desensitization of PRLR in response to chronic hyperprolactinemia. We conclude that PRL inhibits lactotrophs by two distinct mechanisms: (a) indirectly by activation of hypothalamic dopamine neurons and (b) directly within the pituitary in a dopamine-independent fashion.

**YU-LEE L., JEAY S.**

Prolactin and growth hormone receptors.

in: *Hormone Signalling*. (Goffin V., Kelly P.A. eds.)

Kluwer Academic Publishers (London), 2002, pp.121-143.

(Services cités : U344)

**2001**

**AMMARGUELLAT F., LLOVERA M., KELLY P.A., GOFFIN V.**

Low doses of epo activate map kinases but not jak2-stat5 in rat vascular smooth muscle cells.

*Biochem. Biophys. Res. Commun.*, 284 (4), 1031-1038, 2001

(Services cités : U344)

Previous reports have shown a direct effect of erythropoietin (Epo) on vascular smooth muscle cells (VSMCs). Our aim was to assess expression of the Epo receptor (EpoR) on VSMCs and to study the activation of two major signaling cascades activated by Epo, namely JAK2/STAT5 and MAPK pathways. All experiments were performed in parallel using the Epo-responsive UT7 cell line. From semiquantitative RT-PCR experiments, VSMCs were estimated to express similar to 30-fold less EpoR mRNA than UT7 cells. Epo-induced phosphorylation of proteins involved in the EpoR/JAK2/STAT5 cascade could not be detected in VSMCs, even using pharmacological doses of Epo (250 IU/ml). In contrast, a strong activation of MAP kinase pathway was detected with as low as 10 IU/ml Epo. We suggest that MAPK activation reflects a physiologically relevant effect of Epo on VSMCs that may be correlated to cell proliferation. (C) zool Academic Press. [References: 46]

**BAIXERAS E., JEAY S., KELLY P.A., POSTEL-VINAY M.C.**

The proliferative and antiapoptotic actions of growth hormone and insulin-like growth factor-1 are mediated through distinct signaling pathways in the pro-b ba/f3 cell line.

*Endocrinology*, 142 (7), 2968-2977, 2001

(Services cités : U344)

Biological actions of GH can be direct or mediated through insulinlike growth factor I(IGF-I). In the interleukin-3 (IL-3)-dependent Ba/F3 cell line, IGF-I induces cell cycle entry and proliferation. Ba/F3 cells expressing the rat GH receptor (Ba/F3 GHR cells) have been shown to escape from apoptosis and to proliferate under GH stimulation. Using the Ba/F3 GHR cell model, we sought to dissect the signals elicited specifically by IGF-I or GH. In contrast to IGF-I or IL-3, GH is able to maintain cell cycle entry of Ba/F3 GHR cells cultured for 7 days in the absence of serum. The presence of IGF-I messenger RNA was not detected by RT-PCR, and by RIA, IGF-I was not found in culture medium of Ba/F3 GHR cells, unstimulated or stimulated by GH.

Moreover, the addition of an anti-IGF-I antibody that blocks IGF-I effects suggests that the actions of GH are not mediated by IGF-I, but appear to be direct. GH or IGF-I stimulation increased expression of cyclins A and D-1 with comparable kinetics, whereas expression of pal(waf1/cip1) seemed delayed in IGF-I-stimulated cells compared with that in GH-stimulated cells. Contrary to GH or IL-3, IGF-I did not induce nuclear factor-kappaB DNA-binding activity in Ba/F3 cells. Inhibition of nuclear factor-kappaB through expression of the mutant I kappaB alpha (A32/36) abrogated the GH-mediated survival signal, but did not result in alterations of the cell cycle in Ba/F3 GHR cells treated with IGF-I. Phosphatidylinositol 3-kinase was required for both survival and proliferative responses to IGF-I. Transfection of a dominant negative form of AKT (AH-AKT) resulted in suppression of IGF-I-mediated cell survival, but not of the antiapoptotic effect of GH in Ba/F3 GHR cells. Thus, GH and IGF-I are able to promote cell survival and proliferation through independent and different pathways in Ba/F3 cells.

[References: 36]

**BERNICHTEIN S., KINET S., JEAY S., LLOVERA M., MADERN D., MARTIAL J.A., KELLY P.A., GOFFIN V.**

S179d-human prl, a pseudophosphorylated human prl analog, is an agonist and not an antagonist.

*Endocrinology*, 142 (9), 3950-3963, 2001

(Services cités : U344)

For many years, our group has been involved in the development of human PRL antagonists. In two recent publications, S179D-human PRL, a human PRL analog designed to mimic a putative S179-phosphorylated human PRL, was reported to be a highly potent antagonist of human PRL-

induced proliferation and signaling in rat Nb2 cells. We prepared this analog with the aim of testing it in various bioassays involving the homologous, human PRL receptor. In our hands, S179D-human PRL was able to stimulate 1) the proliferation of rat Nb2 cells and of human mammary tumor epithelial cells (T47D), 2) transcriptional activation of the lactogenic hormone response element-luciferase reporter gene, and 3) activation of the Janus kinase/signal transducer and activator of transcription and MAPK pathways. Using the previously characterized antagonist G129R-human PRL as a control, we failed to observe any evidence for antagonism of S179D-human PRL toward any of the human PRL-induced effects analyzed, including cell proliferation, transcriptional activation, and signaling. In conclusion, our data argue that S179D-human PRL is an agonist displaying slightly reduced affinity and activity due to local alteration of receptor binding site 1, and that the antagonistic properties previously attributed to S179D-human PRL cannot be confirmed in any of the assays analyzed in this study. [References: 58]

**BINART N., KELLY P.A.**

Transgenic and knockout models of prolactin action in female reproduction.

*Endocrine Updates Series*, 13 123-141, 2001

(Services cités : U344)

**BOUCHARD B.**

Termination of pregnancy in teenage girls: worrying figures.

*M S-Méd. Sci.*, 17 (3), 350-351, 2001

(Services cités : U344)

**CAMARILLO I.G., THORDARSON G., MOFFAT J.G., VAN HORN K.M., BINART N., KELLY P.A., TALAMANTES F.**

Prolactin receptor expression in the epithelia and stroma of the rat mammary gland.

*J. Endocrinol.*, 171 (1), 85-95, 2001

(Services cités : U344)

The importance of prolactin (PRL) in regulating growth and differentiation of the mammary gland is well known. However, it is not well established whether PRL acts solely on the mammary epithelia or if it can also directly affect the mammary stroma. To determine where PRL could exert its effects within the mammary gland, we investigated the levels of expression and the localization of the PRL receptor (PRLR) in the epithelia and stroma of the rat mammary gland at different physiological stages. For these studies, we isolated parenchymal-free 'cleared' glands from virgin, 18-day-fat pads and intact mammary glands from virgin, 18-day-pregnant and 6-day-lactating rats. In addition, intact mammary tissues were enzymatically, digested to obtain epithelial cells, free of stroma. The mammary tissues, intact gland, stroma and isolated epithelia, were then used for immunocytochemistry, protein extraction and isolation of total RNA. PRLR protein was detected in tissues using specific polyclonal antisera (PRLR-1) by immunocytochemistry and Western blot analysis. Messenger RNA for PRLR, was measured by ribonuclease protection assay. Immunocytochemistry and Western blots with the PRLR-1 antisera detected PRLR in wild-type rat and mouse tissues, whereas the receptor protein was absent in tissues from PRLP, gene-deficient mice. PRLP, was found to be present both in the epithelia and stroma of mammary glands from virgin, pregnant and lactating rats, as determined by immunocytochemistry and Western blotting. Western blots revealed the predominance of three bands migrating at 88, 90 and 92 kDa in each of the rat mammary samples. These represent the long form of the PRLR. During pregnancy and lactation, PRLR protein increased in the

epithelial compartment of the mammary gland but did not change within the stromal compartment at any physiological stage examined. We also found PRLR, mRNA in both the epithelia and stroma of the mammary gland. Again, the stroma contained lower levels of PRLR, mRNA compared with the epithelia at all physiological stages examined. Also, the PRLR, mRNA levels within the stroma did not change significantly during pregnancy or lactation, whereas PRLR mRNA within the epithelia increased twofold during pregnancy and fourfold during lactation when compared with virgin rats. We conclude from this study that PRLR is expressed both in the stromal and epithelial compartment of the mammary gland. This finding suggests PRL may have a direct affect on the mammary. stroma and by that route affect mammary gland development. [References: 44]

**CRAVEN A.J., ORMANDY C.J., ROBERTSON F.G., WILKINS R.J., KELLY P.A., NIXON A.J., PEARSON A.J.**

Prolactin signaling influences the timing mechanism of the hair follicle: analysis of hair growth cycles in prolactin receptor knockout mice.

*Endocrinology*, 142 (6), 2533-2539, 2001

(Services cités : U344)

Pituitary PRL regulates seasonal hair follicle growth cycles in many mammals. Here we present the first evidence implicating PRL in the nonseasonal, wave-like pelage replacement of laboratory mice. In this study we show that messenger RNA transcripts encoding the one long and two short forms of PRL receptor are present in the skin of adult and neonate mice. The receptor protein was immunolocalized to the hair follicle as well as the epidermis and sebaceous glands. Furthermore, PRL messenger RNA was detected within skin extracts, suggesting a possible autocrine/paracrine role. Analysis of the hair growth phenotype of PRL gene-disrupted mice (PRLR-/-) revealed a change in the timing of hair cycling events. Although no hair follicle development differences were noted in PRLR-/- neonates, observations of the second generation of hair growth revealed PRLR-/- mice molted earlier than wild types (PRLR+/+). The advance was greater in females (29 days) than in males (4 days), resulting in the elimination of the sexual dimorphism associated with murine hair replacement. Heterozygotes were intermediate between PRLR-/- and PRLR+/+ mice in molt onset. Once initiated, the pattern and progression of the molt across the body were similar in all genotypes. Although all fiber types were present and appeared structurally normal, PRLR-/- mice had slightly longer and coarser hair than wild types. These findings demonstrate that PRL has an inhibitory effect on murine hair cycle events. The pituitary PRL regulation of hair follicle cycles observed in seasonally responsive mammals may be a result of pituitary PRL interacting with a local regulatory mechanism. [References: 47]

**DE BAERE E., DIXON M.J., SMALL K.W., JABS E.W., LEROY B.P., DEVRIENDT K., GILLEROT Y., MORTIER G., MEIRE F., VAN MALDERGEM L., COURTENS W., HJALGRIM H., HUANG S., LIEBAERS I., VAN REGEMORTER N., TOURAINÉ P., PRAPHANPHOJ V., VERLOES A., UDAR N., YELLORE V., CHALUKYA M., YELCHITS S., de PAEPE A., KUTTENN F., FELLOUS M., VEITIA R., MESSIAEN L.** Spectrum of foxl2 gene mutations in blepharophimosis-ptosis-epicanthus inversus (bpes) families demonstrates a genotype-phenotype correlation.

*Hum. Mol. Genet.*, 10 (15), 1591-1600, 2001

(Services cités : Endocrinologie & Médecine de la Reproduction, U344)

Mutations in FOXL2, a forkhead transcription factor gene, have recently been shown to cause blepharo-phimosis-ptosis-epicanthus inversus syndrome (BPES) types I and II, a rare genetic

disorder. In BPES type I a complex eyelid malformation is associated with premature ovarian failure (POF), whereas in BPES type II the eyelid defect occurs as an isolated entity. In this study, we describe the identification of novel mutations in the FOXL2 gene in BPES types I and II families, in sporadic BPES patients, and in BPES families where the type could not be established. In 67% of the patients studied, we identified a mutation in the FOXL2 gene. In total, 21 mutations (17 of which are novel) and one microdeletion were identified. Thirteen of these FOXL2 mutations are unique. In this study, we demonstrate that there is a genotype-phenotype correlation for either types of BPES by the finding that mutations predicted to result in a truncated protein either lacking or containing the forkhead domain lead to BPES type I. In contrast, duplications within or downstream of the forkhead domain, and a frameshift downstream of them, all predicted to result in an extended protein, cause BPES type II. In addition, in 30 unrelated patients with isolated POF no causal mutations were identified in FOXL2. Our study provides further evidence that FOXL2 haploinsufficiency may cause BPES types I and III by the effect of a null allele and a hypomorphic allele, respectively. Furthermore, we propose that in a fraction of the BPES patients the genetic defect does not reside within the coding region of the FOXL2 gene and may be caused by a position effect. [References: 36]

**DIF F., SAUNIER E., DEMENEIX B., KELLY P.A., EDERY M.**

Cytokine-inducible sh2-containing protein suppresses prl signaling by binding the prl receptor.  
*Endocrinology*, *142* (12), 5286-5293, 2001  
(Services cités : U344)

Inhibition of PRL hormone signaling by suppressor of cytokine signaling (SOCS)/cytokine-inducible SH2-containing protein (CIS) was investigated in transfected HEK 293 cells. We used the physiologically relevant wild-type beta -casein promoter as a target gene for PRL action. We demonstrate that CIS produces a 70% inhibition of PRL signaling by a mechanism distinct from, and downstream of, the effect of SOCS-1 on JAK2. This inhibition involves association with the PRL receptor (PRLR), resulting in the inhibition of signal transducer and activator of transcription 5 (STAT5) activation. Further, we show that SOCS-3 coimmunoprecipitates with the PRLR. These data suggest that SOCS-3 involves a second pathway for the inhibition of PRL signaling other than JAK2 inhibition. Additional results indicate that SOCS-2 can play a more important potentiator role on PRL signaling, resulting in a restoration of 50% of transcriptional inhibition induced by SOCS-3 and a restoration of 100% of transcriptional inhibition induced by CIS. SOCS-2 was able to block the inhibitory effect of SOCS-1. These results indicate that SOCS-2 seems to be an antagonist of the other SOCS. SOCS-1 binds JAK2 and inhibits its phosphorylation; SOCS-3 does not bind JAK2 but binds the PRLR that may mediate its inhibition of JAK2; and finally, CIS binds the PRLR but inhibits signal transducer and activator of transcription 5 rather than JAK2. [References: 38]

**EDERY M., BINART N., BOUCHARD B., GOFFIN V., KELLY P.A.**

Prolactin receptors.  
*Endocrine Updates Series*, *12* 341-353, 2001  
(Services cités : U344)

**FREEMARK M., FLEENOR D., DRISCOLL P., BINART N., KELLY P.A.**

Body weight and fat deposition in prolactin receptor-deficient mice.  
*Endocrinology*, *142* (2), 532-537, 2001  
(Services cités : U344)

To explore the roles of the lactogens in adipose tissue development and function, we measured body weight, abdominal fat content, and plasma leptin concentrations in a unique model of lactogen resistance: the PRL receptor (PRLR)-deficient mouse. The absence of PRLRs in knockout mice was accompanied by a small (5-12%), but progressive, reduction in body weight after 16 weeks of age. Females were affected to a greater degree than males. The reduction in weight in female PRLR-deficient mice (age 8-9 months) was associated with a 49% reduction in total abdominal fat mass and a 29% reduction in fat mass expressed as a percentage of body weight. Lesser reductions were noted in male mice. Plasma leptin concentrations were reduced in females but not in males. That the reductions in abdominal fat may reflect in part the absence of lactogen action in the adipocyte is suggested by the demonstration of PRLR messenger RNA in normal mouse white adipose tissue. Nevertheless, steady state levels of PRLR messenger RNA in mature adipocytes are very low, suggesting that the effects of lactogens might be mediated by other hormones or cellular growth factors. Our observations suggest roles for the lactogens in adipose tissue growth and metabolism in pregnancy and postnatal life. [References: 71]

**GALLEGO M.I., BINART N., ROBINSON G.W., OKAGAKI R., COSCHIGANO K.T., PERRY J., KOPCHICK J.J., OKA T., KELLY P.A., HENNIGHAUSEN L.**

Prolactin, growth hormone, and epidermal growth factor activate stat5 in different compartments of mammary tissue and exert different and overlapping developmental effects.

*Dev. Biol.*, 229 (1), 163-175, 2001

(Services cités : U344)

Prolactin (Prl)-induced phosphorylation of Stat (signal transducer and activator of transcription) 5 is considered a key event in functional mammary development and differentiation. We now demonstrate that not only Prl, but also growth hormone (GH) and epidermal growth factor (EGF), can activate Stat5 in mammary tissue. We investigated the roles of these hormones in mammary development using mice in which the respective receptors had been inactivated. Although Prl receptor (PrIR)-null mice are infertile, we were able to maintain pregnancies in a few mice by treatment with progesterone. Mammary tissue in these mice was severely underdeveloped and exhibited limited differentiation as assessed by the phosphorylation status of Stat5 and the expression of milk protein genes. PrIR +/- mice showed impaired mammary development and alveolar differentiation during pregnancy, which corresponded with reduced phosphorylation levels of Stat5a and 5b, and impaired expression of milk protein genes. Development of the glands in these mice was arrested at around day 13 of pregnancy. While Prl activated Stat5 only in the epithelium, GH and EGF activated Stat5 preferentially in the stroma. To assess the relevance of the GH receptor (GHR) in the mammary gland, we transplanted GHR-null epithelium into cleared fat pads of wild-type mice. These experiments demonstrated that the GHR in the epithelium is not required for functional mammary development. Similarly, the EGFR in the epithelium is not required for alveolar development. In contrast, epithelial PrIR is required for mammary development and milk protein gene expression during pregnancy. Although GH is not required for alveolar development, we were able to demonstrate its lactogenic function in cultured mammary epithelium from PrIR-null mice. However, ductal development in GHR-null mice was impaired, supporting the notion that GH signals through the stromal compartment. Our findings demonstrate that GH, Prl, and EGF activate Stat5 in separate compartments, which in turn reflects their specific roles in ductal and alveolar development and differentiation. [References: 46]

**GLASOW A., HORN L.C., TAYMANS S.E., STRATAKIS C.A., KELLY P.A., KOHLER**

**U., GILLESPIE J., VONDERHAAR B.K., BORNSTEIN S.R.**

Mutational analysis of the prl receptor gene in human breast tumors with differential prl receptor protein expression.

*J. Clin. Endocrinol. Metabol.*, 86 (8), 3826-3832, 2001

(Services cités : U344)

PRL is a major growth and differentiating hormone in the human breast, with activation of the PRL-PRL receptor complex increasingly recognized as an important mechanism in the induction and progression of mammary tumors. Although constitutive activation of various hormone and growth factor receptors is newly recognized as a common cause of tumor development, the PRL receptor gene has not been analyzed for similar aberrations in breast and other tumors. Therefore, using bacterial artificial chromosomes containing the PRL receptor gene and intron-spanning PCR, we determined the exon-surrounding intron sequences providing primers for the first analysis of the entire coding region of the human PRL receptor gene. We examined the presence of PRL receptor in 41 breast tumors by immunohistochemistry and attempted a correlation of its expression to pathological grading of the disease. Then tumor cells were isolated by laser capture microdissection to examine DNA from 30 patients for PRL receptor mutations. The PRL receptor immunoreactive score did not correlate to the tumor size, histopathological grading, age, or family history of patients. PRL receptor immunoreactivity was predominantly found in steroid hormone receptor-positive tumors, but without overall correlation of immunoreactive score. In both PRL receptor-positive and PRL receptor-negative breast cancer cells, direct sequencing of the coding sequence of the PRL receptor gene did not detect any somatic or hereditary gene aberrations. In conclusion, PRL receptor mutations do not appear to be common in human breast cancer, suggesting that constitutive activation of the PRL receptor can be excluded as a major cause of mammary tumor genesis. The molecular structure of the PRL receptor seems to remain intact in tumor tissue, and systemic and local production of PRL may participate in tumor cell growth and proliferation through functional receptors. [References: 68]

**GOFFIN V., TOURAINE P., BINART N., KELLY P.A.**

Vers une nouvelle perception de la prolactine en physiopathologie humaine.

*Actual. Méd. Int. Métab. Horm. Nutr.*, 5 (6), 269-277, 2001

(Services cités : U344)

**JEAY S., SONENSHEIN G.E., KELLY P.A., POSTEL-VINAY M.C., BAIXERAS E.**

Growth hormone exerts antiapoptotic and proliferative effects through two different pathways involving nuclear factor-kappa b and phosphatidylinositol 3-kinase.

*Endocrinology*, 142 (1), 147-156, 2001

(Services cités : U344)

Dependence of murine pro-B Ba/F3 cells on interleukin-3 can be substituted by GH when cells are stably transfected with the GH receptor (GHR) complementary DNA. Recently, we demonstrated that Ba/F3 cells produce GH, which is responsible for the survival of cells expressing the GHR. This GH effect involves the activation of nuclear factor-kappaB (NF-kappaB). Here, we examined the signaling pathways mediating proliferation of growth factor-deprived Ba/F3 GHR cells. Exogenous GH stimulation of Ba/F3 GHR cells induced cyclins E and A and the cyclin-dependent kinase inhibitor p21(waf1/cip1) and repressed cyclin-dependent kinase inhibitor p27(kip1). The presence of the phosphatidylinositol 3-kinase (PI 3-kinase) inhibitor Ly 294002 abolished proliferation induced by GH, arresting Ba/F3 GHR cells at the G(1)/S boundary, but did not promote apoptosis. Thus, the proliferative effect of GH is closely

related to PI 3-kinase activation, whereas PI 3-kinase is not essential for GH-induced cell survival. Addition of Ly 294002 resulted in a moderate decrease in NF-kappaB activation by GH, suggesting a possible link between PI 3-kinase and NF-kappaB signaling by GH. Expression of c-myc was also induced by GH in Ba/F3 GHR cells, and inactivation of either PI 3-kinase or NF-KB reduced this induction. Overexpression of the dominant negative repressor mutant c-Myc-RX resulted in an inhibition of the GH proliferative effect, suggesting the involvement of c-myc in GH-induced proliferation. Taken together, these results suggest that the effects of GH on cell survival and proliferation are mediated through two different signaling pathways, NF-kappaB and PI 3-kinase, respectively; although cross-talk between them has not been excluded. NF-kappaB, which has been shown to be responsible for the antiapoptotic effect of GH, could also participate in GH-induced proliferation, as c-myc expression is promoted by PI 3-kinase, in an NF-kappaB-dependent and -independent manner. [References: 56]

**KELLY P.A., BINART N., FREEMARK M., LUCAS B., GOFFIN V., BOUCHARD B.**

Prolactin receptor signal transduction pathways and actions determined in prolactin receptor knockout mice.

*Biochem. Soc. Trans.*, 29 (2), 48-52, 2001

(Services cités : U344)

Prolactin-receptor-deficient mice are a good model in which to study the various actions of prolactin. Female homozygous knockout mice are completely infertile and show a lack of mammary development, while hemizogotes are unable to lactate following their first pregnancy. Male and female homozygotes have markedly elevated serum prolactin levels, and in some instances pituitary hyperplasia is present. Maternal behaviour is severely affected in both hemizygous and homozygous animals. Bone formation is reduced in young animals and in adults (males and females). Finally, older males and females show a slight reduction in body weight, which seems to be due to reduced abdominal fat deposition in the knockout animals. [References: 21]

**KELLY P.A., BINART N., LUCAS B., BOUCHARD B., GOFFIN V.**

Implications of multiple phenotypes observed in prolactin receptor knockout mice.

*Front. Neuroendocrinol.*, 22 (2), 140-145, 2001

(Services cités : U344)

The development of a mouse line deficient in the PRL receptor (PRLR) would be an ideal means to better understand the multiple functions of prolactin. We were worried initially that removal of the PRLR from the mouse genome might be lethal and were surprised to find this not to be the case. We identified numerous deficiencies in PRLR knockout (KO) animals. Female homozygous mice are completely infertile and lack normal mammary development, while hemizogotes are unable to lactate following their first pregnancy. PRLR KO males and females have markedly elevated (30- to 100-fold) serum prolactin levels and in some instances pituitary hyperplasia is present. Maternal behavior is severely affected in both hemizygous and heterozygous animals. Bone formation is reduced in young animals and adults (males and females). Recently, we noticed that older KO animals show a slight reduction in body weight which appears to be due to reduced abdominal fat deposition. [References: 21]

**KELLY P.A., FINIDORI J., MOULIN S., KEDZIA C., BINART N.**

Growth hormone receptor signalling and actions in bone growth.

*Hormone Res.*, 55 (Suppl 2), 14-17, 2001

(Services cités : [U344](#))

Growth hormone (GH) acts by binding to a membrane receptor that is part of the cytokine receptor superfamily. Ligand binding induces receptor dimerization leading to activation of the associated tyrosine kinase, Janus kinase (Jak) 2. Transphosphorylation of Jak2 occurs followed by tyrosine phosphorylation of the receptor, and numerous cytoplasmic proteins. Among these are the signal transducers and activators of transcription (Stat) proteins, as well as adaptor proteins leading to the activation of the Ras/mitogen-activated protein (MAP) kinase and the phosphatidylinositol-3'-kinase (PI 3-kinase) pathways. Activation of the GH receptor system is relatively transient, with several mechanisms being involved in downregulation: internalization and degradation of the receptor and recruitment of phosphatases or specific inhibitors of the Jak-Stat pathway, the suppressors of cytokine signalling (SOCS) proteins. Finally, the use of the GH receptor knock-out mouse model has allowed us to dissect the role of this hormone in post-natal body growth and homeostasis. Copyright (C) 2001 S. Karger AG, Basel. [References: 25]

**KINET S., BERNICHTEIN S., LLOVERA M., KELLY P.A., MARTIAL J.A., GOFFIN V.**  
Molecular basis of the interaction between human prolactin and its membrane receptor : a ten year study.

*Rec. Res. Dev. Endocrinol.*, 2 1-24, 2001

(Services cités : [U344](#))

**LLOVERA M., PEARSON J.D., MORENO C., RIVEROS-MORENO V V.**

Impaired response to interferon-gamma in activated macrophages due to tyrosine nitration of stat1 by endogenous nitric oxide.

*Br. J. Pharmacol.*, 132 (2), 419-426, 2001

(Services cités : [U344](#))

Inducible NO synthase (iNOS) expression and activity were measured in the mouse macrophage cell line J774 after exposure to bacterial lipopolysaccharide (LPS) with or without interferon-gamma (IFN-gamma). Inhibition of NOS activity by concomitant N(G)-monomethyl-L-arginine (L-NMMA) treatment further increased iNOS protein levels, with a substantial increase in iNOS half-life. Western blotting and ELISA demonstrated that several cell proteins were tyrosine-nitrated when iNOS activity was high. Rapid IFN-gamma-induced phosphorylation of STAT1 was reduced by about 40% when cells were pretreated to induce iNOS, unless L-NMMA was present during the pretreatment period. 2D gel electrophoresis demonstrated the presence of nitrotyrosine in STAT1 after iNOS induction, and confirmed the reduction in phospho-STAT1 on subsequent stimulation with IFN-gamma for 15 min and its partial restoration when L-NMMA was present during the pretreatment period. We did not detect tyrosine nitration of the upstream kinase JAK2 in LPS+IFN-gamma pretreated cells, but JAK2 activity was also impaired, and was partially restored by concomitant L-NMMA pretreatment. We conclude that endogenous production of NO induces feedback inhibition of signalling pathways activated by IFN-gamma, at least in part by nitrating tyrosine residues in STAT1 which prevents phosphorylation.

**MAAMRA M., BIDLINGMAIER M., POSTEL-VINAY M.C., WU Z., STRASBURGER C.J., ROSS R.J.M.**

Generation of human soluble leptin receptor by proteolytic cleavage of membrane-anchored receptors.

*Endocrinology*, 142 (10), 4389-4393, 2001

(Services cités : [U344](#))

The leptin receptor (ObR) exists in multiple isoforms. In rodents, a soluble isoform is generated by alternative splicing; but in humans, there is no mRNA encoding soluble receptor (leptin binding protein). We investigated the hypothesis that human leptin binding protein can be generated by proteolytic cleavage of membrane-anchored leptin receptors (ObRb and ObRa). Leptin binding protein of similar size to that previously detected in human serum was detected by HPLC in medium of cells transfected with ObRa. ObRa exhibited higher expression at the cell surface than ObRb and generated greater levels of leptin binding protein. Ligand-mediated immunofunctional and immunofluorometric assays revealed that the leptin binding protein in medium bound both leptin and an ObR-specific antibody and that the level of leptin binding protein correlated with receptor expression at the cell surface. Phorbol 12-myristate-13-acetate and N-ethylmaleimide increased the accumulation of leptin binding protein, an indication that the production of leptin binding protein was up-regulated by PKC and sulfhydryl group activation. The protease inhibitors, TNF alpha protease inhibitor I and Immunex compound 2, could inhibit the production of leptin binding protein, indicating that the enzyme responsible for leptin binding protein cleavage belongs to the metalloprotease family. In conclusion, human leptin binding protein is generated by proteolytic cleavage of membrane-anchored leptin receptor by a metalloprotease. [References: 39]

**MCCLELLAN K.A., ROBERTSON F.G., KINDBLOM J., WENNBO H., TORNELL J., BOUCHARD B., KELLY P.A., ORMANDY C.J.**

Investigation of the role of prolactin in the development and function of the lacrimal and Harderian glands using genetically modified mice.

*Invest. Ophthalm. Vis. Sci.*, 42 (1), 23-30, 2001

(Services cités : U344)

**PURPOSE.** TO determine whether prolactin receptor is essential for normal development and function of the lacrimal gland and whether hyperprolactinemia can alter lacrimal development. **METHODS.** Lacrimal gland morphology and function were examined in two genetic mouse models of prolactin action: a prolactin receptor knockout model that is devoid of prolactin action and a transgenic model of hyperprolactinemia. **RESULTS.** Image analysis of lacrimal and Harderian gland sections was used to quantify glandular morphology. In females, lacrimal acinar area decreased by 30% and acinar cell density increased by 25% over control subjects in prolactin transgenic animals, but prolactin receptor knockout mice showed no changes, in males, transgenic animals showed no changes, but prolactin receptor knockout mice showed a 5% reduction in acinar area and an 11% increase in acinar cell density, which was lost after castration. The morphology of the Harderian glands underwent parallel changes but to a lesser degree. A complete loss of porphyrin accretions was seen in the Harderian glands of male and female knockout animals. No differences in tear protein levels were seen in knockout animals by two-dimensional gels. Enzyme-linked immunosorbent assay (ELISA) and Western blot analysis showed that the level of secretory component and IgA in knockout mouse tears remained unchanged. There was no change in the predisposition of the 129 mouse strain to conjunctivitis in the knockout animals. **CONCLUSIONS.** Prolactin plays a small role in establishing the sexual dimorphism of male Lacrimal glands. In females, hyperprolactinemia causes a hyperfemale morphology, suggesting a role in dry eye syndromes. Prolactin is required for porphyrin secretion by the Harderian gland but plays no essential role in the secretory immune function of the lacrimal gland. [References: 36]

**SANTOS C.R.A., INGLETON P.M., CAVACO J.E.B., KELLY P.A., EDERY M., POWER**

## **D.M.**

Cloning, characterization, and tissue distribution of prolactin receptor in the sea bream (*Sparus aurata*).

*Gen. Comp. Endocrinol.*, 121 (1), 32-47, 2001

(Services cités : U344)

The prolactin receptor (PRLR) was cloned and its tissue distribution characterized in adults of the protandrous hermaphrodite marine teleost, the sea bream (*Sparus aurata*). An homologous cDNA probe for sea bream PRLR (sbPRLR) was obtained by RT-PCR using gill mRNA. This probe was used to screen intestine and kidney cDNA libraries from which two overlapping clones (1100 and 2425 bp, respectively) were obtained. These clones had 100% sequence identity in the overlapping region (893 bp) and were used to deduce the complete amino acid sequence of sbPRLR. The receptor spans 2640 bp and encodes a protein of 537 amino acids. Features characteristic of PRLR, two pairs of cysteines, WS box, hydrophobic transmembrane domain, box 1 and box 2, were identified and showed a high degree of sequence identity to PRLRs from other vertebrate species. SbPRLR is 29 and 32% identical to tilapia (*Oreochromis niloticus*) and goldfish (*Carassius auratus*) PRLRs, respectively. In the sea bream two PRLR transcripts of 2.8 and 3.2 kb were detected in the intestine, kidney, and gills and a single transcript of 2.8 kb was detected in skin and pituitary by Northern blot. Spermiating gonads (more than 95% male tissue; gonado-somatic index of 0.6) contained, in addition to the 2.8-kb transcript, three more transcripts of 1.9, 1.3, and 1.1 kb. RT-PCR, which is a far more sensitive method than Northern blot, detected PRLR mRNA in gills, intestine, brain, pituitary, kidney, liver, gonads, spleen, head-kidney, heart, muscle, and bone. Immunohistochemistry using specific polyclonal antibodies raised against an oligopeptide from the extracellular domain of sbPRLR detected PRLR in several epithelial tissues of juvenile sea bream, including the anterior gut, renal tubule, choroid membrane of the third ventricle, saccus vasculosus, branchial chloride cells, and branchial cartilage, (C) 2001 Academic Press. [References: 61]

## **TOURAINÉ F., PLU-BUREAU G., BEJI C., MAUVAIS-JARVIS F., KUTTENN F.**

Long-term follow-up of 246 hyperprolactinemic patients.

*Acta Obstet. Gynecol. Scand.*, 80 (2), 162-168, 2001

(Services cités : Endocrinologie & Médecine de la Reproduction, U344, URC)

Background. We wanted to evaluate the very long-term effects of bromocriptine on prolactin (PRL) levels and pituitary tumor size in a large cohort of hyperprolactinemic patients. Methods. We conducted a retrospective cohort study in the Department of Endocrinology from Necker Hospital in Paris, France. Two hundred and forty-six patients consulted primarily for menstrual disorders, with diagnosis of hyperprolactinemia. Patients were followed-up for 99.9±3.6 months. One hundred and ninety-one were treated with bromocriptine, 32 underwent surgery, and 23 received no treatment. Results. The mean initial plasma PRL level was 135.0±20.2 ng/ml. Presence of an adenoma was detected in 60% of our patients and comprised a microadenoma in 64% of cases. Compared to oligomenorrheic women, amenorrheic patients had significantly higher levels of PRL and larger pituitary tumor size. In the bromocriptine group, PRL levels decreased from 99.6±7.9 to 20.0±1.5 ng/ml (p=0.00001). The medical treatment was associated with disappearance of the adenoma in 45% of the women and with stabilization of pituitary tumor size in 40% of patients. Surgery led to disappearance of the adenoma in almost all cases, but failed to definitively cure hyperprolactinemia. Conclusion. In this large-scale retrospective study, the medical treatment of mild hyperprolactinemia was shown to be effective and sufficient after 9 years of follow-up. [References: 43]

**TOURAINÉ P., PLU-BUREAU G., BERESSI N., DECQ P., THALABARD J.C., KUTTENN F.**

Resumption of luteinizing hormone pulsatility and hypogonadotropic hypogonadism after endoscopic ventriculocisternostomy in a hydrocephalic patient.

*Fert. Steril.*, 76 (2), 390-393, 2001

(Services cités : Endocrinologie & Médecine de la Reproduction, U344, URC)

OBJECTIVE: To study gonadotropin pulsatility before and after surgical cure of hydrocephalus.

DESIGN: Case report. SETTING: Department of Endocrinology and Centre d'Investigations

Cliniques, Necker Hospital, Paris, France. PATIENT(S): A 29-year-old woman who presented

with secondary amenorrhea. INTERVENTION(S): The patient underwent an endoscopic

ventriculocisternostomy that led to restoration of normal menses and resolution of

hypogonadism. MAIN OUTCOME MEASURE(S): A gonadotropin pulse study was performed

before and 2 and 5 months after surgery. RESULT(S): No LH pulse was observed before surgery.

Emergence of pulsatility was observed 2 months after surgery, and pulses became clearly

individualized after 5 months. CONCLUSION(S): This observation strongly suggests that

amenorrhea, in case of chronic hydrocephalus, is indeed due to a hypothalamic dysfunction of the GnRH pulse generator.

**2000**

**BINART N., HELLOCO C., ORMANDY C.J., BARRA J., CLEMENT-LACROIX P., BARAN N., KELLY P.A.**

Rescue of preimplantary egg development and embryo implantation in prolactin receptor-deficient mice after progesterone administration.

*Endocrinology*, 141 (7), 2691-2697, 2000

(Services cités : U344)

PRL, a hormone secreted essentially by the pituitary and other extrapituitary sources such as decidua, has been attributed regulatory roles in reproduction and cell growth in mammals. These effects are mediated by a membrane PRL receptor belonging to the cytokine receptor superfamily. Null mutation of the PRL receptor gene leads to female sterility due to a severely compromised preimplantation development and a complete failure of the implantation of the few embryos reaching the blastocyst stage, strongly implicating PRL in the maternal control of implantation. We measured the hormonal status of *-/-* mice, which confirmed that the corpus luteum is unable to produce progesterone. Progesterone administration to *-/-* mice completely rescued the development of preimplantary eggs and embryo implantation. Pregnancy could be maintained to 19.5 days postcoitum, with about 22% of resulting embryos reaching adulthood. Although progesterone and perhaps PRL appear to facilitate mouse preembryo development throughout the preimplantation stages, other factors as well as a possible direct effect of PRL on the uterus are probably necessary to fully maintain pregnancy. Finally, reduced ductal side-branching in the mammary gland can be rescued by progesterone treatment, but females exhibit reduced alveolar formation. Our model establishes the PRL receptor as a key regulator of reproduction and provides novel insights into the function of lactogenic hormones and their receptor. [References: 36]

**BOLE-FEYSOT C., PERRET E., ROUSTAN P., BOUCHARD B., KELLY P.A.**

Analysis of prolactin-modulated gene expression profiles during the Nb2 cell cycle using differential screening techniques.

*Genome Biology*, 1 (4), 0008.1-.15, 2000  
(Services cités : U344)

**BOUCHARD B.**

Estrogen receptors and sexual differentiation.

*M S-Méd. Sci.*, 16 (5), 705-706, 2000

(Services cités : U344)

**COLSON A., LE CAM A., MAITER D., EDERY M., THISSEN J.P.**

Potential of growth hormone-induced liver suppressors of cytokine signaling messenger ribonucleic acid by cytokines.

*Endocrinology*, 141 (10), 3687-3695, 2000

(Services cités : U344)

Endotoxin and proinflammatory cytokines such as interleukin-1 beta (IL-1 beta) and tumor necrosis factor-alpha (TNF alpha) induce a state of GH resistance. A new family of suppressors of cytokine signaling (SOCS), induced by cytokines activating the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, has been recently identified as a negative feedback loop of intracellular signaling. Overexpression of some SOCS (SOCS-3, CIS, and SOCS-2) has been reported to inhibit the JAK-STAT pathway stimulated by GH. To assess the possible role of these three SOCS proteins in the GH resistance induced by endotoxin and cytokines, we investigated the regulation of their gene expression by endotoxin and GH in rat liver and by proinflammatory cytokines and GH in primary culture hepatocytes. Both GH and lipopolysaccharide induced the three SOCS messenger RNAs (mRNAs) in vivo. In vitro, GH also increased the liver mRNAs encoding SOCS-2, SOCS-3, and CIS. Although IL-1 beta and TNF alpha alone induced only weakly the expression of SOCS-3 and CIS, these cytokines strongly potentiated the induction of these two SOCS by GH. In contrast, IL-6 alone markedly induced SOCS-3 mRNA, but did not potentiate the GH action on SOCS-3 and CIS mRNAs. The GH induction of SOCS-2 was not potentiated by any of these cytokines. Considering the ability of these SOCS to inhibit the JAK-STAT pathway induced by GH, these results suggest that the overexpression of SOCS-3 and CIS mRNAs induced by IL-1 beta and TNF alpha or by endotoxin in vivo may play a role in the GH resistance induced by sepsis. [References: 51]

**DALRYMPLE A., EDERY M., JABBOUR H.N.**

Sequence and functional characterisation of the marmoset monkey (*callithrix jacchus*) prolactin receptor: comparative homology with the human long-form prolactin receptor.

*Mol. Cell. Endocrinol.*, 167 (1-2), 89-97, 2000

(Services cités : U344)

This study demonstrates the cloning and in-vitro characterisation of the marmoset monkey (*Callithrix jacchus*) prolactin receptor cDNA. The marmoset prolactin receptor cDNA was generated by reverse transcription-polymerase chain reaction using adrenal RNA and primers designed from prolactin receptor conserved regions. Sequence analysis predicts a mature protein of 598 amino acids exclusive of the 24 amino acid signal peptide. The marmoset prolactin receptor cDNA shares 93 and 61% base pair, and 89 and 61% amino acid sequence homologies with the long form human and rat prolactin receptor cDNA, respectively. The marmoset prolactin receptor cDNA sequence retains all the receptor sequences that have been shown previously to be essential for ligand binding, structural integrity and signal transduction. Transfection of human 293 fibroblast cells with the marmoset prolactin receptor cDNA (three independent experiments)

confirmed the expression of a receptor that has high, binding affinity to human growth hormone ( $K_a = 3.6 \pm 0.07 \text{ nM}^{-1}$ ) and B-max =  $7.55 \pm 2.06 \times 10^{-11} \text{ M}$ ) and human prolactin ( $K_a = 3.1 \pm 0.12 \text{ nM}^{-1}$ ) and B-max =  $2.87 \pm 0.66 \times 10^{-11} \text{ M}$ ). Functionality of the receptor was assessed by co-transfection of 293 fibroblast cells with marmoset prolactin receptor cDNA and the Jak2 cDNA, or marmoset prolactin receptor and a Stat5 responsive element linked to the luciferase coding sequence. Incubation of the cells with 18 nM ovine prolactin resulted in rapid phosphorylation of Jak2 as ascertained by Western blotting. In addition, the marmoset prolactin receptor cDNA led to  $9.06 \pm 0.47$ -fold induction of luciferase gene activity: This was comparable with the induction observed following transfection with the human prolactin receptor cDNA ( $8.55 \pm 0.5$ -fold). In-vivo prolactin receptor expression in the marmoset monkey was assessed by ribonuclease protection assay and detected in a number of tissues including female reproductive organs. These data confirm the cloning and functionality of the marmoset prolactin receptor cDNA. The marmoset prolactin receptor shares a high sequence homology with the long-form human prolactin receptor, and both receptors bind hormones with comparable affinity and confer a similar intracellular response. The marmoset monkey may provide a useful tool to investigate the role of prolactin in primate reproduction. (C) 2000 Elsevier Science Ireland Ltd. All rights reserved. [References: 41]

**DAS T., JOHNS P.W., GOFFIN V., KELLY P., KELDER B., KOPCHICK J., BUXTON K., MUKERJI P.**

High-level expression of biologically active human prolactin from recombinant baculovirus in insect cells.

*Protein Expr. Purif.*, 20 (2), 265-273, 2000

(Services cités : [U344](#))

We examined the feasibility of high-level production of recombinant human prolactin, a multifunctional protein hormone, in insect cells using a baculovirus expression system. The human prolactin cDNA with and without the secretory signal sequence was cloned into pFastBac1 baculovirus vector under the control of polyhedrin promoter. Prolactin was produced upon infection of either Sf9 or High-Five cells with the recombinant baculovirus containing the human prolactin cDNA. The production of recombinant prolactin varied from 20 to 40 mg/L of monolayer culture, depending on the cell types. The prolactin polypeptide with its own secretory signal was secreted into the medium. N-terminal amino acid sequence analysis of the recombinant polypeptide purified from the culture medium indicated that the protein was processed similar to human pituitary prolactin. Carbohydrate analysis of the purified protein indicated that a fraction of the recombinant prolactin made in insect cells appeared to be glycosylated. Also, both secreted and nonsecreted forms of the recombinant prolactin in insect cells were biologically equivalent to the native human prolactin (pituitary derived) in the Nb2 lymphoma cell proliferation assay.

**DINERSTEIN-CALI H., FERRAG F., KAYSER C., KELLY P.A., POSTEL-VINAY M.C.**

Growth hormone (gh) induces the formation of protein complexes involving stat5, erk2, shc and serine phosphorylated proteins.

*Mol. Cell. Endocrinol.*, 166 (2), 89-99, 2000

(Services cités : [U344](#))

The aim of this study was to investigate the interaction of Stat5 with key effector proteins Erk2 and She after activation by growth hormone (GH), using Chinese Hamster Ovary (CHO) cells stably expressing the wild type rabbit growth hormone receptor (GHR). In

coimmunoprecipitation experiments. we show GH-induced formation of complexes consisting of Stat5a and Erk2, and Stat5a and Stat5b association with the protein adaptor. She. In CI-IO cells treated with GH, a rapid association of tyrosine and serine phosphorylated Stat5a with activated Erk2 is observed. In contrast, She proteins interact with non-phosphorylated forms of Stat5. Using truncated and tyrosine mutants of the GI-TR, we identify a carboxy-terminal domain of the receptor, which is critical for serine phosphorylation of Stat5a and Stat5a/Erk2 complex Formation. In addition, tyrosine residues of this region of the GHR are not required for Stat5/Erk2 interaction but are essential for Stat5a serine phosphorylation, Moreover, we detect serine phosphorylated proteins associated with Erk2, She and Stat5: both Stat5 isoforms interact with ii serine phosphorylated protein of 63 kDa, which is shown to be related to the serine-threonine kinase Akt-1. Our results support the importance of serine phosphorylation cascades in GH signaling and open another pathway of GH signal transduction. (C) 2000 Elsevier Science Ireland Ltd. All rights reserved. [References: 26]

**FAVRE YOUNG H., DIF F., ROUSSILLE F., DEMENEIX B.A., KELLY P.A., EDERY M., de LUZE A.**

Cross-talk between signal transducer and activator of transcription (stat5) and thyroid hormone receptor-beta 1 (tr beta 1) signaling pathways.

*Mol. Endocrinol.*, 14 (9), 1411-1424, 2000

(Services cités : U344)

PRL and T-3 are involved in antagonistic regulations during various developmental processes in vertebrate species. We have studied cross-talk between transcription factors activated by these signaling pathways, ie. signal transducer and activator of transcription 5 (Stat5) and thyroid hormone receptor beta 1 (TR beta 1). Liganded TR beta 1 in the presence of its heterodimeric partner, retinoid X receptor gamma (RXR gamma), inhibited the PRL-induced Stat5a- and Stat5b-dependent reporter gene expression by up to 60%. This T-3-inhibitory effect studied on Stat5 activity was partly reversed by overexpression of a TR beta 1 dominant negative variant mutated within its nuclear localization signal (TR2A). We next showed that TR beta 1 and TR2A in the presence of RXR gamma increased and decreased, respectively, Stat5 localization into the nucleus regardless of hormonal stimulation. Thus, our data suggest that TRP1 can be associated with Stat5 in the cytoplasm and may be involved in Stat5 nuclear translocation. In PRL-treated cells overexpressing TR beta 1/RXR gamma, both Stat5 and TR beta 1 were coimmunoprecipitated, indicating physical association of the two transcription factors. In these cells, addition of T-3 with ovine (o)PRL decreased the amounts of total and tyrosine-phosphorylated Stat5 in the cytoplasm compared with oPRL-treated cells. In the nucleus, no clear difference was observed on Stat5 DNA-binding after treatment with PRL and T-3, vs. PRL alone in TR beta 1/RXR gamma transfected cells. However, antibodies directed against TRP1 lowered Stat5-DNA binding and addition of the deacetylase inhibitor trichostatin A (TSA) relieved T-3, inhibition on Stat5 transcriptional activity. Thus, we postulated that the negative cross-talk between TR and Stat5 on target genes could involve histone deacetylase recruitment by liganded TR beta 1. [References: 56]

**FINIDORI J.**

Regulators of growth hormone signaling.

*Vitam. Horm.*, 59 71-97, 2000

(Services cités : U344)

Growth hormone acts through binding to membrane receptors that belong to the cytokine receptor

superfamily. Ligand binding induces receptor dimerization and activation of the receptor-associated kinase: JAK2; this results in phosphorylation of the kinase itself, of the receptor, and of many cellular proteins. Among these are the Stat proteins as well as adaptors leading to the activation of the Ras/MAP kinase pathway and of the PI-3 kinase pathway. Activation by growth hormone is very transient and several mechanisms are involved in this downregulation: internalization and degradation of the receptor and recruitment of phosphatases or of specific inhibitors of the JAK/Stat pathway, the SOCS proteins. [References: 157]

**JEAY S., SONENSHEIN G.E., POSTEL VINAY M.C., BAIXERAS E.**

Growth hormone prevents apoptosis through activation of nuclear factor-kappa b in interleukin-3-dependent ba/f3 cell line.

*Mol. Endocrinol.*, 14 (5), 650-661, 2000

(Services cités : U344)

The pro-B Ba/F3 cell line requires interleukin-3 and serum for growth, and their removal results in cell apoptosis. Ba/F3 cells transfected with the GH receptor (GHR) cDNA become able to proliferate in response to GH. To investigate the role of GH in the control of apoptosis, Ba/F3 cells expressing either the wild-type rat GHR (Ba/F3 GHR) or a mutated rat GHR (Ba/F3 ILV/T) were used. We show that Ba/F3 GHR cells, but not parental Ba/F3 or Ba/F3 ILV/T cells, were able to survive in the absence of growth factor. Furthermore, an autocrine/paracrine mode of GH action was suggested by the demonstration that Ba/F3 cells produce GH, and that addition of GH antagonists (B2036 and G120K) promotes apoptosis of Ba/F3 GHR cells. Consistent with survival, the levels of both antiapoptotic proteins Bcl-2 and Bag-1 were maintained in Ba/F3 GHR cells, but not in parental Ba/F3 cells upon growth factor deprivation. Constitutive activation of the transcription factor nuclear factor-kappa B (NF-kappa B), which has been shown to promote cell survival, was sustained in Ba/F3 GHR cells, whereas no NF-kappa B activation was detected in parental Ba/F3 cells in the absence of growth factor. Furthermore, addition of GH induced NF-kappa B DNA binding activity in Ba/F3 GHR cells. Overexpression of the mutated I kappa B alpha (A32/36) protein, known to inhibit NF-kappa B activity, resulted in death of growth factor-deprived Ba/F3 GHR cells, and addition of GH was no longer able to rescue these cells from apoptosis. Together, our results provide evidence for a new GH-mediated pathway that initiates a survival signal through activation of the transcription factor NF-kappa B and sustained levels of the antiapoptotic proteins Bcl-2 and Bag-1. [References: 55]

**LLOVERA M., TOURAINÉ P., KELLY P.A., GOFFIN V.**

Involvement of prolactin in breast cancer: redefining the molecular targets.

*Exp. Gerontol.*, 35 (1), 41-51, 2000

(Services cités : U344)

The mammary gland is the major target tissue of prolactin (PRL) in mammals. Although this pituitary hormone has been long suspected to be involved in the progression of human breast cancer, the failure or clinical improvement by treatment with dopamine agonists (which lower circulating levels of PRL) rapidly reduced the interest of oncologists concerning a potential role of PRL in the development of breast cancer. Within the last few years, however, several studies reported first, that PRL is also synthesized by mammary epithelial cells, and second that it may exert a proliferative action in an autocrine/paracrine manner. In agreement with a recent epidemiological study, these observations have led to a reconsideration of the role of PRL as an active participant in breast cancer, and are an impetus to redefine the molecular targets of anti-prolactin strategies since dopamine analogs are assumed to be inefficient on extrapituitary PRL

synthesis. In this review, we briefly summarize the current knowledge of PRL effects on both normal and tumor mammary cells, and we discuss the most relevant articles supporting the autocrine-paracrine action of PRL in the breast. With the aim of defining putative new molecular targets, we propose an overview of the main PRL receptor signaling cascades known to be triggered by PRL in mammary epithelial cells or, when not available, in other cell types. Finally, because proteolytic fragments of rat PRL have been shown to inhibit the angiogenic process, which may be relevant for preventing the progression of solid tumors such as breast tumors, we discuss the hypothesis that the enzymatic cleavage of human PRL could also represent a new molecular target in the search for alternative strategies in the treatment of breast cancer. (C) 2000 Elsevier Science Inc. All rights reserved. [References: 51]

**LLOVERA M., PICHARD C., BERNICHTTEIN S., JEAY S., TOURAIN P., KELLY P.A., GOFFIN V.**

Human prolactin (hprl) antagonists inhibit hprl-activated signaling pathways involved in breast cancer cell proliferation.

*Oncogene*, 19 (41), 4695-4705, 2000  
(Services cités : U344)

The involvement of human prolactin (hPRL) in breast cancer has been recently reconsidered based on its autocrine/paracrine proliferative effect described in human mammary tumor epithelial cells. Therefore, there is growing interest in the development of potent hPRL antagonists that may inhibit this effect. We previously designed hPRL analogs displaying antagonistic properties in a human transcriptional bioassay. We now report that the most potent of those analogs, G129R-hPRL, antagonizes all hPRL-induced effects analysed in various breast cancer cell lines, including cell proliferation. The analog per se lacks intrinsic agonistic activity on PRL receptor-activated signaling cascades, cell proliferation and apoptosis, indicating that its mode of action only occurs through competitive inhibition of hPRL. We provide some molecular basis of this antagonistic effect by demonstrating that G129R-hPRL competitively inhibits hPRL-activation of the JAK-STAT and MAPK pathways, two signaling cascades involved in the mitogenic effect of hPRL in mammary epithelial cells. This competitive inhibition persists for at least 48 h, as evidenced by long term analysis of STAT5b activation or of progression through cell cycle. These results are the first demonstration at the molecular level that hPRL antagonists interfering with receptor dimerization disrupt signaling events in breast cancer cells, which prevents hPRL-induced cell proliferation. [References: 54]

**REESE J., BINART N., BROWN N., MA W.G., PARIJA B.C., DAS S.K., KELLY P.A., DEY S.K.**

Implantation and decidualization defects in prolactin receptor (PRLR)-deficient mice are mediated by ovarian but not uterine PRLR.

*Endocrinology*, 141 (5), 1872-1881, 2000  
(Services cités : U344)

PRL and its homologs accomplish their biological effects through the PRL receptor (PRLR). We evaluated the expression and function of PRLR in the embryo and uterus during the periimplantation period because PRLR deficiency results in implantation failure. In wild-type mice, PRLR expression was localized to undecidualized stromal cells in the antimesometrial border on days 6-8 of pregnancy. A small population of PRLR-expressing cells was observed adjacent to the ectoplacental cone in the mesometrial stroma. Low levels of PRLR expression were also detected in the developing embryo on days 6-8. To determine the significance of PRLR

expression in this distribution, we examined implantation and decidualization in PRLR<sup>-/-</sup> mice. Progesterone (P4) administration rescued infertility in PRLR<sup>-/-</sup> mice from the periimplantation period to midgestation. Artificially induced decidualization was absent in pseudopregnant PRLR<sup>-/-</sup> mice but was identical to wild-type in P4-treated PRLR<sup>-/-</sup> mice. Furthermore, wild-type and P4-treated PRLR<sup>-/-</sup> mice had similar expression of the implantation-specific genes, LIF, amphiregulin, HB-EGF, COX-1, COX-2, PPARdelta, Hoxa-10, cyclin-D3, VEGF, and its receptors, Flk-1 and neuropilin-1. Together, these results show that luteal P4 production via ovarian PRLR signaling is required for implantation and early pregnancy. The function of uterine PRLR remains unclear. However, the eventual loss of pregnancy in P4-treated PRLR<sup>-/-</sup> mice suggests that uterine PRLR may be essential for the support of late gestation.

**SANDRA O., LE ROUZIC P., CAUTY C., EDERY M., PRUNET P.**

Expression of the prolactin receptor (tiprl-r) gene in tilapia oreochromis niloticus: tissue distribution and cellular localization in osmoregulatory organs.

*J. Mol. Endocrinol.*, 24 (2), 215-224, 2000

(Services cités : U344)

The expression of the prolactin receptor (PRL-R) gene has been investigated in various tissues of tilapia (*Oreochromis niloticus*) reared in fresh or brackish water. Using a cDNA probe spanning the extracellular domain of the tilapia PRL-R and Northern blot analysis, the presence of tilapia PRL-R mRNA has been confirmed in the osmoregulatory organs and has been detected in other tissues, including the skin, the brain, the reproductive organs, and the two major hematopoietic organs (spleen and head kidney), as well as circulating lymphocytes. These findings suggest a conservation of the physiological processes regulated by prolactin throughout the vertebrates, including immunity and central nervous activity. A non-radioactive in situ hybridization procedure has allowed us to detect the expression of the tilapia PRL-R in the branchial chloride cells and the intestinal mucosal layer of fresh water animals, confirming the direct control exerted by prolactin on the water and ionic exchanges in tilapia. In all the tissues examined one unique PRL-R transcript has been detected with a similar size (3.2 kb) whatever the salinity conditions. Thus, the transcriptional expression of the tilapia PRL-R strongly differs from the complex RNA pattern reported for the higher vertebrates PRL-R and provides an additional argument for the existence of a single PRL-R for both prolactin isoforms in this fish species. [References: 41]

**SIMS N.A., CLEMENT-LACROIX P., DA PONTE F., BOUALI Y., BINART N., MORIGGL R., GOFFIN V., COSCHIGANO K., GAILLARD KELLY M., KOPCHICK J., BARON R., KELLY P.A.**

Bone homeostasis in growth hormone receptor-null mice is restored by igf-i but independent of stat5.

*J. Clin. Invest.*, 106 (9), 1095-1103, 2000

(Services cités : U344)

Growth hormone (GH) regulates both bone growth and remodeling, but it is unclear whether these actions are mediated directly by the GH receptor (GHR) and/or IGF-I signaling. The actions of GH are transduced by the Jak/Stat signaling pathway via Stat5, which is thought to regulate IGF-I expression. To determine the respective roles of GHR and IGF-I in bone growth and remodeling, we examined bones of wild-type, GHR knockout (GHR<sup>-/-</sup>), Stat5ab<sup>-/-</sup>, and GHR<sup>-/-</sup> mice treated with IGF-I. Reduced bone growth in GHR<sup>-/-</sup> mice, due to a premature reduction in chondrocyte proliferation and cortical bone growth, was detected after 2 weeks of age. Additionally, although trabecular bone volume was unchanged, bone turnover was

significantly reduced in GHR(-/-) mice, indicating GH involvement in the high bone-turnover level during growth. IGF-I treatment almost completely rescued all effects of the GHR on both bone growth and remodeling, supporting a direct effect of IGF-I on both osteoblasts and chondrocytes. Whereas bone length was reduced in Stat5ab(-/-) mice, there was no reduction in trabecular bone remodeling or growth-plate width as observed in GHR-/- mice, indicating that the effects of GH in bone may not involve Stat5 activation. [References: 48]

**VON LAUE S., ROSS R.J.M.**

Inflammatory cytokines and acquired growth hormone resistance.

*Growth Horm. IGF Res.*, 10 (Suppl B), 9-14, 2000

(Services cités : U344)

Resistance to growth hormone (GH)-mediated induction of insulin-like growth factor I (IGF-I) is a common complication of catabolic diseases, including critical illness and post-surgical conditions. This resistance to GH is believed to be permissive to the development of protein catabolism, cachexia and wasting, which are associated with an increased mortality rate. Data from in vitro studies and animal models suggest that increased levels of inflammatory cytokines can induce cachexia and might inhibit the effects of GH on target tissues. The molecular mechanisms involved are unclear, although an effect of cytokines on GH receptor signalling has been suggested. The GH-activated pathways that mediate the increase in IGF-I levels are not well understood, thereby impeding the elucidation of the effect of inflammatory cytokines. Several signalling cascades, like the JAK-STAT and MAP kinase pathways, have been shown to be activated by GH and some inflammatory cytokines, hence raising the possibility of crosstalk on this level. Our data, however, indicate that inflammatory cytokines have little or no effect on GH-mediated JAK-STAT signalling. In this review, we discuss these results and the possibility that secondary changes in the structure of chromatin are likely to be involved in the induction of IGF-I gene transcription by GH. (C) 2000 Harcourt Publishers Ltd. [References: 66]

**VON LAUE S., FINIDORI J., MAAMRA M., SHEN X.Y., JUSTICE S., DOBSON P.R.M., ROSS R.J.M.**

Stimulation of endogenous gh and interleukin-6 receptors selectively activates different jaks and stat5, with a stat5 specific synergistic effect of dexamethasone.

*J. Endocrinol.*, 165 (2), 301-311, 2000

(Services cités : U344)

The interaction of GH, interleukin (IL)-6 and glucocorticoids is likely to be important in regulating the GH-insulin-like growth factor (IGF)-I axis. The signalling cascades activated by GH and IL-6 appear to be very similar, as demonstrated by studies using overexpression of the receptor and other components of the Jak-Stat and mitogen-activated protein (MAP) kinase pathways. Here we show that the human embryonic kidney cell line 293 (HEK293) expresses GH and IL-6 receptors endogenously. To determine which specific pathways might be activated by the two cytokines, at physiological levels of all components, we studied GH and IL-6 mediated signal transduction both under basal conditions and in the presence of overexpressed receptors and Stat proteins. Our results suggest a receptor specificity of Jak2 for GH receptors, and Jak1 for IL-6 receptors. Stat activation in response to GH and IL-6 was determined by reporter gene induction. Both GH and IL-6 were able to induce the reporter gene containing the Stat5 responsive element (LHRE) but the IL-6 response appeared to be mediated mainly through Stat3 activation. In contrast, the reporter gene containing the Stat3 responsive element (SIE) was IL-6 specific. The levels of gene induction by GH and IL-6 were not altered by the co-stimulation

with GH and IL-6, suggesting that there is little cross-talk at the Jak-Stat activation level between the two cytokines. Neither GH nor IL-6 activated the MAP-kinase responsive serum response element (SRE), unless GH receptors or gp130 were overexpressed. Transfection of Stat3 or Stat5 expression vectors enhanced the response to GH and IL-6. Stimulation with dexamethasone synergistically enhanced GH activation of the LHRE reporter gene but had no effect on the IL-6 activation of the same reporter or on the SIE reporter gene. Thus, our studies suggest that while each cytokine, GH and IL-6, may activate various members of the Jak-Stat pathway in overexpression studies, specific activation of Stat3 by IL-6 and of Jak2 and Stat5 by GH can be observed in HEK293 cells and that in this system the synergistic effect of dexamethasone appears specific for Stat5. [References: 43]

**1999**

**ALLAMANDO A., LUCAS B.K., EVRA C., BINART N., KELLY P.A.**

Prolactin receptor knockout model: Study of maternal behavior.

*Sci. Techn. Anim. Lab.*, 24 (3), 191-197, 1999

(Services cités : U344)

We have studied pup-directed maternal behavior in mice carrying a germ line null mutation of the prolactin receptor gene. Heterozygous nulliparous and primiparous females and homozygous nulliparous females exhibit a profound deficit in maternal care when challenged with foster pups. Morris maze studies revealed normal configural learning in heterozygous and homozygous animals. Olfactory function was tested in an aversive conditioning paradigm, confirming that heterozygous and homozygous prolactin receptor mutant mice are not anosmic. This studies clearly establish the prolactin receptor as a regulator of maternal behavior. [References: 24]

**AYLING R.M., ROSS R.J.M., TOWNER P., VON LAUE S., FINIDORI J., MOUTOUSSAMY S., BUCHANAN C.R., CLAYTON P.E., NORMAN M.R.**

New growth hormone receptor exon 9 mutation causes genetic short stature.

*Acta Paediatr.*, 88 (Suppl 428), 168-172, 1999

(Services cités : U344)

A novel form of congenital growth hormone insensitivity syndrome (GHIS), which lacks the classic phenotype associated with this condition, is described. Dominant inheritance is shown to result from a heterozygous 876-1 G to C transversion of the 3' splice acceptor site preceding exon 9 in the growth hormone receptor (GHR) gene. The result of this mutation is a severely truncated cytoplasmic domain of the GHR. which is incapable of transmitting a signal. The mutant receptor is shown to form a heterodimer with the wild-type GHR, the activity of which is inhibited in a dominant-negative manner. [References: 16]

**BOUCHARD B., ORMANDY C.J., DI SANTO J.P., KELLY P.A.**

Immune system development and function in prolactin receptor-deficient mice.

*J. Immunol.*, 163 (2), 576-582, 1999

(Services cités : U344)

Prolactin (PRL) is the primary lactogenic pituitary hormone that plays an essential role in many aspects of reproduction, from fertilization to mammary gland development and maternal behavior. PRL has also been reported to play a role in immunoregulation, Because initial observations indicated that hypophysectomized rats present abnormalities of the immune system, including increased thymic atrophy and lymphopenia, a number of studies have focused on the potential immunomodulatory roles of PRL, This hormone exerts its biological activities

following binding to specific cell surface PRL receptors (PRLRs), In this report, we have characterized the development and function of the immune system in PRLR-deficient mice. Compared with wild-type control mice, PRLR<sup>-/-</sup> mice demonstrate no alterations in thymic or splenic cellularity or in the composition of the lymphocyte subsets present in primary (bone marrow and thymus) or secondary (spleen and lymph nodes) lymphoid organs. Lymphocytes from PRLR<sup>-/-</sup> mice are functional in vitro, as they can proliferate normally to mitogens, cytokines, and allogeneic cells. PRLR<sup>-/-</sup> splenocytes display normal NK-mediated cytotoxicity to YAC-1 target cells. In vivo studies have revealed that PRLR<sup>-/-</sup> mice are able to 1) generate normal steady-state Ig levels, 2) mount a normal specific Ig response following immunization with a T-dependent Ag, 3) eliminate injected allogeneic tumor cells, and 3) effectively control *Listeria monocytogenes* infection. Taken together, these results show that immune system development and function proceed normally in the absence of PRL-mediated signaling and suggest that PRLR pathways are not essential for immunomodulation in vivo. [References: 43]

**BRESSON J.L., JEAY S., GAGNERAULT M.C., KAYSER C., BERESSI N., WU Z., KINET S., DARDENNE M., POSTEL-VINAY M.C.**

Growth hormone (GH) and prolactin receptors in human peripheral blood mononuclear cells: relation with age and GH-binding protein.

*Endocrinology*, 140 (7), 3203-3209, 1999

(Services cités : U344, UMR 8603, Département de Pédiatrie)

GH receptors (GHRs) and PRL receptors (PRLRs) were studied in human peripheral blood mononuclear cells (PBMC) using flow cytometry, biotinylated anti-GH receptor monoclonal antibody 10B8, and biotinylated human PRL. Variations of GHR and PRLR expression and the relationship of plasma GHBP and GH receptor in PBMC subsets were examined as a function of age and sex. By double immunofluorescence staining, we show that about 30% of total cells express GH receptors, with a low expression in T cells, whereas almost all B cells and monocytes are GH receptor positive. Four age groups were defined among the 64 normal volunteers, aged 12 to 85 yr, who were included in the study. The percentage of PBMC expressing GH receptors is significantly lower in group 2 (20-40 yr) than in group 1 (12-20 yr) and group 4 (>60 yr). In T cells, monocytes and B cells, no significant changes are detected in either the percentage of GH receptor positive cells or in the GH receptor level per cell. The level of PRLRs expressed in PBMC is significantly higher in age group 2 than in age group 4. A negative correlation is observed between plasma GHBP and the percentage of PBMC expressing GH receptors. These results suggest that regulation of GH receptors in lymphocytes and in other target cells could be different. [References: 33]

**BRISKEN C., KAUR S., CHAVARRIA T.E., BINART N., SUTHERLAND R.L., WEINBERG R.A., KELLY P.A., ORMANDY C.J.**

Prolactin controls mammary gland development via direct and indirect mechanisms.

*Dev. Biol.*, 210 (1), 96-106, 1999

(Services cités : U344)

The inactivation of the prolactin receptor gene by homologous recombination has made it possible to investigate the role of prolactin signaling in mammary gland development without resort to ablative surgery of the endocrine glands. In knockout mice lacking the prolactin receptor, mammary development is normal up to puberty. Subsequently, the ducts branch less frequently than those of wild-type animals. While terminal end buds differentiate to alveolar buds in wild-type females by the end of puberty, in knockout females terminal end bud-like structures

persist at the ductal ends. To distinguish between the developmental defects that are intrinsic to the epithelium and those that result from systemic endocrine alterations in prolactin receptor knockout mice, mammary epithelium from prolactin receptor knockouts was transplanted into mammary fat pads of wild-type mice. In virgin mice, the knockout epithelial transplants developed normally at puberty, indicating an indirect effect of prolactin on ductal development. Prolactin receptor knockout females are infertile due to multiple reproductive defects, but epithelial transplants allowed us to assess the extent to which the absence of prolactin receptor is limiting, under systemic conditions that allow full mammary gland development. During pregnancy, the prolactin receptor knockout transplants showed normal side branching and the formation of alveolar buds, but no lobuloalveolar development. Thus, prolactin affects mammary morphogenesis in two different ways: it controls ductal side branching and terminal end bud regression in virgin animals via indirect mechanisms, but acts directly on the mammary epithelium to produce lobuloalveolar development during pregnancy. (C) 1999 Academic Press. [References: 33]

**CLEMENT-LACROIX P., ORMANDY C., LEPESCHEUX L., AMMANN P., DAMOTTE D., GOFFIN V., BOUCHARD B., AMLING M., GAILLARD-KELLY M., BINART N., BARON R., KELLY P.A.**

Osteoblasts are a new target for prolactin: analysis of bone formation in prolactin receptor knockout mice.

*Endocrinology*, 140 (1), 96-105, 1999

(Services cités : U344)

Bone development is a multistep process that includes patterning of skeletal elements, commitment of hematopoietic and/or mesenchymal cells to chondrogenic and osteogenic lineages, and further differentiation into three specialized cell types: chondrocytes in cartilage and osteoblasts and osteoclasts in bone. Although PRL has a multitude of biological actions in addition to its role in the mammary gland, very little is known about its effect on bone. Mice carrying a germline null mutation for the PRL receptor gene have been produced in our laboratory and used to study the role of PRL in bone formation. In -/- embryos, we observed an alteration in bone development of calvaria. In adults, histomorphometric analysis showed that the absence of PRL receptors leads to a decrease in bone formation rate using double calcein labeling and a reduction of bone mineral density, measured by dual energy x-ray absorptiometry. In addition, serum estradiol, progesterone, testosterone, and PTH levels were analyzed. We also established that osteoblasts, but not osteoclasts, express PRL receptors. This suggests that an effect of PRL on osteoblasts could be required for normal bone formation and maintenance of bone mass. Thus, the PRL receptor knockout mouse model provides a new tool to investigate the involvement of PRL in bone metabolism.

**COSTOYA J.A., FINIDORI J., MOUTOUSSAMY S., SENARIS R., DEVESA J., ARCE V.M.**

Activation of growth hormone receptor delivers an antiapoptotic signal: Evidence for a role of Akt in this pathway.

*Endocrinology*, 140 (12), 5937-5943, 1999

(Services cités : U344)

A signaling pathway was delineated by which GH promotes cell survival. Experiments were performed in human leukemic cells (HL-60) and Chinese hamster ovary (CHO) cells. In HL-60 cells, GH treatment reduced starvation-induced cell death. In contrast, when HL-60 cells were

treated with an anti-GH antibody, cell survival was sharply reduced. In CHO cells stably expressing either the wild-type (wtGHR) or a truncated form (Delta 454GHR) of the GH receptor in which GH induces a sustained activation of the receptor-associated tyrosine kinase JAK2, we found that GH stimulation inhibited programmed cell death induced by withdrawal of survival factors. This effect was enhanced in cells expressing the truncated form. In contrast, GH did not affect cell survival in CHO cells transfected with either the empty vector or a mutated GHR unable to transduce the signal (4P/AGHR). We also showed that the inhibitory action of GH on apoptosis is probably mediated via stimulation of the serine-threonine kinase Akt, as 1) GH treatment induces a prompt phosphorylation of Akt; and 2) GH effects on cell survival are abolished by transfection of an Akt mutant that exhibits dominant negative function. Experiments with pharmacological inhibitors demonstrated that GH-induced Akt phosphorylation is dependent on phosphoinositide 3-kinase activation. In contrast, we found no changes in Bcl-2 levels secondary to GHR activation. [References: 48]

**EDERY M., KELLY P.A.**

Structure, function and expression of prolactin receptors in mammals.

*Mieux Comprendre*, 183-200, 1999

(Services cités : [U344](#))

**FAVRE H., BENHAMOU A., FINIDORI J., KELLY P.A., EDERY M.**

Dual effects of suppressor of cytokine signaling (SOCS-2) on growth hormone signal transduction.

*FEBS Lett.*, 453 (1-2), 63-66, 1999

(Services cités : [U344](#))

A family of suppressors of cytokine signaling (SOCS) has recently been identified of which two members have been shown to block growth hormone (GH) signaling. Dose-response experiments were conducted in 293 cells and SOCS-1 and SOCS-3 were shown to inhibit the transcriptional activation of a GH-responsive element and suppressed Jak2 tyrosine kinase activity. SOCS-2 had two opposite effects: at low concentrations it inhibited GH-induced STAT5-dependent gene transcription, but restoration of GH signaling was observed at higher concentrations. In cotransfection studies, SOCS-2 was able to block the inhibitory effect of SOCS-1 but not that of SOCS-3 on GH signaling. These findings suggest that a major function for SOCS-2 is to restore the sensitivity to GH by overcoming the initial inhibitory effects of other endogenous SOCS molecules. (C) 1999 Federation of European Biochemical Societies.

[References: 23]

**GOFFIN V., BERNICHTEIN S., CARRIERE O., BENNETT W.F., KOPCHICK J.J., KELLY P.A.**

The human growth hormone antagonist B2036 does not interact with the prolactin receptor.

*Endocrinology*, 140 (8), 3853-3856, 1999

(Services cités : [U344](#))

The human growth hormone (hGH) antagonist B2036 combines a single amino acid substitution impairing receptor binding site 2 (G120K) with eight additional amino acid substitutions that improve binding site 1 affinity. This hGH antagonist is being tested for treating pathologies linked to excess hGH levels. B2036-PEG is a polyethylene glycol (PEG) conjugated form of B2036 that has an increased half-life due to reduced renal clearance. It is currently in phase III trials for acromegaly. Human GH is also able to bind to the receptor of prolactin (PRLR). Since

activation of PRLR can promote an array of pathological states (reproduction disorders, breast cancer), the ability of B2036-PEG to interact with the PRLR had to be determined. In this study, we compared four hGH antagonists (G120K, G120K-PEG, B2036 and B2036-PEG) in three bioassays : proliferation of rat Nb2 cells, binding to the human PRLR and activation of human PRLR-mediated signaling in a cell line stably expressing this receptor and a luciferase reporter gene. Agonistic and antagonistic properties were characterized. Our data show that B2036-PEG does not bind, activate or antagonize PRLRs, either from rat or human origin. These observations further demonstrate that the eight amino acid substitutions within binding site 1 provide binding specificity directed towards the human GH receptor. [References: 32]

**GOFFIN V., BINART N., CLEMENT-LACROIX P., BOUCHARD B., BOLE-FEYSOT C., EDERY M., LUCAS B.K., TOURAINE P., PEZET A., MAASKANT R., PICHARD C., HELLOCO C., BARAN N., FAVRE H., BERNICHTEIN S., ALLAMANDO A., ORMANDY C., KELLY P.A.**

From the molecular biology of prolactin and its receptor to the lessons learned from knockout mice models.

*Genet. Anal.- Biomol. Eng.*, 15 (3-5), 189-201, 1999

(Services cités : U344)

Prolactin (PRL), a polypeptide hormone secreted mainly by the pituitary and, to a lesser extent, by peripheral tissues, affects more physiological processes than all other pituitary hormones combined since it is involved in > 300 separate functions in vertebrates. Its main actions are related to lactation and reproduction. The initial step of PRL action is the binding to a specific membrane receptor, the PRLR, which belongs to the class 1 cytokine receptor superfamily. PRL-binding sites have been identified in a number of tissues and cell types in adult animals. Signal transduction by this receptor is mediated, at least in part, by two families of signaling molecules: Janus tyrosine kinases and signal transducers and activators of transcription (STATs). Disruption of the PRLR gene has provided a new mouse model with which to identify actions directly associated with PRL or any other PRLR ligands, such as placental lactogens. To date, several different phenotypes have been analyzed and are briefly described in this review. Coupled with the SAGE technique, this PRLR knockout model is being used to qualitatively and quantitatively evaluate the expression pattern of hepatic genes in two physiological situations: transcriptomes corresponding to livers from both wild type and PRLR KO mice are being compared, and following statistical analyses, candidate genes presenting a differential profile will be further characterized. Such a new approach will undoubtedly open future avenues of research for PRL targets. To date, no pathology linked to any mutation in the genes encoding PRL or its receptor have been identified. The development of genetic models provides new opportunities to understand how PRL can participate to the development of pathologies throughout life, as for example the initiation and progression of breast cancer. [References: 98]

**GOFFIN V., TOURAINE P., PICHARD C., BERNICHTEIN S., KELLY P.A.**

Should prolactin be reconsidered as a therapeutic target in human breast cancer ?

*Mol. Cell. Endocrinol.*, 151 (1-2), 79-87, 1999

(Services cités : U344)

Although prolactin (PRL) has been long suspected to be involved in the progression of human breast cancer, the failure of clinical improvement by treatment with dopamine agonists, which lower circulating levels of PRL, rapidly reduced the interest of oncologists concerning a potential role of this pituitary hormone in the development of breast cancer. Within the last few years,

however, several studies reported first, that PRL is also synthesized in the mammary gland, and second that it exerts its proliferative action in an autocrine/paracrine manner. These observations have led to a reconsideration of the role of PRL as an active participant in breast cancer and are an impetus to search for alternative strategies aimed at inhibiting the proliferative effects of PRL on tumor mammary cells. In this report, we discuss the three possible levels that can be targeted for this purpose: the mammary synthesis of PRL, the interaction of the hormone with its receptor at the surface of mammary cells, and the intracellular signaling cascades triggered by the activated receptor. For each of these steps, we discuss the molecular event(s) that can be targeted, our understanding of the mechanisms involving these putative targets as well as the tools currently available for their inhibition. Besides its proliferative effect, PRL is also involved in the control of angiogenesis through one of its cleaved fragments, named PRL 16K, which has been shown to inhibit the angiogenic process. In view of this biological activity, we discuss first the cleavage of PRL with respect to the human mammary gland and, second, the hypothesis speculating that a balance between the proliferative effect of intact PRL and the anti-angiogenic activity of its 16K-like fragments might be physiologically relevant in the evolution of mammary tumors. If true, our hypothesis would suggest that the enzymatic cleavage of PRL could represent a new molecular target in the search for alternative strategies in the treatment of breast cancer. (C) 1999 Elsevier Science Ireland Ltd. All rights reserved. [References: 71]

**KINET S., BERNICHTEIN S., KELLY P.A., MARTIAL J.A., GOFFIN V.**

Biological properties of human prolactin analogs depend not only on global hormone affinity, but also on the relative affinities of both receptor binding sites.

*J. Biol. Chem.*, 274 (37), 26033-26043, 1999

(Services cités : U344)

Zinc increases the affinity of human growth hormone (hGH) for the human prolactin receptor (hPRLR) due to the coordination of one zinc ion involving GIU-174(hGH) and His-18(hGH) In contrast, binding of h:PRL to the hPRLR is zinc-independent. We engineered in binding site 1 of hPRL a hGH-like zinc coordination site, by mutating Asp-183(hPRL) (homologous to Glu-174(hGH)) into Glu (D183E mutation). This mutation was also introduced into G129R hPRL, a binding site 2 mutant (Goffin, V., Kinet, S., Ferrag, F., Binart, N., Martial, J. A., and Kelly, P, A. (1996) *J. Biol. Chem.* 271, 16573-16579), These analogs were characterized using a stable clone expressing both the hPRLR and a PRLR-responsive reporter gene. The D183E mutation per se decreases the binding affinity and transcriptional activity of hPRL. However, this loss is partially rescued by the addition of zinc and the effect is much more marked on bioactivity than on binding affinity. These data indicate that the D183E mutation confers zinc sensitivity to hPRL biological properties. Due to an impaired site 2, the agonistic activity of G129R analog is almost nil. Although the double mutant D183E/G129R displays lower affinity (similar to 1 log) compared with G129R hPRL, it unexpectedly recovers partial agonistic activity in the absence of zinc. Moreover, whereas zinc increases the affinity of D183E/G129R, it paradoxically abolishes its agonistic activity. Our results demonstrate that the biological properties of hPRL analogs do not necessarily parallel their overall affinity. Rather, the relative affinities of the individual binding sites 1 and 2 may play an even more important role. [References: 50]

**MAAMRA M., FINIDORI J., VON LAUE S., SIMON S., JUSTICE S., WEBSTER J., DOWER S., ROSS R.**

Studies with a growth hormone antagonist and dual-fluorescent confocal microscopy demonstrate that the full-length human growth hormone receptor, but not the truncated isoform, is very

rapidly internalized independent of Jak2-Stat5 signaling.

*J. Biol. Chem.*, 274 (21), 14791-14798, 1999

(Services cités : U344)

We have investigated trafficking of two negative regulators of growth hormone receptor (GHR) signaling: a human, truncated receptor, GHR1-279, and a GH antagonist, B2036, Fluorescent-labeled growth hormone (GH) was rapidly internalized by the full-length GHR, with >80% of the hormone internalized within 5 min of exposure to GH, In contrast, <5% of labeled GH was internalized by cells expressing truncated GHR1-279, Using another truncated receptor, GHR1-317 fused to enhanced green fluorescent protein (EGFP), we have exploited fluorescence energy transfer to monitor the trafficking of ligand-receptor complexes. The data confirmed that internalization of this truncated receptor is very inefficient, It was possible to visualize the truncated GHR1-317-EGFP packaged in the endoplasmic reticulum, its rapid movement in membrane bound vesicles to the Golgi apparatus, and subsequent transport to the cell membrane, The GH antagonist, B2036, blocked Jak2-Stat5-mediated GHR signaling but was internalized with a similar time course to native GH, The results: 1) demonstrate the rapid internalization of GH when studied under physiological conditions; 2) confirm the hypothesis that internalization of cytoplasmic domain truncated human GHRs is very inefficient, which explains their dominant negative action; and 3) show that the antagonist action of B2036 is independent of receptor internalization. [References: 26]

**MULLIS P.E., EBLE A., MARTI U., BURGI U., POSTEL-VINAY M.C.**

Regulation of human growth hormone receptor gene transcription by triiodothyronine (T-3).

*Mol. Cell. Endocrinol.*, 147 (1-2), 17-25, 1999

(Services cités : U344)

In this study the hypothesis that triiodothyronine (T-3) and growth hormone (GH) may have some direct or indirect effect on the regulation of GH-receptor/GH-binding protein (GHR/GHBP) gene transcription was tested. Different concentrations of T-3 (0, 0.5, 2, 10 nmol/l) and GH (0, 10, 150 ng/ml) were added to human hepatoma (HuH7) cells cultured in serum-free hormonally-defined medium for 0; 1 and 2 h. Thereafter GHR/GHBP mRNA expression was quantitatively assessed by using PCR amplification. GH at a concentration of 10 ng/ml resulted in a significant increase of GHR/GHBP gene expression whereas a supraphysiological concentration of GH (150 ng/ml) caused a significant decrease of GHR/GHBP mRNA levels. The simultaneous addition of 0.5 nmol/l T-3 to the variable concentrations of GH did not modify GHR/GHBP mRNA levels whereas the addition of 2 nmol/l up-regulated GHR/GHBP gene expression already after 1 h, an increase which was even more marked when 10 nmol/l of T-3 was added. Interestingly, there was a positive correlation between the increase of GHR/GHBP mRNA levels and the T-3 concentration used (r: 0.8). In addition, nuclear run-on experiments and GHBP determinations were performed which confirmed the changes in GHR/GHBP mRNA levels. Cycloheximide (10  $\mu$ g/ml) did not alter transcription rate following GH addition but blocked GHR/GHBP gene transcription in T-3 treated cells indicating that up-regulation of GHR/GHBP gene transcription caused by T-3 requires new protein synthesis and is, therefore, dependent on indirect mechanisms. In conclusion, we present data showing that T-3 on its own has a stimulatory effect on GHR/GHBP gene transcription which is indirect and additive to the GH-induced changes. (C) 1999 Elsevier Science Ireland Ltd. All rights reserved. [References: 29]

**PEZET A., FAVRE H., KELLY P.A., EDERY M.**

Inhibition and restoration of prolactin signal transduction by suppressors of cytokine signaling.

*J. Biol. Chem.*, 274 (35), 24497-24502, 1999

(Services cités : U344)

Prolactin (PRL) has been shown to activate the cytoplasmic tyrosine kinase Janus kinase 2 (Jak2) and the subsequent recruitment of various signaling molecules including members of the signal transducer and activator of transcription family of transcription factors. Recently, an expanding family of cytokine-inducible inhibitors of signaling has been identified that initially included four members: suppressor of cytokine signaling (SOCS)-1, SOCS-2, SOCS-3, and cytokine-inducible src homology domain 2 (SH-2) proteins. The present study analyzes the role of these members in PRL signaling. Constitutive expression of SOCS-1 and SOCS-3 suppressed PRL-induced signal transducer and activator of transcription B-dependent gene transcription, and Jak2 tyrosine kinase activity was greatly reduced in the presence of SOCS-1 or SOCS-3. SOCS-1 was shown to associate with Jak2, whereas SOCS-2 was associated with the prolactin receptor. Co-transfection studies were conducted to further analyze the interactions of SOCS proteins. SOCS-2 was shown to suppress the inhibitory effect of SOCS-1 by restoring Jak2 kinase activity but did not affect the inhibitory effect of SOCS-3 on PRL signaling. Northern blot analysis revealed that SOCS-3 and SOCS-1 genes were transiently expressed in response to PRL, both in vivo and in vitro, whereas the expression of SOCS-2 and CIS genes was still elevated 24 h after hormonal stimulation. We thus propose that the early expressed SOCS genes (SOCS-1 and SOCS-3) switch off PRL signaling and that the later expressed SOCS-2 gene can restore the sensitivity of cells to PRL, partly by suppressing the SOCS-1 inhibitory effect. [References: 26]

**QUINTON N.D., SMITH R.F., CLAYTON P.E., GILL M.S., SHALET S., JUSTICE S.K., SIMON S.A., WALTERS S., POSTEL-VINAY M.C., BLAKEMORE A.I.F., ROSS R.J.M.**

Leptin binding activity changes with age: The link between leptin and puberty.

*J. Clin. Endocrinol. Metabol.*, 84 (7), 2336-2341, 1999

(Services cités : U344)

The timing of the physical transition from child to adult is determined by a biological clock that switches off the pituitary gonadal axis during infancy until puberty. Body composition (and in particular, fat mass), through leptin, are critical signals to this clock. However, no direct relationship between leptin and puberty has been demonstrated. Leptin is bound in the circulation by a high-affinity binding protein, which has been identified as a soluble leptin receptor. We found circulating levels of leptin binding activity (LBA) to be low at birth, to be high in the prepubertal years, to fall through puberty, and then to remain stable during adult life. LBA correlated with pubertal status in both boys and girls. We postulate that the fall in LBA, associated with increasing age and puberty, reflects a reduction in expression of truncated leptin receptors, and leptin is then available to the full-length receptor, which transmits the biological signal for leptin. The high levels of LBA occur during the years when the pituitary gonadal axis is quiescent. Thus, the change in LBA could explain how leptin regulates puberty. [References: 26]

**SAVAGE M.O., WOODS K.A., JOHNSTON L.B., POSTEL-VINAY M.C., AMSELEM S., CLARK A.J.L.**

Defects of the growth hormone receptor and their clinical implications.

*Growth Horm. IGF Res.*, 9 (Suppl A), 57-61, 1999

(Services cités : U344)

**VLEURICK L., KUHN E.R., DECUYPERE E., BURNSIDE J., PEZET A., EDERY M.**

Generation of chicken growth hormone-binding proteins by proteolysis.

*Gen. Comp. Endocrinol.*, 113 (2), 283-289, 1999

(Services cités : U344)

A soluble protein that specifically bound growth hormone (GH) was characterized in culture medium of a COS-7 cell line transfected with the cDNA of the full-length chicken GH receptor (cGHR). Incubation of culture medium with I-125-labeled human GH resulted in the formation of a single specific complex with high affinity ( $K_D = 0.36$  nM) and apparent molecular weight of 75 kDa. The production of large quantities of GH-binding protein (GHBP) amounting to, per hour, 23% of the cell's GHR, points to the importance of partial proteolysis for GHR turnover. Considerable amounts of GHBP were also detected in a cytosolic fraction. These results strongly suggest that in chicken, as in rabbit and monkey, the GHBP is generated, at least partially, by proteolytic cleavage of the membrane-anchored GHR. (C) 1999 Academic Press. [References: 32]

**VLEURICK L., PEZET A., KUHN E.R., DECUYPERE E., EDERY M.**

A beta-turn endocytic code is required for optimal internalization of the growth hormone receptor but not for alpha-adaptin association.

*Mol. Endocrinol.*, 13 (11), 1823-1831, 1999

(Services cités : U344)

Intracellular trafficking of GH and its receptor, more particularly the chicken GH receptor (cGHR), was examined in COS-7 cells using biochemical and structural studies. Internalization of radioactive GH by the cGHR is reduced as compared with the rat GHR. On the contrary, activation of gene transcription through Janus kinase-2 was similar for both species. Secondary structures of the cytoplasmic domain of chicken and rat GHR were compared, since beta-turns were reported as internalization signals. The substitution of Pro335-Asp335, present in mammalian GH receptors, with Thr307-Gln308 in the cGHR leads to the loss of a beta-turn within a conserved cytoplasmic region. Mutational analysis indicated that the lower rate of internalization of cGHR, as compared with mammalian GHR, was due to this motif. Our data further show that alpha-adaptin, a subunit of adaptor protein AP-2, associates with the GHR upon hormone stimulation. The clathrin-coated pit pathway therefore seems to be involved in the endocytosis of cGHR, as AP-2 is known to intervene in the recruitment of receptors to these pits. Interaction with alpha-adaptin may occur through a common epitope of the chicken and mammalian GHR, since receptors from both species bind similar amounts of alpha-adaptin; alternatively, two different epitopes with similar affinity may be involved. Therefore, not alpha-adaptin but an uncharacterized factor, presumably interacting with the identified beta-turn endocytic code, is responsible for the difference in internalization kinetics. Finally, the present study illustrates that functional amino acid motifs of receptors can be derived from comparative studies.

**WOJCIK J., POSTEL-VINAY M.C.**

Signal transduction of the growth hormone (GH) receptor, and GH-binding protein.

*Growth Horm. IGF Res.*, 9 (Suppl.A), 51-55, 1999

(Services cités : U344)

**WOJCIK J., MORNON J.P., CHOMILIER J.**

New efficient statistical sequence-dependent structure prediction of short to medium-sized protein loops based on an exhaustive loop classification.

*J. Mol. Biol.*, 289 (5), 1469-1490, 1999

(Services cités : U344)

A bank of 13,563 loops from three to eight amino acid residues long, representing motifs between two consecutive regular secondary structures, has been derived from protein structures presenting less than 95 % sequence identity. Statistical analyses of occurrences of conformations and residues revealed length-dependent over-representations of particular amino acids (glycine, proline, asparagine, serine, and aspartate) and conformations (alpha(L), epsilon, beta(P) regions of the Ramachandran plot). A position-dependent distribution of these occurrences was observed for N and C-terminal residues, which are correlated to the nature of the flanking regions. Loops of the same length were clustered into statistically meaningful families on the basis of their backbone structures when placed in a common reference frame, independent of the flanks. These clusters present significantly different distributions of sequence, conformations, and endpoint residue C-alpha distances. On the basis of the sequence-structure correlation of this clustering, an automatic loop modeling algorithm was developed. Based on the knowledge of its sequence and of its flank backbone structures each query loop is assigned to a family and target loop supports are selected in this family. The support backbones of these target loops are then adjusted on flanking structures by partial exploration of the conformational space. Loop closure is performed by energy minimization for each support and the final model is chosen among connected supports based upon energy criteria. The quality of the prediction is evaluated by the root-mean-square deviation (rmsd) between the final model and the native loops when the whole bank is re-attributed on itself with a Jackknife test. This average rmsd ranges from 1.1 Angstrom for three-residue loops to 3.8 Angstrom for eight-residue loops. A few poorly predicted loops are inescapable, considering the high level of diversity in loops and the lack of environment data. To overcome such modeling problems, a statistical reliability score was assigned for each prediction. This score is correlated to the quality of the prediction, in terms of rmsd, and thus improves the selection accuracy of the model. The algorithm efficiency was compared to CASP3 target loop predictions. Moreover, when tested on a test loop bank, this algorithm was shown to be robust when the loops are not precisely delimited, therefore proving to be a useful tool in practice for protein modeling. (C) 1999 Academic Press. [References: 71]