

Publications de l'U 530 (Inserm) (1999-2003)

2003

VILLEVALOIS-CAM L., RESCAN C., GILOT D., EZAN F., LOYER P., DESBUQUOIS B., GUGUEN-GUILLOUZO C., BAFFET G.

The hepatocyte is a direct target for transforming-growth factor beta activation via the insulin-like growth factor II/mannose 6-phosphate receptor.

J. Hepatol., 38 (2), 156-163, 2003

(Services cités : U530)

BACKGROUND/AIMS: The cation-independent mannose 6-phosphate receptor (CIMPR) is overexpressed in hepatocytes during liver regeneration and has been implicated in the maturation of latent pro-transforming growth factor beta (TGFbeta). In this study, we have: (1) kinetically characterized the changes in CIMPR expression in regenerating liver and cultured proliferating hepatocytes; and (2) assessed the contribution of hepatocyte via the CIMPR to latent pro-TGFbeta activation.**METHODS:** The expression of CIMPR protein and mRNA in livers collected after partial hepatectomy and hepatocyte primary cultures was analyzed by Western and Northern blotting. Activity of latent pro-TGFbeta was assessed by inhibition of [3H] methylthymidine incorporation into DNA.**RESULTS:** The expression of the CIMPR protein and/or mRNA progressively increased after 8 h in regenerating liver and 42-46 h in cultured hepatocytes, prior to the onset of DNA replication. Both mature TGFbeta and latent pro-TGFbeta inhibited epidermal growth factor-stimulated DNA synthesis in hepatocytes in a dose-dependent manner. The effect of latent pro-TGFbeta was reversed by two ligands of the CIMPR: beta-galactosidase, a mannose 6-phosphate containing protein, and a CIMPR antibody.**CONCLUSIONS:** (1) The induction of the CIMPR gene during liver regeneration and hepatocyte culture occurs in mid G1 phase; and (2) the CIMPR mediates latent proTGFbeta activation and thus may act, by targeting TGFbeta to hepatocytes, as a negative regulator of hepatocyte growth.

2002

BENELLI C., FOUQUE F., REDONNET-VERNHET I., MALGAT M., FONTAN D., MARSAC C., DEY R.

A novel Y243S mutation in the pyruvate dehydrogenase E1 alpha gene subunit: correlation with thiamine pyrophosphate interaction.

J. Inherit. Metab. Dis., 25 (4), 325-327, 2002

(Services cités : U530, CERTO)

We identified a new Y243S mutation in the X-linked E1 alpha-PDH gene in a patient with pyruvate dehydrogenase complex (PDHc) deficiency. The activity in cultured fibroblasts was very low even in the presence of high thiamine pyrophosphate (TPP) concentrations, indicating that the defect could be due to decreased affinity of PDHc for TPP.

2001

AUTHIER F., DANIELSEN G.M., KOUACH M., BRIAND G., CHAUVET G.

Identification of insulin domains important for binding to and degradation by endosomal acidic insulinase.

Endocrinology, 142 (1), 276-289, 2001

(Services cités : U530)

The endosomal compartment of hepatic parenchymal cells contains an acidic endopeptidase, endosomal acidic insulinase (EAI), which hydrolyzes internalized insulin at a limited number of sites. Although the positions of these cleavages are partially known, the residues of insulin important in its binding to and proteolysis by EAI have not been defined. To this end, we have studied the degradation over time of native human insulin and three insulin-analog peptides using a soluble endosomal extract from rat liver parenchyma followed by purification of the products by HPLC and determination of their structure by mass spectrometry. We found variable rates of ligand processing, i.e. high([Asp(B10)]- and [Glu(A13),Glu(B10)] insulin), moderate (insulin) and low (the H2-analog). On the basis of IC₅₀ values, competition studies revealed that human and mutant insulins display nearly equivalent affinity for the EAI. Proteolysis of human and mutant insulins by EAI resulted in eight cleavages in the B-chain which occurred in the central region (GIU(B13)-Leu(B17)) and at the C-terminus (Arg(B22)-Thr(B27)), the latter region comprising the initial cleavages at phe(B24)-phe(B25) (major pathway) and phe(B25)-Tyr(B26) (minor pathway) bonds. Except for the [Glu(A13),Glu(B10)] insulin mutant, only one cleavage on the A-chain was observed at residues Gln(A15)-Leu(A16). Analysis of the nine cleavage sites showed a preference for hydrophobic and aromatic amino acid residues on both the carboxyl and amino sides of a cleaved peptide bond. Using the B-chain alone as a substrate resulted in a 30-fold increase in affinity for EAI and a 6-fold increase in the rate of hydrolysis compared with native insulin. A similar role for the C-terminal region of the B-chain of insulin in the high-affinity recognition of EAI was supported by the use of the corresponding B-22-B-30 peptide, which displayed an increase in EAI affinity similar to the entire B-chain us. wild-type insulin. Thus, we have identified a highly specific molecular interaction of insulin with EAI at the aromatic locus phe(B24)-phe(B25)-Tyr(B26). Analytical subfractionation of a postmitochondrial supernatant fraction showed that a pulse of internalized [I-125]Tyr(A14)-H2-analog, a protease-resistant insulin analog, undergoes a greater lysosomal transfer and lesser degradation than [I-125]Tyr(A14)-insulin, confirming that endosomal sorting is regulated directly or indirectly by endosomal proteolysis. [References: 44]

CLOT J.P., CROSNIER H., GUEST G., SAUCET C., SOUBERBIELLE J.C., ANDRE J.L., BROYER M., RAPPAPORT R., BENELLI C.

Effects of growth hormone on growth factors after renal transplantation.

Pediat. Nephrol., 16 (5), 397-403, 2001

(Services cités : Endocrinologie et Croissance, Néphrologie Pédiatrique, U530)

Growth retardation occurs frequently in renal transplanted children (RTx) and can be improved by growth hormone (GH) treatment. This study retrospectively examines the insulin-like growth factor-1 (IGF-1) and IGF binding protein (IGFBP) profile of ten growth-retarded children previously given renal allografts, after 1 year of GH treatment period. Ten prepubertal patients (nine boys and one girl) were investigated. They had a mean chronological age (CA) of 11.4 +/- 1.1 years and a mean bone age (BA) of 7.3 +/- 0.9 years. Mean height was -3.9 +/- 0.4 SD units below the mean for CA. The mean body mass index (BMI) was 16.9 +/- 0.6 and the mean insulin clearance was 36.5 +/- 4.9 ml/min/1.73 m². Recombinant hGH was given at 4 IU/m²/day. Plasma GH, total and free IGF-1, IGFBP-2 and -3 were measured by specific radioimmunoassay (RIA). IGFBPs were characterized by SDS PAGE techniques and ligand and immunoblot analyses. Mean velocity was markedly increased (P < 0.01) after 1 year of GH therapy, expressed as SD score for BA. The range of growth response was wide. The total and free plasma IGF-1 increased (P < 0.01) by about 100% (mean values after GH therapy: 95.9 <plus/minus> 2.1 nM and

165 +/- 29 pM, respectively). Plasma IGFBP-3 concentrations increased by about 40% (mean value: 148 +/- 18 pM, P <0.01), with a concomitant increase in both intact IGFBP-3 and its 30-kDa proteolytic fragment. There was no change in plasma IGFBP-2 concentration. Both mean values of inulin clearance and BMI were unchanged during the treatment. In view of the IGF-1/IGFBP concentration changes, there should have been an even better growth response to GH therapy in these patients. This strongly suggests IGF-1 insensitivity, probably as a result of corticosteroid therapy. [References: 30]

CORVOL M.T.

La therapie cellulaire dans ses applications cliniques therapie cellulaire du cartilage, present et futur.

J. Soc. Biol., 195 (1), 79-82, 2001

(Services cités : U530)

Articular cartilage has a very poor capacity for repair. In order to get a normal functional efficacy, the replaced tissue has to reproduce the structure, composition and physico-chemical properties of native cartilage tissue. The transplantation of cultured autologous chondrocytes into chondral defects is currently applicable only in the case of young sportive people with a limited lesion in an otherwise relatively normal joint. Recent experimental studies have shown that pluripotent mesenchymal cells from bone marrow could also repair experimental osteochondral defects. An advantage of this grafting procedure is that large areas of cartilage surface could be covered. Bone marrow cells are not so difficult to get, they have a high potency to divide and they can develop in vitro as chondrogenic, osteogenic or adipogenic cells. The present ways of research are: to characterize one or several growth factors capable to specifically induce the chondrogenic lineage; to determine nutrient and environmental conditions allowing the cultured chondrogenic cells to undergo a maturation process within the cell pellet; to elaborate three-dimensional synthetic, biodegradable polymeric scaffolds assessed with respect to chondrogenic cell adhesion, proliferation, maturation and cartilage matrix secretion; finally, to elaborate a mixed biomaterial composed of chondrogenic and osteogenic cells selectively distributed within polymeric scaffolds in order to get a better adherence of the implanted cells to the lesion sites.

DE LONLAY P., BENELLI C., FOUQUE F., GANGULY A., ARAL B., DIONISI-VICI C., TOUATI G., HEINRICHS C., RABIER D., KAMOUN P., ROBERT J.J., STANLEY C., SAUDUBRAY J.M.

Hyperinsulinism and hyperammonemia syndrome: report of twelve unrelated patients.

Pediat. Res., 50 (3), 353-357, 2001

(Services cités : Biochimie Médicale, Fédération de Pédiatrie, U530, Génétique Médicale Pédiatrique)

Hyperinsulinism and hyperammonemia syndrome has been reported as a cause of moderately severe hyperinsulinism with diffuse involvement of the pancreas. The disorder is caused by gain of function mutations in the *GLUD1* gene. resulting in a decreased inhibitory effect of guanosine triphosphate on the glutamate dehydrogenase (GDH) enzyme. Twelve unrelated patients (six males, six females) with hyperinsulinism and hyperammonemia syndrome have been investigated. The phenotypes were clinically heterogeneous, with neonatal and infancy-onset hypoglycemia and variable responsiveness to medical (diazoxide) and dietary (leucine-restricted diet) treatment. Hyperammonemia (90-200 μ mol/L, normal < 50 μ mol/L) was constant and not influenced by oral protein, by protein- and leucine-restricted diet, or by sodium benzoate or N-carbamylglutamate administration. The patients had mean basal GDH activity (18.3 +/- 0.9

nmol/min/mg protein) not different from controls (17.9 +/- 1.8 nmol/min/mg protein) in cultured lymphoblasts. The sensitivity of GDH activity to inhibition by guanosine triphosphate was reduced in all patient lymphoblast cultures (IC50, or concentrations required for 50% inhibition of GDH activity, ranging from 140 to 580 nM, compared with control IC50 value of 83 +/- 1.0 nmol/L). The allosteric effect of ADP was within the normal range. The activating effect of leucine on GDH activity varied among the patients, with a significant decrease of sensitivity that was correlated with the negative clinical response to a leucine-restricted diet in plasma glucose levels in four patients. Molecular studies were performed in 11 patients. Heterozygous mutations were localized in the antenna region (four patients in exon 11, two patients in exon 12) as well as in the guanosine triphosphate binding site (two patients in exon 6, two patients in exon 7) of the GLUD1 gene. No mutation has been found in one patient after sequencing the exons 5-13 of the gene. [References: 22]

DOFFINGER R., SMAHI A., BESSIA C., GEISSMANN F., FEINBERG J., DURANDY A., BODEMER C., KENWRICK S., DUPUIS-GIROD S., BLANCHE S., WOOD P., RABIA S.H., HEADON D.J., OVERBEEK P.A., LE DEIST F., HOLLAND S.M., BELANI K., KUMARARATNE D.S., FISCHER A., SHAPIRO R., CONLEY M.E., REIMUND E., KALHOFF H., ABINUN M., MUNNICH A., ISRAEL A., COURTOIS G., CASANOVA J.L.

X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired nf-kappa b signaling.

Nat. Genet., 27 (3), 277-285, 2001

(Services cités : U393, U429, U530)

The molecular basis of X-linked recessive anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) has remained elusive. Here we report hypomorphic mutations in the gene *IKBKG* in 12 males with EDA-ID from 8 kindreds. and 2 patients with a related and hitherto unrecognized syndrome of EDA-ID with osteopetrosis and lymphoedema (OL-EDA-ID). Mutations in the coding region of *IKBKG* are associated with EDA-ID. and stop codon mutations, with OL-EDA-ID. *IKBKG* encodes NEMO, the regulatory subunit of the IKK (I kappaB kinase) complex, which is essential for NF-kappaB signaling. Germline loss-of-function mutations in *IKBKG* are lethal in male fetuses. We show that *IKBKG* mutations causing OL-EDA-ID and EDA-ID impair but do not abolish NF-kappaB signaling. We also show that the ectodysplasin receptor, DL, triggers NF-kappaB through the NEMO protein, indicating that EDA results from impaired NF-kappaB signaling. Finally, we show that abnormal immunity in OL-EDA-ID patients results from impaired cell responses to lipopolysaccharide, interleukin (IL)-1 beta, IL-18, TNF alpha and CD154. We thus report for the first time that impaired but not abolished NF-kappaB signaling in humans results in two related syndromes that associate specific developmental and immunological defects. [References: 50]

FRAGNER P., LEE S.L., ARATAN de LEON S.

Differential regulation of the *trh* gene promoter by triiodothyronine and dexamethasone in pancreatic islets.

J. Endocrinol., 170 (1), 91-98, 2001

(Services cités : U530)

TRH was initially found in the hypothalamus and regulates TSH secretion. TRH is also produced by insulin-containing beta -cells. Endogenous TRH positively regulates glucagon secretion and attenuates pancreatic exocrine secretion. We have previously shown that triiodothyronine (T-3)

down-regulates pre-pro-TRH gene expression in vivo and in vitro. The present study was designed to determine the initial impact of T-3 on rat TRH gene promoter and to compare this effect with that of dexamethasone (Dex). Primary islet cells and neoplastic cells (HIT T-15 and RIN m5F) were transiently transfected with fragments of the 5' -flanking sequence of TRH fused to the luciferase reporter gene. The persistence of high TRH concentrations in fetal islets in culture, probably due to transactivating factors, allowed us to explore how T-3 and Dex regulate the TRH promoter activity in transfected cells and whether the hormone effect is dependent on the cell type considered. TRH gene promoter activity is inhibited by T-3 in primary but not neoplastic cells and stimulated by Dex in both primary and neoplastic cells of islets. These findings validate previous in vivo and in vitro studies and indicate the transcriptional impact of these hormones on TRH gene expression in the pancreatic islets. [References: 40]

GEOFFROY V., FOUQUE F., LUGNIER C., DESBUQUOIS B., BENELLI C.

Characterization of an in vivo hormonally regulated phosphodiesterase 3 (pde3) associated with a liver golgi-endosomal fraction.

Arch. Biochem. Biophys., 387 (1), 154-162, 2001

(Services cités : U530)

The biochemical properties of an in vivo hormonally regulated low K-m cAMP phosphodiesterase (PDE) activity associated with a liver Golgi-endosomal (GE) fraction have been characterized. DEAE-Sephacel chromatography of a GE fraction solubilized by a lysosomal extract resulted in the sequential elution of three peaks of activity (numbered I, II, and III), while ion-exchange HPLC resolved five peaks of activity (numbered 1, 2, 3, 4, and 5). Based on the sensitivity of the eluted activity to cGMP and selected phosphodiesterase inhibitors, two phosphodiesterase isoforms were resolved: a cGMP-stimulated and EHNA-inhibited PDES, eluted in DEAE-Sephacel peak I and HPLC peak 2 and a cGMP-, a cilostamide-, and ICI 118233-inhibited PDE3, eluted in DEAE-Sephacel peak III and HPLC peaks 3, 4, and 5. GE fractions isolated after acute treatments with insulin, tetraiodoglucagon, and growth hormone displayed an increase in phosphodiesterase activity relative to saline-injected controls, as did GE fractions from genetically obese and hyperinsulinemic rats relative to lean littermates. In all experimental rats, an increase in PDE3 activity associated with DEAE-Sephacel peak III and HPLC peaks 4 and 5 was observed relative to control animals. Furthermore, in genetically obese Zucker rats, an increase in the sensitivity of PDE activity to cilostamide and in the amount of PDE activity immunoprecipitated by an antibody to adipose tissue PDE3 was observed relative to lean littermates. These results extend earlier studies on isolated hepatocytes and show that liver PDE3 is the main if not sole PDE isoform activated by insulin, glucagon, and growth hormone in vivo. (C) 2001 Academic Press. [References: 43]

PEQUIGNOT M.O., DESGUERRE I., DEY R., TARTARI M., ZEVIANI M., AGOSTINO A., BENELLI C., FOUQUE F., PRIP-BUUS C., MARCHANT D., ABITBOL M., MARSAC C.

New splicing-site mutations in the surf1 gene in leigh syndrome patients.

J. Biol. Chem., 276 (18), 15326-15329, 2001

(Services cités : CERTO, U030)

The gene SURF1 encodes a factor involved in the biogenesis of cytochrome c oxidase, the last complex in the respiratory chain. Mutations of the SURF1 gene result in Leigh syndrome and severe cytochrome c oxidase deficiency. Analysis of seven unrelated patients with cytochrome c oxidase deficiency and typical Leigh syndrome revealed different SURF1 mutations in four of

them. Only these four cases had associated demyelinating neuropathy. Three mutations were novel splicing-site mutations that lead to the excision of exon 6. Two different novel heterozygous mutations were found at the same guanine residue at the donor splice site of intron 6; one was a deletion, whereas the other was a transition [588+1.G>A]. The third novel splicing-site mutation was a homozygous [516-2_516-1delAG] in intron 5. One patient only had a homozygous polymorphism in the middle of the intron 8 [835+25C>T]. Western blot analysis showed that Surf1 protein was absent in all four patients harboring mutations. Our studies confirm that the SURF1 gene is an important nuclear gene involved in the cytochrome c oxidase deficiency. We also show that Surf1 protein is not implicated in the assembly of other respiratory chain complexes or the pyruvate dehydrogenase complex. [References: 13]

RANNOU F., CORVOL M., REVEL M., POIRAUDEAU S.

Disk degeneration and disk herniation: the contribution of mechanical stress.

Joint Bone Spine, 68 (6), 543-546, 2001

(Services cités : U530)

Experimental studies on the role for mechanical stresses in the genesis of disk degeneration and herniation are reviewed. Simple mechanical stimulations of functional vertebral segments cannot cause a disk herniation: a complex mechanical stimulation combining forward and lateral bending of the spine followed by violent compression is needed to produce posterior herniation of the disk. Intervertebral disk degeneration seems to influence the development of posterior disk herniation or foraminal disk protrusion. Furthermore, direct mechanical stimulation of the disk tissue or cells generates complex metabolic and cellular responses that lead to qualitative and quantitative modulation of disk matrix proteins. Thus, it is becoming increasingly likely that physical and metabolic factors act in concert to produce disk herniation. (C) 2001 Editions scientifiques et medicales Elsevier SAS. [References: 27]

TAHIRI K., CAM L., DESBUQUOIS B., CHAUVET G.

Processing of the insulin-like growth factor-II-mannose 6-phosphate receptor in isolated liver subcellular fractions.

Biochem. Cell Biol.-Biochim. Biol. Cell., 79 (4), 469-477, 2001

(Services cités : U530)

A truncated, soluble form of the insulin-like growth factor-II-mannose 6-phosphate (IGF-II-M6P) receptor has been identified in serum and shown to be released from cultured tissues and cells, liver being the main contributor to serum receptor in adult rats. In the present study, the processing of the IGF-II-M6P receptor has been characterized in isolated liver subcellular fractions using ligand binding, affinity crosslinking, and Western immunoblotting techniques. The receptor in plasma membrane fractions differed from that in Golgi-endosomal fractions by: (i) a lower molecular size upon reducing polyacrylamide gel electrophoresis (245 vs. 255 kDa); (ii) a less tight membrane association as judged upon extractibility by NaCl; and (iii) the inability to recognize antibody anti-22C, directed against the cytoplasmic domain of the receptor. Incubation of cell fractions at 30 degreesC led to a pH- and time-dependent release of the receptor into the medium. The pH optimum for release was 5.5 in the Golgi-endosomal fraction and 7.5 in plasma membrane fractions; at this pH, approximately 2% and 20%-30% of total receptors were released per hour, respectively. Receptor release was inhibited in a dose-dependent manner by aprotinin, benzamidine, and leupeptin in the Golgi-endosomal fraction, and by 1,10 phenanthroline in plasma membrane fractions, although high concentrations were required for inhibition. The receptor released from Golgi-endosomes showed a 5-10 kDa

reduction in size and a loss of ability to recognize antibody anti-22C, but that released from plasma membranes showed little or no changes in size. We conclude that soluble, carboxy-terminally truncated forms of the IGF-II-M6P receptor are generated from the intact receptor in isolated Golgi-endosomal and plasma membrane fractions. However, receptor processing in these fractions exhibits different properties, suggesting the involvement of different proteases. [References: 36]

2000

CORVOL M.T.

The chondrocyte: from cell aging to osteoarthritis.

Joint Bone Spin, 67 (6), 557-560, 2000

(Services cités : U530)

RANNOU F., CORVOL M.T., HUDRY C., ANRACT P., DUMONTIER M.F., TSAGRIS L., REVEL M., POIRAUDEAU S.

Sensitivity of anulus fibrosus cells to interleukin 1 beta. Comparison with articular chondrocytes.

Spine, 25 (1), 17-23, 2000

(Services cités : U030)

STUDY DESIGN: Anulus fibrosus cells from rabbits were grown in primary culture 1) to study their ability to produce prostaglandin E2 and Type II phospholipase A2, and to express stromelysin-1 messenger ribonucleic acid; and 2) to study the effect of interleukin 1 beta on this production and on proteoglycan aggregation. **OBJECTIVES:** To investigate the potency of anulus fibrosus cells to respond to interleukin 1 beta by producing degradative and inflammatory agents as compared with the potency of articular chondrocytes in the same animal. **SUMMARY OF BACKGROUND DATA:** Interleukin 1 beta has been implicated in the degradation of intervertebral discs. The way anulus fibrosus cells differ from articular chondrocytes in their responses to interleukin 1 beta remains to be established. **METHODS:** Anulus fibrosus cells and articular chondrocytes were obtained from young rabbits, grown in primary culture, and incubated with interleukin 1 beta. The newly synthesized proteoglycan was measured by labeling with [35S]-sulfate. Proteoglycan aggregation was analyzed by the elution profile on Sepharose 2B columns. The contents of collagen Type II and stromelysin-1 messenger ribonucleic acid were assessed by Northern blot analysis. The Type II phospholipase A2 activity was measured using a fluorometric substrate. Prostaglandin E2 production was evaluated by radioimmunoassay. **RESULTS:** Anulus fibrosus cells had 2.5-fold less Type II collagen messenger ribonucleic acid than articular chondrocytes, and interleukin 1 beta had no significant effect on this. Anulus fibrosus cells synthesized and secreted four-fold less proteoglycan than articular chondrocytes. Interleukin 1 beta reduced the anulus fibrosus content of total [35S]-sulfated proteoglycan by 35% ($P < 0.01$), and that of articular cells by 41% and decreased proteoglycan aggregation. Interleukin 1 beta induced the production of stromelysin-1 messenger ribonucleic acid in both cell types. The stromelysin-1 messenger ribonucleic acid content of anulus fibrosus cells was one half that of articular cells. Interleukin 1 beta increased the production of prostaglandin E2 and caused a dose-dependent secretion of Type II phospholipase A2 activity in both cell types. Its effect was 2.5-fold lower in anulus fibrosus cells than in articular chondrocytes. **CONCLUSION:** Anulus fibrosus cells can be stimulated by interleukin 1 beta to produce factors implicated in local degradative and inflammatory processes. This production is associated with decreased proteoglycan aggregation. Anulus fibrosus cells respond slightly less well to interleukin 1 beta in vitro than do articular cells.

RANNOU F., POIRAUDEAU S., FOLTZ V., BOITEUX M., CORVOL M., REVEL M.

Monolayer anulus fibrosus cell cultures in a mechanically active environment: local culture condition adaptations and cell phenotype study.

J. Lab. Clin. Med., 136 (5), 412-421, 2000

(Services cités : U530)

Intervertebral disc cells can be cultured in vitro. Several culturing systems in a mechanically active environment have been developed to study the relationship between mechanical stimulations and biochemical events. The aim of this study was to assess the phenotype of rabbit intervertebral disc cells from the anulus fibrosus (AF) region cultured on flexible substrate before and after application of cyclic tensile stretch (CTS) and to control culture conditions during application of CTS. CTS was applied with a pressure-operated instrument, inducing the deformation of flexible-bottomed culture plates (Flexercell) at 20% and 5% stretch, at a frequency of 1 Hz, during 30 minutes to 24 hours. A significant decrease in culture medium volume and temperature was observed (52% and 2.1 degreesC at 20% stretch and 24 hours' application of CTS). These phenomena were inhibited by adding culture medium around culture wells and by a culture medium temperature control system. Like AF cells cultured in plastic wells, AF cells cultured on flexible substrate expressed collagen type II, but collagen type I mRNA was not detected. In both culture conditions, neosynthesized proteoglycans had the same aggregating properties. CTS at 20% stretch during 12 hours did not induce cell detachment from the substrate and did not modify aggregating properties of neosynthesized proteoglycans; AF cells continued to express collagen type II but not collagen type I mRNA. In conclusion, the Flexercell system appears to be appropriate for studying, at the cellular level, the metabolic responses to CTS. [References: 42]

VILLEVALOIS-CAM L., TAHIRI K., CHAUVET G., DESBUQUOIS B.

Insulin-induced redistribution of the insulin-like growth factor II/mannose 6-phosphate receptor in intact rat liver.

J. Cell. Biochem., 77 (2), 310-322, 2000

(Services cités : U030)

The ability of acute insulin treatment to elicit a redistribution of the liver insulin-like growth factor-II/ mannose 6-phosphate (IGF-II/M6P) receptor has been studied in rats, using cell fractionation. Injection of insulin (0.4-50 microg) led to a time- and dose-dependent decrease in IGF-II binding activity in Golgi-endosomal (GE) fractions, along with an increase in activity in the plasma membrane (PM) fraction; only receptor number was affected. Quantitative subfractionation of the microsomal fraction on sucrose density gradients showed that IGF-II binding activity distributed similarly to galactosyltransferase (a Golgi marker), at slightly higher densities than in vivo internalized (125)I-insulin, and at lower densities than 5' nucleotidase and alkaline phosphodiesterase (two plasma membrane markers). Insulin treatment led to a slight time-dependent and reversible shift of IGF-II binding activity toward higher densities. Subfractionation of the GE fraction on Percoll gradients showed that IGF-II binding activity was broadly distributed, with about 60% at low densities coinciding with galactosyltransferase and early internalized (125)I-insulin and with 40% at high densities in the region of late internalized (125)I-insulin. Insulin treatment caused a time-dependent and reversible shift of the distribution of IGF-II binding activity toward low densities. On SDS-PAGE, the size of the affinity-labeled IGF-II/M6P receptor was comparable in GE and PM fractions (about 255 kDa), but on Western blots receptor size was slightly lower in the latter (245 kDa) than in the former (255 kDa). Insulin

treatment did not affect the size, but modified the abundance of the IGF-II/M6P receptor in a manner similar to that of IGF-II binding. In vivo chloroquine treatment fully suppressed the changes in IGF-II binding activity in liver GE and PM fractions observed in insulin-treated rats. We conclude that insulin elicits a time-dependent and reversible redistribution of liver IGF-II receptors from Golgi elements and endosomes to the plasma membrane, presumably via early endosomes. Copyright 2000 Wiley-Liss, Inc.

1999

**AMESSOU M., BORTOLI S., LIEMANS V., COLLINET M., DESBUQUOIS B.,
BRICHARD S., GIRARD J.**

Treatment of streptozotocin-induced diabetic rats with vanadate and phlorizin prevents the over-expression of the liver insulin receptor gene.

Eur. J. Endocrinol., 140 (1), 79-86, 1999

(Services cités : U030)

Administration of vanadate, an insulinomimetic agent, has been shown to normalize the increased number of insulin receptors in the liver of streptozotocin-induced diabetic rats. In the present study, the effects of vanadate on various steps of expression of the liver insulin receptor gene in diabetic rats have been analyzed and compared with those of phlorizin, a glucopenic drug devoid of insulinomimetic properties. Livers of rats killed 23 days after streptozotocin injection showed a 30-40% increase in the number of cell surface and intracellular insulin receptors, a 50-90% increase in the levels of 9.5 and 7.5 kb insulin receptor mRNA species, and a 20% decrease in the relative abundance of the A (exon 11-) insulin receptor mRNA isotype. Daily administration of vanadate or phlorizin from day 5 to day 23 prevented the increase in insulin receptor number and mRNA level, and vanadate treatment also normalized receptor mRNA isotype expression. Unlike observations in vivo, vanadate and phlorizin differentially affected the expression of the insulin receptor gene in Fao hepatoma cells. Vanadate treatment (0.5 mmol/l for 4 h) decreased the levels of the 9.5 and 7.5 kb insulin receptor transcripts by at least twofold, without affecting the relative abundance of the A insulin receptor mRNA isotype. In contrast, phlorizin treatment (5 mmol/l for 4 h) slightly increased or did not affect the levels of the 9.5 and 7.5 kb insulin receptor transcripts respectively, and increased by twofold the relative expression of the A insulin receptor mRNA isotype. It is suggested that, although mediated in part by a reversal of hyperglycemia, normalization of liver insulin receptor gene expression by vanadate treatment in diabetic rats may also involve a direct inhibitory effect of this drug on gene expression.

AUTHIER F., CHAUVET G.

In vitro endosome-lysosome transfer of dephosphorylated EGF receptor and Shc in rat liver.

FEBS Lett., 461 (1-2), 25-31, 1999

(Services cités : U030)

We have studied the endosome-lysosome transfer of internalized epidermal growth factor receptor (EGFR) complexes in a cell-free system from rat liver. Analytical subfractionation of a postmitochondrial supernatant fraction showed that a pulse of internalized [I-125]EGF was largely associated with a light endosomal fraction devoid of lysosomal markers. After an additional 30 min incubation in vitro in the presence of an ATP-regenerating system, the amount of [I-125]EGF in this compartment decreased by 39%, with an increase in I-125]EGF in lysosomes. No transfer of I-125]EGF to the cytosol was detected. To assess the fate of the internalized EGFR protein over the time course of the endo-lysosomal transfer of the ligand, the effect of a saturating dose of native EGF on subsequent lysosomal targeting of the EGFR was

evaluated by immunoblotting, A massive translocation of the EGFR to the endosomal compartment was observed in response to ligand injection coincident with its tyrosine phosphorylation and receptor recruitment of the tyrosine-phosphorylated adaptor protein Shc, During cell-free endosome-lysosome fusion, a time-dependent increase in the content of the EGFR and the two 55- and 46-kDa Shc isoforms was observed in lysosomal fractions with a time course super-imposable with the lysosomal transfer of the ligand; no transfer of the 66-kDa Shc isoform was detected. The relationship between EGFR tyrosine kinase activity and EGFR sorting in endosomes investigated by immunoblot studies with anti-phosphotyrosine antibodies revealed that endosomal dephosphorylation of EGFR and Shc preceded lysosomal transfer. These results support the view that a lysosomal targeting machinery distinct from the endosomal receptor kinase activity, such as the recruitment of the signaling molecule Shc, may regulate this sorting event in the endosome. (C) 1999 Federation of European Biochemical Societies. [References: 39]

COTTART C.H., DO L., BLANC M.C., VAUBOURDOLLE M., DESCAMPS G., DURAND D., GALEN F.X., CLOT J.P.

Hepatoprotective effect of endogenous nitric oxide during ischemia-reperfusion in the rat.

Hepatology, 29 (3), 809-813, 1999

(Services cités : U030, Biochimie Générale)

The aim of this study was to evaluate the protective or deleterious effects of endogenous nitric oxide (NO) on liver cells during hepatic ischemia-reperfusion (IR) in the rat. Injury to hepatocytes and endothelial cells was evaluated by determining cytolysis-marker activity in plasma (alanine transaminase [ALT]; aspartate transaminase [AST]) and plasma hyaluronic acid (HA) concentration. Clamping the hepatic pedicle for 45 minutes caused a significant increase in plasma AST and ALT activity after 30 minutes of reperfusion, which reached a maximum (+270% and +740%, respectively) after 6 hours of reperfusion. Plasma HA concentration was significantly higher (+130%) only after 6 hours of reperfusion. Administration of a nonselective NO synthase (NOS) inhibitor, N omega-nitro-L-arginine (L-NNA; 10 mg/kg iv), 30 minutes before IR, caused marked aggravation of postischemic liver injury, as shown by plasma ALT and AST activity and HA concentration. This deleterious effect was partially prevented by the simultaneous injection of L-arginine, the endogenous NO precursor (100 mg/kg iv). Interestingly L-arginine alone limited postischemic damage (AST, -25%; ALT, -45%; HA, -21% vs, untreated IR rats at 6 hours reperfusion). Pretreatment with the Guanosine 3':5'-cyclic monophosphate-independent vasodilator, prazosin, partially reversed L-NNA effects, but it did not protect untreated IR animals. Pretreatment with aminoguanidine, a selective inhibitor of inducible NOS, did not aggravate hepatic IR injury. Thus, endogenous NO, probably produced by an early and transient activation of a constitutive NOS, protects both hepatocytes and endothelial cells against liver ischemia-reperfusion injury, and this effect is not entirely a result of vasorelaxation.

[References: 25]

FRAGNER P., LADRAM A., ARATAN de LEON S.

Triiodothyronine down-regulates thyrotropin-releasing hormone (TRH) synthesis and decreases pTRH-(160-169) and insulin releases from fetal rat islets in culture.

Endocrinology, 140 (9), 4113-4119, 1999

(Services cités : U030)

TRH is localized with insulin in beta-cells. It is synthesized as a prohormone containing five copies of TRH and seven cryptic peptides, including pro (p)-TRH-(160-169). Thyroid hormone

regulates the expression of several genes encoding peptide hormones. We found that circulating T3 concentrations were inversely correlated with TRH levels in two physiopathological situations. There are low concentrations of circulating thyroid hormone and very high concentrations of TRH and pTRH-(160-169) during development, and experimental hypothyroidism results in higher concentrations of prepro (pp)-TRH messenger RNA (mRNA) and TRH content in the adult rat pancreas than are present in the euthyroid pancreas. The present study was carried out to investigate the interaction between T3 and pancreatic TRH during the functional maturation of islets in culture and to validate the data obtained in vivo. T3 decreases ppTRH mRNA in islets in a dose-dependent manner. The primary impact of T3 on islet function may be mediated by ppTRH mRNA, as short term T3 treatment had no effect. Long term T3 treatment reduced the islet TRH content and the amounts of pTRH-(160-169) and insulin released. This secretory pattern and coordinated regulation of pTRH-(160-169) and insulin suggests that pTRH-(160-169) plays a specific role in the regulation of insulin secretion.

GALEN F.X., COTTART C.H., SOUIL E., DINH-XUAN A.T., VAUBOURDOLLE M., NIVET V., CLOT J.P.

Implication of nitric oxide synthase-III and guanosine 3':5'-cyclic monophosphate in the cytoprotective effects of nitric oxide against hepatic ischemia-reperfusion injury.

C. R. Acad. Sci. Sér.III Sci. Vie, 322 (10), 871-877, 1999

(Services cités : U030)

Nitric oxide (NO) exerts cytoprotective effects against hepatic ischemia-reperfusion damage. This study was designed to evaluate which isoform of NO synthase (NOS) is implicated in the generation of cytoprotective NO and to investigate whether NO effects are mediated by cyclic CMP (cGMP). After partial ischemia for 45 min, liver damage was estimated by the release into plasma of cytolytic enzymes. Ischemia-reperfusion induced marked increases in plasma creatine kinase and lactate dehydrogenase after 1 h of reperfusion and of aminotransferases after 6 h of reperfusion. The pretreatment of ischemied rats with 8-bromo-cGMP (16 mg/kg iv 30 min before ischemia) or with L-arginine (the endogenous precursor of NO, 100 mg/kg iv) significantly diminished the ischemia-reperfusion-induced release of all these enzymes. This demonstrates that cGMP possesses hepatoprotective properties. By immunohistochemistry, we observed, after 6 h of reperfusion, an increase in endothelial NOS-III immunoreactivity, particularly in the small arteries and sinusoids. This NOS-III accumulation in endothelial cells could protect the liver against ischemia-reperfusion by the local generation of NO probably via cGMP. (C) 1999 Academie des sciences / Editions scientifiques et medicales Elsevier SAS. [References: 12]

GEOFFROY V., FOUQUE F., NIVET V., CLOT J.P., LUGNIER C., DESBUQUOIS B., BENELLI C.

Activation of a cGMP-stimulated cAMP phosphodiesterase by protein kinase C in a liver Golgi-endosomal fraction.

Eur. J. Biochem., 259 (3), 892-900, 1999

(Services cités : U030)

The ability of Ca²⁺/phospholipid-dependent protein kinase (protein kinase C, PKC) to stimulate cAMP phosphodiesterase (PDE) activity in a liver Golgi-endosomal (GE) fraction was examined in vivo and in a cell-free system. Injection into rats of 4 beta-phorbol 12-myristate 13-acetate, a known activator of PKC, caused a rapid and marked increase in PKC activity (+ 325% at 10 min) in the GE fraction, along with an increase in the abundance of the PKC alpha-isoform as seen on Western immunoblots. Concurrently, 4 beta-phorbol 12-myristate 13-acetate treatment caused a

time-dependent increase in cAMP PDE activity in the GE fraction (96% at 30 min). Addition of the catalytic subunit of protein kinase A (PKA) to GE fractions from control and 4 beta-phorbol 12-myristate 13-acetate-treated rats led to a comparable increase (130-150%) in PDE activity, suggesting that PKA is probably not involved in the in-vivo effect of 4 beta-phorbol 12-myristate 13-acetate. In contrast, addition of purified PKC increased (twofold) PDE activity in GE fractions from control rats but affected only slightly the activity in GE fractions from 4 beta-phorbol 12-myristate 13-acetate-treated rats. About 50% of the Triton-X-100-solubilized cAMP PDE activity in the GE fraction was immunoprecipitated with an anti-PDE3 antibody. On DEAE-Sephacel chromatography, three peaks of PDE were sequentially eluted: one early peak, which was stimulated by cGMP and inhibited by erythro-9 (2-hydroxy-3-nonyl) adenine (EHNA); a selective inhibitor of type 2 PDEs; and two retarded peaks of activity, which were potently inhibited by cGMP and cilostamide, an inhibitor of type 3 PDEs. Further characterization of peak I by HPLC resolved a major peak which was activated (threefold) by 5 μ M cGMP and inhibited (87%) by 25 μ M EHNA, and a minor peak which was insensitive to EHNA and cilostamide. 4 beta-Phorbol 12-myristate 13-acetate treatment caused a selective increase (2.5-fold) in the activity associated with DEAE-Sephacel peak I, without changing the K-m value. These results suggest that PKC selectively activates a PDE2, cGMP-stimulated isoform in the GE fraction. [References: 56]

POIRAUDEAU S., MONTEIRO I., ANRACT P., BLANCHARD O., REVEL M., CORVOL M.T.

Phenotypic characteristics of rabbit intervertebral disc cells. Comparison with cartilage cells from the same animals.

Spine, 24 (9), 837-844, 1999

(Services cités : U030)

STUDY DESIGN: Intervertebral disc cells were extracted from the surrounding matrix, and their metabolic activities and phenotypes were studied. **OBJECTIVES:** To compare the metabolic activities and phenotypes of cell populations extracted from the intervertebral discs of young rabbits with those of articular and growth plate chondrocytes from the same animals.

SUMMARY OF BACKGROUND DATA: The phenotype of intervertebral disc cells has been poorly studied and still is debated. **METHODS:** The intervertebral discs as well as articular and vertebral growth plate cartilage of rabbits were digested enzymatically. The morphology of freshly isolated cells was examined. Their contents of collagen II and X mRNAs were determined by Northern blot analysis, and their sulfation activity by ³⁵S-sulfate incorporation as chondrocytic markers. Cells were cultured at high density or low density and grown in primary culture. The stability of their phenotype was monitored by evaluating the collagen I and II mRNA ratio. The proteoglycans newly synthesized by the cells also were quantified, and their elution profile analyzed on Sepharose 2B columns. **RESULTS:** The anulus fibrosus cells were morphologically undistinguishable from articular chondrocytes. The nucleus pulposus contained mainly large vacuolated cells and a few smaller cells. All freshly extracted cells expressed different levels of collagen II mRNA. Anulus fibrosus and nucleus pulposus cells contained, respectively, 22% and 8% of collagen II mRNA compared with that found in articular or growth plate chondrocytes from the same animal. Only growth plate chondrocytes expressed collagen X. When anulus fibrosus cells were incubated for 48 hours at high density, they had collagen II mRNA contents similar to those of articular and growth plate chondrocytes, but synthesized five to six times fewer sulfated proteoglycans. When seeded at low density, anulus fibrosus cells divided more slowly than articular chondrocytes and incorporated four times fewer ³⁵S-sulfate

into proteoglycans. Their collagen II mRNA content was 2.75-fold lower than that of chondrocytes, and the procollagen alpha 1II/alpha 1I mRNA ratio was 3.1 for annulus fibrosus cells and 7 for chondrocytes. No collagen X mRNA was detected. When incubated for 48 hours at high density, the nucleus pulposus giant cells had four times less collagen II mRNA content than cartilage cells but synthesized the same amounts of sulfated proteoglycans. They did not divide during 21 days in culture and still contained collagen II mRNA but no collagen X mRNA. CONCLUSIONS: Findings showed that intervertebral disc cells all express cartilage-specific matrix proteins with quantitative differences, depending on their anatomic situation. It is suggested that annulus fibrosus cells are chondrocytic cells at a different stage of differentiation than articular and growth plate chondrocytes. The phenotype of nucleus pulposus cells still is unclear. They could be chondrocytic or notochordal. A definitive answer to this important question requires differentiating markers of notochordal cells.

ZANZE M., ROSSIGNOL C., KINDERMANS C., SOUBERBIELLE J.C.

Bsm I polymorphism in the vitamin D receptor gene is related to bone collagen turnover in healthy infants.

Scand. J. Clin. Lab. Invest., 59 (6), 467-474, 1999

(Services cités : U030, Explorations Fonctionnelles)

We examined the vitamin D receptor genotypes (BB, Bb and bb) defined by the BsmI restriction endonuclease in relation to biochemical indices of bone metabolism in healthy Caucasian infants. We measured the serum concentrations of the carboxy-terminal propeptide of type I procollagen (PICP) and the urinary excretion of total pyridinoline, free, total and bound deoxypyridinoline, the type I collagen N-terminal and C-terminal cross-linked telopeptides. The concentrations of the urinary indices are expressed relative to creatinine. Subjects with BE genotype had the highest mean concentrations of free, total and bound deoxypyridinoline and of the N-terminal cross-linked telopeptide (P-ANOVA = 0.0016, 0.0004, 0.0002 and 0.0053, respectively). BE boys had a higher excretion of the C-terminal cross-linked telopeptide than the other genotypes (P-ANOVA = 0.0253). In a subgroup of homozygotes aged 10 (1) months, BE subjects had the highest levels of the C-terminal cross-linked telopeptide (p = 0.03), and of total deoxypyridinoline (p = 0.02) and pyridinoline (p = 0.06) concentrations. No significant association between the vitamin D receptor genotype and PICP was found. These data suggest that there may be a contribution of the vitamin D receptor genotype to skeletal metabolism in early childhood. [References: 31]