from the vitreous, indicating that it was not secreted from ocular tissue, consistent with its lack of a signal peptide sequence. Most of the scGH immunoreactivity detected was associated with a protein of 31KDa, which likely reflects dimerization of the monomer variant. The staining of this protein was lost following the preincubation of the primary antibody with excess N-terminal scGH, but not after its preincubation with full-length GH. Translation of the scGH transcript was also demonstrated by the presence of immunoreactivity for 31 KDa scGH and for trace amounts of 16.5 KDa scGH in extracts (but not media) of HEK cells transfected with a scGH-expressing vector. These results therefore demonstrate, for the first time, the expression, translation and distribution of this novel GH variant in ocular tissues of the chick embryo.

P-04

MORPHINE-INDUCED CELL DAMAGE IS REVERSED BY HUMAN RECOMBINANT GROWTH HORMONE – AN EXPERIMENTAL STUDY USING MICE PRIMARY HIPPOCAMPAL NEURONAL CELL-CULTURES

Fred J Nyberg*, Nora Bucht, Mathias Hallberg, Anne-Lie Svensson. *Uppsala University, Department of Pharmaceutical Biosciences, Sweden*

Previous works on growth hormone (GH) have shown that this hormone is involved in many functions related to the CNS. It is well known that GH-deficient patients exhibit lack of concentration, memory and cognitive disabilities and replacement therapy with recombinant human GH (rhGH) in these patients is shown to improve their memory and cognitive efficiency. The hormone has also been demonstrated to affect learning and memory in rats. These beneficial effects of GH are likely to be mediated through GH receptors in the hippocampus. In this study we have used primary hippocampal cell cultures derived from 16-days old foetal mouse neurons. Cells were treated with morphine for seven days during growth and with rhGH after drug withdrawal. In parallel experiments cells were treated with morphine without the addition of rhGH and with the hormone alone. Untreated cells were used as controls. After completed experiments the number of survived cells were counted and the cell lysis were determined by measure lactate dehydrogenase (LDH) release. Results indicated that the opiate decreased the cell content in a concentration dependent manner and increased the LDH release. Thus, foetal mouse neurons show less viability compared to controls when treated with morphine (15?M), whereas results after rhGH (1?M) treatment indicated augmented viability and preservation from morphine toxicity. Preservative effects were shown when adding rhGH and morphine simultaneously and re-growth when adding the hormone after drug withdrawal. Consistent results were shown in measurements of LDH release in media and western blotting of cleaved the enzyme Caspase 3, indicating increased apoptosis in opiate treated cells. These results are consistent with the ability of GH to prevent opiate-induced damage to these kinds of cells.

GH secretion

P-06

POSSIBLE INVOLVEMENT OF THE INDUCIBLE TRANSCRIPTION FACTOR EGR1 IN THE TRANSCRIPTIONAL REGULATION OF GROWTH HORMONE GENE

Yasumasa Iwasaki[1]*, Mitsuru Nishiyama[1], Takafumi Taguchi[1], Kinji Inoue[2], Kozo Hashimoto[1]. *1Department of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University; [2]Saitama University, Japan*

Although the transcription factor Pit-1 is known to play an indispensable role in the pituitary-specific expression of GH gene, factor(s) involved in the short-term regulation of the gene is not clarified yet. In this study, we examined the possible involvement of the inducible transcription factor Egr1 (NGFI-A) in the transcriptional regulation of GH gene. In the somatotroph cell line MtT/S, GRH and high potassium-induced depolarization increased the transcriptional activity of GH gene, and simultaneously induced intrinsic expression of Egr1, that was completely abolished by p42/44 MAPK inhibitors such as PD98059. Furthermore, overexpression of Egr1 potently stimulated the 5′-promoter activity of GH gene in a dose-dependent fashion. Interestingly, in a heterologous CHO cell line, combined expression of Egr1 and Pit-1, but not Egr1 alone, potently enhanced GH gene expression, suggesting a co-operative role of the two factors. Since Egr1 knockout mouse is dwarf with pituitary hypoplasia and decreased somatotroph cell number, we assume that Egr1 plays a crucial role in the transcriptional regulation of GH gene.

P-08

ANALYSIS OF THE HESX1 AND THE LHX4 GENE IN JAPANESE PATIENTS WITH COMBINED PITUITARY HORMONE DEFICIENCY (CPHD)

Katsura Ishizu[1]*, Wakako Jo[1], Fumie Fujiwara[1], Junko Tsubaki[1], Kenji Fujieda[2], Toshihiro Tajima[1]. *[1]Department of Pediatrics, Hokkaido University School of Medicine; [2]Department of Pediatrics, Asahikawa Medical College, Japan*

HESX1 is one of the earliest transcription factors expressed during pituitary development. So far, several mutations of HESX1 have been identified. There is a phenotypic variability ranging from combined pituitary hormone deficiency (CPHD) and optic nerve dysplasia to isolated GH deficiency. The other transcriptional factor, LHX4 is characterized by two LIM domains N-terminus to a homeodomain. Mutations of the LHX4 gene have been also reported to cause in CPHD in human.

To elucidate the molecular basis of CPHD, we screened mutations of the HESX1 and the LHX4 gene in sporadic patients with congenital hypopituitarism (9 CPHD and 1 isolated GH deficiency). One patient had ins 306/307 AG of the HESX1 gene. This patient showed an ectopic posterior pituitary, small anterior pituitary, and left optic nerve hypoplasia with CPHD. This patient presented at birth with hypoglycemia and respiratory distress complicated by shock. Transient transfection assay demonstrated that 306/307 ins AG mutants abolished the repressing activity to PROP-1 mediated PIT-1 promoter activation compared to the wild-type HESX1. In addition, this mutant HESX1 could not localize to the nucleus, different from the wild-type HESX1. In the other nine patients, we did not identify any mutations of the HESX and the LHX4 gene.

In conclusion, (1) 306/307 ins AG is the cause of CPHD. (2) Mutations of the HESX1 and the LHX4 gene are rare in our small study, however further studies are warranted.

GH signaling

P-10

REGULATION OF GROWTH HORMONE-INDUCED [CA2+]C OSCILLATIONS IN RAT HEPATOCYTES BY PANCREATIC HORMONES

Isabel Marrero[1]*, Antonio S Deniz[2]. *[1]Department of Physiology, Faculty of Health Sciences, University of Las Palmas de Gran Canaria, Las Palmas, Canary Islands, Spain, [2]Dept. of Medical and Surgical Sciences, ULPGC, Spain*

Oscillations in cytosolic free Ca2+ concentration ([Ca2+]c) are a fundamental mechanism of intracellular signalling in hepatocytes. Glucogenolitic agonists, acting via the phosphoinositide signalling pathway (phenilephrine-Phe, vasopressin-Vp, growth hormone-GH) induce oscillations in [Ca2+]c. The aim of this study was to examine the ability of the main pancreatic hormones to induce changes in the [Ca2+]c or to modify the glucogenolitic agonists effect in [Ca2+]c

oscillations at the single cell level in rat hepatocytes microinjected with the photoprotein aequorin.
Glucagon, insulin and somatostatin (SST) did not themselves produce modifications in [Ca2+]c. However, glucagon enhanced oscillatory response induced by agonists acting via the phosphoinositide signalling pathway (Phe, Vp and bGH). Elevation of intracellular cyclic AMP concentration produced similar response than this generated by glucagon. In contrast, insulin attenuated cytosolic Ca2+ oscillations through a decrease in their frequency. Somatostatin had no effect on Phe-induced oscillations, however inhibited bGH-induced Ca2+ oscillations, suggesting a selective peripheral inhibitory action of SST on GH.
Our data provide a possible mechanism for regulation of GH hepatic action by pancreatic hormones more related to an interactive control of nutrients and hormones involved in metabolic homeostasis. They may mediate the effects of GH in hepatic protein anabolism and glucose homeostasis by directly influencing GH transduction.

P-12

INFLUENCE OF EXON 3-DELETED GROWTH HORMONE RECEPTOR VARIANT ON CLINICAL PARAMETERS OF PATIENTS WITH TYPE 2 DIABETES MELLITUS

Rona J Strawbridge[1]*, Lars Karvestedt[2], Harvest G Gu[2], Chunde Li[1], Kerstin Brismar[2]. [1]*Cancer Centrum Karolinska, Karolinska Institute;* [2]*Molecular Medicine and Surgery, KI, Sweden*

Growth hormone (GH) signaling via the growth hormone receptor (GHR) forms a major part of the GH–IGF-I axis which is crucial for controlling metabolism. Two common variants of the GHR differ by the presence or absence of exon-3 (GHRfl and GHRd3, respectively), which encodes a region close to the GH-binding domain. Functional studies report no difference between the ability of the two variants to bind or internalise GH and a single copy of either variant is sufficient for normal growth. However, differential response to GH treatment has been observed, with the GHRd3 variant conferring a greater growth rate. This study investigates the role of these variants in Type 2 Diabetes Mellitus, which is characterized by aberrant metabolic control.
DNA was extracted from peripheral blood samples from healthy controls, T2DM and Late Autoimmune Diabetes of the Adult patients. Genotyping and analysis by gel electrophoresis was carried out as previously described. No significance difference in genotype frequency was observed between diabetic subjects and controls. CRP levels were significantly different and correlated with the number of exon-3 alleles present, whereas IGF-I and free IGF-I levels were significantly higher in GHd3/d3 than GHRfl/fl, with GHRfl/d3 having the lowest levels. GHRd3/d3 subjects were also associated with increased occurrence of CVD, nephropathy and dyslipidemia.
Two interesting trends in metabolic parameters and diabetes complications were observed, which depend on exon-3 dosage or matched receptors, indicating both IGF-I- dependant and independent GHR signaling pathways.

IGF physiology/biology

P-14

EFFECT OF cAMP STIMULATORS ON IGF-II SECRETION FROM HepG2 CELLS

Henrik von Horn*, Inga-Lena Wivall-Helleryd, Kerstin Hall, Moira S Lewitt. *Department of Molecular Medicine and Surgery, Karolinska Institutet, Sweden*

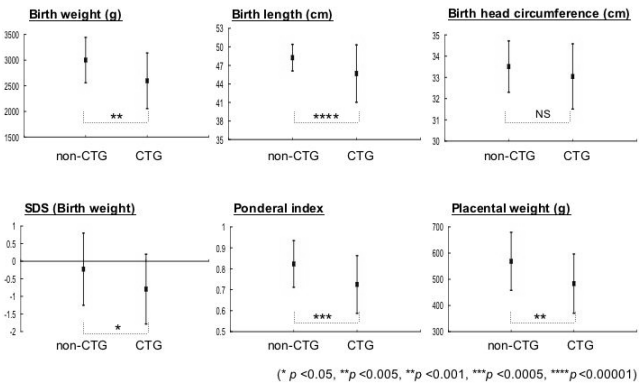
Insulin-like growth factor II (IGF-II) can act via insulin/insulin-like growth factor I hybrid receptors in HepG2 cells, regulating growth and metabolic actions. We have previously shown that hypoxia increases IGF-II and IGFBP-1 mRNA expression but in contrast to IGFBP-1, which increases 5-fold, immunoreactive IGF-II secretion was reduced approx 50%.
There is an additive effect on IGFBP-1 mRNA expression and protein secretion with hypoxia and cAMP (Sugawara et al, JCEM 85:3821–7, 2000). The aim of this study was to determine the effect of stimulating intracellular cAMP on IGF-II secretion. IGF-II was measured by radioimmunoassay using the Upstate monoclonal IGF-II antibody (clone S1F2 1:500,000) and iodinated des (1–6) IGF-II as tracer. Cell viability was determined by MTT assay, and the IGF-II values were corrected accordingly.
During a 24-h incubation under normoxic conditions, forskolin increased IGFBP-1 2-fold, and stimulated IGF-II secretion in a dose-dependent fashion. The percent increase was 38% ($p < 0.01$), 65% ($p < 0.001$) and 38% ($p < 0.01$), for 5 and 50 μM forskolin and 5 μg/ml glucagon, respectively. Under hypoxic conditions basal levels of IGF-II decreased to 44%, the stimulatory effect of forskolin diminished, and the effect of glucagon was lost.
In summary, in our HepG2 cell line, IGF-II secretion is stimulated by agents that increase intracellular cAMP, and this effect is lost under hypoxic conditions. The role of these changes in hepatocellular growth and metabolism will be determined in future studies.

P-16

POLYMORPHISM IN PATERNAL ALLELE OF *IGF2* GENE ASSOCIATED WITH FETAL AND PLACENTAL GROWTH IN JAPANESE

Ken Nagaya*, Yoshio Makita, Genya Taketazu, Eiki Nakamura, Tokitsugi Hayashi, Kenji Fujieda. *Department of Pediatrics, Asahikawa Medical College, Japan*

Objective: Insulin-like growth factor 2 (IGF2) is dipensible for placental and fetal growths in rodent. We hypothesized that polymorphism of IGF2 gene could alter birth weight and placental weight in human.
Subject and methods: The healthy 134 infants without congenital anomaly, who were born at >35 weeks of gestational age between October 2004 and August 2005 in our hospital and Mori obstetrics gynecology hospital, and their mothers were examined about *IGF2* gene. We used three tag-SNPs (rs1003484C/T, rs3741211T/C and rs3741206A/G) on *IGF2* gene and carried out haplotype analysis between fetal placental growth and paternal allele of *IGF2* gene.
Result: Frequency of CTG haplotype of paternal allele in small for date infants was significantly higher than in appropriate for date or heavy for date infants (37.9% vs. 14.3%, $p < 0.05$). CTG haplotype infants were significantly lower gestational age, shorter birth length, and lower birth weight and placental weight, and Ponderal index at birth than non-CTG infants.



Clinical characteristics by offspring IGF2 haplotypes from paternal allele.

Conclusion: CTG haplotype of paternal allele on *IGF2* gene may have some relationship with reduction of feto-placental growth.