IV Zjazd Polskiego Towarzystwa Neuroendokrynologii

10-11 października 2014 r.

Centrum Kongresowe – Hotel Andel's
Łódź, ul. Ogrodowa 17

Główne tematy Zjazdu

I Sesja: NEUROENDOKRYNOLOGIA STARZENIA

II Sesja: NEUROENDOKRYNOLOGIA A METABOLIZM

III Sesja: „III ŁÓDZKIE SPOTKANIA PRZYSADKOWE"

1. **Immunohistochemia guzów przysadki**
2. **Diagnostyka i leczenie guzów przysadki**
3. **Postępy w leczeniu farmakologicznym i operacyjnym**
4. **Radioterapia guzów przysadki – dyskusja Okrągłego Stołu**

IV Sesja: NEUROENDOKRYNOLOGIA ROZRODU

V Sesja: SESJA POSTEROWA

Uroczyste Otwarcie Zjazdu – połączone
z Jubileuszem 80-Lecia Urodzin Profesora Marka Pawlikowskiego,
10.10.2014r od godz. 19:00, Pałac Poznańskich, Łódź, ul. Ogrodowa 15

Walne Zgromadzenie Członków PTNE
Centrum Kongresowe, Hotel Andel's – 10.10.2014r, godz. 16.45 (I), 17.00 (II)

Komitet Naukowy

Przewodniczący: *prof. Marek Pawlikowski*
Kierownicy Sesji Zjazdowych: (I) *prof. Bogusława Baranowska*
(II) *prof. Krystyna Pierzchała-Koziec*
(III) *prof. Jolanta Kunert-Radek*
(IV) *prof. Alina Gajewska*

Komitet Organizacyjny

Przewodnicząca: *prof. Jolanta Kunert-Radek*
Vice Przewodnicząca: *prof. Katarzyna Winczyk*
Członkowie: *dr Karolina Beda-Maluga, dr Julita Fuss-Chmielewska, dr hab. Anna Gruszka, mgr Maria Jaranowska, prof. Marlena Juszczak, dr hab. Hanna Ławnicka, mgr Jacek Świętosławski*

Streszczenia

The diagnostic significance of copeptin

Bogusława Baranowska[1], Wojciech Bik[2], Jan Kochanowski[1]
[1]*Neurology Department, Medical University, Warsaw*
[2]*Neuroendocrinology Department, Medical Center of Postgraduate Education, Warsaw*

Introduction: Copeptin is a 39 aminoacid peptide that shares precursor preprovasopressin, both with vasopressin and neurophysin II. The evaluatio0n of copeptin is useful in the differential diagnosis of polydipsia — polyuria syndrome. Copeptin is also a good diagnostic and prognostic marker in myocardial infarction, heart failure, myocarditis, degenerative aortic stenosis, ischemic cerebral stroke, subarachnoid hemorrhage, sepsis, metabolic syndrome, diabetes with albuminuria. Besides antidiuretic effects, copeptin stimulates ACTH secretion. The activation of the hypothalamo-pituitary-adrenal axis is the early response of cerebral ischemic stroke and also it may influence the intensity of immunological processes in multiplex sclerosis (MS).
Material and methods: The aim of this study is to evaluate plasma copeptin concentrations in 150 patients with acute ischemic stroke and 29 with multiple sclerosis, in comparison to control groups.
Results: In the patients with acute ischemic stroke plasma copeptin levels were significantly higher ($p < 0.001$) as compared with the controls. The correlations of copeptin with metabolic markers and severity of stroke (NIHSS) scale were evaluated. In the patients with MS plasma copeptin levels were higher compared to the control group but the difference was not significant. However, the values of copeptin in overweight/obese patients with MS were significantly higher than those found in overweight/obese and lean subjects of the control group ($p < 0.05$, $p < 0.01$) and lean MS ($p < 0.05$). The correlations between copeptin and metabolic markers were investigated.
Conclusions: Copeptin is a potential prognostic marker in acute ischemic stroke. Elevated copeptin levels in patients with MS may be partly connected with obesity and some components of metabolic syndrome.
Grant CMKP 501-1-31-22-14.

Neuroendocrine and metabolic aspects of selected central nervous system diseases

Agnieszka Baranowska-Bik[1], Wojciech Bik[2], Maria Barcikowska[3], Jan Kochanowski[4], Bogusława Baranowska[4]
[1]*Department of Endocrinology Centre of Postgraduate Medical Education Warsaw, Poland*
[2]*Department of Neuroendocrinology Centre of Postgraduate Medical Education Warsaw, Poland*
[3]*Department of Neurodegenerative Disorders, Medical Research Centre, Polish Academy of Science Warsaw, Poland*
[4]*Department of Neurology; Second Faculty of Medicine, Medical University Warsaw, Poland*

Introduction: An ageing society results in the growing incidence of dementia and cardiovascular diseases. Several studies indicate an essential role of obesity and obesity-related metabolic disturbances, including insulin resistance, in the etiology of the above mentioned diseases. Adipokines, peptides produced by the adipose tissue, may play an important role in the pathogenesis of dementia and ischemic stroke. Adipokines possess pleiotropic properties, including the immunomodulation and modification of insulin sensitivity.
To assess the concentration of selected adipokines (adiponectin and its fractions, leptin and soluble leptin receptor, resistin) and insulin-resistance parameters in Alzheimer's disease (AD) and in individuals with a newly diagnosed brain ischemic stroke.
Material and methods: A group of AD patients consisted of 58 women. A control group comprised of 42 non-demented women matched for age and BMI. 150 women were amongst ischemic stroke patients. A control group consisted of 75 women matched for age and BMI. Selected adipokines and insulin-resistance parameters were measured once in dementia individuals and those results were correlated with dementia index (MMSE). In the women with ischemic stroke the measurements of adipokines were performed twice, on the 1st and 10th day. The results were correlated with stroke severity index (NIHSS).
Results: Among the women with AD we noticed the decreased levels of leptin and the increase in soluble leptin receptor concentration as well as in adiponectin levels in comparison with the results of the controls. In the women with brain ischemic stroke we found an altered adipokine profile (higher levels of leptin and resistin, the decreased concentrations of adiponectin and soluble leptin receptor), remaining in the 10th day of the stroke. The correlation between NIHSS and total adiponectin, HMW adiponectin and resistin was observed.
Conclusions: Alterations in adipokine concentrations in the presented diseases of the central nervous system may reflect the intensity of metabolic disturbances in the course of those disorders.
The study was supported by grants: MNiSW 2794/B/P01/2009/36; MNiSW 5484/B/P01/2011/40

Alzheimer's disease — challenge of 21-st century

Maria Barcikowska
Department of Neurodegenerative Disorders, Medical Research Centre, PAS, Warsaw, Poland

The development of basic research in the field of neurodegenerative diseases resulted in the need of a radical revaluation of methods of diagnosis, which also includes Alzheimer's disease (AD). The results of neuropathological tests indicated that in case of AD the presymptomatic stage can last even 20 years. The process of Alzheimer's type brain degeneration, which is the accumulation of toxic forms of amyloid beta (Ab) and the release of phospho-tau and also total tau protein from the damaged neutrons, starts long before the critical number of neutrons is damaged. This has been officially accepted in the NIA/AA criteria for AD diagnosis (2011), which establish the rules for recognizing AD while still in the

preclinical stage. This phase is crucial for treatment that can slow down the pathological process before the irreversible brain damage occurs. Clinically, during this phase of the disease a patient shows only slight symptoms of the ongoing process which can be recognized only using highly sophisticated examination. The clinical tests of medications that can be used in this stage of AD are currently already carried out. They are aimed at the population of persons who are the carriers of the gene mutations (APP and two presenilins) that are causative agents for AD. The so called sporadic cases in which the genetic background of the disease has still not been defined despite the ongoing GWAS examination will not be routinely diagnosed and treated in the preclinical phase for a long time. Apart from the genetic research aimed at diagnosing AD also biochemical biomarkers (Ab, phospho- and total tau CSF concentration) are widely used to diagnose the already ongoing pathological degeneration of Alzheimer's type, as well as more and more sophisticated methods of neuroimaging, such as using PIB in the PET as a marker of the presence of Ab in the living brain.

Macroprolactine detection with precipitation method — the evaluation of different technical variants

Karolina Beda-Maluga, Aleksandra Adamusiak, Hanna Pisarek, Jacek Świętosławski, Katarzyna Winczyk
Department of Neuroendocrinology, Chair of Laboratory Diagnostics, Medical University of Lodz

Introduction: Macroprolactin (MaPRL) is a large-molecule form of prolactin (PRL). It has limited biological activity but reacts with immunotests for PRL measurement and, therefore, in about 20% of cases may be the cause of the elevated hormone level. To detect macroforms, precipitation with polyethylene glycol (PEG) is used. This method involves the incubation and centrifugation of serum with PEG, then the measurement of PRL in supernatant and the evaluation of real PRL concentration or calculating the percent recovery of hormone. The parameters of centrifugation described in literature differ significantly. For this reason the aim of the study was to assess whether reducing the time and increasing the speed of rotation, allowing the quicker obtainment of the result, affects the amount of precipitate MaPRL, and, thus, the value of real PRL concentration and hormone recovery.
Material and methods: The study involved 56 sera obtained from patients treated in the Department of Endocrinology, Medical University of Lodz. Prolactin was measured with the use of enzyme-amplified chemiluminescent method (EACLIA) on the analyzer Immulite 1000 (Siemens). The sera, depending on the native PRL concentration were classified into the following groups: group 1: 10 sera with normal PRL level, group 2: 34 sera with hyperprolactinaemia below 100 ng/mL, group 3: 12 sera with hyperprolactinaemia above 100 ng/mL. Each serum was mixed with PEG, incubated and centrifuged according to the three options: variant I: 3000 rpm/30 min., variant II: 6000 rpm/8 min., variant III: 9000 rpm/4 min. In the obtained supernatants the PRL was measured and then, the concentrations of the real PRL concentration (hormone level after precipitation) were assessed as well as recovery values calculated by comparing the concentration of the hormone before and after precipitation.
Results: Average differences between the real PRL concentrations obtained with the use of the different variants of centrifugation were in: group 1 — 0.65 ng/mL, group 2 — 1.30 ng/mL, and in group 3 — 16.54 ng/mL. In turn, the recovery values of the hormone differed by an average of 3.18% in group 1, 2.76% in group 2 and 4.23% in group 3. The analysis showed that in all the groups the obtained differences are statistically significant, but the majority of them (87%) is within the error of the EACLIA method.
Conclusions: Modification of the parameters of centrifugation, such as speed and time, affect the amount of precipitated PRL macroforms, and, therefore, the value of the real concentration of the hormone and the percentage recovery. The differences between the values obtained in three PRL variants of rotation are statistically significant, but small differences do not affect the clinical interpretation of the results. The shortening of the time of precipitation using the appropriate speed lets obtain the similar, from a clinical point of view, efficiency of precipitation for each of the tested variants.
This study was supported by grant no 503/5-020-02/503-01.

Evaluation of pituitary function in patients with *macroprolactinoma* treated surgically

Karolina Beda-Maluga, Justyna Janocha, Hanna Pisarek, Julita Fuss-Chmielewska, Katarzyna Winczyk
Department of Neuroendocrinology, Chair of Laboratory Diagnostics, Medical University of Lodz

Introduction: The first-line treatment of *prolactinoma* is pharmacotherapy with dopamine agonists. However, in patients with poor tolerance, drug resistance or in case of a giant adenoma surgery is performed. The outcomes of surgical treatment are dependent mainly on the size of the tumour as well as on the experience of the surgeon. The aim of this study was to evaluate the pituitary function in patients after *prolactinoma* surgery both in terms of the treatment effectiveness and complications due to the surgical procedure.
Material and methods: A study group consisted of 27 patients (11 women and 16 men, aged 22–69 years) after *macroprolactinoma* surgery that had control test in the Clinic of Endocrinology, Medical University of Lodz. As the biochemical marker of cure, we assumed the normalization of PRL level — that means hormone concentration within reference range. We also evaluated the concentrations of other pituitary hormones and the hormones secreted by peripheral glands.
Results: The normalization of PRL concentration was achieved in 20/27 (74%) patients — a correct pituitary function was restored in five of them, hypopituitarism was noted in 15 persons. In seven subjects PRL level after the surgery was still elevated (two patients with proper pituitary function, five persons with hypopituitarism). Transsphenoidal surgery was performed in 12 patients - the normalization of PRL level was achieved in ten of them, an incorrect pituitary function was observed in nine. In all the persons after craniotomy hypopituitarism occurred and normal PRL level was achieved in half of them.
Conclusions: The surgical resection of *macroprolactinoma* leads to the normalization of PRL in almost 3/4 of patients. Hypopituitarism incidence after surgery is high — occurs in, approximately, 70% of persons. Transsphenoidal operation is associated with a lower risk of hypopituitarism than transcranial resection of the tumour.

Evaluation of pituitary function in patients with *corticotropinoma* treated surgically

Karolina Beda-Maluga, Ewelina Ślusarczyk, Hanna Pisarek, Julita Fuss-Chmielewska, Katarzyna Winczyk
Department of Neuroendocrinology, Chair of Laboratory Diagnostics, Medical University of Lodz

Introduction: In patients with Cushing's disease caused by *corticotropinoma* the treatment of choice is the surgical removal of the adenoma with an access via the sphenoid sinus. The aim of the study was to

evaluate the pituitary function in patients after *corticotropinoma* resection both in terms of efficacy and complications after the surgery.
Material and methods: The study group consisted of 21 patients (16 women and 5 men aged 28-68 years) operated due to *corticotropinoma* and, in order to perform control tests, hospitalized in the Department of Endocrinology, Medical University of Lodz. The effectiveness of neurosurgical treatment was assessed on the basis of morning concentrations of ACTH and cortisol, circadian secretion of these hormones and/or dexamethasone suppression test results. Postoperative pituitary hormonal function was assessed on the basis of concentrations of hormones secreted by the pituitary and peripheral endocrine glands.
Results: The majority of the operated patients (18/21) had *microadenoma*. The cure of Cushing's disease was achieved in 15/21 patients (71%) — in 4 of them (including two after reoperation) postoperative hypopituitarism was noted. In 6 patients (29%) the neurosurgical procedure did not lead to the normalization of cortisol levels — only one of these persons (after reoperation) had hypopituitarism.
Conclusions: The surgical resection of *corticotropinoma* is an effective method of Cushing's disease treatment and quite rarely — usually after reoperation — leads to hypopituitarism.

Advances in diagnostics and therapy of acromegaly

Marek Bolanowski
Department of Endocrinology, Diabetes and Isotope Therapy, Medical University Wrocław

Significant advances in the diagnostics and therapy of acromegaly have been noticed recently. There are reports on the administration of new formulations (octreotide implant and oral form), a new analogue (pasireotide) in the direct comparison to octreotide, and the efficacy of first-line lanreotide medical therapy regarding tumour volume decrease and hormonal normalization (clinical study PRIMARYS). Current guidelines (Polish and international) were published. They focus on severe acromegaly complications and the precise rules of the management of patients. IGF-1 concentration is crucial for screening and monitoring of the diseases course.

Neuroendocrine responses to opioid receptor blockade during repeated forced exercise in pigs with mutated ryanodine receptor gene

Ziemowit Ciepielewski, Wojciech Stojek, Dorota Myślińska, Edyta Jurkowlaniec-Kopeć
Department of Animal and Human Physiology, Faculty of Biology, University of Gdansk, Gdansk, Poland

Introduction and Aim: The aim of the study was to determine the role of the opioid receptor system during rest and exercise in pigs with a mutation in the ryanodine receptor gene (*RYR1*). In the present study, in 60 catheterized male pigs (Polish synthetic line 990), the relation of the mutation in *RYR1* gene with the neuroendocrine responses during the repeated exercise (forced walking, FW) was investigated after the opioid receptor blockade with non-specific antagonist naloxone (NX, 1 mg/kg BW).
Methods: The pigs were divided (molecular assay screening mutation of the *RYR1* gene) into three groups: stress susceptible homozygotes-nn; and stress-resistant: heterozygotes-Nn and homozygotes-NN were subjected to FW twice (20-min, with an hour rest between FWs) in the treadmill. Plasma concentrations of adrenaline (A), noradrenaline (NA), (HPLC) and prolactin (PRL), growth hormone (GH), beta-endorphin (BEND), adrenocorticotropin (ACTH) and cortisol (COR) (RIA) were measured in the presence or absence of NX.
Results: It was found that FW alone increased ($p < 0.05$) plasma concentrations of all the measured hormones and the effects were dependent on the genotype of the pig. The greatest concentrations of A, PRL and BEND were observed in nn homozygous pigs, and the greatest concentration of NA and GH in NN homozygous pigs. Plasma ACTH and COR did not differ between *RYR1* genotypes. Moreover, the increases in PRL and GH were observed only after the first FW. During FW preceded with NX the increases in plasma concentrations of A, PRL, BEND were diminished ($p < 0.05$), in all three genotypes and plasma GH and ACTH were augmented ($p < 0.05$) as compared to the pigs exercised without NX. The NX-induced diminishing effects were more pronounced in nn pigs and augmenting effects in NN pigs.
Conclusion: The results support a regulatory effect of the opioid receptor system activation on neuroendocrine responses during exercise and the intensification of the opioid activation may depend on *RYR1* gene status.

Galanin-like peptide modifies feeding behavior, water intake and the hypothalamo-neurohypophysial system function of Wistar rats

Joanna Ciosek
Department of Neuropeptides Research, Chair of General and Experimental Pathology, Medical University of Lodz, Poland

Introduction: Galanin-like peptide (GALP), being an important member of galanin neuropeptides family, is present in some areas of the central nervous system. GALP may be involved in the mechanisms controlling some processes connected to energy metabolism. Some previous findings indicate the ability of GALP to alter vasopressin (AVP) and oxytocin (OT) secretion from the posterior pituitary gland.
Aim of the study: This experiment was performed to study the effects of GALP, injected intravenously (*iv*), on food and water intake as well as body weight changes of experimental animals; AVP and OT release from the neurohypophysis (NH) into blood was also estimated.
Material and methods: Male adult Wistar rats were divided into two series: series A — rats injected *iv* with 0.9% NaCl; series B — rats injected *iv* with GALP in a dose of 50 ng/100 g b.w. The appropriate solutions were administered twice a day: in the morning (8.30–9.00 a.m.) and in the evening (5.30–6.00 p.m.); the number of injections: group I, II and III — 3, 5 or 7, respectively. AVP and OT content in the hypothalamus, neurohypophysis and the blood were estimated by radioimmunoassay.
Results: GALP was the reason of distinctly increased water and food intake as well as body weight of the experimental animals. In all the groups the tendency to impair AVP and OT release into the blood connected with the increase of both neurohormones content in the neurohypophysis has been observed.
Conclusions: It may be assumed that GALP, injected intravenously, modifies such systemic mechanisms which are engaged in the changes of energy metabolism. Simultaneously, GALP plays the role of inhibitory neuromodulator of AVP and OT release.
This study was supported by grant of Medical University of Lodz no 503/6-103-02/503-01.

Melatonin exerts regulatory effect on oxytocin release in rats with myocardial infarction

Joanna Ciosek, Anna Krzymińska, Jacek Drobnik
Department of Neuropeptides Research, Chair of General and Experimental Pathology, Medical University of Lodz, Poland

Introduction: The increased secretion of both vasopressin (AVP) and oxytocin (OT) is frequently observed in subjects with heart failure. The pineal gland and melatonin regulatory effect on the hypothalamo-neurohypophysial system has been reported.
The present study is aimed at verifying whether melatonin (MLT) exerts a regulatory effect on the secretion of oxytocin from the hypothalamo-neurohypophysial system in rats with myocardial infarction (MI).
Material and Methods: Myocardial infarction was induced by the ligation of the left coronary artery. OT content was radioimmunoasayed in control groups, rats treated with vehicle (2% ethanol solution in 0.9% NaCl), MLT injected animals (60 μg/100 g body weight), sham-operated or pinealectomized rats as well as pinealectomized rats treated with vehicle or melatonin.
Results: Elevated OT level in the serum of rats with MI was linked with the lower content of this hormone in the hypothalamus and neurohypohysis. MLT decreased concentration of OT in the serum but both in the hypothalamus and neurohypophysis content of this neurohormone was elevated. Contrary to melatonin, pinealectomy increases OT level in blood plasma and decreases the content of this hormone in the neurohypohysis. The application of MLT to the pinealectomized rats results in the lowering of OT concentration in blood plasma and increases this neurohormone content in the neurohypophysis and hypothalamus.
Conclusions: Melatonin inhibits the release of oxytocin from the neurohypophysis and decreases the concentration of this hormone in the serum of rats with myocardial infarction. The results of this study show that melatonin turns away the effect of myocardial infarction and restricts the increased oxytocin release.
This study was supported by grant of Medical University of Lodz no 503/6-103-02/503-01.

Neurohormonal changes after surgical treatment for obesity

Michał Dyaczyński[1], Grzegorz Kowalski[1], Krystyna Pierzchała-Koziec[2], Joanna Zubel[2], Ewa Ocłoń[2], Anna Latacz[2], Klaudia Pałka[2]
[1]Department of General Surgery, Subdepartment of Metabolic and Bariatric Surgery, District Railway Hospital, Katowice, Poland
[2]University of Agriculture, Krakow, Poland

Introduction: The effectiveness of surgical treatment for obesity is much higher than standard methods. However, surgery has side effects by itself and also the postbariatric convalescence affects the activity of the hypothalamo-intestinal axis. Clinical investigations showed serious changes of nervous, immune and endocrine systems in patients after the surgery.
The aim of the experiment was to estimate the changes of neurotransmitters and hormones in animal models (pigs and rats) after bariatric surgery. Additionally, the effects of synbiotics supplementation were tested in these animals.
Material and methods: Two weeks (pigs) or four weeks (rats) after bariatric surgery blood and fragments of tissues-duodenum, stomach, jejunum, pancreas and brain-were taken. The concentration, secretion and synthesis of Met-encephalin (neurotransmitter) were estimated by RIA method. Also, the levels of ghrelin, insulin, glucagon and the lipid and glycemic profiles were measured.
Results: The results clearly showed the changes in the synthesis and secretion of Met-encephalin in the gastrointestinal and brain fragments. The levels of all the tested parameters were significantly changed by the bariatric procedures. Synbiotic modulated the hormonal and biochemical changes.
Conclusion: Bariatric surgery has a long term impact on the activity of hypothalamo-intestinal axis, particularly on the neurohormonal parameters.
Supported by Grant NCBiR 12006406, DS3243/KFiEZ

Review of fractionated radiotherapy and stereotactic radiosurgery for medically and surgically refractory pituitary adenomas

Jacek Fijuth
Department of Radiotherapy, Chair of Oncology, Medical University of Lodz

Pituitary adenomas are relatively common tumors, comprising approximately 10-20% of all primary intracranial lesions, with a reported incidence of up to 20% within the general population. For decades, radiation therapy has served as an adjuvant treatment for residual or recurrent pituitary adenomas or as a primary treatment in patients who are not candidates for surgery. While surgical intervention remains the first-line therapy, stereotactic radiosurgery is increasingly recognized as a viable treatment option for these often challenging tumors. Despite advances in surgical techniques and medical therapies, a significant proportion of pituitary adenomas remain hormonally active, demonstrate persistent radiographic disease, or recur when followed for long periods of time. Stereotactic radiosurgery (SRS) has recently received a great deal of attention in the secondary treatment of pituitary adenomas. The ability to deliver the full treatment dose within one session, and the theoretically reduced risk of injury to nearby neural structures on account of the conformal nature of the beams gained SRS immediate popularity. The hormonal remission rates vary by tumor type: from 20-30% in prolactinoma, ~50% in growth hormone secreting adenomas, to 40-65% in adrenocorticotrophic hormone (ACTH)-secreting adenomas. The time to remission radiographic control rates is almost universally greater than 90% with long-term follow-up. However, given the relative novelty of SRS compared to conventional radiotherapy, long-term studies on the efficacy and complications of SRS are still being acquired. The authors stratify the outcomes by tumor type, review the importance of prognostic factors (particularly, pre-treatment endocrinologic function and tumor size), and discuss the complications of treatment with special attention to endocrinopathy and visual complications. The authors conclude that the literature supports the use of radiosurgery for treatment-refractory pituitary adenomas, providing the patient with a minimally invasive, safe, and effective treatment option for an otherwise resistant tumor. The authors present their own experience and provide literature-based treatment considerations, including radiosurgical dose, endocrinologic, radiographic, and medical considerations for each adenoma type.

New aspects of Parkinson's disease

Urszula Fiszer[1], Małgorzata Michałowska[1], Bogusława Baranowska[2]
[1]Department of Neurology and Epileptology, Medical Centre of Postgraduate Education, Warsaw
[2]Department of Endocrinology, Medical Centre of Postgraduate Education, Warsaw

Introduction: Parkinson's disease (PD) is a progressive multisystem disorder that includes motor and non-motor symptoms. Recently, several PD subtypes have been identified but the pathogenic mechanisms underlying the observed clinicopathologic heterogeneity in PD are still not well understood. Inflammation bas been proposed

as one of the factors contributing to the onset and progression of neuronal death in PD. Studies have shown that PD patients present lower body weights in comparison with age-matched subjects. The mechanism underlying the weight loss in PD is unknown.
To investigate the role of leptin, ghrelin, GH and IGF-1 in energy balance disturbances in Parkinson's disease (PD). Adipokines are of great interest since they are involved in inflammation and other physiological processes.
Materials and methods: The study included 39 patients: 11 PD patients with unintentional weight loss, 16 PD patients without weight loss and 12 controls. UPDRS, MMSE, MADRS, appetite scale, BMI, adipose tissue content, plasma leptin and active ghrelin concentrations and serum GH, IGF-1, TSH, T3 and T4 concentrations were evaluated.
Results: A lower plasma leptin concentration and a higher serum IGF-1 concentration were found in PD patients with weight loss. BMI and the content of adipose tissue were positively correlated with leptin concentration in all PD patients. However, the lower BMI was, the lower plasma active ghrelin concentration was in PD patients with weight loss.
Conclusion: These findings confirm that the changes of plasma leptin concentration occur in PD patients with the loss of weight.
This work was supported by Medical Centre of Postgraduate Education in Warsaw (grant no. 501-1-1-13-5428-05).

Biochemical evaluation of treatment effectiveness in patients with acromegaly

Julita Fuss-Chmielewska, Aleksandra Rosiak, Hanna Pisarek, Karolina Beda-Maluga, Katarzyna Winczyk
Department of Neuroendocrinology, Chair of Laboratory Diagnostics, Medical University of Lodz

Introduction: In patients with *somatotropinoma* the surgical removal of the tumour is routinely performed. Pharmacotherapy, usually with somatostatin analogues (SSA), is used as the preliminary and/or complementary therapy. In cases of ineffective surgical and pharmacological treatment, though less frequently, radiotherapy is applied. The aim of this study was to evaluate the concentration of growth hormone (GH) and insulin-like growth factor 1 (IGF-1) — biochemical parameters of acromegaly treatment efficacy.
Material and methods: The study group consisted of patients after the removal of the pituitary *somatotropinoma* — 27 women and 13 men aged 25 to 83 years — hospitalized in the Clinic of Endocrinology, Medical University of Lodz. The concentrations of GH and IGF-1 in blood serum were determined with the use of the immunochemical analyzer IMMULITE 1000 (Siemens). As the criteria of acromegaly cure, we assumed the normalization of serum IGF-1 concentration and GH level after the administration of glucose (OGTT) less than 1 ng/mL.
Results: In the study group, 25 of 40 patients (63%) fulfilled the cure criteria. In 14 patients, the normalization of IGF-1 and GH were obtained as a result of neurosurgery only, 11 patients achieved normal laboratory parameters after surgery and additional treatment — including five after the SSA administration, four after radiotherapy, and two after pharmaco- and radiotherapy together. The normalization of GH and/or IGF-1 was not observed in 15 operated patients: 13 were treated with SSA after the operation and four of them also before the surgery. The group of 21 patients was treated with SSA before surgery and among them only five were not cured.
Conclusions: In most patients with acromegaly after neurosurgical resection of the pituitary adenoma the complementary therapy — mainly pharmacological — is needed. The preoperative administration of somatostatin analogues significantly increases the effectiveness of the treatment.

Regulatory role OF Wnt/B-catenin and BMPs signaling in gonadotropin network genes expression

Alina Gajewska, Marlena Zielińska-Górska
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jablonna n. Warsaw

Upon binding to its G-protein coupled receptor, gonadoliberin (GnRH) induces a signal which then flows through MAPK cascades leading to the phosphorylation of a number of downstream targets including several DNA-binding proteins and the regulation of gonadotropin gene transcription and secretion. The effort to identify the full spectrum of signal transduction pathways targeted by the type I GnRHR revealed a crucial role of Wnt/β-catenin transduction in gonadotrope cells. β-catenin is a transcriptional coactivator associated with T cell factor/Lymphoid enhancer factor — responsive genes regulated by the WNT family of secreted glycoproteins. The dependence of Wnt/β-catenin signaling on GnRHR activation was shown in the studies on gonadotroph-derived LβT2 cell line according to GnRH-stimulated nuclear localization of β-catenin and parallel increases in GnRH-dependent mRNAs specific for Jun, Fra1 and Myc. Wnt/β-catenin signaling regulates steroidogenic transcription factor (SF-1) gene activity. SF-1 response elements are found in the promoter regions of four gonadotrope signature genes: LHβ, FSHβ α-GSU and GnRH-R. β-catenin and Dax-1 act as SF-1 transcriptional coactivator/repressor, respectively, to affect SF-1 mediated transcription of its target genes. The studies on LβT2 cells also evidenced that locally produced bone morphogenetic proteins (BMPs) form a signaling system that acts as a major functional regulator of pituitary cells function. Accumulating evidence indicates that BMPs operate through Smad-independent pathways such as MAPK signaling molecules and convergent effects of independent pathways elicited by BMPs/activin and GnRH are likely linked to the combined response leading to the effective amplification of FSHβ transcription. Our *in vivo* studies revealed that the central and steroidal modulation of endogenous GnRH neurons activity had an impact on Wnt/βcatenin and BMPs signaling pathways and these effects were exerted at specific SF-1/βcatenin/Dax-1 and selected BMPs genes transcriptional level.

cAMP/PKA pathway signaling in the anterior pituitary cells *in vitro*: intracellular mechanisms of Cu-GnRH complex activity

Alina Gajewska[1], Marlena Zielińska-Górska[1], Ewa Wolinska-Witort[2], Marta Baran[1], Grzegorz Kotarba[2], Kazimierz Kochman[1]
[1]The Kielanowski Institute of Animal Physiology and Nutrition, PAS, Jablonna n. Warsaw
[2]Medical Centre of Postgraduate Education, Warsaw

Introduction: Complex Cu-GnRH is a gonadoliberin analog which preserves an amino acid sequence identical like in native decapeptide but contains Cu^{2+} ion bound to the histidine (His^2). In previous studies we found that Cu-GnRH stimulates gonadotropin release more potently, exhibits the reduced susceptibility to proteolytic cleavage and is able to activate cAMP/PKA signaling in the anterior pituitary cells.
The determination whether specific receptors (GnRHR and/or PAC-1) and protein kinases C and/or A are involved in Cu-GnRH-

induced cAMP accumulation. The evaluation of Cu-GnRH effect on selected gonadotrophic network genes mRNA expression.
Material and Methods: 5×10^5/mL anterior pituitary cells were incubated (37°C) for 0.5–1–3 h in DMEM with 10^{-7}M Cu-GnRH with/without GnRHR antagonists (antide, cetrorelix), PAC-1 receptor ($PACAP_{6-38}$), PKA (H89, KT 5720) and PKC (GF 109203X) inhibitors, Ca^{2+}-selective ionophore A23187 and cycloheximide. Intracellular cAMP level and LH medium concentration were determined by RIA. 60 anterior pituitary glands from diestrus females were incubated for 2 and 5 h (37°C) in M199 medium with 10^{-6}M Cu-GnRH in the presence of KT 5720. Egr-1, LHβ, GnRHR PAC-1 and NOS1 mRNA expression were analyzed by qRT-PCR method.
Results: Cu-GnRH-induced cAMP accumulation was dependent on GnRHR as well as PAC-1 receptors activation. Although *de novo* protein synthesis was not required, an intracellular, Ca^{2+} and Ca^{2+}/calmodulin-dependent adenylate cyclase might be involved in this process. Complex activity was mediated by PKA however, no impact of PKC was found. PKA activation inhibited mRNA expression of two crucial gonadotropic network genes: Egr-1 and LHβ. A pro-gonadotropic Cu-GnRH effect was exerted via stimulation of GnRHR and NOS-1 mRNA expression.
Conclusions: Cu-GnRH conformation enables the interaction with GnRHR and PAC-1 receptors. Complex-induced cAMP/PKA signaling is involved in gene-specific impact exerted by Cu-GnRH on gonadotrophic network transcriptional activity.
Supported by grant KBN: N N311 406739

Oxytocin receptor gene expression in anterior pituitary of lactating ewe in the aspect of prolactin secretion regulation

Konrad Górski, Elżbieta Marciniak, Małgorzata Hasiec, Tomasz Misztal
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jablonna n. Warsaw

Introduction: Prolactin (PRL) secretion is regulated by the complex network of hormones and neurotransmitters. Numerous data indicate that one of them is oxytocin (OT) which shows stimulating action on the PRL secretion. Furthermore, in rat anterior pituitary (AP) the expression of oxytocin receptor (OTR) occurs and this expression increases during lactation. Another novel PRL stimulating factor is salsolinol, a dopamine derived compound, synthesized in hypothalamic dopaminergic neurons. Salsolinol was demonstrated to play a pivotal role in the mechanism of regulation of the PRL secretion in a lactation period. Our previous studies showed that salsolinol also stimulates OT expression and secretion in lactating sheep.
The aim of the present study was to compare OTR expression in AP between anestrous and lactating ewes and investigate the effect of salsolinol on this expression.
Material and methods: The experiment was conducted on anestrous ewes and lactating ewes during a period of weaning (9th week of lactation). Two days before the experiment lambs were separated from their mothers to avoid the stimulation of the teats. 5-hour infusions of salsolinol (5×30 μg/60 μl/30 min) or Ringer-Locke's (control) solutions into the III ventricle of the brain were performed. After the infusions the animals were sacrificed and AP was dissected. Total RNA from each tissue was extracted and converted to cDNA. The relative amount of specific OTR transcript was determined by the real-time qPCR.
Results: In the lactating ewes OTR gene expression was significantly ($p < 0.01$) upregulated when compared to the anestrous ewes. Administered salsolinol evoked a significant ($p < 0.05$) increase of OTR gene expression in the anestrous ewes. In turn, in the lactating sheep the effect of infused salsolinol on OTR gene expression was not observed.
Conclusions: The upregulation of OTR gene expression in AP of lactating ewes may have an effect on the increased sensitivity of lactotrophs to the OT action and, in consequence, the increase of PRL release. Salsolinol may also contribute to this action.
Work was supported by NCN — grant NN 311 5171 40.

The effect of 1-MeDIQ (salsolinol's analogue) on the release of corticotrophin and cortisol in lactating sheep

Małgorzata Hasiec, Elżbieta Marciniak, Magdalena Cieślak, Konrad Górski, Katarzyna Romanowicz, Tomasz Misztal
Department of Endocrinology, The Kielanowski Institute of Animals Physiology and Nutrition PAS, Jabłonna

Introduction: Our recent studies show that salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroiso-quinoline), present in the hypothalamus of lactating sheep, inhibits stress-induced hypothalamic-pituitary-adrenal (HPA) axis activity. To study the physiological functions of salsolinol its structural analogue 1-MeDIQ (1-methyl-3,4-dihydroisoquinoline) has been used which has antagonistic properties to salsolinol especially with regard to the prolactin release.
The purpose of the research was to investigate the effect of intracerebroventricularly infused 1-MeDIQ on the basal activity of the HPA axis in lactating sheep.
Material and methods: The experiment was performed on lactating sheeps (n = 8) implanted with two guide cannulae: one into the third ventricle of the brain (IIIv) and the other one into the hypothalamus (infundibular nucleus/median eminence, IN/ME). Every animal was used twice at three day interval. During the 4-hour experiments the animals were infused with Ringer-Locke's (control) or 1-MeDIQ (1 μg/μL) solutions, with flow rate of 2 μL/min. The treatment was performed in a series of four 30-min infusions at 30-min intervals. Simultaneously, push-pull perfusions of the IN/ME were performed to measure corticoliberin (CRH) concentrations. Moreover, using a catheter inserted into the jugular vein, blood samples were collected every 10 minutes to determine corticotrophin (ACTH) and cortisol concentrations.
Results: The hourly distribution of hormone concentrations shows that 1-MeDIQ-infused group had significantly higher plasma ACTH concentrations during the 2nd, 3rd and 4th hours, as well as plasma cortisol concentration during the 4th hour of the experiments. No significant differences were observed in perfusate CRH concentrations between 1-MeDIQ and control groups.
Conclusion: On the basis of the results obtained after the central 1-MeDIQ treatment in lactating sheep, we suggest that salsolinol inhibits the basal activity of the HPA axis at least at the pituitary level.

Effect of IL-1β on melatonin secretion from sheep pineal explants — *ex vivo* study

Andrzej Przemysław Herman, Karolina Król, Agata Krawczyńska, Elżbieta Marciniak, Hanna Antushevich, Marlena Zielińska-Górska, Dorota Tomaszewska-Zaremba
The Kielanowski Institute of Animal Physiology and Nutrition PAS, Jablonna

Introduction: The pineal hormone melatonin plays an important role in the neuroendocrine control of reproductive physiology. The

importance of melatonin secretion for reproduction in seasonally breeding animals has been well established. It could be speculated that inflammation may also disturb the reproduction process indirectly affecting the melatonin secretion from the pineal tissue. The study was designed to determine the effect of interleukin (IL)-1β on the melatonin secretion and the expression of enzymes: arylalkylamine N acetyltransferase AA-NAT and O-methyltransferase (HIOMT) involved in melatonin synthesis from the pineal explants and to explain whether the inflammation induced by lipopolysaccharide (LPS) treatment affects the response to IL-1β

Material and Methods: The experiment was carried out during a short-day period. Animals were intravenously injected by LPS (400 ng/mL) or NaCl, 2 hours after sunset. The sheep were euthanized 3 h after the injection. Four fragments of the pineal gland dissected from each ewe were divided into four groups incubated with: (1) 'pure' medium 199, (2) 10 pM of Noradrenaline (NE), (3) 75 pg/mL of IL-1β and (4) NE and IL-1β at 37°C and 95% and 5% CO_2. The melatonin concentration in the media was assayed using RIA, the gene and protein expression of AA-NAT and HIOMT was determined using Real-Time PCR and Western Blot.

Results: IL-1β completely reduced ($p < 0.01$) NE stimulated the secretion of melatonin and the expression of AA-NAT from all the explants. Moreover, NE induced release of melatonin was significantly ($p < 0.01$) lower in the endotoxin treated group. No effect of NE or IL-1β on HIOMT gene and protein expression was found.

Conclusion: The obtained results suggest that IL-1β is a potent modulator of melatonin secretion. Some differences in the secretion of melatonin suggest that inflammation influences the pineal gland activity in a prolonged manner and it could affect its responsiveness even after the deprivation of inflammatory signals.

This work was supported by grant NCN UMO-2011/03/D/NZ9/04021

The influence of melatonin receptors antagonists, luzindole and 4-P-PDOT, on melatonin-dependent vasopressin release from the rat hypothalamo--neurohypophysial system: *in vitro* studies

Marlena Juszczak, Magdalena Roszczyk
Department of Pathophysiology and Experimental Neuroendocrinology, Medical University of Lodz, Poland

Introduction: The aim of the present investigation was to study the effect of melatonin (MT) membrane receptors antagonists, i.e. luzindole (a non-selective antagonist of MT_1 and MT_2 receptors) and 4-phenyl-2-propionamidotetralin (4-P-PDOT - a selective antagonist of MT_2 receptor), on MT-dependent vasopressin (AVP) secretion from the rat hypothalamo-neurohypophysial (H-N) system *in vitro*.

Material and methods: Male rats served as donors of the H-N explants, which were placed in 1 mL of Krebs-Ringer fluid (KRF) heated to 37°C. Each explant was incubated successively in: 1 — normal KRF {fluid B1}, 2 — the incubation fluid as B1 alone or containing the following substances: MT (at the concentrations of 10^{-9}, 10^{-7} or 10^{-3} M) and luzindole or 4-P-PDOT, or their vehicles {fluid B2}. After 20 min incubation, each medium was collected and immediately frozen before AVP estimation by the RIA.

Results: MT, at the concentrations of 10^{-9} and 10^{-7} M, significantly inhibited AVP secretion from the isolated rat H-N explants. Luzindole significantly suppressed MT-dependent effect, while 4-P-PDOT did not eliminate the inhibitory influence of MT on AVP secretion *in vitro*. At the concentration of 10^{3} M, MT significantly stimulated AVP release when H-N explants were incubated in the medium containing luzindole or 4-P-PDOT.

Conclusions: The present study demonstrates that MT_1 receptor may contribute to the inhibitory effect of MT (at a concentration close to its physiological level in the blood) on AVP secretion in the rat. However, for the stimulatory effect of pharmacological dose of the hormone on AVP release, mechanisms different from MT_1/MT_2 receptors are involved.

This study was supported by Medical University of Lodz (grant No 502-03/6-103-01/502-64-013).

The estrogen-dependent modulation of prolactin secretion by orexins in the pituitary primary culture mature female rats

Malgorzata Kalisz[1], Lidia Martynska[1], Ewa Wolinska-Witort[1], Magdalena Chmielowska[1], Anna Litwiniuk[1], Wojciech Bik[1], Boguslawa Baranowska[2]
[1]*Department of Clinical Neuroendocrinology, Centre of Postgraduate Medical Education, Warsaw*
[2]*Department of Neurology, Second Faculty of Medicine Medical University of Warsaw*

Introduction: Orexin A (OxA) and orexin B (OxB) are 33 and 28 amino acid hypothalamic neuropeptides involved in the regulation of the neuroendocrine and the reproductive system. Prolactin (PRL) release from the pituitary is regulated both by local factors and by factors originating from the hypothalamus via the pituitary portal circulation. Estrogen is known to have a stimulatory role in PRL synthesis and release.

To investigate the effect of OxA and OxB on PRL secretion in the pituitary primary culture mature female rat.

Material and method: Pituitary cells were isolated from three-month-old mature ovariectomized female rats (OVX) and female OVX subcutaneous estradiol supplementation (OVX + E_2). OxA and OxB were tested at escalating doses evaluating PRL secretion after 1 and 4 hours. PRL concentration was measured in the culture medium by radioimmunoassay (RIA).

Results: It was found that after 1 hour incubation with OxA and OxB the secretion of PRL increased in OVX group, however, decreased in OVX + E_2 group compared with the control group. After 4 h incubation with OxB (1 nM; $p < 0.05$) PRL secretion was decreased in OVX group. In the OVX+E_2 group incubation with OxA (100 nM $p < 0.05$) and OxB (1 nM; $p < 0.05$) decreased PRL secretion compared with the control group.

Conclusion: It was demonstrated that OxA and OxB may modulate prolactin secretion depending on estrogen status of female rats and the duration of pituitary incubation.

This study was supported by CMKP grants 501-1-31-22-13 and 501-1-31-22-13.

Giant prolactinomas (GP) therapeutic and diagnostic challenges

Aldona Kowalska[1] Iwona Pałyga[1] Danuta Gąsior-Perczak[1] Wiesław Bonicki[2]
Department of Endocrinology Holycross Cancer Centre, Kielce, Poland, Department of Neurosurgery, The Maria Skłodowska-Curie Memorial Cancer Centre and the Institute of Oncology, Warsaw, Poland

Introduction: GP are rare pituitary tumors. The prevalence ranges between 0.5–4.4% of all pituitary tumors and exceeds about 4% of prolactinomas. Due to the different directions of expansion GP can manifest in a diverse set of symptoms. Very large sizes of GP and infiltration of relevant structures of the brain cause a therapeutic challenge.

The aim of this work is to present our own experience with patients with GP. The characteristic of the patients: there has been 7 patients treated with GP in our centre: 2 women and 5 men. Women aged 42 and 45: hormonal disturbances (primary or secondary amenorrhoea) 2/2, without an impairment of other tropic functions 0/2. Mass effect symptoms: persistent headaches 2/2 with no visual disturbances 0/2. Prolactin (PRL) level at diagnosis — 62000-100933 mIU/L (N: < 540 mIU/mL). Maximal dimension of the tumors were 70 and 61 mm. Treatment with dopamine agonists (AD) in the first patient caused reduction in tumor size and normalization of PRL level. In the second patient: combined treatment with neurosurgery (NS) + AD -macroscopic removal of the tumor and AD caused normalization of prolactin levels and restoration of the menses but after AD treatment. Men in the age of 23 to 53 years. Hormonal disturbances: pituitary multiaxial 2/5, mass effect symptoms: headaches 5/5, visual acuity 5/5, temporal visual field defect 2/5, diplopia 1/5. PRL levels from 189900 mIU/L to 230269 mIU/L (N: < 360 mIU/mL). Maximal tumor size from 45mm to 55 mm. Treatment only with AD: 4/5, associated Nch AD-1/5. Reducing tumor size: 5/5 normalization Prl 3/5. Diagnostic trouble: 2/7 initial laryngological diagnosis as sphenoid and ethmoid sinus tumors with the destruction of bone.
Conclusions:
1. Due to the different clinical picture GP may cause a diagnostic problem
2. There is need for combined therapy in many cases of GP.

Influence of thyreoliberin (TRH) on the regulation of the myofibroblasts isolated from cardiac infarction scar

Anna Krzymińska, Jacek Drobnik, Alicja Szczepanowska, Joanna Ciosek
Department of Neuropeptides Research Chair of General and Experimental Pathology, Medical University of Lodz, Poland

Introduction: Thyreoliberin (TRH), synthesized in the hypothalamus, is also produced peripherally where it exerts its effects via TRH receptors which have been detected, inter alia, in the heart. It has shown a positive inotropic effect on the cardiovascular system in myocardial infarction scar which is probably due to TRH protective influence on the contractility of the necrotic area. Fibroblasts migration and proliferation as well as their transformation into myofibroblasts are crucial for a post-infarction scar formation. For strengthening and remodeling of a scar, the deposition of extracellular matrix produced by myofibroblasts particularly of collagen fibers, is essential and associated with the increased area rigidity and the progressive development of the heart failure. Controlling the activity of myofibroblasts will allow defining the antiremodeling treatment of myocardial infarction.
The aim of the study was to clarify whether and in what way the action of myofibroblasts is regulated by TRH as well as by TRH analog (taltirelin).
Material and methods: Myofibroblasts were obtained from rats with the induced myocardial infarction. The influence of TRH was investigated in cultures with the addition of TRH and taltirelin. The metabolism of myofibroblasts was evaluated by measuring the concentration of total collagen.
Results: The TRH concentration range from $1*10^{-11}$ to $1*10^{-7}$M similarly to taltirelin increases the total cells number but contrary to TRH analog it inhibits collagen synthesis.
Conclusions: The reported data indicate that taltirelin acting in CNS via TRH-R1 receptors shows (like TRH) antinociceptive and antidepressant influence. This effect differs from the results obtained from myofibroblasts culturing.
The research was funded by the Medical University of Lodz (grant No 503/6-103-02/503-01).

The effects of somatostatin analogues treatment in patients with non-functioning pituitary adenomas and in acromegaly as primary and secondary therapy

Jolanta Kunert-Radek[1], Natalia Zawada[1], Marek Pawlikowski[2], Hanna Pisarek[3], Maciej Radek[4]
[1]Clinic of Endocrinology, Medical University of Lodz
[2]Department of Immunoendocrinology, Medical University of Lodz
[3]Department of Neuroendocrinology, Medical University of Lodz [4]Clinic of Neurosurgery and Peripheral Nerve Surgery, Medical University of Lodz

Introduction: Somatostatin analogues (SSA) are used in the treatment of somatotropinomas and thyreotropinomas. The latest publications focus on the use of these drugs in the therapy of clinically non-functioning pituitary adenomas (NFPA), especially in recurrent tumours after incomplete surgical resections.
Material and methods: The effectiveness of SSA therapy in patients treated in The Department of Clinical Endocrinology in Lodz was assessed. The material involved 40 acromegalic patients and 22 patients with NFPA treated by SSA up to ten years. Hormonal profile, the dynamics of tumour size changes, ophthalmic exam with the assessment of visual field, somatostatin receptor scintigraphy, the immunohistochemistry of operated tumours and of somatostatin receptor subtypes as well as patients' quality of life and undesirable effects were analysed. The effects of SSA treatment in patients with NFPA in comparison to patients with acromegaly.
Results: The significant decrease of growth hormone and IGF-1 concentrations was noticed in 95% of the patients with acromegaly (16 recurrent tumours and 24 primary tumours). The complete normalisation of hormone levels was observed in 50% of patients. The evaluation of tumours' size in MRI revealed the significant reduction of tumour volume in 40% of patients. Most of tumour regression was noticed in primary adenomas. There was the improvement of quality of life and clinical condition in nearly all the patients. 5% of the patients developed symptomatic *cholelithiasis* that regarded an operation. Considering NFPA (17 — recurrent tumours and 5 primary tumours), the stabilization of tumour size and visual field was observed in 68% of the patients while the reduction of tumour volume was noticed in 9% of the cases. In 23% of the patients there was the increase of adenoma size that was qualified for operation.
Conclusions: Generally, somatostatin analogues are effective in the treatment of acromegaly. In the patients with NFPA the effectiveness of SSA is much lower. However, in the most cases of NFPA the pharmacotherapy with SSA leads to the stabilisation of the disease and can be considered as an alternative for the next neurosurgery.

Endoscopic endonasal transsphenoidal approaches for pituitary adenomas invading the cavernous sinus

Jacek Kunicki, Wiesław Bonicki
Department of Neurosurgery, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw

Introduction: The endoscopic pituitary surgery offers technical possibilities to visualize and access better the tumor extensions invading parasellar areas. The significant number of pituitary adenomas present an invasive growth pattern. The cavernous sinus invasion is a significant predictor of an unfavorable outcome
Aim of the study was to report the results of a consecutive series of patients who underwent an endoscopic endonasal approach (both transsellar or extended — direct) for the resection of pituitary adenomas invading the cavernous sinus.
Material and methods: The retrospective review of clinical and radiographic outcomes of a consecutive series of patients operated

at our center between 2008 and 2013 was performed. The cavernous sinus invasion was defined preoperatively according to Knosp scale III and IV, only cases with intraoperative endoscopic inspection confirmed the invasion of CS were included into the study.
There were 168 patients with the confirmed cavernous sinus (28% macroadenomas), there were 42 cases with Knosp IV, and 126 cases defined as Knosp III. The tumors included non-functioning adenomas in 94 cases (54%), growth hormone-secreting adenomas in 46, prolactin-secreting adenomas in 24, and adrenocorticotropic hormone-secreting adenomas in 4 cases.
Results: The radical resection of cavernous sinus invasive NFPAs was observed in 41 (44%) cases. Normal growth hormone and insulin-like growth factor 1 levels were achieved in 19 patients (41%) with growth hormone adenomas. The endocrinological remission was achieved in 13 patients with prolactinomas and 2 patients with adrenocorticotropic hormone-secreting adenomas. There were no mortalities in the series, the transient oculomotor nerve palsies were observed in 8 patients (4.7%).
Conclusions: Endoscopic endonasal approach offers a chance for radical cure to the patients with adenomas cavernous sinus invasion with a minimal risk of surgical complications.

Clinicopathological analysis of hormone immunonegative silent gonadotroph adenomas (so called null cell — gonadotroph adenomas)

Jacek Kunicki[1], Maria Maksymowicz[2], Wiesław Bonicki[1]
[1]*Department of Neurosurgery, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw*
[2]*Department of Pathology and Laboratory Diagnostics, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw*

Introduction: The gonadotroph adenomas (GA) are one of the most common non-functioning pituitary adenomas treated surgically. There is a subpopulation of GA with low morphological and functional differentiation with lack of immunoreactivity to anterior pituitary hormones, in previous decades those tumors were classified as null cell adenomas. The ultrastructural evaluation with electron microcopy or immunohistochemical characterization of pituitary transcription factors in majority of cases of the so called null cell adenomas show the differentiation into GA.
The aim of the study was to define the morphology and clinical presentation of this subgroup of GA.
Material and methods: Out of 447 non-functioning pituitary tumors treated surgically within last 5 years, 38 (8.5%) hormone immunonegative silent gonadotroph adenomas were diagnosed using immunohistochemical and electron microscopic evaluation.
Results: There was significant women predominance 23/38 (60%). All of those tumors were diagnosed as macroadenomas, in 7 cases (18.4%) the tumors were classified as giant adenomas (> 4 cm).The main clinical presentation were progressive visual worsening and headaches. The MRI and intraoperative inspection showed that 19/38 (50%) of HNGA were invasive adenomas. The cavernous sinus invasion was the most common direction of invasion — 12 cases (31.5%). Coexisting different routes of invasion were observed in 44% of cases. The morphometric evaluation of HN GA did not show significant differences between immunopositive and immunonegative gonadotroph adenomas. The results of endoscopic surgical resection were better for HN GA than for HIP GA (80% GTR *vs.* 72% GTR) but the difference was not statistically significant (Fisher exact test $p = 0.17$)
Conclusions: Results of analysis show that clinical course and morphological appearance did not significantly differ hormone immunonegative silent gonadotroph adenomas (so called null cell — gonadotroph adenomas) from other GA. There was significant female predominance for those tumors.

Endoscopic endonasal resection of giant pituitary adenomas

Jacek Kunicki, Paweł Poppe, Wiesław Bonicki
Department of Neurosurgery, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw

Introduction: The advances of an endoscopic technique allow the resection of pituitary tumors previously operated by transcranial approaches.
The aim of the study was to review the early surgical results in a series of patients with giant pituitary adenomas operated with endoscopic endonasal approach.
Material and methods: The study is a retrospective analysis of a series of 72 patients with giant pituitary adenomas operated at the Department of Neurosurgery Memorial Oncological Center in Warsaw from 2008 to 2014.
Results: The gross total resection was accomplished in 35 out of 72 cases (48.6%), the subtotal resection was achieved in 30 of 72 patients (42%), in 7 cases only the partial resection was possible. There were no mortalities in the series. Postoperatively 86% of the patients showed varying improvement in visual field defects and visual acuity. There was no visual function deterioration in the series. Two patients presented early postoperative CSF leak and were reoperated. During a mean 42 month observation there were 5 cases of recurrences and 8 cases of residual tumor progression.
Conclusions: Our results indicate that resection with endoscopic endonasal approach can be a safe and effective method for the treatment of patients with giant pituitary adenomas and it is the alternative for transcranial approaches.

Are the "classic" and the "modern" forms of Nelson's syndrome the same or different disorders?

Maria Kurowska, Joanna Malicka, Jerzy S. Tarach
Department of Endocrinology, Medical University, Lublin

Introduction: Nowadays a great heterogeneity in the diagnostic criteria and clinical picture of Nelson's syndrome (NS) is observed and a formal consensus about its current definition does not exist. Aim was to answer the title question by the presentation of 8the clinical and therapeutic differences of both forms of NS based on 2 case reports.
Material and methods: Retrospective analysis of clinical picture and diagnostic and therapeutic procedures.
Case 1. A 54 year-old woman. Cushing's disease caused by microadenoma was diagnosed and bilateral adrenalectomy was performed 30 years ago. 5 years later she developed NS [pituitary macroadenoma and skin hyperpigmentation]. In the consecutive imaging studies the increasing of the tumor size was observed. Adenoma apoplexy evoked a considerable but transient tumor shrinkage 8 years ago. The re-growth of the tumor was an indication to neurosurgery 5 years ago. Since this moment a slow progression of tumor size has been observed and the patient needs only a hydrocortisone substitution.
Case 2. A 56-year-old woman with pituitary microadenoma and Cushing's disease diagnosed 7 years ago. She underwent transsphenoidal adenomectomy followed by a 27-month-long remission. Due to a tumor re-growth consecutive 2 transsphenoidal reoperations and stereotactic radiotherapy were conducted. Afterwards, because of sustained hypercortisolism, bilateral adrenalectomy was performed. Then the patient developed Nelson`s syndrome. Propter a rapid tumor's recurrence a 4th transsphenoidal surgery

and right fronto-temporal craniotomy were performed. Only 9 cycles of temozolomide resulted in marked clinical and radiological improvement. After a pause in chemotherapy a consecutive relapse occurred. Then bevacizumab, resulting in the clinical stabilization of disease, was introduced. Propter next tumor re-growth she underwent left fronto-temporal craniotomy.
Conclusion: It appears evident that the "classic" and "modern" form of Nelson's syndrome are different diseases.

Astrocytes GFAP mRNA expression under different glycemic conditions

Anna Latacz, Ewa Ocłoń, Joanna Zubel-Łojek, Krystyna Pierzchała-Koziec
Department of Animal Physiology and Endocrinology, Faculty of Animal Sciences, University of Agriculture Krakow, Poland

Introduction: Astrocytes are the main homeostatic cells of the central nervous system and play a wide role in the physiology and pathology of the CNS. Astrocytes activity is controlled by several kinases e.g. mammalian target of rapamycin — mTOR and can be modified in pathological conditions like metabolic changes. Reactive astrocytes are capable of producing a variety of active molecules e.g. pro-inflammatory mediators.
The aim of this study was to evaluate the effect of various glucose concentrations on astrocyte functions, estimated by mRNA expression of glial fibrillary acidic protein - GFAP, cells proliferation rate and the level of visfatin secretion.
Material and methods: Rat primary cortical astrocytes were incubated for 24 hours in medium with different concentrations of glucose (lower: 5 mM, higher: 67.5 mM) and mTOR inhibitor — rapamycin (10 nM). Culture medium was collected and visfatin concentration was measured by commercial enzyme-linked immunosorbent assay. The activity of astrocytes was evaluated by quantitative PCR measurements of GFAP mRNA expressions and cells proliferation rate.
Results: Significant differences between control and experimental groups have been detected. Lower and higher glucose conditions increased visfatin release by 235% and 49% respectively. Rapamycin addition caused the increase of GFAP mRNA expression and, at the same time, reduced the proliferation rate of the cells incubated in medium with abnormal glucose concentration.
Conclusions:
1. Astrocytes are the central source of visfatin release in pathological conditions.
2. The changes of astrocytes activities showed a modulating effect of mTOR inhibitor depending also on the glucose conditions.
3. Astrocytes are the cells that are able to react in response to peripheral glucose abnormalities and their biochemical features are related to mTOR pathway.
Supported by: DS3243/KFiEZ

The effect of sodium valproate (VPA) on LH secretion from rat pituitary cells after the stimulation with endothelin-1 (ET-1) in primary culture

Anna Litwiniuk[1], Malgorzata Kalisz[1], Justyna Skrzypska[1], Ewa Wolinska-Witort[1], Lidia Martynska[1], Magdalena Chmielowska[1], Alina Gajewska[2], Elzbieta Wasilewska-Dziubinska[1], Wojciech Bik[1]
[1]*Department of Clinical Neuroendocrinology, Centre of Postgraduate Medical Education, Warsaw, Poland*
[2]*Department of Neuroendocrinology, The Kielanowski Institute of Animal Physiology and Nutrition Polish Academy of Sciences, Jabłonna, Poland*

Introduction: The presence of ET-1, a peptide having a vasoconstrictor activity, in the hypothalamus and pituitary, suggested the possibility of the regulatory action of ET-1 in neuroendocrine cells. It has been shown, that ET-1 and GnRH induced the comparable secretion of LH. However, the dynamics of action of ET-1 is different from the dynamic action of GnRH. Previously we have shown that VPA inhibits GnRH-stimulated LH release from pituitary cells. At present it is not known whether VPA affects also the secretion of LH stimulated by ET-1. The aim of the study was to evaluate the effect of VPA on the secretion of LH from the pituitary cells stimulated by ET-1.
Material and methods: The pituitary were taken to the *in vitro* study from sexually mature male Wistar rats. The primary pituitary cells culture ($5x10^5$ cells/well) was incubated 0,5, 1 and 3 h with alone ET-1 (100 nM) or combined with different doses of (100 nM, 1 μM and 10 μM). LH concentrations in medium were assayed by RIA method.
Results: It was observed that after 0.5 h ($p < 0.05$) and 1 h ($p < 0.001$) incubation with ET-1 (100 nM) the secretion of LH was increased. In turn, after 1 h incubation VPA (1 μM; 10 μM, $p < 0.001$) abolished the stimulated by ET-1 secretion of LH from the pituitary cells.
Conclusions: VPA in a dose and time-dependent manner inhibits the stimulated by ET-1 secretion of LH from the pituitary cells.
This study was supported by CMKP grants No 501-1-31-22-13 and 501-1-31-22-14.

A case report of a patient with congenital hypopituitarism, in whom a dysfunction of particular axis appeared in different times

Katarzyna Lizis-Kolus[1], Aldona Kowalska[1], Ewa Piątkowska[2]
[1]*Department of Endocrinology, Holy Cross Cancer Centre, Kielce, Poland*
[2]*Department of Endocrinology and Diabetology, Regional Children's Hospital, Kielce, Poland*

Introduction: Congenital combined pituitary multihormone deficiency is usually diagnosed in childhood. In the natural history of the disease the occurrence of subsequent tropic hormone deficiency is possible at different times.
Aim of the study: Description of a patient aged 43 with congenital hypopituitarism and a gradual falling tropic function.
Material and methods: At the age of nine growth hormone deficiency was diagnosed. For two years the patient was treated with rhGH. Achieved height of 167 cm.
MRI of the pituitary: Empty sella syndrome-the greater part of the anterior lobe flattened, pressed against the bottom of the saddle; a part of the nervous ectopic located in the hypothalamus. At 28 years of age the patient was diagnosed with the deficiency of pituitary axes in the range of gonadotropic and corticotropic with hyperprolactinaemia. Enabled substitution steroid testosterone formulation, a dopamine agonist. Because of the elevated TSH to normal fT4, fT3 and a/TPO, TRH test was performed. Diagnosed with primary hypothyroidism. Thyroid ultrasound: PP — 9 × 11 × 24 mm, LP — 8 × 7 × 28 mm; hypoechoic thyroid gland without focal lesions. Enabled substitution of L-thyroxine. After one year, the decline in the concentrations of TSH and fT4, fT3 was observed. TRH test was repeated. Diagnosed with an underactive pituitary thyrotropic axis and modified L-thyroxine therapy for normalization of fT4, fT3. The patient remains in the observation of the substitution treatment continuation because of multihormonal hypopituitarism accompanied by hyperprolactinaemia.
Conclusions:
1. Patients with congenital hypopituitarism require chronic endocrine observations.
2. In the case of hypopituitarism in the range of one axis, developing deficiency of other axes is possible later.

3. The occurrence of primary hypothyroidism in patients with hypopituitarism, does not preclude the development of secondary hypothyroidism in the course of the deficiency of pituitary thyrotropic axis.

The role of pathomorphological diagnosis of pituitary tumors in patients with clinical symptoms of hyperprolactinemia

Maria Maksymowicz[1], Grzegorz Zieliński[2], Przemysław Witek[3], Ewa Matyja[4, 5], Renata Jonasz[1], Monika Pękul[1], Jacek Kunicki[5], Wiesław Bonicki[5]

[1]*Department of Pathology, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw*

[2]*Department of Neurosurgery, Military Institute of Medicine, Warsaw*

[3]*Department of Endocrinology and Isotope Therapy, Military Institute of Medicine, Warsaw*

[4]*Department of Experimental and Clinical Neuropathology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw*

[5]*Department of Neurosurgery, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw*

Introduction: Prolactinomas are the most frequent tumors of adenohypophysis, but surgical treatment is usually reserved for patients with resistance or intolerance for medical therapy. It is important to differentiate hyperprolactinemia due to the "stalk effect" (the compression of the infundibulum) from the one caused by the pituitary tumor secretion. An accurate diagnosis with the application of immunohistochemistry (IHC) and electron microscopy (EM) makes possible a proper prognosis and appropriate postoperative management (e.g. discontinuation of medical treatment).

The aim of the study was the evaluation of the practical implementation of IHC and EM techniques in the diagnosis of surgically treated prolactinoma.

Material and methods: At the Department of Pathology of Cancer Center 3660 cases of consecutive surgically resected pituitary tumors were evaluated between 1998 and 2014. Among them there were 158 patients with hyperprolactinemia. All the cases were diagnosed by IHC and EM. The MIB-1 proliferative index was determined.

Results: In our material only 67.1% patients with clinically diagnosed hyperprolactinemia (prolactin values of more than 200 ng/mL) have PRL-cell adenoma. In contrast, 26 gonadotroph, 9 mixed GH/PRL, 9 silent adenomas, 5 somatoptroph and 1 acidophil stem cell adenoma were diagnosed. Among nonadenomatous tumors 1 xanthogranuloma and 1 case of lymphocytic hypophysitis were revealed.

Conclusions: Our results confirmed the practical value of complex histopathological diagnosis in recognizing the cases of pseudoprolactinoma. In the cases of overlapping immunohistochemical profiles an ultrastructural analysis is valuable to provide useful information. Proliferative markers, like MIB-1, may be helpful in the diagnosis of invasive, low differentiated tumors.

Newly identified neuropeptides involved in regulation of body weight and energy homeostasis

Ludwik K. Malendowicz, Marcin Ruciński, Agnieszka Ziołkowska

Department of Histology and Embryology, Poznan University of Medical Sciences

Neuropeptides regulate energy homeostasis primarily in response to signals from both the adipose tissue and the gastrointestinal tract. Under normal conditions the interrelationship between the adipose tissue and neuropeptides maintains proper body weight. In recent years only a few novel neuropeptides have been identified and it has been suggested that some of them are involved in the regulation of body weight and energy homeostasis. Regarding this, available data on three neuropeptides will be presented. Spexin (SPX) is a neuropeptide closely related to galanin (GAL) and interacts with GALR2/3 receptors. Because of its widespread expression, SPX is probably involved in many different physiological functions. It is interesting that the expression of SPX gene is markedly down-regulated in the obese human adipose tissue. In rodents SPX protein induces weight loss in DIO (diet-induced obesity) rodents. Neuropeptide QRFP (26RFa) is a 26-amino acid residue peptide that was originally identified from the frog brain. It is highly expressed in the hypothalamus of vertebrates. QRFP has been shown to exert orexigenic activity in mammals, acting throughout GPR103 (QRFPR). Augurin is a product of a esophageal cancer-related gene-4 (ECRG4). ECRG4 mRNA is widely expressed in normal human tissues. The intra-paraventricular nucleus (iPVN) administration of augurin (71–148) stimulates food intake in male Wistar rats.

This study was supported by a grant IP2011 046671 from the Ministry of Science and Education in Poland.

Acromegaly as the first manifestation of MEN 1 syndrome — case report

Joanna Malicka, Maria Kurowska, Patrycja Nowicka-Stążka, Jerzy S. Tarach

Department of Endocrinology, Medical University, Lublin, Poland

Introduction: Pituitary adenomas occur with an incidence of 77.6/100 000, with adenomas derived from somatotrophs and secreting GH accounting for 50–70 cases/1 million. About 5% of GH-secreting adenomas occur with a familial aggregation, as a part of MEN-1, MEN-4, Carney complex or family bound isolated pituitary adenomas-FIPA. Until now, both the clinical picture and the genetic background of MEN-1 have been well recognized. MEN-1 syndrome is inherited in an autosomal dominant pattern. Its most frequent clinical components are: hyperparathyroidism (95%), endocrine enteropancreatic tumors (60%) and pituitary adenomas (42%). Among pituitary adenomas, 9% secrete growth hormone and cause acromegaly with mean recognition between 30 and 50 years of age.

The aim of this study was to present the patient in whom acromegaly was the first manifestation of MEN-1 syndrome.

Case report. A 65-year-old woman with acromegaly recognized in September 2009 (GH > 40 ng/mL, n < 5 ng/mL; IGF1–1127 ng/mL, n: 75–212). MRI revealed 13 × 16mm pituitary macroadenoma. In August 2010 CT confirmed 18 × 12 mm adenoma of the left adrenal gland and 36 × 26 × 54 mm retroperitoneal tumor localized in the pancreatic area with elevated Ca19–9 marker. Three months later, because of laboratory features of primary hyperparathyroidism (Ca = 10.5–11.0 mg/dL, n: 8.7–10.4 mg/dL; PTH = 110–124 pg/mL, n: 14–72) MIBI scintigraphy was performed and confirmed the enlargement of the right inferior parathyroid gland. Nodular goiter, uterine myoma and skin fibroma were also present. The patient didn`t accept the proposal of surgery and now she receives somatostatin analog and remains in follow-up.

Conclusion: Our patient presents the clinical features typical of MEN-1, with the rare type of pituitary adenoma as a part of it. What is more, acromegaly was the first symptom of the syndrome. However, in order to confirm the diagnosis, conducting genetic tests should be considered.

The incidence of neoplasms in acromegaly — own observation

Joanna Malicka, Maria Kurowska, Jerzy S. Tarach
Department of Endocrinology, Medical University, Lublin, Poland

Introduction: Pituitary adenomas derived from somatotrophs occur with an incidence of 50-70/million. The excessive secretion of GH leads to the increased production of growth factors, (mainly insulin-like growth factor-1) in the liver and other tissues. IGF-1 stimulates cell division that, in turn, increases the risk of benign and malignant tumors and leads to the higher mortality rate of patients. The aim of the study was to estimate the prevalence of various tumors in acromegaly patients in own material.
Material and methods: The material covered 57 acromegalics (35 F and 22 M) treated from 2000 in Department of Endocrinology and Endocrinological Outpatient Clinic. We studied the medical histories and the results of patients` diagnostic investigations.
Results: There were 85 benign and 4 malignant tumors in our group. Nodular goiter was the most common (39 cases) with 13 cases of hyperthyroidism and 26 with euthyroidism. Gastrointestinal tract endoscopic examination was performed in 23 patients (40.4%) with 20 colonoscopies and 9 gastroduodenoscopies. 16 colorectal polyps, 1 gastric polyp, 1 polyp of gallbladder, 1 colorectal cancer and 1 gastric cancer were found. Uterine myoma were detected in 6 women, endometrial carcinoma in 1 and uterine cervix polyps in 3. Breast carcinoma was found in 1 patient and breast fibroma in 2 cases. 8 men were treated because of prostatic adenoma. Benign skin neoplasms were diagnosed in 7 cases. There was 1 case of pancreatic and adrenal tumor and 1 case of parathyroid adenoma. In 23 patients we diagnosed one tumor, in 18-two, in 5-three, in 2-four and in 1 woman-seven various tumors. Only in 8 patients from the whole group we did not find such pathologies.
Conclusion: The great number of tumors in acromegaly patients requires precise diagnostics. Gastrointestinal endoscopic procedures, thyroid testing, gynecological and urologic examination should be obligatory.

The influence of Orexin B on LH secretion from mature rat pituitary cells in primary culture

Lidia Martyńska[1], Ewa Wolińska-Witort[1], Magdalena Chmielowska[1], Małgorzata Kalisz[1], Anna Litwiniuk[1], Wojciech Bik[1], Bogusława Baranowska[2]
[1]Department of Clinical Neuroendocrinology, Centre of Postgraduate Medical Education, Warsaw
[2]Department of Neurology, Second Faculty of Medicine Medical University of Warsaw, Warsaw

Introduction: Orexins A and B (OxA and OxB) are peptides synthesized mainly in the lateral hypothalamus. They show a wide effect in central nervous systems as well as in peripheral tissues. Orexins are involved not only in the regulation of food intake, locomotor activity and energy expenditure but also can influence pituitary hormones secretion. Orexin B acts through orexin receptor type two (OX2R) which is found in the pituitary.
The aim of the study was to examine the direct impact of OxB on LH secretion from pituitary cells of sexually mature rats.
Material and methods: Pituitaries for *in vitro* study were taken from 3-months old mature female rats, one week after surgical removal of the ovaries (OVX), as well as from females OVX treated with estradiol given subcutaneously (OVX + E_2). The primary pituitary cells culture (5×10^5 cells/well) was incubated with different doses of OxB (0,1 nM, 1 nM, 10 nM and 100 nM) for 1 h and 4 hs. The cultured cells without OxB constituted the control group. LH concentrations in medium were assayed by RIA method.
Results: We found that after 1 h incubation OxB did not change LH secretion in OVX + E_2 group, and increased the secretion of LH by 18% (0.1 nM OxB; $p < 0.05$) in OVX group when compared to the control group. After 4 h OxB decreased LH secretion by 31% in both OVX + E_2 (10 nM, $p < 0.05$) and OVX group (100 nM, $p < 0.05$) as compared to the control group.
Conclusions: The obtained results indicate that OxB could slightly modify LH secretion and its effect depends on the dose and duration of action.
The study was supported by CMKP grants No 501-1-31-22-13 and 501-1-31-22-14.

Course of prolactinoma in pregnancy in patients treated in the Department of Endocrinology, Holy Cross Cancer Center in Kielce

Estera Mikina, Katarzyna Lizis-Kolus, Aldona Kowalska
Department of Endocrinology, Holy Cross Cancer Center in Kielce

Introduction: Prolactinoma can often be the cause of infertility. The use of dopamine agonists inhibits the excessive production of prolactin and allows many patients to become pregnant. Proceedings of prolactinomas in pregnancy, despite many years of experience, can be a problem.
Aim of the study: The authors present a different course of the disease during pregnancy in women with prolactinoma. We observed 6 pregnant women with prolactinoma. 3 patients had pituitary macroadenoma prior to pregnancy, in the remaining 3 — microadenoma was diagnosed. 5 patients were receiving treatment with bromocriptine before pregnancy; 1 was treated with chinagolid. In all the women drugs were discontinued when pregnancy was detected. 1 patient had the progression of tumor size and cranial nerve III paralysis. After restarting treatment with bromocriptine, the complete resolution of symptoms was achieved and the treatment was maintained until the end of pregnancy. In 2 patients with macroprolactinoma a pituitary hemorrhage apoplexy occurred leading to the cure of the prolactinoma in one of them. No patients required surgical treatment of prolactinoma during pregnancy. In all the described patients the pregnancy ended at the time of birth of a healthy baby. In 2 patients a caesarean section terminated the pregnancy due to obstetric indications, the other four had natural vaginal birth.
Conclusions:
1. Women with prolactinomas in pregnancy require careful endocrinological observation.
2. Progression of tumor size and tumor stroke are the most common complications of prolactinomas in pregnancy.
3. In the case of the size of the tumor progression, the return to the treatment with dopamine agonists give rapid clinical improvement.

The role of salsolinol in regulating physiological processes in mammals

Tomasz Misztal
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland

Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) is synthesized in the central nervous system by dopaminergic neurons. Early reports equated the presence of this compound

with various types of functional disorders of the dopaminergic system, characteristic, in particular, of Parkinson's disease or alcohol addiction. Opinions as to the harmful effects of salsolinol changed when its role in lactating females was discovered. Studies on rats and ewes showed its involvement in stimulating prolactin secretion. Salsolinol is currently believed to be one of the hypothalamic factors stimulating both the synthesis and the release of prolactin. The stimulus that releases salsolinol during lactation is sucking. The presence of salsolinol in the hypothalamus and median eminence of ewes during lactation also suggests a role for this compound in mechanisms regulating other processes in which dopamine has been found to play a part, i.e., in processes determining adaptation of a female to lactation and rearing of an offspring. Especially noteworthy is the inhibition of reproductive activities and the reduction of lactating females' sensitivity to stress. The studies conducted at the Kielanowski Institute of Animal Physiology and Nutrition on ewes *in vivo* and *in vitro*, using exogenous salsolinol or its analogue with antagonistic properties, show that during lactation, salsolinol is a modulator of secretory activity not only of pituitary lactotrophs, but of entire hypothalamic-pituitary axes, including gonadotropic (GnRH-LH) and corticotropic (CRH-ACTH) axes. It has also been suggested that salsolinol participates in the regulation of oxytocin secretion. The development of methods for experiments on the central nervous system of sheep may predispose this species for use as an experimental model for the studies on the activity of neuronal systems in various physiological states or induced pathological ones.

Changes in plasma corticosterone and IL-1β during the stimulation of the bed nucleus of the *stria terminalis* and the central nucleus of the amygdala in rats

Dorota Myślińska[1], Ziemowit Ciepielewski[1], Mateusz Karnia[2], Agnieszka Wądołowska[1], Katarzyna Czerwiec[1], Jan Ruciński[1], Karolina Plucińska[1], Magdalena Podlacha[1], Kacper Ptaszek[1]

[1]*Department of Animal and Human Physiology, University of Gdansk*
[2]*Department of Biochemistry, Academy of Physical Education and Sport, Gdansk*

Introduction: The central nucleus of the amygdala (CeA) is a limbic structure involved in the fear conditioning and modulation of cognitive functions. The bed nucleus of the *stria terminalis* (BST) is a part of "the extended amygdala", a formation responsible for emotional aspects of behavior. On the basis of our previous study we assumed that BST and CeA also influenced the primary antitumor immune response. In the present study we investigated the influence of 14-day electrical stimulation of the CeA and BST on corticosterone (COR) and IL-1β level and behavioral response.

Material and methods: Male Wistar rats implanted with electrodes into CeA or BST were divided into groups: CeA or BST stimulated, CeA or BST sham. Current intensity (70–160 μA; 50 Hz) was raised incrementally in 30-s trials until a behavioral reaction in Opto Varimex Minor actometer was observed. Blood samples were collected by heart puncture. COR level was determined by radioimmunoassay, IL-1β was quantified using ELISA.

Results and conclusion: The stimulation of the CeA caused the decrease in IL-β concentration, the augmentation of COR ($p < 0.001$) correlated with an increase in the average number of movements in vertical plane imitating escape behavior. Contrary to CeA, BST stimulation results in the increase in IL-β level and locomotor activity, without any effect on COR. We suggest that the hormonal outcome of the CeA and BST stimulation plays a crucial role in the regulation of behavioral and immune effects.

Supported by: NN303819040.

The role of orexins in the regulation of energetic metabolism

K.W. Nowak[1], M. Skrzypski[1,2], E. Pruszyńska-Oszmałek[1], P Kaczmarek[1], P. Kołodziejski[1,2], T. Wojciechowicz[1], D. Szczepankiewicz[1], M. Sassek[1], M.Z. Strowski[2]

[1]*Department of Animal Physiology and Biochemistry, Poznań University of Life Science*
[2]*Department of Hepatology and Gastroenterology and Interdisciplinary Centre of Metabolism: Endocrinology, Diabetes and Metabolism, Charité-University Medicine Berlin*

The neuropeptide orexin stimulates food intake and energy expenditure by acting on two different receptor isoforms OXR1 and OXR2. Orexin exists in two isoforms, both of which bind to OXR1 and OXR2. The studies on genetic engineered animals which lack or overexpress orexins or its cognate receptors revealed that orexin may play a role in controlling glucose homeostasis and body weight changes. Observational studies in narcoleptic humans who display orexin deficiency also indicate that orexin plays a role in the pathophysiology of type 2 diabetes mellitus and obesity. The results implicate that orexin may become an interesting therapeutic tool in alleviating metabolic diseases and in controlling body weight. Indeed, the application of exogenous orexin in narcoleptic humans alleviates the symptoms associated with the disease. Therefore, it is important to broaden our understating about the physiological role of orexin as well as to evaluate further its potential in the context of the pathophysiology of metabolic diseases. Our investigation focuses on the orexin role in the regulation of endocrine and metabolic functions *in vivo* and *in vitro*, as well as, on its possible involvement in the pathophysiology of type 2 diabetes mellitus and obesity.

The usefulness of MIB1 proliferation index in functioning and nonfunctioning pituitary adenomas, especially in invasive tumors

Karolina Nowakowska[1], Maria Maksymowicz[2], Renata Jonasz[2], Monika Pękul[2], Wiesław Bonicki[3], Jacek Kunicki[3]

[1]*Department of Pathology, Children's Hospital Medical University of Warsaw, Warsaw,*
[2]*Department of Pathology and Laboratory Diagnostics, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw*
[3]*Department of Neurosurgery, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw*

Introduction: Pituitary adenomas are the most common intracranial tumors and usually are regarded as benign. According to the 2004 WHO classification, tumors of the anterior pituitary are divided into adenomas, atypical adenomas and cancers. The atypical pituitary adenomas are defined as pituitary adenomas with the elevated proliferative index (> 3%). In the last few years there have been reports undermining the value of assessing the proliferative index MIB1 as a prognostic factor in those tumors.

The aim of this study was to determine the proliferative index MIB1 in pituitary adenomas with particular regard to invasive adenomas.

Material and methods: 153 cases of surgically treated pituitary adenomas were examined. Clinically 49 patients had hormonally active adenomas, and 104 patients had nonfunctioning adenomas (among which were both tumors: null cell adenoma and silent adenomas — positive by immunohistochemistry).

In all the cases anterior pituitary hormones were determined by immunohistochemistry and the proliferative index MIB1 was evaluated. All the tumors were studied by electron microscopy.

Results: Among 153 examined pituitary adenomas, 18 atypical tumors were found. In 47 tumors clinical invasiveness was shown,

among which 5 cases of atypical adenomas were found. Among them, 2 sparsely granulated somatotroph adenomas, 1 densely granulated corticotroph adenoma, 1 sparsely granulated lactotroph adenoma and 1 gonadotroph adenoma were diagnosed.
Conclusion: On the basis of the evaluated material we cannot confirm the assessment of the proliferative index MIB1 as a prognostic factor in invasive pituitary adenomas.

Resistin as marker of endothelial cells (HAEC) dysfunction under hyperglycemic stress

Ewa Ocłoń, Joanna Zubel-Łojek, Anna Latacz, Klaudia Pałka, Krystyna Pierzchała-Koziec
Department of Animal Physiology and Endocrinology, Faculty of Animal Sciences, University of Agriculture in Krakow

Introduction: Hyperglycemia is a risk factor for cardiovascular diseases. Resistin, a newly discovered adipocyte-derived cytokine, may play an important role in insulin resistance, diabetes, and cardiovascular disease.
The aim of this study was to determine the effect of resistin on endothelial cell function in response to hyperglycemic stress. Furthermore, the adipokine was estimated as an indicator of endothelium dysfunction.
Materials and methods: Human aortic endothelial cells (HAEC) were cultured in a standard medium (M 199). The experiment was performed two times on the forth passage of HAEC culture. The cultures were incubated for 48 hours in four treatment groups: control (5.5 mmol/L glucose), hyperglycemia (22.2 mmol/L glucose), resistin (500 nM) and resistin + hyperglycemia (500 nM resistin + 22.2 mmol/L glucose). The activity of HAEC was evaluated by measurement endothelin secretion 1 and proliferation rate.
Results: The secretion of resistin was increased by 42.1% during hyperglycemia ($p < 0.05$). Resistin caused the increase in endothelin 1 secretion by 43.5% ($p < 0.05$) and the decrease of cell proliferation rate by 44.2% ($p < 0.05$).
Conclusions:
1. The activity of aortic endothelial cell negatively changed in response to hyperglycemic stress.
2. Hyperglycemia increases resistin secretion which induces endothelial dysfunction by reducing proliferation and increasing the secretion of ET-1.
3. Resistin seems to be a potential prognostic marker for endothelium dysfunction under hyperglycemic stress.
Supported by DS 3243/KEiEZ/

The role of opioid peptides in the modulation of gonadotropin and prolactin secretion from the anterior pituitary of cyclic gilts — the *in vitro* studies

Stanisław Okrasa[1], Bartosz Wylot[1, 2], Kinga Tworus[1], Mariusz Dziekoński[1]
[1]*Department of Animal Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury, Olsztyn, Poland*
[2]*Department of Cell Biology, Nencki Institute of Experimental Biology, Warsaw, Poland*

Endogenous opioid peptides (EOP) derive from high-molecular opioid precursors: proopiomelanocortin (POMC), proenkephalin (PENK), prodynorphin (PDYN), and act through three major types of opioid receptors: *mu*, *delta* and *kappa*. They are involved in the regulation of reproductive processes in females, participating in the control of gonadotropin and prolactin secretion. These effects might be implemented through their action at the hypothalamic and pituitary levels. Genes cording for opioid precursors and receptors are expressed in the anterior pituitary. This presentation involves data on: 1) the effects of potential gonadotropin (GnRH, estradiol, progesterone, inhibin and activin) and prolactin (TRH, dopamine, oxytocin, kisspeptin and PGF2α or PGE2) secretagogues on the gene expression of opioid precursors and receptors *in vitro* in the anterior pituitary cells of gilts in the luteal and follicular phase of the estrous cycle as well as 2) the influence of opioid receptor agonists (*mu*, *delta* and *kappa*) on the basal and GnRH-stimulated gonadotropin secretion (LH and FSH), and also the basal and in the presence of dopamine or TRH prolactin secretion by these cells. The presented data have confirmed the variable pituitary expression of genes coding for opioid precursors and receptors in response to tested factors. Moreover, the inhibitory effects of *kappa* and *delta* opioid receptor activation on the LH and FSH secretion *in vitro* by porcine pituitary cells were noted. In turn, the influence of opioid receptor agonists on prolactin secretion was more variable and dependent on the stage of the estrous cycle. During the luteal phase, its secretion was reduced after activation of *mu*, *delta* and *kappa* opioid receptors under all tested conditions. In the early follicular phase, the activation of *mu*, *delta* and *kappa* opioid receptors increased prolactin secretion under basal conditions, and *delta* receptor — in the presence of TRH, whereas the stimulation of *mu* and *kappa* receptors inhibited its secretion in the presence of dopamine. In the late follicular phase, *kappa* receptor agonist stimulated prolactin secretion under all tested conditions. In turn, the activation of *mu* and *delta* receptors increased its secretion under basal conditions and in the presence of dopamine, but decreased — in the presence of TRH.
The presented data indicate the participation of endogenous opioid peptides in the modulation of gonadotropin (mainly dynorphins and enkephalins) and prolactin (all families of EOP) secretion at the pituitary level in gilts during the estrous cycle.

Phoenixin — a new player in the gonadal axis

Artur Pałasz[1], Ewa Rojczyk-Gołębiewska[1], Rafał Skowronek[2]
[1]*Department of Histology, Chair of Histology and Embryology, Medical University of Silesia in Katowice*
[2]*Department of Forensic Medicine and Forensic Toxicology, Medical University of Silesia in Katowice*

Introduction: The dynamic development of biotechnology results in the discovery and description of new neuropeptides, localized in different areas of the brain which have a brought, multidirectional spectrum of activities performed at the level of different neuronal pathways.
Aim of the study: 1) The review of literature concerning with newly-discovered neuropeptide — phoenixin (PNX) and 2) the assessment of its distribution in hypothalamic structures of adult rats.
Material and methods: 1) The search of available databases for articles about PNX; 2) evaluation of the distribution of PNX in hypothalamic structures of adult Sprague-Dawley (SD) rats with immunohistochemistry (IHC) and immunofluorescence (IFC), using the original antibody from Phoenix Pharmaceuticals.
Results: 1) We found only 2 original papers and one proceeding; 2) we confirmed the presence of PNX in various structures of the hypothalamus of SD rats, both by IHC and IFC.
Conclusions: PNX is a newly-discovered and still extremely poorly known neuropeptide, representing a unique class of hypothalamic regulatory factors. So far we know only that it regulates the secretion of pituitary gonadotropins by modulating the expression of the receptor for gonadotropin-releasing hormone (GnRH-R). An

initial study suggests that PNX sensitizes the pituitary to the action of releasing factors, rather than directly stimulates the exocytosis of secretory vesicles to pituitary endocrine cells. Immunohistochemical studies revealed PNX immunoreactivity in the rat hypothalamus, superficial dorsal horn, spinal trigeminal tract, nucleus of the solitary tract; and in the population of dorsal root, trigeminal and nodose ganglion cells. It was also observed that exogenously administered PNX may preferentially suppress visceral pain as opposed to thermal pain. Recent reports suggest that the mechanism of signal transduction activated by PNX is MAPK/ERK pathway.

„Clinically" non-functioning pituitary adenomas (CNFPA)-heterogeneous group of pituitary tumors. Is their hormonal phenotype clinically relevant?

Marek Pawlikowski
Department of Immunoendocrinology, Chair of Endocrinology, Medical University of Łódź

Introduction: Pituitary adenomas, recognized before surgery as hormonally non-functioning, in great majority reveal the expression of pituitary hormones or their subunits in the post-operative immunohistochemical examination. A question arises, whether the hormonal phenotype recognized by immunohistochemistry is connected with their clinical outcome.
Material and methods: One hundred seventy six surgically removed pituitary adenomas were examined. Eighty nine of them did not exhibit detectable hormonal activity before surgery („clinically" non-functioning pituitary adenomas, CNFPA). In all samples immunohistochemical examination of pituitary hormones or their subunits was performed. A part of tumors were also examined with antibodies against Ki67 and prothymosin alpha (PTalfa).
Results: Majority of CNFPA (67.4%) expressed gonadotropins or their free subunits. Other hormones were detected as follows: GH — 45.9%; ACTH — 31.7%; PRL — 22.5%. Only two tumors expressed TSH. The hormonal phenotype of CNFPA seems to be connected with their recurrence rate. The recurrence rate is the highest in "pure" " gonadotropinomas (33.3%) and „silent" corticotropinomas (31.5%). The medium frequency concerns the „silent" prolactinomas (20%), and the lowest the „silent" somatotropinomas (4.8%). Almost all CNFPA are invasive tumors and have high Ki67 index (6.4 ± 2.3%). Ki67 index does not differ in silent" somatotropinomas in comparison with active acromegaly, but is significantly higher in „silent" corticotropinomas *vs.* Cushing's disease (4.8 ± 2.1 *vs.* 1.9 ± 0.65 %). ProTα index is higher in CNFPA in comparison to any type of functioning adenomas. The ProTα-positive nuclei are more abundant in "silent" corticotropinomas than in corticotropinomas manifesting with Cushing's disease (19.4 ± 6.6% *vs.* 2.2 ± 0.6%). Similarly, "silent somatotropinomas" reveal a higher ProTα index as compared with acromegaly (32.8 ± 9.9% *vs.* 16.7 ± 7.7% respectively).
Conclusions: The data presented above indicate that the hormonal phenotype is important for the outcome of the non-functioning pituitary tumor.

Central and peripheral responses of sympathetic nervous system to hand grip maneuver stress (HGME)

Krystyna Pierzchała-Koziec[1], Michał Labus[2], Michał Furdal[2], Lesław Szydłowski[3], Sebastian Stec[4], Ewa Ocłoń[1], Joanna Zubel[1], Anna Latacz[1]
[1]Department of Animal Physiology and Endocrinology, University of Agriculture, Krakow
[2]The Regional Specialist Hospital in Wrocław, R&D Centre, Wrocław
[3]Clinic of Children Cardiology, Silesian Medical University, Katowice, [4]Clinic of Cardiology, Centre of Postgraduate Medical Education, Warsaw

Introduction: The sympathetic nervous system responds to stress by releasing catecholamines, mainly adrenaline and noradrenaline, from the central and peripheral nervous systems. Hand grip maneuver (HGME) test is used for the diagnosis of arrhythmia and the localization of ablation place. The activity of the sympathetic nervous system may affect the electrophysiological properties of the atrioventricular nodus and cause problems for the ablation procedure.
The aim of the study was to estimate the response of the sympathetic nervous system to HGME measured by cerebral and peripheral circulation catecholamines.
Material and methods: Hand grip maneuver (30-50% max) was applied to patients prepared to the ablation procedure. Blood was taken simultaneously from the internal jugular vein (I), the coronary sinus (II), the femoral vein (III) and the femoral artery (IV) at 5 and 10 min of calm lying, one min of stress and 2 and 3 min after HGME had been finished. Plasma catecholamines levels were estimated by RIA method.
Results: HGME increased adrenaline levels in the blood taken from internal the jugular vein and the coronary sinus (by 38 and 34% respectively). Unexpectedly, adrenaline levels in the blood from the femoral vein and the artery were decreased (by 36 and 40%, respectively). Stress HGME increased noradrenaline levels in the blood taken from the internal jugular vein (by 49%) and the coronary sinus (by 28%). Simultaneously, noradrenaline levels were decreased in the coronary sinus blood (by 15%, delayed response) and the quick fall (after 1min of stress) of noradrenaline level was observed in the blood from the femoral artery. It must be pointed out that noradrenaline levels in the blood taken from all intakes were higher at 10 min of calm lying.
Conclusion: The stress responses of the sympathetic nervous system measured by adrenaline and noradrenaline levels depend on a blood drawing place.
Supported by: DS 3243/KFiEZ

Stress and pain coping strategies versus the intensity of premenstrual syndrome symptoms

Leszek Putyński[1], Mariusz Paciorek[2]
[1]Zakład Psychopatologii i Psychologii Klinicznej, Instytut Psychologii UŁ, Łódź
[2]Poradnia „K" SPZOZ, Szczerców

Introduction: Premenstrual Syndrome (PMS) is a condition characterised by a variety of psychical and somatic symptoms which occur before the menstrual period and cease after the arrival of the period. Hormonal disturbances are often given as the major cause of PMS. The role of psychological influences in the aetiology, process and treatment of PMS is often forgotten or ignored.
The aim of the present study was the analysis of the influence of stress and pain coping strategies on the intensity of emotional and somatic symptoms in PMS period.
Material and methods: The research was conducted in a group of 50 women aged 20 – 35 years. The self developed inventory (IONP) was used to evaluate the intensity of PMS symptoms. The mini-COPE questionnaire was applied to detect stress coping strategies. Pain coping strategies were assessed using CSQ questionnaire.
Results: The results revealed the significant correlations between the level of PMS symptomatology and stress and pain coping strategies. The women with the higher level of emotional PMS symptoms such as dysphoria and depression manifest more maladaptive stress

coping strategies than the women with the higher level of somatic symptoms such as pain and vasomotor symptoms. The women who revealed the higher level of PMS symptoms are likely to use filed pain coping strategies.
Conclusions: The results suggest that stress coping strategies and pain coping strategies are the most important psychological factors influencing the intensity of PMS symptoms.
Various medical treatment strategies for PMS may be the reinforcement to psychological treatment. The psychotherapy may help women develop appropriate ways to cope with frustrations and discomfort of daily life in PMS time.

Melatonin mt1 receptor is involved in melatonin--dependent inhibition of forskolin-stimulated vasopressin secretion from the rat hypothalamo--neurohypophysial system *in vitro*

Magdalena Roszczyk, Marlena Juszczak
Department of Pathophysiology and Experimental Neuroendocrinology, Medical University of Lodz, Poland

Introduction: Previous *in vitro* studies have shown that melatonin (MT), at a concentration of 10^{-7} M, significantly inhibits forskolin-stimulated vasopressin (AVP) release. The aim of the present investigation was, therefore, to study the effect of melatonin membrane receptors antagonists, i.e. luzindole (a non-selective antagonist of MT_1 and MT_2 receptors) and 4-phenyl-2-propionamidotetralin (4-P-PDOT — a selective antagonist of MT_2 receptor), on MT-dependent inhibition of forskolin-stimulated AVP secretion from the rat hypothalamo-neurohypophysial (H-N) system *in vitro*.
Material and methods: Male rats served as donors of the H-N explants which were placed in 1 mL of Krebs-Ringer fluid (KRF) heated to 37°C. The H-N explants were first preincubated (for 30 min) in KRF containing DMSO (antagonists solvent) or luzindole, or 4-P-PDOT and next they were incubated successively in: 1 — normal KRF {fluid B1}, 2 — the incubation fluid as B1 enriched with MT (10^{-7} M) and/or forskolin (10^{-5} M), or their vehicles {fluid B2}. After 20 min incubation in each medium (B1 and B2), they were collected and immediately frozen before AVP estimation by the RIA.
Results: Forskolin significantly stimulated AVP release from isolated H-N system which was inhibited by MT when the explants were preincubated in DMSO or 4-P-PDOT. The preincubation of the explants with luzindole significantly suppressed the MT-dependent effects *in vitro*.
Conclusion: The present results demonstrate that the inhibitory influence of MT on cAMP-dependent activation of AVP-ergic neurons in the rat is mediated through membrane MT_1 receptor.
This study was supported by Medical University of Lodz (grant No 502-03/6-103-01/502-64-013).

Current methods of gene expression microarrays analysis for genes identification

Marcin Ruciński, Marcin Trejter, Anna Hochół-Molenda, Karol Jopek, Marta Szyszka, Marianna Tyczewska, Ludwik K. Malendowicz
Department of Histology and Embryology, Poznan University of Medical Sciences

Gene expression microarrays constitute a popular tool for the determination of expression profile for multiple genes simultaneously. This method is based on the hybridization of labeled target genetic materials to probes, fixed on a solid surface. However, the large number of identified genes requires special analytical tools, some of them will be presented.

In this review, we present the overview of some methods of microarray data analysis of gene expression in rat adrenal glands under various experimental conditions (enucleation-induced adrenal regeneration, rat adrenal primary cell culture, chronic and acute ACTH administration). All the experiments were performed by Rat Gene 2.1 ST Array from Affymetrix.
The values of the signal intensity of individual probes in relation to their position on microarray were saved as files with CEL extension. Most of the presented results were obtained by Bioconductor package, which is a component of R programing language. The first step of the microarray analysis is the general determination of expression changes in experimental groups. Usually differentially expressed genes were selected by differences in their mean expression levels more than two fold (fold2) and $p < 0.05$. General expression profile may be shown as a scatter plot or a volcano plot. These graphs present the normalized mean values of the log signal intensity from each of the analyzed genes. Another valuable type of graph is a Venn diagram. This plot is particularly useful in the studies with more than two experimental groups. Such graphs clearly show how many genes are regulated in the same way between multiple groups and also demonstrate how many genes are specific only to one particular group. The hierarchical clustering with a heat map graph is another approach that allows determining groups of genes whose expression is regulated in a similar manner. Another type of analysis utilizes genes annotation tags stored in Gene Ontology Database (GO). By GeneAnswers package and online Database for Annotation, Visualization and Integrated Discovery (DAVID). Few functional groups of genes were obtained. The final step of the microarray data analysis is the validation of obtained results. Usually only few of the most interesting genes are selected for validation. The validation of the results may be performed on mRNA level (QPCR) as well as on a protein level.
This study was supported by a grant 2013/11/B/NZ4/04746 from National Science Centre in Poland.

Prenatal stress (PNS) and programming of the hypothalamo-pituitary-adrenal (HPA) axis — mechanisms and consequences

John A. Russell[1], Paula J. Brunton[2]
[1]Centre for Integrative Physiology, College of Medicine and Veterinary Medicine, University of Edinburgh, United Kingdom
[2]Division of Neurobiology, The Roslin Institute and Royal (Dick) School of Veterinary Studies

Introduction: During pregnancy stress responses of the HPA axis and adrenal medulla are attenuated. In pregnancy the high levels of allopregnanolone (AP), a neurosteroid progesterone metabolite, inhibit HPA stress responses [1]. Nonetheless, social stress in pregnancy adversely programmes offspring behaviours and physiological processes [2].
Aim of the study: To establish whether disrupted neurosteroid mechanisms underlie the programmed phenotype in PNS offspring.
Material and method: Pregnant Sprague-Dawley rats were stressed in the last few days of pregnancy by exposure to an aggressive lactating rat. Anxious behaviour of adult offspring was tested on the elevated plus maze and underlying changes in corticotropin releasing hormone (CRH) and CRH receptor (CRHR) gene expression in amygdala measured by in situ hybridisation (also in PNS pigs [4]; metabolic function was assessed by glucose tolerance tests and RT-PCR for a panel of enzymes in liver and fat [3]. HPA axis responses to interleukin-1β (IL-1β) were measured as ACTH and corticosterone secretion, and as hypothalamic CRH gene activation; neurosteroid deficiency was tested by pre-treating with AP (females)

or androstanediol (AS; males), and by assessing mRNA expression of 5α-reductase (5αR; rate-limiting synthesising enzyme), and estradiol receptorβ (ERβ; AS target) in brain regions; a viral vector was used to up-regulate 5αR mRNA in the brainstem.
Result: PNS male rats, and PNS female pigs were more anxious, with increased CRH and CRHR1:2 expression in the amygdala. PNS rats had enhanced HPA stress responses, and adversely modified metabolism. Exaggerated HPA axis stress responses were normalised by neurosteroid and by up-regulating 5α-R gene expression in the brainstem (females). PNS males had reduced ERβ mRNA expression in brain regions regulating the HPA axis.
Conclusions: PNS has widespread adverse, sex-dependent, effects on offspring: deficient neurosteroid production/action is an important factor.
Supported by: Biotechnology and Biological Sciences Research Council; British Council; Polish School of Medicine Memorial Fund.

Access of molecules from the periphery to the brain: barriers and gates of the hypothalamus

Janina Skipor
Department of Local Physiological Regulations, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland

The access of circulating factors to the brain is regulated by brain barriers: 1) the blood-brain barrier located in the endothelium of brain microvessels, 2) the blood-cerebrospinal fluid (CSF) barrier that consists of two main anatomical components, the internally situated epithelial cells of the choroid plexus and the externally located arachnoid membrane and 3) the CSF-brain barrier located in specialized ependymal cells lining the third ventricle — the tanycytes. Therefore, circumventricular organs (CVO) located in certain regions of the ventricular system of the brain play an important role in blood–brain communication. The blood-brain tissue exchange of molecules in CVO is enabled by the leaky endothelium, however, CVO are not a path across the blood-brain barrier. They are separated from the brain by an external glial barrier and from the CSF by a barrier at the ependyma. Fenestrated endothelial cells of median eminence (ME) together with ME and arcuate hypothalamus tanycytes (β2 and β1, respectively) constitute a highly sensitive "supply chain" for the distribution of blood-born metabolic signals. Leptin-mediated activation of hypothalamic neurons in the mediobasal hypothalamus seems to require a leptin receptor signaling in tanycytes and a passage of leptin to the CSF. The access of molecules to the hypothalamus is also modulated by the level of glucose that can be detected by tanycytes. The decrease in blood glucose levels during fasting alters the structural organization of blood-brain barrier in the area of the arcuate hypothalamus throughout the mechanism that requires the expression of vascular endothelial growth factor (VEGF) in tanycytes. The expression of several genes linked to body weight and energy balance has been demonstrated in tanycytes, wherein the expression of certain genes is regulated by food restriction and day length.

Comparison of the influence of sodium valproate (VPA) and nifedipine (NIF) on LH release stimulated by GnRH and Bay K 8644 from rat anterior pituitary cells culture *in vitro*

Justyna Skrzypska[1], Anna Litwiniuk[1], Malgorzata Kalisz[1], Ewa Wolinska-Witort[1], Lidia Martynska[1], Magdalena Chmielowska[1], Alina Gajewska[2], Elzbieta Wasilewska-Dziubinska[1]

[1]Department of Clinical Neuroendocrinology, Centre of Postgraduate Medical Education
[2]Department of Neuroendocrinology, The Kielanowski Institute of Animal Physiology and Nutrition Polish Academy of Sciences

Introduction: Previously we reported that VPA suppressed only GnRH and KCl stimulated but not basal LH secretion from rat primary anterior pituitary cells culture. Although the mechanism involved in mediating VPA effects in the pituitary gland is not well recognized, its potential impact on intracellular calcium metabolism cannot be excluded. It seems that VPA may act in a similar manner as dihydropyridine calcium channel antagonists because NIF and nitrendipine inhibited GnRH stimulated LH secretion from rat anterior pituitary cells and did not alter basal LH release *in vitro.* It was also stated that nitrendipine blocked the potentiation of GnRH- induced LH secretion by calcium channel agonist Bay K 8644 (BK 8644). If VPA acts in a similar manner as nitrendipine it may also suppress the ability of BK 8644 to potentiate LH responses to submaximal doses of GnRH.
The aim of the study was to compare the influence of VPA and NIF on the potentiation of GnRH and KCl induced LH secretion by BK 8644.
Material and methods: The primary pituitary cells culture was incubated 3 h with: GnRH 1 nM, GnRH 1 nM + BK 8644 1 μM, GnRH 1 nM + BK 8644 1 μM + VPA 10 μM, GnRH 10 nM, GnRH 10 nM + BK 8644 , GnRH 10 nM + BK 8644 1 μM + VPA 10 μM, GnRH 10 nM + BAY 10 μM + NIF 1 μM, GnRH 10 nM + BK 8644 1 μM + NIF 10 μM, KCl 30 mM, KCl 30 mM + BK 8644 1 μM, KCl 30 mM + BK 8644 1 μM + VPA 10 μM, KCl 30 mM+ BK 8644 1 μM + NIF 10 μM. Medium rLH concentration were determined by RIA method.
Results: Contrary to NIF the administration of VPA did not suppress the potentiation by BK 8644 of GnRH– and KCl–stimulated LH secretion from the rat anterior pituitary cell culture.
Conclusion: The mechanism of the inhibition by VPA stimulated by GnRH and KCl secretion of LH from the anterior pituitary cells is different from the mechanism action of NIF.
This study was supported by CMKP grants No 506-1-31-01-13 and 506-1-31-01-14.

Lymphocitic hypophysitis — case study

Dorota Szyska-Skrobot, Aldona Kowalska
Clinical Endocrinology and Department of Nuclear Medicine Holycross Cancer Center, Kielce, Poland

Introduction: Lymphocitic hypophysitis is numbered among autoimmune diseases with female predisposition.
Aim of the study: The presentation of the case of a female patient in whom the symptoms of diabetes insipidus appeared as the first and only manifestation of lymphocytic hypophysitis.
Case description: A 26-year-old woman was admitted to hospital to have the function of the pituitary assessed. In an interview a few months earlier she had reported excessive thirst, increased diuresis, weakness, lack of appetite, weight loss. She was hospitalized in a local hospital, where she was diagnosed with diabetes insipidus and where the treatment with Adiuretin was applied. In the performed MR, the pituitary stalk thickened to 5.5 mm was described (n. to 3.5 mm), undergoing strong contrast enhancement which might represent inflammatory infiltration as well as decreased signal from the neural part of the pituitary, characteristic of diabetes insipidus. On the basis of the performed hormone determination, the proper tropic function of the pituitary gland was found, hypothyroidism in the course of Hashimoto's disease was diagnosed and the differential diagnosis of hypophysitis was carried out

(chest X-ray and HRCT, BAL bronchoscopy, tuberculin test, ACE test, lumbar puncture) without finding irregularities. Based on the whole, lymphocytic hypophysitis was suspected and the treatment with Encorton was included at a dose of 60 mg per day and, while observing the improvement of the clinical condition, the dose of Adiuretin was reduced. In an imaging examination there was the normalization of the pituitary gland, and after a few years, the features of empty sella partially syndrome. The patient remains in observation, she takes Minirin and thyroxine.
Conclusions: Although lymphocytic hypophysitis is a rare disease, because of its potential life-threatening property it should be considered, particularly in women of child bearing age with the symptoms of hypopituitarism, diabetes insipidus and/or headache and disorders of the visual field in conjunction with the abnormal image of the gland in MR examination.

Integration of robotized radiotherapy in treatment of pituitary tumors

Rafał Tarnawski
III Clinic of Radiotherapy and Chemotherapy, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland

Radiotherapy has a long history of use in the treatment of pituitary tumors. Old irradiation techniques resulted in the relatively high incidence of side effects due to the high volume of irradiated critical structures. The development of new techniques, including Image Quided Radiotherapy (IGRT) and Intensity Modulated Radiotherapy (IMRT), resulted in the tremendous increase of treatment precision. Several clinical cases of pituitary tumors treated with robotized radiotherapy will be discussed.

The role of IL-1β in the regulation of hypothalamic-pituitary-gonadal axis in ewe during immunological stress — the influence of central anti IL-1β antibody and IL-1R antagonist injection on the GnRH/LH secretion

Dorota Tomaszewska-Zaremba, Andrzej Przemysław Herman, Karolina Król, Karolina Haziak
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, Poland

Introduction: Bacterial or viral infections have an inhibitory effect on female reproduction influencing the hypothalamic-pituitary-gonadal axis activity. Interleukin-1β (IL-1β) plays the main role in this process. The aim of the study was to investigate the effect of intracerebroventricular (icv) administration of anti-IL-1β antibody and IL-1R antagonist during immune stress induced by the intravenous (iv) administration of bacterial endotoxin — LPS on GnRH/LH secretion.
Material and methods: The study was performed on 20 anestrous ewes divided into 4 groups: control (NaCl iv, Ringer-Locke icv), LPS (LPS iv, NaCl icv), anti-IL-1β (iv LPS. anti IL-1β icv), IL-1R antagonist (iv LPS., IL-1R antg icv). The central injections were given 0.5 h before iv administration of LPS/NaCl. Blood samples were collected every 15 min for 6 h. After 2 weeks an analogous experiment was conducted. The animals were euthanized 2 hours after the administration of LPS/NaCl. The structures of the hypothalamus: the preoptic area (POA), anterior hypothalamus (AHA), medial basal hypothalamus (MBH), medial eminence (ME) and anterior pituitary (AP) were taken and the expression of GnRH and LHβ genes by Real Time PCR, GnRH concentration by ELISA and LH level in plasma by RIA were assayed.
Results: It was shown that the injection of LPS caused a significant reduction in mRNA level of GnRH in POA, AHA, MBH, and ME, and LHβ in AP, and the expression of the GnRH peptide in POA. The intraventricular administration of anti IL-1β antibody and IL-1R antagonist reversed the inhibitory effect of endotoxin on GnRH gene expression without affecting the GnRH peptide level in all studied structures; the strongest effect was observed in POA and ME. In AP, only IL-1R antagonist reversed the suppressive effect of LPS on LHβ gene expression.
Conclusion: The results confirm the hypothesis that the immune stress induced by LPS inhibits the secretion of GnRH/LH at the CNS level at least in part by IL-1β.
This work was supported by the National Science Centre, grant NN 31151644

Germline aryl hydrocarbon receptor interacting protein (AIP) gene mutations in patients with apparently sporadic pituitary macroadenomas (PMA) — a pilot study

Małgorzata Trofimiuk-Müldner, Anna Skalniak, Jakub Piątkowski, Grzegorz Sokołowski, Alicja Hubalewska-Dydejczyk
Chair and Department of Endocrinology, Jagiellonian University Medical College, Krakow, Poland; Department of Endocrinology, University Hospital in Krakow

Introduction: Germline AIP gene mutations have been linked with familial isolated pituitary adenomas (FIPA) and are responsible for about 15–30% of such cases. The inactivating mutations of AIP have also been reported in seemingly sporadic pituitary adenomas (in about 3–4% of all cases), particularly of early onset, aggressive, and growth hormone secreting.
Aim of the study: to assess the frequency and type of germline AIP gene mutations in patients with apparently sporadic PMAs.
Material and methods: The study included 31 consecutive patients with pituitary macroadenoma (17 males, 14 females; median age at diagnosis 43 years), followed in the Outpatient Clinic of the Endocrinology Department, University Hospital in Krakow. 13 subjects were diagnosed (based on hormonal results and, if feasible, on pituitary tumor histopathology) with non-functioning pituitary adenoma (NFPA), 10 — acromegaly, 4 — prolactinoma, 2 — Cushing's disease, 1 — TSH-oma, and 1 with gonadotropinoma. Median tumor size at diagnosis was 30 mm.
DNA of all participants was isolated from whole peripheral blood. The 6 exons of the AIP gene were sequenced using sanger sequencing (ABI 3500). The sequences were compared to reference data at NCBI, accordingly: NM_003977.2 and NP_003968.2. Nucleotide conservativity was estimated by Phyolop. The mutation influence on protein was estimated by PROVEAN Protein.
Results: 2 patients (9.8%) have been suggested to harbor a germline missense mutation in exon 3 of the AIP gene c.[377A > T];[=], p.[Q126L]; [=] (patient 1 — a 53 year old female with suprasellar NFPA, operated due to pituitary apoplexy symptoms — harboring also SNP rs2276020/c.[516C > T];[=], p.[D172 =]; [=]; patient 2 — a 41 year old female with 17 mm ACTH-secreting macroadenoma). The mutation, to our knowledge so far unreported, is localized in a highly conservative region of the gene and the substitution of Gln with Leu at codon 126, most probably leads to the abnormal function of AIP suppressor gene.
Conclusions: As both mutation carriers would not be selected for AIP gene sequencing based on clinical features, it seems feasible to search for germline AIP gene mutations in every patient with PMA. The presented report is an initial one, the recruitment of patients is still pending.

New prognostic classification of endocrine pituitary tumors useful in clinical practice

Jacqueline Trouillas
INSERM U1028; CNRS UMR5292; Université Lyon1; Centre de Pathologie Est, Hospices Civils de Lyon, Lyon, France

Endocrine pituitary tumors are frequently occurring intracranial tumors that are historically considered as benign. However, various pieces of clinical evidence and data obtained from recent advances in pathological and molecular analyses, favor their consideration as more than an endocrinological disease despite the low incidence of metastasis. These tumors are currently classified by histological, immunohistochemical and numerous ultrastructural characteristics, without prognostic clinical correlations.
Recently, we validated a new prognostic clinicopathological classification of these pituitary tumors by a retrospective multicentric case-control study comprised 410 patients who had surgery for a pituitary tumor with long-term follow-up. Using pituitary magnetic resonance imaging for diagnosis of cavernous or sphenoid sinus invasion, immunohistochemistry, markers of the cell cycle (Ki-67, mitosis) and p53, the tumors were classified according to size (micro, macro and giant), immunohistochemical type (PRL, GH, FSH/LH, ACTH and TSH) and grade (grade1a: non-invasive; 1b: non-invasive and proliferative; 2a: invasive; 2b: invasive and proliferative; and 3: metastatic. Analyses of disease-free and recurrence/progression status revealed the significant prognostic value ($p < 0.001$; $p < 0.05$) of age, tumor type, and grade across all tumor types and for each tumor type. Invasive and proliferative tumors (grade 2b) had a poor prognosis, with an increased probability of tumor persistence or progression of 25 or 12 fold respectively compared to non-invasive tumors (grade 1a). On the basis of six out of the eight carcinomas of this series being grade 2b at the first surgery, we propose that grade 2b or aggressive-invasive tumors are actually malignant tumors without metastasis. Until now, metastasis is required to give the diagnosis of carcinoma. However, the association of the following pathological signs: invasion, neoangiogenesis, vascular invasion, abnormal mitoses, very high indices for Ki-67 (i.e. > 10%) and p53 (i.e. > 5%), and genomic alteration (chromosome 11 deletion in PRL tumors), which combined might suggest malignancy.
In addition, pathological subtypes (silent ACTH or PRL tumors in men) appear to pose a higher risk of recurrence and resistance to conventional treatments. Moreover, molecular markers (endocan, somatostatine receptors) should improve the characterization of tumor behavior and prognosis.
This pathological information should further help the clinician to select patients presenting pituitary tumors with a high risk of recurrence or malignancy and to propose personalized therapies.

Cushing's syndrome — diagnostic problems

Tomasz Trybek, Aldona Kowalska
Department of Endocrinology, Holycross Cancer Center, Kielce

Most cases of endogenous Cushing's syndrome are the result of the presence of corticotropin secretion pituitary adenomas. The diagnosis of ACTH-dependent Cushing's syndrome requires a differentiation between corticotropinomas and ectopic secretion of ACTH or CRH syndrome. There are no pathognomonic symptoms of diseases. It is difficult to prove the relationship between the tumor and the presence of sometimes increasing concentrations of ACTH. The location of the source of the pathological secretion of adrenocorticotropic hormone may be a problem with the diagnosis and treatment. In this paper we present two cases of patients with the symptoms of hypercortisolism in ectopic Cushing's syndrome and describe the problems with the location of the source of ACTH secretion. The first case refers to a 26 year old man who had rapidly developed typical clinical symptoms of hypercortisolism, with deep hypokalemia and markedly elevated plasma levels of ACTH and cortisol. Diagnostic problems appeared to differentiate the causes of hypercortisolism. We obtained suppression to > 50% of baseline of urinary 17-OHCS on day 2 in high-dose (8-mg) dexamethasone suppression test and ACTH and cortisol response (approximately 30%) in CRH stimulation test. Plasma ACTH levels after CRH during external jugular vein catheterization did not indicate ACTH source of pituitary secretion. MRI scans of the pituitary were correct. CT scan revealed the bilateral thickening of adrenal glands, but chest CT scans were correct initially. The octreotide scintigraphy did not reveal the source of ACTH. We obtained a partial but significant inhibition of the synthesis of cortisol after the administration of a somatostatin analogue. High Resolution CT of the whole body revealed the left lung nodule. A left thoracotomy was performed and a bronchial neuroendocrine tumor was diagnosed. Postoperative hormonal tests and clinical improvement revealed a complete cure. The second case refers to a 66 year old man, also with typical clinical symptoms, with a deep hypokalemia and markedly elevated plasma levels of ACTH and cortisol. Unlike the previous patient we did not obtain the suppression of baseline of urinary 17-OHCS on day 2 in high-dose (8-mg) dexamethasone suppression test and lack of response ACTH and cortisol to CRH stimulation test. The results of the tests indicated hypercortisolism by ectopic ACTH secretion. CT scan revealed a tumor of the pancreas. We obtained the normalization of cortisol levels using somatostatin analogs. The patient currently has qualified for surgery. Despite the important progress in diagnosis, the ACTH-dependent Cushing's syndrome still remains a big challenge for clinicians and each case requires individual approach.

The patient with *prolactinoma* symptoms with no pituitary tumor on imaging and macroprolactinemia (case report)

Agnieszka Walczyk, Aldona Kowalska
Department of Endocrinology, Holy Cross Cancer Centre, Kielce, Poland

The lactotroph adenoma (*prolactinoma*) is the most frequent pituitary tumor. Supranormal serum prolactin (PRL) concentrations arising from the autonomous secretion by tumor cells result in hypogonadotropic hypogonadism presented in a reproductive female with *amenorrhoea-galactorrhoea* syndrome. It is known that there is a positive correlation between elevated serum PRL levels and *prolactinoma* size but the coexistence of macroprolactinemia may interfere in a correct diagnostic approach. In March 2014 a 43-year-old woman was admitted to the Department of Endocrinology, she presented with secondary amenorrhoea lasting 6 months and quite severe spontaneous galactorrhoea with no neurological disorders. An initial hormonal testing showed the level of estradiol less than 10 pg/mL, FSH 4.46 mIU/mL, LH 3.39 mIU/mL and hyperprolactinemia: 3858.0 mIU/L (reference range: 40-530) with no dysfunction of maintain tropic axes. The elevated PRL concentrations were detected in all consecutive measurements: 4304…3562…3710…3689 mIU/L, with no response in the metoclopramid-provocation test (PRL in 60′ — 3901, in 120′ — 3430 mIU/L). Drug-induced hyperprolactinemia and systemic diseases were excluded. The initial diagnosis of *prolactinoma* was established and magnetic resonance imaging (MRI) focused on the sellar region was performed but no pituitary

pathology was shown. The diagnostic process was extended and the detection of macroprolactin (BB-PRL) by 25% polyethylene glycol (PEG) precipitation method revealed the predominance of BB-PRL in each serum sample ranging more than 95% of assessed PRL complex with normal concentrations of monomeric prolactin. Nonetheless, a dopamine agonist — bromocriptine has been administered due to clinically symptomatic hyperprolactinemia. Currently we are waiting for the effects of the recommended therapy which will be studied during a control visit in our outpatient clinic soon. In the reported case the greatest challenge for an endocrinologist is to determine a source of clinical symptoms uniquely dependent on hyperprolactinemia. Despite no evidence for the presence of a pituitary tumor in MR imaging we cannot exclude the possibility of the existence of *microprolactinoma* but too small for imaging MR technique. Additionally, macroprolactinemia may hinder to establish an appropriate diagnosis. Moreover, in the presented case low concentrations of bioactive monomeric PRL were detected so we should consider the presence of intermediate molecular mass prolactin complexes able to connect to PRL-receptors with all known biological consequences.

Gonadotropinoma and macroprolactinemia in a 40-year-old woman — diagnostic pitfalls (case report)

Agnieszka Walczyk[1], Aldona Kowalska[1], Wiesław Bonicki[2]
[1]Department of Endocrinology, Holycross Cancer Centre, Kielce, Poland
[2]Department of Neurosurgery, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland

Pituitary tumors in reproductive females may cause menstrual disturbances. However, secondary amenorrhea is usually the manifestation of *prolactinoma*, it also may be present as a symptom due to the 'mass effect' of pituitary tumors arising from other but lactotroph cells.
In December 2011 a 40-year-old woman was admitted to the Department of Endocrinology. She presented with secondary amenorrhea lasting 9 months and preceded by a 3-month *oligomenorrhea, a* severe headache lasting 3 months, anisocoria and a right eye visual field defect. Prior to the admission, a gynecologist consulted her and premature menopause was established, however, no therapy was administered. Physically galactorrhea was not observed. Her endocrine profile showed no tyreo-, cortico- and somatropic dysfunction but total prolactin (PRL) concentrations in consecutive measurements were elevated: 1217…1878…1696 mIU/L (reference range: 40-530). The testing of gonadotropic axis: supressed LH (< 0.1 mIU/mL) and normal value of FSH (6.54 mIU/mL) with estradiol 60 pg/mL. Magnetic resonance imaging (MRI) focused on the sellar region showed a large solid tumor (33x30x25 mm) extending into the suprasellar region with fields of cystic degenerations, the invasion of the cavernous sinus and compressing the optic chiasm. The initial diagnosis of *prolactinoma* with a possible 'hook effect' occurring in the prolactin immunoassay was established. Nonetheless, the diagnosis was verified due to the retesting of the original serum prolactin samples with a serial dilution revealing the similar results to the previous prolactin levels. Moreover, free PRL turned out to be 318 mIU/L, macroprolactin (BB-PRL) content was 67.8% of the total PRL concentration of 1113 mIU/L. Finally, the patient diagnosed of non-functioning pituitary adenoma was recommended to surgery. On 11 January 2012, she underwent endoscopic transsfenoidal adenomectomy with no post-operative complications. Histopathological examination revealed the gonadotropic pituitary adenoma: FSH (+), alfa-subunit (+/–), LH (–) PRL (–).In February 2012 the restoration of menstruation was achieved, the visual problems totally improved, whereas, a temporary headache is present. In the diagnostic approach of menstrual disturbances in women clinicians should consider gonadotropic insufficiency due to pituitary tumors with possible coexistent macroprolactinemia which may lead to misdiagnosis and difficulties to apply an appropriate therapeutic strategy.

The assessment of the effect of histone deacetylases (HDAC) inhibitors: Trichostatin A (TSA), Scriptaid (SCP) and sodium valproate (VPA) on GnRH-stimulated secretion of LH from rat anterior pituitary cells in primary culture

Elzbieta Wasilewska-Dziubinska[1], Alina Gajewska[2], Justyna Skrzypska[1], Anna Litwiniuk[1], Malgorzata Kalisz[1], Ewa Wolinska-Witort[1], Lidia Martynska[1], Magdalena Chmielowska[1]
[1]Department of Clinical Neuroendocrinology, Centre of Postgraduate Medical Education
[2]Department of Neuroendocrinology, The Kielanowski Institute of Animal Physiology and Nutrition Polish Academy of Sciences

Introduction: Previously we reported that VPA — an antiepileptic drug- suppresses only GnRH stimulated but not basal LH release from rat anterior pituitary cells *in vitro.* The mechanism involved in mediatory VPA effects in pituitary cells is not well recognized. VPA is included in the group of HDAC. Recently it has been found that three different HDAC inhibitors — TSA, SCP and VPA have similar inhibitory effects on LH–induced androstendione secretion by bovine theca cells and FSH — induced estrogen secretion- by bovine granulosa cells. With regard to the mechanism through which VPA suppresses androgen secretion by theca cells, the findings reported by Glister et al. (2012) support the notion that the action of VPA was due to its activity as a type 1 HDAC inhibitor. In available literature the research concerning TSA and SCP effects on LH secretion from pituitary *in vitro* has not been found.
Aim of the study: To explore the possibility that also the suppressive action of VPA on GnRH stimulated secretion of LH from anterior pituitary cells of rat reflects its HDAC inhibiting properties, the aim of this study was to compare the effects of VPA with those two highly potent HDAC inhibitors, TSA and SCP.
Material and methods: The experiments were performed on the primary culture of anterior pituitary cells of the rat. The effects of TSA 1 μM, SCP 1 μM and VPA 1 μM and 10 μM (as a control) on GnRH (100 nM)-stimulated LH release from anterior pituitary cells were examined after 3 h of incubation. Medium LH concentrations were determined by RIA method.
Results: TSA and SCP as a VPA also suppressed GnRH stimulated LH secretion suggesting that the action of VPA reflects its HDAC inhibiting properties.
Conclusion: It seems that VPA inhibits GnRH dependent LH stimulation likely by inhibition of HDAC.
This study was supported by CMKP grants No 501-1-31-22-13 and 501-1-31-22-14.

Galanin and galanin-like peptide participate in the mechanisms of vasopressin release from the rat hypothalamo-neurohypophysial explant *in vitro:* the role of galanin receptors

Justyna Wodowska, Joanna Ciosek
Department of Neuropeptides Research, Chair of General and Experimental Pathology, Medical University of Lodz, Poland

Introduction: Galanin (Gal) and galanin-like peptide (GALP) are widely distributed in the different areas of the central nervous system where they exert their distinct biological actions. Some previous findings indicate the ability of Gal and GALP to alter vasopressin (AVP) secretion from the posterior pituitary gland.
Aim of the study: These experiments were performed to study the effects of Gal and GALP on AVP release from the incubated rat hypothalamo-neurohypophysial explant (Hth-NH) or neurohypophysis (NH) *in vitro*.
Material and methods: Male adult Wistar rats as the donors of appropriate nervous tissues were divided into two series: series 1 — the incubation of Hth-NH with Gal tested at the concentrations 10^{-10} and 10^{-8} M, series 2 — the incubation with GALP (10^{-10} and 10^{-9} M). To some incubations galantide — an antagonist of galanin receptors were added. After each incubation, the samples were stored at –25°C until AVP estimation by radioimmunoassay.
Results: Both concentrations of Gal were found to inhibit the basic as well as K^+-stimulated AVP release separately from the NH and Hth-NH; galantide at a concentration of 10^{-10} M blocked such Gal action. The presence of GALP in the incubative media was the cause of intensified AVP release from both NH and Hth-NH. Galantide did not block GALP influence on AVP secretion into incubative media.
Conclusions: It may be assumed that in the rat central nervous system, Gal acts as an inhibitory neuromodulator for AVP release *via* its galanin receptors. On the other hand, the stimulatory effect of GALP on AVP release is likely to be mediated *via* an unidentified specific GALP receptor(s).
This study was supported by grant of Medical University of Lodz no 502-03/6-103-02/502-64-045.

Prevalence of goiter, benign and malignant tumors in acromegalic patients

Kosma Woliński, Adam Stangierski, Edyta Gurgul, Marek Ruchała
Department of Endocrinology, Metabolism and Internal Medicine Poznan University of Medical Sciences

Introduction: Acromegaly is a quite rare disease caused by the increased secretion of growth hormone. The most commonly observed complications of acromegaly include cardiovascular and respiratory system diseases and also the increased risk of benign and malignant tumors.
The aim of the study was the retrospective analysis of the prevalence of benign and malignant neoplasms in patients with acromegaly, with particular emphasis on thyroid morphological changes.
Material and methods: Medical documentation of patients treated in one medical center from the years 2004-2013 was searched. The data on the prevalence of benign and malignant neoplasms were assessed using the latest available records of particular patients.
Results: 180 patients with acromegaly were identified (108 women, 72 men). The mean age at the time of the last available assessment was 52.5 years (standard deviation 12.2, median — 54.0). 140 patients (77.8%) had focal lesions in thyroid, including 110 patients with multinodular goiter (MNG) (61.1%) or resection of MNG in the past medical history. Thyroid cancer was diagnosed in 11 patients (6.1%), including 10 papillary and one follicular thyroid cancer. Thirty-two patients (17.8%) had thyroid surgery or radioiodine treatment in the past; among the rest of the patients a mean thyroid volume was 35.3 ± 27.3 cm^3 in men (median 30.6) and 32.5 ± 29.7 in women (median 22.0). Among other malignancies, the most common were breast cancer- in 5 patients (4.6% of women with acromegaly) and colonic cancer- in 4 patients (2.2%).
Conclusions: Patients with acromegaly have the high risk of goiter, thyroid lesions and thyroid cancer, which was present in the medical histories of over 6% of patients. Among other malignancies, breast and colonic cancer were most frequent. According to our results, an active screening for potential malignancies should be an important part in the management of acromegaly.

The effect of intracerebroventricular infusions of obestatin on the secretory activity of luteinizing hormone in the pituitary in prepubertal female lambs (preliminary results)

Anna Wójcik-Gładysz, Alina Gajewska, Tomasz Misztal, Katarzyna Romanowicz, Michał Szlis, Jolanta Polkowska
Department of Endocrinology, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Instytucka 3, Jabłonna, Poland

Introduction: Obestatin is an anorexigenic peptide engaged in the integration of information about the organism nutritional status with the reproductive processes. Obestatin acting both at the periphery and at the central nervous system level, along with ghrelin, leptin and neuropeptide Y can form a neuromodulatory network that modulates the gonadotrophic axis activity. The mechanism of obestatin action on the hypothalamic-pituitary system related to reproduction processes is not fully elucidated, and, especially in ruminants, requires intensive research.
The aim of the present study was to investigate the effect of intracerebroventricular (ICV) infusion of obestatin on the secretory activity of LH cells in the pituitary gland in prepubertal lambs.
Material and methods: Immature female lambs were randomly divided into two groups. One control group (vehicle infusion) and an experimental group (obestatin infusion). The infusions were performed during three consecutive days; blood samples were collected during the "day 0" and "day 3" After the experiment the sheep were slaughtered and the brains were protected for the further analysis.
Results: In the sheep ICV infused with obestatin the decrease in the secretory activity of the LH cells in the pituitary gland was observed. This was manifested by a reduction in the amount of immunoreactive LH accumulated in the secretory granules of the pituitary cells. The mean LH plasma concentrations decreased in the obestatin infused animals between day 0 and day 3 of the infusions ($p < 0.01$) compared to the values noted in the standard fed group and this decrease is related to a lower pulse amplitude ($p < 0.05$). However, ICV administered obestatin did not cause any changes in the mRNA for LHβ subunit level in the gonadotrophic cells.
Conclusions: It can be suggested that obestatin takes part in the mechanisms linking the nutritional status of an organism with the reproductive function affecting the LH - producing cells secretory activity in the pituitary gland.

Pituitary adenomas — pharmacological or surgical treatment?

Wojciech Zgliczyński[1], Grzegorz Zieliński[2], Jolanta Kunert-Radek[3]
[1]Department of Endocrinology, Medical Centre of Postgraduate Education, Warsaw
[2]Department of Neurosurgery Military Medical Institute, Warsaw, [3]Clinic of Endocrinology Medical University of Lodz

Pituitary adenomas are benign, slowly growing neoplasms deriving from adenocytes of pituitary gland, presenting mostly hormonal activity. They consist of very diverse group of tumours localized in

sella turcica region that can lead not only to hyperactivity of pituitary (excessive secretion of PRL, GH, ACTH, TSH, gonadotropins and their subunits), but also to its hypoactivity. Those tumours vary in size, localization, as well as infiltration of surrounding structures and speed of growth — invasiveness. Likewise, they may differ from one another in expression of dopamine and somatostatin receptors (including all of the subtypes). Therefore all mentioned factors should be considered during planning treatment schedule side by side with precising main goal of treatment.

In case of treatment of secretory pituitary adenomas a main objective is inhibition of their hormonal activity, which in prolactinomas improves gonads function, in acromegaly restores expected lifespan as well as improves its quality, in Cushing's disease significantly decreases mortality. The next aim of therapy is reduction of size of a tumour that normally leads to remission of neurological disorders and improvement of pituitary hormonal function.

In case of prolactinomas, treatment of first choice is a use of dopamine agonists. These drugs do not only block PRL secretion, but also result in regression of tumour, which convinces to use conservative treatment as the one of choice, even in patients with big tumours and visual field deficits. Surgical treatment should be conducted in patients presenting resistance to pharmacological therapy or intolerating it. Women with prolactinomas that are planning a pregnancy should be considered individually concerning a purpose of surgery. Patients with acromegaly, who prognose recovery after surgery should be prepared preoperatively with somatostatin analogues. Rest of patients, including those who already underwent surgery without expected remission of disease should be subjected to chronic treatment with long-acting analogues of somatostatin.

Treatment of Cushing's disease, which ought to be radical therapy, despite constant progress in medicine is still challenging for endocrinologists and neurosurgeons. Surgical treatment (treatment of choice) may be difficult in cases of microadenomas that are not previously localized in MRI (approximately 40% of cases). Relatively high percentage of recurrence of hypercortisolism is an additional problem that may appear even many years after effective surgery. The advancement in Cushing's disease therapy includes undertaking surgical treatment (*sella turcica* exploration) in cases of hormonally confirmed disease with negative pituitary magnetic resonance imaging (MRI), as well as greater possibilities of pharmacological therapy. Among drugs presenting causal action, inhibiting hormonal activity of corticotroph adenomas, it is worth mentioning somatostatin analogue — pasireotide, being effective in approx. 20% of cases and dopamine agonist — cabergoline with its effectiveness in approx. 40% of patients.

Most of clinically non-functioning pituitary adenomas (NFPA) are characterised by the high density of somatostatin receptors (SSTR). Last data focus on the clinical trials evaluating somatostatin analogues (SSA) in the treatment of NFPA, especially in the cases of recurrent adenomas after the incomplete neurosurgeries of invasive tumours. The pharmacotherapy of primary tumours is a therapeutic option for patients who do not agree for neurosurgery and for those who are disqualified from neurosurgical procedures. The effects of SSA treatment in primary and recurrent NFPA as well as in cases of coincidence of other brain tumours were discussed.

All things considered, it is worth emphasizing to remember during planning a treatment of pituitary tumour that its results should not be worse than the disease itself.

Therefore decision about a choice of proper therapy should be made individually depending on type of adenoma, its size and localization with correlation to expected respond to pharmacological treatment (expression of proper receptors).

Is chromogranin A a specific marker of adrenal chromaffin cells?

Agnieszka Ziolkowska, Anna Hochół-Molenda, Marcin Trejter, Paulina Grabowska, Marianna Tyczewska, Marcin Ruciński, Ludwik K. Malendowicz

Department of Histology and Embryology, Poznan University of Medical Sciences

Chromogranin A (CgA) is a member of granins — a protein family that controls the formation and secretion of hormones from granules in endocrine and neuroendocrine cells. Regarding adrenal gland, CgA is known as an important marker of tumors derived from chromaffin cells (*pheochromocytoma*). CgA is also a prohormone that gives rise to several bioactive peptides such as vasostatins 1 and 2, chromofungin, chromacin, pancreastatin, catestatin, WE14, chromostatin, GE25, parastatin, and serpinin. CgA expression has been demonstrated also e.g. in the adenohypophysis, islets of Langerhans, follicular and parafollicular cells of the thyroid gland, DNES cells of the gastrointestinal tract and in many other endocrine cells. These observations led us to the precise analysis of CgA expression in particular components of the rat adrenal gland.

In mammals, adrenal glands are composed of a centrally placed medulla and peripherally situated cortex. The cortex and medulla arise from different germ layers, however these two components (with different structure and function) — even in a mature gland — are not strictly separated from each other.

It has been shown using QPCR, that adrenal glands express CgA from the earliest stages of postnatal development. The highest CgA expression level was observed in neonates. The expression level of many bioactive peptides and neuropeptides in adrenal glands increases and could be related to the stress occurring during delivery. In the subsequent periods of ontogenesis the significant decrease of CgA expression was observed, followed by a slight increase during puberty period. The expression profile of CgA was similar in both sexes. CgA expression at the mRNA level was observed in all adrenal components, however, the expression in the cortex was several times lower than in the medulla. CgA expression was investigated during enucleation-induced adrenal regeneration. From the first day of the experiment CgA expression was significantly lower than in the control adrenal cortex, and temporarily increased on day 8.

CgA expression has been also demonstrated in rat adrenocortical cells, both freshly isolated as well as in primary culture. In cultured adrenocortical cells ACTH increases CgA expression.

CgA expression at protein level was confirmed by immunofluorescence. Using this method also a co-localization of CgA and steroidogenic acute regulatory protein (StAR) was demonstrated.

CgA expression in rat adrenocortical cells was significantly lower than in medullary chromaffin cells, but simultaneously higher than in the cells of other examined endocrine glands (pituitary, thyroid, ovary and testis). In contrast to the medulla, where CgA is easily detectable at protein level, in the cortical cells CgA protein expression is very low. The enzymatic modifications of CgA that - potentially - occurs in adrenocortical cells may prevent the binding of the antibody - used in immunohistochemical and immunofluorescence method — to the native protein. It may suggest the rapid metabolism of CgA in steroidogenic cells and indicates a different function of this peptide in adrenocortical cells when compared to the medulla.

This study was supported by a grant N N401 334639 from the Ministry of Science and Education in Poland.

Acute and chronic inflammation modulate hypothalamic ghrelin secretion in piglets

Joanna Zubel-Łojek, Ewa Ocłoń, Anna Latacz, Klaudia Pałka, Krystyna Pierzchała-Koziec
Department of Animal Physiology and Endocrinology, University of Agriculture, Krakow, Poland

Introduction: Ghrelin was primarily isolated from the stomach but is also distributed in the central nervous fragments. Ghrelin plays an important role in energy homeostasis, the control of food intake and immunity. There is evidence suggesting that ghrelin is associated with immune cells activity.
Aim of study: The present study was performed to examine the effect of TNF and dexaven on the hypothalamic ghrelin secretion during acute and chronic inflammation.
Material and methods: The experiment was carried out on 10-week-old piglets (n = 24). The animals (females) were kept in standard conditions and divided into 4 experimental groups: I — control, II — chronic inflammation (overweight), III — acute inflammation and IV — overweight with acute inflammation. The piglets from groups I and III were fed with commercial food, whereas the animals from groups II and IV received high-calories diet in order to develop overweight. For inducing acute inflammation the animals received a single i.p. injection of streptozotocin (100 mg/kg b.w.). 24 hours after the injection the hypothalamus was quickly removed. The tissue fragments were placed in Eagle'a medium and incubate 20 min without (basal release) or with the addition of exogenous TNF or dexaven (DEX). The media were directed to the estimation of ghrelin secretion (RIA, Millipore).
Results: It was observed that inflammatory process (acute and chronic inflammation) changed *in vitro* ghrelin release from the hypothalamus. Also *in vitro* addition of TNF increased (84–172%), whereas DEX decreased (16–82%) ghrelin secretion.
Conclusions: The obtained results indicate that hypothalamic ghrelin secretion is modulated by the immune system activity.
Supported by: NN 311 227 138, DS 3243/KFiEZ

Survivin expression in invasive pituitary gland adenomas with a diameter exceeding 20 mm

Ariadna Zybek[1] , Joanna Waligórska-Stachura[1], Nadia Sawicka-Gutaj[1], Mirosław Andrusiewicz[2], Marek Ruchała[1]
[1]Department of Endocrinology, Metabolism and Internal Medicine, Poznan University of Medical Sciences, Poznan, Poland
[2]Department of Cell Biology, Poznan University of Medical Sciences, Poznan, Poland

Introduction: Survivin is a multifunctional protein and acts as the inhibitor of apoptosis. Its expression occurs in nearly all human cancers. In some tumors survivin expression correlates with the malignant behavior and the diminished response to cytotoxic therapy. The data concerning survivin expression in invasive pituitary gland adenomas are contradictory.
Material and methods: Survivin expression was assessed in 38 invasive pituitary gland adenomas (31 non-functioning tumours, seven somatotroph adenomas) with a diameter exceeding 20 mm, removed during transsphenoidal surgery. 12 control samples of the normal pituitary tissue were obtained post-mortem. The tumour size was assessed by preoperative MRI scan. The amplification of survivin gene using sequence specific primers and qRT-PCR method was performed.
Results: The mean age of patients was 54 ± 14 years. The mean tumour size was 33.8 mm ± 7.8 mm (Min. 20 mm, and Max. 55 mm). Survivin expression was found in 31 out of 38 tumours and in ten out of 12 control samples. There was no difference between the level of survivin expression in the pituitary adenomas and in the normal pituitary tissue samples.
Conclusions: Survivin expression in invasive pituitary adenomas varies greatly. Our results suggest that survivin expression in invasive pituitary adenomas is comparable to the healthy tissue. Since a considerable effort has been made in recent years to explore new therapeutic options based on survivin counteracting chemicals, its potential role in pituitary adenomas needs further evaluation.