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Late abstracts

PGE2 as a possible molecular mechanism of compensation lack of immunesensory function of the vagus nerve during infection

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The vagus nerve constantly monitors the state of the body's internal environment. It is also one of the crucial routes of communication between the peripheral immune system and the central nervous system (CNS). It provides information about the local excitation of the immune system to the CNS. This information allows to start centrally controlled processes to prevent the development of infection. The nerve itself, trough inflammatory reflex, controls and limits excessive local inflammatory response.

Aim of the study was to propose a possible mechanism explaining the changes in the activity of central neurotransmission systems observed in the context of the increased activity of the humoral route of communication between the immune and nervous systems.

As we have shown (using HPLC-ED with Reversed Phase, Agilent 1100 chromatographic system) in the situation of vagal dysfunction, central neurotransmission activity changes within the brain structures associated with stress, emotions or pain feeling. These changes concern in particular in the activity of the dopaminergic system. We have shown that these changes are largely eliminated during peripherally experimental inflammation. These observations suggest existence of the molecular mechanisms that compensate lack of immunesensory and immunesuppressive functions of the vagus nerve which are activated in the face of infection. The results of our research suggest that this effect may be associated with increased peripheral synthesis of prostaglandin E2 (LC-MS, Eksigent microLC 200 System coupled with AB Sciex 4500 QTRAP mass spectrometer). Based on our results and available literature, we proposed possible explanation of the communication mechanisms between the immune system and the CNS during vagus nerve dysfunction.

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The influence of interleukin 15 and metabolic inhibitors on lipid deposition in 3T3-L1 adipogenic cultures

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Introduction: Interleukin 15 (IL-15) plays an important role in the lipid metabolism and the regulation of lean/fat body composition in animals with obesity or type 2 diabetes. However, the underlying molecular mechanisms responsible for the beneficial effects of IL-15 remain largely unknown.

The aim of the study was to evaluate the molecular mechanism of IL-15 influence on the adipocyte differentiation, lipid deposition, and to assess the ability of IL-15 to modulate the expression of interleukin 10 (IL-10) and uncoupling protein (UCP-1) in 3T3-L1 cells at different stages of differentiation.

Materials and methods: We examined 3T3-L1 preadipocytes a standard differentiation protocol. The 3T3-L1 preadipocytes were treated with DMEM (control) or experimental factors: IL-15 alone or with LPS and metabolic inhibitors (PD98059; LY294002; Compound C; AG490; LiCl). The quantitative analysis of adipocyte differentiation was done by oil red O staining. Immunoreactivity of IL-10, UCP-1 proteins was analyzed by Western Blot.

Results: IL-15 treatment decreased ($p < 0.001$) lipid deposition on the 2nd and 8th day of differentiation. Combined treatment with PD98059 increased the effect of IL-15 ($p < 0.001$), while LY294002 attenuated the effect of IL-15 ($p < 0.01$) on the 8th day. IL-15 increased expression of IL-10 and UCP-1 in the selected days of differentiation.

Conclusion: Our results suggest that IL-15 by PI-3-K/AKT and MEK/ERK signaling pathways exerts important effects on lipid deposition in the mature adipocyte. Moreover, IL-15 can modulate expression of IL-10 and UCP-1.

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The influence of orexin A and endogenous cannabinoids on the activity of GnRH neurons in female rat hypothalamus

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It has been known that in vitro OX1R and CB1 receptors can interact with each other to form heterodimers.
The aim of the study was to examine the in vivo effect of orexin A (OxA) acting through the OX1R receptor on the activity of GnRH neurons with blocked CB1 receptors of the cannabinoid system in female ovariectomized (OVX) rats.
The experiment was conducted on 3-month-old OVX female Wistar rats which underwent two short-term infusions into the third ventricle of the brain: firstly, the CB1 receptor antagonist (AM281) or 10% DMSO in artificial cerebrospinal fluid (CSF) was infused, and after an hour, OxA or CSF. The experiment was carried out in the following groups: 1. AM281 + CSF; 2. AM281 + OxA; 3. DMSO + OxA; 4. DMSO + CSF–control.
One hour after the end of the infusion, blood was collected from the heart. The concentration of plasma LH was determined using RIA method. The brains were removed and fixed for tests of GnRH neurons in the median eminence of the hypothalamus using the immunohistochemical method.
There were no statistically significant differences in plasma LH concentrations between the groups. In the control group (DMSO + CSF) the LH concentration was 7.9 ± 3.5ng/ml, in the DMSO + OxA group the LH concentration increased (11.2 ± 3.6 ng/ml), similarly to the AM281 + CSF group (19.3 ± 14.9 ng/ml) and the AM281 + OxA group (15.6 ± 7.6 ng/ml).
The analysis of histological preparations did not show any significant differences in the image of GnRH neuron endings (accumulation of irGnRH immunoreactive material) in the hypothalamus of females from particular groups.
Our results indicate that the activity of GnRH neurons, under the adopted in vivo conditions, is not significantly dependent on the interaction of the OxA system and the cannabinoid system in mature female OVX rats.

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A neurosteroid, allopregnanolone, inhibits the endocrine pituitary response to stress

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Acute stressors induce an increase in pituitary hormone secretion to the blood. However, in pregnant females, the endocrine stress response is decreased and it is thought that a neurosteroid, allopregnanolone (AL), plays a crucial role in this process. Thus, we have investigated the central effects of AL on the secretion of adrenocorticotropin (ACTH), prolactin (PRL) and gonadotropins (LH and FSH) in sheep subjected to stressful stimuli.
Adult, intact sheep (n = 24) were implanted with stainless steel guide cannula into the third brain ventricle and divided into 4 groups: i. infused for 3 days with Ringer-Locke (RL) solution (C group); ii. infused with RL and treated in the third day with stressful stimuli (isolation and partial immobilization; S group); iii. infused with AL (4 × 15 μg/60 μl/ 30 min for 3 days) and treated with stressful stimuli (AS group); and iv. infused with AL alone (A group). Plasma hormones concentrations were assayed in samples taken every 10 min., for 4 h, by the RIA method.
Plasma ACTH, PRL and LH concentrations, except FSH, increased ($p < 0.001$) in response to stressful stimuli and maintained at a high levels by the end of experiment in comparison to C group. Infusion of AL in the AS group decreased ($p < 0.001$) the levels of these hormones within 2 h in comparison to S group. AL evoked a decrease in FSH concentrations in both AS ($p < 0.05$) and A ($p < 0.001$) groups, while administered alone it did not affect ACTH, PRL and LH as compared to controls. In conclusion, AL is a potent inhibitor of the endocrine pituitary response to stress. Moreover it may block the maturation of ovarian follicles during pregnancy.

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Influence of allopregnanolone on the expression of selected genes in the anterior pituitary of stressed and non-stressed sheep

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Allopregnanolone (AL) is a neurosteroid which exerts an inhibitory mode of action through interaction with

the GABAA receptor. The GABAergic projections reach the hypothalamus and may indirectly affect secretory activity of the anterior pituitary (AP). The aim of this study was to investigate whether central administration of AL would modulate the expressions of follicle stimulating hormone (FSH), luteotropic hormone (LH), prolactin (PRL) and proopiomelanocortin (POMC) genes in the AP of stressed and non-stressed sheep.
Adult, intact sheep (n = 24) with stainless steel guide cannula implanted into the third brain ventricle were divided into 4 groups: i. infused for 3 days with Ringer-Locke (RL) solution (C group); ii. infused with RL and treated in the third day with stressful stimuli (isolation and partial immobilization; S group); iii. infused with AL (4 x 15 μg/60 μl/ 30 min for 3 days) and treated with stressful stimuli (AS group); and iv. infused with AL alone (A group). The animals were euthanized after the last infusion and the expressions of selected genes were determined in the AP by Real Time-PCR.
It was demonstrated that both stress and AL decreased the expression of FSH mRNA in comparison to controls ($P < 0.05$). The expression of LH gene increased in S group ($P < 0.05$) but AL prevented this increase in AS group ($P < 0.05$) with no effect in A group. The amounts of PRL mRNA increased in response to AL in AS and A groups in comparison to controls ($P < 0.05$). Neither stress nor AL influenced the expression of POMC gene in the AP. In conclusion, AL may differently modulate the expression of selected genes of the AP hormones in stressed and non-stressed sheep.

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The influence of subdiaphragmatic vagotomy on the activity of selected neurotransmission systems in the rats' striatum after intraperitoneal administration of lipopolysaccharide

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Information exchange between the immune and central nervous systems (CNS) is possible due to the presence of a neural and humoral pathways. The neuronal pathway is represented by peripheral sensory nerve endings, e.g. the vagus nerve. In contrast, the humoral pathway acts via signaling molecules (cytokines, prostaglandins) released into the bloodstream by activated immunocompetent cells. The main goal of the study was to determine the effect of subdiaphragmatic vagotomy and peripheral inflammation on the activity of the neurotransmitter systems in rat striatum. The next aim of the study was to examine changes in the density of EP3 receptors for PGE-2 occurring as a potential mechanism to compensate for the lack of vagus nerve. Determination of the activity of neurotransmission systems in the rats striatum was performed using HPLC-ED (Agilent 1100). To determine the density of the EP3 receptors, an indirect immunohistochemical reaction was made, and then, using confocal macroscope (Leica LSI), scans of stained brain sections were obtained. The obtained data indicate the effect of subdiaphragmatic vagotomy on the activity of neurotransmission systems in the CNS. Bilateral section of the vagus nerve, below diaphragm, increases the activity of the dopaminergic and noradrenergic system in the right while serotonergic one in the left striatum. The present data shows the influence of LPS-induced peripheral inflammation on the increase of serotonergic system activity in the left striatum and dopaminergic in the right. Additionally we observed decreased density of EP3 receptors in vagotomized rats. Moreover, the results confirm the hypothesis about the neurochemical lateralization of striatum.

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Pituitary and adrenal responses to vagotomy in rat

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Vagus nerve is the most important part of the parasympathetic system. The role of vagus nerve is particularly important during the stress response modulating the interaction of nervous, endocrine and immune system activities. Stress responses are manifested by hormones synthesis and release changes from central and peripheral levels of hypothalamic-pituitary-adrenal (HPA) axis. Endogenous opioid peptides, widely distributed in the body, are involved in the modulation of the systems activity in the physiological and pathological situations. Thus, the aim of the study was to evaluate the effect of vagotomy on the Adrenocorticotropic hormone (ACTH), corticosterone and Metenkephalin concentrations in the rat pituitary and adrenal gland. Experiment was carried out on adult, male Wistar rats

divided into intact, NaCl injected, sham operated and vagotomy treated groups. Met-enkephalin, ACTH, corticosterone were measured by radioimmunoassay (RIA) in the pituitary and adrenal. Vagotomy significantly increased Met-enkephalin concentrations in the both glands as well as corticosterone level in the adrenal. Unexpectedly, ACTH pituitary level was not strongly affected by vagotomy. Interestingly, injection of NaCl was the most stressful factor for pituitary ACTH and Met-enkephalin increasing their levels by 63 and 206%, respectively. It must be pointed out that increased concentration of corticosterone in adrenal gland was in parallel with higher level of Met-enkephalin what may suggest attenuating effect of opioid on the glucocorticoid secretion from the adrenal cortex. Changes in the synthesis and release of corticosterone and Met-enkephalin from the adrenal suggest the direct effect of parasympathetic system on the adrenals. Increased concentration of Met-enkephalin in the pituitary probably potentiated the release of ACTH from the gland after vagotomy.

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Age-dependent changes in the proenkephalin mRNA expression in the HPA axis in stressed lambs

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Endogenous opioid peptides are involved into regulation of the hypothalamo-pituitary-adrenal (HPA) axis during stress responses. However, the synthesis, secretion and concentration of Met-enkephalin, one of the most important peptide, in the brain and peripheral tissues are associated with the gestation period in human and animals.

The objective of the study was to investigate whether the proenkephalin mRNA (PENK) expression is associated with the stressful responses in growing lambs.

The experiment was carried out on 3, 6 and 9 months old female lambs divided into control and stressed by 30 min of isolation from the herd. Sixty min after the onset of isolation (30 min after the end of stress), fragments of hypothalamus, pituitary and adrenals were taken out and the PENK mRNA expression was measured by in situ hybridization. Additionally, the levels of Adrenocorticotropic hormone (ACTH), cortisol and Met-enkephalin were evaluated. The results showed the increased level of mRNA expression in each level of HPA axis after isolation. Interestingly, the most pronounced changes were noticed in the HPA of 6 months old lambs. Unexpectedly, at this age, hypothalamus mRNA expression was the highest and increased above the control value by 58% compare to pituitary (increase by 25%) and adrenal (increase by 35%). Thus, the obtained results clearly showed that the response of PENK mRNA expression to the isolation stress at each level of HPA axis are strongly dependent on the age of lambs. It seems probable that the hormones of hypothalamo-pituitary-gonadal (HPG) axis interact with the opioid system at the hypothalamic level. The question arises whether opioid peptides serve as stimulatory or inhibitory factors to the HPG axis.

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