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ABSTRACTS

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THE CREB CO-ACTIVATOR, TORC 2: A MASTER CONTROLLER OF HPA AXIS ACTIVITY DURING STRESS

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Adequate regulation of hypothalamic corticotropin releasing hormone (CRH) secretion and expression is essential for stress adaptation. Transcriptional regulation of the CRH gene depends on cyclic AMP/protein kinase A-depending signaling and binding of phospho-CREB to the CRH promoter. Increasing evidence indicates that initiation of CRH transcription requires activation and translocation of the CREB co-activator, transducer of regulated CREB activity (TORC), from the cytoplasm to the nucleus. In basal conditions, TORC is located in the cytoplasm in the phosphorylated state by the Ser/Thr protein kinase, salt inducible kinase (SIK). In vitro, TORC overexpression potentiates cyclic-AMP-stimulated CRH transcription, while TORC blockade using silencing RNA blunts it. Immunohistochemistry show irTORC2 in the cytoplasm of all CRH neurons of the PVN in basal conditions. Staining shifted to the nucleus, exclusively in CRH neurons (about 60%), at 30 min restraint stress and returned to the cytoplasm by 3 h. Activation of CRH transcription, in vitro and in vivo, was associated with recruitment of phospho-CREB and TORC 2 by the CRH promoter. These data show that TORC2 is essential for activation of CRH transcription. In addition, there is marked induction of the TORC kinase, SIK in CRH neurons, concomitantly with the declining phase of CRH transcription, in vivo and in vitro. Over-expression of SIK reduces TORC translocation to the nucleus and CRH transcription, while blockade of SIK using staurosporin or SIK 1 and 2 silencing RNA results in TORC translocation to the nucleus and activation of CRH transcription. These findings demonstrate that regulation of SIK expression and activity can act as a sensitive switch mechanism for rapid activation and inactivation of CRH transcription during stress, by controlling the nuclear translocation of the CREB coactivator, TORC.

WHAT CAN WE KNOW FROM THE HYPOTHALAMUS-PITUITARY-ADRENAL AXIS ABOUT THE NATURE AND CONSEQUENCES OF EXPOSURE TO EMOTIONAL STRESSORS?

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Although exposure to stress elicits a wide range of physiological and behavioral responses, only a few physiological responses has been found to be consistently related to the intensity of stressors and also to reflect the process of habituation to a daily repeated stressor. Among them, we have particularly focused on peripheral hormones of the hypothalamus-pituitary-adrenal (HPA) axis: ACTH and corticosterone. In the last years we have re-evaluated their usefulness studying whether or not HPA hormones are able to reflect several underlying emotional processes. HPA activation in response to predominantly emotional stressors neither predicts long-lasting changes in anxiety-like behavior they elicit nor reflects trait or state anxiety as evaluated with classical tests such as the elevated plus-maze or the light-dark. Confirming previous data, HPA activation is unable to discriminate between controllable and uncontrollable electric shock paradigms even after chronic exposure. In contrast, HPA activation does reflect fear conditioning provided that testing lasts more than the 5 min period typically used in behavioral studies. Finally, HPA hormones, mainly ACTH, are able to reflect habituation to a wide range of stressors, but with some particular stressors (i.e. exposure to forced swim in relatively cold water-25ºC) or conditions (i.e. irregular exposure) no reduction of the ACTH response to the daily repeated stressor is observed, whereas such reduction was observed with glucose, that likely reflects adrenaline release. In conclusion, a better understanding of cognitive and emotional factors involved in the control of the HPA axis can better predict the potential negative impact of acute and chronic exposure to stressors.

MILD STRESS COMBINED WITH MEMANTINE TREATMENT HAS A NEGATIVE EFFECT ON THE HIPPOCAMPUS

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Glutamatergic neurotransmission plays an key role in cognitive processes such as learning and memory. However, excessive release of glutamate may be excitotoxic and harmful. Increased activation of glutamatergic system may be induced by both acute and chronic stress stimuli and it may impair neural plasticity. The aim of the present studies was to verify the hypothesis that pharmacological blockade of excessive glutamatergic neurotransmission can alleviate negative effects of stress on brain plasticity and adult neurogenesis. Adult male Wistar rats received daily injections of vehicle or memantine, a glutamate antagonist that blocks effects of excessive amount of glutamate. Following drug administration rats were exposed to a mild stress paradigm (limitation of movement – hypokinesis) for 2 h daily for 8 days. On the day 7 rats were injected with 5-brom-2-deoxyuridine (BrdU), a marker of cell proliferation. The stress model used resulted in only mild activation of the hypothalamo-pituary-adrenocortical axis. Hippocampal concentrations of brain derived neurotrophic factor (BDNF), an indicator of brain plasticity, did not differ between the groups. Number of BrdU positive cells in subgranular zone of the hippocampus was found to be decreased in stressed rats. Incorporation of BrdU into the DNA, which reflects cell proliferation, was found to be unchanged in the hippocampus of memantine-treated control rats but it was decreased in stressed rats treated with memantine. In contrast to our expectations, memantine treatment decreased rather than increased cell proliferation in the hippocampus of stress-exposed rats. Thus, memantine administration potentiated negative effects of stress on brain plasticity. These findings warn of possible undesired effects of memantine, a drug used in the treatment of Alzheimer disease, during stress exposure. Supported by grant Vega 2/0118/11

EFFECT OF OXYTOCIN ON NEURONAL VIABILITY

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Oxytocin is released in response to different physiological stimuli and it could play a key role in reducing stress reaction. It was suggested that oxytocin has protective effect against inflammation and consequences of oxidation stress. Mechanisms, how are oxytocin effects mediated in the brain tissue are unclear. Oxytocin affects cell growth, mitochondrial activity and cytoskeleton organisation. In the present study, oxytocin effects on neuronal viability were examined. Neuroblastoma cells (SH-SY5Y) were exposed to different concentrations of oxytocin for 24 or 48 hours . Potential protective effect of oxytocin treatment was investigated after exposing cells to oxidation stress using hydrogen peroxide (2h, 50mM) or hypoxia (2h). Cell proliferation was measured by cell counting, and cell viability was examined by MTT assay. Protein expression of selected neurotrophic factors was also measured as additional parameter. Oxytocin (1μM) significantly increased the number of neuronal cells. Cell viability was increased in the presence of oxytocin without the significant effect of dose (0.01-1μM). Cell death induced by hydrogen peroxide was not prevented by incubation with oxytocin. Hypoxia increased mitochondrial activity of neuronal cells without any additional effect of oxytocin. In conclusion, oxytocin might influence the viability of neuronal cells in vitro by changes of expression of neurotrophic factors. Supported by grants of VEGA 2/0094/09 and APVV-0253-10.

SEX DIFFERENCES IN PRENATALLY PROGRAMMED ANXIETY BEHAVIOUR AND CORTICOTROPIN RELEASING HORMONE (CRH) RECEPTOR MRNA EXPRESSION IN THE AMYGDALA IN RATS

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Exposure to stress during pregnancy can permanently 'programme' abnormal adult behaviour. Social stress (10min/day exposure to a lactating rat, gestational days 16-20) heightens anxiety-like behaviour in male, but not female, offspring [Brunton & Russell 2010; J Neuroendocrinol. pp 258]. The amygdala organises anxious behaviour and involves the actions of the neuropeptide, CRH. The CRH type-1 receptor (CRH-R1) promotes anxiety-like behaviour, while the type-2 receptor (CRH-R2) may dampen anxiety. Here we sought changes in basal CRH and CRH receptor mRNA expression in the amygdaloid nuclei of adult male and female prenatally stressed (PNS) rats (n=5-8/group) using quantitative in situ hybridisation. CRH mRNA expression in the central amygdala (CeA) was significantly greater in male (25%) and female (36%) PNS rats compared with controls. CRH-R1 mRNA expression was significantly greater in the CeA (35%) and basolateral amygdala (BLA; 19%) in male PNS rats compared with controls, with no change in the basomedial (BMA) or medial amygdala (MeA). In PNS females CRH-R1 mRNA expression was greater than controls only in the MeA (33%). Conversely, CRH-R2 mRNA expression was significantly lower in the BMA (32%) of male PNS rats compared with controls, but greater (21%) in female PNS rats, with no change in the BLA or MeA in either sex. Overall, the relative expression of CRH-R1:CRH-R2 mRNA in the amygdaloid nuclei was increased in PNS males, but not females. In conclusion, sex differences in anxiety-type behaviour in PNS rats may be explained by differential mRNA expression for CRH-R1 (pro-anxiogenic) and CRH-R2 (pro-anxiolytic) in the amygdaloid complex, without sex differences in CRH mRNA expression. [Funding: BBSRC/CAPES]

FROM BONE TO THE NERVOUS SYSTEM: OSTEOCALCIN AS A NEUROPEPTIDE

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Osteocalcin (OC) is a small, acidic extracellular protein synthesized by osteoblasts during bone formation. 3 residues of gamma-carboxy glutamic acid, formed in a vitamin K dependent process, enable highly specific binding to ionic or bone mineral calcium. A series of experiments indicated that OC is stress-responsive in ways that vary with the type of stressor. Glucocorticoid excess slowly decreases OC, while sympathetic activation (foot restraint immobilization, Immo) rapidly increases OC. Mice have 3 homologous genes in an OC gene cluster. Two of these (OG1 and OG2) are expressed in bone and have been deleted in a null mutant strain (KO). The third, termed ORG, remains, but is apparently only expressed in kidney. Comparison of KO and wild type (WT) indicated that OC might regulate energy metabolism by stimulating insulin and adiponectin synthesis. We have determined that KO mice are also less sensitive to thermal, pain, touch and balance stimuli. Because previous reports showed immunoreactive OC in rat trigeminal (TG) and dorsal root (DRG) ganglia associated with nociceptive and mechanoreceptive responses, we have analyzed KO and WT mice for both OC gene expression and OC protein content in TG, DRG, and brain. WT express both OC mRNA and protein, while KO do not, confirming that the genes deleted from KO are active in the neural tissues of WT in keeping with our behavioral observations. We have begun a series of specific stimuli to test interaction of OG1 with neuropeptides and receptors associated with the behavioral responses we observed. A small pilot experiment indicated that capsaicin receptor VR1 in TG of WT C57Bl/6J mice was more sensitive to capsaicin than in KO. We have begun a series of larger experiments including Immo to determine time course of OC gene expression and interactions between OC and VR1 as well as Tac1 (substance P), CGRP, and TRPM8.

SPLENECTOMY: ITS EFFECT ON THE STRESS RELATED BRAIN AREAS ACTIVATED BY LIVER ISCHEMIA-REPERFUSION INJURY

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Liver ischemia reperfusion injury (LIRI) induces Fos expression in several brain structures involved in stress response including the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei, the nucleus of the solitary tract (NTS), and catecholaminergic A1/C1 cells. Splenectomy, performed prior to LIRI, ameliorates pathological changes in the liver morphology and reduces the elevated levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and proinflammatory cytokines (TNF-α, IL-1β, IL-6). The aim of this study was to reveal whether splenectomy may reduce Fos expression stimulated by LIRI in selected brain structures. Splenectomy was performed 14 days prior to LIRI (ligation of the portal triade for 15 min). Animals were sacrificed by transcardial perfusion with fixative 90 min after reperfusion. Splenectomized, intact, and sham rats served as controls. Fos expression (general neuronal activity marker) was counted in the hypotlalamic PVN and SON, NTS, and A1/C1 areas. Levels of proinflammatory cytokines (TNF-α, IL-1β, IL-6) and hepatal enzymes (ALT, AST) were measured in plasma. LIRI increased plasma levels of cytokines and hepatal enzymes. This increase was partially lowered by splenectomy. Sham operation and LIRI elevated Fos expression in the PVN, SON, and A1/C1 area almost to the same extent, the splenectomy did not reduce the elevated Fos expression induced by LIRI. In the NTS, LIRI significantly increased Fos occurrence compared to shams. Splenectomy markedly reduced Fos amount augmented by LIRI. Our data indicate that NTS, which is the primary recipient of afferent signals from visceral organs, clearly reflects the peripheral changes evoked by LIRI and splenectomy. Although effect of LIRI is very markant it partially interferes with some nonspecific interventions accompanying the LIRI process. Supported by MZ 2006/19-SAV-01

STRESS-INDUCED IMMUNIZATION AGAINST HELPLESSNESS INVOLVES STRIATAL CIRCUITS SUBSERVING HABITUAL RESPONDING

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In some individuals stress experiences increase the resistance to subsequent stress challenge. The phenomenon, known as "immunizing" or "steeling" effect, could favor development of stress resilience. The present study investigated the neural bases of the phenomenon in an animal model. An experience of reduced food availability (12 days, NFood), ending 3 days before testing, prevented acquisition of immobility in the Forced Swimming Test (FST) by mice of the DBA/2J inbred strain and reduced anxiety in the elevated Plus Maze. NFood mice showed low FST-induced c-Fos expression within the infralimbic cortex, the nucleus accumbens shell, the amygdala and the paraventricular nucleus of the hypothalamus. In the left dorso-lateral Striatum c-Fos activation promoted by FST in free-feeding (Food) mice was absent in NFood. Temporary inactivation of this brain area, by local infusion of lidocaine during or immediately after FST, prevented acquisition of immobility in Food mice. NFood mice not exposed to FST showed increased FosB/DeltaFos immunostaining in the ventral tegmental area and substantia nigra, supporting adaptation of the so-called „spiraling" striato-nigro-striatal circuitry. Finally, when trained in a water Cross Maze NFood DBAs quickly acquired an escape strategy as a habit-like response. Together, these results support the view that a temporary experience of restricted feeding protects DBA/2J mice against helplessness by promoting a bias toward active coping strategies through adaptation of the brain circuit that mediate habit-like responding.

TRANSCRIPTIONAL REGULATION OF THE HUMAN GLUCOCORTICOID RECEPTOR TRANSCRIPT 1F

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The human glucocorticoid receptor (GR) promoter 1F is susceptible to methylation during stressful early life events. Methylation interferes directly with the transcriptional machinery by preventing the binding of transcription factors. It was reported that methylation of a hypothetical NGFI-A binding site in promoter 1F resulted in lower 1F transcript levels. In this study, the role of NGFI-A in regulating promoter 1F was analysed using transient transfection in 293FT cells. Here we showed that over-expression of NGFI-A did not affect promoter 1F activity in the reporter gene assay. Several other transcription factors predicated by using in silico phylogenetic footprinting (ISPF) were examined. We found that transcription factor E2F1 was identified to bind and strongly up-regulate promoter 1F. The result of E2F1 siRNA knockdown suggests direct regulation of 1F transcript by E2F1. To investigate the relationship between the cytosine methylation and transcription factor binding, we employed the single nucleotide methylation approach. However in our study, the single cytosine methylation on E2F1 binding site or the combined several cytosines methylation did not significantly reduce the promoter activity. This finding suggests that single or key methylcytosine(s) in the promoter may not prevent the binding of transcription factor.

DISSECTING THE CENTRAL STRESS RESPONSE USING SITE-SPECIFIC GENETIC MANIPULATION - FOCUS ON CRF/UROCORTIN SYSTEMS

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The biological response to stress is concerned with the maintenance of homeostasis in the presence of real or perceived challenges. This process requires numerous adaptive responses involving changes in the central nervous and neuroendocrine systems. When a situation is perceived as stressful, the brain activates many neuronal circuits linking centers involved in sensory, motor, autonomic, neuroendocrine, cognitive, and emotional functions in order to adapt to the demand. However, the details of the pathways by which the brain translates stressful stimuli into the final, integrated biological response are presently incompletely understood. Nevertheless, it is clear that dysregulation of these physiological responses to stress can have severe psychological and physiological consequences, and there is much evidence to suggest that inappropriate regulation, disproportional intensity, or chronic and/or irreversible activation of the stress response is linked to the etiology and pathophysiology of anxiety disorders and depression. Understanding the neurobiology of stress by focusing on the brain circuits and genes, which are associated with, or altered by, the stress response will provide important insights into the brain mechanisms by which stress affects psychological and physiological disorders. The CRF/Urocortin system is fundamental in orchestrating the organisms stress response. In addition to its hypophysiotropic action, CRF integrates the behavioral responses to stress within the central nervous system. This lecture will present an integrated multidisciplinary approch from gene to behavior using mouse genetics and animal models aim in elucidating the contribution of different members of the CRF/Urocortin family of peptides and receptors to the central stress response.

INTERACTION BETWEEN SUBSTANCE P AND SEROTONIN IN THE BRAIN AND THE REGULATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS (HPA) UNDER STRESS

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Substance P (SP) in the brain participates in the initiation and regulation of stress responses and has been implicated in the pathophysiology of stress-related disorders, such as anxiety and depression. Stimulation of forebrain NK1 (substance P) receptors activates the sympathoadrenal system, but inhibits the release of ACTH, indicating an inhibitory role of the peptide in the regulation of the hypothalamic-pituitary-adrenal axis (HPA). In line with this assumption, the ACTH response to acute immobilization stress (IMO) and the expression of corticotropin-releasing hormone (CRH) in the hypothalamic paraventricular nucleus (PVN) was augmented in NK1 receptor knock-out (NK1-KO) mice compared to wild-type (WT) mice. Serotonin (5-HT) is a potent activator of the HPA. Although IMO increased the 5-HT turnover in the dorsal and median raphe nuclei in both, NK1-KO and WT mice, the increases in 5-HT and 5-hydroxyindoleacetic (5-HIAA) acid concentrations were more pronounced in NK1-KO mice. IMO accelerated the 5-HT turnover in the prefrontal cortex, amygdala and some hypothalamic nuclei, but not in the PVN. No differences in 5-HT and 5-HIAA concentrations were observed between NK1-KO and wild-type mice in these areas. Our results demonstrate that stimulation of NK1 receptors in the brain inhibits the expression of CRF in the PVN and attenuates the activation of 5-HT cell bodies in the dorsal and median raphe nuclei.

MIFEPRISTONE, A GLUCOCORTICOID AND PROGESTERONE RECEPTOR BLOCKER, INDUCES APOPTOSIS IN RAT CARDIOMYOCYTES: ROLE OF THE BCL-2 GENE FAMILY.

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Mifepristone is a synthetic antagonist of glucocorticoid and progesterone receptors that is being widely used for the treatment of several therapeutic uses such as the treatment of endometriosis, glaucoma, meningiomas, as well as breast, ovarian and prostate cancer. In those tissues, mifespristone has growth inhibitory activity that includes estrogen and progesterone receptors down-regulation, induction of bax and p53 expression, and reduction of bcl-2 expression. However, the effect of mifepristone on other organs was not investigated. The goal of this study was to verify whether the treatment with mifepristone modulates apoptosis in the myocardium. The effects of mifepristone on the histopathological aspect and on immunohistochemistry for p53, bcl-2 and bax proteins in the heart tissue were evaluated. Male Wistar rats (n=10) were distributed in two groups: naive control rats and rats exposed to mifepristone (3.7 mg/kg/day, ip, during 5 days). The results pointed out no remarkable differences between groups of the myocardium histology nor for p53 or bcl-2 immunomarkers. However, mifepristone induced an overexpression of bax protein in the cardiomiocytes. These results suggest that mifepristone induces apoptosis in rat heart cells all the way through bax protein upregulation and that its use as a therapeutic agent may interfere with cardiac tissue remodelling. The effect of mifepristone on apoptosis markers in other tissues deserve to be investigated. Financial support: CNPq and FAPESP.

STRIATAL-ENRICHED PROTEIN TYROSINE PHOSPHATASE (STEP) IS A KEY REGULATOR OF SYNAPTIC PLASTICITY IN CRF NEURONS OF THE RAT BNST

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STEP is a neuron specific tyrosine phosphatase, which plays a critical role in the NMDA receptor-mediated regulation of the ERK signaling. Hence, STEP is involved in induction of long-term potentation (LTP) and the formation of fear memory. Stress facilitates fear memory, a process partially mediated by activation of corticotrophin releasing factor (CRF) neurons in the anterolateral BNST (BNSTALG). Here, we used a repeated restraint stress (RRS) paradigm to examine the effects of chronic stress on STEP expression in the BNSTALG. Immunofluorescence studies revealed that STEP shows somatodendritic expression in the CRF rich oval nucleus of the BNSTALG and dual labeling studies revealed that STEP expression overlaps with CRF, NMDAR1, and ERK1/2 in BNSTALG neurons, suggesting that STEP could play a major role in regulating synaptic plasticity in these neurons. Significantly, RSS decreased levels of both STEP mRNA and protein expression in the BNSTALG. RSS also significantly facilitated the induction of LTP in BNSTALG neurons compared to controls. To determine if these RRS-induced changes were correlated, we examined the effects of inclusion of exogenous STEP (TAT-STEP) in the recording pipette on the induction of synaptic plasticity in BNSTALG neurons. Inclusion of TAT-STEP did not affect LTP induction in control neurons, but significantly attenuated the RRS-induced facilitation of LTP. We propose that in response to acute stress, STEP expression in CRF neurons acts to buffer this cell population against excessive activation. However, the chronic stress-induced reduction in STEP expression results in a pathological enhancement of the activation of CRF neurons in the BNST. Thus, targeted manipulations of STEP activity might represent a novel treatment strategy for stress-induced anxiety disorders. NIH (MH072908) to DGR and National Primate Research Center grant #RR-00165

OBESITY INDUCED BY STRESS AND THE EMOTIONAL NERVOUS SYSTEM.

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Stress and limbic brain networks foster eating behaviors that can lead to obesity. The neural networks underlying interactions among stressors, body, brain and food intake are now better understood. Stressors, by activating a neural stress-response network, bias cognition toward increased emotional activity and decreased executive function, causing formed habits rather than cognitive appraisal of rewards to be employed with stress. Stress increases glucocorticoids which increase motivation for food, and glucocorticoids also increase insulin, which determines which foods are eaten. Together, the hormones promote abdominal obesity. However, this feedforward hormonal mechanism serves the purpose of damping activity in the stress-response network, resulting in feeling better and reinforcing the feeding habit. Employing mental reappraisal techniques may help to shift habitual responses of palatable, fattening food intake that occurs with stressors to more thoughtful appraisal of the need, rather than the desire, for food.

CARDIOVASCULAR RESPONSES EVOKED FROM THE PERIAQUEDUCTAL GREY REQUIRE NEURONAL ACTIVITY IN THE AMYGDALA.

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The periaqueductal gray matter (PAG) is involved in the integration of specific cardiovascular changes associated with emotional behaviors. Moreover, microinjection of excitatory aminoacids (EEA) into the lateral/dorsolateral column of the PAG (l/dlPAG) evokes increases in the heart rate (HR) and in the mean arterial pressure (MAP) in a pattern similar to those observed during emotional stress. This pattern of cardiovascular changes can also be attained by activation of the basolateral amygdala (BLA) neurons. Therefore, the aim of this study was to evaluate the role of the BLA in the cardiovascular responses evoked by the stimulation of PAG. Male Wistar rats were anesthetized with ketamine-xylazine (80 mg/kg – 11.5 mg/kg, i.p.), and ipsilateral guide cannulae were implanted into the l/dlPAG and into the BLA. Six days latter, the rats were anesthetized again (Isoflurane 2% in 3l of O2) and a catheter was inserted into the femoral artery for recording of MAP and HR. Experiments were performed 48 hr later. Each rat was subjected to two different trials, 24hr apart, and in random order in which either saline vehicle (100 nl) or muscimol (100 pmol/100 nl) was microinjected into the BLA followed, 5 minutes later, by the microinjection of the EEA, NMDA (6pmol/100nl), into the l/dlPAG. Microinjection of muscimol into the BLA attenuated the increase in HR evoked by the microinjection of NMDA into the l/dlPAG (20 ± 10 bpm, n=5) when compared with the saline treatment (80 ± 12 bpm, n=5, p= 0.0001). Likewise, the microinjection of muscimol into the BLA reduced the increase in MAP produced by the microinjection of NMDA into the l/dlPAG (4 ± 2 vs 12 ± 3 mmHg after saline, n=5, p=0.033). These results indicate that the neuronal activity in the region of the BLA plays a role in the generation of physiological response induced by activation of the l/dlPAG neurons.

BRAIN CORTICOSTEROID BALANCE AND RESILIENCE TO PSYCHOPATHOLOGY

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Environmental stimuli perceived as stressors and processed in higher brain circuits including the hippocampus activate the release of several neuropeptides eventually leading to the secretion of adrenal cortisol which feeds back on the brain. To exert this feed back cortisol action is mediated by nuclear receptors that operate as gene transcription factors and surprisingly also directly can affect neurotransmission. There are two types of receptors, the mineralocorticoid (MR) and glucocorticoid receptors (GR), which serve together as master switch in the control of neural network responses that facilitate in complementary fashion the defense and recovery of homeostasis underlying behavioural adaptation. Imbalance in MR:GR driven pathways caused either by genetic receptor variants or by experience-related factors compromises processing of stressful information. Therapies are therefore envisioned to rebalance the stress system for protection or repair from damaging signaling pathways that can introduce a bias towards stress-related brain disease. Here I will focus on (i) functional implications of basal pulsatility for stress-induced HPA axis responsiveness (ii) functional implications of genetic receptor variants of MR and GR dispositional optimism and protection against negative mood. (iii) the programming effects of adverse early life / adolescent experiences in animals genetically selected for surrogate endpoints of psychosis (iv) a brief synthesis of cortisol actions for clinical neurobiology and some thoughts on potential treatment strategies of stress-related mental disorders conclude the contribution. Supported by the Royal Netherlands Academy of Sciences.

IS INTERLEUKIN-1 THE MEDIATOR OF DEPRESSIVE ILLNESS

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Interleukin-1 injected into animals induces neurochemical, behavioural, and endocrine effects. Noradrenergic and serotonergic systems in the brain are activated and tryptophan is increased. IL-1 also activates the HPA axis. Behavioural changes include decreased locomotor activity and increased immobility in the forced swim test in rats and mice, and in the tail-suspension test in mice. IL-6 and TNFα activate the HPA axis, but are far less potent than IL-1, and IL-6 activates tryptophan and 5-HT metabolism, TNFα at higher doses activates NE and 5-HT metabolism. It has been suggested that IL-1 may be the mediator of these depression-like behavioural effects. But there is no substantial evidence that IL-1 is elevated in the circulation of depressed patients. Maes et al. (widely miscited) showed that white cells from depressed patients synthesized more IL-1 after in vitro challenge by LPS. A natural IL-1-receptor antagonist (IL-1ra) has been available for more than 20 years, but there are no reports of its clinical antidepressant activity. However, several studies have indicated increased circulating concentrations of IL-6. It has recently, been suggested that it is IL-1 in the brain that is responsible for the depression-like effects. Certainly intracerebral IL-1 can induce behavioral, neurochemical and HPA activation. This is supported by one small study in which CSF concentrations of IL-1 were shown to be elevated in depressed patients (Levine ea, 1999). There are several reports that intracerebral injection of IL-1 can induce depression-like behavior in animals, but, needless to say, there have been no human studies. Animal studies have shown that IL-1 may be able to penetrate the blood-brain barrier, and activation of endothelial cells, such as occurs with injection of large peripheral doses of LPS may result in the appearance of IL 1.

PRO-AND ANTI-APOPTOTIC EFFECTS OF STRESS AND GLUCOCORTICOIDS IN THE BRAIN: FUNCTIONAL IMPLICATIONS

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Severe stress and high levels of glucocorticoids cause neuronal damage and death through apoptosis that may contribute to psycho-behavioral disorders. We have analyzed effects of stress and glucocorticoids on the expression of pro- and anti-apoptotic proteins in the brain in relation to the behavioral effects of these treatments in newborn and adult rats. Glucocorticoid action during early-life depends on animal's developmental stage and treatment duration. Dexamethasone and hydrocortisone induced a significant increase in the anti-apoptotic protein Bcl-xL and its mRNA levels in the cortex and hippocampus of 8-day-old rats in 6 h after injection. In contrast to the acute, repeated treatment of neonatal rats with glucocorticoids caused pro-apoptotic responses in the brain and impaired locomotor activity of these pups 3-5 days later. Balance between pro- and anti-apoptotic proteins was also important for adult behaviors. Stress-induced increase in Bcl-xL expression in the hippocampus was implicated in the resilience to the development of behavioral despair in response to forced swimming. This stress also caused an increase in the expression of, for example, pro-apoptotic protein Bax in the frontal cortex and chronic pretreatment with fluoxetine abolished this increase. Thus, regulatory apoptotic proteins may be involved in pathogenesis of stress-induced depression as well as in the mechanism of antidepressant action. In general, data suggest that activation of anti-apoptotic pathways in the brain is associated with behavioral resilience to stress and glucocorticoids. This work was supported by RFBR grant N 11-04-00375.

PACAP: A STRESS RESPONSE REGULATOR ACTING IN BOTH CENTRAL AND PERIPHERAL NERVOUS SYSTEMS

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The HPA (hypothalamo-pituitary-adrenal) axis mediates general adaptation to stress, while the HSA (hypothalamo-sympatho-adrenal) axis can mobilize metabolic resources in response to threat somewhat more acutely. The propagation of signaling through both circuits, however, may elicit a cellular stress response (CSR) within the components of each multi-cellular pathway. Thus, the CSR is a point of entry to identifying targets for pharmacological modulation of organismic stress perception and processing. The neuropeptide PACAP is now recognized as an informational molecule released from stress-transducing neurons that exerts post-synaptic effects required for completion of hypothalamo-pituitary-adrenocortical and sympathoadrenal circuits activated by psychogenic and metabolic stressors (Stroth and Eiden, Neurosci. 165: 1025, 2010; Stroth et al., Ann. N. Y. Acad. Sci. in press, 2011). Genes responsive to PACAP in cultured neuronal and neuroendocrine cells, and to PACAP in vivo in PACAP-deficient mice in various stress paradigms, have been identified as encoding potentially neuroprotective proteins that may be involved in the organismic stress-associated CSR. These include members of the Nr4a protein family that act as transcription factors in the nucleus and also control Bcl-2-dependent triggering of apoptosis at the mitochondrion, and modulators of intracellular calcium disposition such as stanniocalcin 1 and selenoprotein T (see Grumolato et al., FASEB J. 22: 17526, 2008). The signal transduction pathways through which PACAP signaling to these genes occurs, their likely function in stress-transducing neurons, and discussion of the possibility that PACAP is involved in a neuroendocrine-specific CSR triggered by response to organismic stress will be the subject of this presentation.

CATECHOLAMINE METABOLOMIC AND SECRETORY PHENOTYPES IN PHEOCHROMOCYTOMA

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Phaeochromocytomas and paragangliomas (PPGLs) show different catecholamine phenotypes, locations, ages of presentation and propensities for malignancy depending on the presence of underlying germline mutations. This analysis examined relationships among the above variables in 365 patients with PPGLs, including 38 with multiple endocrine neoplasia type 2 (MEN 2), 10 with neurofibromatosis type 1 (NF1), 66 with von Hippel-Lindau (VHL) syndrome and 59 with succinate dehydrogenase type B (SDHB) or D (SDHD) gene mutations. Patients with MEN 2 and NF1 presented with tumours characterized by increases in plasma metanephrine (indicating adrenaline production) at a later age than patients with tumours due to VHL, SDHB and SDHD gene mutations, which lacked significant adrenaline production (noradrenergic phenotype). Patients without evidence of disease-causing mutations also presented with adrenaline-producing tumours at a later age than those with noradrenergic tumours. Furthermore, among patients with hereditary noradrenergic tumours, those with multifocal tumours had a younger age of disease presentation than those with solitary tumours. Adrenaline-producing tumours contained higher concentrations of catecholamines, but exhibited lower rates of catecholamine secretion than noradrenergic tumours. Increased plasma concentrations of methoxytyramine, the O-methylated metabolite of dopamine, presence of SDHB mutations, extra-adrenal locations and a large size of primary tumours were inter-related predictors of malignancy. The different catecholamine metabolomic profiles, ages of disease presentation and tumour locations provide potentially useful predictive information about underlying mutations and the presence of metastases, and suggest origins from different chromaffin progenitor cells with variable susceptibility to disease causing mutations and potential for malignancy.

MENTAL STRESS ORIGINS OF ESSENTIAL HYPERTENSION: NEURAL MECHANISMS

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Although still subject to dispute, the basis in epidemiological research for chronic mental stress causation of essential hypertension has strengthened in the past decade. Baker IDI research has taken a different tack, in searching for biological rather than epidemiological evidence. We have studied patients with hypertension and panic disorder in parallel, using the latter as an explicit clinical model of recurring stress responses. Our argument for mental stress causation of essential hypertension runs this way: (i) There is common clinical comorbidity; panic disorder prevalence is increased 3-fold in essential hypertension, (ii) Plasma cortisol is elevated in both, (iii) In panic disorder and hypertension, but not in health, single sympathetic nerve fibres commonly fire repeatedly, in salvos within an individual cardiac cycle, which is a "signature" of mental stress exposure, (iv) For both, adrenaline cotransmission is present in sympathetic nerves, (v) There is induction of the adrenaline synthesizing enzyme, PNMT, in sympathetic nerves, demonstrated with Western blot analysis of sympathetic nerve proteins extracted from forearm vein biopsies. Hypertension commonly arises from societal ills, including from chronic mental stress in personal life, in the workplace and in the life of nations. This elevates blood pressure primarily through persistent activation of the renal sympathetic outflow, commonly demonstrable in patients with hypertension. No doubt it would be better to catch this fault at its roots, by changing people and the world they live in, but for the moment the interest of my colleagues and I is in changing the renal sympathetic nerves, ablating them using an intravascular radiofrequency catheter. In patients with drug-resistant hypertension this technique produces substantial and durable blood pressure lowering.

STRESS AND THE STOMACH: CORTICOTROPIN-RELEASING FACTOR MAY PROTECT THE GASTRIC MUCOSA IN STRESS THROUGH INVOLVEMENT OF GLUCOCORTICOIDS

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The results of our previous investigations suggest that glucocorticoids released during stress act as gastroprotective hormones, and not as ulcerogenic agents as has been generally accepted for a long time. In the present study we investigated whether corticotropin-releasing factor (CRF) may protect the gastric mucosa against stress-induced gastric injury through involvement of glucocorticoids. CRF administration (1.25 mkg/kg, i.p., 30 min before onset of stress) markedly increased plasma corticosterone level and significantly suppressed the occurrence of gastric erosion induced by 3-h cold-restraint stress (at 10°C) in control rats. To estimate the role of glucocorticoids in CRF-induced gastroprotection the effect of CRF on the stress-induced gastric erosion was studied after acute reduction of corticosterone release by metyrapone (30 mg/kg, i.p., 30 min before CRF) or occupation of glucocorticoid receptors by the antagonist RU-38486 (20 mg/kg, i.p., 2 h before CRF). Metyrapone injected shortly before CRF administration caused a fast inhibition of corticosterone response to CRF and prevented its protective effects on the gastric mucosa against the stress-induced erosion formation. The gastroprotective effect of CRF was also eliminated by the pretreatment rats with glucocorticoid receptor antagonist RU-38486. The results obtained suggest that CRF may protect the gastric mucosa against stress-induced gastric injury through involvement of glucocorticoids. This study was supported by grants from RFBR-10-04-00605; FNM RAS-2010-2011; DBS RAS-2011.

STRESS MODULATES TAU PROTEIN PHOSPHORYLATION: EFFECT IN CRH DEFICIENT MICE

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Stress most probably plays an important role in the etiopathogenesis of Alzheimer's disease (AD). Although the exact molecular link between the stress and neurodegeneration is still missing a possible negative influence of repeated stress on the onset and progression of AD have already been stated. We have focused on examination of the influence of acute and chronic stressors in intact animals (WT) and mice deficient in CRH. Specifically, we have analyzed the levels of hyperphosphorylated, AD-specific epitopes on the molecule of tau, which is the major molecular player in AD type of neurodegeneration. Immobilization stress (IMO) induced a transient hyperphosphorylation of tau proteins in WT and also in CRH (-/-) mice. Tau protein phosphorylation was quantified in several AD specific epitopes (pS202/pT205, pT181, pS396-pS404). The changes were similar in several brain regions: frontal and temporal cortices, hippocampal C1 region, dentate gyrus, and amygdala. The intensity of hyperphosphorylation induced by acute IMO depends on the duration of stress in both, WT and CRH (-/-) mice. The strongest tau phosphorylation was observed after 30' of IMO. The IMO for 120' followed by 3 hours of rest lead to the disappearance of hyperphosphorylation in all investigated epitopes. Interestingly, the magnitude of pathological phosphorylation of tau protein in CRH (-/-) mice was much lower in comparison to WT animals – pointing to the role of CRH and glucocorticoids in pathogenesis of dementia. Thus, our results suggest that pathological phosphorylation of tau protein induced by stress may lead to misfolding of tau protein and eventually to initiation of neurodegeneration. The CRH plays an important role in stress induced phosphorylation of tau protein, which might be a direct effect of CRH innervations or an effect mediated via HPA axis. (Supported by APVV-0088-10, APVV-0634-07).

SUB-CHRONIC TRYPTOPHAN DEPLETION EFFECTS ON BEHAVIOUR, HORMONE, CENTRAL AND PERIPHERAL TRYPTOPHAN METABOLIC PATHWAY MEASURES – IS THIS A BETTER MODEL OF DEPRESSION?

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Background: Dietary induced-tryptophan (TRP) deficiency has been proposed as an animal model for depression. Studies have mostly tested acute TRP deficiency (ATD) with inconsistent results. Sub-chronic TRP deficiency (SCTD) may prove more reliable and sensitive. The aim was to test the hypothesis and to optimise the time of SCTD-induced depression-related behaviour with biochemical change. Methods: Sprague Dawley (SD) rats were treated with a low TRP-containing diet for 0, 7 or 14 days. Peripheral and central neurochemical markers were measured. SCTD-induced depression-related behaviour was assessed by the forced swim test (FST). Sensitivity to antidepressants was tested by concomitant paroxetine treatment. Results: SCTD induced significant reductions in weight gain and measures of peripheral and central TRP change. Corticosterone, aldosterone, and kynurenine (K), which has NMDA-ergic activity, increased, whilst kynurenic acid (KA), an NMDA receptor antagonist decreased. Corticosterone was significantly negatively correlated to weight gain. 5-HT2 receptor binding was up-regulated. SCTD increased floating time and reduced swimming time in the FST which were reversed by paroxetine. Aldosterone was significantly and similarly increased at 7 and 14 days, whereas other changes maximised at 14 days SCTD. Conclusion: Findings indicate that 14 days SCTD was optimal in this proposed model which resembles human depression in terms of behavioural and neurochemical attributes. Aldosterone may be an early marker or causal link for depression development, whereas increased cortisol and hippocampus 5-HT-receptor density could be correlates of depressive behaviour. Consequential increases in NMDA signalling through increased K/KA ratios suggests the model may be useful for testing novel antidepressants which transcend the monoamine theory of depression.

LONG-TERM EFFECTS OF SEVERE STRESSORS IN RATS ARE GENDER-DEPENDENT

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Acute exposure to some traumatic stressors, such as immobilization (IMO) on boards, induces a long-term reduction of the response of the hypothalamic-pituitary-adrenal (HPA) axis to a second exposure to the same stressor given some days after. This homotypic desensitization is accompanied by a sensitization of the HPA axis to a novel (heterotypic) mild stressor (novel environment). However, little is known if these phenomena are gender-dependent. To address this issue, we performed an experiment with male and female Sprague-Dawley rats. On day 1, part of the animals were exposed to IMO for 60 min and blood sampled by tail-nick immediately after the stressor and at 45 and 90 min after its end to study plasma ACTH and corticosterone levels by radioimmunoassay. The rest of the animals (control group) were only sampled. IMO-exposed males showed an increase in resting levels of HPA hormones in the day after, whereas females did not. IMO-induced sensitization of the response to a novel environment and hypoactivity were similar in both sexes, but the degree of reduction of the HPA response to a second exposure to the stressor was greater in females. Therefore, females appear to better cope with IMO than males.

STRESS, ALLOSTATIC LOAD, AND CATECHOLAMINES, AND OTHER NEUROTRANSMITTERS IN NEURODEGENERATIVE DISEASES

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As populations age, the prevalence of geriatric neurodegenerative diseases will increase. These diseases generally are multifactorial, arising from complex interactions among genes, environment, concurrent morbidities, treatments, and time. This essay provides a concept for the pathogenesis of Lewy body diseases such as Parkinson disease, by considering them in the context of allostasis and allostatic load. Allostasis reflects active, adaptive processes that maintain apparent steady states, via multiple, interacting effectors regulated by homeostatic comparators—"homeostats." Stress can be defined as a condition or state in which a sensed discrepancy between afferent information and a setpoint for response leads to activation of effectors, reducing the discrepancy. "Allostatic load" refers to the consequences of sustained or repeated activation of mediators of allostasis. From the analogy of an idling car, the revolutions per minute of the engine can be maintained at any of a variety of levels (allostatic states). Just as allostatic load (cumulative wear and tear) reflects design and manufacturing variations, byproducts of combustion, and time, eventually leading to engine breakdown, allostatic load in catecholaminergic neurons might eventually lead to Lewy body diseases. Central to the argument is that monoamines in the neuronal cytoplasm are autotoxins and that monoaminergic neurons leak vesicular contents into the cytoplasm continuously during life. The neurons therefore depend on vesicular sequestration to limit autotoxicity of cytosolic transmitter. Parkinson disease might be a disease of the elderly because of allostatic load, reflecting genetic predispositions and environmental exposures and continual episodes of stress-related catecholamine release and reuptake over time.

OXYTOCIN: NEUROANATOMICAL AND PHYSIOLOGICAL BASIS FOR ATTENUATION OF STRESS AND FEAR RESPONSES

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The hypothalamic neuropeptide oxytocin (OT) controls parturition and lactation in mammals and centrally orchestrates various types of social behaviors. In addition, OT exerts pronounced anxiolytic effects, acting on the central nucleus of amygdala (CeA) – the key brain region controlling fear and stress responses. Up to now the sites and cellular structures of OT release within the CeA have remained unknown. We therefore gained genetic access to hypothalamic OT neurons in live rats by viral vectors to dissect the connectivity of OT neurons with the CeA and control the activity of these neurons by optogenetic means. Anatomical anterograde and retrograde tracings, employing recombinant adeno-associated (rAAV) and deletion-mutant pseudotyped rabies viruses, revealed that a subset of magnocellular OT neurons, preferentially residing in the paraventricular and accessory magnocellular nuclei, monosynaptically project to the CeA. To study the functional role of OT in the CeA we infected OT neurons by an rAAV expressing channelrhodopsin2 (ChR2). Local blue light stimulation in the CeA of OT fibers containing ChR2 evoked robust OT-dependent cellular responses within the CeA, which led to the increased inhibition of GABA-ergic neurons projecting from the CeA to brainstem nuclei, subsequently controlling autonomic and behavioral outcomes of stress and fear responses. In conclusion, our results demonstrate OT release in the CeA from long-range axons, suggesting the existence of a new pathway for anxiolytic action of this neuropeptide in the brain.

PROGRAMMING EFFECTS OF EARLY DEVELOPMENTAL STRESS ON CARDIOVASCULAR AND METABOLIC RISK IN THE OFFSPRING: ROLE OF NEUROPEPTIDE Y

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Both stress and obesity are on the rise, and obesity affects not only adults but, alarmingly, also children, some of them progeny of parents, who suffered from psychosocial, nutritional or other severe stress. Supported by animal studies, a concept of transgenerational inheritance, non-genomic and epigenetic, is gaining momentum. Here we studied long-term effects of prenatal and postnatal stress on a stress-activated system - sympathetic co-transmitter, neuropeptide Y (NPY), a growth factor, potentially epigenetically regulated due to CpG-rich promoter. In adult mice fed high fat diet (HF), cold stress activated, in a glucocorticoid-dependent way, NPY and its Y2 receptor (R), specifically in the abdominal fat and adipose stem cells (ASCs). This increased de-novo adipogenesis and angiogenesis leading to abdominal obesity and metabolic-like syndrome which were inhibited by intra-fat Y2R inactivation. Circulating NPY and its Y2R in the fat were also up-regulated in ApoE-/- mouse offspring prenatally stressed by chronic cold stress, which accelerated atherosclerosis. To determine if ASCs carry stress memory, we "stressed" murine embryonic stem cells with epinephrine during their adipogenic differentiation. This "stress" dramatically up-regulated adipogenesis and NPY mRNA while decreasing DNA methylation of its promoter. In vivo, offspring of mice fed low protein diet in pregnancy+lactation, initially smaller, grew faster than controls when weaned to HF (females>males). Surprisingly, while females developed abdominal adiposity, Y2R up-regulation and glucose intolerance, males showed lower fat Y2R and improved metabolic health. Thus, stress may induce gender- and/or sex-hormone-specific epigenetic changes in the NPY and/or its receptor genes, specifically in ASCs of the visceral fat, and thus program for future development of abdominal obesity and metabolic syndrome.

ADMINISTRATION OF THE MDR1 PGP ANTAGONIST ELACRIDAR INDUCES NEUROENDOCRINE AND BEHAVIOURAL CHANGES IN MICE SELECTIVELY BRED FOR EXTREMES IN STRESS REACTIVITY

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It has been suggested that mdr1 Pgp plays a role in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis. Here, we used an animal model of affective disorders consisting of three independent mouse lines selectively bred for high (HR), intermediate (IR) or low (LR) corticosterone (CORT) secretion in response to stressors in order to investigate the impact of mdr1 Pgp on HPA axis regulation. We were particularly interested in i) the gene expression profile of mdr1 Pgp in the brain of these animals and ii) whether pharmacological mdr1 Pgp antagonism influences the neuroendocrine and behavioural endophenotypes of the three mouse lines. Our gene expression studies in the hippocampus revealed no significant differences in mRNA levels of the mdr1a gene, whereas clear differences between the three lines were observed concerning the expression of the mdr1b gene (HR>IR>LR). Acute treatment with the mdr1 Pgp antagonist Elacridar resulted in a significant increase of stress-induced CORT concentrations in the plasma of HR mice, while a trend was observed in the animals of the IR line. The HPA axis reactivity of the LR group, however, remained unaffected. Furthermore, Elacridar treatment induced significant behavioural changes regarding locomotor activity and exploration in the open field test (all groups: treat,veh) and Elacridar-treated HR and LR mice showed an increase in passive stress-coping behaviour, i.e. less struggling in the forced swim test. In summary, our results indicate that mdr1 Pgp is not only involved in regulating the access of CORT to hippocampal neurons, but also participates in the modulation of emotional behaviours in mice selectively bred for extremes in stress reactivity. Hence, mdr1 Pgp could be one of the key players in the mechanisms underlying a pathophysiologically altered HPA axis function prominent in depressed patients.

TRANSLATIONAL STRESS MEDICINE. A VIEW FROM THE BEDSIDE

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Several research programs are currently underway to support the development of a brain-based classification of mental illness. Such translational classifications may improve diagnostic assessments and, consequently, therapeutic outcome in patients with mental disorders. So far, however, preclinical translational research seems to rarely result in a higher efficacy in clinical routine. This called for another translational approach, also referred to as "integrated knowledge translation". Knowledge translation is a dynamic and iterative process that includes synthesis, dissemination, and exchange of knowledge, strengthening the health care system and engaging knowledge users in the research process. The primary knowledge users in stress medicine are family physicians, which treat up to 90% of these patients. We here introduce "Neuropattern", a newly developed diagnostic tool for clinical practice, allowing an individualized detection of stress effects in bodily diseases. Neuropattern analyses stress effects on neurobiological interfaces, participating in the communication between the brain and the body. Functional changes of each of these interfaces are assessed by characteristic patterns of concomitant biological, psychological, and symptomatic measures, occurring in consequence of stress. Stressed patients may differentially qualify for one or more of these patterns, thus facilitating individualized indications for therapeutic treatments. Data will be presented, showing that the therapeutic efficacy can be significantly improved, once the physicians have access to data, generated by such a translational diagnostic tool.

ORCHESTRATING THE STRESS RESPONSE: A SYMPHONY IN B (OR F)

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The mammalian stress response is an integrated physiological and psychological reaction to real or perceived adversity. Glucocorticoids (GCs)(corticosterone (also called 'B') in rats and mice, cortisol (F) in humans) are an important component of this response, acting to redistribute energy resources to both optimize survival in the face of challenge and restore homeostasis after the immediate challenge has subsided. Release of GCs is mediated by the HPA axis, driven by a neural signal originating in the paraventricular nucleus (PVN). Stress levels of glucocorticoids bind to glucocorticoids receptors (GRs) in multiple body compartments, including brain, and consequently have wide-reaching actions. For this reason, glucocorticoids themselves serve a vital function in feedback inhibition of their own secretion. Using corticolimbic GR knockout mice and local lentiviral GR knockdown, we have revealed that GCs act via the limbic system, specifically the medial prefrontal cortex, to shut-off of their own secretion. In contrast, fast feedback inhibition of the HPA axis is mediated by GC signaling in the PVN itself, acting by a cannabinoid-dependent mechanism to rapidly reduce both neural activity and glucocorticoid release. Studies in knockout mice suggest that PVN GC effects are mediated by membrane-associated GRs. Finally, HPA axis responses are also controlled by signals in other body compartments. Recent work from our lab demonstrates that knockout of GR in mouse adipose tissue enhances HPA axis responses to stress, likely mediated by reduced secretion of inhibitory lipid messengers. Thus, rather than having a defined feedback 'switch', control of the stress response requires a wide-reaching feedback 'network' that coordinates HPA activity to suit the overall needs of multiple body systems. Support: MH049698, MH069725 and MH069860.

LISTENING MUSIC CAN MODIFY NEUROENDOCRINE RESPONSE DURING STRESS EXPOSURE IN HEALTHY MEN

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Several studies have indicated the influence of acoustic stimuli on physiological response during rest and/or stress conditions. It is however not clear, which kind of acoustic stimuli have positive impact on the stress response. The aim of this study was to test how the degree of familiarity of acoustic stimuli applied during stress modifies neuroendocrine response. The study was performed in 14 young healthy males in a crossover design. We employed popular rock music familiar to the volunteers played in the regular mode or played backwards, thus representing unfamiliar disturbing sounds. The stress procedure was of a mild intensity and consisted of the combination of mental tasks (Stroop word color test + mental arithmetic) and handgrip exercise. The music was played before, during and after the stress procedure. Stress exposure resulted in increased state anxiety under the conditions when music was played in backwards but not in the regular mode indicating a higher perception of stress in case of disturbing sounds. Accordingly, stress-induced increases in plasma aldosterone and testosterone levels evaluated as areas under the curve were more pronounced when music was presented in backwards mode. On the contrary, stress-induced ACTH and diastolic pressure responses were significantly lower when music was applied in the backwards mode. Stress-induced increases in plasma epinephrine and dopamine were similar in both music modes. Thus, enhanced perception of stress is associated with inadequate increase in ACTH but higher aldosterone and testosterone release during stress. The results allow us to suggest that the familiarity of acoustic stimuli has positive influence on stress perception but different impact on individual parameters of the stress response. Supported by grants of VEGA 2/0118/11 and APVV-0028-10.

HYPOXIC STRESS MODULATES GENE EXPRESSION AND PROTEIN LEVELS OF THE SODIUM CALCIUM EXCHANGER IN HEK 293 CELL LINE VIA HIF 1 ALPHA

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Up to now a little is known about the effect of hypoxia on the sodium calcium exchanger type 1 (NCX1) expression and function. Therefore we studied, how dimethyloxallyl glycine (DMOG), an activator and stabilizator of the hypoxia-inducible factor (HIF)-1α, could affect expression of the NCX1 in HEK 293 cell line. We also tried to determine whether this activation can result in the induction of apoptosis in HEK 293 cells. We have found that DMOG treatment for 3 hours significantly increased gene expression and also protein levels of the NCX1. This increase was accompanied by a decrease in intracellular pH. Wash-out of DMOG did not result in reduction of the NCX1 mRNA and protein to original - control levels, although pH returned to physiological values. Using luciferase reporter assay we observed increase in the NCX1 promoter activity after DMOG treatment and using wild-type mouse embryonic fibroblast (MEF)-HIF-1+/+ and HIF-1-deficient MEF-HIF-1-/- cells we have clearly shown that in the promoter region, HIF-1 alpha is involved in DMOG induced upregulation of the NCX1. Moreover, we also showed that an increase in the NCX1 mRNA due to the apoptosis induction is not regulated by HIF-1 alpha. This work was supported by grant VEGA 2/0082/10

NEUROCIRCULATORY FUNCTION IN PATIENTS WITH PRIMARY SJÖGREN'S SYNDROME

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Several studies showed signs of autonomic dysfunction (AD) in patients with primary Sjögren's syndrome (pSS). The aim of the study was analyze neurociculatory function in patients with pSS. Reflexive cardiovagal gain (decrease in interbeat interval per unit decrease in systolic pressure during the Valsalva maneuver) and reflexive sympathoneural function (orthostatic increment in plasma norepinephrine (NE)) were evaluated in 21 pSS patients (mean±SE age 44±3) and in 7 healthy controls (51±3 years). A noninvasive real-time digital autonomic nervous system monitoring was performed using ANSAR™ device during device-guided slow breathing with target respiratory frequency 0.1 Hz, Valsalva maneuver and active standing. There were no significant differences in mean baroreflex gain and mean NE increment between the groups. One (N=1) pSS patient and none of the controls had orthostatic hypotension during passive tilting. Five (N=5) pSS patients and two (N=2) controls had baroreflex gain over 3 ms/mmHg (p=0.58). Sixteen (N=16) pSS patients and four (N=4) controls had over 300 pg/mL increment in plasma NE during tilt (p=0.31). Diastolic blood pressure decrease was smaller in pSS patients than in controls during slow breathing (p=0.005). Unlike in controls, systolic blood pressure did not significantly decrease in pSS patients during slow breathing (p=0.014). Indices of heart-rate variability in time and frequency domains did not differ between patients and controls. A comparable frequency of subjects with subnormal cardiovagal and sympathoneural function among patients with pSS and healthy controls suggests normal neurocirculatory function in pSS. Abnormal blood pressure response to device-guided slow breathing may suggest only subtle changes in autonomic control of vascular tonus. The research project was supported by the intramural research program of the NIH.

SPECIFIC REGULATION OF ACTH SECRETION UNDER THE INFLUENCE OF LOW AND HIGH AMBIENT TEMPERATURE APPLIED ACUTELY OR INTERMITTENTLY

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The response of hypothalamo-pituitary-adrenocortical (HPA) axis to different stressors depends on numerous stimulatory and inhibitory signals arriving from various parts of the brain to the hypothalamic paraventricular nucleus (PVN). We examined the role of catecholamines and vasopressin (VP) in the specific adrenocorticotropic hormone (ACTH) secretion under the influence of thermal stressors, cold (+4°C) and heat (+38°C), applied acutely during 60 min or repeatedly during 7 and 14 days (60 min daily). The results obtained show that hypothalamic dopamine (DA), noradrenalin (NA) and adrenaline (A) concentration significantly decrease in the same manner, as compared to non stressed controls. The quantity of hypothalamic VP significantly increases under the effect of both cold and heat, suggesting the intense synthesis of this neuropeptide as compared to controls, with much stronger effect of heat in comparison to cold. Applied stressors significantly increase the V1b receptor (V1bR) amount mainly present at the corticotrophes, depending on both duration of exposure and nature of stressor. The level of ACTH synthesis, estimated by the changes of this protein content in the pituitary, corresponds to changes in VP and V1bR as well as to circulating ACTH. Concluding, in signal transmission of thermal stress to HPA axis, catecholamines have inhibitory or no effect, rather than stimulatory one, independent of both nature and duration of applied stressor. It seems that hypothalamic VP can directly influence ACTH synthesis and secretion as judged by its quantity as well as V1bR amount.

STRESS HORMONES IN RELATION TO DEPRESSION AND ANXIETY

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Animal models of depression and anxiety are inevitable with respect to understanding the pathophysiology and the development of new drug treatments. Classical models are based on unpredictable stress stimuli. Chronic mild stress model of depression results in an anhedonic state and in changes in gene expression of corticotrophin-releasing hormone or tyrosine hydroxylase in the brain. In another model, the olfactory bulbectomy, we observed a reduction in plasma noradrenaline levels normalized by treatment with a drug with antidepressant action (1). Many patients with depression are treatment-resistant to presently utilised antidepressants. It is therefore necessary to search for new approaches. According to our recent findings, such new target may be the stress hormone aldosterone. Mineralocorticoid aldosterone mainly controls water-electrolyte balance, but we have recently provided evidence on its role in stress response, anxiety and depression (2). Treatment with aldosterone via osmotic minipumps induced an anhedonic state manifested by decreased sucrose preference, depression-like behavior and increased anxiety. Hippocampal gene expression profiling revealed a number of genes significantly altered by aldosterone treatment. The main transcriptional change was identified in genes related to inflammation, glutamatergic activity and synaptic and neuritic remodeling. Moreover, an overlap between aldosterone- and stress-regulated genes was observed. Thus, aldosterone treatment induced changes relevant to the etiology of major depressive disorder. Treatment with aldosterone may serve as a new animal model of depression. *Supported by Vega 2/0118/11.*

(1) Pistovcakova J, Dostalek M, Sulcova A, Jezova D.: Pharmacopsychiatry 41, 54-59, 2008.
(2) Hlavacova N, Wes PD, Ondrejcakova M, Flynn ME, Poundstone PK, Babic S, Murck H, Jezova D.: Int J Neuropsychopharmacol 2011 (in press)

STRESS AND THE BRAIN: FROM CALCIUM TO MEMORY

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Corticosteroid hormones have clear effects on many cognitive processes, such as decision making or emotional, spatial and working memory. The nature of the effects strongly depends on the time at which corticosteroid are present relative to the performance of the cognitive task. This is also reflected in the activity of the brain areas involved in the task and their connectivity, as shown with fMRI in the human brain. These changes in brain activity can be understood from the underlying neuronal substrate. One pathway by which corticosteroids affect activity involves the influx of calcium, subsequent activation of intracellular signaling molecules, eventually changing the functionality of glutamate receptors and hence both basal transmission and synaptic plasticity. This pathway is linked to glucocorticoid receptors and seems important for adequate consolidation of stressful information. More recently it has become evident that additionally corticosteroids impinge on a rapid modulatory pathway of glutamate transmission, a process that depends on mineralocorticoid receptors and is probably important for appraisal of and response selection to the situation. Balanced activity between these two systems and other hormones and transmitters involved in the stress response is necessary for optimal performance in cognitive tasks involved in adaptation to the stressful environment.

EFFECTS OF ACUTE STRESSORS ON THE EXPRESSION OF OXYTOCIN RECEPTOR MRNA IN HEARTS OF RATS WITH DIFFERENT ACTIVITY OF HPA AXIS

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Oxytocin (OT) is known to regulate cardiovascular homeostasis by central as well as peripheral mechanisms. It was reported that this neuropeptide decreases heart rate and force of contraction; these effects are also involved in the stress responses. The role of oxytocin appears to be mediated and regulated by oxytocin receptors (OTR) expression, which was reported in all rat heart compartments. The aim of this investigation was to study the expression of OTR mRNA in rat heart in dependence on two types of restraint stress in animals with different activity of HPA axis. In Sprague-Dawley (SD) and Lewis (LE) rats we investigated the effects of a single exposure to 60 min lasting restraint stress (IMO) or to this stress combined with the immersion of rats into water (21°C) (IMO+C); these stresses differ in their proportion of psychogenic and physical components of action. Expression of OTR mRNA was followed 1 or 3 h after stress termination. Expression of OTR mRNA in heart atria and ventricles were analyzed by quantitative real time RT-PCR where beta-actin was used as housekeeping gene and SYBR green as a marker. Relative expression (RE) of OTR mRNA was calculated according to the formula $2(-\Delta\Delta CT)$. There is no statistical difference between the expressions in control heart compartments of SD and LE rats but the OTR mRNA expression is higher in atria than in ventricles. RE of OTR mRNA induced by the exposure to restraint stresses differ significantly between SD and LE rats. Differences in the effects of IMO and IMO+C may be caused by participation of higher proportion of physical component in IMO+C stress. Our findings support the notion that oxytocin and its receptors have regulatory role in cardiovascular system under stress, and that the overall effect is dependent on the activity of HPA axis of the used rat strains. Supported by MSM 0021620806 and MSM 0021620819.

MITOCHONDRIA AND STRESS (MAL)ADAPTATION

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Tissues with high energy costs, such as the brain, are susceptible to reductions in metabolism, especially in states of high energy expenditure, such as in adverse life events („stress"). Mitochondria enable the brain to meet this high energy demand. Neuronal networks in the brain modify their structure and communication in response to stress, and therefore, the major functions of mitochondria in neurons include the regulation of neuronal plasticity. Thus, any degree of energy disturbance in the brain might lead to alterations of the local neuronal circuitries, and eventually to dysregulation of biological systems leading to e.g. the development of stress-related mood disorders, such as depression. Interestingly, patients diagnosed with inborn errors of oxidative phosphorylation (OXPHOS) present with a markedly higher rate of depressive behavior compared to the general population. In my talk I will present several line of evidence on stress-induced neuronal plasticity in the brain concomitant with altered mitochondrial functioning as reflected in alterations in functioning of OXPHOS as well as that in mitochondrial networks in response to environmental stimuli. Since adaptation to adverse life experiences requires a tightly coordinated harmony of the principle stress-mediators (corticotropin releasing factor and urocortins), and consequently, failure in the operation of these stress-mediators enhances vulnerability to depression, in my presentation I will focus on the interplay of mitochondrial (dys)functioning and these principle stress mediators. At the end of my talk I will propose a model, emphasizing that the interaction of such interwoven biological processes could produce a vulnerable phenotype characterized by an imbalance in the action of these stress mediators that in time may culminate in the precipitation of depression.

IP3 RECEPTORS, HYPOXIC STRESS AND APOPTOSIS

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Inositol 1,4,5-trisphosphate receptors (IP3Rs) as the intracellular calcium channels play a crucial role in variety of physiological and pathophysiological processes. Among stimuli that can result in a serious damage of the cell, hypoxia plays an important role. We have found that gene expression of the type 1 and 2 IP3 receptors is significantly increased after the exposure of mice to hypoxic stimulus for 6 hours, and also in rat cerebellar granular cells. Increased gene expression of IP3 receptors was reflected in increased protein levels of these channels as well. In this process, reactive oxygen species are most probably involved, since antioxidant quercetin abolished hypoxia-induced increase of both, type 1 and 2 IP3 receptor. Increased levels of IP3Rs is known to be involved in the induction of apoptosis. Thus, we tested a hypothesis whether hypoxia-induced increase in IP3Rs can induce the apoptosis. We have found that under certain conditions, severe hypoxic stress can activated mitochondrial pathway of the hypoxic stress. Deeper understanding of mechanisms, through which hypoxia regulates intracellular calcium could point towards the development of new therapeutic approaches to reduce or suppress the pathological effects of cellular hypoxia, such as those seen in stroke or ischemia. This work was supported by grant VEGA 2/0049/10.

ADIPOCYTES AS A NEW SOURCE OF CATECHOLAMINE PRODUCTION IN STRESSED RATS

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The sympathoadrenal system plays a key role in the regulation of adipose tissue metabolism. During emotional or physical stress exposure there is an increase in the production of norepinephrine (NE) and epinephrine (EPI) also in the adipose tissue. We investigated NE and EPI concentrations in five types of rat adipose tissue (mesenteric, subcutaneous, epididymal, retroperitoneal and brown). All adipose tissues were found to contain both NE and EPI. To find the source of observed EPI level, we examined catecholamine (CA) levels and gene expression of CA biosynthetic enzymes – tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DBH), and phenylethanolamine N-methyltransferase (PNMT) - in whole adipose tissues and in isolated adipocytes. Gene expression of TH, DBH and PNMT was detected and quantified in all five mentioned types of rat adipose tissue. To find the cells expressing these enzymes we separated mesenteric adipose tissue to adipocytes and stromal/vascular fraction. In both cell fractions we detected EPI and NE levels and gene expression, as well as protein levels of TH, DBH, and PNMT. Immobilization stress, especially repeated, caused a significant increase in NE, EPI, and TH, PNMT mRNA and protein levels in isolated adipocytes. Our data indicate that adipocytes accommodate gene machinery able to synthetise NE and EPI, which is highly activated under stress. This finding suggests the functionality of CA biosynthetic machinery found in adipocytes. Stress activation of sympathetic innervation in various adipose tissues is not the only source of CAs, since adipocytes are also able to produce NE and EPI. Thus, adipocyte catecholamines might contribute to the regulation of lipolysis and other metabolic processes via different way than only the NE released locally from the sympathetic system. Supported by grants VEGA 2/0188/09 , 2/0036/11 and APVV-0088-10

THE SYMPATHETIC NERVOUS SYSTEM POTENTIATES FIBROSARCOMA DEVELOPMENT IN RATS

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The sympathetic nervous system (SNS) represents one of the most important nervous pathways that is widely discussed in context with cancer. In the present study we used three different approaches to investigate the role of the SNS in tumor growth. An impact of the chemical sympathectomy induced by 6-hydroxydopamine (6-OHDA) pretreatment, on tumor development and progression as well as survival of tumor-bearing rats was investigated. Four experimental groups of animals were used: I/ animals with tumors, II/ animals with tumors and the chemical sympathectomy, i.e. intraperitoneally pretreated with 6-OHDA, III/ animals with no tumors pretreated with 6-OHDA, and IV) absolute controls. One week after the chemical sympathectomy the intra-abdominal tumor growth was induced by an intraperitoneal injection of BP6-TU2 fibrosarcoma cells in male Wistar rats. Significant reduction in the incidence of intraperitoneal tumors was observed in animals that underwent the chemical sympathectomy compared to rats having preserved sympathetic innervation. Moreover, 6-OHDA pretreatment significantly improved the survival of tumor-bearing rats in comparison with the non-sympathectomized animals. Moreover, presence of neural tissue in tumors was examined and proven by immunohistochemistry for neuronal marker neuron-specific enolase. Finally, parallel in vitro study showed elevated proliferation of BP6-TU2 fibrosarcoma cells after addition of the SNS neurotransmitter, norepinephrine, into the medium. Our data indicate that sympathetic nerves stimulate the proliferation of fibrosarcoma tumor cells and reduce the survival of tumor-bearing rats. Supported by the Slovak Research and Development Agency under the contract No. APVV-0045-06 and APVV-0007-10.

DIFFERENT CHANGES IN GENE EXPRESSION OF CATECHOLAMINE BIOSYNTHETIC ENZYMES IN SPLENIC T AND B-CELLS INDUCED BY STRESS

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Catecholamines (CA) regulate various functions of the organism including the immune organs and cells. Besides circulating CAs and those released from sympathetic nerves, spleen, as an important immune organ, contains the machinery to produce CAs de novo. However, the cell type involved in this endogenous CAs production, mainly in stress, is not clearly appointed. Therefore, our aim was to analyze CAs, mRNA and protein of CA biosynthetic enzymes in T and B-cells isolated from rat spleen after acute and repeated immobilization stress (IMO). In T-cells, acute IMO induced an increase of tyrosine hydroxylase (TH) mRNA and protein while repeated IMO returned them to baseline levels. In B-cell, TH gene expression was very low, close to the detection limit. Despite low mRNA level, the TH protein was detectable in B-cells and was decreased after a single while increased after repeated IMO. Phenylethanolamine-N-methyltransferase (PNMT) mRNA showed similar changes as TH. Acute IMO induced a rise of PNMT mRNA in T-cells but a significant decrease in B-cells. On the other hand, repeated IMO increased PNMT mRNA especially in B-cells. Moreover, B-cells showed a significantly higher level of PNMT mRNA at basal conditions than T-cells. Nevertheless, the PNMT protein was present at detection limit of Western blot in both cell types. CA levels found in T and B-cells reflected changes observed in CA biosynthetic enzymes. Taken together, this is the first report about CA biosynthesis in T and B-cells during acute and repeated stress. Different changes in expression of CA biosynthetic enzymes and CA levels in T and B-cells during acute and chronic stress are most probably involved in stress-mediated changes of immune response. Nevertheless, exact functional consequences of observed changes remain to be elucidated. (Supported by VEGA 2/0036/11, 2/0188/09, APVV 0088-10, NFM/EEA SK0095)

STRESS-RELATED SEROTONERGIC SYSTEMS: IMPLICATIONS FOR AFFECTIVE AND ANXIETY DISORDERS

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Previous studies have suggested that serotonergic neurons in the midbrain raphe complex have a functional topographic organization. Our recent studies support the hypothesis that stimulation of a central nucleus of the amygdala-dorsal raphe nucleus pathway by anxiety and fear-related stimuli activates a subpopulation of serotonergic neurons in the dorsal part of the dorsal raphe nucleus (DRD), which participates in facilitation of anxiety responses. On the other hand, our recent studies support the hypothesis that activation of a spinoparabrachial pathway by peripheral thermal or immune stimuli activates subpopulations of serotonergic neurons in the ventrolateral part of the dorsal raphe nucleus/ventrolateral periaqueducal gray (DRVL/VLPAG) and interfascicular part of the dorsal raphe nucleus (DRI), which participate in antidepressant-like effects. Understanding the anatomical and functional properties of these distinct serotonergic systems may lead to novel therapeutic strategies for the prevention and/or treatment of affective and anxiety disorders. The project described was supported by Award Number R01MH086539 to CAL from the National Institute of Mental Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Mental Health or the National Institutes of Health.

CONGENITAL ABSENCE OF VASOPRESSIN RESULTS IN AGE DEPENDENT ALTERATION OF HORMONAL STRESS RESPONSE

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The hypothalamo-pituitary-adrenal axis is controlled by corticotropin-releasing hormone (CRH) and vasopressin. To test the hypothesis that the regulation changes with age we compared ether stressor and bacterial lipopolysaccharide (LPS) injection induced stress reaction in adult and 10-day-old Brattleboro rats, naturally lacking vasopressin (di/di). The LPS stimulus was used with V1b antagonist pretreatment (SSR149415), too. In adult di/di or pretreated animals we observed normal pituitary and adrenocortical reaction, while in the 10-day-old rats stress-induced plasma corticosterone rises were marked with significantly smaller adrenocorticotropin elevations. Compared to control pups the adenohypophysis of the 10-day-old di/di rats was responding normally to CRH and their adrenal glands were hyperresponsive to adrenocorticotropin, while in adult there was a higher response at both level with no difference between the genotypes. The plasma corticosterone binding capacity (CBG) was higher in adults than pups, with the di/di pups having higher CBG than controls. We assume that during the perinatal period vasopressin might be the predominant secretagogue for adrenocorticotropin, while in adult the regulation changes to CRH dominance. The marked discrepancy in stressor-induced adrenocorticotropin and corticosterone rises in pups suggests that without functional vasopressin the circulating corticosterone levels are regulated by alternative factors different from immunoreactive adrenocorticotropin.

EFFECTS OF NITRIC OXIDE ON THE PREFRONTAL CORTEX IN STRESSED RATS

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Background: Nitric oxide (NO) exhibits both protective and detrimental effects in the central nervous system. Objective: To investigate the effect of NO on the prefrontal codex in neonatal stressed rats. Design, time nad setting: A randomized, controlled, animal study was performed at the Anatomical Department of Iran University of Medical Sciences from May 2007 to August 2008. Materials: Forty-eight male Wistar rats were obtained from Pasteur‘s Institute, Tehran, Iran. Methods: Rat stress models were established by immobilization and randomly received intraperitoneal injection of 2 ml physiological saline, L-arginine (200 mg/kg) as a NO precursor, N(G)-nitro-L-arginine methyl ester (20 mg/kg), or subcutaneous injection of 7-nitroindazole (25 mg/kg) as a NO synthase inhibitor. Main outcome measures: After the rats were treated for 4 weeks, the frontal cortex was harvested for histological observation and NO detection. Results: Subcutaneous administration of N(G)-nitro-L-arginine methyl ester or 7-nitroindazole resulted in significantly lower prefrontal cortex thickness and NO production compared with subcutaneous administration of L-arginine ($p<0.05$). Prefrontal cortex thickness significantly increased in rats following L-arginine treatment as compared to physiological saline intervention ($p<0.05$). Conclusion: NO exhibited protective effects on the prefrontal codex of stressed rats.

REGULATION OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS BY PHARMACOLOGICAL TOOLS: DISSECTION OF RECEPTORS AND INPUT PATHWAYS

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The corticotrophin releasing hormone (CRH) neurons in the hypothalamic paraventricular nucleus (PVN) are the major positive drivers of the hypothalamo-pituitary adrenocortical (HPA) axis. These neurons are the down-stream component of parallel network of neuronal structures coordinating the hormonal output by various stressors. Inhibitory feedback loops, mainly mediated via glucocorticoid and mineralocorticoid receptor activation of the hippocampus and GABAergic pathways also regulate these neurons. It is also well known that systemic administration of pharmacological agents can both increase (i.e. serotonin, nicotine, and catecholamine receptor agonists) and inhibit (GABA-AR agonists) HPA activity, but the neuronal systems involved in these effects are not understood. The aims of our studies have been to dissect the neuronal pathways and the receptors involved in both positive and negative regulation of the HPA. We have used a number of compounds selective for nicotine (nAChR), serotonin (5-HTR), and GABA-A receptor subtypes to differentiate the receptor subtypes involved in HPA activation, both in terms of hormonal release and immediate-early gene expression in the PVN. In addition, we have used neuronal tracing to identify the neuronal structures projecting to the PVN that are activated after systemic administration of pharmacological agents. We have found that α4*nAChR, α7nAChR, 5-HT1A, 5-HT2A, and GABAα1 receptors activate, whereas GABAα2/3 inhibits HPA activity. These effects are mediated both directly on the CRH neurons, but also indirectly via distinct neurons in structures upstream of the PVN that are also considered to be involved in both psychological and physical stress activation and inhibition.

PARAVENTRICULAR HYPOTHALAMIC NUCLEUS AND PREFRONTAL CORTEX DEAFFERENTATION AFFECTS NEUROENDOCRINE STRESS RESPONSE IN RATS

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The paraventricular hypothalamic nucleus (PVN) and media prefrontal cortex (mPFCx) play an important role in regulation of neuroendocrine stress reaction. Therefore, we investigated the effect of PVN and mPFCx deafferentation on stress response in Sprague-Dawley rats exposed to immobilization stress. Chronic cannula was implanted into the tail artery one day before the experiment. At the day of experiment, blood was collected before exposure of rats to immobilization as well as at 5, 30, 60, and 120 min interval of immobilization. We found increased immobilization-induced release of epinephrine and norepinephrine in rats with posterolateral deafferentation of PVN. In contrary, stress-induced release of corticosterone in these animals was reduced. In rats with posterior mPFCx transection we found increased release of epinephrine, whereas norepinephrine was increased only later at 60 and 120 min after beginning of immobilization. Plasma glucocorticoids levels were not affected by transection of mPFCx. Our data showed that transection of nervous pathways interconnecting the PVN with brainstem or forebrain structures alters immobilization stress-induced activation of sympathoadrenal system and hypothalamic-pituitary-adrenal axis in rats. Exaggerated stress-induced increase of sympathoadrenal system activity in rats with PVN or mPFCx deafferentation indicates that both interventions interrupt pathways inhibiting activity of sympathetic preganglionic neurons. However, different time-course of immobilization-induced release of catecholamines in rats with PVN and mPFCx deafferentation indicates different role of pathways interconnecting PVN with forebrain and brainstem structures in modulation of neuroendocrine stress response. Supported by the Slovak Research and Development Agency under the contract No. APVV-0148-06 and APVV-0088-10 and VEGA grant No. 2/0010/09.

THE ROLE OF CHOLINERGIC RECEPTOR SYSTEM IN RESPONSE TO STRESS

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In general, the stimuli that elicit stress (i.e. stressors) can be divided into some categories: physical, chemical, psychological, social, stressors that affect cardiovascular and metabolic homeostasis and mixed stressors. Catecholamines and adrenoceptors are considered as first molecules acting in response to stress. Between the other neuromediator systems, cholinergic system can be also activated by stress. As a result, the hyperexcitation of cholinergic circuits, both peripherally and in the central nervous system (CNS) occurs. Although there are reports about the activation of cholinergic system in response to stressors, it is not known if the cholinergic system is activated in parallel to catecholamine system or secondarily as a result of primary catecholamine system activation. We investigated the effects of different stressors (immobilization, restraint, cold, chemicals) on peripheral and central cholinergic system in rats and mice. In addition to that, animals with targeted disruption of muscarinic M2 receptors (main heart cholinergic receptors) and with disrupted HPA axis (corticotropin releasing hormone knockout mice) were used. The changes in gene expression, the number of binding sites and functional parameters (heart rate, animal activity and body temperature) were followed. In general, treatment with different stressors resulted in decreased amount of muscarinic receptor on the level of gene expression, protein as well as in decreased binding. On the other hand, the function of respective organ was not affected in large extent. This suggests the new balance between affected neurotransmitter systems in stress. The time sequence of changes implies the role of adrenergic neurotransmitter system as principal. In conclusion, the cooperation of multiple neurotransmitter systems is needed for appropriate reaction to stress in order to maintain homeostasis.

STRESS DUE TO PERIPHERAL LIPOPOLYSACCHARIDE INDUCES APOPTOSIS IN THE SUBVENTRICULAR ZONE

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Peripheral administration of the endotoxin lipopolysaccharide (LPS), the major lipid constituent of the cell wall of gram-negative bacilli, is an acute stress to invoke the acute-phase immune response. LPS stimulates the production and secretion of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6 in various brain regions as well as in peripheral tissues and plasma. Peripheral LPS treatment has been observed to affect norepinephrine metabolism in the locus coeruleus and dopamine system in the olfactory system. We previously reported that peripherally injected LPS induced apoptotic cell death in the olfactory bulb (OB) in young adult mice. The subventricular zone (SVZ) constantly supplies newly generated neurons to the OB along the rostral migratory stream (RMS) in adult brain. Since AVZ-RMS-OB is thought to work as a unit, we have examined whether peripherally injected LPS induces apoptotic cell death also in the SVZ in young adult mice. Two mouse strains were used: C3H/HeN and Toll-like receptor 4-mutated C3H/HeJ, and wild- type C57BL/6 and TNFR1-/- –2-/-, in which the genes TNF receptor (TNFR)1 and TNFR2 are knocked out. Immunohistochemical study and terminal deoxynucleotididyl transferase-mediated dUDP nick-end labeling assay were carried out on the SVZ-RVZ pathway. These in vivo studies provided the following information: first, peripherally injected LPS switches on apoptotic signals in cells of the SVZ-RMS pathway; second, activation of the cascade triggered by TNF-α-TNFR system plays a critical role in fully inducing apoptosis in this pathway; third, TNF-α may be released mainly from astrocytes in the pathway. These results suggest that stress produced by peripheral LPS causes deterioration of catecholamine system in the brain.

STRESS-INDUCED CHANGES IN TAU PROTEINS AND CATECHOLAMINES IN ANIMAL MODEL OF ALZHEIMER'S DISEASE

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Psychological stress is a known risk factor for numerous disorders; in the brain, it was shown to impair memory function and long-term potentiation, neuronal morphology and neurogenesis in the hippocampus, a region affected early in Alzheimer's disease. The present study seeks to investigate the relationship between stress, catecholamines and pathological modifications of tau proteins. The influence of stress on progression of neurodegeneration has been investigated using transgenic rats expressing truncated tau protein derived from PHF core tau of Alzheimer's disease. Furthermore, the role of the HPA axis in the impact of stress on tau protein modifications was elucidated in CRH-knockout mice. A total of 15 brain areas have been analyzed for pathological tau protein alterations, catecholamine levels and catecholamine biosynthetic enzyme expression. In both experimental models we found significant hyperphosphoryalation of several Alzheimer's disease associated epitopes on tau proteins (pT181, AT8, PHF). The phosphorylation response to stress was found to be biphasic and transient. The neuropathological localization of degenerative changes in the transgenic rat (Locus coeruleus), when coupled with depletion of noradrenalin in stress, may constitute a mechanism for strongly increased inflammation and accelerated neurodegeneration in cerebral areas and brainstem of the affected animals. The HPA axis has been found to be an important mediator of the acute hyperphosphorylation response of tau proteins to stress. Our results suggest that pathological phosphorylation of tau proteins induced by stress represents one of the potential mechanisms, which can lead to misfolding of tau proteins and to initiation or acceleration of neurodegeneration (Supported by APVV-0088-10, APVV–0634–07).

SUBDIAPHRAGMATIC VAGOTOMY EXAGGERATES THE CATECHOLAMINE RELEASE INDUCED BY IMMOBILIZATION STRESS IN RATS

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Signals transmitted via the vagal afferent pathways are one of the factors regulating the activity of the sympathoadrenal system. Therefore, we investigated the effect of the subdiaphragmatic vagotomy on the sympathoadrenal system activity during immobilization of Sprague Dawley rats. Three or 14 days respectively, after the subdiaphragmatic vagotomy a permanent cannula was inserted into the jugular vein to allow repeated blood sampling from freely moving rats. One day after cannulation rats were exposed to immobilization stress and blood samples were collected. Immediately after the baseline blood collection, animals were exposed to stress. Subsequently, blood samples were collected after 5, 15, and 60 minutes of immobilization and 60 minutes after the end of stress stimuli. Blood plasma was separated and plasma concentrations of epinephrine and norepinephrine were determined by a commercial ELISA kit. We found exaggerated immobilization-induced epinephrine and norepinephrine release in vagotomized rats exposed to stress on the 3th and 14th days following vagotomy. The subdiaphragmatic vagotomy-induced enhancement of catecholamine release was the highest in vagotomized rats immobilized on the 3th day after vagotomy. Our data showed that transection of vagal pathways at subdiaphragmatic level potentiated the neuroendocrine stress response. We suggest that the vagal afferent pathways activated by peripheral catecholamines serve as a part of the negative feedback inhibiting the stress-induced activation of the sympathoadrenal system. Supported by the VEGA grant No. 2/0010/09.

SIMULATION OF PROLONGED STRESS-INDUCED OXYTOCIN RELEASE INDUCES MYOCARDIAL PROTECTION AGAINST INFARCTION

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Oxytocin is a hormone, which is released into the circulation in response to acute or chronic stress stimuli and has a great array of physiological activities in the periphery. One of the important targets of oxytocin action is cardiovascular system. The aim of the present studies was to test the hypothesis that a prolonged increase in circulating oxytocin levels exerts cardioprotective effects and that such levels of oxytocin stimulate intracellular signaling pathways playing a role in ischemia/reperfusion injury. Rats were treated with oxytocin or vehicle continuously via osmotic minipumps for 14 days. Oxytocin concentrations in plasma were analysed by radioimmunoassay and the activation of proteins and peptides by immunoblotting assay. We used an isolated rat heart model of global ischemia and reperfusion. The infarct size was determined by a computerized planimetric method. Oxytocin treatment led to an increase in plasma oxytocin levels approaching those observed during immobilization stress. Infarct size was significantly reduced and the recovery of functional parameters after ischemia was significantly better in oxytocin-treated rats. Treatment with oxytocin resulted in a significant increase in activation of p38-MAPK, Akt kinase, Hsp27 and an elevation in atrial natriuretic peptide concentrations in left heart ventricle. In conclusion, prolonged increase in circulating oxytocin levels leads to a reduction of the infarct size and improvement of postischemic recovery of heart function. Activation of p38-MAPK and PI3K/Akt kinase appears to mediate oxytocin effects on the tolerance of the heart to ischemia/reperfusion injury. We may hypothesize that oxytocin released during stress may ameliorate the negative consequences of stress on the heart. Supported by Vega 2/0118/11 and SAS-NSC JRP 2010/07.

PHEOCHROMOCYTOMA: A CATECHOLAMINE AND OXIDATIVE STRESS DISORDER

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The WHO classification defines pheochromocytoma as a tumor arising from chromaffin cells in the adrenal medulla. Tumors of extra-adrenal sympathetic and parasympathetic paraganglia are classified as extra-adrenal paragangliomas. Practically all pheochromocytomas and paragangliomas produce catecholamines. Catecholamine concentrations in tumor tissue are enormous, creating a volcano that can erupt at any time. The production of norepinephrine and epinephrine is optimally assessed by the measurement of their O-methylated metabolites, normetanephrine or metanephrine, respectively. Methoxytyramine, O-methylated metabolite of dopamine is the marker of dopamine secreting tumors. The presence of either constitutive or regulated secretory pathways contributes further to the very unique mutation-dependent catecholamine production and release. Oxidative stress results from a significant imbalance between levels of prooxidants that are generated during oxidative phosphorylation and antioxidants. The gradual accumulation of prooxidants due to metabolic oxidative stress results in proto-oncogene activation, tumor suppressor gene inactivation, and genomic instability. Since the mitochondria serves as the main source of prooxidants, any mitochondrial impairment leads to severe oxidative stress and glycolysis, a major outcome of which is tumor development. Pheochromocytomas and paragangliomas are practically the only known tumors where the oxidative phosphorylation defect due to the mutation of succinate dehydrogenase is the cause, not a consequence, of tumor development. Targeting mTORC, IGF-1, HIF, topoisomerases, proteosome, balancing activity of protein kinases and phosphatases, or even synchronizing the cell cycle before any exposure to any kind of therapy will soon become a reality.

INTERPLAY BETWEEN MOLECULAR RESPONSES OF TUMOR CELLS TO HYPOXIA AND STRESS MEDIATED BY CATECHOLAMINES

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Tumor progression is a multistep process driven by genetic, epigenetic as well as physiologic alterations. Adaptations to physiologic factors in tumor microenvironment (particularly hypoxia and acidosis), deregulation of metabolism, ion transport and cell adhesion as well as neo-angiogenesis are basic components of this clinically well-characterized process. Epidemiologic and experimental data suggest that in addition to these factors of tumor microenvironment, also stress mediators (glucocorticoids and catecholamines) excreted in organism of patient contribute to tumor progression. Most information in this area comes from investigations of glucocorticoids, that show differential and antagonistic effects on outcome of chemotherapy and in some tumor types can contribute to treatment resistance and increased metastatic propensity. On the other hand, mechanisms of pro-oncogenic effects of stress-induced catecholamines (i.e. adrenergic hormones) remain unclear and information on their link to clinical parameters is only sporadic. Their effects are usually attributed to negative influence of chronic stress and/or depression on anticancer cellular immunity. Character of other biological mechanisms remains mostly unexplored. In particular, mutual cross-talk between stress-activated signaling and hypoxia-stimulated molecular pathways have been insufficiently studied and their cooperative effect on tumor progression awaits better elucidation.

ADVERSE EARLY LIFE EXPERIENCE INCREASES REACTIVE COPING BEHAVIOR DURING SOCIAL DEFEAT IN ADULTHOOD

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Stressful life events, whether early in life or in adulthood, confer vulnerability to developing psychiatric diseases, including anxiety and depression. An organism's behavioral coping strategy during a stressful event can modulate the physiological severity of that stressor and subsequent stressors. We exposed male Long Evans rats to either 180 min of daily maternal separation or normal animal facility rearing for two weeks early in life (postnatal days 2-14) followed by exposure to social defeat stress or home cage control conditions in adulthood (postnatal day 60). In addition, we videotaped and quantified the duration and frequency of the animals' behavior during social defeat. We report that social defeat induced a change in behavioral coping styles in maternally separated rats, resulting in a more reactive (passive-submissive) coping strategy when compared to rats that received normal animal facility rearing. This shift in coping behavior was mainly due to an increase in the amount of freezing behavior and a reduction in rearing behavior in maternally separated animals. These data are consistent with the idea that adverse early life experiences alter an organism's coping behavior to subsequent stress.

HOW THE SELFISH BRAIN ORGANIZES ITS SUPPLY AND DEMAND

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During acute mental stress, the energy supply to the human brain increases by 12%. To determine how the brain controls this demand for energy, 40 healthy young men participated in two sessions (stress induced by the Trier Social Stress Test and non-stress intervention). Subjects were randomly assigned to four different experimental groups according to the energy provided during or after stress intervention (rich buffet, meager salad, dextrose-infusion and lactate-infusion). Blood samples were frequently taken and subjects rated their autonomic and neuroglycopenic symptoms by standard questionnaires. We found that stress increased carbohydrate intake from a rich buffet by 34 g (from 149 ± 13 g in the non-stress session to 183 ± 16 g in the stress session; $P < 0.05$). While these stress-extra carbohydrates increased blood glucose concentrations, they did not increase serum insulin concentrations. The ability to suppress insulin secretion was found to be linked to the sympatho-adrenal stress-response. Social stress increased concentrations of epinephrine 72% (18.3 ± 1.3 vs. 31.5 ± 5.8 pg/ml; $P < 0.05$), norepinephrine 148% (242.9 ± 22.9 vs. 601.1 ± 76.2 pg/ml; $P < 0.01$), ACTH 184% (14.0 ± 1.3 vs. 39.8 ± 7.7 pmol/l; $P < 0.05$), cortisol 131% (5.4 ± 0.5 vs. 12.4 ± 1.3 μg/dl; $P < 0.01$) and autonomic symptoms 137% (0.7 ± 0.3 vs. 1.7 ± 0.6; $P < 0.05$). Exogenous energy supply (regardless of its character, i.e., rich buffet or energy infusions) was shown to counteract a neuroglycopenic state that developed during stress. Exogenous energy did not dampen the sympatho-adrenal stress-responses. We conclude that the brain under stressful conditions demands for energy from the body by using a mechanism, which we refer to as "cerebral insulin suppression" and in so doing it can satisfy its excessive needs.

DOES HIGH FAT DIET INCREASE THE SENSITIVITY OF NEURONS TO COLCHICINE STRESS IN OVARIECTOMIZED C57BL/6 MICE? DUAL FOS/NEUROPEPTIDE IMMUNOHISTOCHEMISTRY

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Employing dual Fos/neuropeptide immunohistochemistry, activity of neuropeptide Y (NPY), tyrosine hydroxylase (TH), corticoliberine (CRH), and oxytocin (OXY) producing cells was investigated in response to colchicine stress in ovariectomized female C57BL/6 (with standard or high fat diet) mice in selected brain areas including the hypothalamic paraventricular (PVN), dorsomedial (DMN), A1/C1 catecholaminergic cell groups, and the nucleus of the solitary tract (NTS, A2/C2). Colchicine stress was induced by an intracerebroventricular injection of 3 μl (3mg/500 μl) colchicine. The animals were sacrificed by transcardial perfusion with fixative 20 h after the drug administration. In control mice, no Fos expression was found in NPY and TH cells in A1/C1 and NTS areas. High fat status itself did not provoke any increase in Fos expression in any of the structures studied. However, prominent Fos expression was achieved by the colchicine administration in the NTS of both groups of mice where many NPY and TH cells showed Fos presence. Although less extensive Fos response to colchicine was seen in the A1/C1 cells, some Fos/TH colocalizations clearly occurred. In the PVN only a little Fos presence was observed after colchicine treatment and neither CRH, OXY, nor TH cells revealed colocalizations with Fos. In the ARC and DMN areas many TH perikarya occurred after colchicine treatment however, they revealed only rarely Fos immunoreactivity. The present pilot study indicate that hight fat status does not: 1/ represent an incessant stimulus for neurons in the stress sensitize brain structures (PVN, DMN, C1/A1, NTS) and 2/ sensitize the neurons in the above mentioned structures to colchicine stress more prominently as does colchicine in the mice feed with standard diet. (supported by GAČR 303/09/0744 grant)

DISSECTING ANXIOGENIC AND ANXIOLYTIC PROPERTIES OF THE CRH/CRHR1-SYSTEM USING GENETIC MOUSE MODELS.

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The corticotropin-releasing hormone (CRH) and its type 1 high affinity receptor (CRHR1) are widely distributed throughout the CNS. Together they orchestrate the neuroendocrine and behavioral adaptations to stress. Chronic stress-associated dysfunction of CRH/CRHR1-related neuronal circuitries has been implicated in the onset and maintenance of mood and anxiety disorders. To address the function of the CRH/CRHR1 system in vivo, we have generated i) a collection of GFP and LacZ reporter mice, ii) conditional CRH over-expressing mice and iii) conditional knockout mice where the deletion of CRHR1 is restricted to neurons of a specific neurotransmitter identity. These models allowed us on the one hand to elucidate the neurochemical identities of the CRH/CRHR1 system and on the other hand to dissect its functional properties in different brain areas and circuits. With this approach we were able to further substantiate and specify the anxiogenic properties of the CRH/CRHR1 system. Moreover, these mouse models allowed us to uncover previously unknown anxiolytic effects of the CRH/CRHR1-system.

STRESS, GABA AND PTSD

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Studies have shown that stress can affect neural plasticity. Most studies dealing with the effects of stress on neural plasticity focused on synaptic plasticity and long-term potentiation (LTP) of principle cells. However, following stress, modifications may also take place at the level of complex interactions with interneurons, i.e. at the local circuit level. We set out to examine the possible impact of a traumatic experience (i.e. underwater trauma) on the plasticity of the principle cells but also on local circuit activity within the dentate gyrus (DG). To analyze the effects of re- exposure to stress, rats were first exposed to underwater trauma and 24 hrs later were examined. Compared to control rats, rats that had been exposed to underwater trauma and had been re-exposed to its context 24 hr. later showed increased anxiety behavior in the elevated plus maze and in the open field tests. Frequency-dependent inhibition (FDI) and paired-pulse inhibition (PPI) were employed to reflect the activity of GABAergic interneurons in the DG. Measuring both population spike amplitude and field-EPSP, all groups showed similar input-output and baseline responses. Rats that had been exposed to underwater trauma and had been re-exposed to its context 24 hr. later showed significantly higher inhibition in both PPI and FDI protocols compared to control rats. Additionally, compared to control rats, traumatized rats showed reduced level of dentate gyrus LTP. Taken together, these findings indicate that the re-exposure to a contextual reminder of a trauma affects not only aspects of plasticity of principle cells, but also aspects of local circuit activity in the DG. These alterations may underlie some of the behavioral consequences of the traumatic experience.

A ROLE OF CART AND AGRP AS MODULATORS OF FUNCTIONAL ACTIVITY OF DOPAMINE NEURONS OF BRAIN

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As known the CART (cocaine - and amphetamine-related transcript/peptide) - and AGRP (agouti related protein)-immunoreactive processes are present in the different brain dopaminergic (DA) structures. But the functional role of the peptides on DA neurons is unknown. We had been conducted immunohistochemical investigations (western blotting, biotin-streptavidin technique, double fluorescence staining and confocal microscopy) on the Wistar rats and mice C57J/Bl the purpose to find the functional relations CART/AGRP and their influences on DA neurons. CART neurons from nucleus accumbens (nAcc) send processes in the substantia nigra (SN) to DA neurons. Immunochistochemically the significant increase of optical density (OD) of CART-immunoreactivity was detected in nAcc and in SN after 4 h blockade of tyrosine hydroxylase (TH - a key enzyme of DA synthesis) in experiment in vitro by alfa-metyl-para-tyrosine. Destruction of 30 % DA neurons of SN after lactacystin (inhibitor of proteosomes) influence via cannula in SN it is not shown the reduction of TH-OD in dorsal striatum, but the significant increase of CART-OD both in the neurons of nAcc (on 53 %) and in the SN (on 60 %) was indicated. In experiment in vitro after an incubation of a rat SN brain slices in the media with a CART-peptide (100 nM) the significant increase of TH-OD was indicated in DA neurons (on 57 %). In experiment in vitro after 4 h an incubation of a mice brain slices from ventral tegmental area in the media with AGRP (200 nM) the significant reduction of TH-OD (on 50%) was shown. Our data testifies to antagonistic functional interrelations CART/AGRP and shows activating influence of CART and inhibitor influence of AGRP on the functional activity of DA neurons. Our data shows participation of CART from nAcc in compensatory brain mechanisms at DA lack.

MODULATION OF NEUROENDOCRINE STRESS RESPONSES: NEUROSTEROID – OPIOID – NORADRENALINE INTERACTIONS IN RATS

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In late pregnant rats hypothalamo-pituitary-adrenal (HPA) axis responses to stressors are strongly reduced [1]. This is a result of presynaptic inhibition of noradrenaline release in the paraventricular nucleus (PVN), from projections of nucleus tractus solitarii (NTS) neurones, via activation of an opioid mechanism in pregnancy. This opioid mechanism, in the NTS neurones, is driven by high levels of the neurosteroid, allopregnanolone (AP), produced from progesterone in late pregnancy by sequential actions of 5α-reductase (5α-R) and 3α-hydroxysteroid dehydrogenase (3α-HSD). Moreover, expression of 5α-R and 3α-HSD is up-regulated in the hypothalamus and brainstem in late pregnancy [1]. Despite reduced HPA axis responses to stressors in pregnancy, repeated social stress in late pregnancy programmes offspring HPA axis hyper-responsiveness to stress, including to interleukin-1β challenge (IL-1β, 500 ng i.v.) [2]. As we found NTS 5α-R mRNA expression was reduced in PNS rats, we tested whether their increased HPA axis responses to stress might result from deficient neurosteroid production. We found that exaggerated HPA axis responses to IL-1β in prenatally stressed (PNS) males are reduced by pre-treatment (overnight) with 5α-androstane-3β,17β-diol (3β-diol), the 5α-R/ 3α-HSD neurosteroid product of testosterone. In PNS females, AP pre-treatment normalised HPA axis responses. Up-regulation of 5α-R and 3α-HSD gene expression in the NTS, by injecting adeno-associated virus vectors, also normalised HPA axis responses to IL-1β. Hence up- and down-regulation of neurosteroid production in the brain has key roles in HPA axis hypo-responsiveness in pregnancy and in hyper-responsiveness in programmed offspring, respectively. Supported by the BBSRC. [1] Brunton PJ et al J Neurosci 2009, 29:6449-6460. [2] Brunton PJ et al J Neuroendocrinol 2010, 22:258-271.

NEUROPROTECTIVE EFFECTS OF SARTANS (ANGIOTENSIN II AT1 RECEPTOR BLOCKERS, ARBS)

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Poor adaptation to stress, alterations in cerebrovascular function and excessive brain inflammation play critical roles in the pathophysiology of many psychiatric disorders. Treatment for these conditions is at present very limited and many times inefficient, and the search for novel therapeutic options is of major importance. Recently, attention has been focused on the role of a brain regulatory peptide, Angiotensin II, and in the translational value of the blockade of its physiological AT1 receptors. Angiotensin II, through AT1 receptor stimulation, is a pleiotropic brain modulatory factor controlling the reaction to stress, in the regulation of cerebrovascular flow and the response to inflammation. Excessive brain AT1 receptor activity associates with exaggerated sympathetic and hormonal response to stress, vulnerability to cerebrovascular ischemia and brain inflammation, leading to neuronal injury. Inhibition of brain AT1 receptor activity with systemically administered Angiotensin II receptor blockers (sartans) is neuroprotective, reducing stress responses, anxiety, stress-induced gastric ulcerations, vulnerability to ischemia and stroke, chronic cerebrovascular inflammation, and acute inflammatory responses produced by bacterial endotoxin. Neuronal protection contributes to increase lifespan. Sartans are safe compounds widely used in the treatment of hypertension and their anti-inflammatory and vascular protective effects reduce renal and cardiovascular failure. Inhibition of brain AT1 receptors in humans is also neuroprotective, reducing the incidence of stroke, improving cognition and decreasing the progression of Alzheimer's disease. Blockade of AT1 receptors offers a novel and safe therapeutic approach for the treatment of illnesses of increasing prevalence and socioeconomic impact, such as mood disorders and neurodegenerative diseases of the brain.

NEW INSIGHTS INTO ADAPTATION OF THE ADRENOMEDULLARY SYSTEM TO STRESS

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The role of the adrenal medullary (AM) catecholamines (CA) as key mediators of the response to stress is well established. However, mechanisms mediating transition from acute to chronic responses, associated with stress related disorders, are less clear. Following up on results from microarray and bioinformatics analyses have uncovered important new aspects to the response of the AM to stress. These include: 1. Differences between Epi and NE synthesizing chromaffin cells. The two vesicular monoamine transporters (VMAT) were differentially expressed – VMAT2 only in NE-cells, and VMAT1 in both. Chronic stress altered the distribution, with VMAT2 presence also in Epi- synthesizing chromaffin cells. 2. Indications of enhanced CA storage with repeated immobilization stress (IMO). This includes induction of gene expression for VMAT2, the more efficient VMAT, as well as chromogranin A and chromogranin B, important protein components of neurosecretory vesicles implicated in their biogenesis. 3. Elevated gene expression of CRH related peptides. Enormous rise in urocortin II and CRH transcripts, especially following single IMO, which may regulate CA synthesis and secretion. 4. Changes in expression of renin angiotensin (RAS) /kallikrein-kinin systems, key cardiovascular regulators. We found IMO triggered elevated angiotensinogen and angiotensin converting enzyme transcripts, but AT2R, the major Ang II receptor in AM, is down regulated, especially with acute stress and AT1R unchanged. At the same time mRNA for bradykinin receptor (BK2R) was higher. The findings indicate redistribution and up-regulation of neurosecretory components with repeated stress, which may provide a mechanism to facilitate utilization of the enhanced catecholaminergic capacity. They also suggest involvement of CRH/urocortin and RAS systems in modulating the AM response to stress.

ADENOSINE A1 AND A2A RECEPTORS IN MOUSE ADRENAL GLAND: LOCALIZATION AND CATECHOLAMINE RELEASE MODULATION AFTER CHRONIC STRESS

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Adenosinergic system is a neuromodulatory system able to control the activity of others neurotransmitters and hormones strongly related to stress responses. Rat and bovine adrenal gland express adenosine receptors subtypes (A1, A2A, and A3 receptors) (2;3) However, the role of adenosine receptors and their distribution in mouse adrenal gland are not completely well known. The aim of this work was to study the precise localization of A1 and A2A adenosine receptors in mouse adrenal gland and their role on the modulation of catecholamines release in mouse after a chronic stress. Mice were submitted to Chronic Unpredictable Stress (CUS for 21 days) and exposed to caffeine (1g/L, a non-selective A2A receptor antagonist) or KW6002 (3 mg/kg, a selective A2A receptor antagonist) in the drinking water 4 weeks before the stress and during CUS. The levels of norepinephrine (NE) and epinephrine (EP) in plasma and in adrenal glands were determined by HPLC-ED. By immunohistochemistry adenosine A2A receptors were found in the mouse adrenal while adenosine A1 receptors in the adrenal cortex. CUS enhanced plasma costicosterone levels and decreased the plasma levels of EP and NE and increased their content in the adrenal gland. Caffeine or KW6002 chronic consumption prevented catecholamine increase effects induced by CUS. In conclusion, our results showed that A2A receptor antagonists modulate catecholamine release upon chronic stress, supporting the potential of A2A receptor antagonists to manage modifications induced by chronic stress. (1) Fredholm, BB. Cell Death Differ, 14, 1315-23, 2007.

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PROLACTIN ACTIONS ON CORTICOTROPHIN-RELEASING HORMONE NEURONS IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS DURING LACTATION: LACK OF A DIRECT ACTION INVOLVING THE LONG-FORM OF THE PROLACTIN RECEPTOR

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Prolactin secretion from the anterior pituitary is markedly increased during pregnancy and lactation to co-ordinate important changes in the maternal rat brain. One potential action of prolactin is dampening the responsiveness of the hypothalamic-pituitary-adrenal axis to stress during lactation. We hypothesised that prolactin acts directly on corticotrophin-releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN) through the long-form of the prolactin receptor, the predominant isoform found in the brain. We injected diestrous and lactating rats with 100 ng or 500 ng of ovine prolactin, or vehicle, icv and examined prolactin induced-signalling in the PVN by immunohistochemistry for pSTAT5. We also used dual-label in situ hybridisation to investigate whether CRH neurons express prolactin receptors in the PVN. Nuclear pSTAT5 labelling was increased in the PVN of lactating rats by the 100 ng prolactin dose compared with diestrous rats ($p < 0.05$), although there was no further difference in response to the 500 ng dose ($p > 0.05$). Contrary to our hypothesis, very few (< 5%) pSTAT5-labelled nuclei co-localised with CRH in response to either prolactin dose during diestrus or lactation. This was consistent with in situ hybridisation data indicating that CRH mRNA and prolactin receptor mRNA were not co-expressed in the PVN of diestrous rats. Oxytocin neurons, however, did co-localise with prolactin-induced pSTAT5 in the PVN. Magnocellular oxytocin neurons were more responsive to prolactin during lactation (100 ng, $p < 0.05$ versus diestrus), but the total number of magnocellular oxytocin neurons that responded was the same (500 ng, $p > 0.05$). These data indicate that prolactin does not directly influence CRH neuronal activity through the long-form prolactin receptor. It could potentially exert indirect actions, for example, involving oxytocin.

UNRAVELING THE MOLECULAR AND GENETIC MECHANISMS OF INDIVIDUAL STRESS VULNERABILITY

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Chronic stress is widely regarded as a key risk factor for a variety of diseases, including depression. Genetic predispositions are thought to interact with environmental demands such as chronic stress. However, the molecular mechanisms underlying individual susceptibility or resilience to chronic stress are still poorly understood. Corticotropin-releasing hormone (CRH) and CRH receptor 1 (CRHR1) are key candidate genes for modulating the risk for stress-related disorders. Polymorphisms in the CRHR1 gene have been shown to increase the risk for depression in traumatized individuals. To test the interaction of the CRH-CRHR1 system with individual stress vulnerability, we used two different approaches. First, we utilized animals with a forebrain specific deletion of the CRHR1 gene or with a conditional overexpression of CRH in combination with an exposure to early life stress. We could show that deletion of CRHR1 in forebrain regions was protective for a number of molecular, structural and behavioural consequences of early life stress, while CRH overexpression mimicked the phenotype of early life stress. In a second approach, we identified a mouse single nucleotide polymorphism (SNP) in the CRHR1 gene, which affects individual stress vulnerability. Risk allele carriers of this SNP display an enhanced CRHR1 expression, increased anxiety and a more robust and longer lasting response to chronic social stress exposure. Taken together, our results strongly support the important role of the CRH-CRHR1 system in stress vulnerability and depression.

EFFECTS OF SWIM STRESS AND FLUOXETINE ON 5-HT1A RECEPTOR GENE EXPRESSION AND MONOAMINE METABOLISM IN THE RAT BRAIN

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Expression of serotonin 1A receptors (5-HT1AR) is an object of numerous studies because these receptors are implicated in mood disorders, including depression. Given that stress can precipitate depressive-like symptoms through still not fully understood mechanisms, the aim of this study was to investigate the effects of repeated forced swim (FS) stress and chronic fluoxetine treatment on the brain 5-HT1AR mRNA levels as well as 5-HT and dopamine (DA) metabolism. Stress increased 5-HT1AR mRNA in the brainstem (BS) and frontal cortex (CORT) at 24 h. However, in the CORT both basal and stress 5-HT1AR mRNA levels were significantly reduced by fluoxetine. In the hippocampus (HIPP), stress-induced increase in the receptor expression was prevented by fluoxetine. This effect together with a positive relationship between HIPP 5-HT1AR mRNA levels and immobility in the FS test suggests involvement of these receptors in the mechanism of antidepressant action. Metabolism of 5-HT in the BS decreased by fluoxetine was further reduced by FS at 24 h, showing a certain degree of its independence from the receptor expression that was increased in the BS only after the stress. FS also decreased BS DA metabolism, the level of which unexpectedly positively correlated with the 5-HT1AR mRNA expression in the CORT. This correlation could be provided by catecholaminergic projections to the CORT from BS. The results indicate that mechanisms of stress-induced depression and antidepressant action may involve long-term alterations in the brain 5-HT1AR gene expression, some of which may be interrelated with concomitant changes in 5-HT and DA metabolism. Supported by RFBR N 09-04-00284

THE ROLE OF CARDIAC BETA-ADRENOCEPTOR SYSTEMS IN STRESS

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The actions of the catecholamines in the heart are mediated by, at least, three beta-adrenoceptors subtypes (β-AR) coupled to Gs, and adenilil cyclase (AC) causing positive inotropic, chronotropic and lusitropic effects. β2-AR may also couple to Gi that inhibits AC. The effect of β3-AR vary from stimulation (through cyclic AMP-dependent pathway and L type Ca2+ current activation) to inhibition mediated by Gi/o proteins, NO synthases (NOS) activation and NO production; and depends on the chamber, the amount of endothelial cells, and the NOS activity. The proportion of β-AR subtypes expressed in the heart tissue and their coupling may vary under sympathetic overstimulation and several pathologies. Mice not expressing the LDL receptor (LDLr-/-) show ventricular hypertrophy, supersensitivity to norepinephrine and underexpression of NOS. When fed a high cholesterol diet, these animals also present atherosclerosis and dislypidemia, characterizing a model of metabolic stress. The left atrium of LDLr-/- mice display a contractile deficit due to β2-AR coupling to Gi, since it is eliminated by ICI188,551, a β2-AR antagonist; or Pertussis toxin. In the right atria, both treatment leads to arrhythmia, thus suggesting that β2-AR coupling to Gi is part of an adaptive remodelling of the β-AR population aimed to protect the heart from arrhythmia and from the consequences of the metabolic stress. In the heart of foot-shock stressed rats, β1-AR mRNA and protein were reduced; β2-AR protein was enhanced due to post-transcriptional mechanisms, and β3-AR expression was not altered. eNOS, eNOS-Ser (1177) and iNOS expression were reduced. As a consequence, the atria of those rats were subsensitive to the effects of β1-AR agonists and supersensitive to β2-AR agonists. The physiological meaning of altered NOS expression is still under investigation. Financial Support: CAPES and FAPESP

MOLECULAR BASIS OF CHRONIC STRESS-INDUCED HIPPOCAMPAL LATERAL ASYMMETRY IN RATS

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Right-left asymmetry of human brain function has been known of for a century, although molecular basis of brain laterality has been less known about. The aim of this study was to analyze the effect of social isolation on catecholamine stores and regulation of synthesis and uptake of catecholamines in right and left hippocampus. Therefore, we investigated changes in gene expression and protein levels of tyrosine hydroxylase (TH) and vesicular monoamine transporter 2 (VMAT 2) in right and left hippocampus of socially isolated adult male rats during 12 weeks, by Taqman RT-PCR and Western blot analysis. Asymmetric right-left distribution of the dopamine and norepinephrine content in hippocampus of control rats was absent. Chronic isolation stress reduced norepinephrine content in right hippocampus, but cancelled this right-left asymmetry in dopamine content. The levels of mRNA and protein of TH was increased in left hippocampus of socially isolated rats, whereas gene expression of this enzymes was unchanged in right hippocampus. However, social isolation produced marked elevation of VMAT 2 mRNA and proteins in both right and left hippocampus. Our study has revealed the lateralization of stress regulatory system and demonstrated that long-term isolation stress produced right-left asymmetry of hippocampus norepinephrine and different regulation catecholamines synthesis between right and left hippocampus. Our findings confirm previous report of hippocampal asymmetry in the rat brain and suggest that this might be results of molecular laterization

EXTRASYNAPTIC (VOLUME) TRANSMISSION IN HEALTH AND DISEASE

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Although synaptic transmission is an important means of communication between neurons, neurons themselves and neurones and glia also communicate by extrasynaptic "volume" transmission, which is mediated by diffusion in the extracellular space (ECS). The ECS of the central nervous system (CNS) is the microenvironment of neurons and glial cells. The composition and size of ECS changes dynamically during neuronal activity, physiological states as development, aging, learning, memory formation, stress, lactation as well as during pathological states e.g. ischemia, Alzheimer´s diseade, demyelination diseases, epilepsy and tumors. Following their release, a number of neuroactive substances, including ions, mediators, metabolites, neuropeptides and neurotransmitters, diffuse via the ECS to targets distant from their release sites. Glial cells affect the composition and volume of the ECS and therefore also extracellular diffusion. An increase in diffusion barriers, manifested also as a decrease in both ADCs, due to astrogliosis as well as due to an increase in extracellular matrix e.g. chondroitin sulphate proteoglycans. Measurements in mice deficient for the ECM glycoprotein Tenascin-R revealed not only an increase in ADCs, but also a smaller ECS volume fraction, while APP23 mice with excessive amyloid plaque deposition had a larger ECS volume fraction. The ECM, besides its apparent importance in tissue anisotropy, is therefore important for maintaining a relatively large ECS volume and effective extracellular transmission(Sykova and Nicholson, Physiol. Rev., 2008).

STRESS AND GLUCOCORTICOIDS IMPAIR HIPPOCAMPUS-DEPENDENT EMOTIONAL MEMORY RETRIEVAL VIA GI/O-COUPLED BETA2-ADRENERGIC SIGNALING

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Stress and glucocorticoids impair the retrieval of hippocampus-dependent emotional memory. It has been proposed that glucocorticoids act by promoting the release of norepinephrine (NE), which impairs retrieval by stimulating beta-adrenergic receptors and cAMP signaling. In contrast, evidence indicates that NE, beta1 and cAMP signaling are transiently required for the retrieval of hippocampus-dependent emotional memory. Here we report that glucocorticoids do not require NE to impair retrieval. Despite this, stress- and glucocorticoid-induced impairment of retrieval depends on the activation of beta2 adrenergic receptors (not beta1). Importantly, the effects of stress, glucocorticoids and beta2 agonists are blocked by pertussis toxin, suggesting that they act through Gi/o proteins. In support of this, beta2 signaling in isolation decreases cAMP in the hippocampus, and when combined with beta1 signaling, prevents the increase in cAMP elicited by beta1 receptors. The results demonstrate that beta1 and beta2 receptors can have quite distinct roles in CNS signaling and in cognition.

MICE SELECTED FOR EXTREMES IN STRESS REACTIVITY: MODELLING CLINICALLY RELEVANT ENDOPHENOTYPES OF MAJOR DEPRESSION

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Alterations of the stress hormone system, particularly dysregulation of the HPA axis, play a prominent role in the aetiology of major depression. We therefore aimed to generate a new animal model comprising the neuroendocrine core symptoms of increased or decreased stress reactivity. Utilising a selective breeding approach, three independent mouse lines were established from an outbred population of CD-1 mice according to the outcome of a 'stress reactivity test' (SRT) consisting of a 15-minute restraint period and tail blood samplings immediately before and after exposure to the stressor. Mice showing a very high, an intermediate or a very low secretion of corticosterone in the SRT were selected for the 'high reactivity' (HR), 'intermediate reactivity' (IR) and the 'low reactivity' (LR) breeding line, respectively. Already in the first generation, significant differences in HPA axis reactivity between HR, IR and LR mice were observed. These differences remained stable across all subsequent generations and could be increased by selective inbreeding, indicating a genetically linked trait. In addition to pronounced differences in neuroendocrine functions, extensive characterisation of the three mouse lines revealed effects on emotional behaviours, cognitive functions, sleep measures, neurophysiological parameters and molecular markers, pointing to further similarities with depressed patients. Our results indicate that distinct mechanisms influencing the function and regulation of the HPA axis seem to mediate the respective behavioural and neurobiological endophenotypes. Thus, the generated HR/IR/LR mouse lines are a highly valuable model to elucidate the molecular pathways and genetic underpinnings of altered stress reactivity seen in affective disorders. It is only with deep insight into these mechanisms that novel treatment strategies can be developed in the future.

DOES STRESS-INDUCED RELEASE OF INTERLEUKIN-1 CAUSE LIVER INJURY?

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It is well established that repeated immobilization stress (RIS) is inducing increased levels of cytokines and the emergence of lesions in the liver. Our data prove that interleukin-1 (IL-1) causes liver lesions in stressed Wistar rats. In essence, the relationship between IL-1 and stress-induced liver injury is based on the findings that: 1) IL-1 treatment causes liver inflammation, consisting of infiltration monocytes and appearance of necrosis by increased lipid peroxidation and protein carbonylation. Positive correlation between the content of heptane-soluble Schiff bases and the area of necrosis, as well as between content of carbonylated proteins and an area of necrosis, were found after injection of IL-1 to unstressed rats 2) RIS is accompanied by increased levels of circulating IL-1, corticosterone and lesion of the HPA axis in the dexamethasone test. In the liver, stress causes an emergence of foci of necrosis with perivascular and lobular infiltration of mononuclear cells and increased free radical oxidation. Moreover, there was observed down regulation of CYP450 dependent enzymes and decreased CYP1A1 mRNA content. Positive correlation between the level of circulating IL-1 and necrosis area, as well as between circulating IL-1 and the content of heptane-soluble Schiff bases were observed in stressed rats. 3) Use of IL-1 antagonist Anakinra administration (dose 2mg/kg) before stress prevents infiltration of mononuclear cells and reduces the level of free radical oxidation, as well as necrosis of lesions. 4) The use of glucocorticoid antagonist RU 3486 (dose20 mg / kg) failed to prevent stress-induced liver injury and oxidative stress in the liver. Thus, repeated stresses affect liver injury in vivo via IL-1-dependent mechanism that is associated with high free-radical oxidation level. Supported by RFBR-Ural 10-04-96091

CARDIAC AND VASCULAR MOLECULAR RESPONSES IN EMOTIONAL STRESS-INDUCED ACUTE HEART FAILURE

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Emotional stress triggers acute cardiovascular accidents and heart failure such as Takotsubo cardiomyopathy. However, the etiology and pathophysiology, especially molecular aspects of this syndrome have not been fully investigated. In this study, we investigated the early alterations of cardiac and aortic mRNA expression in the rat exposed immobilization stress (IMO), an animal model of emotional stress-induced takotsubo cardiomyopathy using microarray analysis, followed by re-confirmation with real-time RT-PCR. Expression levels of the identified genes were further estimated by pretreatment with an α1-adrenoceptor blocker and/or a β1-adrenoceptor blocker. In response to IMO, expression of 46 genes was significantly altered in the heart and that of 49 genes was significantly altered in the aorta. Pathway analysis with DAVID Bioinformatics Resources indicated that regulation of transcription and response to endogenous stimulation were the top-two scoring pathways in the heart. Pathway analysis indicated that regulation of transcription were also the top scoring pathways in the aorta. Though precise functional implications of these transcription-related genes in the cardiovascular system are not clearly demonstrated, some of them have been shown to be involved in the adaptive and protective responses to vascular and cardiac insults. Altered expression of cardiac genes was blunted by pretreatment with a β1-adrenoceptor blocker or α1+β1-adrenoceptor blockers. In contrast, that of aortic genes was blunted by pretreatment with an α1-adrenoceptor blocker or α1+β1-adrenoceptor blockers. Activation of α1-adrenoceptor in the blood vessels or activation of β1-adrenoceptors in the heart were mainly responsible for emotional stress-induced alteration of cardiac and vascular genes profiles.

CENTRAL STRESS RESPONSES TO ANGIOTENSIN AND SUBSTANCE P: WHERE IS THE PATHWAY TO HYPERTENSION ?

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Stress, however defined, has long been accused to represent one of the main contributers to arterial hypertension. Bjorn Folkow stated already decades ago that repeated sympathetically mediated hypertensive stress responses to unpleasant stimuli will lead to changes in the vasculature, manifesting high blood pressure disease. We investigated two centrally acting peptides, angiotensin II and substance P, in central stress responses and their potential role in establishing hypertension. We have demonstrated that stimulation of central neurokinin receptors by intracerebroventricular (icv) substance P induces a classical defense reaction with marked pressor- and tachycardic responses mediated by a generalized sympathetic stimulation and corresponding changes in splanchnic-, renal- and peripheral muscle blood flow. We could further show that the sympathetic stress response to unpleasant (pain) stimuli involves substance P receptors in the forebrain and oxytocinergic pathways in the hypothalamic paraventricular nucleus. In contrast, the central pressor responses to icv angiotensin II were characterized by initial reductions of heart rate as well as splanchnic-, renal- and adrenal nerve activity and plasma noradrenaline, but by a stimulation of the neuro-endocrine axis involving ACTH and vasopressin. Our findings suggest that stress-induced mechanisms potentially leading to hypertension involve central tachykinin receptor stimulation and brain oxytocin as a mediator, whereas angiotensin is not directly involved in these classical stress responses, contributing to hypertension via different mechanisms. The clinical relevance of these findings I highlighted by most recent successful trials in patients with drug-resistant hypertension in whom renal sympathetic nerve ablation has been shown to persistently lower blood pressure.

SEX DIFFERENCES IN SENSE OF DIRECTION AT THE LEVEL OF THE CELL

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Affective disorders are more prevalent in women than in men. Dysfunctions of the stress neuromediator, corticotropin-releasing factor (CRF), and the locus coeurleus (LC)-norepinephrine system, a target of CRF, have both been implicated in these disorders. Here we describe sex differences in CRF receptor (CRFr) signaling, CRFr trafficking and LC dendritic structure that may underlie the sex bias in mood disorders. In rats, immunoprecipitation studies revealed greater CRFr coupling to its signaling molecule, Gs, in females compared to males. This translated to increased LC activation by CRF. CRFr association to β-arrestin, a step necessary for CRFr internalization, was less in females, resulting in failure to internalize CRFr after acute stress. Similarly, in CRF-transgenic mice that overexpress CRF, CRFr was internalized in males, but not females and this was associated with elevated LC firing rates in female mice. Importantly, sex differences in CRFr signaling and trafficking render LC neurons of females more sensitive to low levels of CRF and less adaptable to high levels of CRF. In addition to affecting LC neuronal activity, CRF can increase LC dendritic length. Morphological studies demonstrated that LC dendrites of female rats were longer and more complex, extending further into the peri-LC compared to those of male rats. This structural feature would increase the probability of contacting limbic afferents, some of which contain CRF, that convey emotion-related information to the LC. Thus, sex differences in structure allow LC neurons of female rats to receive and process more emotion-related information. As excessive LC activity is thought to underlie hyperarousal and attentional impairments of mood disorders, the present findings provide novel molecular and cellular mechanisms that may contribute to sex differences in affective arousal and mood disorders.

TRANSCRIPTOME ANALYSIS OF THE LEFT CARDIAC ATRIUM AND VENTRICLE IN RATS CHRONICALY EXPOSED TO COLD AS HOMOTYPIC OR HETEROTYPIC STRESSOR

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Chronic or intermittent activation of sympathoadrenal system (by cold, exercise, emotional stressors) results in pathophysiological changes in the heart leading to hypertension, hypertrophy or other cardiac disorders. In this study, exon expression analysis (Affymetrix Rat GeneChip 1.0 ST) was applied to elucidate global changes of transcriptome in left cardiac atrium and ventricle of rats exposed to 1 or 7 day cold with/without prior 7 day exposure to repeated immobilization stress (2h daily, IMO). In the left atrium, 1d and particularly 7d cold upregulated ($P < 0.05$, fold change ≥1.5): prostaglandin synthesis, cell cycle, inflammatory response, while downregulated: calcium regulation and myometrial relaxation and contraction pathway. In the left ventricle, 1d (more intensively than 7d) cold upregulated: prostaglandin synthesis, p38 MAPK signaling, TGF-β receptor signaling, TNFα and NFκB signalling and downregulated: LC-fatty acid oxidation, oxoacid metabolic processes, tryptophan and retinol metabolism processes. Cold-induced changes observed were related to hypertension (angiotensin system, nitric oxide, endothelin-1, natriuretic peptides) or cardiac hypertrophy (myc-1, trh, tgfβ 1, fgf2, Hmox1). Prior exposure of rats to repeated IMO potentiated (apoptosis, hypertension and hypertrophy-related genes, catabolic processes) or reduced (glycerophospholipid metabolism, endocytosis, β1-adrenergic receptor) many cold-regulated genes in ventricle. In the atrium, only few transcripts were potentiated (circadian genes), while the majority of transcripts (cell cycle, striated muscle contraction, matrix metalloproteinases) were reduced. This suggests that exposure to the repeated emotional stress prior to cold exposure could potentiate pathological changes related to hypertension and cardiac hypertrophy in left ventricle. Supported by VEGA 2/0036/11, 2/0188/09, APVV 0088-10.

AUTONOMIC NERVOUS SYSTEM FUNCTION IN RHEUMATOID ARTHRITIS

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Introduction: The autonomic nervous system (ANS) is an important regulatory system participating in maintaining of homeostasis. The sympathetic branch of the ANS is also involved in regulation of immune system and inflammation. Thus, impairments of the sympathetic nervous system have been suspected in the pathogenesis of rheumatoid arthritis (RA). The aim of our study was to evaluate sympathoneural and adrenomedullary function in premenopausal women with RA. Methods: The insulin-induced hypoglycemia (0.1 IU/kg) was performed in 15 glucocorticoid-naive patients with long term RA and low disease activity and in 14 healthy women matched for age and body mass index. The orthostatic test (10 minutes of 60 degrees passive head-up tilt) was performed in 11 glucocorticoid-naive and 11 glucocorticoid-treated patients with long term RA with low/moderate disease activity and in 15 healthy women matched for age and body mass index. Concentrations of glucose, epinephrine (EPI), norepinephrine (NE) were analyzed in plasma. Results: RA patients had comparable responses of glucose, and lower EPI ($p = 0.005$) and NE (p 0.001) responses to the insulin-induced hypoglycemia. Orthostasis induced increase in EPI and NE without differences between patients and controls, only with a small trend for higher baseline NE levels in glucocorticoid-treated patients ($p=0.08$). Conclusions: Significantly lower responses of EPI and NE to hypoglycemia may suggest sympathoadrenal hyporeactivity in patients with RA. Orthostasis, which mainly activates sympathoneural response, suggest normal reactivity of the sympathetic nervous system in RA patients. Our results indicated stressor-specific alteration of the sympathetic nervous system affecting predominantly adrenomedullary, but not sympathoneural function.

AGING IN PATIENTS WITH POSTTRAUMATIC STRESS DISORDER

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Objective: Post-Traumatic Stress Disorder (PTSD) develops after exposure to particularly traumatic events. The present study was carried out to find out whether PTSD, resulting from deportation to Siberia in the patients‘ childhood (from 1940 to 1946), has any association on the physical disability, cognition function impairment and depression of these persons in advanced age. Methods: 80 patients with PTSD and 70 subjects without PTSD followed up in primary care setting were enrolled in the study. PTSD was diagnosed according to the DSM-IV criteria. All patients were subject to a standardized interview including demographic data, Activities of Daily Living (ADL), Instrumental Activities of Daily Living (IADL), modified Mini-Mental State Examination (MMSE), Geriatric Depression Scale (GDS) questionnaires. The results were compared applying t-test and Chi2 test. Results: Parameter PTSD (+) PTSD (-) n=80 n=70 Age [years] 69,3 ± 5,9 70,8 ± 4,9 Men [%] 50,0 50,0 modified MMSE mean ± SD [pts]25,6 ± 3,7 26,8 ± 2,0 normal [%] 37,5 34,3 mild cognitive impairment [%]38,8*** 62,9 dementia [%] 23,8*** 2,9 GDS mean ± SD [pts] 10,9 ± 3,5** 4,43 ± 2,8 no depression [%] 11,3*** 68,6 mild depression [%] 25,0 30,0 severe depression [%] 63,8*** 1,4 ADL mean ± SD [pts] 5,12 ± 1,23 5,94 ± 0,2 normal [%] 78,8*** 100 mild disability [%] 17,5*** 0 severe disability [%] 3,8*** 0 IADL mean±SD [pts.] 21,6 ± 3,96* 26,2 ± 1,33, *= p less than 0,05, ***= p less than 0,001. Conclusions: Several-year long deportation in childhood was associated with severe trauma and development of PTSD. Cognitive function impairment, higher freqency of depression and physical disability were found in the group of former deportees compared to the group of persons without history of such traumatic experience.

CHEMICAL NEUROANATOMY OF SYMPATHETIC NEURONS AND CHROMAFFIN CELLS: IMPLICATIONS FOR SPECIES-SPECIFIC STRESS TRANSDUCTION?

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Environmental and metabolic signals leading to activation of the autonomic nervous system are transmitted to heart, blood vessels, sweat glands and other end organs via post-ganglionic sympathetic neurons, and to the adrenal medulla via the splanchnic nerve. Post-ganglionic sympathetic neurons and adrenal chromaffin cells are differentially stimulated for secretion by cold, immobilization, and hypoglycemia. We and others have investigated the chemical neuroanatomy of these two sympathetic circuits in several mammalian species: some surprising findings about the nature of the transmitters released by each system have emerged. Sweating is generally considered to be cholinergically mediated because sympathetic post-ganglionic projections to rodent sweat glands produce and release acetylcholine rather than norepinephrine. However, it is now known that human eccrine sweat glands like human arteriovenous anastomoses are innervated by sympathetic neurons that co-express cholinergic and noradrenergic biosynthetic enzymes and vesicular transporters (Weihe et al., J. Comp. Neurol. 492:370, 2005). Furthermore, a major subpopulation of intrinsic human cardiac neurones codes for a cholinergic/noradrenerg cophenotype. In rodents, sympathetic neurons express the vesicular transporter VMAT2, while chromaffin cells of the adrenal medulla express VMAT1. However, during immobilization stress, VMAT2 is expressed in rat PNMT-negative chromaffin cells (Tillinger et al., Cell. Mol. Neurobiol. 30:1459, 2010), increasing the potential for a more norepinephrine-biased hormonal response. In contrast, human chromaffin cells co-express both VMAT1 and VMAT2 regardless of their PNMT phenotype. This suggests a need to re-evaluate translational expectations about rodent models for human stress responses, and re-assess therapeutic strategies for stress management on which they are based.

STRESS CARDIOMYOPATHY: A SYNDROME OF CATECHOLAMINE MEDIATED MYOCARDIAL STUNNING

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Stress cardiomyopathy (SCM) is a syndrome of transient left ventricular dysfunction precipitated by acute emotional or physiologic stress. While the clinical features of SCM have become increasingly well recognized during the past several years, the precise pathophysiologic mechanism of stress induced myocardial stunning remains unknown. There is increasing evidence that exaggerated sympathetic stimulation may be central to the pathogenesis of SCM. Plasma catecholamine levels have been found to be markedly elevated in some patients with this syndrome, and SCM has been observed in other clinical states of catecholamine excess, such as pheochromocytoma and central neurologic injury. Further, intravenous catecholamines and beta-agonists can precipitate SCM in humans and can reproduce apical ballooning in animal models. The precise mechanism in which excessive sympathetic stimulation may result in transient left ventricular dysfunction remains controversial. Abnormal myocardial blood flow due to sympathetically mediated microvascular dysfunction has been suggested, and this is supported by the decrease in coronary flow reserve observed during the acute phase of the syndrome, despite the absence of obstructive epicardial disease. Catecholamines may alternatively have a direct effect on cardiac myocyte contractility, possibly through cyclic AMP mediated calcium overload. The evidence supporting these and other potential mechanisms of SCM will be reviewed. Further, psychological, hormonal, and genetic risk factors that may increase susceptibility to catecholamine mediated myocardial dysfunction in patients with SCM will be highlighted.

CHRONIC STRESS AND ADRENERGIC DRIVE: IMPLICATIONS FOR ILLNESS

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Epinephrine (epi) is associated with short-term stress and initiates physiological and behavioral changes to counter stress and ensure survival. The epi-producing enzyme, phenylethanolamine N-methyltransferase (PNMT, EC.2.1.1.28), is markedly altered by chronic, repeated stress. Changes appear mediated by Hypoxia Inducible Factor 1α (HIF1α). While HIF1α binding elements exist in the PNMT gene promoter, they appear unresponsive to stress despite a marked rise in HIF1α. HIF1α seems to work as "on-off switch", inducing Egr-1 and Sp1 to stimulate PNMT mRNA and enzyme. HIF1α is activated by hypoxic stress, immobilization stress (IMMO) and PACAP. Stress and PACAP have been linked to depression, bipolar disorder, post-traumatic stress disorder (PTSD) and schizophrenia. Epi is produced in adrenal medulla and brainstem neurons (C1, C2 and C3 nuclei). Via spinal intermediolateral projections, it activates vagal noradrenergic afferents to stimulate basal lateral amygdala. Brainstem adrenergic neurons send caudal projections to forebrain and midbrain, which may modulate activity of prefrontal cortex, striatum and locus coerulus. To show that stress-induced adrenergic drive contributes to long-term illness, Fear Potentiated Startle (FPS, test of stress/anxiety) was used to assess IMMO effects on behavior and adrenergic indices. Fear extinction was delayed and adrenergic indices markedly elevated.

INVOLVEMENT OF CANNABINOIDS IN FUNCTIONING OF THE NON-PREGANGLIONIC EDINGER-WESTPHAL UROCORTIN 1 SYSTEM

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The non-preganglionic Edinger-Westphal nucleus (npEW) in the midbrain, main site of urocortin-1 (Ucn1) synthesis, plays an important role in stress and related psychiatric diseases in sex-specific manner. Because the endocannabinoid signaling has also been heavily implicated in these pathological conditions, we hypothesized that endocannabinoids would modulate the functions of the npEW during acute and chronic stress. First, we showed that the CB1 receptor mRNA was present in the npEW. Acute restraint (1h) and even more the chronic mild stress (2 weeks) resulted in Ucn1 elevation in npEW. These effects were smaller in cannabinoid receptor 1-knockout mice (CB1-/-) in a sex-dependent manner. Specifically, the lack of CB1 receptor diminished the stressor-induced changes more expressly in males than in females. With respect to stress-induced physiological parameters, we found that basal stress-hormone levels were higher in CB1-/- compared to wild-types, and the lack of CB1 receptor resulted in more pronounced stress-induced changes in males vs. females. Collectively, our results show for the first time that a subset of npEW neurons is sensitive to endocannabinoids. We also provide novel evidence that the lack of endocannabinoid signaling interferes with the animal's stress response, concomitant with alterations in the expression of Ucn1 in npEW neurons. Finally, we show that the above actions of endocannabinoid are sex-dependent.

ENDOGENOUS ADRENALINE PROTECTS AGAINST HYPERTENSION AND DIABETES

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Adrenaline is synthesized by the enzyme PNMT and we have previously reported that only half of PNMT is in the adrenal medullae. We created a C57BL/6 mouse in which the PNMT promoter was linked to Cre rather than to PNMT exons. We crossed this mouse with mice that activate green fluorescent protein (GFP) and found that about 10% of adult cardiac myocytes isolated from the left ventricle expressed GFP. The cardiac myocytes contained mRNA of the catecholamine biosynthetic enzymes aromatic-L-amino-acid decarboxylase, dopamine β-hydroxylase, and PNMT, but not tyrosine hydroxylase. When these mice are homozygous, they lack adrenaline (PNMT-/-). They had normal resting heart rate and blood pressure (BP) by telemetry. Their basal plasma norepinephrine level was normal. Exercise caused an 11% greater increase in the BP of PNMT-/- mice. During stress, echocardiography revealed decreased cardiac filling and a 23% reduction in their cardiac output, suggesting that the blood pressure increase was due to increased peripheral vascular resistance. C57BL/6 mice are prone to overfeeding induced type 2 diabetes. When C57BL/6 mice lacking adrenaline were fed a normal diet containing 14% fat they had glucose tolerance similar to control animals and were slightly, but not significantly more sensitive to insulin. On a 40.6% fat diet they gained slightly more weight than control animals, and had similar overall fat and muscle mass by DEXA measurement. The PNMT-/- mice on a high fat diet became diabetic and had a greater blood glucose response to a glucose tolerance test than control animals ($p = 0.0002$). They were also resistant to the glucose lowering effects of insulin ($p< 0.05$). We conclude that endogenous adrenaline improves cardiac output, decreases peripheral vascular resistance and protects against both exercise induced hypertension and overfeeding induced diabetes.

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