Pharmacological Reports
2011, 63, 1393–1403
ISSN 1734-1140

Effect of prior stress on interleukin-1β and HPA axis responses to acute stress

Anna Gądek-Michalska, Joanna Tadeusz, Paulina Rachwalska, Jadwiga Spyrka, Jan Bugajski

Department of Physiology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Correspondence: Anna Gądek-Michalska, e-mail: gadek@if-pan.krakow.pl

Abstract:
Interleukin-1β (IL-1β) level is modulated during multiple stress reactions both in brain structures involved in hypothalamic-pituitary-adrenal (HPA) axis regulation and peripheral systems. Multiple distinct stressors induce different IL-1β and HPA axis responses. The purpose of the present study was to determine if the effect of prior repeated restraint stress on IL-1β levels in prefrontal cortex, hippocampus, hypothalamus and plasma may have an impact on alterations induced in HPA axis responses. Experiments were performed on male Wistar rats which were exposed to 10 min restraint stress twice a day for 3 days. Twenty four hours after the last stress period rats were restrained for 10 min and decapitated at 0, 1, 2 or 3 h after cessation of stress. Control rats were injected *ip* with saline and some of experimental groups with IL-1β receptor antagonist (IL-1ra). After rapid decapitation, trunk blood was collected and prefrontal cortex, hippocampus and hypothalamus were excised and frozen. Interleukin-1β, adrenocorticotropic hormone (ACTH) and corticosterone (CORT) levels were determined in plasma using commercially available kits and IL-1β levels in brain structures samples were analyzed by western blot procedure. Repeated restraint for 3 days alone did not alter resting plasma levels of IL-1β, and moderately augmented plasma ACTH and CORT levels and IL-1β content in brain structures 24 h after the last restraint. IL-1β antagonist abolished the increase in plasma levels of IL-1β, ACTH and CORT as well as IL-1β in brain structures in response to repeated stress and also reduced these changes induced by 10 min stress. This suggests the selectivity of IL-1β receptors in central and peripheral mechanisms modulating the stress-induced HPA axis responses. These results indicate that repeated stress markedly increases IL-1β production in brain structures involved in HPA axis regulation. The present results support the role of brain and peripheral IL-1β in adaptation of HPA response during prolonged stress.

Key words:
Interleukin-1β, limbic-hypothalamic-adrenal axis, IL-1β in stress responses, immuno-endocrine responses, ACTH, corticosterone

Introduction

Pro-inflammatory cytokine interleukin 1β is an endogenous signaling molecule in the brain and the first cytokine associated with modulation of the hypothalamic-pituitary-adrenal (HPA) axis. During stimulation by different stressors IL-1β interacts with neurotransmitter and neuropeptide regulatory systems [31, 35–37]. This cytokine is a critical mediator of adaptive stress response and stress associated psycho- and neuropathology [11, 12, 24].

The brain cytokines network can interact with their receptors in regions involved in HPA axis regulation, including the hypothalamus paraventricular nucleus (PVN) and the hippocampus, which provides negative feedback regulation on PVN activity [20, 21]. Brain regions involved in signaling the message of circulating cytokine to the PVN express cytokine receptors.

Interleukin-1β significantly modulates central synaptic transmission and is modulated by stress [26, 27]. In well established potent stress-induced activation of the HPA axis, interleukins can act at the level of the hypothalamus, to induce expression and release of corticotropin-releasing hormone (CRH), and at the pituitary to release ACTH or through both of these mechanisms [20]. Interleukins can also directly stimulate the adrenal gland [8] to stimulate glucocorticoid release.

Cytokines are too large to readily pass the blood-brain barrier (BBB) to activate CRH-producing neurons in the PVN [1, 4, 7]. Cytokines could act on the brain regions that lack a functional BBB, the circumventricular organs (CVO's), such as the median eminence, organum vasculosum laminae terminalis (OVLT), or the area postrema (AP) [22]. This structure has a close relationship with the nucleus of the tractus solitarius (NTS), and dorsal motor nucleus of the vagus nerve (DMX), plays a role in controlling the entry of blood-borne substances to neurons of brainstem and may convey IL-1-mediated signals [30, 34, 37]. IL-1 in the general circulation may also act directly on CRH-containing terminals in the median eminence to initiate HPA axis activation [37]. Local application of IL-1 in the median eminence elevates plasma ACTH and corticosterone in rats.

Interleukin-1 may stimulate perivascular cells in the medulla oblongata, in which IL-1 receptors are abundantly expressed [34], and these perivascular cells may activate ascending aminergic neurons to eventually stimulate CRH neurons. Induction of cyclooxygenase (COX)-2 expression by IL-1 in perivascular cells may mediate IL-1 effects in the hypothalamus and medulla and the HPA axis [14].

An important role in cytokine to brain communication and endocrine hypothalamus stimulation have afferent vagal system [6, 10] and visceral sensory inputs [3, 29]. Interleukin 1β-induced corticosterone secretion accompanied by hypothalamic noradrenaline depletion is vagally mediated. Vagotomy can also block the induction of IL-1β mRNA in the brain of rats in response to systemic IL-1β. Electrical stimulation of afferent vagus nerve induces IL-1β expression in the brain and stimulates HPA axis [15]. Subdiaphragmatic vagotomy affects dorsal motor nucleus of the vagus and inhibits intra-abdominal stimulation of ACTH secretion [18–20]. Interleukin-1 binding sites are present in peripheral afferent vagus nerve fibers that relay cytokine signals to nucleus of the solitary tract and hypothalamus [28] and in vagus nerve paraganglia, which may be stimulated by circulating endogenous or exogenously administered IL-1β and signal transmitted by afferents of vagus nerve to its respective brain regions [5]. The inflammatory signaling molecule nuclear factor kappa B (NF-κB), a primary transcription factor in the initiation of the inflammatory response is an essential mediator at the blood-brain barrier interface that communicates peripheral inflammatory signals to the central nervous system (CNS).

It is unlikely that blood-borne IL-1 contributes to the majority of IL-1 detected in the hypothalamus. Since the hypothalamus IL-1 is produced in neurons, microglia, and astrocytes [38] and the bioactive IL-1 was present in brain under non-pathological conditions [9]. Also *icv* IL-1 causes c-fos expression in CRH-producing parvocellular neurons in the PVN, indicating that stress-induced intrinsic IL-1β production in the PVN evidently contributes to the activation of HPA axis. The potential relationship between stress and brain IL-1β has not been elucidated [25].

Pro-inflammatory cytokines often sensitize various neuronal, hormonal, and behavioral responses to subsequent stimulation [23, 24]. Single administration of IL-1 increased CRH mRNA in the hypothalamic PVN which paralleled long-lasting sensitization to emotional stress [32]. HPA axis responsiveness to IL-1β undergoes individual variation. Prior stressor exposure enhances peripheral and central pro-inflammatory cytokine and HPA axis responses to subsequent immune challenge [16, 17]. Stressful life events can sensitize various neuronal and hormonal responses, sympathetic nervous system activation and hypothalamic-pituitary-adrenal responses. Central and systemic administration of IL-1β increased release of ACTH and corticosterone upon subsequent IL-1β stimulation 11–22 days later [34]. Elevated levels of central IL-1β during stress exposure are necessary for pro-inflammatory cytokine sensitization. Systemic administration of human recombinant interleukin-1 beta (hrIL-1β) in animals under basal conditions results in sensitization of IL-1β responses in limbic structures, hypothalamus, hippocampus, and cortex involved in HPA axis activity to subsequent cytokine challenge. Systemic and central IL-1β administration augments the response to stressors or further cytokine exposure and IL-1ra attenuated the effects of the stressor [15, 17]. Stressors, and IL-1 increased time-dependently co-expression of CRH and AVP within the external zone of the median eminence which syn-

ergistically stimulated ACTH secretion from the anterior pituitary [33]. Thus exposure to stressful events may sensitize animals to greater vulnerability to stressor related pathology.

The purpose of the present study was to determine if the changes in IL-1β levels in plasma and prefrontal cortex, hippocampus and hypothalamus, reflect the alterations in HPA axis activity expressed by ACTH and corticosterone secretion induced by acute restraint stress. The further purpose was to examine if the effect of prior repeated restraint stress for 3 days affects the plasma and brain IL-1β and ACTH and corticosterone response to acute restraint stress.

Materials and Methods

Experiments were performed on male Wistar rats (6 weeks old, 190–220 g). The animals were housed in groups of 5 per cage (52 × 32 × 20 cm) under controlled conditions (12 h light/dark cycle; a constant temperature 22 ± 2°C) with free access to a standard laboratory diet and tap water. The rats were allowed 1 week of habituation to the animal room before the onset experimentation. Experiments were performed on 4 groups of rats in which the changes in plasma levels of IL-1β, ACTH and corticosterone were determined and IL-1β content in brain structures, prefrontal cortex, hippocampus and hypothalamus involved in HPA axis regulation were measured.

In the first group, the effect of restraint stress for 10 min on the above parameters was determined immediately after restraint termination and 1, 2 and 3 h later. The restraint was performed using metal tubes (length = 16 cm; inside diameter = 4.5 cm) with ample holes for ventilation. In the second group, the effect of prior 10 min restraint twice a day at 8 a.m. and 4 p.m. repeated for 3 consecutive days on 10 min restraint stress 24 h after the last restraint on the above listed parameters was examined. In the third group, the effect of IL-1ra 50 μg/kg given 15 min earlier on 10 min restraint stress induced IL-1β, ACTH and corticosterone responses were investigated. In the fourth group, the influence of prior repeated restraint stress for 3 days and IL-1ra on the 10 min restraint stress induced responses were determined. Respective control group was examined with each of experimental group. All experimental protocols were approved by the Local Bioethics Commission of the Institute of Pharmacology, Polish Academy of Sciences in Kraków and met internationally accepted principles for the care and use of laboratory animals.

Blood and tissue collection

Animals were sacrificed by rapid decapitation 0 h, 1 h, 2 h or 3 h after restraint. Trunk blood for plasma determinations was collected in the presence of EDTA (10% w/v; Merck; 25 μl/ml of blood) and for ACTH immunoassay in the presence of EDTA and aprotinin (0.6 TIU/ml of blood; Sigma-Aldrich). After decapitation, the brain was removed from the skull and three structures (prefrontal cortex, hippocampus, hypothalamus) were excised on a cold plate and snap frozen at –70°C until assayed.

Plasma hormones and IL-1β measurement

Total corticosterone, ACTH and IL-1β levels were measured in plasma obtained from blood by centrifugation within 30 min after collection. There were used commercially available Rat/Mouse Corticoterone Enzyme Immuno Assay (EIA) kit (Immunodiagnostic Systems), ACTH Rat/Mouse EIA kit (Phoenix Pharmaceuticals) and Rat IL-1β enzyme-linked immunosorbent assay (ELISA) kit (BioVendor).

Preparation of protein extracts

Proteins were extracted from frozen tissues by homogenization (Ultra-turax, 10,000 rpm) in a solution of Radio-Immunoprecipitation Assay (RIPA) buffer (Sigma-Aldrich) and freshly added Protease Inhibitor Cocktail, Phosphatase Inhibitor Cocktail 2 and 3 (1:100, Sigma-Aldrich), then centrifuged at 14,000 rpm for 20 min at 4°C and supernatant collected. After determining of protein levels using BCA™ Protein Assay Kit (Thermo Scientific) and adjusting concentrations, homogenates were mixed 1:1 with Laemmli sample buffer (Bio-Rad) and β-mercapthoethanol (50 μl per 950 ml of Laemmli; Sigma-Aldrich) and boiled for 5 min.

Sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) and western blot

Denaturated proteins (30 μg per lane for hippocampus samples, 40 μg for prefrontal cortex, 25 μg for hypo-

thalamus) were separated on a 7.5% SDS-polyacrylamide gel (90 V in stacking gel; 150 V in resolving gel) and electrotransferred (90 V, 1 h) onto a nitrocellulose membrane (0.45 μm, Bio-Rad). The membranes were blocked with 5% nonfat milk (Bio-Rad) in Tris-buffered saline (TBS, pH 7.5) containing 0.05% Tween20 (Sigma-Aldrich) for 1 h at room temperature, and then incubated overnight at 4°C with anti-IL-1β antibody (1:750, Thermo Scientific) in 1% non-fat dry milk in TBS with 0.05% Tween20. The membranes were then washed four times for 10 min with TBST (TBS-0.1% Tween20) and finally incubated with the respective horseradish peroxidase-conjugated secondary antibody (1:10,000 in TBS-0.05% Tween20) for 1 h at room temperature. After washing the membranes four times for 15 min with TBST, the proteins were detected using Immun-Star HRP Chemiluminescence Kit (Bio-Rad) and visualized by a computerized video-densitometry (FujiLas 1000 Imager). The optical density of appropriate bands was quantified by densitometric analysis of blots using Image Gauge V4.0 Software (Fujifilm). Results were normalized to β-actin and the values are expressed as a percentage of controls.

Statistical analysis

Data in text and figures are expressed as the mean ± SEM (n = 6–8 rats per group). For multiple comparisons, a one-way ANOVA followed by the Dunnett's *post-hoc* test to compare means of all groups to controls was made. Differences with p value < 0.05 was considered statistically significant.

Results

Effect of prior repeated restraint stress on acute restraint-induced plasma IL-1β, ACTH and corticosterone levels

Restraint stress for 10 min considerably increased plasma IL-1β level immediately after termination of restraint and more strongly 1 h later, whereas no substantial increase was observed 2 or 3 h after stressor termination. Likewise, the increase in plasma ACTH and corticosterone levels were most potent immediately after cessation of restraint and were absent 1, 2 and 3 h later. (Fig. 1A, B, C). These results show a rapid and strong increase of plasma IL-1β, ACTH and corticosterone levels induced by acute short lasting restraint stress.

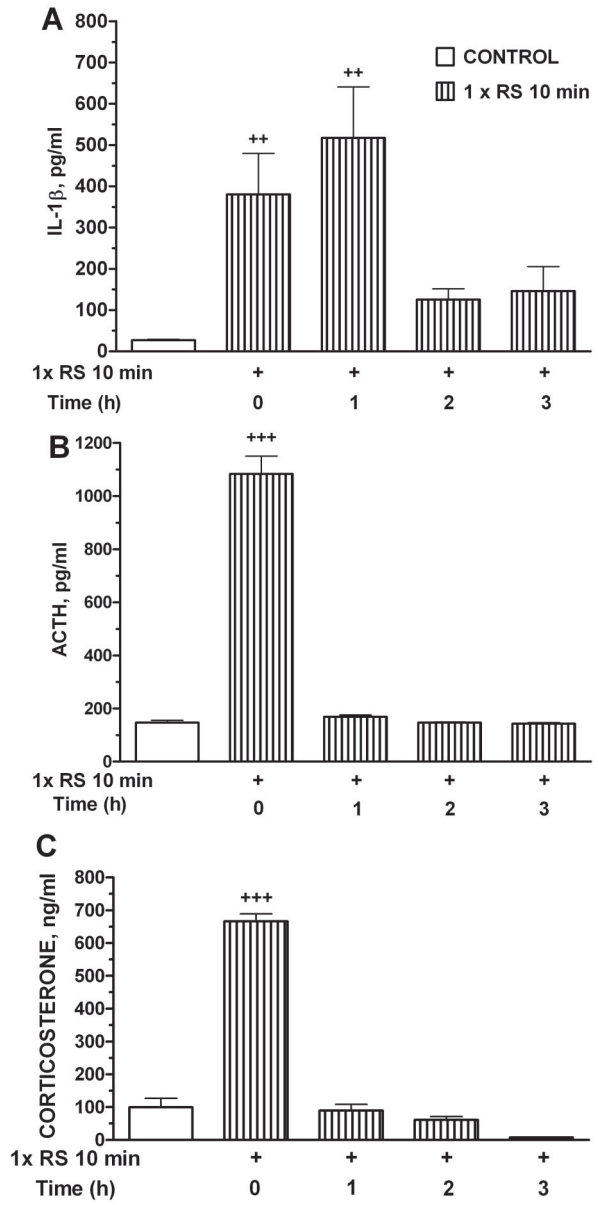

Fig. 1. Effect of restraint stress on plasma levels of interleukin-1β (IL-1β) (**A**), ACTH (**B**) and corticosterone (**C**). Rats were restrained in metal tubes for 10 min and decapitated at the termination of restraint 0, and 1, 2 and 3 h later. In Figs. 1–7, 4–6 rats per group were used. $^{+}$ $p < 0.05$, $^{++}$ $p < 0.01$ and $^{+++}$ $p < 0.001$ *vs.* saline control group

Since prior stressor exposure may alter peripheral and central pro-inflammatory cytokine levels and HPA axis response to subsequent stimulation, we in-

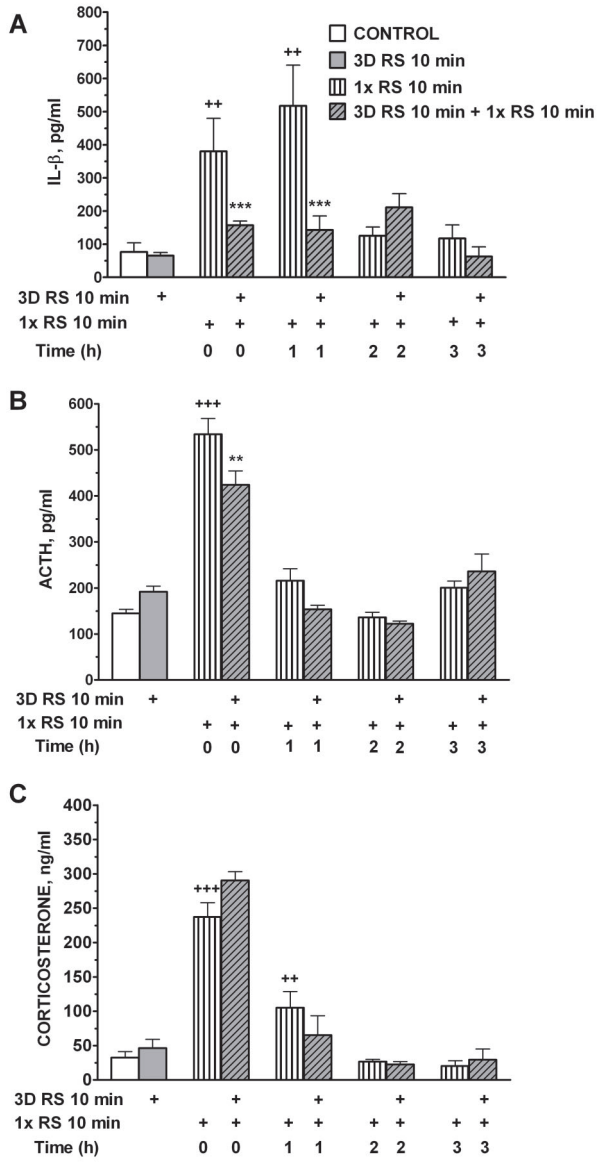

Fig. 2. Effect of prior repeated restraint 2 × 10 min per day for 3 days on 10 min restraint stress-induced plasma IL-1β (**A**), ACTH (**B**) and corticosterone (**C**) levels. In Figures 2, 4, 6, 7 rats were restrained 24 h after the last prior restraint. ** $p < 0.05$, *** $p < 0.001$ *vs.* group restrained for 10 min. See legend to Figure 1

vestigated weather and how prior repeated restraint stress (2 × 10 min/day for 3 days) influences plasma IL-1β levels and HPA axis responses to subsequent 10 min restraint stress applied 24 h after the last restraint. Repeated stress alone (2 × 10 min/day for 3 days) induced a moderate increase in plasma ACTH and corticosterone levels, measured 24 h after the last restraint compared to the corresponding levels in control rats without prior stress exposure.

Prior, repeated restraint significantly reduced the plasma IL-1β response to 10 min restraint immediately after termination of stress and 1–3 h later (Fig. 2A). Prior repeated stress also markedly impaired the potent increase of plasma ACTH level at the end and

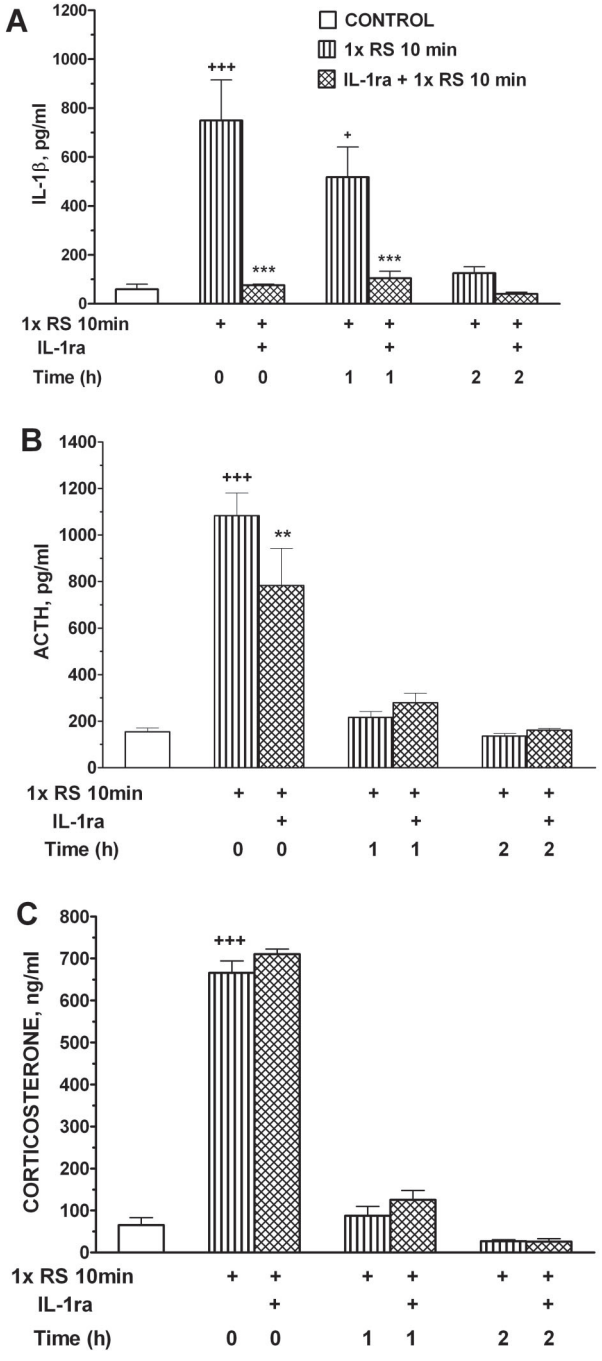

Fig. 3. Influence of IL-1β receptor antagonist (IL-1ra) given *ip* 15 min earlier on 10 min restraint stress-induced plasma levels of IL-1β (**A**), ACTH (**B**) and corticosterone (**C**). See legend to Figure 1

1 h after termination of 10 min restraint stress (Fig. 2B). Prior stress did not diminish but substantially enhanced the 10 min restraint-induced corticosterone response immediately after terminating of restraint period and markedly impaired this response 1 h later compared with control levels (Fig. 2C).

Influence of IL-1β receptor antagonist on stress-induced plasma IL-1β, ACTH and corticosterone levels

Functional specificity of IL-1β in the stimulation of HPA axis by acute restraint stress was verified by pretreatment of rats with antagonist of its receptor (IL-1ra) 15 min before stress exposure. Interleukin-1β receptor antagonist 5 μg/kg administered *ip* 15 min earlier totally abolished the 10 min restraint-induced potent increase in plasma IL-1β level immediately after stress termination and 1 and 2 h later (Fig. 3A). Interleukin-1β receptor antagonist also significantly diminished the 10 min RS-induced ACTH response immediately after cessation of restraint (Fig. 3B). Significantly increased corticosterone response at the end of 10 min RS period was not altered by pretreatment of rats with IL-1β receptor antagonist (Fig. 3C). These results show that selective functional antagonist of IL-1β receptor totally abolishes the acute restraint stress-induced plasma IL-1β response, significantly diminishes ACTH response and does not alter corticosterone response immediately after restraint termination when the increase of plasma IL-1β, ACTH and corticosterone were most strongly manifested.

Effect of IL-1β receptor antagonist on restraint-induced plasma IL-1β, ACTH and corticosterone response after prior stress

In rats exposed to prior repeated stress IL-1ra reduced the 10 min-restraint stress-evoked increase in plasma IL-1β levels 1 and 2 h after termination of restraint (Fig. 4A). In repeatedly restrained rats IL-1ra markedly enhanced plasma ACTH response 1 and 2 h after termination of 10 min restraint stress and insignificantly altered corticosterone response (Fig. 4B, 4C). The lack of diminution of plasma ACTH and insignificantly altered corticosterone response parallel with IL-1β response is not clear and needs further elucidation.

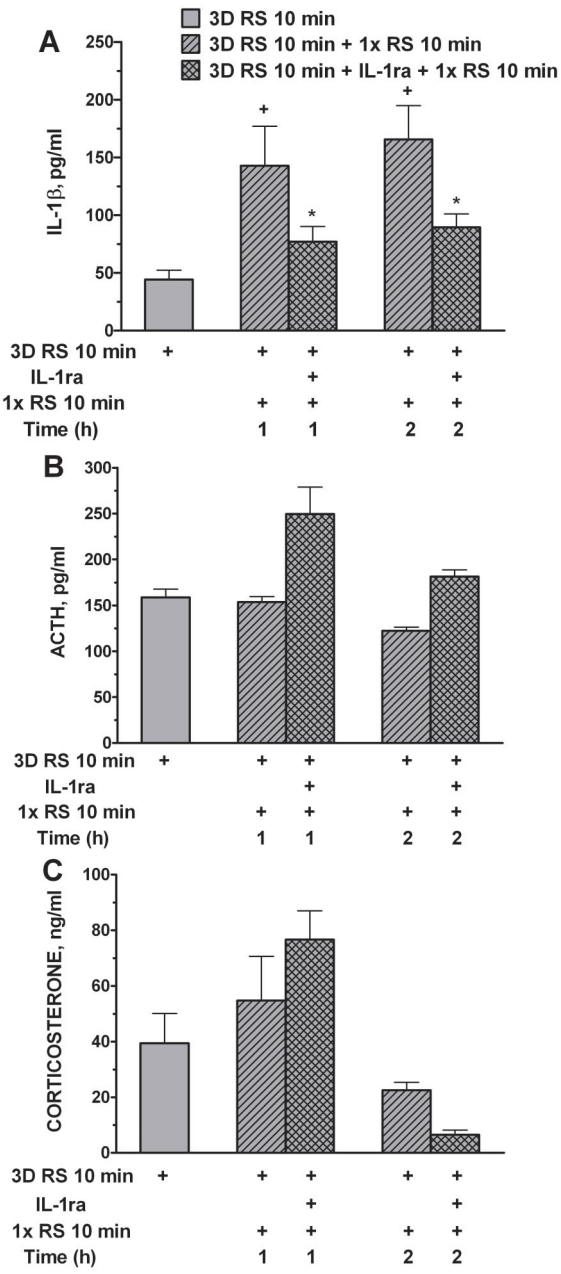

Fig. 4. Effect of prior repeated restraint 2 × 10 min per day for 3 days and IL-1β receptor antagonist (IL-1ra) on the 10 min restraint induced plasma IL-1β (**A**), ACTH (**B**) and corticosterone (**C**). See legend to Figure 2

Effect of restraint stress on IL-1β level in brain structures

Immediately after termination of 10 min restraint stress, IL-1β content increased significantly and most markedly in the prefrontal cortex, hippocampus and to a lesser extent in the hypothalamus. One hour later,

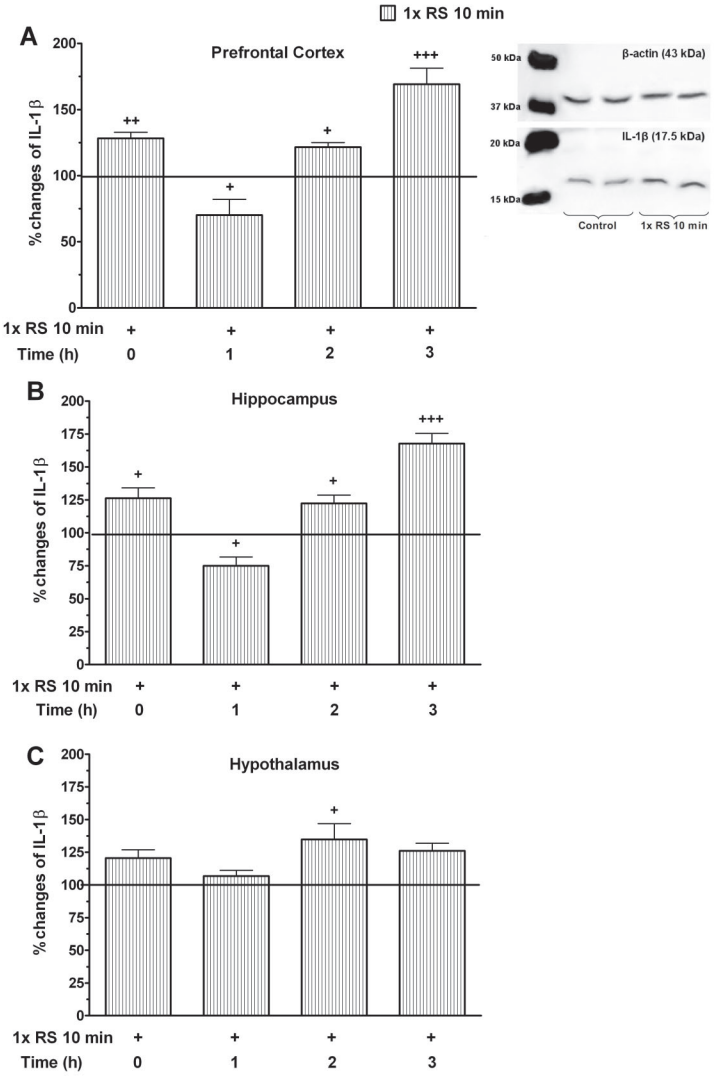

Fig. 5. IL-1β content in prefrontal cortex (**A**), hippocampus (**B**) and hypothalamus (**C**) in rats restrained for 10 min. See legend to Figure 1. Right panel: representative immunoblot showing the expression of IL-1β in studied brain regions

IL-1β content significantly decreased in prefrontal cortex and hippocampus and 2 and 3 h after restraint this content gradually and significantly increased compared with respective control levels in non-stressed rats. These alterations were most pronounced in the prefrontal cortex, and least marked in the hypothalamus (Fig. 5A, B, C).

Effect of prior stress on the restraint-induced IL-1β level in brain structures

In rats exposed to repeated restraint stress (10 min/day for 3 days twice a day) acute stress for 10 min 24 h after the last restraint significantly increased IL-1β content in all brain structures examined. Prior stress enhanced the acute restraint-induced IL-1β content immediately after termination of restraint (Fig. 6A, B) and 2 and 3 h later. The strongest increase appeared 2 h after termination of restraint in the prefrontal cortex and the hippocampus and weaker effect was observed in the hypothalamus (Fig. 6A, B and C).

IL-1β receptor antagonist-induced inhibition of stress-evoked changes of IL-1β in brain structures

Interleukin-1β receptor antagonist, given 15 min before 10 min restraint in prior stressed rats abolished the stress-induced significant increase of IL-1β content in the prefrontal cortex 1 h after restraint termination and further diminished that content below control level 2 h after restraint (Fig. 7A). In the hippocampus IL-1ra also suppressed the IL-1β content below the control level 1 h after termination of restraint (Fig. 7B).

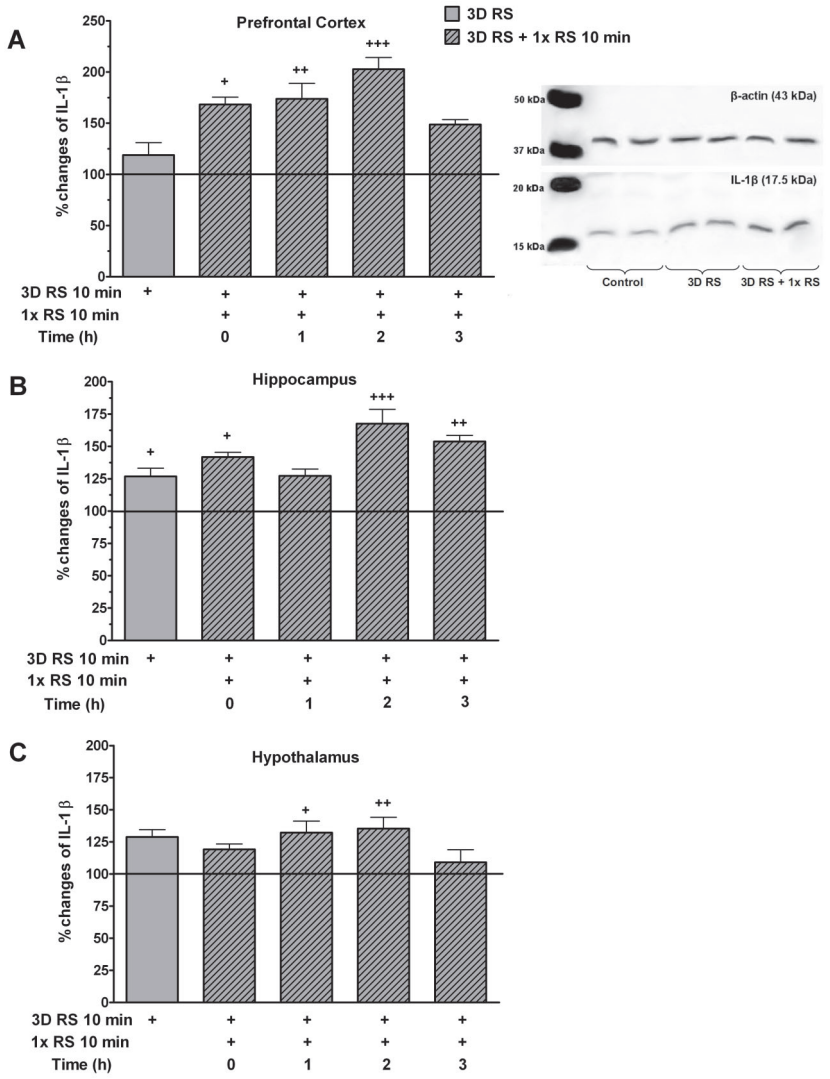

Fig. 6. Effect of prior restraint 2 × 10 min per day for 3 days on 10 min restraint stress-induced IL-1β content in the prefrontal cortex (**A**), hippocampus (**B**) and hypothalamus (**C**). See legend to Figure 1. Right panel: representative immunoblot showing the expression of IL-1β in studied brain regions

The restraint-induced significant increase of IL-1β content in the hypothalamus was not substantially affected by pretreatment with IL-1β receptor antagonist (Fig. 7C). This finding indicates a selective involvement of IL-1β receptors in the stress-induced increase of IL-1β levels in brain structures involved in the regulation of HPA axis.

Discussion

In the present experiment, acute 10 min restraint stress induced rapid significant and transient increase in plasma IL-1β which coincided with changes in plasma ACTH and corticosterone levels. Considerable increases of these components in plasma immediately after termination of restraint were practically absent 1, 2 and 3 h later. The changes in plasma IL-1β and ACTH and corticosterone levels coincided in time and magnitude, which suggests interaction of their mechanisms of release. The results evidently indicate that IL-1β is an essential mediator in the rapid stimulation of HPA axis during acute stress. The specificity of the functional involvement of IL-1β in ACTH secretion was confirmed by total reduction of plasma IL-1β and significant diminution of ACTH levels by selective IL-1β receptor antagonist during acute restraint stress.

In the present experiment, 10 min restraint stress immediately after termination, significantly increased IL-1β content in brain structures involved in HPA axis regulation, prefrontal cortex and hippocampus. One

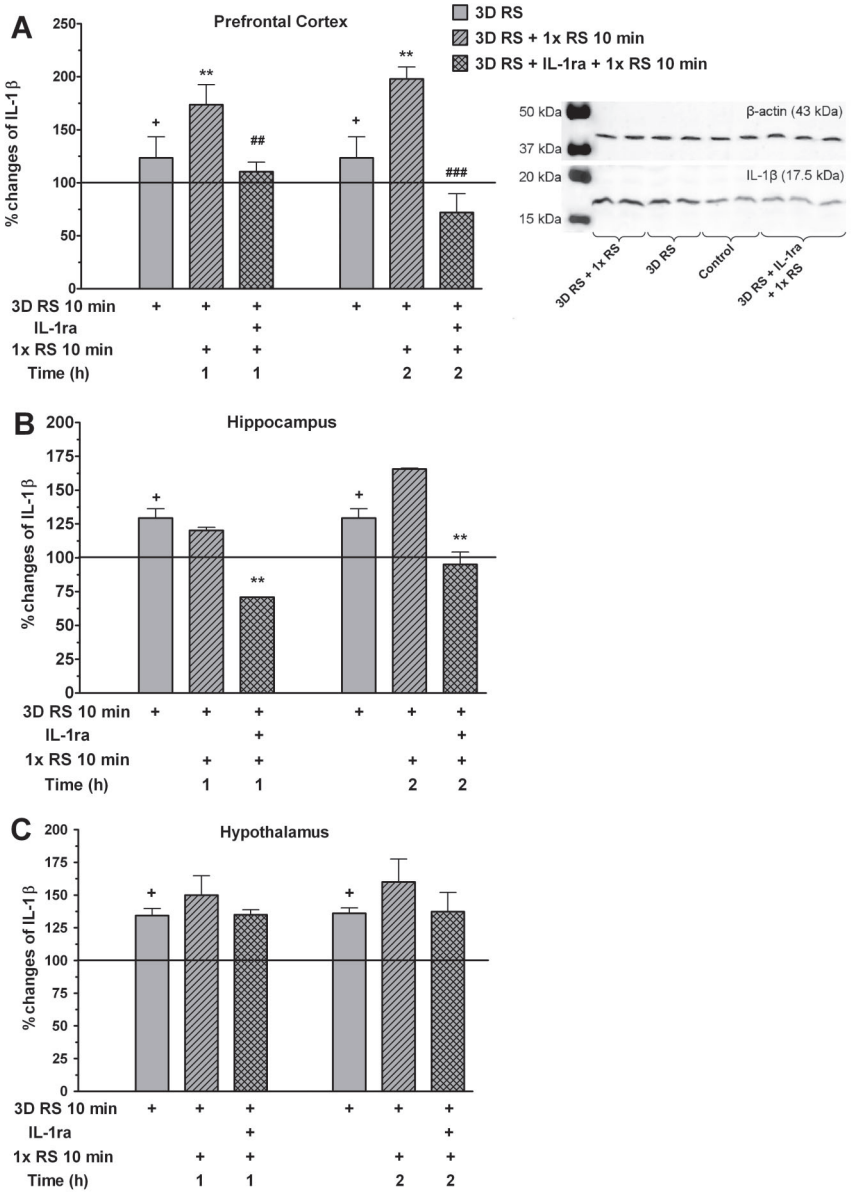

Fig. 7. Effect of prior restraint 2 × 10 min per day for 3 days and IL-1β receptor antagonist on the 10 min restraint stress-induced IL-1β content in prefrontal cortex (**A**), hippocampus (**B**) and hypothalamus (**C**). See legend to Figure 2. Right panel: representative immunoblot showing the expression of IL-1β in studied brain regions

hour later IL-1β content in these structures decreased below control levels and gradually returned to its higher levels 2 and 3 h later. The fall of IL-1β content 1 h after stress termination may result from the stress-induced IL-1β utilization which is compensated by increased synthesis 2–3 h later.

Interleukin-1β is particularly inducible in CNS by stressors, both at the mRNA or protein levels [2, 24]. Immobilization and psychological stressors can augment hypothalamic IL-1 production [13], while restraint and social isolation did not markedly affect hypothalamic IL-1 levels in some earlier report. Our data show much weaker, insignificant increase of IL-1β content in hypothalamus in comparison with its considerable increase in prefrontal cortex and hippocampus at the termination of acute restraint in rats.

However, different kinds of stress differently affect IL-1β levels in brain structures. Stressors such as footshock, tailshock, immobilization and psychological stressors can induce hypothalamic IL-1 production [13], while other stressors such as restraint, maternal separation and social isolation have no marked effect on hypothalamic IL-1 levels. Stress-induced intrinsic IL-1β production in the PVN contributes to the activation of HPA axis since *icv* IL-1 causes c-fos expression in CRH producing parvocellular neurons in the

PVN. The potential relationship between stress, brain IL-1β and HPA axis activity has not been elucidated [24]. In our experiment the moderate rise of IL-1β level by chronic stress and further significant increase by acute restraint stress in brain structures involved in regulation of HPA axis activity indicate important mediation of IL-1β in these stress-induced reactions and HPA axis response. The robust increase of IL-1β in brain structures, parallel with similar changes in plasma levels as well as effective or almost total reduction of the stress-induced reactions in peripheral and brain components of HPA axis by IL-1β receptor antagonist in our present experiment indicate the involvement of central and systemic IL-1β in regulation of HPA axis activity.

The induction of IL-1β by stressors is accompanied by an increase in IL-1β mRNA in hypothalamus and an increase in IL-1β protein levels in a number of brain regions 2 h after inescapable tailshock. Diverse stressors such as inescapable tailshock, social isolation, immobilization and restraint increase IL-1 in both peripheral tissues such as blood, pituitary and spleen as well as in the CNS hypothalamus, hippocampus and cortex. The regional specificity of these effects within the CNS depends upon the nature of the stressor employed [12]. Psychological stressors can induce pro-inflammatory cytokine production both centrally and peripherally. Proinflammatory cytokines often sensitize various neuro-hormonal and behavioral responses to subsequent stimulation [22, 23]. Single administration of IL-1β increased CRH mRNA in the hypothalamic PVN which paralleled long-lasting sensitization to emotional stress [30]. Prior stressor exposure enhances peripheral and central cytokine and HPA axis up to 4 days later [15, 16].

Elevations in central IL-1β induced by stress or exogenous administration are necessary and sufficient for sensitizing central IL-1β and corticosterone responses to subsequent stress challenge. Our data shows that exposure of rats to repeated restraint stress, for 10 min 2 × daily per 3 days, itself substantially increased plasma IL-1β, ACTH and corticosterone levels, measured 24 h after the last restraint. Previous repeated restraint also intensified markedly IL-1β content in brain structures, involved in HPA axis regulation, prefrontal cortex, hippocampus and hypothalamus 24 h after the last restraint, compared to control levels in non-stressed rats. This suggests the selectivity of IL-1β systems in central and peripheral mechanisms modulating the stress-induced HPA axis responses.

It is known that the IL-1β -induced stimulation of central and peripheral components of HPA axis may depend on both direct stimulation of anterior pituitary corticotrophs and adrenal gland and by activation of central limb of HPA regulatory systems. Systemic IL-1β may do so by penetration of brain through leaking parts of the BBB in the circumventricular organs or by afferent sensory nerves, particularly the vagal afferents.

The present results indicate time-related similarities in the potent alterations in both plasma and brain structures IL-1β levels and pituitary-adrenal hormones. This suggests that both peripheral and central IL-1β is involved in inducing IL-1β and HPA axis activity alterations under basal and stress conditions.

Acknowledgment:
This research was supported by grant: POIG 01.01.02-12-004/09-00 financed by European Regional Development Fund as well as by the statutory funds from the Institute of Pharmacology, Polish Academy of Sciences.

References:

1. Banks WA, Farr SA, Morley JE: Entry of blood-borne cytokines into the central nervous system: effects on cognitive processes. Neuroimmunomodulation, 2002, 10, 319–327.
2. Barrientos RM, Sprunger DB, Campeau S, Higgins EA, Watkins LR, Maier JW: BDNF mRNA expression in rat hippocampus following contextual learning is blocked by intrahippocampal IL-1β administration. J Neuroimmunol, 2004, 155, 119–126.
3. Berthoud H-R, Neuhuber WL: Functional and chemical anatomy of the afferent vagal system. Auton Neurosci, 2000, 85, 1–17.
4. Capuron L, Miller AH: Immune system to brain signaling: Neuropsychopharmacological implications. Pharmacol Ther, 2011, 130, 226–238.
5. Dantzer R, Konsman JP, Bluthe RM, Kelly KW: Neural and humoral pathways of communication from the immune system to the brain: parallel or convergent? Auton Neurosci, 2000, 85, 60–65.
6. De Herdt V, Puimege L, De Waele J, Raedt R, Wyckhuys T, El Tahry R, Libert C et al.: Increased rat serum corticosterone suggests immunomodulation by stimulation of the vagal nerve. J Neuroimmunol, 2009, 212, 102–105.
7. Dunn AJ: Effects of cytokines and infections on brain neurochemistry. Clin Neurosci Res, 2006, 6, 52–68.
8. Engstrom L, Rosen K, Angel A, Fyrberg A, Mackerlova L, Konsman JP, Engblom D, Blomqvist A: Systemic immune challenge activates an intrinsically regulated local

inflammatory circuit in the adrenal gland. Endocrinology, 2008, 149, 1436–1450.
9. Flugge G, Van Kampen M: Perturbations in brain monoamine systems during stress. Cell Tissue Res, 2004, 315, 1–14.
10. Follesa P, Biggio F, Gorni G, Caria S, Talani G, Dazzil L, Puligheddu M et al.: Vagus nerve stimulation increases norepinephrine concentration and the gene expression of BDNF and bFGF in the rat brain. Brain Res, 2007, 1179, 28–34.
11. Gądek-Michalska A, Bugajski J: Interleukin-1 (IL-1) in stress-induced activation of limbic-hypothalamic-pituitary arenal axis. Pharmacol Rep, 2010, 62, 969–982.
12. Goshen I, Yirmiya R: Interleukin-1 (IL-1): A central regulator of stress responses. Front Neuroendocrinol, 2009, 30, 30–45.
13. Hayley S: Toward an anti-inflammatory strategy for depression. Behav Neurosci, 2011, 5, 1–7.
14. Hayley S, Mangano E, Strickland M, Anisman H: Lipopolysaccharide and a social stressor influence behaviour, corticosterone and cytokine level. Divergent actions in cyclooxygenase-2 deficient mice and wild type controls. J Neuroimmunol, 2008, 197, 29–36.
15. Hosoi T, Okuma Y, Nomura Y: Electrical stimulation of afferent vagus nerve induces IL-1β expression in the brain and activates HPA axis. Am J Physiol Regul Integr Comp Physiol, 2000, 279, R141–R147.
16. Johnson JD, O'Connor KA, Deak T, Spencer RL, Watkins LR, Maier SF: Prior stressor exposure primes the HPA axis. Psychoneuroendocrinology, 2002, 27, 363–365.
17. Johnson JD, O'Connor KA, Watkins LR, Maier SF: The role of IL-1β in stress-induced sensitization of proinflammatory cytokine and corticosterone responses. Neuroscience, 2004, 127, 569–577.
18. Kapcala LP, He JR, Gao Y, Pieper JO, DeTolla LJ: Subdiaphragmatic vagotomy inhibits intra-abdominal interleukin-1β stimulation of adrenocorticotropin secretion. Brain Res, 1996, 728, 247–254.
19. Karalis KP, Venihaki M, Zhao J, van Vlerken LE, Chandra C: NF- β participates in the corticotropin-releasing hormone-induced regulation of the pituitary proopiomelanocortin gene. J Biol Chem, 2004, 279, 10839–10840.
20. Kariagina A, Romanenko D, Ren SG, Chesnokova V: Hypothalamic-pituitary cytokine network. Endocrinology, 2004, 145, 104–112.
21. Kim YK, Maes M: The role of the cytokine network in psychological stress. Acta Neuropsychiatr, 2003, 15, 148–155.
22. Maolood N, Meister B: Protein components of the blood-brain barrier (BBB) in the brainstem area postrema-nucleus tractus solitarius region. J Chem Neuroanat, 2009, 37, 182–195.
23. MohanKumar SMJ, Smith CL, MohanKumar PS: Central adaptation to chronic administration of interleukin-1β (IL-1β) in rats. Brain Res Bull, 2003, 62, 71–76.
24. Mormede C, Castanon N, Medina C, Moze E, Lestage J, Neveu PJ, Dantzer R: Chronic mild stress in mice decreases peripheral cytokine and increases central cytokine expression independently of IL-10 regulation of the cytokine network. Neuroimmunomodulation, 2002, 10, 359–366.
25. Nguyen KT, Deak T, Will MJ, Hansen MK, Hunsaker BN, Fleshner M, Watkins LR, Maier SF: Timecourse and corticosterone sensitivity of the brain, pituitary, and serum interleukin-1β protein response to acute stress. Brain Res, 2000, 859, 193–201.
26. O'Connor KA, Johnson JD, Hansen MK, Frank JLW, Maksimowa E, Watkins LR, Maier SF: Peripheral and central proinflammatory cytokine response to severe acute stressor. Brain Res, 2003, 991, 123–132.
27. Pace TWW, Hu F, Miller AH: Cytokine-effects on glucocotricoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression. Brain Behav Immun, 2007, 21, 9–19.
28. Quan N, Herkenham M: Connecting cytokines and brain: a review of current issues. Histol Histopathol, 2002, 17, 273–288.
29. Rinaman L: Visceral sensory inputs to the endocrine hypothalamus. Front Neuroendocrinol, 2007, 28, 50–60.
30. Rivest S, Lacroix S, Vallieres L, Nadeau S, Laflamme N: How the blood talks to the brain parenchyma and the paraventricular nucleus of the hypothalamus during systemic inflammatory and infectious stimuli. Proc Soc Exp Biol Med, 2000, 223, 22–38.
31. Rogóż Z, Kubera M, Rogóż K, Basta-Kaim A, Budziszewska B: Effect of co-administration of fluoxetine and amantadine on immunoendocrine parameters in rats subjected to a forced swimming test. Pharmacol Rep, 2009, 61, 1051–1060.
32. Schmidt ED, Aguilera G, Binnekade R, Tilders FJ: Single administration of interleukin-1 increased corticotropin releasing hormone and corticotropin releasing hormone-receptor mRNA in the hypothalamic paraventricular nucleus which paralleled long-lasting (weeks) sensitization to emotional stressors. Neuroscience, 2003, 116, 275–283.
33. Schmidt ED, Binnekade R, Janszen AW, Tilders FJ: Short stressor induced long-lasting increases of vasopressin stores in hypothalamic corticotropin-releasing hormone (CRH) neurons in adult rats. Neuroendocrinology, 1996, 8, 703–712.
34. Schmidt ED, Janszen AW, Wouterlood FG, Tilders FJ: Interleukin-1-induced long-lasting changes in hypothalamic corticotropin-releasing hormone (CRH)-neurons and hyperresponsiveness of the hypothalamus-pituitary-adrenal axis. J Neurosci, 1995, 15, 7417–7426.
35. Schulte K, Kumar M, Zając JM, Schlicker E: Noradrenaline release in rodent tissues is inhibited by interleukin-1β but is not affected by urotensin II, MCH, NPW and NPFF. Pharmacol Rep, 2011, 63, 102–111.
36. Smith EM: Neuropeptides as signal molecules in common with leukocytes and the hypothalamic-pituitary-adrenal axis. Brain Behav Immun, 2008, 22, 3–14.
37. Szelenyl J: Cytokines and the central nervous system. Brain Res Bull, 2001, 54, 329–338.
38. Theodosis DT, MacVicar B: Neurone-glia interactions in the hypothalamus and pituitary. Trends Neurosci, 1996, 19, 363–367.

Received: July 26, 2011; **in the revised form:** October 26, 2011; **accepted:** October 28, 2011.