EXOGENOUS IL-1β INDUCES ITS OWN EXPRESSION, BUT NOT THAT OF IL-6 IN THE HYPOTHALAMUS AND ACTIVATES HPA AXIS AND PROLACTIN RELEASE

MARTINA SKURLOVA[1], ANDREA STOFKOVA[1], JANA JURCOVICOVA[1,2]

[1]Institute of Normal, Clinical and Pathological Physiology, Third Medical Faculty, Charles University, 120 00 Prague, Czech Republic
[2]Institute of Experimental Endocrinology, Slovak Academy of Sciences, 833 06 Bratislava, Slovakia
e-mail:ueenjurc@savba.sk

Objective: Proinflammatory cytokines IL-1ß, and IL-6 are synthesized in the brain, where they exert local regulatory functions. Our aim was to find out whether, along with the activation of hypothalamo-pituitary-adrenocortical (HPA) axis and prolactin (PRL), the acute systemic enhancement of IL-1ß affects its own production in the hypothalamus as well as that of IL-6.

Method: Forty five minutes after a single i.p. administration of recombinant rat IL-1ß (5 μg/kg) to male Long Evans rats we estimated the expression of IL-1ß and IL-6 mRNA in the hypothalamus by real time PCR, ACTH, corticosterone (CORT), and PRL by RIA

Results: IL-1ß administration stimulated the expression of IL-1ß mRNA in the hypothalamus by 99 %, but not that of IL-6. It also significantly activated plasma levels of ACTH, PRL, CORT, and CORT production in adrenal gland.

Conclusion: These results indicate that acute peripheral enhancement of IL-1ß may induce neuroendocrine changes also via the immediate activation of its own expression in the hypothalamus, but not that of IL-6 expression in the hypothalamus was found.

Keywords: IL-1ß administration – IL-1ß – IL-6 mRNA expression – Hypothalamus – HPA axis – Prolactin

Interleukins IL-1ß and IL–6 are considered the main mediators of inflammation and exert a variety of regulatory functions in the brain. Their neuroendocrine effects have been repeatedly described after the first report on the activation of growth hormone, TSH and PRL by a single intracerebroventricular (icv) administration of IL-1ß into the third ventricle (RETTORI et al.1987). They are also produced in the brain where they are involved in physiological processes such as the regulation of hypothalamic–brainstem circuits implicated in sleep-wake behavior (OPP 2005), genes associated with sleep and learning (TANG et al, 2005), sickness behavior in rats resembling human depression (DUNN et al. 2005). Under several pathological conditions excessive amount of IL-1ß contributes to CNS injury resulting in neuronal loss (GIBSON et al. 2004).

Systemic IL-1ß administration has been shown to enhance its mRNA expression in the hypothalamus, hippocampus and brain stem already two hours after the injection and such effect is attenuated by a subdiaphragmatic vagotomy (HANSEN et al., 1998). In this study we followed the acute effect of a single i.p. administration of IL-1ß on the IL-1ß, and also on IL-6 mRNA expression in the hypothalamus, and on the activity of HPA axis and prolactin release.

Materials and methods

We used male Long Evans rats bred in the Department of Normal, Pathological, and Clinical Physiology of the Third Medical Faculty (Prague) weighing about 180g. They were kept under standard laboratory

conditions: 12/12 h light/dark cycle, controlled humidity and temperature, free access to standard pelleted diet and tap water. We applied 5 μg/kg of recombinant rat IL-1ß (AR&D Abington, Oxon,UK) i.p. to the experimental group, while the control group received saline. Forty-five minutes later the rats were decapitated**.** Blood was collected into cooled EDTA-NA_2 and centrifuged. Hypothalamus and adrenal gland was dissected, snap frozen in liquid nitrogen and stored at –70 °C. We performed the experiment between 8:00 and 9:30 a. m., in accordance with national law of the Czech Republic on the Use of the Laboratory Animals # 167/1993.

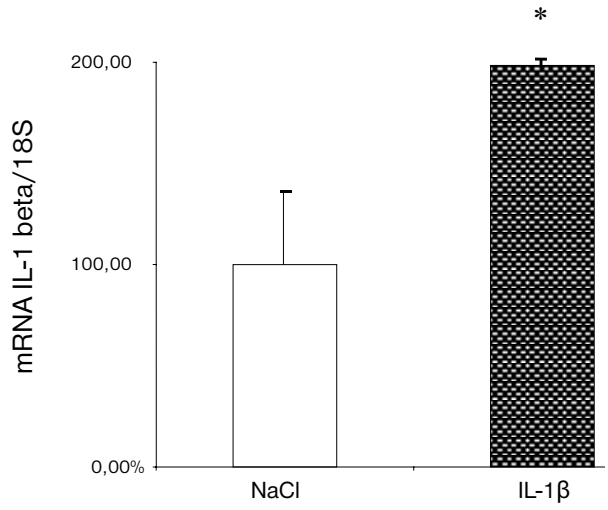
Plasma PRL and ACTH were determined directly using specific radioimmunoassays. Adrenal glands were homogenized in saline and delipidated with N-heptane (Sigma, Aldrich, Deisenhofen, Germany), CORT was extracted from aqueous phase or from plasma with methylenechloride and determined by specific radioimmunoassay. Proteins were estimated by Bradford method. The amount of 1.5 μg of total RNA extracted from hypothalamus was reverse transcribed into complementary DNA using transcription beads (Ready-To-Go™ , You-Prime First-Strand Beads, Amersham Pharmacia Biotech, Buckinghamshire, UK). To quantify the expression of IL-1ß and IL-6, we applied ABI PRISM 7000 Sequence Detector (Applied Biosystems, Foster, CA, USA). The reactions were performed with commercial Taq Man® Assays-on-Demand™ Expression Products, as detailed previously (Seres et al. 2004). The results were analyzed using unpaired Student t-test, and in the case of PRL values the Welch correction was applied to level the differences between SD.

Results

The expression of mRNA for IL-1ß and IL-6 in the hypothalamus is depicted in Fig.1. The amount of mRNA in controls was assessed as 100 %. IL-1ß-treated animals showed approximately 99% increase in IL-1ß mRNA expression compared to controls, wheras IL-6 mRNA remained unchanged. Single dose of IL-1ß significantly increased plasma ACTH levels compared to saline (427.38± 36.08 vs. 238.97± 47.36 pg/ml) which was reflected by circulating CORT levels (21.8±1.67 vs. 12.41±2.46 μg /100 ml) and its production by adrenal gland (63.96±7.15 vs. 11.37±3.73 ng per mg of proteins in the organ). IL-1ß also activated PRL release from 1.94± 0.24 to 9.41± 2.97 ng/ml (Fig. 2).

Discussion

Our results have clearly shown that a single acute i.p. administration of IL-1ß activates its own expression, but not that of IL-6 in the hypothalamus followed by the ACTH, CORT, and PRL release in male Long Evans rats. We have characterized the changes that occurred in response to the dose capable to increase CRH



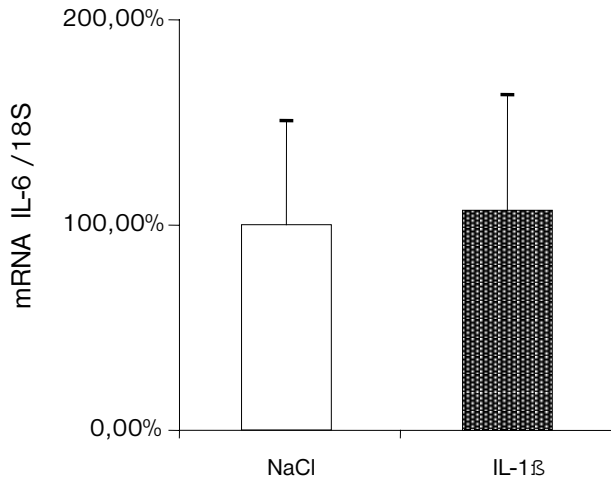


Fig.1 Real-time PCR expression of IL-1ß, and IL-6 mRNA in rat hypothalamus in control and and IL-1ß treated rats. The expression of the signals was normalized to 18S. The values of control rats are considered as 100 %. The results are expressed as percent of the controls. Means ± S.E.M of 8 rats in each group is presented. Statistical significance: *P<0.05.

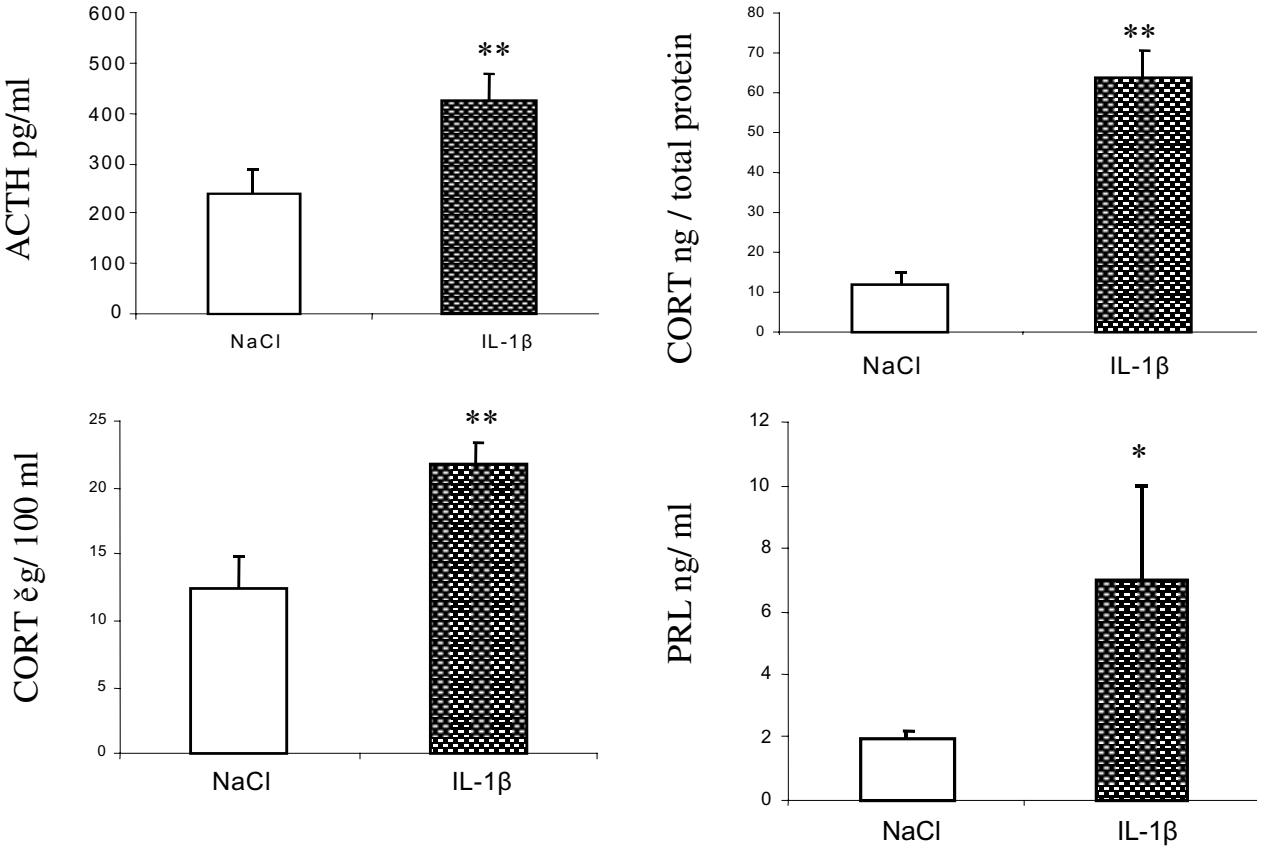


Fig.2 Levels of HPA-axis hormones, circulating ACTH, CORT, and CORT in adrenal gland, and circulating PRL in control and IL-1ß treated rats. Control rats were administered with saline i.p. and experimental rats with IL-1ß 5m/kg i.p. Eight rats per group were used. The results are expressed as means ±S.E.M. Statistical significance: ** P<0.01; *P<0.05

levels, and mRNA for CRH receptors in PVN of Wistar rats (SCHMIDT et al., 2003).

These results are supported by earlier findings by GAYLE et al. (1999) who described an up regulation of IL-1ß and IL-1 receptors type 1 as well as that of IL-1 receptors antagonist mRNAß in hypothalamus after a chronic 7-day icv. infusion of IL-1ß. Here we have shown that an immediate peripheral peak of IL-1ß can induce similar activation which may show further neuroinflammatory implications. Interestingly, in this experiment the expression of IL-6 which is known to be IL-1 ß related under vitro conditions (SPANGELO et al. 1991), remained unaffected. For its activation different timing may be needed, or also a different sensitivity to endogenous CORT levels may play a role. The parallel stimulation of PRL and HPA axis by IL-1ß may be of physiologicel relevance to counter-regulate the immunosuppressive effect of CORT and maintain the immune balance. Since IL-1ß was shown to increase central noradrenaline levels (MOHANKUMAR, and MOHANKUMAR 2005) which has been involved in CRH activation**,** and central noradrenaline is known to regulates also secretion of PRL (JURCOVICOVA et al. 1989), the stimulation of ACTH and PRL by IL-1ß may share a common regulatory pathway.

In conclusion, our results have shown that a single acute peripheral injection of IL-1ß activates its own expression in the hypothalamus which further triggers neuroendocrine changes. In addition, peripherally increased IL-1ß may induce brain inflammation that can be further exacerbated by its enhanced local IL-1ß production.

Acknowledgements

We wish to thank Mrs. Hana Stastna, for her excellent technical assistance. This work was supported by VZ 002162018, MSM 00216208 and VEGA 304/0214/2004.

References

DUNN AJ, SWIERGIEL AH, DE BEAUREPAIRE R: Cytokines as mediators of depression: what can we learn from animal studies? Neurosci BioBehav Rev **29,** 891-909, 2005

GAYLE D, ILYIN SE, ROMANOVITCH AE, PELOSO E, SATINOFF E, PLATA-SALAMAN CR: Basal and IL-1ß-stimulated cytokine and neuropeptide mRNA expression in brain regions of young and old Long-Evans rats. Mol Brain Res **70,** 92-100, 1999

GIBSON RM, ROTHWELL NJ, LEFEUVRE RA: CNS injury: the role of cytokine IL-1. Vet J **168,** 230-7 2004

HANSEN MK, TAISHI P, CHEN Z, KRUEGER JM: Vagotomy blocks the induction of interleukin-1beta (IL-1beta) mRNA in the brain of rats in response to systemic IL-1beta. J Neurosci **18,** 2247-53, 1998

JURCOVICOVA J, LE T, KRULICH L: The paradox of alpha 2 adrenergic regulation of prolactin (PRL) secretion. I. The PRL-releasing action of the alpha 2 receptor agonists. Brain Res Bull **23,** 417-24, 1989

MOHANKUMAR SM, MOHANKUMAR PS: Systemic Interleukin-1 beta stimulates the simultaneous release of norepinephrine in the paraventricular nucleus and median eminence. Brain Res Bull **65,** 451-456, 2005

OPP MR: Cytokines and sleep. Sleep Med Rev **9,** 355-364, 2005

RETTORI V, JURCOVICOVA J, MCCANN SM: Central action of interleukin-1 altering the release of TSH, growth hormone, and prolactin in the male rat. J Neurosci Res **18,** 179-183, 1987

SCHMIDT ED, AGUILERA G, BINNEKADE R, TILDERS FJH: 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 **116,** 275-283, 2003

SERES J, HERICHOVA I, ROMAN O, BORNSTEIN S, JURCOVICOVA J: Evidence for daily rhythms of the expression of proopiomelanocortin, interleukin-1-beta and interleukin-6 in adenopituitaries of male Long Evans rats: Effect of adjuvant arthritis. Neuroimmunomodulation **11,** 316-322, 2004

SPANGELO BL, JUDD AM, ISAKSON PC, MACLEOD R.M: Interleukin-1 stimulates interleukin-6 release from rat anterior pituirary cells in vitro. Endocrinology **128,** 2685-2692, 1991

TANG C, SULA MJ, BOHNET S, REHMAN A, TAISHI P, KRUEGER JM: Interleukin-1beta induces CREB-binding protein (CBP) mRNA in brain and the sequencing of rat CBP. Mol. Brain.Res. **13,** 213-222, 2005

Corresponding author: Jana Jurcovicova, PhD
Institute of Experimental Endocrinology
Slovak Academy of Sciences
Vlárska 3
83306 Bratislava
Slovakia
tel.: +421 2 5477 2800
fax: +421 2 5477 4247
e-mail:ueenjurc@savba.sk