S. HENRYK, J. JOŚKO[1], H. JĘDRZEJOWSKA-SZYPUŁKA[1], B. JARZĄB[2], K.D. DÖHLER[3]

CORTICOTROPIN RELEASING HORMONE (CRH) INCREASES β-ENDORPHIN (β-END LIKE) CONCENTRATION IN CEREBROSPINAL FLUID OF RATS WITH VASOSPASM FOLLOWING SUBARACHNOID HEMORRHAGE

Department of Neurosurgery and Neurotraumatology in Bytom, [1]Department of Physiology in Zabrze, [2]Laboratory of Nuclear Medicine, II Department of General Surgery in Bytom, Silesian Medical University in Katowice, Poland, and [3]Bisserdorf Peptide Corticobios, Germany

The chronic stage of vasospasm occurring several days after subarachnoid hemorrhage (SAH) is characterized by the development of histopathologic changes in cerebral arteries causing cerebral ischemia. Numerous experimental data indicate the involvement of immune mechanisms in the angiopathy caused by SAH. Endogenous opioids play also an important role in the ischemic lesions of the brain. Corticotropin releasing hormone (CRH) induces the release of β-endorphin (β-END) from hypothalamic neurons and also from mononuclear white blood cells. The function of CRH and β-END in vasospasm following SAH and the interrelationship between neuroendocrine and immune changes requires further elucidation.
In the present study we investigated the influence of CRH injected into cerebral cisterna magna (CM) of rats on β-END-like level in cerebrospinal fluid (CSF) in acute and chronic phase of cerebral vasospasm following artificial SAH. Acutely CRH induced a significant rise of β-END-like in CSF both in SAH and sham SAH rats. However, in rats subjected to SAH, a single injection of CRH caused a prolonged rise of β-END in CSF, which was also seen 2 days after SAH, during the chronic phase of vasospasm. The obtained results indicate that CRH increases neuroendocrine changes induced by SAH, probably by an activation of immune cells involved in the patomechanism of chronic vasospasm.

K e y w o r d s: *vasospasm after SAH, influence of the CRH on the β-END-like concentration in rats CSF*

INTRODUCTION

SAH may be complicated by cerebral vasospasm, in the course of which two phases: the early, acute and the late phase, so called chronic vasospasm, can be distinguished (1). The very same two-phased course of vasospasm is observed in animal experimental models with its maximal intensity in 10—20 minutes and in 2 days after hemorrhage (2, 3, 4). Following spontaneous and

experimental SAH erythrocytes lysis in subarachnoid space, and the brain and its vascularity are exposed to many different spasmogenic substances. Their rapid metabolism may prove to be beneficial after SAH (5,6).

The initial vasospastic response to SAH probably involves simple, reversible smooth muscles contraction caused by a variety of angioactive substances as: F2-α-prostaglandin, serotonine, oxyhemoglobin or bilirubin, released from blood clots to the subarachnoid space (2,3,4,5,7). The chronic stage of vasospasm, occurring several days after hemorrhage, is characterized by the development of histopathologic changes of vascular walls including corrugation of the elastic lamina, detachment of endothelial cells and vascular formation in smooth muscle layer (1,8,9,10,11). In result of these changes, the proliferative angiopathy develops and causes considerable narrowing of vessels and in consequence cerebral ischemia (9,10,12). The mechanism of origin of angiopathy is still uncertain, although there are many proofs of its immunologic background (10,13,14—17). However, neuroendocrine pathways have also to be considered. Serotonine and prostaglandins, which increase in CSF during SAH, stimulate hypothalamic secretion of CRH and cause the release of β-END (18,19). These neuropeptides events may also contribute to the development of angiopathy (18,19). Several reports suggest that cerebral ischemic disease is associated with increased levels of β-END immunoreactivity (20,21,22) and β-END contributes to pial artery constriction (23).

Endogenous opioids play an important role in the development of ischemic lesions of the brain and the spinal cord, as proven in many studies investigating the actions of naloxone, an opiate antagonist (18,20,24,25,26). In humans naloxone has been reported to attenuate the neurological deficits after cerebral ischemia and to have the beneficial effect on the blood flow and on the sequelae of spinal injury (20,25). Adenohypophysis is the main source of peripheral β-END and its production is stimulated by hypothalamic CRH, but β-END present in CSF comes almost entirely from the neurons of hypothalamic arcuate nuclei (27,28,29). CRH induces also the release of β-END from mononuclear white blood cells (30,31,32).

The role of β-END and CRH in vasospasm following SAH is unclear. It has been shown that CRH decreases permeability of chemically injured peripheral vessels and cold-injured cerebral vessels and reduces blood-brain barrier's damage after SAH (33,34,35). It was found that its concentration in CSF patients with vasospasm following SAH is higher and significantly rises in the chronic phase while the cerebral ischemia develops (36). It is unknown whether any release of β-END to the perivascular space occurs in the vasospasm after SAH. In the present study we investigate the changes of β-END secretion to CSF in the acute and chronic phase of cerebral vasospasm and estimate the influence of CRH injected to the CSF on β-END release in rats with vasospasm following SAH.

MATERIAL AND METHODS

The study was performed on Wistar male rats (250—300 g. b.w.) were housed in cages in-groups of six under controlled standard microclimate condition (18° ± 2°C temp. and 50—60% humidity) and illumination (light-dark cycle of 12:12h) and had free access to standard food and water. All the experiments were carried out between 2 and 4 p.m. on animals anesthetized with intraperitoneally injected Ketamine in dose of 100 mg × kg $^{-1}$. The Bioethical Committee of Silesian Medical University granted the permission for the study.

The technique of SAH induction and cannulation of cisterna magna (CM):

All the experiments were carried out in the sterile condition. The trephination window was drilled over the right hemisphere 3-mm caudally from the coronals suture and 2 mm laterally from the sagittal suture. The Neoflon 0.6-mm needle punctured the dura mater and its cannula was inserted into the subdural space forward and a little down over the brain surface. The entrance to the subarachnoid space was recognized by the CSF flow out. The trephination hole was protected with osseous wax and the cannula was fixed to the bone with the cyanoacrilic glue. Animals were injected with either a 0.9% saline solution in the sham SAH groups or 250 μl of autologous non-heparinized blood obtained from orbital arterio-venous plexus to induce the SAH.

The cannulation of CM was performed using the original technique described by Solomon (1985) (37) with minor modification. Briefly: the trephination hole was burred sagitally over the parieto-occipital suture. The cannula prepared from Abbot Venocath—18 was placed via the hole in the CM. The correct position of the cannula was maintained by observing a) the localization of its end in the CM through the atlanto-occipital membrane, b) the easy outflow of CSF through the cannula. Cannula was attached to the parietal bone using cyanoacrilic glue. The trephination hole was protected from the CSF leakage with osseous wax and cyanoacrilic glue.

Experiments were conducted 1 week after surgery. This period assured lack of surgical procedure consequence (38). The 50 μl samples of CSF obtained directly after the cannulation were established for initial β-END-like concentrations. CSF was aspirated in the same time through the cannula inserted into CM using Hamilton syringe in the course of 1—5 minutes and was immediately cooled down to the temperature −4°C and centrifuged (at 3000 rpm for 10 min) and kept frozen at −25°C until assayed. The CSF samples for β-END-like concentration assay were collected 20 minutes after induction of SAH or saline application (acute phase of vasospasm) and on the two days after SAH (chronic vasospasm). CRH (10 μg in 100μl of diluent — Bissendorf Peptide's Corticobios) was injected in two groups of rats into the CM after the drainage of the same CSF volume.

The animals were divided into three groups: 1) — with CRH and SAH (CRH/SAH group) (n = 15), 2) — with CRH and sham SAH (CRH/NaCl group) (n = 10), 3) — without CRH and with SAH (NaCl/SAH group) (n = 9).

B—END-like immunoconcentration was measured with commercially available RIA KIT (Allegro^R, Nichols Institute Diagnostics B.V., Bad Nauheim, Germany). Sensitivity of the assay was 14 pg/ml and was 16% cross reactivity between β-endorphin and β-lipotropin at highest doses (500 pg/ml) studied. The results obtained in the groups were calculated as the mean with a standard deviation; and, subsequently, an analysis of the results was performed using Student's unpaired t-test, with the level of significance — $p < 0.05$.

RESULTS

The mean β-END-like concentration in rats' CSF obtained directly after CM cannulation was 98,0 ± 33,4 pg/ml. A significant increase of CSF β-END-like level was observed on 20' after SAH or its imitation in both CRH pretreated groups: either CRH/SAH (p<0,01) and CRH/NaCl (p<0,05) (*Fig. 1*). The values observed in both groups did not differ significantly. A smaller and statistically insignificant rise was stated in the NaCl/SAH group. These was, however no significant difference between both SAH groups: CRH pretreated and without CRH group (NaCl/SAH) (*Fig. 1*).
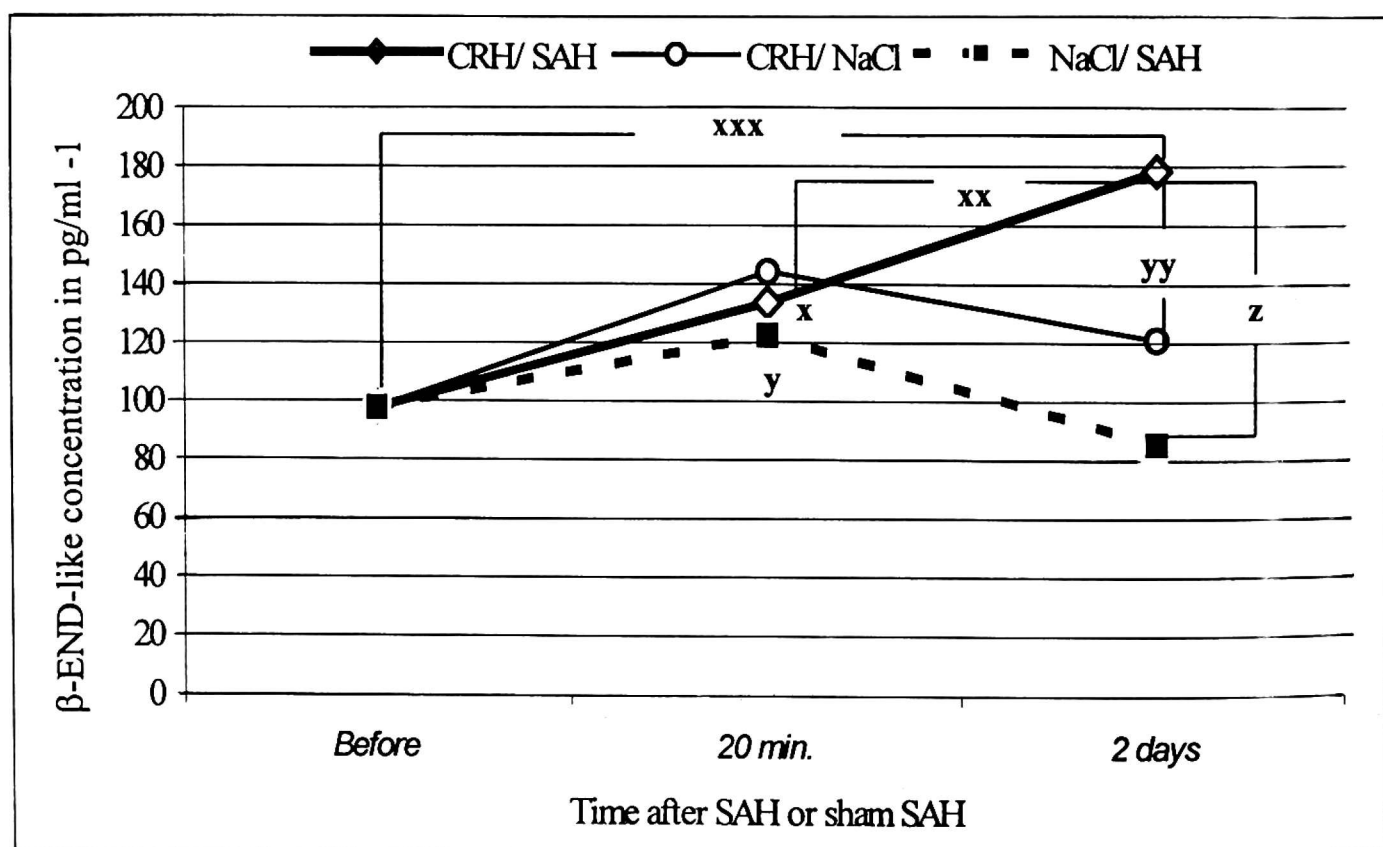

Fig. 1 Graph showing the change in β-END-like concentration in CSF after 10 μg CRH injection into CM followed by 20' later subarachnoid blood injection (group CRH/SAH) and in two control groups. First-after 10 μg CRH injection into CM followed by sham SAH with 0,9% NaCl (group CRH/NaCl) and second — after 0,9% NaCl injection into CM followed by subarachnoid blood injection (group NaCl/SAH).

The statistically significant increase of CSF β-END-like concentration is found between sample 0 day and 20' after CRH in both SAH and sham SAH groups (x-p<0,01 and y-p<0,05 respectively), while SAH without CRH induced only an insignificant β-END-like rise (group NaCl/SAH).

In the experimental group (CRH/SAH) the concentration of β-END-like on day 2 after SAH was significantly different from the response to SAH after 20' (xx-p<0,05) and from initial concentration of β-END-like on day 0 (xxx-p<0,001). This value was also significantly higher than β-END-like level seen on day 2 in control animals, which received SAH without CRH pretreatment (z-p<0,001) and sham SAH with pretreatment CRH (yy-p<0,01)

The second estimation of β-END-like, carried out on day 2, during the chronic phase of vasospasm, revealed significant differences between groups. In the CRH/SAH group a further increase of CSF β-END-like level was seen, while a decrease was observed in CRH/NaCl and NaCl/SAH groups (*Fig. 1*). The increase of β-END-like in CRH/SAH rats was statistically significant both in comparison with the initial β-END-like level (p < 0,001) and with the values seen during acute phase of vasospasm (p < 0,05) (*Fig. 1*). The difference between CRH pretreated and control animals subjected to SAH was statistically significant (p < 0,001) (*Fig. 1*). β-END-like CSF level in the CRH/NaCl group behaved intermediately — a small decrease was seen and this difference was statistically significant in comparison with the CRH/SAH group (p < 0,01) (*Fig. 1*).

DISCUSSION

We observed an increase of CSF β-END-like 20' after SAH, but it was not statistically significant and transient. The values observed on day 2 were similar to the initial ones — it means, SAH by itself had no significant influence on β-END-like level. CRH induced a significant rise of CSF β-END-like investigated 20' after control NaCl injection and this effect was related to the known actions of neuropeptide. The early response seen in the animals subjected to SAH was very similar. However, the combined treatment with CRH and SAH resulted in a prolonged CSF β-END-like increase. The values observed on day 2 were significantly higher than in the NaCl/SAH group. This increase should not be interpreted as caused by CRH pretreatment only, as no rise was observed in the CRH/NaCl group and it is improbable, that β-END secretion would be increased 2 days after CRH administration without any intensifying mechanism. It should by related to an interaction between SAH-induced changes and CRH injection, which caused a prolonged β-END-like rise in CSF in CRH/SAH, treated animals. SAH-induced inflammatory response was probably enhanced at the early stage by CRH and the following cytokine cascade resulted in prolonged β-END increase in CSF.

In the patients with cerebral infarctions (4—48h) and in rats after brain ischemia (15—90 min) the increase of β-END concentration in the acute phase has been described only in CSF, with no changes in plasma (20, 39). After this period the significant decrease of β-END concentration in CSF occurred. Circulating β-END in plasma has been shown to arise largely from the pituitary (40), while brain β-END derives almost exclusively from neurons in the arcuate nucleus of the hypothalamus (41, 42).

Thus, the effect in the acute phase of vasospasm might be interpreted not as a result of β-END transudation from the serum to the CSF through the

damaged blood-brain barrier, but as the effect of the augmented production in the hypothalamus. That is secondary to the activation of neurons in the arcuate nucleus (28,29,43). We have seen a similar influence of SAH on β-END secretion to CSF in experimental animals but it was transient and not statistically significant. In CRH pretreated animals SAH augmented the secretion of β-END-like in the chronic phase of vasospasm. An interaction between CRH action and SAH is to be responsible for the effect.

CRH plays an important role in the mutual influences between immunological, nervous and endocrine system, mainly through the influence on the production and release of β-END from pituitary and hypothalamus (19,30,44,45). A CRH induced release of interleukins from lymphocytes and macrophages, stimulation of lymphocytes proliferation and increase of receptors for IL-2 (IL-2-R) expression on lymphocytes leading to increased β-END production by lymphocytes may contribute to the observed β-END-like CSF level, as lymphocytes are capable to produce and release β-END (19,46). In turn, β-END stimulates the IL-2 production and lymphocyte blastic transformation (19).

It has become evident, that in the pituitary the cytokines IL-1, IL-2 and IL-6 can induce the secretion of POMC peptides (19,47,48). IL-1 can exert its effect either via a direct interaction with the pituitary or via induction of release of CRH from cells of hypothalamus (36,49,50). Next, IL-1 may induce the synthesis and secretion of CRH of neurons of periventricular hypothalamic nuclei (32,51,52,53,54). CRH increases β-END production, so the loop closes and the cascade may be repeated amplifying the effect on CSF β-END level.

The cytokines are involved in the pathology of several central nervous system diseases, particularly in cerebral inflammatory responses (55,56,57). Astrocytes are known to produce and/or respond to a variety of cytokines (58). IL-1 has been reported to act on endothelial cells and to induce cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) (59), that promote the infiltration of neutrophils into the brain parenchyma and to accelerate ischemic brain damage (60). So it might influence proliferative change in vessels wall.

In humans significantly higher concentrations of IL-1 α and β in CSF on day 3 and especially day 5 were reported during SAH (9). Either neurons or neuroglia cells were considered to secrete IL-1 and prostaglandins were found to be one of stimulating factors (7,19,61,62). Svendgaard et al. already in 1985 suggested, that a substance liberated either by the hypothalamus or by the pituitary after SAH be involved in the occurrence of vasospasm (3).

CRH and β-END strongly stimulate the immunologic system in a positive feedback circle via induction of the production of interleukins (19,30,51,61,63) and may participate in SAH induced brain damage. Prostaglandins and serotonine released after SAH from blood cloths may be other promoters of the

mechanisms leading to the increase of IL-1 production. Simultaneously they stimulate CRH and β-END synthesis and secondarily influence the increase IL-1 secretion in to CSF. The intensity of these processes may be dependent on the strength of the SAH and duration of such stimulation, what may finally lead to the development of chronic vasospasm as the result of proliferative angiopathy.

REFERENCES

1. Matsui T, Kaizu H, Itoh S, Asano T. The role of active smooth-muscle contraction in the occurrence of chronic vasospasm in the canine two-hemorrhage model. *J Neurosurg* 1994; 80: 276—282.
2. Delgado TJ, Brismar J, Svendgaard NA. Subarachnoid hemorrhage in the rat: Angiography and fluorescence microscopy of the major cerebral arteries. *Stroke* 1985; 16: 595—601.
3. Svendgaard NA, Brismar J, Delgado TJ, Rosengren E. Subarachnoid hemorrhages in the rat: Effect on the development of vasospasm of selective lesions of the catecholamine systems in the lower brain stem. *Stroke* 1985; 16: 602—608.
4. Svendgaard NA, Brismar J, Delgado TJ, Rosengren E, Stenevi U. Subarachnoid hemorrhage in the rat: Effect on the development of cerebral vasospasm of lesions in the central serotoninergic and dopaminergic systems. *Stroke* 1986; 17: 86—90.
5. Matz PG, Massa SM, Weinstein PR, Turner Ch, Panter SS, Sharp FR. Focal hyperexpression of hemeoxygenase-1 protein and messenger RNA in rat brain caused by cellular stress following subarachnoid injections of lysed blood. *J Neurosurg* 1996; 85: 892—903.
6. Onoue H, Kaito N, Akiyama M, Tomii M, Tokudome S, Abe T. Altered reactivity of human cerebral arteries after subarachnoid hemorrhage. *J Neurosurg* 1995; 83: 510—515.
7. O'Banion MK, Miller JC, Chang JW, Kaplan MD, Coleman PD. Interleukin-1β induces prostaglandin G/H synthease-2 (cyclooxygenase-2) in primary murine astrocyte cultures. *J Neurochem* 1996; 66: 2532—2540.
8. Mayberg MR, Okada T, Bark DH. The significance of morphological changes in cerebral arteries after subarachnoid hemorrhage. *J Neurosurg* 1990; 72: 626—633.
9. Nagata K, Sasaki T, Iwama J, Mori T, Ivamoto S, Nirei H, Hamada K, Kirino T. Failure of FK—506, a new immunosuppresant, to prevent cerebral vasospasm in the canine two-hemorrhage model. *J Neurosurg* 1993; 79: 710—715.
10. Nashizawa S, Peterson JW, Shimoyama I, Iwasaki K, Uemura K. Therapeutic effect of new immunosuppressant, FK—506, on vasospasm after subarachnoid hemorrhage. *Neurosurgery* 1993; 32: 986—992.
11. Vorkapic P, Bevan RD, Bevan J.A. Longitudinal time course of reversible and irreversible components of chronic cerebrovasospasm of the rabbit basilar artery. *J Neurosurg* 1991; 74: 951—955.
12. Kassell NF, Sasaki T, Colohan AR, Nozar G. Cerebral vasospasm following aneurysmal subarachnoid hemorrhage. *Stroke* 1985; 16: 562—572.
13. German JW, Gross CE, Giclas P, Watral W, Bednar MM. Systemic complement depletion inhibits experimental cerebral vasospasm. *Neurosurgery* 1996; 39: 141—146.

14. Peterson JW, Kwun BD, Teramura A, Hackett JD, Morgan JA, Nishizawa S, Bun T, Zervas NT. Immunological reaction against the aging human subarachnoid erythrocyte. *J Neurosurg* 1989; 71: 718—726.

15. Peterson JW, Kwun BD, Hackett JD, Zervas NT. The role of inflammation in experimental cerebral vasospasm. *J Neurosurg* 1990; 72: 767—774.

16. Peterson JW, Nishizawa S, Hackett JD, Bun T, Teramura A, Zervas NT. Cyclosporine A reduces cerebral vasospasm after subarachnoid hemorrhage in dogs. *Stroke* 1990; 21: 133—137.

17. Peterson JW, Roussos L, Kwun BD, Hackett JD, Owen CJ, Zervas NT. Evidence of the role of hemolysis in experimental cerebral vasospasm. *J Neurosurg* 1990; 72: 775—781.

18. Haba K, Ogawa N, Asanuma M, Hirata H, Sora YH, Mori A. Changes of neuropeptides and their receptors in experimental stroke gerbil brains. *J Neurological Sci* 1992; 108: 88—92.

19. Heijnen CJ, Kavelaars A, Ballieux RE. β-Endorphin: Cytokine and Neuropeptide. *Immunological Reviews* 1991; 119: 41—63.

20. Furi T, Satoh K, Asano Y, Shimosawa S, Hasuo M, Yaksh TL. Increase of β-endorphin levels in cerebrospinal fluid but not in plasma in patients with cerebral infarction. *J Neurosurg* 1984; 61: 748—751.

21. Hosobuchi Y, Baskin DS, Woo SK. Reversal of induced ischemic neurologic deficit in gerbils by the opiate antagonist naloxone. *Science* 1982; 215: 69—71.

22. Matsumiya N, Dohi S. Effects of intravenous or subarachnoid morphine on cerebral and spinal cord hemodynamics antagonism with naloxone in dogs. *Anesthesiology* 1983; 59: 175—181.

23. Babuna O, Gao GC, Wei ET, Chan P, Weinstein PR. Corticotropin-Releasing Factor; a powerful inhibitor of vasogenic edema in cold-traumatized rat brain. *10-th International Symposium on Microvascular Surgery for Cerebral Ischemia*, 1990; San Francisco, California, USA, July 13—15.

24. Baskin DS, Widmayer MA, Browning JL, Heizer ML, Schmidt WK. Evaluation of delayed treatment of focal cerebral ischemia with three selective ϰ-opioid agonists in cats. *Stroke* 1994; 25: 2047—2054.

25. Faden AI, Jacobs TP, Holoday JW. Opiate antagonist improves neurologic recovery after spinal injury. *Science* 1981; 211: 493—494.

26. Molina PE, Abumrad NN. Metabolic effects of opiates and opioid peptides. *Advances in Neuroimmunology* 1994; 4: 105—116.

27. Ibata Y, Kawakami F, Okamura HE, Obata-Tsuto HL, Morimoto N, Zimmerman EA. Light and electron microscopic immunocytochemistry of β-endorphin/β-LPH-like immunoreactive neurons in the arcuate nucleus and surrounding areas of the rat hypothalamus. *Brain Res* 1985; 341: 233—242.

28. Kerdelhue B, Bethea CL, Ling N, Chretien M, Weiner RI. β-endorphin concentrations in serum, hypothalamus and central grey of hypophysectomized and mediobasal hypothalamus lesioned rats. *Brain Res* 1982; 231: 85—91.

29. Schlachter LB, Wardlaw SL, Tindall GT, Frantz AG. Persistence of β-endorphin in human cerebrospinal fluid after hypophysectomy. *J Clin Endocrinol Metab* 1983; 57: 221—224.

30. Sacerdote P, Manfredi B, Bianchi M, Panerai AE. Intermittent but not continuous inescapable footshock stress affects immune responses and immunocyte beta-endorphin concentrations in the rat. *Brain, Behavior, and Immunity* 1994; 8: 251—260.

31. Smith EM, Morrill AC, Meyer WJ, Blalcok JE. Corticotropin releasing factor induction of leukocyte-derived immunoreactive ACTH and endorphins. *Nature* 1986; 32: 881—882.

32. Spangelo BL, Judd A.M, Isakson PC, MacLeod RM. Interleukin—6 stimulates anterior pituitary hormone release *in vitro*. *Endocrinology* 1989; 125: 575—577.

33. Armstead WM. Role of vasopresin in altered pial artery responses to dynorphin and β-endorphin following brain injury. *J Neurotrauma* 1996; 13: 115—123.

34. Olejnik I, Hendryk S, Pieniążek W, Jędrzejowska-Szypułka H. Corticotropin-Releasing Factor's influence on blood-brain barrier permeability after subarachnoid hemorrhage in rats. *Folia Neuropathol* 1994; 32: 182.

35. Hendryk S, Jędrzejowska-Szypułka H, Jarząb B, Jośko J. CRF influence on brain-blood barier permeability in acute, and chronic phases of cerebral ischemia in rats. *4th International Symposium on Intracranial Hypertension and Cerebral Ischemia in Clinical Practice*, 1997, Warsaw, Poland.

36. Hendryk S, Jarząb B, Łątka D, Mrówka R. CRF levels in cerebrospinal fluid in patients with vasospasm after subarachnoid haemorrhage. *Proc. of the Symposium of Polish Neurosurgeons Society* 1994, Łódź 1994, p. 25.

37. Solomon RA, Antunes JL, Chen RYZ, Bland L, Chien S. Decrease in cerebral blood flow in rats after experimental subarachnoid hemorrhage: A new animal model. *Stroke* 1985; 16: 58—64.

38. Barna I, Sweep CGJ, Veldhuis HD, Wiegant VM. Different effects of cisterna magna cannulation on β-endorphin levels in rat plasma and cerebrospinal fluid. *Acta Endocrinologica (Copenh)* 1988; 117: 517—524.

39. Furi T, Tanaka I, Iwata K. Alterations in $Na^+ - K^+ -$ ATPase activity and β-endorphin content in acute ischemic brain with and without naloxone treatment. *J Neurosurg* 1990; 72: 458—462.

40. Guillemin R, Vargo T, Rossier J, Minick S, Ling N, Rivier C, Vale W, Bloom F. β-endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. *Science* 1977; 197: 1367—1369.

41. Gramsch C, Hollt V, Mahraein P, Pasi A, Herz A. Regional distribution of methionine-enkephalin and beta-endorphin-like immunoreactivity in human brain and pituitary. *Brain Res* 1979; 171: 261—270.

42. Stengaard-Pedersen K, Larsson LI. Comparative immunocytochemical localization of putative opioid ligands in the central nervous system. *Histochemistry* 1981; 73: 89—114.

43. Young RF, Bach FW, Van Norman AS, Yaksh TL. Release of β-endorphin and methionine-enkephalin in to cerebrospinal fluid during deep brain stimulation for chronic pain. Effects of stimulation locus and site of sampling. *J Neurosurg* 1993; 79: 816—825.

44. Bidzinska B, Petraglia F, Angioni S, Genazzani AD, Criscuolo M, Ficarra G, Gallinelli A, Trentini GP, Genazzani AR. Acetyl-L-Carnitine effect on pituitary and plasma β-endorphin responsiveness to different chronic intermittent stressors. *J Neuroendocrinology* 1993; 5: 151—155.

45. De Souza EB, Webster EL, Grigoriadis DE, Tracey DE. Stress, neuroendocrine, and immune relationships. Corticotropin-Releasing Factor (CRF) and interleukin-1 (IL-1) receptors in the brain-pituitary-immune axis. *Psychopharmacol Bull* 1989; 25: 299—304.

46. Kavelaars A, Berkenbosch F, Croiset C, Ballieux RE, Heijnen C.J. Induction of beta-endorphin secretion by lymphocytes after subcutaneous administration of CRF. *Endocrinology* 1990; 126: 759—764.

47. Bernton EW, Beach JE, Holaday JW, Smallridge RC, Fein HG. Release of multiple hormones by a direct action of interleukin-1 on pituitary cells. *Science* 1987; 238: 519—521.

48. Brown SL, Smith LR, Blalock JE. Interleukin-1 and interleukin-2 enhance pro-opiomelanocortin gene expression in pituitary cells. *J Immunol* 1987; 139: 3181—3183.

49. Berkenbosch F, Van Oers J, Del Rey A, Tilders F, Besedovsky H. Corticotropin-releasing factor producing-neurons in the rat activated by interleukin-1. *Science* 1987; 238: 524—526.

50. Xia L, Matera C, Ferin, Wardlaw SL. Interleukin-1 stimulates the central release of corticotropin-releasing hormone in the primate. *Neuroendocrinology* 1996; 63: 79—84.

51. Dorn LD, Chrousos GP. The endocrinology of stress and stress system disorders in adolescence. *Adolescent Endocrinology* 1993; 22: 685—699.

428

52. Rivier C, Rivest S. Mechanisms mediating the effects of cytokines on neuroendocrine functions in the rat. In: D.J. Chadwick, J. Marsh, & K. Ackrill (Eds.) *Corticotropin-releasing factor. Ciba foundation symposium. Wiley: Chichester* 1992, 172: 204—225.
53. Sapolsky R, Rivier C, Yammamoto G, Plotsky P, Vale W. Interleukin-1 stimulates the secretion of hypothalamic corticotropin releasing factor. *Science* 1987; 238: 522—524.
54. Turnbull AV, Rivier C. Regulation of the HPA axis by cytokines. *Brain, Behavior, and Immunity* 1995; 9: 253—275.
55. Saito K, Suyama K, Nishida K, Się Y, Basile AS. Early increases in TNF-α, IL-6 and IL—1β levels following transient cerebral ischemia in gerbil brain. *Neurosci Lett* 1996; 206: 149—152.
56. Schotanus K, Meloen RH, Puijk WC, Berkenbosch F, Binnekade R, Tidlers FJH. Effects of monoclonal antibodies to specific epitopes of rats interleukin-1 beta (IL-1β) on Il—1β-induced ACTH, corticosterone and IL-6 responses in rats. *J Neuroendocrinology* 1995; 7: 255—262.
57. de Vries HE, Blom-Roosemalen MCM, van Oosten M, de Boer AG, van Berkal TJC, Breimer DD, Kuiper J. The influence of cytokines on the integrity of the blood-brain barrier in vitro. *J Neuroimmunol* 1996; 64: 37—43.
58. Kikuchi, T, Okuda Y, Kaito N, Abe T. Cytokine production in cerebrospinal fluid after subarachnoid haemorrhage. *Neurol Res* 1995; 17: 106—108.
59. Lane TA, Lamkin GE, Wancewicz EV. Protein kinase c inhibitors block the enhanced expression of intracellular adhesion molecule-1 on endothelial cells activated by interleukin-1, lipopolisaccharide and tumor necrosis factor. *Biochem Biophys Res Commun* 1990; 172: 1273—1281.
60. Shiga Y, Onodera H, Kogure K, Yamasaki Y, Yashima Y, Syozuhara H, Sendo F. Neutrophils as a mediator of ischemic edema formation in the brain. *Neurosci Lett* 1991; 125: 110—112.
61. Kimura T, Yamamoto T, Ota K, Shoji M, Inoue M, Sato K, Ohta M, Funyu T, Yoshinaga K. Central effects of interleukin-1 on blood pressure, thermogenesis, and the release of vasopressin, ACTH, and atrial natriuretic peptide. The neurohypophysis: A window on brain function. *Ann New York Acad Sci* 1993; 689: 330—345.
62. Oka T, Aou S, Hori T. Intracerebroventricular injection of interleukin-1β induces hyperalgesia in rats. *Brain Res* 1993; 624: 61—68.
63. Yabuuchi K, Minami M, Katsumata S, Yamazaki A, Satoh M. An in situ hybridization study on interleukin-1β mRNA induced by transient forebrain ischemia in the rat brain. *Molecular Brain Res* 1994; 26: 135—142.

Author's address: S. Hendryk, Department of Neurosurgery and Neurotraumatology, Poland, 41—902 Bytom, Al. Legionów 10, tel. +48(32) 2810271, fax. +48(32) 2810270, E-mail: neuro @zeus.polsl.gliwice.pl