Neuroendocrinology Letters ISSN 0172–780X

ORIGINAL ARTICLE

Expression of NADPH-diaphorase (NOS) in rat hypothalamo-neurohypophysial system after ischemic and traumatic brain injury

Barbara Gajkowska, Michal Walski & Hanna Olszewska-Badarczuk

Laboratory of the Cell Ultrastructure, Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Correspondence to: Barbara Gajkowska, Ph.D. D.Sci., Laboratory of the Cell Ultrastructure, Medical Research Centre, Polish Academy of Sciences, 5 Pawińskiego Str., 02-106 Warsaw, Poland
TEL.: +48 22 6086445, 6685277; FAX: +48 22 6685532
E-mail: gajk@cmdik.pan.pl

Submitted: *March 3, 1999*
Accepted: *October 22, 1999*

Key words: **hypothalamo–neurohypophysial system; rat; ultrastructure; NOS-histochemistry; brain injury**

*Neuroendocrinology Letters 1999; **20**:365–372 pii: NEL200699A01*

Abstract

To investigate the sites of nitric oxide synthase (NOS) expression after brain injury, NADPH-d histochemistry was performed on the hypothalamo-neurohypophysial system (HNS) of adult rats several days after both ischemia and trauma. Electron-microscopic examination revealed the sites of formazan end-product of the diaphorase reaction in some neurons, astrocytes, cells and vascular pericytes, and in all activated microglial/macrophagal cells in perivascular and juxtaneuronal regions of hypothalamus. In neurohypophysis, positive NADPH-d staining was present in cytosol of many pituicytes, endothelial cells, pericytes and corresponding axonal endings. NADPH-d activity was also present in perivascular macrophages or microglial cells of neurohypophysis. Finally, we suppose that NOS expression and the consequent productions of nitric oxide could be involved in pathophysiology of HNS injury by ischemia and trauma, where activations inducible isoform of NOS especially, may contribute to a variety of neurogenerative processes. The imbalance in regulation of nitric oxide could disturb the physiological function of this neuroendocrine system.

Introduction

Reports about the expression of nitric oxide synthase (NOS) and the role of nitric oxide (NO) in the pathophysiology of the endocrine system are scarce. In the hypothalamo-neurohypophysial system (HNS), the activity of NOS has been found in the hypothalamus, particularly in supraoptic and paraventricular nuclei and throughout the neurohypophy-sis, suggesting a neuromodulatory role of NO in vasopressin and oxytocin secretion [1, 2]. NOS inhibitors augment the corticotropin-releasing hormone, adrenocorticotropic hormone, oxytocin, luteinizing hormone and growth hormone release induced by a variety of stimuli. Thus, NO seems to inhibit hormone secretion [3]. However, in other experiments, NO was proposed to mediate release of corticotropin-releasing factor and luteinizing hormone-releasing hormone. Some reports showed that NO mediated vassopresin and oxytocin release in vitro and in vivo [4, 5]. Our previous studies have revealed up–regulation of NOS in HNS of rat after ischemic injury [6, 7]. These observations suggest the involvement of NO in the pathophysiology of HNS injury.

Material and Methods

Animals were handled according to the ethical guidelines of our Institution. 15 male rats, weighing 180–250g were used for this study. 5 rats were used as a control. 10 rats were subjected to total brain ischemia by 10 min cardiac arrest according to the method described earlier by Korpatchev [8]. Then the animals were resuscitated and kept alive up to 7 days. Then, in halothane anesthesia the cranium was trepanned by removing a 2 mm fragment of the bone in the fronto-temporal region. Mechanical trauma was applied to the hemisphere through the trepanned opening. The skin was sutured and the animals were sacrificed for morphological studies after 4 days. Morphological studies and NADPH-d histochemistry were performed as described earlier by Gajkowska and Walski [6]. The hypothalamo–neurohypophysial sections containing supraoptic (SO) and paraventricular (PV) nuclei and neurohypophysis were examined by the JEOL 1200EX electron microscope.

Results

The reaction for NADPH-d was performed to determine NO production in HNS. The distribution of NADPH-d in HNS of control animals was described in our earlier paper [6]. Here, we did not observe any differences in analyzed material.

Distribution of NADPH-d in nucleus supraopticus (SO) and nucleus paraventricularis (PV) after ischemic and traumatic injury.

We observed positive reaction for NADPH-d in many neurons of SO and PV (Fig. 1). The reaction product (15 nm granules) was associated with rough endoplasmic reticulum and ribosomes. We did not show any staining for other neuronal organella. The strong accumulation of NADPH-d deposit, clustered, was in cytosol of endothelial cells in association with ribosomes or mitochondria, and in some region of cytoplasm of pericytes (Fig. 2). The richest accumulation of NADPH-d deposit distributed in patched in cytoplasm was seen in perivascualr astrocytes and smooth muscle cells. A striking finding was the presence of NADPH-d reaction product in many microglial or macrophagal cells in perivascular and juxtaneuronal regions in whole HNS (Figs. 3, 4).

Distribution of NADPH-d in neurohypophysis (NH) after ischemic and traumatic injury.

A population of distinctly NADPH-d stained axonal endings was observed (Fig.5). Dense deposits of reaction product was seen in axoplasm between neurosecretory granules. The strong accumulation of NADPH-d deposit was found in many pericytes and pituicytes (Fig. 6,7). The deposits were distributed in patched in cytoplasm. NADPH-d expression was strong in perivascualr microglial cells and in macrophages (Fig. 8). The distribution and intensity of NADPH-d deposit in endothelial cells of neurohypophysis was similar to that described in SO and PV.

Discussion

The present study revealed expression of NOS in hypothalamo- neurohypophysial system in the rat brain subjected to a combined ischemic and traumatic brain damage. Electron-microscopic examination revealed the sites of formazan end-product of the diaphorase reaction in many neurons of supraoptic and paraventricular nuclei and their axonal endings in the neurohypophysis. In several recent studies using this histochemical marker (NADPH-d), it was discovered that these neurons are resistant to death in a wide variety of neurologic disorders, including stroke, Alzheimer's disease and Huntington's disease [9, 10, 11]. Additionally, these neurons are resistant to excitotoxic neuronal death mediated by glutamate or N-methyl-D-asparate (NMDA)in

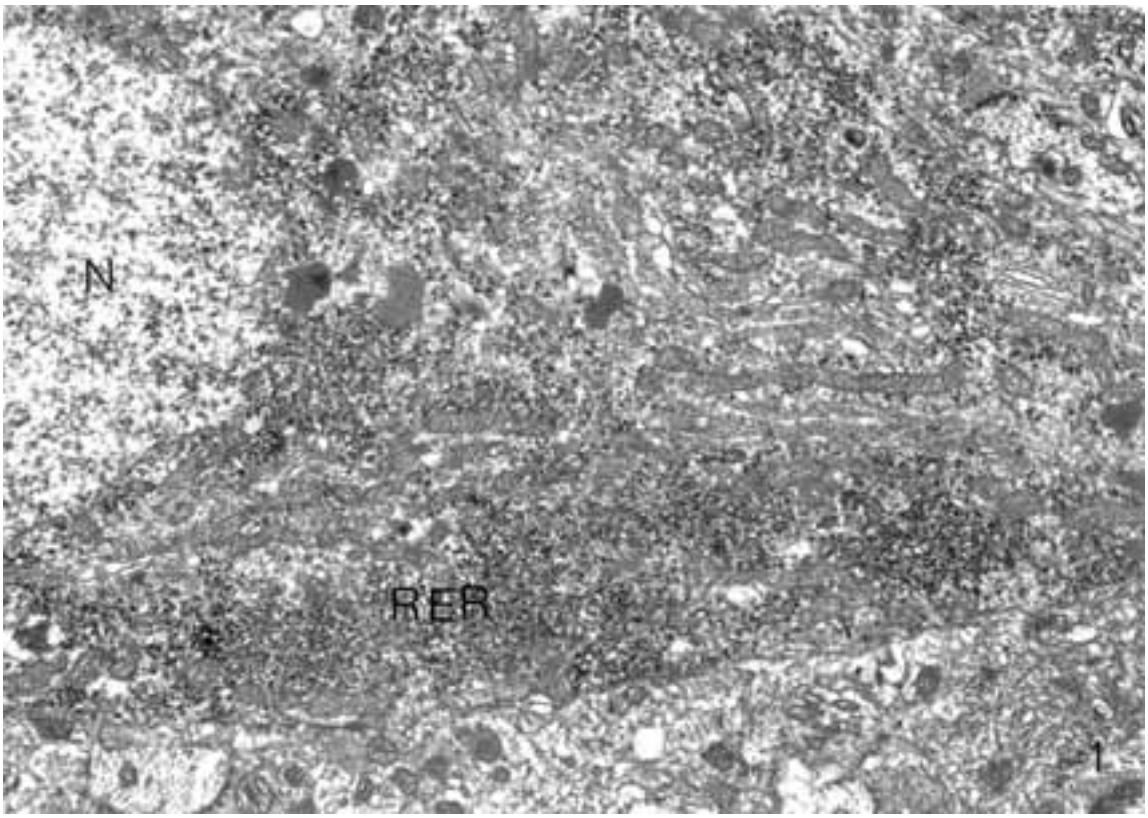

Fig. 1. Distribution of NADPH-d in nucleus supraopticus after ischemic and traumatic injury. Note strong accumulation of NADPH-d deposit in rough endoplasmic reticulum (RER) of the neuron (N) and non-stained neuropil. x 16,000

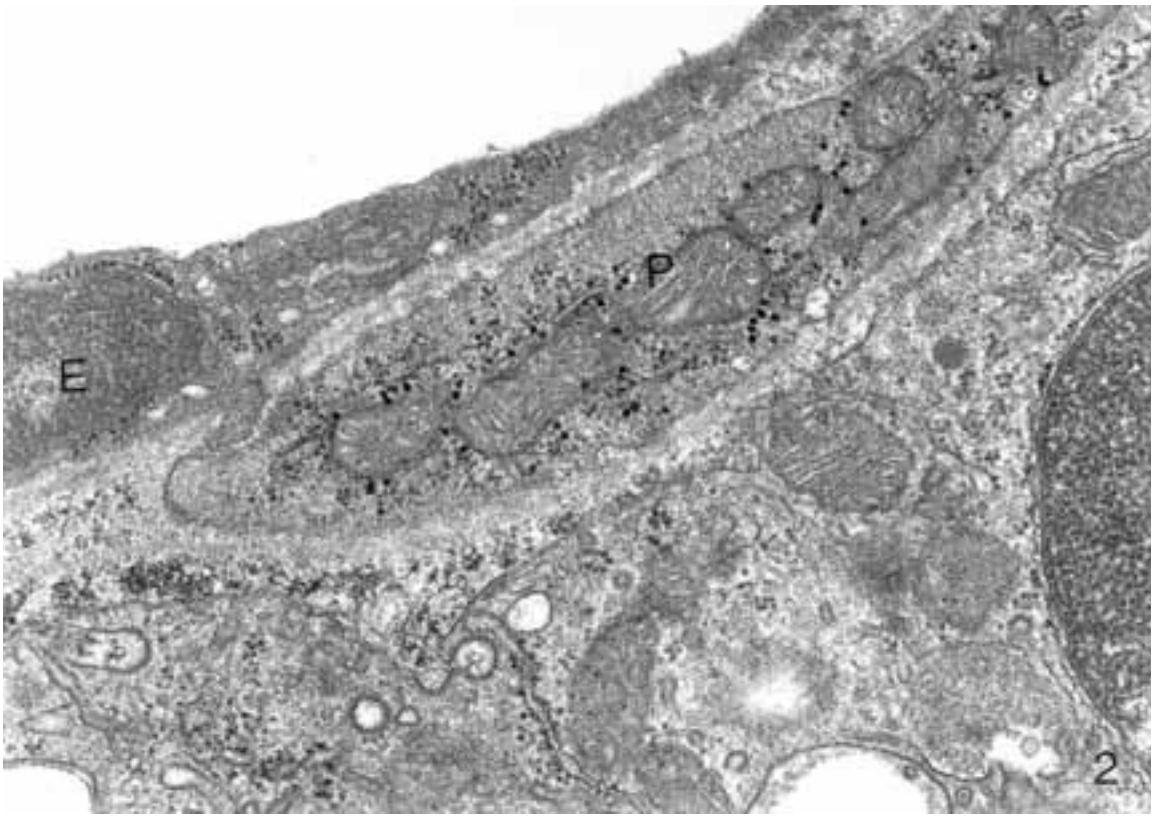

Fig. 2. Distribution of NADPH-d in nucleus paraventricular after ischemic and traumatic injury. NADPH-d deposit in cytosol of endothelial cell (E) and in pericyte (P) is present. x 50,000

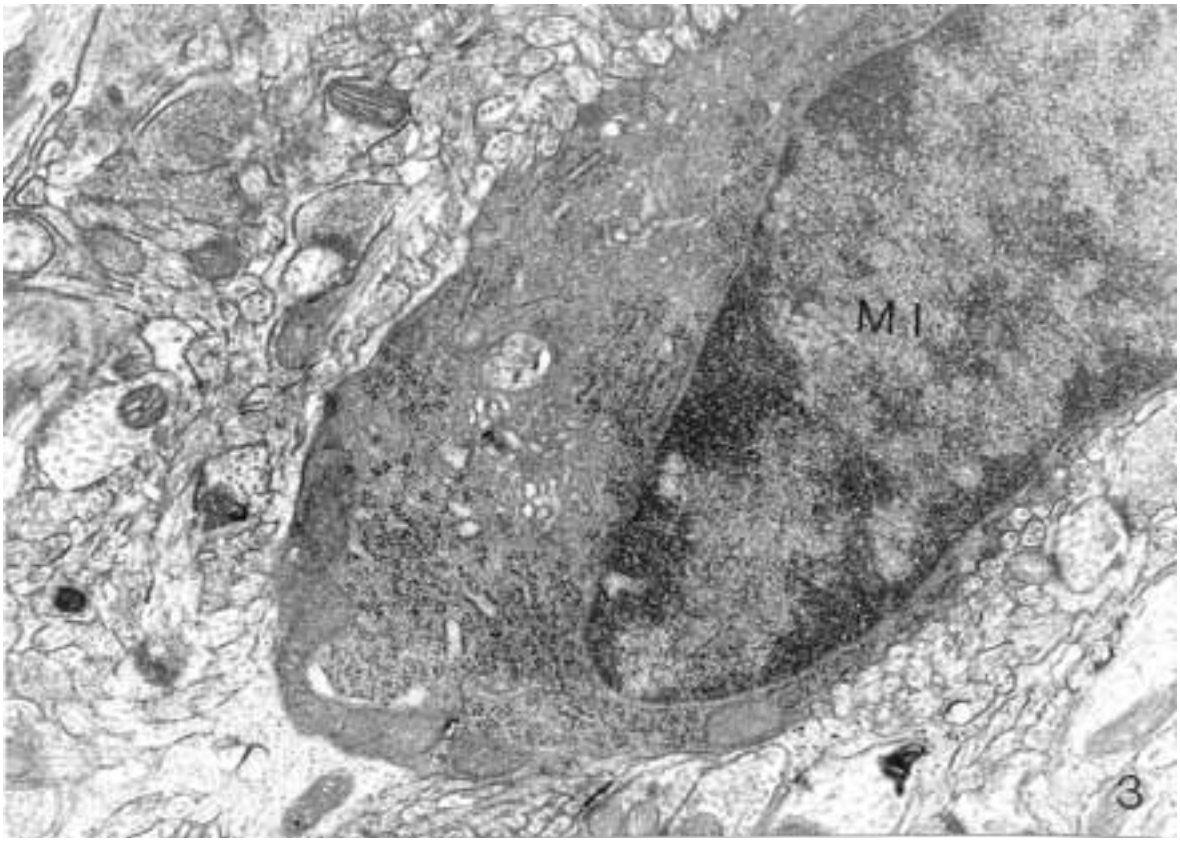

Fig. 3. Distribution of NADPH-d in nucleus supraopticus after ischemic and traumatic injury. Deposit of NADPH-d in cytosol of microglial cell (Mi) is present. x 30,000

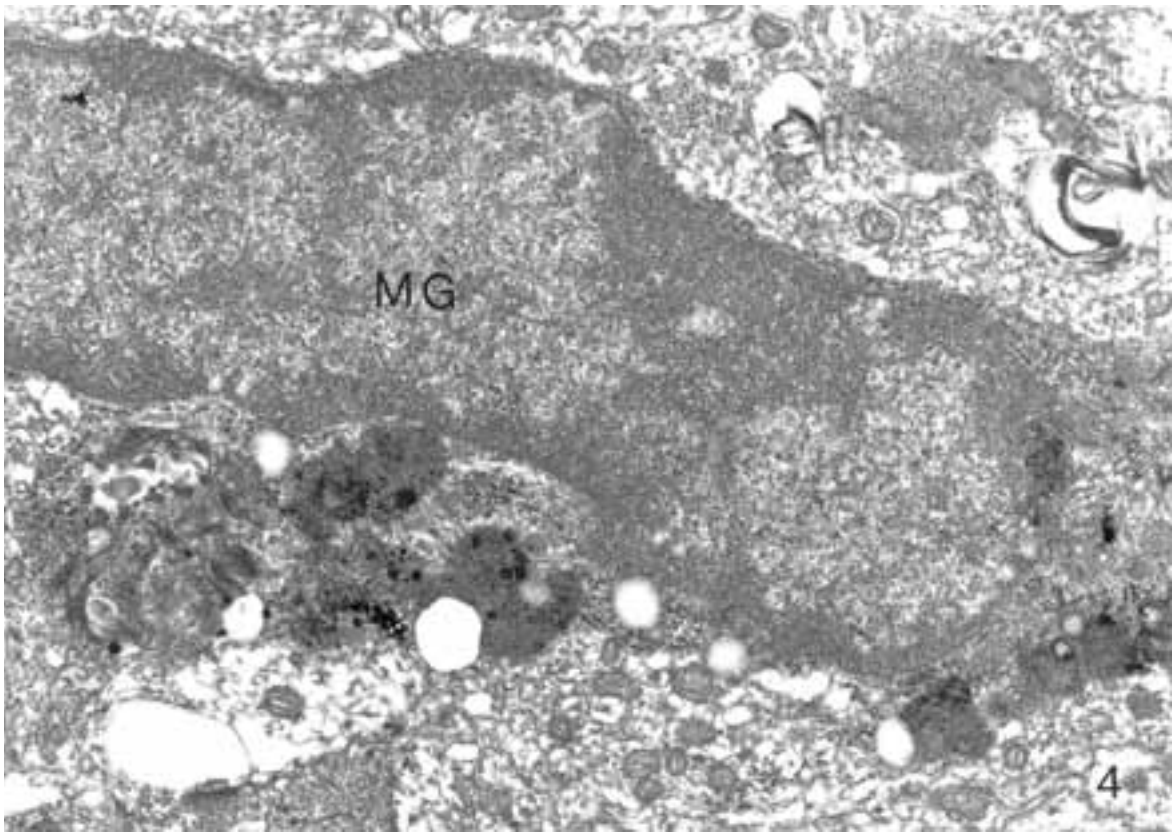

Fig. 4. Distribution of NADPH-d in nucleus supraopticus after ischemic and traumatic injury. Note deposit of NADPH-d in macrophages (MG). x 25,000

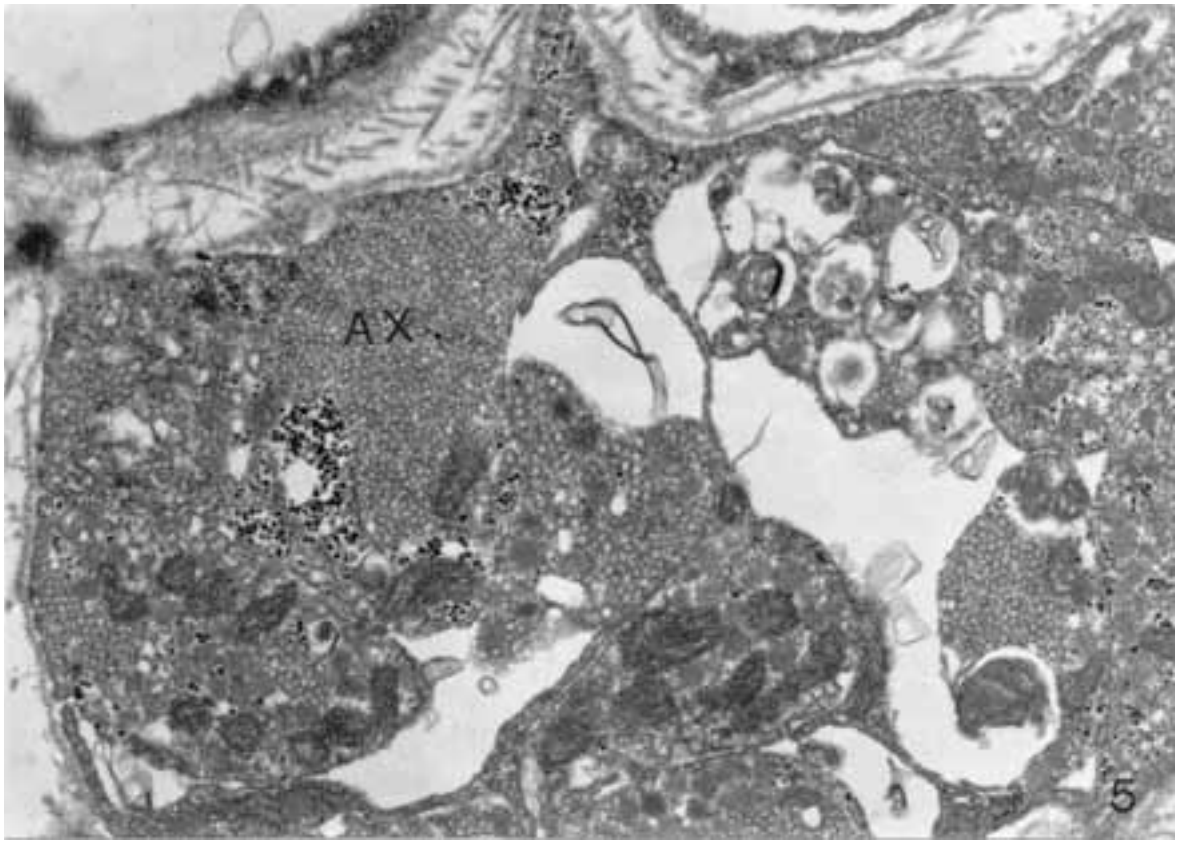

Fig. 5. Distribution of NADPH-d in neurohypophysis after ischemic and traumatic injury. Note NADPH-d deposit in cytoplasm axonal endings (Ax). x 37,000

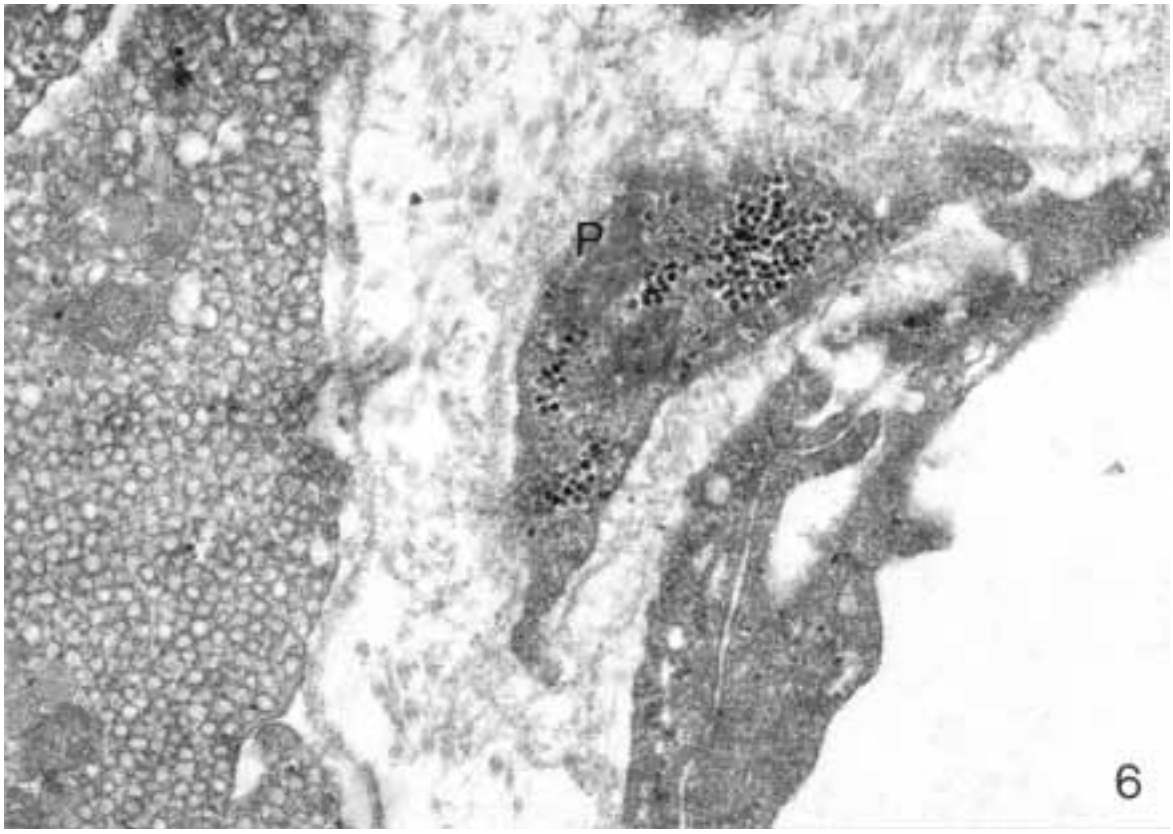

Fig. 6. Distribution of NADPH-d in neurohypophysis after ischemic and traumatic injury. Strong NADPH-d stained the perivascular pericyte (P). x 45,000

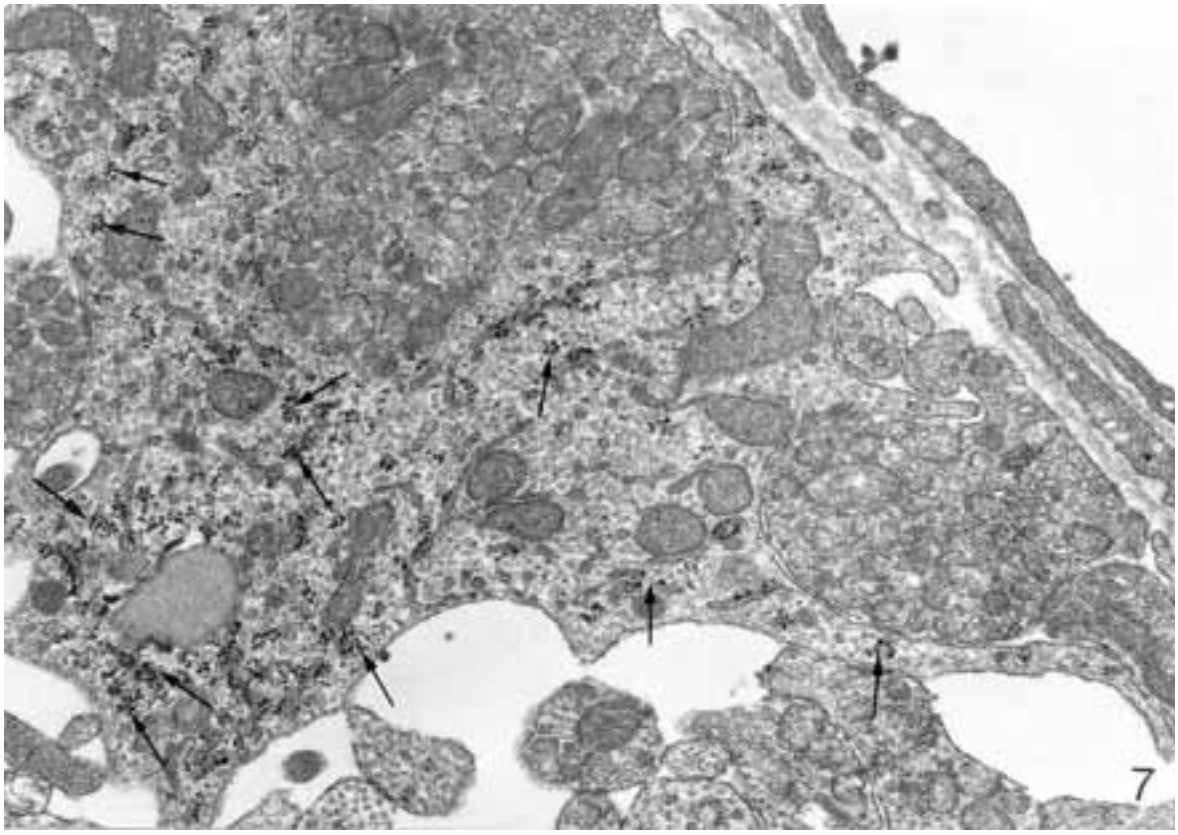

Fig. 7. Distribution of NADPH-d in neurohypophysis after ischemic and traumatic injury. The reaction product(arrows) in cytoplasm of pituicyte is seen. x 40,000

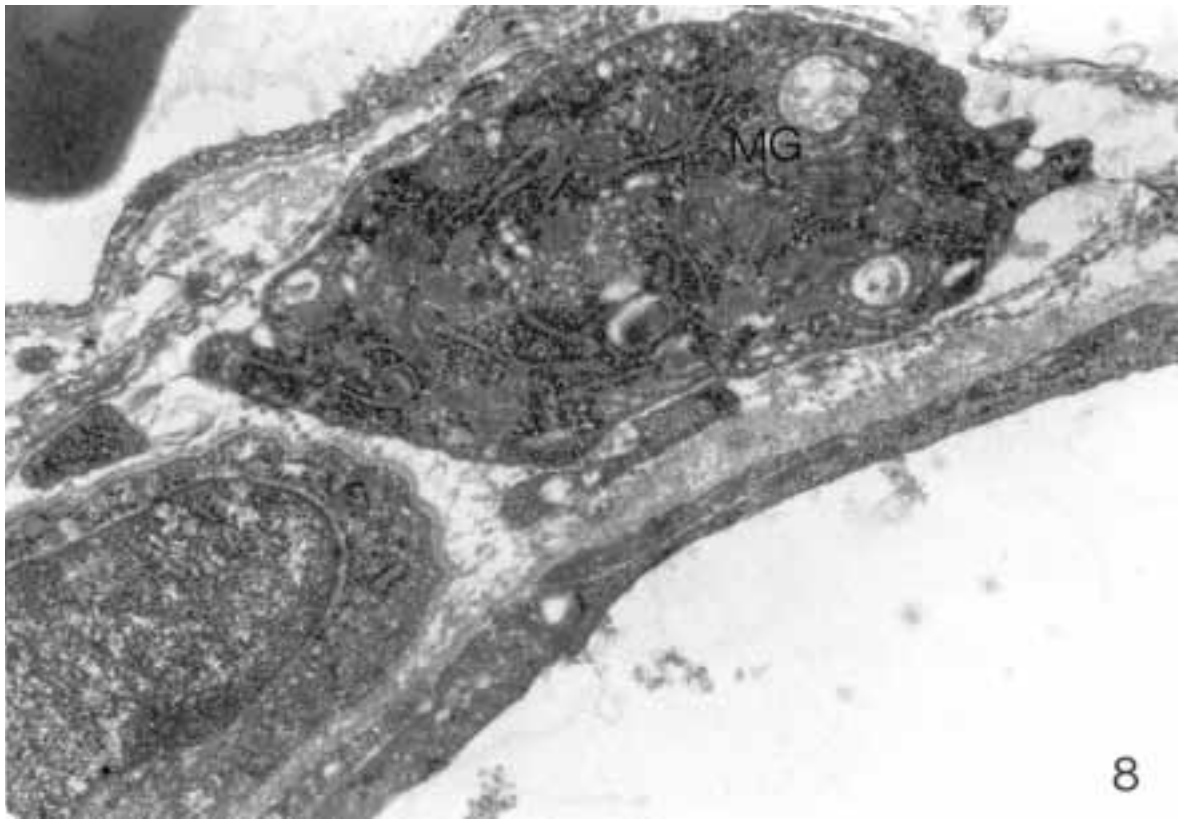

Fig. 8. Distribution of NADPH-d in neurohypophysis after ischemic and traumatic injury. Note perivascular macrophage (MG) with strong deposit NADPH-d in cytoplasm. x 30,000

experimental animals and in culture [12]. However NADPH-d positive neurons have been hypothesized to be lethal to adjacent cells [13]. Besides neuronal sites, NOS was concentrated in vascular endothelium in HNS. The increased NADPH-d staining was significant after ischemic and traumatic brain injury. In pathological states including traumatic brain injury and ischemia, over-production of NO in the brain may lead to vasodilatation and abnormal permeability [14, 15, 16]. Such enhanced expression of NOS in microvasculature may potentiate the vasogenic edema and hyperemia that is seen in human brain injury. However the role that NO plays in the mechanism of cerebral ischemia is multifaceted. Immediately after ischemia, NOS may be an important mechanism alleviating the deleterious effects of ischemia on brain tissue and limits the degree of ischemia [17]. More then 2 hr after ischemia, the vascular effects of NO are no longer beneficial [18]. Our recent immunocytochemical findings are also in general agreement with their reports. We showed that ischemia caused an increase e-NOS in labelling in capillary endothelial cells and additionally an increase in expression of e-NOS in perivascular mast cells, in a short time after ischemia [19, 7]. We propose that e-NOS induced in perivascular mastocytes mediated NO production in the early post-ischemic period and thus constitutes an important defense mechanism counteracting the effects of brain ischemia. Another potential source for NO under our experimental pathological conditions was expression of NOS in a variety of cell types in HNS including astrocytes, pituicytes, microglia, macrophages and smooth muscle cells encircling the blood vessels. All these cells contain inducible NOS (i-NOS) [20, 21, 7]. The i-NOS differs from the constitutive NOS of neurons and endothelial cells in that it is calcium independent, produces NO for extended periods, probably mediates most of the toxic actions of NO and is induced on stimulation with a variety of cytokines. We observed the increased NADPH-d staining intensities in many glial (astrocytes and pituicytes), microglial and macrophagal cells especially in perivascular and juxtaneuronal regions of HNS. Our findings are also in agreement with reports which clearly show NADPH-d activity in astroglial cells following brain injury [22, 23]. Also, immunocytochemical data revealed that activated glia or macrophages produce NO via i-NOS in various pathological conditions [24, 7]. In contrast to other isoforms of NOS, i-NOS is likely to cause dysfunction of HNS during ischemia and trauma and therefore observed expression of this isoform in glia and macrophages entering in the brain may be deleterious rather than protective. In conclusion, ultrahistochemical analysis revealed the expression of NADPH-d product reaction in most glial cells and macrophages frequently present in HNS after injury of the brain by ischemia and trauma. The imbalance in regulation of NO could disturb the physiological function of this neuroendocrine system.

Acknowledgments:

We thank Mrs. M. Zawistowska for her help in preparing the manuscript.

REFERENCES.

1 Bredt DT, Hwang PM, Snyder SH. Localization of nitric oxide synthase indicating a neural role of nitric oxide. Nature 1990; **347**: 768–770.
2 Vincent SR, Kimura H. Histochemical mapping of nitric oxide synthase in the brain. Neuroscience 1992; **46**:755–784.
3 Garthwaite J. Glutamate, nitric oxide and cell-cell signaling in the nervous system. Trends Neurosci 1991; **14**:60–67.
4 Yasin S, Costa A, Trainer P, Windle R, Forsling ML, Crossman A. Nitric oxide modulates the release of vasopressin from rat hypothalamic expants. Endocrinology 1993; **133**:1466–1469.
5 Ota M, Crotton JT, Festavan GT, Share L. Evidence that nitric oxide can act centrally to stimulate vasopressin release. Neuroendocrinology 1993; **57**:955–959.
6 Gajkowska B, and Walski M. Ultrastructrural demonstration of NADPH-diaphorase, a marker for nitric oxide synthase in hypothalamo-neurohypohysial system, hippocampus and cerebral cortex of the rat after ischemia. Neuroendocrinology Letters 1998; **19**:2:79–85.
7 Gajkowska B, Viron A, Cholewiñski M. Immunocytochemical localization of endothelial nitric oxide synthase (e-NOS) and inducible nitric oxide synthase (i-NOS) in rat neurohypophysis after cerebral ischemia. Folia Neuropathol 1999; **37**:1: 11–20.
8 Korpathev VG, Lysenko SP, Tiel LZ Models of clinical death and postreanimational disease in rats. Pathol Fizjol Exp Terapia 1982; **3**:78–80.
9 Ferrante RJ, Kowall NW, Richardson EP Jr, Bird ED, Martin JB. Selective sparing of a class of striatal neurons in Huntington's disease. Science 1985; **230**:561–563.
10 Uemura Y, Kowall NW, Beal MF. Selective sparing of NADPH-diaphorase-somatostatin-neuropeptide Y neurons in ischemic gerbil striatum. Ann Neurol 1992; **27**:620–625.
11 Hyman BT, Harzloff K, Wenniger JJ, Dawson TM, Bredt DS, Snyder SH. Relative sparing of nitric oxide synthase-containing neurons in the hippocampal formation in Alzhaimer's disease. Ann Neurol 1992; **32**:818–820.
12 Koh JY, Peters S, Choi DW. Neurons containing NADPH-diaphorase are selectively resistant to quinolinate toxicity. Science 1986; **234**:73–76.
13 Dalkara T, Yoshida T, Irikura K, Moskowitz MA. Dual role of nitric oxide in focal cerebral ischemia. Neuropharmacology 1994; **33**: 1447–1452.
14 Moncada S and Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993; **329**:2002–2012.
15 Cobs Ch, Fenoy SA, Bredt DS, Noble LJ. Expression of nitric oxide

synthase in the cerebral microvasculature after traumatic brain injury in the rat. Brain Research 1997; **751**:336–338.
16 Gajkowska B, Mossakowski MJ. Endothelial nitric oxide synthase in vascular endothelium of rat hippocampus after ischemia: evidence and significance. Folia Neuropathol 1997; **353**:171–180.
17 Zhang F, White JG, Iadecola C. Nitric oxide increase blood flow and reduce brain damage in focal ischemia: evidence that nitric oxide is beneficial in the early stages of cerebral ischemia. Journal of Cerebral Blood Flow and Metabolism 1994; 14: **2**:217–226.
18 Zhang F, Xu S, Iadecola C. Time dependence of effect of nitric oxide synthase inhibition on cerebral ischemic damage. Journal of Cerebral Blood Flow and Metabolism 1995; **15**:4:595–601.
19 Gajkowska B, Gniadecki R. Induction of endothelial nitric oxide synthase in perivascular mast cells in rat neurohypophysis after ischemia. Neuroendocrinal Letters 2000; in press.
20 Dawson VL, Dawson TM, Bartley DA, Uhl GR, Snyder SH. Mechanism of nitric oxide-mediated neurotoxicity in primary brain cultures. J Neurosci 1993; **13**:2651–2661.
21 Murphy S, Simmons ML, Agullo L, Garcia A, Feinstein DL, Galea E, et al. Synthesis of nitric oxide in CNS glia cells. Trends Neurosci 1993; **16**:323–328.
22 Wallace MN, Fredens K. Activated astrocytes of the mouse hippocampus contain high levels of NADPH-diaphorase. Neuro Report 1992; **3**:953–956.
23 Redigor J, Montesdeoca J, Ramirez-Gonzales JA, Hernandez-Urquia CM, Divac I. Bilateral induction of NADPH-diaphorase activity in neocortical and hippocampal neurons by unilatelar injury. Brain Res 1993; **631**:171–174.
24 Lorsbach RB, Murphy WJ, Lowenstein ChJ, Snyder SH, Russell SW. Expression of the nitric oxide synthase gene in mouse macrophages activated for tumor cell killing. Molecular basis for the between interferon- and lipopolysaccharide. The Journal of Biological Chemistry 1993; **268**: 3:1908–1913.

Neuroendocrinology Letters ISSN 0172–780X