FOLIA NEUROPATHOL. 1997, 35, 1
PL ISSN 0028-3894

BARBARA GAJKOWSKA[1], MIROSŁAW J. MOSSAKOWSKI[2]

ENDOTHELIN-LIKE IMMUNOREACTIVITY IN HIPPOCAMPUS FOLLOWING TRANSIENT GLOBAL CEREBRAL ISCHEMIA. II. THE BLOOD-BRAIN INTERPHASE

[1] Laboratory of the Ultrastructure of the Nervous System and [2]Department of Neuropathology, Medical Research Centre, Polish Academy of Sciences, Warszawa

The effect of transient, global cerebral ischemia on the distribution of endothelin (ET) in blood-brain barrier (BBB) in CA1 area of hippocampus long-time after ischemia was estimated using post-embedding immunogold technique.
ET-like immunoreactivity as a gold particles was localized in all compartments of the blood-brain barrier e.g. in endothelial cells, in pericytes, in periendothelial space including basement membrane, and in astroglial processes. In control animal the density of labelling in all elements of BBB in CA1 area of hippocampus was moderate. ET-like immunoreactivity (ET-like IR) was estimated 1 week – 12 months after ischemia. Intense ET-like IR in all elements of BBB was noted 2 and 6 months after ischemia. A potential pathophysiological role of endothelin in cerebral vasospasm in long-time after ischemia is well documented.

Key words: *ischemia, endothelin, immunocytochemistry, blood-brain barrier, hippocampus*

The possibility that endothelins have a role in the development of neuronal cell death following transient forebrain ischemia inclined us to study the morphological and immunocytochemical properties of the blood-brain interphase (Fuxe et al. 1989; Yamashita et al. 1993; Gajkowska, Mossakowski 1995).

The potential role of endothelin (ET) in pathomechanism of cerebral ischemia is supported by both animal studies and clinical observations in patients. Endothelins are potent vasoconstrictors of large and small cerebral arteries both *in vivo* and *in vitro*, and the long-lasting and extensive vasoconstriction produced by exogenously administrated peptides causes tissue damage similar to that observed following ischemia in rat, cat and dog brain (Kurosawa et al. 1991; Robinson et al. 1991; Fuxe et al. 1992).

Bilateral carotid artery occlusion (cerebral ischemia) followed by reperfusion is associated with elevated plasma immunoreactivity in ET-1 in gerbils (Wilette et al. 1993) and in anesthetized rabbits intracerebro-ventricular injection of ET-1 causes significant reduction of CSF production *via* sustained vasoconstriction in the choroid plexus (Schalk et al. 1992).

Transient forebrain ischemia significantly augmented ET-1, and ET-3-like immunoreactivity in hippocampus of stroke-prone spontaneously hypertensive rats (Yamashita et al. 1993) and in normal rats (Gajkowska, Mossakowski 1995).

This brief review focuses on immunocytochemical evidences of long-lasting vasoconstrictor properties of endothelin in the narrowing of cerebral arteries.

Material and methods

We used 20 adult male Wistar rats (250-300 g). Sixteen of them were subjected to experimental 10 min cerebral ischemia. This procedure was described in the previous works (Gajkowska et al. 1989; Gajkowska, Mossakowski 1995).

For ultrastructural and immunocytochemical studies the animals were decapitated 1 week, 2 weeks, 2, 6 and 12 months after ischemia. The rats were anesthetized with ether and perfused intracardially with 0.9% NaCl (1 min) followed by 0.5% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M of sodium phosphate buffer (PBS) of pH 7.4 (30 min). Blocks of the tissue were taken from CA1 area hippocampus, rinsed for 2 hours in PBS, treated with 1% osmium tetroxide for 1 hour, dehydrated in increasing gradients of ethanol and finally embedded in Epon. Ultrathin sections were processed according to the post-embedding immunogold procedure. Briefly, the section were mounted on the formwar-coated golden grids, placed in 10% hydrogen peroxide for 10 min, rinsed in PBS for 15 min

and exposed for 15 min to 5% bovine serum albumin in PBS.

Monoclonal antibody to endothelin 1, 2, 3(human) (Biogenesis, UK, Cat. No 4113-0957) was diluted 1:100 in PBS and applied on the slices for 3 hours in 37°C. Then the grids were washed in PBS for 30 min and exposed to goat anti-rat IgG(H+L) (Human ABS) conjugated with colloidal gold particles of 10 nm in diameter (Janssen Pharmaceutica, Beerse, Belgium) diluted 1:50 PBS. After incubation for 30 min

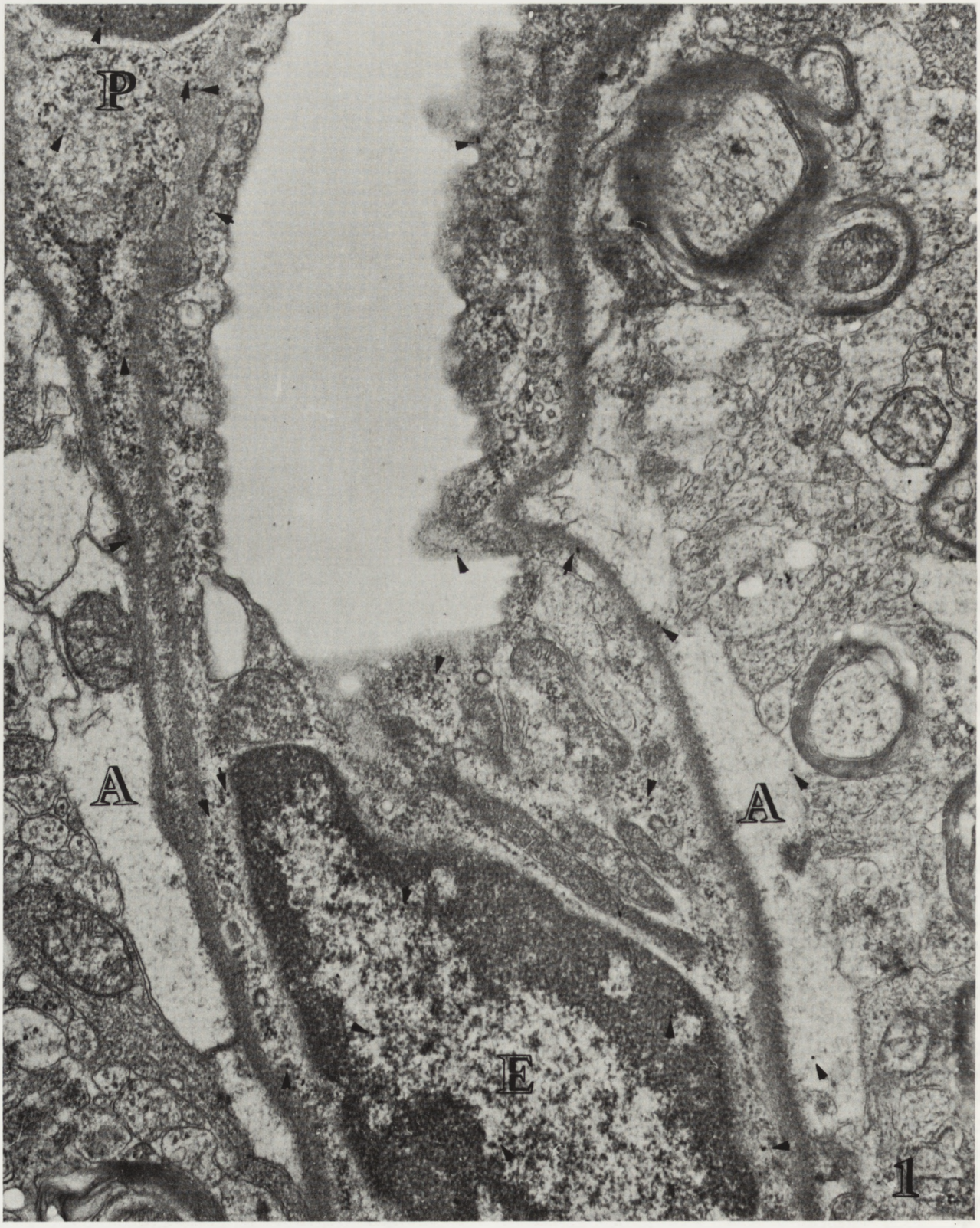

Fig. 1. Control animals. Immunocytochemical localization of ET-like immunoreactivity in microvessels of CA1 area of hippocampus. Note localization of golden particles in all compartments of the blood-brain barrier; endothelial cell (E), basement membrane, pericyte (P) and very low labelling in perivascular astroglial processes (A). ×26 500

in darkness, the grids were washed with PBS for 15 min, followed by distilled water for 15 min. Tissue slices were air-dried, stained for 10 min with 4.7% uranyl acetate and for 2 min with lead citrate. Control slices were prepared using normal murine serum instead of anti-endothelin antibody. The sections were examined and photographed using JEOL 1200 EX electron microscope.

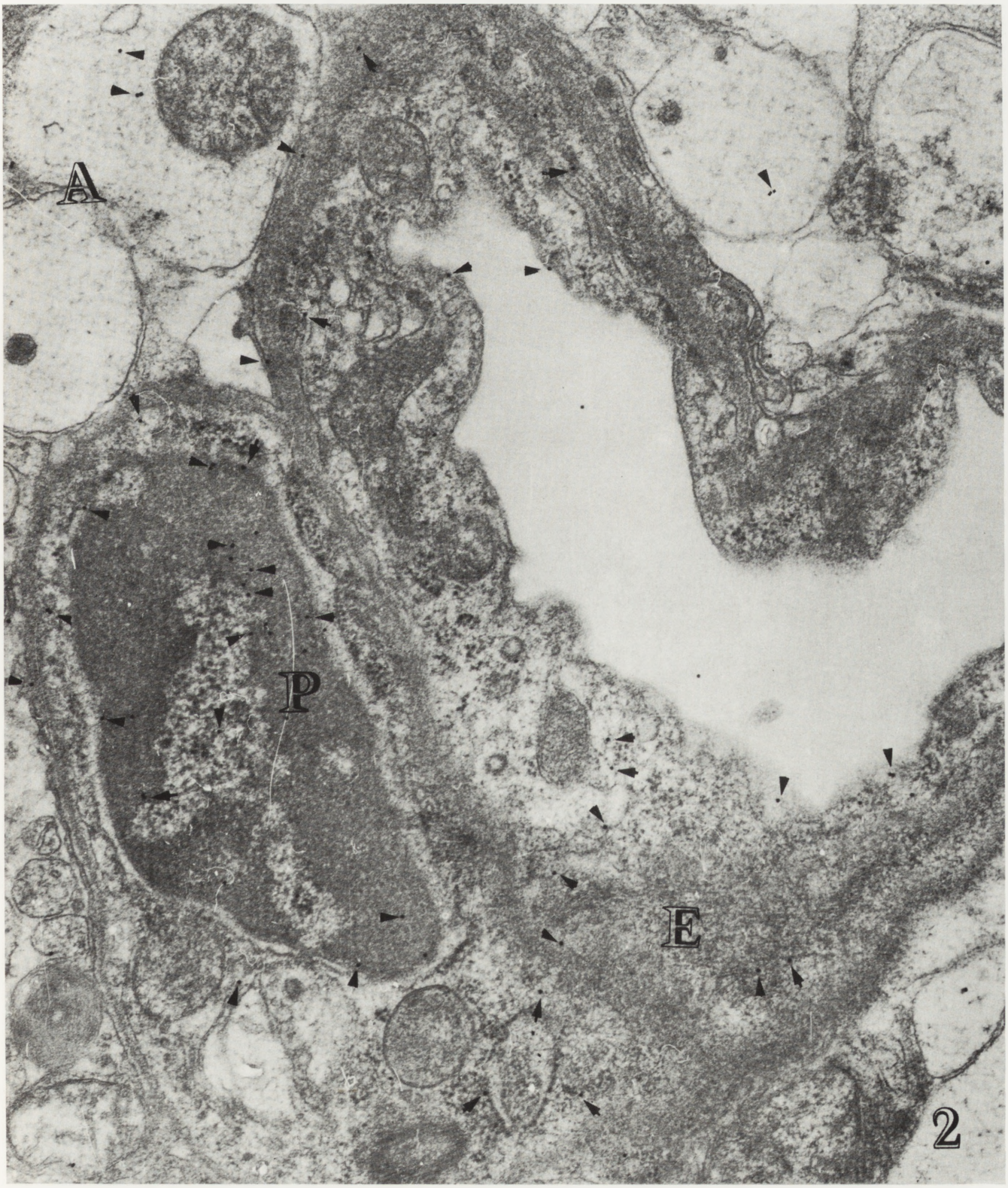

Fig. 2. Experimental animal. One week after ischemia. As indicated by arrowheads, slightly intense immunoreactivity to ET in endothelial cell (E) and pericyte (P) is present. ×42000

Results

Control animal. In CA1 area of hippocampus ET-like immunoreactivity was apparent in four compartments of the blood-brain barrier; endothelial cells (E), subendothelial (perivascular) space including basement membrane, in pericytes (P) and in perivascular astroglial (A) processes. In endothelial cells the gold particles were diffusely distributed over the nucleus and cytoplasm in moderate density (Fig. 1).

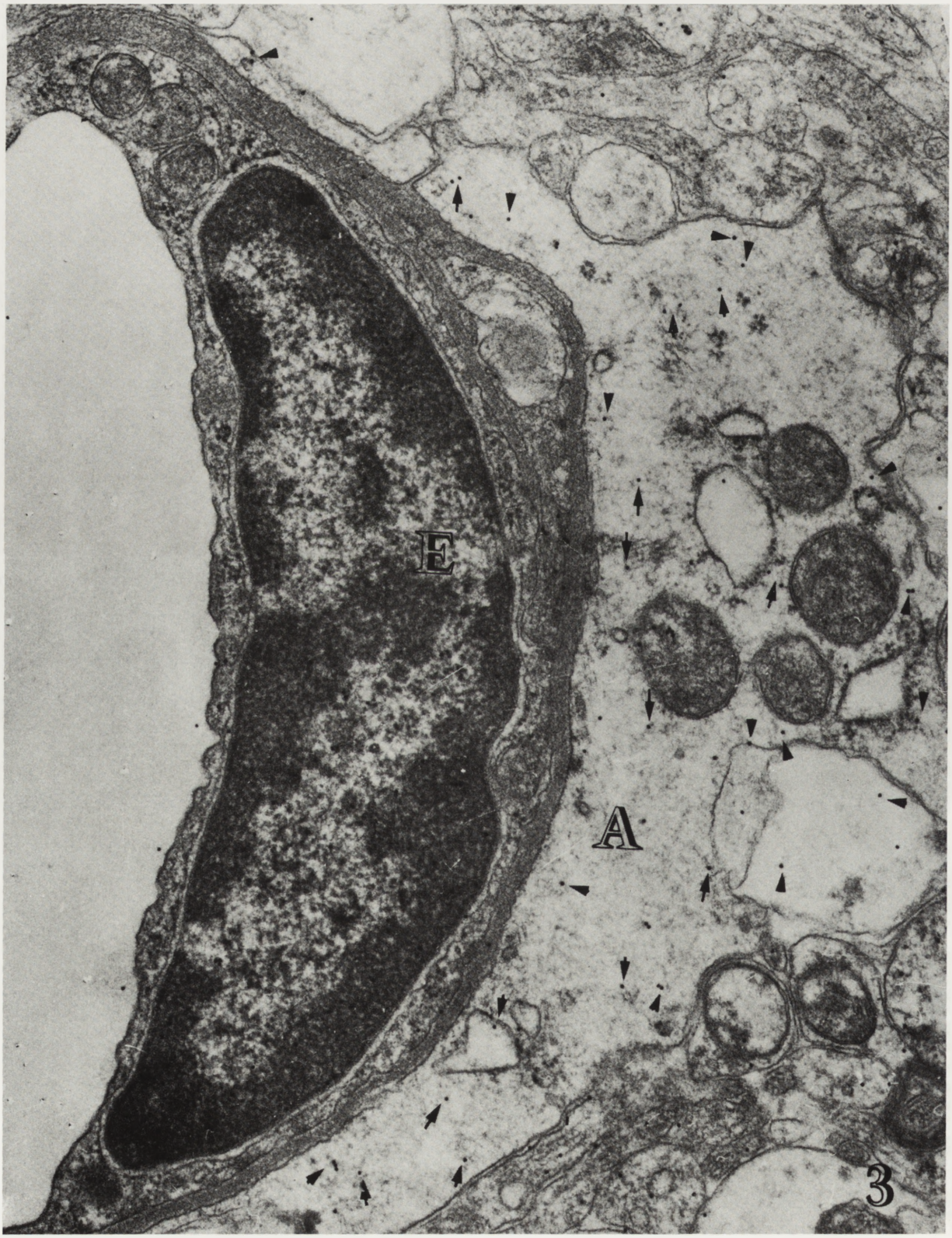

Fig. 3. Experimental animal. One week after ischemia. Intense immunoreactivity to ET in the perivascular astroglial processes is seen. ×42 000

Lower labelling density was found in the sub-endothelial space and in perivascular astroglial cells or in axonal endings in the brain parenchyma.

The labelling density of pericytes was comparable to that of the endothelial cells.

Experimental animal.

One week after ischemia many capillary profiles showed slight increase in the labelling density over endothelial cells (E) and over pericytes (P). High density of gold particles over astroglial (A) processes

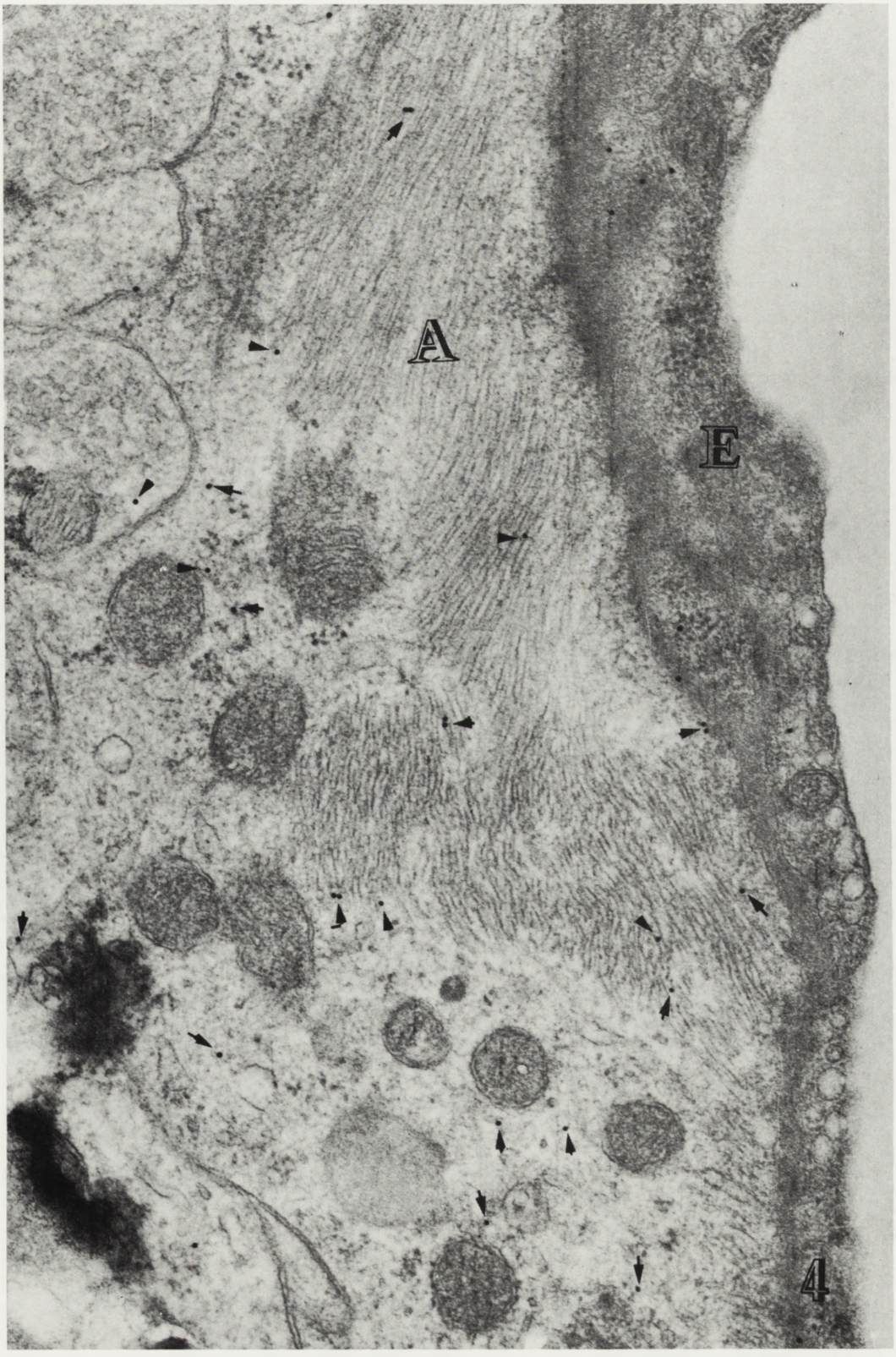

Fig. 4. Experimental animal. Two weeks after ischemia. A marked endothelin-like immunoreactivity is present in perivascular astroglial cell. × 51 000

was seen, but ET-like immunoreactivity in subendothelial space including basement membrane was similar to those found in the control animals (Fig. 2, 3).
Two weeks after ischemia. ET-like immunoreactivity in all compartments of blood-brain barrier was similar to those found in the group described above. The labelling density of the perivascular astroglial (A) cells was highest (Fig. 4).
Two and six months after ischemia. In the majority of the examined capillaries significant increase in the labelling density in four compartments of blood-brain barrier was observed. The labelling density of endothelial cells

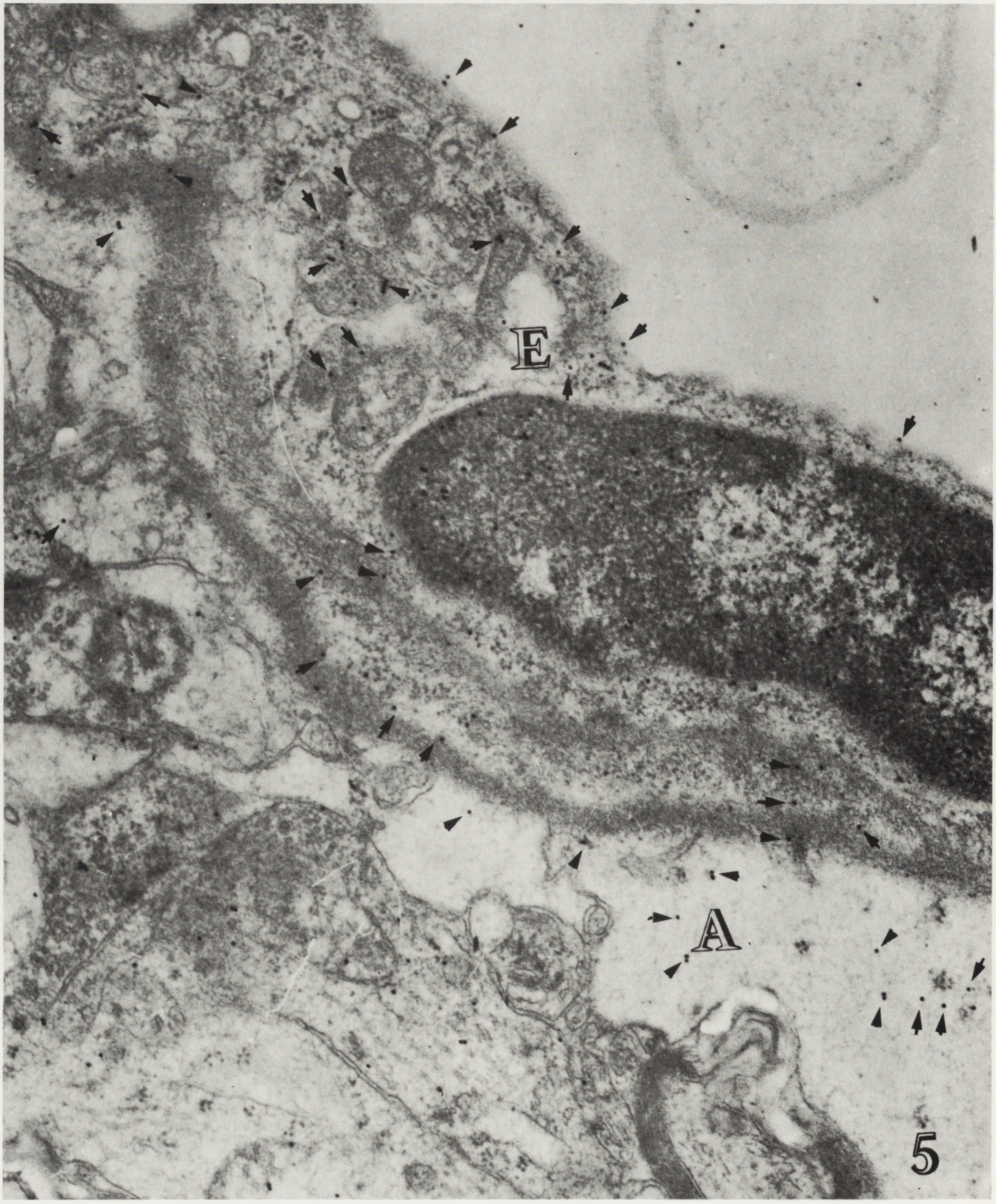

Fig. 5. Experimental animal. Two months after ischemia. Note a significant increase in the labelling density in all compartments of blood-brain barrier and in some elements of brain parenchyma. ×42 000

(E), pericytes (P) and astroglial (A) processes was significantly higher than that of the previously described in control and experimental groups. Additionally, the strong ET-like- immunoreactivity in macrophages (M) neighboring blood vessels was observed (Fig. 5, 6, 7).
Twelve months after ischemia. Many capillary profiles showed ET-like immunoreactivity in all compartments of the blood-brain barrier similar to those found in the control animals (Fig. 8). Occasionally, strong ET-like immunoreactive microglial cells and macrophages were present in neighboring blood vessels, or in close proximity to blood vessels.

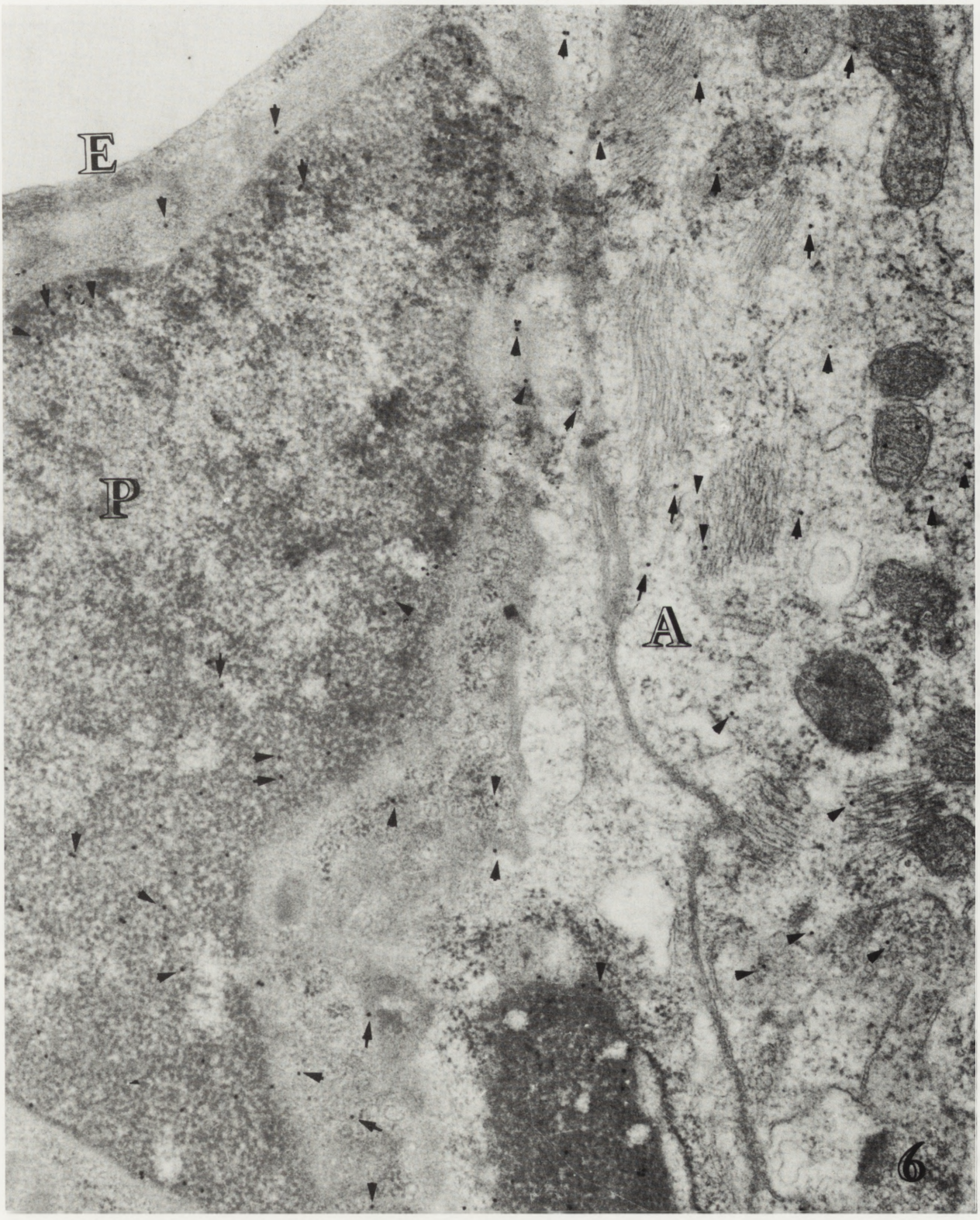

Fig. 6. Experimental animal. Six months after ischemia. Intense immunoreactivity to ET is demonstrated in pericyte and astroglial cell. × 26 500

Discussion

The potent and long-lasting vasoconstriction produced by endothelin (ET) raised the possibility that the peptide may be contribute to a reduction of blood flow (ischemia) to several organs, including brain, heart, lung and kidney.

Our immunocytochemical investigation suggests that even 6 months after ischemia ET-like immunoreactivity in all compartments of the blood-brain barrier (BBB) is higher to that found in the normal BBB in many blood vessels of CA1 area of hippocampus. It is widely assumed that endothelin (ET) must contribute to many pathological events

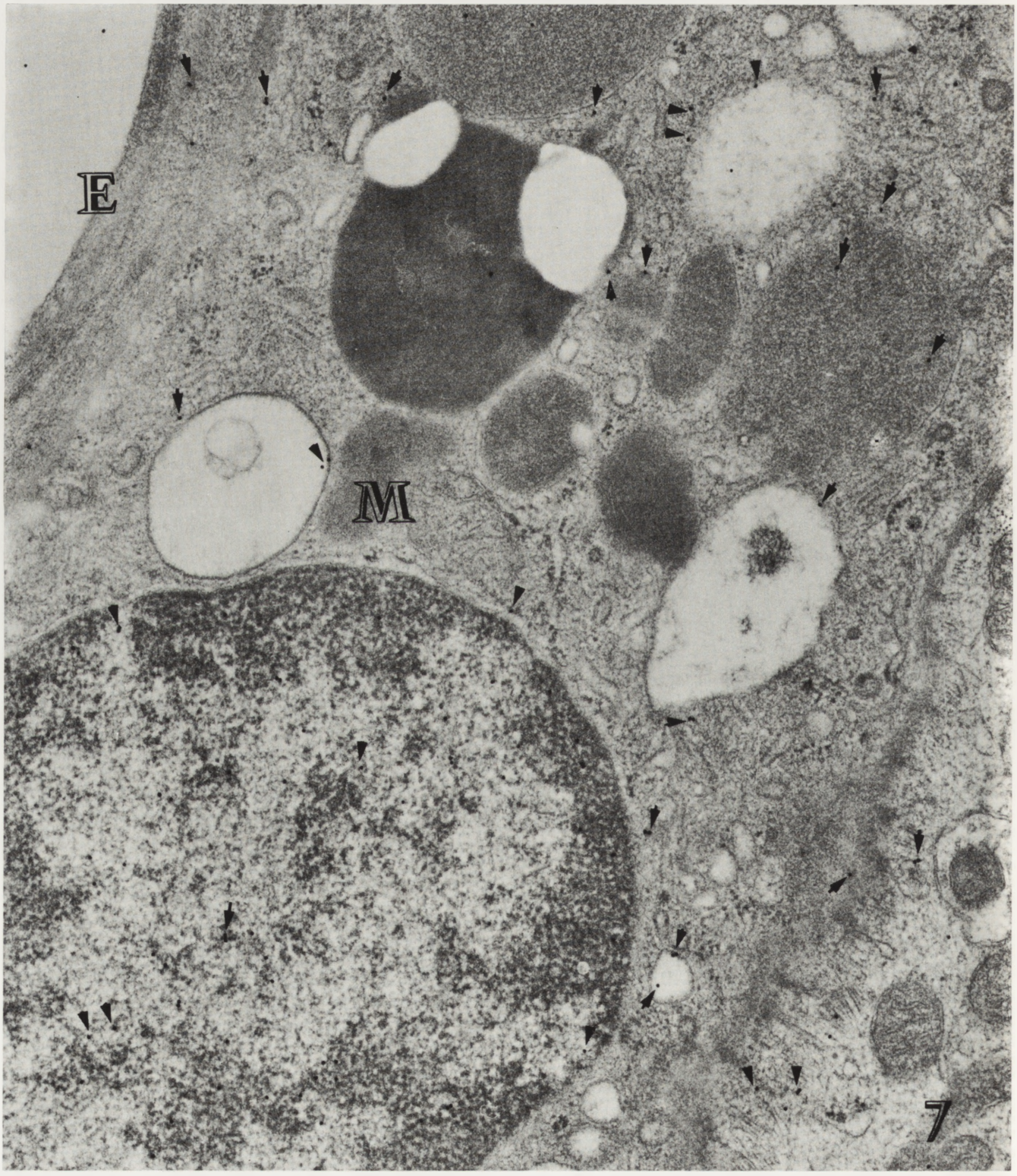

Fig. 7. Experimental animal. Two months after ischemia. Strong endothelin-like immunoreactivity is observed in perivascular macrophages. × 28 000

rather than to play a role in physiological homeostatic mechanism.

The potential role of ET in the pathomechanism of cerebral ischemia is supported by studied animals as well as clinical observations in patients. Topical application of ET-1 to the middle cerebral artery in the rat *in vivo* causes a reduction in blood flow and ischemic cell damage similar to that observed after occlusion of the same arteries (Robinson et al. 1991).

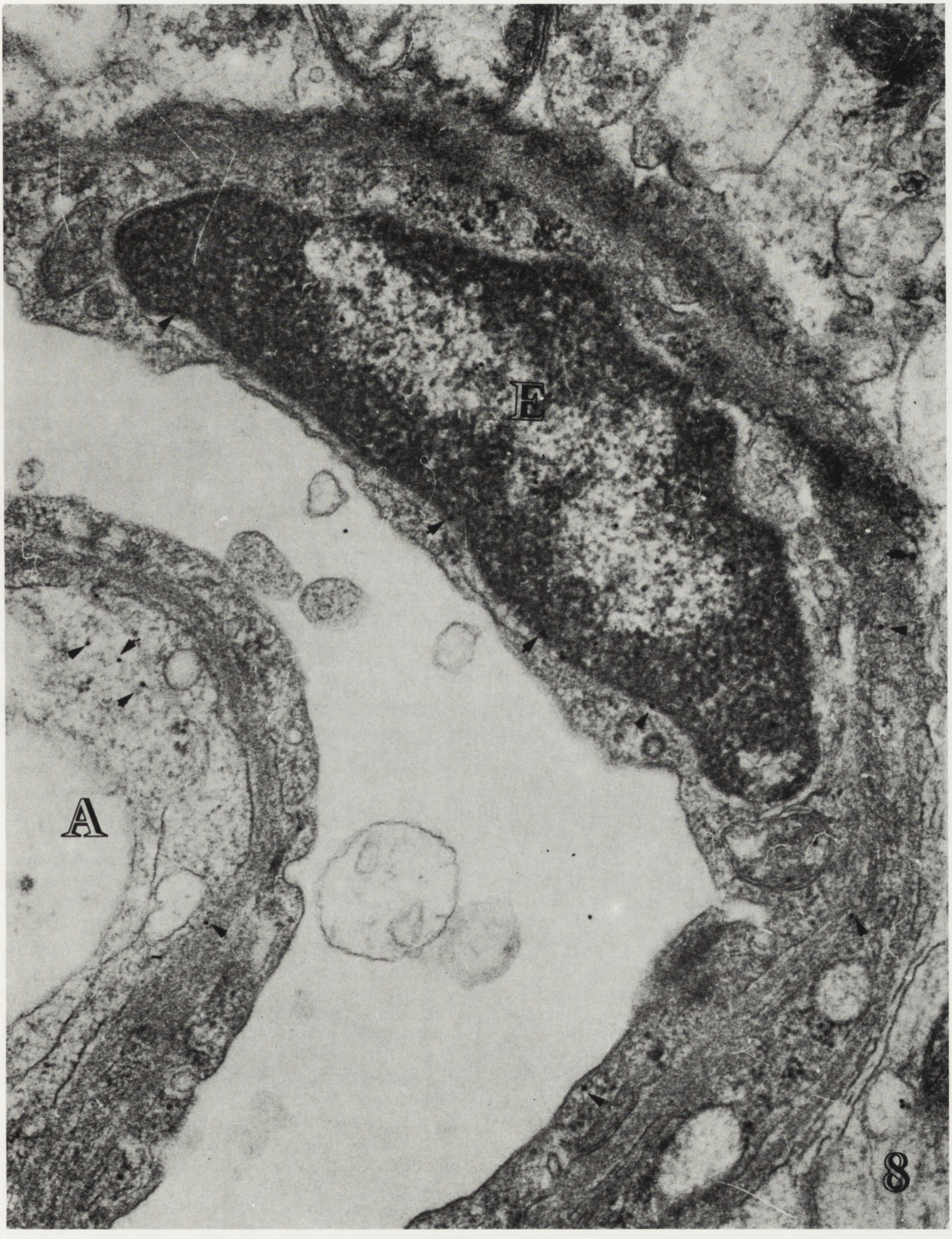

Fig. 8. Experimental animal. Twelve months after ischemia all compartments of microvessel are decorated with gold particles with moderate density. ×26 000

These findings in animal experiments were supported by clinical studies demonstrating significant elevation of plasma in ET-1 in patients with cerebral ischemia. Ziv et al. (1992) postulated that excessive production of ET-1 may cause vasoconstriction in the collateral circulation, thereby enlarging the area of tissue damage.

In addition, to promoting ischemia through vasoconstriction, ET might contribute to the pathogenesis of stroke by direct effects on neurons or glial cells. Damage of neurons after ischemia is thought to result mainly from increases in Ca^{2+} (Choi, Rothman 1990). Indeed, ET increases Ca^{2+} in cultured glial cells and neuroblastoma cells (Yue et al. 1990, Masault et al. 1990). ET produced in astrocytes, cells which are interposed between neurons and blood vessels, can influence regional cerebral flow, by interacting with specific receptors in the vascular smooth muscles. Furthermore, functions of blood-brain barrier composed of astrocytic processes and cerebral capillaries may be regulated by ET, as ET receptors are present in capillary endothelial cells (Vigne et al. 1990, 1991).

Thus, it is also postulated that astrocytic ET participate in the pathophysiology related to neuronal death caused by transient cerebral ischemia, as vasoactive regulators of neuronal environment (Lustig et al. 1992). We found ET-like immunoreactivities to be increased not only in astrocytes but even in microglial and macrophages in BBB environment in long-time after ischemia.

The phagocytic capabilities of these cells suggest that they are involved in physiological regulation of their microenvironment and often reside within or in close proximity to blood vessels. In conclusion, our result provide immunocytochemical evidence that ET, one of the most known potent endogenous vasoconstrictor, is produced by BBB in endothelial cells, pericytes, astroglial cells and macrophages. Its long-lasting vasoconstrictor action after ischemia may influence the microcirculation.

This brief review concerns the potential pathophysiological role of ET in cerebral vasospasm after ischemia.

Immunoreaktywność dla endoteliny w hipokampie szczura po przemijającym całkowitym niedokrwieniu mózgu. II. Bariera krew-mózg

Streszczenie

Oceniano wpływ przejściowego całkowitego niedokrwienia na rozmieszczenie endoteliny w obrębie bariery krew-mózg w sektorze CA1 hipokampa przy użyciu pozatopieniowej immunocytochemicznej metody znakowania złotem. Immunoreaktywność dla endoteliny pod postacią cząsteczek złota była widoczna we wszystkich elementach bariery: pericytach, komórkach śródbłonka, przestrzeni okołoendotelialnej łącznie z błoną podstawną i w wypustkach astrocytów. U zwierząt kontrolnych gęstość tego typu wyznakowania w elementach bariery sektora CA1 hipokampa była umiarkowana. Immunoreaktywność dla endoteliny była badana od pierwszego tygodnia do dwunastego miesiąca po niedokrwieniu. Jako intensywna była stwierdzona w drugim i szóstym miesiącu po niedokrwieniu. Przypuszcza się, że endotelina odgrywa rolę w skurczu naczyń mózgowych występującym przez długi okres po niedokrwieniu.

References

1. Choi DW, Rothman SM: The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. Ann Rev Neurosci, 1990, 13, 171-182.
2. Fuxe K, Cintra A, Andbjer B, Angsard E, Goldstein M, Agnati LF: : Centrally administered endothelin 1 produced lesions in the brain of the male rat. Acta Physiol Scand, 1989, 137, 155-156.
3. Fuxe K, Kurosawa N, Cintra A, Hallstrom A, Goiny M, Rosen L, Agnati LF, Ungerstedt U: Involvements of local ischemia in endothelin 1 induced lesions of the neostriatum of the anesthetized rat. Exp Brain Res, 1992, 88, 131-139.
4. Gajkowska B, Gadamski R, Mossakowski MJ: Influence of short-term ischemia on the ultrastructure of hippocampal gyrus in Mongolian gerbils. Part II. Electron microscope picture of synapses in early postischemic period. Neuropatol Pol, 1989, 27, 359-366.
5. Gajkowska B, Mossakowski MJ: Localization of endothelin in the blood-brain interphase in rat hippocampus after global cerebral ischemia. Folia Neuropathol, 1995, 33, 221-230.
6. Kurosawa M, Fuxe K, Hallstrom A, Goiny M, Cintra A, Ungerstedt: Responses of blood flow, extracellular lactate and dopamine in the striatum to intrastriatal injection of endothelin 1 in anesthetized rats. J Cardiovasc Pharmacol, 1991, 17 (Suppl 7), s 340-342.
7. Lustig HS, Chan J, Greenberg DA: Comparative neurotoxic potential of glutamate, endothelins, and platelet-activating factor in cerebral cortical cultures. Neurosci Lett, 1992, 139, 15-18.
8. Marsault R, Vigne P, Brettmayer JP, Frelin C: Astrocytes are target cells for endothelins and sarafotoxin. J Neurochem, 1990, 54, 2142-2144.
9. Robinson MJ, Macrea JM, Todd M, Reid JL, McCulloch J: Reduction in local cerebral blood flow induced by endothelin 1 applied topically to the middle cerebral artery in the rat. J Cardiovasc Pharmacol, 1991, 17, Suppl 7, 354-357.
10. Schalk KA, Faraci FM, Heistad DD: Effect of endothelin on production of cerebrospinal fluid in rabbits. Stroke, 1992, 23, 560-563.
11. Vigne P, Breittmayer JP, Marsault R, Freling C: Endothelin mobilizes Ca^{2+} from a caffeine and ryanodine intensive intracellulare pool in rat atrial cells. J Biol Chem, 1990, 265, 6782-6787.
12. Vigne P, Ladoux A, Freling C: Endothelins activate Na^+/H^+ exchange in brain capillary endothelial cells via a high affinity endothelin 3 receptor that is not coupled to phospholipase C. J Biol Chem, 1991, 266, 5925-5926.
13. Willete RN, Ohlstein EH, Pullen M, Sauermelch CF, Cohen A, Nambi P: Transient forebrain ischemia alters acutely endothelin receptor density and immunoreactivity in gerbil brain. Life Sci, 1993, 52, 35-40.
14. Yamashita K, Kataoka Y, Niwa M, Shigematsu K, Himen A, Koizumi S, Taniyama K: Increased production of endothelins in the hippocampus of stroke prone spontaneously hypertensive rats following transient forebrain ischemia: histochemical evidence. Cell Mol Neurobiol, 1993, 13, 15-23.
15. Yamashita J, Ogawa M, Egami H, Matsuo S, Kiyohara H, Inda K, Yamashita S, Fujita S: Abundant expression of immunoreactive endothelin 1 in mammary phyllodes tumor:

possible paracrine role of endothelin 1 in the growth of stromal cells in phyllodes tumor. Cancer Res, 1992, 52, 4046-4049.

16. Yue TL, Nambi P, Wu HL, Feuerstein G: Effect of endothelins on cytosolic free calcium concentration in neuroblastoma NG 108 15 and NG 20 cells. Neuropeptides, 1990, 17, 7-12.
17. Ziv J, Fleminger G, Djaldetti R, Achiron A, Melamed E, Sokolovsky M: Increased plasme endothelin 1 in acute ischemic stroke. Stroke, 1992, 23, 1014-1016.

Authors' address: Laboratory of Ultrastructure of the Nervous System, Medical Research Centre Polish Academy of Sciences, 5A Pawińskiego St., Warszawa