EFFECT OF NOOTROPIL AND CONTRYKAL ON STRUCTURAL CHANGES IN THE CNS IN HYPOXIC CEREBRAL EDEMA

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In 27 experiments on rabbits the cerebral cortex was investigated after ischemia for 15 min associated with controlled hypotension. The aim of the investigation was to test the efficacy of Nootropil and Contrykal as drugs for the treatment of posthypoxic cerebral edema. The effects of treatment were judged on the basis of light- and electron-microscopic observations. Nootropil had a favorable effect on the ultrastructure of intraneuronal organelles (mitochondria, lysosomes, ribosomal apparatus, nuclear membrane, and so on). During treatment with Nootropil and Contrykal the changes in the microcirculatory system after hypoxia showed signs of compensation and were reversible. The manifestations of posthypoxic cerebral edema were less marked. The efficacy of the suggested scheme of treatment is attributed to the normalizing effect of Nootropil on metabolic and intracellular repair processes in neurons and cells belonging to the microcirculatory system and also to the direct action of Contrykal on local capillary permeability factors.

KEY WORDS: cerebral edema; Nootropil; Contrykal; CNS.

With the accumulation of clinical and experimental data in recent years views on the formation of posthypoxic cerebral edema have undergone certain changes. The reasons must be sought in the frequent disparity between the neurological disturbances, sometimes leading to a fatal outcome, and the histological findings [3, 5-8].

It was found previously that the characteristic structural changes of cerebral edema appear later than signs of hypoxic damage to neurons [1, 14, 15, 18, 19].

In the opinion of Fox [11] and Paulson et al. [17], the main causes of cerebral edema are disturbances of the functions of the blood-brain barrier. It has also been shown [4, 10] that substantial disturbances take place in the microcirculatory system during the formation of posthypoxic cerebral edema.

One of the most important tasks in the treatment of hypoxic cerebral edema is the treatment of disturbances in the microcirculatory system and stabilization of the cerebral circulation. Treatment aimed at normalizing metabolism in the neurons, which must have a beneficial effect on the resistance of the brain to hypoxia, and at stimulating repair processes also is important.

The object of this investigation was to study structural changes in the CNS after hypoxia and treatment of hypoxic cerebral edema with Nootropil and Contrykal.

The use of natural protease inhibitors (Contrykal) for the treatment of microcirculatory disturbances arising in various pathological states is well known [2].

Nootropil (piracetam), the first member of a new group of nootropic drugs synthesized by the Belgian firm UCB, chemically speaking is a cyclic derivative of gamma-aminobutyric acid, and is 2-oxo-1-pyrrolidineacetamide.

The principal pharmacological properties of Nootropil are: it improves glucose utilization, lowers the lactate level, increases the synthesis and normalizes consumption of ATP, and stimulates the synthesis of

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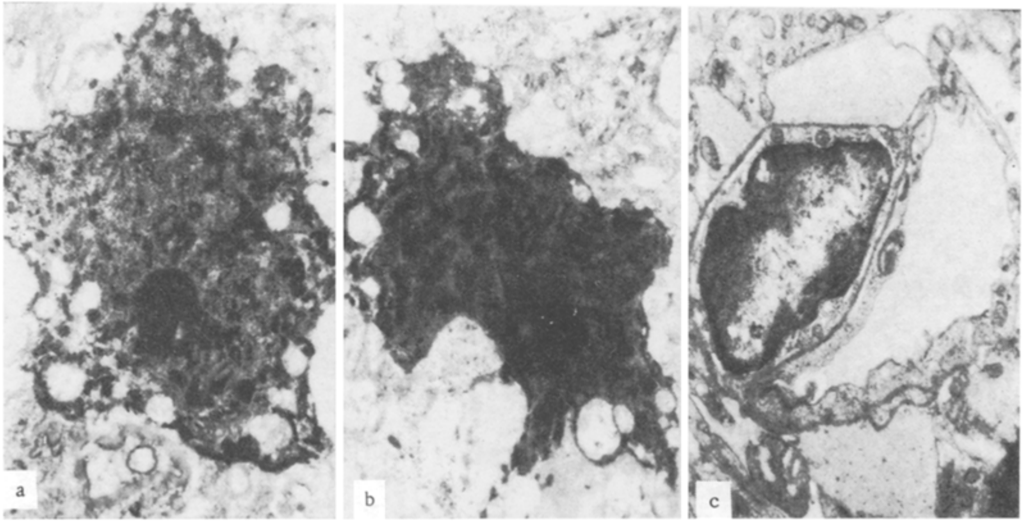

Fig. 1. Ultrastructural changes in CNS of rabbit with hypoxic cerebral edema: a) pycnomorphic neuron from layer V of the cerebral cortex, dense osmiophilic cytoplasm, mitochondria destroyed, their cristae cannot be made out. 25,000×; b) rugose neuron from cerebral cortex, features of neuronophagy by glial cells. 25,000×; c) fine structure of capillary in cerebral cortex, edema of cytoplasm of endothelial cell, many vacuoles, swelling and edama of pericyte nucleus. 25,000×.

organophosphorus derivatives, phospholipids, and ribonucleic acids in hypoxic and toxic lesions of the CNS [9, 12, 13, 16, 20].

EXPERIMENTAL MATERIAL AND METHOD

Experiments were carried out on 27 rabbits weighing 2670-3140 g. Group 1 comprised six control animals. The 21 rabbits of group 2 were treated with Contrykal and Nootropil. Cerebral ischemia was induced in the animals of both groups for 15 min by compression of the carotid arteries during control hypotension. The hypotension was maintained by administration of the ultrashortacting ganglion blocker Arfonad by intravenous drip and monitored by means of a catheter introduced into the femoral artery and connected to a pressure transducer, an electronic recorder, and automatic writer. The rabbits of group 2 received an injection of 2000 antitrypsin units of Contrykal and 300 mg Nootropil intravenously, 90 min before compression of the carotid arteries, and a further 500 mg Nootropil immediately after ischemia for 15 min.

On the subsequent days Nootropil was injected intramuscularly twice in a dose of 500 mg. The animals were decapitated under hexobarbital anesthesia 4 or 5 days after experimental cerebral ischemia.

Brain tissue for morphological examination was fixed in 10% neutral formalin and embedded in paraffin wax; serial frontal sections stained by Nissl's method were studied. For electron-microscopic examination with the JEM-100B instrument pieces of brain from the motor cortex were fixed in osmium and embedded in Epon. Ultrathin serial sections were stained with uranyl acetate and lead acetate.

EXPERIMENTAL RESULTS

The brain of the rabbits of group 1 was macroscopically cyanosed, the meninges were stretched, and the blood vessels distended with blood.

Microscopically, marked edema of the meninges and brain tissues was observed. Pathomorphological changes in the CNS were mosaic in character. For instance, cells with a reduced content of Nissl's substance were observed in the cerebral cortex. Large and small vacuoles were found in the cytoplasm of some neurons, i.e., the cells were in various stages of hydropic degeneration. However, most cerebral cortical neurons of the rabbits of group 1 showed evidence of severe disturbances, in the form of commencing swelling of the cell bodies and processes with manifestations of tigrolysis, rhexis, and pycnosis. The outlines of the nerve cells were irregular and the cell membrane was difficult to distinguish. The nucleus of such neurons was deformed and became basophilic. In the field of vision of the light-optical microscope ischemized triangular or polygonal nerve cells with homogeneous cytoplasm, completely free from hydrolysis, with a distinctly hyperchromic and

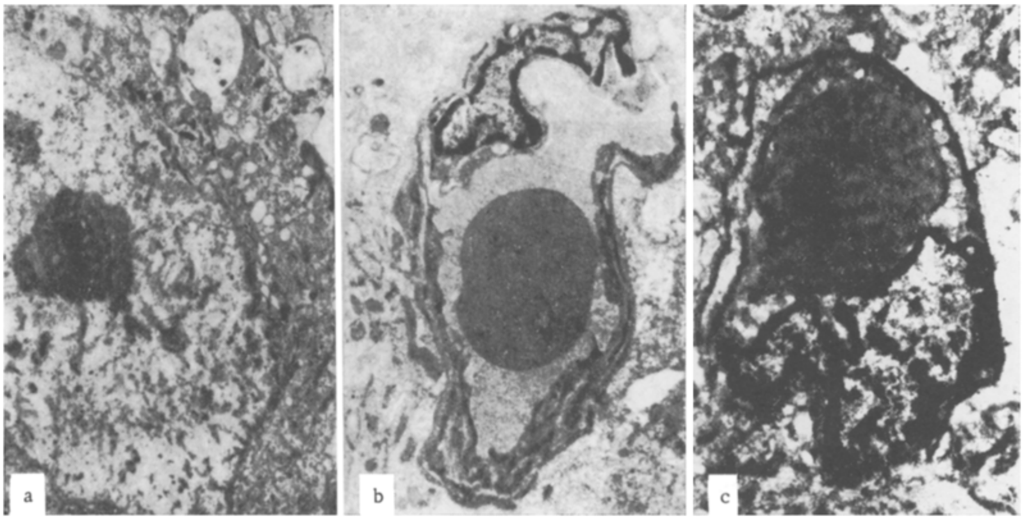

Fig. 2. Ultrastructural changes in CNS of rabbit during treatment of cerebral edema by Nootropil and Contrykal. a) Nuclear membrane clearly distinguishable, no pathological changes in structure of nucleus and nucleolus, cytoplasm of cortical neuron contains mitochondria in "energized" state. 25,000 ×; b) normal structure of capillary wall, active state of nucleus of endothelial cell. 25,000 ×; c) constriction of capillary lumen in brain and activation of endothelial cell. 25,000 ×.

deformed nucleus, were observed in sections through the cerebral cortex. Some neurons showed homogenizing necrosis. In the field of vision of the electron microscope these neurons (Fig. 1a, c) were reduced in size, their cytoplasm and karyoplasm were more osmiophilic, and the outlines of the nuclear membrane were difficult to distinguish. Mitochondria of neurons in cortical layer V were pale and their cristae completely destroyed. As a rule the nerve cells were surrounded by processes of astrocytes and oligodendrocytes. The latter, like their processes, were edematous and swollen. Many vacuoles were observed in the cytoplasm of the glial cells, their mitochondria contained destroyed cristae, and ribosomes were almost absent.

Investigation of the cortical blood vessels revealed the typical morphological picture of a hypoxic state. The capillaries in the brain substance were dilated throughout their length, distended with blood cells, and surrounded by spaces indicating pericapillary edema. Ultrastructural formations of the capillary wall were involved in the hydropic process (Fig. 1c). As these electron micrographs show, the pericyte nucleus was round, slightly elongated, and vesicular, and the cytoplasm of the epithelial cell contained many vacuoles. Very often the cytoplasm of these cells gave off outgrowths into the capillary lumen.

The observations showed that during treatment of hypoxic cerebral edema with Nootropil and Contrykal the morphological picture of the rabbits' brains was considerably altered (group 2). Macroscopically the brain substance of the animals of this group preserved its normal structure, the pattern of the cortical convolutions was distinct and regular, and there were no visible changes in the meninges and blood vessels. On light-optical examination the cortical neurons had a pale cytoplasm, a clearly distinguishable nuclear membrane, and a round nucleus; as a rule the nucleolus was central. However, a few intensively hyperchromic neurons could be observed. The pattern of the capillaries was tortuous, and pericapillary edema was mild in degree.

The ultrastructural characteristics of most neurons showed compensatory features: the nucleus was pale and round, with a small content of heterochromatin, the nuclear membrane was clearly outlined, and the nucleolus hypertrophied and located in the center of the nucleus. The cytoplasm of the nerve cells consisted of a rough endoplasmic reticulum with many ribosomes attached to it; the mitochondria were small, with tightly packed cristae, and with pale areas in places ("energized" mitochondria). The processes of the astrocytes and oligodendrocytes surrounding the neuron were normal in structure (Fig. 2a).

The capillaries and their component parts showed no considerable changes and their ultrastructure was almost normal. Moderate constriction of the capillary lumen was observed and the cytoplasm of the endothelial cells had a narrow zone above an unchanged basal layer. The processes of the astrocytes, surrounding nearly all the brain capillaries like a muff, were normal in structure. The very active state of the endothelial cell nuclei, which were curiously twisted in shape, was very noticable (Fig. 2b, c).

It can be concluded from the results of these experiments that Nootropil, as a drug for the treatment of hypoxic (ischemic) states of the brain, has a beneficial effect on neuronal structure, especially on the cell and nuclear membranes, ribosomal apparatus, and the structure of the mitochondria and lysosomes. From the writers' point of view it is an exceptionally important fact that the respiratory activity of the mitochondria was preserved, or was rapidly restored after a reversible "failure," in neurons subjected to hypoxia and treatment with Nootropil, as shown by their virtually normal ultrastructural picture. In the writers' view, it was because of the functional integrity of the mitochondria and their adequate energy supply to the neurons, that the ultrastructure of their organelles also was preserved. Conditions for autolysis were unable to develop.

The less severe changes in the microcirculatory system found in the animals of group 2 can be attributed both to the direct action of Contrykal on local permeability factors and to the action of Nootropil, normalizing the metabolism and functions of the cells composing this system.

LITERATURE CITED

1. A. M. Gurvich, I. S. Novoderzhkina, N. P. Romanova, et al., in: Proceedings of the 1st All-Union Congress of Neurosurgeons [in Russian], Vol. 1, Moscow (1971), pp. 193-197.
2. V. P. Kaznacheev and A. A. Dzizinskii, Clinical Pathology of Transcapillary Metabolism [in Russian], Moscow (1975).
3. V. P. Kurkovskii, in: Collection of Articles Commemorating the 60th Birthday and 35 Years of Scientific, Teaching, and Public Activity of Academician N. N. Anichkov [in Russian], Moscow (1946), pp. 221-228.
4. G. I. Mchedlishvili, in: Oxybiotic and Anoxybiotic Processes in Experimental and Clinical Pathology [in Russian], Kiev (1975), pp. 157-158.
5. V. P. Tumanov and M. D. Malamud, Changes in the Central Nervous System after Thermal, Radiation, and Combined Trauma [in Russian], Kishinev (1977).
6. J. H. Adams, J. B. Brierley, R. C. R. Conner, et al., Brain, 89, 935 (1968).
7. L. Bakay and J. C. Lee, Cerebral Edema [Russian translation], Moscow (1969).
8. J. Brierley, B. S. Meldrum, and A. W. Brown, Arch Neurol. (Chicago), 29, 367 (1973).
9. P. E. Burnotte, J. G. Golbert, and J. J. Temmerman, Biochem. Pharmacol., 22, 811 (1973).
10. E. G. Fisher and A. Ames, Stroke, 3, 538 (1972).
11. J. L. Fox, J. Neurosurg., 21, 909 (1964).
12. J. Golbert, J. Pharm. Belg., 27, 281 (1972).
13. J. Golbert and J. Temmerman, Report of 4th Meeting of the International Society for Neurochemistry, Tokyo (1973), pp. 26-31.
14. A. Hirano, S. Levine, and H. M. Zimmerman, J. Neuropathol. Exp. Neurol., 26, 200 (1967).
15. M. J. Mossakowsky, D. M. Long, R. E. Myers, et al., J. Neuropathol. Exp. Neurol., 27, 500 (1968).
16. V. J. Nickolson and O. L. Wolthuis, Biochem. Pharmacol., 25, 41 (1976).
17. O. Paulson, N. Lassen, and E. Skinhoj, Neurology (Minneapolis), 20, 125 (1970).
18. F. Plum, J. B. Posner, and E. C. Alvord, Arch. Neurol. (Chicago), 9, 563 (1963).
19. A. Steegman, in: Pathology of the Nervous System, edited by J. Minckler, Vol. 1, New York (1968), pp. 1005-1029.
20. H. Vial, G. Guillemin, and H. Pacheco, J. Pharmacol., 7, 177 (1976).