EFFECT OF HIGH POTASSIUM ION CONCENTRATION ON NEURONAL AND SYNAPTIC ULTRASTRUCTURE OF *Helix pomatia* CNS

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UDC 616.83-091.81-02:615.31:546.32]-076.5

KEY WORDS: *Helix pomatia*; CNS; extracellular potassium

Increased neuronal activity in the CNS leads to a marked increase in the local extracellular potassium ion concentration and a decrease in calcium ion concentration [3]. The rise of extracellular potassium is due to its voltage-dependent or Ca-dependent release through K channels along the concentration gradient. In some pathological states of the CNS such as hypoxia or ischemia and spreading depression a considerable rise of extracellular potassium also takes place. Nonsynaptic information exchange of ions has a particularly important role in the regulation of activity of neuronal modules, thereby ensuring the cooperative and hierarchical working of single neurons. However, information in the literature on the effect of potassium ions on neuronal and synaptic ultrastructure is very limited [5]. The isolated CNS of *Helix pomatia* is a very convenient model for neuromorphological research in vitro [2].

The aim of this investigation was to study the ultrastructure of neurons and synapses in the CNS of *Helix pomatia* under the influence of high extracellular potassium concentrations.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated CNS of *Helix pomatia* in vitro after removal of the compact membrane. The isolated ganglia were incubated for 0.5 h in media of the following composition: KCl 80 mM, NaCl 4 mM, $CaCl_2$ 7 mM, $MgCl_2$ 5 mM, Tris-HCl 10 mM (experimental group of 5 animals) and NaCl 80 mM, KCl 4 mM, $CaCl_2$ 7 mM, $MgCl_2$ 5 mM, Tris-HCl 10 mM (control group of 5 animals). After incubation the ganglia were fixed in 10% paraformaldehyde solution in 0.1 M S-collidine buffer with the addition of 10 mM $CaCl_2$ [4]. The material was fixed for 2 days at +2 to +4°C. After washing in fresh 0.1 M S-collidine buffer it was cut on a freezing microtome into 100-μ sections, which were then postfixed in 1% OsO_4 in 0.1 M collidine buffer for 1 h. Next, after rinsing to remove the osmium (1 h) in the same buffer the sections were dehydrated in increasing concentrations of alcohols. They were then stained with 2% uranyl acetate in 70% alcohol overnight. The sections were next taken through propylene oxide and embedded in a mixture of Epon and Araldite. All procedures on the experimental and control specimens were carried out synchronously. Ultrathin sections were cut on an LKB-IV Ultrotome, stained with lead citrate by the usual method, and examined in the EM-10 CR electron microscope ("Opton," Germany).

EXPERIMENTAL RESULTS

Morphometric investigation of the neurons was conducted on semithin sections through the snail's CNS. The results are given in Table 1. They show that in the experimental group of animals, whose CNS was incubated in a medium with a high potassium ion concentration, all the parameters studied were increased (area of cross section, perimeter, maximal and minimal diameters), except the shape factor, both of the neuron and of the nucleus and nucleolus ($p < 0.001$).

Moscow Institute of Radio-Engineering, Electronics, and Automation. (Presented by Academician of the Russian Academy of Medical Sciences N. K. Permyakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 8, pp. 117-120, August, 1992. Original article submitted January 9, 1992.

 0007-4888/92/0008-1064$12.50

TABLE 1. Morphometric Parameters of *H. pomatia* Neurons after K-Depolarization (M ± m)

H. pomatia neurons	Area	Perimeter	Length	Maximal diameter	Shape factor	Minimal diameter
Neuron	1868,72	161,834	80,917	55,372	0,7736	40,170
experiment	±164,40	±5,359	±2,680	±1,828	±0,00503	±1,338
control	1281,7	134,845	67,423	46,689	0,7834	32,875
	±72,87	±3,826	±1,913	±1,327	±0,00397	±0,9700
Nucleus	592,703	90,226	45,113	31,352	0,8033	21,939
experiment	±38,408	±3,151	±1,576	±1,153	±0,00567	±0,686
control	429,61	78,203	39,102	27,610	0,7745	18,531
	±26,19	±2,349	±1,175	±0,843	±0,00516	±0,565
Nucleolus	37,508	21,208	10,604	7,123	0,8201	5,593
experiment	±9,876	±2,027	±1,013	±0,7538	±0,00542	±0,482
control	17,328	16,075	8,037	5,310	0,8158	4,279
	±1,148	±0,494	±0,247	±0,171	±0,00444	±0,129

The ultrastructural study of the neuropil revealed a marked increase in size of the outgrowths of the glial cells invading it, and especially into large axons. There was a marked increase in the number of intercellular spaces filled with vesicular material. Enlargement of the myelinlike bodies in the axons was observed, and sometimes they were formed by groups surrounded by a common membrane. Synaptic vesicles in large outgrowths were fused together (Fig. 1a), and quite often complex convergent junctions were formed, where two or more axon endings were Present on a single spine (Fig. 1b).

Changes observed in glial cells merit special attention. Very often gliocytes were found in the neuropil in groups of 3-5 cells (Fig. 1c), and in a few cases satellite gliocytes were closely apposed to the neuronal membrane (Fig. 1d). The smooth endoplasmic reticulum was considerably widened, creating the impression of a honeycomb. Myelinlike bodies were very often present in the gliocytes. The mitochondria in them had clearly outlined cristae, with a matrix of average electron density.

Investigation of the neurons revealed many energized mitochondria (Fig. 2a). The nuclear membrane in the large neurons was very tortuous, and in some cases circular nucleoli were present (Fig. 2b). The rough endoplasmic reticulum was located mainly in the perinuclear region of the cytoplasm, whereas the Golgi apparatus was located peripherally, near the cytoplasmic membrane (Fig. 2c). The cytoplasm of the neurons contained many electron-translucent regions (Fig. 2d), possibly indicating the presence of general cytoplasmic edema.

During this investigation an attempt was made to evaluate the ultrastructural changes arising as a result of modification of the ionic environment of the neuronal modules. Such a situation arises quite often during neuronal activity, and as a result local potassium ion concentrations may rise by an order of magnitude.

Several publications have been devoted to the study of the normal ultrastructure of the nervous system of *Helix pomatia* [1, 2] and have given a detailed examination of its structural features. Long exposure to potassium ions, however, significantly modifies the structure, above all, of cells: neurons and gliocytes, and affects the synaptic apparatus by a much lesser degree. It is perfectly possible that this effect may be due to the unequal buffer capacity of different gliocyte subpopulations: satellite gliocytes surrounding neurons have a smaller capacity for potassium than gliocytes located in the neuropil. The results of the present investigation also demonstrate that more marked ultrastructural changes after exposure to potassium are to be found in gliocytes located in the neuropil. These findings may also be evidence that potassium ions are more important from the standpoint of information at the neuronal than at the synaptic level. Death of neurons also has been observed after direct contact however, the situation was complicated by the hyperosmolality of the medium. It has to be said that an increase in extracellular potassium, caused either by its equivalent replacement in the physiological saline or by intensification of neuronal activity or by electrical stimulation, leads to a fall of calcium in the surrounding medium [6], due to increased inflow into the neurons. Unfortunately, under our experimental conditions we could not distinguish the effect of a raised potassium level in the extracellular space on ultrastructure from that of a raised calcium concentration in the neurons. It seems likely that experiments with the calcium ionophore A23187 may shed light on this problem.

Increased vacuolation during electrical stimulation of dorsal roots in frogs has been observed in Ranvier nodes and was independent of the method of fixation [7]. In this case vacuolation was not observed if the nerve, after stimulation, was immersed in physiological saline for 30 min before the beginning of fixation. The authors cited consider that these changes were caused by accumulation of potassium ions in the intercellular space.

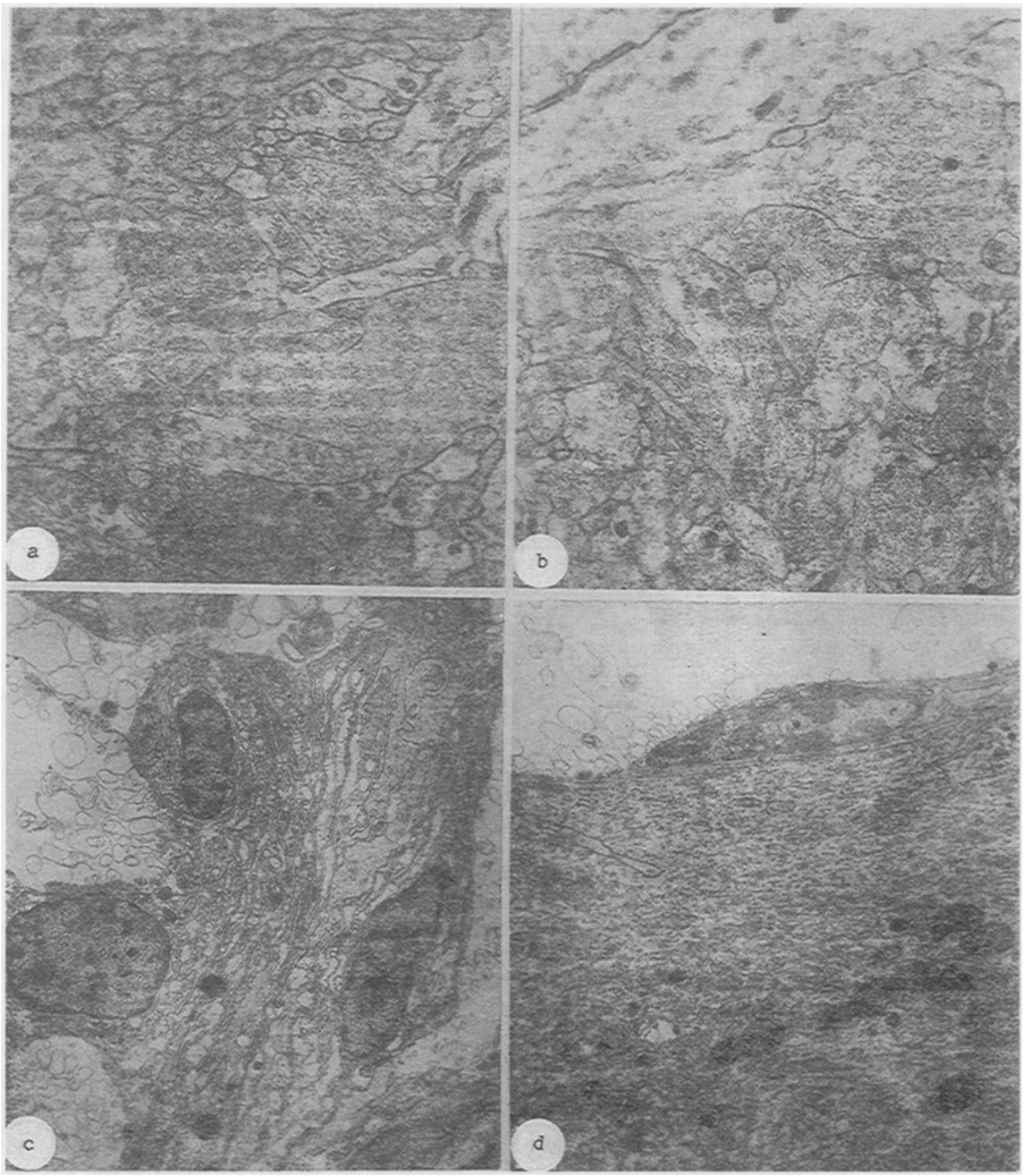

Fig. 1. Neuropil of ventral nerve ganglion of *Helix pomatia* during incubation in medium with high potassium ion concentration: a) fusion of synaptic vesicles in large outgrowths of neuropil in ventral nerve ganglion of *Helix pomatia* (10,000×); b) convergent junctions between neurons (10,000×); c) grouping of gliocytes in neuropil (3270×); d) close apposition of satellite gliocytes to neuronal membrane (4050×).

Investigations [7] on neurons and glial cells of the leech CNS showed that with an increase in the extracellular potassium concentration the K^+ concentration rises not only in gliocytes, but also in neurons. However, the Na/K ratio in the gliocytes changes significantly, whereas in the neurons it remains constant.

The extent to which exposure to high potassium ion concentrations adequately reflects physiological stimulation of neurons remains an unsolved problem. However, an increase in potassium ion concentration in the intercellular space is not only the result of more intensive neuronal activity, but also the cause of changes in the activity of other neighboring neurons, connected into a module with the given neuron. Thus potassium ions can play the role of

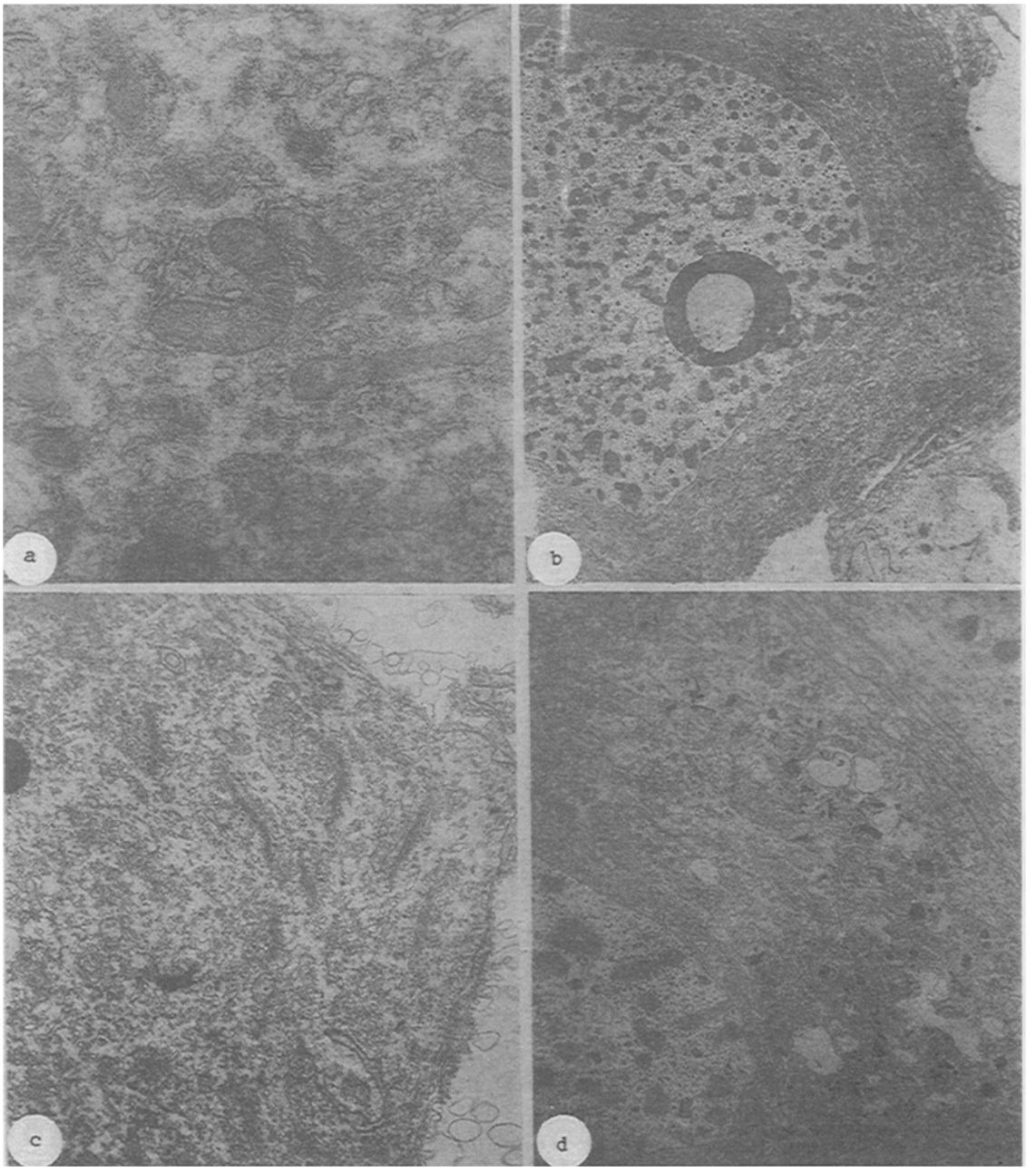

Fig. 2. Neuronal ultrastructure of ventral nerve ganglion of *Helix pomatia* during incubation in medium with high potassium ion concentration: a) energized mitochondria in a neuron (20,000×); b) circular nucleolus of neuronal nucleus (1600×); c) arrangement of Golgi apparatus near neuronal plasmalemma (6400×); d) areas of low electron density in cytoplasm of neurons (3270×).

humoral regulator in a neuronal module system. The importance of potassium relative to information also is determined by its action on the neuron, which, as a result of its own activity, caused an increase in the K^+ concentration in the intercellular space, i.e., potassium ions act by a feedback mechanism.

It can be concluded that cells of the nervous system exhibit structural plasticity during brief exposure to potassium ions. However, further morphometric studies are needed in order to clarify its mechanisms and to obtain quantitative data.

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METABOLISM OF CONNECTIVE TISSUE BIOPOLYMERS IN THE AORTA AFTER INTRAVENTRICULAR NEUROPEPTIDE INJECTION

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UDC 616.132-008.9.-02:615.175.82

KEY WORDS: aorta; myocardium; connective tissue; metabolism; neuropeptides

There is only limited information about the role of some physiologically active peptides in the metabolism of connective tissue biopolymers. In particular, it has been shown that repeated microinjections of angiotensin II and substance P into the mesencephalic reticular formation leads to collagen accumulation in the aorta and myocardium [4]. Neuropeptides are known to play an essential role in the realization of various bodily functions [5, 6, 11], including the regulation-of metabolism [3, 8].

The aim of this investigation was to study metabolism of connective tissue biopolymers in the aorta in response to repeated intraventricular injection of substance P, Leu-enkephalin, and beta-endorphin.

EXPERIMENTAL METHOD

Experiments were carried out on 40 adult Chinchilla rabbits weighing 3.0-3.5 kg. Taking coordinates from an atlas of the brain [1], and using a stereotaxic apparatus, cannulas were inserted into the left lateral ventricle of experimental and control animals (AP = −1, V = 1.5, S = 2.7). Substance P in a dose of 100 ng, Leu-enkephalin in a dose of 150 ng, and beta-endorphin in a dose of 150 ng in 10 μl of physiological saline were injected intraventricularly on alternate days for 30 days. Control animals received the same volume of physiological saline. Intact rabbits also were used as the control. Each experimental series and the control group consisted of eight animals, which were used in a chronic experiment 7-8 days after implantation of the cannulas. At the end of the experiment the animals were killed by air embolism under short-term ether anesthesia. The accuracy of location of the cannulas was verified histologically. Blood was taken in the course of the experiment on the 10th, 20th, and 30th days from the marginal vein of the ear 30 min after intraventricular injections of the neuropeptides, for determination of concentrations of glycosaminoglycans based on the hexuronic acid (HUA) level [10], sialic acids (SA) [9], and 11-hydroxycorticosteroids (11-HCS) [13] and urea, using a kit from "Lachema" (Czechoslovakia). Parameters of collagen metabolism – serum levels of free (FH), peptide-bound (PBH), and protein-bound (PrBH) hydroxyproline were analyzed by the aid of

Department of Normal Physiology, Izhevsk Medical Institute. (Presented by Academician of the Russian Academy of Medical Sciences K. V. Sudakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 8, pp. 120-122, August, 1992. Original article submitted January 10, 1992.

 0007-4888/92/0008-1068$12.50