LITERATURE CITED

1. M. A. Azhigirova, E. P. Vyazova, M. G. Vashkevich, et al., Byull. Éksp. Biol. Med., No. 9, 302 (1988).
2. M. A. Azhigirova, M. G. Vashkevich, E. P. Vyazova, and P. A. Khachatur'yan, Gematol. Transfuziol., No. 7, 27 (1988).
3. E. P. Vyazova, M. A. Azhigirova, A. L. Shuvalova, et al., Byull. Éksp. Biol. Med., No. 10, 446 (1988).
4. E. P. Vyazova and M. A. Azhigirova, Khim.-Farm. Zh., No. 6, 645 (1989).
5. T. M. C. Chang, M. Farmer, R. P. Geyer, and G. F. Moss, Trans. Am. Soc. Artific. Intern. Organs, **33**, 819 (1987).
6. Y. Funakoshi, T. Agishi, H. Honda, et al., Yinko Zoki, **17**, 725 (1988).
7. D. H. Marks, J. E. Lynett, R. M. Letscher, et al., Military Med., **152**, 265 (1987).
8. G. S. Moss, S. A. Gould, L. R. Sehgal, et al., Biomater. Artif. Cells Artif. Organs, **16**, 57 (1988).
9. B. Teisseire, C. Ropars, C. Vieilledent, and C. Nicolau, Adv. Biosci., **54**, 153 (1986).

THE Ca^{2+}-PUMP IN SMALL INTESTINAL MYOCYTES

V. K. Rybal'chenko

UDC 577.352.3

KEY WORDS: plasma membrane; smooth-muscle cell; Ca^{2+}-pump; calmodulin; oxytocin

The total calcium content in smooth-muscle cells can be regarded as the resultant of three components: binding and release of the cation by chelating components of the cell and by subcellular structures, and exchange of Ca^{2+}-ions between the cell and the extracellular medium, realized through channel conductance and diffusion of ions along the electrochemical gradient, and also countergradient transport as a result of functioning of sodium-calcium exchange and the Ca-pump of the plasma membrane (PM). Particular attention is currently being paid to the latter, because establishment of the principles governing the functioning of the Ca-pump may provide the basis for the development of new approaches and methods of overcoming muscular function and correcting disturbances of motor activity in the digestive system.

This paper gives the results of experimental studies of the Ca-pump of PM of smooth-muscle cells and its regulation by calmodulin and oxytocin.

EXPERIMENTAL METHOD

The PM fraction was isolated from smooth-muscle tissue of the rabbit small intestine by differential centrifugation in a sucrose density gradient, and characterized with respect to marker enzymes and by electron microscopy; calcium accumulation by membrane vesicles was studied with the aid of a Ca-selective electrode and by the isotopic method [3].

EXPERIMENTAL RESULTS

The study of ATP-dependent accumulation of Ca^{2+} ions by vesicles of the PM fraction by means of a Ca-selective electrode showed that the membrane vesicles can accumulate the cation rapidly with an initial velocity of about 2 nmoles Ca^{2+}/mg protein/sec. Calcium accumulation by PM vesicles measured by the isotopic method after incubation for 30 min amounted to 20.0 ± 2.3 nmoles Ca^{2+}/mg protein, but in the presence of calmodulin (20-25 μg/ml) in the incubation medium, it rose to 27.5 ± 1.5 nmoles Ca^{2+}/mg protein (Figs. 1 and 2). Activation of the Ca-pump by calmodulin is dependent on concentra-

Laboratory of Membranology, Kiev University. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 4, pp. 376-377, April, 1991. Original article submitted July 6, 1990.

 0007-4888/91/0004-0476$12.50

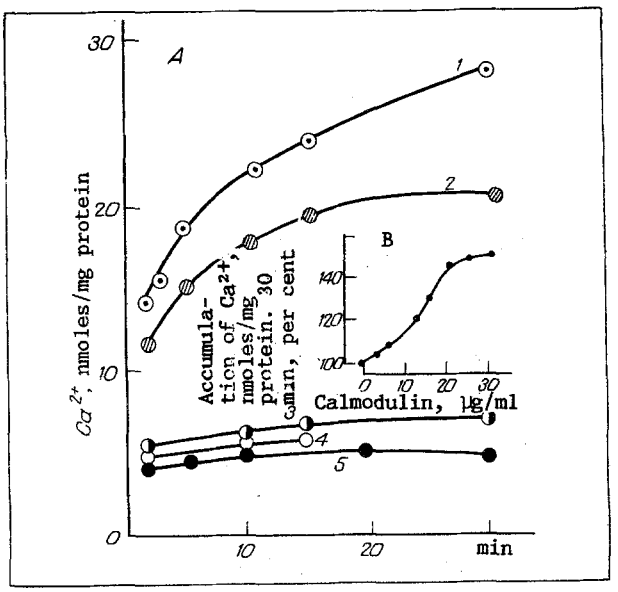

Fig. 1. Accumulation of Ca^{2+} ions by PM vesicles. Medium (in mM): sucrose 250, imidazole-HCl 50, ATP 5, $MgCl_2$ 5, and $CaCl_2$ 20 μM (4-6 μCi/ml), pH 7.2, 37°C (curve 2), and also 20 μg/ml calmodulin (1), calmodulin plus $5 \cdot 10^{-5}$ M oxytocin (3), oxytocin without calmodulin (4), and medium with ATP (5). Incubation time 30 min, 100 μg PM protein/ml.

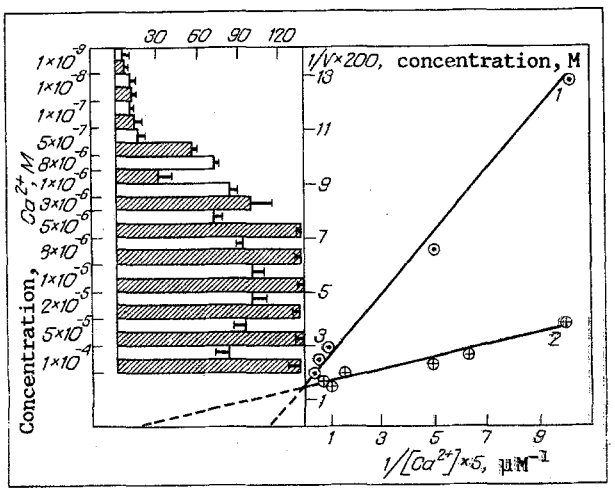

Fig. 2. Dependence of accumulation of Ca^{2+} ions by PM vesicles on calcium concentration. Medium indicated in caption to Fig. 1. 1 (and unshaded columns) — without calmodulin; 2 (and shaded columns) — in presence of 20 g/ml calmodulin.

tion, a characteristic feature also of PM of smooth-muscle cells from other organs [5, 6], thereby increasing the affinity of the pump for calcium fivefold (Fig. 2). ATP-dependent Ca^{2+} transport was blocked by oxytocin in the same concentrations as Ca,Mg-ATPase [4]. In the presence of the hormone, the quantity of calcium accumulated by the vesicles corresponded to binding of the cation by the membrane [2].

The data relating to the mechanisms of binding of the activator and inhibitor of the Ca-pump of PM of the smooth-muscle cells are not to be found in the literature. We accordingly studied the surface activity of calmodulin and oxytocin and their interaction with the membranes, using for this purpose mono- and bimolecular lipid layers, liposomes, and proteoliposomes, possessing Mg^{2+} ions and Ca,Mg-ATPase activity, and capable of accumulating Ca^{2+} ions. It was found that calmodulin does

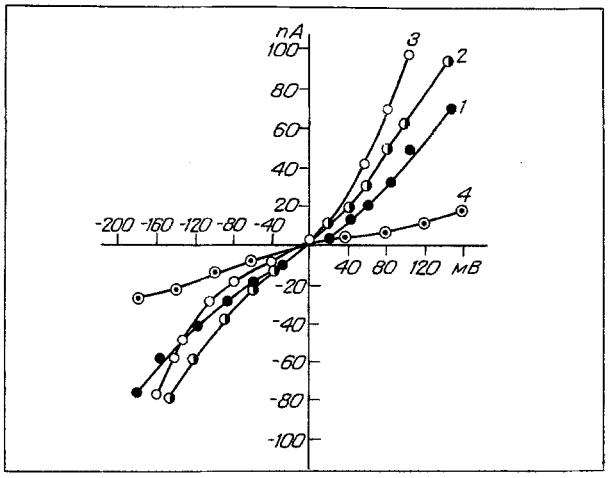

Fig. 3. Current—voltage characteristic curves of azolectin bilayer lipid membranes modified from the cis-side by 10^{-5} M oxytocin. 1) 100 mM NaCl plus 0.1 mM $CaCl_2$; 2,3) 70 mM KCl plus 10 mM $CaCl_2$ or $BaCl_2$ respectively; 4) 70 mM KCl plus 10 mM $CaCl_2$ plus 0.5 mM $MnCl_2$, pH 7.3.

not exhibit surface activity, does not form monolayers at the phase separation boundary, does not modify lipid mono- and bilayers, and does not change the permeability to calcium of azolectin liposomes and PM vesicles. It can therefore be postulated that calmodulin activates the Ca-pump as a result of direct binding with transport Ca,Mg-ATPase — the main structure of the Ca-pump of PM.

As regards oxytocin, the situation was found to be much more complex: from the traditional view of binding of the hormone by membrane receptors it is impossible to explain blocking of the Ca-pump of the vesicles oriented with the cytoplasmic surface of PM toward the surrounding medium. We accordingly postulated that the primary processes of binding of the hormone by the membranes are interaction of peptide molecules with molecules of the peptide matrix of PM. Oxytocin was found to have surface activity, to form a monolayer on the phase separation boundary, to modify liposomes, and to inhibit Ca,Mg-ATPase of the PM vesicles when turned inside out [4]. In the presence of the hormone the outflow of calcium from membrane vesicles into the medium with sucrose or KCl was increased by 50-60%, and contraction of strips of small intestine also was intensified (unpublished data).

Further investigations showed that oxytocin in concentrations of 10^{-9}-10^{-5} M inhibits channel conductance of azolectin bilayer lipid membranes (BLM) and BLM with implanted fragments of PM. Ionic channels of BLM induced by oxytocin varied in conductance from 10 to 140 pCm, depending on the voltage on the membrane. A negative potential (—50 to —120 mV) on BLM, at the side of introduction of the hormone, did not significantly change the life span of the channels, but a positive potential, if gradually increased, reduced the life span of the channels in the open state and blocked them at +100 mV. Current voltage characteristic curves of azolectin BLM, modified by oxytocin, were nonlinear and asymmetrical (Fig. 3), which is qualitatively in agreement with the results of investigations [1] conducted on phosphatidylserine BLM. Similar current—voltage characteristic curves were obtained in the presence of oxytocin also on BLM with implanted fragments of PM of smooth-muscle cells.

Thus the Ca-pump of PM of the small intestinal myocytes carries out active transport of calcium ions against the concentration gradient on account of energy of ATP hydrolysis by Ca,Mg-ATPase, the "power" of which (calculated data based on the initial velocity of the process) is sufficient to maintain 70% homeostasis of calcium in the course of cell function. Calmodulin activates the transport process by 35-40% on account of a fivefold increase in affinity of the pump for calcium. Oxytocin inhibits ATP-dependent transport, initially by forming a transmembrane potential-dependent ionic channel in the lipid matrix. These peptide-lipid structures are linked with receptors (or with Ca,Mg-ATPase molecules) and they exhibit a regulatory effect on the Ca-pump which depends on the voltage on the membrane.

LITERATURE CITED

1. O. V. Krasil'nikov, R. Z. Sabirov, and B. A. Tashmukhamedov, Dokl. Akad. Nauk SSSR, **272**, No. 1, 220 (1983).

2. N. E. Kucherenko and V. K. Rybal'chenko, Byull. Éksp. Biol. Med., **103**, No. 1 (1990).
3. V. K. Rybal'chenko, V. I. Karamushka, P. V. Pogrebnoi, et al., Byull. Éksp. **97**, No. 1, 106 (1984).
4. V. K. Rybal'chenko, G. V. Ostrovskaya, and N. E. Kucherenko, Byull. Éksp. Biol. Med., **97**, No. 12, 687 (1989).
5. N. Morel, M. Wibo, and T. A. Godfraind, Biochim. Biophys. Acta, **649**, No. 1, 82 (1981).
6. H. N. Nellans and J. E. Popovitch, J. Biol. Chem., **256**, 9932 (1981).

PARAMETERS OF METABOLISM OF HEXOSAMINE-CONTAINING BIOPOLYMERS IN EXPERIMENTAL TOOTH REIMPLANTATION

P. N. Sharaev, A. I. Pantyukhin, and O. N. Zubarev

UDC 547.917:516.314-089.843

KEY WORDS: hexosamine-containing biopolymers; reimplantation of teeth

Renewed interest has recently been shown in a surgical method of treatment of chronic periodontitis, namely the reimplantation of teeth [1, 6]. However, the mechanisms of wound healing under these circumstances, and changes in metabolism of connective-tissue biopolymers, including hexosamine-containing biopolymers (HCB), glycosaminoglycans (GAG), and glycoproteins, have still received only little study. Accordingly it was decided to study parameters of HSB metabolism in the periodontal tissues and blood during the course of healing of uninfected wounds associated with one-stage reimplantation of teeth.

EXPERIMENTAL METHOD

Experiments were carried out on 16 male mongrel dogs aged 2-3 years. After preliminary examination by a veterinary surgeon the teeth were removed by the most sparing method under thiopental anesthesia. Four teeth were immediately reimplanted, namely the lateral incisor on both sides of each jaw. Neither curettage of the alveolus nor endodontic treatment of the teeth was carried out. After extraction of the teeth the alveoli were covered with sterile gauze swabs. The extraalveolar period amounted to 15-18 min. During this time the teeth were kept in physiological saline (18-22°C) with penicillin (100,000 U/ml) and streptomycin (100 mg/ml), and with observance of the rules of asepsis. After reimplantation of the teeth in the appropriate alveoli, they were fixed to neighboring teeth by means of quick-hardening plastic.

Of the 16 experimental animals, normal healing of all the reimplanted teeth took place in 14 animals (87.5%), and these were further investigated.

After 14 (Group 1, seven dogs) and 28 (Group 2, seven dogs) days of observation the animals were killed under short-term ether anesthesia. Fragments of the upper and lower jaws were cut with a saw to include experimental (reimplanted) and intact (control, next to the reimplanted) teeth. The fragments of the jaws were freed from soft tissues mechanically, and the roots of the teeth, tissues of the periodontium, and bony tissue of the alveoli were isolated. The total content of HCB [3, 4] and GAG [5] in these tissues was determined and expressed in millimoles hexosamines and hexuronic acids per kilogram dry weight of defatted tissue respectively. Hexosamine synthetase activity (HSA) was determined in the supernatant (3000 rpm, 10 min) of periodontal tissue homogenate and expressed in nanomoles/mg protein/h [7]. Every 7 days during healing, the GAG level was determined in citrated plasma from blood of both groups of animals [5].

Department of Biochemistry and Surgical Stomatology, Izhevsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 4, pp. 377-379, April, 1991. Original article submitted September 4, 1990.

0007-4888/91/0004-0479$12.50