ISOLATION OF PLASMA MEMBRANES OF SMOOTH MUSCLE CELLS OF THE RABBIT SMALL INTESTINE

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UDC 611.341-018.61:57.6.314

KEY WORDS: plasma membranes; subcellular fractions; ATPase.

The plasma membrane (PM) of the smooth muscle cell has recently attracted the closest attention of research workers because of the view that it plays an essential role in regulating the intracellualr concentration of ionized Ca^{++} [1]. Definite progress in this direction has been achieved by the study of functioning of membrane structures of the myometrium [1, 5, 9, 11], but other types of smooth muscles have received less study.

The object of this investigation was to develop a technique of obtaining subcellular fractions of smooth-muscle cells of the rabbit small intestine rich in PM, in order to study the role of the sarcolemma in maintenance of intracellular calcium homeostasis. During development of the technique, attention was paid to the most recent investigation in this field [2, 4, 5, 7, 9, 12].

EXPERIMENTAL METHOD

The recently isolated small intestine of the decapitated animal was washed with cold water, freed from outer connective tissue, and the mucosa was curetted. About 50 g of native tissue from two animals was used in the experments. Minced tissue was homogenized for 35-45 sec in a homogenizer of "Politron" type in medium A, containing 0.25 M sucrose, 0.1 mM EDTA, 1 mM dithiothreitol, and 5 mM imidazole-HCl, pH 7.2. The volume of homogenate was made up to 550 ml and it was centrifuged for 10 min at 11,000g. The resulting supernatant was filtered through three layers of gauze and centrifuged for 10 min at 38,000g. By the first two procedures fragments of tissues and cells and large cellular organelles (nuclei and mitochondria) were removed. The supernatant after the second centrifugation was used to obtain microsomes (145,000g, 60 min). The residue was resuspended in medium A and centrifuged for 120 min at 95,000g in a stepwise sucrose density gradient. For this purpose, 25, 30, 35, and 40% solutions of sucrose containing 5 mM imidazole-HCl, pH 7.2, were used. Suspensions of subcellular fractions located in the upper layers of sucrose solutions were separated, diluted 20-25 times with medium A, and recentrifuged for the purpose of concentration and replacement of the high-density medium by keeping medium (60 min, 145,000g). The latter contained 25% (by volume) glycerin, 0.2 mM $CaCl_2$, 0.1 mM EDTA, and 5 mM imidazole-HCl, pH 7.2. The preparations were kept at between 0 and −5°C.

Quantitatively the fractions were characterized relative to their protein content [10]. ATPase activity was recorded at 37°C potentiometrically [3] or depending on the rate of increase of inorganic phosphate, determined by the method in [6], in the course of hydrolysis of ATP. 5'-Nucleotidase activity was recorded by the method in [11]. The composition of the incubation medium for determination of enzyme activities is indicated in Table 1.

EXPERIMENTAL RESULTS

The distribution of enzyme activities among subcellular fractions is shown in Fig. 1. Clearly activities of enzymes which are plasmalemma markers, namely 5'-nucleotidase [11] and Mg^{++}-ATPase [1], were maximal in fractions obtained in 35% sucrose solutions, but activity of another PM marker, Na,K-ATPase [1, 9], reached a maximum in a lighter fraction of membranes, as was observed also in [5]. Meanwhile the level of inhibition of total ATPase

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(Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 1, pp. 106-108, January, 1984. Original article submitted April 8, 1983.

 0007-4888/84/9701-0134$08.50

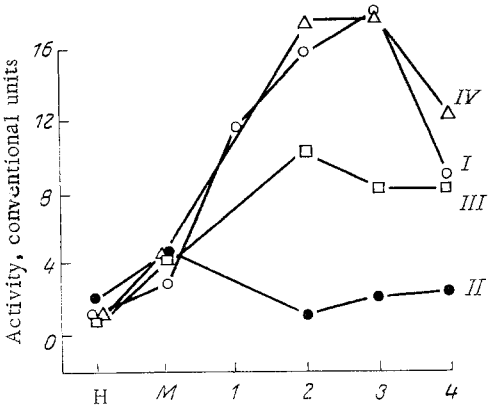

Fig. 1

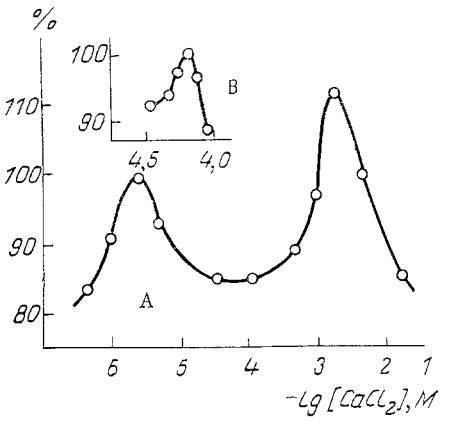

Fig. 2

Fig. 1. Distribution of enzyme activities among subcellular fractions. Abscissa: H) Homogenate, M) microsomes, 1, 2, 3, 4) membrane fractions obtained in 25, 30, 35, and 40% sucrose solutions respectively; ordinate, enzyme activity (in conventional units). I) 5'-Nucleotidase, II) % inhibition of total ATPase activity by sodium azide, III) Na,K-ATPase, IV) Mg-ATPase. Values of corresponding enzyme activity of fraction whose absolute activity is minimal taken as unity.

Fig. 2. ATPase activity of PM fraction as a function of $CaCl_2$ concentration. Incubation medium contains 1.25 mM imidazole-HCl, pH 7.2, 100 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, 3 mM ATP (A), and also 60 μM EGTA (B).

TABLE 1. Enzyme Activities of Fractions of Plasma Membranes of Smooth-Muscle Cells (M ± m)

Fraction	Medium and method of determination	Enzyme activity, nmoles P_i/mg protein/min	P
5'-Nucleotidase	50 mM Tris-HCl, pH 7.4-7.8, 1 mM $MgCl_2$, 4 mM AMP, 100-500 μg protein	142±13	<0,05
Total ATPase	100 mM NaCl, 20 mm KCl, 3 mM $MgCl_2$, 3 mM ATP, 1.5 mM imidazole-HCl, pH 7.2	1350±80	<0,05
Mg-ATPase	5 mM $MgCl_2$, 3 mM ATP, 100 μM ouabain, 1.5 mM imidazole-HCl, pH 7.2	1200±70	<0,05
Na,K-ATPase	According to level of inhibition of total ATPase activity by ouabain	215±40	>0,05
Mg,Ca-ATPase	According to increase in total ATPase activity in the presence of $CaCl_2$ (5-15 μM)	370±45	<0,05

activity by sodium azide, evidence of the presence of mitochondrial contamination [8], was 9 ± 3% for preparations obtained in 30% sucrose solution, and it increased in the heavier fractions. Consequently, the subcellular fraction obtained in 30% sucrose solution was chosen for the subsequent work (conventionally described as PM). Compared with the homogenate, the PM fraction was on average 15 times richer in sarcolemma according to 5'-nucleotidase, 17 times according to Mg-ATPase, and 10 times according to Na,K-ATPase activity.

Values of enzyme activities of PM determined under optimal conditions are given in Table 1. The graph of total ATPase of PM as a function of pH is bell-shaped with a maximum at pH 7.2. Optimal substrate concentrations are 3-4 mM ATP.

It was shown that total ATPase activity of PM is increased in the presence of Ca^{++}. The graph of this function is not linear but has two maxima — in the region of micromolar and millimolar concentrations of Ca^{++} respectively (Fig. 2). In the presence of EGTA the maximum shifts toward higher concentrations. Values of K_m (Michaelis constant) for Ca^{++}, calculated by the double reciprocal method, are 0.29 μM and 1.67 mM. Essentially Mg^{++} also activates ATPase, but activity reaches a maximum only in the region of millimolar Mg^{++} concentrations (K_m = 1.20 mM). Considering the high affinity of Mg-dependent, Ca-activated ATPase for Ca^{++} it can be tentatively suggested that the enzyme participates in regulation of the ionized Ca^{++} concentration in the cell, i.e., that it is a transport Ca-ATPase.

Correlation between the presence of transport Ca-ATPase activity and ability of microsomal membrane vesicles to accumulate Ca^{++} in the presence of ATP has been demonstrated for sarcolemma-enriched membrane fractions of myometrium [1], small intestine [7], and blood vessels [8]. It is possible that ATP hydrolysis and Ca^{++} transport are coupled or performed by the same system. Such a system, in PM of smooth-muscle cells of rabbit small intestine could be Mg-dependent Ca-activated ATPase, activated by micromolar calcium concentrations.

LITERATURE CITED

1. M. D. Kurskii, S. A. Kosterin, N. F. Braktova, et al., Biokhimiya, 46, 1435 (1981).
2. P. V. Pogrebnoi, V. K. Rybal'chenko, and A. I. Ferents, in: Fundamental Problems in Gastroenterology [in Russian], Kiev (1981), p. 196.
3. V. B. Ritov, Biokhimiya, 36, 393 (1971).
4. D. M. Bers, Biochim. Biophys. Acta, 555, 131 (1979).
5. M. E. Carsten and J. D. Miller, Arch. Biochem., 204, 404 (1980).
6. C. H. Fiske and Y. Subarrow, J. Biol. Chem., 66, 375 (1925).
7. A. K. Grover, C. L. Kwan, R. E. Larfield, et al., Canad. J. Physiol. Pharmacol., 58, 1102 (1980).
8. U. Klinner, D. Ehlers, and G. Bartsch, Acta Biol. Med. Ger., 37, 1 (1978).
9. J. F. Krall and S. G. Kornman, Biochim. Biophys. Acta, 556, 105 (1979).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
11. M. A. Matlie, G. Crankshaw, R. E. Larfield, et al., J. Biol. Chem., 254, 1834 (1979).
12. E. Van Alsteine, D. K. Bartschat, N. V. Wellsmith, et al., Biochim. Biophys. Acta, 553, 388 (1979).

EFFECT OF EXCESS VITAMIN A INTAKE ON STATE OF THE EPITHELIUM OF THE RAT SMALL INTESTINE

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UDC 615.356:577.161.11].015.34:616.341-018.73

KEY WORDS: small intestine; vitamin A.

There is much evidence in the literature of the important role of vitamin A in the regulation of differentiation and also, evidently, proliferation of epithelial cells [3, 8, 11]. The effects of vitamin A are linked mainly with its influence on the epithelium of the skin, the upper respiratory passages, and the genitourinary tract [3, 9]. The mucous membrane of the small intestine is considered to be a tissue with low sensitivity to this vitamin. In recent years, however, evidence has been obtained that may compel a revision of this outlook. It has been shown that vitamin A deficiency in experimental animals leads to depression of synthesis in the intestinal mucosa of glycoproteins, which play an important role in intercellular interaction and cellular differentiation processes [9, 10], activation of the enzymes of their catabolism [5], a decrease in the number of goblet cells [9, 10, 12], and lengthening of the cell cycle of the epithelium of the crypts [15]. Only isolated communications have been published on structural changes in the intestinal mucosa following administration of large doses of vitamin A [6, 7]. Meanwhile biochemical studies undertaken previously by one of us (I. Ya. K. [4]) revealed significant changes in glycoprotein metabolism in the intestinal mucosa of rats with hypervitaminosis A.

With the above facts in mind it was decided to study the effect of large doses of vitamin A on the structure and cellular proliferation processes in the mucous membrane of the rat small intestine.

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(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 1, pp. 108-110, January, 1984. Original article submitted May 11, 1983.

0007-4888/84/9701-0136$08.50