EFFECT OF PHENOTHIAZINES ON Ca-ATPase OF THE SARCOPLASMIC RETICULUM IN RABBIT SKELETAL MUSCLES

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The calcium-binding protein calmodulin (CaM) is a modulator of the activity of many key enzymes of metabolism, such as adenylate cyclase, phosphodiesterase, myosin light chain kinase, phosphorylase kinase, etc. [11]. CaM is a regulator of the Ca pump of the plasma membranes of erythrocytes [6, 12] and the sarcoplasmic reticulum (SR) of the heart [10]. It has recently been shown that one member of the phenothiazines, namely trifluoperazine, inhibits activity of the skeletal muscle Ca pump [7]. The phenothiazines are widely used to study the properties of CaM-sensitive enzymes, for they can inhibit the action of CaM [15]. The inhibitory effect of trifluoperazine on the Ca pump of skeletal muscle SR has served as indirect evidence of the presence of CaM in the SR preparations used, and of its regulatory role in operation of the Ca pump.

This paper describes an attempt to study whether the inhibitory action of the phenothiazines on Ca-ATPase in skeletal muscle SR is connected with their ability to abolish the activating action of CaM, or whether their action is aimed directly at the enzyme.

EXPERIMENTAL METHOD

Fragments of SR from rabbit muscles were obtained by differential centrifugation [2]. To abolish the Ca-accumulating ability of the vesicles the SR was treated with a solution of EDTA [8]. Ca-ATPase was purified by the method in [4, 9]. CaM was obtained from the bovine brain [3]. Dansyl labeling of CaM was carried out by the method in [14]. Fluorescence of the dansyl-labeled CaM was measured on a Hitachi 650-60 spectrofluorometer (Japan) in a quartz cuvette with a capacity of 2 ml, containing 100 mM Tris-HCl, pH 7.2. The wavelength of the exciting light was 330 nm, the wavelength of emission 490-510 nm. Activity of Ca-ATPase was determined from the increase in the inorganic phosphate concentration in medium of the following composition (in mM): ATP 3, $MgCl_2$ 3, KCl 100, $CaCl_2$ 0.5, imidazole 30, pH 7.0 (25°C), or from acidification of a weakly buffered medium, using the method of continuous pH recording. In this case the composition of the medium was as follows (in mM): ATP 3, $MgCl_2$ 3, KCl 100, oxalate ion 5, imidazole 3, pH 7.0 (25°C), and 20 μM Ca^{++} [2].

EXPERIMENTAL RESULTS

The study of the effect of phenothiazines on Ca-ATPase of skeletal muscle SR showed that ethmozine, chlorpromazine, and trifluoperazine inhibit the activity of this enzyme. The inhibitory action diminishes in the order: trifluoperazine > chlorpromazine > ethmozine (Fig. 1). The effectiveness of the action of trifluoperazine in these experiments agreed with data in the literature [7].

It was next shown that, after addition of trifluoperazine to the medium, CaM was able to reactivate Ca-ATPase up to the initial level. This fact can be explained in two different ways: 1) Inhibition of Ca-ATPase by phenothiazines is connected with abolition of the activating effect of CaM, present initially in SR preparations [7]; 2) CaM is not concerned in the regulation of the Ca pump of skeletal muscle SR, and the effect of the phenothiazines is aimed directly at the Ca-sites of the enzyme in question, namely Ca-ATPase [1]. To determine which of these hypotheses is correct, the action of CaM itself on the Ca pump was analyzed. The

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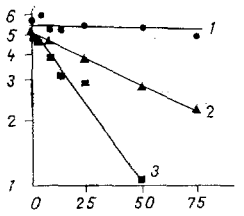

Fig. 1

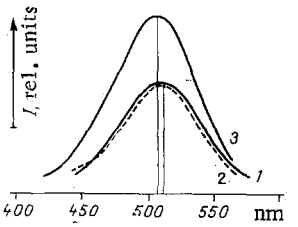

Fig. 2

Fig. 1. Dependence of logarithm of SR Ca-ATPase activity on phenothiazine concentration. 1) Ethmozine; 2) chlorpromazine; 3) trifluoperazine. Abscissa, concentration (in μM); ordinate, log of Ca-ATPase activity (in μmoles P_i/mg protein/min).

Fig. 2. Fluorescence spectra of dansyl-labeled calmodulin. Wavelength of exciting light 330 nm. 1) Control; 2) in presence of SR fragments (170 μg protein); 3) in presence of troponin I (160 μg protein).

results showed that CaM does not induce changes either in the velocity of ATP hydrolysis or the Ca-accumulating capacity of skeletal muscle SR. There is correspondingly no change in the effectiveness of its action, expressed as the value of the Ca/ATP ratio. However, this could mean that the necessary quantity of intrinsic CaM was present initially in the SR preparations.

To obtain direct information on CaM-dependent regulation of skeletal muscle Ca-ATPase, similar investigations were carried out on a preparation of purified Ca-ATPase.

The method of obtaining a preparation of purified C-ATPase includes a stage of solubilization of the SR membrane with Triton X-100 followed by fractionation of the material on a column packed with Sepharose 4B. This treatment of the SR membrane guarantees freedom of the Ca-ATPase preparation from contamination by CaM. In fact, according to electrophoretic data, in polyacrylamide gel and in the presence of sodium dodecylsulfate, protein with a molecular weight of 100 kilodaltons, corresponding to Ca-ATPase, accounts for more than 95% in the purified enzyme preparation. Protein corresponding in molecular weight to CaM is not found in this preparation [3]. Just as with native SR preparations, addition of CaM to the medium in which activity of the purified Ca-ATPase was measured did not lead to activation of the enzyme.

Further evidence of the absence of molecular interaction between SR and CaM was given by experiments with dansyl-labeled CaM. The CaM-binding protein troponin I is known to modify the parameters of fluorescence of dansylated CaM [13]. In fact, on addition of troponin I to dansyl-labeled CaM a considerable increase was observed in the intensity of fluorescence in the 500-510 nm region, reflecting interaction between troponin I and CaM. Meanwhile, addition of SR fragments to dansyl-labeled CaM did not change the parameters of its fluorescence (Fig. 2).

It is a noteworthy fact that, in the present experiments, trifluoperazine, chlorpromazine, and ethmozine inhibited activity of Ca-ATPase of the purified preparation just as effectively as the enzyme preparation before purification. Addition of an excess of CaM, compared with phenothiazines, also led to complete reactivation of the purified preparation.

It can be concluded from the results that the inhibitory action of phenothiazines on Ca-ATPase of skeletal muscles is connected with their direct effect on Ca-ATPase. The reactivating effect of CaM against the background of phenothiazines is probably due to its ability to bind phenothiazines and thus to abolish their inhibitory action on Ca-ATPase of skeletal muscle SR.

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LITERATURE CITED

1. A. A. Boldyrev, Nauch. Dokl. Vyssh. Shkoly, Biol. Nauki, No. 4, 5 (1982).
2. A. A. Boldyrev, ed., Transport Adenosine Triphosphatases [in Russian], Moscow (1977).
3. A. A. Boldyrev and A. M. Rubtsov, in: Functional Activity of Enzymes and Ways of Regulating It [in Russian], Moscow (1981), pp. 82-97.

4. O. D. Lopina, A. A. Boldyrev, and O. V. Esyrev, Dokl. Akad. Nauk SSSR, 230, 240 (1976).
5. M. Yu. Men'shikov and V. A. Tkachuk, Dokl. Akad. Nauk SSSR, 246, 240 (1979).
6. G. H. Bond and D. L. Clough, Biochim. Biophys. Acta, 323, 592 (1973).
7. M. Chiesi and E. Carafoli, J. Biol. Chem., 257, 984 (1982).
8. P. B. Duggan and A. Martonosi, J. Gen. Physiol., 56, 147 (1970).
9. N. Ikemoto, G. M. Bhatnager, and I. Gergely, Biochem. Biophys. Res. Commun., 44, 1510 (1971).
10. S. Katz and M. A. Remtulla, Biochem. Biophys. Res. Commun., 83, 1373 (1978).
11. C. B. Klee, T. H. Crouch, and P. G. Richman, Ann. Rev. Biochem., 49, 498 (1980).
12. M. G. Luthra, G. R. Hildenbrandt, and D. J. Hanahan, Biochim. Biophys. Acta, 419, 164 (1976).
13. D. A. Malencik and S. R. Anderson, Biochemistry (Washington), 21, 3480 (1982).
14. J.-P. Van Ferd and J. Kawazaki, Biochemistry (Washington), 12, 4972 (1973).
15. B. Weiss, W. Prozialeck, M. Cimino, et al., Ann. New York Acad. Sci., 356, 319 (1980).

LOCATION OF DOPAMINE RECEPTORS ON THE NERVE CELL MEMBRANE

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It is generally accepted that mediators of synaptic transmission interact with specific receptors located on the outer surface of nerve cells. Recently, however, evidence of the effectiveness of various mediators when introduced intracellularly has been published [5-7]. This has raised the urgent question of whether the effects of these mediators are the result of their penetration inside the neuron through the cell membrane.

To study this problem, it is convenient to use immobilized mediators, i.e., mediators chemically bound with a certain carrier substance which prevents their intracellular penetration. For this purpose we have used dopamine (DA), bound with a high-molecular-weight polymer. The choice of DA was determined by the similarity of its membrane effects (membrane depolarization) when applied to neurons extracellularly and intracellularly [4, 6]. DA bound with polymer (DA-P) and low-molecular-weight DA were applied to the outer surface of the membrane of sensomotor cortical neurons of a rabbit by microiontophoresis.

EXPERIMENTAL METHOD

Experiments were carried out on 11 adult rabbits weighing 3.0-4.0 kg, anesthetized with urethane (750 mg/kg) and chloralose (30 mg/kg). Altogether 49 sensomotor cortical neurons were tested.

Action potentials (AP) of the neurons were recorded extracellularly through one barrel of a seven-barreled glass microelectrode, filled with 3 M NaCl. The other barrels of the microelectrode were filled with aqueous solutions of the following substances: DA hydrochloride (0.14 M, pH 4.0), DA bound with polymer (0.14 M calculated as low-molecular-weight dopamine, pH 4.0), droperidol (0.02 M, pH 4.5), sodium glutamate (1.0 M, pH 7.3), and the carrier polymer (38%, pH 4.0). One barrel of the microelectrode was filled with 3 M NaCl as control for polarization effects of the current and to compensate current artifacts.

DA-P was purified twice by gel chromatography on Sephadex G-50. Absence of contamination with low-molecular-weight DA was verified by thin-layer chromatography on Silufol in a system of methanol—acetic acid (100:1). The DA content in DA-P, measured spectrophotometrically, was 7.0%.

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