Mg, Ca-ACTIVATION OF ATPase OF PACCINIAN CORPUSCLES

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An ATPase activated by Mg^{++} or Ca^{++} ions was found in single mechanoreceptors (Paccinian corpuscles) of cats; the addition of Ca^{++} (10^{-5} M) to the Mg-ATPase increased its activity by 1.6 times. The optimum for activity of Mg- or Ca-ATPase lies in the alkaline pH region. High substrate specificity of Mg,Ca-ATPase was demonstrated. Parachloromercuribenzoate (5 mM) considerably reduced Mg,Ca-ATPase activity whereas ouabain (10^{-5} M) had no significant effect on its activity. It is suggested that the Mg,Ca-ATPase of the Paccinian corpuscles is closely related to actomyosin-like proteins.

KEY WORDS: tissue mechanoreceptors; ATPase; actomyosin-like proteins.

The reception of an external stimulus by the sense organs takes place through the utilization of energy of the high-energy bond of ATP. The act of reception is considered to be based on a universal mechanism of interaction between proteins of the actomyosin complex and the energy of the external stimulus [9, 11]. For example, motion of the olfactory hairs of the frog [1] and of the kinocilia of the hair cells of the labyrinth and lateral line of fishes [2] depends on the ATP concentration in the fluid which bathes them. ATP increases the activity of the taste receptors of frogs [10]. The function of some tissue mechanoreceptors (Paccinian corpuscles) has also been shown to depend on the presence of ATP in the surrounding medium: ATP increases the receptor and spike potentials in response to mechanical stimulation [8]. Attempts have been made previously to detect ATPase activity in Paccinian corpuscles by histochemical methods [4, 6, 7].

The biochemical investigation of the ATPase properties of these mechanoreceptors is of considerable interest.

EXPERIMENTAL METHOD

Paccinian corpuscles were isolated from the mesentery and pancreas of adult cats under superficial ether anesthesia. The receptors were dissected under a binocular microscope in tris-HCl buffer solution. Protein was determined by Lowry's method. ATPase activity was determined in the homogenate by the increase in inorganic phosphorus (P_i) by the method of Fiske and Subbarow and expressed in μmole P_i/mg protein/h. The incubation medium contained 60 mM tris-HCl buffer (pH 7.4) and 2.5 mM ATP-Na_2. To study the effect of bivalent cations on enzyme activity, $MgCl_2$ and $CaCl_2$ were added to the medium. The substrate specificity of the enzyme was investigated by replacing the ATP by equimolar amounts of ADP, AMP, and β-glycerophosphate.

EXPERIMENTAL RESULTS AND DISCUSSION

Investigations on 22 animals showed that the protein content in the Paccinian corpuscles is 74.4 ± 1.1 μg/mg wet weight, i.e., 7.44%, in agreement with data in the literature [13]. The level of ATPase activity of the homogenate varied only slightly in the pH region from 6.0 to 9.8 (Fig. 1, 1). On the addition of Mg^{++} or Ca^{++} ions to the medium the enzyme activity was increased. Activation of the enzyme by bivalent cations differed at different pH values of the medium and was maximal in the alkaline region (Fig. 1, 2 and 3).

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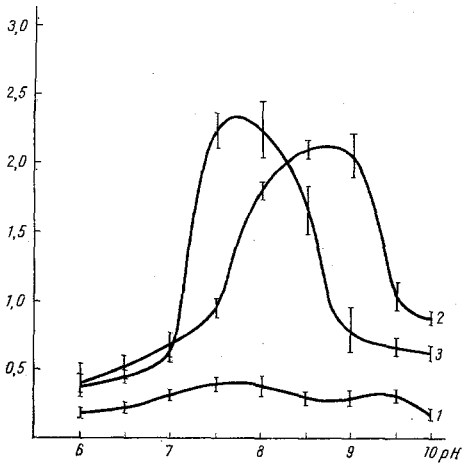

Fig. 1.

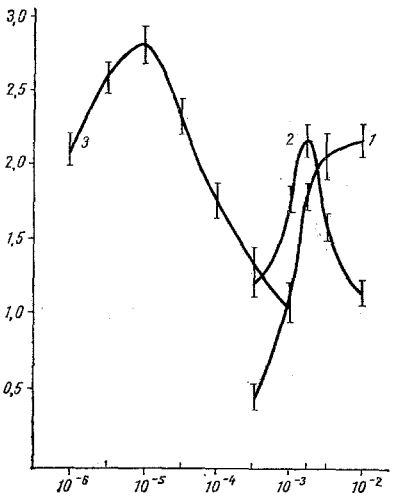

Fig. 2.

Fig. 1. Effect of pH of incubation medium on ATPase activity of Paccinian corpuscles: 1) activity of enzyme in medium without cations; 2) in the presence of 2.5 mM $MgCl_2$; 3) in the presence of 2.5 mM $CaCl_2$. Abscissa, pH of incubation medium; ordinate, ATPase activity (in μmole P_i/mg protein/h in the presence of 2.5 mM ATP).

Fig. 2. Effect of concentration of bivalent cations on ATPase activity of Paccinian corpuscles: 1) enzyme activity in relation to $MgCl_2$ concentration; 2) to $CaCl_2$ concentration; 3) to $CaCl_2$ concentration in the presence of 2.5 mM $MgCl_2$. Abscissa, concentration of bivalent cations (in moles); ordinate, ATPase activity (in μmole/mg protein/h in the presence of 2.5 mM ATP).

Characteristically the maximum of enzyme activation by Ca^{++} ions was shifted into the neutral pH region.

Activation of the enzyme by bivalent cations varied differently as a function of their concentration. With an increase in the concentration of Mg^{++} ions in the medium, activity of the enzyme increased up to a certain, relatively constant level (Fig. 2, 1). As regards Ca^{++} ions, an increase in their concentration above a certain optimum led to a decrease in the activation of the enzyme (Fig. 2, 2). This difference may perhaps be explained by different mechanisms of enzyme activation by bivalent cations. According to Ikemoto [12], Mg^{++} activates the enzyme by forming a complex with the substrate, whereas Ca^{++}, depending on its concentration, binds with sites on the ATPase molecule that are more or less accessible for it, thereby inducing conformational changes which initially activate the enzyme but later inhibit it.

Since activation by Mg^{++} and Ca^{++} ions is a characteristic feature of proteins of actomyosin type, it is particularly interesting to study the effect of Ca^{++} ions on enzyme activity in the presence of an optimal concentration of Mg^{++} ions. Experiments showed that the activity of Mg-ATPase was increased by 1.6 times in the presence of Ca^{++} ions. The increase in activity depended on the concentration of Ca^{++} ions and was maximal when the Ca^{++} concentration was 10^{-5} M. However, a concentration of $2.5 \cdot 10^{-3}$ M, optimal for Ca-ATPase, inhibited Mg-ATPase (Fig. 2, 3). The sensitivity of Mg-ATPase to Ca^{++} can perhaps be explained by the presence of troponin–tropomyosin-like proteins in Paccinian corpuscles [3].

The study of the substrate specificity of Mg,Ca-ATPase relative to different esters of phosphoric acid showed that this enzyme hydrolyzes ATP most actively and ADP less actively. Activity of the enzyme with respect to AMP and β-glycerophosphate was much lower than for ATP, namely 0.14 ± 0.01 and 0.18 ± 0.01 μmole P_i/mg protein/h, respectively. The enzyme discovered in Paccinian corpuscles in the present experiments was highly sensitive to p-chloromercuribenzoate (5 mM), which inhibited it by 75%. Ouabain, in a concentration of 10^{-5} M, did not affect Mg, Ca-ATPase activity. The results accordingly show that the properties of Mg,Ca-ATPase of the Paccinian corpuscles are similar to the properties of proteins of the actomyosin complex found in various tissues, including nerve tissue [5].

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