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The effects of calcium on the ATPase activity of electric tissue extracts

The existence of an ATPase (ATP phosphohydrolase, EC 3.6.1.3), or ATPase system, that requires the presence of both Mg^{2+} and Ca^{2+} for full activity has been demonstrated in muscle sarcosomes[1,2] as well as in red cell ghosts[3,4]. In both cases, it appears that the activity of the enzyme is involved in active transport of Ca^{2+} (refs. 1, 5). Evidence has been presented[6] indicating the presence of an analogous ATPase system in brain microsomes; this may be related to the fact that brain microsomes can accumulate Ca^{2+} in the presence of Mg^{2+} and ATP[7,8]. Such findings may be important in view of the essential role played by Ca^{2+} in excitation and conduction.

In the present work, we show the presence of a $(Mg^{2+} + Ca^{2+})$-requiring ATPase activity in particulate fractions obtained from a tissue which is extremely rich in postsynaptic membranes, the electric organ of *Torpedo marmorata* L.

The freshly dissected organ (about 40 g wet tissue usually) was forced through holes (1 mm in diameter) in a tissue press (Harvard Apparatus Co., Dover, Mass.). The resulting paste was diluted with 2 vol. of 0.3 M sucrose and was either directly centrifuged or homogenized in a Potter–Elvehjem homogenizer with a loose-fitting Teflon pestle (clearance about 1 mm). The pestle was passed up and down the tube only twice. The homogenate was diluted again with 2 vol. of 0.3 M sucrose and centrifuged for 20 min at 3000 rev./min. The pellet (P_1 fraction) was resuspended in 0.3 M sucrose and filtered through gauze before use. The protein concentration was approx. 5–10 mg/ml. The supernatant was centrifuged at 11000 × *g* for 20 min, yielding a relatively small pellet (P_2). The remaining supernatant (S_2) was used as such.

The effects of increasing concentrations of Ca^{2+} on the ATPase activity of different fractions are shown in Fig. 1. It can be seen that Ca^{2+} induces about 50 % stimulation in the presence of Mg^{2+} and ouabain. When the tissue has been passed through the press but not homogenized, the curves obtained (a and b) generally have a simple shape, the apparent K_m being relatively high (0.05–0.2 mM). However, if the tissue has been homogenized or frozen, the curves are more complex and generally exhibit 2 maxima. The first corresponds to a Ca^{2+} concentration of less than 0.01 mM; the activity at this concentration is very near the maximal values. It appears, therefore, that a more extensive fragmentation of the cellular structures induces a considerable decrease in the apparent K_m values for calcium. Ageing of the preparation at −20° or repeated freezing and thawing generally results in the appearance of even more than two peaks in the curve, together with a marked decrease in the activity ratio $(Mg^{2+} + Ca^{2+})/(Mg^{2+}$ alone) (data not presented). The Ca^{2+}-stimulated system thus appears to be very labile in this tissue.

If the incubation medium contained relatively large amounts of monovalent cations (Fig. 1, d), a decrease in the total ATPase activity was observed, concomitant with an increase in the activity ratio $(Mg^{2+} + Ca^{2+})/(Mg^{2+}$ alone). Little or no specificity was noted as far as the monovalent cation was concerned, *i.e.* the same effect was observed with K^+, Na^+, Rb^+ and NH_4^+.

Another remarkable effect of Ca^{2+} was observed in the presence of both Na^+ and K^+ in the medium (Fig. 2). No ouabain was present. Under these conditions,

Abbreviation: EGTA, ethyleneglycol-bis-(2-aminoethyl)-*N*,*N*,*N*,*N*-tetraacetate (Tris salt).

a 5-fold higher activity was measured in the P_1 fraction, in the absence of Ca^{2+}, thus indicating that some 80% of the total ATPase activity of the tissue is due to the (Na^+-K^+)-ATPase. This was also the case in electric organ of *Electrophorus electricus*[9].

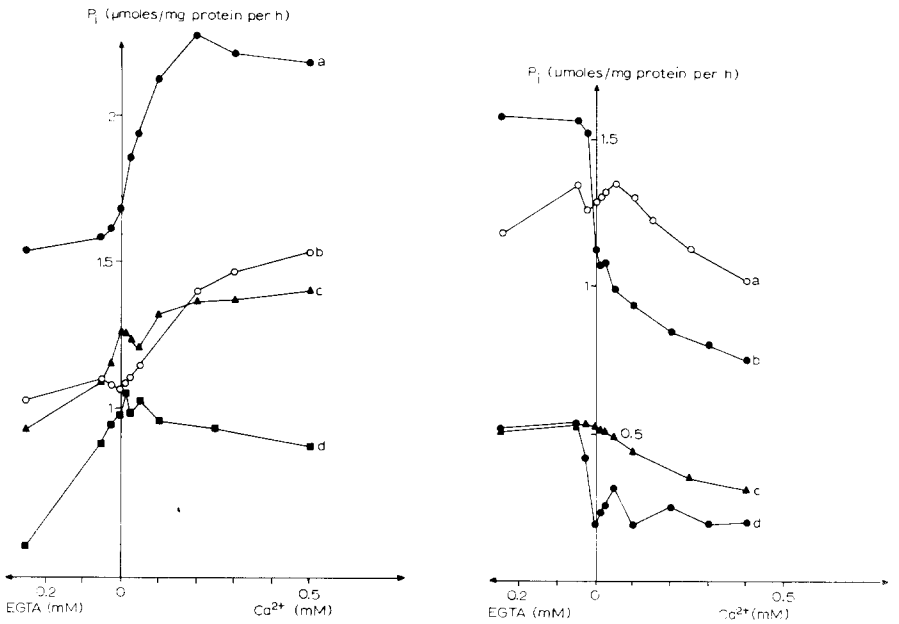

Fig. 1. Effect of ethyleneglycol-bis-(2-aminoethyl)-*N*,*N*,*N*,*N*-tetraacetate (Tris salt) (EGTA) and Ca^{2+} on the ATPase activity of particulate fractions of electric tissue extracts under different conditions. Since some contaminating Ca^{2+} was always present in the incubation medium, it was necessary to use EGTA in order to measure the activities in the presence of less than 0.01 mM Ca^{2+}. A calculation of the concentrations of free Ca^{2+} in the presence of limited amounts of EGTA was not attempted in view of the high Ca^{2+}-buffering capacity of the enzymatic preparation used. The composition of the incubating medium was: 4 mM Na_2ATP (Sigma), 5 mM $MgCl_2$, 25 mM Tris–HCl buffer (pH 7.1), 0.05 mM ouabain. The incubation was run at 37° for 20 min. P_i was estimated by the Fiske and SubbaRow method after deproteinization with trichloroacetic acid. Curve a, a freshly prepared P_2 fraction was used. The tissue was only filtered through the press without further homogenization; Curve b, P_1 fraction: no homogenization; Curve c, P_1 fraction: the tissue was ground with the Potter–Elvehjem homogenizer after filtration through the press. Curve d, P_1 fraction prepared as in c but 0.3 M KCl was added to the incubation medium.

Fig. 2. See Fig. 1, but the incubation medium contains 150 mM NaCl, 50 mM KCl and no ouabain. Curve b, P_1 fraction freshly prepared: no homogenization; Curve a, see b, but the preparation was frozen and thawed twice; Curve c, S_2 fraction: the tissue was ground with the Potter–Elvehjem homogenizer after filtration through the press; Curve d, S_2 fraction: no homogenization.

However, in the presence of less than 0.01 mM Ca^{2+} in the medium, a considerable drop in the activity was observed (Curves b and d). This was true for both P_1 and S_2 fractions, provided the preparation was fresh and obtained from a tissue that had not been strongly ground. Extensive homogenization as well as repeated freezing and thawing (Curves a and c) resulted in the loss of this property. Under these conditions, much larger amounts of Ca^{2+} were required to obtain an appreciable inhibition of the ATPase activity.

These results illustrate the fact, already observed in red cell ghosts[10], that the

breaking up of cellular or subcellular structures may considerably affect the properties of membrane ATPases. This fact must be borne in mind in any attempt to interpret the regulatory effects of calcium on ion exchanges by an approach at the enzymatic level.

The (Mg^{2+}-Ca^{2+})-activated ATPase described here is not likely to be bound to mitochondria since mitochondrial ATPase was never found to require both Mg^{2+} and Ca^{2+} for full activity. However, it is not possible to decide whether the ($Mg^{2+} + Ca^{2+}$)-stimulated enzyme system originates from plasma membranes or from endoplasmic reticulum of electroplaxes. On the other hand, the above findings suggest that low amounts of Ca^{2+} can exert a regulating effect on the ($Na^+ + K^+$)-stimulated ATPase provided subcellular structures are preserved.

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Laboratoire de Biochimie, P. WINS
Université de Liège, Liège (Belgium) M.-L. DARGENT-SALÉE

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Sugar uptake in acutely denervated levator ani muscle of rat

Previous experiments[1] showing that acute denervation specifically blocks testosterone effect on xylose uptake in the rat levator ani, a completely white muscle[2], prompted us to study further the regulation of sugar transport in this muscle after denervation.

No effect on the basal transport rate was shown after 16 h (ref. 1), but a rise in sugar uptake could be expected, at least in the white muscle, after more than 1 day, on the basis of the control of sugar transport by the energy metabolism[3]. In fact, at that time the denervated white muscle showed an increase in active transport of amino acids[4] and in O_2 consumption (see ref. 4). Decreases of glycogen (see ref. 5) and phosphagen content[6] and an increase of glycogen phosphorylase activity[7] have also been reported.