PN 1290

The relationship between phosphorus metabolism and the sodium pump in intact crab nerve

A number of workers have shown that the microsomal fraction of cells contains an ATPase which requires Mg^{2+}, Na^+ and K^+ for maximal activity[1-10]. In the red cell, evidence has been presented that the enzyme is located in the cell membrane and that activation is brought about by internal Na^+ and external K^+ (see ref. 11, 12), but comparable evidence for other cells is lacking.

In the present work, intact nerves were obtained from the walking legs of the spider crab *Maia squinado*[13]. This preparation consists largely of two cell types: nerve cells and Schwann cells. The total P_i content was assayed, without breakdown of energy-rich phosphate compounds, by the following method. Nerves were first frozen by laying them on a copper boat floating on a mixture of acetone and solid CO_2 and then rapidly homogenised in a 5% solution (w/v) of trichloroacetic acid containing crystals of ice. After centrifuging the samples, P_i was estimated in the supernatant by a micro modification of the method of BERENBLUM AND CHAIN[14]. The extracellular space was estimated from the uptake of ^{131}I to be 30% of the recorded weight and the results were corrected accordingly. Before commencing the experiment, all nerves were soaked for at least 2 h in a NaCl artificial sea water containing 10 mM K^+ (10K(Na)ASW)[15]. Stimulation experiments were performed at 18–20° and all other treatments at 16.5°. None of the experimental procedures irreversibly impaired the electrical excitability of the nerves. All solutions were approx. pH 8.0.

In resting nerve in 10K(Na)ASW the P_i content was 91 ± 22 μg P per g which rose during stimulation for 10 min at 30 impulses/sec to 162 ± 23 μg P per g. Addition of 1 mM ouabain to the sea water abolished the rise in P_i on stimulation. Stimulation in 10K(Li)ASW also caused no rise in P_i; but stimulation in 1 part 10K(Li)ASW plus 1 part 10K(Na)ASW caused the normal increase. The main result of stimulation on the ionic content of nerve cells is an increase in internal Na^+ and a decrease in internal K^+ (ref. 16). In LiASW the fall in K^+ still occurs, but the nerve accumulates Li^+ and not Na^+. The fact that stimulation in LiASW causes no change in P_i, whilst stimulation in a 1 + 1 mixture of Li and NaASW causes a large increase in P_i, is strong evidence that internal Li^+ is entirely passive—causing neither activation nor inhibition—and that the key factor in initiating the increase in P_i is the increase in internal Na^+ content of the nerve.

In all subsequent experiments involving stimulation, the nerves were placed in 10K(Na)ASW and tetanised for 10 min at 30 impulses/sec.

After stimulation, the P_i concentration of nerves kept in 10K(Na)ASW fell back to the original level over a period of about 4 h. 80% of this fall occurred in the first hour after cessation of stimulation and its time course closely resembled the time course of "recovery heat" production in Maia nerves which had been subjected to prolonged stimulation[17]. In 100K(Na)ASW, the P_i content rose rapidly and then declined until after about 3 h it was below the unstimulated level. In 0K(Na)ASW and 10K(Na)ASW plus 1 mM (or 0.1 mM) ouabain, the P_i content fell rapidly (10 min) and remained low as long as the solution was applied. Returning the nerve to 10K(Na)ASW resulted in an increase in P_i to the stimulated level in the 0K-treated

Abbreviation: ASW, artificial sea water.

nerve, but only a small increase in the ouabain treated nerve. The marked reduction in P_i concentration following transfer from 10K(Na)ASW to oK(Na)ASW occurred in artificial sea waters and whose pH ranged from 6 to 9, in the presence or absence of Ca^{2+} and Mg^{2+} and with a variety of anions (chloride, nitrate, acetylglycine, propionate and sulphate).

The changes in P_i level reflect changes in the activity of an intracellular energy-rich phosphatase. The amount of P_i in an unpoisoned cell depends on the balance between the rate of synthesis and breakdown of energy-rich phosphate compounds. Any alteration in this balance results in a change in the level of P_i; for instance, acceleration of breakdown raises the P_i level whereas inhibition lowers it. The breakdown reactions can be separated from synthesis by poisoning the cell with 2 mM cyanide. This markedly reduces the rate of synthesis of energy-rich phosphate compounds and under these conditions the intitial rate of P_i release gives a measure of the total energy-rich phosphatase activity of the tissue. The measured initial rates were in the order 10K(Na)ASW (stimulated) $\gg$ 10K(Na)ASW (unstimulated) $>$ oK(Na)ASW (stimulated) $=$ 10K(Na)ASW + ouabain (stimulated). The substrate acted on is not known. The major energy-rich phosphate compounds in Maia nerve are ATP and arginine phosphate. Estimation of the arginine content of the nerves by the method of ROSENBERG *et al.*[18] indicated that two-thirds of the change in P_i was due to breakdown of arginine phosphate. There was a slow and very variable breakdown of externally applied ATP. This ATPase activity was unaffected by ouabain, but inhibited by fluoride and most probably resulted from damaged cells (but see ref. 19).

The results show that there is in the axon and possibly in the Schwann cell an enzyme system which utilises intracellular energy-rich phosphate and is activated by internal Na^+ and external K^+ and is inhibited by external application of ouabain. Thus it has both the main properties of the crab-nerve microsomal preparation of SKOU[1] and the orientation in ion specificity of the sodium pump[20,21].

Evidence that elevation of the P_i content of the nerves reflected an increased activity of the sodium pump was obtained by measuring the concentration of Na^+ and K^+ by flame photometry. The nerves were first washed in oK(choline)ASW for 10 min to remove extracellular Na^+ and K^+. In comparison with unwashed nerves in which a correction for extracellular space was made, this choline wash caused negligible changes in internal Na^+ and K^+. On stimulation in 10K(Na)ASW, the Na^+ content of the nerve rose and the K^+ content fell. This was reversed on soaking in 10K(Na)ASW or 100K(Na)ASW, but in oK(Na)ASW or 10K(Na)ASW + ouabain the Na^+ continued to rise and the K^+ to fall. Poisoning the nerves with cyanide in 10K(Na)ASW also resulted in a continued accumulation of N^+ and loss of K^+. Thus the pump is activated following stimulation of the nerve in 10K(Na)ASW, but is inhibited by removal of external K^+, addition of ouabain or poisoning with cyanide.

Following stimulation, replacement of 10 mM K^+ in the artificial sea water by an equal concentration of other cations gave the following series listed in order of effectiveness in ability to maintain an elevated P_i level: $K^+ > Rb^+ > NH_4^+ > Cs^+ = Li^+ = Na^+ = Rb^+ + 1$ mM ouabain. At a concentration of 10 mM, Rb^+ but not Cs^+ or Li^+ was able to produce a net efflux of Na^+ from the nerve.

Complete replacement of external Na^+ by Li^+, choline or sucrose resulted in a marked elevation of the P_i level, even in oKASW. This effect required previous

stimulation of the nerve (*i.e.* internal Na^+) and was inhibited by ouabain. One possible explanation is that normally Na^+ competes with K^+ at the external K^+ activation site and that in absence of Na^+ the energy-rich phosphatase is maximally activated by the traces of K^+ (0.1 mM) always present in "oK" sea waters. WHITTAM AND AGER[22] and POST *et al.*[3] have observed a similar effect in red cells.

The conclusion from these experiments is that intact crab nerve contains an enzyme system which can utilise energy-rich phosphate compounds, but requires the presence of both internal Na^+ and external K^+ for activity. When activated the enzyme system accounts for a substantial fraction of the total energy-rich phosphatase activity of the tissue and under normal conditions its activity is associated with a net outward movement of Na^+ and a net inward movement of K^+. Rb^+, but not Cs^+ is able to substitute for K^+ in the external solution; but Li^+ is unable to replace internal Na^+. Although the substrate is not known, it is most likely to be either ATP or arginine phosphate. In recent unpublished experiments with the perfused giant nerve fibre of the squid[23] the breakdown of internally perfused ATP, in presence of K^+ and Mg^{2+}, was greatly stimulated by addition of Na^+ to the perfusion fluid and was inhibited by external application of ouabain. By analogy with these experiments, it seems probable that ATP is the substrate for the Na^+–K^+ activated energy-rich phosphatase of intact Maia nerve. As the properties of this system are very similar to those of the ATPase preparation isolated by SKOU from Carcinus nerve, it seems likely that SKOU's preparation is involved in the sodium pump.

I wish to thank the Director of the Laboratory of the Marine Biological Association, Plymouth, for providing facilities for much of this work; the Medical Research Council for a Scholarship and Mr. A. E. NUTTY for valuable technical assistance.

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Received May 27th, 1963