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PRELIMINARY STUDIES ON THE CHARACTERIZATION OF Na-K-ATPase AND ITS RELATIONSHIP TO ACTIVE Na TRANSPORT IN THE URINARY BLADDER OF THE WINTER FLOUNDER *Pseudopleuronectes americanus*

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Skou originally proposed (BBA, 23, 394, 1957) that a Mg-ATPase and a Na-K-ATPase might be involved in the active pumping of sodium from crab axon. His idea fostered studies which linked ATPase activity to active cation transport in a variety of tissues (Bonting in Membranes and Ion Transport, E.E. Bittar, editor, Wiley-Interscience, London, 1970, pp. 270-277). The present study was initiated to determine if such a relationship exists in the urinary bladder of the winter flounder *Pseudopleuronectes americanus*.

Na-K-ATPase activity was determined in freeze-dried homogenates of flounder bladder by a modification of the procedure of Bonting (*op. cit.* pp. 260–264). To define the optimal conditions for maximal Na-K-ATPase activity freeze-dried homogenates were reconstituted and incubated with increasing concentrations of ATP, Mg^{++}, K^{+}, and Na^{+} (Figure 1). In each case Michaelis-Menton kinetics are obeyed. The pH optimum for this enzyme lies between 7.2 and 7.9. This finding is in excellent agreement with pH optima found for Na-K-ATPase (7.0-8.0) in all other tissues studied (Bonting, *op. cit.*, p. 267). Further we have determined the inhibitory effects of varying concentrations of ouabain (10^{-9}–10^{-5}M) on flounder bladder Na-K-ATPase and have found maximum inhibition for effector concentrations $\geqslant 10^{-5}$M.

In a separate set of bladders Na reabsorption was compared with Na-K-ATPase activity. Tissue preparation procedures and the protocol for measuring sodium fluxes have been reported elsewhere (Renfro, Bull. MDIBL, this issue). Immediately following sodium flux determinations, each bladder was homogenized and freeze-dried in preparation for ATPase assays. Na reabsorption, mucosa to serosa flux, which is the predominately active transport in flounder bladder (Renfro, *op. cit.*) correlates well (r = 0.81) with Na-K-ATPase activity in whole bladder homogenates (Figure 2). Variations in Na flux and ATPase activity were spontaneous. Indeed Van Os (Biochim, Biophys. Acta, 241, 89, 1971) obtained a similar correlation for Na-K-ATPase and net water reabsorption (used as an indicator of net Na reabsorption) in guinea pig and rabbit gall bladders.

In conclusion the effect of ouabain on active Na transport reported earlier (Bull. MDIBL, 12, 81, 1972) and the relationship of Na-K-ATPase to active Na transport provide preliminary evidence that Na-K-ATPase plays a role in transport processes in the teleost urinary bladder. It remains to be established whether or not this enzyme is directly involved with transepithelial ion movement or indirectly involved through its known role in maintenance of cellular ion gradients.

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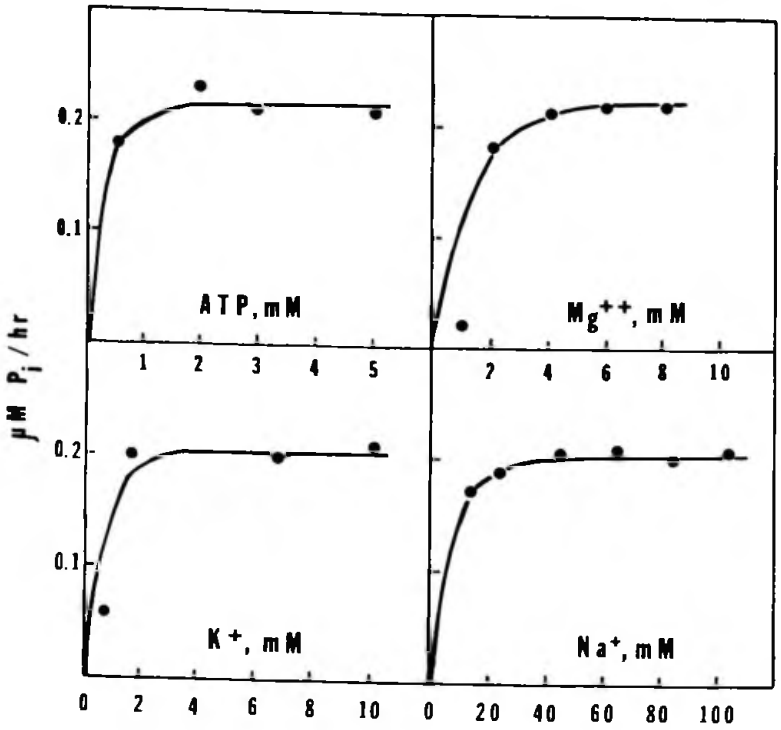
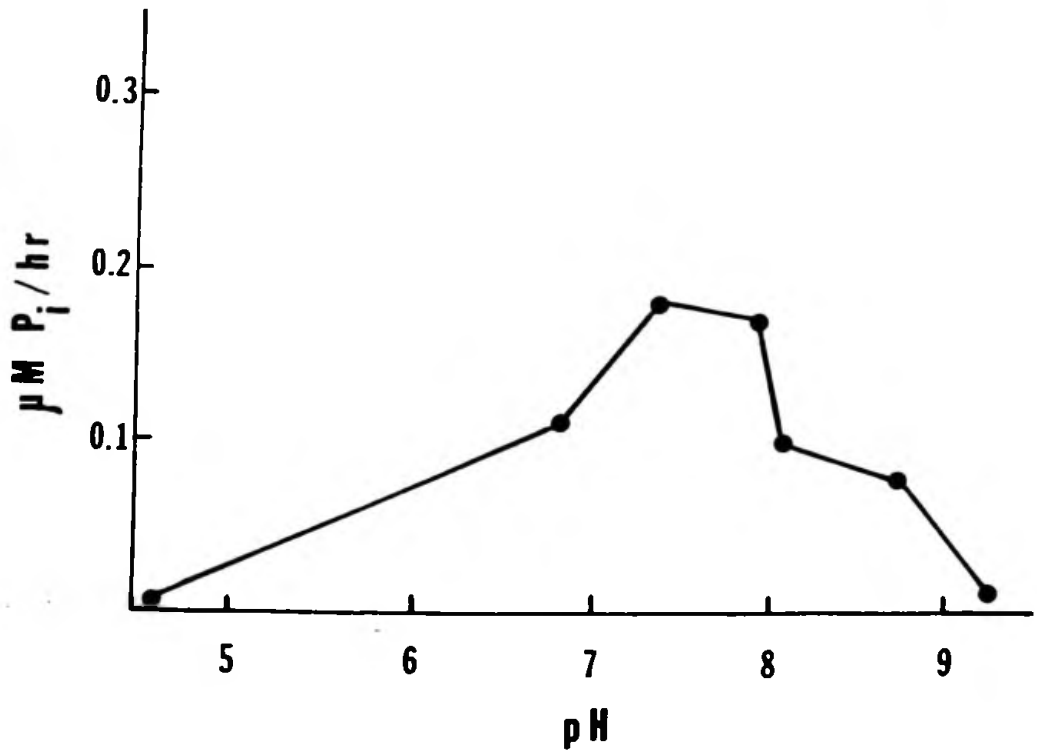


Figure 1. Effect of independently varying ATP, Mg^{++}, K^+, and pH on the Na-K-ATPase (ouabain sensitive) activity in flounder urinary bladder. Each graph shows the effect of varying the concentration of one constituent of the following standard reaction mixture: 60 mM Na, 5 mM K, 2 mM ATP, 4 mM Mg, 92 mM Tris (pH 7.5). Tissue homogenates were preincubated for 10 min. at 37°C in the appropriate reaction mixture (without ATP or Mg). The assay was started by addition of a standard volume of a solution of $MgCl_2$ and disodium-ATP. Incubation time was 15 min. Each point represents the mean of duplicate assays. Data are presented in relative units.



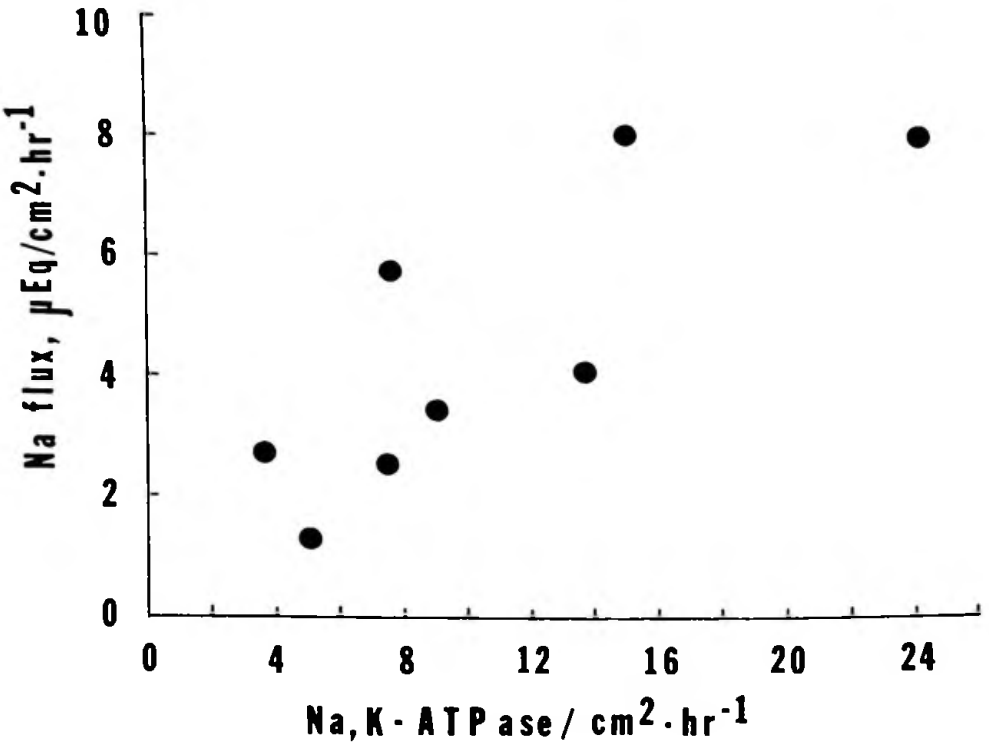


Figure 2. Relationship between Na-K-ATPase activity and unidirectional mucosal to serosal sodium flux in the isolated perfused urinary bladder of the winter flounder. ATPase assays (standard reaction mixture) as described in Figure 1. Sodium flux was determined with identical solutions (modified Forster's saline) bathing serosa and mucosa under open circuited conditions. Units of Na-K-ATPase activity are μM P_i liberated/$cm^2 \cdot hr^{-1}$.

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VOLTAGE CLAMP STUDIES IN VENTRICULAR TRABECULAE FROM DOGFISH *Squalus acanthias.*

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The link between excitation of the myocardium and the initiation of contraction has been extensively studied in various mammalian and amphibian species. The general picture emerging from a combination of electrophysiological and electron microscopic studies is that specific subcellular structures can account for specialized functional developments in the myocardium. For instance in the amphibian (frog) heart, which has a relatively simple, subcellular structure with no t-tubules and little sarcoplasmic reticulum, the surface membrane electrical activity (action potential) directly controls the availability of the activator Ca^{+2} and therefore the development and maintenance of tension. On the other hand the mammalian (cat, sheep, dog) heart, which shows an extensive and complex intracellular membrane system (t-tubules and sacroplasmic reticulum), responds indirectly to the surface membrane electrical activity. That is the action potential serves: 1) as a "trigger"