## Thermal Activation of the Cyclic AMP Stimulated Sodium Transport Across Isolated Toad Bladder and $Na^+/K^+$ ATPase in Toad Bladder Homogenates

Our analysis of the temperature coefficients for sodium transport across the isolated toad bladder[1-3] has shown that although passive mucosal permeability and active serosal transport may each involve several stages they have characteristic activation energies of 13.5 kcals/mole for mucosal permeability and 9 kcals/mole for the active pump. In the present work we have investigated the thermal activation of the $Na^+/K^+$ ATPase measured in toad bladder homogenates using the BONTING and CARAVAGGIO[4] method and of the cyclic AMP stimulated $Na^+$ transport across the isolated toad bladder.

The toads (*Bufo marinus*) were killed and the bladders quickly exised and prepared as a 10% homogenate in a 0.6% saline containing 0.2 m*M* EDTA pH 7.4. Two incubation media were prepared, solution A containing 2 m*M* ATP, 1 m*M* $MgCl_2$,. 5 m*M* KCl, 58 m*M* NaCl, 10 m*M* KCN, 0.1 m*M* EDTA, 92 m*M* *tris* and solution B containing 2 m*M* ATP, 1 m*M* $Mg\,Cl_2$, 58 m*M* NaCl, 10 m*M* KCN, 0.1 m*M* EDTA, 92 m*M* *tris* and $10^{-4}M$ ouabain. A 0.15 ml aliquot of the toad bladder homogenate was added to 2.25 ml of solution A and 50 µl of this solution was quickly added to 6 small tubes and incubated in a bath at a temperature between 5° and 35°C. A similar routine was carried out using 0.15 ml of the same homogenate with 2.25 ml of solution B. At each temperature there were 6 samples of homogenate incubated in solution A and 6 samples incubated in solution B. After incubation for 1 h 250 µl of a 10% TCA solution was added to all solutions. They were spun in an Eppendorf centrifuge and 250 µl taken into fresh tubes to which were added 250 µl aliquots of the colour reagent containing 400 mg of ferrous sulphate in 10 ml of 1% ammonium molybdate in 1.15 normal sulphuric acid. The colour was allowed to develop for 20 min and the absorbency read at 700 nm. The difference in absorbency at this wavelength was determined for solutions A and B and the relative $Na^+/K^+$ ATPase calculated by considering the maximum difference as equal to 100. A logarithm of this relative $Na^+/K^+$ ATPase against the reciprocal temperature is shown (Figure 1). The activation energy determined from such plots gave a value of about 9 kcals per mole corresponding to activation of the serosal pump. We have suggested that under normal conditions the rate of ion transport is controlled by the rate at which sodium can enter across the mucosal permeability barrier and that aldosterone, vasopressin and amphotocin B (Table) act to lower this barrier to an extent that makes the pump rate limiting. Any agent that predominantly affects pump activity may increase $Na^+$ transport but the temperature coefficient will remain unaltered. We have determined the activation energies for the $Na^+$ transport stimulated (Figures 2 and 3) in the presence of cyclic AMP ($10^{-3}M$) and theophylline ($10^{-2}M$) using the method described previously[3]. The values obtained in both cases are close

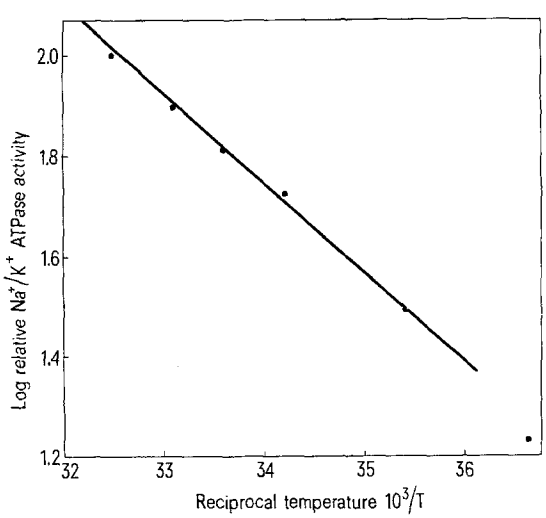


Fig. 1. Thermal activation of the $Na^+/K^+$ ATPase measured in toad bladder homogenates.

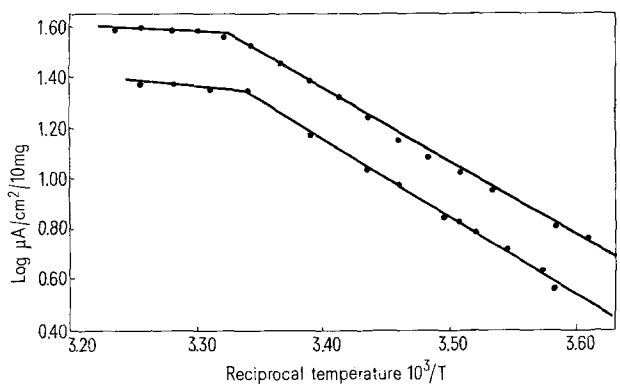


Fig. 2. Thermal activation of the short circuit current measured across toad bladder stimulated by $10^{-2}M$ theophylline (upper plot) and $10^{-3}M$ cyclic AMP (lower plot).

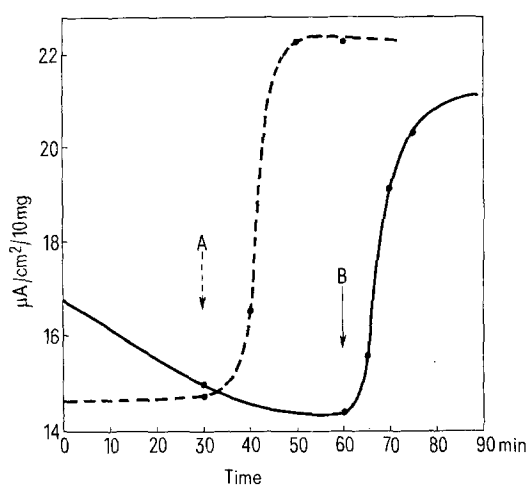


Fig. 3. Effect of $10^{-2}M$ theophylline (A) and $10^{-3}M$ cyclic AMP (B) on the short circuit current measured across toad bladder.

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to 13.5 kcals per mole unlike the activation energy determined in the presence of vasopressin.

There is a considerable amount to evidence supporting a separation of water and $Na^+$ transport effects of vasopressin[6–9]. The observation that 10 m*M* $Ca^{2+}$ inhibits the vasopressin stimulated water transport but has no effect on vasopressin stimulated sodium transport led PETERSEN and EDELMAN[9] to suggest that sodium and water transport effects of vasopressin may be due to to separate adenylate cyclase systems. However, we have studied the vasopressin stimulation of cyclic AMP levels in the toad bladder and have shown a dose response characteristic of a single activation process[10]. The dose response characteristic of respiration increases following vasopressin treatment shows evidence of two activation processes[4] one of which is entirely insensitive to theophylline, the second is theophylline sensitive. The first of these can be related to the dose response characteristic for vasopressin stimulated $Na^+$ transport. The second can be related to the effects of vasopressin on the stimulated adenylate cyclase[6].

We believe that interaction of vasopressin with membrane receptor sites[11] leads directly to an increase in the mucosal permeability to $Na^+$, to a release of membrane bound $Ca^{2+}$ ions and activation of the adenylate cyclase. The release of $Ca^{2+}$ from the membrane and its subsequent mobilization is believed to mainly affect water transport. Such a mechanism of action involving 2 types of permeability effects would provide an alternative basis for understanding the separate effects of the hormone on $Na^+$ and water transport. Although cyclic AMP may help to mobilize tissue $Ca^{2+}$ probably as a result of a biochemical effect on glycolysis[10] and mitochondrial bound calcium, the principle effect of cyclic AMP on glycolysis is possibly to increase the available supply of ATP to the ion pump. Cyclic AMP may affect membrane bound calcium ion and thereby increase the number of 'pores'. This effect it not equivalent to the hormone permeability effect which is believed to have an affect on the size of effective 'pores'.

*Zusammenfassung.* Der Temperaturkoeffizient für den durch das zyklische AMP und Theophyllin stimulierten Natriumtransport in der isolierten Krötenblase wurde analysiert. Die Enzympumpe wurde stärker beeinflusst als die mukosale Permeabilität und die Aktivität von $Na^+/K^+$ ATPase korrespondiert mit der Energie der Pumpenaktivität.

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Activation energy EA of the $Na^+$ transport across the isolated toad bladder stimulated by various agents including results of ref.[3] and present work

| Treatment | EA (kcal/mole) |
|---|---|
| Control | 13.6 |
| Cyclic AMP ($10^{-3}M$) | 13.8 |
| Theophylline ($10^{-2}M$) | 13.4 |
| Aldosterone ($10^{-7}M$) | 9.4 |
| Vasopressin ($10^{-6}M$) | 9.0 |
| Amphotericin B ($10^{-7}M$) | 9.0 |
| $Na^+/K^+$ ATPase | 8.9 |

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## Morphological Evidence for Electrical Synapse of 'Gap' Junction Type in Another Vertebrate Receptor

'Gap' junctions[1] – synapses presumed to be electrically mediated[2] – are such cases of close apposition of synaptic membranes as show a 20–30 Å gap[3], traversed by a hexagonal array of subunits[4], in between. The over-all thickness of the synaptic junction is 135–180 Å[1,5] and, for a given synapse, is not affected by differences in methods of treatment[1,6]. This last feature, therefore, serves as a good criterion for locating and identifying a 'gap' junction[6].

Using the above criterion, it is possible to demonstrate the presence of 'gap' junction between the sensory cells and afferent nerve endings in large tuberous organ[7,8] of *Sternarchus albifrons*, a gymnotid weakly electric fish. For electron microscopy, the material was fixed in osmium tetraoxide, acetate veronal buffered, and sections were double stained with uranyl acetate and lead citrate. Each sensory cell receives at its basal end a single large bouton terminal of an afferent nerve fibre (Figure 1). The latter carries its myelin sheath very close to the base of the bouton[8]. The cell membranes of the sensory cell and the nerve termination are separated by an interspace of 300–450 Å, except where they enter into formation of synapses. Two types of synapses are found at this interface. One type (Figure 1) is of what has come to be known as chemical synapses[9] which are readily recognized by the presynaptic structures, viz., electron dense body surrounded by vesicles. The synaptic membranes in this case are separated by a uniform cleft of 200 Å, and vesicles and mitochondria are found close to the synapse in the nerve ending.

The other type of synapses, at once conspicuous by the closeness of the 2 junctional membranes, appears in section as a single dark line in low magnification electron micrographs (Figure 1), as if a fusion has occurred here

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