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THE TWO STAGE NATURE OF THE ALDOSTERONE RESPONSE

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SUMMARY

Metabolic changes associated with the aldosterone stimulated sodium transport across the isolated toad bladder show a two stage dose response characteristic, which may be related to the saturation of two specific binding sites, identified in an analysis of the hormone-tissue binding. The mechanism of action of the hormone has been interpreted in terms of the stimulation of two systems of control, involving increases in mitochondrial enzyme activity and the mucosal sodium permeability of the transport epithelial cells. In such a mechanism both ion pump and mucosal permeability barriers are affected. Evidence is presented in terms of temperature coefficients measured for the short circuit current across toad bladder and the Na^+/K^+ ATPase activity, supporting the idea that sodium transport across the toad bladder is normally limited by mucosal sodium permeability and that the major effect of aldosterone is to increase this permeability. The second effect of the hormone allows for an increased supply of high energy intermediate to the pump in conditions where the pump becomes rate limiting.

INTRODUCTION

ACTIVE sodium transport across the isolated toad bladder is believed to involve certain mucosal epithelial cells that allow passive sodium entry into the cell at the mucosal surface and pump sodium actively at the serosal surface. This transport might be stimulated by increasing either mucosal permeability or the activity of the enzyme pump. Considerable attention has been given to the problem of which process normally limits sodium transport, particularly in relation to the mechanism of action of aldosterone [1, 2]. Sharp *et al.* [3] and Snart [4] favour the idea that sodium transport across the toad bladder is normally limited by the permeability of the mucosal surface and that the effect of aldosterone is to increase this permeability. Fanestil *et al.* [5, 6] on the other hand support the view that the pump is normally rate limiting and that aldosterone acts to increase the activity of the enzyme pump independently of any effect on mucosal permeability. In a recent review Edelman and Fanestil [7] have presented most of the scientific background to these opposing views. It is the purpose of the present paper to review our own approach to this problem and present our latest results and opinions. Our work supports a two stage mechanism of action of the hormone in which both ion pump and mucosal permeability barrier are affected.

Temperature dependence of the short circuit current and of the Na^+/K^+ATPase *system*

The effect of aldosterone on the rate limiting step for sodium transport across the isolated toad bladder (*Bufo marinus*) has been investigated [8] by measuring the temperature dependence of the short circuit current across the bladder in order to obtain an activation energy E_a. Linear activation energy plots obtained over a temperature range of 5–25°C gave rise to a value for E_a of about 13·5

kcal/mole. The effect of amphotericin B, vasopressin and aldosterone was to reduce the value of E_a to about 9 kcal/mole in all cases. It was argued that because amphotericin B and vasopressin are agents accepted as causing an increase in mucosal permeability then a similar reduction in E_a following aldosterone treatment probably corresponded to a lowering of the energy associated with mucosal permeability.

It may be argued that the rate at which sodium ions enter the epithelial transport cell across the mucosal permeability barrier can be represented by $k_1 n_1$ where k_1 corresponds to the rate constant for this passive process and n_1 corresponds to the number of ions available for such transport. At the serosal surface the rate of ion transport out of the cell may be represented by $k_2 n_2$ where k_2 corresponds to the rate constant for the active process and n_2 corresponds to the number of ions available for active transport. These rate constants may be related to the corresponding activation energies E_1 (permeability barrier) and E_2 (ion pump) by the Arrhenius equation. The short circuit current (s.c.c.) measured across the toad bladder will be equivalent to $k_1 n_1$ and $k_2 n_2$. If the permeability barrier limits the s.c.c. then a plot of log s.c.c. against reciprocal temperature will give rise to a single activation energy $E_a = E_1$ provided that the pump can move sodium at catalytic rates relative to the permeability barrier, i.e. thermal activation of the pump will not affect the measured temperature coefficient. Using a similar argument we expect $E_a = E_2$ if the ion pump normally limits the s.c.c..

The observed linearity (Fig. 1) of the activation plots within the temperature range considered is taken as justification for the use of this model in our analysis. The measured values of E_a were 13·5 kcal/mole in the absence and 9 kcal/mole in the presence of aldosterone, vasopressin and amphotericin B.

It seems most probable that 9 kcal/mole, the value of E_a observed in the presence of agents accepted as causing an increase in mucosal permeability corresponds either to a lowering of the permeability activation energy E_1 from 13·5 kcal/mole to 9 kcal/mole or to E_2 the pump activation energy. We have carried out experiments[9] in which the pump activity has been limited by increasing conditions of anoxia and have shown that the activation energy for

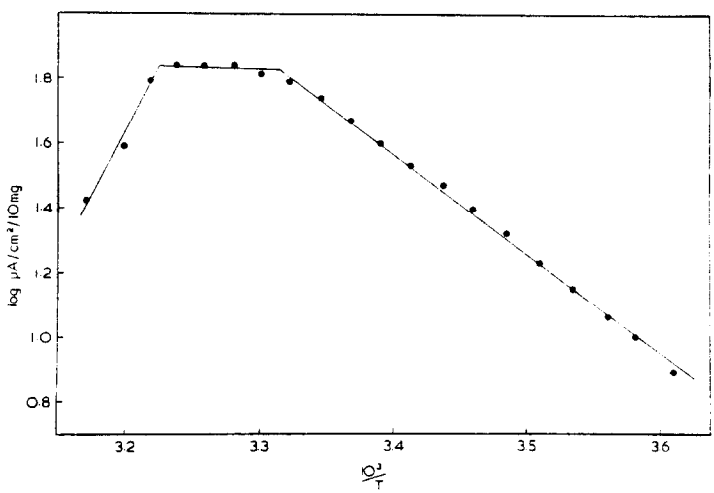

Fig. 1. Shows the activation energy plot for the temperature dependence of the short circuit current measured across the isolated toad bladder[8].

the short circuit current is thus reduced from 13·5 kcal/mole to about 9 kcal/mole. As this case represents one in which there should be no effect on mucosal permeability, then 9 kcal/mole probably corresponds to the activation energy of the pump and 13·5 kcal/mole probably represents the activation energy of the permeability barrier. In these experiments we obtained intermediate conditions which gave rise to inflection points in the short circuit current activation plot, at lower temperatures E_a equalled 9 kcal/mole, whereas at higher temperatures E_a equalled 13·5 kcal/mole. It was suggested that any effect that predominantly increases mucosal permeability will give rise to an activation energy of 9 kcal/mole whereas any effect that predominantly increases the activity of the pump will give rise to an activation energy of 13·5 kcal/mole.

In order to investigate the possibility that the activation of the enzyme pump corresponds to 9 kcal/mole we have studied the effects of temperature on the Na^+/K^+ATPase activity in toad bladder homogenates, using the method described by Bonting and Caravaggio[10]. We found a good linear relationship for the activation energy plot obtained over the temperature range studied (Fig. 2), which gives rise to an energy of 8·5 kcal/mole using a least mean square analysis, a value which agrees with an earlier value reported by Bonting and Canady (8·2 kcal/mole). They and others[10–12] have studied the temperature dependence of the Na^+/K^+ATPase system isolated from various tissues, all of which have similar values for the activation energy, well below the 16 kcal/mole suggested by Fanestil[5]. Similar studies using rat kidney homogenates at temperatures of 22°C and 43°C have been carried out. These results give rise to an activation energy of 8·9 kcal/mole. This evidence is taken to support the idea that sodium transport across the isolated toad bladder is normally limited by the rate at which sodium can cross the mucosal permeability barrier and that the effect of aldosterone is to increase this permeability.

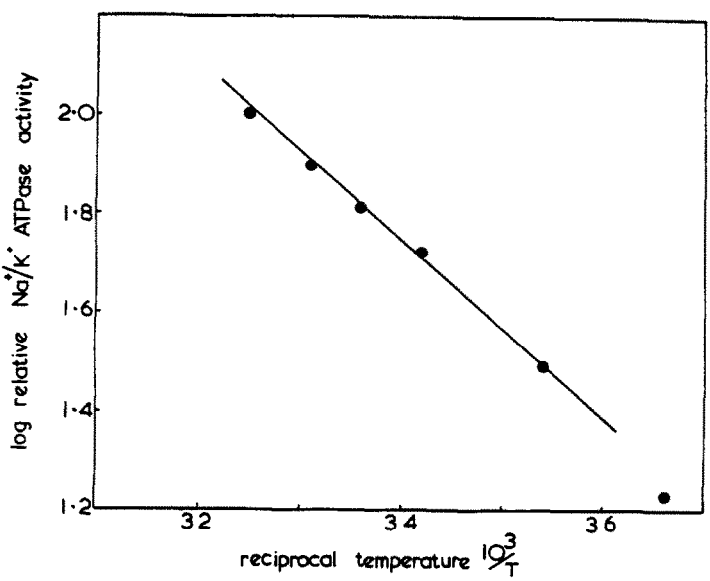

Fig. 2. Plot of log relative Na^+/K^+ATPase activity measured at various temperatures, using method [16] in toad bladder homogenate, against reciprocal absolute temperature. The activity at 35° has been considered equivalent to 100% and other values determined on this relative scale.

Metabolic effects of aldosterone in the toad bladder

We have followed the displaceable binding technique used by Sharp *et al.* [13] in order to extend the binding study of aldosterone in the toad bladder. We were particularly concerned to obtain more points for the second set of binding sites on the Scatchard plot, drawn from the hormone tissue binding results. Our results[14] confirm the analysis of the binding characteristic in terms of two sets of binding sites one with a K value of the order 10^{10} l/mole, the second with a K value equal to 10^{8} l/mole.

The dose response curve for aldosterone stimulation of sodium transport across the toad bladder has a characteristic which would correspond to the saturation of 10^{8} l./mole sites, which are therefore considered to represent physiological receptors. The problem remained as to the nature of the 10^{10} l/mole sites. We believed that a hormone such as aldosterone should not only show specific binding in target tissue but also give rise to an increased metabolic activity associated with any physiological response. We therefore studied changes in the rate of oxygen consumption in isolated toad bladder[15] in response to addition of aldosterone and have found (Fig. 3) that a significant increase in respiration occurs after a lag period of 90 min following addition of the hormone. A dose response curve for the maximum increase associated with each dose of hormone is shown in Fig. 4, which indicates that oxygen consumption is increased by doses of hormone that do not stimulate sodium transport to a comparable extent. The nature of this aldosterone response over the lower dose range would correspond to the saturation of 10^{10} l/mole sites.

As aldosterone is able to maintain the stimulated sodium transport across the isolated toad bladder for a considerable period during which the pump is rate determining, it is believed that the effect of aldosterone on the toad bladder represents a two stage process, the major effect of the hormone is to lead to an

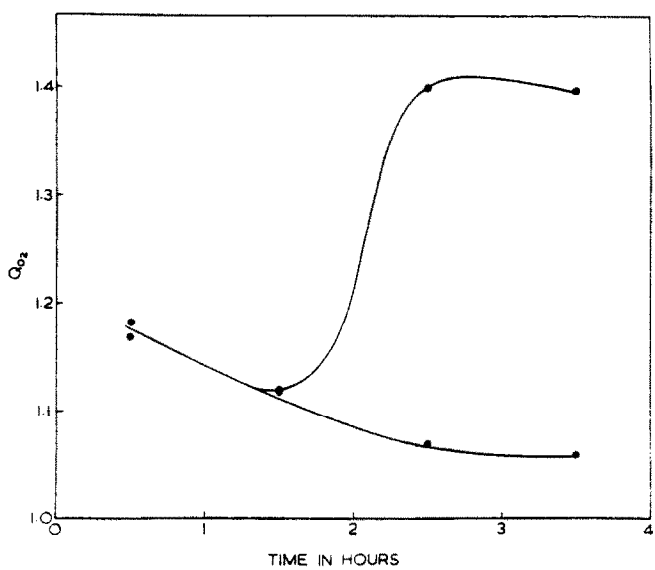

Fig. 3. Aldosterone (10^{-6} M) stimulation of the oxygen consumption (μl O2 consumed/mg protein/h) by toad bladder (upper curve) relative to controls (lower curve). Each point represents the mean of ten values[15].

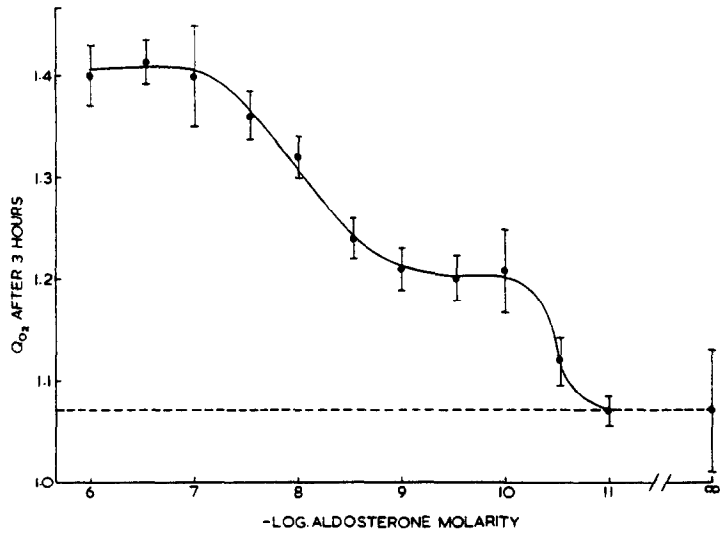

Fig. 4. Dose response curve obtained for aldosterone stimulated oxygen consumption in toad bladder. Each point represents the mean of ten values ± S.E. obtained for the 3–4 h period following aldosterone treatment.

increase in mucosal permeability to sodium. However, a lower dose effect serves to increase the respiratory capacity of the tissue in preparation for the increased activity of the enzyme pump. Several workers[16, 17] have shown that there was no increase in the amount of Na^+/K^+ATPase in the tissue following aldosterone treatment. We have investigated this possibility allowing a full 3 h for hormone stimulation. Our work confirms the observation that there is no effect of aldosterone on the amount of Na^+/K^+ATPase (Table 1). Any increase in activity of the enzyme pump is therefore probably due to increased availability of high energy intermediates[18]. Kirsten *et al.*[19] have shown that aldosterone leads to a stimulation of several tricarboxylic acid cycle enzymes, though it was uncertain whether this represented an effect of aldosterone in stimulating the amount of these enzymes. We have compared the effect of aldosterone in increasing succinate dehydrogenase activity in mitochondrial and homogenate preparations with the increased activity that occurs following freeze thaw procedures, Fig. 5, designed to break down any permeability barriers. The effect of aldosterone in stimulating SDH activity was found to be equivalent to the increase following our freeze thaw procedures. It is therefore believed that the hormonal effect may be to stimulate an enzyme or carrier system associated with the decrease of tricarboxylic acid enzyme inhibitors within the mitochondria. This may be more probable than an increase in mitochondrial substrate permeability as it is generally accepted that the rate limiting step for aerobic respiration is the rate of which ADP becomes available to the mitochondria rather than substrate permeability. The changes in oxygen consumption that occur in response to low doses of hormone and preceed the demand for increased ion transport could correspond to hormonal stimulation of another energy requiring process. However, it is believed that this observation may be taken to indicate that aldosterone stimulates the respiratory capacity for ATP turnover in the tissue in preparation for the increased activity associated with ion transport. The low dose increase in

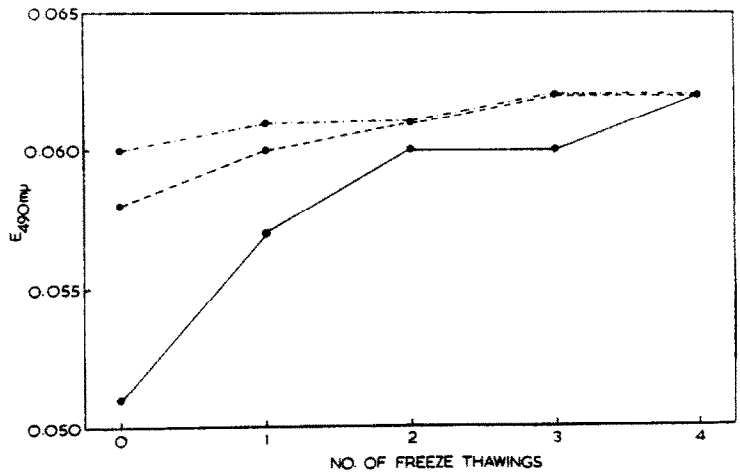

Fig. 5. Succinate dehydrogenase activity (E_{490}/mg protein) determined in toad bladder homogenates using method [20]. The curves show values obtained after stimulation of the tissue with 10^{-7} M aldosterone (–·–·–), 10^{-9} M aldosterone (– – –) and in controls (———).

Table 1. ATPase activity (mmoles ATP split/kg wet wt/h) measured at 22°C using method [16] in toad bladder homogenate prepared following preincubation in frog Ringer's solution of the isolated bladder 1–3 h in the presence and absence of 10^{-7} M aldosterone. Means ± S.D.

	Total ATPase	Residual ATPase	Na^+/K^+ATPase
1 h preincubation			
Control	104·4 ± 9·7	88·9 ± 10·5	15·5
Aldosterone	103·0 ± 7·5	86·6 ± 7·9	16·4
2 h preincubation			
Control	104·9 ± 8·1	89·2 ± 7·4	15·8
Aldosterone	105·2 ± 7·4	90·3 ± 8·7	14·9
3 h preincubation			
Control	103·3 ± 6·3	87·5 ± 7·6	15·7
Aldosterone	101·8 ± 5·0	85·5 ± 5·1	16·3

oxygen consumption could be associated with a controlled change from glycolytic to aerobic respiration, resulting in a change in the P/O ratio for the respiration or with a change in the ATP/ADP ratio for the tissue. This last possibility seems unnecessary as the tissue needs only to allow for an increased turnover, i.e. supply of ATP not an increased concentration. These changes are not associated with any increased ion transport and the level of ATP is expected to be carefully controlled. We are investigating the biochemical aspects of this hormonal control system.

CONCLUSIONS

Evidence presented in this paper concerning the mechanism of action of aldosterone in stimulating sodium transport across the isolated toad bladder is taken to support the idea that both ion pump and mucosal permeability barriers are affected. We believe that sodium transport across the isolated toad bladder is normally limited by mucosal permeability and that aldosterone acts to increase this permeability.

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DISCUSSION

Handler: Over the last few years we have presented evidence regarding the role of cyclic AMP that we interpreted in a manner that differs from your interpretation of the role of the nucleotide. I wonder whether you have considered alternate explanations for your observations. For instance, there are several steps involved in sodium transport, including the generation of energy to support the transport. Any of these or the mechanism involved in the stimulation of transport by cyclic AMP or by aldosterone may have different temperature sensitivity. In other words, the rate limiting step may change with temperature, in which case measurements of activation energy are impossible to interpret.

Snart: I think that the interpretation of our results should be kept as simple as possible. The two processes revealed by our straight line activation plots are believed to represent mucosal permeability and serosal pump. These processes may each involve several stages but are shown by our work to have characteristic activation energies. We do not believe that effects of cyclic AMP and vasopressin are identical. They do not give rise to similar effects on the temperature coefficients for Na^+ or H_2O transport. We believe that interaction of vasopressin with membrane receptor sites leads directly to an increase in the mucosal permeability to Na^+ and to a release of membrane bound Ca^{2+}. Such an interaction leads to the activation of the adenylate cyclase system. The release of Ca^{2+} from the membrane and its subsequent mobilisation is believed to affect mainly H_2O transport. There is a considerable amount of evidence supporting a separation of H_2O and Na^+ transport effects of vasopressin. We have done quite a lot of work on cyclic AMP and vasopressin. The vasopressin stimulation of cyclic AMP levels in toad bladder has a dose response characteristic of a single activation process. Our model provides a basis for understanding the separate effects of the hormone on Na^+/H_2O transport. We have evidence of a selective cation permeability effect of vasopressin on artificial lipid bilayers, associated with the formation of effective pores, and suppose that this property is important for the Na^+

transport effect of the hormone. The hormonal stimulation of cyclic AMP is believed to help to mobilize tissue Ca^{2+} possibly as a result of its biochemical effect on glycolysis. This effect of cyclic AMP on glycolysis probably also helps the supply of ATP to the pump. The low dose respiratory effect of aldosterone may similarly make more ATP available to the pump as required. This would not imply any increased level of ATP in the tissue. The magnitude of the sodium permeability effects of aldosterone and vasopressin may differ but the result in both cases is to make the pump rate limiting. We suggest that the second effect of aldosterone is to help maintain pump activity; in a similar way we suppose that the supporting biochemical role of cyclic AMP is to help maintain pump activity. Although cyclic AMP alone may affect membrane bound Ca^{2+} and thereby increase the number of pores, this is not equivalent to the hormone permeability effect.

Fanestil: Were the ATPase preparations on which you determined the activation energies from toad bladder and what ATPase method did you use?

Snart: We used Bonting's method in which there are $2ATP : 1Mg^{2+}$, but other workers using different ATPase methods also get these lower activation energies.

Fanestil: Was this a ouabain-sensitive ATPase?

Snart: Yes.

Wiederholt: Did you use homogenates of the whole kidney or cell membrane preparations?

Snart: We have used homogenate.

Edelman: Did you use an inhibitor of mitochondrial oxydative phosphorylation to block resynthesis of ATP?

Snart: Yes we used cyanide.