EFFECT OF ALDOSTERONE ON Na EFFLUX IN SINGLE MAIA MUSCLE FIBRES

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Unpublished studies on the natriferic action of insulin suggested that the changes in the response pattern of the Na pump of single muscle fibres from the crab Maia squinado induced by this hormone after a latent period of 15 hours in vivo were attributable to DNA polymerase activity. This work has been extended to include aldosterone but the results described here in contradistinction to those obtained with insulin indicate that the steroid is an inhibitor of sodium transport across the fibre membrane.

The procedures used for the isolation and cannulation of single muscle fibres and for micro-injection were those described by Caldwell and Walster (1963). A full account covering the methodology has been given elsewhere (Bittar, 1966).

Results and Discussion. Neither internal nor external application of aldosterone to Maia fibres (6 experiments) had a significant effect on Na efflux. Fig. 1 which shows this result is that of an experiment involving a muscle fibre whose rate constant for ^{22}Na loss was about double that of most Maia fibres (see Bittar, 1966). The inability of aldosterone to alter the Na efflux is a finding that raises doubts about the legitimacy of regarding steroidal effects on the movement of Na^+ out of the liquid crystals of lecithin (Bangham, Standish and Weissman, 1965) as being of biological import.

Fifteen hours following a single injection of 10 μg of aldosterone into a crab in vivo, Na efflux from Maia fibres was found to be sensitive to aldosterone. In Fig. 2 is shown that injected 1.3×10^{-3}M aldosterone (i.e. an intrafibre concentration of ca. 10^{-5}M) caused a gradual but prompt fall in efflux; similar

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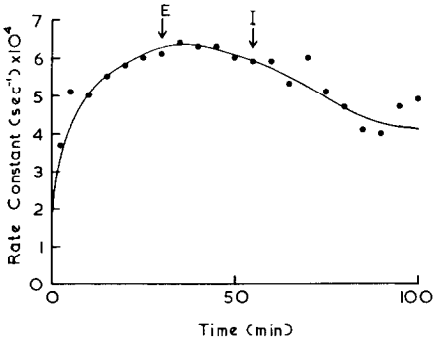

Fig. 1. Absence of an effect on Na efflux following external application of 2.6×10^{-6}M aldosterone (indicated by E) and injection of 1.3×10^{-3}M solution of the steroid. The resting potential of the fibre at the start and end of the experiment was 51 mV and 65 mV respectively. Temperature 19°C; pH of crab saline 7.4 (N.B. d-aldosterone was supplied in isotonic saline whose pH was 6.2; injection of isotonic saline into fibres was without effect on Na efflux, the threshold concentration for auto-inhibition being 5M).

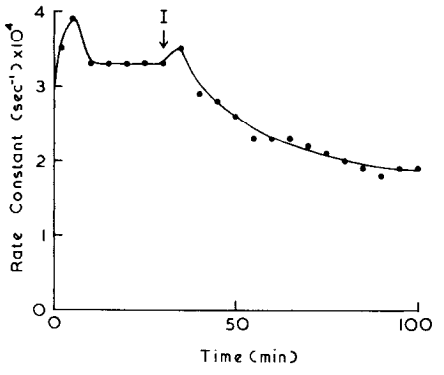

Fig. 2. Inhibitory influence on Na efflux of injected 1.3×10^{-3}M aldosterone in a fibre previously exposed in vivo to the steroid over a period of 15 hours. The resting potential of the fibre at the start and end of the experiment was 50 mV and 59 mV respectively. Temperature 20°C; pH of crab saline 7.5.

results were obtained in the other five experiments. This effect of aldosterone probably implies (i) that its immediate and primary site of action is on or near

the membrane and (ii) that any theory which is to explain in detail the nature of its action must take into account the presence in the membrane of a protein (or enzyme) with a particular conformation which can be altered by long-term exposure to the steroid (see Warner, 1965; Hechter, 1965).

Two days after the daily injection of 10 μg of aldosterone into a crab in vivo the Maia fibre exhibited a decreased response to internally applied 1.3×10^{-3}M aldosterone (3 experiments). Partial escape of Maia fibres from the effect of aldosterone illustrated by Fig. 3 constitutes a mechanism which is also known to develop in dogs, rats and human subjects.

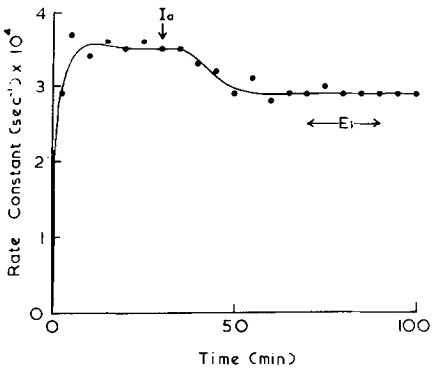

Fig. 3. Partial escape from the effect of aldosterone occurring two days after daily injection of 10 μg of aldosterone into a crab in vivo. The fall in efflux accompanying the injection of 1.3×10^{-3}M aldosterone into this fibre is small. External application of 1 unit/ ml of insulin (indicated by the horizontal arrows) failed to stimulate the Na pump. The resting potential of the fibre at the start and end of the experiment was 48 mV and 53 mV respectively. Temperature 21°C; pH of crab saline 7.4.

Partial escape of fibres isolated from crabs previously exposed in vivo to aldosterone over a period of two days from the inhibitory influence of directly injected aldosterone was also noticed when the external pH was 8.0. Fig. 4 is that of an experiment performed in duplicate in which the fibre had been pre-treated with aldosterone in vivo for two days. The first injection of 1.3×10^{-3}M aldosterone into the fibre can be seen to be followed by slight

reduction in the efflux of sodium. The second injection, using the same concentration of the steroid, was carried out ten minutes after adjusting the external pH to 7.4. The result was a slightly more pronounced inhibition even though the efflux of sodium had already been affected by the first injection of

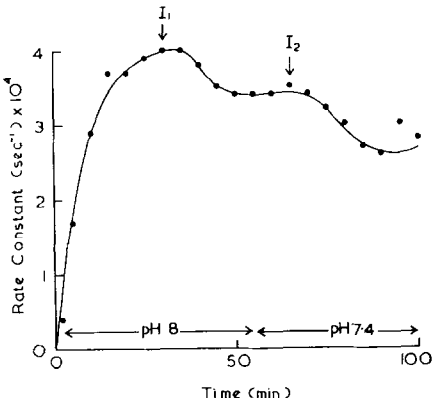

Fig. 4. Possible relationship between the escape mechanism and the H^+ ion gradient across the fibre membrane. When the external pH was 8.0, internal application of 1.3×10^{-3}M aldosterone (indicated by I_1) into a fibre previously exposed in vivo to aldosterone for a period of about 15 hours caused slight inhibition of Na efflux. Ten minutes after restoring the external pH to 7.4, the injection of 1.3×10^{-3}M aldosterone (indicated by I_2) resulted in further slowing of Na loss from this fibre. The resting potential of the fibre at the start and end of the experiment was 54 mV and 58 mV respectively. Temperature 23°C.

aldosterone. From these results it seems possible that there may be a direct connexion between extracellular alkalosis brought on by long-term administration of aldosterone in animals and the escape mechanism.

Thirty minutes after injecting phleomycin, a known DNA polymerase inhibitor (Falaschi and Kornberg, 1964) into fibres that had been exposed earlier to aldosterone in vivo for some 15 hours, internal or external application of the steroid (3 experiments in each case) produced a drastic fall in the efflux of sodium. Fig. 5 which illustrates this result is that of an experiment in which the efflux followed a linear rather than an exponential process suggesting that in this case the Na pump was mainly electrogenic (see Bittar, 1966).

The addition of 5.2×10^{-6}M aldosterone to the bathing fluid promptly reversed the linear rise of the efflux of Na, while subsequent injection of aldosterone hardly showed potentiation of this inhibitory effect.

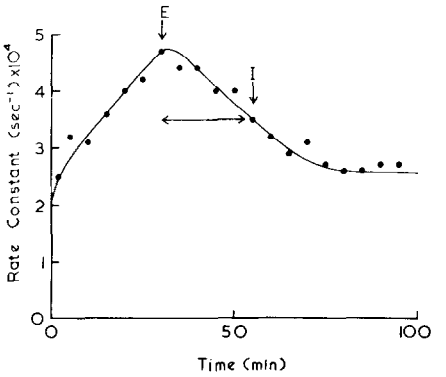

Fig. 5. Failure of phleomycin to abolish the aldosterone effect. About 30 minutes after injecting a solution of 5 mg/ml of phleomycin into an aldosterone-pretreated fibre, the external medium was replaced by one containing 5.2×10^{-6}M aldosterone for a period of 25 minutes. The result was a substantial fall in efflux. Injection of aldosterone (indicated by I) was without effect. The resting potential of the fibre at the start and end of the experiment was 60 mV and 66 mV respectively. Temperature 22°C; pH of crab saline 7.4.

Working with a different target organ, e.g., the urinary bladder of Bufo marinus, Edelman, Bogoroch and Porter (1963) have been able to show that aldosterone acts by inducing the synthesis of m-RNA. More recent results indicate that before Na transport from mucosal to serosal surface speeds up, m-RNA stimulates ATP formation (Fimognari, Kasbekar and Edelman, 1965). Other lines of evidence point to increased decarboxylation of pyruvate in the presence of aldosterone (Sharp and Leaf, 1965). The findings of these various workers are germane in that the Na pump of Maia fibres appears to be characterised by an Na^+-K^+-ATPase system driven by ATP and ArP and an electrogenic mechanism driven by respiration and glycolysis (Bittar, 1966). Conceivably the next problem here is to determine whether ATP or pyruvate → lactate or pyruvate → alanine turnover rates are diminished by aldosterone following a latent period

in vivo as well as after blocking DNA polymerase with phleomycin. Fortunately techniques devised by Caldwell and Walster (1962) and by Ashley (1965) are available for a direct attack on this very problem.

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