# ON THE NATRIFERIC ACTION OF 8-LYSINE VASOPRESSIN

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A question of much interest is whether vasopressin influences the efflux of sodium in skeletal muscle. The following work which was undertaken in search of an answer describes the effect of 8-Lysine vasopressin on the loss of $^{22}Na$ from single muscle fibres of the crab Maia squinado. The evidence put forward is that inhibition of the Na pump by this hormone is decisively governed by the $H^+$ ion gradient across the fibre membrane.

Methods (i) Single fibres from the flexor muscles of the leg or claw of male crabs were isolated and cannulated according to the method of Caldwell and Walster (1963).

(ii) The resting potential of these fibres was measured and recorded with a Ling and Gerard glass micro-electrode filled with 0.6M KCl and with a Vibron electrometer, model 33B.

(iii) Using a microsyringe, as devised by Hodgkin and Keynes (1956) $^{22}Na$ was injected directly into and along the axis of each fibre. Only fibres having resting potentials over 45 mV were used in this study.

(iv) The emerging $^{22}Na$ was collected in the bathing crab Ringer solution, and counting of the activity of the isotope was carried out in a scintillation counter, type D 657, Panax Equipment Ltd.

(v) Measurement of the pH of crab Ringer solutions was performed with a Pye pH meter.

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8-Lysine vasopressin was supplied by Sandoz Products Ltd., London. The solution (1 ml) had a pH of 4.0 $\pm$ 0.3 and contained chlorbutol 5 mg, a small amount of sodium acetate-acetic acid as buffer, and ethyl alcohol, 93% as preservative (5 mg).

Results and Discussion. In Fig. 1 is shown the result of an experiment in which 8-Lysine vasopressin in a concentration of 100 mU/ml in crab Ringer (pH adjusted to 7.0) was applied externally to a Maia fibre followed 30 mins later by the injection of a solution of 10 I.U./ml of the hormone. Under these conditions 8-Lysine vasopressin was without effect on Na efflux. In five other experiments of identical design,

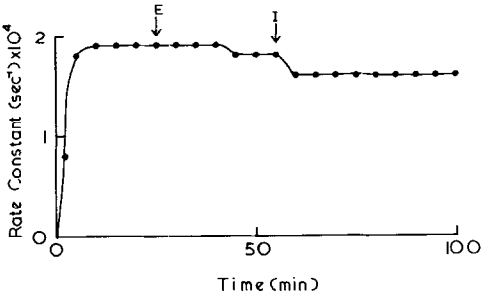


Fig. 1. Lack of effect on Na efflux of internally and externally applied 8-Lysine vasopressin (10 I.U./ml and 100 mU/ml respectively) to a Maia fibre at a time when the external pH was 7.0. Abscissa: time in mins. Ordinate: $^{22}Na$ collected per sec per $^{22}Na$ in fibre. The resting potential of the fibre at the start and end of the experiment was 49 mV and 56 mV respectively. Temperature 22°C.

externally applied vasopressin was similarly unaccompanied by a change in efflux, but in two of the fibres, injected vasopressin caused delayed inhibition of the Na pump. This result could have been associated, among other things, with (i) mitochondrial swelling (Lehninger and Neubert, 1961) (ii) an appreciable alkaline pH of the sarcoplasm of the fibres in question, and (iii) the presence of ethanol, or possibly metallic contaminants in the vehicle used (Cash, Gardy, Amend and Evans, 1964). Micro-injection of the vasopressin vehicle into Maia fibres was found to lead to small irregular inhibitory effects on the Na efflux mechanism. These were thought to be due to chlorbutol and ethanol in the solvent. In view of these uncertainties,

no further attempts at injecting the hormone were made.

Fig. 2 indicates the effect on Na efflux of 8-Lysine vasopressin following its external application to a Maia fibre when the bathing fluid pH was 8.4. It can be seen that there was a drastic and almost immediate fall in efflux. In addition, the Na pump failed to recover after restoring the external pH to 7.4, as well as removing the hormone from the medium. These observations were substantiated in five other fibres while in a separate set of experiments

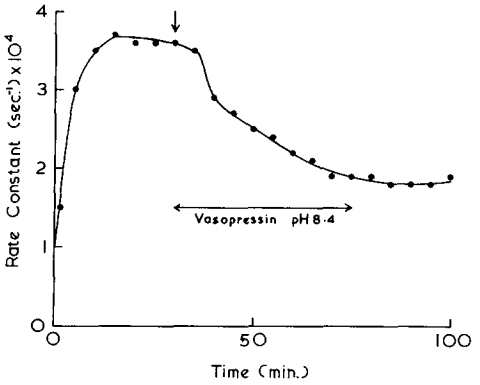


Fig. 2. Effect on Na efflux of externally applied 8-Lysine vasopressin (100 mU/ml) after adjusting the external pH to 8.4. Arrow designates the time at which the hormone was applied to the fibre; it also designates the time at which the pH adjustment was made. The resting potential of the fibre at the start and end of the experiment was 57 mV and 59 mV respectively. Temperature 19°C.

the threshold external pH for natriferic action by vasopressin was determined to be near 7.4.

In order to confirm that the observed fall in Na efflux was the result of externally applied vasopressin, and not of sudden alkalinisation of the bathing medium, three experiments were done where the external pH was 8.4 throughout the experiment. In each case, inhibition of the Na pump occurred right after external application of the hormone. Fig. 3 illustrates this inhibitory effect of vasopressin on the emergence of sodium from a Maia fibre. Admittedly in both types of experiments involving alkalinisation of the external medium, swelling of the plasma membrane could have taken place. In the presence of vasopressin a structural change in the membrane might have

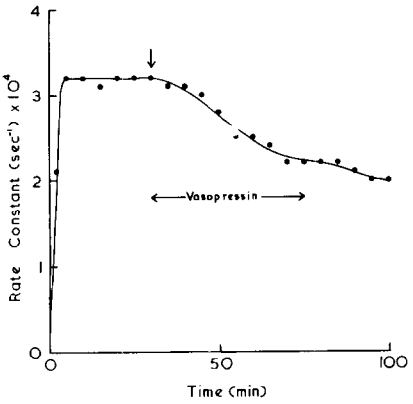


Fig. 3. Effect on Na efflux of externally applied 8-Lysine vasopressin (100 mU/ml). The external pH was 8.4 during the entire course of the experiment. Arrow designates the time at which vasopressin was applied to the fibre. The resting potential of the fibre at the start and end of the experiment was 60 mV. Temperature 20°C.

contributed in part to inhibition of the efflux of sodium.

The above results are in keeping with those of Friedman and Sreter (1961) who showed that vassopressin causes an increase in the $Na^+$ and water content of rat skeletal muscle. These workers, however, interpreted their findings as indicating a rise in influx of sodium rather than a decrease in efflux. The inward movement of water would in any case be considered as the outcome of the altered osmotic gradient. When frog muscle is treated with pitressin it also shows an uptake of water (Zadunaisky, Parisi and Montereano, 1963). Among the lower vertebrates there are several examples of vasopressin influencing water or sodium movements (Maetz, 1963; Morel, 1965), thus strengthening the view of there being more than one type of receptor that can interact with the hormone. Other authors (Heller and Bentley, 1965) have hinted that the natriferic effect of vasopressin probably antedates phylogenetically the control of water flow by this hormone.

Working with toad bladder mucosa, Leaf, Keller and Dempsey (1964) have demonstrated that alkalinisation of the medium on the serosal side of the membrane augments the stimulatory action of vasopressin on $Na^+$ transport from the mucosal to the serosal surface. Under similar circumstances increased

water flow in this tissue has been noticed by Gulyassy and Edelman (1965). Such results together with those obtained with Maia fibres are reasonably explained firstly, by an increase in the inward diffusion of uncharged vasopressin molecules upon elevating the external pH. This is in line with a vast body of evidence in the literature showing that neutral molecules penetrate membranes more readily than the charged species. It has been suggested that a folding of the side-chains of the uncharged vasopressin molecule towards its ring facilitates diffusion of the hormone through membranes (Craig, Harfenist and Paladini, 1964). Secondly, by diminished competition between the $H^+$ ions and vasopressin for the anionic sites on the receptor. That is, if a raised external pH gives rise to hydroxylation of the internal fluid, as has been revealed to be the case in rat skeletal muscle (Adler, Roy and Relman, 1965) then binding of vasopressin by the receptor would be expected to develop readily. That complementarity between the hormone and the region of the receptor has first to be achieved is implied by the finding that a "type" of SH group is essential for the activation of the $Na^+$-$K^+$-ATPase (Skou, 1964). Since vasopressin by itself fails to have an effect on the transport enzyme (Bonting and Canady, 1964), it is not unlikely that the role of the SH group is one of inducing conformity or non-conformity of $Na^+$ to the active centre of the $Na^+$-$K^+$-ATPase. This concept appears to have the ability to account for the dualistic natriferic action of vasopressin, namely that of stimulating the Na pump in the epithelial cell, and of inhibiting it in skeletal muscle.

Finally, it is perhaps fair to say that by virtue of its size the Maia fibre is an ideal subject for a direct attack on the problem of determining the pK of the vasopressin receptor (or receptors) by means of the pH micro-electrode technique (Caldwell, 1958). Research in this direction would not be without a rationale, as the eyestalk of the crab does produce and store an antidiuretic-like principle (Carlisle, 1956; Passano and Jyssum, 1963).

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