Inhibition of the CO_2-Sensitive Na Efflux in Barnacle Muscle Fibers by Micro-Injection of Ethacrynic Acid

It now appears, as explained by us in a recent paper[1], that ethacrynic acid is a potent inhibitor of the CO_2-sensitive component of the Na efflux in barnacle muscle fibers. In view of the importance attached to the part played by CO_2 in the mechanism of Na transport, and in view of the possibility that the point of action of ethacrynic acid is not the external side of the plasma membrane, the following experiments were undertaken to see whether the microinjection of ethacrynic acid affects the Na efflux only in the presence of external acidification.

The experiments were done using single muscle fibers approximately 5 cm in length and 1.5 mm in diameter from the barnacle *Balanus nubilus* or *B. aquila*. The fibers were cannulated and then loaded with ^{22}Na by microinjection, employing a microinjector similar to that devised by HODGKIN and KEYNES[2] as modified by CALDWELL and WALSTER[3]. The microinjector discharged a volume of ca. 0.1 µl of fluid per 1 cm excursion of the micromanipulator. The bathing medium was artificial sea water the composition of which was the same as that used by BITTAR and TONG[4]. Ethacrynic acid was a gift from Merck Sharp and Dohme, Rahway, N. J. A neutral solution was prepared by adding equivalent amounts of $NaHCO_3$.

The methods of measuring ^{22}Na in the washout specimens and in the fiber were essentially the same as described by BITTAR[5] and BITTAR, CALDWELL and LOWE[6]. [^{22}Na]Cl was supplied by Amersham-Searle Corp. (SKS-1). All experiments were carried out between 22 and 24 °C.

In the first group of experiments (7 fibers) the effect of internal application of $2 \times 10^{-2} M$ ethacrynic acid on the Na efflux into artificial sea water at pH 7.8 was examined. A typical experiment is recorded in Figure 1, where it is shown that ethacrynic acid failed to alter the course of the Na efflux.

In the second group of experiments the fibers were bathed in artificial sea water at pH 6.3. The change in external pH was effected some 20 min before the microinjection of radio-sodium. As illustrated in Figure 2, injection of $2 \times 10^{-2} M$ ethacrynic acid caused a pronounced fall in the rate coefficient for Na efflux. An estimate of the size of this effect gave an average value of 58% ($\eta = 4$), which is not very different from that obtained following external application of ethacrynic acid at pH 6.3[1].

The results reported here are in accord with the theory that ethracrynic acid is a powerful blocking agent of the CO_2-sensitive component of the Na efflux and that its site of action is not the external surface of the plasma membrane. That internal application of the diuretic at pH 7.0 failed to reduce the Na efflux into artificial sea water at pH 7.8 suggests that the internal pH of barnacle muscle fibers may be higher than that of crab muscle fibers[5], i.e. above 7.0, or that the action observed in this instance may be independent of the degree of ionization of the drug. Furthermore, if it be true that ethacrynic acid is an inhibitor of ATP-ases[7], then the present results are a telling argument against this idea. Failure of ethacrynic acid to reduce the Na efflux in the presence of an external pH of 7.8 does not necessarily imply that glycolysis is not inhibited. What it does suggest is that barnacle muscle fibers have some reserves of high-energy phosphate compounds[8].

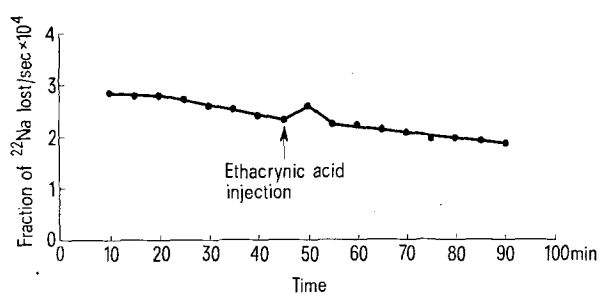

Fig. 1. Lack of effect of internal application of $2 \times 10^{-2} M$ ethacrynic acid on the Na efflux into artificial sea water at pH 7.8.

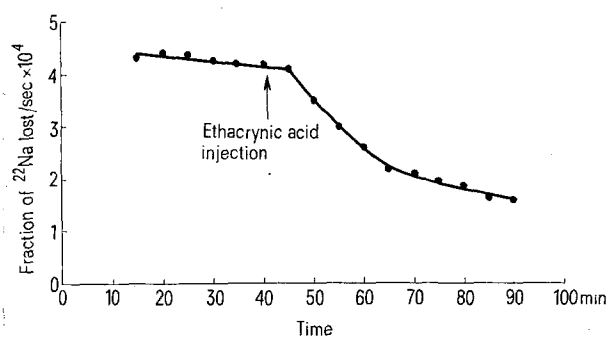

Fig. 2. Reduction of the Na efflux into artificial sea water at pH 6.3 by internal application of $2 \times 10^{-2} M$ ethacrynic acid.

Zusammenfassung. Es wird gezeigt, dass die Mikroinjektion von Äthacrynsäure [2, 3-Dichloro-4 (2-methylenbatyryl)-phenoxyacetsäure] in die isolierte Muskelfaser der Entenmuschel nur dann den Natriumausstrom herabsetzt, wenn die Badelösung vorher angesäuert wird.

B. G. DANIELSON[9], E. E. BITTAR, S. S. CHEN and E. Y. TONG[10]

Department of Physiology, University of Wisconsin, Madison (Wisconsin 53706, USA), 17 April 1972.

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[9] B. G. D. is a visiting Scientist of the American Heart Association whose permanent address is: Institute of Physiology and Medical Biophysics, University of Uppsala, Uppsala (Sweden). E. Y. T. is a postdoctoral Fellow of the Wisconsin Heart Association.
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