would be to decrease the permeability barrier to water and ions. However, this order of permeability change is not as significant as that produced by the steroid-induced protein that causes a lowering of the permeability barrier to the same extent as vasopressin and amphotericin B. In artificial systems[8] the nature of the effective "pores" produced by these polypeptides has been suggested, and it would seem that, for water transport, vasopressin removes the permeability barrier of the toad bladder membrane completely[11].

The results are taken as strong evidence in support of the idea of a permeability effect of aldosterone in the toad bladder. The magnitude of the maximum increase in short-circuit current obtainable with aldosterone is greater than that obtaineable with vasopressin. As the final permeability effect is similar with both hormones, the aldosterone-induced proteins must also have an effect on the Na^+ pump or the supply of energy to the pump.

This work was supported by a grant from the U.K. Science Research Council, and by a grant from the British Empire Cancer Campaign which provides a studentship for T.D.

Department of Zoology,
The University,
Sheffield (Great Britain)

T. DALTON
R. S. SNART

1 G. W. G. SHARP AND A. LEAF, *Nature*, 202 (1964) 1185.
2 D. D. FANESTIL AND I. S. EDELMAN, *Federation Proc.*, 25 (1966) 912.
3 G. W. G. SHARP AND A. LEAF, *J. Clin. Invest.*, 42 (1963) 978.
4 J. CRABBE, *Nature*, 202 (1963) 787.
5 D. D. FANESTIL, G. A. PORTER AND I. S. EDELMAN, *Biochim. Biophys. Acta*, 135 (1967) 74.
6 V. KOEFOED-JOHNSEN AND H. M. USSING, *Acta Physiol. Scand.*, 28 (1953) 60.
7 A. LEAF AND E. DEMPSEY, *J. Biol. Chem.*, 235 (1960) 2160.
8 N. N. SANYAL AND R. S. SNART, *Nature*, 213 (1967) 798.
9 R. S. SNART, *Proc. 3rd Jenaer Symp., 1965*, Akademie Verlag, Berlin, No. 4, p. 281.
10 R. S. SNART AND M. J. WILSON, *Nature*, 215 (1967) 964.
11 R. M. HAYS AND A. LEAF, *J. Gen. Physiol.*, 45 (1962) 933.

Received July 10th, 1967

Biochim. Biophys. Acta, 135 (1967) 1059–1062

BBA 73031

Dose–response characteristics of deoxycorticosterone-stimulated Na^+ transport by the isolated toad bladder

Studies have been made of the effect of deoxycorticosterone on the active ion transport by the isolated urinary bladder of the American toad, *Bufo marinus*. The toad bladder consists essentially of a single layer of epithelial cells supported by a small amount of connective tissue[1]. The bladder exhibits a characteristic transmembrane potential with the serosal surface electrically positive to the mucosal surface. The transport of Na^+ can be resolved into two components: the entry of

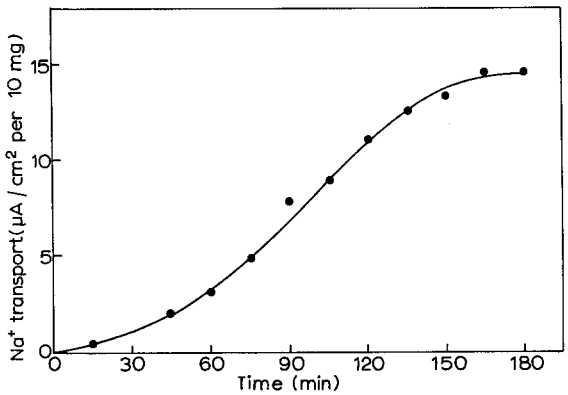

Fig. 1. The effect of deoxycorticosterone (10^{-7} M) on the Na^+ transport across the toad bladder, showing the stimulated Na^+ transport ($\mu A/cm^2$ per 10 mg) shown by the treated side of one half bladder relative to that of the control side. Showing a typical initial response reaching a maximum after 180 min.

Na^+ from the mucosal medium into the bladder epithelial cells, and the extrusion of Na^+ from the cells into the serosal medium. The stimulation of Na^+ transport in the bladder by deoxycorticosterone is well established[2,3].

The present work was undertaken in order to determine the dose–response characteristic of deoxycorticosterone-stimulated Na^+ transport by the toad bladder as a means of reflecting the characteristics of the active binding sites in the tissue.

The toads were placed in 0.11 M NaCl and carefully handled before use, in order to reduce endogenous mineralocorticoid. The toads were rapidly pithed and the bladders removed. Each half bladder was stretched across a double chamber, similar to that described by SHARP, KOMACK AND LEAF[4], in aerated Ringer solution. The bladders were short circuited and the variation of short-circuit current with time

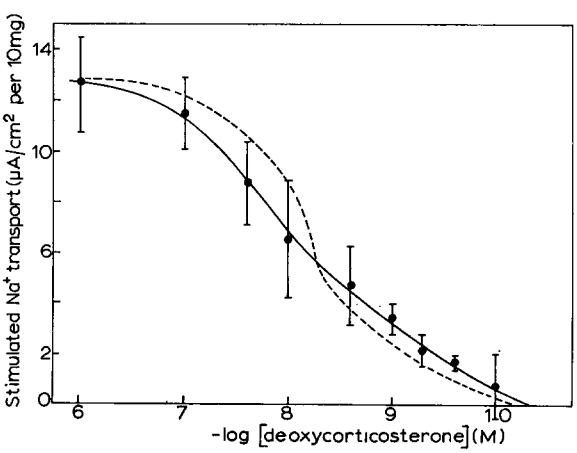

Fig. 2. The dose–response characteristic of deoxycorticosterone-stimulated Na^+ transport by the isolated toad bladder, showing the maximum increase in short-circuit current ($\mu A/cm^2$ per 10 mg) as a function of steroid concentration; standard deviations are shown for each point. The dotted line represents the equivalent activity curve where the activity is proportional to the saturation of active sites having an association constant $2.1 \cdot 10^8$ l/mole.

measured over a period of 4 h. After 1 h a standard amount of deoxycorticosterone in aqueous ethanol was added to the serosal surface of one section of bladder and a similar amount of solvent added to the control side. The steroid-treated side showed a stimulated Na^+ transport at concentrations above $5 \cdot 10^{-11}$ M. After a run of 4 h the section of bladder in each half of the double chamber was weighed and the short-circuit current corrected to unit weight and unit area. The increase in Na^+ transport ($\mu A/cm^2$ per 10 mg) shown by the treated side, above that of the control, has been plotted as shown in Fig. 1. It was found that very consistent results were obtained for given dose and it was possible to get a dose–response curve (Fig. 2) showing the maximum response obtained for a given concentration, using four half bladders for each concentration of steroid. In the case of 10^{-7} M 7 values were obtained and for 10^{-10} M 8 values were obtained. The standard deviations were calculated for each point using standard formulae. Correction of the short-circuit current to unit weight of bladder is a procedure recommended for such comparative work as variability was considerably reduced.

The range of activity of deoxycorticosterone in the toad bladder varies from $5 \cdot 10^{-11}$ M to 10^{-6} M, being a slightly wider range than that proposed for aldosterone[4]. The binding characteristics of aldosterone in the toad bladder[4,5] have shown that a set of binding sites exists in the membranes, with an association constant of $2.1 \cdot 10^8$ l/mole. If it is assumed that the stimulated active transport can be correlated with the saturation of this set of binding sites, the dose–response curve would follow the dotted line (Fig. 2). It is therefore, concluded that these sites are probably the active binding sites and that the association between deoxycorticosterone and these active sites parallels that of aldosterone.

This work was supported by a grant from the U.K. Science Research Council, and by a grant from the British Empire Cancer Campaign which provides a studentship for T.D.

Department of Zoology,
The University,
Sheffield (Great Britain)

T. DALTON
R. S. SNART

1 A. LEAF, in J. DE GRAEFF AND B. LEIJNSE, *Water and Electrolyte Metabolism II*, Elsevier, Amsterdam, 1964, p. 20.
2 G. A. PORTER AND I. S. EDELMAN, *J. Clin. Invest.*, 43 (1964) 611.
3 G. W. G. SHARP AND A. LEAF, *Nature*, 202 (1964) 1185.
4 G. W. G. SHARP, C. L. KOMACK AND A. LEAF, *J. Clin. Invest.*, 45 (1966) 450.
5 R. S. SNART, *Biochim. Biophys. Acta*, 135 (1967) 1056.

Received July 10th, 1967