BBA 73030

Effect of hormones on the permeability of toad bladder (*Bufo marinus*)

Measurement of the short-circuit current across the toad bladder *(Bufo marinus)* has shown that an aldosterone response has a 90-min lag period[1] during which time protein induction occurs[2]. On the basis of radio sodium labelling, SHARP AND LEAF[3] and CRABBE[4] have suggested that aldosterone increases Na^+ transport by affecting the permeability of the mucosal surface. However, FANESTIL, PORTER AND EDELMAN[5] have concluded that aldosterone increases the output of the Na^+ pump independently of an effect on the permeability of the mucosal surface. The present work has been carried out in order to try to resolve this conflict.

All toads used in this work were soaked in 0.11 M NaCl for two days prior to use, in order to reduce endogenous mineralocorticoids. They were rapidly pithed and the bladders excised. Each half bladder was stretched across a double chamber similar to that described by SHARP AND LEAF[1] and incubated in aerated Ringer (Na^+ 113.5 mequiv/l, K^+ 3.5 mequiv/l, Cl^- 116.5 mequiv/l, HCO_3^- 2.5 mequiv/l, Ca^{2+} 0.89 mequiv/l).

After incubation for 1 h at 22°, small volumes of ice-cooled Ringer at 0° were used to reduce the temperature, in one half of the double chamber, by 1 or 2° at a time. A constant temperature (± 0.1°) was maintained for 3–5 min by adding further cold Ringer as required. The membrane potential and short-circuit current were then measured. Heated Ringer was used in the other half of the double chamber to increase the temperature. The temperature dependence of the short-circuit current was followed between 4 and 42°. After each run 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 ($\mu A/cm^2$ per 10 mg).

The effect of vasopressin (10^{-6} M), amphotericin B ($3 \cdot 10^{-5}$ M), aldosterone (10^{-7} M) and deoxycorticosterone (10^{-6} M) was investigated using Ringer containing hormone, the temperature dependence being followed after a maximum response was obtained.

A plot of the short-circuit current ($\mu A/cm^2$ per 10 mg) against reciprocal temperature in the presence or absence of hormone gave rise to a very reproducible profile as shown (Fig. 1). The short-circuit current increases with temperature up to point I, obeying an exponential relationship. As the rate-limiting factor for Na^+ transport is believed to be determined by the number of ions crossing the mucosal permeability barrier then a Boltzmann distribution law should be obeyed and the slope of this line will determine the energy of the permeability barrier E_a. The Na^+ will be pumped out at the serosal surface at catalytic rates and not affect the temperature dependence of the short-circuit current. At temperature I the permeability may no longer be a limiting factor and the pumping efficiency will determine the rate of movement. Eventually a temperature is reached(II) at which the system will undergo temperature inactivation.

Response curves for deoxycorticosterone over a 3-h period were obtained taking short-circuit current ($\mu A/cm^2$ per 10 mg) readings every 15 min. The response of the toad bladder to deoxycorticosterone (Fig. 2) shows an immediate increase in short-circuit current, which could correspond to an initial slight lowering of the per-

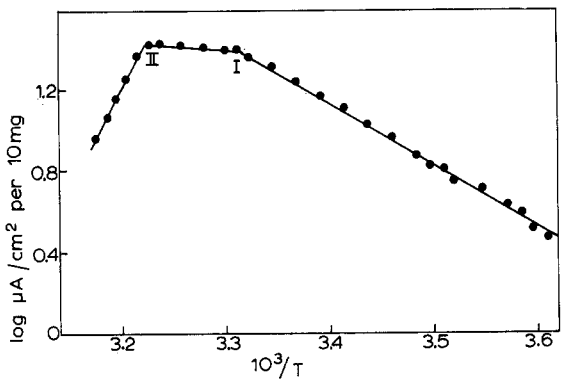

Fig. 1. A plot of the logarithm of the short-circuit current (μA/cm^2 per 10 mg) against reciprocal temperature measured across toad bladder membrane.

meability barrier. This is followed after a 90-min lag period by a further increase similar to that observed with aldosterone[1]. There is no initial response with aldosterone.

As the initial response differs from that of aldosterone the temperature dependence of the short-circuit current across the toad bladder during the first 90-min steroid treatment was followed by adding deoxycorticosterone or aldosterone to all 4 compartments of the double chamber. Using one half of the chamber as control, the temperature in the other half was varied. The change in short-circuit current relative to the control was followed as function of temperature within a 90-min period. The results were then treated as described (Fig. 3) and the Q_{10} and E_a values are shown in Table I. These indicate that vasopressin and amphotericin which are believed to produce effective "pores" in the mucosal surface[6-8] of the bladder membrane, lower the permeability activation energy considerably. Such a lowering is also evident after the 90 min lag period with aldosterone and deoxycorticosterone although within the first 90 min aldosterone had no effect on the permeability barrier whereas deoxycorticosterone showed a lowering.

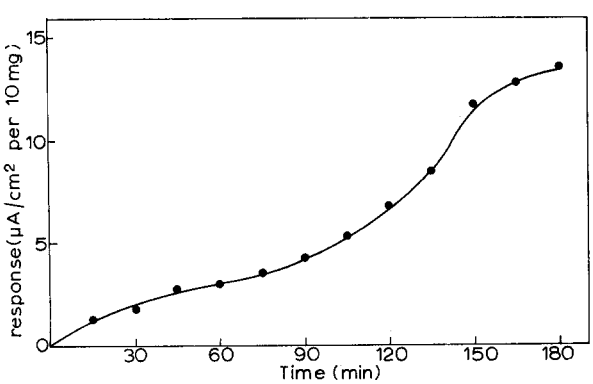

Fig. 2. The stimulated active transport (μA/cm^2 per 10 mg) by the toad bladder in response to 10^{-7} M deoxycorticosterone. Showing a typical initial response followed by a further increase after a 90-min lag period.

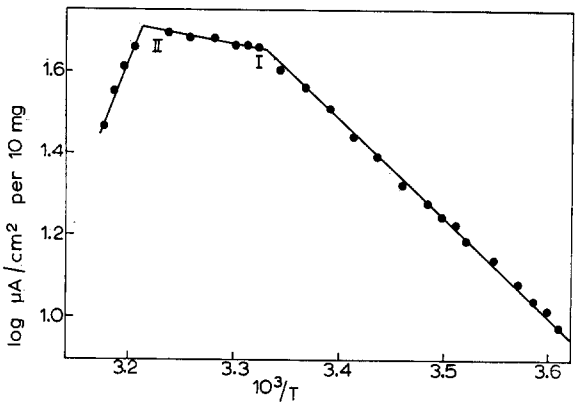

Fig. 3. A plot of the logarithm of the short-circuit current (μA/cm^2 per 10 mg) against reciprocal temperature measured across toad bladder membrane in the presence of 10^{-6} M deoxycorticosterone within the first 90 min.

TABLE I

A LIST OF THE TEMPERATURES CORRESPONDING TO POINTS I AND II FROM GRAPHS SUCH AS FIG. 1, ALONG WITH THE Q_{10} AND E_a VALUES DETERMINED IN THE PRESENCE OR ABSENCE OF HORMONES

Treatment	T_I	T_{II}	E_a	Q_{10} 10°–20°
Hormone				
1	27.7°	37.5°	13.45	2.27
2	28.7°	37.2°	13.80	2.29
Vasopressin				
1	29.6°	39.5°	9.1	1.74
2	27.9°	37.1°	9.05	1.74
Amphotericin B				
1	28.7°	37.4°	9.35	1.78
2	27.6°	37.0°	8.70	1.66
Aldosterone: 3 h				
1	28.5°	36.8°	9.90	1.82
2	28.4°	37.0°	9.45	1.78
Deoxycorticosterone: 3 h				
1	28.2°	36.6°	9.65	1.80
2	27.9°	37.2°	9.43	1.80
Aldosterone: 0–90 min				
1	28.0°	36.6°	13.60	2.29
2	28.7°	38.0°	13.10	2.29
Deoxycorticosterone: 0–90 min				
1	27.5°	37.3°	11.50	2.04
2	27.7°	36.0°	10.80	2.00

The direct effect of deoxycorticosterone on the permeability of the membranes possibly results from the potential stabilisation of steroid in lipid membranes[9]. It has been shown[10] that deoxycorticosterone is more soluble in artificial lipid membranes than aldosterone. The effect of introducing polar groups within the lipid membrane

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