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HORMONAL INFLUENCES ON TRANSEPITHELIAL SODIUM TRANSPORT: ALDOSTERONE vs. INSULIN

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SUMMARY

Aldosterone and insulin are both capable of stimulation of transcellular transport by amphibian epithelia. Arguments are reviewed for considering insulin as active close to the cell "pump", while aldosterone would act as the apical border of these specialized epithelial cells. Strikingly, after several hours of incubation, glucose enhances the activity of aldosterone-treated preparation only; this observation and other data briefly discussed point at (an) influence(s) of aldosterone on the energy-requiring step in addition to its apical effect.

APPRAISAL of hormonal effects on sodium movement across the cell membrane—a phenomenon of utmost biological importance[1]—requires adequate control of the electrochemical potential of this ion in compartments on either side of the membrane. This usually represents an insuperable challenge for the investigator who therefore often chooses to examine the issue on *epithelia* specialized in *transcellular* transport of sodium. From electrical measurements it appears that, for sodium to move across such epithelia, the ion has to overcome 2 main "barriers", supposedly located at the apical (outward-facing) and basal (inward-facing) poles of the epithelial cells, respectively. There are reasons to believe that the energy-requiring process, dubbed the "pump", operates at the basal border (and, likely, at the cell borders facing interspaces). Sodium entry at the apical border, while considered exergonic, at least in some experimental conditions, would require an interaction of the ion with some sort of carrier. It is this structure that is held responsible for the lack of direct proportionality between the concentration of sodium in the external solution and the rate at which the "pump" functions, as recalled in Fig. 1.

When amphibian tissues such as bladder, colon and skin are selected and when transepithelial sodium transport is studied according to Ussing and Zerahn[2], aldosterone and insulin are found capable of stimulation of sodium transport [3–6]. This is illustrated in Fig. 2 in which data collected with the ventral skin of *Bufo marinus* are summarized. There is a striking difference, however: insulin effect is fully apparent within 1 h after its introduction into the incubation fluid while, with aldosterone, 2–6 h have to go by before one can detect sodium transport stimulation. This latency period does not depend on the hormonal concentration[7].

Should one immediately come to the conclusion that both hormones increase sodium transport because they both stimulate the "pump"? Although this is a possibility, it should be kept in mind that the entry step represents, in some circumstances at least, a limiting factor which could be influenced by hormones in a way such that sodium be allowed easier access to the "pump" proper that would merely react in proportion[8].

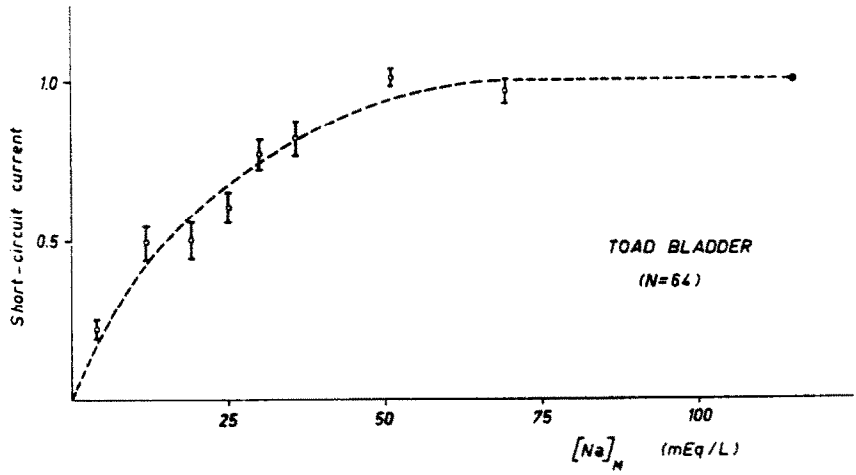

Fig. 1. Repercussions on short-circuit current of dilution of sodium in the solution on the outside of a sodium-transporting epithelium.

This graph deals with the behaviour of fresh toad (*Bufo marinus*) bladder. In each of the 64 experiments performed, Ringer's was replaced to a variable extent on the outside with an equivalent, isosmotic solution, for 45–60 min. Short-circuit current during this phase of incubation was expressed as a function of what it was before *and after* exposure to the modified solution; in each instance, the concentration of sodium was assessed by flame photometry.

Each experimental point summarizes 8 experiments; the curve was drawn by visual inspection across means ± S.E.

Ringer's composition was (mM): NaCl, 115; $KHCO_3$, 2·5; $CaCl_2$, 1·0. Sodium-free fluid contained (mM): $MgCl_2$, 57·5; $KHCO_3$, 2·5; $CaCl_2$, 1·0; sucrose, 57·5.

Toad colon and skin yield analogous data when examined this way [4].

The following experiments indicate that the mechanisms (i.e. sites) of action of aldosterone and insulin are quite distinct indeed; the data are interpreted as meaning that insulin acts close to, or at the "pump" proper, whereas aldosterone exerts its effect at least in part at the apical cell border.

(1) As already pointed out there are arguments for ascribing to the apical cell border the curvilinear shape of the relationship describing the repercussions of changes of external sodium concentration on transepithelial sodium transport. It was therefore considered worthwhile to examine whether this relationship is modified for preparations stimulated with insulin or aldosterone.

When incubations are carried out as indicated in the legend to Fig. 1, once the hormonal effects are obtained (see legend to Fig. 2), it appears that short-circuit current is more influenced by dilution of external sodium in the case of treatment with insulin than with matched control; on the other hand, influence of this type of manipulation is *decreased* when aldosterone is used instead [9]. Relevant data are summarized in Table 1. Complementary experiments, performed to check the relationship of sodium influx vs. short-circuit current, confirmed the reliability of the latter variable in these circumstances.

If indeed the curvilinear relationship describing short-circuit current as a function of sodium concentration on the outside depends on the existence of a saturable structure located apically, one would be led to conclude that aldosterone improves interaction at that cell border, unlike insulin.

It should be added, however, that when similar experiments are performed in

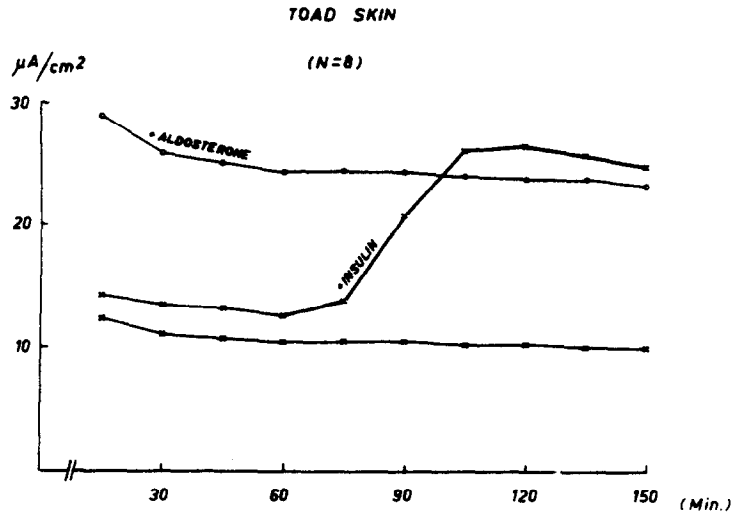

Fig. 2. Stimulation of active sodium transport across the isolated ventral skin of *Bufo marinus* upon exposure to aldosterone and insulin.

Three fragments of the skin preparation of a given toad were incubated simultaneously in 8 instances. Incubation started in the evening, one of the pieces being exposed from the outset to aldosterone, 5×10^{-6}M. The following morning, all preparations were short-circuited and insulin (125 mU/ml.) was introduced in the solution bathing one of the 2 other pieces 1 h later.

The effect of aldosterone added the preceding evening, was apparent as soon as short-circuit current measurements were performed. Within a short period of time, insulin brought about a stimulation, usually of like amplitude.

Hormones were present only in the solution to which the inner surface of the skin was exposed. No energy-providing substrate was present.

Table 1. Repercussions of dilution of external sodium on short-circuit current in the case of ventral toad skin treated or not with aldosterone vs. insulin

		Untreated	+ Aldosterone ($5 \cdot 10^{-6}$M)	+ Insulin (125 mU/ml)
Baseline activity (μA/cm² ± S.E.)		9·2 ± 1·0	17·8 ± 1·8	18·4 ± 1·9
	$[Na]_E$ (mEq/L)			
Residual activity* upon dilution of external sodium	0–5	0·362	0·569	0·291
	5–10	0·448	0·767	0·351
	10–15	0·677	0·874	0·461
	15–20	0·793	0·964	0·772
	20–25	0·867	0·927	0·717

*= SCC during/½(SCC before + SCC after) in which short-circuit current (s.c.c) is measured before, during and after exposure to low-sodium solution on the outside.

Approximately 40 experiments were performed, each time on 3 fragments of the ventral skin of a given *Bufo marinus*. They were almost evenly distributed between these 5 subgroups.

The incubations were carried out overnight in the absence of energy-providing substrate. Aldosterone was added at the outset; insulin, the following morning.

the presence of glucose, these differences tend to decrease on account of a shift towards the behaviour of insulin-treated preparations. As is the case for toad bladder[10], glucose can enhance remarkably the activity of aldosterone-treated skin preparations[7]; this is illustrated in Fig. 3.

(2) Such experiments suggest that aldosterone modifies the initial step of transepithelial sodium transport. Additional evidence would be welcome, however: amiloride is though to provide it. Amiloride is a guanidine derivative that inhibits swiftly, profoundly yet reversibly, transepithelial sodium transport when applied to the outward-facing surface of the preparation[11, 12]. The same is seen with triamterene. Both drugs apparently prevent sodium from entering the epithelial cells.

It so happens that aldosterone-stimulated preparations are *less* sensitive to the drug than matched untreated ones. In the case of insulin, there even are indications that the sensitivity, to triamterene at least, is *increased* (Table 2).

If amiloride and triamterene inhibit transepithelial sodium transport at the site of ion interaction with the carrier molecules the existence of which is postulated at the apical cell border, such data again provide evidence for *qualitative* changes induced at that border by aldosterone.

As was the case when sodium transport was lowered by decreasing external sodium concentration, addition of glucose resulted in a narrowing of the differ-

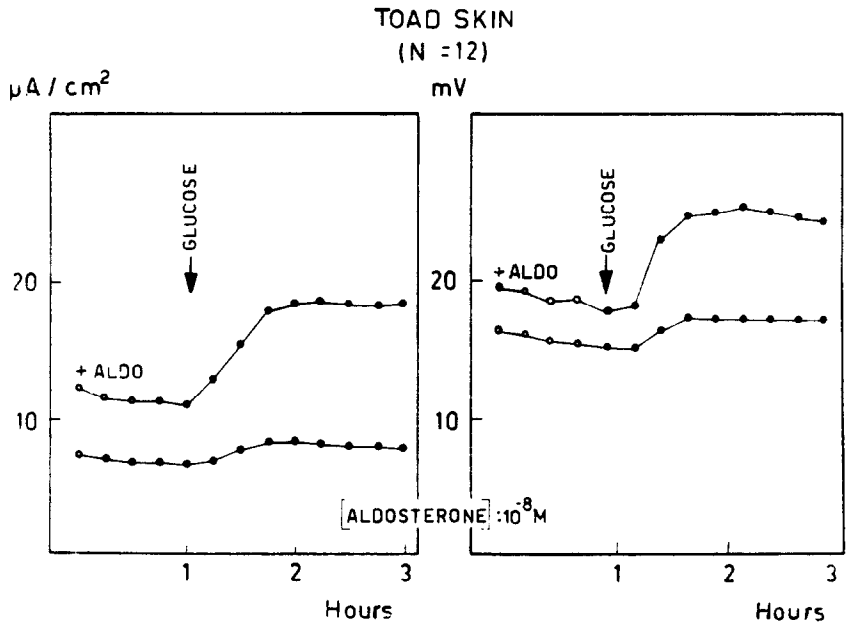

Fig. 3. Enhanced activity of aldosterone-treated preparations upon addition of glucose to the incubation fluid.

These experiments were performed with matched pieces of toad (*Bufo marinus*) ventral skin incubated overnight in the presence or absence of aldosterone. The following morning, after 1 h of short-circuiting (and intermittent measurements of transmembrane electrical potential), glucose, 10 mM, was introduced in the solution on the inside.

Glucose brought about a sizable increase in electrical activity of the aldosterone-treated preparations only. It is noteworthy that, when insulin was used instead, *no* such increase resulted[6].

Table 2. Influence of amiloride and triamterene on sodium transport by ventral skin of *Bufo marinus* stimulated by hormonal treatment

	Baseline short-circuit current (μA/cm^2 ± S.E.)	Residual activity during exposure to drug*
Experiments with Amiloride, $4 \cdot 10^{-7}$ M ($N = 8$)		
Untreated	9·4 ± 1·8	0·533
+ Aldosterone	15·1 ± 2·3	0·681
+ Insulin	20·6 ± 4·7	0·533
Experiments with Triamterene, $2 \cdot 10^{-4}$M (N = 8)		
Untreated	8·7 ± 1·2	0·619
+Aldosterone	14·9 ± 2·5	0·827
+ Insulin	18·9 ± 5·0	0·517

*= SCC during/½(SCC before + SCC after) incubation setting and hormone concentrations were as detailed in the comments to Table 1.

ence between the response to amiloride of untreated and aldosterone-exposed preparations.

(3) A less indirect approach consisted in attempts at measuring, at steady state, the amount of sodium drawn from the solution on the outside into the tissue—toad bladder was studied for this purpose[8]. The results indicate that aldosterone failed to modify the relationship between tissue sodium pool size and sodium transport, while insulin stimulated the latter variable significantly more than the former one (Table 3). Thus, insulin was considered as increasing, one way or another, the affinity of the "pump" for sodium—which has, as a consequence, a departure from the baseline relationship between pool size and transport

Table 3. Changes in the size of tissue (toad bladder) sodium pool involved in transepithelial sodium transport, as a function of hormonal effects on the latter*

	N	Sodium transport (μEq/h)	Size of tissue sodium pool (μEq)	Pool half-time* (min.)	Dry weight residue (mg)
Untreated	20	3·58 ± 0·32	0·221 ± 0·023	2·7 ± 0·2	11·6 ± 1·0
+ Aldosterone	15	4·96 ± 0·80	0·314 ± 0·064	2·6 ± 0·4	10·8 ± 1·2
+ Insulin	18	4·12 ± 0·67	0·185 ± 0·042	1·8 ± 0·3	8·8 ± 0·8

*Mean values ± S.E.

†$T_{1/2}$ = (Pool × ln2)J_{Na} where pool size and J_{Na} (sodium transport) are experimental data; it is assumed that sodium is distributed homogeneously in the pool as measured[8].

rate. This, however, did *not* happen when aldosterone was used: in this instance, both variables increased in proportion.

There are uncertainties regarding the exact location of this tissue sodium pool [8], which is slightly larger than that studied by Finn and Rockoff[13] by means of a kinetic method: but data of these investigators as well as unpublished data of ours, would lead one to assume that a significant fraction of the tissue sodium pool, as measured, provides reliable information on the function of the sodium-transporting cells. Actually Handler *et al.* just reported that the sodium content of isolated epithelial cells of the toad bladder is augmented by aldosterone[14].

For these reasons, one would like to consider that insulin acts close to, or at, the "pump" proper, while aldosterone facilitates sodium entry into the cell, at its apical border. But the enhancing effect exerted by glucose on sodium transport by aldosterone-treated epithelia is associated with qualitative changes in behaviour, as mentioned; it seems difficult therefore not to admit that changes are induced by aldosterone at the vicinity of the "pump" as well. Whether the increased permeability apically is the "primum movens" or whether a dual effect of aldosterone has to be postulated could not be resolved so far.

At any rate, it is tempting to reflect on the fact that insulin administered systemically brings about hypokalemia acutely, which phenomenon could be ascribed to a stimulation of sodium transport in tissues such as striated muscle[15]; as a result, hyperpolarization of these cell-membranes would set in, providing a driving force for additional potassium moving into cells.

Such does *not* take place with aldosterone[16] the action of which being possibly limited to those cells specialized in transepithelial sodium transport and, among them, to these equipped so as to be able to develop steep concentration gradients for sodium, thereby reducing the losses from the milieu intérieur to trivial amounts. The distal parts of the renal tubule and of the gut, the excreting ducts of saliva and sweat glands behave that way: these are all well-known targets for aldosterone. Their efficiency as sodium-transporting tissues might in turn reside in characteristics of the apical pole of their cells.

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DISCUSSION

Hviid Larsen: Usually you represent the transport parameters in the toad skin after overnight incubation of the skin with aldosterone. Do you see any stimulation or inhibition of the short-circuit current or a resistance drop during the hours following aldosterone treatment?

Crabbé: I am afraid we didn't have as much patience as you... With fresh tissue there are indications – corresponding to some of Dr. Porter's recent observations (*Gen. comp. End.* **16** (1971) 443) – that a response to aldosterone could be observed after 2 h of incubation. But when the skin preparation is exposed to the hormone after 15-odd h of preincubation, 4–6 h have to elapse before a stimulation of the sodium-transporting activity can be detected. Glucose has no influence on this. Neither Dr. Porter nor myself noticed during the latency period this remarkable transient depression of the electrical activity of aldosterone-treated preparations which you and other investigators, e.g. Eigler (*Pflüg. Arch.* **317** (1970) 236) regularly observe with frog skin.

Hviid Larsen: Did you study the effect of insulin right after addition of aldosterone?

Crabbé: Yes: when insulin is introduced 30–60 min after addition of aldosterone to the bathing solution, it brings about an increase in sodium-transporting activity of normal amplitude and duration.

Hviid Larsen: In toad skin stimulation of the active sodium transport by oxytocin is progressively blocked during the hours following aldosterone treatment. Did you notice a reduction of the insulin response in the aldosterone-treated skins as compared to the response in their matched control skins during the first six hr after aldosterone treatment?

Crabbé: It's a matter of definition, but as you know I'd rather speak of a latency period. During the latter, as I just said, it seems that the response to insulin (and to vasopressin, incidentally) is normal.

Edelman: Whatever the mechanism of the substrate effects in the response of the toad bladder to aldosterone, the effect depends on substrate utilisation, by either the glycolytic or the oxidative pathways. If the apical boundary constitues a Na^+ diffusion barrier, the permeability to Na^+ may be regulated by the local ATP: ADP concentration ratio. I don't know whether Dr. Ussing would conclude that changes in Na^+ transport produced by shifts in extracellular osmolality are necessarily mediated by changes in apical Na^+ conductance.

Ussing: Well, I think that it would take too much time at this moment to go seriously into this question. We had better postpone this to the next meeting here!