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Rapid Report

Aldosterone and chloride conductance of amphibian skin

Olivier Devuyst, Viviane Beaujean and Jean Crabbé

Department of Physiology, U.C.L. Medical School, Brussels (Belgium)

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Chloride influx (J_{Cl}) across the skin of toads maintained in dilute $MgCl_2$ or Na_2SO_4 was determined after overnight incubation with(out) aldosterone, and related to mitochondria-rich cell (MRC) density of the preparations. Adaptation to $MgCl_2$ vs. Na_2SO_4 was reflected by higher plasma aldosterone in the former group (17 vs. 3 nmol/l, respectively) while J_{Cl} was lower, even after overnight incubation (172 vs. 318 pmol cm^{-2} s^{-1}). Incubation with aldosterone induced a more pronounced increase in J_{Cl} in the case of Na_2SO_4- vs. $MgCl_2$-adapted toads (ΔJ_{Cl}: 242 vs. 25 pmol cm^{-2} s^{-1}, respectively), which could be related to difference in MRC density between these two groups (1078 vs. 615 cells/mm^2, respectively). On the other hand, the *in vitro* effect of aldosterone on Na^+ transport (assessed by I_{sc}) was equally pronounced in both groups, and thus independent of MRC density. These data suggest that aldosterone, rather than being involved in MRC proliferation, stimulates Cl^- conductance by influencing the functional state of MRC.

Amphibian skin has been used extensively for the study of the action of aldosterone as a regulator of extracellular fluid volume. Aside from the stimulation of Na^+ transport by principal cells of this tissue, the hormone has been found to increase its Cl^- permeability [1]. There are indications that the latter is conductive and involves another cell-type, the mitochondria-rich cells (MRC) [2]. However, plasma aldosterone concentration was correlated neither with MRC density nor with Cl^- conductance across toad skin, after adaptation of the animals to Cl^--free vs. Na^+-free salts; furthermore, MRC density in this tissue did not increase despite long-term treatment of the animal with aldosterone [3]. This apparent discrepancy might result from the fact that the hormone, rather than being involved in MRC proliferation, could somehow activate Cl^- conductance in this type of cell. We decided therefore to investigate the effect of *in vivo* treatment of toad skin with aldosterone, as a function of MRC density.

The experimental methods were as follows. Toads (*Bufo marinus*), from the Dominican Republic, were maintained on moist peat at room temperature for at least one month before experiments, and they were fed once weekly. Adaptation consisted in keeping animals ($n = 6$ in each group) half-immersed for at least two weeks in dilute (30 mmol/l) Na_2SO_4 or $MgCl_2$ aqueous solution renewed every other day. After killing by double pithing, the abdominal skin was dissected free and divided longitudinally in two parts for overnight (ON) incubation of matched pieces at 25°C in aerated Ringer's solution (NaCl 115 mmol/l, $KHCO_3$ 2.5 mmol/l, $CaCl_2$ 1.0 mmol/l), with or without aldosterone, 50 nmol/l. The following morning, the skin pieces were mounted in Ussing-type chambers (incubation area: 3.14 cm^2) filled with aerated Ringer's, for measurement of transepithelial potential difference (V) and for short-circuiting (I_{sc}). Thereafter, transepithelial Cl^- influx (J_{Cl}) was evaluated after replacement of Na^+ with K^+ in the solutions on both sides, and replacement of Cl^- with gluconate on the chorial side [2]. J_{Cl} was measured in the short-circuit state by means of ^{36}Cl counted by liquid scintillation spectrometry. The influence of long-term incubation on electrical parameters was also studied for toads acclimated for 2 weeks in distilled water vs. dilute (30 mmol/l) NaCl. Transepithelial conductance (G_t) and I_{sc} were determined in fresh preparations and following 20-h incubation in Ringer's solution. In this case, 'shunt' conductance (G_{sh}) was calculated after exposure to amiloride [4]. MRC density of the abdominal skin preparations studied was determined by silver staining [3]. Plasma aldosterone concentration was measured by radioimmunoassay (Abbott, Wiesbaden, F.R.G.), on

Correspondence: J. Crabbé, Département de Physiologie, Unité ENDO, Avenue Hippocrate 55, B-1200 Brussels, Belgium.

TABLE I

Influence of acclimation to $MgCl_2$ vs. Na_2SO_4 on transepithelial potential difference (V) and conductance (G_t), and on short-circuit current (I_{sc}), for matched toad skin preparations incubated overnight under control conditions (C) or with aldosterone 50 nmol/l (A) (n = 6 in each group)

Preparations were studied with Ringer's solution on both sides. Data are means ± S.E. * indicates that the hormonal effect is statistically significant ($P < 0.05$).

		V (mV)	I_{sc} (μA cm^{-2})	G_t (mS cm^{-2})
$MgCl_2$	C	49 ± 7	21 ± 4	0.47 ± 0.09
	A	48 ± 3	38 ± 8 *	0.75 ± 0.14 *
Na_2SO_4	C	24 ± 7	21 ± 4	1.25 ± 0.35
	A	23 ± 5	37 ± 6 *	1.82 ± 0.22 *

blood collected on heparin by cardiac puncture of the pithed animal. All data are means ± S.E.; Student's *t*-test was used for statistical analysis.

The term 'adaptation' of the animals studied is justified by the fact that, after 2 weeks in the solutions selected, urine composition reflected that of these solutions [3], and MRC density was stabilized (unpublished data). Plasma aldosterone, determined at the time of killing, averaged 17 ± 5 nmol/l in $MgCl_2$-adapted animals, against 3 ± 1 nmol/l in Na_2SO_4-adapted toads ($P < 0.01$).

The Na^+-transporting activity of toad skin incubated ON with(out) aldosterone was monitored according to Ussing and Zerahn [5], and the hormonal effect was related to prior adaptation. Table I summarizes the pertinent electrical data. The influence of environment on Na^+-transporting activity was no longer detectable after ON incubation, as control I_{sc} values were identical in both groups. On the other hand, adaptation was still reflected by differences in G_t, the latter being higher for the skin of toads maintained in Na_2SO_4. Incubation with aldosterone led to an increase in I_{sc} of similar magnitude whether the skin preparations were obtained from $MgCl_2$- or $NaSO_4$-adapted toads; by contrast, the increase in G_t was twice as pronounced in the latter group (ΔG_t: 0.57 vs. 0.28 mS cm^{-2}). Influence of long-term incubation itself (i.e. without aldosterone) on electrical parameters was also investigated on NaCl- vs. water-adapted toads: the increase in I_{sc}, classically induced by salt-deprivation, was no longer observed after 20-h incubation in Ringer's solution, while G_{sh} was still higher in this condition (Table II).

Conductive (I_{sc}) and total unidirectional transepithelial Cl^- flux (J_{Cl}) were also measured in $MgCl_2$ vs. Na_2SO_4 preparations, as was MRC density. J_{Cl} was increased after adaptation to Na_2SO_4 vs. $MgCl_2$ (318 ± 70 vs. 172 ± 36 pmol cm^{-2} s^{-1}; $P < 0.05$); so was MRC density (1078 ± 37 vs. 615 ± 15 cells/mm^2, $P <$ 0.001). After ON incubation with aldosterone, the increase in J_{Cl} was more pronounced across the skin of Na_2SO_4- vs. $MgCl_2$-adapted toads (ΔJ_{Cl}: 242 ± 87 vs. 25 ± 50 pmol cm^{-2} s^{-1}), as a likely reflection of difference in MRC density between these preparations (Fig. 1); so was the conductive counterpart of Cl^- flux (ΔI_{sc}: 20.1 ± 6.8 vs. 4.7 ± 3.2 μA cm^{-2}) (Table I).

The data presented here confirm that aldosterone secretion by the interrenal tissue, as well as MRC density in amphibian skin epithelium, can easily be regulated by exposing toads to Na^+- vs. Cl^--containing salts: aldosterone secretion was stimulated in Na^+-free conditions, while MRC density increased in a Cl^--free habitat. The change in opposite directions of these parameters, as well as the failure to influence MRC density by *in vivo* treatment with the hormone [3], seem to rule out aldosterone as the factor responsible for MRC proliferation. As previous data indicated that Cl^- conductance was increased after incubation of toad skin with aldosterone [1], the question was raised of a role played by the hormone in MRC *activation* (in terms of Cl^- conductance) - rather than in *proliferation* of this cell type.

Thus, J_{Cl} and MRC density were determined after ON incubation of matched toad skin preparations with(out) aldosterone, in experimental conditions such that Na^+ transport was blocked. Conceptually,

$$G_t = G_{cell} + G_{sh}$$

where cell conductance (G_{cell}) is largely determined by apical conductance of the principal cells [6], and shunt conductance (G_{sh}) stands for the conductance of MRC together with that of the paracellular pathway. Since

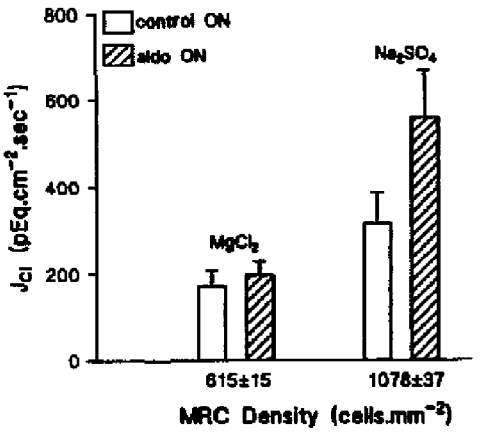

Fig. 1. Mitochondria-rich cell (MRC) density in toad skin and aldosterone-mediated increase in transepithelial chloride influx (J_{Cl}). Preperations were incubated overnight in control conditions, or with aldosterone 50 nmol/l. MRC density (mean ± S.E.) was determined on the abdominal skin preparations of animals maintained for 2 weeks in $MgCl_2$ or Na_2SO_4, 30 mmol/l ($n = 6$ in each group).

the apical conductance of principal cells is a Na^+ conductance [7], G_{cell} reduces to near-zero when Na^+ is replaced with an impermeant ion on the apical side; in these conditions G_t provides an estimate of G_{sh} – largely a reflection of transepithelial Cl^- movement [8].

Adaptation to the environments selected led to a significant difference both in J_{Cl} (reflected by G_t) and MRC density, still apparent after ON incubation under control conditions. A spontaneous loss of Cl^- permeability resulted from ON incubation itself, as the J_{Cl} data reported here in baseline conditions are half those obtained for fresh tissue [3]. When toad skin was incubated ON with aldosterone, J_{Cl} increased, even though MRC density was uninfluenced. The hormonal effect on J_{Cl} was more pronounced after Na_2SO_4 adaptation, i.e. at higher MRC density. These data indicate that the hormone might influence MRC function, such that Cl^- conductance increased; they are in keeping with previous reports on aldosterone-induced morphological changes in MRC [3,9] and stimulation of carbonic anhydrase activity in this cell-type [10].

Despite the large difference in plasma aldosterone concentration between $MgCl_2$- and Na_2SO_4-adapted toads, I_{sc} was similar for both groups after ON incubation under control conditions (Table I). The same was observed in the case of toads adapted to either distilled water or dilute saline (Table II). The level of endogenous aldosterone, which can be regulated by acclimation of the toad, thus appears not to exert its effect on Na^+-transporting activity of amphibian skin beyond a few hours. By contrast, G_t was still significantly higher in Na_2SO_4- or water-adapted toads. As Na^+ transporting activity was similar in both sets of preparations, and given the difference in J_{Cl} between $MgCl_2$ and Na_2SO_4 toads, one could assume that G_{sh} stands for the difference between G_t values. This assumption is confirmed by the determination of G_{sh} by means of amiloride (Table II). Interestingly, although stimulation of J_{Cl} by aldosterone *in vitro* seems to be related to MRC density, the hormone-induced increase in Na^+ transport – mediated by principal cells – was of the same magnitude in both groups of toads (Table I).

In conclusion, (i) the *in vitro* effect of aldosterone on transepithelial Cl^- pathway seems to be quantitatively related to MRC density; this can be interpreted as an influence exerted by the hormone on the functional state of the MRC. On the other hand, MRC density does not seem to play a role in the stimulation of Na^+ transport by aldosterone; (ii) following long-term incubation of the preparations, previous adaptation of the animals is no longer reflected in terms of I_{sc}, but well of G_t: this reflects a difference in G_{sh} – thus in MRC density.

TABLE II

Influence of toad's acclimation to dilute NaCl vs. distilled water on short-circuit current (I_{sc}), transepithelial (G_t) and shunt (G_{sh}) conductance, determined on fresh tissue ('fresh') and following 20-h incubation in Ringer's fluid ('overnight')

Data are means ± S.E. All the differences in terms of 'fresh' I_{sc}, G_t and G_{sh} between NaCl- and water-adapted toads are significant ($P < 0.05$). Following overnight incubation, I_{sc} data are similar in both groups, while the differences in terms of G_t and G_{sh} remain significant ($n = 10$ in each group).

	NaCl 30 mmol/l		Distilled water	
	fresh	overnight	fresh	overnight
I_{sc} ($\mu A\ cm^{-2}$)	19.6 ±2.9	15.4 ±1.2	32.8 ±3.1	14.1 ±1.3
G_t (mS cm^{-2})	0.77±0.11	0.40±0.03	1.77±0.17	0.68±0.09
G_{sh} (mS cm^{-2})	0.37±0.07	0.32±0.04	0.74±0.11	0.54±0.14

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