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Regulation of sodium transport by alteration of chloride conductance

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SUMMARY

A previously undescribed *in vivo* Na^+ transport regulatory mechanism is demonstrated in skin of frogs. This adaptive response, produced by preconditioning in high salt environment, is mediated by decrease in passive Cl^- permeability or conductance. It is independent of aldosterone suppression and of the presence of Na^+ in the conditioning medium.

Research emphasis in NaCl transport across epithelial tissues and its regulation has usually centered upon humoral alteration of active Na^+ transport. Aldosterone and its effect on active Na^+ transport has been of major interest. I now report an Na^+ transport regulatory system independent of aldosterone and mediated by change in passive Cl^- conductance.

Previously, I demonstrated that preconditioning of frogs in a high concentration of NaCl produces not only the expected decrease in active Na^+ transport but also a decrease in passive Cl^- conductance or permeability[1]. The suppression of active Na^+ transport is due, at least in part, to decrease in blood aldosterone[2,3]. Since the work of Ussing and co-workers[4] and Linderholm[5], it has been clear that alteration of passive Cl^- conductance or permeability could serve as an *in vivo* mode of Na^+ transport regulation. Therefore, I postulated that the unexplained Cl^- conductance decrease produced by NaCl preconditioning represented such an *in vivo* mechanism of Na^+ transport regulation independent of direct active Na^+ transport alteration.

Na^+ is actively transported inward in isolated frog skin, associated with a potential difference (PD) positive on the inside. Cl^- moves inward under the "passive" force of the PD or electrical gradient[4]. If the conductance or permeability of the passive Cl^- is decreased, this should increase the work of the active Na^+ transport mechanism. Thus, the alteration in passive Cl^- conductance described above should decrease Na^+ transport by a Cl^- "drag"

effect[5] and be a means of NaCl transport regulation independent of a direct effect on active Na^+ transport. Study of Na^+ and Cl^- transport under these conditions, the open-circuit state, will not distinguish between the role of alteration in active Na^+ transport and passive Cl^- conductance in a given Na^+ transport change.

The short-circuit current technique is an artifice which will distinguish between a direct alteration of either active Na^+ transport or passive Cl^- conductance. In the short-circuited state the skin PD is nulled or clamped to approximately zero. There is no longer an electrical gradient so that the Cl^- flux is approximately equal in both directions and is electrically uncoupled from Na^+ transport. The Cl^- flux in either direction then represents passive Cl^- conductance, provided the concentration of ions on both sides of the skin are equal[5]. There is still net ion flow of Na^+ inwards or short-circuit current (I_S) which is a measure of active Na^+ transport.

To demonstrate that an Na^+ transport regulatory system exists which is mediated by Cl^- conductance change the following must be done. First, Cl^- flux in the short-circuited state (Cl^- conductance) must be adaptively altered without change in I_S (active Na^+ transport). Second, net Na^+ transport or flux must be appropriately altered when the ions are electrically coupled (the open-circuit state). A non-Na^+ salt, arginine chloride, was used to selectively suppress passive Cl^- conductance since NaCl preconditioning also suppresses active Na^+ transport, as noted above.

Frogs of the species *Rana pipiens* were maintained for 5 days prior to transport experiments in 1-quart Mason jars containing 50 ml tap water or a salt (100 mM arginine chloride or 100 mM NaCl) in tap water. The solutions were changed twice daily. At the end of conditioning, isolated abdominal skin was mounted between two Lucite hemi-chambers (skin area of 1.32 cm^2) in modified Ringer's solution. For Cl^- flux determination, they were continuously short-circuited according to the method of Ussing and Zerahn[4] except for periodic determination of the open-circuit PD. Details of the electrical and isotopic ion flux methods will be described elsewhere[6]. Flux periods of 1 h were performed in the second hour after mounting the skin in both the ^{36}Cl and the ^{22}Na studies. Unidirectional Na^+ influx and outflux, performed in the open-circuit state, were usually measured simultaneously on skin halves from the same animal. Cl^- fluxes were both influx and outflux, measured alternately on different skins. The reported PD and I_S were values taken at the mid-point of the Cl^- flux period.

Table IA shows the effect of pre-conditioning of frogs in arginine chloride on skin PD, I_S, and Cl^- flux. In the short-circuit state Cl^- flux was significantly decreased but there was no decrease in I_S or active Na^+ transport. This selective effect of arginine chloride is in contrast to the effects of NaCl pre-conditioning previously described[1-3]. The increase in PD, although not statistically significant, is also compatible with a decreased passive Cl^- conductance[4]. Under open-circuit conditions, when the effect of "drag" of the Cl^- on Na^+ transport should be manifest (Table IB), net Na^+ flux was decreased 28%. This indicates an Na^+ transport alteration *via* Cl^- conductance change. The net Na^+ flux change was primarily a consequence of decrease in Na^+ influx.

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TABLE I

ELECTRICAL AND ION FLUX MEASUREMENTS ON ISOLATED SKINS OF FROGS MAINTAINED IN TAP WATER AND ARGININE·Cl OR NaCl IN TAP WATER

N is the number of observations. *P* is probability of statistical significance on comparing the series using the Student's *t*-test. All flux values in Series B are expressed as μequiv·cm^{-2}·h^{-1}. Net flux is the difference in unidirectional Na^+ influx and outflux in those cases in which they were performed on skin halves of the same animal. See text for timing of flux experiments. Experiments were performed in the open-circuit state. N.S., not significant.

	Conditioning								
	Tap water			*100 mM arginine chloride in tap water*				*100 mM NaCl in tap water and aldosterone**		
	N	*Mean ± S.E.*		*N*	*Mean ± S.E.*		*P*	*N*	*Mean ± S.E.*	*P*
A. PD (mV)	28	18	± 1.7	27	25	± 3.4	<0.10			
I_s (μA·cm^{-2})	28	43	± 3.1	27	40	± 3.7	N.S.			
Cl^- flux (μequiv·cm^{-2}·h^{-1})	28	2.00	± 0.18	27	1.34	± 0.21	0.025			
B. Na^+ influx	30	2.49	± 0.173	28	1.75	± 0.183	<0.005			
Na^+ outflux	30	0.412	± 0.073	26	0.552	± 0.150	N.S.			
Na^+ net flux	29	2.08	± 0.170	24	1.50	± 0.185	<0.025			
C. PD (mV)	31	17	± 1.6					25	24 ± 2.2	<0.02
I_s (μA·cm^{-2})	31	59	± 3.9					35	63 ± 4.0	N.S.
Cl^- flux (μequiv·cm^{-2}·h^{-1})	20	3.44	± 0.38					25	2.29 ± 0.26	<0.02

* 7 μg aldosterone administered daily. See text for details.

Decreased circulating aldosterone causes at least part of the I_S decrease associated with pre-conditioning in NaCl (ref.3). Also, aldosterone produces transient increases in Cl^- flux in amphibian skin *in vitro*[7,8]. To evaluate the role of aldosterone in the Cl^- conductance adaptation, sufficient aldosterone was administered during pre-conditioning in NaCl to prevent a major decrease in circulating aldosterone and a consequent decrease in I_S. D-Aldosterone in 95% ethanol was injected daily into the dorsal lymph sac of animals maintained in 100 mM NaCl for 5 days. Control animals received ethanol alone. A dose of 70 μg per 100 g frog daily caused a marked increase in I_S. However, 7 μg per 100 g frog daily maintained I_S at control levels (Table IC). Yet, Cl^- flux under these short-circuit conditions was decreased. The PD also increased. This series again demonstrates a selective decrease in Cl^- conductance. Thus, the change is unlikely to be dependent on decreased circulating aldosterone.

These data indicate the presence of an Na^+ transport regulatory mechanism which has the following qualities: (1) It is mediated *via* change in passive Cl^- conductance. (2) It is operative independently of direct alteration of active Na^+ transport. (3) It is independent of aldosterone suppression. (4) It is independent of the presence of Na^+ in the conditioning medium. The mechanism may be humorally regulated as previously suggested[1]. Additional investigation is necessary to determine whether this regulatory system is dependent on the presence of Cl^- in the conditioning medium.

This additional NaCl transport regulatory system could be operative in the mammalian renal tubule and be a means of maintenance of extracellular fluid volume. Also the system could be active in the human disease states associated with abnormal volume regulation and edema, such as congestive heart failure, nephrosis and cirrhosis of the liver[8].

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