BBA Report

BBA 71102

Regulation of NaCl transport: Relation to chloride conductance

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(Received August 30th, 1971)

SUMMARY

Preconditioning of frogs in high salinity environment produced a suppression of skin Cl^- conductance as well as active Na^+ transport. The Cl^- transport effect is not readily explained by alteration in the known humoral substances vasopressin and aldosterone and could reflect a heretofore undescribed mechanism of NaCl transport regulation. A spontaneous time-dependent decrease in Cl^- conductance occurred in isolated skin of unconditioned animals, possibly from inactivation or loss of an endogenous factor(s) which increases Cl^- conductance. This spontaneous decrease was inhibited for two successive 0.5-h periods by plasma from non-conditioned animals placed in the inside chamber. The decrease was prevented only in the second 0.5-h period by addition of plasma from animals conditioned to high salinity. The spontaneous decrease in Cl^- permeability in isolated skin and reversal by frog plasma are compatible with the presence of a humoral substance which regulates Cl^- conductance.

In recent years NaCl transport across epithelial tissues and its regulation has been extensively studied. Research emphasis has usually centered upon humoral alteration of active Na^+ transport in such tissues as the isolated frog skin and toad bladder and the mammalian renal tubule. However, an alteration in the permeability or conductance of Cl^-, usually a major passive co-ion, could be an additional means of regulating NaCl transport by alteration of the electrochemical gradient which the active Na^+ transport mechanism must overcome[1]. For instance, an increase in Cl^- conductance should decrease the electrical "drag" effect of the passive anion and increase NaCl transport. To investigate this possibility, the adaptation of frog skin Na^+ and Cl^- transport to high salinity environment was examined. The short-circuit current technique was used since it is an artifice which allows electrical uncoupling of NaCl transport and thus evaluation of active Na^+ transport and passive Cl^- flux or conductance separately[1]. Also, in a preliminary search for a humoral regulatory factor frog plasma was tested for its effects on Cl^- flux.

To assess the effect of high salinity conditioning, short-circuit current (I_S) and Cl^- flux were determined as measures of net active Na^+ transport and Cl^- conductance, respectively. Frogs of the species *Rana pipiens* were maintained for 5 days prior to transport experiments in 0.5 gal Mason jars containing 50 ml tap water or 60 mM NaCl in tap water. The solutions were changed twice daily. At the end of conditioning, isolated abdominal skin was mounted between two lucite hemichambers in modified Ringers' solution and continuously short-circuited according to the method of Ussing and Zerahn[1], except for periodic determination of the open-circuit potential (PD). Details of the electrical and isotopic ion flux methods have been previously described[2,3]. 1-h flux periods were performed in the 4th h after mounting the skin in both the initial ^{36}Cl and ^{22}Na studies (Table I,A and B). Unidirectional Na^+ influx and outflux were measured simultaneously on skin halves from the same animal. Cl^- fluxes in this series 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 flux period. To compare I_S to ion flux, I_S was expressed as the amount of positive charge, in cation equivalents, that moved inward across 1 cm^2 of skin per h. It is designated as the "short-circuit current equivalent".

For collection of frog plasma, animals were anesthetized with 10% urethane (0.03 ml/g frog) injected into the dorsal lymph sac. Blood was removed by cardiac puncture after opening the thoracic cavity in the midline.

TABLE I

ELECTRICAL AND ION FLUX MEASUREMENTS ON ISOLATED SKINS OF FROGS MAINTAINED IN TAP WATER OR HIGH SALINITY MEDIUM

N is the number of observations, *P* is probability of statistical significance on comparing the two series using the "Student" *t* test. See text for method of unidirectional flux measurements.

	Conditioning				
	Tap water		*60 mM NaCl in tap water*		
	N	*Mean ± S.E.*	*N*	*Mean ± S.E.*	*P*
A. PD (mV)	29	32 ± 2.6	19	37 ± 3.1	N.S.
I_S ($\mu A \cdot cm^{-2}$)	29	24 ± 1.2	19	18 ± 1.5	< 0.005
Cl^- flux ($\mu equiv \cdot cm^{-2} \cdot h^{-1}$)	29	0.372 ± 0.087	16	0.153 ± 0.021	< 0.02
B. Na^+ influx*	15	1.67 ± 0.24	14	1.01 ± 0.18	< 0.05
Na^+ outflux	15	0.254 ± 0.099	14	0.223 ± 0.079	N.S.
Na^+ net flux	15	1.41 ± 0.22	14	0.78 ± 0.20	< 0.05
Short-circuit current equivalent**	15	1.46 ± 0.26	14	0.75 ± 0.07	< 0.01

*All flux values in Series B are expressed as $\mu equiv \cdot cm^{-2} \cdot h^{-1}$. Net flux is the difference in unidirectional Na^+ influx and outflux performed separately on skin halves of the same animal. See text for timing of flux experiments.

**Short-circuit current equivalent is the short-circuit current from influx experiments expressed as cation equivalents, that moved inward across 1 cm^2 of skin per h.

Table IA shows the effect of high salinity environment on PD, I_S, and Cl^- flux. Both I_S and Cl^- flux were significantly decreased in the skin from animals conditioned to 60 mM NaCl. In untreated frogs, I_S is equivalent to net Na^+ transport and Cl^- transport is

passive[1], *i.e.* there is no net Cl^- flux in the short-circuited state. Therefore, Cl^- flux represents passive conductance of the Cl^-. If the same conditions hold true in the skin of animals adapted to high salinity, then the treatment suppressed both net Na^+ transport (I_S) and passive Cl^- conductance (Cl^- flux). Table IB demonstrates that this is so. Net Na^+ transport is reduced in the skins of conditioned animals and remains approximately equal to the short-circuit current equivalent. Also, the decrease in Na^+ influx accounts for the net flux and the short-circuit current equivalent decrease, as there is no significant difference in Na^+ outflux in the two series.

TABLE II

SEQUENTIAL Cl^- FLUX IN ISOLATED SKIN OF NON-CONDITIONED FROGS AND EFFECT OF FROG PLASMA

N is the number of observations. Values are expressed as mean ± S.E. *P* is probability of statistical significance using the "Student" *t* test and comparing the two series which received plasma to the control series. See text for method of flux measurements and details of plasma administration.

Conditioning of animal source of plasma	*N*	*Cl^- flux as % of Period 1*	
		Period 2	*Period 3*
Control (no plasma)	8	69 ± 2.0	57 ± 2.6
Tap water	9	99 ± 2.8	99 ± 13
P		< 0.001	< 0.01
60 mM NaCl in tap water	12	62 ± 4.4	113 ± 10
P		N.S.	< 0.001

Sequential Cl^- influx experiments were performed on isolated skin of non-conditioned frogs under short-circuit conditions (Table II). Cl^- (ref. 36) was added at the time of mounting the skin. After 1 h, two 45-min flux periods (Periods 1 and 2) followed by a 60-min flux period (Period 3) were performed. The results were corrected to flux/h and expressed as percent of the first flux period. At the end of flux Period 1, the control series received Ringers' solution in the inside chamber and the other two series received plasma from frogs maintained in tap water or high salinity environment as described above. The plasma concentration in the inside chamber varied from 3 to 13% in both experimental series. In the control series, the Cl^- flux or conductance spontaneously decreased. After plasma from frogs maintained in tap water was placed in the inside chamber, Cl^- flux increased relative to control series in both periods. After plasma from conditioned animals, Cl^- flux increased in Period 3 only.

The equivalence between the short-circuit current and Na^+ decrease after high salinity conditioning confirms previous work[4], but the Cl^- conductance alteration has not been previously described. The decrease in frog skin Na^+ transport after high salinity conditioning is probably, at least in part, due to decreased endogenous aldosterone secretion[5]. However, the decrease in Cl^- conductance cannot be easily explained by alteration in the two humoral agents known to affect transepithelial Na^+ transport. Vasopressin does not alter active or passive Cl^- flux[6]. Aldosterone only transiently affects Cl^- flux[7,8]. The decrease in Cl^- conductance could represent a mode of NaCl transport

regulation unrelated to aldosterone and vasopressin. Alteration of passive Cl^- conductance or permeability should alter the electrochemical gradient which the active Na^+ transport mechanism must overcome[1] and thus alter the rate of NaCl transport. Of course, if parallel active transport of Cl^- also occurred, as demonstrated in the isolated skin of *Leptodactylus ocellatus*[6] and as proposed for *Rana pipiens in vivo*[17], such a mechanism could also regulate the active Cl^- transport mechanism by Cl^- conductance alteration. It is possible that the Cl^- flux in the short-circuited state does not represent, even in part, Cl^- conductance through the pathways involved in open-circuit NaCl transport. Thus, experimental proof of an effect of change in Cl^- conductance on open-circuit net Na^+ transport must ultimately be directly demonstrated experimentally.

The spontaneous decline in Cl^- flux in skins of non-conditioned frogs (Table II) could be due to inactivation or loss of a Cl^- permeability stimulating substance(s) from the site of Cl^- transport regulation, similar to the time-related decrease in Na^+ transport in isolated toad bladder which is ascribed to loss of aldosterone effect[9]. The relative increase in Cl^- flux after exposure to frog plasma is compatible with the presence of a circulating substance which regulates Cl^- conductance or permeability. However, the interpretation must be viewed with some caution. The plasma was obtained by cardiac puncture of anesthetized frogs,and relatively non-specific bioactive substances could have been released by this stress. For instance, catecholamines increase Cl^- flux, presumably by stimulation of mucous glands[10] and could be the factor in question.

A factor important in volume regulation which increases NaCl transport by increasing Cl^- conductance would be expected to be suppressed by high salinity conditioning. Indeed, the plasma from high salinity animals did not stimulate Cl^- flux in the 1st period after administration but in the 2nd period, produced a relative increase to the level found with the non-conditioned plasma. This is disturbing but the detection system is extremely crude at present.

A hypothetical scheme of three possible sites of regulation of NaCl transport is presented in Fig. 1 and is based on the simple model often used to describe ion transport in frog skin and toad bladder. Site A is probably the locus of action of vasopressin which increases Na^+ transport[11]. Aldosterone also increases active Na^+ transport and both A and

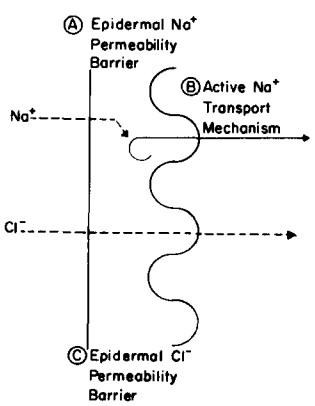

Fig. 1. Possible sites of regulation of transcellular NaCl transport in epithelial membranes. This diagram represents frog skin epidermis.

B are proposed sites of its action[12-14]. A third humoral agent has been proposed which is natriuretic in mammals[15]. Indeed, a dialysable factor has been found in volume expanded animals which inhibits Na^+ transport in toad bladder and thus would act at Site A and/or B[16]. Site C represents the passive transport of Cl^- which is here proposed as an additional site and mechanism of regulation of NaCl transport. 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[15].

This work was supported by research grant AM-13693 from the National Institutes of Health.

REFERENCES

1 H.H. Ussing and K. Zerahn, *Acta Physiol. Scand.*, 23 (1951) 110.
2 C.O. Watlington, *Am. J. Physiol.*, 214 (1968) 1001.
3 C.O. Watlington and W.R. Harlan, Jr., *Am. J. Physiol.*, 214 (1969) 1004.
4 R. Hornby and S. Thomas, *J. Physiol. London*, 200 (1969) 321.
5 J. Crabbé, *The Sodium-Retaining Action of Aldosterone*, Editions Arsica, Bruxelles, 1963, pp. 35–74.
6 J.A. Zadunaisky and F.W. DeFisch, *Am. J. Physiol.*, 207 (1964) 1010.
7 R. Nielsen, *Acta Physiol. Scand.*, 77 (1969) 85.
8 H.E. Larsen, *Acta Physiol. Scand.*, 81 (1971) 254.
9 G.A. Porter and I.S. Edelman, *J. Clin. Invest.*, 43 (1964) 611.
10 V. Koefoed-Johnson, H.H. Ussing and K. Zerahn, *Acta Physiol. Scand.*, 27 (1953) 38.
11 F.C. Herrera and P.F. Curran, *J. Gen. Physiol.*, 46 (1963) 999.
12 J. Crabbé, *Nature*, 200 (1963) 787.
13 G.W.G. Sharp and A. Leaf, *J. Clin. Invest.*, 42 (1963) 978.
14 D.D. Fanestil, G.A. Porter and I.S. Edelman, *Biochim. Biophys. Acta*, 135 (1967) 74.
15 N.S. Bricker, *Am. J. Med.*, 43 (1967) 313.
16 V.M. Buckalew, Jr., J. Martinez and W.E. Green, *J. Clin. Invest.*, 49 (1970) 926.
17 C.O. Watlington, P.K. Burke, A.D. Campbell and E.G. Huf, *J. Cell. Comp. Physiol.*, 64 (1964) 389.