See related article, pages 1030–1038

Local Sodium, Global Reach

Filling the Gap Between Salt and Hypertension

Mordecai P. Blaustein, W. Gil Wier

The plasma membrane (PM) Na^+/Ca^{2+} exchanger (NCX) in vascular smooth muscle is an unique link between the trans-PM Na^+ electrochemical gradient and intracellular Ca^{2+} and, therefore, between Na^+ ions and Ca^{2+} signaling, vascular tone and blood pressure.[1] The mechanisms by which Na^+ normally enters the myocytes and influences the Na^+ gradient and NCX activity are, however, incompletely understood. Our view of how Na^+ ions help regulate sarco-/endoplasmic reticulum (S/ER) Ca^{2+} stores and contractility in arteries has now been signally enhanced by Poburko and colleagues.[2] Using CoroNa green, a Na^+-sensitive fluorochrome, they observed local Na^+ concentration transient increases ("LNats") in cultured arterial myocytes. The LNats were generated by Na^+ entry through cation-selective TRPC6 channels, a member of the TRP (transient receptor potential) channel family.[2] This is direct, dynamic evidence for a predicted sub-PM compartment with greatly restricted Na^+ diffusion[3,4] in which the local rise in Na^+ concentration should drive Ca^{2+} into the myocytes via NCX.

The present study has broad implications for Ca^{2+} homeostasis and signaling. Earlier vascular smooth muscle studies indicated that other members of the TRP channel family might also admit Na^+ to sub-PM domains.[3,5] Indirect evidence,[6] as well as an electron microprobe study, indicate that cardiomyocytes, too, can exhibit elevated local sub-PM Na^+ concentrations ($[Na^+]_{SPM}$).[7] Moreover, comparable diffusion-restricted, sub-PM cytosolic compartments may also be present in other types of cells (e.g., astrocytes[8]).

To explain how S/ER Ca^{2+} stores in smooth muscles could refill from the extracellular fluid without inducing contractions,[9,10] van Breeman and colleagues postulated a "privileged pathway" (the Ca^{2+} "buffer barrier"), through which Ca^{2+} could move directly between the extracellular fluid and the sub-PM ("junctional") S/ER, jS/ER.[9] One mechanism purportedly involved in this Ca^{2+} transfer was the NCX.[9]

This model was supported by the discovery that NCX in smooth muscles (and neurons and astrocytes) is confined to PM microdomains that overlie closely-apposed jS/ER,[11,12] as are Na^+ pumps with an $\alpha 2$ or $\alpha 3$ catalytic subunit.[13–15] In contrast, coexpressed Na^+ pumps with an $\alpha 1$ subunit, the predominant "housekeepers" that maintain the low bulk cytosolic Na^+ concentration ($[Na^+]_{CYT}$), are excluded from these microdomains.[13,15] Cation-selective TRPC-containing store- or receptor-operated channels,[3,5] which also are located in these PM microdomains,[15–17] are, therefore, key Na^+ entry pathways. The jS/ER, the PM microdomains, and the tiny volume of cytosol between them (perhaps 10^{-19} to 10^{-18} l), form a structural and functional unit, the "PLasmERosome" (Figure).[3]

LNats,[2] which presumably arise in PLasmERosomes, are surprisingly long-lasting, on the order of 1 minute. Thus, Na^+ diffusion between the PLasmERosomes and bulk cytosol must be markedly restricted. The nature of the diffusion barrier is unknown, but intracellular Na^+ gradients[2] could not be sustained even for 1 second if Na^+ diffusivity was comparable to that measured in muscle cytoplasm.[18] This helps explain how Na^+ pumps with an $\alpha 2$ or $\alpha 3$ subunit can function in cells that also express 4 times as many pumps with an $\alpha 1$ subunit,[19,20] which have a much higher affinity for intracellular Na^+.[21] The implication is that the membrane potential and the balance between Na^+ entry through receptor- and store-operated channels, and Na^+ extrusion via the $\alpha 2/\alpha 3$ Na^+ pumps, control $[Na^+]_{SPM}$ and the local Na^+ electrochemical gradient. This gradient drives Ca^{2+} either into or out of the myocytes via NCX, and thereby controls the local sub-PM Ca^{2+} concentration, $[Ca^{2+}]_{SPM}$. Indeed, $[Ca^{2+}]_{SPM}$ transients have been observed in arterial smooth muscle.[15,22,23] The $[Ca^{2+}]_{SPM}$, in turn, influences the transport of Ca^{2+} into the jS/ER (mediated by SERCA pumps), and thereby helps regulate Ca^+ signaling,[5,8,17,24] vascular tone and blood pressure.[20,24]

Mitochondria accumulate Ca^{2+} when global $[Ca^{2+}]_{CYT}$ rises, and mitochondrial NCX may then help the mitochondria extrude Ca^{2+}. When mitochondrial NCX was inhibited by CGP37157,[25] ATP-stimulated global $[Na^+]_{CYT}$ rose, as did the frequency of LNats.[2] The structural and functional details of the PLasmERosome/SR/mitochondria and bulk cytosol interrelationships are yet to be fully elucidated.

The present work advances the concept that local $[Na^+]_{SPM}$ controls vascular tone by directly demonstrating local $[Na^+]_{SPM}$, and by identifying a key cation channel that may be involved, TRPC6. Nevertheless, the mechanisms of activation of LNats in arteries may differ from those in cultured cells; different GPCRs (G protein–coupled receptors) and different receptor-operated channels/TRPCs may be involved. It seems unlikely that LNats will be activated by ATP in intact arteries. In the cultured smooth muscle cells used by Poburko,[2] ATP (1 mmol/L) activated metabotropic purinergic receptors. But in isolated mouse mesenteric arteries, the effects of bath-

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

From the Departments of Physiology (M.P.B., W.G.W.) and Medicine (M.P.B.) and the Center for Heart, Hypertension, and Kidney Disease (M.P.B., W.G.W.), University of Maryland School of Medicine, Baltimore, Md.

Correspondence to Mordecai P. Blaustein, MD, Department of Physiology, University of Maryland School of Medicine, 655 W. Baltimore Street, Baltimore, MD 21201. E-mail mblaustein@som.umaryland.edu

(*Circ Res*. 2007;101:959-961.)

***Circulation Research* is available at http://circres.ahajournals.org**
DOI: 10.1161/CIRCRESAHA.107.164459

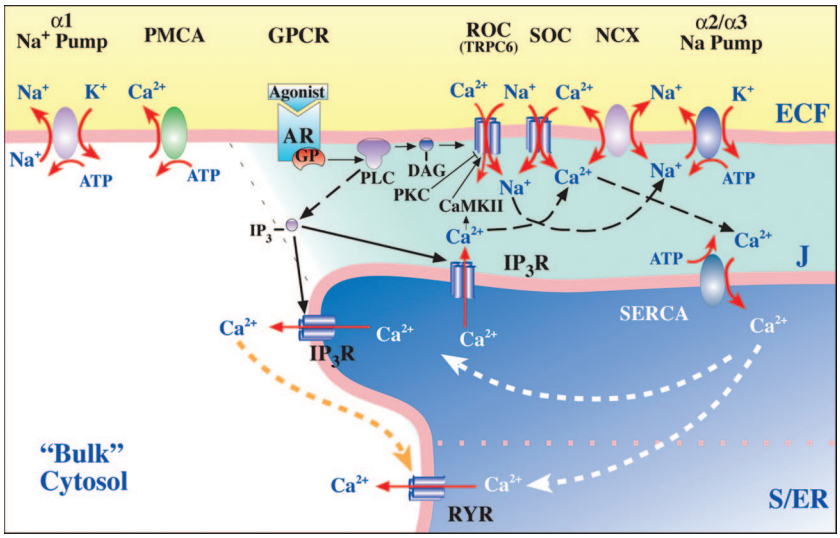

Figure. Model of PM-jS/ER region (PLasmERosome) showing location of key transport proteins involved in local control of jS/ER Ca^{2+} stores and Ca^{2+} signaling. The PLasmERosome consists of a PM microdomain, the adjacent jS/ER (with SERCA, IP_3R and RYR), and intervening "diffusion-restricted" junctional space ("J"). The PM microdomain contains agonist receptors, ARs (GPCRs), ROCs and SOCs (receptor- and store-operated channels; composed of various TRP channels), $\alpha2/\alpha3$ Na^+ pumps, and NCX. Activation of GPCRs and release of G proteins (GPs) stimulates phospholipase C (PLC) to produce diacylglycerol (DAG) and inositol trisphosphate (IP_3). DAG may activate ROCs (TRPC6) directly, to generate LNats, which then promote Ca^{2+} entry via NCX. Shading indicates relative Na^+ and/or Ca^{2+} concentrations. ECF indicates extracellular fluid; PKC, protein kinase C; CaMK II, Ca^{2+}-calmodulin dependent kinase II. Other regions of the PM contain $\alpha1$ Na^+ pumps and PM Ca^{2+} pumps (PMCA). Other abbreviations defined in text.

applied ATP (0.1 mmol/L) are entirely dependent on a different (ionotropic) purinergic receptor, P2X1. Both the vasoconstrictor effect and an endothelium dependent vasodilator effect of ATP are completely absent in mesenteric arteries of P2X1 receptor–deficient mice.[26] It seems much more likely that TRPC6-dependent LNats would be activated physiologically in arteries after norepinephrine binding to well-known GPCRs (*viz.* α_1-adrenoceptors, or α_1-ARs). In freshly dispersed rabbit mesenteric artery myocytes, the vasoconstrictor, angiotensin II, acting on AT1 GPCRs, triggers a cation conductance that likely is mediated by TRPC6.[27] In intact arteries, however, the role of Na^+ or Ca^{2+} entry through TRPC6 has proven difficult to evaluate; aortas of mice deficient in TRPC6 display enhanced, not reduced, contractile responses to α_1-AR activation.[28] In the myocytes from these $TRPC6^{-/-}$ animals, the enhanced cation influx associated with the potentiated contraction seems to be attributable to enhanced constitutive activity of a closely related channel, TRPC3. Expression of TRPC6 and GPCR-stimulated currents are clearly enhanced in the mesenteric arteries of DOCA-salt hypertensive rats, however,[29] implicating TRPC6 in the altered agonist responsiveness of these arteries. TRPC6 is also implicated in the production of myogenic tone.[30] Nevertheless, caution should be used in extrapolating results from cultured myocytes[2] to intact arterial smooth muscle. In cultured cells, TRPC6 and NCX appear to have dominant roles in controlling intracellular Na^+ and Ca^{2+}. In many arteries however, voltage-gated Ca^{2+} channels play major roles in myogenic tone and agonist-induced Ca^{2+} entry.

Now that LNats can be observed experimentally, with a molecular identity reasonably well established, we should be able to obtain more mechanistic information. The details of activation are still uncertain, although Ca^{2+} and calmodulin are likely involved, and either Ca^{2+}-calmodulin dependent kinase II or myosin light chain kinase.[31] TRPC6 channels heterologously expressed in HEK293 cells are activated by diacylglycerol and Ca^{2+}-calmodulin dependent kinase II, but are subsequently inactivated by protein kinase C (Figure).[32] Interestingly, most LNats occur early during the response to ATP, at a time when release of S/ER Ca^{2+} causes a large increase in cytosolic $[Ca^{2+}]$. Perhaps this Ca^{2+} activates the TRPC6 through Ca^{2+}-calmodulin dependent kinase II (Figure). These unresolved details notwithstanding, the LNats[2] shed new light on the key roles of TRP channels and NCX in regulating $[Na^+]_{SPM}$ and global Ca^{2+} signals in vascular smooth muscle. This opens significant opportunity for investigating the links between salt and vascular contractility and hypertension.

Sources of Funding

The authors were supported by research grants from the National Heart Lung and Blood Institute and the National Institute of Neurological Diseases and Stroke.

Disclosures

None.

References

1. Blaustein MP, Zhang J, Chen L, Hamilton BP. How does salt retention raise blood pressure? *Am J Physiol Regul Integr Comp Physiol*. 2006; 290:R514–R523.
2. Poburko D, Liao CH, Lemos VS, Lin E, Maruyama Y, Cole WC, van Breemen C. Transient receptor potential channel 6 mediated, localized cytosolic $[Na^{+}]$ transients drive Na^+/Ca^{2+} exchanger mediated Ca^{2+} entry in purinergically stimulated aorta smooth muscle cells. *Circ Res*. 2007; 101:1030–1038.
3. Arnon A, Hamlyn JM, Blaustein MP. Na^+ entry via store-operated channels modulates Ca^{2+} signaling in arterial myocytes. *Am J Physiol Cell Physiol*. 2000;278:C163–C173.
4. Arnon A, Hamlyn JM, Blaustein MP. Ouabain augments Ca^{2+} transients in arterial smooth muscle without raising cytosolic Na^+. *Am J Physiol Heart Circ Physiol*. 2000;279:H679–H691.
5. Lee CH, Rahimian R, Szado T, Sandhu J, Poburko D, Behra T, Chan L, van Breemen C. Sequential opening of IP(3)-sensitive Ca^{2+} channels and SOC during alpha-adrenergic activation of rabbit vena cava. *Am J Physiol Heart Circ Physiol*. 2002;282:H1768–H1777.
6. Leblanc N, Hume JR. Sodium current-induced release of calcium from cardiac sarcoplasmic reticulum. *Science*. 1990;248:372–376.
7. Wendt-Gallitelli MF, Voigt T, Isenberg G. Microheterogeneity of subsarcolemmal sodium gradients. Electron probe microanalysis in guinea-pig ventricular myocytes. *J Physiol*. 1993;472:33–44.

8. Golovina VA, Song H, James PF, Lingrel JB, Blaustein MP. Na^+ pump alpha 2-subunit expression modulates Ca^{2+} signaling. *Am J Physiol Cell Physiol*. 2003;284:C475–C486.
9. Van Breemen C, Aaronson P, Loutzenhiser R. Sodium-calcium interactions in mammalian smooth muscle. *Pharmacol Rev*. 1978;30:167–208.
10. Casteels R, Droogmans G. Exchange characteristics of the noradrenaline-sensitive calcium store in vascular smooth muscle cells or rabbit ear artery. *J Physiol*. 1981;317:263–279.
11. Juhaszova M, Ambesi A, Lindenmayer GE, Bloch RJ, Blaustein MP. Na^+-Ca^{2+} exchanger in arteries: identification by immunoblotting and immunofluorescence microscopy. *Am J Physiol*. 1994;266:C234–C242.
12. Moore ED, Etter EF, Philipson KD, Carrington WA, Fogarty KE, Lifshitz LM, Fay FS. Coupling of the Na^+/Ca^{2+} exchanger, Na^+/K^+ pump and sarcoplasmic reticulum in smooth muscle. *Nature*. 1993;365:657–660.
13. Juhaszova M, Blaustein MP. Distinct distribution of different Na^+ pump alpha subunit isoforms in plasmalemma. Physiological implications. *Ann N Y Acad Sci*. 1997;834:524–536.
14. Shelly DA, He S, Moseley A, Weber C, Stegemeyer M, Lynch RM, Lingrel J, Paul RJ. Na^+ pump alpha 2-isoform specifically couples to contractility in vascular smooth muscle: evidence from gene-targeted neonatal mice. *Am J Physiol Cell Physiol*. 2004;286:C813–C820.
15. Lee MY, Song H, Nakai J, Ohkura M, Kotlikoff MI, Kinsey SP, Golovina VA, Blaustein MP. Local subplasma membrane Ca^{2+} signals detected by a tethered Ca^{2+} sensor. *Proc Natl Acad Sci U S A*. 2006;103:13232–13237.
16. Golovina VA. Visualization of localized store-operated calcium entry in mouse astrocytes. Close proximity to the endoplasmic reticulum. *J Physiol*. 2005;564:737–749.
17. Eder P, Poteser M, Romanin C, Groschner K. Na(+) entry and modulation of Na^+/Ca^{2+} exchange as a key mechanism of TRPC signaling. *Pflugers Arch*. 2005;451:99–104.
18. Kushmerick MJ, Podolsky RJ. Ionic mobility in muscle cells. *Science*. 1969;166:1297–1298.
19. James PF, Grupp IL, Grupp G, Woo AL, Askew GR, Croyle ML, Walsh RA, Lingrel JB. Identification of a specific role for the Na,K-ATPase alpha 2 isoform as a regulator of calcium in the heart. *Mol Cell*. 1999;3:555–563.
20. Zhang J, Lee MY, Cavalli M, Chen L, Berra-Romani R, Balke CW, Bianchi G, Ferrari P, Hamlyn JM, Iwamoto T, Lingrel JB, Matteson DR, Wier WG, Blaustein MP. Sodium pump alpha2 subunits control myogenic tone and blood pressure in mice. *J Physiol*. 2005;569:243–256.
21. Zahler R, Zhang ZT, Manor M, Boron WF. Sodium kinetics of Na,K-ATPase alpha isoforms in intact transfected HeLa cells. *J Gen Physiol*. 1997;110:201–213.
22. Marsault R, Murgia M, Pozzan T, Rizzuto R Domains of high Ca^{2+} beneath the plasma membrane of living A7r5 cells. *EMBO J*. 1997;16:1575–1581.
23. Matchkov VV, Gustafsson H, Rahman A, Briggs Boedtkjer DM, Gorintin S, Hansen AK, Bouzinova EV, Praetorius HA, Aalkjaer C, Nilsson H. Interaction between Na^+/K^+-pump and Na^+/Ca^{2+}-exchanger modulates intercellular communication. *Circ Res*. 2007;100:1026–1035.
24. Iwamoto T, Kita S, Zhang J, Blaustein MP, Arai Y, Yoshida S, Wakimoto K, Komuro I, Katsuragi T. Salt-sensitive hypertension is triggered by Ca^{2+} entry via Na^+/Ca^{2+} exchanger type-1 in vascular smooth muscle. *Nature Med*. 2004;10:1193–1199.
25. Cox DA, Conforti L, Sperelakis N, Matlib MA. Selectivity of inhibition of Na^+-Ca^{2+} exchange of heart mitochondria by benzothiazepine CGP-37157. *J Cardiovasc Pharmacol*. 1993;21:595–599.
26. Harrington LS, Evans RJ, Wray JA, Norling LV, Swales KE, Vial C, Ali F, Carrier MJ, Mitchell JA P2X1 receptors mediate endothelial dependent vasodilatation to ATP. *Mol Pharmacol.* In press.
27. Saleh SN, Albert AP, Peppiatt CM, Large WA. Angiotensin II activates two cation conductances with distinct TRPC1 and TRPC6 channel properties in rabbit mesenteric artery myocytes. *J Physiol*. 2006;577:479–495.
28. Dietrich A, Mederos YSM, Gollasch M, Gross V, Storch U, Dubrovska G, Obst M, Yildirim E, Salanova B, Kalwa H, Essin K, Pinkenburg O, Luft FC, Gudermann T, Birnbaumer L. Increased vascular smooth muscle contractility in $TRPC6^{-/-}$ mice. *Mol Cell Biol*. 2005;25:6980–6989.
29. Bae YM, Kim A, Lee YJ, Lim W, Noh YH, Kim EJ, Kim J, Kim TK, Park SW, Kim B, Cho SI, Kim DK, Ho WK. Enhancement of receptor-operated cation current and TRPC6 expression in arterial smooth muscle cells of deoxycorticosterone acetate-salt hypertensive rats. *J Hypertens*. 2007;25:809–817.
30. Welsh DG, Morielli AD, Nelson MT, Brayden JE. Transient receptor potential channels regulate myogenic tone of resistance arteries. *Circ Res*. 2002;90:248–250.
31. Albert AP. Activation of TRPC6 channel proteins: evidence for an essential role of phosphorylation. *J Physiol*. 2004;561:354.
32. Shi J, Mori E, Mori Y, Mori M, Li J, Ito Y, Inoue R. Multiple regulation by calcium of murine homologues of transient receptor potential proteins TRPC6 and TRPC7 expressed in HEK293 cells. *J Physiol*. 2004;561:415–432.

KEY WORDS: sodium ■ subplasma membrane microdomains ■ TRPC6 ■ receptor-operated channels ■ Na^+/Ca^{2+} exchanger

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

Local Sodium, Global Reach: Filling the Gap Between Salt and Hypertension

Mordecai P. Blaustein and W. Gil Wier

Circ Res. 2007;101:959-961
doi: 10.1161/CIRCRESAHA.107.164459
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571