

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

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The plasma membrane (PM) $\text{Na}^+/\text{Ca}^{2+}$ 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 ($[\text{Na}^+]_{\text{SPM}}$).⁷ Moreover, comparable diffusion-restricted, sub-PM cytosolic compartments may also be present in other types of cells (e.g., astrocytes⁸).

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 ($[\text{Na}^+]_{\text{CYT}}$), 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^+ gradients² 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 $[\text{Na}^+]_{\text{SPM}}$ 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, $[\text{Ca}^{2+}]_{\text{SPM}}$. Indeed, $[\text{Ca}^{2+}]_{\text{SPM}}$ transients have been observed in arterial smooth muscle.^{15,22,23} The $[\text{Ca}^{2+}]_{\text{SPM}}$, in turn, influences the transport of Ca^{2+} into the jS/ER (mediated by SERCA pumps), and thereby helps regulate Ca^{2+} signaling,^{5,8,17,24} vascular tone and blood pressure.^{20,24}

Mitochondria accumulate Ca^{2+} when global $[\text{Ca}^{2+}]_{\text{CYT}}$ rises, and mitochondrial NCX may then help the mitochondria extrude Ca^{2+} . When mitochondrial NCX was inhibited by CGP37157,²⁵ ATP-stimulated global $[\text{Na}^+]_{\text{CYT}}$ 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 $[\text{Na}^+]_{\text{SPM}}$ controls vascular tone by directly demonstrating local $[\text{Na}^+]_{\text{SPM}}$, 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-

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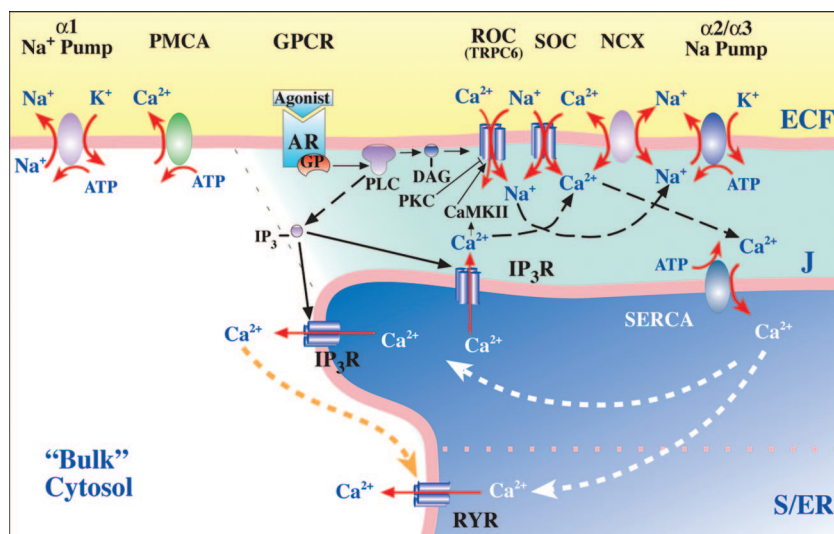


Figure. Model of PM-jS/ER region (PLasmERosome) showing location of key transport proteins involved in local control of jS/ER Ca^{2+} stores and Ca^{2+} signaling. The PLasmERosome consists of a PM microdomain, the adjacent jS/ER (with SERCA, IP_3R and RYR), and intervening “diffusion-restricted” junctional space (“J”). The PM microdomain contains agonist receptors, ARs (GPCRs), ROCs and SOCs (receptor- and store-operated channels); composed of various TRP channels), $\alpha 2/\alpha 3$ 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 $\alpha 1$ 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 myocytes² 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 $[\text{Ca}^{2+}]$. Perhaps this Ca^{2+} activates the TRPC6 through Ca^{2+} -calmodulin dependent kinase II (Figure). These unresolved details notwithstanding, the LNats² shed new light on the key roles of TRP channels and NCX in regulating $[\text{Na}^+]_{\text{SPM}}$ and global Ca^{2+} signals in vascular smooth muscle. This opens significant opportunity for investigating the links between salt and vascular contractility and hypertension.

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Disclosures

None.

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