
**Question:** How does blocking tetrodotoxin-resistant (TTX-R) Na⁺ channels with multivalent cations alter channel gating and permeation?

**Background:** Na⁺ channels are classified as being either TTX-sensitive (TTX-S) or TTX-R. TTX-R channels are more susceptible as being either TTX-sensitive (TTX-S) or TTX-R channels. The amino acid at position 374 in the outer pore region of Na⁺ channels is critically important for both TTX and Cd²⁺ sensitivity. In the pore region of Na⁺ channel there is a ring of highly conserved aspartate, glutamate, lysine, and alanine residues (the “DEKA” ring), which are implicated in the formation of the channel selectivity filter, slightly deeper into the pore. The amino acid at position 374 is in close proximity to the aspartate residue in the DEKA ring, and the TTX- and Cd²⁺-blocking sites are hypothesized to be near, but external to, the selectivity filter. To probe whether the extracellular pore entrance could be involved in channel gating, Kuo and colleagues explored whether transitional metal ions that block ion permeation also alter channel gating.

**Observations:** Several multivalent transitional metal ions blocked TTX-R channels in a current-dependent manner, and this block correlated with channel fast inactivation gating. Thus the current-dependent blocking effects of the ions were associated with a conformational change and altered inactivation kinetics. The multivalent ions induced blockade more markedly when there was an inward Na⁺ current, implying a nonselective cation-binding site at the external mouth of the channel, close to the single-file selectivity filter. Ion binding at this site altered channel inactivation, which suggests a conformational coupling between the extra- and intracellular pore entrances.

**Significance:** These results provide further evidence that the DEKA selectivity filter is near the sites of TTX- and transitional metal ion-induced pore blockade. These data support the notion that the processes of permeation and gating of ion channels are interrelated, that the channel structure is flexible, and that current- and voltage-dependent channel inactivation is a more universal process than previously appreciated.


**Question:** Do protons play a role in the chemotactic signaling cascades of sperm from two species of sea urchins?

**Background:** Eggs release peptides that form concentration gradients to attract and accumulate spermatozoa, a process known as chemotaxis. In sea urchin, the peptide-induced stimulation of sperm results in a variety of intracellular events, including an increase in intracellular pH (pHi) and intracellular Ca²⁺ concentration. It has been hypothesized that the increase in pHi evokes and is necessary for the influx of Ca²⁺, which somehow controls the swimming behavior of sperm. A recent report cast doubts on the validity of the standard model, although the possibility remained that different species use distinct signaling pathways. As such, it became necessary to reexamine the role of protons in sperm chemotaxis.

**Observations:** Solzin et al., using kinetic measurements, demonstrated that the peptide-induced increase of intracellular Ca²⁺ precedes the rise in pHi and that preventing this rise in pHi did not affect the opening of Ca²⁺-permeable channels in sperm of either of two species. The rise in pHi was not a prerequisite for chemotactic behavior. Importantly, the peptide concentrations selected are physiologically relevant; higher concentrations used in other studies, and confirmed here, result in an initial increase in pHi, followed by the increase in intracellular Ca²⁺.

**Significance:** Solzin et al. demonstrated that not only are the signal transduction pathways more similar than distinct between species, but that a change in pHi was not required to open Ca²⁺-permeable channels. These results demand rethinking about the signaling pathways involved in sperm chemotaxis and highlight the importance of using physiologically relevant concentrations. This represents a significant advancement in our understanding of the molecular mechanism of sperm chemotaxis in sea urchin; this advance may also provide insight into sperm chemotaxis in mammals, which is not well understood.


**Question:** Does the protein spinophilin affect arrestin-dependent regulation of G protein-coupled receptor-mediated (GPCR) signaling and trafficking?

**Background:** The ubiquitous GPCRs mediate signaling in myriad cells, where they regulate various physiological functions and are therefore targets of many therapeutic compounds. GPCR activation and trafficking are determined by the initiation of signaling cascades and by regulatory mechanisms, such as arrestins, that control signal extent and duration. Arrestin binds to agonist-occupied GPCRs that have been phosphorylated by GPCR kinases (GRKs) to mediate desensitization. Arrestin interacts with GPCRs at the same regions as other GPCR-protein interactions, such as spinophilin, but the functional significance of this overlapping site of action is unknown.

**Observations:** Wang et al. demonstrated that spinophilin antagonizes GRK2-GPCR interactions by binding to the Gγ
complex of the receptor in vitro. Moreover, they established a reciprocal interaction between arrestin and spinophilin in regulating the phosphorylation of agonist-occupied GPCRs, which also suggests that arrestin activity is antagonized by spinophilin. In addition, downstream effects of the arrestin-GPCR interaction were affected by spinophilin because it attenuated arrestin-dependent receptor endocytosis and mitogen-activated protein kinase (MAPK) activity. Finally, the functional relevance of this reciprocal relationship between arrestin and spinophilin in regulating GPCRs was demonstrated in vivo.

Significance: The balance of activation and desensitization associated with arrestin-spinophilin interactions represents a previously unknown mechanism in the regulation of a cell’s ultimate physiological response to both endogenous and exogenous receptor stimuli. Unexpectedly, arrestins were also determined to have a more significant role in promoting signal activation than in terminating signal transduction in GPCR-induced sedation, indicating that the signal-promoting role of arrestin is more important for certain pathways. Therapeutic benefits may be realized from this because modulators of arrestin activity may have beneficial effects in the treatment of cardiac diseases. Therefore, spinophilin may represent a future target to mitigate cardiovascular dysfunction.

**HIGHLIGHTS FROM THE LITERATURE**

Nkx2-5 gene, which has a role in cardiac morphogenesis, cause myriad complex congenital heart malformations that do not respond to surgical interventions. Trabeculation, the process of cell differentiation that forms a meshwork in the ventricles, is associated with the Nkx2-5-BMP-10 axis; therefore, a dysfunction of this growth process may be involved in congenital heart disease phenotypes. Unraveling the contribution of the Nkx2-5 gene in congenital heart defects has proven challenging because Nkx2-5 knockout mice do not survive past the embryonic stages. The goal then is to delineate the molecular mechanisms associated with Nkx2-5 in dysfunctional cardiac phenotypes. Observations: Mice with a ventricular-restricted knockout of Nkx2-5 were generated to illuminate associated mechanisms that may lead to myocardial disease. Nkx2-5 mutants were determined to have defects in the formation of the AV nodal and ventricular muscle cell lineages and aberrant expression of downstream target genes. In addition, the overexpression of the growth factor BMP-10 was associated with hypertrabeculation. Significance: This represents a new molecular paradigm for understanding congenital heart defects, because the Nkx2-5 mutant mice displayed many of the ventricular muscle cell phenotypes seen in patients with a similar dysfunctional Nkx2-5 pathway. These data support the contention that attempts to surgically correct various structural heart abnormalities will be futile because the underlying mechanisms of these deficits are molecular. Understanding the molecular pathways of the defects in ventricular maturation and in the differentiation of muscle cells will aid in the development of novel therapeutic approaches for congenital heart disease.

**Question:** What role does the Nkx2-5 pathway have in congenital heart disease? **Background:** Human mutations of the Nkx2-5 gene, which has a role in cardiac morphogenesis, cause myriad complex congenital heart malformations that do not respond to surgical interventions. Trabeculation, the process of cell differentiation that forms a meshwork in the ventricles, is associated with the Nkx2-5-BMP-10 axis; therefore, a dysfunction of this growth process may be involved in congenital heart disease phenotypes. Unraveling the contribution of the Nkx2-5 gene in congenital heart defects has proven challenging because Nkx2-5 knockout mice do not survive past the embryonic stages. The goal then is to delineate the molecular mechanisms associated with Nkx2-5 in dysfunctional cardiac phenotypes. Observations: Mice with a ventricular-restricted knockout of Nkx2-5 were generated to illuminate associated mechanisms that may lead to myocardial disease. Nkx2-5 mutants were determined to have defects in the formation of the AV nodal and ventricular muscle cell lineages and aberrant expression of downstream target genes. In addition, the overexpression of the growth factor BMP-10 was associated with hypertrabeculation. Significance: This represents a new molecular paradigm for understanding congenital heart defects, because the Nkx2-5 mutant mice displayed many of the ventricular muscle cell phenotypes seen in patients with a similar dysfunctional Nkx2-5 pathway. These data support the contention that attempts to surgically correct various structural heart abnormalities will be futile because the underlying mechanisms of these deficits are molecular. Understanding the molecular pathways of the defects in ventricular maturation and in the differentiation of muscle cells will aid in the development of novel therapeutic approaches for congenital heart disease.

**Question:** Does altering the activity of embryonic neurons in vivo change neuronal phenotype? **Background:** The specification of neurotransmitters is achieved during embryonic stages of development. Traditionally, the neurotransmitter expressed by a neuron has been thought to be dictated by the neuron’s genetic profile. However, intrinsic transcription factors, cytokines, and neurotrophic factors and alterations in Ca²⁺ influx have all been shown to affect, differentially, the transmitter phenotype of neurons. The spinal cord of the *Xenopus laevis* embryo contains only eight classes of neurons, four of which have distinct Ca²⁺ spike patterns and express different transmitter phenotypes, and it is thus an attractive model for studying activity-induced changes in transmitter phenotype. Observations: Borodinsky et al. found that when they modulated the Ca²⁺ spike activity of *Xenopus* embryos by over-expressing potassium or sodium channels in vivo, there was a phenotypic change in the transmitters without a change in neuronal identity. Suppressing spike frequency increased the incidence of expression of the excitatory transmitters acetylcholine and glutamate and concomitantly decreased expression of the inhibitory transmitters GABA and glycine. Conversely, when spike activity was enhanced, the numbers of neurons expressing excitatory transmitters decreased while the numbers of neurons expressing inhibitory transmitters increased. The modified expression patterns of transmitters were also seen in isolated neurons in vitro, which suggests that these effects may be due to an intracellular feedback loop as opposed to extrinsic factors. **Significance:** These results indicate that Ca²⁺ spike activity patterns regulate the expression of a neurotransmitter for...
particular neurons; modifying these patterns alters the neurotransmitter phenotype. Thus not only can activity change the level of neurotransmitter expression, but it can also alter the specific neurotransmitter expressed without changing a neuron's identity. This finding emphasizes the need to explore patterned activity in the development of neuronal phenotypes and suggests that transcriptional codes reveal only part of the mechanism by which neuronal differentiation unfolds.


Question: Is the gene for the Na+/K+-ATPase α3 subunit (ATP1A3) a cause of rapid-onset dystonia-parkinsonism (RDP)?

Background: RDP is an autosomal dominant disorder characterized by the acute onset of some Parkinson-like symptoms, including bradykinesia and postural instability. However, unlike Parkinson's disease, the pathogenesis of RDP is rapid, nonprogressive, characterized by dystonia, and not hallmarked by dopaminergic neurodegeneration. The P-type Na+/K+ ATPases catalyze the active transport of cations to maintain ionic gradients across cell membranes. There are three isoforms of the P-type ATPase catalytic α-subunits (α1, 2, and 3) expressed in brain, and although their physiological significance is unknown, mutations of the α2 isoform are associated with hemiplegic migraine in humans and akinesia in knockout mice. Moreover, mutations in P-type sodium pumps are known to cause neuronal dysfunctions and neurodegeneration. Thus an association between the α3-subunit and RDP was explored.

Observations: Six missense mutations located in highly conserved regions of the ATP1A3 gene are reported in RDP patients from seven families. A structural model of the α3-subunit was generated based on its homology with other P-type ATPases, which predicted the localization of the mutants (5 of the 6 mutations occurred in the transmembrane domain) and the resultant loss of activity and/or stability. The loss of function predicted from the modeling was confirmed in vitro by expressing each mutant human α3 in human embryonic kidney 293T cells. All presented significantly lower survival than wild-type α3, indicating that these mutations impaired the function of the α3-ATPase.

Significance: This report provides evidence that mutations of the ATP1A3 gene are involved in the pathogenesis of RDP. Moreover, these data highlight the unequivocal role of a relatively low-abundance pump isoform in normal brain activity and prove (medically) that the isoform is physiologically significant. Future investigations are sure to elucidate whether ATP1A3 gene mutations are associated with the genesis of other forms of dystonia and/or other disease states.


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Question: What are the conformational changes associated with the sarcoplasmic reticulum Ca2+-ATPase (SERCA) binding ATP?

Background: P-type ATPases are ion pumps that establish concentration gradients by pumping cations across a lipid bilayer. The SERCA is a P-type ATPase consisting of an actuator domain (A), a nucleotide-binding domain (N), a phosphorylation domain (P), and 10 transmembrane helixes that form two high-affinity Ca2+–binding sites. SERCA catalyzes the uptake of cytosolic Ca2+ into the sarcoplasmic reticulum via the formation of an intermediate state that occludes Ca2+ ions, making them inaccessible from the cytoplasmic side. This occlusion requires Mg2+ and phosphorylation of the ATPase. Crystal structures of the SERCA with bound Ca2+ (E1-2Ca2+) and unbound Ca2+ (E2(TG)) have been described, but the state with ATP, Mg2+, and two Ca2+ ions bound has not been observed.

Observations: The binding of a non-hydrolyzable ATP analog to SERCA (Ca2+-E1-adenosine (β,γ-methylene)-triphosphate (AMPPCP)) induced the reorganization of the A, N, and P domains by bridging the N and P domains. The modeling of this structure revealed that the P domain was altered by the binding of the ATP analog, which induced Mg2+ to bind and caused a reorientation of the A domain.

Significance: This study describes a mechanism that begins to account for the occlusion of bound Ca2+ ions before their release into the sarcoplasmatic reticulum. The AMPPCP complex is insufficient to occlude bound Ca2+, and the authors noted that the phosphorylated state is necessary. This phosphorylated conformational state is described by Sorensen et al. (Science 304: 1672–1675, 2004), which is also highlighted in this issue of Physiology. Understanding the fundamental processes associated with SERCA, which is present in skeletal muscle, may help to elucidate the underlying dysfunctions in the pathogenesis of movement disorders and allow the identification of therapeutic targets for the development of selective strategies to prevent or mitigate these disorders.


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Question: What is the mechanism that allows the SERCA calcium pump to couple phosphoryl transfer and calcium ion occlusion?

Background: P-type ATPases are cation pumps that use energy derived from ATP to transport ions across membranes against a concentration gradient. SERCA is a P-type ATPase that catalyzes the...
reuptake of cytosolic Ca\textsuperscript{2+} into the sarcoplasmic reticulum after a muscle contraction. This active transport cycle is dependent on the formation of a high-energy intermediate state formed as the result of a phosphoryl transfer from ATP. This conformational state is associated with the essential occlusion of intramembranously bound Ca\textsuperscript{2+}, which prevents its backflow into the cytosol. Previous crystal structures of SERCA have described conformational states associated with calcium binding of the SERCA functional cycle and established the presence of 10 transmembrane helices and 3 cytoplasmic domains, which include an anchor domain (A), a phosphorylation domain (P), and a nucleotide-binding domain (N). The structural basis of ATP binding and the phosphoryl transfer reaction coupled to Ca\textsuperscript{2+} occlusion remains unresolved.

**Observations:** Complexes of AMPPCP, as an ATP substitute, and Ca\textsubscript{2+}-E1-ADP:AlF\textsubscript{4}– to mimic the phosphorylated state, were crystallized, crystallographic data were collected, and an electron density map was produced that allowed complete tracing and model building. The structures of the AMPPCP and Ca\textsubscript{2+}-E1-ADP:AlF\textsubscript{4}– complexes were found to be almost identical, with the noted exception that the AMPPCP complex is not adequate to occlude the Ca\textsuperscript{2+} ions. The mechanism that underlies the phosphorylation of the SERCA was described, as were two distinct Mg\textsuperscript{2+} sites that stabilized the transition state as mimicked by Ca\textsubscript{2+}-E1-ADP:AlF\textsubscript{4}–. **Significance:** Sorensen et al. provide structural evidence to explain the previously perplexing coupling mechanism of SERCA. These results are in agreement with the study from Toyoshima and Mizutani (Nature 430: 529–535, 2004. First published June 30, 2004; 10.1038/nature02680) also highlighted in this month’s journal. Notably, the present study describes a second Mg\textsuperscript{2+} site in Ca\textsubscript{2+}-E1-ADP:AlF\textsubscript{4}– complex that is not evident from the AMPPCP complex but presumably is necessary for stabilization of the complex and Ca\textsuperscript{2+} occlusion.


**Question:** Do guanylate cyclases mediate oxygen sensation or associated feeding behaviors?

**Background:** Specialized cells in the nervous system have evolved to sense oxygen levels rapidly enough to induce a behavioral response. The behavioral avoidance of hypoxic conditions in *Drosophila* is mediated by a nitric oxide (NO)-sensitive cGMP-dependent pathway. NO is best known for activating cGMP production via a soluble guanylate cyclase (sGC). The sGC contains a heme cofactor that is activated by the gaseous ligand NO. There are seven sGC homologs (gcy-31 through gcy-37) predicted for the *C. elegans* genome, but their functional roles are unknown.

**Observations:** Oxygen sensing and related feeding behaviors were explored by using genetic mutants of *C. elegans*. All of the sGCs were expressed in sensory neurons. A sensory cGMP-gated channel (tax-2/tax-4) and the sGC homolog gcy-35 were determined to be prerequisite for hyperoxia avoidance. The heme domain of gcy-35 bound oxygen, and it, along with tax-4, mediated oxygen sensing in four sensory neurons that served as quantitative regulators of feeding behaviors.

**Significance:** Acute oxygen sensing is of major physiological relevance, but the underlying mechanisms are still only partially understood. Some elucidation at the molecular level is presented here. The polymorphic social feeding behaviors of *C. elegans* required cGMP-gated channel activation by cGMP generated from gcy-35, which formed a ferrous oxygen complex that acted as an oxygen sensor. This suggests that gcy-35 represents a new class of oxygen-sensitive sGCs that mediates a prophylactic response. This is the first sGC activated by a ligand other than NO. Future efforts should undoubtedly be aimed at determining if sGCs act as oxygen sensors in other animal species, particularly in mammals.
Paracrine effects are associated with similar expression of the von Willebrand factor, organized basement membrane, and of SECs to a vascular phenotype, resulting in enhanced circulation of plasma to endothelium and lack of an organized phenotype characterized by a fenestrated phenotype maintained by paracrine and autocrine regulation.

Significance: This study suggests that the responses of LMAN neurons to a previously acquired auditory/vocal memory are effectively replaced by a subsequently imitated TS. Interestingly, this result appears to be in contrast to other sensory systems that develop multiple distinct representations to conflicting inputs, all of which are maintained into adulthood. These findings provoke many intriguing questions about processing and encoding of early auditory and vocal motor experiences and whether their neurophysiological representations persist into adulthood.

**HIGHLIGHTS FROM THE LITERATURE**


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**Question:** How is the phenotype of sinusoidal endothelial cells (SECs) maintained?

**Background:** Liver SECs have a unique phenotype characterized by a fenestrated endothelium and lack of an organized basement membrane that allows an enhanced circulation of plasma to hepatocytes. Capillarization is the change of SECs to a vascular phenotype, resulting in a loss of fenestration, formation of an organized basement membrane, and expression of the von Willebrand factor. Paracrine effects are associated with similar phenotypic changes in other endothelial cells, although the roles of paracrine and autocrine effects in governing SEC phenotype are unknown. Additionally, there are conflicting reports concerning the expression of the adhesion molecule CD31 by normal SECs. Because CD31 is a defining feature of vascular endothelial cells, understanding its expression by and distribution within SECs is of importance.

**Observations:** CD31 expression and localization were explored by von Willebrand factor expression and confocal and electron microscopes. In normal SECs, CD31 expression was localized to the cytoplasm; conversely, CD31 was expressed on cell-cell borders of capillarized SECs. Vascular endothelial growth factor (VEGF) released by hepatocytes or stellate cells stimulated SECs to produce nitric oxide. Both the paracrine-released VEGF and the subsequent formation of nitric oxide by SECs were necessary to suppress surface development of CD31 expression and the capillarized phenotype. Dissimilar from macrovascular and central nervous system endothelial cells where heterotypic contact is the major determinant of EC phenotype, direct contact between SECs and either hepatocytes or stellate cells did not add to the paracrine effect of stellate cells or hepatocytes.

**Significance:** This study delineated mechanisms that control SEC phenotypic changes and clarified inconsistencies concerning CD31 expression. Interestingly, both paracrine and autocrine pathways regulated SEC differentiation as characterized by subcellular localization of CD31. Understanding the regulation of SEC phenotype has important implications for liver function, because capillarization inhibits accessibility of oxygen and solutes to the hepatocytes, resulting in impaired drug metabolism.


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**Question:** Does interleukin-6 (IL-6) or tumor necrosis factor-α (TNF-α) directly affect fatty acid (FA) metabolism of skeletal muscle?

**Background:** Abnormalities in muscle FA metabolism and the cytokines leptin, IL-6, and TNF-α are implicated in skeletal muscle insulin resistance. TNF-α stimulates diacylglycerol (DAG) production, which is also implicated in the pathogenesis of insulin resistance. IL-6 is secreted by adipocytes and released from contracting skeletal muscles. Although IL-6 and TNF-α are associated with insulin resistance, there is a paucity of direct evidence linking them to FA metabolism.

**Observations:** Bruce and Dyck examined the effects of leptin, IL-6, and TNF-α on endogenous and exogenous skeletal muscle FA metabolism. IL-6 stimulated exogenous and endogenous FA oxidation and attenuated insulin-induced lipogenesis.


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**Observations:** Bruce and Dyck examined the effects of leptin, IL-6, and TNF-α on endogenous and exogenous skeletal muscle FA metabolism. IL-6 stimulated exogenous and endogenous FA oxidation and attenuated insulin-induced lipogenesis.

important implications for understanding the etiology of insulin resistance and type 2 diabetes and could provide targets for alternative therapies.


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**Question:** Can a superior technique be developed to monitor Na+/H+ exchanger (NHE) activity?

**Background:** Mammalian NHEs catalyze the one-for-one exchange of intracellular H+ for extracellular Na+. Consequently, members of the mammalian NHE family participate in the regulation of pH, Na+ concentration, and fluid volume. To date, eight NHE (NHE1–NHE8) isoforms have been identified and cloned. NHE1 expression is localized to plasma membranes, where it regulates pH and cell volume, whereas the NHE3 isofrom is present on endosomal and plasma membranes of epithelial cells, where it regulates Na+ concentrations. Monitoring the activity of these NHEs by traditional methods is associated with several caveats, including low sensitivity, poor time resolution, an inability to monitor changes in ion concentrations, and limited control of the intracellular milieu.

**Observations:** Fuster et al. monitored the activity of NHE1 and NHE3 with a novel approach that used “self-referencing” pH microelectrodes during whole cell patch-clamp recording. This technique allowed manipulations of the cytoplasmic milieu, quantification of H+ vacillations induced by NHE1, and examination of low-activity NHE mutants and revealed previously unappreciated lipid- and mechanosensitive properties of NHE1 and NHE3.

**Significance:** Using an innovative method that permitted monitoring of NHE1 and NHE3 activities in several cell lines, Fuster et al. verified and extended earlier inferences. For example, NHE1 and NHE3 are both susceptible to phosphatidylinositol-induced effects and NHE1 is sensitive to modulations of its cell volume. This methodological approach will allow a more detailed characterization of wild-type and mutant NHE activity.

**Wamhoff BR, Bowles DK, McDonald OG, Sinha S, Somlyo AP, Somlyo AV, and Owens GK.** L-type voltage-gated Ca²⁺ channels modulate expression of smooth muscle differentiation marker genes via a Rho kinase/myocardin/SRF-dependent mechanism. *Circ Res.* First published July 15, 2004; 10.1161/01.RES. 0000138582.36921.9e.

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**Question:** Does activation of L-type voltage-gated Ca²⁺ channels (VGCCs) affect smooth muscle cell (SMC) differentiation marker gene expression, thereby modulating smooth muscle plasticity?

**Background:** Despite compelling evidence that the phenotypic modification of vascular SMCs plays a pivotal role during normal vascular development and in the development of vascular diseases such as atherosclerosis, little is known about how this process is regulated in vivo. L-type VGCCs activate RhoA (a Rho GTPase) and its downstream effector Rho kinase (ROK) to mediate, in part, vascular SMC contraction. In addition to mediating contraction, the RhoA/ROK pathway also regulates transcription of SMC differentiation marker genes. Evidence that L-type VGCCs can regulate SMC gene expression has been shown whereby activation of VGCCs increases the immediate early growth-response gene, c-fos, via phosphorylation of the cAMP-responsive element-binding protein (CREB). However, it is unknown whether there is a cooperative interaction of these pathways to regulate SMC differentiation marker gene expression, i.e., the subset of genes that differentiate a SMC from all other cell types.

**Observations:** Wamhoff et al. demonstrated that depolarization-induced activation of L-type VGCCs in SMCs induces an increase in the expression of SMC differentiation marker genes (SMGX) and c-fos. Unlike VGCC-induced c-fos expression, which is dependent on CREB, VGCC-induced activation of SMC differentiation marker genes was dependent on RhoA/ROK signaling, myocardin [a SMC-selective coactivator of serum response factor (SRF)], and increased binding of SRF to endogenous CaTG cis-regulatory elements of SMC differentiation marker gene promoters: VGCC → RhoA/ROK → myocardin/SRF → SMGX.

**Significance:** These results provide evidence for a novel mechanism whereby Ca²⁺ influx via VGCCs stimulates expression of SMC differentiation marker genes. Interestingly, VGCC-mediated activation is associated with two distinct gene subsets that have divergent consequences: activation of c-fos (SMC growth) and SMC differentiation marker gene expression (SMC differentiation). The novel mechanism described here could have a significant impact on our understanding of vascular remodeling associated with pathological processes such as hypertension, in which SMC hypertrophy and increased SMC differentiation marker gene expression occurs, and, conversely, atherosclerosis, in which cellular proliferation is required for SMC migration/proliferation in lesion development.


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**Question:** What role does calmodulin (CaM) play in the activation of a novel cGMP- and Ca²⁺-dependent Ca²⁺ channels of mesenteric arteries?

**Background:** In many smooth muscle preparations, agonist-induced depolarization and contraction (SMC) is mediated by Ca²⁺-dependent Ca²⁺ channels [CaT(Ca)] expressed in the plasma membrane.
However, a novel Ca\textsuperscript{2+}-dependent Cl\textsuperscript{–} conductance that requires cGMP and Ca\textsuperscript{2+} ions for Cl\textsuperscript{–} channel activation \( I_{\text{Cl(cGMP, Ca)}} \) has recently been described in smooth muscle cells of mesenteric arteries. Classic \( I_{\text{Cl}} \) is simply activated by a rise in intracellular Ca\textsuperscript{2+}, but cGMP is essential for activation of \( I_{\text{Cl(cGMP, Ca)}} \) and Ca\textsuperscript{2+} simply increases open channel probability. This study describes the role of CaM in mediating the effect of Ca\textsuperscript{2+} on \( I_{\text{Cl(cGMP, Ca)}} \).

**Observations:** Using inside-out patches from mesenteric artery smooth muscle cells, Piper and Large demonstrate a physiologically relevant cGMP- and Ca\textsuperscript{2+}-dependent CaM-induced potentiation of \( N_{\text{P}} \). They also report that intracellular cGMP was necessary to activate the Cl\textsuperscript{–} channel and could do so alone but that Ca\textsuperscript{2+} and CaM were needed for full channel activation. Interestingly, Ca\textsuperscript{2+}- and CaM-dependent protein kinase II did not mediate these effects.

**Significance:** This work further describes a novel Cl\textsuperscript{–} conductance in terms of the biophysical properties of the channel, its activation mechanisms (cGMP and Ca\textsuperscript{2+}), and its pharmacology. Perhaps one of the more intriguing aspects of this channel is that cGMP activation produces vasorelaxation; normally, cGMP activation is associated with vasodilatation. This paradox will undoubtedly receive attention in future experiments, as will the mediator of this Ca\textsuperscript{2+}/CaM-dependent modulation.


Nominated by Jeff M. Sands

**Question:** What role does the toxicity-responsive enhancer-binding protein (TonEBP) play in the development of urinary concentrating ability?

**Background:** Osmolarity in the mammalian kidney medulla is very high. In cultured cells, TonEBP is stimulated by hypertonicity (hyposmotic salt concentration) via several pathways, including nuclear translocation and induction (increased abundance of protein), which protects cells from hypertonicity-induced stress. TonEBP stimulates genes involved in the cellular accumulation of protective organic osmolytes, such as inositol, betaine, and sorbitol. Interestingly, TonEBP also stimulates the gene encoding the vasopressin-stimulated urea transporters (UT-A1 and -A3) of the inner medullary collecting duct and thereby contributes to the urea accumulation in the medullary interstitium. Thus TonEBP is an important regulator in the hypotonic renal medulla. However, neonatal animals cannot concentrate urine, and thus osmolality of the renal medulla is hypothesized to increase with the increased expression of TonEBP after birth.

**Observations:** The expression of TonEBP was monitored in the developing rat kidney using immunohistochemistry and in situ hybridization. TonEBP expression was detected as early as fetal day 16 in the cytoplasm of endothelial cells surrounding the medullary collecting ducts and increased until postnatal day 21, when it shifted to the nucleus of the tubules, which Correlates to adult expression patterns of the renal medulla and the ability to concentrate urine. TonEBP expression in the developing kidney preceded expression of its target genes.

**Significance:** This study illustrates how the hyperosmolality of the renal medulla acts as a signal to differentiation of the renal medulla, i.e., specific pattern of gene expression that leads to development of specific function (urine concentration). Hypertonicity drives the high concentration of urea in the medulla that accounts for the urine-concentrating ability in rat via activation of TonEBP. Timing of the rise in expression and nuclear translocation of TonEBP is consistent with the view that local hypertonicity is a major stimulus for TonEBP in developing rat kidney. This is the first experimental evidence, albeit indirect, that hypertonicity drives the medullary accumulation of urea in the early postnatal period via activation of TonEBP.


Nominated by Michael Welsh

**Question:** What is the mechanism of the large increase in TRP current induced by growth factors?

**Background:** The TRP superfamily of proteins is comprised of cation channels that have diverse cellular functions. In the central nervous system, TRPs participate in neurite outgrowth, receptor signaling, and excitotoxic cell death. The TRP ion channels, TRPC5 and TRPC1, are expressed centrally, where they form homomeric and heteromeric channels (TRCP1+TRCP5) that have distinct properties and distributions. Although both are found in soma dendrites and axons, TRPC5 is selectively expressed in nascent synapses and neuronal growth cones. TRPC5 is thus positioned and known to affect the growth of neuronal processes and sensory structures that are effectors of cellular motility; however, their regulatory mediators are unclear.

**Observations:** Using Evansen field microscopy, confocal microscopy, a biotinylation assay, and electrophysiology, Bezzerides et al. observed and quantified the growth factor-induced rapid translocation of functional TRPC5 from intracellular vesicles to the plasma membrane in transfected cells and primary hippocampal neurons. This incorporation of TRPC5 into the plasma membrane was mediated by a tyrosine kinase receptor-dependent signal transduction pathway involving Rac1 and phosphoinositide (4) phosphate 5-kinase (PI4P5Kc). Notably, this was selective for TRPC5, because TRPC1 and the TRPC1+TRPC5 heteromer were not sequestered to the growth cone plasma membrane.

**Significance:** This research suggests a novel mechanism for regulating ion channels, which the authors term the “rapid insertion of vesicular intracellular TRPs” (RIVIT). This physiologically relevant mechanism will be of interest to scientists who study morphological changes associated with cellular responses to stimuli, such as cell migration, synaptic plasticity, and the targeting of growth cones.