
Nominated by Alberto Nasjletti
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Question: What role does calcineurin signaling have in cardiac maturation and function?

Background: Calcineurin (Cn) is a calcium-calmodulin-regulated, serine-threonine phosphatase that regulates gene transcription in multiple cell types via activation of the nuclear factor of activated T-cell family of transcription factors. In cardiomyocytes, Cn signaling has been implicated in the regulation of pathological hypertrophic growth and remodeling. However, the role of Cn and its downstream targets under normal physiological conditions in the adult mammalian heart is not well defined.

Observations: Borboue and colleagues compared coronary blood flow in normally fed swine to those fed an excess caloric ketogenic diet, which induces many of the features of metabolic syndrome. Their experiments revealed significant impairments in BK channel-mediated coronary vasodilation in vivo and in vitro. Whole cell patch-clamp recordings showed decreased BK channel current, while BK channel protein was increased, suggesting defective trafficking of BK channels to the plasma membrane. Further studies revealed an increase in L-type Ca²⁺ channel-mediated coronary vasoconstriction.

Significance: These findings provide some insight into the molecular and functional changes associated with obesity-related coronary vascular disease. The apparent discordant results between decreased BK channel current and increased protein suggest highly specific regulation of BK channels in disease. Given the pervasiveness of obesity and MetS, these results make an important contribution toward elucidating pathophysiological mechanisms and novel therapeutic targeting of BK channels to reduce the incidence of cardiovascular complications in obese patients.


Nominated by Ole Petersen
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Question: Is there a third hormone released from the posterior pituitary that affects water homeostasis?

Background: The pituitary gland can be subdivided into posterior (neurohypophysis) and anterior (adenohypophysis) regions. It is currently thought that the neurohypophysis releases two hormones, vasopressin (Vp) and oxytocin, which are synthesized in the paraventricular nucleus and supraoptic nucleus. Vp is an antidiuretic that acts primarily in the renal collection ducts where it functions, in part, by inserting additional water channels (aquaporins) into the apical membrane. However, accumulating evidence suggests that there are Vp-independent mechanisms in the kidneys that also affect water homeostasis.

Observations: Based on their previous findings, Chow and colleagues explored the contribution of secretin (SCT) in regulating responses to perturbations in water balance and osmotic stability. They determined that, in contrast to SCT inducing the expression of the immediate early gene c-fos in the hypothalamus, it also induced the expression of Vp genes and release of Vp from the hypothalamo-pituitary axis. Additionally, they found that, under conditions of hyperosmolality, SCT is released into circulation by the neurohypophysis.

Significance: These collective findings support the notion that SCT directly affects renal water reabsorption by interacting with the hypothalamus, pituitary, and kidneys. Thus these findings challenge previously held beliefs as they implicate a third hormone expressed by neurons. In fact, these findings represent an important insight into elucidating the multi-faceted role of the type D syndrome in both short-term and long-term regulation of water homeostasis.
HIGHLIGHTS FROM THE LITERATURE


**Nomination by Ole Petersen**
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**Question:** What is the major mechanism(s) that regulate cellular levels of magnesium?

**Background:** Magnesium (Mg²⁺), one of the most abundant and ubiquitous cellular cations, serves a variety of essential functions in prokaryotic and eukaryotic cells. Although recent reports have characterized the structure of Mg²⁺ channels in bacteria, they suggest that Mg²⁺ channels are significantly different than other cation channels. In vertebrates, although several genes have been implicated in Mg²⁺ homeostasis, the molecular machinery necessary for Mg²⁺ transport into cells has remained elusive.

**Observations:** Zhou and Clapham took advantage of the Mg²⁺ transporter mutant ab/lA, which results in a growth arrest phenotype in the yeast Saccharomyces cerevisiae. By performing a complementary screen to this mutant, they were able to identify two human genes, MagT1 and TUSC3, as major players in Mg²⁺ influx. They found that MagT1 is expressed in all human tissues, whereas expression of TUSC3 is not. Further studies identified a role for these Mg²⁺ transporters in embryonic development in vertebrates.

**Significance:** Astutely aware of the inherent difficulties in attempting to identify Mg²⁺ transporters through homology searches, the investigators used an ingenious approach to identify two mammalian genes, MagT1 and TUSC3, that are essential for Mg²⁺ uptake into human cells and embryonic development. Although there is still much to elucidate about the molecular mechanisms that control homeostasis of Mg²⁺ levels, this report is an important step toward that end. Thus MagT1 and TUSC3 are essential for the transport of Mg²⁺ across plasma membranes.


**Nomination by Ole Petersen**
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**Question:** How can the visualization of ATP in single cells be achieved?

**Background:** Adenosine-5'-triphosphate (ATP) is a nucleotide whose energy is provided by photosynthesis and cellular respiration and is consumed by many enzymes and during various cellular processes. However, an understanding of ATP regulation at the single cell level has not been possible because the available methods of quantification could only provide averaged ATP levels of an ensemble of cells. Thus the intracellular distribution of ATP was also not distinguishable with previous methods.

**Observations:** Zhou et al. generated fluorescent ATP biosensors that encode ATP fluorescent resonance energy transfer (FRET)-based indicators for ATP called AATFs (adenosine 5'-triphosphate indicator that is encoded on episomal subunit for analytical measurements). Importantly, these ATP probes are highly selective for ATP over adenosine diphosphate (ADP) and other similar nucleotides. These AATFs have different affinities for ATP, ranging from 7.4 µM to 3.3 mM, which allowed them to quantify ATP concentrations in various cellular compartments and make some novel observations about ATP concentrations in mitochondria, cytosol, and nucleus. They found that, when cytosolic ATP was depleted, a partial recovery ensued. They also found that mitochondrial ATP was significantly lower than cytosolic ATP.

**Significance:** This research provides a promising new methodological approach for quantifying ATP not only in single cells but within various compartments of those cells. This ability led the authors to suggest, based on their observations, that additional ATP-generating pathways are activated when cytosolic ATP is depleted and that mitochondria have a high activity of adenine nucleotide translocators. Although these findings need further verification and exploration, this unique approach to measuring ATP is sure to be useful for a number of important studies.


**Nomination by Tuilo Pozzan**
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**Question:** Is the synaptic dysfunction associated with Alzheimer’s disease a presynaptic or postsynaptic phenomenon?

**Background:** Some cases of Alzheimer’s disease have a strong genetic component. In these cases, mutations in the presenilin genes (PS1 and PS2) cause the pathology of Alzheimer’s disease. Presenilins form part of a gamma secretase complex that cleaves proteins including the amyloid precursor protein (APP); cleavage of APP by gamma secretase gives rise to amyloid-beta peptides. Although presenilins have several other functions, it is currently thought that the pathogenic mechanism leading to Alzheimer’s disease is increased accumulation of amyloid-beta peptides, which leads to dysfunction of excitatory synapses, memory impairments, and neurodegeneration. The authors previously demonstrated that loss of the presenilin genes impairs synaptic function and leads to deficits in memory formation and neurodegeneration in the absence of amyloid peptide accumulation. However, the precise site where presenilin mutations induce synaptic dysfunction is not known.

**Observations:** Zhang et al. inactivated presenilins in either presynaptic (CA3) neurons or postsynaptic (CA1) neurons in mouse hippocampi. They determined that mice with none released that affects water homeostasis. Thus GnB1 mice are a more com¬mon-
selectively lacking PS1 and PS2 presynaptically at the CA3-CA1 hippocampal synapse resulted in alterations in long-term potentiation (LTP) and activity-dependent neurotransmitter release, processes essential to neuronal computation, learning, and memory. They also found that presynaptic presenilins are required for glutamate release regulated by internal calcium stores via ryanodine receptors.

**Significance:** These results expand on earlier findings that associated dysfunction of intracellular calcium release with Alzheimer’s disease. In addition, and unexpectedly, these findings also raise the novel possibility that mutations in presenilins may contribute to memory impairment and neurodegeneration in Alzheimer’s disease by selectively affecting presynaptic function. Although disruption of some of the other functions of presenilins may also contribute to Alzheimer’s disease, these findings represent an important contribution toward understanding the pathogenesis of familial Alzheimer’s disease.


**Question:** Can breathing patterns of preterm infants be stabilized by a drug-free approach?

**Background:** One common medical complication associated with premature babies is apnea, an interruption in breathing that lasts at least 20 s. Although this may be benign, it can also be clinically significant and require stimulation to prevent the deleterious effects of hypoxia. A number of studies have elucidated factors that promote unstable and irregular breathing patterns and suggest that nonlinear input-output properties at many levels contribute to the destabilized breathing in preterm infants. However, the underlying physiological mechanisms are still unknown, and caffeine therapy continues to be the most utilized approach to stabilizing apnea in preterm infants.

**Observations:** Building on the knowledge that noisy inputs can enhance the stability of nonlinear control systems, Bloch-Salisbury et al. used a novel technique of low-level exogenous stochastic stimulation to try to improve breathing stability in preterm infants. Using a specially constructed mattress that contained an actuator mounted to a sounding board imbedded within the mattress foam, they measured how intermittent vibrations affected respiration. Their results indicate that breathing patterns, described in terms of interbreath interval variance and the incidence of breath periods >5 s become more stable by stochastic mechano-sensory stimulation.

**Significance:** These findings suggest that the irregular breathing patterns associated with some preterm infants can be made regular by using stochastic mechanosensory stimulation applied to an infant’s mattress. Although this has been reported anecdotal-ly, this is the first scientifically sound evidence to support this approach. Nonetheless, perhaps the most important contribution of this work comes from finding a non-drug treatment for this condition.