PHYSIOLOGY 21: 229–232, 2006; 10.1152/physiol.00026.2006

Mechanisms underlying heterogeneous Ca^{2+} sparklet activity in arterial smooth muscle. Navedo MF, Amberg GC, Nieves M, Molkentin JD, and Santana LF. *J Gen Physiol* (May 19, 2006); doi:10.1085/jgp.09519.2006.

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Question: Do "elementary" Ca^{2+} signals occur in plasmalemmal Ca^{2+} channels?

Background: Ca^{2+} release from the sarco(endo)plasmic reticulum is due to the activation of inositol trisphosphate receptors (IP_3R) and ryanodine receptors (RyR). When several of these discretely clustered receptors are opened in a concerted manner, they form Ca^{2+} signals known as "puffs" (IP_3R) or "sparks" (RyR). These "elementary" signals were thought to occur only at intracellular/organellar membranes and not during Ca^{2+} influx across the plasma membrane. Recently, however, Navedo and colleagues reported that small clusters of L-type Ca^{2+} channels (LTCC) could function in a "high-activity" gating mode, which produced nearly continual Ca^{2+} influx: "persistent Ca^{2+} sparklet sites."

Observations: In the present work, the CaV1.2 α subunit of LTCC and PKCα were determined to be the minimal molecular components necessary for persistent sparklet activity. Protein phosphatase 2A and 2B were identified as inhibitory regulators of the number and activity of persistent sparklet sites, which opposed PKC-mediated phosphorylation.

Significance: Collectively, these studies unequivocally demonstrate that persistent Ca^{2+} sparklet activity is a fundamental property of LTCC-PKCα interactions. That these localized Ca^{2+} signals resulted from the concerted openings of adjacent plasmalemmal LTCC suggests that the persistent sparklets are a member of the elementary Ca^{2+} signal family. In contrast to the "puffs" and "sparks," however, this is the only known member of the family that involves plasmalemmal Ca^{2+} channels.

SV2 is the protein receptor for botulinum neurotoxin A. Dong M, Yeh F, Tepp WH, Dean C, Johnson EA, Janz R, and Chapman ER. *Science* 312: 592—596, 2006.

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Question: How does botulinum toxin A (BoNT/A) gain access to neurons?

Background: Botulinum toxin can paralyze and kill if consumed in contaminated food. The bacterial toxin is also safely used, in a purified form, as a medicine to control certain conditions marked by involuntary muscle contractions. BoNT/A is perhaps best known for its cosmetic application; dermatologists can quickly and safely inject botulinum toxin to diminish wrinkles. These effects are accomplished by locally producing a long-lasting neuromuscular block that leads to muscular paralysis. The mechanism by which BoNT/A blocks transmission has been delineated, but the method of neuronal entry is not as clear. Although gangliosides have been shown to bind, with low affinity, to BoNT/A, and depletion of gangliosides prevents BoNT/A entry in neuroblastoma cells, the high-affinity protein receptor required for BoNT/A entry has not been identified.

Observations: Dong et al. discovered that BoNT/A gains entry to neurons by binding to the synaptic vesicle protein SV2. Reduction of SV2 expression in cell lines inhibited BoNT/A entry, and this was restored by expressing any of the SV2 isoforms (A, B, and C). Similarly, when the expression of the A and B isoforms of SV2 were inhibited in cultured hippocampal neurons, BoNT/A binding was abolished; binding was restored when any of the three SV2 isoforms were expressed. Finally, Dong et al. showed that knockout mice lacking the SV2B isoform were less sensitive to BoNT/A.

Significance: These results suggest that the SV2 membrane protein is a receptor for BoNT/A. This finding may be of particular importance as BoNT/A is a potential agent of biological warfare. Knowing the protein receptor could lead to the development of therapeutic agents that block the entry of the toxins into cells.

Novel sub-cellular locations and functions for secretory pathway Ca^{2+}/Mn^{2+} ATPases. Southall TD, Terhzaz S, Cabrero P, Chintapalli VR, Evans JM, Dow JA, and Davies SA. *Physiol Genomics* (April 11, 2006); doi:10.1152/physiolgenomics.00038.2006.

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Question: Do secretory pathway Ca^{2+}/Mn^{2+} ATPases (SPCAs) exist somewhere other than the Golgi apparatus?

Background: Ca^{2+} signaling plays an important role in the regulation of numerous cellular functions. The plasma membrane Ca^{2+} pump (PMCA) and sarco(endo)plasmic reticular Ca^{2+} pump (SERCA) are important components of the Ca^{2+} homeostasis system. The SPCA family forms the third class of Ca^{2+} pump, which has been localized to the Golgi apparatus where it is important for Ca^{2+} and Mn^{2+} homeostasis.

Observations: Southall and colleagues identified the SPCA gene of *Drosophila* (*SpoCk*) and three alternatively spliced transcripts (SpoCk-A, -B, and -C). SpoCk-A expression was determined to be localized to the Golgi apparatus. Surprisingly, however, because the SERCA pump is known to pump Ca^{2+} in the endoplasmic reticulum (ER) they did not anticipate that SpoCk-B would be localized to the ER. Further studies revealed the expression of SpoCk-B in the ER may be important for inositol trisphosphate-mediated Ca^{2+} signaling. Another unexpected finding was the sexually dimorphic expression of SpoCk-C in peroxisomes, where it impacts Ca^{2+} storage and transport.

Significance: This is the first report to describe localization of SPCAs to the ER and peroxisomes. Further elucidation of the physiological role of the SPCA family in cellular homeostasis may provide insight into the pathology of diseases such as Hailey-Hailey disease, which is due to a primary defect in human SPCA1. Likewise, the role of the peroxisomal SPoCk-C may prove to be important for understanding peroxisome biogenesis disorders.

Mechanical ventilation promotes redox status alterations in the diaphragm. Falk DJ, DeRuisseau KC, Van Gammeren DL, Deering MA, Kavazis AN, and Powers SK. *J Appl Physiol* (March 4, 2006); doi:10.1152/japplphysiol.00104.2006.

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Question: What is the mechanism responsible for mechanical ventilation (MV)-induced oxidative stress in the diaphragm?

Background: MV is used in medicine to maintain adequate alveolar ventilation when patients cannot sufficiently breathe for themselves. It is clear that locomotor skeletal muscle experiences both oxidative stress and

atrophy during periods of disuse (e.g., immobilization), and this phenomenon is also present in the primary inspiratory muscle, the diaphragm, during MV. Unfortunately, the underlying mechanisms that produce skeletal muscle atrophy remain unclear. Nonetheless, recent observations implicate oxidative stress as a key regulatory signaling pathway for disuse muscle atrophy. This notion is supported by the observation that Trolox, a vitamin E analog, retards MV-induced diaphragmatic proteolysis. However, whether disuse-induced oxidative stress in skeletal muscles is due to increased oxidant levels or depletion of cellular antioxidant defenses is unknown.

Observations: Falk et al. set out to determine whether MV-induced oxidative stress in the diaphragm results from increased oxidant production and/or impaired antioxidant capacity. Their results reveal that MV-induced diaphragmatic oxidative stress occurs due to both an increase in oxidant production and a decrease in muscle antioxidant capacity. Moreover, this investigation also highlights the disconcerting notion that, although skeletal muscle may adapt to oxidative stress by increasing antioxidant mRNA, this increase in mRNA does not always translate into increased muscle antioxidant protein levels.

Significance: These experiments reveal that as few as 12 h of MV cause an increase in oxidant production, altered antioxidant gene expression, and diminished antioxidant capacity in diaphragm muscle. Recently, there has been a heightened interest in the relevance of oxidative stress and its impact in disuse skeletal muscle atrophy. Therefore, elucidating the mechanisms responsible for disuse-induced oxidative stress in skeletal muscle may lead the way to novel therapeutic approaches to relieve MV-induced diaphragm failure and assist in weaning patients from the ventilator.

Startle response elicited by whiplash perturbations. Blouin JS, Inglis JT, and Siegmund GP. *J Physiol* (March 31, 2006); doi:10.1113/jphysiol.108274.2006.

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Question: Does the "startle response" have a role in whiplash injuries?

Background: Low-speed rear-end collisions are associated with whiplash injuries, which are exacerbated when the victim is unprepared for the collision. The reason for the injury is not known, but it is generally thought that an overreaction (i.e., startle response) along with the sudden recoil movement of the head damages the joints and muscles of the neck. Acoustic startle evokes synchronous electromyographic (EMG) activity between 10 and 20 Hz in upper limb muscles, which is thought to represent increased reticulospinal activity. However, reticulospinal drive is also important for the control and regulation of posture. Thus postural responses, rather than a startle response, could underlie any observed synchrony in activity between the neck muscles during an impact.

Observations: Blouin and colleagues exposed subjects to rear-end collisions that were sometimes accompanied by loud sounds. They showed that an increased synchrony between the necks muscles in the 10- to 20-Hz bandwidth occurred during the first exposure to a collision. Moreover, because the increased activity elicited by an impact was attenuated on repeated exposures and the local peak in synchronized activity subsequently reappeared in habituated subjects who were simultaneously exposed to a loud acoustic stimulus, the reticulospinal control of the neck muscles during posturing was determined not to be responsible for the synchronized EMG activity.

Significance: These studies suggest that the startle response forms part of the neuromuscular response to low-intensity collisions, which may play a role in whiplash injuries. Habituation to the impact appears to manifest as attenuation of the startle response and suggests a shift with experience to a tuned muscle response. This new finding promises a new understanding of the causes of this common injury, potentially leading to new ways to prevent and manage it.

The antibacterial activity of human neutrophils and eosinophils requires proton channels but not BK channels. Femling JK, Cherny VV, Morgan D, Rada B, Davis AP, Czirják G, Enyedi P, England SK, Moreland JG, Ligeti E, Nauseef WM, and DeCoursey TE. *J Gen Physiol* (May 19, 2006); doi:10.1085/jgp.09504.2006.

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Question: Do large conductance, Ca^{2+}-activated K^+ (BK) channels play a role in antibacterial activity?

Background: Optimal antibacterial activity of neutrophils is dependent on the respiratory burst, the production of superoxide anions ($O_2^{\cdot-}$) and other reactive oxygen species by the NADPH oxidase complex. Because NADPH oxidase is electrogenic, the electrical consequences of its activity must be offset to prevent membrane depolarization (which, otherwise, would inhibit its function). Numerous studies have suggested that proton channels mediate this charge compensation. However, a recent report concluded that BK channels are essential for bacterial killing and that proton channels do not contribute to the respiratory burst. Those conclusions deviated radically from prevailing views of the respiratory burst and were therefore reexamined in the current studies.

Observations: In human neutrophils and eosinophils, Femling and colleagues were unable to detect any BK channel currents, any prevention of bacterial killing by BK inhibitors, any expression of BK channel protein, or other evidence that would support a role for BK channels in antibacterial activity. New evidence that voltage-gated proton channels play an essential role in sustaining the respiratory burst was provided by the finding that zinc inhibited NADPH oxidase activity, which was assayed by following H_2O_2 (the immediate product of $O_2^{\cdot-}$). This supported previous work, which showed that zinc inhibited $O_2^{\cdot-}$ production as detected by cytochrome *c* reduction, while avoiding the criticism that zinc might interfere with cytochrome *c*.

Significance: The current studies provided no evidence that BK channels are involved in bacterial killing; nor did they uncover any evidence for the presence of BK channels in human neutrophils or eosinophils. This work introduces the use of H_2O_2 production to demonstrate the importance of proton channels to the respiratory burst. This measurement validated the earlier conclusion that proton channels are required for the respiratory burst because they compensate for the charge moved across the membrane by the NADPH oxidase.

Alveolar type I cells protect rat lung epithelium from oxidative injury. Chen J, Chen Z, Chintagari NR, Bhaskran M, Jin N, Narasaraju T, and Liu L. *J Physiol* 572: 625—638, 2006.

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Question: What is the physiological function of alveolar type I cells in the lungs?
Background: The lung alveolar surface is covered by alveolar epithelial cell types I and II (AEC I and II). AEC II covers ~5% of the alveolar surface where they synthesize, secrete, and recycle lung surfactant and transdifferentiate into AEC I. By contrast, type I cells cover ~95% of the alveolar surface and have long been considered to function primarily as a thin barrier for efficient gas exchange. However, recent breakthroughs in isolating and culturing AEC I have allowed further analysis of the physiological functions of AEC I.
Observations: Chen et al. make novel use of microarray analysis to deduce an important function for this cell type. Gene expression profiles revealed that, compared with neighboring type II cells, type I cells are particularly rich in transcripts for apolipoprotein (ApoE), transferrin, and other proteins involved in responses to oxidative stress. These proteins were specifically localized in AEC I and were upregulated in hyperoxic lungs. Studies in an animal model demonstrated that they function to protect the lungs from oxidative stress injury.
Significance: In addition to their role in gas exchange, type I cells may be an important local source of ApoE, transferrin, and other proteins that help to protect the alveolus against the reactive oxygen species generated by inhaled pollutants or inflammatory cells. If similar results are obtained for AEC I of human lung, some AEC I-specific genes identified in this study might be useful markers of lung injury. The present findings may also lead to the development of new therapeutic targets for pulmonary diseases.

Postinfarct cytokine therapy regenerates cardiac tissue and improves left ventricular function. Dawn B, Guo Y, Rezazadeh A, Huang Y, Stein AB, Hunt G, Tiwari S, Varma J, Gu Y, Prabhu SD, Kajstura J, Anversa P, Ildstad ST, and Bolli R. *Circ Res* 98: 1098—1105, 2006.

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Question: How do cytokines improve cardiac function following an acute myocardial infarction (MI)?
Background: Acute MI is defined as death or necrosis of myocardial cells. MI occurs when myocardial ischemia exceeds a critical threshold and overwhelms repair mechanisms. If the ischemia persists at this critical threshold level for an extended time period, myocardial cell damage or death occurs. There are reports that cytokine-induced mobilization of bone marrow cells (BMCs) causes the regeneration of cardiac tissue after MI, which produces functional improvement, but data on this subject are inconsistent and the model systems demonstrating this phenomenon are not clinically relevant.
Observations: Using a more clinically relevant experimental design, Dawn et al. determined the optimal combination of cytokines to repair damaged cardiac cells. First, they found that a distinct combination of cytokines synergistically improved left ventricle (LV) remodeling and function by inducing the mobilization of BMCs. Next, they found that this recovery resulted from the ability of the cytokine-induced mobilization of BMCs to generate new cardiac tissue. Finally, the benefit of the cytokines was also due to modulation of adhesion molecules expressed by the BMCs.
Significance: These results address several issues concerning the ability of hematopoietic cytokines to limit the adverse consequences of infarct. Although initial clinical trials using cytokine therapy were not successful, this preclinical research provides important insights for future clinical trials.

Hypoxia promotes relaxation of bovine coronary arteries through lowering cytosolic NADPH. Gupte SA and Wolin MS. *Am J Physiol Heart Circ Physiol* 290: H2228—H2238, 2006.

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Question: What mechanism underlies the relaxation of arteries during hypoxic conditions?
Background: It has been known for some time that hypoxia induces a dilation of systemic arteries; however, the mechanism underlying this phenomenon has not been clearly delineated. The response of isolated endothelium-intact arteries to hypoxic conditions has provided several mechanisms that could contribute to the metabolic regulation of blood flow. These mechanisms include ionic factors, such as loss of ATP production, lactic acid elevation, lowered pH, and increased adenosine levels. More recently, endothelial factors have gained notoriety as possible dilator responses to hypoxia. In contrast, the relaxation of endothelium-denuded arteries (EDA) in response to hypoxia appears to occur via alternative mechanisms that depend on oxygen-dependent or ATP-dependent changes in Ca^{2+} permeability.
Observations: Previous work by Gupte and colleagues demonstrated that inhibitors of the pentose phosphate pathway (PPP) induce EDA to relax with an associated coordination of multiple mechanisms that decrease intracellular Ca^{2+}. Thus, here, they determined that both PPP inhibition and hypoxia induced a relaxation mechanism in EDA that was associated with the oxidation of cytosolic NADPH and glutathione, but not alterations in ATP levels. They also demonstrated that pyruvate, which potentially stabilizes or enhances tissue levels of NADPH, could attenuate the hypoxia-induced relaxation.
Significance: The response of EDA to inhibition of the PPP and hypoxic conditions was not due to changes in the opening of K^+ channels or altered levels of ATP, prostaglandins, or NO. Thus control of the PPP on the generation of NADPH appears to represent a novel process by which EDA relaxes in response to hypoxic conditions. A model is proposed that is independent of mitochondrial energy metabolism but is associated with lowering of intracellular Ca^{2+}.

Intrauterine ethanol exposure results in hypothalamic oxidative stress and neuroendocrine alterations in adult rat offspring. Dembele K, Yao XH, Chen L, and Nyomba BLG. *Am J Physiol Regul Integr Comp Physiol* (April 13, 2006); doi:10.1152/ajpregu.00633.2005.

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Question: Does prenatal ethanol (EtOH) exposure cause insulin resistance and other associ-

ated anomalies by inducing hypothalamic oxidative stress?

Background: EtOH exposure during pregnancy is associated with intrauterine growth restriction, followed by increased appetite, catch-up growth, insulin resistance, and impaired glucose tolerance in rat offspring. EtOH is known to induce oxidative stress, which can interfere with normal cell physiology. Recent studies suggest that oxidative stress is a contributing mechanism to insulin resistance and β-cell failure characteristic of Type 2 diabetes. Because of the central role of the hypothalamus in the regulation of energy homeostasis and insulin action, the authors hypothesized that prenatal EtOH exposure results in oxidative damage to the hypothalamus.

Observations: In *postnatal day 7* (juvenile) and 3-mo-old (adult) rat offspring of dams exposed to EtOH, Dembele et al. demonstrated the presence of oxidative stress in the hypothalamus, which is associated with neuroendocrine damage. In both juvenile and adult rats, prenatal EtOH exposure was associated with decreased levels of glutathione (GSH), an endogenous antioxidant. However, only adult rats preexposed to EtOH had increased hypothalamic tissue damage, as determined by lipid and protein oxidation. In addition, these adult rats had decreased levels of pro-opiomelanocortin (POMC), which could impair melanocortin signaling.

Significance: These data suggest that prenatal EtOH exposure induces hypothalamic oxidative stress in *postnatal day 7* rats. This persists into adult life and is accompanied by damage of hypothalamic tissue. The reduction in POMC protein concentration may explain the weight gain and insulin resistance in rats exposed to EtOH early in life. This highlights the importance of maintaining a healthy lifestyle during pregnancy.

Postnatal environment overrides genetic and prenatal factors influencing offspring obesity and insulin resistance. Gorski JN, Dunn-Meynell AA, Hartman TG, and Levin BE. *Am J Physiol Regul Integr Comp Physiol* (April 13, 2006); doi:10.1152/ajpregu.00138.2006.

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Question: Nature versus nurture: which is more predictive of obesity and insulin resistance in adult offspring?

Background: It is well documented that maternal intake of a high-fat diet and the presence of obesity during pregnancy and lactation promote obesity in offspring. This is particularly true in individuals who are genetically prone to obesity. However, there is growing evidence that various postnatal, environmental manipulations can impact the development of obesity and insulin resistance. For example, in a rat model of diet-induced obesity (DIO) in which obesity is inherited as a polygenetic trait, when DIO rats are raised by obese dams they become more obese and insulin resistant than DIO rats raised by lean rats.

Observations: This interesting paper examines the effect of cross-fostering the offspring of obese DIO dams with lean diet-resistant (DR) dams and of cross-fostering the offspring of DR dams with obese and lean DIO dams. Although DIO offspring cross-fostered to DR dams remained obese, they did improve their insulin sensitivity as adults. In contrast, DR pups cross-fostered with obese DIO dams displayed an increase in adiposity, reduction in insulin sensitivity, and associated changes in hypothalamic circuits involved in energy homeostasis. Increased insulin and reduced polyunsaturated fatty acid content of obese DIO dam milk may have contributed to the increased obesity of the fostered DR offspring. Surprisingly, when DR offspring were fostered with lean DIO dams, they ate less and were leaner than those fostered by DR or obese DIO dams.

Significance: These results demonstrate that postnatal environment can overcome both genetic predisposition and prenatal factors in determining the development of adiposity, insulin sensitivity, and the brain pathways that mediate these functions. It is well known that the intrauterine environment is a critical period for the development of obesity. This work emphasizes the importance of the postnatal maternal environment in determining the metabolic outcome in offspring.

A voltage-gated proton-selective channel lacking the pore domain. Ramsey IS, Moran MM, Chong JA, and Clapham DE. *Nature* 440: 1213—1216, 2006.

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A voltage sensor-domain protein is a voltage-gated proton channel. Sasaki M, Takagi M, and Okamura Y. *Science* 312: 589—592, 2006.

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Question: Has the elusive voltage-dependent proton channel finally been identified?

Background: Voltage-gated ion channels are constructed of six transmembrane domains (S1—S6). S1 through S4 form the voltage-sensor domain (VSD), whereas S5 and S6 make up the hydrophobic pore region. Traditionally, these two domains are thought to exist together. Recently, Okamura and colleagues identified a voltage-sensor-containing phosphoinositide phosphatase protein (VSP) that consists of a VSD and a phosphate domain, the first case in which a VSD was shown to regulate the activity of something other than an ion channel. A voltage-dependent proton channel is known to exist and function in mammalian cells, but the gene encoding this channel was unidentified until now.

Observations: In two reports, Sasaki et al. and Ramsey et al. independently identified and characterized a protein similar to the VSDs of ion channels with a notable difference: it lacks a pore-forming domain. Despite not having a pore-forming domain, this protein is proton-permeable. Several lines of evidence support this conclusion: depolarization induced outward currents, which were accompanied by inward tail currents during repolarization; reversal potentials occurred at equilibrium potentials for protons; currents were pH-dependent; and currents were sensitive to Zn ions. Finally, as expected for voltage-gated channels, positively charged residues in S4 functioned as the primary voltage sensors.

Significance: Collectively, these findings have identified and characterized the voltage-dependent proton channel and established a new family of mammalian VSD proteins. Voltage-dependent proton channels are used in diverse physiological processes, such as the respiratory burst pathway and bone resorption by osteoclasts. These novel findings provide new avenues to pursue in elucidating the events related to these processes and identifying potential defects associated with human diseases. ■