

Conformational switch of syntaxin-1 controls synaptic vesicle fusion. Gerber SH, Rah JC, Min SW, Liu X, de Wit H, Dulubova I, Meyer AC, Rizo J, Arancillo M, Hammer RE, Verhage M, Rosenmund C, Südhof TC. *Science* 321: 1507–1510, 2008.

Nominated by Michael Caplan
Associate Editor, *Physiology*
Yale University School of Medicine
michael.caplan@yale.edu

Question: What is the functional significance of syntaxin-1 in the closed conformation?

Background: The soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein syntaxin-1 catalyzes intracellular membrane fusion reactions that result in exocytosis. The process of exocytosis results from the formation of a SNARE complex, which requires the binding of synaptobrevin, SNAP-25 (synaptosome-associated protein of 25 kDa) and the Munc18-1 protein to the H3, or SNARE domain, of syntaxin-1. When syntaxin-1 is not in the SNARE complex, it assumes a closed formation; however, when it is in the SNARE complex, it assumes an open formation. In the closed formation, syntaxin-1 binds to the Munc18-1 protein alone, but the significance of the closed formation has been unexplored, until now.

Observations: In an attempt to understand what function syntaxin-1 has in the closed formation, Gerber et al. generated mutant mice with one syntaxin isoform deleted (syntaxin-1A) and the other isoform (syntaxin-1B) mutated to predominately render it in the open conformation. Although overt abnormal phenotypes were not evident in homozygous 1A mutants, the syntaxin-1B mutant mice were ataxic and went on to develop lethal seizures 2–12 weeks after birth. The syntaxin-1B mutation also resulted in a decrease in syntaxin-1B levels, decreased binding of Munc18-1, and a decrease in the readily releasable vesicle pool. However, the rate of synaptic vesicle fusion was greatly enhanced.

Significance: These findings suggest that syntaxin-1, in the closed conformation, regulates the fusion of synaptic vesicles. Thus, under normal physiological conditions, syntaxin-1 is not only required for the fusion process, it also controls the rate at which the vesicles fuse to the intracellular membrane. This adds to the increasing complexity of

interactions known to govern synaptic exocytosis but more importantly contributes to a greater understanding of the complex mechanism of Ca²⁺-triggered neurotransmitter release.

Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs.

Rogers CS, Stoltz DA, Meyerholz DK, Ostedgaard LS, Rokhlina T, Taft PJ, Rogan MP, Pezzulo AA, Karp PH, Itani OA, Kabel AC, Wohlford-Lenane CL, Davis GJ, Hanfland RA, Smith TL, Samuel M, Wax D, Murphy CN, Rieke A, Whitworth K, Uc A, Starner TD, Brogden KA, Shilyansky J, McCray PB Jr, Zabner J, Prather RS, Welsh MJ. *Science* 321: 1837–1841, 2008.

Nominated by Michael Caplan
Associate Editor, *Physiology*
Yale University School of Medicine
michael.caplan@yale.edu

Question: Is the pig a better animal to model cystic fibrosis than the mouse?

Background: The ABC transporter class protein and ion channel, cystic fibrosis (CF) transmembrane conductance regulator (CFTR), transports chloride ions across epithelial cell membranes. Mutations of the CFTR gene leads to cystic fibrosis, but mutant CFTR mice do not display the full spectrum of phenotypes that are observed in humans. This is an important distinction because most of the morbidity and mortality in CF patients is associated with airway disease, which mutant mice do not develop. In contrast, many aspects of the physiology, genetics, and other indexes of pigs more closely resemble those of humans.

Observations: Based on many factors, including the fact that a large effort to develop pigs as a source of organs for xenotransplantation, Rogers et al. used pigs with mutant CFTR alleles to determine how it would affect physiological functions. Mutant newborn pigs shared several defects common to humans, including defects in the pancreas, liver, and intestines. However, there was no evidence of lung defects.

Significance: These results provide exciting new possibilities that suggest the pig model may be useful for the development of treatments for those who suffer from CF. The fact that lung defects were not observed is not unexpected, since human CF lung disease

does not appear until months or even years after birth. However, because most patients eventually develop lung disease, this will undoubtedly be something that future studies will address in the pig model, which may help to elucidate whether the pathogenesis requires bacterial infection, as some have speculated.

Relation between obesity and blunted striatal response to food is moderated by Taq1A A1 allele. Stice E, Spoor S, Bohon C, Small DM. *Science* 322: 449–452, 2008.

Nominated by Michael Caplan
Associate Editor, *Physiology*
Yale University School of Medicine
michael.caplan@yale.edu

Question: Can a polymorphism in the dopamine D2 receptor contribute to a dysfunctional reward system that underlies obesity?

Background: Dopamine (DA) is known to function in cognitive and motivated behaviors. These functions of DA are perhaps directly related to its function in the pleasure and reward systems of the CNS. As such, it should come as no surprise that, whenever someone eats, it is dorsal striatum DA release that correlates with the amount of pleasure one receives. Alleles of the DA D2 receptor (DRD2) have been associated with a number of disorders, including obesity. Thus, in the present studies, the influence of the DRD2 Taq1A A1 allele, which results in hypofunction, on the striatal response to food was investigated.

Observations: Stice et al. utilized BOLD (blood oxygen level-dependent) fMRI and determined that obese women presented with abnormal activation of the dorsal striatum following consumption of a highly palatable food (chocolate milkshake). Additionally, they determined that expression of the A1 allele resulted in an amplification of the blunted striatal response to the milkshake. Interestingly, the relationship between striatal activation and the expression of an A1 allele correctly predicted weight gain when measured a year later.

Significance: Although the hypothesis that obesity may be related to a dysfunctional dopamine reward system, which results in compensatory overeating, has been around for some time, this is the first prospective

evidence to support such a hypothesis. Astutely, the authors are quick to point out that overconsumption of high-fat and high-sugar foods could have preceded a hypo-functioning striatum. Nonetheless, the finding that this relationship is even stronger for individuals expressing an A1 allele suggests that it is an important biological factor that increases the risk for obesity.

H₂S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, Snyder SH, Wang R. *Science* 322: 587–590, 2008.

Nominated by Michael Caplan
Associate Editor, *Physiology*
Yale University School of Medicine
michael.caplan@yale.edu

Question: Is hydrogen sulfide a gaseous transmitter?

Background: Gasotransmitters, i.e., endogenous gaseous signaling molecules, such as nitric oxide are known to diffuse across the cell membrane where they have important roles in many physiological and pathological processes. Just like neurotransmitters, gasotransmitters must display several properties to meet this classification. They must be small gas molecules that are produced and regulated by endogenous and enzymatic processes. In addition, they must be able to pass freely across membranes where they have defined cellular and molecular targets that affect physiological functions. Hydrogen sulfide (H₂S) has been implicated as a potential gasotransmitter, and this report provides further evidence to support this classification.

Observations: Yang et al. generated mice that had the gene encoding cystathionine gamma-lyase (CSE), which is one of the enzymes known to produce H₂S in cardiovascular system, deleted. This deletion resulted in a decrease in endogenous H₂S by ~80% in cardiac tissue and ~50% in serum levels in CSE^{-/-} mice. CSE mutants also developed age-dependent perturbations in physiological functions associated with H₂S, hypertension, and diminished endothelium-dependent vasorelaxation, which is in accord with the ontogeny of CSE in mice. Finally, they

determined that CSE in endothelium is activated by calcium-calmodulin.

Significance: These findings suggest that CSE is involved in the physiological production of H₂S, which provides further evidence supporting the role of H₂S as a gaseous transmitter. Interestingly, the deletion of CSE induces effects that are similar to endothelial nitric oxide (NO) synthase knockout mice. However, unlike NO, H₂S does not appear to have the capacity to form a toxic metabolite. As such, because H₂S regulates blood pressure, the CSE/H₂S system may become a novel pharmacological target for the treatment of hypertension.

Regulation of smooth muscle contractility by the epithelium in rat vas deferens: role of ATP-induced release of PGE₂. Ruan YC, Wang Z, Du JY, Zuo WL, Guo JH, Zhang J, Wu ZL, Wong HY, Chung YW, Chan HC, Zhou WL. *J Physiol* 586: 4843–4857, 2008.

Nominated by Hsiao Chang Chan
Chinese University of Hong Kong
hsiaocchang@cuhk.edu.hk

Question: What is the underlying mechanism of epithelial-dependent regulation of smooth muscle contraction?

Background: When muscles contract, a calcium-regulated phosphorylation of myosin allows the myosin and actin filaments to slide over each other in an ATP-dependent manner. The cells surrounding the muscles have a role in regulating this phenomenon. For example in endothelial cells, nitric oxide (NO) and prostacyclin are both released and regulate the tone of smooth muscle. Epithelial cells have also been implicated in the regulation of smooth muscle contraction. However, the mechanisms underlying epithelial cell regulation of contraction are not well defined.

Observations: Ruan et al. hypothesized that modulation by epithelial cells may result in alterations in the contractile response to neurotransmitters, such as ATP and adrenaline. They found that exogenously applied ATP inhibited electrical field-stimulated smooth muscle contraction, which depended on the epithelium and prostaglandin synthesis but not on NO synthesis. Further studies were completed to elucidate the cascade of events related to this regulation.

Significance: These findings suggest that epithelial cells regulate smooth muscle contraction via an ATP-dependent mechanism, which activates P2Y purinergic receptor-coupled Ca²⁺ mobilization. The subsequent release of the prostaglandin E₂ (PGE₂) activates cAMP-dependent K⁺ channels, which induces hyperpolarization of the membrane and inhibition of contraction in the vas deferens. This mechanism of epithelial-dependent inhibition of smooth muscle contraction may also be relevant to other tissues. These results provide insight into the novel process of how epithelial tissue regulates smooth muscle tone.

Mechanisms of active laryngeal closure during noninvasive intermittent positive pressure ventilation in nonsedated lambs. Roy B, Samson N, Moreau-Bussière F, Ouimet A, Dorion D, Mayer S, Praud JP. *J Appl Physiol* 105: 1406–1412, 2008.

Nominated by Jerry Dempsey
Editor, *Journal Applied Physiology*
University of Wisconsin
jdempsey@wisc.edu

Question: Does the glottal closure associated with noninvasive intermittent positive pressure ventilation result from activation of receptors from upper or lower airways?

Background: Noninvasive intermittent positive pressure ventilation (nIPPV) assists patient breathing without the use of an endotracheal tube. In contrast to breathing assistance mediated by the use of endotracheal tubes, nIPPV was developed to minimize patient discomfort and mitigate the complications associated with invasive ventilation. However, the use of nIPPV can be complicated if the larynx is closed, which prevents the lungs from receiving the necessary oxygen. However, the mechanism(s) underlying nIPPV-induced glottal closure remains unresolved.

Observations: Roy et al. set out to determine whether reflexive glottal narrowing in response to nIPPV results from activation of receptors in the upper airways or the lower airways. They found that when neonatal lambs were subjected to a bilateral thoracic vagotomy (i.e., the vagus nerve was sectioned), nIPPV-induced glottal narrowing was prevented. Similarly, when a separate cohort of lambs was subjected to a laryngotracheal

separation, which isolates the upper airway, the nIPPV-induced glottal narrowing did not develop.

Significance: These findings suggest that the interaction between bronchopulmonary receptors and upper airway regulation in nIPPV is complex. Hence, the glottal narrowing is not regulated via upper airway receptors but by bronchopulmonary receptors. While we await further studies to determine whether a similar mechanism is activated in adults, the implied clinical relevance is clear; a pharmacological approach to improve the effectiveness of nIPPV, by decreasing the glottal closure, in newborn animals should prove highly beneficial.

The ion pathway through the opened Na⁺,K⁺-ATPase pump. Takeuchi A, Reyes N, Artigas P, Gadsby DC. *Nature* 456: 413–416, 2008.

Nominated by Baruch Kanner
Hebrew University Hadassah Medical School
kannerb@cc.huji.ac.il

Question: What pathway do ions follow when they traverse Na⁺/K⁺ pumps?

Background: The Na⁺-K⁺-ATPase is a P-type pump (so called because it becomes phosphorylated) that exchanges three intracellular Na⁺ ions for two extracellular K⁺ ions across the plasma membrane for each ATP molecule it hydrolyzes. When the pump is in the phosphorylated state, known as E2P, the ion binding sites are accessible from the extracellular medium. In contrast, when the pump is not phosphorylated, in the state known as E1, the binding sites are accessible from the intracellular milieu. The alternating access associated with cyclical phosphorylation and dephosphorylation underlies active ion transport. However, the paths that the ions follow when approaching and leaving the binding sites are unknown.

Observations: Building upon previous findings, Takeuchi et al. used an agent known to disrupt the strict alternating access of the pump, leaving the binding sites sometimes simultaneously accessible from both sides of the membrane. This allowed them to perform electrophysiological recordings on the resulting ion channels and to add to their previous findings. In short, they determined the location of the cation pathway that traverses the pump, from one side of the membrane to the other.

Significance: These findings provide structural insights into the molecular pathway along which ions move during their transport by P-type pumps. This active transport is essential for maintaining the high concentrations of K⁺ ions and low concentrations of Na⁺ ions in cells. Elucidating elements of this fundamental physiological process has implications for most biological scientists, and especially for neuroscientists, since the difference in the concentrations of these ions between the outside and inside of nerve cells allows them to generate electrical impulses.

Altered free radical metabolism in acute mountain sickness: implications for dynamic cerebral autoregulation and blood-brain barrier function. Bailey DM, Evans KA, James PE, McEneny J, Young IS, Fall L, Gutowski M, Kewley E, McCord JM, Møller K, Ainslie PN. *J Physiol* (October 27, 2008); doi:10.1113/jphysiol.159855.2008.

Nominated by Michael Kjaer
Associate editor, *Journal of Physiology*
European College of Sport Science
m.kjaer@mfi.ku.dk

Question: Does acute mountain sickness (AMS) develop because hypoxia-induced changes in free radical metabolism impair cerebral blood flow control, which disrupts the blood-brain barrier?

Background: AMS, a disorder of the CNS thought to be due to a mild form of cerebral edema, produces headache and other symptoms in nonacclimatized travelers to altitudes above 2,500 m. The edema is thought to result from disruption of the blood-brain barrier due to intracranial hypertension produced by hypoxic cerebral vasodilatation not countered by the cerebral blood flow autoregulatory system. The impairment of the autoregulatory system may be caused by an altered interplay between oxidative stress and nitric oxide (NO) metabolism.

Observations: Of 18 healthy low-land subjects exposed to 6 h of 12% inspired oxygen, one-half developed AMS with increased headache scores. Tests of cerebral autoregulation (CA) showed that it was impaired in the AMS group with the test results linearly related to the increase in headache scores. Furthermore, the AMS group was more hypoxemic, had a greater increase in free

radical-mediated lipid peroxidation, and a selective reduction in the antioxidants ascorbate and γ -tocopherol. However, the declines in NO with hypoxia were not different between groups, and no evidence for cerebral hyper-perfusion, blood-brain barrier disruption, or neuronal-parenchymal damage was detected.

Significance: This study represents the first comprehensive examination of systemic circulatory NO metabolites in AMS. Although the findings implicate an altered redox homeostasis as the primary event producing AMS, they also question whether the consequent impairment of dynamic CA is pathophysiologically significant. The observations are also consistent with the notion that the metabolites of oxidative-nitrosative stress may act to change pain perception by sensitizing perivascular sensory nerves and activating trigeminovascular nociceptors. These findings have important implications for further studies of AMS.

Glucose transporter isoform-3-null heterozygous mutation causes sexually dimorphic adiposity with insulin resistance. Ganguly A, Devaskar SU. *Am J Physiol Endocrinol Metab* 294: E1144–E1151, 2008.

Nominated by Amira Klip
Hospital for Sick Children
amira@sickkids.ca

Question: Do perturbations of the glucose transporter in utero affect metabolic functions in the offspring?

Background: The transport of glucose across most cell membranes is essential for metabolism. Additionally, it is involved in clearing glucose from the blood, a process facilitated by insulin in mammals. Glucose transporter protein isoform-3 (GLUT3) mediates facilitated transport in neurons, placenta, and testes, and although the GLUT3^{-/-} mutation is lethal, the heterozygous mutation causes a decline in glucose transport across the placenta, resulting in stunted fetal growth. To date, studies of the GLUT3^{+/-} mutant have not found any overt metabolic changes in mice up to 2 mo old.

Observations: Ganguly and Devaskar hypothesized that metabolic changes in GLUT3^{+/-} mice would be measurable but not until adulthood. Thus they determined

whether metabolic changes were evident in the mutant mice at several stages during postnatal development up to 11 mo old. They found that males, but not females, had increased adiposity. Although the increased fat mass was not due to increased caloric intake, it was the likely result of decreased basal metabolism and insulin resistance.

Significance: Similar to in utero protein or calorie-restricted rats that develop metabolic perturbations between 15 and 21 mo of age, GLUT3^{+/-} male mice also develop metabolic perturbations at a later adult stage. Although in contrast to the protein/calorie restrictions, which led to glucose intolerance, the GLUT3^{+/-} mice were insulin resistant. Collectively, studies of nutrient deficiencies in utero suggest that metabolic programming in the fetus may result in sex- and age-dependent phenotypes and underscores the importance of the prenatal environment to metabolic functions of the offspring.

Exenatide can reduce glucose independent of islet hormones or gastric emptying. Ionut V, Zheng D, Stefanovski D, Bergman RN. *Am J Physiol Endocrinol Metab* 295: E269–E277, 2008.

Nominated by Amira Klip
Hospital for Sick Children
amira@sickkids.ca

Question: Do the physiological effects of exenatide on glycemia occur through a similar mechanism to the endogenous glucagon-like peptide-1?

Background: Glucagon-like peptide-1 (GLP-1) affects glycemia via pancreatic mechanisms, including increasing insulin secretion, in a glucose-dependent manner, and concomitantly decreasing glucagon secretion. GLP-1 also affects glycemia by inhibiting acid secretion and gastric emptying. Recently, accumulating evidence has suggested that GLP-1 also influences

glycemia via receptors on the portal vein. Exenatide is a medication approved for the treatment of diabetes mellitus Type 2 because of its ability to mimic GLP-1, but whether the glycemia-reducing effect of exenatide is independent of gastric and pancreatic effects is unknown.

Observations: Ionut et al. determined that exposure to exenatide before the consumption of food reduced glycemia but did not increase insulin secretion. However, the reduction in plasma glucose was associated with delayed gastric emptying and lower glucagon. Using matched intraportal infusion, they found that exenatide's effect is independent of gastric emptying and glucagon lowering, and by using a GLP-1 receptor antagonist, suggested the effect might be mediated via receptors in the porto-hepatic area.

Significance: These results support the theory that exenatide lowers glycemia via a portal GLP-1 receptor and by a mechanism that is independent of effects on islet hormones and gastric emptying. Although additional research is necessary to elucidate the exact location of receptors that mediate exenatide effects on glycemia, these findings will be useful for future studies aimed at identifying treatments for diabetes.

Measurements of the BK_{Ca} channel's high-affinity Ca²⁺ binding constants: effects of membrane voltage. Sweet TB, Cox DH. *J Gen Physiol* 132: 491–505, 2008.

Nominated by Edward Pugh, Jr.
Associate Editor, *Journal of General Physiology*
University of Pennsylvania, School of Medicine
pugh@mail.med.upenn.edu

Question: What are the affinities of the two high-affinity binding sites of BK_{Ca}, and is Ca²⁺ binding voltage dependent?

Background: Calcium-activated potassium

channel (BK_{Ca}) opening is regulated by changes in the electrical potential across the cell's membrane and by changes in the intracellular concentration of Ca²⁺. The latter has three binding sites, one of low affinity and two types of high affinity; the high-affinity ones are known as the Ca²⁺ bowl and the cytoplasmic RCK1 site. Although the binding properties of the two high-affinity sites have been estimated, discrepancies between these estimates make them uncertain.

Observations: Sweet and Cox have attempted to circumvent the uncertainty of estimating binding properties by not employing a voltage-sensing model as previous scientists have done. Instead, they estimated the affinity constants of the channel's Ca²⁺ binding sites by studying the effects of Ca²⁺ on channel opening at various intracellular Ca²⁺ concentrations at a single voltage, which allows the effect of voltage on channel opening to be treated as a constant. This novel approach, coupled with mutations that functionally eliminate one or more of the binding sites, allowed them to determine that the sites have very different affinities. Moreover, they found that Ca²⁺ binding at the RCK1 site is voltage dependent, which is in contrast to Ca²⁺ binding at the Ca²⁺ bowl.

Significance: Understanding the mechanism by which BK channels sense and respond to cytoplasmic [Ca²⁺] is underscored by the fact that they regulate several key physiological processes, such as smooth muscle tone and neuronal excitability. The surprising finding that the RCK1 site is voltage dependent, whereas binding to the Ca²⁺ bowl was not, should aid in elucidating a structural basis for interactions between the voltage sensor and the RCK1 site. Since BK channels are pharmacological targets for mitigating the effects of strokes by preventing excessive Ca²⁺ from entering neurons, these findings may be of particular interest to pharmaceutical companies.