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Daily electrical silencing in the mammalian circadian clock. Belle MD, Diekman CO, Forger DB, Piggins HD. *Science* 326: 281–284, 2009.

AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. Lamia KA, Sachdeva UM, DiTacchio L, Williams EC, Alvarez JG, Egan DF, Vasquez DS, Juguilon H, Panda S, Shaw RJ, Thompson CB, Evans RM. *Science* 326: 437–440, 2009.

Nominating editor Michael Caplan
michael.caplan@yale.edu

Question: How does the differential expression of proteins entrain circadian rhythms in disparate organs?

Background: Circadian rhythms are endogenously generated ~24-h cycles that control or initiate various biological processes. The suprachiasmatic nucleus (SCN) of the hypothalamus is the master pacemaker of circadian rhythms. The zeitgeber, or the environmental event, that entrains the rhythmic firing of the SCN is light sensed by the retinohypothalamic pathway. In addition to the SCN, molecular pacemakers exist in almost every cell, including the liver. However, in contrast to the SCN, the zeitgeber of the liver pacemaker is feeding-fasting cycles. The molecular mechanisms that underlie the rhythmic firing patterns of these pacemakers are thought to be proteins that encode components of the circadian clock and are expressed more during the day than at night. Thus, in the SCN, light is thought to induce an increase in the expression of the *period 1* (*per1*) gene that subsequently entrains the rhythmic firing pattern of cells in the SCN. In contrast, although feeding is thought to induce an increase in the expression of core clock proteins, such as *cryptochrome 1* (*cry1*), the biochemical basis of entrainment in liver is unknown.

Observations: Belle et al. measured and compared the electrical activity of two populations of cells in the SCN: those that do vs. those that do not make period 1 proteins. They determined that neurons without period 1 followed the predicted firing pattern. In contrast, the cells expressing the protein displayed a moderate firing rate in the morning followed by an overexcited state in the afternoon that persisted until dusk, during which they would not fire. In the second report, Lamia et al. identified two sites on cry1 that correspond to phosphorylation targets of an enzyme that responds to nutrient availability, adenosine monophosphate-activated protein kinase (AMPK). They subsequently determined that AMPK activity was rhythmic and inversely correlated with cry1 protein levels and that AMPK-induced phosphorylation of cry1 leads to its degradation.

Significance: The report by Belle et al. confirmed their predictions made using mathematical models, which suggested the SCN contains cells that could experience this type of overexcitedness. Although the findings of Lamia et al. provide a clearer picture of how nutrition affects oscillations in gene and protein expression that ultimately impinge on cellular functions, it remains to be determined how altered period 1 expression affects firing patterns and what other mechanisms are involved in communicating nutritional status to peripheral clocks; elucidating the normal homeostatic mechanisms that control behaviors is essential for understanding how dysfunction of these pathways could lead to various diseases.

The Ca^{2+} channel beta subunit determines whether stimulation of Gq-coupled receptors enhances or inhibits N current. Heneghan JF, Mitra-Ganguli T, Stanish LF, Liu L, Zhao R, Rittenhouse AR. *J Gen Physiol* 134: 369–384, 2009.

Orientation of palmitoylated CaVbeta2a relative to CaV2.2 is critical for slow pathway modulation of N-type Ca^{2+} current by tachykinin receptor activation. Mitra-Ganguli T, Vitko I, Perez-Reyes E, Rittenhouse AR. *J Gen Physiol* 134: 385–396, 2009.

Nominating editor Edward Pugh
enpugh@ucdavis.edu

Question: What are the underlying causes of the different patterns of activity observed in voltage-gated calcium (Ca^{2+}) channels (VGCC)?

Background: Depolarization of VGCC, such as the neuronal (N)-type channel $Ca_v2.2$, results in an influx of Ca^{2+} ions that functionally links the electrical and biochemical signals between neurons by modulating ion channels, enzyme activity, and gene transcription. Therefore, variations in the levels and sites of expression and/or the kinetics of these channels directly affect the location and levels of Ca^{2+} influx into a neuron and, in turn, impact the strength of the synaptic connections formed by the neurons. N-channel activity is regulated by several converging signal transduction cascades from G-protein-coupled receptors (GPCRs). Although the molecular mechanisms (including the signaling molecules and sites of action) that modulate some N-type VGCCs have been reported, the mechanisms that alter the function of other channels [e.g., M1 muscarinic acetylcholine receptor (M1-mAchR) and the neurokinin-1 receptor (NK-1R)], remain controversial.

Observations: Two reports from the Rittenhouse laboratory build on their previous findings and further support the hypothesis that arachidonic acid (ArA) regulates the activity of M1 and NK-1 receptors. Using a heterologous expression system, they determined that, when $Ca_v2.2$ $\alpha1$ subunits were coexpressed with $Ca_v\beta3$ subunits, stimulation of M1 or NK-1 receptors inhibited N-channel activity, an effect that was attenuated by exogenous ArA application. In contrast, stimulation of M1 or NK-1 receptors, or exogenous application of ArA, enhanced N-channel activity when palmitoylated $Ca_v\beta2a$ subunits were coexpressed with $Ca_v2.2$. Further studies revealed that the palmitoyl groups of $Ca_v\beta2a$ occupy an inhibitory site on $Ca_v2.2$, which blocks ArA from inhibiting N-current activity.

Significance: These findings reveal a novel regulatory molecular mechanism (protein palmitoylation) underlying the different endogenous patterns of gating activity observed in N-channel currents. In fact, lipid modulation of VGCC is a relatively new area of research, and that different β-subunits can provide regulatory plasticity to Ca^{2+} channel function provides novel insight into how this phenomenon occurs. Thus it appears that this regulatory mechanism allows VGCCs to select between stimulation and inhibition by GPCRs depending on β-subunit expression. An overall understanding of the basic principles of channel modulation may reveal insights into disorders that are associated disruption of these signaling pathways, such as Alzheimer's disease, schizophrenia, and stroke.

Control of iron homeostasis by an iron-regulated ubiquitin ligase. Vashisht AA, Zumbrennen KB, Huang X, Powers DN, Durazo A, Sun D, Bhaskaran N, Persson A, Uhlen M, Sangfelt O, Spruck C, Leibold EA, Wohlschlegel JA. *Science* 326: 718–721, 2009.

An E3 ligase possessing an iron-responsive hemerythrin domain is a regulator of iron homeostasis. Salahudeen AA, Thompson JW, Ruiz JC, Ma HW, Kinch LN, Li Q, Grishin NV, Bruick RK. *Science* 326: 722–726, 2009.

Nominating editor Michael Caplan
michael.caplan@yale.edu

Question: How is the synthesis of iron metabolism proteins regulated?

Background: Iron homeostasis is controlled by the iron regulatory proteins (IRP1 and IRP2), which respond to cytosolic iron concentrations by binding to iron-responsive elements (IRE) found in the mRNA of genes that modulate the synthesis of iron-metabolizing proteins. Thus, when iron availability is high, IRP1 loses its affinity for IREs, and IRP2 is degraded by proteasomes. Proteins targeted for ubiquitin-dependent degradation by E3 ubiquitin ligases. The E3 ubiquitin ligase subsequently attaches ubiquitin monomers to the lysines of the target protein, which marks it for degradation by proteasomes. The two reports in this highlight sought to determine the ligase responsible for sensing iron bioavailability and the subsequent mechanism responsible for the degradation of IRP2.

Observations: The Wohlschlegel laboratory used a combination of biochemistry and proteomic mass spectrometry to evaluate novel roles of F-box proteins, including FBXL5. IRP1 and IRP2 were determined to be substrates of a mutated FBXL5 that retained the ability to interact with target proteins but were prevented from catalyzing the degradation of these target proteins. Additional experiments confirmed that the interaction between FBXL5 and IRP2 was regulated by an iron-binding hemerythrin-like domain on FBXL5 and that FBXL5 regulated the iron-dependent ubiquitination and proteasomal degradation of IRP2. The Bruick consortium used a small interfering RNA (siRNA) screen to identify an E3 ubiquitin ligase complex that targets IRP2 for proteasomal degradation. This vastly different approach determined that FBXL5 expression increased when iron and oxygen levels were high and decreased when iron levels were low. In addition, FBXL5 was found to contain a hemerythrin-like iron- and oxygen-binding domain that regulated ligand binding, underlying its differential stability.

Significance: Collectively, the unique approaches used by these different groups to identify the same protein strongly suggests that there is a mechanistic link between the hemerythrin-like domain of the E3 ligase FBXL5, IRP2 regulation, and cellular responses to maintain iron homeostasis. The ability to sense and respond to changes in iron and oxygen availability is critical for many developmental and physiological processes. Therefore, these findings may have implications for understanding the pathogenesis of many diseases including anemia, neurodegeneration, cancer, and immune system disorders.

The taste of carbonation. Chandrashekar J, Yarmolinsky D, von Buchholtz L, Oka Y, Sly W, Ryba NJP, Zuker CS. *Science* 326: 443–445, 2009.

Nominating editor Michael Caplan
michael.caplan@yale.edu

Question: How do taste receptor cells (TRCs) detect and respond to carbonation?

Background: Mammals detect carbonation by a number of sensory systems including nociception, olfaction, and chemoreception (related to respiratory control). However, although humans are known to distinguish sweet, sour, bitter, salty, and umami taste qualities, accumulating evidence suggests that CO_2 is also perceived. To that end, it is generally believed that carbonic anhydrases (CAs) have a role in how TRCs detect and respond to CO_2, but the molecular mechanisms that contribute to this experience are not well understood.

Observations: Chandrashekar et al. determined that the ability of CO_2 to stimulate the TRCs was dose-dependent and saturable. Moreover, when the sour-sensing cells of mice were genetically ablated via diphtheria toxin, the responses to gaseous or dissolved CO_2 were abolished. Further studies identified *Car4*, a gene that is selectively expressed in sour-sensing cells and encoded by the extracellular carbonic anhydrase CA-IV, as necessary for CO_2 taste sensation. Finally, by examining mice whose sour cells are present but silenced by expression of tetanus toxin, the authors found that intact signaling from the sour receptor cells to the taste nerves is essential for CO_2 detection.

Significance: This study suggests that sour-cells are necessary for CO_2 detection and identifies the cellular and molecular mechanisms underlying the taste of carbonation in mammals. CO_2 detection by the chemosensory system may have evolved as a means to recognize fermentation or other CO_2-producing sources to prevent the ingestion of spoiled foods. Alternatively, the authors suggest that CA-IV may be playing a role in maintaining the pH balance of taste buds and that the taste of CO_2 arises as an accidental consequence of the presence of this enzyme on the surface of sour-sensing cells. Nonetheless, these findings provide evidence that, in addition to the well known taste qualities we can sense, CO_2 is also sensed by our TRCs.

Physiological roles of endogenous ouabain in normal rats. Nesher M, Dvela M, Igbokwe VU, Rosen H, Lichtstein D. *Am J Physiol Heart Circ Physiol.* doi:10.1152/ajpheart.00734.2009.

Nominating editor Alberto Nasjletti
alberto_nasjletti@nymc.edu

Question: What is the physiological role of endogenous ouabain (EO)?

Background: EO is the most frequently studied of the digitalis-like compounds (DLC), which are a family of steroids released from the adrenal gland. EO has been implicated in numerous pathologies including the regulations of body and organ weight gain, hypertension, and sodium and vascular tone homeostasis. Furthermore, mutations in the ouabain-binding site of the cell surface enzyme Na^+-K^+-ATPase alter adrenocorticotropic hormone-induced hypertension and sodium homeostasis. Despite the known roles of EO and DLC in pathologies and related cellular mechanisms, its physiological role in normal animals has not been elucidated.

Observations: Nesher et al. compared rats treated for 28 days with antiouabain antibodies (Oua-Ab) or with rabbit IgG as a

control on numerous physiological indexes. Oua-Ab rats showed reduced plasma EO concentrations and increased absolute and relative adrenal cortex mass compared with IgG rats, although no difference was observed in adrenal medulla mass, body weight gain, or blood pressure. Oua-Ab rats showed a correlation (r^2 = 0.78) between adrenal mass and plasma EO but not corticosterone or aldosterone. The maximal contraction response (MR) of thoracic aortic rings to phenylephrine (PE)-induced vasoconstriction was reduced, whereas the MR to atrial natriuretic peptide-induced vasodilation was elevated in Oua-Ab rats. Finally, the authors observed a significant reduction in sodium excretion (natriuresis) after the second day of Oua-Ab administration, as well as reduced basal natriuresis in actively immunized rats.

Significance: These novel findings identify EO as a regulator of sodium homeostasis and vasculature tone in normal animals. In addition, the finding of adrenal cortex hypertrophy after long-term reductions in EO supports previous research that suggests the adrenal cortex is a major synthesizer of EO. By shedding necessary light on the synthesis pathway of EO and DLC in general, these findings will enable further research targeted at understanding the molecular mechanisms underlying the role of EO in natriuresis and vascular reactivity, as well as at developing treatments for related pathologies.

Impaired overload-induced hypertrophy in obese Zucker rat slow-twitch skeletal muscle. Paturi S, Gutta AK, Kakarla SK, Katta A, Arnold EC, Wu M, Rice KM, Blough ER. *J Appl Physiol.* doi:10.1152/japplphysiol.00330.2009.

Nominating editor Jerry Dempsey
jdempsey@wisc.edu

Question: Does insulin resistance (IR) influence skeletal muscle hypertrophy after mechanical overload?

Background: Exercise is commonly prescribed for the treatment of IR and Type 2 diabetes mellitus, and its benefits appear to be mediated through the activation of signaling cascades involved in the regulation of gene expression. However, it is unknown how IR affects muscle adaptation. Previous studies have suggested that increased muscle loading increases the rate of protein synthesis, which is controlled in part by the mammalian targets of rapamycin (mTOR) and p70 ribosomal protein S6 kinase (p70s6k) signals. The authors of this study previously found that IR impairs the activation of mitogen-activated protein kinase (MAPK) by skeletal muscle after increased muscle loading, but questions remain regarding the ability of IR muscles to undergo hypertrophy and the signaling mechanisms involved in this process.

Observations: Paturi et al. studied 4-wk-old male lean (LZ) and obese (OZ) Zucker rats, removing each animal's left gastrocnemius muscle while leaving the right muscle intact. After muscle loading for 8 wk, the authors observed 12% less soleus muscle mass compared with LZ rats and no increase in type I fiber cross-sectional area (as determined by ATPase staining), thereby demonstrating decreased hypertrophy in IR animals. Further studies revealed that OZ rats had less activated p70s6k^{Thr389} and mTOR^{Ser2448} in the overloaded muscle than LZ rats and increased phosphorylation of the mTOR inhibitors AMPK and Tuberin/TSC2.

Significance: Although exercise is known to result in a number of adaptations in healthy muscles, this important work sheds light on the adaptive abilities of IR muscles. This study is the first to identify deficiencies in skeletal muscle hypertrophy in IR animals, concomitant with alterations in the activation of signaling pathways involved in protein synthesis. Given the potential for muscle growth to regulate glucose homeostasis, these findings have important implications for treating patients with Type 2 diabetes and other associated comorbidities.

Ribosomal protein S6 kinase 1 signaling regulates mammalian lifespan. Selman C, Tullet JMA, Wieser D, Irvine E, Lingard SJ, Choudhury AI, Claret M, Al-Qassab H, Carmignac D, Ramadani F, Woods A, Robinson ICA, Schuster E, Batterham R, Kozma SC, Thomas G, Carling D, Okkenhaug K, Thornton JM, Partridge L, Gems G, Withers DJ. *Science* 326: 140–143, 2009.

Nominating editor Michael Caplan
michael.caplan@yale.edu

Question: How does ribosomal protein S6 kinase 1 (S6K1) signaling regulate mammalian lifespan?

Background: The nutrient-responsive mammalian target of the rapamycin (mTOR) pathway has recently been identified as a regulator of longevity in mammals. S6K1 is a downstream target of mTOR that indicates an organism's nutritional status and regulates cell size, growth, and metabolism by affecting mechanisms that are regulated, in part, by the activity of adenosine monophosphate (AMP)-activated protein kinase (AMPK). Additionally, S6K1 serine phosphorylates insulin receptor substrate protein (IRS) 1 and 2, which decreases insulin signaling. However, the molecular and cellular mechanisms by which S6K1 may play a role in the regulation of mammalian lifespan have not been elucidated.

Observations: Selman et al. compared male and female wild-type (WT) and *S6K1*$^{-/-}$ mice on a C57BL/6 background and found that median lifespan in *S6K1*$^{-/-}$ mice increased 9% relative to WT mice. Interestingly, however, this effect was evident for females only, in which *S6K1*$^{-/-}$ lifespan was increased by 19% relative to WT lifespan. Older female *S6K1*$^{-/-}$ mice exhibited improvements in several age-sensitive biomarkers of aging. Increased insulin sensitivity and reduced adiposity, which are traits also present in WT mice under caloric restriction (CR), were observed in older female *S6K1*$^{-/-}$ mice, as were improvements in T cell and fat mass in male *S6K1*$^{-/-}$ mice despite the lack of any lifespan effect in males. Selman et al. also observed highly significant overlaps between hepatic gene expression in *S6K1*$^{-/-}$ mice and categories overrepresented among CR-regulated genes as well as significant correlations in the directions of transcriptional changes. Finally, the authors found that enhancing AMPK activity may contribute to the longevity of both *C. elegans* and mice lacking S6K1.

Significance: These intriguing findings indicate that S6K1, acting indirectly through enhanced AMPK activity (via reduced ATP and increased AMP levels), may mediate the responses to CR. As such, it will be interesting to see whether compounds can be developed that selectively target S6K1 and AMPK to achieve improved health later in life. More importantly, these results point to the possibility of manipulating mTOR/S6K1 signaling in the treatment of age-related disease.

NOS inhibition blunts and delays the compensatory dilation in hypoperfused contracting human muscles. Casey DP, Joyner MJ. *J Appl Physiol*. 107: 1685–1692, 2009.

Nominating editor Jerry Dempsey
jdempsey@wisc.edu

Question: Does nitric oxide (NO) contribute to recovery of blood flow during acute hypoperfusion?

Background: The contraction of a skeletal muscle evokes an increase in blood flow that is proportional to the force generated by the contraction. When hypoxic conditions exist in an active muscle, due to conditions such as ischemia or hypoperfusion, the accumulation of metabolites induces either an increase in blood pressure or vasodilation to restore blood flow. Casey and Joyner previously reported restoration of blood flow in contracting skeletal muscle following empirically induced hypoperfusion. Their findings suggest that the restoration of blood flow was due to vasodilation. The current report explored the role of NO in this compensatory vasodilation.

Observations: Importantly, Casey and Joyner utilized the same experimental protocol to measure forearm blood flow (FBF) before, during, and after a balloon was inflated in the subject's brachial artery to induce hypoperfusion (this methodology eliminates the possible confounding affects of previous approaches that also caused venous obstruction). They found that NO plays an intensity-dependent role in the compensatory vasodilation that occurs in contracting muscles subjected to hypoperfusion. In addition, administration of a NO synthase (NOS) inhibitor into the brachial artery delayed the vasodilator response caused by hypoperfusion during muscle contraction.

Significance: These novel findings are the first to demonstrate that NO contributes to the marked forearm skeletal muscle vasodilation seen during exercise stress in humans. However, despite NOS inhibition, recovery of FBF was still substantial (77%). Therefore, as the authors note, they expect that additional vasodilator mechanisms are involved in the recovery of FBF. Future studies are necessary to determine these additional mechanisms and whether NO is released from autonomic nerves or vascular endothelium. Nevertheless, these findings have important implications for understanding conditions associated with endothelial dysfunction or reduced NO bioavailability.

Genome-wide RNAi screen identifies Letm1 as a mitochondrial Ca^{2+}/H^{+} antiporter. Jiang D, Zhao L, Clapham DE. *Science* 326: 144–147, 2009.

Nominating editor Michael Caplan
michael.caplan@yale.edu

Question: What is the molecular identity of the mitochondrial Ca^{2+}/H^{+} exchanger?

Background: Mitochondria are the primary energy-producing organelles found in most eukaryotic cells and are structurally defined by a solute-permeable outer membrane and an inner membrane that is compartmentalized into cristae. Mitochondria actively sequester Ca^{2+} and therefore have extrusion and uptake mechanisms. Although Ca^{2+} readily diffuses through the large pores of the outer membrane, it can only traverse the inner mitochondrial membrane via a Ca^{2+} uniporter, a Na^{+}/Ca^{2+} exchanger, or a Ca^{2+}/H^{+} exchanger. The uniporter mediates the uptake of Ca^{2+}, whereas the exchangers function to extrude Ca^{2+}. However, despite decades of research, the molecular identity of these inner membrane proteins remained unresolved, in part because of confounds associated with previous approaches.

Observations: To circumvent limitations of other techniques, Jiang et al. first performed RNA interference (RNAi)-based genome-wide screens to identify Ca^{2+} transport proteins in mitochondria. Utilizing *Drosophila* S2 cells that expressed the mitochondrial Ca^{2+} and H^{+} detector pericam (a chimeric fluorescent protein that simultaneously detects Ca^{2+} and H^{+}), they identified a homolog of the human *Letm1* gene that fulfilled the criteria. Subsequent experiments characterizing the functional role and transport mode of the Letm1 protein determined it was dependent on transmembrane voltage, pH gradient, and cytosolic Ca^{2+}, all of which support the idea that Letm1 is a mitochondrial Ca^{2+}/H^{+} exchanger in mammalian cells.

Significance: These findings reveal the unusual properties of Letm1, a novel antiporter that mediates the slow uptake of one Ca^{2+} ion in exchange for one H^{+} ion. Thus, when submicromolar increases in cytosolic Ca^{2+} concentrations result in greater levels of Ca^{2+} in the cytoplasm than in a mitochondrion (due to either Ca^{2+} release from intracellular stores or Ca^{2+} entry from the extracellular milieu), Letm1 drives Ca^{2+} entry and H^{+} efflux. Decades in the making, this represents a significant step toward elucidating the molecular identification of all the proteins involved in mitochondrial Ca^{2+} homeostasis. Although several challenges remain, including elucidation of the molecular identity of the Ca^{2+} uniporter and clarification of whether the transporters are differentially expressed and interact within a mitochondrion, this report will facilitate the development of selective pharmacological inhibitors of this Ca^{2+}/H^{+} exchanger.

The normal mechanisms of pregnancy-induced liver growth are not maintained in mice lacking the bile acid sensor Fxr. Milona A, Owen BM, van Mil S, Dormann D, Mataki C, Boudjelal M, Cairns W, Schoonjans K, Milligan S, Parker M, White R, Williamson C. *Am J Physiol Gastrointest Liver Physiol*. doi:10.1152/ajpgi.00336.2009.

Nominating editor P. Kay Lund
empk@med.unc.edu

Question: What is the mechanism of pregnancy-induced liver enlargement in rodents?

Background: During the normal course of pregnancy, alterations occur in a number of metabolic indexes to meet the demands of the growing fetus, such as levels of insulin, growth hormones, and caloric intake. These metabolic alterations are thought to contribute to maternal hepatomegaly in rodents (an enlargement of the liver), although cause and effect has not been proven. Interestingly, bile acids are necessary for liver regeneration following partial hepatectomy. In addition, bile acids are elevated during pregnancy, although in a tightly controlled manner since long-term elevation of bile acids can result in hepatocellular carcinoma. However, the potential role of bile acids in the cellular mechanisms and signaling pathways that underlie hepatomegaly are not known.

Observations: Milona et al. first characterized pregnancy-induced liver growth and found that it occurred independently of gains in maternal body weight but was proportional to the number of fetuses.

Subsequently, they determined that the hepatomegaly was due to hypertrophy and not hyperplasia. In addition, they found that elevated reproductive hormones did not play a role, whereas increased levels of serum bile acids did have a role in the hepatomegaly. Finally, mice lacking the primary bile acid receptor farnesoid X receptor (FXR) did not display the pregnancy-associated increase in hepatocyte size as wild-type mice.

Significance: This paper makes a novel link between bile acids and the FXR, rather than reproductive hormones, as mediators of pregnancy-induced hepatomegaly. Although humans do not exhibit hepatomegaly during pregnancy, they do face enhanced hepatic metabolic demand. Therefore, these findings have translational relevance as they suggest a role of bile acids and the FXR in meeting the increased metabolic demand of pregnancy. In addition, a more detailed analysis of the signaling mechanisms and pathways that affect bile acid homeostasis may prove beneficial for understanding the etiology of diseases associated with pregnancy, such as obstetric cholestasis, which is associated with FXR mutations.

Spinal endocannabinoids and CB1 receptors mediate C-fiber-induced heterosynaptic pain sensitization. Pernía-Andrade AJ, Kato A, Witschi R, Nyilas R, Katona I, Freund TF, Watanabe M, Filitz J, Koppert W, Schüttler J, Ji G, Neugebauer V, Marsicano G, Lutz B, Vanegas H, Zeilhofer HU. *Science* 325: 760–764, 2009.

Nominating editor Julie Kauer
julie_kauer@brown.edu

Question: What role do endocannabinoids play in secondary pain amplification (hyperalgesia)?

Background: The nociceptors of unmyelinated slow conduction C fibers, whose cell bodies are located in the dorsal root ganglion, transmit burning throbbing pain sensations. Following inflammation, neuropathy or intense nociceptive input, primary and secondary (touch-evoked pain surrounding the primary site) pain amplification can occur through various mechanisms; however, the mechanism of activity-dependent secondary hyperalgesia is not well defined. Thus, in contrast to primary hyperalgesia, secondary hyperalgesia is not associated with alterations in peripheral sensitivity. Building on the knowledge that endocannabinoids (eCBs) decrease GABA inhibition when released and that CB1 receptors (the targets of eCBs) densely populate the dorsal horn, Pernia-Andrade et al. sought to determine the role of eCBs in secondary hyperalgesia.

Observations: The authors first determined that activation of CB1 receptors in dorsal horn neuronal circuits inhibited $GABA_A$ receptor- and glycine-mediated inhibitory postsynaptic currents, a well characterized phenomenon known as depolarization-induced suppression of inhibition (DSI). They then used electron microscopy to demonstrate the presence of CB1 receptors on the presynaptic terminals of inhibitory dorsal horn neurons. Subsequently, they found that secondary hyperalgesia induced by intense nociceptive stimulation was inhibited by a CB1 receptor antagonist and absent in global as well as inhibitory interneuron-specific CB1 receptor-deficient mice.

Significance: These results suggest that eCBs are released in the dorsal horn during intense nociceptor stimulation and act on dorsal horn CB1 receptors to promote activity-dependent secondary hyperalgesia. Therefore, it appears that the DSI following the release of eCBs prevents the inhibitory interneurons from separating touch and pain circuits. Although these findings provide a novel explanation for touch-evoked pain following a peripheral injury, they are somewhat surprising since, until now, endogenous and exogenous CB1 receptor agonists have been associated with pain relief (although analgesic action of CB1 receptor antagonists has been reported previously). Nonetheless, identification of a compound that selectively targets peripherally expressed CB1 receptors may prove beneficial to those suffering from chronic pain and circumvent untoward side effects associated with central CB1 receptors. ■