molecules to coincide with M current suppression. Although demanding in terms of the amount of experimental information needed, a similar quantitative modeling system will prove useful in predicting and elucidating other signaling cascades of complex in vivo systems.


Question: Can a complex signaling pathway be quantitatively modeled?

Background: The muscarinic acetylcholine receptor (mACHR) modulates the "M current," a neuronal K⁺ conductance, which activates the G₄/₁₁ family of G proteins. This induces a cascade of intracellular events that do not use PKC or Ca²⁺ as the primary mediators for signaling muscarinic inhibition of the M current. Some observations implicate membrane transport proteins, such as phosphatidylinositol 4,5-bisphosphate (PIP₂), as potent regulators of PLC, and it has been hypothesized that its depletion may be the second messenger signal of the muscarinic receptor.

Observations: Suh et al. found, as others have, that G₄ signaling can be described by the scheme developed for G₄ and G₄/₁₁, and that the extent of activation was dependent on the balance between formation of nucleotide-free G protein, followed by binding of GTP and Mg²⁺, versus the hydrolysis of GTP to GDP. When cast as a kinetic model in a virtual cell-modeling program, many aspects of the observations could be explained within a coherent framework.

Significance: There are few precedents in attempting to construct a complex signaling pathway with an unusual hybrid of biochemistry and electrophysiology and a detailed kinetic model. This modeling is very important because it successfully explains some experimental observations that initially were surprising, for example that suppression of current continues after agonists are removed. Moreover, the model successfully predicted empirical observations, including perturbations of the system with ions, analogs, and mutants and that occupancy of mACHRs activates enough G proteins to hydrolyze enough PIP₂
findings was that a significant oncotic pressure difference could still be generated across the microvascular wall even when the vascular and tissue concentrations of albumin were equivalent.

**Significance:** The fact that tissue protein concentrations contributed less to fluid balance than would be expected from the Starling equation provides evidence for an osmotic asymmetry across the endothelial barrier. This elegant study supports a novel filtration model that can account for the inability to sustain fluid reabsorption across the capillary wall as well as the apparent discrepancy in net fluid filtration and lymph formation. Hence the gradient that determines fluid movement is not between blood and tissue but between blood and the immediate environment of the cleft, resolving the paradox associated with the Starling equation. This will undoubtedly revolutionize the philosophy and thinking concerning tissue fluid balance.


**Editor, Physiological Genomics**

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**Question:** Can viral vector manipulation of the tightly restricted gene-modification system Cre-loxP serve to elucidate the pathophysiological processes of the central renin-angiotensin system (RAS)?

**Background:** The multigene RAS that coordinates central cardiovascular and body fluid homeostasis is made up of multiple cell types and molecules. Because the RAS has complex regional and cellular expression patterns, it has proven difficult to dissect the functional contribution of a single protein in a specific cell type.

Understanding central nervous system (CNS) regulation of the cardiovascular system through the Cre-loxP method of gene modification has been problematic due to a lack of brain region-specific promoters. However, viral vectors have recently been reported as an efficient and selective technique for targeting gene delivery to discrete brain nuclei.

**Observations:** The in vitro and in vivo delivery of Cre recombinase from two recombinant viral vectors [adenovirus-Cre (Ad-Cre) and feline immunodeficiency virus-Cre (FIV-Cre)] induced selective gene recombination in key cardio-vascular networks of the CNS. It was determined that Ad-Cre was susceptible to retrograde transport and induced recombination in neuronal and non-neuronal cells, whereas FIV-Cre-induced recombination was localized to neuronal cells.

**Significance:** These findings establish the feasibility of elucidating the physiological processes of RAS genes in maintaining fluid and cardiovascular homeostasis by inducing differential cell-selective gene deletions through direct, discrete microinjections into cardiovascular regulatory nuclei or lateral ventricles or by the retrograde transport of the Ad-Cre virus to secondary neuronal nuclei. The ability to control the onset of gene recombination, along with the time- and resource-saving aspects of this technique, represents a significant advancement in the tools required to illuminate the functional roles of RAS genes. Improved medications that target discrete nuclei are certainly foreseeable as research exploits these methodologies.


Nominated by Reiko Fitzsimonds

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**Question:** What is the functional relevance of the protein-protein interactions between synaptotagmin and t-SNAREs in the process of exocytosis?

**Background:** The microsecond time scale of neurotransmitter release from a nerve terminal to the extracellular milieu involves the Ca2+-dependent fusion of synaptic vesicles with the presynaptic plasma membrane. The synaptotagmin I protein (syt) is a vesicular Ca2+ sensor hypothesized to interact directly with a set of proteins essential for intracellular membrane-fusion events (soluble N-ethylmaleimide-sensitive factor attachment protein receptors, or SNAREs). SNAREs can be subdivided into t-SNAREs [the membrane-targeting proteins syntaxin and synaptosome-associated protein of 25 kDa (SNAP-25)] that form homo- and heterodimers, and v-SNAREs (the vesicle-associated protein synaptobrevin). Although several biochemical studies have demonstrated a Ca2+-dependent interaction between syt and t-SNAREs, it is unclear what functional relevance this has on the opening and dilation of fusion pores, i.e., if these molecular events happen on a time scale amenable with excitation-secretion coupling events.

**Observations:** Ca2+-dependent interactions were observed between the various dimers of the t-SNAREs and syt. This Ca2+-triggered interaction between syt and the t-SNAREs occurred on a millisecond time scale and involved conformational changes to the syt-SNAP-25 complex. Moreover, by modulating these protein-protein interactions in a Ca2+-independent manner, Bai et al. were able to provide evidence that change in t-SNARE binding activity correlates with exocytosis rates.

**Significance:** These data demonstrate that the process of Ca2+-triggered exocytosis is regulated by interactions between syt and the t-SNAREs. Kinetic analysis suggests that disruption of these interactions favors the closed state of a fusion pore. This fundamental physio-logical event of exocytosis is of universal interest and importance to researchers and may have particular relevance to those investigating the pathology of seizures.
linked to the Gq pathway. In addition, the GPR99 receptor was exclusively sensitive to the Gi/Go pathway and a pertussis toxin-insensitive role. A pertussis toxin-induced receptor internalization. The stimulation of both GPR91 and GPR99 receptors, which are linked to various signaling pathways, including regulation of CAMP and activation of the GTPase RhoA. Serum response factor (SRF) is a key regulator of the proliferative phenotype of VSMC. The synthetic phenotype is characterized by decreased CAMP production, increased rates of proliferation, and migration, resulting in thickening of the medial layer of blood vessels. A growing literature documents the key importance of extracellular ATP as a modulator of vascular smooth muscle cells (VSMCs) changes from contractile to synthetic. The synthetic phenotype is characterized by a decrease in the expression of cytoskeletal proteins and increased rates of proliferation and migration, resulting in thickening of the medial layer of blood vessels. A growing literature documents the key importance of extracellular ATP as a modulator of vascular smooth muscle cells (VSMCs) changes from contractile to synthetic. The synthetic phenotype is characterized by a decrease in the expression of cytoskeletal proteins and increased rates of proliferation and migration, resulting in thickening of the medial layer of blood vessels.

Regulation of blood flow to tissues, particularly during ischemia or with tissue injury. Indeed, succinate induces hypertension in rats, an effect that involves the RAS and is mediated by the GPR1. Perhaps most exciting about linking cellular metabolism with blood pressure is the possible facilitation of developing novel therapeutic interventions for cardiovascular disorders by specifically targeting these ligands and/or their receptors.


**Significance:** Varying concentrations of ATP can occur in the extracellular environment as a function of cell injury, autonomic nervous system activation, or platelet activation or with deformation of the cell surface. These results define what may be a key mechanism in the modulation of phenotype and function of VSMCs in health and disease states, such as atherosclerosis, neointimal proliferation, and transplant rejection.


**Question:** How do alterations in extracellular ATP result in the phenotypic modulation of vascular smooth muscle cells?

**Background:** Under the pathological conditions of certain cardiovascular diseases, the phenotype of vascular smooth muscle cells (VSMCs) changes from contractile to synthetic. The synthetic phenotype is characterized by a decrease in the expression of cytoskeletal proteins and increased rates of proliferation and migration, resulting in thickening of the medial layer of blood vessels. A growing literature documents the key importance of extracellular ATP as an agonist that regulates the plasma membrane P2 (nucleotide, purinergic) receptors, which are linked to various signaling pathways, including regulation of CAMP and activation of the GTPase RhoA. Serum response factor (SRF) is a transcription factor activated by a RhoA-dependent mechanism and implicated in the proliferative phenotype of VSMC. The intermediate steps and role of the cAMP pathway in this sequence of events were heretofore unknown.

**Observations:** In their study, Hogarth et al. demonstrate a striking concentration-dependent effect of ATP on VSMC, whereby low concentrations of ATP promoted expression of the contractile phenotype and higher ATP concentrations promoted expression of the synthetic phenotype. Key to this shift was an ATP-mediated, transient activation of PKA, which inhibited the activation of SRF. The duration and extent of ATP-induced PKA activation appears to contribute to the bidirectional effects of the nucleotide on VSMC phenotype.

**Question:** Are extracellular tricarboxylic acid (TCA) cycle intermediates physiologically active ligands for orphan G protein-coupled receptors?

**Background:** The mitochondrial TCA cycle contributes to cell metabolism by extracting high-energy electrons for the generation of ATP, i.e., ensuring energy homeostasis. Although the intermediates of the TCA cycle exist in micromolar concentrations in blood, their potential roles as specific receptor protein ligands are unexplored.

**Observations:** The ability of TCA intermediates to evoke an intracellular increase in Ca2+ concentrations was used to identify potential natural ligands for orphan G protein-coupled receptors (GPCR). Two compounds were identified, and mass spectrometry results coupled with in vitro and biochemical assays confirmed that succinic acid is a selective ligand for the orphan GPCR, GPR91, and that α-ketoglutarate is a selective ligand for GPR99. Examination of the signaling pathways induced by succinate binding to GPR91 revealed both a pertussis toxin-sensitive Gq/Gi pathway and a pertussis toxin-insensitive Gq pathway, whereas the GPR99 receptor was exclusively linked to the Gq pathway. In addition, stimulation of both GPR91 and GPR99 induced receptor internalization. The physiological function of succinate was determined by administering it to rats. It activated plasma renin and dose-dependently increased arterial pressure. This effect could be abolished pharmacologically, genetically (GPR91 knockout mice), or by nephrectomy.

**Significance:** The TCA cycle intermediates succinic acid and α-ketoglutarate are natural ligands for GPR91 and GPR99, respectively. These GPCRs could provide a link between local metabolism and tissue-specific activity of the renin-angiotensin system (RAS), providing a mechanism for regulation of blood flow to tissues, particularly during ischemia or with tissue injury. Indeed, succinate induces hypertension in rats, an effect that involves the RAS and is mediated by the GPR1. Perhaps most exciting about linking cellular metabolism with blood pressure is the possible facilitation of developing novel therapeutic interventions for cardiovascular disorders by specifically targeting these ligands and/or their receptors.
country skiers were simultaneously monitored for changes in blood flow to the arms and legs, intra-arterial blood pressure, and cardiac output during three different exercise movements: combined arm and leg, arm-only, or leg-only exercises. A muscle’s vasodilatory response was found to adjust according to its demand for oxygen, and the arm muscles were less efficient than the legs at extracting oxygen from the blood and therefore received greater blood flow. In addition, the combined maximal vascular conductance of arms and legs was greater than the output capacity of the heart.

**Significance:** The most significant discovery from these studies is that during heavy exercise, blood flow (and therefore oxygen uptake) to active muscles is restrained to prevent hypotension. This suggests a compensatory vasoconstriction in some of the active muscles induced by the sympathetic nervous system. Therefore, as previously theorized, blood flow is managed in the context of blood pressure regulation.

Arvanian VL, Bowers WJ, Petruska JC, Motin V, Manuzon H, Narrow WC, Fedoroff HJ, and Mendell LM. Viral delivery of NR2D subunits reduces Mg²⁺ block of NMDA receptor and restores NT-3-induced potentiation of AMPA/kainate responses in maturing rat motoneurons. *J Neurophysiol* (May 19, 2004). 10.1152/jn.00278.2004

Nominated by Eve Marder
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**Question:** Can enhancement of a specific N-methyl-D-aspartate (NMDA)-glutamate receptor (NMDAR) subunit restore properties characteristic of younger animals to spinal cord motoneurons?

**Background:** Monosynaptic inputs of rat spinal cord motoneurons are subject to many neurotrophin-induced changes that occur during late embryonic and early postnatal stages. Some of the responses of these motoneurons are mediated by NMDARs, but their sensitivity declines in the first few weeks of life because of an increasing Mg²⁺ blockade. Besides the knowledge that the NR2D subunit of the NMDAR confers resistance to this Mg²⁺ blockade, little else is understood about this arrangement.

**Observations:** Using electrophysiology, gene chip analysis, RT-PCR, and immunocytochemistry, Arvanian et al. demonstrated that NMDARs become subjected to a Mg²⁺ blockade between embryonic day 18 and the second postnatal week because of a concurrent downregulation of the NR2D subunits of NMDAR. The delivery of the NR2D subunit, via viral vectors, resulted in a decrease of the motoneuron’s sensitivity to Mg²⁺, potentiation of responses (increased excitatory postsynaptic potentials), and increases in the magnitude of the NMDAR-mediated response.

**Significance:** Most notable was the finding that the Mg²⁺ blockade of NMDARs associated with postnatal development and driven by a decrease in NR2D expression was prevented by the enhancement of NR2D subunits. Artificially enhancing motoneuron NR2D protein levels reduced the Mg²⁺ blockade and restored NMDAR function in motoneurons from postnatal day 12 levels to levels observed at embryonic day 18. This ability to extend the critical period for synaptic plasticity by using this approach might be useful for enhancing the function of surviving fibers after a spinal cord injury.


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**Question:** Can a high-resolution fluorescence imaging system be developed that allows access to the deep recesses of the brain in vivo?

**Background:** The imaging of fluorescent probes tagged with specific molecular markers enables scientists to analyze biochemical signaling pathways, map gene and protein expression patterns, determine cellular morphologies, and visualize neuronal dynamics. However, issues surrounding the accessibility of many neurons in vivo have limited the use of fluorescence imaging techniques to superficial tissues or in vitro preparations.

**Observations:** A one-photon microendoscope (optical fiber) was developed that allows the user to image individually labeled cells, including the ability to make video-rate (30-frames/s) movies of cerebral blood flow and red blood cell dynamics. A two-photon microendoscope was also developed that allows the optical sectioning of tissue deep within the brain.

**Significance:** Complementary one- and two-photon fluorescence microendoscopes were developed that facilitate the visualization of cellular-level fluorescence imaging in vivo, providing the user with resolution on a micrometer scale without grossly disrupting overlying tissues. The ability to visualize a wide variety of cell types deep within individual vessels of the living rodent brain by using the one-photon microendoscope will be an invaluable tool to study cellular dynamics with video-rate time resolution. The two-photon endoscope provides threedimensional sectioning and better lateral optical resolution than the one-photon endoscope, allowing a scientist to isolate individual signals from dendrites. These methodological advancements will open new and exciting possibilities for researchers in evaluating the in vivo physiology of cells on a micrometer scale.

Rivard B, Li Y, Pierre-Pascal LS, Poucet B, and Muller RU. Representation of objects in space by two classes of hippocampal pyramidal cells. *J Gen Physiol*. In press.

Nominated by Olaf Anderson, Editor, *Journal of General Physiology*
Communicated by Angus Nairn
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**Question:** Are barriers in an environment represented by separate classes of hippocampal pyramidal cells?

**Background:** The cognitive map theory postulates that the hippocampus is the center of a memory system that provides the spatial framework for everyday
situations. The identification of “place cells,” cells that correspond to an animal’s location in a given environment, supports this theory. Although the ability of barriers/objects to influence the firing of place cells has been known for some time, the manner of cellular representation for these barriers/objects is vague.

**Observations:** Electrodes recorded from single CA1 pyramidal cells in rat hippocampus and place fields were determined in an environment with reference to a barrier (near or far with respect to the barrier). When the barrier was fixed in position, all of the cells appeared to be ordinary place cells. However, when the barrier was relocated the far place fields were unaffected but the near place fields were relocated in such a way that they were apparently coupled with the barrier. Similarly, when the barrier was removed, these cells stopped firing. Finally, if the barrier was put into a novel environment, place cell activity was remapped but the barrier-associated cells continued to correspond to that barrier in the new environment.

**Significance:** This demonstration implies that, in addition to hippocampal cells representing spatial locations, a portion of these cells represents objects within that same space. Place fields are remapped when an animal is put into a novel environment; interestingly, Rivard et al. found that there was no remapping of barrier cells, suggesting that their role was the identification of the barrier rather than representation of the environment. Thus the combined place and barrier cell activity of the hippocampus appears to pragmatically imitate an environment and objects in that environment.

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**Question:** What is the molecular basis for interactions between the cystic fibrosis transmembrane conductance regulator (CFTR) and members of the SLC26 transporter family, and do these interactions regulate CFTR activity?

**Background:** Cystic fibrosis (CF) is a genetic disorder attributed to inherited mutations of a gene for a chloride channel (i.e., CFTR) that plays a critical role in epithelial chloride transport. Recent studies have shown that CFTR also plays a crucial role in bicarbonate-driven fluid secretion in epithelia (e.g., pancreatic duct) by activating electrogenic chloride/bicarbonate exchange that is mediated by bicarbonate transporters of the SLC26 family. Before this study, it was not known how CFTR interacts with the SLC26 transporters and whether the interaction influences CFTR activity.

**Observations:** Using molecular, biochemical, and electrophysiological techniques in cultured and native epithelial cells, the authors demonstrate physical and functional interactions between CFTR and two SLC26 transporters (SLC26A3 and SLC26A6). The CFTR regulatory (R) domain binds to the sulfate transporter and antisigma antagonist (STAS) domain of SLC26. This association is facilitated by phosphorylation of the R domain and binding of the two transporters to PDZ scaffolding proteins. The authors also demonstrate a reciprocal regulation of CFTR activity, in which expression of SLC26 (or just its STAS domain) enhances CFTR activity via a gating effect.

**Significance:** The results identify where the molecular interactions between CFTR and SLC26 transporters occur and how the interactions influence CFTR function. The authors also propose a model that relates how these protein-protein interactions can be responsible for the characteristic high bicarbonate and low chloride concentrations in pancreatic secretions and how disruptions of this interaction may contribute to some of the symptoms associated with CF. Ultimately, the results from this study provide a new level of understanding of the mechanisms responsible for the pathologies of CF and should provide new strategies for future therapies.

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Nominated by Stewart Sage, Chair, Editorial Board, *Journal of General Physiology*

Communicated by Quentin Pittman

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**Question:** What effect does a bacteria-free environment have on stress-related physiological functions of adult mice, and are these effects reversible?

**Background:** There are more bacteria in the gastrointestinal tract than there are eukaryotic cells in the human body. In fact, the symbiotic relationship between postnatal bacterial colonization and the host is well documented. In addition, although some early life events have long-lasting effects on stress responses, it is not known whether bacterial colonization of a host is fundamental for the appropriate physiological responses to stress.

**Observations:** After ensuring that maternal interactions were similar, mice raised in a germ-free (GF) or specified pathogen-free environment were moni-tored for a number of different behavioral, endocrinological, and morphological changes associated with exposure to an emotional stressor (confinement). GF mice displayed an enhanced hypothalamic-pituitary-adrenal (HPA) response to stress, including elevated plasma corticosterone and ACTH and regionally selective alterations in corticotropin-releasing factor, glucocorticoid receptors, glutamate receptor subunits, and brain-derived neurotrophic factor expression in brain. These effects could be reversed by inoculating the gut with an innocuous flora, but only if this were done at an early age. Successful re colonization manifested as c-fos activation in the paraventricular nucleus and a concomitant increase in plasma corticosterone; however, there was no correlation between c-fos activation and plasma cytokines. In contrast, neither stressor induced differential effects between groups, suggesting a cognitive component to the exaggerated HPA response in GF mice.
**Significance:** These findings clearly show that visceral signals affect brain processes and that this visceral information is transmitted to the brain, at least in part, through a humoral cytokine-independent pathway. These data also indicate that even innocuous gut flora can alter the brain in a way that can have an impact on diverse neuronal functions. This leads to the clinically important possibility that neonatal infection with pathological bacteria may alter the development of neural systems that govern the endocrine response to stress and thereby predispose the individuals to stress-related pathologies later in life.


Nominated by Ulrich Pohl
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**Question:** Is there an association between the genes expressed during cellular activation and the phases of the cell cycle?  

**Background:** Cell proliferation is controlled by the transient expression of cell cycle-specific genes coordinated with cell cycle phases. The pathologies of proliferative cardiovascular diseases are associated with a change in the cell cycle of vascular smooth muscle cells (VSMCs). In fact, development of atherosclerotic plaques is associated with changes of VSMC phenotypes. These cells, which contribute to the deleterious formation of blood vessels (neointima), are in a state of “activation.” This change in phenotype goes along with the expression of adhesion molecules, which may be directly implicated in the development of cardiovascular diseases because they are observed in human plaques and in animal models of atherosclerosis. Recent studies purport that vascular cell cycle arrest inhibits proliferation and neointima formation and ameliorates changes in vascular cell phenotype, but whether this proangiogenic effect involves the modulation of adhesion molecules is undetermined.

**Observations:** VSMC stimulation induced a proliferative response with no concurrent change in gene expression; however, when the VSMC activator TNF-α was introduced there was robust up-regulation of adhesion molecules. TNF-α alone or stimulation plus TNF-α induced VSMC proliferation and gene expression, although the kinetics of this induced expression was divergent. Delving further into the mechanics of this phenomenon revealed that the time-associated resistance to TNF-α-induced effects was related to the stage of the cell cycle, i.e., gene expression was prevented during the G, and S phases of the cell cycle.

**Significance:** The inability of TNF-α to stimulate adhesion molecule expression during protein synthesis phases indicates that the activities during progression of the cell cycle are highly organized. The cell cycle then is not only important for proliferation but represents a system that can be modulated to express a particular cellular phenotype. As cell cycle regulation and control of other cellular processes are exposed, so will novel preventions and treatments for vascular proliferative diseases as well as other pathologies be developed.


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**Question:** Does hyperglycemia, by activating the antiangiogenic protein angiostatin, regulate the development of blood vessels that bypass arterial obstructions?  

**Background:** An adaptive response of blood vessels to mitigate myocardial ischemia is to develop bypass (coronary) collaterals capable of providing normal blood flow to distal vascular sites. Formation of coronary collaterals involves the proliferation of capillaries in the ischemic area (angiogenesis) and/or maturation/recruitment of preexisting collateral vessels (arteriogenesis). A dysfunctional collateral response is associated with the morbidity and mortality of cardiovascular diseases such as myocardial infarction, stroke, and diabetes. Pharmacologically manipulating the growth inhibitor angiostatin or the gelatinase metalloproteinase-9 (MMP-9; responsible for angiostatin formation) inhibits coronary angiogenesis. However, the role of angiostatin in regulating coronary collateralization during hyperglycemic conditions is poorly understood.

**Observations:** Coronary collateral development was promoted by repetitive ischemic stimuli in hyperglycemic and control dogs. Several hemodynamic markers of coronary collateral development were unaffected, whereas MMP-9 activity and angiostatin expression were upregulated in the myocardial interstitial fluid (MIF) of hyperglycemic animals. In addition, the MIF from the hyperglycemic dogs did not induce cell proliferation or angiogenesis in vitro.

**Significance:** These results suggest that hyperglycemia may impair the formation of coronary collaterals because of increased MMP-9 activity and angiostatin expression. This mechanistic dysfunction may be responsible for the increased morbidity and mortality rates of patients with diabetes and coronary artery disease. This research offers several new approaches for pharmacologically controlling the hyperglycemia-induced attenuation of coronary collateral development; in particular, the control of antiangiogenic factors is a new option.

Nominated by Pontus Perrson, Editor, American Journal of Physiology-Regulatory, Integrative, and Comparative Physiology
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Question: Are genes associated with circadian rhythms involved in the homeostatic regulation of sleep?

Background: The suprachiasmatic nucleus (SCN) is the center of the circadian system, where a set of genes [3 period (mPer 1, 2, and 3) and 2 cryptochrome (mCry 1 and 2) genes] interact to regulate the timing of sleep and wakefulness. There is some evidence supporting the theory that circadian clock genes regulate the duration of sleep/wakefulness periods. However, other homeostatic factors are hypothesized to determine the amount of time spent sleeping, because lesions of the SCN do not alter time spent sleeping.

Observations: Various mPer mutant mice were monitored during various sleep/wakefulness conditions and light-dark cycles for electroencephalogram activity, muscle activity, and temperature. The mPer 2 gene mutation affected the timing of wake/sleep periods and temperature peaks; mPer 1- and mPer 3-deficient mice were similar to wild-type mice. However, mPer genes were not integral to the regulation of sleep/wakefulness periods, nor did their mutations affect homeostatic sleep responses.

Significance: No genotype-specific alterations were observed in the homeostatic regulation of daily amounts of waking, slow wave sleep, or REM sleep. These data suggest that the daily cycling of sleep-wake periods is more susceptible to cellular metabolic demands than circadian rhythms. However, exploitation of this arrhythmic rodent model, which requires the same amount of sleep as wild-type mice, will help to circumvent the confounding influence of circadian rhythms on activity when evaluating homeostatic influences on sleep. Moreover, these findings may have relevance to sleep disorders, in particular familial advanced sleep phase syndrome, which is associated with a Per2 gene mutation in humans.