iting. If the e37 region contributes to an interface that binds the N channel to other proteins, there is reason to be optimistic about both these questions.

Targeting a nociceptor-specific calcium channel is not the only strategy for making a calcium antagonist that is analgesic. The other is to block N channels only when they are hyperactive. This should selectively relieve intense, persistent pain because it is then that calcium channels are most active on nociceptors. The devil is in the kinetic details of the channel and of the putative drug, but the concept of state-dependent block is central to drug design against ion channels, in general, and is the basis of the most widely used calcium channel antagonists, dihydropyridines (Triggle, 1999). These block L-type calcium channels on vascular smooth muscle, thereby relaxing blood vessels and making it easier for a weak heart to perfuse them. The problem is that cardiac and smooth muscle calcium channels are virtually identical and the heart stops if its calcium channels are blocked. Preferential block occurs only because smooth muscle has a lower rest potential than cardiac muscle, and this puts its calcium channels into a gating state to which dihydropyridines bind with particular strength. Thus, state-dependent binding turns this particular drug from lethal to useful.

There is a holy grail of analgesic drug design. It is not just to inhibit pain without side effects; it is to inhibit *pathological* pain without side effects. Acute pain is useful because it tells us of danger. But pain becomes counterproductive when it is excessive, lasts long after an injury, or becomes completely dissociated from sensory input. The success of calcium channel blockers in the cardiovascular field, the possibility of making state-dependent blockers against neuronal calcium channels, and this new evidence for nociceptor-specific calcium channel variants are all reasons for enthusiasm about creating useful analgesics by targeting the N-type calcium channel.

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A Constraint on cAMP Signaling

Studies in invertebrates and vertebrates have demonstrated a critical role for cAMP signaling and adenylyl cyclase (AC) activity in learning and memory. In this issue of *Neuron*, Pineda et al. show that in the hippocampus, reduction of AC activity via the inhibitory G protein G_i is critical for memory formation, suggesting that a balance of inhibitory and stimulatory regulators of AC is required for optimal cAMP signaling.

Neuronal signaling is regulated by multiple pathways that convey signals through cells independently and/or via cross-talk with neighboring pathways. The cAMP-dependent pathway is a ubiquitous communication device within neurons that is extremely efficient, versatile, and multimodal, and is able to integrate two major cellular messengers, cAMP and Ca^{2+}. Because cAMP-dependent signaling is so prominent and essential to cells, multiple means of regulation have evolved to insure its proper functioning. One layer of control is at the level of adenylyl cyclase (AC), the first enzyme in the pathway that synthesizes cAMP from ATP, and that together with phosphodiesterase (PDE) regulates the turnover of cAMP. AC is controlled spatially by confinement to specialized cellular compartments such as caveolaes and lipid rafts and attachment to specific scaffolding proteins. Likewise, cAMP itself is confined to local microdomains in close vicinity with AC, PDE, and multiple cAMP-dependent effectors. Biochemically, the activity of AC is modulated by several regulators, which include inhibitory and activating G proteins and Ca^{2+}. The concerted action of these mechanisms of regulation provides a tight positive and negative control over cAMP signaling, which is critical for many brain functions including learning and memory. For instance, in *Aplysia*, *Drosophila*, or mice, inactivation or stimulation of AC, PDE, or the downstream cAMP-dependent protein kinases PKA or transcription factor CREB severely affects learning and memory.

A new article in this issue of *Neuron* (Pineda et al., 2004) provides evidence for an essential role of inhibitory G proteins (G_i) in the control of AC activity in learning and memory. By eliminating G_i activity through pertussis toxin injection, genetic knockout, or antisense oligonucleotides treatment, Victor Pineda and colleagues show

that a selective decrease in G_i increases cAMP signaling and enhances synaptic plasticity while impairing certain forms of memory. Specifically, the inhibition of $G_{i\alpha}$ by pertussis toxin in area CA1 of the hippocampus is shown to perturb passive avoidance and long-term memory for contextual information. Interestingly, this effect was specific to area CA1 and did not occur when $G_{i\alpha}$ was blocked in CA3. The negative impact of G_i blockade was confirmed by disruption of one or two copies of the $G_{i\alpha}$ gene in knockout mice. This genetic manipulation also impaired long-term retention of information on the passive avoidance and object recognition test. However, it did not perturb spatial learning and memory or the acquisition and the extinction of cued fear conditioning, suggesting a selective effect of $G_{i\alpha}$ disruption on some, but not all, forms of memory. Contrary to its inhibitory effect on memory, however, lack of $G_{i\alpha}$ facilitated synaptic plasticity and prolonged long-term potentiation (LTP). It turned a decremental form of LTP into a robust PKA- and protein synthesis-dependent form, indicating that an increase in cAMP strengthens synaptic plasticity.

These results are novel in that they demonstrate the existence of a tonic inhibitory constraint applied by G_i on cAMP production in the context of learning and memory. cAMP is therefore not only modulated by PDE-mediated degradation, but also by restraint on its synthesis via ACs. This inhibitory constraint seems to complement and not simply duplicate the action of PDE-dependent mechanisms because inactivating G_i or PDEs appears to have a different impact on cAMP signaling and learning and memory. Thus, spatial learning is altered by PDE1B knockout while it is intact in the $G_{i\alpha}$ mutant mice (Reed et al., 2002). Further, the inhibition of PDE4 by specific antagonists improves rather than impairs learning and memory (Barad et al., 1998; Bourtchouladze et al., 2003). These discrepant results suggest that PDEs may be differentially involved in learning and memory, similar to ACs, which are known to be distinctly regulated in specific brain areas depending on the type of learning (Guillou et al., 1999; Mons et al., 2003). Such specificity may partly account for the different impact of G_i inactivation in CA1 versus CA3, or on hippocampus-dependent versus -independent memory in the $G_{i\alpha}$ mutant mice.

However, the divergent impact of cAMP modulation in the different models could also be explained by a disparity in the extent, localization, or duration of the increase in cAMP. Quantitatively, it is recognized that optimal levels of cAMP are required for efficient learning and memory. While a mild increase in cAMP may enhance performance, a large increase may impair it. Further, the cellular compartment in which cAMP is released is of prime importance as it determines which downstream effectors are activated. Since cAMP has a low diffusion rate ($<1\ \mu m$) due to its compartmentalization, a change in its synthesis by local G protein-dependent transmembrane ACs is likely to affect mostly targets near or at the membrane. Many cAMP-dependent and cAMP binding effectors have been identified, and recently, several alternative and unconventional pathways have been discovered. For instance, direct cAMP binding is now known to regulate cyclic nucleotide-gated (CNG) channels such as pacemaker voltage-gated potassium channels or other channels involved

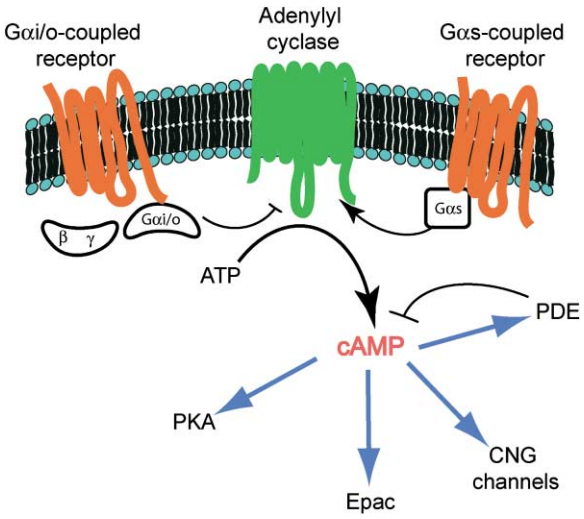

Figure 1. The Activation of G Protein-Responsive Transmembrane AC by $G_\alpha s$ and Their Inhibition by $G_{i\alpha}$ Control the Level of cAMP and the Recruitment of Downstream Effectors

Abbreviations: AC, adenylyl cyclase; CNG, cyclic nucleotide gated; Epac, exchange protein activated by cAMP; PDE, phosphodiesterase.

in the transduction of sensory signals. cAMP also controls a newly identified family of proteins, the Epacs (exchange protein directly activated by cAMP) (Bos, 2003). Epacs contain domains homologous to the guanine nucleotide exchange factors (GEFs), and cAMP-regulated GEFs selectively activate the Ras superfamily of small G proteins, Rap1 and Rap2. They can therefore initiate active cross-talk between the cAMP and the Ras/mitogen-activated protein kinase (MAPK) pathways, providing an additional layer in cAMP signaling. Several PDEs are known as well to be activated by cAMP and thereby contribute to the homeostatic regulation of cAMP. This diversity of targets makes cAMP a pleiotropic messenger whose action is therefore not limited to the control of PKA activity as initially thought (Figure 1). Differential modulation of these targets may explain the divergent phenotypes observed in the various pharmacological and genetic mouse models.

Finally, the $G_{i\alpha}$ mutant mice add to the list of mouse models in which opposite effects on memory and plasticity are observed. The most recent examples of a dissociation between memory/LTP phenotypes are mice lacking tropomodulin-2, syndecan-3, or Fmr2 in which LTP is enhanced but learning and memory are impaired (Gu et al., 2002; Cox et al., 2003; Kaksonen et al., 2003). Additional examples include mice with enhanced LTP but normal learning and memory (Jun et al., 1998) or mice with defective plasticity but improved learning (Collinson et al., 2002). Rather than shadowing the potential link between synaptic plasticity and memory, such dissociation highlights the existence of multiple forms of LTP whose relevance is likely specific to certain forms of learning and memory. The physiological environment during LTP recordings is also important, and LTP measures in the intact animal may best reveal changes correlating with behavior. Additional forms of plasticity like LTD and depotentiation may also be relevant.

In summary, great progress has been made in the understanding of cell signaling since the first discoveries of cAMP by Earl Sutherland and of G proteins and their role in signal transduction by Alfred Gilman and Martin Rodbell. Much progress still remains to be made, however, to fully uncover the mechanisms of cAMP-mediated pathways, a step that is essential to the elucidation of many brain pathologies.

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