CHAPTER

22

Cyclic Nucleotides in the Nervous System

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OUTLINE

INTRODUCTION: SECOND MESSENGERS

The mechanisms whereby extracellular agents, such as neurotransmitters and circulating hormones, can produce alterations in intracellular and nuclear processes have been intensively investigated for several decades. A seminal advance came in the late 1950s (Sutherland et al., 1992), when Earl Sutherland and his colleagues demonstrated that epinephrine induces glycogenolysis in the liver by stimulating the intracellular synthesis of adenosine 3′5′-cyclic monophosphate (cyclic AMP, or cAMP).

This finding led to the formulation of the 'second messenger hypothesis.' cAMP, and subsequently discovered intracellular signaling small molecules such as cyclic GMP (cGMP), Ca^{2+}, nitric oxide, and metabolites of inositol lipids and arachidonic acid, are called 'second messengers' because they communicate signaling by extracellular molecules such as neurotransmitters—the first messengers—to intracellular signaling mechanisms (Fig. 22-1). However, second messengers do not simply convey an extracellular message unchanged to intracellular effectors. They also provide spatial and temporal integration, gating and filtering, and state-dependent regulation of such signals, potently modulating a cell's response to receptor activation. This chapter reviews signaling by the cyclic nucleotide second messengers, cAMP and cGMP, in the nervous system. Other second-messenger signaling systems are reviewed elsewhere in this volume. We describe the synthesis and degradation of cyclic nucleotide second messengers, spatial and temporal regulation and integration of their signaling, and downstream cyclic nucleotide-regulated effector molecules.

ADENYLYL CYLCASES

Biochemistry of cAMP production

cAMP is formed from ATP through the action of adenylyl cyclases (ACs). The energy for this catalytic reaction derives

from the substrate ATP molecule, which is complexed with Mg^{2+}; pyrophosphate is released (Fig. 22-2). An additional divalent cation, beyond the Mg^{2+} that is complexed with the ATP, is required for this reaction. The result is a cyclic phosphodiester bond between the α phosphate group and the 3′ hydroxyl of the ribose moiety.

The mammalian genome contains multiple AC genes. Across phylogeny, ACs have been divided into six classes; all eukaryotic ACs belong to Class III (Kamenetsky et al., 2006). They are divided into two families, the nine membranous ACs (AC1-AC9) and the more divergent soluble AC (sAC). The membranous ACs are divided into four groups on the basis of phylogenic and biochemical similarity (Fig. 22-3).

The membranous ACs are embedded in the cell membrane by two discrete domains, each containing six transmembrane alpha helices (Fig. 22-4). There are two cytoplasmic lobes. The C1 lobe lies between the two membranous domains, while the C2 lobe is at the carboxy-terminus of the protein; C1 and C2 are roughly 40% homologous among all mammalian membranous ACs. Catalysis occurs in a crevice between subdomains of both lobes, the structurally similar C1a and C2a domains, which are >50% conserved among the various membranous ACs. C1a and C2a are both necessary for catalysis. X-ray crystal structure of the active domain shows critical side-chain interactions from both of these domains with the substrate ATP, with phosphate binding being coordinated by two divalent cations (typically Mg^{2+}), a hydrogen-bonding purine ring binding site, and a hydrophobic pocket (Tesmer et al., 1997; Zhang et al., 1997).

All membranous ACs are activated by stimulation of G protein–coupled receptors and liberation of GTP-$G\alpha_s$ (see Ch. 21). Gα binds to both cytoplasmic domains, but primarily C2; this binding is thought to result in a rotation of the C1 domain, presumably positioning it relative to C2 to form the active catalytic site (Kamenetsky et al., 2006). ACs 1-8 are also activated by the drug forskolin, which has been used

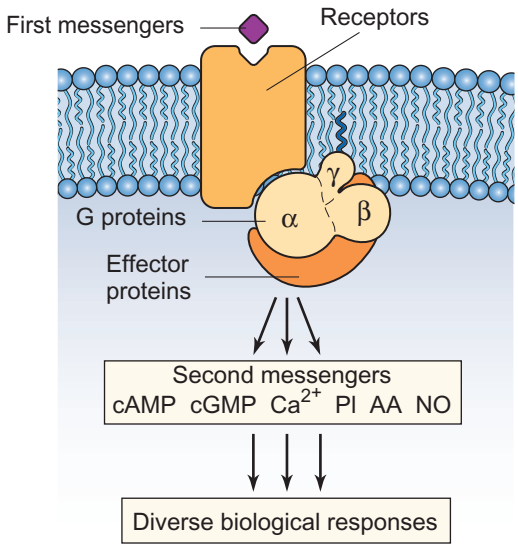

FIGURE 22-1 Schematic illustration of the 2nd-messenger hypothesis. This hypothesis, supported by decades of research, states that many types of 1st messengers in the brain, through the activation of specific plasma membrane receptors and G proteins (shown), as well as other effectors/channels, stimulate the formation of intracellular 2nd messengers, which mediate many of the biological responses of the 1st messengers in target neurons. Prominent 2nd messengers in the brain of G proteins include cAMP, and of other effectors/channels include cGMP, Ca^{2+}, the metabolites of phosphatidylinositol (PI) (e.g., inositol triphosphate and diacylglycerol) and of arachidonic acid (AA) (e.g., prostaglandins, prostacyclins, thromboxanes, leukotrienes), and nitric oxide (NO).

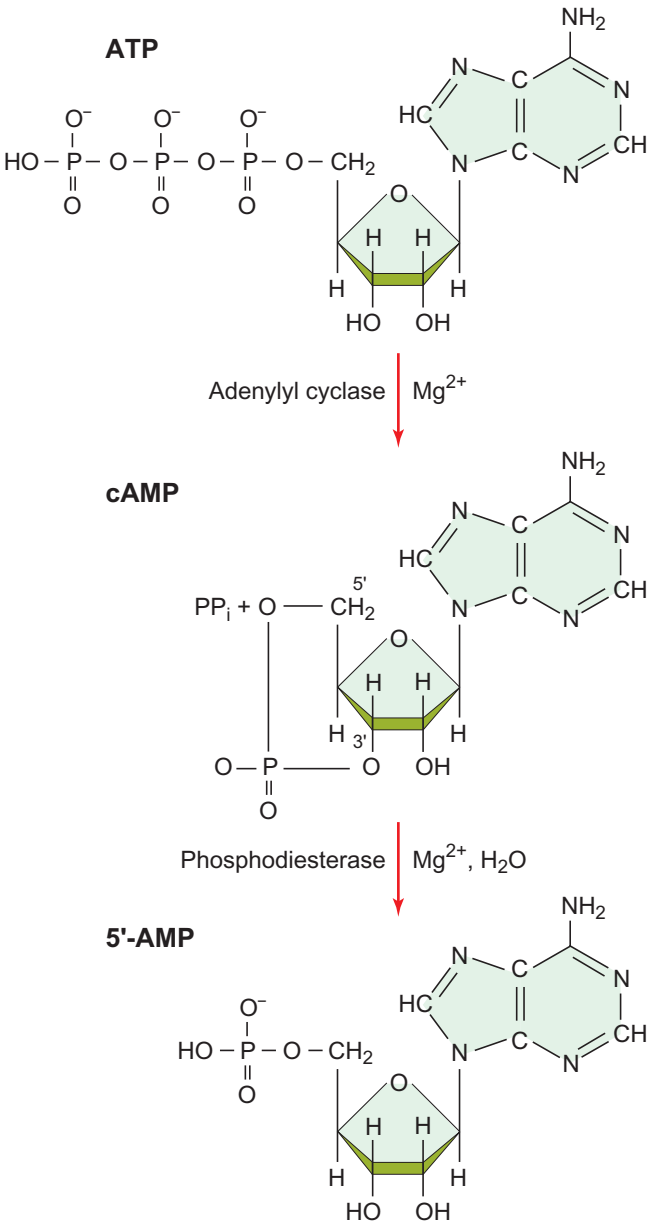

FIGURE 22-2 Chemical pathways for the synthesis and degradation of cAMP. cAMP is synthesized from ATP by the enzyme adenylyl cyclase with the release of pyrophosphate, and is hydrolyzed into 5′-AMP by the enzyme phosphodiesterase. Both reactions require Mg^{2+}. Analogous reactions underlie the synthesis and degradation of cGMP (not shown).

extensively in their study. Regulation by other signaling mechanisms varies among the different ACs and is reviewed below.

Mammals also express a soluble adenylyl cyclase (sAC), whose catalytic domain is evolutionarily related to AC1-9 but which is otherwise less homologous. There is a single sAC gene; however, it is subject to extensive alternative splicing, and variation at the protein level has yet to be fully characterized. sAC is thought to be the most phylogenetically ancient of mammalian ACs; it is the most similar at the sequence level to ACs from other phyla. sAC has been less intensively studied than AC1-AC9, especially in neuronal cells.

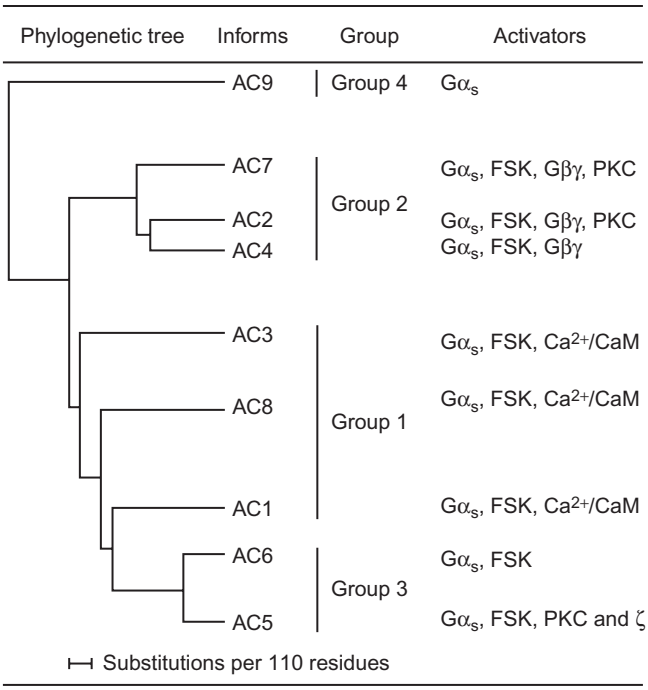

FIGURE 22-3 **Phylogenetic tree depicting relationship of different isoforms of membranous adenylyl cyclase.**

Adenylyl cyclase isozymes: expression and regulation

The 10 mammalian ACs (membranous AC1-9 and sAC) are differentially expressed and regulated, both in the nervous system and in other tissues, and over the course of ontogeny. AC1 has been the most extensively studied in the central nervous system; however, most of the other ACs are also expressed in brain. Differential regulation and differential subcellular localization of different ACs permits cAMP signaling to serve different functions in different cell types and even to serve multiple distinct functions in the same cell.

Group 1 adenylyl cyclases

The group 1 adenylyl cyclases include ACs 1, 3, and 8. As a group, they are characterized by their dual activation by $G\alpha_s$ and Ca^{2+}/calmodulin.

ADENYLYL CYCLASE 1

AC1 is the paradigmatic Group 1 membranous adenylyl cyclase (see Figure 22-3). It is expressed at high levels in brain and is also expressed in adrenal gland and retina (Defer et al.,

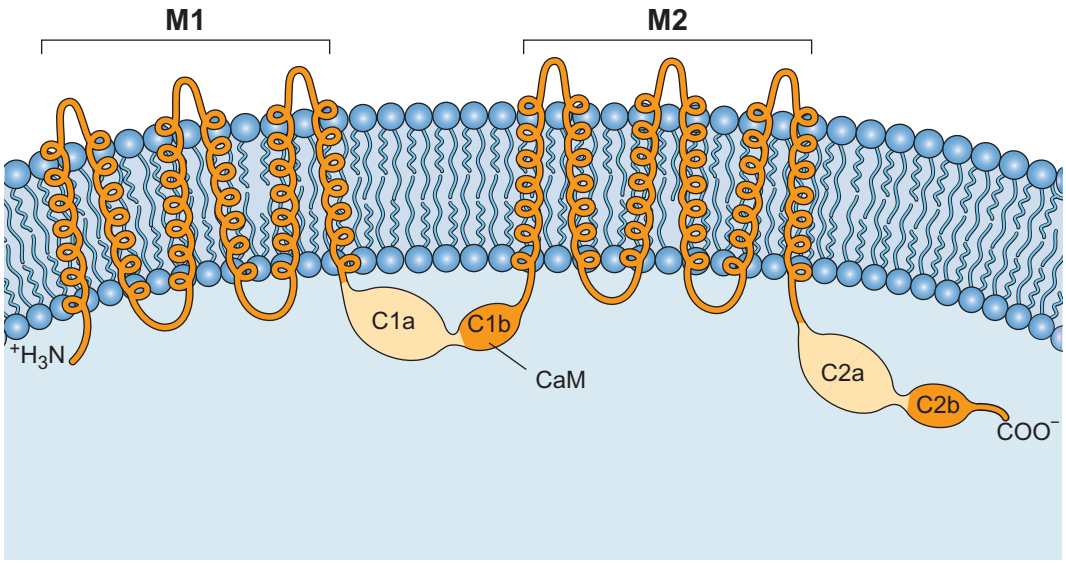

FIGURE 22-4 **Schematic illustration of the proposed topographical structure of adenylyl cyclases**. Hydropathicity profiles predict that adenylyl cyclases contain two hydrophobic regions (M1 and M2), each of which contain six membrane spanning regions, and two relatively less hydrophobic regions (C1 and C2), which are thought to be located in the cytoplasm. The catalytic domains may be located within C1 and C2, and both are necessary for functional activity of the enzyme. The carboxy (COO^-) portion of the C1 and C2 domains determines whether $\beta\gamma$ subunit complexes inhibit (type I) or stimulate (types II and IV) adenylyl cyclases. The C1b domain contains a calmodulin-binding site and is thought to mediate Ca^{2+}/calmodulin activation of certain forms of the enzyme.

2000). Expression is particularly strong in cerebral cortex, in granule cells of the cerebellum, in pyramidal and granule cells of the hippocampus, and in the olfactory system (Matsuoka et al., 1992).

AC1 is activated by $G\alpha_s$ and is thereby coupled to a variety of endocrine and neurotransmitter receptors, such as the D1 dopamine receptor and the β-adrenergic receptor. As described in Chapter 21, binding of a ligand to such a receptor catalyzes the exchange of GDP for GTP in the α subunit of the associated heterotrimeric G protein. This allows dissociation of the α and the βγ subunits, which diffuse within the cell membrane. The α subunit has inherent GTPase activity; upon GTP hydrolysis the Gα subunit dissociates from AC and reassociates with the βγ subunits and with the membrane receptor. The GTPase activity of the Gα subunit therefore provides an inherent time switch for G protein-coupled signaling. This GTPase activity can be enhanced by specific GTPase Activator Proteins (GAPs), which thereby attenuate G protein–mediated signaling.

The group 1 ACs are also activated by Ca^{2+}/calmodulin; half-maximal activation is achieved at a Ca^{2+} concentration of approximately 150 nM, well within the physiological range. Optimal activation is thus achieved by a simultaneous increase in cytoplasmic [Ca^{2+}] (often consequent to depolarization) and activation of a $G\alpha_s$-coupled membrane receptor. Activation of AC1 by Ca^{2+}/calmodulin appears to be mediated by the C1b domain of the cyclase; this region binds to Ca^{2+}/calmodulin *in vitro*, and application of a peptide fragment of the C1b region blocks the activation of AC1 by Ca^{2+}/calmodulin. Activation by $G\alpha_s$ and Ca^{2+} is synergistic.

Phosphorylation by protein kinase C (PKC) activates AC1; phosphorylation by CaMKIV inactivates it. This regulation by phosphorylation adds another dimension to the modulation of AC1 activity. Many PKC isoforms are activated by calcium (see Ch. 24); a local increase in calcium can therefore increase AC1 activity both through direct binding of Ca^{2+}/calmodulin and through phosphorylation by PKC. In contrast, elevated calcium can activate CaMKIV, thereby inhibiting AC1. This may provide an important counterbalance, keeping AC1 activation regulated within an appropriate range.

AC1 is also inhibited by heterotrimeric G proteins of the $G\alpha_i$ family—specifically, $G\alpha_{i1}$, $G\alpha_{i3}$, $G\alpha_o$, and $G\alpha_z$—and therefore by membrane receptors such as the D2 dopamine receptor. $G\alpha_i$ binding noncompetitively inhibits AC activation by both $G\alpha_s$ and forskolin. Regulation of Gα through GTPase-activating proteins (GAPs) can differentially modulate AC regulation by $G\alpha_s$ and $G\alpha_i$.

The βγ subunits of the heterotrimeric G proteins, which also diffuse within the plane of the membrane when G protein–coupled receptors are activated (see Ch. 21), also inhibit AC1. This inhibition is less potent than the stimulation by $G\alpha_s$; binding of both species in the 1:1 stoichiometric ratio expected after activation of a $G\alpha_s$-coupled membrane receptor therefore leads to net activation. However, liberation of βγ from other heterotrimeric G-proteins, including those containing $G\alpha_i$ or $G\alpha_o$, can also inhibit AC1. The differential regulation of AC activity by different subtypes of βγ represents another level of complexity in this regulatory network, but it remains to be elucidated in detail.

ADENYLYL CYCLASES 3 AND 8

AC3 shares many characteristics with AC1. It is expressed in brain, at lower levels than AC1, and in testis, brown fat and uterus (Defer et al., 2000). Its highest expression is in the olfactory neuroepithelium; knockout results in olfactory deficits (Wong et al., 2000).

AC3 is activated by $G\alpha_s$ and by Ca^{2+}/calmodulin, similarly to AC1 (Defer et al., 2000). However, it has not been shown to be inhibited by $G\alpha_i$ or by βγ subunit binding. It is inhibited by CaMKII, which may play a role in limiting excessive activation upon Ca^{2+} elevation similar to that played by CaMKIV in the case of AC1.

AC8 is the third member of the calcium-activated Group 1 ACs. In the brain, AC8 expression overlaps to a significant extent with that of AC1, especially in the medial temporal lobe; it is prominent in hippocampal pyramidal and granule cells, entorhinal and piriform cortex, with lower levels in neocortex, amygdaloid complex, thalamus and hypothalamus (Cali et al., 1994). It appears to be the only calcium-stimulated AC in the hypothalamus (Defer et al., 2000).

AC8 is stimulated by $G\alpha_s$ and by Ca^{2+}/calmodulin, like AC1 and AC3. However, it is approximately five-fold less sensitive to Ca^{2+} than is AC1. Activation of AC8 by $G\alpha_s$ and Ca^{2+}/calmodulin is not synergistic. None of the negative regulators of AC1 or AC3—$G\alpha_i$, βγ subunit binding, or phosphorylation by any of the Ca^{2+}/calmodulin kinases—have been shown to negatively regulate AC8.

AC1 and AC8 are thought to be partially functionally redundant in some contexts; this has been particularly clearly demonstrated in studies of hippocampal AC in synaptic plasticity and learning, in which a knockout of both AC1 and AC8 is required to abolish long-lasting plasticity and profoundly disrupt memory (Wong et al., 1999).

Group 2 adenylyl cyclases

The Group 2 enzymes consist of ACs 2, 4 and 7. These ACs are insensitive to Ca^{2+} and are activated, rather than inhibited, by βγ subunit binding. All of the Group 2 ACs are activated by forskolin.

ADENYLYL CYCLASE 2

AC2 is the paradigmatic Group 2 AC. It is expressed in brain and olfactory bulb, as well as in skeletal muscle and uterus (Defer et al., 2000).

AC2 is activated by $G\alpha_s$ but is not inhibited by $G\alpha_i$ or $G\alpha_o$. Activation by βγ subunit binding depends on and is synergistic with $G\alpha_s$ binding. Activation by βγ is less potent than that by $G\alpha_s$. The βγ binding site has been localized to amino acid residues 956–982 of AC2, near the middle of the C2a catalytic domain; a βγ-binding motif in this region of the protein resembles that found in other proteins, including G protein–coupled receptor kinases, inward-rectifying K^+ channels, and phospholipase Cβ. This highlights how regulation by βγ subunits may integrate across disparate intracellular signaling pathways. AC2 can also be regulated by phosphorylation and is activated by PKC.

ADENYLYL CYCLASE 4 AND 7

AC4 and AC7 are closely related to AC2. Both are expressed at low levels in brain. AC4 is expressed in heart, liver, lung,

brown fat, and uterus; AC7 is broadly expressed outside the central nervous system (Defer et al., 2000). Both of these ACs are activated by $G\alpha_s$ and $\beta\gamma$; both are insensitive to $G\alpha_i$, $G\alpha_o$, and calcium. AC7 is activated by PKC, like AC2, while AC4 is not.

Group 3 adenylyl cyclases

AC5 and AC6 constitute the Group 3 ACs. They are characterized by activation by $G\alpha_s$, dramatic inhibition by $G\alpha_i$, and inhibition by ionic Ca^{2+}; they are not regulated by calmodulin. Like most of the membranous ACs, these enzymes are activated by forskolin.

ADENYLYL CYCLASE 5

AC5 is expressed in brain, with highest levels in the striatum, as well as broadly outside the central nervous system. AC5 is the dominant adenylyl cyclase, of any class, in the striatum and is the primary transducer of changes in cAMP concentration after activation of both D1 and D2 dopamine receptors (Iwamoto et al., 2003). Consequently, knockout of AC5 produces marked motor abnormalities reminiscent of Parkinson's disease (Iwamoto et al., 2003).

AC5 is activated by $G\alpha_s$, and by phosphorylation by PKC and PKζ. It is inhibited by $G\alpha_i$ and $G\alpha_o$—indeed, inhibition of AC5 and AC6 by these G proteins is substantially more potent than inhibition of any of the other $G\alpha_i$-regulated ACs. $G\alpha_i$ binds to the C1 domain, inhibiting the interaction of the C1 and C2 domains and the formation of the catalytic site (Beazely et al., 2006).

The unique characteristic of AC5 and AC6 is their inhibition by Ca^{2+}. All ACs can be inhibited by Ca^{2+} *in vitro*, but only at supraphysiologic concentrations (10–25 µM). AC5 and AC6, in contrast, are inhibited by concentrations of Ca^{2+} as low as 0.2–0.6 µM (Beazely et al., 2006). This inhibition, which is not mediated by calmodulin, appears to involve the direct interaction of Ca^{2+} with the catalytic domain of the ACs (Hu et al., 2002).

ADENYLYL CYCLASE 6

AC6 shares many properties with AC5, with which it is the most closely evolutionarily related (Fig. 22-3). It is expressed in brain, at higher levels and more broadly than AC5, as well as in heart (Defer et al., 2000). AC6 is regulated similarly to AC5. It is activated by $G\alpha_s$ and is potently inhibited by $G\alpha_i$ and by ionic calcium at low concentrations; it does not interact with calmodulin. AC6 is inhibited by phosphorylation by PKC, providing a potential negative feedback regulatory mechanism. It has not been shown to be regulated by $G\beta\gamma$.

Group 4 adenylyl cyclase

AC9 is distinct from the other membranous ACs in several respects and is commonly classified as the single member of a distinct Group 4 (Defer et al., 2000; Premont et al., 1996). It is most prominently expressed in skeletal muscle and heart but is also expressed in brain and lung. It is broadly expressed in the brain, with particularly high expression in hippocampus, cerebellum and neocortex (Premont et al., 1996).

AC9 is activated by $G\alpha_s$, like all of the other membranous ACs, but its regulation is otherwise quite different. It is the only membranous AC not activated by forskolin; this is due to the switch of a single residue (Y1082) from leucine in all other membranous ACs to tyrosine in AC9 (Yan et al., 1998). AC9 has not been shown to be regulated by $G\alpha_i$, $G\alpha_o$, $G\beta\gamma$ or interaction with either ionic or calmodulin-bound calcium. It is, however, indirectly inhibited by calcium through negative regulation by the phosphatase calcineurin. Regulation by phosphorylation is not well understood but is likely to be complex: at least 12 potential phophorylation sites have been identified. AC9 activity is inhibited by PKC, although the mechanism remains unclear (Sadana et al., 2009).

Soluble adenylyl cyclase

Soluble adenylyl cyclase (sAC, sometimes called AC10) is evolutionarily more distant than the nine membranous ACs and is more closely related to the soluble adenylyl cyclases of prokaryotes (Kamenetsky et al., 2006). sAC is expressed at high levels in testes, where it has been most intensively studied; it has a critical role in sperm capacitation and fertilization (Esposito et al., 2004). It is also expressed in brain, where it has been found to have a critical role in signaling in growth cones during development (Wu et al., 2006). sAC has two well-characterized splice variants, the full-length ~187 kDa protein and a testis-specific splice variant of ~50 kDa.

As its name implies, sAC is not associated with the membrane; it is therefore not regulated, either positively or negatively, by G protein subunits. It is, however, regulated by calcium (Jaiswal et al., 2003) and, uniquely, by bicarbonate (Chen et al., 2000). This suggests a role in acid/base homeostasis; however, the extent to which sAC in brain plays such a mechanistic role remains to be elucidated.

Models for cellular regulation of the different types of adenylyl cyclase

The differential sensitivity of ACs 1-8 to various G protein α and $\beta\gamma$ subunits and to Ca^{2+} provides potentially complex mechanisms for their regulation of cAMP formation. While attempts at integration of these mechanisms in different cell types remain necessarily somewhat hypothetical, they serve an important illustrative purpose (Fig. 22-5).

In cells that contain Group 1 ACs (AC1, 3 and 8), cAMP formation will be stimulated by extracellular signals that activate receptors coupled to $G\alpha_s$ as well as by those that increase Ca^{2+} entry into cells. Neurons expressing AC1 may have inherently high tonic levels of cAMP formation due to basal stimulation by Ca^{2+}/calmodulin. This effect may explain the high levels of basal adenylyl cyclase enzymatic activity found in brain extracts, relative to other tissues.

This high level of basal enzyme activity may necessitate the multiple mechanisms whereby different ACs can be inhibited. In such Group 1–expressing neurons, cAMP formation could be inhibited not only by signals that activate receptors coupled to $G\alpha_i$, but also by signals activating receptors coupled to other G proteins (e.g., $G\alpha_o$ or $G\alpha_q$) through the release of $G\beta\gamma$ subunits. Such regulation may provide a mechanism for keeping the brain's high capacity for cAMP synthesis in check, while providing multiple pathways for the regulation of cAMP formation by a variety of extracellular signals.

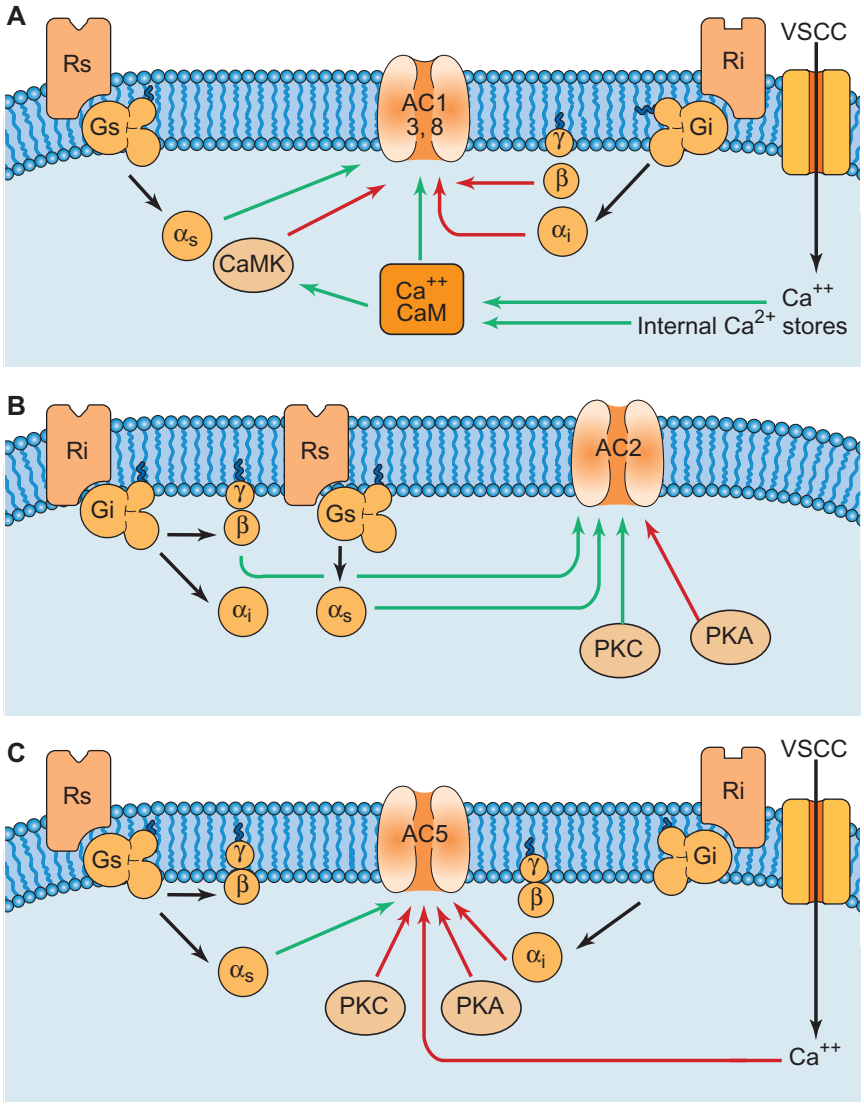

FIGURE 22-5 **Schematic illustration of the mechanisms by which the activity of adenylyl cyclases may be regulated**. Whereas all forms of adenylyl cyclase are activated by Gαs (αs) and forskolin, different types of the enzyme can be distinguished by their regulation by Ca^{2+} and by other G protein subunits. **(A)** Since adenylyl cyclase types I, III and VIII are stimulated by Ca^{2+}/calmodulin, an increase in cellular Ca^{2+} levels, which can result from either increased entry of Ca^{2+} into the cell or increased release of Ca^{2+} from internal stores, would be expected to activate these enzymes. The actions of Ca^{2+}/calmodulin are synergistic with Gαs. In addition, in the presence of activated Gαs, type I adenylyl cyclase is inhibited by βγ subunits. (The effect of βγ on the type III and VIII enzymes remains unknown.) The potency of Gαs to activate the enzyme is some 10- to 20-fold higher than that of βγ complexes to inhibit it, so that activation of enzyme activity is the predominant effect when only stimulatory receptors and Gs are activated. Gαi (αi) mediates neurotransmitter inhibition of these adenylyl cyclases. This is particularly well established for the type I enzyme. **(B)** Adenylyl cyclase types II and IV are not sensitive to Ca^{2+}/calmodulin and, in the presence of activated Gαs, are stimulated by βγ complexes. The receptors (Rx) and G protein a subunits that provide the βγ subunits for this type of regulation could conceivably involve receptors coupled to several types of G proteins (e.g., Gi, Go, Gq, etc.). The activation by βγ is synergistic with Gαs. Note: while the same βγ complexes are shown for all the G proteins listed, there are several known subtypes of β and γ subunits, which may well influence the various types of adenylyl cyclase in different ways. **(C)** Adenylyl cyclases types V and VI are inhibited by free Ca^{2+}. In addition, these enzymes are inhibited upon phosphorylation by protein kinase A (PKA) or protein kinase C (PKC). Types V and VI adenylyl cyclases are also inhibited by Gαi, but are not influenced by βγ subunits.

A very different situation would exist in cells that primarily express Group 2 ACs (AC2, 4 and 7), which are activated by $G\alpha_s$ but are insensitive to calcium. In such cells, enzyme activity would not be stimulated by Ca^{2+}/calmodulin, but it would be synergistically activated by $G\alpha_s$ and $G\beta\gamma$ subunits. Activation of receptors coupled to $G\alpha_q$—or even to $G\alpha_o$ or $G\alpha_i$—would thereby synergize with AC activation by $G\alpha_s$-coupled receptors, rather than inhibiting it. Indeed, there are several examples of such synergistic activation. For example, stimulation of adenylyl cyclase in cerebral cortex by activation

of β-adrenergic receptors (which are coupled to $G\alpha_s$) can be potentiated by activation of α_1-adrenergic or $GABA_B$ receptors (both coupled to other G proteins), which by themselves have little or no effect on cAMP formation (Duman et al., 1987). This potentiation may result from the release of free $G\beta\gamma$ complexes from the G proteins coupled to the α_1-adrenergic or $GABA_B$ receptors, which would synergize with $G\alpha_s$ in the activation of Group 2 membranous ACs.

The third situation would be in cells that express primarily the Group 3 ACs, AC5 and AC6 (such as neurons in the striatum). In these cells, adenylyl cyclase activity would be potently activated by $G\alpha_s$-coupled receptors, but this activity would be tightly controlled, since the same signals would lead to activation of protein kinase A (PKA), which would phosphorylate AC5/6 and inhibit further cAMP production. Activation of AC5/6 would be further controlled by receptors coupled to $G\alpha_i$, which potently inhibits these ACs, and by those that increase free Ca^{2+}. Receptors coupled to $G\alpha_q$, which can activate PKC, would also be expected to inhibit AC5/6. Such mechanisms of inhibition may have particular relevance in understanding the downstream effects of psychotropic drugs that affect the striatum, such as first-generation antipsychotic drugs and psychostimulants, since AC5 is the principal cyclase in this region.

Long-term regulation of adenylyl cyclases

The above discussion focuses on molecular mechanisms that acutely activate or inhibit the activity of the various adenylyl cyclases. However, these molecules are also subject to longer-term regulation, especially in response to chronic pharmacological exposure or other long-term experimental or clinical manipulations.

It has been known for many years that prolonged exposure of cells to a receptor agonist often leads to receptor desensitization, whereas prolonged exposure to an antagonist can lead to receptor sensitization. Such desensitization and sensitization can be achieved either through changes in receptors themselves or through alterations in signaling proteins distal to the receptor that mediate its signaling function, such as adenylyl cyclase (Watts, 2002). Mechanisms involved are not fully understood but could entail phosphorylation, alterations in transcription or translation, proteolytic processing, or other posttranslational modifications.

An example is found in the study of opiate tolerance and dependence. Chronic exposure to opiates leads to coordinated upregulation of the cAMP cascade, including higher levels of AC, increased levels of PKA, and in some cases lower levels of inhibitory G proteins, in opiate-responsive brain regions (Watts, 2002; Nestler et al., 1997; Zachariou et al., 2008). Upregulation of cAMP signaling contributes to opiate tolerance and dependence by opposing the acute actions of opiates (which stimulate receptors coupled to $G\alpha_i$ and thereby reduce cAMP levels). Increased activity of adenylyl cyclase (typically AC1 and AC8) is partly due to increased transcription through activation of regulated transcription factors such as the cAMP-response element-binding protein (CREB) (Nestler et al., 1997; Cao et al., 2010)—which is itself activated by cAMP, as its name suggests. This feedback loop leads to increased tonic cAMP in certain neurons upon chronic opiate exposure.

Molecular targets of cAMP

cAMP produces downstream effects through activation of a variety of effectors. The best studied of these is the activation of a protein kinase, PKA. More recently, a variety of other targets have been identified. We do not attempt here to exhaustively review these downstream effectors, some of which are reviewed elsewhere in this volume (Chapter 25), but simply to highlight the most important ones.

Protein kinase A

Protein kinase A (PKA) is activated by elevations in cAMP and is a prominent and well-studied effector of cAMP signaling. PKA exists as a heterotetramer of two catalytic and two regulatory subunits. This holoenzyme complexes with other proteins, including the A-kinase anchoring proteins (AKAPs), which thereby localize and further regulate its activity (Taylor et al., 2008). In the absence of cAMP, the regulatory subunits mask the catalytic domains and prevent kinase activity. cAMP binds to the regulatory subunits and causes the complex to dissociate, unmasking the catalytic subunits, which are thereby activated.

PKA has been implicated in numerous signaling processes and has innumerable downstream targets (Taylor et al., 2008). As noted above, it phosphorylates AC5 and AC6, thereby inhibiting them in a form of negative feedback. PKA also phosphorylates neurotransmitter receptors, including the AMPA receptor; transcription factors, such as CREB; cytoskeleton-associated proteins; ion channels; and components of other intracellular signaling pathways (Kinasource, 2010).

Cyclic nucleotide-gated channels

Cyclic nucleotide-gated (CNG) channels, and the related hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, compose a family of nonspecific cation channels that are directly gated by cAMP or cGMP (Biel et al., 2009; Kaupp et al., 2002). CNG channels are heterotetrameric complexes consisting of α, β, and γ subunits, while HCN channels, also tetrameric, are composed of products of four genes, HCN1-4. Both the ligand specificity (cAMP vs. cGMP) and the ionic permeability of these channels are determined by specific combinations of subunits. The channel subunits CNGA2, A4, and B1b, for example, are expressed in olfactory receptors and form a cAMP-gated channel that is critical for olfactory signal transduction. HCN channels are responsible for the pacemaker activity of many central neurons. In frontal cortex, modulation of cAMP-gated HCN channels by β-adrenergic signaling regulates working memory (Wang et al., 2007). The CNG and HCN channels thus represent a mechanism whereby intracellular cAMP signaling can dynamically modulate neurons' electrical properties.

Most CNG channels are opened by cAMP; all have a higher affinity for cGMP than for cAMP. cAMP is only a partial agonist of the channels CNGA1 and CNGA3, and HCN3 is unaffected by or even inhibited by cAMP (Zufall et al., 2010).

Epac

The Exchange Proteins directly Activated by cAMP (Epac1 and Epac2) represent a more recently characterized third molecular target of neuronal cAMP (Gloerich et al., 2010). Epac is also known as cAMP-GEF (guanosine exchange factor); in the presence of cAMP, it catalyzes the exchange of GMP for GTP in the signaling proteins Rap1 and Rap2. This represents a mechanism whereby cAMP signaling can directly couple to MAP kinase signaling (see Chs. 25, 26). Epac1 and Epac2 are expressed broadly in the central nervous system, as well as in numerous peripheral tissues. Extensive studies have implicated Epac as an important regulator of cardiac function and insulin secretion (Gloerich et al., 2010). CNS functions are less clear. Epac has been found to be elevated in the brains of patients with Alzheimer's disease, though its pathophysiological role, if any, has yet to be elucidated (McPhee et al., 2005). A role in axon path finding during neural development is increasingly clear (Peace et al., 2011). In the adult brain, Epac has been shown in animal models to modulate synaptic spines and to promote certain forms of learning (Penzes et al., 2011). Further elucidation of the mechanisms and role of this cAMP effector is an area of active research.

Functions of cAMP signaling in the brain

The functions of specific ACs in particular brain processes have been extensively investigated using knockout mice and other experimental approaches. Some functions of individual ACs have been mentioned in the discussion above; here we briefly review areas in which substantial research has been done. This treatment is necessarily incomplete (for a more thorough recent review of this topic see Sadana et al., 2009) and is provided to give a sense for the breadth of brain and behavioral processes in which these signaling molecules are implicated.

Synaptic plasticity, learning, and memory

An association of cAMP-mediated signaling with learning and memory was first shown through classical genetic screens in *Drosophila*. Mutations in the *dunce* gene, which was found to encode a phosphodiesterase, and in the *rutabaga* gene, which was found to encode a calcium-regulated adenylyl cyclase, both impair flies' ability to learn olfactory-guided tasks. Extending this work to rodents, knockout of AC1 produced impaired plasticity of barrel cortex, impaired hippocampal and cerebellar synaptic plasticity and impairments in spatial learning (Wu et al., 1995). These impairments were more profound in double knockout mice in which AC8 was also disrupted, suggesting partially overlapping function of these two Ca^{2+}-regulated cyclases (Wong et al., 1999).

Pain

The analgesic properties of opiate drugs derive, at least in part, from their inhibition of AC activity through their binding of $G\alpha_i$-coupled μ-opiate receptors. AC1 knockout mice show abnormal responses to painful stimuli (Sadana et al., 2009). Knockout of AC1 (or of both AC1 and AC8) was found to lead to a reduced response to both acute and chronic pain. This modulation appears to be at the level of the spinal cord: injection of forskolin into the spinal cord could rescue the hypoalgesic phenotype. AC5 knockout also modulates pain processing, reducing responses to inflammatory and visceral pain and to neuropathic allodynia. The neuroanatomical circuitry underlying this phenotype remains to be elucidated.

Dopamine signaling in the striatum

AC5 is the principal cyclase in the striatum; it is coupled to both D1 and D2 receptors. Knockout of this cyclase disrupts dopamine signaling and produces bradykinesia and dyscoordination that may recapitulate symptoms of Parkinson's disease. These knockout mice also show an attenuation of behavioral responses to antipsychotic drugs, which are D2 antagonists (Sadana et al., 2009).

Neurodegeneration

Knockout of AC1 significantly attenuates the excitotoxic effects of injection of NMDA (Sadana et al., 2009). Conversely, knockout of either AC1 or AC8 potentiates degeneration after administration of ethanol or phenobarbital to neonatal mice (Sadana et al., 2009). These isoforms are therefore implicated in various forms of neurodegeneration, but the details are complicated and remain to be fully elucidated.

Drugs of abuse

Long-term modulation of AC activity may underlie some aspects of drug dependence and withdrawal. Chronic treatment of cells in culture with morphine produces supersensitivity of ACs 1, 5, 6 and 8 to subsequent stimulation. As described above, chronic morphine treatment leads to upregulation of ACs 1 and 8 in specific brain regions; knockout of these two isoforms attenuates some of the behavioral effects of morphine in mice. AC5 is thought to be critical for morphine's effects on the striatum, in keeping with its dense expression there; knockout of AC5 attenuates all major effects of morphine administration, including acute effects on locomotion, analgesia, tolerance and withdrawal. Conversely, transgenic overexpression of AC7 in mouse brain leads to an enhancement of the behavioral effects of morphine (Sadana et al., 2009).

The sedative effects of ethanol are thought to derive, in part, from its inhibition of cyclases. Correspondingly, AC1 knockout mice show an enhancement in ethanol-induced sedation (Sadana et al., 2009).

Olfaction

AC2, 3 and 4 are prominent in the olfactory system. In the mouse, AC3 is most prominent in the main olfactory system, while AC2 predominates in the vomeronasal system. AC3 knockout mice are anosmic for most odorants. Interestingly, AC3 knockouts also show deficits in the formation of peripheral olfactory projections and the formation of glomeruli (Sadana et al., 2009).

GUANYLYL CYCLASES

Guanylyl cyclases (GCs) catalyze the synthesis of cGMP from GTP in a reaction closely analogous to the conversion of ATP to cAMP by adenylyl cyclase. Like cAMP, cGMP is

known to regulate many cellular proteins, including protein kinases, ion channels and phosphodiesterases (see below). Two major classes of GC have been characterized, identified originally on the basis of their subcellular distribution: membrane-bound and soluble. These classes of cyclase differ in their molecular structure and regulation (Domek-Lopacinska et al., 2005). The general mechanisms by which these GC types are regulated by extracellular signals are shown in Figure 22-6.

Membrane-bound guanylyl cyclase

There are seven isoforms of transmembrane GC, denoted GC-A through GC-G. All share the same structure (Fig. 22-6). They differ from adenylyl cyclase, and from soluble GCs, in that they include a receptor domain for extracellular messengers. This contrasts with ACs, which are indirectly activated (or inhibited) by extracellular signals. This arrangement means that ligand binding is more directly coupled to cGMP

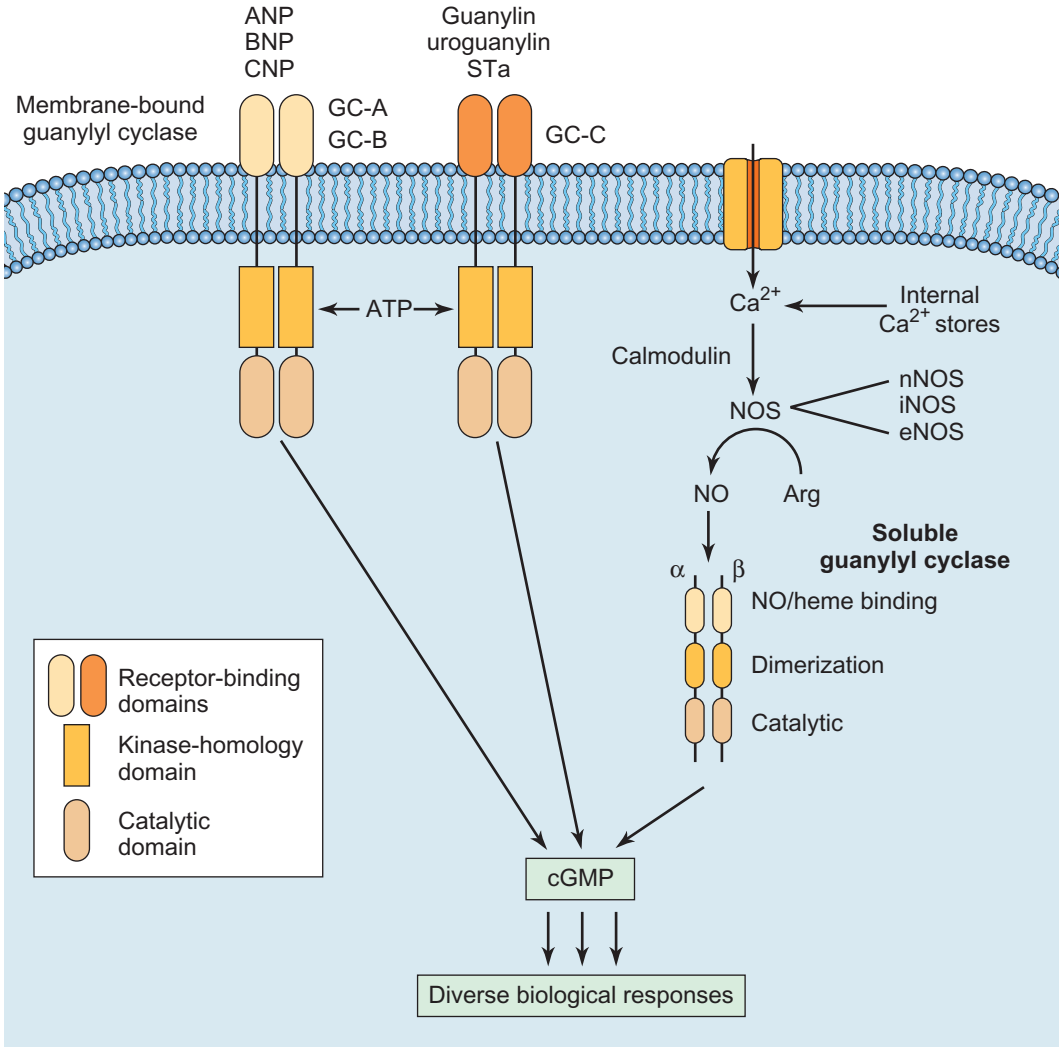

FIGURE 22-6 **Schematic illustration of the mechanisms by which 1st messengers stimulate guanylyl cyclase**. Two major classes of guanylyl cyclase are known: membrane-bound and soluble. The membrane-bound forms (GC-A, GC-B and GC-C) contain extracellular receptor domains that recognize specific peptide ligands (atrial natriuretic peptide and related peptides): GC-A binds atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) and GC-B binds C-type natriuretic peptide (CNP). GC-C binds the endogenous peptide guanylin, as well as a heat stable bacterial enterotoxin (STa). The membrane bound receptors contain an intracellular kinase-like domain that binds ATP and a catalytic domain that synthesizes cGMP from GTP. The soluble forms contain the catalytic domains only (a and b subunits), and are activated by nitric oxide (NO). Catalytic activity of soluble guanylyl cyclase is dependent on the presence of both a and b subunits. 1st messengers lead to activation of NO synthesis by increasing cellular levels of Ca^{2+}, which in conjunction with calmodulin activates NO synthase. 1st messengers increase cellular Ca^{2+} levels in most cases by depolarizing neuronal membranes and thereby activating voltage-dependent Ca^{2+} channels and increasing the flux of Ca^{2+} into the cell (e.g., nerve impulses, glutamate, acetylcholine, substance P). In some cases, Ca^{2+} can enter the cell directly via ligand-gated ion channels (e.g., as with NMDA glutamate receptors). 1st messengers can also regulate cellular Ca^{2+} levels by stimulating Ca^{2+} release from intracellular stores.

production; as a corollary, there are fewer opportunities for complex regulation and cross-talk between different signaling systems.

Membranous GCs have a single transmembrane domain, an extracellular ligand-binding domain, and intracellular kinase homology and catalytic domains. Receptor binding by an extracellular ligand leads to receptor oligomerization (similarly to the activation of membrane tyrosine kinase receptors by growth factors; see Chs. 26 and 28). This leads to phosphorylation and ATP binding by the intracellular kinase homology domains. This ATP binding plays a critical role in enzyme activation, functionally homologous to the role of GTP-binding to G proteins in transducing the binding of ligand to G protein-coupled receptors in the regulation of adenylyl cyclase. Oligomerization, phosphorylation and ATP binding permit the intracellular catalytic domain to catalyze the conversion of GTP to cGMP (Domek-Lopacinska et al., 2005).

GC-A, -B and -C are receptors for natriuretic peptides

GC-A and GC-B serve as receptors for atrial natriutic factor (ANP) and related peptides. GC-A binds both ANP and brain natriuretic peptide (BNP); it is located in vascular tissue and kidney. GC-B is expressed in neuronal tissue and binds C-type natriuretic peptide (CNP). There is also a truncated form of GC-B that lacks the intracellular kinase and catalytic domains. The truncated form may act to bind and clear the peptide ligands. Increasing evidence suggests that these peptides play important roles as extracellular signals in diverse tissues, including brain. Activation of GC, and the subsequent increase in cellular cGMP levels, mediates some of the cellular actions of these peptides on target tissues. A third and related GC type, GC-C, is localized to the intestine and binds the endogenous peptides guanylin and uroguanylin; it is activated by a bacterial enterotoxin. These three GC isoforms have been best functionally characterized outside the central nervous system and regulate diverse aspects of cardiac, smooth muscle, intestinal and kidney function (Kuhn, 2009).

GC-D and GC-G are implicated in olfaction

GC-D is expressed in the main olfactory epithelium of the rodent, in a small subset of olfactory sensory neurons (OSNs) (Zufall et al., 2010). Olfaction is further discussed in Chapter 52. These OSNs appear to be distinct from the majority, which express AC3 and signal primarily through cAMP. GC-D–expressing OSNs also express the cGMP-specific phosphodiesterase PDE2 and cGMP-specific cyclic nucleotide-gated (CNG) channels (see below), emphasizing the centrality of cGMP-mediated signaling to their function. This signaling system, in rodents, has been found to respond to the natriuretic peptides guanylin and uroguanylin and to components of urine (Zufall et al., 2010). Activation of GC-D through binding of these ligands leads to cGMP accumulation, activation of the CNG channel CNGA3, and consequent neuronal excitation. GC-D has also been reported to be activated by CO_2, via a carbonic anhydrase (CA)–dependent mechanism; CA catalyzes the conversion of CO_2 to bicarbonate, which appears to directly activate GC-D. The functional relationship of these two mechanisms of activation is an area of active research (Zufall et al., 2010).

GC-G is less well understood. It is expressed in the Grueneberg ganglion of rodents, an accessory olfactory sensory structure; it is also expressed elsewhere in brain and in peripheral tissues, including testis, sperm, intestine, lung, kidney and skeletal muscle. The specific ligands that activate GC-G have not been clearly characterized; data from rodents suggests that these cells may respond to alarm pheromones and to cool temperatures (Zufall et al., 2010).

It is unclear whether these functions of GC-D and GC-G are shared throughout mammalian phylogeny; the homologous genes appear to be pseudogenes in humans (Kuhn, 2009).

GC-E and GC-F are involved in photoreceptor signal transduction

GC-E and GC-F are expressed in photoreceptor cells in the retina and are critical in phototransduction (Wensel, 2008); GC-E is also expressed in olfactory epithelium, pineal, cochlear nerve and organ of Corti, though its function in those tissues is not well understood. A full review of photoreceptor signal transduction is beyond our scope; a brief sketch must suffice. No extracellular ligand for these GCs has been identified. They are instead regulated by intracellular Ca^{2+}-sensing GC activator proteins, GCAP-1 and GCAP-2. In the presence of Ca^{2+}, these GACPs bind to and inhibit GC-E and GC-F, limiting cGMP production. Photoreceptor activation entails activation of phosphodiesterase (PDE6; see below), reduction in cGMP, closure of CNG channels, and a consequent decline in intracellular Ca^{2+}. This leads to the disinhibition of GC-E and GC-F, triggering the reaccumulation of cGMP, opening of CNG channels, and the termination of signaling (Kuhn, 2009).

The importance of this process for normal vision is emphasized by the observation that humans with a mutation in GC-E have congenital blindness. Similarly, GC-E knockout produces visual deficits in mice, and GC-E/F double knockout produces total blindness (Kuhn, 2009).

Soluble guanylyl cyclases

Soluble guanylyl cyclases are structurally similar to the membranous GCs but, as the name implies, lack the transmembrane domain that tethers the former enzymes to the membrane. sGCs appear to function under physiological conditions as heterodimers of α and β subunits, each with a MW of 70–80 kDa. The α and β subunits are structurally similar, with each containing three primary domains: an amino-terminus heme domain that binds nitric oxide (NO; see below), a dimerization domain, and a carboxy terminus catalytic domain (Fig. 22-6). The $\alpha\beta$ heterodimer is required for enzyme activity (Domek-Lopacinska et al., 2005). This is analogous to membranous ACs, in which the catalytic pocket is formed by two discrete domains—though in the case of ACs, these two domains are parts of the same long polypeptide chain.

There are multiple isoforms of the α and β subunits of sGC, with distinct tissue and cellular distributions. The predominant isoforms in brain are $\alpha1$ and $\beta1$, although $\alpha2$, $\alpha3$ and $\beta3$ are also found; $\beta2$ is expressed primarily in the lung. $\beta2$ contains a consensus sequence at its carboxy terminus for

isoprenylation or carboxymethylation, which could serve to anchor the protein to the plasma membrane. It has been proposed that specific isoforms of the α and β subunits of GC may form heterodimers with distinct functional and regulatory properties, although this remains to be established with certainty. At the mRNA level, at least one α and one β subunit is expressed in virtually every part of the brain; the distribution of protein is less well characterized. The $\alpha 2$ subunit has a C-terminal domain that permits interaction with the postsynaptic density protein PSD95 and may therefore be localized to the synapse (Kleppisch et al., 2009).

sGC is regulated by nitric oxide (NO)

Soluble guanylyl cyclase is potently activated by NO, which binds the heme group located at the amino terminus of both α and β subunits. Removal of this heme group abrogates the ability of NO to activate cGMP synthesis. (Hope et al., 1991; Bredt et al., 1992).

A full review of NO signaling in the brain is beyond our scope here; we briefly review its essential characteristics. NO is produced by NO synthase (NOS), which is regulated by Ca^{2+}-calmodulin. The systemic importance of this signaling system is shown by the potency of organic nitrate drugs used in the treatment of heart disease (e.g., nitroglycerine). These drugs induce vasodilation by activating sGC and increasing [cGMP].

NOS converts L-arginine into free NO and citrulline in a reaction that requires a tetrahydrobiopterin cofactor and NADPH (Hope et al., 1991; Bredt et al., 1992). Three forms of NOS have been characterized. Type I NOS, also known as neuronal NOS (nNOS), is found at high levels in neuronal tissue. It directly binds and is activated by Ca^{2+}-calmodulin. Type II NOS is also known as macrophage NOS or inducible NOS (iNOS); it is present at low levels at baseline but is dramatically increased in response to immune challenge and other stimuli. Type III NOS, also known as endothelial NOS (eNOS), is also activated by Ca^{2+}-calmodulin. It is associated with the plasma membrane and is the primary transducer of the endothelial response to endothelium-derived relaxing factor (EDRF). Despite their names, which derive from the tissues in which they were first identified and studied, iNOS and eNOS are also expressed in brain.

Free NO has a short half-life but diffuses rapidly. It can readily cross the plasma membrane and therefore can serve both as an extracellular and an intercellular second messenger. Indeed, NO generated in a postsynaptic cell after synaptic stimulation appears to serve as a retrograde messenger, permitting presynaptic responses to postsynaptic events (Feil et al., 2008).

Molecular effectors of cGMP signaling

Like cAMP signaling, cGMP signaling can affect neuronal function through several molecular mechanisms. The best studied is the activation of the kinase PKG; but effects on ion channels and on phosphodiesterases have also been well characterized. Again, our review of these effector mechanisms is necessarily brief.

Protein kinase G

The first characterized effector mechanism for cGMP signaling was protein kinase G [PKG; (Francis et al., 2010)]. The mammalian genome contains two PKG isoforms, PKGI and PKGII. Both are expressed in brain and peripherally. PKGI is more prominent in the central nervous system, where it is expressed in Purkinje cells, hippocampus, amygdala and olfactory bulb. PKGII is expressed in the suprachiasmatic nucleus and various other brain nuclei (Hofmann et al., 2009). PKGI exists in two alternatively spliced forms, PKGIα and PKGIβ; they have similar cGMP affinity but somewhat different substrate specificity. The PKGs are homologous to PKA; while each kinase has substantial specificity for the cyclic nucleotide for which it is named, they can each be activated to an extent by the other cyclic nucleotide, within the physiological range (Francis et al., 2010).

PKGs function as homodimers; dimerization is achieved via a leucine zipper domain. Each PKG molecule consists of this leucine zipper, a regulatory domain with two cGMP binding sites, and a catalytic domain. These domains are analogous to the catalytic and regulatory subunits of PKA, but the two domains lie on a single polypeptide chain. Binding of cGMP to a binding pocket in the regulatory domain (which is homologous to the cAMP binding sites on the PKA regulatory domain and on the Epacs) leads to a conformational change that unmasks the catalytic domain, giving it access to substrates and thereby activating the kinase (Hofmann et al., 2009). PKGII, but not PKGI, is bound to the cell membrane by myristoylation at the N-terminus.

Diverse substrates of PKGs have been characterized, including regulators of intracellular kinase and phosphatase signaling, regulators of intracellular calcium signaling, regulators of vesicle trafficking and cytoskeletal dynamics, and ion channels (Hofmann et al., 2009). PKG was identified and its importance realized more recently than PKA; its substrates have correspondingly been less thoroughly characterized, and further details of this signaling system and its effects in the brain remain an area of active research.

cGMP-gated ion channels

Like cAMP, cGMP directly opens CNG ion channels (Biel et al., 2009; Kaupp et al., 2002). The structure and regulation of these channels has been summarized above.

Phosphodiesterases

As further discussed below, cGMP and cAMP are broken down by a large family of phosphodiesterases (PDEs). Some of these are directly regulated by cGMP, creating a mechanism whereby cGMP can directly regulate the kinetics of its own degradation. Interestingly, the cGMP binding sites of the various cGMP-regulated phosphodiesterases are evolutionarily unrelated to the binding sites found on PKG and on the CNG channels (Francis et al., 2010). Further details of the regulation of PDEs by cGMP are provided below.

Functions of cGMP signaling in the brain

The prominent role of cGMP-mediated signaling in phototransduction in the retina and in olfactory perception

in defined subsets of olfactory receptor neurons has been described above. Neuronal cGMP signaling is also implicated in a variety of other processes.

Synaptic plasticity, learning, and memory

As noted above, NO has been hypothesized to serve as a retrograde messenger at synapses and to participate importantly in the regulation of synaptic plasticity (Feil et al., 2008). Soluble guanylyl cyclase, which is regulated by NO, is critical to this process. Mice deficient in the α_1 or α_2 subunit of sGC have a deficit in synaptic plasticity in the visual cortex; this can be rescued by the application of exogenous cGMP (Haghikia et al., 2007). A similar deficit is seen in hippocampal LTP in both knockout mice (Taqatqeh et al., 2009). cGMP-mediated signaling also has important roles in modulating synaptic plasticity in amygdala and cerebellum. sGC knockout mice have a variety of memory deficits (Kleppisch et al., 2009).

Interestingly, aging attenuates cGMP signaling through the increased expression of phosphodiesterases, and the β-amyloid protein that builds up in Alzheimer's disease has recently been shown to further impair cGMP-mediated signaling. This has led to increasing interest in the possible application PDE inhibitors as a pharmacotherapeutic option both in normal aging and in Alzheimer's disease (Domek-Lopacinska et al., 2010).

Cognition and mood

Studies in knockout mice in which guanylyl cyclases and other components of the cGMP–signaling cascade have been disrupted have revealed an important contribution of this signaling mechanism to the regulation of diverse aspects of behavior. For example, elevations of cGMP can produce aggression in mice. cGMP elevations also appear, in rodent studies, to have an anxiolytic effect, and to interact with the reinforcing effects of drugs of abuse. In accordance with this influence on a broad range of higher CNS functions, it is unsurprising that various components of the cGMP signaling system have been implicated by genetic studies in a variety of psychiatric conditions, including bipolar disorder, schizophrenia and major depression (Kleppisch et al., 2009).

Pain

NO signaling in the spinal cord has long been associated with pain sensitivity, possibly through its participation in synaptic plasticity in dorsal horn nociceptive cells. sGC β_1 knockout mice exhibit a reduction in nociceptive responses to both inflammatory and neuropathic pain, though not to acute thermal or mechanical pain (Schmidtko et al., 2008). These and related findings suggest that cGMP-mediated signaling may be critically involved in chronic pain and in allodynia but not in normal pain perception, making it a potentially attractive target for pharmacological treatments for chronic pain conditions.

PHOSPHODIESTERASES

Given the importance of cyclic nucleotides in signal transduction pathways, it is not surprising that the degradation of these second messengers, like their synthesis, is highly regulated. cAMP and cGMP are broken down by members of a large family of enzymes, the phosphodiesterases (PDEs), which catalyze the conversion of cAMP and cGMP into 5′-AMP and 5′-GMP, respectively, via hydrolysis of the 3′-phosphoester bonds.

Structure of phosphodiesterases

The many PDEs are structurally related; they generally function as homodimers. PDEs have three distinct functional domains. The N-terminal regulatory domain is quite variable among different PDEs, endowing them with their distinct regulatory profiles (discussed below); this is the region of the most extensive alternative splicing. The central catalytic domain is highly conserved; in particular, it contains a glutamine residue that is required for cyclic nucleotide binding. The highly conserved nature of this domain has made the development of isozyme-specific inhibitors challenging. The spatial orientation of this glutamine determines substrate specificity. A number of conserved histidine residues within the catalytic domain appear to be necessary for appropriate protein folding and stability of the catalytic pocket. The carboxy-terminal domain is shorter; it is also a region of some alternative splicing (Menniti et al., 2006).

The association of some PDE isozymes with the cell membrane is mediated by a conserved, hydrophobic sequence in the amino terminus of the proteins. This has been most convincingly demonstrated for PDE4A: when the amino terminus is removed PDE4A is no longer localized to the membrane fraction. A similar amino terminus sequence is found in the membrane-bound forms of PDE2, and may also mediate the membrane-association of certain PDE3 isozymes.

Families of phosphodiesterases

The mammalian PDEs are commonly divided into 11 major families, listed in Table 22-1 and schematized in Figure 22-7; these families are delineated on the basis of substrate specificity, sensitivity to pharmacological agents, mechanisms of regulation, and relatedness of amino acid sequence. They are encoded by 21 genes that, through alternative splicing, can produce more than 50 unique enzyme subtypes (Menniti et al., 2006). Subtypes of PDE vary in their expression, regulation, distribution, and kinetics. We group them here on the basis of their most prominent mode of regulation; alternatively, they can be categorized on the basis of their substrate specificity into cGMP-specific (PDE5, 6, and 9), cAMP-specific (PDE3, 4, 7 and 8), and dual-specificity (PDE 1, 2, 10, and 11) enzymes.

Ca^{2+}/calmodulin-stimulated PDEs (PDE1)

The PDE1 family consists of several soluble subtypes of PDE in brain and peripheral tissues. These enzymes are characterized by their regulation by Ca^{2+}/calmodulin. They are highly expressed in neural tissue: two isozymes of PDE1, PDE1A (61 kDa) and PDE1B (63 kDa), account for more than 90 percent of total brain PDE activity. They are encoded by separate genes. PDE1A and B are dual-substrate PDEs; they have a relatively low affinity for substrate, with affinity for cGMP somewhat higher than for cAMP.

PDE1A is expressed at highest levels in cerebral cortex and hippocampus and at moderate levels in amygdala (Yan et al.,

TABLE 22-1 Classification and Selected Properties of Cyclic Nucleotide Phosphodiesterases

	Family regulatory/kinetic characteristics	Genes described	Inhibitors*
1	Ca^{2+}/calmodulin-stimulated PDEs PDE1A/1B, low affinity, cAMP/cGMP PDE1C, high affinity for cAMP	PDE1A PDE1B PDE1C	Trifluoperazine Vinpocetine SCH51866† (0.1 μmol/l)
2	cGMP-stimulated PDEs regulated by cGMP low affinity for cAMP and cGMP	PDE2 (soluble, particulate)	EHNA, erytho-9-(2-hydroxy-3-nonyl) adenine
3	cGMP-inhibited PDEs	PDE3A	Milrinone
	Regulated by phosphorylation and cGMP high affinity, cGMP > cAMP	PDE3B	Enoximone Amrinome‡
4	cAMP-specific PDEs regulated by phosphorylation and cAMP high affinity, cAMP >>> cGMP	PDE4A PDE4B PDE4C PDE4D	Rolipram Ro20–1724
5	cGMP-binding, cGMP-specific PDEs regulated by phosphorylation, cGMP high affinity, cGMP >>> cAMP	PDE5A	Sildenafil Zaprinast Dipyridamole SCH51866† (0.1 μmol/l)
6	Retina cGMP specific PDEs regulated by transducin high affinity, cGMP >>> cAMP	PDE6A PDE6B PDE6C	Zaprinast Zaprinast Zaprinast
7	cAMP-specific PDE rolipram-insensitive	PDE7A PDE7B	–
8	cAMP-specific PDEs IBMX- and rolipram-insensitive	PDE8A PDE8B	–
9	cGMP-specific PDE IBMX-insensitive	PDE9	SCH51866† (1.5 μmol/l)
10	Dual substrate, cAMP-inhibited PDE high affinity, cAMP >>> cGMP	PDE10	SCH51866† (1.0 μmol/l) Dipyridamole
11	Dual substrate PDE cGMP > cAMP	PDE11A	Dipyridamole

*In addition to the relatively specific inhibitors listed in this table, there are a number of compounds, particularly the methylxanthines (e.g., theophylline, isobutylmethylxanthine (IBMX), papaverine, caffeine), that inhibit most major forms of PDE except the newer forms).
†SCH51866 is not selective and inhibits PDE1, PDE5, PDE9, and PDE10 with IC50 values shown.
‡The compounds listed here are among a large number that have been developed as specific inhibitors of PDE3.

1994). Expression of PDE1B is high in brain regions innervated by dopamine (e.g., striatum, nucleus accumbens, olfactory tubercle), with moderate levels of expression found in hippocampus and cerebral cortex (Yan et al., 1994). An additional isozyme, PDE1C (73 kDa), encoded by a third distinct gene, has been identified in brain and testes; in the brain, it is expressed primarily in the olfactory bulb. PDE1C has a higher affinity for substrate than the other PDE1s and differs from them in having approximately equal affinity for cAMP and cGMP (Menniti et al., 2006; Yan et al., 1995).

The PDE1 isozymes are primarily regulated under physiological conditions by intracellular Ca^{2+} levels. Such activation may parallel the activation of Ca^{2+}-activated Group 1 adenylyl cyclases (AC1, 5, and 8), meaning that when these ACs and PDEs are co-expressed (as they are in, for example, hippocampus), the same intracellular signaling can activate both the synthesis and breakdown of cAMP. These enzymes are regulated by protein phosphorylation (Sharma et al., 1985; Hashimoto et al., 1989): the 61 and 63 kDa isozymes are good substrates for protein kinase A and Ca^{2+}/calmodulin-dependent protein kinase, respectively. Such phosphorylation decreases the activity of the enzymes by decreasing their affinity for Ca^{2+}/calmodulin.

cGMP-regulated PDEs (PDE2, PDE3, and PDE11)

There are three PDE families that are regulated primarily by cGMP, two that are stimulated (PDE2 and 11) and one that is inhibited (PDE3). These PDEs exist in both soluble and membrane-associated forms.

cGMP-stimulated PDE2 is a dual-substrate PDE with a low affinity for both cAMP and cGMP, like PDE1A and PDE1B. As its regulation by cGMP implies, there are at least two cyclic nucleotide-binding sites on PDE2: the catalytic site, and a high-affinity site that allosterically regulates the catalytic activity of the enzyme (Fig. 22-7). The regulatory site is not homologous to the cyclic nucleotide-binding sites on other cyclic nucleotide effectors, like PKA, PKG and Epac; rather, it has homology with an evolutionarily conserved sequence known as a GAF domain, named after different classes of proteins that contain this site, including cGMP-specific and cGMP-regulated PDEs, cyanobacterial adenylyl cyclase, and *E. coli* transcription factor FhlA (Martinez et al., 2002). PDE2 can be activated 10- to 50-fold by physiological concentrations of cGMP. However, the enzyme limits its own activation, since cGMP is metabolized rapidly by the activated phosphodiesterase, leading to reduced binding at the GAF site and consequent reduced PDE activity. PDE2 expression is largely restricted to brain and adrenal gland. Within brain, highest levels of the enzyme are seen in cerebral cortex, striatum and hippocampus. PDE2 is phosphorylated by PKA, but this phosphorylation does not appear to significantly affect enzyme activity.

In contrast to PDE2, PDE3 is inhibited by cGMP. There are two closely related PDE3s, PDE3A and PDE3B, transcribed from distinct genes. They are relatively specific for cAMP.

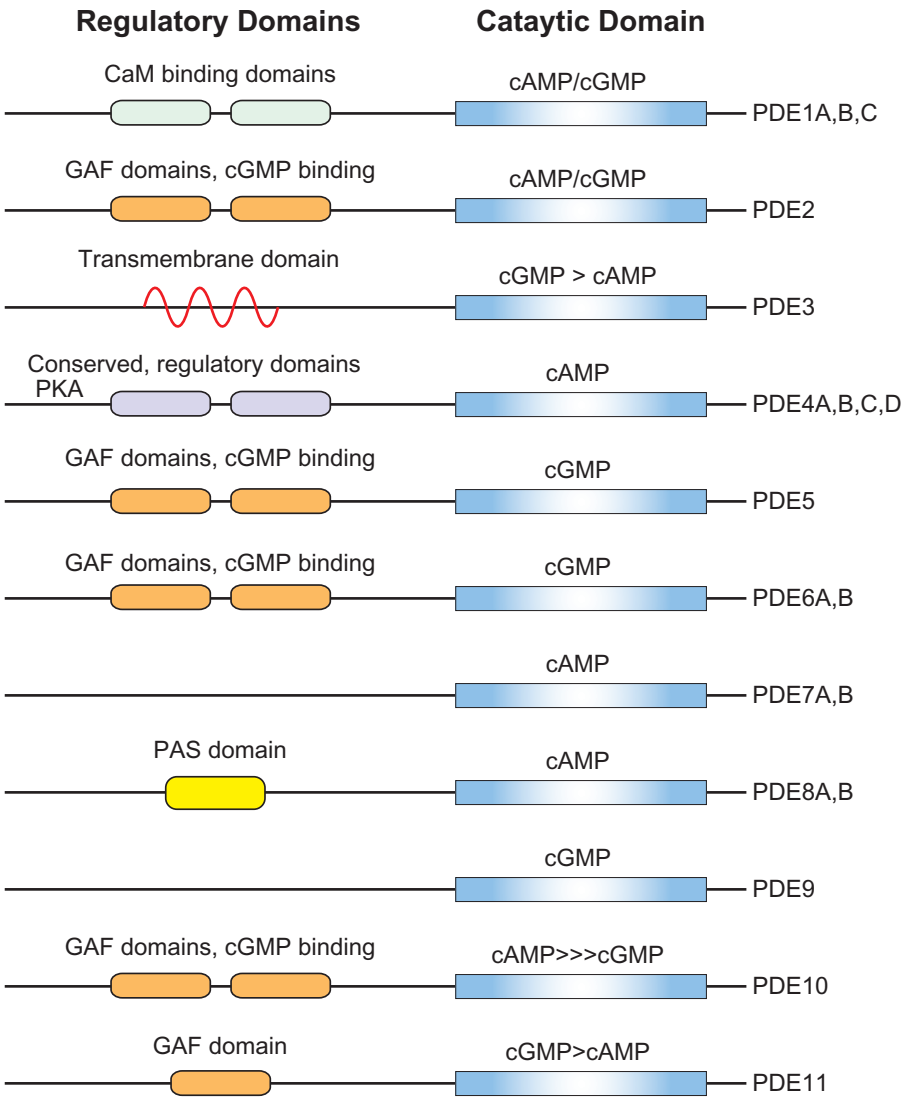

FIGURE 22-7 Schematic illustration of the overall structure and regulatory sites of representative phosphodiesterase subtypes. The catalytic domain of the phosphodiesterases are relatively conserved, and the preferred substrate(s) for each type is shown. The regulatory domains are more variable and contain the sites for binding of Ca^{2+}/calmodulin (CaM) and cGMP. The regulatory domains also contains sites of phosphorylation by cAMP-dependent protein kinase (PKA), protein kinase C (PKC), a cGMP-dependent protein kinase (PKG). In addition, the amino terminus contains a hydrophobic sequence in certain forms of cGS-PDE (PDE2), cGI-PDE (PDE3) and cAMP-PDE (PDE4) that could act to anchor the enzyme to the membrane. CaM-PDE, calmodulin-stimulated PDE; cGS-PDE, cGMP-stimulated PDE; cGI-PDE, cGMP-inhibited PDE; cAMP-PDE, cAMP-specific PDE; cGMP-PDE, cGMP-specific PDE; NH2, amino terminus; COOH, carboxy terminus.

PDE3A is expressed in a complex spatiotemporally regulated pattern during brain development. It is expressed early on in neuroepithelial germinal zones, in a variety of thalamic, brainstem, and cerebellar nuclei during development, and in subpopulations of hippocampal and striatal neurons into adulthood. Outside the CNS, PDE3A is most prominently expressed in adipocytes, liver, and spermatocytes. PDE3B is expressed more broadly through the developing and adult brain, with its mRNA density generally reflecting cell density; it is also prominently expressed in heart, smooth muscle, oocytes and megakaryocytes (Reinhardt et al., 1995; Reinhardt et al., 1996). PDE3 is activated by phosphorylation by PKA, though this has been studied primarily in peripheral tissues and its relevance in the brain remains unclear.

PDE11 is a dual-specificity phosphodiesterase activated by cGMP. It is expressed from a single gene, with at least four splice variants. PDE11 is expressed in skeletal muscle, prostate, liver, kidney, pituitary and salivary gland, with relatively low levels in brain.

G protein–activated phosphodiesterase in retinal phototransduction: PDE6

PDE6 is specific to the rods and cones of the retina and is critical to the process of phototransduction (as described above). PDE6 has a high specificity for cGMP. It is a multimeric enzyme complex, consisting of catalytic PDE6α and PDE6β subunits and a regulatory PDE6γ subunit (the stoichiometry

of these subunits is generally $\alpha\beta\gamma_2$, though it differs in details between rods and cones). The enzyme complex is localized to the cell membrane by farnesylation and geranylgeranylation of the α and β subunits. A fourth subunit, PDEδ, inhibits the activity of the complex. PDE6 is activated by a photoreceptor-specific G protein, transducin, which is activated upon absorption of a photon by photorhodopsin. This rapidly activates its catalytic activity, quickly reducing the high [cGMP] that prevails in the dark and thereby closing CNG cation channels and repolarizing the photoreceptor (Wensel, 2008).

PDEs regulated primarily by phosphorylation: PDE4, 5 and 10

PDEs 4, 5 and 10 are regulated primarily by phosphorylation; they are also regulated by cyclic nucleotide binding.

The four members of the PDE4 family are encoded by four different genes, each of which has alternative transcriptional start sites and multiple splice variants that can produce at least 17 different transcripts. The PDE4s are found in many tissues in both soluble and membrane-associated forms, and are abundant in the central nervous system. They have a high specificity for cAMP.

The ability of alternative transcriptional start sites and splicing to influence the regulation of PDE4 isozymes has been studied in some detail. Short and long forms of PDE4D are generated by alternative splicing. Expression of the short forms (D1 and D2, 67–72 kDa) results from activation of an intronic promoter, whereas expression of the long form (D3, 93 kDa) results from activation of another promoter located further upstream. The long form, but not the short forms, contains a site for phosphorylation and is regulated by protein kinase A (Sette et al., 1994). The short forms, however, are upregulated at the transcriptional level by chronic elevations in cAMP. This dual regulation may provide both short-term (via PKA-mediated phosphorylation) and long-term (via transcriptional upregulation) negative feedback to chronic elevations in cAMP. The PDE4A-C genes also have splice variants that give rise to short and long forms that may be regulated in a similar manner. The activity of the long, but not the short, forms is enhanced by phosphatidic acid and phosphatidylserine.

PDE4A and PDE4B are expressed at relatively high levels in hippocampus, cerebral cortex, and striatum, and represent the majority of the membrane-bound form of PDE4 in these brain regions (McPhee et al., 1995). PDE4D represents the majority of the soluble PDE4 in brain. Expression of PDE4C is relatively low in nervous tissue. PDE4 activity has been particularly implicated in synaptic plasticity and learning; rolipram, an inhibitor of all 4 PDE4 isozymes, has been explored as a potential cognitive enhancer, in normal subjects, aging, and dementia (Blokland et al., 2006). There has also been great interest in the role of PDE4 as a regulator of mood-regulating cAMP signaling and a potential novel antidepressant target.

In addition to their well-characterized regulation by PKA, certain PDE4 isozymes have been reported to be phosphorylated by protein kinase C and MAP-kinases, and in response to insulin, although this regulation by phosphorylation is incompletely characterized and the functional consequences are not yet well-established.

PDE5 has a high specificity for cGMP (a property it shares with PDE6 and PDE9). It became famous in recent years as the target of the erectile dysfunction drugs such as sildenafil (Viagra). PDE5 is regulated by phosphorylation; it also contains an N-terminal GAF domain that binds and allows coordinate regulation by cAMP. In addition to its expression in smooth muscle and in erectile tissue, PDE5 is expressed at lower levels in brain and has been implicated in cognitive functions, including learning and memory (Van Staveren et al., 2003). Interestingly, sildenafil treatment can lead to aggression in mice, emphasizing the role of PDE5 in regulating complex CNS functions (Kleppisch et al., 2009).

PDE10 is a dual-specificity phosphodiesterase with a higher affinity for cAMP than for cGMP (K_m values are 0.05 and 3.0 µM, respectively) (Soderling et al., 1999). However, while it has a higher affinity for cAMP, it has a five-fold greater V_{max} for cGMP, suggesting that cAMP may function as an inhibitor of cGMP hydrolysis. PDE10A (along with PDE11A) has a GAF domain at its N-terminus homologous with that found in PDE2, PDE5, and PDE6. As described above, these GAF domains may serve as noncatalytic cGMP binding sites for allosteric modulation of enzyme activity. In the brain, PDE10 is expressed in striatum, globus pallidus and substantia nigra (Coskran et al., 2006); outside the CNS, expression is most prominent in testes. The ability of this PDE to regulate cAMP signaling in the basal ganglia has led to substantial interest in it as a potential drug target in schizophrenia (Menniti et al., 2006; Siuciak, 2008).

THE MECHANISMS OF ANTIDEPRESSANT ACTION

Christopher Pittenger

Given the centrality of cyclic nucleotide signaling in many aspects of neuronal signaling and brain function, it is not surprising that alterations in cAMP and cGMP signaling are implicated in a variety of neuropsychiatric disorders and in the actions of numerous medications that influence the central nervous system.

For example, increasing evidence suggests that upregulation of cAMP-mediated signaling is central to the mechanism of action of many antidepressant drugs (Pittenger et al, 2008). Most conventional antidepressants act by increasing levels of the monoaminergic neurotransmitters serotonin (5-HT), norepinephrine (NE) and dopamine (DA) at synapses in the brain, most commonly by blocking their reuptake into neurons. Among the downstream effects is an enhancement of cAMP signaling in the hippocampus through increased activation of G protein–coupled receptors and membrane-bound adenylyl cyclase activity. This leads to an increase in cAMP, PKA levels, and activation of downstream targets such as the transcription factor CREB

THE MECHANISMS OF ANTIDEPRESSANT ACTION (*cont'd*)

(Pittenger et al., 2008). These changes are likely to be important for antidepressant efficacy: enhancement of CREB function in transgenic mice leads to an antidepressant response in animals, independent of any pharmacological treatment (reviewed in Pittenger et al., 2008).

Because of the association of this signal transduction cascade with antidepressant response, there has been significant interest in the potential antidepressant efficacy of pharmacologically inhibiting phosphodiesterases. These efforts have focused primarily on the type 4 phosphodiesterase, which is highly expressed in hippocampus and other limbic brain regions implicated in depression. Rolipram, an inhibitor of the PDE4, has been found to have some antidepressant efficacy in a controlled clinical trial (Fleischhacker et al., 1992). Unfortunately, rolipram produced significant side effects—chiefly nausea—that have limited its potential as an antidepressant treatment. Ongoing pharmacological studies are seeking to identify more specific inhibitors of PDE4 isozymes in an effort to identify a pharmacological strategy that will produce a novel antidepressant that is better tolerated by patients.

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PDE7, 8 and 9

The regulation of PDE families 7, 8 and 9 is not yet well characterized. PDEs 8 and 9 are unusual in that they are not inhibited by isobutylmethylxanthine (IBMX), a broad-specificity PDE inhibitor that affects most other phosphodiesterases.

PDE7 and PDE8 are high-affinity cAMP-specific phosphodiesterases. There are two members of each family, PDE7A and PDE7B, and PDE8A and PDE8B. Unlike the PDE4s, the other cAMP-specific phosphodiesterase family, PDE7A-B and PDE8A-B are not inhibited by rolipram (see Table 22-1).

PDE7A is expressed at high levels in the immune system, but is also expressed throughout the brain, with highest levels seen in hippocampus, olfactory system, thalamus and certain brainstem nuclei (Miro et al., 2001). PDE7B is expressed at relatively high levels in portions of the basal ganglia, including the striatum, olfactory tubercle and islands of Calleja, as well as in dentate gyrus, thalamus and Purkinje cells of the cerebellum (Reyes-Irisarri et al., 2005), and in pancreas, heart, thyroid, skeletal muscle and liver.

PDE8A is expressed at low levels in brain; it is expressed at high levels in liver and testis. PDE8B, in contrast, is expressed primarily in brain, with high levels in hippocampus and cerebral cortex. Interestingly, PDE8B expression is increased in both cortex and hippocampus in Alzheimer's disease (Perez-Torres et al., 2003). The amino terminus of PDE8A and B contains a PAS domain, a conserved motif that can mediate homomeric and heteromeric protein–protein interactions; this domain may influence the subcellular distribution and regulation of these PDEs (Wu et al., 2004).

There is a single PDE9 gene, with five characterized splice variants. PDE9 is a high-specificity cGMP phosphodiesterase. It has the highest affinity for cGMP of any of the PDEs. Expression is widespread in the brain, with highest levels of expression in cerebellar Purkinje cells, striatum, nucleus accumbens, hippocampus, and cerebral cortex (Van Staveren et al., 2003). Expression parallels that of soluble, NO-activated guanylyl cyclase (see above); together with the high affinity and specificity of PDE9 for cGMP, this fact suggests functional coupling between these enzymes in the regulation of cGMP signaling throughout much of the central nervous system. Peripherally, PDE9 is expressed in the immune system, intestine and prostate.

Phosphodiesterases as pharmacological targets

The universality of cyclic nucleotide signaling in the brain, together with the molecular diversity and differential expression of the phosphodiesterases, makes them attractive potential therapeutic targets (see Table 22-1). An early example of such pharmacological inhibition is provided by the methylxanthines, including caffeine and theophylline, which have been used therapeutically in the treatment of chronic obstructive pulmonary disease and are the mild stimulants present in coffee, tea, and related substances. Inhibition of PDE contributes to some of the clinical effects of these drugs, particularly at high doses. The PDE5 inhibitor sildenafil, and related compounds, are used in the treatment of erectile dysfunction, as discussed above.

Other potential applications of specific PDE inhibitors in a variety of contexts remain theoretical but represent an area of active research. Several have been mentioned above. For example, inhibitors of PDE4 have been explored as potential cognitive enhancers and memory promoters (Blokland et al., 2006). PDE4 inhibitors have also been explored as potential antidepressants, because of the observation that the cAMP cascade implicated in synaptic plasticity and learning is also upregulated by a variety of clinically used antidepressant treatments (Pittenger et al., 2008).

SPATIOTEMPORAL INTEGRATION AND REGULATION OF CYCLIC NUCLEOTIDE SIGNALING IN NEURONS

The complex geometry of neurons creates unique opportunities for restriction of second-messenger signaling

to functional microdomains. This has been best characterized in the case of calcium signaling, in part because of the availability of calcium-sensitive fluorescent dyes that permit the optical detection of calcium transients in dendritic spines and other microdomains, both *in vitro* and, more recently, *in vivo*. But it is no less true of cyclic nucleotide signaling; and imaging technologies that allow visualization of cAMP and cGMP concentrations with subcellular resolution, analogous to calcium imaging, have recently led to enhanced appreciation of some of the details of spatiotemporal integration of these signals (Baillie, 2009). It has become clear that cAMP and cGMP can produce multiple, discrete subcellular gradients that may carry information. This makes the subcellular localization of cyclases and phosphodiesterases of critical importance. Specific protein–protein interactions provide for discrete localization of these enzymes.

As summarized above, adenylyl cyclases AC1-9 are intrinsic membrane proteins that are activated by G protein signaling; cAMP production is therefore primarily localized to the plasma membrane. Subcellular targeting of ACs can influence the source of cAMP gradients upon their activation. For example, in mouse brain, AC1 has been found to be enriched in postsynaptic density fractions, while AC8 is enriched presynaptically (Conti et al., 2007). Such differential targeting of differentially regulated cyclase isozymes may provide subcellular specificity to cAMP production. Membrane-bound guanylyl cyclase is also subject to subcellular targeting within the membrane. For example, GC-D and GC-E, which are central to phototransduction, are specifically targeted to the photoreceptor outer segment membrane (Wensel, 2008).

The phosphodiesterases may be the primary determinants of the geometry of cAMP and cGMP signaling. Subcellularly targeted PDEs have the capacity to produce local 'sinks' of cyclic nucleotides, limiting the spatial extent of their effect and potentially producing the spatial boundaries of functional microdomains (Baillie, 2009). Such subcellular targeting has been particularly well described for the PDE4 isozymes. For example, the PDE4D5 splice variant appears to be exquisitely targeted to β-adrenergic receptors, producing a local cAMP sink and truncating the spatial and temporal extent of signaling after activation of these receptors (Baillie, 2009). The wealth and variety of the PDEs provides enormous scope for such regulation; the details of this dimension of cyclic nucleotide signaling are only now beginning to emerge.

CONCLUSION AND FUTURE PERSPECTIVE

Cyclic AMP was the first second messenger to be characterized. Decades of work have refined the details of cAMP and cGMP signaling, both within and outside the central nervous system, and have revealed more and more areas in which such signaling is central to neuronal differentiation, function, adaptation, and pathology. Correspondingly, the variety of enzymes involved in cyclic nucleotide synthesis, regulation, degradation, and downstream effector responses is enormous. A central current research need is a careful characterization of the subcellular localization of these enzymes within specific neuronal types and how they interact in space and time to provide integration and specificity to the cellular response to impinging signals. In this regard, the recent development of fluorescent sensors that can reveal subcellular gradients and microdomains of cyclic nucleotides is an exciting advance (Baillie, 2009).

Ultimately, better understanding of the function and interaction of these signaling pathways will lead to a fuller appreciation of their contribution not only to normal neuronal function, but to neuropsychiatric disease. Another exciting direction of recent research is the development of new and more specific pharmacological agents to target specific isozymes, especially the phosphodiesterases. In light of the wealth of neurobiological processes that are regulated by cyclic nucleotide signaling, there is optimism for an advancing capacity to pharmacologically manipulate specific cyclases and phosphodiesterases that will permit new therapeutic approaches to a variety of neurological and psychiatric conditions.

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