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MINIREVIEW

Calmodulin-Regulated Adenylyl Cyclases: Cross-Talk and Plasticity in the Central Nervous System

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ABSTRACT

Gene disruption studies have shown that the Ca^{2+}-stimulated adenylyl cyclases, AC1 and AC8, are critical for some forms of synaptic plasticity, including long-term potentiation as well as long-term memory formation (LTM). It is hypothesized that these enzymes are required for LTM to support the increased expression of a family of genes regulated through the cAMP/Ca^{2+} response element-binding protein/cAMP response element transcriptional pathway. In contrast to AC1 and AC8, AC3 is a Ca^{2+}-inhibited adenylyl cyclase that plays an essential role in olfactory signal transduction. Coupling of odorant receptors to AC3 stimulates cAMP transients that function as the major second messenger for olfactory signaling. These cAMP transients are caused, at least in part, by Ca^{2+} inhibition of AC3, which is mediated through calmodulin-dependent protein kinase II. The unique structure and regulatory properties of these adenylyl cyclases make them attractive drug target sites for modulation of a number of physiological processes including memory formation and olfaction.

Cross-talk between the cAMP signal transduction system and other signaling pathways is important for several forms of neuroplasticity, including long-term potentiation (LTP) and memory formation. The Ca^{2+}-regulated adenylyl cyclases are important for adaptive changes in neurons because they provide a critical linkage between Ca^{2+} and cAMP signaling. This review focuses on the physiological roles of three Ca^{2+}-regulated adenylyl cyclases, AC1, AC3, and AC8. AC1 and AC8 are Ca^{2+}/CaM-stimulated enzymes, whereas Ca^{2+} inhibits AC3. Gene disruption studies have revealed that these enzymes play critical roles in several physiological processes, including olfaction, development of the sensory motor cortex, and hippocampus-dependent memory formation.

Distribution and Regulatory Properties of AC1

The existence of distinct forms of Ca^{2+}-sensitive and -insensitive adenylyl cyclases was first demonstrated using CaM-Sepharose affinity chromatography (Westcott et al., 1979). Partially purified adenylyl cyclase from bovine brain was resolved into two forms: a Ca^{2+}/CaM stimulated activity and a CaM-insensitive activity. In addition, polyclonal antibodies that distinguish between CaM-sensitive and -insensitive adenylyl cyclases in brain were isolated (Rosenberg and Storm, 1987). CaM-stimulated adenylyl cyclase was subsequently purified extensively using either CaM-Sepharose affinity chromatography (Yeager et al., 1985) or forskolin-Sepharose affinity chromatography (Pfeuffer et al., 1985; Smigel, 1986). The availability of purified adenylyl cyclase led to the isolation of a cDNA clone encoding AC1 (Krupinski et al., 1989).

Although the sequence of AC1 and other adenylyl cyclases predicts a membrane topology reminiscent of transporters or ion channels, there is no evidence that these enzymes function as membrane transporters or channels. However, it is interesting that cultured neurons express a voltage-sensitive adenylyl cyclase activity that is Ca^{2+}-independent (Reddy et al., 1995). This suggests the interesting possibility that neurons contain an adenylyl cyclase that is sensitive to the membrane potential or that an adenylyl cyclase is associated with a regulatory protein that is sensitive to the membrane potential.

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ABBREVIATIONS: LTP, long-term potentiation; LTM, long-term memory; CaM, calmodulin; IQ domain, protein motif mediating interaction with calmodulin, usually consisting of consensus sequence as IQXXXRGXXXR; CRE, cAMP/Ca^{2+} response element; CREB, cAMP/Ca^{2+} response element-binding protein; PKA, cAMP-dependent protein kinase; NMDA, *N*-methyl-D-aspartate; Erk/MAP, extracellular signal-regulated kinase/mitogen-activated protein; L-LTP, long-lasting long-term potentiation.

Of the adenylyl cyclase genes cloned, AC1 is the only neurospecific adenylyl cyclase identified thus far; it is expressed in brain, adrenal medulla, and retina (Xia et al., 1993). AC1 mRNA is expressed in the hippocampus (dentate gyrus, CA1-CA3), neocortex, entorhinal cortex, cerebellar cortex, and the olfactory system as well as the pineal (Xia et al., 1991; Tzavara et al., 1996). In monkey brain, AC1 protein is detectable in the mossy fibers as well as the molecular layers of both the dentate gyrus and fields CA1, CA2, and CA3 of the hippocampus (Kumar et al., 2001). Because AC1 is neurospecific, it provides a "pharmacological window of opportunity" to increase cAMP in specific areas of brain without the side affects associated with cAMP increases in other tissues.

AC1 is directly stimulated by Ca^{2+} and CaM in vivo (Choi et al., 1992a; Wu et al., 1993) with half-maximal stimulation at 150 to 200 nM free Ca^{2+}, concentrations just above resting free Ca^{2+} in neurons. Several point mutations within the CaM binding domain of AC1 were made to determine whether its Ca^{2+} sensitivity can be modified by mutagenesis and to verify assignment of the CaM binding domain. Replacement of Lys-504 with Asp causes a 4-fold decrease in sensitivity to Ca^{2+}. Ca^{2+} and CaM stimulation are abolished by substitution of Phe-503 with Arg-503. Stimulation of AC1 activity in vivo by intracellular Ca^{2+} is also greatly diminished with the Arg-503 mutant. This indicates that Ca^{2+} stimulation of the enzyme in vivo is caused primarily by direct interactions with CaM and Ca^{2+}. Direct interaction between the catalytic subunit of the adenylyl cyclase and CaM was also demonstrated by ^{125}I-CaM gel overlays (Minocherhomjee et al., 1987).

Although AC1 is not stimulated by activation of G_s-coupled receptors alone, it is stimulated by receptor activation when paired with Ca^{2+}(Wayman et al., 1994). Consequently, AC1 is synergistically stimulated by Ca^{2+} and β-adrenergic agonist and functions as a coincidence factor to integrate Ca^{2+} and receptor-mediated signals. AC1 is inhibited by G_i-coupled receptors, including somatostatin and dopamine D_2 receptors (Nielsen et al., 1996). It is also inhibited by CaM kinase IV in vivo (Wayman et al., 1996). The enzyme has two CaM kinase IV consensus phosphorylation sequences near its CaM binding domain at Ser-545 and Ser-552. Conversion of either serine to alanine by mutagenesis abolishes CaM kinase IV inhibition of AC1, suggesting that the activity of this enzyme may be directly inhibited by CaM kinase IV phosphorylation. These inhibitory constraints on AC1 activity may be required to prevent spurious cAMP increases caused by Ca^{2+} transients and to insure a significant signaling differential. Because synaptic plasticity may depend upon optimal cAMP levels, or transient cAMP increases, mechanisms for attenuation of AC1 may be just as important as stimulatory mechanisms.

Distribution and Properties of AC8

Although AC8 is expressed in brain, it is not neurospecific and is found in other tissues, including lung (Muglia et al., 1999) and parotid gland (Watson et al., 2000). In brain, the highest levels of AC8 mRNA are within the olfactory bulb, thalamus, habenula, cerebral cortex, and hypothalamic supraoptic and paraventricular nuclei. AC8 is also expressed in the hippocampus and cerebellum. AC8 is stimulated by CaM but its Ca^{2+} sensitivity is approximately 5-fold lower than AC1 (half-maximal activation by Ca^{2+} is 150 nM for AC1 and 800 nM for AC8) (Nielsen et al., 1996). The Ca^{2+} activation curve for adenylyl cyclase activity in hippocampal membrane preparations reflects the presence of a mixture of AC1 and AC8. The CaM binding domain of AC8, which is localized to the C-terminal end of AC8, resembles an IQ domain. Interestingly, IQ domains generally mediate Ca^{2+}-inhibited or Ca^{2+}-independent binding of proteins to CaM (Alexander et al., 1988). Studies with the P/Q-type calcium channels suggested a novel Ca^{2+}-dependent calmodulin binding site in AC8 (Lee et al., 1999). The C-terminal region of AC8 shares significant sequence similarity to the novel CaM binding domain in α_{1A} subunit of P/Q-type calcium channels. Peptides corresponding to the CaM binding domain in AC8 and α_{1A} subunit inhibited calmodulin stimulation of AC8 activity (S. Wong and D. R. Storm, unpublished data). In another line, Gu and Cooper showed that the IQ domain in the C terminus of AC8 interacted with CaM and modulate Ca^{2+} stimulation (Gu and Cooper, 1999). Like AC1, AC8 is not stimulated by Gs-coupled receptors in vivo (Nielsen et al., 1996), even though it is stimulated by G_S-α complexed to guanosine 5′-*O*-(3-thio)triphosphate in vitro (Cali et al., 1994). In contrast to AC1, AC8 is not synergistically stimulated by G_s-coupled receptors and Ca^{2+} in vivo. Although serotonin stimulates AC8 activity in vivo, this stimulation is mediated by serotonin-induced increases in intracellular Ca^{2+} (Baker et al., 1998). AC8 is not inhibited by G_i-coupled receptors in vivo or by CaM kinase IV; it is a pure Ca^{2+} detector that responds to relatively high concentrations of Ca^{2+} compared with AC1.

Distribution and Regulatory Properties of AC3

Although AC3 is expressed in a number of tissues including heart, vascular smooth muscle, germ cells, brain, and lung (Xia et al., 1992; Defer et al., 1998; Ishikawa et al., 2000), it was first discovered in the olfactory epithelium (Bakalyar and Reed, 1990). AC3 in membrane preparations is stimulated by Ca^{2+} and CaM, when it is concomitantly activated by Gs (Choi et al., 1992b). However, submicromolar concentrations of intracellular free Ca^{2+} inhibit receptor-stimulated AC3, suggesting that it may be inhibited by a Ca^{2+}-stimulated kinase (Wayman et al., 1995b). Indeed, CaM kinase inhibitors antagonize Ca^{2+} inhibition of AC3 and coexpression of constitutively activate CaM kinase II completely inhibits isoproterenol-stimulated AC3 activity. Furthermore, AC3 is phosphorylated in human embryonic kidney-293 cells when intracellular Ca^{2+} is increased. This phosphorylation is prevented by CaM-kinase inhibitors (Wei et al., 1996). Site-directed mutagenesis of a CaM-kinase II consensus site (Ser-1076 to Ala-1076) in AC3 blocks Ca^{2+}-stimulated phosphorylation and inhibition of AC3 in vivo. Cells expressing AC3 exhibit hormone-stimulated Ca^{2+} and cAMP oscillations. Activation of AC3 by hormones through G protein increases cAMP level and stimulates PKA activity. Consequently, PKA phosphorylates and activates inositol trisphosphate receptors, which are responsible for Ca^{2+} release from internal pool. This leads to activation of CaMKII and inhibition of AC3, and intracellular cAMP level drops because of cAMP phosphodiesterase activity. Once cAMP level decreases below a certain threshold point, the inositol trisphosphate receptors are dephosphorylated, and Ca^{2+} gets resequestered (Wayman et al., 1995a). Consequently, cAMP and Ca^{2+} will continue to oscillate as long as an adenylyl

cyclase activator stimulates the enzyme. Ca^{2+} oscillations in cells expressing AC3 show a periodicity of 3 to 5 min. The distribution and regulatory properties of AC1, AC3, and AC8 are summarized in Table 1.

AC1 is the Gatekeeper for Pineal Melatonin Synthesis

The circadian organization of behavior determines how complex organisms respond to social and light/dark cues encountered on a daily and seasonal basis. The daily cycle of melatonin synthesis in the pineal is controlled by the circadian clock in the suprachiasmatic nucleus (Chang and Reppert, 2001; Dunlap, 1999). Melatonin biosynthesis in the pineal gland is regulated by cAMP, which stimulates transcription of genes encoding enzymes important for circadian expression of melatonin (Borjigin et al., 1995).

Stimulation of adenylyl cyclase activity in the pineal in response to activation of β- and α1-adrenergic agonists suggest that AC1 plays a major role in generating cAMP signals in response to nocturnal norepinephrine. Increases in pineal intracellular cAMP caused by activation of β-adrenergic receptors are greatly enhanced by costimulation of α_1-adrenergic receptors (Vanecek et al., 1985). Because activation of α1-adrenergic receptors increases intracellular Ca^{2+}, this cAMP increase is very probably caused by synergistic stimulation of AC1 by Ca^{2+} and β-adrenergic receptors (Wayman et al., 1994). Ca^{2+}-stimulated adenylyl cyclase activity in the pineal shows a circadian oscillation with maximal levels during subjective night (Tzavara et al., 1996). On the other hand, AC1 mRNA shows maximum expression at midday with a minimum at night; synthesis of mRNA for AC1 and translation to protein are separated by 12 h. An analysis of the promoter for the AC1 gene provides an explanation for the circadian expression of AC1 mRNA (Chan et al., 2001). A 280-bp fragment from the AC1 promoter region that contains the transcription start site directs reporter gene expression in cultured pinealocytes and CNS neurons. Interestingly, pinealocyte expression of the reporter gene is inhibited by increases in cAMP. This cAMP-inhibitable element may explain why AC1 mRNA in the pineal gland is low at night, when cAMP is elevated, and high during the day, when cAMP signals drop.

Calcium-Stimulated Adenylyl Cyclase Activity is Critical for Hippocampus Dependent Long-Term Memory and Synaptic Plasticity

The physiological roles of Ca^{2+}-stimulated adenylyl cyclases have been evaluated by generation of $AC1^{-/-}$ mice (Wu et al., 1995), $AC8^{-/-}$ mice (Wong et al., 1999; Schaefer et al., 2000; Watson et al., 2000), and $AC1^{-/-} \times AC8^{-/-}$ double knockout mice (Wong et al., 1999). $AC1^{-/-}$ mice have normal growth, motor coordination, and longevity. They show no detectable anatomical or morphological differences in the brain except that they lack barrel patterning in the sensory motor cortex (Abdel-Majid et al., 1998). Compared with wild-type mice, Ca^{2+}-sensitive adenylyl cyclase activities in the hippocampus and cerebellum are decreased approximately 50% in $AC1^{-/-}$ mice. Because there are comparable levels of AC1 and AC8 in the hippocampus, this indicates that there are not compensating increases in AC8 to adjust for the loss of AC1. Similar observations have been made with AC8 mutant mice; there is no compensating increase in AC1 with $AC8^{-/-}$ mice. Membranes isolated from the hippocampus of $AC1^{-/-} \times AC8^{-/-}$ mice show no Ca^{2+}-stimulated adenylyl cyclase activity (Wong et al., 1999).

$AC1^{-/-}$ Mice Exhibit Defects in Several Types of LTP

Although they exhibit long-lasting LTP (L-LTP) in area CA1 of the hippocampus that persists for greater than 3 h, there are quantitative differences between the mutant and wild-type mice (Wu et al., 1995). For example, the rate of increase of the excitatory postsynaptic potential slope in hippocampal slices from the mutant mice is half that of the wild-type mice. The maximal field excitatory postsynaptic potential slope above baseline is also significantly reduced in mutant mice. In contrast to NMDA receptor-dependent LTP in area CA1, which is dependent upon increased postsynaptic Ca^{2+}, mossy fiber LTP requires an increase in presynaptic Ca^{2+} (Zalutsky and Nicoll, 1990; Johnston et al., 1992). Evidence from several laboratories suggests that PKA activation is obligatory for the induction and maintenance of mossy fiber LTP (Weisskopf et al., 1994; Huang and Kandel, 1996), and it is hypothesized that mossy fiber LTP is caused by stimulation of an adenylyl cyclase by presynaptic Ca^{2+} increases (Weisskopf et al., 1994; Villacres et al., 1998). Subsequent activation of PKA may stimulate prolonged glutamate release. To test this hypothesis, mossy fiber LTP was examined in $AC1^{-/-}$ mice. Although the mutant mice exhibit normal paired pulse facilitation, mossy fiber LTP is significantly impaired in $AC1^{-/-}$ mice. High concentrations of forskolin induce mossy fiber LTP to comparable levels in wild-type and AC1 mutant mice. This indicates that signaling components downstream from the adenylyl cyclase, including PKA, ion channels, and secretory machinery, are not affected by disruption of the AC1 gene. These data indicate that coupling of Ca^{2+} to activation of AC1 is crucial for mossy fiber LTP, most likely through activation of PKA and enhancement of excitatory amino acid secretion (Trudeau et al., 1996; Castillo et al., 1997; Geppert et al., 1997). Because

TABLE 1
Distribution and regulatory properties of Ca^{2+}-sensitive adenylyl cyclases

	Distribution	Modulation by G Proteins and in Vivo Effects	Modulation by Ca^{2+}/Half-Maximal Concentration	Phosphorylation and In Vivo Effects
AC1	Brain (abundant in dentate gyrus and cerebral cortex), adrenal medulla, and retina	G_s/activation when paired with Ca^{2+} G_i/inhibition	Activation/150 to 200 nM	CamK IV/inhibition PKC/activation
AC3	Olfactory epithelium, retina, brain, spinal cord, adrenal medulla, adrenal cortex, germ cells, smooth muscle, heart, and lung.	G_s/activation	Inhibition/100 to 200 nM	CamK II/inhibition PKC/activation
AC8	CNS, lung, and parotid gland; low in heart and ovary.	Not regulated by G_i or G_s.	Activation/800 nM	Not determined

there are noradrenergic projections from the locus ceruleus to the dentate gyrus and to the stratum lucidum of the CA3, where the glutamatergic mossy fibers terminate, modulation of mossy fiber LTP by β-adrenergic input (Huang and Kandel, 1996) may be attributable to synergistic stimulation of AC1 by β-adrenergic receptors and Ca^{2+}.

The presynaptic LTP mechanism described above may not be unique to the mossy fiber/CA3 synapse. AC1 is expressed at relatively high levels in cerebellar granule cells (Xia et al., 1991). Cerebellar parallel fibers exhibit an LTP with properties similar to hippocampal mossy fiber LTP (Salin et al., 1996). It is independent of NMDA receptors but dependent on extracellular Ca^{2+} and adenylyl cyclase activation. Interestingly, $AC1^{-/-}$ mice show a number of defects in cerebellar physiology, including the complete lack of parallel fiber/Purkinje cell LTP induced by 4- to 8-Hz parallel fiber stimulation (Storm et al., 1998; Lev-Ram et al., 2002). This blockade is not accompanied by alterations in a number of basal electrophysiological parameters and is bypassed by application of an exogenous cAMP analog, suggesting that it results specifically from deletion of AC1. However, cerebellar LTP induced by 1 Hz parallel fiber stimulation for at least 300 s is not reduced in $AC1^{-/-}$ mutant mice (Lev-Ram et al., 2002).

$AC1^{-/-}$ mice show normal LTM for several forms of fear-associated learning including contextual, passive avoidance, and cued training. However, they do not show normal spatial memory when examined in the Morris water task, a test that measures the ability of a mouse to navigate by means of direct and indirect visual cues (Wu et al., 1995). Although $AC1^{-/-}$ mice learn to find the hidden platform in the Morris water task as well as wild-type mice, they do not show a preference for the region where the platform was during training. This suggests that AC1 may be important for spatial memory.

Because $AC1^{-/-}$ and $AC8^{-/-}$ mice both exhibit L-LTP as well as fear-associated learning and memory, transgenic mice were prepared that lacked both AC1 and AC8 ($AC1^{-/-} \times AC8^{-/-}$ mice). Although the single mutants exhibit normal LTM for contextual and passive avoidance learning, the $AC1^{-/-} \times AC8^{-/-}$ mice do not (Wong et al., 1999). However, $AC1^{-/-} \times AC8^{-/-}$ mice are able to learn and exhibit short-term memory that lasts only 5 to 10 min after training (Fig. 1). This indicates that the $AC1^{-/-} \times AC8^{-/-}$ mice are memory mutants but learn normally and with normal short-term memory. To determine whether this defect in passive avoidance LTM is caused by a loss of cAMP increases in the hippocampus, $AC1^{-/-} \times AC8^{-/-}$ mutant mice were unilaterally cannulated to administer forskolin, an adenylyl cyclase activator. Administration of forskolin to area CA1 of the hippocampus 15 min before training restores normal memory for passive avoidance learning. The defect in L-LTP is also reversed by application of forskolin to $AC1^{-/-} \times AC8^{-/-}$ hippocampal slices. These data indicate that Ca^{2+}-stimulated adenylyl cyclase activity is essential for L-LTP as well as some forms of LTM and that either AC1 or AC8 can produce the critical cAMP signal.

Why is Ca^{2+}-stimulated adenylyl cyclase activity required for LTM? Transcriptionally dependent L-LTP in area CA1 of the hippocampus and hippocampus-dependent LTM are initiated by activation of NMDA receptors and postsynaptic Ca^{2+} increases. Both of these processes depend on cAMP signaling and de novo transcription. The transcriptional pathway most strongly implicated in LTM formation is the CREB/CRE-transcriptional pathway (Athos et al., 2002; Bourtchuladze et al., 1994; Impey et al., 1998b; Pittenger et al., 2002). It is hypothesized that long-term increases in synaptic plasticity and LTM depend, at least in part, on increased transcription of a family of genes regulated through CREs in their promoters. Ca^{2+} increases generated through NMDA receptors stimulate CRE-mediated transcription in the hippocampus by stimulating Erk/MAP kinase, CaM-stimulated adenylyl cyclases, and CaM kinase IV (Fig. 2). The major Ca^{2+}-stimulated CREB kinase in hippocampal neurons is rsk2, which is activated by Ca^{2+} through the Erk/MAP kinase family, a process that requires the nuclear translocation of Erk/MAP kinase (Impey et al., 1998a). The nuclear translocation of Erk/MAP kinase depends upon a cAMP signal, which, we hypothesize, arises from CaM-stimulated adenylyl cyclases.

AC1 Is Required for Development of the Mouse Somatosensory Cortex

The somatosensory cortex of mice contains a patterned distribution of neurons in layer IV termed the barrelfield (Woolsey and Van der Loos, 1970). Thalamocortical afferents terminating in layer IV are organized such that each barrel structure, a group of neurons surrounding a cell-sparse center, represents a specific receptive field. Mice homozygous for the barrelless (brl) mutation, a spontaneous mouse mutant, do not develop barrel structures in the somatosensory cortex even though the size of individual whisker representations is comparable with those wild-type mice (Welker et al., 1996). A genetic analysis revealed that the AC1 gene (Adcy1) is disrupted in brl mutant mice (Abdel-Majid et al., 1998), a discovery that was confirmed by showing that *AC1*$^{-/-}$ mice are also barrelless. This was the first evidence that the cAMP signal transduction pathway is important for pattern formation in the brain. The specific role of AC1 for pattern formation has not been defined but is presumably dependent upon Ca^{2+} -stimulated cAMP increases in the somatosensory cortex.

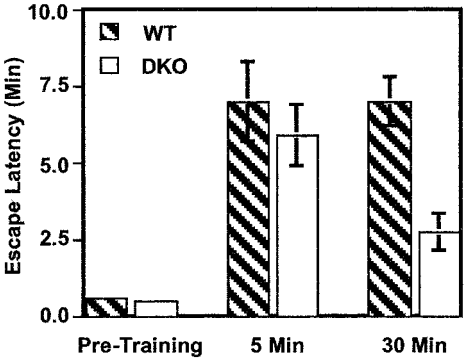

Fig. 1. Mice lacking both AC1 and AC8 have short-term memory but show memory loss within 30 min after training for passive avoidance learning and memory. During passive avoidance training, mice are placed into a chamber that has lighted and dark sides. Mice have a natural tendency to crossover into the dark chamber. During training, mice were shocked when they crossed over to the dark. Memory for passive avoidance training is manifested as an increase in the crossover latency when they are placed into the training chamber later. Wild type (n = 9) and $AC1^{-/-} \times AC8^{-/-}$ (n = 14) mutant mice were submitted to passive avoidance training and analyzed for memory at 5 and 30 min. $AC1^{-/-} \times AC8^{-/-}$ mice exhibit comparable crossover latencies at 5 min, whereas they exhibit a significant deficit in passive avoidance memory at 30 min.

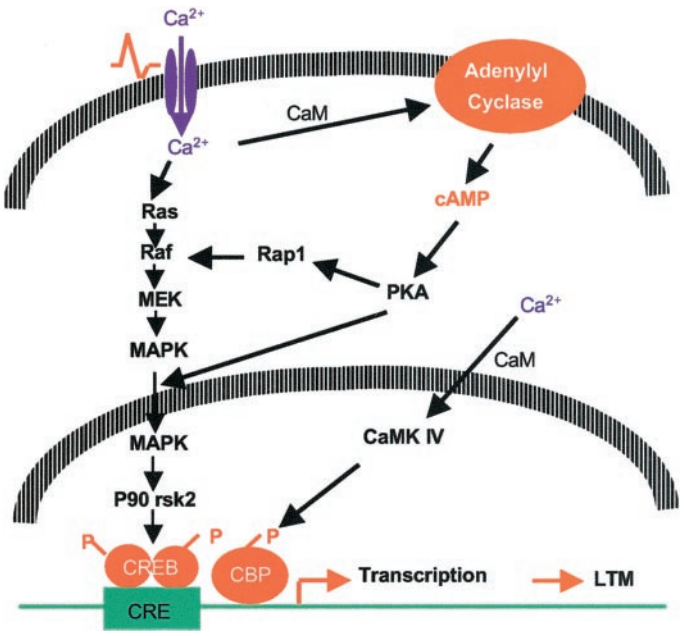

Fig. 2. Calmodulin-stimulated adenylyl cyclases provide a critical cAMP signal required to support Ca^{2+} activation of CRE-mediated transcription in the hippocampus. It is hypothesized that postsynaptic Ca^{2+} increases generated through NMDA receptors activate several signal transduction pathways including the Erk/MAP kinase and cAMP regulatory pathways. It is proposed that either AC1 or AC8 can provide the cAMP signal necessary for activation of CRE-mediated transcription for L-LTP and LTM. Convergence of these pathways at the level of the CREB/CRE transcriptional pathway may increase expression of a family of genes required for LTM.

AC3 is Required for Detection of Odorants in the Main Olfactory Epithelium

Many odorants stimulate cAMP levels in cilia preparations from the olfactory epithelium, and it has been hypothesized that cAMP is a major secondary messenger for olfaction (Pace et al., 1985; Pfeuffer et al., 1989). The main olfactory epithelium contains several adenylyl cyclases, including AC2, AC3, and AC4. To evaluate the role of AC3 for olfactory responses and to determine whether cAMP signaling is required for olfaction, the gene for AC3 was disrupted in mice (Wong et al., 2000). Interestingly, electro-olfactogram responses in the main olfactory bulb are completely ablated in AC3 mutants, despite the presence of AC2 and AC4 in olfactory cilia. Furthermore, AC3 mutants fail several odorant-based behavioral tests, indicating that AC3 and cAMP signaling are critical for olfactory-dependent behavior mediated through the main olfactory epithelium. This was the first direct evidence that cAMP signaling is required for olfaction.

Desensitization of olfactory signaling is a critical property of the olfactory system that allows animals to respond to odorants. An important feature of odorant-stimulated cAMP increases is their transient nature, which may be attributable, at least in part, to the unique regulatory properties of AC3. Because odorant-stimulated cAMP increases are accompanied by elevated intracellular Ca^{2+} (Tareilus et al., 1995), CaM kinase II inhibition of AC3 may contribute to termination of olfactory signaling. To test this hypothesis, phosphorylation of AC3 at Ser-1076 was monitored using a polyclonal antibody specific for AC3 phosphorylated at Ser-1076 (Wei et al., 1998). A brief exposure of olfactory cilia or primary olfactory neurons to odorants stimulates phosphorylation of AC3 at Ser-1076. This phosphorylation is blocked by inhibitors of CaM kinase II, which also ablate cAMP decreases associated with odorant-stimulated cAMP transients.

What is the physiological significance of multiple cAMP transients caused by odorants? An animal cannot sense an olfactory gradient unless it can turn off the olfactory signal and re-sample during movement through an odorant gradient. It is hypothesized that rodents may need multiple peaks of odorant-stimulated cAMP increases to detect odorant gradients. The initial rapid cAMP increase is crucial because it alerts the animal to the presence of a specific odorant in its environment. The animal may then react by moving toward or away from the odorant source, if and only if it can compare a second odorant signal within the time scale that it takes to move through a gradient. An animal may determine whether it is moving up a positive odorant gradient by comparing the amplitude of the first cAMP transient with subsequent signals.

Collectively, these data indicate that AC3 is ideally suited to couple odorant receptors to cAMP increases and to provide a mechanism that contributes to the rapid decline in intracellular cAMP. This general model is supported by data showing that treatment of olfactory sensory neurons with CaM kinase II inhibitors impairs odor adaptation (Leinders-Zufall et al., 1999).

Pharmacological Considerations

Although the catalytic subunits of AC have not received serious attention as drug target sites, the distinct properties and their different expression patterns make them worthy of consideration. A considerable body of evidence indicates that increases in cAMP are required for, or positively modulate, various forms of synaptic plasticity in the central nervous system. This suggest that drugs that increase cAMP in specific areas of brain in response to synaptic specific signaling may enhance synaptic plasticity and memory formation. AC1 is an attractive drug target site for this purpose because it is neurospecific and expressed in specific areas of brain important for learning and memory. Drugs that specifically enhance AC1 activity, only when it is activated by Ca^{2+}, may be preferable to those that cause sustained increases in cAMP, such as inhibitors of the cyclic nucleotide phosphodiesterases. Because of the pivotal role played by AC3 in olfaction, it may be a useful drug target site for enhancement or inhibition of olfaction.

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