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The role of Ca^{2+}/calmodulin-stimulable adenylyl cyclases as molecular coincidence detectors in memory formation

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Abstract. Evidence from systems as diverse as mollusks, insects and mammals has revealed that adenylyl cyclase, cyclic adenosine 3′,5′-monophosphate (cAMP) cascade, cAMP-dependent protein kinases and their substrates are required for the cellular events underlying the short-term and long-term forms of memory. In *Aplysia* and *Drosophila* models, the coincident activation of independent paths converge to produce a synergistic activation of Ca^{2+}/calmodulin-stimulable adenylyl cyclase, thereby enhancing the cAMP level that appears as the primary mediator of downstream events that strengthen enduring memory. In mammals, in which long-term memories require hippocampal function, our understanding of the role of adenylyl cyclases is still fragmentary. Of the differently regulated isoforms present in the hippocampus, the susceptibility of type 1 and type 8 to stimulation by the complex Ca^{2+}/calmodulin and their expression in the hippocampus suggest a role for these two isoforms as a molecular coincidence device for hippocampus-related memory function. Here, we review the key features of Ca^{2+}/calmodulin stimulable adenylyl cyclases, as well as the involvement of cAMP-regulated signaling pathway in the processes of learning and memory.

Key words. Memory; adenylyl cyclase; cAMP; calcium; calmodulin; cellular signaling.

Introduction

Adenylyl cyclase: a short-term and long-term memory device from mollusks to mammals

A feature of memory formation, ranging from a simple form of implicit memory in invertebrates to more complex forms of explicit learning in mammals, is its progression from a labile and highly sensitive to a longer-lasting and stable form. One approach to identifying key molecular elements implicated in short and long forms of memory processes has emerged from studies in invertebrates. Work in several systems, including *Drosophila* and *Aplysia*, has provided evidence for an important role for cyclic adenosine 3′,5′-monophosphate (cAMP) in learning. In *Aplysia*, sensitization of the gill-withdrawal reflex requires stimulation of adenylyl cyclase (AC) via stimulation of serotoninergic receptors. In *Drosophila*, deficits in olfactory associative learning and memory storage are observed in mutant flies that are affected in the cAMP-signaling cascade. In both of these models, the coincident activation of two input paths converge to produce a synergistic activation of Ca^{2+}/calmodulin-stimulable ACs, thereby enhancing the cAMP level that appears as the primary mediator of downstream events that strengthen enduring memory. As with cAMP, Ca^{2+}-initiated phosphorylation cascades also represent a major mechanism by which external stimuli modulate functions, and it has been suggested that both Ca^{2+} and cAMP, acting as synarchist messengers, may constitute a crucial intracellular cascade for the establishment of memory traces. The likely involvement of Ca^{2+}/calmodulin-sensitive AC in the molecular mechanism underlying learning in invertebrates raises the question of its role in mammals, in which more complex forms of learning and memory exist. In mammals, the hippocampal formation is critical in encoding many types of information into memory storage. Interestingly, hippocampal connections possess an unusual ca-

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pacity for expressing long-lasting increases in synaptic strength. This phenomenon, known as long-term potentiation (LTP), is hypothesized to represent a central mechanism for memory storage. Because LTP and memory share many characteristics, much attention has focused on the role of cAMP and AC in mediating elements of LTP. Although differently regulated ACs are present in the hippocampus, the susceptibility of type 1 and type 8 to stimulation by the complex Ca^{2+}/calmodulin and their abundant expression in the hippocampus strongly suggest a role for these isoforms as a molecular device for hippocampus-related memory function. The fact that these Ca^{2+}/calmodulin-stimulable ACs are subject to a range of influences has given rise to the notion that they can act as 'coincident signal detectors' capable of yielding a unique response when simultaneously exposed to two regulatory cues. In this review, we will focus on many of the important regulatory properties and distribution patterns of mammalian Ca^{2+}/calmodulin-stimulable ACs and then discuss the role of Ca^{2+}/calmodulin-stimulable ACs as coincidence detectors in the formation of short- and long-term memory.

Heterogeneity of mammalian adenylyl cyclases

Since complementary DNA (cDNA) clones for type 1 adenylyl cyclase have been cloned from bovine and human brain cDNA libraries, messages for eight isoforms of mammalian AC (designated types 2–9) have, to date, been identified in mammalian tissues [1–4]. All isoforms are predicted to be transmembrane glycoproteins, and there are considerable similarities between mammalian ACs and a number of ion channels and adenosine triphosphate (ATP)-dependent transporters. Although mammalian AC isoforms so far identified share similarities in their cytoplasmic domains (up to 92%), the transmembrane domains are not highly conserved between ACs.

All of the isoforms characterized thus far are regulated in type-specific patterns by Ca^{2+}, $\beta\gamma$ subunits of G-proteins ($G\beta\gamma$) and protein kinase C (PKC), and thus each AC appears to function as a uniquely designed molecular signal. Mammalian ACs have been divided into four subfamilies, based on their sequences and their susceptibility to regulation by Ca^{2+}-signaling pathways: (i) types 1, 3 and 8 are stimulated by Ca^{2+}/calmodulin; (ii) types 5 and 6 are Ca^{2+}-inhibitable; (iii) types 2, 4 and 7 are insensitive to changes in intracellular Ca^{2+} concentrations; and (iv) type 9 is sensitive to indirect effects of Ca^{2+} and is affected by α subunit of Gs ($G\alpha s$). As the regulatory properties of the nine isoforms have been discussed in earlier reviews, these data will not be presented here. The regulatory susceptibilities coupled with distinct tissue expression of each of these species provides opportunities for the generation of unique patterns. The key point is that the cAMP signal is potentially subject to dynamic control by a variety of neurotransmitters and hormone receptors acting via activation of G-proteins and also via cross-talk with other signaling pathways (fig. 1). Such mechanisms of regulation are often highly synergistic or conditional, conferring on ACs a function as coincidence detectors [5, 6].

Distribution pattern of Ca^{2+}/calmodulin-stimulable adenylyl cyclases in mammalian brain

The isoforms differ in their tissue expression, and the brain is the source with the greatest variety of isoforms expressed. To gain insight into the functional role played by individual isoforms in brain, expression of individual isoforms has been determined at the message level because of the lack of isoform-specific antibodies capable of detecting the low native protein concentra-

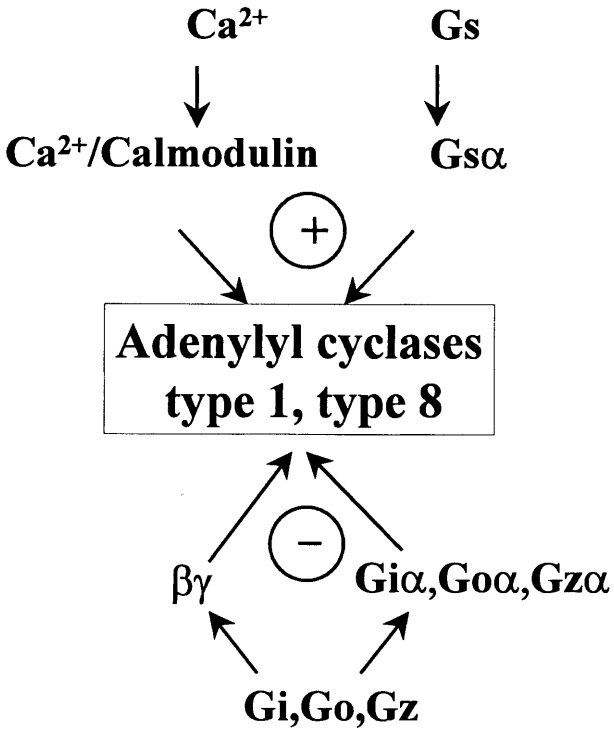

Figure 1. Summary of the regulatory susceptibility of mammalian Ca^{2+}/calmodulin-stimulable type 1 and type 8 adenylyl cyclases by Ca^{2+} and G-protein subunits (see [1–4]). The regulatory properties summarized emanate largely from studies of cDNAs expressed in intact cells that have been subject to physiological elevation of $[Ca^{2+}]i$ or hormonal activation. Both isoforms are stimulated by micromolar concentrations of Ca^{2+}. Both endogenous and transfected Ca^{2+}-sensitive ACs have been shown to be regulated by capacitative Ca^{2+} entry rather than by Ca^{2+} released from internal stores (see [2]).

tion of AC in mammalian tissues. Individual AC has a quite specific expression in particular regions of rat brain [7–11]. Although all nine isoforms can be detected in the brain, messages for types 3 and 6 occur at very low levels in most brain regions. The messages for Ca^{2+}/calmodulin-stimulable AC (types 1, 8), Ca^{2+}-inhibitable (type 5) and Ca^{2+}-insensitive (type 7) are remarkably discretely expressed, whereas other isoforms are broadly distributed in the brain. These studies suggest that by their restricted distribution, many species may play specific roles in the regulation of physiological functions in the nervous system.

The susceptibility of type 1 and type 8 to stimulation by the complex Ca^{2+}/calmodulin and their abundant expression in hippocampus strongly suggests a role for these isoforms as a molecular device for hippocampus-related memory function. The pattern of expression of type 1 provides a good example of both regional and cell type-specific expression of an individual AC isoform in the brain [7–9]. The highest expression of type 1 messenger RNA (mRNA) is specifically restricted to regions that are associated with learning and memory, including the hippocampus, cortex and cerebellum. In the hippocampus, type 1 is present predominantly in the glutamatergic pyramidal neurons of both CA1–CA2 fields and the granule cells of the dentate gyrus, whereas it is barely above background in the CA3 field. The high level of type 1 mRNA in the CA1 field and the dentate gyrus is consistent with the involvement of a Ca^{2+}/calmodulin adenylyl cyclase in *N*-methyl-D-aspartate (NMDA)- and Ca^{2+}/calmodulin-dependent long-term potentiation (LTP) occurring in these areas. Compared with type 1, the level of expression of type 8 in hippocampus is weaker [11]. The pattern of expression of type 8 in all pyramidal cells of CA1–CA3 fields and dentate granular cells is consistent with its active role in hippocampal memory formation. Whether or not types 1 and 8 mediate distinct functions has not been demonstrated.

Two other isoforms (Ca^{2+}-insensitive type 2 and Ca^{2+}/calmodulin phosphoprotein-inhibitable type 9) are also highly expressed in the hippocampal formation [8, 10]. Both types are expressed ubiquitously in the hippocampal CA1–CA3 layers and dentate gyrus, and they showed overlapping distribution patterns, suggesting that two or more differently regulated AC isoforms are simultaneously expressed in the same area.

Variations in the relative levels and the subcellular distributions of the Ca^{2+}/calmodulin-sensitive and Ca^{2+}-insensitive ACs need to be determined to understand the specific contributions of each AC to hippocampal cellular processes.

Adenylyl cyclases and learning in invertebrate models

The *Aplysia* system

Sensitization and habituation processes have been extensively studied in *Aplysia*. Classical conditioning of the gill- and siphon-withdrawal reflex is mediated by activation of a modulatory presynaptic input to the afferent siphon sensory neurons. A weak conditioned stimulus (CS; a weak touch to the siphon), leads to a brisk reflex of its gill. In contrast, an unconditioned stimulus (US; an electric shock to the tail), produces a strong withdrawal reflex. When CS to the siphon and US to the tail are repeatedly paired with each other, CS greatly potentiated the gill-withdrawal reflex elicited by a touch to the siphon [12]. In this case, the reflexes of *Aplysia* that can be depressed by habituation can be strengthened by sensitization [13]. This sensitization is graded, and retention overtime is proportional to the level of initial training [14]. Both habituation and sensitization produce short-term forms (that last minutes) and long-term forms (>1 day) of memory [15].

An analysis of this system demonstrated that CS is induced by Ca^{2+} influx through voltage-gated ion channels activated by the action potential within the sensory neuron [16]. In contrast, the US is induced by exciting facilitatory interneurons, which leads to the release of modulatory transmitters, such as serotonin (5HT), from sensory neurons [17]. The effect of 5HT can be mimicked by application of cAMP analogs into the presynaptic sensory neuron. Based on these findings, it has been proposed that a single pulse of 5HT triggers the activation of αs guanosine triphosphate (αs-GTP)-coupled 5HT receptors that activate the cAMP cascade [18, 19]. Hence, activation of cAMP-dependent protein kinase A (PKA) results in the phosphorylation of substrates responsible for modification of preexisting proteins and thus contributes to presynaptic facilitation [20].

When CS to the siphon and US to the tail are repeatedly paired, CS elicits potentiated siphon- and gill-withdrawal reflexes [12]. Ocorr et al. [21] showed that depolarization of the sensory neurons prior to exposure to 5HT increases levels of cAMP over those seen when CS and US are unpaired. It has been suggested that Ca^{2+} influx resulting from CS could converge upon Ca^{2+}/calmodulin sensitive-AC and increase the cAMP level produced by 5HT (fig. 2). In this case, the *Aplysia* adenylyl cyclase is activated by both Ca^{2+}/calmodulin and GTPγs (a GTP analog that acts by binding to αs), and therefore acts as a coincidence detector that is sensitive to the timing and order of stimuli [22–24].

Ample evidence exists implicating the cAMP/PKA cascade and a specific class of transcription factors, cAMP-response element binding proteins (CREBs) in the induction of long-term cellular and behavioral modifi-

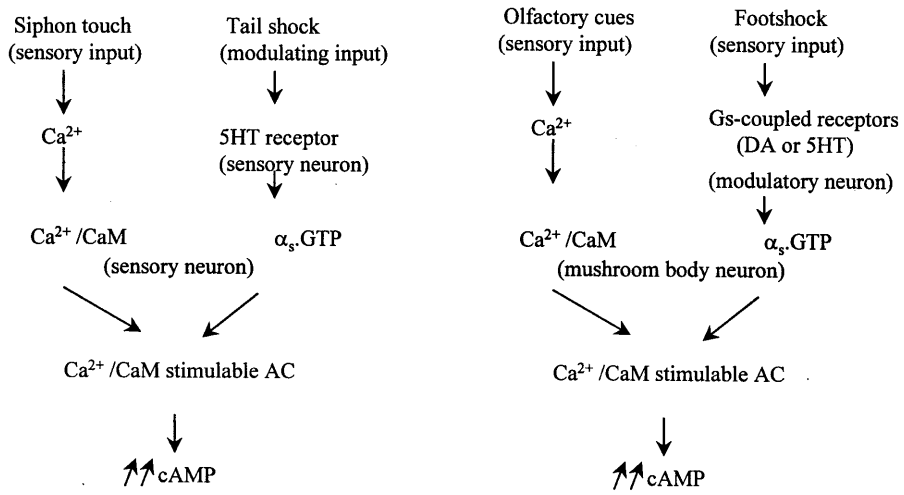

Figure 2. Adenylyl cyclases act as coincidence detector in two invertebrate forms of learning. Coincident activation of two separate signals—Ca^{2+} and αs.GTP—leads to a synergistic increase of intracellular cAMP (see [5]).

cations in *Aplysia* [25]. In this system, enhanced neurotransmitter release and long-term synaptic changes are regulated by the synergistic interaction between Ca^{2+}/calmodulin and 5HT-αs.GTP pathways that coactivate AC to produce exceptionally strong or prolonged cAMP signals required for stimulation of transcription. Specifically, long-term facilitation produced in isolated coculture of sensory and motor neurons by repeated spaced pulses of 5HT causes an increase in cAMP and gives rise to the translocation of the catalytic subunit of PKA to the nucleus, where it phosphorylates CREB-related transcription factors (CREB proteins and immediate early genes) that regulate expression of a second class of genes termed late response genes. Late response genes encode new proteins that are critical for the persistent changes known to occur in the development of more stable and durable forms of memory [26]. The convergence of Ca^{2+}/calmodulin and cAMP pathways at the level of gene expression for long-term classical conditioning in *Aplysia* suggests that CREB might be a second molecular site of convergence for the CS and US, with adenylyl cyclase being the first one [27].

The *Drosophila* system

The cAMP signaling cascade has a crucial role in long-term memory of olfactory avoidance learning in which the fruit fly *Drosophila melanogaster* is presented with two novel odors, and then trained to avoid a particular odor by pairing that odor with an electric shock [28]. With repeated, temporally spaced training trials, an enduring memory requiring protein synthesis is formed [29, 30]. The ability of *Drosophila* to learn and retain simple associative tasks has enabled the identification of mutants that are affected in the acquisition and/or storage of information [30–32]. From the various mutant strains with deficits in olfactory associative learning and/or memory formation, four have been shown to have impaired cAMP-signaling cascades. These include mutant flies with disruption of a G-protein α-subunit (Gs), an adenylyl cyclase gene (*rutabaga*) [33, 34], a cAMP phosphodiesterase gene (*dunce*) [31], a catalytic subunit of PKA [35] and a cAMP-responsive transcription factor [36,37, for review see [30]. An interesting behavioral difference exists between the cAMP mutants (*dunce* and *rutabaga*) and the PKA mutants. The latter seem to disrupt conditioned behavior immediately after training with no effect on memory decay rates, although the former disrupt initial learning levels and memory decay rates. Such findings suggest the direct involvement of cAMP in (short-term) memory processing. Interestingly, the *rutabaga* AC is structurally, biochemically and functionally conserved with its mammalian Ca^{2+}/calmodulin-stimulable AC homolog (type 1) [33]. Heterologous expression of *rutabaga* in mammalian cells indicates that its activity is also responsive to activation by both G-proteins and Ca^{2+}/calmodulin. Moreover, the *dunce*$^{-}$, *rutabaga*$^{-}$ double-mutant flies, while exhibiting approximatively normal levels of cAMP [34], still fail to learn, suggesting that memory requires complex spatial and temporal regulation of cAMP as opposed to absolute levels of cAMP.

As in *Aplysia*, repeated, temporally spaced training trials produced an enduring memory that requires protein

synthesis and implicates a signaling pathway by which elevated cAMP can trigger new gene transcription [29]. A neuronal model of associative learning has been recently proposed [35]. In this model, mushroom bodyneurons integrate sensory inputs from both olfactory cues (that produce an increase in intracellular Ca^{2+}) and footshock (that activates Gs-coupled receptor). It is likely that the coincident activation of these two input paths may converge to produce a synergistic activation of Ca^{2+}/calmodulin-stimulable ACs, thereby enhancing the cAMP level (fig. 2). Therefore, elevated cAMP could be the primary mediator of downstream events that strengthen the enduring memory. Increased cAMP levels may cause the translocation of PKA to the nuclei where it phosphorylates CREB and then initiates a cascade of gene expression responsible for long-term functional and structural changes at synaptic sites [35]. It has been shown that overexpression of a CREB repressor in *Drosophila* blocks the formation of long-term memory, whereas transgenic flies expressing an activating isoform of CREB showed an enhancement of long-term memory [36, 37].

A specific role for mammalian Ca^{2+}/calmodulin-stimulable adenylyl cyclases in the learning and memory function?

The long-term potentiation hypothesis

Particular attention has been directed towards understanding the role of cAMP pathways in hippocampal LTP, which is the best-studied model of the cellular mechanisms that underlying memory formation [38]. The mechanisms that generate LTP in the three major pathways in the hippocampus, including perforant, mossy fiber and Schaffer collateral, are quite distinct [39]. Of these, the associative NMDA receptor-dependent LTP present in the Schaffer collateral and the perforant pathway is the most widely described. It is well established that this form is initiated postsynaptically by an elevation of intracellular Ca^{2+}, mainly by Ca^{2+} influx via the associative activation of the NMDA receptors [39–42]. By contrast, LTP at the mossy fiber CA3 synapse is associated with a low level of NMDA-receptor binding, and repetitive activation of these synapses is an NMDA receptor-independent form that is initiated by the entry of Ca^{2+} into the presynaptic terminal [43, 44].

There is ample evidence that the cAMP-signaling cascade is strongly implicated in the cellular mechanisms that underly the distinct temporal phases of hippocampal LTP. The early transient phase (< 3 h), whose induction is unaffected by protein synthesis inhibitors, requires activation of Ca^{2+}-sensitive adenylyl cyclase and an enhancement of cAMP levels to activate PKA activity. The late, persistent phase (days or weeks) that is blocked by protein-synthesis inhibitors requires the cAMP pathway to regulate cAMP-dependent transcription [27, 45–47].

A mechanism of cAMP dependence in the early stage of Schaffer collateral LTP in the CA1 region has been recently proposed [48]. The induction of early LTP requires a postsynaptic elevation of intracellular Ca^{2+}, via activation of the NMDA receptors, that leads to the activation of Ca^{2+}/calmodulin-stimulated adenylate cyclase and an increase in cAMP levels [39]. It has been proposed that the cAMP signaling pathway, instead of transmitting signals for the generation of LTP, may act as a 'gate' in the development of LTP (fig. 3) by regulating the activity of the Ca^{2+}/calmodulin-activated phosphatase 2B (calcineurin) [48, 49]. This role is dependent upon cAMP that is synthesized postsynaptically, and the activation of the postsynaptic cAMP/PKA pathway may be an essential step for expression of early LTP, because inhibitors of PKA block LTP. However, cAMP by itself does not induce LTP, whereas activated Ca^{2+}/calmodulin protein kinase (CaMKII) is necessary and sufficient to generate LTP [48, 50]. As shown by Raman et al. [51] in cultured hippocampal neurons inhibition of PKA prevented recovery of NMDA receptors from calcineurin-mediated dephosphorylation induced by synaptic activity. Conversely, elevation of PKA activity by forskolin, cAMP analogs or the β-adrenergic receptor agonists can antagonize the effects of calcineurin. Taken together, the findings indicate that the level of phosphorylation of NMDA receptors in the early stage of LTP is subject to a complex system of interactions between the cAMP/PKA- and the Ca^{2+}/calcineurin-pathways (fig. 3).

Much less is known about the molecular mechanisms underlying mossy fiber LTP. There is pharmacological evidence that the cAMP/PKA pathway is critically important presynaptically for the induction and maintenance of LTP [52, 53]. For example, forskolin evokes mossy fiber LTP whereas the inactivated forskolin homolog does not [53].

The long-lasting stage of LTP, which requires both transcription and protein synthesis, is entirely dependent on an elevation in cAMP levels that activates PKA and triggers phosphorylation of the CREB [54]. Long-lasting LTP requires both transcription [46] and protein synthesis and can be blocked by PKA inhibitors. In contrast, application of PKA activator is sufficient to induce late LTP in the absence of electrical stimuli [55]. Recent studies showed that perfusion of D1/D5 dopamine receptor agonists without any tetanus can itself imitate the late phase of LTP, an effect that is blocked by inhibitors of protein synthesis [47]. The D1/D5 modulation seems to result from the functional coupling with AC, since activating AC (forskolin) or mimicking

cAMP has similar effects. As Bourne and Nicoll [5] discussed the importance of the temporal coincidence of intracellular signals in regulatory processes, coincident activation of DA and glutamatergic inputs may contribute to the elevation of cAMP because tetanization that establishes late LTP can be blocked by application of D1 inhibitors and of NMDA-R antagonists. Together, these findings highlight an important feature of the Ca^{2+}/calmodulin-stimulated AC that can act as coincidence detector of convergent signals delivered by independent receptors acting on αs.GTP- and on the Ca^{2+}/calmodulin-pathways to generate cAMP increases greater than that produced by Ca^{2+} or neurotransmitter alone.

Of the mammalian adenylyl cyclases, two Ca^{2+}/calmodulin-stimulable adenylyl cyclases, type 1 and type 8, seem particularly important in the regulation of hippocampal LTP, based on their regulatory properties, their expression pattern in the hippocampus and the strong homology between their amino acid sequence and that of the *Drosophila rutabaga* gene [33]. In a mouse type 1 mutant in which the amount of Ca^{2+}/calmodulin-stimulated AC activity in hippocampal membrane is approximatively half that in a wild mouse [56], presynaptic forms of LTP at hippocampal mossy fiber were impaired significantly [57], whereas wild-type and type 1 mutants mice exhibited comparable postsynaptic LTP at the Schaffer collateral and the perforant path synapse [56]. Furthermore, LTP in the cerebellar parallel fiber-Purkinje cell synapses, which exhibit properties similar to mossy fiber LTP, also showed serious impairment in type 1 mutants [58].

Other neurotransmitter receptors acting on Gs, Gi or G$\beta\gamma$ can also modulate LTP in hippocampal CA1 neurons. In pyramidal neurons, enhancement of cAMP production via β-adrenergic receptors is potentiated by agonists of $GABA_B$ or $5HT_{1A}$ receptors through the release of $\beta\gamma$ complexes from Gi [59]. Thus, by acting as a molecular coincidence detector [5] to integrate signals from PKC- and Gs/Gi-protein-regulated pathways [60, 61], it is possible that the cAMP cascade arising from activation of Ca^{2+}-insensitive type 2 also participates in the molecular events that trigger LTP. Of further interest is the high expression of Ca^{2+}-inhibitable type 9 in the hippocampal formation [9]. As type 9 is inhibited by Ca^{2+}/calmodulin-activated phosphatase (calcineurin), AC9 also appears as a good candidate trigger for the molecular events underlying LTP processes.

The behavioral view

To date, the hypothesis for a role of ACs in learning and memory in mammals has focused on type 1 for the following reasons: (i) within the mammalian brain type 1 is located in brain regions (hippocampus and neocortex) that are involved in cognitive memory (termed declarative and/or relational); (ii) these forms of memory are thought to involve the binding of geographically

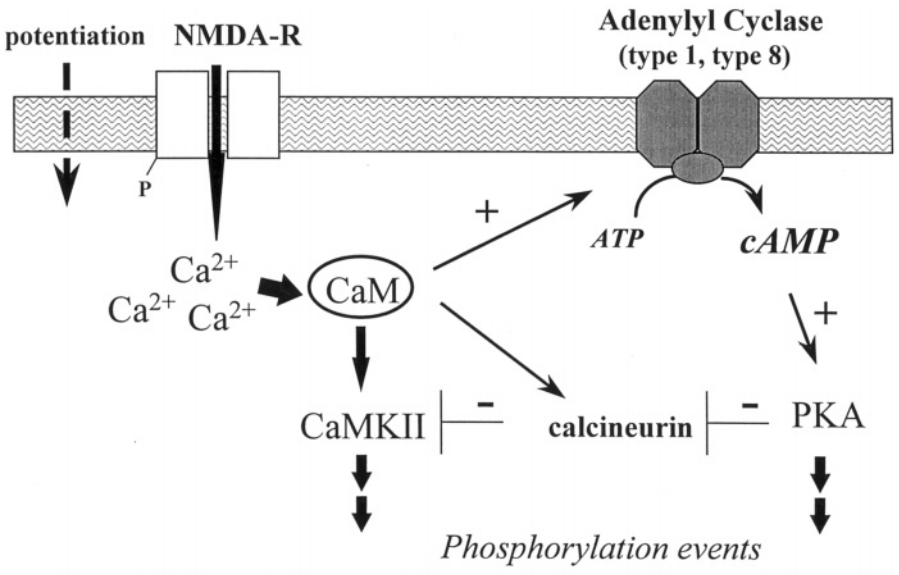

Figure 3. Role of Ca^{2+}/calmodulin-stimulable type 1 and/or type 8 adenylyl cyclases in the early phase of LTP. In CA1 pyramidal cells of hippocampus, an increase in the concentration of Ca^{2+} arising from either glutamatergic NMDA-R or voltage-gated Ca^{2+} channels elevates cAMP via the activation of Ca^{2+}/calmodulin-stimulable AC isoforms. Ca^{2+}/calmodulin also activates CaMKII, which is the primary pathway in signaling LTP. An increase in the cAMP level activates PKA activity that can enhance signal flow through the CaM kinase pathway by inhibiting the Ca^{2+}/calmodulin phosphoprotein phosphatase (calcineurin) (see [48, 49]).

separate cortical representations by the hippocampus, a process that would involve type 1 AC acting as a conjoncture detection device.

However, there are at least two reports that weight against this hypothesis. First, type 1 null-mutant mice have been found to be only marginally deficient in the Morris water maze task [56]. Second, it was found that spatial discrimination training in the radial maze actually induced a decrease in hippocampal adenylyl cyclase activity in mice; moreover, counteracting the testing-induced decrease in AC activity by subcutaneous injections of cysteamine resulted in dramatic impairment of learning [62]. These findings are in fact far from being conclusive to reject the hypothesis for a role of type 1 in cognitive memory. Specifically, the type 1 null-mutant mice still displayed substantial, and thus possibly sufficient, levels of type 1 expression in their telencephalic brain regions to quite normally master the water maze task [56]. Moreover, results from the experiment by Guillou et al. [62], only provided evidence for spatial memory testing-induced decreases in AC activity but did not address the question of whether this change differentially affected each of the three isoforms expressed in the hippocampal formation. In other words, one can speculate that the observed overall decrease in hippocampal AC activity actually resulted from a massive decrease in Ca^{2+}-insensitive type 2 and a more discrete increase in Ca^{2+}/calmodulin -stimulable type 1 AC.

In line with the current hypothesis, the role of type 1 AC would be to play a role in the establishment of hippocampal representations (engrams). According to many authors this would involve strengthening a minuscule and distributed fraction of the cell population [63]. It is thus likely that, in the case where such specific testing-induced changes for type 1 would occur, their magnitude should be very weak. Interestingly, whereas spatial memory testing induced a decrease in hippocampal AC activity, the same report [62] indicated that an opposite effect (i.e. an increase in hippocampal AC activity) was observed when mice were trained in a bar-pressing task and that increasing hippocampal AC activity by subcuteanous injections of cysteamine resulted in a facilitation of learning. Importantly for our present concern, facilitation of learning in the bar-pressing task may also be obtained using lesions of the hippocampal formation; this suggests, as is the case for other 'hippocampus-independent' tasks, that in normal mice the hippocampus exerts an inhibitory influence on the brain systems that subserve acquisition of these tasks (see [63]). We have postulated that part of the observed bar-pressing task testing-induced upregulation of hippocampal AC activity would be associated with inhibition of hippocampal processing functions [64]. Conversely, these functions (i.e. processing information whether spatial or nonspatial in nature) would be absolutely necessary for mastering hippocampus-dependent tasks involving, in the broad sense, the establishment of relational representations. It is thus conceivable that bringing into play this task-specific hippocampal processing function may be conditional upon an overall decrease in hippocampal cAMP levels (i.e. 'noise'), allowing a more specific process involving type 1 AC (i.e. 'signal') to operate in order to establish specific representations. This hypothesis may be tested. Whatever the case, it appears that a complete understanding of the role of ACs in learning and memory in the mammalian brain cannot be achieved without considering not only the existence of separate memory and brain systems but the way these systems interact in the normal brain.

Ca^{2+}/calmodulin-stimulable ACs and synapse specificity

Recent studies on the spatiotemporal dynamics of the cAMP cascade have provided evidence for distinct regulatory roles that are played by dynamically changing levels of cAMP in an intact somatogastric ganglion of the lobster circuit [65]. Low frequency stimulation of neuromodulatory afferent fibers is accompanied by cAMP increases in fine neurite branches of identified neurons. After prolonged electrical stimulation periods, the local increase of cAMP produced in neurite branches diffuses retrogradely throughout the neuritic tree and eventually to the cell body, reflecting the role of cAMP as a cellular spatiotemporal integrator [66]. In remarkable parallel, ultrastructural analysis of the hippocampal molecular layer of the CA1 field revealed a striking concentration of AC immunoreactivity near postsynaptic densities in dendritic spines [67]. This observation places Ca^{2+}/calmodulin-sensitive ACs precisely where they are most efficient in the propagation of NMDA-mediated LTP. Synaptic activation of NMDA receptors triggers a rise in Ca^{2+} principally in the apical dendrites and dendritic spines of a CA1 pyramidal neuron. Thus, it can be anticipated that Ca^{2+} entry, through voltage or ligand-gated ion channels, would readily activate Ca^{2+}/calmodulin-stimulable AC in dendritic spines following synaptic stimulation. Dendritic spines are areas of high concentration of Ca^{2+} channels and pumps [68, 69], as well as of PKA and CaMKII [70, 71]. We then might expect that cAMP would need to diffuse only a short distance before activating the anchored PKA, thereby greatly facilitating the local downstream phosphorylation steps that are responsible for the short-term modifications. Thus it can be speculated that, during the early period immediately after LTP induction, individual synapses or a group of synapses that contain proteins directly involved in the reception and propagation of the Ca^{2+} and cAMP cascades may be capable of being specifically activated.

Conclusions

Our understanding of the role of ACs is still fragmentary. However, the past few years have revealed that members of the mammalian AC family are much more diverse than initially predicted. ACs can be divided in four groups with distinct functional regulatory properties. Based on their expression pattern in the hippocampus, the Ca^{2+}/calmodulin-stimulable ACs (types 1 and 8), but also the Ca^{2+}-insensitive type 2 and calcineurin-inhibitable type 9, have the potential to modulate short and long forms of memory processes. A challenge for future studies will be to resolve how the different hippocampal ACs are targeted to different neuronal compartments, and to determine whether the establishment of hippocampal representation maps alterations in specific isoforms. It will also be important to clarify how the synaptic and subsynaptic distribution of the differently regulated ACs matches the distribution of other signal transduction molecules that are involved in formation of learning and memory.

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