that the 5-HT_{1B} receptor is at least in part responsible for the antiaggressive properties of the serenics, but do not rule out a participation of other receptors with a high affinity for these compounds, such as the 5-HT_{1A} receptor. The antiaggressive properties of the serenics are not affected by serotonin depletions and are therefore most likely mediated by postsynaptic receptors (*19*). 5-HT_{1B} receptors are expressed in a variety of brain structures, but predominantly on the terminals of projecting neurons where they often inhibit transmitter release (*3*). Candidate structures could be the amygdala and the central gray, which express 5-HT_{1B} mRNA and 5-HT_{1B} binding sites, respectively (*3*, *4*), and which are two regions involved in the response to fear and defensive behaviors (*20*).

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8. Preparation of the brains, horizontal sections, preincubation of the sections, washing, and exposure to film were as described (*3*). Incubations were done with 82 pM ^{125}I-CYP in the presence of 30 μM isoproterenol and 100 nM (±)-8-hydroxydipropylaminotetralin (8-OH-DPAT) (RBI) to mask β-adrenergic and 5-HT_{1A} receptors, respectively. Nonspecific binding was determined with 10 μM serotonin. Quantitative analysis of the autoradiograms was carried out for the different anatomical structures with a computer device for image analysis (Biolab); [L. Segu, P. Rage, P. Boulenguez, *J. Neurosci. Methods* **31**, 197 (1990)].
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10. Twelve-week-old male mice were housed alone in standard cages and kept on a 12:12-hour light-dark cycle with light onset at 0700 hours. The mice were tested between 1000 hours and 1600 hours during the light phase. Mice were placed in a circular open field (70 cm in diameter), and the distance traveled by the animal was recorded by a videotracking apparatus (Videotrack, Viewpoint; Lyon, France). The locomotor activity was scored over a 30-min period divided into 1-min intervals and was analyzed both in the central part of the open field and in the periphery. Ten days after the first test, mice were injected with RU24969 before a second test. RU24969 [5-methoxy-3-(1,2,5,6-tetrahydropyrid-4-yl)-1H-indole] was dissolved in saline and administered intraperitoneally, 40 min before testing, at a concentration of 5 mg per kilogram of body weight in a volume of 10 ml/kg.
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Mediation of Hippocampal Mossy Fiber Long-Term Potentiation by Cyclic AMP

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Repetitive activation of hippocampal mossy fibers evokes a long-term potentiation (LTP) of synaptic responses in pyramidal cells in the CA3 region that is independent of *N*-methyl-D-aspartate receptor activation. Previous results suggest that the site for both the induction and expression of this form of LTP is presynaptic. Experimental elevation of cyclic adenosine 3′,5′-monophosphate (cAMP) both mimics and interferes with tetanus-induced mossy fiber LTP, and blockers of the cAMP cascade block mossy fiber LTP. It is proposed that calcium entry into the presynaptic terminal may activate Ca^{2+}-calmodulin–sensitive adenylyl cyclase I which, through protein kinase A, causes a persistent enhancement of evoked glutamate release.

Long-lasting modifications of the strength of synaptic signals after repetitive stimulation of synapses is a common property of excitatory synapses in the central nervous system. This use-dependent synaptic plasticity provides a possible cellular basis for many types of learning and memory. One of the most studied forms of synaptic plasticity is LTP, which in most cases requires the activation of postsynaptic *N*-methyl-D-aspartate (NMDA) glutamate receptors (*1*). In contrast to this pattern, the LTP evoked at mossy fiber synapses of CA3 hippocampal pyramidal cells is entirely independent of NMDA receptors (*2*). Most evidence favors a presynaptic site for both the induction (*3–5*) [but see (*6*)] and expression (*3*,

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7) of mossy fiber LTP. What remains obscure, however, is the mechanism underlying the enhanced release of glutamate, the transmitter at this synapse. Here we present evidence that the cAMP cascade plays a pivotal role in mossy fiber LTP (8).

Hippocampal slices were prepared as described previously (9). Extracellular field potential recordings were used to compare the effect of forskolin, a diterpene that directly activates adenylyl cyclase (10), on synaptic responses evoked by stimulating either mossy fibers or associational-commissural (assoc-com) fibers (Fig. 1A). Application of forskolin (1 to 50 μM) caused a dramatic potentiation of mossy fiber responses in every preparation tested (Fig. 1, B and C) [see also (11)]. This enhancement was found to be entirely independent of synaptic stimulation during the application (n = 4). Forskolin had little effect, however, on the simultaneously recorded assoc-com responses (Fig. 1, B and C). This differential effect of forskolin on the two types of synaptic input could also be seen in whole-cell recording (WCR) (Fig. 1B). An isomer of forskolin, 1,9-dideoxyforskolin, has no effect on adenylyl cyclase (10) but does mimic many of the cAMP-independent actions of forskolin, including the blockade of some types of K^+ channels (12). This compound had no effect on mossy fiber responses, whereas subsequent application of forskolin caused the usual enhancement (Fig. 1D). In addition, the protein kinase A (PKA) antagonist Rp-8-CPT-cAMPS (13) reduced the action of forskolin (Fig. 1E). We also examined the action of analogs of cAMP that have been shown in other systems to mimic the action of cAMP. The analogs Sp-cAMPS (Fig. 1F) and Sp-8-CPT-cAMPS (n = 3) (13), activators of PKA (14), also reproducibly enhanced mossy fiber responses. As expected if Sp-cAMPS and forskolin enhance transmission by means of the same mechanism, Sp-cAMPS did not produce any enhancement of responses already enhanced by forskolin and only a transient depression was seen (Fig. 1F) (15).

The enhancement evoked by manipulations that elevated cAMP was long lasting. Thus, after the application of Sp-cAMPS (3 to 10 min) (Fig. 2A) or forskolin (3 min) (Fig. 2B), responses remained potentiated for the duration of the experiment. Because it was possible that forskolin washed out slowly from the slice, we monitored its effects on the Ca^{2+}-activated slow after-hyperpolarization recorded in pyramidal cells, which has previously been shown to be a target of cAMP (16). In contrast to the mossy fiber response, the action of forskolin on the after-hyperpolarization completely reversed within an hour of a 3-min application (Fig. 2, C and D). These results suggest that a brief elevation of cAMP triggers a persistent enhancement in synaptic transmission that does not depend on the continued presence of cAMP.

We next examined the site at which forskolin exerts its effect. It has been shown that CA3 pyramidal cells express mRNA for the GluR6 subtype of glutamate receptor (17), which is up-regulated by cAMP-dependent phosphorylation (18). In addition, in a number of systems, elevation of cAMP can enhance the release of transmitter (19–22). In a first set of experiments, a double-barreled pipette was positioned in the stratum lucidum, glutamate was released from one of the barrels, and the responses to the local action of glutamate and to mossy fiber stimulation were recorded from the other barrel and compared (23). During the enhancing action of forskolin on the mossy fiber responses (Fig. 3A), there was no change in the response evoked by the iontophoretic pulse of glutamate (Fig. 3A). The results from four experiments are graphed in Fig. 3B. We have also monitored paired pulse facilitation (PPF) during the action of forskolin. When a synapse is activated twice at short intervals (~40 ms), the second response is typically larger because of a facilitation of transmitter release, and manipulations that increase transmitter release decrease the magnitude of PPF (24). During the action of forskolin, there was a clear decrease in the degree of PPF. An example of the effect of forskolin is shown in Fig. 3C, and the results from seven experiments are plotted in Fig. 3D. Forskolin decreased PPF to 61 ± 9% of baseline val-

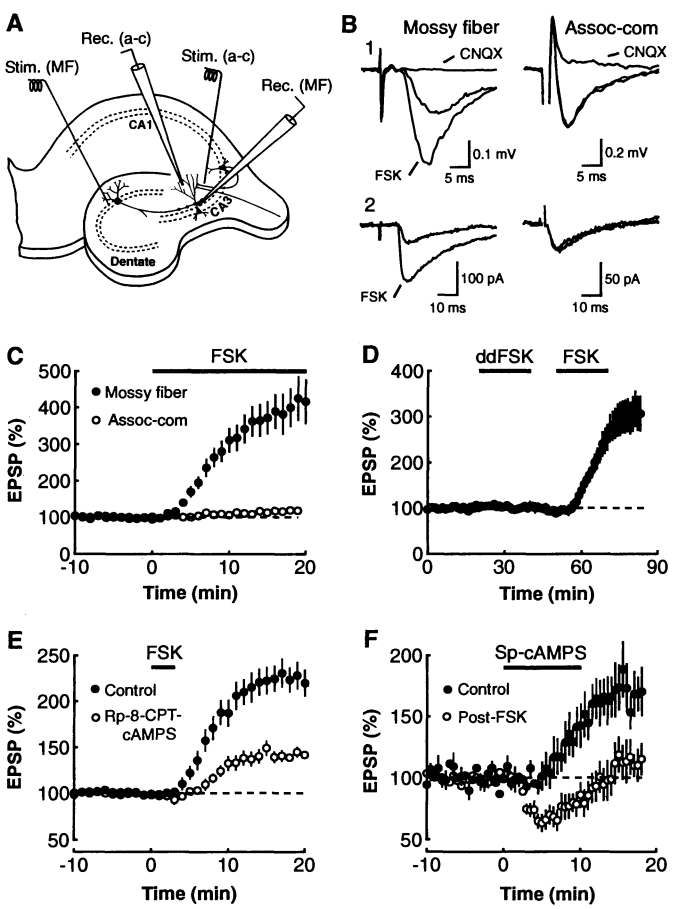

Fig. 1. Selective enhancement of mossy fiber synaptic responses by elevation of cAMP. (**A**) Schematic diagram of the arrangement of electrodes for stimulating and recording. To activate mossy fibers, a stimulating electrode was placed near the granule cell bodies in the dentate gyrus [stim. (MF)]. An extracellular field electrode placed in the stratum lucidum [rec. (MF)] was used to monitor monosynaptic mossy fiber synaptic responses in area CA3. To record assoc-com synaptic responses in area CA3 of the same slice, a second stimulating electrode [stim. (a-c)] and recording electrode [rec. (a-c)] were placed in the stratum radiatum of area CA3. In some experiments, whole-cell patch recordings were made to record mossy fiber and assoc-com responses in the same cell. (**B**) Sample superimposed traces from typical experiments using extracellular field recordings (1) and whole-cell recordings (2), showing traces before, during 50 μM forskolin (FSK), and after the addition of CNQX (20 μM). Each trace is the average of 10 and 8 excitatory postsynaptic potential (EPSP) and EPSC responses, respectively. (**C**) Comparison of the effect of forskolin (FSK, 50 μM) on mossy fiber (filled circles) and assoc-com (open circles) responses in the same slice (n = 7). (**D**) A 20-min application of 50 μM 1,9-dideoxyforskolin (ddFSK) had no effect on mossy fiber responses. This was followed 10 min later by a 20-min application of 10 μM forskolin (FSK), which produced enhancement (n = 4). (**E**) Comparison of the effect of a 3-min application of 10 μM forskolin (FSK) on control mossy fiber responses (filled circles; n = 8) and on responses from slices incubated in Rp-8-CPT-cAMPS (open circles; n = 7). In the latter case, slices were incubated for at least 2 hours in 100 μM Rp-8-CPT-cAMPS and then transferred to the recording chamber where the concentration was 10 to 30 μM. (**F**) Comparison of the effect of Sp-cAMPS (100 μM) on control mossy fiber responses (filled circles; n = 6) and on responses previously enhanced by 50 μM forskolin (open circles; n = 5). In the latter case, the post-forskolin stimulation strength was reduced so that responses were approximately at control sizes.

ues. When the stimulus strength was reduced so that responses were similar to control responses, the decrease in PPF was unaffected (to 62 ± 8%). The decrease in PPF could also clearly be seen with excitatory postsynaptic currents (EPSCs) recorded in the whole-cell mode (48 ± 12% of control) (n = 4) (Fig. 3C). Finally, the whole-cell recordings revealed that forskolin caused an increase in $1/CV^2$ (289 ± 90%) (CV is the coefficient of variation) that was similar to the increase in the mean EPSC amplitude (296 ± 31%), again suggesting a presynaptic mechanism according to classical interpretations. These results indicate that the enhancement of synaptic transmission caused by forskolin is due to an enhancement of transmitter release (25).

How might this cAMP-dependent enhancement of transmitter release normally be activated? First, we tested a number of agonists that activate receptors positively coupled to adenylyl cyclase, but none enhanced mossy fiber responses, presumably because mossy fiber terminals lack receptors for these agonists (26). Second, because the cAMP-induced enhancement and mossy fiber LTP both appear to be long-lasting presynaptic phenomena, the two processes may involve a common mechanism and, therefore, one might expect that maximal activation of one process would occlude the other. The design of this experiment is shown in Fig. 4A. It examined two independent mossy fiber pathways, so that in the same slice, the action of forskolin on a control pathway (S2; 408 ± 56%) and on a pathway expressing LTP (S1; 251 ± 28%) could be compared (Fig. 4B). In addition, LTP in a control pathway (S1; 176 ± 16%) could be compared to LTP evoked in the presence of forskolin (S2; 111 ± 14%) (Fig. 4C). Traces from a typical experiment are shown in Fig. 4D. Forskolin completely occluded LTP, and the action of forskolin was greatly reduced after LTP ($P < 0.01$, n = 12) (27).

These experiments clearly indicate that forskolin and LTP interact with one another and suggest that they share a common process. This could occur in one of two possible ways. Either the cAMP cascade is an independent parallel process that converges on some step in the LTP pathway, or the cascade is positioned in series with the LTP pathway and is an essential step in the expression of LTP. One way to distinguish between these two alternatives is to determine whether blockade of the cAMP cascade interrupts LTP. H-89 and KT 5720, at concentrations of 10 and 1 μM, respectively, are selective blockers of the catalytic site of PKA (28, 29). At these concentrations, neither antagonist had any effect on baseline synaptic transmission, but both H-89 (Fig. 5A) and KT 5720 (Fig. 5B) did antagonize LTP. Rp-cAMPS, a blocker of the regulatory site of PKA, superfused at 100 μM (and in most cases preincubated for more than 2 hours at 1 mM), had no effect on either the forskolin-induced enhancement (n = 5) or on LTP (n = 10), which

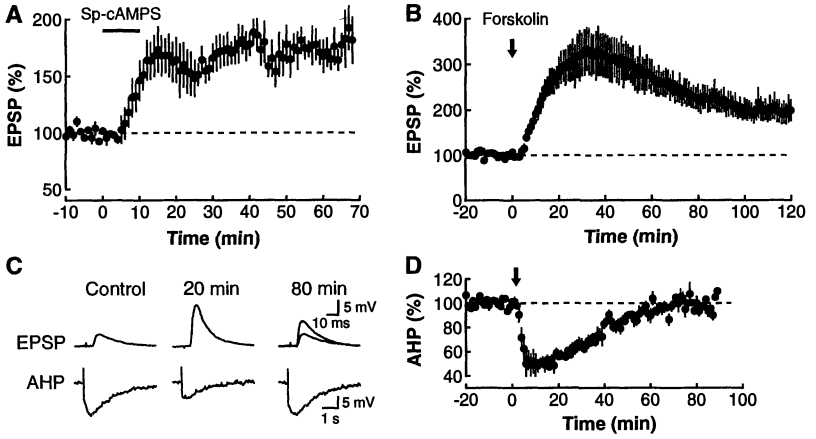

Fig. 2. Long-lasting effects of cAMP. (**A**) Effect of a 3- to 10-min application of Sp-cAMPS (1 mM) (n = 10) on mossy fiber responses. (**B**) Effect of a 3-min application of forskolin (50 μM; arrow) (n = 6). (**C**) Sample intracellular recordings obtained with sharp electrodes in the same CA3 pyramidal cell. Each trace represents the average of six and three EPSP and after-hyperpolarization (AHP) responses, respectively. (**D**) Time course of after-hyperpolarization amplitude recorded from CA3 pyramidal cells (n = 5). Forskolin was bath-applied at the same concentration and for the same duration as in (B).

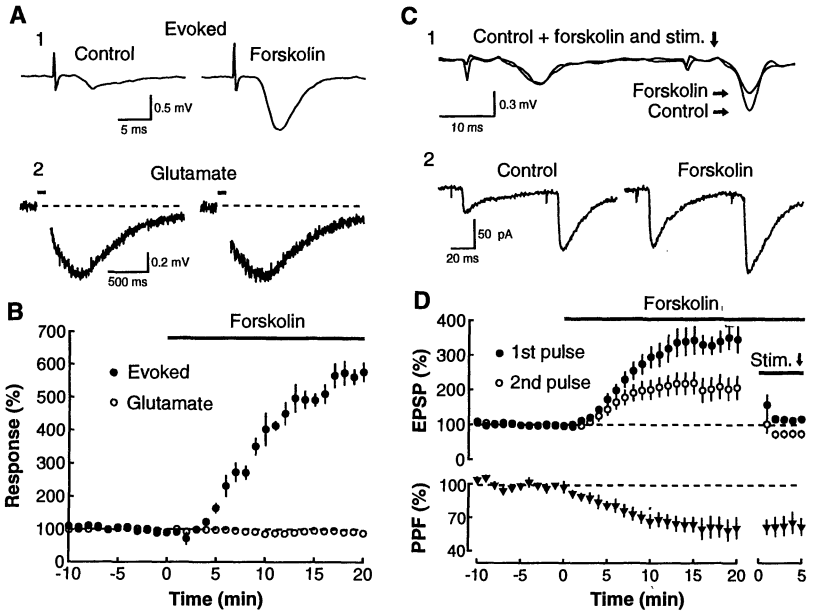

Fig. 3. The forskolin-mediated enhancement is presynaptic. (**A**) Sample traces showing (1) mossy fiber synaptic responses in area CA3 and (2) responses to iontophoretically applied glutamate (solid bars; 200 mM in H_2O, pH 8, 600 nA, 100 ms, no retaining current) before (control) and after (forskolin) the addition of forskolin to the bath. Each trace is the average of 15 and 5 EPSP and iontophoretic responses, respectively, and the responses remaining in the presence of 40 μM CNQX and 50 μM D-APV have been subtracted. (**B**) Comparison of the effect of forskolin on evoked synaptic responses (filled circles) and on responses to iontophoretically applied glutamate (open circles; 400 to 600 nA, 50 to 100 ms, no retaining current). (**C**) (1) Sample extracellular mossy fiber responses to paired pulses recorded before the addition of forskolin to the bath (control). Superimposed is the response after the addition of forskolin and reduction of the stimulus strength so that the response to the first of the two pulses was similar to that in control conditions (forskolin). (2) Sample mossy fiber responses recorded using the whole-cell recording mode both before (control) and after (forskolin) the addition of forskolin to the bath. All traces are the average of 5 (1) and 10 (2) responses. (**D**) The top panel shows the average of seven experiments as in the top panel of (C), comparing the effect of forskolin (long bar) on the response to the first (filled circles) and second (open circles) of paired pulses both before and after the reduction in stimulus strength (short bar; stim.), so that the responses to the first pulse were similar to those in control conditions. The bottom panel shows the concurrent changes in the PPF ratio (second pulse/first pulse; normalized to pre-forskolin levels). In all cases, forskolin was applied at a concentration of 50 μM.

was presumably due to inadequate concentrations entering the presynaptic terminal. However, the more membrane-permeating analog Rp-8-CPT-cAMPS (*13*) did antagonize LTP (Fig. 5C) (*30*). Rp-8-CPT-cAMPS often caused some depression of baseline synaptic responses, but this cannot explain the block of LTP, because Rp-cAMPS, which had no effect on LTP, had a similar effect on baseline responses. In contrast to the blockade of mossy fiber LTP, Rp-8-CPT-cAMPS had no effect on NMDA-receptor–dependent LTP recorded at 70 min in the CA1 region of the hippocampus (Fig. 5D). This result is in general agreement with results obtained from study of the effects of other antagonists of the cAMP cascade on LTP in the CA1 region, although some disagreement exists concerning the extent of the blockade of LTP at 1 hour that was induced by a stronger tetanus protocol than the one used in the present study (*31*).

The present results are similar to previous results obtained at a number of synapses in the peripheral autonomic nervous system. At these synapses, tetanization of preganglionic fibers results in a nonassociative presynaptic form of LTP (*32, 33*), and manipulations that increase cAMP also potentiate these synapses (*21*). Furthermore, a similar series of findings has been made at the crustacean neuromuscular junction (*19, 34, 35*), where it is possible to inject inhibitors of the cAMP cascade directly into the presynaptic axon and block the long-lasting potentiation (*19*). One difference in this form of potentiation is that it does not require the entry of Ca^{2+} into the presynaptic terminal (*34*) as mossy fiber LTP does (*5, 36*). Our results also have features in common with those of other behavioral models of learning and memory. The process of sensitization of the sensory fibers in the abdominal ganglion of *Aplysia* involves changes in presynaptic cAMP (*37*), and *Drosophila* learning mutants have defects in adenylyl cyclase activity (*38*). Although there are marked differences in the mechanisms underlying mossy fiber LTP and NMDA receptor–dependent LTP, the proposed involvement of cAMP in a late phase of LTP in the CA1 region (*31*) and in LTP of the dentate gyrus (*39*) suggests that similarities may also exist.

In summary, this study suggests that a brief elevation of cAMP in mossy fiber terminals causes a large and persistent increase in evoked glutamate release. The observations that the action of forskolin and mossy fiber LTP interact with each other and that blockers of PKA block mossy fiber LTP indicate that PKA activation is an essential step in mossy fiber LTP. Consistent with this finding, forskolin binding sites are present in high concentrations in the mossy fiber pathway (*40*). Furthermore, the mRNA for adenylyl cyclase I (AC I), a subtype of cyclase that is activated by Ca^{2+} by means of calmodulin (*41*) and is specifically implicated in learning and memory (*37, 38*), is expressed in large amounts in

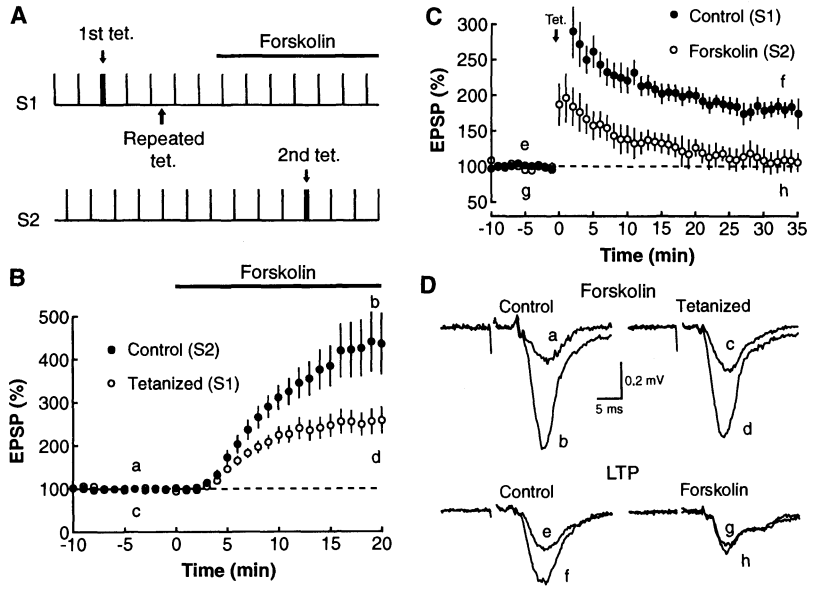

Fig. 4. Interaction between forskolin-mediated enhancement and mossy fiber LTP. (**A**) Schematic diagram of the experimental protocol. Two independent mossy fiber synaptic inputs in area CA3 were monitored (S1 and S2). A single tetanic stimulus was given to S1 (1st tet.), and after at least 40 min, repeated tetanic stimuli were given to S1 (four stimuli at 100 Hz for 1 s, separated by 20 s) in an attempt to maximize LTP. Subsequently, the strength of the stimulus given to S1 was reduced so that the two inputs gave similar responses, and 50 μM forskolin (bar) was added to the bath. After at least 30 min, the strength of the stimulus given to S2 was reduced so that the response of S2 was similar to that of S1 before the first tetanus. A single tetanic stimulus was then given to S2 (2nd tet.). (**B**) Comparison of the effect of forskolin (bar) on control (filled circles) and previously tetanized (open circles) responses (n = 12). (**C**) Comparison of the effect of a tetanic stimulus (tet.) on control responses (filled circles) and on responses previously enhanced by forskolin (open circles; n = 5). (**D**) Sample traces from a single experiment as described in (A). The enhancement by forskolin is greater for control responses than for those previously tetanized (compare a to b with c to d). The effect of a tetanic stimulus is greater on control responses than on those previously enhanced by forskolin (compare e to f with g to h). All traces are the average of 10 responses. The letters refer to the times at which the responses were taken as shown in (B) and (C).

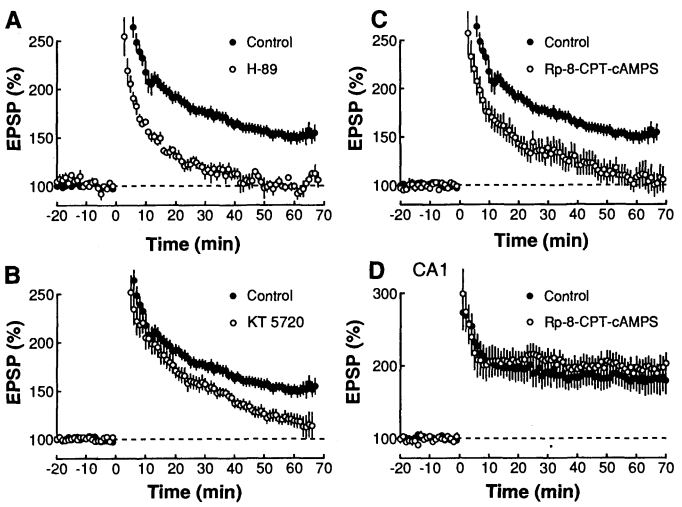

Fig. 5. Inhibition of mossy fiber LTP but not of NMDA-receptor–dependent LTP by PKA inhibitors. (**A** and **B**) Ten μM H-89 (n = 6) and 1 μM KT 5720 (n = 7), concentrations that selectively inhibit the catalytic site of PKA, inhibit LTP at the mossy fiber synapse (open circles). The slices were incubated in the drug for at least 2 hours before being transferred to the recording chamber where the drug was present at the same concentration. (**C**) The regulatory site inhibitor Rp-8-CPT-cAMPS (n = 8) also antagonized mossy fiber LTP (open circles). These slices were incubated for at least 2 hours in 100 μM Rp-8-CPT-cAMPS before being transferred to the recording chamber where the concentration was 10 to 30 μM. For all three antagonists, the slices remained in the inhibitors for the duration of the experiment. LTP in control conditions (n = 23) obtained during the same period as that of the experimental cases is superimposed on each panel (filled circles). (**D**) Rp-8-CPT-cAMPS applied in the same manner as in (C) has no effect on NMDA-receptor–dependent LTP in the CA1 region recorded for 70 min. Control slices (n = 9) were interleaved with experimental slices (n = 7). LTP was induced by a 1-s, 100-Hz tetanic stimulus repeated four times at 20-s intervals.

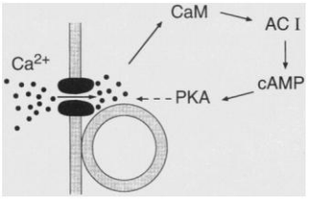

Fig. 6. Schematic diagram of proposed mechanism for mossy fiber LTP. The entry of Ca^{2+} into the presynaptic terminal through Ca^{2+} channels is responsible for the release of glutamate from synaptic vesicles. In addition, it is proposed that the tetanus-induced increase of Ca^{2+} above a threshold binds to calmodulin (CaM), which in turn stimulates AC I, thereby generating cAMP and activating PKA. PKA (dashed arrow) either causes a long-lasting increase in Ca^{2+} channel activity or a long-lasting increase in the sensitivity of the release process to Ca^{2+}.

dentate granule cells, which give rise to the mossy fibers (*42*). Indeed, estimates of Ca^{2+} concentrations in mossy fiber terminals during a tetanus (*43*) and those necessary to fully activate AC I (*44*) are similar. This raises the intriguing possibility, as summarized in Fig. 6, that a tetanus-induced rise in intraterminal Ca^{2+}, an essential step in mossy fiber LTP (*5*), activates, by means of calmodulin, AC I. The activation of AC I produces a transient rise in cAMP, which through the activation of PKA enhances the Ca^{2+}-triggered release process, resulting in a persistent enhancement of evoked glutamate release.

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