PKA and PKC Enhance Excitatory Synaptic Transmission in Human Dentate Gyrus

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Chen, Huan-Xin and Steven N. Roper. PKA and PKC enhance excitatory synaptic transmission in human dentate gyrus. J Neurophysiol 89: 2482–2488, 2003. First published January 15, 2003; 10.1152/jn.01031.2002. cAMP-dependent protein kinase (PKA) and protein kinase C (PKC) are two major modulators of synaptic transmission in the CNS but little is known about how they affect synaptic transmission in the human CNS. In this study, we used forskolin, a PKA activator, and phorbol ester, a PKC activator, to examine the effects of these kinases on synaptic transmission in granule cells of the dentate gyrus in human hippocampal slices using whole-cell recording methods. We found that both forskolin and phorbol ester increased the frequency of spontaneous and miniature excitatory postsynaptic currents (sEPSCs and mEPSCs) but left the amplitude unaffected. Inactive forskolin and phorbol ester had no effect on sEPSCs in human dentate granule cells. Prior application of forskolin occluded the effects of phorbol ester on mEPSC frequency. Tetanic stimulation applied to the perforant path induced short-term depression in dentate gyrus granule cells. Both forskolin and phorbol ester significantly enhanced this short-term depression. Taken together, these results demonstrate that PKA and PKC are involved in up-regulation of excitatory synaptic transmission in human dentate granule cells, primarily by presynaptic mechanisms. In addition, the occlusion experiments suggest that the two kinases may share a common signal pathway.

INTRODUCTION

Modulation of synaptic transmission plays a critical role in the development of the nervous system, mechanisms of learning and memory, and pathological states (Bliss and Collingridge 1993). Of the molecules that modulate synaptic transmission and synaptic plasticity, cAMP-dependent protein kinase (PKA) and protein kinase C (PKC) are two major modulators of synaptic transmission in hippocampus and other regions of the CNS (Carroll et al. 1998; Chavez-Noriega and Stevens 1994; Finch and Jackson 1990; Hori et al. 1999; Parfitt and Madison 1993). This indicates that the enhancement has a presynaptic locus. Additionally, both forskolin and phorbol ester have been reported to decrease paired-pulse facilitation, a presynaptic event (Gustaffson et al. 1988; Huang and Kandel 1998; Lu and Gean 1999), and phorbol ester did not affect the response induced by iontophoretically applied glutamate in hippocampal slices (Malenka et al. 1986), thus arguing against a postsynaptic effect.

How PKA and PKC modulate excitatory synaptic transmission in the human CNS has not been fully studied. We think it is particularly important to examine their actions in human hippocampal synapses because of the crucial roles of human hippocampus in learning and memory (Milner et al. 1998) and in epilepsy (Fischer et al. 1998). PKA and PKC have also been suggested to be involved in epileptogenesis (Osonoe et al. 1994; Tehrani and Barnes 1995; Yechikhov et al. 2001). Previous work found that impaired synaptic plasticity (including forskolin-induced potentiation) in the hippocampus was related to deficient declarative memory in human temporal lobe epilepsy (Beck et al. 2000). In the present study, using whole-cell recordings from the dentate gyrus granule cells (DGCs) in human hippocampal slices, we examined the effect of forskolin and phorbol ester on the frequency and amplitude of sEPSCs and mEPSCs and found that the frequency, but not the amplitude, was enhanced by both kinases. These results are comparable to reports from animal studies and indicate a presynaptic mechanism. We then examined the effects of forskolin and phorbol ester on short-term depression induced by 5-pulse train stimulation at 20 and 50 Hz. We found that both forskolin and phorbol ester enhanced short-term depression, lending further support for a presynaptic mechanism. Finally, we questioned...
whether forskolin and phorbol ester act on a common signal pathway and found that forskolin occluded the effects of phorbol ester on mEPSCs, which implies that PKA and PKC may share a common signal pathway.

**METHODS**

**Human brain slice preparation**

Human hippocampal specimens were obtained from nine patients, aged 9 to 55 yr (mean of 29 yr with 2 patients younger than 18 yr), undergoing a resection of the medial temporal lobe for the surgical treatment of intractable epilepsy. Six patients had hippocampal sclerosis on pathological examination of adjacent hippocampal tissue. Two of these six patients had dual pathology, that is, hippocampal sclerosis in association with a foreign tissue lesion (both were low-grade gliomas). One patient had a small vascular malformation and no hippocampal sclerosis. Two patients had no specific pathological findings in their surgical specimens. Surgically resected tissue was immediately immersed in oxygenated ice-cold artificial cerebrospinal fluid (ACSF) containing the following (in mM): 124 NaCl, 2.5 KCl, 1.25 KH₂PO₄, 6 MgCl₂, 1 CaCl₂, 26 NaHCO₃, 10 glucose (pH 7.4, 300 mosmol/kg). The specimen was transported to the laboratory within 5 min and coronal sections (400 μm) of the middle one-third of the hippocampus were obtained using a Vibratome (Campden Instruments, UK). The slices were incubated on culture inserts covered by a thin layer of ACSF solution and surrounded by a humidified 5% CO₂-95% O₂ atmosphere at room temperature (22°C). Slices were transferred to a submerged recording chamber for electrophysiological recording.

**Electrophysiological recordings**

DGCs were identified using infrared differential interference contrast (IR-DIC) videomicroscopy with a fixed-stage microscope (Axioskop-FS, equipped with a 40×/0.80 W water-immersion lens, Zeiss, Germany). All recordings were made at room temperature (22–25°C) from slices kept under constant (2–3 ml/min) perfusion of ACSF as given above, with the exception that MgCl₂ and CaCl₂ concentrations were 1 and 2 mM, respectively. Tight-seal (>1 GΩ) whole-cell recordings were obtained from the cell body of DGCs. Patch electrodes had a resistance of 3–5 MΩ when filled with the following (in mM): 120 K-gluconate, 8 NaCl, 10 HEPES, 4 MgATP, 0.4 Na₂GTP, 0.2 EGTA, 0.1% biocytin (pH 7.3, 290 mosmol/kg). Neurons were voltage clamped at −68 mV using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA). Access resistance (15–20 MΩ) was regularly monitored during recordings, and cells were rejected if it changed more than 15% during the experiment. Data were filtered at 2 kHz, digitized at 10 kHz, and stored in a computer using pClamp 8 software (Axon Instruments) for off-line data analysis. All EPSCs were isolated by adding picrotoxin (50 μM) to the perfusion solution to block γ-aminobutyric acid-A (GABA_A)-mediated currents. Forskolin, 1,9-dideoxyforskolin, phorbol-12,13-dibutyrate (PDBu), 4α-phorbol-12,13-didecanoate (4αPDD), and 3-isobutyl-1-methylxanthine (IBMX) were first dissolved in 100% DMSO as stock solution and then diluted in bath solution to the desired concentration. The concentration of DMSO in bath solution was 0.01 to 0.03%. At this concentration, DMSO had no effect on synaptic transmission.

In some experiments, a glass electrode filled with ACSF (3–5 MΩ) was placed in the inner molecular layer of the dentate gyrus to evoke synaptic responses with 5-pulse train stimulation. The frequency of pulses was 20 and 50 Hz. Short-term plasticity was evaluated based on the average of 20 to 40 consecutive traces before and after drugs.

**Data analysis and statistics**

sEPSC and mEPSC analysis was performed on continuous segments of spontaneous synaptic current records lasting a period of 5 min in a semi-automated manner. Histograms were made of two parameters extracted from the continuous records, amplitude and inter-event time interval, and these data were converted to probability density functions for comparison. Synaptic events per period were detected using a threshold crossing of the derivative with parameters set for each cell and kept constant for the whole session (Mini Analysis Program, Synaptosoft, Leonia, NJ). The events detected were then visually inspected to remove electrical artifacts prior to final analysis. All results are given as mean ± SE. Short-term plasticity was evaluated by normalizing the amplitudes of all five responses to the first responses based on 20 to 40 trials. The large sample approximation of the Kolmogorov-Smirnov test (K-S test) was used to compare the distributions of EPSC parameters in individual neurons before and after treatments. The paired or unpaired Student’s two-tailed t-test was used to examine the level of significance of group results. Statistical significance was set at $P < 0.05$.

**Histology**

Slices with biocytin-filled cells were stored in 4% paraformaldehyde overnight at 4°C, then rinsed with PBS (0.1 M) three times, and incubated in 0.1% ExtraAvidin peroxidase (Sigma, St. Louis, MO) in PBS solution overnight. Slices were treated with 3% H₂O₂ for 5 min, rinsed two times with PBS and three times with acetate buffer, and then reacted with 0.5% 3,3-diaminobenzidine (Sigma) for 5 to 10 min.

**Drugs**

PDBu, 4αPDD, IBMX, DMSO, 1,9-dideoxyforskolin, 3,3-diaminobenzidine, and picrotoxin were obtained from Sigma. Forskolin and TTX were obtained from Alomone Labs (Jerusalem, Israel).

**RESULTS**

The visualized whole-cell patch-clamp method was performed on granule cells in the dentate gyrus of human hippocampal slices to record sEPSCs, mEPSCs, and evoked EPSCs. DGCs were distinguished from interneurons based on morphology and action potential (AP) firing patterns during suprathreshold depolarizing current injection. APs in DGCs had lower frequency and showed frequency adaptation. Interneurons displayed higher AP frequency and no frequency adaptation. Data from interneurons were not included in this study.

Human DGCs had a resting membrane potential of 69 ± 2 mV, input resistance of 224 ± 16 MΩ, and access resistance of 14 ± 1 MΩ (n = 26). The frequency and amplitude of sEPSCs and mEPSCs in human granule cells showed significant heterogeneity, depending on each individual neuron and individual patient. Among recorded neurons, sEPSC and mEPSC frequency ranged from 0.4 to 5 Hz, similar to the data in animal hippocampus (Parfitt and Madison 1993) and their average amplitude ranged from 14 to 36 pA.

**Forskolin enhances the frequency of sEPSCs and mEPSCs**

We first examined the effect of forskolin on sEPSCs. Forskolin (20 μM) with IBMX (20 μM), an inhibitor of PKA phosphatase, was applied to the slice after stable pretreatment recordings were obtained. The use of forskolin with IBMX has been shown to be more effective in activating PKA than forskolin alone (Chavez-Noriega and Stevens 1994) and, in this paper, the concurrent application of IBMX is implied whenever forskolin is mentioned. As shown in Fig. 1, bath
application of forskolin significantly increased sEPSC frequency. The increase in frequency started about 5 min after the drug was added and stabilized in about 20 min. The increase in sEPSC frequency ranged from 125 to 316% of control values with an average increase of 203 ± 25% (n = 7, P < 0.05) (Fig. 1D). The amplitude, however, was not affected significantly (Fig. 1D). The average sEPSC amplitude after forskolin was 92 ± 4% of control values (P > 0.05). When inactive forskolin, 1,9-dideoxyforskolin, was applied to the slice, no significant effects on sEPSC frequency and amplitude were found (n = 3, data not shown). Because each cell can serve as its own control (i.e., preexposure and postexposure), we could test for statistical significance of forskolin effects in each cell using the K-S test. When analyzed in this fashion, seven of seven DGCs showed decreased inter-event interval (corresponding to an increased frequency) of sEPSCs (P < 0.05) and zero of seven cells showed a change in amplitude after exposure to forskolin. Of these seven cells, four came from specimens with hippocampal sclerosis and three came from specimens without hippocampal sclerosis. Generally, these results are in agreement with previous work in animals (Carroll et al. 1998; Chavez-Noriega and Stevens 1994), indicating that forskolin increases excitatory synaptic transmission through a presynaptic mechanism.

We then examined the effect of forskolin on spontaneous vesicle release without APs. TTX (1 μM) was added in solution to block all APs. As shown in Fig. 1E, forskolin markedly increased the frequency of mEPSCs to 264 ± 30% of control values (n = 7, P < 0.05), but left the amplitude unchanged (99 ± 3% of control values, P > 0.05). Analysis of individual cells with the K-S test showed that inter-event interval of mEPSCs was significantly decreased (corresponding to an increased frequency) in seven of seven cells (P < 0.05) and zero of seven cells showed a change in amplitude. As above, four of these cells were from specimens with hippocampal sclerosis and three were from specimens without hippocampal sclerosis. These results are consistent with those from sEPSCs and also imply a presynaptic enhancement of excitatory synaptic transmission.

Phorbol ester enhances frequency of sEPSCs and mEPSCs

Phorbol esters are analogs of diacylglycerol, the endogenous activator of PKC (Tanaka and Nishizuka 1994). Previous work with whole cell recording in animal hippocampal tissues showed that application of phorbol ester increases the frequency of sEPSCs and mEPSCs, but does not affect the amplitude (Finch and Jackson 1990; Parfitt and Madison 1993). We examined the effect of the phorbol ester, PDBu (5 μM), on both sEPSCs and mEPSCs. We found that bath application of PDBu dramatically enhanced both sEPSC and mEPSC frequency (Fig. 2). sEPSC frequency was increased 298 ± 45% (n = 4, P < 0.05) of control values (Fig. 2D) and mEPSC frequency was increased 636 ± 152% (n = 4, P < 0.05) (Fig. 2E) 20 min after application of PDBu. The amplitude of sEPSCs and mEPSCs was not significantly affected by PDBu (Fig. 2, D and E). The average amplitude 20 min after phorbol ester was 100 ± 8% of control for sEPSCs (n = 4, P > 0.05) and 100 ± 5% of control for mEPSCs (n = 4, P > 0.05). Analysis of individual cells using the K-S test showed that PDBu produced a significant decrease in inter-event interval (corresponding to an increased frequency) of sEPSCs and mEPSCs (P < 0.05) in four of four cells and a change in amplitude of sEPSCs and mEPSCs in zero of four cells. The increase in the frequency of both sEPSC and mEPSC was comparable to what has been observed in rat hippocampus (Carroll et al. 1998; Parfitt and Madison 1993). The inactive phorbol ester, 4αPDD (5 μM), had no effect on sEPSC amplitude or frequency (n = 3, data not shown). These results indicate a presynaptic enhancement of excitatory synaptic transmission by phorbol ester.

Forskolin and phorbol ester enhance short-term depression

Although an increase in postsynaptic current frequency without a change in amplitude is widely interpreted as an
increase in presynaptic release probability (Bekkers and Stevens 1990; Carroll et al. 1998; Manabe et al. 1992; Parfitt and Madison 1993), in some circumstances it can reflect an increase in the number of functional synapses (Isaac et al. 1995; Liao et al. 1995). To help differentiate between these two possibilities as an explanation for the increase in frequency of sEPSCs and mEPSCs by forskolin and phorbol ester, we examined the effect of these two drugs on short-term plasticity (STP) induced by 5-pulse train stimulation at 20 and 50 Hz applied to the inner molecular layer of the dentate gyrus. Previous work showed that STP induced by 5-pulse stimulation is largely a presynaptic event, affected by a change in release probability and not by postsynaptic manipulations (Pananceau et al. 1998). If forskolin and PDBu enhance sEPSC and mEPSC frequency by increasing the number of functional synapses, one would not expect STP to be affected. In agreement with a previous report (Beck et al. 2000), short-term depression was, on average, induced in both inner and outer molecular layers of human dentate gyrus. Short-term depression was influenced by the frequency of pulses in a train. Pulses measuring 50 Hz induced more pronounced depression than 20-Hz stimulation (n = 6, P < 0.05). These findings are similar to previous reports from animal hippocampus and neocortex (Kilbride et al. 2001; Varela et al. 1997).

As illustrated in Fig. 3, both forskolin (n = 5) and phorbol ester (n = 4) significantly enhanced short-term depression induced either by 20- or by 50-Hz trains (P < 0.05). These results are consistent with previous reports that forskolin and phorbol ester reduced paired-pulse facilitation, another form of presynaptic STP, in animals (Gustafsson et al. 1988; Huang and Kandel 1998; Lu and Gean 1999). Therefore our results indicate that an increase in the number of active synapses or release sites is unlikely to account for PKA- and PKC-induced enhancement of sEPSC and mEPSC frequency. They indicate that an increase in release probability is the most likely mechanism for this enhancement.

Forskolin occludes the effects of phorbol ester

Both forskolin and PDBu appear to enhance synaptic transmission by increasing presynaptic release, but it is not known if they share a common signal pathway. We tried to address this question by testing for occlusion of the effects of phorbol ester by prior application of forskolin. As shown in Fig. 4, when forskolin was applied first, mEPSC frequency increased to 320 ± 32% (n = 4) of control values in 20 min. Subsequent application of PDBu produced no further increase in mEPSC frequency. The frequency 20 min after application of PDBu (in addition to forskolin) was 317 ± 35% (n = 4) of control values, and this was not significantly different from the effect of forskolin alone (P > 0.05). Thus forskolin occluded the effect of PDBu on mEPSCs. This occlusion is not likely due to saturation of transmitter release by forskolin. We observed that, in one cell, high-frequency mEPSCs (200% compared with the nonbursting frequency) occurred periodically and this “burst” activity could last ≤2 min. This activity occurred both before and after application of forskolin. This indicates that, even in the presence of forskolin, excitatory synapses on DGCs still have the capacity for significant increases in

FIG. 2. Activation of protein kinase C (PKC) produces increase in frequency of sEPSCs and mEPSCs in human DGCs. A: recordings of sEPSCs from a representative DGC show an increase in frequency after exposure to phorbol-12,13-dibutyrate (PDBu). B: cumulative probability curves from a representative DGC show a significant leftward shift (K-S test, P < 0.05) in the inter-event interval of sEPSCs (decreased interval = increased frequency) in the presence of PDBu (filled circles) in comparison to preexposure values (open circles). C: cumulative probability curves of mEPSC amplitude show no effect of PDBu on this property (control = open circles, after PDBu = filled circles). D: group data from 4 DGCs show a significant increase in sEPSC frequency (left bar) after exposure to PDBu compared with control values (normalized to 100% for each cell). There was no change in amplitude of sEPSCs (right bar) after exposure to PDBu when the group data were analyzed. E: group data from 4 DGCs show an increased frequency of mEPSCs after exposure to PDBu (left bar) compared with control values. Group data show no change in mEPSC amplitude (right bar).

FIG. 3. PKA and PKC enhance short-term depression of stimulus-evoked EPSCs. A: averaged, normalized data from 5 DGCs show that exposure to forskolin (filled squares) enhances short-term depression of EPSCs compared preexposure responses (open squares) at both 20 (left) and 50 (right) Hz stimulation. B: averaged, normalized data from 4 DGCs show that PDBu (filled squares) also enhances short-term depression of EPSCs compared with control values (open squares) at both 20- (left) and 50- (right) Hz stimulation.
Discussion

We have, for the first time, systematically examined the role of activation of PKA and PKC in the regulation of synaptic transmission in human hippocampus. Our results, that bath application of forskolin and phorbol ester selectively enhance the frequency of sEPSCs and mEPSCs without affecting their amplitude, is in agreement with previous work from rodent hippocampus and support the notion that a presynaptic mechanism underlies this enhancement (Chavez-Noriega and Stevens 1994; Malenka et al. 1987; Parfitt and Madison 1993). Because an increase in the number of synapses or the conversion of silent synapse into functional ones could provide an alternative mechanism for an increase in mEPSC frequency (Liao et al. 1995), we studied the effects of forskolin and phorbol ester on short-term plasticity of synaptic transmission. Short-term plasticity, alterations of synaptic strength with repeated stimulation, is largely a presynaptic event. It is affected by presynaptic release probability but not by postsynaptic manipulations (Dobrunz and Stevens 1997; Manabe et al. 1993; Pananceau et al. 1998). Our results, that forskolin and PDBu both enhanced short-term depression induced by 5-pulse trains at 20 and 50 Hz, further support a presynaptic locus for their effects on synaptic transmission. However, these experiments do not eliminate the possibility that an increase in active synapses could also be occurring.

Previous studies have also supported a presynaptic mechanism of forskolin- and phorbol ester-induced enhancement of synaptic transmission. Presynaptic infusion of PKA and PKC inhibitors can block the effects of forskolin and phorbol ester (Hori et al. 1999; Trudeau et al. 1996). Therefore the effects of forskolin and PDBu that we have demonstrated in human dentate gyrus are similar to those that have been reported in animal studies.

Because forskolin activates PKA indirectly through adenylate cyclase, one can question if the forskolin-mediated effects in this study are truly due to actions of PKA. Inactive forskolin had no effect on synaptic transmission, which suggests that the effects of forskolin are specific to the cAMP signal pathway. Previous work in animals showed that specific PKA inhibitors block forskolin’s enhancement of synaptic transmission and application of analogs of cAMP mimic forskolin’s effects (Chavis et al. 1998; Kohara et al. 2001; Lu and Gean 1999). This suggests that the effects of forskolin on synaptic transmission are primarily due to the action of PKA. However, the possibility remains that some other actions of adenylate cyclase are contributing to our results.

Similarly, phorbol esters are not completely specific for activation of PKC. Phorbol ester also acts on the presynaptic Mu-protein, which can also cause an increase in transmitter release (Betz et al. 1998). However, specific inhibitors of PKC block the effects of phorbol ester (Hori et al. 1999; Reymann et al. 1988) and, in rat brain synaptosomes, phorbol ester activates PKC to increase neurotransmitter release (Nichols et al. 1987). Thus a major component of phorbol ester-induced enhancement of synaptic transmission is attributable to the activation of PKC. Taken together, it is most likely that the primary effects of forskolin and phorbol ester on synaptic transmission are due to activation of PKA and PKC, respectively. This indicates that PKA and PKC play a common role in modulating synaptic transmission among different species, including humans.

Although the exact pathways through which PKA and PKC regulate transmitter release is not known, our finding that forskolin occluded the effects of phorbol ester indicates that PKA and PKC pathways may converge onto a common target. This is feasible based on several common actions of PKA and PKC. These include phosphorylation of metabotropic glutamate receptors (Kamiya and Yamamoto 1997; Macek et al. 1998; Schaffhauser et al. 2000) and synapsins (Browning and Dudek 1992; Hilfiker et al. 1999).

In addition, PKA and PKC have been shown to increase the size of the pool of readily releasable vesicles (Gillis et al. 1996; Sakaba and Neher 2001; Stevens and Sullivan 1998) and to
increase Ca\(^{2+}\) influx (Hell et al. 1995; Parfitt and Madison 1993; Yoshihara et al. 2000) in presynaptic terminals. However, Capogna et al. (1995) reported that PDBu and forskolin are additive in enhancing inhibitory synaptic transmission in CA3 pyramidal neurons from cultured rat hippocampal slices. This discrepancy from our results may indicate a different machinery of transmitter release between excitatory and inhibitory synapses.

Our experiments did not show a postsynaptic effect of PKA and PKC on human DGCs. However, it has been reported that PKA and PKC can act on postsynaptic targets (Banke et al. 2000; Greengard et al. 1991; Wang et al. 1994). There are several possible explanations for this discrepancy. One report pointed out that, in conventional whole-cell recording, PKC’s postsynaptic effects may be washed out (due to free communication between the cytoplasm and the filling solution of the recording electrode) because a postsynaptic effect was seen in perforated patch recordings that prevent this communication (Carroll et al. 1998). However, “postsynaptic washout” does not explain previous findings using sharp microelectrodes (which have no “washout”) that phorbol ester did not affect the response induced by exogenous glutamate (Malenka et al. 1986). It also has been reported that the increase in mEPSC amplitude is observed 2 h or more after forskolin application (Bolshakov et al. 1997). This effect would not have been detected in our experiments because most of our recordings lasted less than 1 h. Alternatively, it is possible that bath-applied forskolin and phorbol esters are not accessible to postsynaptic targets for reasons which are not understood.

This study was performed on abnormal human tissue. All patients suffered from intractable temporal lobe epilepsy and all were on antiepileptic medications. Six of the nine patients had hippocampal sclerosis and three did not. We saw no differences in the effects of PKA and PKC on these two groups, although our numbers are too small to clearly exclude such a possibility. Based on our data alone, we cannot know if the responses that we have reported are properties of the normal human hippocampus or some effect of the patients’ underlying pathology. Based on similarities between our findings and animal studies, we feel that it is most likely that our findings represent responses of the normal human hippocampus.

PKA and PKC have been implicated in human memory disorders, such as Alzheimer’s disease (Bonkale et al. 1999; Masliah et al. 1990, 1991), and some investigators have suggested as role for PKA in the declarative memory deficits that occur in people with medial temporal lobe epilepsy. Beck et al. (2000) studied LTP in human hippocampal slices by recording field potentials in the dentate granule cell layer while stimulating the outer molecular layer (lateral perforant path). They found that LTP was impaired in patients with hippocampal sclerosis and that exposure to forskolin/IBMX did not produce long-term enhancement of field potentials. Both LTP and the response to forskolin/IBMX were intact in human hippocampal specimens without hippocampal sclerosis. However, our studies did show an acute effect of forskolin/IBMX on spontaneous excitatory synaptic transmission and short-term plasticity of EPSCs. There are several possible explanations for this discrepancy. Regarding stimulus-evoked responses, we stimulated the inner molecular layer of the dentate gyrus and Beck et al. (2000) stimulated the outer molecular layer. Therefore it is possible that synapses in the two areas do not have the same response to forskolin. In addition, we were looking at two different physiological responses and it is possible that changes in sEPSCs, mEPSCs, and STP do not equate with the forskolin-induced long-lasting potentiation in Beck’s study. Regarding spontaneous currents, we were presumably sampling all of the synaptic inputs onto a given DGC (including both inner and outer molecular layer synapses) and a positive response to forskolin in either group would be recorded as a change in total sEPSCs or mEPSCs, even if the synapses in the outer molecular layer did not respond.

This study shows that PKA and PKC have potent enhancing effects on excitatory synaptic transmission in the human dentate gyrus. These effects are seen even in patients with intractable temporal lobe epilepsy and hippocampal damage. These findings add to our knowledge of the role of PKA and PKC in modulating excitatory synaptic transmission and, coupled with reports that enhancement of PKA can ameliorate memory deficits in aged mice (Bach et al. 1999), support the possibility of manipulating these second messenger systems to treat human memory impairment in the future.

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