Adenylate Cyclase-Mediated Forms of Neuronal Plasticity in Hippocampal Area CA1 Are Reduced With Aging

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Reis, Gerald F., Michael B. Lee, Alex S. Huang, and Karen D. Parfitt. Adenylate cyclase-mediated forms of neuronal plasticity in hippocampal area CA1 are reduced with aging. J Neurophysiol 93: 3381–3389, 2005. First published January 5, 2005; doi:10.1152/jn.00827.2003. Beta-adrenergic receptors and the cyclic AMP signaling pathway play an important role in neuronal plasticity and in learning and memory and are known to change with aging. We examined the effects of β-adrenergic stimulation paired with 5-Hz low frequency stimulation (LFS) of Schaffer collateral-commisural afferents on population spike amplitude in area CA1 of hippocampal slices from young (3 mo) and aged (22 mo) Fischer 344 rats. Application of the β-adrenergic agonist isoproterenol (1 μM) for 10 min followed immediately by 3 min LFS produced long-lasting potentiation in young hippocampi, but the magnitude of potentiation in aged rats was significantly attenuated and was not long-lasting. In slices prepared from young rats, long-term potentiation (LTP) induced by this protocol occludes subsequent attempts to produce conventional high frequency stimulation-induced LTP, and vice versa, suggesting that these two forms of potentiation share one or more molecular mechanisms. Age-related differences in response to LFS alone were not observed, but significant differences in response to β-adrenergic stimulation were apparent. Similarly, significant age-related differences in response to direct activation of adenylate cyclase with forskolin (10 μM) were observed. In both age groups, this enhancement produced by isoproterenol or forskolin is only transient, returning to baseline within 60 or 90 min, respectively. Taken together, these studies of adenylate cyclase-mediated forms of potentiation in area CA1 suggest that there is an age-related defect, either upstream or downstream of adenylate cyclase activation, in this important signaling system. Such changes may contribute to the compromised performance on memory tasks that is often observed with normal aging.

INTRODUCTION

The hippocampus is known to be a critical component of the mammalian CNS for consolidation of experience into long-term memory (Milner 1966; Scoville and Milner 1957; Squire 1982, 1992). The role of the hippocampus in declarative memory is substantiated by a growing body of human (Squire 1982) and animal (Barnes 1979; Rapp and Amaral 1992) research demonstrating that an intact hippocampus is necessary for successful performance of declarative memory tasks. Although aging does not inevitably lead to mnemonic decline, humans (Poon 1985) and rats (Gallagher and Burwell 1989; Jiang et al. 1989) frequently display compromised performance on memory tasks during normal aging.

Such changes in behavior have been correlated with neurophysiological changes in the hippocampus, particularly changes in long-term potentiation (LTP) (Bach et al. 1999). LTP is widely regarded as a cellular substrate for learning and memory (for review, see Bliss and Collingridge 1993) due to its long-lasting nature and associative properties. Interestingly, aged (24-mo-old) rats that have learning and memory deficits also exhibit a higher rate of decay of LTP or synaptic enhancement in the dentate gyrus (Barnes 1979; deToledo-Morrell et al. 1988). The most thoroughly characterized form of LTP to date is that which is induced in vitro at CA1 synapses by brief bursts of high-frequency tetanic stimulation (0.5–1 s of 100- to 200-Hz stimulation) of the Schaffer collateral-commisural pathway. In earlier studies of the effects of aging on this form of LTP in vitro, deficits in the induction or maintenance of LTP were not apparent (Deupree et al. 1991; Landfield et al. 1978; Moore et al. 1993), even in slices prepared from aged rats displaying significant spatial memory deficits (for reviews, see Barnes 1994, 2003; Lynch 1998). More recent studies by Bach et al. (1999) again demonstrated no significant age-related difference in tetanus-induced LTP 1 h poststimulus, which they refer to as early LTP (E-LTP), but they did observe significant reduction in late-phase LTP (L-LTP) measured 3 h poststimulus. This reduction in L-LTP, a form of potentiation that is dependent on the cAMP signaling pathway (Abel et al. 1997; Frey et al. 1993; Bourritchuladze et al. 1994), was associated with an increase in spatial memory errors in aged mice.

Although it is unclear why deficits in E-LTP were not observed in aged animals, it is possible that deficits in potentiation were masked by the supraphysiological 100-Hz stimulation parameters typically used in these studies. Indeed, other studies by Moore et al. (1993), Deupree et al. (1993), and Rosenzweig et al. (1997) have demonstrated that when less intense, more physiologically relevant stimulus parameters are used to induce LTP, significant differences in the magnitude and incidence of LTP are observed in slices from aged versus young rats. One such stimulation pattern is primed burst stimulation, which reliably induces LTP in young but not aged animals (Moore et al. 1993). Alternatively, Thomas et al. (1996) have found that 5-Hz LFS applied for 3 min after β-adrenergic receptor activation induces significant LTP of field excitatory postsynaptic potentials (EPSPs) in area CA1 of young (3- to 5-wk-old) mice. It is unclear why the pairing of these stimuli induces such reliable LTP, whereas either stimulus alone does not (Thomas et al. 1996), but it may be that the LFS-induced rise in intracellular [Ca2+]i may act synergistically with β-adrenergic receptor-coupled Gs to activate adenylate cyclase, thereby producing a persistent form of synaptic potentiation. This prospect of two signaling pathways acting in concert to produce LTP is attractive in that it provides a molecular mechanism of associativity.

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The hippocampus receives a diffuse but substantial projection of norepinephrine (NE)-containing fibers from the locus coeruleus (Fuxe 1965; Loy et al. 1980; Moore 1982). NE has been shown to modulate neuronal function in all three regions of the trisynaptic hippocampal pathway by acting at β-adrenergic receptors. In area CA1, superfusion of NE or the β-adrenergic agonist isoproterenol (ISO) does not seem to have any direct effect on EPSPs (Gereau and Conn 1994a; Heginbotham and Dunwiddie 1991; Mueller et al. 1981; Parfitt et al. 1992; but see Thomas et al. 1996); both agonists, however, markedly potentiate the population spike amplitude (Gereau and Conn 1994a; Heginbotham and Dunwiddie 1991; Dunwiddie et al. 1992; Mueller et al. 1981). NE can modulate the excitability of CA1 pyramidal neurons by decreasing the amplitude and duration of the slow calcium-activated potassium afterhyperpolarization (AHP) that occurs after depolarizing stimuli (Madison and Nicoll 1986a); this is mediated by the cAMP effector pathway (Madison and Nicoll 1986b). This reduction in the AHP blocks the accommodation of pyramidal cell discharge rate, a phenomenon that is also referred to as “spike frequency adaptation.” As a result, NE or elements of the cAMP pathway profoundly affect neuronal excitability and neuronal function. Activation of the cAMP pathway via β-adrenergic stimulation (Gereau and Conn 1994b) or direct stimulation of adenylate cyclase (Chavez-Noriega and Stevens 1994) can also enhance excitatory synaptic transmission.

Previous studies have demonstrated an age-related functional deficit in receptor systems that are coupled to the cAMP effector pathway, including β-adrenergic receptors in the cerebellum (Bickford 1983; Parfitt 1988; Parfitt and Bickford-Wimer 1990; Parfitt et al. 1988, 1990b) and hippocampus (Bickford-Wimer et al. 1987) and dopaminergic receptors in the prefrontal cortex (Parfitt et al. 1990a). In these studies, changes in the responsiveness of receptors coupled to other second-messenger systems were not observed. Such deficits in the hippocampus can lead to reduced cAMP-dependent phosphorylation events (Parfitt et al. 1991). Although there do not appear to be significant age-related changes in β-adrenergic receptor number or affinity in the hippocampus (Miller and Zahniser 1988) or changes in NE levels (Roubein et al. 1981), NE is unable to fully stimulate adenylate cyclase activity in the senescent rat hippocampus (Walker and Walker 1973); as a result, decreases in cAMP levels are observed (Hara et al. 1992). In light of these observations, the goal of the present study was to investigate age-related changes in hippocampal neuronal excitability produced by β-adrenergic receptor stimulation paired with low-frequency electrical stimulation.

METHODS

Fischer 344 rats were obtained from the National Institute of Aging colony at Charles River Breeding Laboratories and maintained according to National Institutes of Health guidelines in a facility accredited by the Association for Accreditation of Laboratory Animal Care (AAALAC). Hippocampal slices from young (3-mo-old) and aged (22- to 24-mo-old) male rats were prepared as previously described (Madison and Nicoll 1986a) and placed in an oxygenated holding chamber at room temperature (22-24°C) for ≥1 h. A single slice was then transferred to a recording chamber, where it was submerged and superfused continuously at a rate of 3-4 ml/min with artificial cerebrospinal fluid (ACSF) containing (in mM) 119 NaCl, 2.5 KCl, 1.3 MgCl₂, 2.5 CaCl₂, 1.0 NaH₂PO₄, 26.2 NaHCO₃, and 11 glucose. This solution was gassed with 95% O₂-5% CO₂, bringing it to pH 7.4. Stimulating electrodes and recording electrodes were placed in stratum radiatum and s. pyramidale, respectively. Stimulating pulses of 0.1-ms duration were delivered to the Schaffer collateral-commissural fibers via bipolar tungsten electrodes (Fred Haer) at a rate of 0.033 Hz. The intensity of stimulation was set so that the amplitude of responses was ~50% of the maximum population spike amplitude. Responses were recorded at room temperature (22-24°C) with extracellular electrodes of 5–10 MΩ resistance, filled with 3.0 M NaCl. Signals were amplified on a Dagan IX1 amplifier and digitized using MacLab/2e data-acquisition hardware. Data were filtered at 1 kHz and analyzed using MacLab Scope software. In some experiments, signals were amplified using Axoclamp 2B and Brownlee amplifiers, filtered at 1 kHz, and analyzed using Labview acquisition and analysis software. The amplitude of population spikes was measured as the length of a vertical line drawn from the minimum of the field response to the midpoint of the line that joined the two positive peaks. In all experiments, manipulations were made only after stable responses had been obtained for ≥20 min. ISO (isoproterenol HCl, Sigma) or forskolin (Calbiochem) were initially dissolved in dH₂O or DMSO, respectively, and then diluted to their final concentration in the ACSF. Final DMSO concentration was 0.1%, a concentration that did not affect the population spike amplitude.

Acute changes in neuronal excitability measured during an appropriate 5-min period after drug application or stimulation were compared in slices from young versus aged rats using repeated-measures ANOVA, with population spike amplitude over time as the repeated measure and age as a factor. Persistent changes in neuronal excitability were also compared by repeated-measures ANOVA, with population spike amplitude over time, measured 55–60 min after completion of a manipulation, as the repeated measure and age as a factor. In a few cases where indicated, population spike amplitudes were compared using a paired Student’s t-test. Where stated, N represents the number of slices used in each experiment, prepared from a minimum of five animals per age group. Results are reported as the means ± SE.

RESULTS

Thomas and colleagues (1996) have reported a form of LTP of field EPSPs in area CA1 of juvenile mouse hippocampus that persisted for >1 h when ISO superfusion was immediately followed by 3 min of low-frequency (5 Hz) electrical stimulation (LFS). In a similar vein, we observed in young adult (3-mo-old) F344 rats that 10-min superfusion of slices with ISO (1 μM), followed by LFS, produces LTP of the CA1 population spike amplitude (Fig. 1A). Because of the presumed dependence of this potentiation on adenylate cyclase, we refer to it as AC-LTP. In aged rats, the acute response to ISO was much lower than that observed in the young rats (see also Fig. 4), and AC-LTP was not observed (Fig. 1B); the population spikes returned to their baseline amplitude within 60 min after the LFS. Data from 10 slices from 9 young adult rats and 7 slices from 5 aged rats are summarized in Fig. 1C. In slices from the young rats, the population spike amplitude was 48.7 ± 12.1% above baseline 50–60 min after LFS, which was significantly different from baseline amplitude (0.0 ± 2.4%; P < 0.01, repeated-measures ANOVA). In slices from aged rats, responses 50–60 min after the LFS were significantly lower than those in slices from young rats (P < 0.01, repeated-measures ANOVA) and had returned to baseline (9 ± 10. vs. 0.0 ± 3.0%; P > 0.05, Student’s paired t-test). In all slices for this and subsequent studies, responses of similar baseline amplitude were used. Although the population spike amplitude for a given stimulus strength was lower in slices from aged as
and persisted for slices from young rats was significantly greater than in slices from aged rats, depression of the population spike was observed. Subsequent potentiation in slices were significantly different in slices from young vs. aged rats. After the LFS, leads to long-term potentiation (LTP) of the population spike amplitude in slices from young adult rat. The slice was exposed to ISO (1 M) for 10 min followed immediately by LFS. B: plot of normalized CA1 population spikes in a slice prepared from an aged rat. The slice was exposed to ISO (1 M) + LFS. A and B: insets: sample population spike waveforms corresponding to times a–c of time plots. Calibration: 2 mV, 10 ms. C: ensemble analysis showing that ISO followed by LFS leads to long-term potentiation (LTP) of the population spike amplitude in young but not aged rats. As demonstrated in Fig. 5, the acute effects of ISO were significantly different in slices from young vs. aged rats. After the LFS, depression of the population spike was observed. Subsequent potentiation in slices from young rats was significantly greater than in slices from aged rats, and persisted for >60 min. In slices from aged rats, population spikes decayed to baseline within 60 min after the LFS.

FIG. 1. β-adrenergic stimulation followed by 5-Hz, 3-min low-frequency stimulation (LFS) produces persistent potentiation of population spike amplitude in slices from 3- but not 24-mo-old F344 rats. A: plot of normalized CA1 population spikes in a slice prepared from a young adult rat. The slice was perfused with isoproterenol (ISO; 1 μM) for 10 min followed immediately by LFS. B: plot of normalized CA1 population spikes in a slice prepared from an aged rat. The slice was exposed to ISO (1 μM) + LFS. A and B: insets: sample population spike waveforms corresponding to times a–c of time plots. Calibration: 2 mV, 10 ms. C: ensemble analysis showing that ISO followed by LFS leads to long-term potentiation (LTP) of the population spike amplitude in both young and aged rats (Fig. 2); neither the magnitude nor the time course of this depression was significantly different in slices from young versus aged hippocampus ($P > 0.05$; repeated-measures ANOVA). In contrast with recent work by Watabe and O’Dell (2003) and Tombaugh et al. (2002), but consistent with Brown et al. (2000), we did not observe potentiation of the EPSP or complex spiking after either short (150 pulses) or long (900 pulses) exposure to this 5-Hz LFS.

To determine whether AC-LTP observed in young adult rats shares some common molecular mechanisms with LTP induced by a 100-Hz tetanus, we investigated whether tetanus-induced LTP occluded AC-LTP and vice versa. LTP induced by two bursts of 100-Hz stimulation (HFS) did occlude subsequent attempts to induce AC-LTP (Fig. 3A); conversely, induction of AC-LTP occluded subsequent HFS-induced LTP (Fig. 3B). In contrast, in aged animals, the transient potentiation induced by ISO/LFS treatment did not occlude subsequent HFS-induced LTP (Fig. 4).

Because the acute effects of ISO in aged rats were markedly attenuated as compared with acute responses in young (Fig. 1C), we decided to examine age-related changes in response to β-adrenergic stimulation alone. As previously reported by Heginbotham and Dunwiddie (1991), we found that exposure of slices from young rats to ISO alone produced an increase in excitability of pyramidal neurons, as demonstrated by the potentiation of the evoked population spike amplitude (Fig. 5); however, as observed by Gereau and Conn (1994a), this potentiation did not persist on washout of the agonist. In slices prepared from young rats, ISO (1 μM) produced significant enhancement (61.9 ± 8.1% above baseline; $P < 0.01$; repeated-measures ANOVA) of the population spike amplitude 3–8 min after beginning the superfusion of the agonist. Although the enhancement in population spike amplitude was not of equal magnitude for each slice studied, all 30 slices displayed potentiation in response to ISO application. This enhancement declined steadily during washout, however, such that the population spike amplitudes were not significantly different from baseline amplitudes 60–65 min after washout (11.6 ± 5.9 vs. 0.0 ± 1.5%; $P > 0.05$, paired Student’s $t$-test).

Slices prepared from aged F344 rats revealed significant susceptibility to β-adrenergic agonist; the magnitude of the acute response (35.9 ± 6.8% above baseline; $n = 8$) was significantly less in the aged as compared with young animals ($P < 0.05$; repeated-measures ANOVA). As in the young animals, the acute effects of ISO (1 μM) were not persistent; the population spike amplitude 60–65 min after washout declined toward baseline (3.3 ± 7.2 vs. 0.2 ± 1.7%). The dose of ISO chosen for these studies (1 μM) produces maximal...
potentiation of the population spike (Mueller et al. 1981) in slices from young rats; increasing the ISO dose to 2 or 10 μM in slices from aged rats did not enhance the maximal increases in population spike amplitude (Fig. 6). Thus in the aged rats the lack of AC-LTP was not due to using a suboptimal dose of the β-adrenergic agonist (i.e., to a rightward shift in the ISO dose-response curve with aging), but to a depression of the maximal enhancement.

The direct activation of adenylate cyclase by forskolin (10 μM) produced an increase in population spike amplitude that was significantly greater than the forskolin-induced potentiation in slices from aged animals (39 ± 7 vs. 14 ± 6%, respectively, measured 20–30 min after forskolin superfusion was stopped; P < 0.05; repeated-measures ANOVA; Fig. 7). When forskolin was initially applied, the population spike amplitude was inhibited somewhat, perhaps due to the transport of cAMP out of cells and activation of adenosine receptors by cAMP-derived adenosine (Chavez-Noriega and Stevens 1994; Dunwiddie et al. 1992; Lu and Gean 1999; Pockett et al. 1993; Rosenberg and Li 1995). This hypothesis is supported by previous observations that forskolin-induced or 8-bromo-cAMP-induced depression is inhibited by the cAMP transport inhibitor probenicid (Lu and Gean 1999), by inhibition of cAMP phosphodiesterase (Lu and Gean 1999), or by inhibition of adenosine receptors (Dunwiddie et al. 1992; Pockett et al. 1993). The brief inhibition in response to forskolin, if it occurs, is followed by enhancement of the population spike amplitude. As reported by Dunwiddie and colleagues (1992), the forskolin-induced potentiation persisted for ~40 min after washout; we have observed, however, that the potentiation decays back to baseline with 50 additional min of washout of this very lipophilic agent in slices from both young and aged rats (Fig. 7B). The inactive forskolin derivative 1,9-dideoxy-forskolin did not significantly change population spike amplitudes (Fig. 7B; paired Student’s t-test).

**DISCUSSION**

The results presented here demonstrate that LTP of population spikes can be induced in area CA1 of hippocampal slices prepared from young adult rats by pairing β-adrenergic receptor stimulation with LFS. This form of LTP, which we refer to as AC-LTP, is significantly reduced in magnitude in aged rats and is not long-lasting. Thomas et al. (1996) hypothesize that potentiation of this kind in young animals is produced by the coincident signals of cAMP (due to β-adrenergic receptor stimulation) and calcium influx (due to the LFS). Thus the deficits in AC-LTP in the aged animals could be due to changes in the β-adrenergic/cAMP signaling pathway, or to changes in calcium signaling. There have been numerous reports of changes in calcium homeostasis in hippocampus from senescent animals, revealing an excess of voltage-dependent calcium influx, perhaps due to increases in voltage-sensitive calcium channels, alterations in calcium buffering, or disturbances in calcium uptake and extrusion (for reviews, see Foster and Kumar 2002; Khachaturian 1984; Landfield 1994). Such changes in calcium signaling contribute to prolonged calcium-dependent AHPs in aged hippocampal neurons (Moyer et al. 1992; Landfield and Pitler 1984). Pretreatment with ISO may further enhance the AHP via the activation of PKA and phosphorylation-induced opening of L-type calcium channels; this would prevent AC-LTP from persisting in slices from aged animals. Further experiments to address this possibility would involve examining whether AC-LTP can be rescued in slices from aged rats pretreated with an L-type channel blocker, or the K⁺ channel blocker apamin, using an approach similar to
that of Norris et al. (1998b). Given the evidence for calcium dysregulation with aging, further experiments should also be done to reveal whether the compromised AC-LTP observed in aged animals here was due to the relatively high Ca\(^{2+}\):Mg\(^{2+}\) ratio that was used (2.5 mM Ca\(^{2+}\):1.3 mM Mg\(^{2+}\)).

Norris et al. (1996) demonstrated an increased susceptibility to long-term depression (LTD) at CA3–CA1 synapses of aged rats, perhaps due to the altered calcium homeostasis in these animals (Landfield 1994; Ouannonou et al. 1999) and an enhancement of protein phosphatase activity (Norris et al. 1998a). In the present study, however, responses to LFS alone were not significantly different in slices from young versus aged rats (Fig. 2). This suggests that the observed differences in AC-LTP may be due primarily to changes in adenylate-cyclase-mediated signaling. Indeed, pyramidal neurons of the aged hippocampi were significantly less responsive to super-

FIG. 3. In young rats, HFS-induced LTP occludes subsequent attempts to induce AC-LTP and vice versa. A: LTP was induced by two 100-Hz tetani (1 s each, separated by 30 s). After 25 min, the stimulus strength was reduced to elicit responses of baseline amplitude. Once a stable baseline was achieved again, ISO (1 μM) was applied for 10 min followed immediately by 5-Hz LFS for 3 min. Responses 60 min after the LFS were not significantly different from baseline (n = 4; Student's t-test). B: conversely, after induction of AC-LTP and allowing ≥90 min for ISO to be washed from the slice, tetanus-induced LTP was blocked; responses returned to baseline within 40 min after tetanus (n = 4; Student's t-test). Insets: sample population spike waveforms corresponding to times a–c of time plots. Calibration: 4 mV, 10 ms.

FIG. 4. In aged rats, transient potentiation induced by ISO + LFS does not occlude subsequent HFS-induced LTP. ISO (1 μM) was applied for 10 min followed immediately by 5-Hz LFS for 3 min. Responses 60 min after the LFS were not significantly different from baseline (n = 6; Student's t-test). LTP was subsequently induced by 2 100-Hz tetani (1 s each, separated by 30 s).
fusion of the β-adrenergic agonist, ISO, or to direct activation of adenylate cyclase with the diterpene, forskolin, as compared with young adult hippocampi (Figs. 5–7). This agrees with previous work demonstrating age-related changes in β-adrenergic modulation of neuronal excitability (Bickford 1983; Bickford-Wimer et al. 1987; Gould and Bickford 1997; Parfitt 1988; Parfitt et al. 1988, 1990b, 1991) and changes in cAMP signal transduction (Hara et al. 1992; Walker and Walker 1973). It is unclear, however, whether the age-related decrease in response to ISO is due to a reduced ability of receptors to activate G proteins, a reduced ability of G proteins to activate adenylate cyclase, an increase in expression of Gi alpha proteins (as described by Bazan et al. 1994 in heart), decreased activity of adenylate cyclase, or increased cAMP phosphodiesterase activity; alternatively or additionally, these changes may be due to a defect(s) downstream of cAMP production. Bach et al. (1999) observed that deficits in spatial learning in aged mice can be ameliorated by treatment with agents that elevate cAMP concentrations, such as D1/D5 receptor agonists and the cAMP phosphodiesterase inhibitor, rolipram; furthermore, deficits in L-LTP, which is dependent on cAMP production, are attenuated by D1/D5 agonists. These experiments support the hypothesis that the age-related defect in the cAMP-PKA signaling pathway is upstream of cAMP production.

Previous investigations by others (Dunwiddie et al. 1992; Heginbotham and Dunwiddie 1991) suggested that long-lasting potentiation of evoked population spike amplitudes in area CA1 occurs after exposure of hippocampal slices to β-adrenergic agonists (such as ISO) alone or to agents that stimulate adenylate cyclase directly (such as forskolin). These effects were reported to persist for ≥30–40 min after the washout of ISO. In our experiments, β-adrenergic potentiation (>12% above control) at 25–30 min into the washout period was observed in only 10 of 30 slices from the young rats. After 55–60 min of washout, the population spike amplitudes in our experiments were not significantly different from control. The direct activation of adenylate cyclase with forskolin produced potentiation that persisted for ≥40 min on washout, as observed by Dunwiddie and colleagues (Dunwiddie et al. 1992; Heginbotham and Dunwiddie 1991).
et al. 1992; Heginbotham and Dunwiddie 1991); we found, however, that the potentiation decays to baseline within 50 additional min of washout of this lipophilic agent. Thus to produce reliable potentiation of population spikes that persists for \( \geq 1 \) h, we found that adenylate cyclase activation must be paired with LFS.

AC-LTP shares some common molecular mechanisms with conventional high-frequency (100 Hz) stimulation-induced LTP as shown by the occlusion experiments. One such common mechanism is likely the activation of \( N \)-methyl-\( D \)-aspartate (NMDA) receptors because both AC-LTP (data not shown) and tetanus induced LTP (Wigstrom and Gustafson 1986) are attenuated or blocked, respectively, by the NMDA receptor antagonist APV. Furthermore, tetanus-induced LTP produces significant elevation of intracellular cAMP (Chetkovich and Sweatt 1996), activation of PKA (Blitzer et al. 1995; Roberson and Sweatt 1996), and phosphorylation of PKA substrates (Blitzer et al. 1998). Phosphorylation of the GluR1 AMPA receptor subunit at S845 can regulate the peak open probability of the AMPA receptor channel (Banke et al. 2000; Roche et al. 1996) and is required for subcellular trafficking of GluR1-containing AMPA receptors to the synaptic membrane (Ehlers 2000). In addition, PKA-mediated phosphorylation of S845 is required for the maintenance of NMDA receptor-dependent LTP (Esteban et al. 2003).

The occlusion of AC-LTP by prior tetanus-induced LTP suggests that prior saturation of one or more of these intracellular events by

![FIG. 7. Effects of forskolin on CA1 population spike amplitude in young vs. aged F344 rats. A: forskolin application (10 \( \mu \)M for 10 min, as denoted by the bar) enhanced the population spike amplitude 39 \( \pm \) 7\% in 3-mo-old rats but only 14 \( \pm \) 6\% in 22- to 24-mo-old rats. A repeated-measures ANOVA supports the hypothesis that there is a significant difference (\( P < 0.05 \)) between the acute responses in slices from young vs. aged rats to direct stimulation of adenylate cyclase. At 50 min after washout, significant potentiation of the population spike was still observed, but within 90 min, the population spike amplitudes decayed back to baseline (see B). B: summary of results of forskolin-induced potentiation of CA1 population spikes. Whereas population spike amplitudes 20 min after washout (“acute” responses) were significantly different from control, responses at 90 min after washout (“persistent”) did not differ significantly from baseline responses (Student’s \( t \)-test, \( P > 0.05 \)). In addition, superfusion of the inactive derivative, 1,9-dideoxyforskolin (10 \( \mu \)M), did not affect the population spike amplitude. \( n = 26 \), forskolin in young; \( n = 4 \), dideoxyforskolin in young; \( n = 10 \), forskolin in aged slices.](http://jn.physiology.org/doi/abs/10.1152/jn.00598.2004)
tetanic stimulation inhibits the production of AC-LTP. Conversely, stimulation of adenylyl cyclase via β-receptor-coupled G_i and LFS-induced rises in intracellular [Ca^{2+}] appear to saturate one or more mechanisms required for subsequent tetanus-induced LTP. Experiments in hippocampal slices from aged rats, in which modest tetanus-induced LTP was still observed after the failed attempts to induce AC-LTP, suggest that it is the more persistent cellular changes, such as those produced by PKA activation, that contribute to the occlusion of subsequent tetanus-induced LTP in young rats. In such cases in aged rats, the tetanus-induced LTP observed after the transient AC-LTP likely depends more heavily on calcium-calcmodulin-triggered mechanisms that are activated by the intense calcium influx that occurs during the high-frequency stimulation.

Noradrenergic modulation of synaptic potentiation after various patterns of LFS has been studied extensively by Katsuki et al. (1997) in 4- to 5-wk-old rats, and by Thomas et al. (1996) in 3- to 5-wk-old mice. In both cases, β-adrenergic activation produced LTP of field EPSPs when accompanied by 900 pulses of LFS (5–10 Hz), whereas neither of these stimuli alone produced potentiation. We observed that ISO followed by 5-Hz (3 min) stimulation produces potentiation of EPSPs in slices from 4-wk-old but not from mature adult rats (6-wk- to 3-mo-old rats; data not shown). For this reason, we decided to study the modulation of population spike amplitude so that comparisons could be made between mature adult and aged adult animals. Nevertheless, further investigation of the differences in noradrenergic modulation of synaptic potentiation of EPSPs in juvenile versus mature adult rodents may provide additional clues as to the molecular requirements for achieving synaptic potentiation.

Although the noradrenergic modulation of excitatory synapses and neuronal excitability in the CNS has yet to be understood, it is likely that noradrenergic input to the hippocampus plays a role in enhancing memory formation. Norepinephrine seems to play an important role in selective attention, arousal, and emotions (Aston-Jones and Bloom 1981). In enhancing memory formation. Norepinephrine seems to play an important role in selective attention, arousal, and emotions (Aston-Jones et al. 1996). Norepinephrine is also present in the amygdala, which is involved in emotional processing. Thus, it is likely that the noradrenergic modulation of hippocampal synaptic plasticity is mediated by the α2-adrenergic receptors in the amygdala. Future studies should investigate the role of these receptors in hippocampal plasticity and their potential contribution to memory formation.

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Nimodipine...H9252


