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Enhanced Long-Term Potentiation During Aging Is Masked by Processes Involving Intracellular Calcium Stores

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Kumar, Ashok and Thomas C. Foster. Enhanced long-term potentiation during aging is masked by processes involving intracellular calcium stores. *J Neurophysiol* 91: 2437–2444, 2004. First published February 4, 2004; 10.1152/jn.01148.2003. The contribution of Ca^{2+} release from intracellular Ca^{2+} stores (ICS) for regulation of synaptic plasticity thresholds during aging was investigated in hippocampal slices of old (22–24 mo) and young adult (5–8 mo) male Fischer 344 rats. Inhibition of Ca^{2+}-induced Ca^{2+} release by thapsigargin, cyclopiazonic acid (CPA), or ryanodine during pattern stimulation near the threshold for synaptic modification (5 Hz, 900 pulses) selectively induced long-term potentiation (LTP) to CA1 Schaffer collateral synapses of old rats. Increased synaptic strength was specific to test pathways and blocked by AP-5. Intracellular recordings demonstrated that ICS plays a role in the augmentation of the afterhyperpolarization (AHP) in old rats. The decrease in the AHP by ICS inhibition was reversed by the L-channel agonist, Bay K8644. Under conditions of ICS inhibition and a Bay K8644–mediated enhancement of the AHP, pattern stimulation failed to induce LTP, consistent with the idea that the AHP amplitude shapes the threshold for LTP induction. Finally, ICS inhibition was associated with an increase in the *N*-methyl-D-aspartate (NMDA) receptor component of synaptic transmission in old animals. This increase in the synaptic response was blocked by the calcineurin inhibitor FK506. The results reveal an age-related increase in susceptibility to LTP-induction that is normally inhibited by ICS and suggest that the age-related shift in Ca^{2+} regulation and Ca^{2+}-dependent synaptic plasticity is coupled to changes in cell excitability and NMDA receptor function through ICS.

INTRODUCTION

Fluctuations in the level of intracellular Ca^{2+} can result in highly localized activation of Ca^{2+}-dependent processes, such as induction of long-term potentiation (LTP) due to the rise in Ca^{2+} within a dendritic spine (Koch and Zador 1993; Muller and Connor 1991; Sabatini et al. 2001; Svoboda et al. 1999; Wickens 1988; Yuste et al. 2000). Alternatively, intracellular Ca^{2+} can have distal influences due to the propagation of Ca^{2+} waves (Jaffe and Brown 1994; Kapur et al. 2001; Miller et al. 1996; Nakamura et al. 1999) or membrane effects that influence cell excitability and the integration of electrical potentials (Disterhoft et al. 1996; Foster and Kumar 2002; Johnston et al. 2003; Landfield and Pitler 1984; Norris et al. 1998a; Sah and Bekkers 1996). Several sources of intracellular Ca^{2+} are available, including ligand-gated glutamate receptors such as the *N*-methyl-D-aspartate (NMDA) receptors, voltage-dependent Ca^{2+} channels (VDCCs), and release from intracellular Ca^{2+} stores (ICS). It is generally accepted that, at CA1 synapses, NMDA receptors provide the foremost source of Ca^{2+} for LTP induction for stimulation frequencies near the threshold for synaptic modification (Cavus and Teyler 1996; Johnston et al. 1992). However, the level of Ca^{2+} in dendritic spines can be influenced by VDCCs, ICS, and other glutamate receptors (Christie et al. 1996a,b, 1997; Dingledine et al. 1999; Jaffe et al. 1994; Korkotian and Segal 1999). Recent studies have suggested that VDCCs and ICS are involved in regulating the threshold for induction of synaptic plasticity in young adult animals (Foster and Kumar 2002; Foster and Norris 1997; Kamsler and Segal 2003; Nishiyama et al. 2000; Norris et al. 1998a; Raymond and Redman 2002; Rose and Konnerth 2001; Wilsch et al. 1998).

The relationship between Ca^{2+} sources and synaptic plasticity thresholds is particularly relevant to studies of aging since changes in synapse modifiability have been linked to dysregulation of Ca^{2+} homeostasis (Foster and Kumar 2002; Foster and Norris 1997). Over the course of aging, there is a shift in the influence of VDCCs on the induction of synaptic plasticity, particularly for stimulation patterns that are near the threshold for synaptic modification (Foster 1999; Norris et al. 1998a; Shankar et al. 1998; Watabe and O'Dell 2003). In addition, L-channels are closely linked to the Ca^{2+}-dependent, K^+-mediated afterhyperpolarization (AHP) (Disterhoft et al. 1996; Kumar and Foster 2002; Landfield and Pitler 1984; Power et al. 2002; Thibault and Landfield 1996; Wu et al. 2002), and the increase in the AHP during senescence is associated with an increased threshold for LTP, possibly due to weakened synaptic integration (Kumar and Foster 2002; Norris et al. 1998a). Studies in neonate and juvenile animals indicate that Ca^{2+} release from ICS contributes to LTP induction for stimulation near the threshold for induction (Behnisch and Reymann 1995; Harvey and Collingridge 1992; Obenaus et al. 1989; Raymond and Redman 2002), possibly by contributing to Ca^{2+} levels in dendritic spines (Emptage et al. 1999; Nishiyama et al. 2000). However, ICS also contribute to the AHP (Shah and Haylett 2000), suggesting that this source of Ca^{2+} may be a factor in altered synaptic plasticity thresholds during aging. Thus this study was designed to investigate the role of ICS in mediating age-related changes in the threshold for LTP induction.

METHODS

Subjects

Procedures involving animal subjects have been reviewed and approved by Institutional Animal Care and Use Committee. Male

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Fischer 344 rats, young (5–8 mo; $n = 20$) and old (22–24 mo; $n = 41$), were group housed (1–2/cage), maintained on a 12:12 h light schedule, and provided ad libitum access to food and water.

Hippocampal slice preparation

Rats were killed with CO_2, hippocampi were dissected, and slices (~400 μm) were cut parallel to the alvear fibers with a Vibratome (Technical Products International, St. Louis, MO). Slices were transferred to a standard interface-recording chamber that was continuously perfused (1 ml/min) with oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 2 KCl, 1.25 KH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose. Slices were maintained at 30–32°C, and humidified air (95% O_2-5% CO_2) was blown over the slices.

Electrophysiological recordings

Extracellular synaptic field potentials from CA3–CA1 synaptic contacts were recorded with glass micropipettes (4–6 MΩ) filled with ACSF. Stimulating electrodes (stainless steel, tip diameter ~0.13 mm) were positioned on either side (~1 mm) of a recording electrode localized to the middle of stratum radiatum, and single diphasic stimulus pulses were alternated between pathways such that each pathway was activated at 0.033 Hz. One stimulating electrode activated a control pathway used to insure that the effects of pattern stimulation were specific to activated synapses and not due to a change in slice health (Norris et al. 1996). Stimulation intensity was set to elicit a 1- to 2-mV excitatory postsynaptic potential (EPSP) response. Drug application to the slices occurred ≥35–120 min prior to pattern stimulation, and stable response baseline in the presence of drug was collected for ≥20 min prior to delivery of pattern stimulation. Following pattern stimulation to induce synaptic modification, the response was recorded for 60 min (Norris et al. 1996, 1998a). Synaptic modification was induced by pattern stimulation (5 Hz, 900 pulses) delivered to the Schaffer collaterals. The signals were amplified, filtered between 1 Hz and 1 kHz, and stored on computer disk for off-line analysis (Data Wave Technologies, Longmont, CO). Two cursors were placed around the initial descending phase of the waveform, and the maximum slope (mV/ms) of the EPSP was determined by a computer algorithm that found the maximum change across all sets of consecutively recorded points (20-kHz sampling rate) between the two cursors. Changes in transmission properties induced by pattern stimulation or application of drug were calculated as the percent change from the averaged baseline response collected 10 min prior to pattern stimulation or drug application.

Intracellular recordings were performed from CA1 pyramidal neurons to record AHPs and EPSPs. Microelectrodes were pulled from thin-wall 1.0-mm microfiber-filled borosilicate capillaries using a Flaming/Brown horizontal micropipette puller (Sutter Instruments, San Rafael, CA). The resistance of microelectrodes when filled with 3 M potassium acetate was 50–100 MΩ. Microelectrodes were visually positioned in the CA1 pyramidal cell layer using a dissecting microscope (SZH10, Optical Elements Corp., Washington, DC). The signals were amplified by an Axoclamp 2B amplifier (Axon Instruments, Union City, CA), and recordings (continuous bridge mode) were sampled at 5 kHz and stored on computer disk for off-line analysis (Data Wave Technologies).

Only neurons with a resting membrane potential less than –57 mV, an input resistance >20 MΩ, and an action potential amplitude rising ≥70 mV above the point of spike initiation were included in the analysis as described earlier (Kumar and Foster 2002). Resting membrane potential was maintained between –57 and –84 mV with current injection. Voltage deflections resulting from hyperpolarizing current pulses (100 ms, 0.2 nA) were used to determine input resistance. Depolarizing current pulses (100 ms, 0.1–0.6 nA) were delivered every 20 s through the intracellular electrode to elicit a sodium spike bursts of five to six action potentials. The AHPs in the control and experimental conditions were elicited at the same membrane potential by manually clamping the potential with DC current injection (0.1–0.5 nA). The AHP amplitude was measured as the difference between the membrane potential during the 100-ms period, immediately before the onset of the depolarizing current and the membrane potential 500 ms after the offset of the depolarizing current. The amplitude and duration of the AHP were compared before and during drug administration in the same cell. For each cell, several (>10) consecutive AHPs were measured in each experimental condition, and the values were averaged for data analysis or statistical comparison. To record the NMDA receptor-mediated component of CA3–CA1 synaptic transmission, slices were incubated in low extracellular Mg^{2+} (0.5 mM), 6, 7-dinitroquinoxaline-2,3-dione (DNQX, 30 μM), and picrotoxin (10 μM).

Cyclopiazonic acid (CPA, Tocris, Ballwin, MO), Bay K8644, thapsigargin, and DNQX (Sigma, St. Louis, MO) were initially dissolved in a small amount of dimethyl sulfoxide (DMSO) and diluted by ACSF to a final DMSO concentration of 0.001% and a final concentration of CPA, Bay K8644, thapsigargin, and DNQX of 3, 1, 1, and 30 μM, respectively. The metabotropic receptor antagonist, (*s*)-α-methyl-4-carboxyphenylglycine (MCPG, 500 μM, Tocris, Ballwin, MO), was initially dissolved in a small amount of 1 M NaOH (150 μl for 50 mg of MCPG) and further diluted with ACSF; pH was adjusted to 7.42 ± 0.2. Picrotoxin (Sigma) was initially dissolved in a small amount of ethanol and further diluted in ACSF to reach final concentration of 10 μM. FK506 (CalBiochem, La Jolla, CA) was initially dissolved in DMSO and diluted in potassium acetate (3 M) to a final DMSO concentration of 0.001% and FK506 of 50 μM. Ryanodine and AP-5 (Sigma) were dissolved directly in ACSF. Suitable precautions were taken to ensure that ryanodine was protected from exposure to light.

For synaptic plasticity studies, the mean percent changes in the slope of the extracellular synaptic response were measured 55–60 min after 5-Hz stimulation for both control and tetanized pathways. ANOVA, repeated across the two pathways, determined effects of patterned stimulation and age. Post hoc analyses were conducted using Scheffe tests, with significance set at $P < 0.05$. Where stated, n represents the number of slices used in each set of experiment.

RESULTS

Inhibition of Ca^{2+} release from intracellular stores facilitates LTP induction in slices from old rats

An episode of pattern stimulation (5 Hz, 900 pulses) produced an initial short-lasting (~10 min) depression in slices from young ($n = 10$) and old ($n = 18$) rats, which returned to baseline over the next 60 min. The percent change for both age groups [young, 108.3 ± 3.9% (SE); old, 101.8 ± 2.4%] was not significantly different from their respective nontest pathway in either group, and the change in the synaptic response was not significantly different between young and old rats.

An age-dependent shift in susceptibility to LTP induction was observed following ICS depletion by thapsigargin. Pattern stimulation in presence of thapsigargin (1 μM) resulted in a significant increase in synaptic strength relative to the control (nontetanized) pathways [$F(1, 15) = 9.85$, $P < 0.01$]. Post hoc comparisons between responses in test and control pathways within each age group indicated that pattern stimulation in slices from old animals ($n = 9$) resulted in an enhancement of synaptic transmission at the 60-min time point (Fig. 1*A*). In contrast, pattern stimulation to slices from young rats treated with thapsigargin did not produce a significant change in synaptic strength ($n = 8$, Fig. 1*B*).

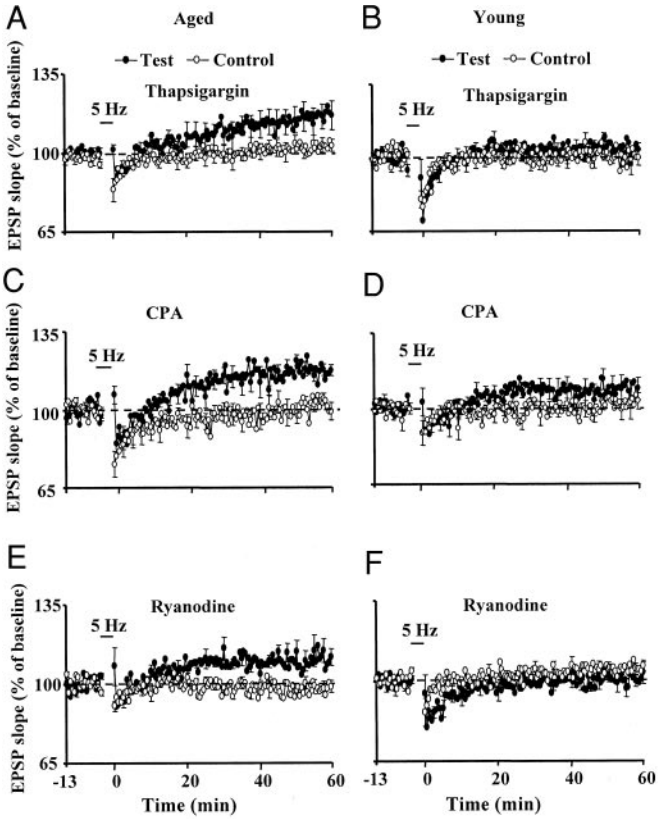

FIG. 1. Inhibition of intracellular Ca^{2+} stores by thapsigargin (1 μM) and cyclopiazonic acid (CPA; 3 μM) reveals an age difference in susceptibility to long-term potentiation (LTP) induction. Illustrations show mean percentage change in the synaptic response during 10 min before and 60 min after delivery of 5-Hz stimulation (5 Hz/3 min, solid horizontal line) to the control (○) and test (●) pathways. Thapsigargin (*A*) and CPA (*C*) facilitate LTP induction in slices from old animals. No effect of pattern stimulation was observed for young animals in the presence of thapsigargin (*B*) and CPA (*D*). Ryanodine (20 μM) facilitated LTP induction in slices from old (*E*) but not young rats (*F*). Error bars equal mean ± SE and alternate every 5 sweeps in this and subsequent figures.

The facilitation of LTP in old animals following depletion of ICS was confirmed with CPA. In the presence of CPA (3 μM), delivery of 5-Hz pattern stimulation resulted in a significant difference across the two pathways [$F(1, 12) = 18.90$, $P < 0.0001$]. Post hoc comparisons indicated that pattern stimulation to slices from old animals resulted in an enhancement of synaptic transmission ($n = 8$, Fig. 1*C*), relative to the control pathway. For young animals, pattern stimulation delivered in the presence of CPA produces only a modest increase (109 ± 6%, $n = 6$, Fig. 1*D*), which was not different from the control pathway. The LTP induced by pattern stimulation in CPA was NMDA receptor sensitive such that addition of the NMDA-receptor antagonist, AP-5 (100 μM), to slices from old rats blocked the induction of LTP (106 ± 5%, $n = 5$).

The specific source of Ca^{2+} from ICS that mediate age-related differences can be investigated by blocking Ca^{2+}-induced Ca^{2+} release from intracellular stores through blockade of ryanodine receptors or blockade of metabotropic receptor-induce inositol 1,4,5-trisphosphate (IP_3) formation, which mobilizes Ca^{2+} from intracellular stores in dendritic spines through IP_3 receptors (Takechi et al. 1998). In the presence of ryanodine (20 μM), 5-Hz pattern stimulation resulted in an age by pathway interaction [$F(1, 8) = 9.77$, $P < 0.05$], and post hoc comparisons indicated that old animals exhibited significant growth in the response of the test pathway relative to the control pathway (Fig. 1*E*). However, pattern stimulation in the presence of ryanodine did not alter the synaptic response in slices from young rats ($n = 5$, Fig. 1*F*). The LTP induced by pattern stimulation in presence of ryanodine was NMDA receptor sensitive such that addition of the NMDA receptor antagonist, AP-5 (100 μM), to slices from old rats blocked the induction of LTP (105 ± 3%, $n = 7$). Finally, LTP induction was not observed in slices of old animals for 5-Hz pattern stimulation in the presence of the metabotropic receptor antagonist, MCPG (500 μM; 108 ± 4%, $n = 7$).

Mechanisms for facilitation of LTP following inhibition of intracellular stores: reduced AHP and increased NMDA receptor function

Previous work suggests that the LTP threshold for older animals may be regulated by the amplitude of the Ca^{2+}-dependent AHP (Kumar and Foster 2002; Norris et al. 1998a). To determine whether a similar mechanism might contribute to facilitation of synaptic enhancement following blockade of ICS, the Ca^{2+}-dependent K^+-mediated AHP was examined. A total of 33 cells from old animals and 11 cells from young animals were acceptable according to our criteria. The input resistance, resting membrane potential, and spike amplitude were not different between age groups, and no significant differences in the intrinsic membrane properties (input resistance, resting membrane potential, and spike amplitude) were observed across the various treatment conditions (Table 1). Nevertheless, in confirmation of previous reports (Disterhoft et al. 1996; Kumar and Foster 2002; Landfield and Pitler 1984; Power et al. 2002), the AHP was significantly increased [$F(1, 23) = 13.67$, $P < 0.001$] in old (6.91 ± 0.39 mV, $n = 19$), relative to young, animals (3.79 ± 0.85 mV, $n = 6$). Figure 2 shows that application of CPA (3 μM) or ryanodine (20 μM) resulted in a rapid (5–10 min) reduction in the AHP in cells from old animals. Compared with control conditions, AHP amplitude decreased 44.5 ± 5.4% and 40.2 ± 4.5% for cells exposed to CPA ($n = 5$) or ryanodine ($n = 5$), respectively, and was significantly different ($P < 0.0001$) from the control condition. The CPA-mediated reduction in the AHP in young animals was highly variable and considerably less than that for old animals (15.57 ± 15.7%, $n = 3$). Finally, the reduction in the AHP was specific to ryanodine receptors, since blockade of

TABLE 1. *Biophysical properties of CA1 neurons recorded from old male F 344 rats*

	IR, MΩ	RMP	Spike Amplitude
Control (19)	62.3 ± 8.45	−64 ± 1.48	79.7 ± 0.38
DMSO (4)	51.3 ± 9.84	−62 ± 2.63	80.0 ± 1.16
CPA (5)	49.4 ± 10.5	−65 ± 0.58	80.0 ± 0.89
Ryanodine (5)	60.6 ± 9.76	−65 ± 2.76	80.4 ± 0.75
CPA + BK (3)	55.0 ± 15.4	−62 ± 2.19	82.7 ± 1.76
MCPG (3)	48.33 ± 3.84	−63 ± 0.58	82.0 ± 0.0

Values are mean ± SE. RMP, resting membrane potential; IR, input resistance; CPA, Cylopiazonic acid; BK, Bay K8644; MCPG, (s)- -methyl-4-carboxyphenylglycine. RMP and spike amplitude values are in mV. Numbers in parentheses indicate number of cells for each condition. In some cases, the same cell was recorded under control conditions and following treatment.

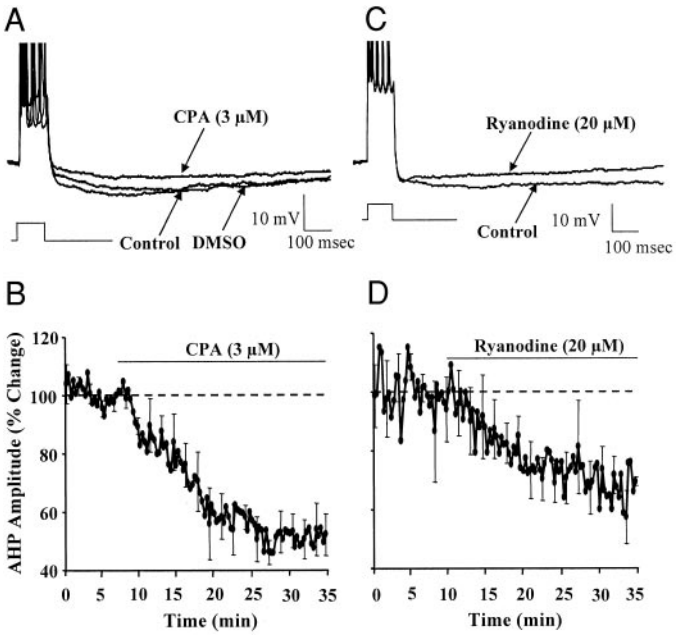

FIG. 2. Inhibition of ICS by CPA reduces the afterhyperpolarization (AHP) in old animals. *A*: representative intracellular voltage records of a single CA1 pyramidal neuron from an old rat are shown after a train of 5 action potentials elicited by a 100-ms pulse of depolarizing current and recorded prior to CPA application (control), in presence of DMSO, and following application of CPA (3 μM). The cell was held at −67 mV. Note that in this and subsequent figures, the action potentials are truncated to better show the AHPs. *B*: time course in reduction of AHP amplitude following CPA application. Data points represent the means of 4 cells recorded from old rats. For each cell, depolarization was set to elicit 5 spikes, and the amplitude of the AHPs collected during the 7 min prior to CPA application was used as a baseline for calculating the percent change over time. In general, cells exhibited a reduction in the AHP compared with baseline (dashed line) within the 1st 5–10 min following CPA application (solid line), and stable AHPs were recorded ~30 min after CPA application. Ryanodine reduced the amplitude of the AHPs recorded from old rats. *C*: representative intracellular records from a CA1 pyramidal neuron of an old rat. The AHP was elicited by a train of 5 action potentials and recorded prior to (control) and following application of ryanodine (20 μM). The cell was held at −69 mV. *D*: time course of AHP reduction relative to baseline (dashed line) following ryanodine application ($n = 5$). In general, cells exhibited a reduction in the AHP within the 1st 10–15 min following ryanodine application (solid line), before stabilizing ~30 min after ryanodine application.

metabotropic glutamate receptors (mGluRs) by application of MCPG (500 μM) resulted in a slight increase in the AHP amplitude in old rats (4.23 ± 2.27%, $n = 3$). Similarly, application of drug vehicle, DMSO ($n = 4$), or NaOH ($n = 2$) had no effect on the AHP amplitude.

The results are consistent with the idea that the ICS influence on the threshold for synaptic plasticity in old animals is mediated by changes in the AHP. To further test this idea, slices from old rats were bathed in medium containing CPA (3 μM) and the L-channel agonist, Bay K8644 (1 μM). Figure 3*A* shows that the AHP is increased [$F(5, 33) = 8.97$, $P < 0.0001$] as the L-channel agonist is added to the bath containing CPA. Indeed, Bay K8644 approximately doubled the amplitude of the AHP (7.05 ± 0.33 mV, $n = 3$) compared with CPA alone (3. 67 ± 0.38 mV, $n = 5$). For seven of eight slices recorded extracellularly, delivery of 5-Hz pattern stimulation in the presence of CPA and Bay K8644 blocked the induction of LTP (105.1 ± 1.3%, $n = 7$) such that the response of the tetanized pathway was not different from the control (nontetanized) pathway (Fig. 3*B*). In one slice, pattern stimulation in CPA + Bay K8644 was associated with a rather large (141%) increase in the synaptic response.

Figure 4 summarizes the results of manipulations of ICS by different drugs on the AHP and the induction of synaptic plasticity following 5-Hz pattern stimulation in slices obtained from old rats. The results suggest that a rise in intracellular Ca^{2+} from ryanodine receptor activation contributes to the AHP in old animals. Moreover, blockade of Ca^{2+}-induced Ca^{2+} release from ICS reduces the AHP and facilitates LTP in old animals.

In another set of experiments, CA3–CA1 synaptic responses were recorded intracellularly, and the NMDA receptor component was isolated by bathing the slices in ACSF, which contained $MgSo_4$ (0.5 mM), DNQX (30 μM), and picrotoxin (10 μM). After stabilization of the initial EPSP, ryanodine (20 μM) was applied to recording medium. Figure 5 shows that inhibition of Ca^{2+}-induced Ca^{2+} release from ryanodine-sensitive ICS enhanced the intracellular NMDA receptor-mediated synaptic response in slices from old rats (121.09 ± 11.5%, $n = 5$). When intracellular recording electrodes were filled with FK506 (50 μM), application of ryanodine resulted in a modest decrease in the synaptic response ($n = 3$) in old animals, which was significantly different [$F(1, 6) = 8.52$, $P < 0.03$] compared with responses obtained in the presence of ryanodine alone. Furthermore, the ryanodine-mediated enhancement of the NMDA receptor response was not observed in slices from young rats (98.47 ± 13.4, $n = 5$). Successive application of AP-5 (100 μM) following ryanodine eliminated the EPSP response in old ($n = 7$) and young ($n = 4$) rats, indicating successful isolation of the NMDA receptor component of synaptic transmission (Fig. 5*C*).

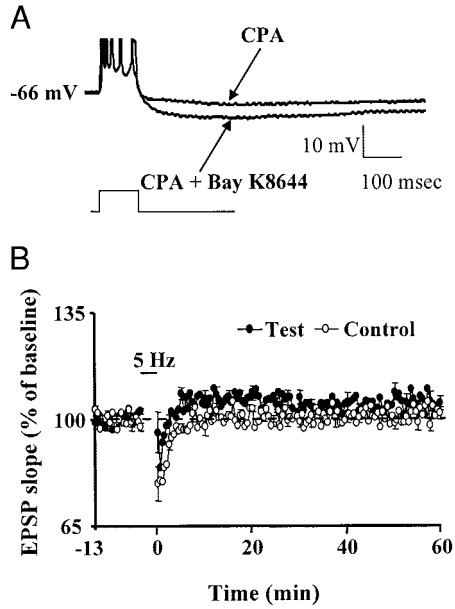

FIG. 3. Enhancement of AHP is associated with impaired LTP induction. *A*: representative intracellular current clamp records from a CA1 pyramidal neuron in an old rat are shown after a train of 5 action potentials elicited by a 100-ms pulse of depolarizing current under conditions of CPA (3 μM) and following bath application of Bay K8644 (1 μM) in the presence of CPA. Note that under conditions of ICS blockade, the L-channel agonist increased the amplitude of Ca^{2+}-dependent AHP. *B*: time course of the synaptic response following 5-Hz pattern stimulation for old rats ($n = 7$) in presence of Bay K8644 + CPA. LTP induced by 5-Hz stimulation was blocked under conditions of Bay K8644 + CPA in which AHP amplitude was increased.

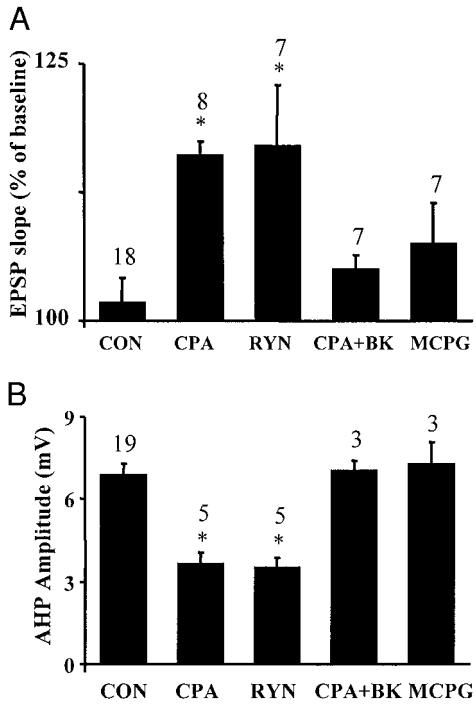

FIG. 4. Bar diagram representing (*A*) mean percent change in the synaptic response following 5 Hz stimulation and (*B*) AHP amplitude (mV) for slices from old rats. Slices were bathed in CPA, ryanodine (RYN), CPA + Bay K8644 (CPA + BK), or MCPG. *Significance difference from control (CON) slices. Number above each bar indicates number of slices recorded in each group.

DISCUSSION

This study revealed three important features relating to the interaction of ICS and synaptic plasticity during aging. First, NMDA receptor-dependent LTP induction, by 5-Hz stimulation, is induced when Ca^{2+}-mediated Ca^{2+} release from intracellular stores is blocked. An ICS-independent LTP is contrary to the finding of LTP inhibition observed in younger animals during ICS blockade and points to a change in the role of this Ca^{2+} source in regulating synaptic plasticity from development to maturation and aging. Second, the mechanism for ICS inhibition of LTP involves a reduction in cell excitability since inhibition of ICS decreased the AHP specifically in old animals. Moreover, the increased susceptibility to LTP induction following ICS blockade can be reversed by increasing the AHP through enhanced VDCCs activation. Together, these results suggest that during aging, ICS and VDCCs act to enhance the AHP and impair LTP, and the shift in these Ca^{2+}-dependent processes is expected to have important implications for the transmission of information through the hippocampus. Finally, inhibition of Ca^{2+} release from ICS was associated with an increase in synaptic NMDA responses. The findings underscore the importance of understanding the interaction of different Ca^{2+} regulatory mechanisms in influencing Ca^{2+}-dependent processes that are thought to underlie age-related cognitive changes.

In contrast to a number of studies that indicate an age-related impairment of LTP induction (Foster 1999), this study demonstrates an age-related facilitation of LTP, which was revealed when release of Ca^{2+} from ICS was pharmacologically blocked through depletion of ICS (thapsigargin, CPA) or blockade of Ca^{2+}-induced Ca^{2+} release (ryanodine). The findings are contrary to those observed for studies examining LTP in young animals, which indicate a contribution of ICS, including ryanodine receptor-gated calcium pools, in the induction of LTP for weak stimulation (Behnisch and Reymann 1995; Harvey and Collingridge 1992; Raymond and Redman 2002; Reyes and Stanton 1996; Wang et al. 1996). One model suggests that, at least for juveniles, Ca^{2+} influx through NMDA receptors and VDCCs, in concert with mGluR-IP_3–mediated Ca^{2+} release from internal stores, is able to access Ca^{2+}-induced Ca^{2+} release at synapses of neonatal animals (Nishiyama et al. 2000). The results of this study indicate that the role of Ca^{2+}-induced Ca^{2+} release in regulating the level of Ca^{2+} at the postsynaptic membrane may be minimized with advanced age. A reduction in the contribution of ICS to the rise in Ca^{2+} in the dendritic spine could result from an age-related decline in NMDA receptor function (Clark et al. 1992; Nicolle

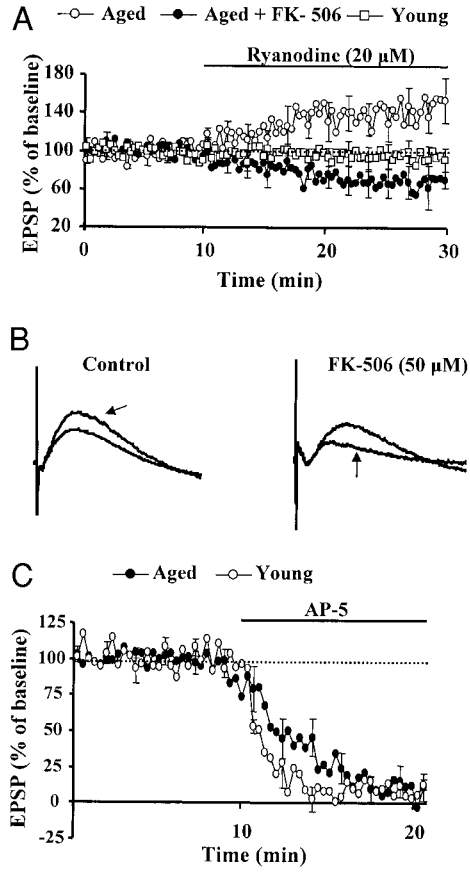

FIG. 5. ICS regulate *N*-methyl-D-aspartate (NMDA) receptor function. *A*: mean percentage change in the amplitude of the synaptic NMDA response recorded intracellularly during 10 min before and 20 min after bath application of ryanodine (20 μM, solid horizontal line). Ryanodine induced an enhancement in synaptic strength in slices obtained from old rats (○, $n = 5$). The enhancement of the synaptic response by ryanodine was not observed in young animals (□, $n = 7$) and was blocked in old animals by including FK-506 (50 μM, ●, $n = 3$) in the recording pipette. *B*: representative excitatory postsynaptic potentials (EPSPs) recorded intracellularly from individual pyramidal neurons before and after application of ryanodine (arrows). Under control conditions (Control), application of ryanodine results in an increase in the EPSP, which is not observed when FK506 is included in the recording pipette (FK506). *C*: subsequent application of AP-5 (100 μM) dramatically reduced the EPSP in old ($n = 7$) and young ($n = 4$) rats, demonstrating successful isolation of the NMDA receptor component of synaptic transmission.

et al. 1996; Wenk and Barnes 2000). Indeed, controversy concerning whether Ca^{2+} influx through NMDA receptors initiates Ca^{2+}-induced Ca^{2+} release for ICS may be due to the experimental variables including developmental state (Emptage et al. 1999; Kovalchuk et al. 2000; Mainen et al. 1999). In addition, changes in the proximity of the endoplasmic reticulum relative to the spine could limit ICS as a Ca^{2+} source during synaptic activation (Nimchinsky et al. 2002; Spacek and Harris 1997; Yuste et al. 1999).

Under similar recording conditions as employed in this study, a slow onset LTP, induced by stimulation patterns near the threshold for LTP (i.e., 900 pulses at 5 Hz) can be observed in old animals following blockade of L-channels (Norris et al. 1998a). It seems counterintuitive that blockade of Ca^{2+} sources, could facilitate LTP, a process that depends on a substantial rise in intracellular Ca^{2+}. One possibility is that the age-related difference in LTP induction following application of Ca^{2+} pump inhibitors may have been due to reduced rate of Ca^{2+} clearance as a result of blockade of Ca^{2+} pumps (Kovalchuk et al. 2000; Markram et al. 1995) combined with increased Ca^{2+} entry through L-channels and impaired Ca^{2+} buffering in old animals (Thibault et al. 1998). However, this does not seem likely since ryanodine, which blocks release rather than the Ca^{2+} pump, also facilitated LTP. Furthermore, the LTP was specific to the pathway that received 5-Hz stimulation and was sensitive to NMDA receptor blockade, suggesting that it was not generalized as would be expected from increased VDCC activation and impaired buffering. Moreover, increasing L-channel activity via the L-channel agonist, Bay K8644, blocked the facilitation of LTP by CPA, demonstrating that increased influx through L-channel did not mediate the induction of LTP.

Voltage-dependent ion channels can control the induction of synaptic plasticity by regulating dendritic excitability (Johnston et al. 2003; Magee and Johnston 1997). The fact that LTP induction was readily modified by manipulations that influence the AHP indicates that regulation of the LTP threshold in old animals is closely coupled to changes in postsynaptic excitability involving Ca^{2+}-dependent K^+ channel activity. The shape of the AHP is influenced by release of Ca^{2+} from ICS (Borde et al. 2000), and this source of Ca^{2+} may contribute to the enhancement of the AHP during senescence (Kumar and Foster 2002; Landfield and Pitler 1984; Power et al. 2002). The results are consistent with the notion that augmentation of the hyperpolarization in old animals limits synaptic integration and depolarization required for NMDA receptor activation and LTP induction (Behnisch and Reymann 1998; Cohen et al. 1999; Foster and Kumar 2002; Foster and Norris 1997; Norris et al. 1998a; Sah and Bekkers 1996). The results suggest that during aging, ICS and VDCCs inhibit LTP induction by increasing the AHP and the level of stimulation required for LTP-induction. In this regard, the decreased excitability imposed by the larger Ca^{2+}-dependent AHP may represent compensatory mechanisms restricting Ca^{2+} influx through NMDA receptors, disallowing enhanced LTP induction, and preventing the accumulation of toxic Ca^{2+} levels in the dendrite.

In this study, inhibition of ICS increased NMDA receptor responses specifically in old animals, and enhancement of the synaptic NMDA receptor response was blocked by including FK506 in the recording pipette. FK506 can influence several cellular processes including inhibiting peptidylprolyl isomerase activity and disrupting Ca^{2+} channels to increase release of Ca^{2+} from ICS (Brillantes et al. 1994). It is unclear how enhanced peptidylprolyl isomerase activity or decreased release for ICS could mediate differences in NMDA receptor function during aging, unless possibly through the regulation of the balance of Ca^{2+}-dependent phosphatase/kinase activity. Indeed, several lines of evidence suggest that the effects are mediated by FK506 effects on the balance of kinase/phosphatase activity possibly involving the inhibition of the Ca^{2+}-dependent phosphatase, calcineurin. First, activation of calcineurin decreases NMDA receptor function in younger animals (Lieberman and Mody 1994; Tong et al. 1995). Second, an increase in phosphatase activity is thought to act on glutamate receptors to reduce synaptic strength in aged animals (Foster et al. 2001; Norris et al. 1998b). While phosphatase activity was not measured in this study, previous work indicates that NMDA receptor function is regulated through ICS by means of calcineurin activation (Tong and Jahr 1994). Moreover, the balance of kinase/phosphatase activity adjusts dendritic excitability (Migliore et al. 1999; Schrader et al. 2002), and augmented calcineurin activity of old animals may underlie increased L-channel activity (Norris et al. 2002). Accordingly, elevated phosphatase activity during aging could explain the hypothesized shift in Ca^{2+} sources, increasing and decreasing the L-channel and NMDA receptor contribution, respectively (Foster 1999). Indeed, the age-associated enhancement of the AHP and decreased NMDA receptor activation are thought to underlie changes in the frequency-response function for synaptic plasticity, resulting in a pronounced plateau region for intermediate stimulation frequencies near the threshold for LTP induction (Foster 1999; Foster and Norris 1997). Together, the results are consistent with the notion that ICS contribute to altered Ca^{2+} regulation, leading to a shift in the balance of phosphatase/kinase activity and decreased NMDA receptor function during aging. As such, 5-Hz stimulation during ICS inhibition may have normalized or stabilized kinase/phosphatase activity, permitting the growth of synaptic strength selectively in old animals. While whole cell patch-clamp studies of synaptic function are extremely difficult in old brains, such studies are needed to confirm the findings and may provide more accurate measures of ICS regulation of NMDA receptor function.

A change in ionotropic glutamate receptor or mGluR function is likely to contribute to altered Ca^{2+} regulation and a shift in synaptic plasticity with age (Carlson et al. 2000; Foster and Kumar 2002; Hof et al. 2002; Pagliusi et al. 1994; Rosenzweig and Barnes 2003). While the results of studies using MCPG do not exclude a role for IP_3 receptors in altered synaptic plasticity with age, the inability of MCPG to facilitate LTP indicates that the mGluR-IP_3 pathway does not participate in inhibiting LTP under our experimental conditions. Other researchers have noted developmental changes in the mGluR-IP_3 pathway such that activation of this pathway can reduced cell excitability, decrease LTP and promote depression of synaptic transmission in neonates (Nishiyama et al. 2000), while in juvenile or young adult animals, the LTP threshold is reduced by activation of mGluRs (Brown et al. 2000; Cohen et al. 1999; Matias et al. 2002; Thomas et al. 1996). Under conditions in which mGluR activation facilitates LTP induction, an increased cell excitability involving a reduction in the AHP and increased current through NMDA receptors is observed as well (Cohen et al.

1999; Madison and Nicoll 1986; Pisani et al. 1997; Thomas et al. 1998). In contrast, this study indicates that, under our experimental conditions, ICS have only a modest influence on the AHP in young animals. It is possible that the effect of ICS on the AHP and LTP may be more relevant under conditions in which the AHP is enhanced, such as increasing the level of Ca^{2+} in the medium (Kumar and Foster 2002; Norris et al. 1996) or increasing L-channel function (Thibault et al. 2001). Regardless, the results emphasize the importance of changes in the role of ICS for Ca^{2+} dysregulations during aging.

In considering the implications of ICS in Ca^{2+} homeostasis, other researchers have noted that overfilling of ICS may represent the fundamental cellular defect linking altered Ca^{2+} signaling to pathogenesis of age-related neurodegenerative diseases (Leissring et al. 2000). For neurons of the basal forebrain, increased intracellular buffering can act as a compensatory mechanism limiting ICS filling in old animals (Murchison and Griffith 1999). ICS of hippocampal neurons may be more susceptible to overfilling since most studies suggest that Ca^{2+} buffering is impaired in the hippocampus (Thibault et al. 1998). Furthermore, the increased in VDCC activity in old hippocampal neurons could increase ICS filling (Garaschuk et al. 1997). Moreover, release of Ca^{2+} through ryanodine receptor activation can contribute to enhanced L-channel activity in old animals (Thibault and Landfield 1996; Chavis et al. 1996) suggesting a positive feedback mechanism. It will be important for future studies to determine whether alterations in ICS include a shift in the location, sink-source activity, or filling of ICS and whether these changes contribute to altered Ca^{2+} homeostasis or provide compensation for Ca^{2+} dysregulation to limit runaway synaptic plasticity and Ca^{2+}-mediated toxicity.

GRANTS

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