Nimodipine Increases Excitability of Rabbit CA1 Pyramidal Neurons in an Age- and Concentration-Dependent Manner

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SUMMARY AND CONCLUSIONS

1. Cellular properties were studied before and after bath application of the dihydropyridine L-type calcium channel antagonist nimodipine in aging and young rabbit hippocampal CA1 pyramidal cells in vitro. Various concentrations of nimodipine, ranging from 10 nM to 10 μM, were tested to investigate age- and concentration-dependent effects on cellular excitability. Drug studies were performed on a population of neurons at similar holding potentials to equate voltage-dependent effects. The properties studied under current-clamp conditions included steady-state current-voltage relations (*I-V*), the amplitude and integrated area of the postburst afterhyperpolarization (AHP), accommodation to a prolonged depolarizing current pulse (spike frequency adaptation), and single action-potential waveform characteristics following synaptic activation.

2. Numerous aging-related differences in cellular properties were noted. Aging hippocampal CA1 neurons exhibited significantly larger postburst AHPs (both the amplitude and the integrated area were enhanced). Aging CA1 neurons also exhibited more hyperpolarized resting membrane potentials with a concomitant decrease in input resistance. When cells were grouped to equate resting potentials, no differences in input resistance were noted, but the AHPs were still significantly larger in aging neurons. Aging CA1 neurons also fired fewer action potentials during a prolonged depolarizing current injection than young CA1 neurons.

3. Nimodipine decreased both the peak amplitude and the integrated area of the AHP in an age- and concentration-dependent manner. At concentrations as low as 100 nM, nimodipine significantly reduced the AHP in aging CA1 neurons. In young CA1 neurons, nimodipine decreased the AHP only at 10 μM. No effects on input resistance or action-potential characteristics were seen.

4. Nimodipine increased excitability in an age- and concentration-dependent manner by decreasing spike frequency accommodation (increasing the number of action potentials during prolonged depolarizing current injection). In aging CA1 neurons, this effect was significant at concentrations as low as 10 nM. In young CA1 neurons, nimodipine decreased accommodation only at higher concentrations ($\geq$1.0 μM).

5. We conclude that aging CA1 neurons were less excitable than young neurons. In aging hippocampus, nimodipine restores excitability, as measured by size of the AHP and degree of accommodation, to levels closely resembling those of young adult CA1 neurons. These actions of nimodipine on aging CA1 hippocampal neurons may partly underlie the drug's notable ability to improve associative learning in aging rabbits and other mammals.

INTRODUCTION

Nimodipine is a 1,4-dihydropyridine L-type calcium channel antagonist that crosses the blood-brain barrier more readily than many dihydropyridines (van den Kerckhoff and Drewes 1989). Nimodipine is of particular interest because it improves learning in different tasks in aging subjects including rats, rabbits and nonhuman primates (Deyo et al. 1989; Sandin et al. 1990; Schuurman and Traber 1989; Scriabine et al. 1989; Straube et al. 1990). The mechanism of this enhancement is unresolved, but recent studies suggest that nimodipine has direct actions on the CNS that may contribute to the drug's ability to improve learning. For example, in awake aging rabbits, intravenous application of nimodipine causes an age- and dose-dependent increase in the spontaneous firing rates of hippocampal CA1 pyramidal neurons (Thompson et al. 1990). Increased hippocampal pyramidal cell excitability also occurs during associative eye-blink conditioning in rabbits. As a rabbit acquires the conditioned response, significant increases in hippocampal firing rates occur in vivo that temporally model the behavioral response (Akase et al. 1988; Berger et al. 1983).

Increased hippocampal pyramidal cell excitability has also been correlated with learning using in vitro recording techniques. One measurement of in vitro excitability is the postburst afterhyperpolarization (AHP). The postburst AHP is largely mediated by a Ca^{2+}-activated K^+ current that clamps the cell membrane at a more hyperpolarized potential (Hotson and Prince 1980; Lancaster and Adams 1986). Conditioning-specific post-burst AHP reductions were observed in CA1 neurons in hippocampal slices from delay (Disterhoft et al. 1986; Sanchez-Andres and Alkon 1991) and trace (de Jonge et al. 1990) eye-blink conditioned but not control rabbits. The conditioning-specific AHP reductions observed in vitro may partly underlie the increased hippocampal excitability observed in vivo during and after eye-blink conditioning in rabbits (Disterhoft et al. 1988).

Hippocampal CA1 neurons of aging rats have longer lasting Ca^{2+}-activated AHPs in vitro (Landfield and Pitler 1984; Pitler and Landfield 1990) and lower spontaneous firing rates in vivo (Lippa et al. 1981) than young CA1 neurons. These data suggest a correlation between hippocampal excitability, learning, and aging-related learning deficits (Disterhoft et al. 1989; Landfield and Pitler 1984). Aging animals whose hippocampal neurons are less excitable may have marked difficulty learning tasks mediated by hippocampal circuitry. We wished to test whether the learning enhancement and the increased hippocampal excitability observed in vivo after nimodipine administration to aging rabbits may be related to nimodipine's direct actions on
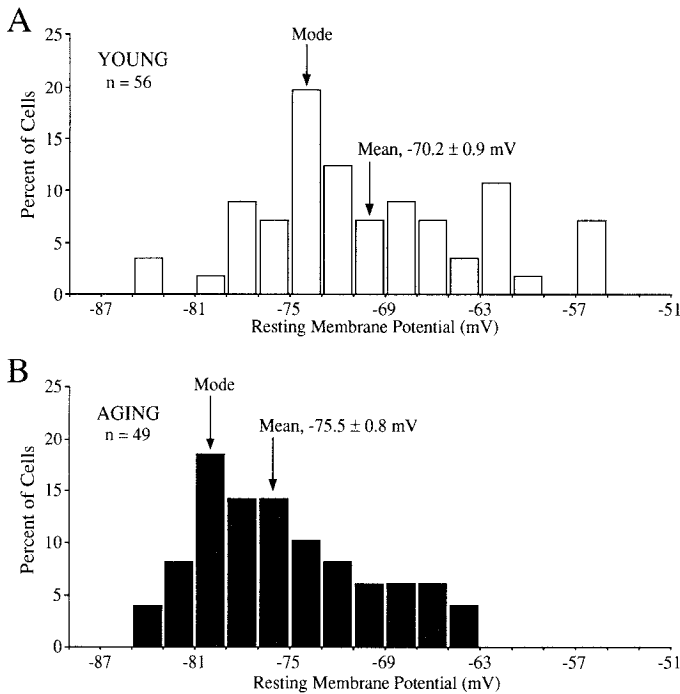
FIG. 1. Aging rabbit CA1 neurons tended to rest at a more hyperpolarized membrane potential than young neurons. *A*: distribution of membrane potentials for young CA1 neurons. Each bar represents the percentage of cells that rested within consecutive 2-mV bins. *B*: distribution of membrane potentials for aging CA1 neurons. Aging neurons had a significantly more hyperpolarized resting membrane potential [$F(1,1) = 19.578$, $P < 0.001$].

hippocampal CA1 pyramidal cells. Therefore we examined several measures of excitability in aging and young rabbit CA1 neurons in vitro and evaluated the effects of nimodipine on these measurements. The preliminary reports of these data have been presented in abstract form (Moyer et al. 1990a, 1991).

METHODS

Slice preparation

Slices were prepared from young adult (3.6 ± 0.3 mo, mean ± SE) and aging (39.3 ± 1.6 mo) New Zealand albino rabbits (*Oryctolagus cuniculus*). Rabbits were killed by decapitation. The brain was exposed and quickly hemisected in vivo, removed (within 45 s) one hemisphere at a time, and placed in ice-cold, oxygenated artificial cerebrospinal fluid (ACSF, composition in mM: 124 NaCl; 3 KCl; 1.3 $MgSO_4$; 1.24 NaH_2PO_4; 2.4 $CaCl_2$; 26 $NaHCO_3$; 10 D-glucose; gassed with 95% O_2-5% CO_2 at pH 7.4) for ~1 min.

The hippocampus from each hemisphere was dissected out over ice, and 4-mm-thick blocks were cut from the dorsal hippocampus. Tissue blocks were glued to a small, chilled chamber, which was then filled with ice-cold oxygenated ACSF. Slices were cut 400 μm thick with the use of a vibratome. Slices were transferred to a holding chamber filled with oxygenated ACSF at room temperature for at least 45 min. The holding chamber was a 100-ml beaker, fitted with a nylon net on which the slices rested, covered with a lid containing a small hole through which a fritted glass bubbler entered for oxygenation. Under these conditions, slices typically remained viable for 10–14 h without displaying epileptiform burst activity or unusual resting membrane potentials. For recording, slices were transferred to a submersion chamber (Medical Systems) and continuously perfused (~1.75 ml/min) with oxygenated ACSF at 31°C. Stock solutions of nimodipine and nifedipine were prepared by dissolving the drugs in 100% ethanol, which were then diluted with ACSF to the desired concentration. The final concentration of ethanol in the bathing medium never exceeded 0.01% (vol/vol). All experiments and dihydropyridine solution preparations were conducted in near darkness.

Electrophysiological recordings

Recordings were made from 89 young and 68 aging CA1 pyramidal cells in slices taken from 34 young and 25 aging rabbits with the use of a Dagan 8100-1 amplifier in current-clamp mode. Fourteen young neurons were recorded from at room temperature and were not included in the present study. Thin-walled glass omega-dot microelectrodes filled with 3 M KCl (20–80 MΩ) were used for recording. Bipolar tungsten stimulating electrodes were placed in the alveus (or fimbria/fornix) and Schaffer collaterals under visual guidance. A CA1 neuron was classified as a pyramidal cell if it exhibited accommodation to a prolonged (800-ms) depolarizing current injection and an action-potential duration >1.2 ms from rise threshold to recrossing of the resting potential. Antidromic activation from the alveus or fimbria/fornix region was attempted in most cells and was successful in >90% of the cells. Cells were included in the study if they exhibited little spontaneous activity at rest, had an action-potential amplitude of at least 70 mV, had an input resistance ≥20 MΩ, and had a stable resting membrane potential of at least −55 mV. Cells that were stable for at least 5 min were studied with the use of the following protocol.

1) Current-voltage (*I-V*) measurements were taken by measuring the plateau voltage deflections during the last 150 ms of a 400-ms current pulse (range, −1.0 to +0.2 nA). Input resistance was calculated from the slope of the lines fitted to both the hyperpolarizing and depolarizing *I-V* relationships.

2) AHP measurements were made after a burst of action potentials elicited by a 100-ms depolarizing pulse, with current adjusted to a minimal level that reliably evoked a burst of four action potentials. The peak AHP amplitude was measured from the baseline membrane potential, and the integrated area of the AHP (expressed in mVms) was calculated from the time of current offset for the next 800 ms.

TABLE 1. *Electrophysiological properties of CA1 neurons*

		Input Resistance		Afterhyperpolarization		Orthodromic AP		Antidromic AP	
	RMP, mV	IR Hyp, MΩ	IR Dep, MΩ	Amp, mV	Area, mVms	Amp, mV	Duration, ms	Amp, mV	Duration, ms
Aging	−55 to −85	32.4 ± 1.3 (49)*	38.9 ± 1.9 (47)*	−4.23 ± 0.13 (49)†	1,820 ± 93 (49)†	97.5 ± 1.9 (26)	1.2 ± 0.1 (26)	93.5 ± 1.5 (40)	1.0 ± 0.1 (40)
Young		37.0 ± 1.4 (56)	44.9 ± 2.1 (49)	−3.47 ± 0.11 (56)	1,346 ± 67 (56)	96.1 ± 2.0 (27)	1.1 ± 0.1 (27)	92.5 ± 1.3 (40)	1.1 ± 0.1 (40)
Aging	−65 to −75	29.8 ± 4.7 (19)	40.3 ± 4.4 (18)	−5.24 ± 0.19 (20)†	2,570 ± 130 (20)†	95.4 ± 2.2 (12)	1.2 ± 0.1 (12)	91.3 ± 1.8 (16)	1.0 ± 0.1 (16)
Young		35.4 ± 2.0 (34)	46.4 ± 3.2 (27)	−2.97 ± 0.10 (33)	1,076 ± 59 (33)	99.9 ± 2.3 (14)	1.1 ± 0.1 (14)	94.5 ± 1.3 (24)	1.1 ± 0.1 (24)

Values represent the means ± SE for the particular measurement; number of neurons are in parentheses. RMP, resting membrane potential; AP, action potential; IR, input resistance; Hyp, hyperpolarizing current pulses; Dep, depolarizing current pulses; Amp, amplitude. Symbols indicate significant difference when compared with young neurons within a given RMP range: *$P < 0.05$, †$P < 0.001$.
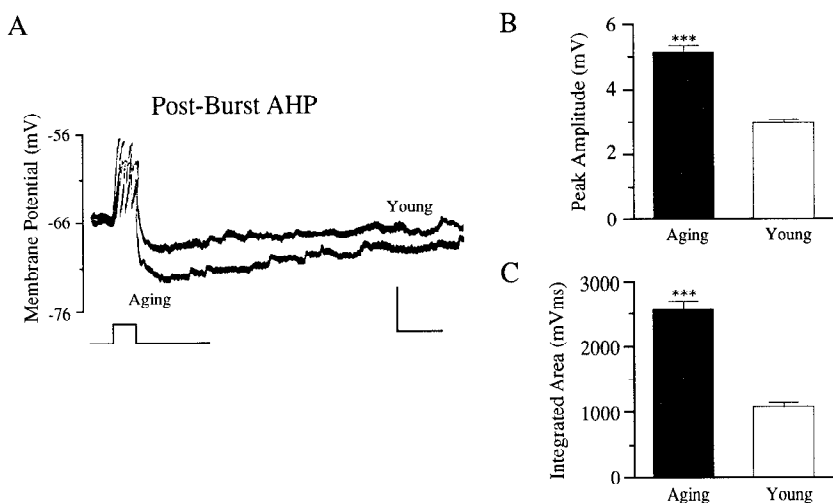
A

Post-Burst AHP

B

C

FIG. 2. Aging rabbit CA1 neurons exhibited larger postburst afterhyperpolarizations (AHPs) than young neurons. An overlay from aging and young CA1 neurons with nearly identical resting membrane potentials indicates the dramatic difference in their AHPs (A). Data from 20 aging and 34 young CA1 neurons at similar membrane potentials were compared. Both the peak amplitude (B) and integrated area (C) of the AHP were significantly enhanced in aging neurons. Significant age effects on amplitude [$F(1,4) - 124.680, P < 0.001$] and on area [$F(1,4) = 138.854, P < .001$] were observed. Scale: 5 mV, 1 nA, and 200 ms (A). ***$P < 0.001$.

3) Accommodation was studied with the use of an 800-ms duration depolarizing current injection of the same current intensity used to study the AHP. The number of action potentials elicited was recorded.

4) Orthodromic and antidromic action potentials were studied separately. Action-potential amplitude was measured from the baseline, and spike width was measured at one-third the maximum amplitude.

5) Nimodipine was bath applied, and spontaneous activity was monitored for 20 min. Then steps 1 through 4 of the experimental protocol were repeated. In one set of experiments, nifedipine, another dihydropyridine calcium channel antagonist, was substituted for nimodipine. In control experiments, ACSF containing ethanol vehicle (0.01%, vol/vol) alone was perfused onto the slices. This vehicle concentration was selected because it was the maximum concentration used during drug perfusions.

6) Resting membrane potential (RMP) was determined as the difference in potential before and after withdrawing the electrode from the cell. Data from cells lost during drug treatment were used only if a reliable membrane potential was obtained. Data from only one stable cell were recorded from a given slice. The slice was changed if a cell was lost during an experiment, or on completion of an experiment.

All data were digitally recorded and controlled on-line by the use of a program run on an LSI 11/23 computer system. Analog-to-digital sampling rates were 1 kHz (for *I-V*, AHP, and accommodation measurements), 20 kHz (for orthodromic and antidromic action-potential measurements), or 100 Hz (for membrane-potential measurements). Data were also stored on videocassette with the use of a digital data recorder (Instrutech) for backup and future playback when necessary. Analysis of results was performed off-line with the use of a Macintosh IIci and custom software developed within our laboratory. Statistical analyses were performed with the use of analyses of variance for repeated measures (Super-ANOVA, Abacus Concepts, Berkeley, CA) to evaluate age and drug effects. Significant main effects were evaluated with the use of Tukey-Kramer post hoc tests. All data are reported as the mean ± the standard error of the mean (mean ± SE).

RESULTS

Aging-related changes in the electrophysiological properties of rabbit CA1 pyramidal neurons

Aging CA1 neurons exhibited altered biophysical properties when compared with young neurons. The RMPs of aging and young cells showed different distributions (see

Fig. 1). Aging CA1 neurons had a slightly more hyperpolarized RMP than young neurons, with a greater percentage of neurons resting between −75 and −81 mV (Fig. 1B), whereas a greater percentage of young neurons rested between −65 and −77 mV (Fig. 1A). Comparisons made between aging ($n = 17$) and young ($n = 21$) neurons that were lost and not included revealed that these aging neurons were also more hyperpolarized (mean RMPs: aging, −72.8 ± 1.6 mV, mean ± SE; young, −68.6 ± 1.7 mV), suggesting that the observed difference in RMPs did not result from cell selection bias. Aging neurons also had a lower input resistance to both hyperpolarizing and depolarizing current injection, and the postburst AHP was significantly larger in aging CA1 neurons. Aging neurons also showed more accommodation to a prolonged depolarizing current injection than young neurons. No differences in action-potential amplitude or duration were observed between groups (for summary see Table 1, top).

To evaluate the properties of aging and young neurons independent of differences in membrane potential, cells with RMPs between −65 and −75 mV were selected for
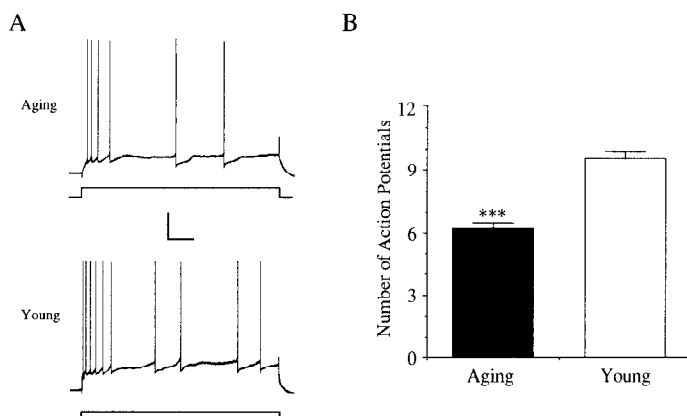
A   B

FIG. 3. Aging CA1 neurons showed more accommodation to a prolonged depolarizing current injection than young neurons. Data from an aging (A, top) and a young (A, bottom) neuron are shown and clearly illustrate the age difference in neuronal excitability. Accommodation was compared between 15 aging and 28 young CA1 neurons whose resting potentials were between −65 and −75 mV (B). Aging neurons fired significantly fewer action potentials than young neurons [$F(1,1) = 43.034, P < 0.001$]. Scale: 20 mV, 1 nA, and 200 ms (A). ***$P < 0.001$.
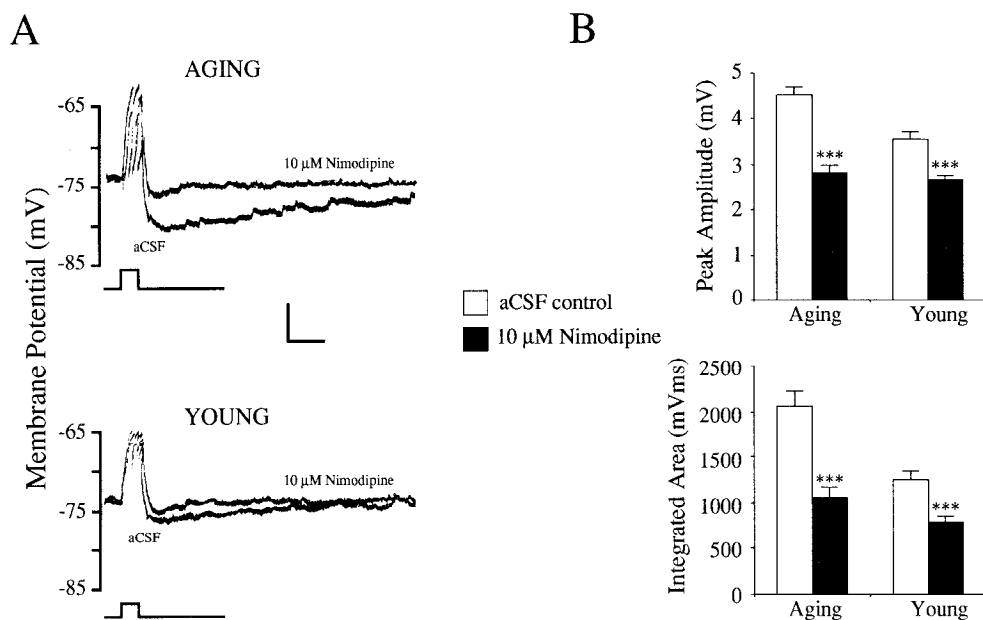
A

B

FIG. 4. Nimodipine (10 μM) reduced the postburst afterhyperpolarization (AHP) in both aging and young CA1 neurons. *A*: overlay records of oscilloscope traces before and after bath-applied nimodipine in an aging (*top*) and a young (*bottom*) CA1 neuron at similar resting potentials. *B*: 10 μM nimodipine significantly reduced both the peak amplitude (*top*) and the integrated area of the AHP in both aging ($n = 13$) and young ($n = 5$) CA1 neurons (***$P < 0.001$). Significant effects of the drug were noted in aging neurons on the amplitude [$F(1,4) = 35.528$, $P < 0.001$] and area [$F(1,4) = 25.406$, $P < 0.001$]. Significant effects of the drug were also noted in young neurons at this dose [amplitude, $F(1,4) = 19.837$, $P < 0.001$; area, $F(1,4) = 16.010$, $P < 0.001$]. Holding potentials were similar in both age groups: aging, -72.13 ± 0.52 mV; young, -72.05 ± 1.33 mV (*B*). Scale: 5 mV, 1 nA, and 200 ms (*A*).

further study (mean RMPs: aging, -70.6 ± 0.7 mV; young, -71.3 ± 0.5 mV). No significant differences in input resistance, action-potential amplitude or action-potential duration were seen. One prominent difference noted was in the size of the postburst AHP. Figure 2 shows that aging CA1 neurons had postburst AHPs that were about twice as large as those of young neurons. Both the peak amplitude and the integrated area of the AHP were significantly larger in aging neurons (see Fig. 2*B*). These data agree with and extend previous observations of prolonged AHP durations in aging rat CA1 neurons (Landfield and Pitler 1984). When accommodation or spike frequency adaptation (a measure of excitability) was examined, it was noted that aging CA1 neurons fired fewer action potentials during a prolonged depolarizing current injection than young neurons (see Fig. 3). Thus, under equivalent resting membrane conditions, aging CA1 neurons were less excitable than young CA1 neurons (for summary see Table 1, bottom). In all pharmacological experiments reported here, only cells with a membrane potential between -65 and -75 mV were compared so that cellular properties could be examined without concern for voltage-dependent differences between groups.

Age- and concentration-dependent effects of nimodipine on the postburst AHP in CA1 neurons

Bath application of 10 μM nimodipine to aging CA1 neurons dramatically decreased the postburst AHP (see Fig. 4*A*). Of 13 aging CA1 neurons tested, nimodipine reduced the AHP in 12 cells. Both the peak amplitude and the integrated area of the AHP were significantly reduced by nimodipine (Fig. 4*B*). Nimodipine (10 μM) also significantly decreased both the peak amplitude and the integrated area of the AHP in young adult CA1 neurons, although the effect was not as large as in aging neurons (Fig. 4). Input resistance was not significantly affected by 10 μM nimodipine in either aging or young cells, and no effects on action-potential amplitude or action-potential duration were seen. An additional experiment was conducted in an aging neu-

ron to evaluate whether the AHP reduction by 10 μM nimodipine could be washed out. Even after a 30-min wash with nimodipine-free ACSF, the postburst AHP was still reduced, although a partial recovery did occur (unpublished observation). This is not surprising because previous studies have demonstrated difficulty in washing nimodipine, presumably because of its lipophilic properties (Docherty and Brown 1986; Kokubun and Reuter 1984; van den Kerckhoff and Drewes 1989).

To ensure that reductions in the AHP observed in aging and young CA1 neurons were specific to the actions of nimodipine, rather than to time-dependent deterioration of the preparation or to actions of the vehicle (ethanol), control experiments were conducted with the ethanol vehicle
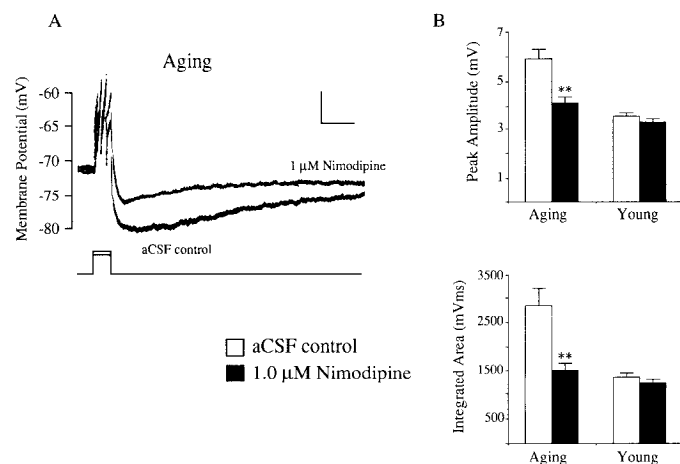
A

B

FIG. 5. Age-specific effects of 1 μM nimodipine were seen on rabbit CA1 neurons. *A*: overlay record of an aging CA1 neuron before and after bath application of 1.0 μM nimodipine. Nimodipine significantly reduced the afterhyperpolarization (AHP) in aging ($n = 5$) CA1 neurons but had no significant effects on the AHP of young ($n = 4$) neurons (*B*). Both the peak amplitude (*top*) and the integrated area (*bottom*) were significantly reduced by 1.0 μM nimodipine. Significant drug effects on the AHP of aging cells were seen on both the amplitude [$F(1,4) = 12.400$, $P < 0.01$] and area [$F(1,4) = 9.207$, $P < 0.01$]. Scale: 5 mV, 0.5 nA, and 200 ms (*A*).
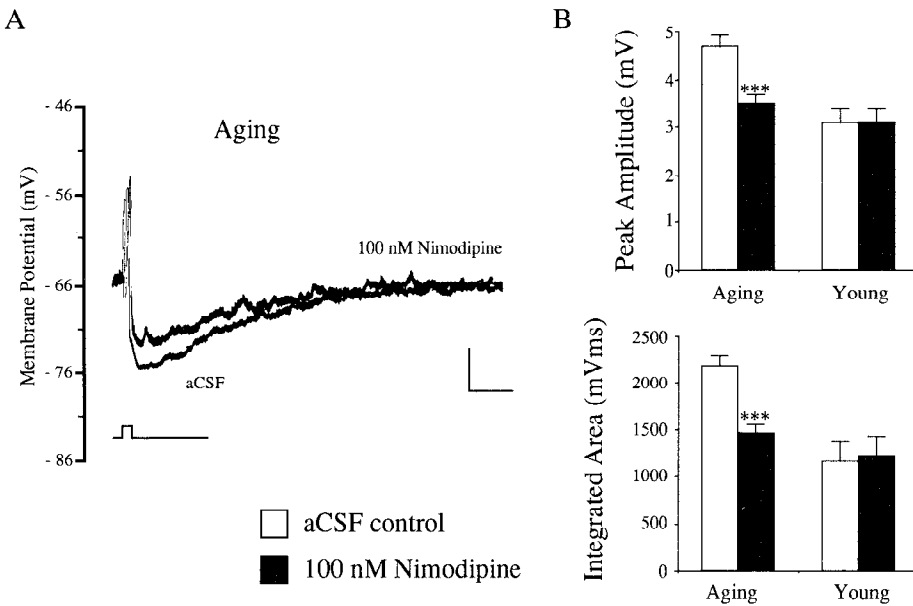
A

B

FIG. 6. Nanomolar concentrations of nimodipine reduced the afterhyperpolarization (AHP) of aging CA1 neurons. *A*: overlay record from an aging CA1 neuron before and after bath application of 100 nM nimodipine. *B*: age-specific reduction of both the peak amplitude (*top*) and the integrated area (*bottom*) of the AHP in aging ($n = 7$) but not young ($n = 4$) CA1 neurons. Significant drug effects on the AHP of aging cells were seen on both the amplitude [$F(1,4) = 15.449$, $P < 0.001$] and integrated area [$F(1,4) = 24.497$, $P < 0.001$]. Scale: 5 mV, 1 nA, and 500 ms (*A*).

substituted for nimodipine in the experimental protocol. In seven of seven cells tested, 0.01% ethanol had no effect on either the AHP or input resistance of aging or young CA1 neurons. None of the other cellular properties studied were altered, indicating that ethanol was a suitable vehicle for use in these experiments.

When a lower concentration of nimodipine (1.0 μM) was tested, the effectiveness of the drug in reducing the AHP was dramatically different. Figure 5 shows that 1.0 μM nimodipine significantly reduced the AHP in aging CA1 neurons but not in young neurons. All five aging neurons tested showed decreased AHPs, with reductions of nearly the same magnitude as seen at 10 μM nimodipine (compare Fig. 5*A* with Fig. 4*A*). Both the peak amplitude and the integrated area of the AHP were significantly decreased in aging CA1 neurons at this lower concentration (see Fig. 5*B*). No significant effects were seen on the input resistance or action-potential characteristics of aging or young neurons.

Nimodipine also significantly decreased both the peak amplitude and the integrated area of the postburst AHP in aging CA1 neurons at a concentration of 100 nM. No effects on the AHP were seen in young CA1 neurons with 100 nM nimodipine. Figure 6*A* shows an overlay recording be-

fore and after bath application of 100 nM nimodipine to an aging CA1 neuron. Even at this low concentration, seven of seven cells tested showed significant AHP reductions. Figure 6*B* summarizes the effects of 100 nM nimodipine on both the peak amplitude and the integrated area of the AHP in aging and young neurons. No significant effects on input resistance or action-potential amplitude or duration were seen at this concentration.

The lowest concentration of nimodipine tested was 10 nM. At this concentration, nimodipine did not significantly reduce the AHP in either aging ($n = 5$) or young ($n = 5$) neurons. No effects on input resistance to either hyperpolarizing or depolarizing current pulses or action-potential characteristics were seen.

Age- and concentration-dependent effects of nimodipine on accommodation in CA1 neurons

High concentrations of nimodipine (1–10 μM) significantly reduced accommodation in both aging and young CA1 neurons. In 11 of 13 aging cells and 10 of 11 young cells, bath application of 10 μM nimodipine significantly decreased accommodation. The number of action potentials elicited by a prolonged depolarizing current injection increased significantly after nimodipine. An additional experiment on an aging neuron showed that 10 μM nimodipine's block of accommodation was partially reversed after a 30-min wash. At a concentration of 1 μM, nimodipine reduced accommodation in five of five aging and three of three young cells tested. Lower nimodipine concentrations yielded quite different age-dependent results. At a concentration of 100 nM, nimodipine partially blocked accommodation in six of six aging cells tested without effects on young cells ($n = 4$). Figure 7 shows that, even with the nimodipine concentration decreased to 10 nM, accommodation was reduced in all four aging neurons tested, without effects on young CA1 neurons. Table 2 summarizes the accommodation data on aging and young neurons at all concentrations of nimodipine tested.
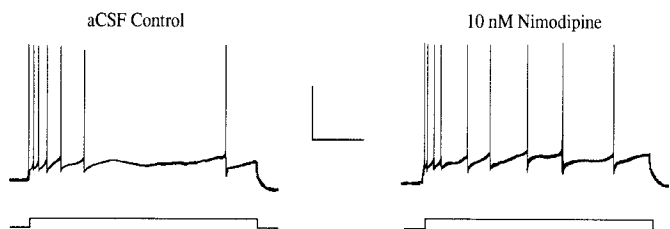

FIG. 7. Nanomolar concentrations of nimodipine decreased accommodation in aging neurons. *Left*: accommodation of an aging neuron to a prolonged depolarizing current injection before nimodipine. *Right*: same aging neuron after bath application of 10 nM nimodipine. In aging neurons ($n = 4$), nimodipine significantly increased the number of action potentials elicited during the accommodation pulse [$F(1,1) = 14.027$, $P < 0.01$]. Scale: 40 mV, 2 nA, and 200 ms.

TABLE 2. *Effects of various concentrations of nimodipine on accommodation*

	ACSF*	Nimodipine Concentration			
		10 μM	1 μM	100 nM	10 nM
Aging CA1	6.84 ± 0.25 (28)	9.54 ± 0.49 (13)†	10.44 ± 1.26 (6)‡	10.73 ± 1.00 (5)§	9.92 ± 0.57 (4)‡
Young CA1	9.75 ± 0.32 (19)	13.71 ± 0.53 (7)†	12.67 ± 0.24 (3)†	10.42 ± 0.47 (4)	10.57 ± 0.88 (5)

Values represent the means ± SE for the number of action potentials elicited during a prolonged depolarizing current injection (see METHODS). Numbers of neurons are in parentheses. Control data from the individual drug populations were collapsed to yield the average ACSF values. This was possible because the control values from each individual drug concentration were not significantly different from each other. *Average number of action potentials of control cells from all dose groups. Other symbols indicate significant difference when compared with controls: †$P < 0.001$, ‡$P < 0.01$, §$P < 0.05$.

Effects of nifedipine on aging and young CA1 neurons

To test whether other dihydropyridines might have similar actions on hippocampal neurons in vitro, where access is not impeded by the blood-brain barrier, a single concentration of another dihydropyridine antagonist, nifedipine, was bath applied to aging and young hippocampal slices. Ten micromolars of nifedipine significantly decreased the peak amplitude and integrated area of the AHP in three of three aging and three of three young cells tested. Nifedipine also reduced spike accommodation in both aging and young neurons. No differences in input resistance, action-potential amplitude, or action-potential duration were seen in either aging or young CA1 neurons with nifedipine treatment. Table 3 summarizes the effects of nifedipine on aging and young neurons. These effects are comparable with those seen with 10 μM nimodipine (compare Fig. 4 with Table 3).

DISCUSSION

Aging-related decreases in rabbit hippocampal CA1 neuronal excitability

Our experiments revealed several electrophysiological properties that differed between aging and young rabbit CA1 neurons. The most profound difference between aging and young neurons was in their level of excitability. Aging CA1 neurons exhibited significantly increased postburst AHPs. This effect was independent of differences in RMP (Table 1). Our observation of an increased AHP extends a previous report that noted aging-related increases in the duration of the AHP in rat CA1 neurons (Landfield and Pitler 1984). Our findings indicate not only that aging rabbit CA1 neurons have a significantly longer AHP duration, but that they also have a larger AHP amplitude than young neurons (see Fig. 2). The major identified component underlying the postburst AHP in hippocampal and neocortical pyrami-

dal neurons is a slow, calcium-activated potassium current known as I_{AHP} (Hotson and Prince 1980; Gustafsson and Wigström 1981; Lancaster and Adams 1986; Schwindt et al. 1992). This current regulates cellular excitability by hyperpolarizing the membrane potential after a burst of action potentials and thus prevents recurrent burst firing and epileptiform activity (for review see Storm 1990). With their larger AHPs of longer duration, aging neurons would be unable to respond to excitatory responses as frequently as young neurons. This decreased responsiveness in aging neurons would lead to a general dampening of neuronal signaling within the hippocampus.

We hypothesize that the larger AHPs of aging neurons may contribute to the learning deficits observed during trace eye-blink conditioning in aging rabbits (Deyo et al. 1989; Graves and Solomon 1985). This hypothesis is supported by observations in hippocampal neurons that decreased AHPs in vitro (Coulter et al. 1989; de Jonge et al. 1990; Disterhoft et al. 1986) and increased firing rates in vivo (Berger et al. 1983) correlate with associative learning in rabbits. One way that the excitability of CA1 neurons can be modulated is through neurotransmitters, such as acetylcholine, that decrease both the AHP and accommodation (Cole and Nicoll 1983). Loss of cholinergic neurons has been implicated in aging-associated Alzheimer's dementia (Bartus et al. 1985; Davies and Maloney 1976; Decker 1987). Cholinergic blockers such as scopolamine impair classical conditioning in rabbits (Harvey et al. 1985) but only when the hippocampus is intact (Solomon et al. 1983). Thus aging-related changes in cholinergic neurotransmission or in other neurotransmitter systems that decrease the AHP, including norepinephrine (Benardo and Prince 1982; Madison and Nicoll 1986), serotonin (Colino and Halliwell 1987), or dopamine (Malenka and Nicoll 1986), may alter postburst AHPs and lead to changes in the level of CA1 neuron excitability.

Aging CA1 neurons showed more accommodation to a

TABLE 3. *Effects of 10 μM nifedipine on CA1 neurons*

	RMP, mV	AHP Amplitude, mV		AHP Integrated Area, mV/ms		Accommodation, Number of APs	
		ACSF	10 μM Nifedipine	ACSF	10 μM Nifedipine	ACSF	10 μM Nifedipine
Aging	−74.90 ± 3.34	−4.43 ± 0.29	−2.49 ± 0.15*	2,143 ± 196	1,022 ± 135*	7.11 ± 0.56	10.44 ± 0.69†
Young	−74.08 ± 0.91	−3.21 ± 0.15	−2.68 ± 0.11‡	974 ± 134	431 ± 44†	9.89 ± 0.95	14.78 ± 0.60†

Values represent the means ± SE for the particular measurement for $n = 3$ cells within each group. Abbreviations as in Table 1. Asterisks indicate significant difference when compared with ACSF controls: *$P < 0.001$, †$P < 0.01$, ‡$P < 0.05$.

prolonged depolarizing pulse than young neurons (see Fig. 3). This finding paralleled the increased AHP observed in aging neurons, further suggesting that aging neurons are less excitable than young neurons. These findings were also consistent with previous in vivo recordings that found lower spontaneous firing rates of aging rat hippocampal neurons (Lippa et al. 1981). Previous studies have shown that hippocampal pyramidal cells accommodate in response to a prolonged depolarizing stimulus, unlike interneurons that show little or no accommodation (Schwartzkroin 1975; Schwartzkroin and Mathers 1978). The precise relationship between the size of the slow AHP and the level of accommodation of hippocampal neurons is unclear, but several studies using drugs to manipulate the slow AHP found corresponding changes in accommodation. For example, block of the calcium-activated AHP by intracellular injection of the calcium chelator ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) also reduced accommodation in rat CA1 neurons (Madison and Nicoll 1984; Schwartzkroin and Stafstrom 1980). Acetylcholine application to hippocampal slices blocked the slow AHP and decreased accommodation, resulting in increased excitability (Cole and Nicoll 1983). Similarly, alaproclate, a 5-hydroxytryptamine uptake inhibitor, blocked the AHP and decreased accommodation (Hedlund and Andersen 1989). Conversely, adenosine application enhanced the slow AHP and increased cell accommodation, which decreased excitability (Haas and Greene 1984). Thus an increased AHP coupled with increased accommodation may be correlated indicators of decreased excitability in aging CA1 neurons.

Aging CA1 neurons also exhibited more hyperpolarized RMPs than young neurons (see Fig. 1). This result was unexpected and has not been consistently observed in aging hippocampal neurons in other species (Barnes and McNaughton 1980; Barnes et al. 1987; Landfield and Pitler 1984). Aging neurons tended to have a lower input resistance, but this significant difference was correlated with the presence of a RMP difference (see Table 1). Other researchers have reported a decreased input resistance and decreased excitability in aging CA1 neurons without significant differences in membrane potential (Turner and Deupree 1991). We cannot at present fully explain the differences between our data and those of other groups, but a number of possibilities exist. For example, differences between species, in cell selection criteria, in bath temperature, or in protocol length, may account for some of the discrepancies between studies. One possibility could be that aging rabbit neurons are more sensitive to in vitro manipulations than young neurons. This would suggest that more resilient young neurons would survive at more depolarizing membrane potentials, whereas less resilient aging neurons would be lost. Our comparison of RMPs between aging and young neurons that were lost argues against this point, because even these unstable aging neurons were more hyperpolarized (see RESULTS).

Our observation of a more hyperpolarized resting potential in aging neurons may reflect greater activation of Ca^{2+}-activated K^+ channels, possibly because of higher intracellular calcium levels. Alternatively, aging neurons may have difficulty inactivating Ca^{2+}-activated K^+ channels. The

prolonged duration of the Ca^{2+}-activated slow AHP in aging neurons observed in the present study supports this hypothesis. Additional evidence comes from the finding that CA1 neurons from aging rats (Pitler and Landfield 1990) and aging rabbits (Moyer and Disterhoft 1992) exhibit prolonged calcium action potentials. Age-dependent changes in calcium-buffering capacity, changes in calcium channel inactivation kinetics, or an inability to rapidly sense changes in calcium influx could contribute to these observations (Khachaturian 1989; Landfield 1987). Other potassium currents in these aging cells might also be prolonged, but our studies were not designed to directly address this issue. The changes in excitability observed here suggest that intracellular calcium levels differ, or influence excitability differently in aging than in young CA1 neurons.

Nimodipine increases hippocampal excitability in an age- and concentration-dependent manner

Another major finding of this study was that the L-type calcium channel antagonist nimodipine reversed the aging-related reductions in CA1 excitability, even at concentrations in the nanomolar range. Our data are the first to show that concentrations of nimodipine as low as 100 nM significantly reduced the AHP of aging hippocampal CA1 neurons (see Fig. 6). Nimodipine's effects at lower concentrations and the larger magnitude of these effects at all concentrations in aging neurons may be reflective of the aging neuron's increased AHP size and duration (see Figs. 2 and 4–6). Another report also suggested that 2 μM nimodipine decreased the amplitude of the AHP to similar extents in both aging and young rat CA1 neurons; experiments at lower concentrations were not reported (Mazzanti et al. 1992). This is consistent with our finding that nimodipine reduced the AHP in both aging and young CA1 neurons at high concentrations (10 μM). Only at lower concentrations ($\leq$1 μM) were the age-specific AHP reductions observed. It is unclear why nimodipine acts preferentially on aging neurons at lower concentrations. No marked difference in the level of membrane depolarization between aging and young neurons that could account for nimodipine's effects on aging neurons at lower concentrations were observed. Perhaps calcium currents in aging neurons have an increased sensitivity to nimodipine, but our data do not specifically address this issue. The effects of nimodipine on the AHP of aging neurons were quite robust and consistent at doses as low as 100 nM.

The observed reduction in the AHP of young CA1 neurons by 10 μM nimodipine corroborates data from other studies demonstrating significant block of L-type calcium currents with high concentrations of nimodipine or other dihydropyridines in various neuronal and nonneuronal cell types (Bean 1991; Black et al. 1990; Fox et al. 1987; Hess et al. 1984; Hirning et al. 1988; Mogul and Fox 1991). Several other studies also reported reductions of L-type calcium currents by high concentrations (3–20 μM) of nimodipine or other dihydropyridine antagonists in hippocampal CA1 neurons (Black et al. 1990; O'Dell and Alger 1991; Regan et al. 1991). Nifedipine (1 μM) also reduced calcium spike potentials in young guinea pig CA1 (Higashi et al. 1990) and rat CA3 neurons (Gähwiler and Brown 1987).

We observed that the AHP of aging neurons was nearly as small as the AHP of young neurons after addition of nanomolar concentrations of nimodipine (see Fig. 6). Thus our data suggest that L-type calcium channels are at least partly responsible for the entry of calcium that activates the slow AHP, especially in aging CA1 neurons. L-Type calcium channels are highly concentrated on the soma and basal dendrites of hippocampal CA1 as well as CA3, the dentate gyrus, and some interneurons (Ahlijanian et al. 1990; Westenbroek et al. 1990). In young guinea pig CA1 neurons, 1 μM nifedipine decreased the AHP after a single action potential (Higashi et al. 1990). Nimodipine (2 μM) also reversed Bay K 8644–induced increases of the AHP, and 5 μM nimodipine decreased the duration of the AHP in rat hippocampal CA1 neurons (Rascol et al. 1991). Previous studies have not looked at the effects of nimodipine on the AHP in aging or young hippocampal CA1 neurons using nanomolar concentrations, which would more likely approximate physiological concentrations seen by neurons after administration to an intact animal (H. P. Krause, personal communication). Other studies, however, demonstrated that nanomolar concentrations of dihydropyridine antagonists reduced Ca^{2+} currents in other cell types such as dorsal root ganglion cells (Bean 1991; Boland and Dingledine 1990; McCarthy 1989; McCarthy and TanPiengco 1992), anterior pituitary cells (Cohen and McCarthy 1987), and vascular smooth muscle cell lines (McCarthy and Cohen 1989).

In addition to the effects on the AHP, nimodipine also reduced accommodation in aging CA1 neurons at concentrations as low as 10 nM (Table 2). This strongly suggests that nimodipine's block of L-type calcium channels also modulates this index of excitability in aging CA1 neurons. Similar effects were seen in young neurons but only at concentrations ≥1 μM (Table 2). Accommodation was decreased at concentrations at least 10-fold lower than those that reduced the AHP in both aging and young neurons. One reason for this may lie in the voltage dependence of dihydropyridine actions (Bean 1991; Hess et al. 1984). L-Type calcium channels are voltage sensitive and yield long-lasting, high-threshold currents with large conductances at strong depolarizations (Fox et al. 1987; Mogul and Fox 1991; Nowycky et al. 1985). Prolonged depolarization during the sequence of action potentials, generated by the long depolarizing current injection used to evaluate accommodation, may enhance nimodipine's ability to act at lower concentrations (than those that reduced the AHP) in both aging and young neurons. This may occur through indirect actions of nimodipine on calcium-activated K$^+$ currents. This is consistent with previous reports of depolarization-dependent effects of dihydropyridines on CA1 neurons (Bean 1991; Docherty and Brown 1986; Meyers and Barker 1989; O'Regan et al. 1990).

Effects of another dihydropyridine on hippocampal CA1 neuron excitability

Previous experiments in our laboratory found that intravenous administration of the dihydropyridine antagonist nifedipine did not significantly alter spontaneous firing rates of rabbit CA1 neurons, whereas administration of ni-

modipine increased firing rates (Thompson et al. 1990). We hypothesized that one reason for the lack of effect in vivo could result from nifedipine's relative inability to cross the blood-brain barrier as compared with nimodipine (van den Kerckhoff and Drewes 1989). When we substituted nifedipine (10 μM) for nimodipine in the slice bathing medium, both the amplitude and the integrated area of the AHP were significantly reduced in aging and young neurons (see Table 3). Nifedipine also decreased accommodation in aging and young neurons. Similar effects with nifedipine and nimodipine in enhancing Schaffer collateral CA1 synaptic transmission in vitro have been reported (O'Regan et al. 1991). Our experiments did not systematically examine the effects of a wide range of nifedipine concentrations on CA1 neuron excitability. However, the effects observed with nifedipine were similar to those seen with the same concentration of nimodipine. Presumably, this is due to the direct access nifedipine has to CA1 neurons in the hippocampal slice because it does not have to cross the blood-brain barrier.

Relationship between calcium, nimodipine, learning, and the aging hippocampus

Modulation of intracellular calcium levels and calcium-dependent cellular processes has been implicated in learning and memory processes (Disterhoft et al. 1986, 1989, 1991a; Olds et al. 1989). Aging-related deficits have been observed in a variety of learning tasks including eye-blink conditioning in rabbits (Disterhoft et al. 1991b; Graves and Solomon 1985; Woodruff-Pak and Thompson 1985). One of the consequences of aging has been suggested to be an increase in intracellular calcium levels either through impaired buffering or increased calcium influx (Khachaturian 1989; Landfield 1987; Pitler and Landfield 1990), which may contribute to toxicity and cell death in aging neurons (Choi and Rothman 1990; Farber 1981; Landfield et al. 1986; Scharfman and Schwartzkroin 1989). Impaired inactivation of calcium currents in aging hippocampal neurons may also contribute to the observed aging-related changes in CA1 neurons (Moyer and Disterhoft 1992; Pitler and Landfield 1990). Our experiments support this hypothesis and suggest that nimodipine can restore the biophysical properties of aging CA1 neurons to a level more closely resembling those of young CA1 neurons. For example, 100 nM nimodipine reduced the AHP in aging CA1 neurons so that the final amplitude and duration of the AHP were similar to young neurons without the drug (see Fig. 6).

Nimodipine treatment improves learning and memory in a variety of cognitive tasks in aging subjects (Bono et al. 1985; Deyo et al. 1989, 1990; Sandin et al. 1990; Schuurman and Traber 1989). Most of these tasks, such as trace eye-blink conditioning (Moyer et al. 1990b), are dependent on an intact hippocampus for successful learning. In awake rabbits, nimodipine increases spontaneous activity of hippocampal pyramidal neurons in an age- and dose-dependent manner (Thompson et al. 1990). These data suggest that nimodipine may exert its physiological effects via direct actions on neurons. We demonstrate here that two measures of neuronal excitability (the AHP and accommodation) are altered in aging rabbit CA1 pyramidal cells in

vitro, resulting in decreased excitability. Nimodipine decreased both the AHP and accommodation, which reversed the loss of excitability in aging neurons. These effects were seen at concentrations of nimodipine as low as 10 (on accommodation) and 100 nM (on the AHP), concentrations that were without effects on young cells. These low concentrations are likely to be comparable with extracellular levels seen by CA1 neurons after administration of doses of nimodipine that facilitate learning (H. P. Krause, personal communication). This suggests that nimodipine may improve learning in some behavioral tasks through its direct actions on hippocampal CA1 neuron excitability in aging animals.

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