Brief Communication

Differential Calcium-Dependent Modulation of NMDA Currents in CA1 and CA3 Hippocampal Pyramidal Cells

Anton A. Grishin, Christine E. Gee, Urs Gerber, and Pascal Benquet
Brain Research Institute, University of Zurich, CH-8057 Zurich, Switzerland

Neuronal Ca^{2+} influx via NMDA receptors (NMDARs) is essential for the development and plasticity of synapses but also triggers excitotoxic cell death when critical intracellular levels are exceeded. Therefore, finely equilibrated mechanisms are necessary to ensure that NMDAR function is maintained within a homeostatic range. Here we describe a pronounced difference in the modulation of NMDA currents in two closely related hippocampal cell types, the CA1 and the CA3 pyramidal cells (PCs). Manipulations that increase intracellular Ca^{2+} levels strongly depressed NMDA currents in CA3 with only minor effects in CA1 PCs. Furthermore, activation of G_q-coupled metabotropic receptors potentiated NMDA currents in CA1 PCs but depressed them in CA3 PCs. Interestingly, the CA3 type modulation of NMDARs could be converted into CA1-like behavior, and vice versa, by increasing Ca^{2+} buffering in CA3 cells or decreasing Ca^{2+} buffering in CA1 cells, respectively. Our data suggest that a differential Ca^{2+} sensitivity of the regulatory cascades targeting NMDARs plays a key role in determining the direction and magnitude of NMDA responses in various types of neurons. These findings may have important implications for NMDA receptor-dependent synaptic plasticity and the differential sensitivity of CA1 and CA3 PCs to NMDAR-dependent ischemic cell death.

Key words: **acetylcholine; ACh; depression; muscarinic; potentiation; glutamate; metabotropic glutamate receptor**

Introduction

NMDA receptors (NMDARs) are glutamatergic ionotropic receptors that act as gatekeepers for the influx of dendritic Ca^{2+} during synaptic activity. Reflecting the importance of Ca^{2+} entry via NMDARs, several regulatory pathways exist that meticulously control NMDAR activity. Potentiation of NMDARs can be achieved by activating one of several transduction cascades that converge on Src kinase resulting in tyrosine phosphorylation of the receptor (Kotecha and MacDonald, 2003). This mechanism, which is triggered by the activation of metabotropic receptors, increases the open probability without changing the unitary conductance of NMDAR channels (Yu et al., 1997; Ali and Salter, 2001). Depression of NMDARs occurs through a negative feedback loop that promotes desensitization and inactivation of NMDARs as intracellular Ca^{2+} rises (Kotecha and MacDonald, 2003). Because the activation of metabotropic receptors induces both Src activation and release of Ca^{2+} from intracellular stores, it is difficult to predict whether the final outcome on NMDARs will be upregulation or downregulation. In fact, depending on the cell type and experimental conditions, either potentiation or depression of NMDA currents has been reported after activation of metabotropic receptors (Benquet et al., 2002; Kotecha and MacDonald, 2003).

In this study, we investigated the interactions between intracellular transduction pathways mediating positive and negative regulation of NMDARs. We found marked differences in the Ca^{2+}-sensitive metabotropic modulation of NMDARs between CA1 and CA3 pyramidal cells (PCs).

Materials and Methods

Slice culture preparation and electrophysiology. Hippocampal organotypic slices were prepared from postnatal day 6 Wistar rats using the roller-tube technique (Gahwiler et al., 1998). After 2–4 weeks *in vitro*, slice cultures were transferred to a 1 ml recording chamber that was perfused continuously with Tyrode's solution (1.5 ml/min, 30°C) containing (in mM): 137 NaCl, 2.7 KCl, 11.6 $NaHCO_3$, 0.4 NaH_2PO_4, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 5.6 D-glucose, (in μM): 5 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX), 100 picrotoxin, 0.1 tetrodotoxin, 0.001% phenol red, pH 7.4, ~305 mOsm. Whole-cell voltage-clamp recordings were obtained from visualized CA1 and CA3 PCs at −50 mV using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Patch pipettes (2–5 MΩ; series resistance 3–15 MΩ) were filled with (in mM): 140 K-gluconate, 10 HEPES, 1 EGTA, 4 Mg-ATP, 0.4 Na-GTP, pH 7.2, ~290 mOsm. Liquid junction potentials (−13 mV) were corrected for. When the Ca^{2+} buffer concentration was increased, K-gluconate was reduced to maintain osmolarity. (*S*)-3,5-dihydroxyphenylglycine (DHPG) experiments were performed with 3 mM extracellular Ca^{2+}. Currents were filtered at 1–2 kHz, stored, and analyzed off-line (pClamp7; Axon Instruments). Intracellular recordings were made using an Axoclamp-2A amplifier (Axon Instruments) and 40–60 MΩ microelectrodes filled with 1 M potassium acetate. Because of the poor voltage clamp in cells recorded with microelectrodes, NMDA responses were measured in current-clamp mode.

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Correspondence should be addressed to Urs Gerber, Brain Research Institute, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. E-mail: gerber@hifo.unizh.ch.

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NMDA current induction. NMDA (100 μM) was pressure ejected (1 bar) for ~200 msec from a pipette positioned ~100 μm from the soma of recorded cells. NMDA-induced currents were completely blocked by 40 μM (E)-4-(3-phosphonoprop-2-enyl)piperazine-2-carboxylic acid (CPP). Peak effects of metabotropic agonists (measured after 1–5 min) were compared with and normalized to the average NMDA current amplitude from three to five NMDA puffs immediately preceding agonist application (referred to as "baseline"). When testing the effects of different extracellular Ca^{2+} concentrations, the $MgCl_2$ concentration was 2 mM. Solutions were changed after NMDA currents reached a steady state for a given test solution.

Ca^{2+} imaging. Oregon Green 488 BAPTA-2 (20 μM) was added to the intracellular solution (K_d ~580 nM) and excited at 488 nm using a TILL Photonics Polychrome I monochromator (Planegg). Emitted images were collected with a cooled CCD camera (Princeton Instruments, Trenton, NJ) after they were passed through a TILL FITC filter set, stored, and analyzed using the Axon Imaging Workbench program (Axon Instruments). Images were collected at 10 sec intervals (exposure time 200–500 msec). Average fluorescence was determined for regions of interest over the soma (avoiding the nucleus), and the average background fluorescence of a region away from the filled cell was subtracted. $\Delta F/F$ was calculated for each image [(average fluorescence − average baseline fluorescence)/average baseline fluorescence].

Statistics. Data are presented as means ± SEM. Potentiation or depression of NMDA currents is given as percentage of the baseline response. Paired Student's *t* tests were used to compare the non-normalized NMDA currents before and after agonist application. To compare effects of changing Ca^{2+} concentration in CA1 and CA3 cells, two-way ANOVAs followed by Tukey's honestly significant difference (HSD) *post hoc* tests were used with normalized data (SPSS, Chicago, IL). Values of $p < 0.05$ were considered statistically significant.

Drugs. CPP was kindly provided by Novartis (Basel, Switzerland). Tetrodotoxin was purchased from Latoxan (Valence, France), NBQX from AG Scientific (San Diego, CA), DHPG from Tocris Cookson (Avonmouth, UK), Oregon Green BAPTA-2 and K_4BAPTA were from Molecular Probes (Leiden, Netherlands). Other chemicals were from Sigma (St. Louis, MO). Stock solutions of NBQX and picrotoxin were prepared in DMSO, which never exceeded a final concentration of 0.02%.

Results

Differential regulation of NMDA responses in CA1 and CA3 PCs by Ca^{2+}

We compared the effect of changing the extracellular Ca^{2+} concentration on NMDA responses in CA1 and CA3 PCs. Pharmacologically isolated NMDA currents were induced in PCs voltage-clamped at −50 mV by pressure application of NMDA at 30 sec intervals. Increasing the extracellular Ca^{2+} concentration affected NMDA currents differently in CA1 and CA3 PCs ($p < 0.001$; two-way ANOVA) (Fig. 1). Ca^{2+} (10 mM) strongly depressed NMDA currents in CA3 cells (41.3 ± 4.2% of response in 0.1 mM Ca^{2+}; $n = 9$; $p < 0.001$; Tukey's HSD) but had much less effect in CA1 cells (80.2 ± 2.4% of response in 0.1 mM Ca^{2+}; $n = 8$; $p < 0.001$; Tukey's HSD) (Fig. 1).

Increasing the extracellular Ca^{2+} concentration is expected to cause a corresponding increase in intracellular Ca^{2+} concentration because of enhanced Ca^{2+} influx through the repetitively activated NMDARs and because of a Ca^{2+} conductance active at a holding potential of −50 mV (Gee et al., 2003). This was confirmed by imaging intracellular Ca^{2+} with Oregon Green 488 BAPTA-2 while simultaneously recording whole-cell currents. We wished to compare both the basal Ca^{2+} rises attributable to changing extracellular Ca^{2+} and the transient Ca^{2+} rises caused by activation of NMDARs in CA3 versus CA1 PCs. To determine the baseline Ca^{2+}, $\Delta F/F$ was calculated from the background-subtracted images taking the value at 0.1 mM Ca^{2+} as the baseline. To calculate the Ca^{2+} transients associated with NMDAR activation, the Ca^{2+} level before each transient was taken as the baseline. Because extracellular Ca^{2+} increased, intracellular Ca^{2+} increased in both CA1 and CA3 PCs ($p < 0.05$; $n = 3$ CA1; $n = 3$ CA3; two-way ANOVA) (Fig. 2*A–C*), but there was no difference between CA1 and CA3 PCs ($p > 0.10$). Likewise, the Ca^{2+} transients evoked by NMDA increased in both CA1 and CA3 PCs, reflecting the increased driving force for Ca^{2+} entry through the NMDA channels ($p < 0.003$; $n = 3$ CA1; $n = 3$ CA3; two-way ANOVA). Again, there was no difference between CA1 and CA3 PCs ($p > 0.5$; two-way ANOVA) (Fig. 2*A*,*B*,*D*,*E*). In contrast, the simultaneously recorded NMDA currents were significantly more depressed in CA3 than in CA1 PCs ($p < 0.5$; $n = 3$; two-way ANOVA) (Fig. 1*A*). Thus, similar manipulation of intracellular Ca^{2+} levels results in markedly different Ca^{2+}-dependent depression of NMDA currents in CA1 and CA3 PCs.

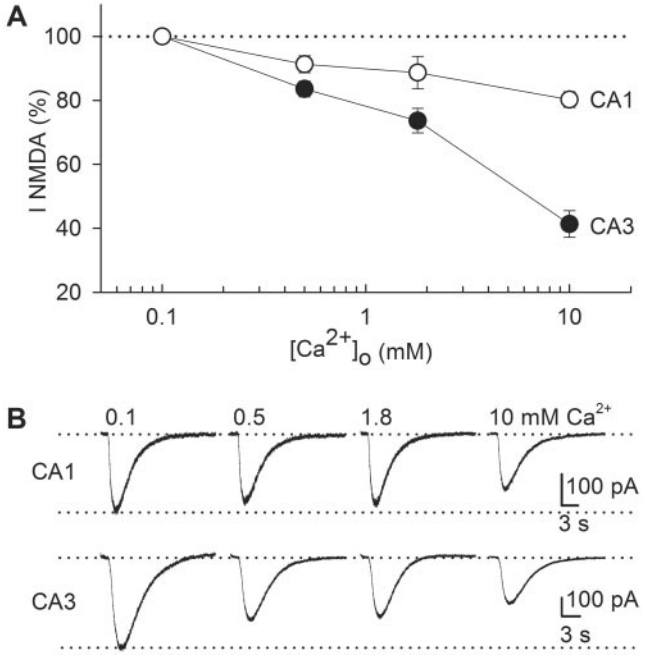

Figure 1. Increasing extracellular Ca^{2+} reduces NMDA currents more strongly in CA3 than in CA1 PCs. *A*, Normalized NMDA current versus extracellular Ca^{2+} concentration. Note the much steeper concentration–response curve for CA3 cells. *B*, NMDA currents in different Ca^{2+} concentrations from a CA1 and a CA3 PC.

NMDA currents are potentiated in CA1 and depressed in CA3 PCs by metabotropic receptors

Because the above experiments show that the increasing intracellular Ca^{2+} depresses NMDA currents, we asked whether similar modulation occurs when intracellular Ca^{2+} levels are raised by activating metabotropic G_q-coupled receptors. Activating metabotropic receptors enhances NMDA currents in hippocampal PCs via a second messenger cascade involving phosphoinositol hydrolysis and subsequent phosphorylation of NMDA channels via the protein tyrosine kinase Src (Kotecha and MacDonald, 2003). As expected, we observed that application of the cholinergic agonist muscarine (10 μM for 2 min) potentiated NMDA currents in CA1 PCs (140.6 ± 2.79%; $n = 5$; $p < 0.01$) (Fig. 3*A*). In contrast, muscarine induced a transient potentiation followed by a strong depression of NMDA currents in CA3 PCs obtained from the same slice cultures (68.2 ± 1.6%; $n = 5$; $p < 0.01$) (Fig. 3*A*). The potentiation of NMDA currents in CA1 and the depression in CA3 were reversible after ~15 min of muscarine washout.

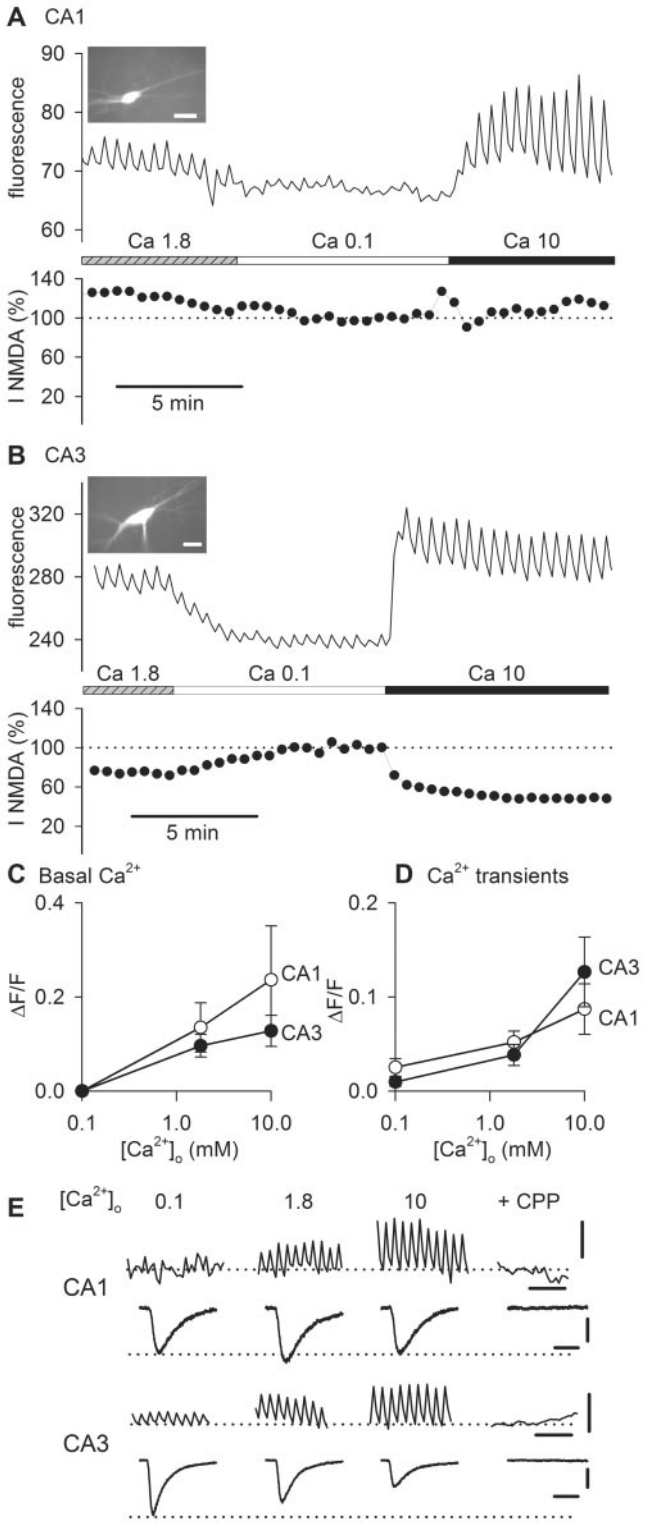

Figure 2. Increasing extracellular Ca^{2+} concentration causes similar increases in intracellular Ca^{2+} levels and NMDA-induced Ca^{2+} transients in CA1 and CA3 PCs, whereas simultaneously recorded NMDA currents are strongly depressed only in CA3. A CA1 (*A*) and a CA3 (*B*) PC were filled with the Ca^{2+} indicator Oregon Green 488 BAPTA-2 and fluorescence images were collected every 10 sec. Top traces show fluorescence signals (background-subtracted arbitrary units) with the simultaneously recorded normalized NMDA-induced currents below. Note the transient rises in the fluorescence signal corresponding to Ca^{2+} influx through the NMDARs. Extracellular Ca^{2+} was changed where indicated. CPP completely blocked responses to NMDA. *C*, The increase in $\Delta F/F$ relative to the basal level in 0.1 mM was the same in CA1 (○) and CA3 (●) PCs when extracellular Ca^{2+} was increased. *D*, The change in fluorescence induced by NMDA application increased with increasing Ca^{2+} concentration in both CA1 and CA3 PCs. *E*, Sample Ca^{2+} transients and NMDA currents from the cells in *A* and *B*. Note that NMDA currents are much more sensitive to changes in Ca^{2+} concentration in CA3 than in CA1 cells. Scale bars: *A*, *B*, 20 μm. *E*, Ca^{2+} transients, 2 min; CA1 0.5 $\Delta F/F$; CA3 0.1 $\Delta F/F$; NMDA currents, 3 sec, 100 pA.

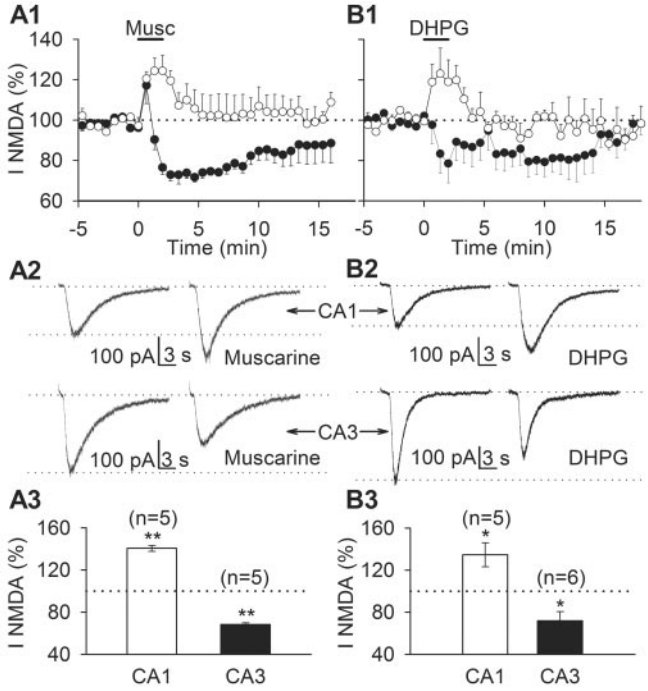

Figure 3. Activating muscarinic or metabotropic glutamate receptors potentiates NMDA currents in CA1 and depresses NMDA currents in CA3 PCs. *A1*, Normalized NMDA currents in CA1 (○) and in CA3 (●) PCs from five slices before and after application of muscarine (Musc; 10 μM). *B1*, Effects of DHPG (50 μM) on normalized NMDA currents. NMDA currents before and after application of muscarine (*A2*) or DHPG (*B2*) are shown. Pooled maximal effects of muscarine (*A3*) or DHPG (*B3*) are shown (*$p < 0.05$; **$p < 0.01$).

The difference in NMDA receptor modulation in CA1 versus CA3 PCs was also observed with activation of group I metabotropic glutamate receptors. Application of DHPG (50 μM for 2 min), a group I metabotropic glutamate receptor agonist, markedly potentiated NMDA responses in CA1 PCs (134.5 ± 11.4%; $n = 5$; $p < 0.05$), as described previously (Valenti et al., 2002; Kotecha and MacDonald, 2003), whereas NMDA responses were depressed in CA3 PCs (71.8 ± 8.9%; $n = 6$; $p < 0.05$) (Fig. 3*B*). These results indicate that increases in intracellular Ca^{2+} levels have opposite effects on the metabotropic transduction mechanisms modulating NMDARs in CA3 versus CA1 PCs. This idea was tested further by examining the effects of changing intracellular Ca^{2+} buffering on the modulation of NMDA currents in these two types of PCs.

Intracellular Ca^{2+} buffering alters metabotropic modulation of NMDA currents

In CA1 PCs, when Ca^{2+} buffering was decreased by lowering intracellular EGTA from 1 to 0.1 mM, muscarine depressed rather than potentiated NMDA currents (83.3 ± 2.7%; $n = 5$; $p < 0.05$) (Fig. 4*A*). Conversely, increasing intracellular Ca^{2+} buffering with 30 mM BAPTA prolonged the muscarinic potentiation of NMDA currents (149.5 ± 11.8%; $n = 5$; $p < 0.05$) (Fig. 4*A*). In CA3 PCs, the depression of NMDA currents seen with 1 mM EGTA intracellularly (71.5 ± 2.0%; $n = 9$; $p < 0.001$) was

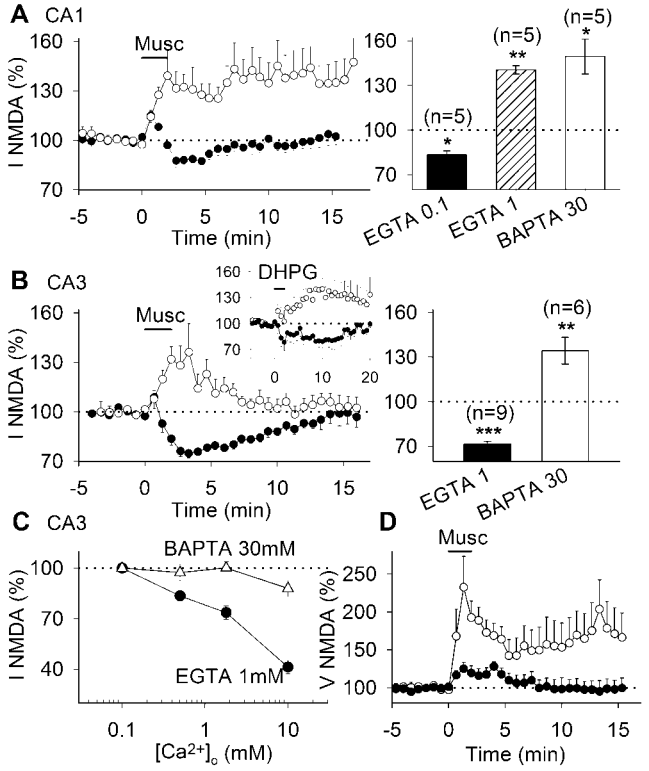

Figure 4. Intracellular Ca^{2+} buffering changes the polarity and amplitude of metabotropic modulation of NMDA currents. *A*, Normalized peak NMDA currents in CA1 PCs with low intracellular Ca^{2+} buffering (0.1 mM EGTA, ●) and high Ca^{2+} buffering (30 mM BAPTA, ○) with pooled maximal potentiation–depression at right. *B*, Normalized peak NMDA currents in CA3 PCs with 1 mM EGTA ● and high Ca^{2+} buffering (30 mM BAPTA, ○). Inset, DHPG (50 μM) effect on NMDA currents in CA3 PCs with 1 mM EGTA (●) or 30 mM BAPTA (○) with pooled maximal potentiation–depression at right. *C*, Effects of changing extracellular Ca^{2+} concentration on NMDA currents in CA3 PCs. (1 mM EGTA, ●; 30 mM BAPTA, △). *D*, Normalized NMDA-induced depolarizations recorded intracellularly in CA1 (○) and CA3 (●) PCs (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

switched to a significant potentiation with 30 mM BAPTA (134.3 ± 8.9%; $n = 6$; $p < 0.01$) (Fig. 4*B*), mimicking the effect of muscarine on NMDA currents in CA1 PCs loaded with 1 mM EGTA (compare Fig. 3*A*). Intermediate Ca^{2+} buffering with 10 mM EGTA resulted in an intermediate degree of muscarinic modulation of NMDA responses (125.0 ± 17.4%; $n = 4$; $p > 0.10$ in CA3; data not shown). Likewise, the DHPG-induced depression of NMDA currents in CA3 PCs was converted into potentiation when intracellular Ca^{2+} buffering was enhanced with 10 mM BAPTA (142.8 ± 9.6%; $n = 5$; $p < 0.05$) (Fig. 4*B*, inset). Thus, the modulation of NMDARs by muscarinic or metabotropic glutamate receptors depends critically on intracellular Ca^{2+}.

We next examined whether the pronounced Ca^{2+}-dependent depression of NMDA currents in response to increased extracellular Ca^{2+} in CA3 PCs is changed by increasing intracellular Ca^{2+} buffering. When 30 mM BAPTA was used as the intracellular buffer, a virtually flat concentration–response curve reminiscent of that in CA1 cells was observed (Fig. 4*C*) (88.0 ± 4.8% of response in 0.1 mM Ca^{2+} at 10 mM Ca^{2+}; $n = 5$; $p < 0.001$ two-way ANOVA) (compare Fig. 1*A*).

To ensure that the differential Ca^{2+}-dependent modulation of NMDA responses occurs in PCs with unmanipulated Ca^{2+} buffering, we recorded NMDA-induced depolarizations with sharp microelectrodes. Under these conditions, Ca^{2+} buffers are not introduced into the neurons, and intracellular dialysis is minimal. Again we found a significant difference in the modulation of NMDA responses between both cell types. In response to 1 μM muscarine, NMDA responses were strongly and irreversibly potentiated in an long-term potential (LTP)-like manner in CA1 (236.8 ± 38.9%; $n = 5$; $p < 0.05$), whereas in CA3 PCs potentiation was significantly less and of short duration (5–6 min; 135.1 ± 5.4%; $n = 5$; $p < 0.01$). The fact that we observe this slight transient potentiation rather than a depression in CA3 PCs when recording with microelectrodes suggests that intrinsic Ca^{2+} buffering in this cell type is greater than that observed with 1 mM EGTA in the patch-clamp experiments and very similar to the modulation obtained with 10 mM EGTA (1 mM EGTA: 71.5 ± 2.0%; $n = 9$; $p < 0.001$; versus 10 mM EGTA: 125.0 ± 17.4%; $n = 4$; $p > 0.10$ in CA3; data not shown). These findings confirm that the differential Ca^{2+}–dependent regulation of NMDA responses in CA1 versus CA3 PCs is inherent to the cell type.

Discussion

Our experiments show that Ca^{2+}-dependent depression of NMDA responses is greater in hippocampal CA3 PCs than in CA1 PCs. This effect was observed by altering intracellular Ca^{2+} levels through changes in extracellular Ca^{2+} concentration or by altering intracellular Ca^{2+} buffers. Strikingly, this Ca^{2+}-dependent mechanism resulted in distinct modulation in the two cell types when activating G_q-coupled metabotropic receptors, such that activation of muscarinic receptors or metabotropic glutamate receptors typically depressed NMDA responses in CA3 PCs but potentiated them in CA1 PCs.

It is well established that Ca^{2+} influx through NMDARs triggers a negative feedback process that inactivates NMDA receptors through the activation of various Ca^{2+}-dependent proteins, including calmodulin and Ca^{2+}-dependent phosphatases (Kotecha et al., 2003). On the other hand, NMDA responses can be potently upregulated by activation of G_q-coupled metabotropic receptors via a protein kinase C (PKC)-dependent (Ben Ari et al., 1992; Lu et al., 1999; Kotecha and MacDonald, 2003) or a PKC-independent pathway (Benquet et al., 2002; Heidinger et al., 2002), both of which culminate in Src-mediated tyrosine phosphorylation of NMDARs (Ali and Salter, 2001). Our data demonstrate that both the positive and negative modulatory pathways are present and converge on NMDARs in CA1 and CA3 PCs but that a difference in the balance between opposing signaling cascades can result in an opposite net modulatory effect. Both potentiation and depression of NMDA responses after metabotropic glutamate receptor activation have been reported (for review, see Benquet et al., 2002; Kotecha and MacDonald, 2003). Likewise, stimulation of muscarinic receptors potentiates NMDARs in CA1 PCs (Markram and Segal, 1990), whereas we found that depression predominated in CA3 PCs. We propose that cell type-specific differences in intrinsic Ca^{2+} signaling or buffering and the intracellular Ca^{2+} concentrations set by the experimental conditions determine which modulatory pathway prevails and results in either potentiation or depression of NMDARs by metabotropic receptors.

Although an increase in intracellular Ca^{2+} concentration primarily depresses NMDARs, very strong Ca^{2+} buffering blocks the potentiation (Calabresi et al., 1998; Skeberdis et al., 2001; Kotecha and MacDonald, 2003). The absolute requirement for Ca^{2+} in the potentiating pathways reflects the presence of the Ca^{2+}-dependent proteins PKC or calmodulin, both of which activate the tyrosine kinase proline-rich tyrosine kinase-Z/cell-adhesion kinase-β that, in turn, induces Src-dependent phosphorylation of NMDARs (Heidinger et al., 2002; Kotecha and

MacDonald, 2003). Therefore, metabotropic receptor activation will maximally potentiate NMDARs under physiological conditions when concentrations of intracellular Ca^{2+} are low, and shifts in intracellular Ca^{2+} concentration in either direction will reduce NMDAR potentiation or induce depression (i.e., a bell-shaped curve).

The signaling pathways leading to potentiation of NMDARs have been mapped out in considerable detail. In contrast, the processes underlying the Ca^{2+}-dependent inactivation of NMDA receptors are less understood. A number of Ca^{2+}-dependent proteins, including calmodulin, calcineurin, PKC, and α-actinin-2, are implicated in the regulation of NMDAR function (Rycroft and Gibb, 2002). Of particular interest in light of our data are the recent reports that striatal-enriched protein tyrosine phosphatase (STEP), which is also expressed in hippocampus, is activated by calcineurin as a result of Ca^{2+} entry through NMDARs (Paul et al., 2003). Thus, the positive and negative signaling pathways modulating NMDARs can target the tyrosine kinase Src and the tyrosine phosphatase STEP, respectively, and bi-directionally control the phosphorylation state of NMDARs (Pelkey et al., 2002). Low intracellular Ca^{2+} could therefore favor the potentiating Src-dependent pathway, increased intracellular Ca^{2+} could favor NMDAR depression via STEP, and a lack of Ca^{2+} would activate neither pathway.

How can the differences in NMDAR modulation in CA1 versus CA3 PCs be explained? Numerous factors could contribute, including (1) differential expression of the metabotropic receptors, (2) differential expression of NMDAR subunits, (3) the presence of different pathways coupling the metabotropic and NMDA receptors, and (4) differential Ca^{2+} handling. The first possibility is unlikely because experimental alteration of Ca^{2+} concentration, thereby bypassing the metabotropic receptors, led to stronger depression of NMDA currents in CA3 than in CA1 cells. With respect to the second possibility, immunohistochemical studies report similar expression patterns of NMDA receptor subunits in CA1 and CA3 PCs in rat hippocampus (Fritschy et al., 1998), including in slice cultures (Gerfin-Moser et al., 1995). In CA3 PCs, however, there is marked heterogeneity in NMDA receptor expression at mossy fiber versus associational fiber synapses (Watanabe et al., 1998) and at apical versus basal dendrites (Kawakami et al., 2003). It will be interesting to compare the consequences of metabotropic modulation of NMDARs at specific synapses in the future. Third, we predict that differences in the Ca^{2+} sensitivity of the signaling cascades coupling metabotropic receptors and NMDARs contribute to the differential responses recorded in CA1 and CA3 PCs. Fourth, our experiments provide direct evidence that differential Ca^{2+} handling is critical in determining the direction of NMDAR modulation in the two cell types. By manipulating intracellular Ca^{2+} buffering we were able, with respect to NMDAR modulation, to convert a CA3 into a CA1 PC, and vice versa. This finding suggests that there may be a substantially higher Ca^{2+} buffering capacity in CA1 PCs. An attractive candidate for endogenous Ca^{2+} buffering is calbindin-D_{28K}, which is expressed in CA1 but not in CA3 PCs (Sloviter, 1989). Furthermore, LTP in CA1 PCs is impaired by a calbindin deficiency but rescued by chelating intracellular Ca^{2+} (Jouvenceau et al., 2002). Our observation of a similar rise in free intracellular Ca^{2+} in CA1 and CA3 PCs in response to raising extracellular Ca^{2+} appears to argue against a difference in endogenous buffering capacity; however, critical signaling events may occur within functional microdomains with localized differences in Ca^{2+} concentration that cannot be resolved against the bulk changes in cytoplasmic Ca^{2+} levels or that are inaccessible to the Ca^{2+} indicators.

Our findings have a number of functional implications. Depending on the intracellular Ca^{2+} availability, metabotropic receptors can potentiate or depress NMDARs and therefore can act as a switch to determine whether synapses undergo long-term potentiation or long-term depression (Bortolotto et al., 1999). This mechanism may explain why the threshold for NMDA receptor-dependent LTP induction is lower at the Schaffer collateral–CA1 synapse than at the associational fiber–CA3 synapse (Moody et al., 1998). Furthermore, the propensity for NMDARs to become potentiated in CA1 PCs may explain, in part, the selective vulnerability of these neurons to ischemic cell death (Schmidt-Kastner and Freund, 1991), whereas in CA3 PCs the excessive Ca^{2+} influx during an excitotoxic insult may be neuroprotective by shutting down NMDARs.

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