$GABA_B$-Receptor-Mediated Inhibition of the *N*-Methyl-D-Aspartate Component of Synaptic Transmission in the Rat Hippocampus

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GABA receptor regulation of NMDA-receptor-mediated synaptic responses was studied in area CA_1 of the rat hippocampus using extracellular and intracellular recording techniques. Picrotoxin (PTX) was used to suppress $GABA_A$ inhibition and 6,7-dinitroquinoxaline-2,3-dione (DNQX) was used to suppress non-NMDA receptor-mediated responses. In this manner, we were able to avoid the complicating factors caused by potentials induced by other excitatory and inhibitory amino acid receptors. Under these conditions, large NMDA-receptor-mediated EPSPs were observed. When paired stimuli were given at interstimulus intervals from 100 to 400 msec, powerful inhibition of the second response was observed. This inhibition was reversed by the $GABA_B$ antagonists phaclofen and 2-hydroxy-saclofen; it was also depressed by removal of Mg^{2+} from the bath. Examination of non-NMDA receptor-mediated synaptic responses (determined in the presence of D-2-amino-5-phosphonovalerate and PTX) showed no such inhibition, thereby supporting the hypothesis that $GABA_B$ inhibition of NMDA EPSPs is postsynaptic. This difference in paired-pulse inhibition of NMDA and non-NMDA EPSPs leads us to conclude that there was no evidence of $GABA_B$-mediated presynaptic inhibition of excitatory transmitter release. Intracellular recordings in the presence of DNQX and PTX revealed a phaclofen-sensitive late IPSP that correlated in time with the period of inhibition of NMDA responses. Taken together, these data suggest that paired-pulse-inhibition of NMDA responses is produced by a $GABA_B$-receptor-mediated hyperpolarization of the postsynaptic membrane, causing an enhanced block of the NMDA channels by Mg^{2+}. Regulation of NMDA-mediated synaptic responses by $GABA_B$ receptors constitutes a powerful mechanism for control of a major excitatory system in hippocampal pyramidal cells.

Induction of neuronal plasticity in many systems is dependent upon activation of the NMDA subtype of excitatory amino acid receptor (Harris et al., 1984; Morris et al., 1986; Collingridge and Bliss, 1987; Morrisett et al., 1989). However, in many cases, the involvement of NMDA receptors in normal synaptic transmission is minimal because the inward currents via NMDA-operated channels are regulated in a voltage-dependent manner by Mg^{2+} (Mayer and Westbrook, 1984; Nowak et al., 1984; Herron et al., 1986). Because of the development of specific non-NMDA receptor antagonists such as 6,7-dinitroquinoxaline-2,3-dione (DNQX; Drejer and Honoré, 1988), the demonstration of NMDA-receptor-mediated synaptic potentials has been facilitated (Blake et al., 1988; Andreasen et al., 1989; Davies and Collingridge, 1989; Lambert and Jones, 1989). When, in the presence of DNQX, synaptic inhibition is reduced or blocked, one can record large depolarizing responses to NMDA receptor activation (Andreasen et al., 1989; Davies and Collingridge, 1989; Lambert and Jones, 1989; Morrisett et al., 1990b).

We have recently utilized excitatory amino acid antagonists in combination with the $GABA_A$ antagonist picrotoxin (PTX) to study NMDA-mediated synaptic potentials (NMDA EPSPs) in area CA_1 of the rat hippocampus in the presence of Mg^{2+} (Morrisett et al., 1990a,b). Very large, long-lasting NMDA-receptor-mediated synaptic potentials were recorded in the presence of these drugs. Indeed, the prominent effect of PTX upon the expression of NMDA EPSPs dramatically points up the apparent role of $GABA_A$-mediated synaptic inhibition in regulating the Mg^{2+} block of NMDA-operated channels. We now report that NMDA-mediated synaptic potentials recorded in dendritic and somatic layers of area CA_1 of the rat hippocampus exhibited marked paired-pulse depression. Investigation of this phenomenon revealed that $GABA_B$ receptors were responsible for paired-pulse inhibition of NMDA EPSPs. By this mechanism, $GABA_B$ receptors could participate in the regulation of the activity of NMDA-receptor-operated channels and, hence, the development of neuronal plasticity.

A preliminary report of these findings has been submitted (Morrisett et al., 1990a).

Materials and Methods

Male Sprague–Dawley rats (150–250 gm) were killed under $CHCl_3$ anesthesia. The hippocampi were rapidly removed, and transverse slices 625 μm thick were prepared. After the slices were allowed to equilibrate for 1 hr in artificial cerebrospinal fluid (ACSF; containing, in mM, NaCl, 120; KCl, 3.3; NaH_2PO_4, 1.23; $NaHCO_3$, 25; $CaCl_2$, 1.8; $MgSO_4$, 1.2; dextrose, 10; continually gassed with 95% O_2:5% CO_2; pH, 7.4; 32°C), a midtemporal slice was transferred to a superfusion chamber (which contained 1.0 mM $MgSO_4$ and 3.0 mM $CaCl_2$ in the ACSF unless otherwise indicated). Monophasic, constant current stimulus pulses (100 μsec, 100–1000 μA) were generated with a Dagan S-900 stimulator and were applied through a tungsten stimulus electrode to the Schaffer collaterals in the stratum radiatum of area CA_1.

Extracellular population field potentials were recorded from the stratum radiatum and stratum pyramidale of area CA_1 using glass microelectrodes filled with 150 mM NaCl (1–3 MΩ; Fig. 1). Extracellular

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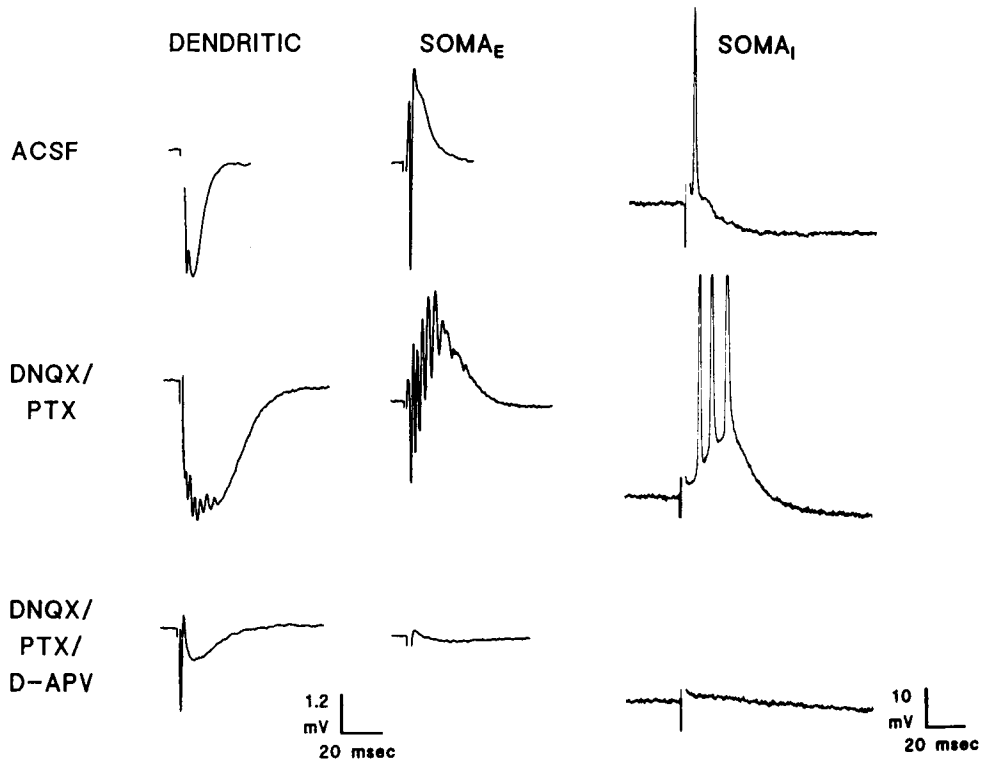

Figure 1. Isolation of NMDA-mediated responses in area CA_1. Population responses recorded from the dendritic and somatic (extracellular) electrodes ($SOMA_E$) are shown in the *first 2 columns.* The *third column* shows responses from the intracellular electrode ($SOMA_I$). In the *top row,* responses recorded in the presence of normal ACSF are presented. In the *bottom 2 rows,* responses recorded in the presence of the non-NMDA antagonist DNQX (10 μM) and the $GABA_A$ antagonist PTX (10 μM) are presented. In the *bottom row,* the effect of the NMDA receptor antagonist D-APV (50 μM) upon the NMDA EPSP induced by DNQX/PTX is shown. Stimulus artifacts were omitted for clarity.

recording from the stratum radiatum in ACSF typically elicited responses (see Fig. 1) consisting of a negative-going presynaptic fiber volley, immediately followed by a negative-going population field potential, reflecting the dendritic excitatory postsynaptic potential. Simultaneous extracellular recording from the stratum pyramidale resulted in a negative-going orthodromic population spike superimposed upon a positive-going EPSP. Slices were monitored until stable input–output curves were established. A slice was rejected if the maximal dendritic EPSP recorded from the stratum radiatum was of 1.0 mV amplitude or less.

Intracellular recordings were made from the stratum pyramidale of CA_1 using microelectrodes filled with 4 M K^+ acetate (approximately 100 MΩ). Intracellular recording typically elicited positive-going EPSPs and action potentials, followed by early and late IPSPs. In every case, CA_1 pyramidal cells were electrophysiologically identified by stimulation of the alveus and intracellular recording of antidromic action potentials. Neurons were considered acceptable for intracellular recording if all of the following criteria were met: (1) The input resistance was greater than 30 MΩ, (2) the action-potential spike height was greater than 50 mV, and (3) the resting membrane potential was less than −60 mV. A total of 11 neurons was utilized for this study.

To elicit the NMDA component of synaptic transmission (NMDA EPSP) in the presence of Mg^{2+}, 10 μM DNQX and 10 μM picrotoxin were added to the ACSF. The response typically became stable within 30–45 min, and input–output curves were obtained for the NMDA EPSPs and intracellular responses.

Extracellular dendritic waveforms were analyzed on a Compaq 386s computer using Waveform Basic software (Blue Feather Software for Nicolet Corp., Madison, WI). A program, written in this laboratory in Waveform Basic language was used to calculate the area under the extracellular dendritic responses potentials. This value, in units of mV·msec, is assumed proportional to the charge entering the population of neurons detected by the recording electrode. In all extracellular experiments, every measurement was made at least 3 times and the mean calculated (1 slice/animal). All drugs and compounds were from Sigma (St. Louis, MO), except DNQX, D-amino-5-phosphonovalerate (D-APV), 2-hydroxy-saclofen, and phaclofen were from Tocris Neuramin (Essex, England).

Results

The top row of Figure 1 depicts typical responses recorded from dendritic and somatic sites in area CA_1. Although not shown in Figure 1, when the non-NMDA receptor antagonist DNQX (10 μM) was added to the bath, postsynaptic responses were completely abolished. As shown in the middle row of Figure 1, when the $GABA_A$ channel antagonist PTX (10 μM) was added in combination with DNQX to the bath, excitatory post synaptic potentials were recorded from the dendritic extracellular electrode. These depolarizations (at maximal stimulus intensity) were about the same amplitude as the control EPSP but of much greater duration; thus, the area under the NMDA EPSP was approximately 3 times that of the control EPSP.

The excitatory responses recorded in the combination of DNQX and PTX were presumed to be initiated by activation of NMDA receptors. NMDA EPSPs were accompanied by repetitive cell firing in both individual cells and in the population response. Addition of the NMDA receptor antagonist D-APV to the bath containing DNQX and PTX almost completely abolished the EPSP recorded extracellularly and blocked cell firing. In separate experiments, these responses have been demonstrated to be almost completely blocked by the NMDA channel antagonist MK-801 (10 μM; data not shown). For these reasons, the response recorded from the dendritic electrode in the presence of DNQX/PTX was termed the NMDA EPSP.

Non-NMDA EPSPs were also studied. These EPSPs were obtained in the presence of D-APV (50 μM) and PTX (10 μM). These non-NMDA responses were not significantly different from control (normal ACSF) responses, except that they were prolonged about 30%, and that somatic responses included re-

petitive cell firing, which was never seen in control responses. Non-NMDA responses were blocked completely by DNQX (10 μM).

We first compared paired stimulation of NMDA and non-NMDA EPSPs as demonstrated in Figure 2*A*. There were marked differences seen when pairing NMDA EPSPs (Fig. 2*A*, left). At 50 msec between stimuli, there was prominent paired-pulse potentiation of the NMDA EPSPs. However, the second response markedly decreased in amplitude and duration at interstimulus intervals between 100 and 400 msec. Maximal paired-pulse inhibition was noted at 200 msec. This inhibitory effect was independent of stimulus intensity and could be produced from both distant and nearby stimulation sites within the stratum radiatum of CA_1 relative to the recording electrode in the stratum radiatum.

There was no apparent paired-pulse inhibition of non-NMDA EPSPs at any interstimulus interval (Fig. 2*A*, right). In fact, at intervals of 50 and 100 msec, the second response was slightly (30%) potentiated.

The effect of varying the interstimulus interval on non-NMDA and NMDA EPSPs is compiled in Figure 2*B*. Paired-pulse potentiation of both NMDA and non-NMDA EPSPs is shown at intervals of 50 and 100 msec, while profound inhibition of only NMDA EPSPs occurred at the 200-msec interval. These 2 curves are significantly different by Kruskal–Wallis ($p < 0.001$).

The time course of paired-pulse inhibition of NMDA EPSPs follows closely the known time course of the late IPSP induced by activation of the $GABA_B$ receptor subtype (Alger, 1984; Dutar and Nicoll, 1988a). We hypothesized that addition of the $GABA_B$ receptor antagonist phaclofen to the bath would reverse the 200-msec inhibition of the NMDA component of synaptic transmission. Figure 3 shows the effect of phaclofen (667 μM) on NMDA EPSPs and somatic responses recorded both intracellularly and extracellularly (left and middle columns). Inhibition of the NMDA EPSP recorded at 200 msec was almost completely abolished in the presence of phaclofen, so that the size of each NMDA EPSP was equivalent.

Intracellular recordings in Figure 3 (right column) are shown at the reversal potential for the $GABA_B$ IPSP at the soma. This allowed us to demonstrate in the clearest manner the ability of phaclofen to prevent the 200-msec inhibition of the NMDA EPSPs. Note that, at this membrane potential, the $GABA_B$ IPSP, as recorded from the somatic site, is not hyperpolarizing; however, the NMDA EPSP and its paired-pulse inhibition are still seen. Presumably, the dendritic site of the NMDA EPSP is quite electrically remote from the cell body, and thus, hyperpolarization of the soma by current injection does not sufficiently hyperpolarize the dendrites to suppress these processes (see Discussion). [Phaclofen also reversed the inhibition of the NMDA EPSPs when recorded at resting membrane potentials (data not shown).]

The input resistance of several cells in DNQX/PTX was measured by current injection at membrane potentials equivalent to the peak of the late IPSP and during the late IPSP itself (data not shown). The input resistance during the late IPSP was only slightly less (20%) than the resistance tested at membrane potentials equivalent to the peak of the late IPSP. This conductance change also occurs following non-NMDA EPSPs, yet no paired-pulse inhibition of these EPSPs was demonstrated. Because it is likely that non-NMDA- and NMDA-receptor-operated channels occupy sites very close to one another (Monaghan and Cotman, 1985; Cotman et al., 1987), one would expect that

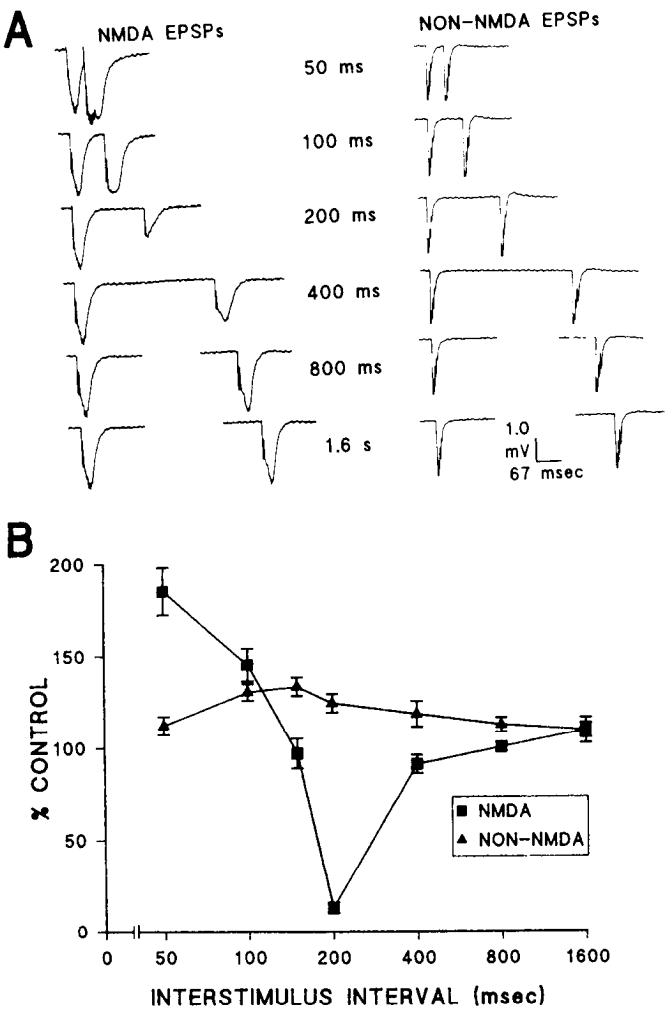

Figure 2. *A*, Paired stimulation of NMDA and non-NMDA EPSPs. Responses are dendritic EPSPs induced by DNQX/PTX treatment (*NMDA EPSPs*) on the *left panel* and APV/PTX treatment (*non-NMDA EPSPs*) on the *right panel*. Paired-pulse potentiation (50–100 msec) and inhibition (100–400 msec) of NMDA EPSPs was apparent, while non-NMDA EPSPs were only slightly enhanced by pairing. Note that the duration of NMDA EPSPs was much longer than non-NMDA EPSPs. *B*, Time course of effect of pairing stimuli on NMDA and non-NMDA EPSPs. Inhibition of NMDA EPSPs was maximal at 200 msec, non-NMDA EPSPs displayed slight increases in response to pairing of stimuli. Data are mean ± SEM; $n = 5$–10 slices; *curves* significantly different by Kruskal–Wallis; $p < 0.001$.

resistance changes, if significant, would affect both responses. Taken together, these data strongly suggest that changes in input resistance during the $GABA_B$ IPSP are not sufficient to explain the paired-pulse inhibition of NMDA EPSPs.

The dose–response relationship of the phaclofen block was studied. Figure 4*A* depicts the effect of varying phaclofen concentrations (333–1000 μM) upon paired-pulse inhibition of NMDA EPSPs induced at varying stimulus intensities. The ability of phaclofen to block inhibition of the NMDA EPSPs was dose related. Also, in the presence of phaclofen, there was a significant relationship between stimulus intensity and the degree of paired-pulse inhibition of NMDA EPSPs (as opposed to the control response). The low concentration of phaclofen tested (333 μM) decreased inhibition at low stimulus intensities by about 50%, while at maximal stimulus intensities, inhibition

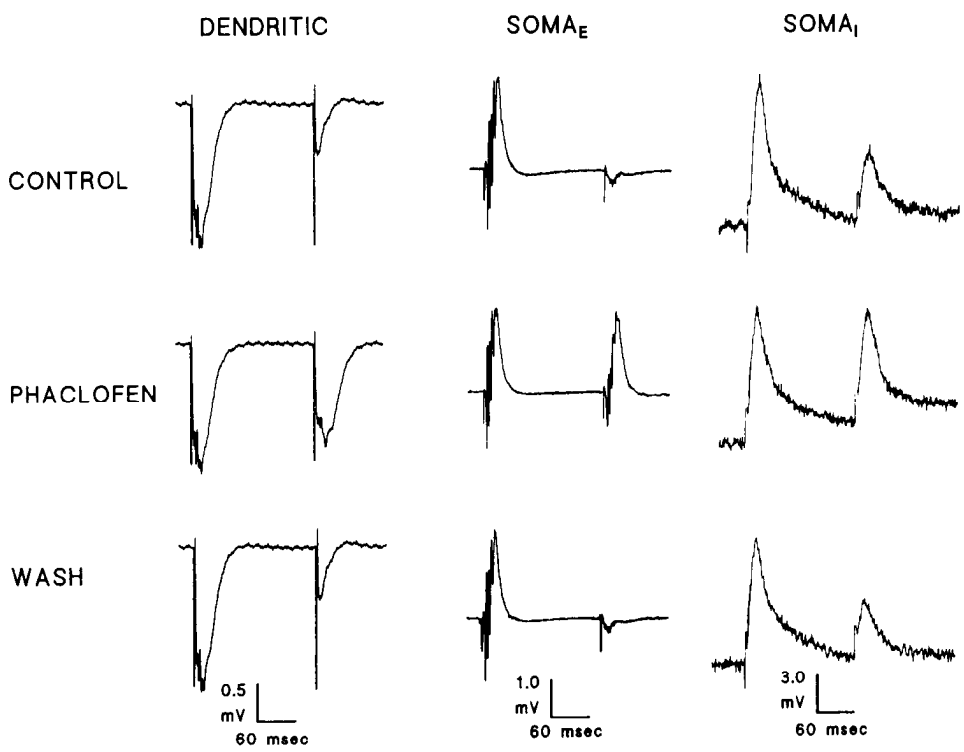

Figure 3. Paired-pulse inhibition of NMDA EPSPs recorded from dendritic and somatic (extracellular *SOMA$_E$*; intracellular, *SOMA$_I$*) layers of CA_1 was blocked by the $GABA_B$ receptor antagonist phaclofen (667 μM). The intracellular data were recorded at the reversal potential for the $GABA_B$ IPSP (−92 mV) for clarity. Phaclofen almost completely blocked paired-pulse inhibition at 200 msec in a manner that was reversible upon washout of the drug. An identical result was obtained in the presence of 2-hydroxy-saclofen.

was completely blocked. Higher concentrations of phaclofen (667 μM, 1 mM) had more pronounced stimulus dependencies. At lower stimulus intensities (<600 μA), these concentrations of phaclofen produced potentiation of the second NMDA EPSP. However, at higher stimulus intensities (>600 μA), there was no potentiation or inhibition of paired responses (see Discussion). In all cases, the maximum areas of the first NMDA component measured in the presence of phaclofen did not differ significantly from control. However, there was a slight rightward shift (approximately 30%) in the input–output relationship caused by phaclofen.

The effect of phaclofen was always reversible within 15 min (Fig. 4*A*, wash). Furthermore, the more potent $GABA_B$ antagonist 2-hydroxy-saclofen (200 μM) completely reversed inhibition of the NMDA EPSPs ($n = 4$). In a separate but related set of experiments, the effect of phaclofen (667 μM) upon non-NMDA EPSPs was studied. In no case did the $GABA_B$ antagonist alter the amplitude or duration of non-NMDA EPSPs, either in single stimulation or in pairs over various interstimulus intervals (50–1600 msec; $n = 4$).

The block of paired-pulse inhibition by $GABA_B$ antagonists suggests that a late IPSP mediated by $GABA_B$ receptors might be responsible for suppression of the paired response. Therefore, we felt that direct examination of the late IPSP following NMDA EPSPs was necessary. Figure 4*B* depicts intracellular recordings from CA_1 pyramidal cells in the presence of DNQX/PTX. In the control response following the EPSP, a prolonged hyperpolarization of approximately 12 mV is present. Addition of phaclofen (667 μM) partially antagonized the late IPSP, leaving the EPSP and action potential unchanged except for a small shift in the input–output relationship. The effect of phaclofen on the late IPSP was completely reversible upon washout. The final column of Figure 4*B* shows the phaclofen-sensitive late inhibition by digital subtraction of the first 2 recordings, thus demonstrating that $GABA_B$-mediated inhibition was present.

Because the NMDA channel is increasingly blocked by Mg^{2+} as the membrane is hyperpolarized (Mayer and Westbrook, 1984; Nowak et al., 1984), we hypothesized that the paired-pulse inhibition of the NMDA EPSP resulted from the hyperpolarizing effects of $GABA_B$ inhibition. If this were the case, then removal of Mg^{2+} from the ACSF would suppress the paired-pulse inhibition of the NMDA EPSP, because the Mg^{2+}-dependent block would be prevented. Therefore, we studied the effect of varying the concentration of Mg^{2+} upon NMDA EPSPs in comparison to non-NMDA EPSPs.

Figure 5, *A* and *B*, depicts the sensitivity of NMDA and non-NMDA EPSPs to Mg^{2+} and shows that the paired-pulse inhibition of NMDA EPSPs was dependent upon Mg^{2+}. As indicated in Figure 5*A*, the NMDA EPSPs were extremely sensitive to Mg^{2+}. As the concentration of Mg^{2+} was increased from no added Mg^{2+} to 3.16 mM, NMDA EPSPs decreased in area approximately 10-fold (from 200 to 20 mV·msec). The apparent median effective concentration (EC_{50}) of the NMDA component to Mg^{2+} was approximately 500 μM. Non-NMDA EPSPs were minimally altered by addition of Mg^{2+}.

Figure 5*B* shows an example of the effect of varying Mg^{2+} concentration upon the 200-msec inhibition. Mg^{2+} appeared to inhibit the 200-msec response more readily than the first response. This difference in Mg^{2+} sensitivity was especially evident at 500 μM and 1 mM Mg^{2+}, where the 200-msec NMDA EPSPs were reduced almost completely, while the initial responses were still quite large. These data are presented cumulatively in Figure 5*C*, showing the Mg^{2+} dependence of the 200-msec inhibition. The size of the second response relative to the first was dose related to Mg^{2+}, with an apparent EC_{50} of approximately 500 μM Mg^{2+}. In the absence of added Mg^{2+}, there is apparently

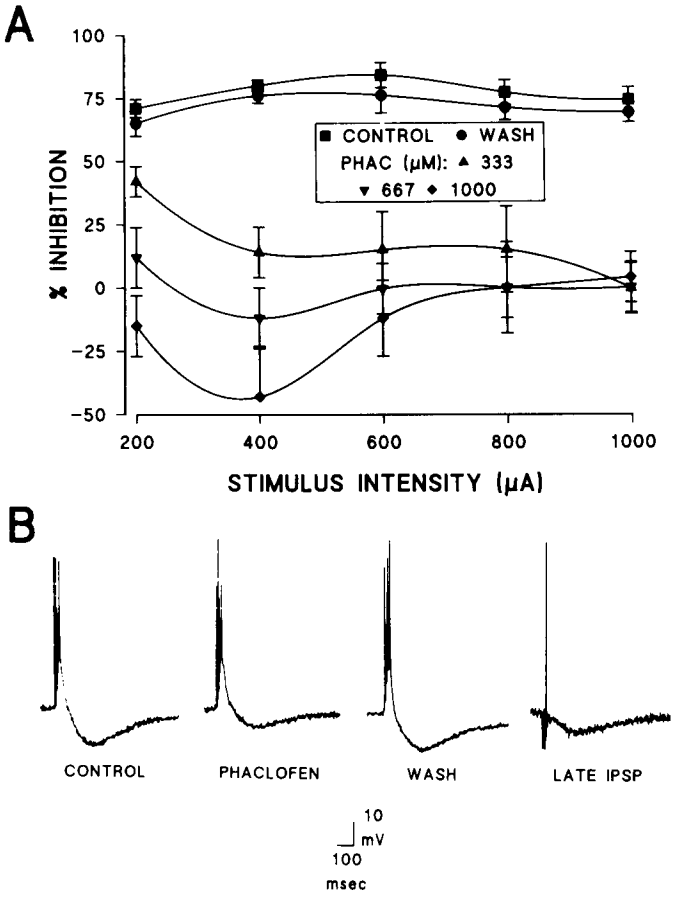

Figure 4. *A*, Dose response of antagonism of paired-pulse inhibition of NMDA EPSPs by phaclofen at different stimulus intensities. In control media (DNQX/PTX), pairing of stimuli at various intensities elicited inhibition of approximately 75%. Addition of phaclofen to the media resulted in a dose-dependent reduction (333–1000 μM) in paired-pulse inhibition. Biphasic effects of phaclofen were observed to be dependent upon stimulus intensity. At low intensity, phaclofen tended to induce paired-pulse potentiation, while at greater intensities, no effect of pairing of stimuli was seen. In every case, the effects were reversible. Data are mean ± SEM; n = 4–8 slices. *B*, Phaclofen-sensitive late IPSP follows NMDA-mediated EPSP and action potentials in pyramidal cell of area CA_1. Control response was recorded in DNQX/PTX; note the prominent IPSP following EPSP, with no early IPSP. Addition of phaclofen (667 μM) resulted in an almost complete block of late IPSP, with no effect upon excitatory response. Response to phaclofen was reversible upon washout. Late IPSP is the digital subtraction of the response recorded in phaclofen from the control response indicating the degree of antagonism of the late IPSP in this cell by phaclofen. Membrane potential, −67 mV. This response was consistent in 5 of 5 cells tested in 5 separate slices.

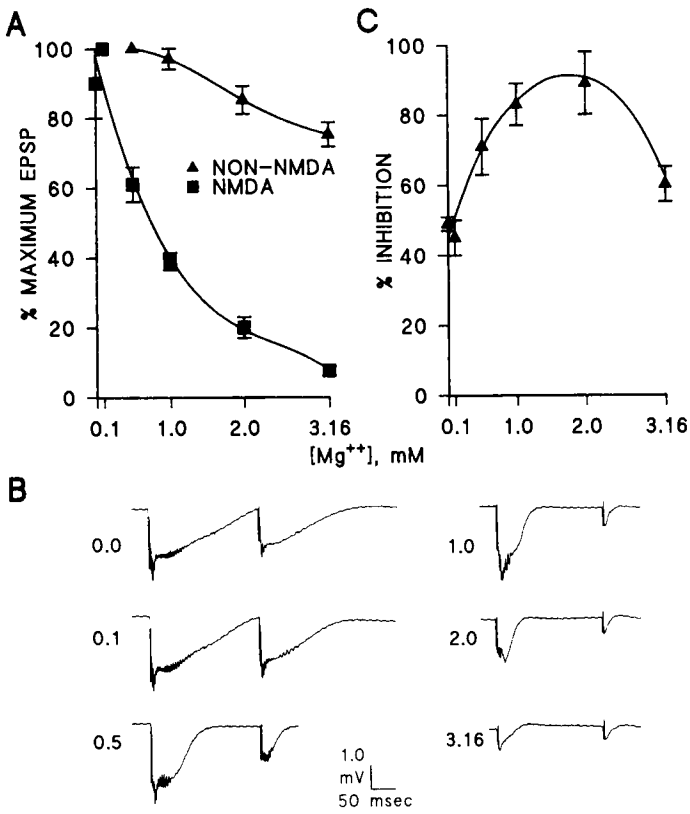

Figure 5. *A*, Mg^{2+} inhibits NMDA but not non-NMDA EPSPs. Dose response of the effect of Mg^{2+} (0.0–3.16 mM) upon NMDA and non-NMDA EPSPs in area CA_1. Data are mean ± SEM; n = 5–12 slices. *B*, Paired-pulse inhibition of NMDA EPSPs is Mg^{2+} dependent (*numbers* at *left* of tracings are $[Mg^{2+}]_{bath}$). All NMDA EPSPs were powerfully inhibited by Mg^{2+}, but the inhibition of the paired response at 200 msec occurred at much lower concentrations of Mg^{2+}. For example, note that the responses in 0.1 mM Mg^{2+} are almost identical, while in 1 mM Mg^{2+}, there was a large decrease in the paired response at 200 msec. *C*, Mg^{2+} dependence of paired-pulse inhibition of NMDA EPSPs. Inhibition of NMDA EPSPs was approximately doubled in the presence of 1 mM Mg^{2+} relative to responses recorded in the absence of Mg^{2+}. Increasing the concentration of Mg^{2+} above 1 mM reduced the apparent inhibition of NMDA EPSPs due to the reduction of the size of the initial response. Data are mean ± SEM; n = 4–8 slices.

sufficient residual Mg^{2+} to block NMDA channels to a significant extent. Above 1 mM Mg^{2+}, no further inhibition could be induced, primarily because all NMDA EPSPs were largely blocked at those concentrations of Mg^{2+}.

Discussion

The primary results from these experiments include the following: (1) NMDA EPSPs induced by DNQX/PTX exhibit sensitivity to competitive and noncompetitive NMDA antagonists, (2) NMDA EPSPs exhibit paired-pulse inhibition of a particularly specific time frame (between 100 and 400 msec, peak at 200 msec) while non-NMDA EPSPs displayed no paired-pulse inhibition, (3) the mechanism of the paired-pulse inhibition of NMDA EPSPs appears to depend upon activation of $GABA_B$ receptors because phaclofen and 2-hydroxy-saclofen reversed it in a dose-dependent manner at pharmacologically effective concentrations, and (4) the mechanism of paired-pulse inhibition appears to be dependent upon Mg^{2+} block of the NMDA-receptor-channel complex, because dependence of inhibition upon Mg^{2+} could be demonstrated.

Reversal of inhibition of NMDA EPSPs with $GABA_B$ antagonists

Evidence indicates that late IPSPs are mediated by activation of $GABA_B$ receptors (Newberry and Nicoll, 1984; Dutar and Nicoll, 1988a,b; Soltesz et al., 1988; Lambert et al., 1989). The late IPSP is likely due to $GABA_B$ receptor regulation of a K^+ channel in a G-protein-dependent mechanism (Andrade et al., 1986; Dutar and Nicoll, 1988b; Nicoll, 1988). Phaclofen and 2-hydroxy-saclofen are specific antagonists of $GABA_B$ receptors (Dutar and Nicoll, 1988; Hasuo and Gallagher, 1988; Kerr et al., 1989).

$GABA_B$ antagonists had potent effects upon paired-pulse inhibition of NMDA EPSPs, while non-NMDA EPSPs were not changed. The relative potencies of these antagonists in blocking paired-pulse inhibition of NMDA EPSPs agrees well with previously published studies (Hasou and Gallagher, 1988; Kerr et al., 1989). As seen in Figure 4*A*, it was readily apparent that higher concentrations of phaclofen (667 and 1000 μM) fully blocked inhibition at higher stimulus intensities. However, at lower stimulus intensities, paired-pulse potentiation was revealed by the highest concentration of phaclofen. This stimulus intensity dependence may be due to several mechanisms: (1) At higher stimulus intensities, the increased release of endogenous GABA may compete more effectively with phaclofen, therefore preventing the effect of phaclofen to fully reverse paired pulse inhibition; (2) $GABA_B$ antagonists may regulate GABA and glutamate release by way of presynaptic $GABA_B$ receptors differently at different stimulus intensities; and (3) the paired-pulse potentiation of the NMDA response might exist only at low stimulus intensities and thus be uncovered as inhibition is blocked.

In most cases, the $GABA_B$ antagonists did not fully suppress the hyperpolarization after a stimulation. We suspect that the residual potential remaining in the presence of $GABA_B$ antagonists is an afterhyperpolarization (AHP) due to the Ca^{2+}-activated K^+ conductance. There is good precedent for such AHPs, especially following repetitive cell firing, which may induce Ca^{2+} spikes and therefore activate Ca^{2+}-dependent K^+ conductances, therefore decreasing cell excitability (Dingledine, 1983).

Mechanism of $GABA_B$-mediated inhibition of NMDA EPSPs

Dendritic $GABA_B$ receptors in area CA_1 have been demonstrated using both electrophysiologic and autoradiographic techniques (Alger et al., 1984; Newberry and Nicoll, 1984; Bowery et al., 1987). $GABA_B$ receptors that exist on afferent terminals may inhibit transmitter release (Hill and Bowery, 1981; Dutar and Nicoll, 1988a,b). In the present study, it is conceivable that $GABA_B$ receptors located on terminals of Schaffer collaterals are activated by inhibitory interneurons and subsequently inhibit transmitter release by regulating Ca^{2+} levels in the terminal. On the other hand, it is also possible that the relevant $GABA_B$ receptors are located postsynaptically on the dendrites of CA_1 pyramidal cells. Activation of these receptors may increase the Mg^{2+} block of the NMDA channel (Nowak et al., 1984; Mayer and Westbrook, 1984; Mayer et al., 1984).

The data presented here suggest that there is a postsynaptic localization of $GABA_B$ receptors that regulate NMDA EPSPs. Inhibition of transmitter release does not appear to be the mechanism of the paired-pulse inhibition of NMDA EPSPs due to the following observations: (1) that NMDA EPSPs and *not* non-NMDA EPSPs displayed paired-pulse inhibition, and (2) that paired-pulse inhibition of NMDA EPSPs was reversed by $GABA_B$ receptor antagonists, while $GABA_B$ antagonists had no effect upon non-NMDA EPSPs. Thus, the mechanism of inhibition of NMDA EPSPs most likely involves systems that are specific to the NMDA receptor channel/complex. This observation supports the hypothesis that the mechanism of inhibition of NMDA EPSPs is dependent upon $GABA_B$ modulation of the voltage-dependent block of NMDA-operated channels by Mg^{2+} because it is well known that non-NMDA-operated channels display little Mg^{2+} sensitivity.

A presynaptic locus of $GABA_B$-mediated inhibition of NMDA EPSPs is conceivable, but unlikely. If NMDA and non-NMDA postsynaptic receptors were separately innervated, with presynaptic terminals of only the NMDA system containing $GABA_B$ receptors, then paired-pulse inhibition of only the NMDA EPSPs would be explained. Nevertheless, it is well known that activation of presynaptic $GABA_B$ receptors with exogenous agonists potently inhibits responses due to release of endogenous excitatory amino acids (Hill and Bowery, 1981; Dutar and Nicoll, 1988a,b). Thus, while there may be evidence for $GABA_B$-receptor-mediated presynaptic regulation of glutamate release, the process does not occur to a significant extent following a single stimulation in this model.

Our intracellular experiments showed that, when the soma of the CA_1 cell was hyperpolarized from the resting potential to the $GABA_B$ reversal potential (about -90 mV), NMDA EPSPs were still present, as was paired-pause inhibition of these EPSPs (see Fig. 3). It is well known that marked inhibition of NMDA-mediated processes occurs when cells are hyperpolarized from about -30 mV (Andreasen et al., 1989; Hestrin et al., 1990). We assume that, in our hyperpolarized cells, there is a minimal contribution to the EPSPs from somatic or electrically nearby (dendritic) NMDA channels. Therefore, our NMDA EPSPs almost certainly result from channels on electrically remote dendrites that are not hyperpolarized by somatic current injection. Indeed, paired-pulse inhibition of these EPSPs is retained during this somatic hyperpolarization, suggesting that the source of $GABA_B$ inhibition is also present locally at the dendrites.

Physiologic significance of $GABA_B$ inhibition of NMDA EPSPs

The findings of this study indicate that pairing of stimuli 200 msec apart can suppress NMDA-mediated depolarizations. However, Lynch and coworkers have shown that a single conditioning stimulus given 200 msec before a weak stimulus train will facilitate NMDA depolarizations and the induction of long-term potentiation (Larson and Lynch, 1986; Muller and Lynch, 1988). They suggest that this occurs because the conditioning stimulus suppresses $GABA_A$-mediated inhibition and thus permits depolarization and opening of NMDA channels by the subsequent stimulus train. Our laboratory and others have shown that this disinhibition may be mediated by $GABA_B$ receptors acting to *promote* NMDA function by decreasing GABAergic inhibition (Mott et al., 1989, 1990a,b; Davies et al., 1990). Nevertheless, in the present study, $GABA_B$ receptors had opposite effects to *suppress* NMDA function.

This work indicates that $GABA_B$-mediated responses can modulate the activity of the NMDA-receptor-channel complex under certain circumstances. This process appears to be related to the proximity of the $GABA_B$ and NMDA channels on pyramidal cell dendrites. It is entirely possible that, during normal (and/or excitable) states of hippocampal activity, $GABA_B$ receptors may locally regulate currents through NMDA channels. Through such a mechanism, it seems that the function of NMDA-mediated processes may be regulated within these discrete dendritic regions. Therefore, $GABA_B$ modulation of NMDA responses is potentially an important mechanism for the regulation of neuronal plasticity and other NMDA-dependent processes.

References

Alger BE (1984) Characteristics of a slow hyperpolarizing synaptic potential in rat hippocampal pyramidal cells *in vitro*. J Neurophysiol 52:892–910.

Andrade R, Malenka RC, Nicoll RA (1986) A G-protein couples

serotonin and $GABA_B$ to the same channels in hippocampus. Science 234:1261–1265.

Andreasen M, Lambert JDC, Jensen MS (1989) Effects of non-*N*-methyl-D-aspartate antagonists on synaptic transmission in the *in vitro* rat hippocampus. J Physiol (Lond) 414:317–336.

Blake JF, Brown MW, Collingridge GC (1988) CNQX blocks acidic amino acid induced depolarizations and synaptic components mediated by non-NMDA receptors in rat hippocampal slices. Neurosci Lett 89:182–186.

Bowery NG, Hudson AL, Price GW (1987) $GABA_A$ and $GABA_B$ receptor site distribution in the rat central nervous system. Neuroscience 20:365–383.

Collingridge GL, Bliss TVP (1987) NMDA receptors—their role in long-term potentiation. Trends Neurosci 10:288–293.

Cotman CW, Monaghan DT, Ottersen OP, Storm-Mathisen J (1987) Anatomical organization of excitatory amino acid receptors and their pathways. Trends Neurosci 10:273–280.

Davies CH, Davies SN, Collingridge GL (1990) Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. J Physiol (Lond) 424:513–531.

Davies SN, Collingridge GL (1989) Role of excitatory amino acid receptors in synaptic transmission in area CA_1 of rat hippocampus. Proc R Soc Lond [Biol] 236:373–384.

Dingledine R (1983) *N*-methyl aspartate activates voltage-dependent calcium conductance in rat hippocampal pyramidal cells. J Physiol (Lond) 343:385–405.

Drejer J, Honoré T (1988) New quinoxalinediones show potent antagonism of quisqualate responses in cultured mouse cortical neurons. Neurosci Lett 87:104–108.

Dutar P, Nicoll RA (1988a) A physiological role for $GABA_B$ receptors in the central nervous system. Nature 332:156–158.

Dutar P, Nicoll RA (1988b) Pre- and postsynaptic $GABA_B$ receptors in the hippocampus have different pharmacological profiles. Neuron 1:585–591.

Harris EA, Ganong A, Cotman CW (1984) Long-term potentiation in the hippocampus involves activation of *N*-methyl-D-aspartate. Brain Res 323:132–137.

Hasuo H, Gallagher JP (1988) Comparison of antagonism by phaclofen of baclofen induced hyperpolarizations and synaptically mediated late hyperpolarizing potentials recorded intracellularly from rat dorsolateral septal neurons. Neurosci Lett 86:77–81.

Herron CE, Lester RAJ, Coan EJ, Collingridge GC (1986) Frequency-dependent involvement of NMDA receptors in the hippocampus: a novel synaptic mechanism. Nature 322:265–268.

Hestrin SR, Nicoll RA, Perkel DJ, Sah P (1990) Analysis of excitatory synaptic action in pyramidal cells using whole-cell recording from rat hippocampal slices. J Physiol (Lond) 422:203–225.

Hill DR, Bowery NG (1981) [^{3}H]baclofen and [^{3}H]GABA bind to bicuculline-insensitive $GABA_B$ sites in rat brain. Nature 290:19–152.

Kerr BIB, Ong J, Johnston GAR, Prager RH (1989) $GABA_B$-receptor-mediated actions of baclofen in rat isolated neocortical slice preparations: antagonism by phosphono-analogues of GABA. Brain Res 480:312–316.

Lambert JDC, Jones RSG (1989) Activation of *N*-methyl-D-aspartate receptors contribute to the EPSP at perforant path synapses in the rat dentate gyrus *in vitro*. Neurosci Lett 97:323–328.

Lambert NA, Harrison NL, Kerr DIB, Ong J, Prager RH, Teyler TJ (1989) Blockade of the late IPSP in rat CA_1 hippocampal neurons by 2-hydroxy-saclofen. Neurosci Lett 107:125–128.

Larson J, Lynch G (1986) Induction of synaptic potentiation in hippocampus by patterned stimulation involves two events. Science 232: 985–988.

Mayer ML, Westbrook GL (1984) Mixed-agonist action of excitatory amino acids on mouse spinal cord neurones under voltage clamp. J Physiol (Lond) 354:29–53.

Mayer ML, Westbrook GL, Guthrie PB (1984) Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. Nature 309:261–263.

Monaghan DT, Cotman CW (1985) Distribution of NMDA-sensitive L-[^{3}H]-glutamate-binding sites in rat brain. J Neurosci 5:2909–2919.

Morris RGM, Anderson EA, Lynch GS, Baudry M (1986) Selective impairment of learning and blockade of long term potentiation by an NMDA antagonist, AP5. Nature 319:774–776.

Morrisett RA, Chow CC, Nadler JV, McNamara JO (1989) Biochemical evidence for enhanced sensitivity to NMDA in the hippocampal formation of kindled rats. Brain Res 496:25–28.

Morrisett RA, Mott DD, Swartzwelder HS, Lewis DV, Wilson WA (1990a) $GABA_B$ receptor mediated inhibition of the NMDA component of synaptic transmission. Soc Neurosci Abstr 16:463.

Morrisett RA, Mott DD, Lewis DV, Wilson WA, Swartzwelder HS (1990b) Reduced sensitivity of the *N*-methyl-D-aspartate component of synaptic transmission to Mg^{2+} in hippocampal slices of immature rats. Brain Res, in press.

Mott DD, Lewis DV (1990) Fading of recurrent inhibition in the rat dentate gyrus is mediated by $GABA_B$ receptors. Soc Neurosci Abstr 16:945.

Mott DD, Ferrari CM, Lewis DV, Wilson WA, Swartzwelder HS (1989) Baclofen facilitates the induction of long term potentiation. Proc $GABA_B$ Symp 1:47.

Mott DD, Bragdon AC, Lewis DV (1990) Phaclofen antagonizes post-tetanic disinhibition in rat dentate gyrus. Neurosci Lett 110:131–136.

Muller D, Lynch G (1988) Long-term potentiation differentially affects two components of synaptic responses in hippocampus. Proc Natl Acad Sci USA 85:9346–9350.

Newberry NR, Nicoll RA (1984) A bicuculline-resistant inhibitory post-synaptic potential in rat hippocampal pyramidal cells *in vitro*. J Physiol (Lond) 348:239–254.

Nicoll RA (1988) The coupling of neurotransmitter receptors to ion channels in the brain. Science 241:545–551.

Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A (1984) Magnesium gates glutamate-activated channels in mouse central neurones. Nature 307:462–465.

Soltesz I, Haby M, Leresche N, Crunelli V (1988) The $GABA_B$ antagonist phaclofen inhibits the late K^+-dependent IPSP in cat and rat thalamic and hippocampal neurones. Brain Res 448:351–354.