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Long-lasting Potentiation and Epileptiform Activity Produced by $GABA_B$ Receptor Activation in the Dentate Gyrus of Rat Hippocampal Slice

Edward C. Burgard[a] and John M. Sarvey

Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799

Bath application of the $GABA_B$ receptor agonist baclofen produced a concentration-dependent long-lasting potentiation (LLP) of the evoked population spike in the dentate gyrus of rat hippocampal slices. A high concentration of baclofen (5 μM) also produced a loss of inhibition that was manifested as the appearance of epileptiform, multiple evoked population spikes and a decrease in paired-pulse inhibition. Both baclofen-induced potentiation and epileptiform activity could be blocked or significantly reduced in slices from pertussis toxin-treated animals (1 μg, intradentate) or in slices pretreated with the NMDA receptor antagonist D-(−)-2-amino-5-phosphonovaleric acid (10 μM). At a concentration that had no significant effect on individual evoked responses (0.1 μM) but still produced a loss in paired-pulse inhibition, baclofen facilitated the induction of β-adrenergic receptor-mediated LLP. LLP was induced in the dentate gyrus by bath application of 1 μM, but not 0.1 μM, isoproterenol. Coapplication of baclofen and isoproterenol, both at a concentration (0.1 μM) that individually had no effect on the population spike, produced a synergistic LLP of the population spike. We propose that baclofen produces a selective disinhibitory effect in the granule cell layer of the dentate gyrus by inhibiting the activity of GABAergic interneurons. At a low concentration, the subtle loss of inhibition can facilitate the induction of isoproterenol-induced LLP. At a high concentration, baclofen can produce an LLP that is probably induced by a loss of inhibition.

In the mammalian CNS, the major inhibitory neurotransmitter is GABA, and receptors for GABA have been divided into two main classes, $GABA_A$ and $GABA_B$ (Bowery, 1989). Since their discovery by Bowery et al. (1980), the study of $GABA_B$ receptors has been facilitated by the availability of the specific $GABA_B$ receptor agonist baclofen (β-*p*-chlorophenyl GABA; Hill and Bowery, 1981). It now appears that $GABA_B$ receptors can be subclassified according to their signal-transduction mechanisms. Different $GABA_B$ receptors may be distinguished in terms of their coupling to either Ca^{2+} (Dunlap, 1981; Dolphin and Scott, 1986) or K^+ channels (Newberry and Nicoll, 1984) or their association with pertussis toxin (PT)-sensitive GTP-binding proteins (G proteins; Andrade et al., 1986; Holz et al., 1986; Dutar and Nicoll, 1988b; Thalmann, 1988). Activation of postsynaptic hippocampal $GABA_B$ receptors results in an increased K^+ conductance (Gähwiler and Brown, 1985; Newberry and Nicoll, 1985; Rausche et al., 1989) that underlies the late IPSP following synaptic stimulation (Dutar and Nicoll, 1988a). The physiological role of this late IPSP is to hyperpolarize the neuron, decrease membrane resistance, and decrease the probability of epileptogenesis (Alger, 1984). Overall, the postsynaptic electrophysiological actions of $GABA_B$ receptor activation appear to be inhibitory. On the other hand, activation of presynaptic $GABA_B$ receptors leads to a decrease in neurotransmitter release (Potashner, 1978; Bowery et al., 1980). If this occurs on excitatory nerve terminals, the result is a net decrease in excitation. However, if this occurs on $GABA_B$ "autoreceptors" located on GABAergic nerve terminals, the result is a decrease in GABA release (Bonanno et al., 1988; Waldemeier et al., 1988) and a net decrease in inhibition. The activation of $GABA_B$ receptors on GABAergic terminals has been shown to lead to a PT-insensitive net loss of inhibition (Harrison, 1990). Both PT-sensitive (Andrade et al., 1986; Xu and Wojcik, 1986; Colmers and Williams, 1988; Dutar and Nicoll, 1988b) and PT-insensitive (Colmers and Williams, 1988; Dutar and Nicoll, 1988b; Harrison, 1990) $GABA_B$ receptor-mediated responses have been demonstrated in the CNS. The pre- and postsynaptic actions of baclofen may also be distinguished on the basis of their antagonism by the $GABA_B$ receptor antagonist phaclofen (Kerr et al., 1987). The postsynaptic late hyperpolarization appears to be more sensitive to phaclofen than is the presynaptic decrease in neurotransmitter release (Dutar and Nicoll, 1988b; Hasuo and Gallagher, 1988; Harrison, 1990).

The electrophysiological actions of baclofen have been well characterized in the hippocampal formation, especially on the pyramidal cells of fields CA1 and CA3, as mentioned above. In these areas, baclofen exhibits net inhibitory (Lanthorn and Cotman, 1981) and antiepileptic (Ault and Nadler, 1983) properties. Granule cells of the hippocampal dentate gyrus also exhibit a

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Correspondence should be addressed to Dr. John M. Sarvey, Ph.D., Department of Pharmacology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814-4799.

[a] Present address: Neurobiology Research Center, Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL 35294.

K^+-mediated late hyperpolarization in response to synaptic activation, as seen in fields CA1 and CA3 (Thalmann and Ayala, 1982; Fricke and Prince, 1984; Rausche et al., 1989) that is presumably $GABA_B$ receptor mediated. However, in the dentate gyrus of hippocampal slices, application of baclofen results in the net disinhibition of granule cell responses (Misgeld et al., 1984, 1989) and, at higher concentrations, the appearance of epileptiform activity (Burgard and Sarvey, 1989; Mott et al., 1989). Similar disinhibitory actions of other proepileptic agents such as penicillin and picrotoxin also result in the appearance of epileptiform activity within the CNS (Prince, 1978; Hablitz, 1984). One characteristic of epileptic activity is the appearance of the intracellularly recorded paroxysmal depolarizing shift (PDS; Matsumoto and Ajmone-Marsan, 1964). The PDS underlying epileptiform bursting is dependent, in part, on the activation of NMDA receptors (Dingledine et al., 1986) due to the voltage dependence of the NMDA receptor (see Mayer and Westbrook, 1987).

The dentate gyrus differs from the rest of the hippocampal formation not only in its response to baclofen, but also in its response to β-adrenergic agonists such as norepinephrine and isoproterenol. In the dentate gyrus, but not in field CA1, β-adrenergic agonists produce a long-lasting potentiation (LLP) of evoked responses (Neuman and Harley, 1983; Lacaille and Harley, 1985; Stanton and Sarvey, 1985b). β-Adrenergic LLP is similar to long-term potentiation (LTP) in the dentate gyrus in that both are forms of NMDA receptor-dependent synaptic plasticity (Burgard et al., 1989; Sarvey et al., 1989; Stanton et al., 1989; Dahl and Sarvey, 1990). β-Adrenergic LLP is also associated with a long-lasting increase in cAMP levels measured in the dentate gyrus (Stanton and Sarvey, 1985a). Biochemical studies indicate that, in brain slices, cAMP accumulation in response to β-adrenergic receptor stimulation can be synergistically increased by $GABA_B$ receptor activation (Hill and Dolphin, 1984; Karbon et al., 1984; Karbon and Enna, 1985). This synergistic activity occurs using concentrations of baclofen alone that have no effect on cAMP accumulation.

In this paper, we have made an attempt to define some of the actions of baclofen in the dentate gyrus. Our first objective was to characterize pharmacologically the disinhibitory effects of baclofen on evoked responses in the rat dentate gyrus and to determine their sensitivities to both phaclofen and PT treatment. These experiments were aimed at determining both the receptor specificity and the mechanism of $GABA_B$ signal transduction responsible for baclofen's effects. Until now, the PT sensitivity of the effects of baclofen in the dentate gyrus has not been determined.

Our second objective was to determine whether baclofen would produce a disinhibition that results in the activation of NMDA receptors and whether NMDA receptor antagonists would affect this activity. Accordingly, because NMDA receptor activation also regulates multiple forms of long-lasting synaptic plasticity (Sarvey et al., 1989), we explored the possibility of a baclofen-induced, NMDA receptor-dependent form of synaptic plasticity in the dentate gyrus.

Our third objective was to determine whether subthreshold concentrations of baclofen plus the β-adrenergic agonist isoproterenol could synergistically produce an LLP similar to what is seen with higher concentrations of isoproterenol alone.

Preliminary results from these studies have appeared in abstract form (Burgard and Sarvey, 1989, 1990; Sarvey and Burgard, 1989).

Materials and Methods

Preparation of hippocampal slices. Male Sprague–Dawley rats (100–200 gm) were anesthetized with the cytoprotectant (see Rothman and Olney, 1987) ketamine hydrochloride (50 mg/kg, i.m.) before decapitation. Slices were routinely incubated in an interface recording chamber for 3 hr before obtaining responses, which allowed for washout of the drug. We found that the effects of ketamine were reversible, because LTP, which can be blocked by ketamine (Stringer and Guyenet, 1983), could be elicited in slices from anesthetized rats within 2 hr after decapitation. Responses obtained in slices from anesthetized rats were qualitatively healthier than those obtained from nonanesthetized rats. This was evident as the improved ability of these slices to exhibit single population spikes and paired-pulse inhibition at interstimulus intervals (ISIs) of up to 30 msec. However, we have seen no differences in slices from anesthetized versus nonanesthetized rats in their responses to a variety of compounds (E. Burgard, D. Dahl, G. Decker, and J. Sarvey, unpublished observations).

The hippocampus was dissected free in a Petri dish filled with oxygenated (95% O_2, 5% CO_2), modified Krebs–Ringer buffer (125 mM NaCl, 3 mM KCl, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 2.4 mM $CaCl_2$, 1.3 mM $MgSO_4$, 10 mM glucose) at room temperature. Five to eight hippocampal slices (400 μm thick) per animal were prepared using a McIlwain tissue chopper (Brinkmann). Slices from the ventral hippocampal pole from non-PT-treated rats were used because this region allows for visual separation of the medial and lateral perforant paths. We have observed no differences in the responses of slices from either dorsal or ventral poles to any of the compounds used in this study. In PT-treated animals, slices were prepared from the region surrounding the injection site and extending 1.6 mm from the site that was located midway between dorsal and ventral poles.

Electrophysiological recordings. Slices were maintained at 35°C in an interface recording chamber. Stimuli consisted of rectangular pulses of constant current and variable duration delivered through monopolar stainless-steel electrodes. The return path for stimulating electrodes was via an Ag/AgCl wire in the bathing medium. Recording electrodes consisted of glass micropipettes filled with 2 M NaCl (3–10 MΩ). Monopolar stimulation of the medial perforant path produced an EPSP recorded extracellularly in the middle third of the molecular layer and a population spike recorded in the granule cell layer of the dentate gyrus (Fig. 1). A perforant path response profile (Dahl and Sarvey, 1989) was obtained in each slice before the experiment was started in order to ensure selective stimulation of only the medial perforant path. Stimulation of the mossy fibers in the hilar region produced an antidromic spike recorded in the granule cell layer and an orthodromic population spike recorded in field CA3 of the hippocampus. After initial responses had stabilized, a series of baseline recordings was taken over a 30-min period. The average of these recordings was referred to as mean baseline amplitude. An input/output (i.e., stimulus duration/response) curve was obtained at least every 10–15 min throughout each experiment. Response amplitudes used for analysis were taken from the linear portion of the input/output curve (30–70% maximum population spike amplitude or EPSP slope). For some time-course experiments, data were sampled every 2 min throughout the experiment. When EPSPs were analyzed, the stimulus strength chosen was always subthreshold for evoking a population spike. The initial negative slope of the EPSP was determined as in Stanton and Sarvey (1987). The criterion for LLP was met if the amplitude of the response measured 30 min after beginning washout of isoproterenol was greater than two standard deviations above the mean baseline amplitude (Stanton and Sarvey, 1985b).

It has been our experience that dentate gyri that do not exhibit paired-pulse inhibition at ISIs greater than 15 msec often develop small second-population spikes during the course of an experiment (E. Burgard and J. Sarvey, unpublished observations). Because we wanted to use only "healthy" responses, and because our results with baclofen displayed changes in net inhibition, only responses that exhibited paired-pulse inhibition at ISIs of 20 msec or more were used. Two paired-pulse stimulus procedures were used as a check for intact inhibition. In the first procedure, both the first (conditioning) and the second (test) pulses were delivered orthodromically at the same stimulus intensity at ISIs of 20–30 msec (ortho/ortho). In this case, if a compound affected the amplitude of the orthodromic population spike, then the intensity of an orthodromic test pulse in the presence of the compound was always adjusted to yield a population spike of the same amplitude as the baseline response to which it was compared. We refer to this procedure as

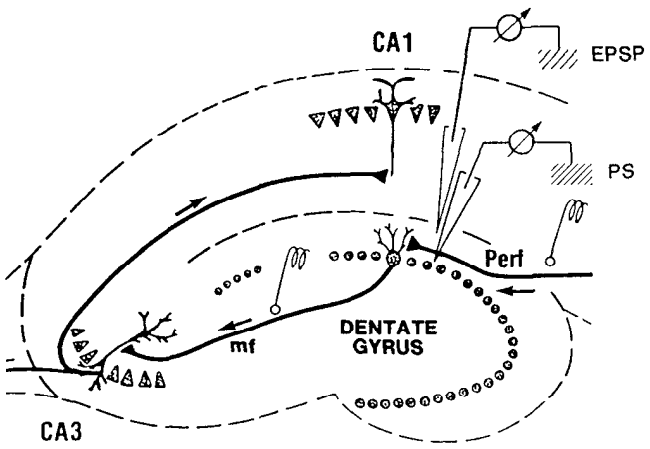

Figure 1. Schematic representation of the hippocampal slice. Stimulating electrodes were placed either in the medial perforant path (*Perf*) or in the hilar region of the dentate gyrus for stimulation of the mossy fibers (*mf*). Recording electrodes were placed in either the granule cell layer for recording the population spike (*PS*) or in the mid-molecular layer of the dentate gyrus for recording the *EPSP*.

"amplitude matching" of paired-pulse responses. In the second procedure, the conditioning pulse was antidromic and the test pulse, delivered 20–30 msec later, was orthodromic (anti/ortho). Because no compounds ever affected the antidromic spike, amplitude matching was not necessary.

Pertussis toxin (PT) injections. Male Sprague–Dawley rats (120–160 gm; Taconic Farms) were anesthetized with halothane and placed in a stereotaxic apparatus (David Kopf). Bilateral intrahippocampal injections of PT (1 μg in 2 μl sterile saline) were made over a 5-min period using a 10-μl Hamilton syringe at the following coordinates (from bregma): caudal, 0.5 cm; lateral, ±0.3 cm; ventral, 0.35 cm; incisor bar, −2.5 mm. These coordinates placed the tip of the needle at the level of the hippocampal fissure just above the dentate gyrus, as confirmed by injections of fast green. The animals were allowed to recover for 3–4 d, then were killed. The injection site was visible on all animals, and slices were prepared from within 1.6 mm dorsal or ventral to the injection site. The slices used for experiments were usually within 800 μm of the injection site and had no visible damage from the injection. A slice was used only if it exhibited healthy responses (a single population spike with paired-pulse inhibition at a minimum ISI of 20 msec).

Drug application. The following compounds were used: (−)- and (+)-baclofen (CIBA, Nutley, NJ), (−)-isoproterenol, D,L-propranolol (Sigma, St. Louis, MO), D-(−)-2-amino-5-phosphonovaleric acid (APV; Cambridge Research Biochemicals, Cambridge, UK), pertussis toxin (List Biologicals, Campbell, CA), diazepam and midazolam (Hoffman-La Roche, Nutley, NJ), and phaclofen and 2-hydroxysaclofen (Tocris Neuramin, Essex, UK).

All compounds were bath applied to hippocampal slices at a flow rate of 3 ml/min. Unless otherwise stated, the (−) isomer of baclofen was used for all experiments. When baclofen was applied alone, the exposure time was typically 10 min, and only one concentration was applied per slice. When applied in combination with isoproterenol, baclofen was applied 10–15 min prior to, and during, a 30-min exposure to isoproterenol. For the induction of LLP, 1 μM isoproterenol was applied alone for 20 min. APV and phaclofen were always added 10 min prior to, during, and for at least 5 min after baclofen application. All experiments were followed by at least a 30-min wash period.

Data analysis. Illustrated traces of evoked responses are the digitized average of four to eight successive responses elicited at a stimulus frequency of 0.2 Hz. Paired-pulse traces are the digitized average of three successive responses elicited at a frequency of 0.033 Hz. Responses were digitized and averaged on line and then stored on disk for later analysis. Data are expressed as mean ± SEM and were analyzed by Student's *t* test or ANOVA plus Student–Newman–Keuls (SNK) test for multiple comparisons; level of significance was set at 0.05.

Results

Disinhibitory effects of baclofen

Bath application of baclofen produced concentration-dependent effects on evoked responses recorded in the hippocampal slice. As shown in Figure 2*A*, a concentration of 0.1 μM baclofen had no effect on the evoked population spike elicited by medial perforant path stimulation (89 ± 8.9% of baseline; $N = 8$). However, increasing the concentration of baclofen produced a potentiation of the population spike amplitude that reached a maximum at 5 μM (222 ± 26% of baseline; $N = 5$). A concentration of 10 μM baclofen did not further increase the amplitude (not shown). At a concentration of 5 μM, baclofen elicited epileptiform activity evident in the appearance of multiple population spikes elicited in response to a single stimulus pulse (Figs. 2*B*, 3). Both the potentiation of the initial population spike amplitude and the appearance of two to four multiple spikes lasted throughout a 30-min wash period (initial spike amplitude, 246 ± 47%; four of four responses potentiated). Often the initial spike continued to increase in amplitude throughout the wash, while the epileptiform activity decreased. This disinhibitory effect was specific to orthodromic stimulation of the perforant path, because baclofen had no effect on the antidromic population spike recorded in the granule cell layer in response to mossy fiber stimulation (Fig. 2*C*). The effects of baclofen were also stereospecific, as shown in Figure 2*D*, where a 10-μM concentration of the less active isomer (+)-baclofen (Haas et al., 1985) had no effect on the evoked population spike. In contrast to the effects seen in the dentate gyrus, orthodromic population spikes recorded in the pyramidal cell layer of hippocampal field CA3 in response to mossy fiber stimulation were depressed to approximately 50% of baseline by 3 μM baclofen (not shown). This is in agreement with other observations in field CA3 (Lanthorn and Cotman, 1981).

The disinhibitory actions of baclofen were restricted to population spikes recorded in the granule cell layer and were not seen in the dendritically recorded EPSPs in the dentate gyrus. Following a 10-min application of 5 μM baclofen, the initial negative slope of the EPSP was 97 ± 8.9% of baseline ($N = 7$). Because we observed no acute effects of baclofen in the molecular layer, we restricted our analysis of baclofen's effects in this manuscript mainly to those produced in the granule cell layer. However, during a 30-min drug-free wash, the EPSP slope significantly increased to 119 ± 12% ($N = 7$; $p < 0.05$, Student's *t* test) of baseline. Thus, though baclofen had no acute effect on the EPSP, it did induce an LLP of the EPSP.

In order to determine if baclofen was producing its effects through activation of $GABA_B$ receptors, we bath applied the $GABA_B$ antagonist phaclofen before and during exposure to baclofen. Phaclofen (200 μM) had no effect on the evoked population spike (99 ± 2.5% of baseline; $N = 2$), but blocked both the potentiation of the population spike (107 ± 0.5%) and the appearance of any multiple spikes (Fig. 2*E*). This block persisted throughout a subsequent 30-min wash period (data not shown). We obtained similar results with 100 μM 2-hydroxysaclofen (not shown). If we applied phaclofen (400 μM) during the wash period following exposure to baclofen, the effects of baclofen were not reversed (Fig. 2*F*), indicating that the long-lasting effects of baclofen are not simply due to incomplete washout of the drug. In addition, the benzodiazepines, diazepam and midazolam (1

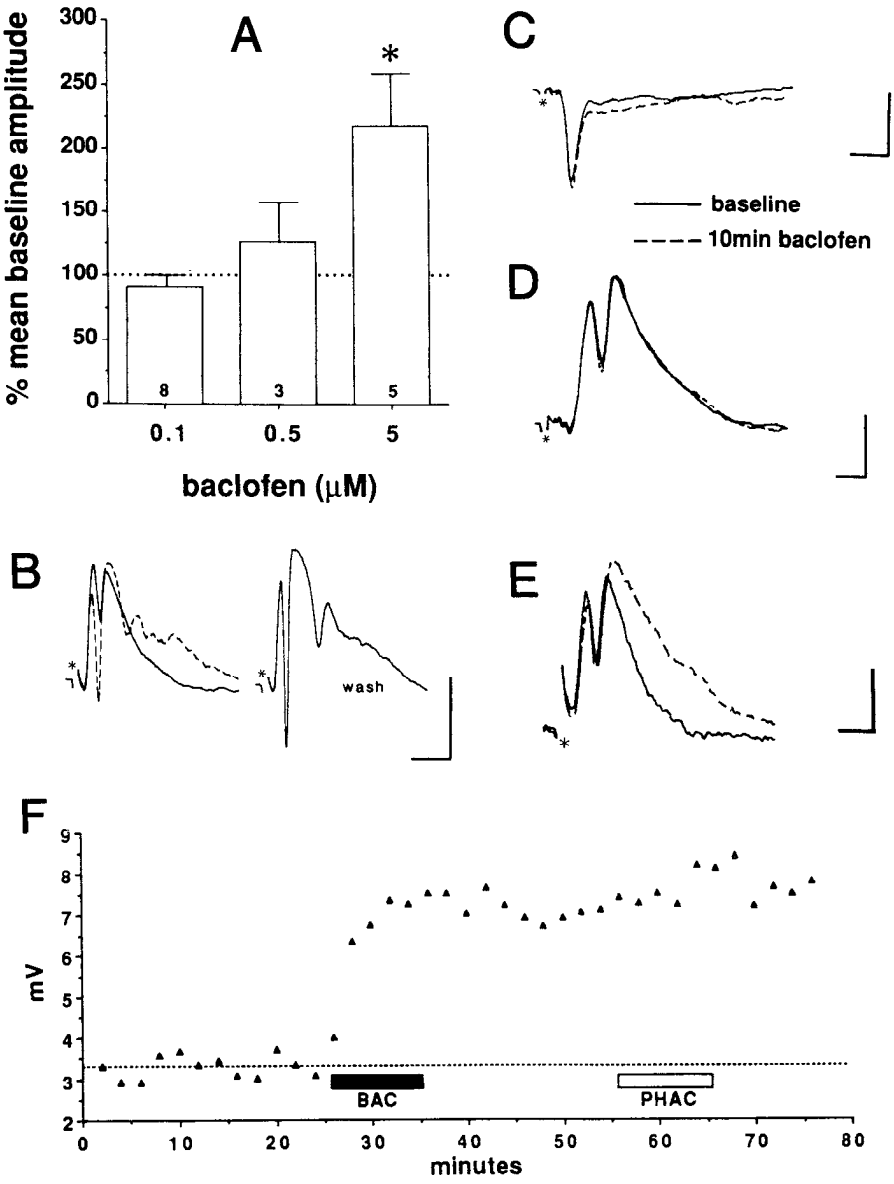

Figure 2. Effects of baclofen in the dentate gyrus. *A,* Dose–response graph showing the effects of a 10-min application of 0.1, 0.5, and 5 μM (−)-baclofen on the amplitude of the initial evoked population spike. The values are expressed as percentage of mean baseline (pretreatment) amplitude, and the *number* of responses is shown within each bar. *Asterisk* denotes $P < 0.05$, Student's t test, compared to baseline. *B,* Series of population spikes recorded before (*solid line*), during (*broken line*), and after (30 min *wash*) a 10-min application of 5 μM (−)-baclofen. Calibration, 5 mV, 5 msec. *C,* Antidromic spikes elicited before (*solid line*) and at the end of (*broken line*) a 10-min exposure to 3 μM (−)-baclofen. *D,* Population spikes elicited before (*solid line*) and at the end of (*broken line*) a 10-min exposure to 10 μM (+)-baclofen (traces are superimposed). *E,* Population spikes elicited before (*solid line*) and at the end of (*broken line*) exposure to 200 μM phaclofen plus 5 μM (−)-baclofen. Phaclofen was added 10 min prior to and during a 10-min application of baclofen. Phaclofen completely prevented the development of epileptiform, multiple population spikes and the increase in amplitude of the initial spike, though the late positive phase of the evoked response was still extended in the presence of baclofen. Calibration, 2 mV, 2 msec for *C–E. F,* Time course of an experiment demonstrating the effect of a 10-min application of baclofen (*BAC*; 5 μM) on the amplitude of the initial population spike. The LLP of the population spike was not reversed by a 10-min application of phaclofen (*PHAC*; 400 μM) during the wash period. Similarly, addition of phaclofen immediately after baclofen did not reverse the potentiation (data not shown). In *B–E, asterisks* denote stimulus artifacts.

μM; not shown), significantly reduced the effects of baclofen. These results suggest that baclofen, while acting directly as an agonist at $GABA_B$ receptors, can produce an epileptic state sensitive to blockade by both $GABA_B$ receptor antagonism and $GABA_A$ receptor activation. This epileptiform activity outlasts the exposure to baclofen.

PT sensitivity of baclofen's effects

In order to determine whether or not the baclofen response seen in the dentate gyrus is regulated by a PT-sensitive G protein, we performed bilateral intrahippocampal injections of PT 3 d prior to the experiment (see Materials and Methods). Animals were generally lethargic at the time of death. A few (three of nine) animals also exhibited seizurelike activity (i.e., eye blinking, rearing, wet dog shakes), at which point they were killed without dissection. Of the remaining six animals, the general electrophysiological condition of slices was slightly worse than controls. The population spikes were smaller, and they occasionally exibited a second population spike. In other words, it was more difficult to obtain a response that met the condition for paired-pulse inhibition described in Materials and Methods. However, we were always able to obtain at least one slice per animal that was close to the injection site and that met our criteria for a "healthy" slice. Animals treated only with a comparable saline injection also displayed a visible injection site. However, the slices were just as healthy and responded to baclofen as did slices from uninjected animals.

In slices from PT-treated animals, the response to application of 5 μM baclofen was significantly decreased as compared to uninjected controls (Fig. 3). In six slices from six PT-treated animals, baclofen did not significantly increase the amplitude of the initial population spike (106 ± 9.3% of baseline; two of six responses potentiated), and the appearance of multiple population spikes was significantly decreased in both frequency of occurrence and amplitude. The mean latency to onset of each consecutive spike was normalized to the latency to onset of the

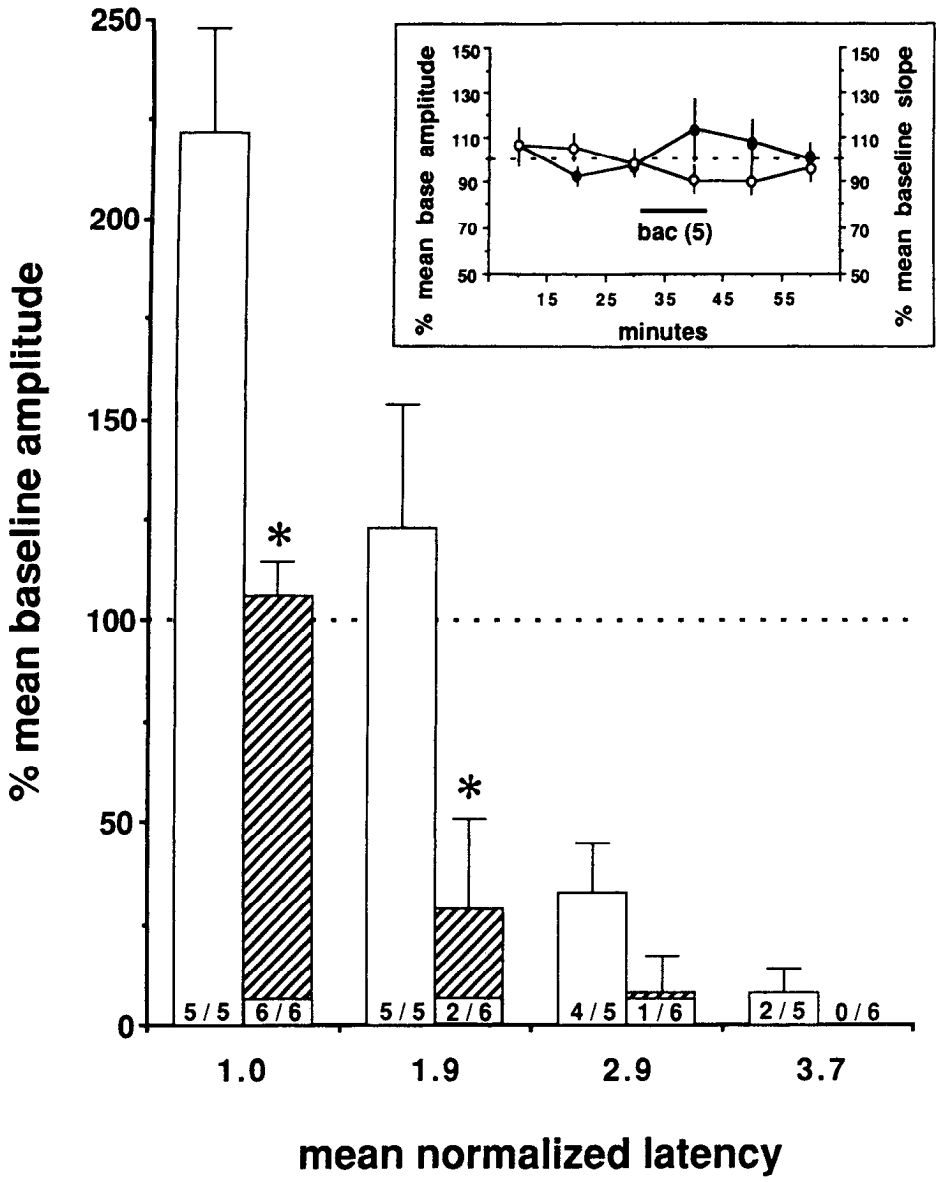

Figure 3. PT treatment blocks the disinhibitory effects of baclofen. The *bar graph* depicts the effects of PT treatment on the amplitude and occurrence of population spikes. The *ordinate* represents the percentage of the mean baseline amplitude of the population spike recorded at the end of a 10-min application of 5 μM baclofen in slices from both uninjected control rats (*open bars*) and in PT-treated rats (*hatched bars*). The *abscissa* represents the mean latency to peak amplitude of each population spike, normalized to the first spike (see Results). The *numbers* within each bar are the ratio of the number of slices exhibiting the spike versus the number of slices studied. *Asterisks* denote significant differences compared to non-PT-treated response ($P < 0.05$, Student's t test). *Inset,* Time course of the effects of 5 μM baclofen in slices from PT-treated rats ($n = 6$ for all time points except 20-min wash, where $n = 3$). The *solid circles* represent percentage of mean population spike amplitude, and the *open circles* represent percentage of mean baseline initial slope of the EPSP.

initial spike. Each consecutive onset latency was approximately the same as that of the initial spike, indicating that population spike firing was occurring synchronously and at approximately equal latencies. The ranges of normalized onset latencies were as follows: population spike 2, 1.6–2.1; 3, 2.8–3.0; 4, 3.7. The inset in Figure 3 shows the effects of baclofen on the time course of the initial population spike and EPSP in PT-treated animals. In contrast to the long-lasting effects of baclofen seen in uninjected and saline-injected animals, PT treatment prevented the potentiation of the initial population spike in response to 5 μM baclofen. As in controls, there was no significant effect of baclofen on the EPSP in PT-treated slices. After washout of baclofen, neither the population spike nor the EPSP was significantly changed. The data displayed in Figure 3 demonstrate the presence of a PT-sensitive component of both the epileptiform activity and the potentiation of the population spike induced by baclofen. Although we often observed a loss of paired-pulse inhibition in the presence of baclofen in slices from PT-treated animals, this loss was much less dramatic than that produced by baclofen in slices from uninjected animals.

Synergistic effects of baclofen plus isoproterenol

An LLP of evoked responses induced by β-adrenergic agonists such as isoproterenol or norepinephrine can be induced in the dentate gyrus. Figure 4*A* demonstrates the effect of bath application of isoproterenol on population spikes evoked in the dentate gyrus. A 20-min exposure to 0.1 μM isoproterenol had no effect on the amplitude of the population spike (106 ± 6.8% of baseline; one of six responses potentiated). At a concentration of 1 μM, however, isoproterenol produced both an acute (192 ± 19%; $N = 5$) and a long-lasting potentiation of the population spike that lasted for a minimum of 30 min in a drug-free wash (191 ± 21%; four of five responses potentiated; Fig. 4*A,B*). Baclofen at the low concentration of 0.1 μM had no effect on the population spike (89 ± 8.9% of baseline; one of eight responses potentiated; Fig. 4*C*). However, concurrent bath application of low concentrations of baclofen (0.1 μM) and isoproterenol (0.1 μM) produced a synergistic potentiation of the population spike that lasted throughout a 30-min wash period (163 ± 17% of baseline; five of six responses potentiated; Fig.

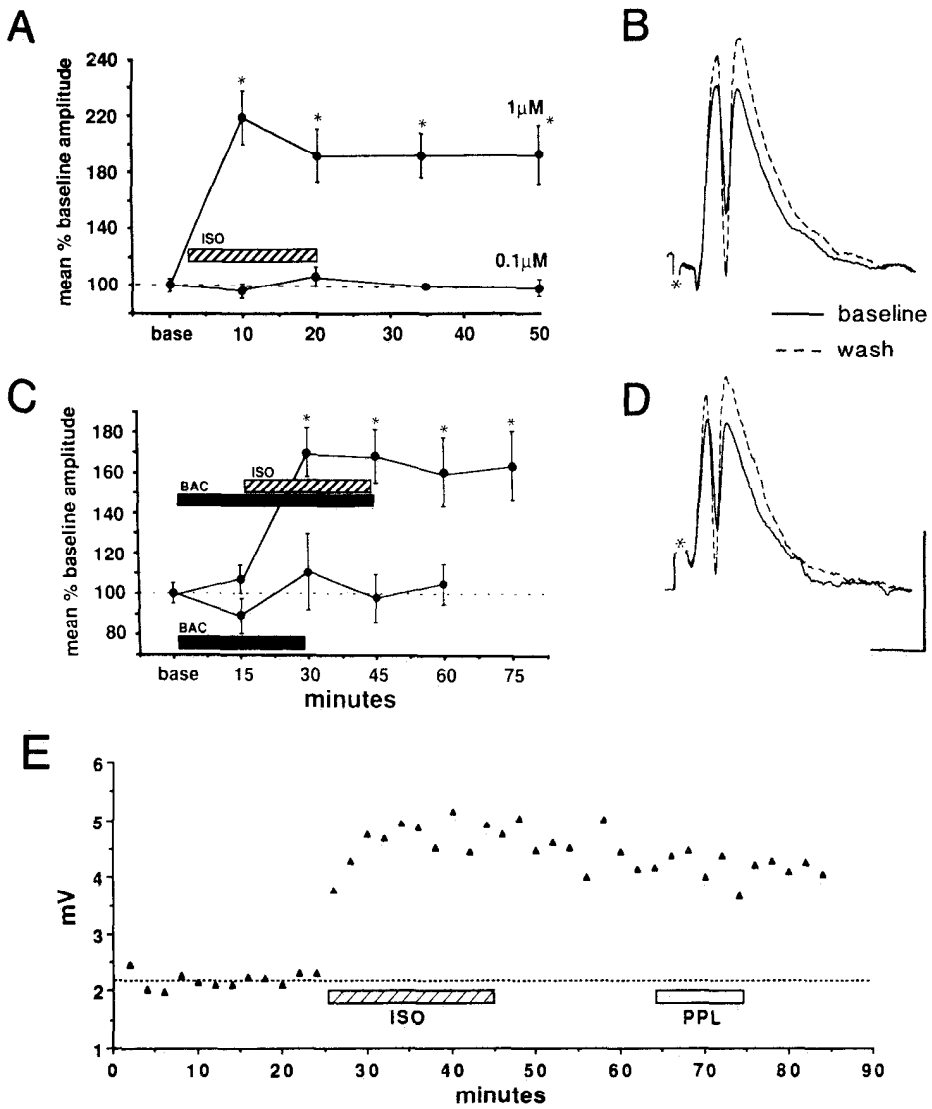

Figure 4. Isoproterenol and baclofen can synergistically potentiate evoked population spikes. *A,* Time course of the effects of two different concentrations of isoproterenol (*ISO*; 0.1 and 1 μM; 20-min exposure indicated by the *hatched bar*) on evoked population spikes. A concentration of 0.1 μM isoproterenol ($N = 8$) had no effect on the amplitude of the population spike. However, increasing the concentration to 1 μM ($N = 5$) induced LLP. *B,* Representative population spikes recorded before (*solid line*) and 30 min after (*broken line*) the induction of LLP by 1 μM isoproterenol. *C,* Time course of the effects of 0.1 μM baclofen alone (*BAC*; $N = 8$) and also in combination with 0.1 μM isoproterenol (*ISO*; $N = 6$). Baclofen was applied during the *solid bar,* and isoproterenol was applied during the *hatched bar* (for the *upper line* only). A synergistic LLP of the population spike amplitude is produced by coapplication of baclofen and isoproterenol. *D,* Representative population spikes recorded before (*solid line*) and at the end of (*broken line*) a 30-min wash following the application of 0.1 μM baclofen plus 0.1 μM isoproterenol. *E,* Time course of a typical experiment demonstrating the effect of a 20-min application of isoproterenol (*ISO*; 1 μM) on the amplitude of the population spike. The LLP induced by isoproterenol was not reversed by a 10-min application of propranolol (*PPL*; 1 μM) during the wash period. In *A* and *C,* baseline values are the average of a 30-min stable recording period, and *Asterisks* indicate significant differences compared to baseline values ($P < 0.05$, ANOVA and SNK). In *B* and *D, asterisks* denote stimulus artifacts; calibration, 5 mV, 5 msec.

4*C,D*). The synergistic potentiation produced by 0.1 μM baclofen plus 0.1 μM isoproterenol was not significantly different from that produced by 1 μM isoproterenol alone ($p > 0.05$, t test) measured after a 30 min wash and was not accompanied by epileptiform activity. As can be seen in Figure 4*E*, the LLP produced by 1 μM isoproterenol alone was not due to incomplete washout of the drug, because a subsequent addition of propranolol (1 μM; a nonspecific β-adrenergic receptor antagonist) did not reverse the established LLP.

Effects of baclofen and isoproterenol on paired-pulse stimulation

The inability of 0.1 μM baclofen to affect significantly the population spike yet still facilitate the induction of β-adrenergic LLP led us to the hypothesis that baclofen at this concentration may be producing subtle effects on recurrent and/or feedforward GABAergic inhibition in the dentate gyrus. As a test for this, two stimulation procedures were used to test for paired-pulse inhibition. Paired orthodromic stimuli gave a measure of mixed feedforward and recurrent inhibition. Using this procedure, the conditioning responses recorded during drug application had to be amplitude matched to those recorded before drug application (see Materials and Methods) in order to compensate for any changes produced by the drugs. Application of an antidromic conditioning pulse before an orthodromic test pulse provided a measure of mainly recurrent inhibition and never had to be amplitude matched. Figure 5 shows the effect of a low concentration of either isoproterenol or baclofen on paired-pulse inhibition. At 0.1 μM, neither drug had an effect on the population spike evoked in response to a single orthodromic stimulus pulse (see Fig. 4*A,C*), so no amplitude matching was necessary. Isoproterenol (0.1 μM) had no effect on paired-pulse inhibition evoked either by ortho/ortho or anti/ortho stimulation (Fig. 5, ISO). In contrast, 0.1 μM baclofen decreased paired-pulse inhibition, as is evident in the increase in the test pulse amplitudes (Fig. 5, BAC).

Effects of APV on evoked responses

In order to examine the relationship between baclofen-induced disinhibition and the activation of NMDA receptors, we studied the effects of the NMDA receptor antagonist APV on a number of parameters. First we found, as previously reported (Burgard et al., 1989; Dahl et al., 1990), that 10 μM APV produced a reversible depression of the evoked population spike (65 ± 11%

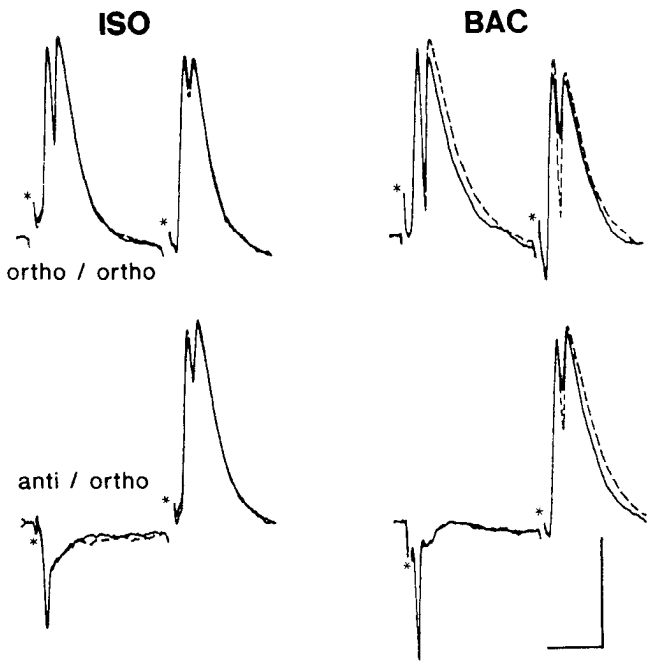

Figure 5. A concentration of 0.1 μM baclofen (*BAC*), but not isoproterenol (*ISO*), reduces paired-pulse inhibition. *ISO column,* Superimposed traces recorded in response to paired-pulse stimulation at 25-msec ISI both before (*solid line*) and in the presence of (*broken line*) 0.1 μM isoproterenol. The *upper pair* was recorded in response to ortho/ortho stimulation, whereas the *lower pair* used anti/ortho stimulation. No change in paired-pulse inhibition was seen. *BAC column,* Superimposed traces recorded at an ISI of 25 msec both before (*solid line*) and in the presence of (*broken line*) 0.1 μM baclofen. Stimulation paradigms were as in *A*. At this concentration, baclofen reduced paired-pulse inhibition, as seen in the increased amplitude of the test pulse. *Asterisks* denote stimulus artifacts. Calibration, 5 mV, 10 msec for both *A* and *B*.

of baseline; $N = 6$; $p < 0.05$, Student's t test). This concentration of APV has been shown to be effective in blocking both LTP and β-adrenergic-induced LLP in the dentate gyrus (Burgard et al., 1989; Dahl and Sarvey, 1990). Second, we found that 10 μM APV could decrease the amplitude of the test response in our paired-pulse stimulation paradigms (Fig. 6). As shown in Figure 6*A*, we analyzed the conditioning: test response ratio. This ratio allows us to compare the amount of inhibition present during the test response both before and during drug application. In baseline recordings of population spikes ($N = 6$), the mean test response was 0.45 times as large as the conditioning response, indicating the presence of intact inhibition. With the application of 10 μM APV, this test response decreased to 0.14 times the amplitude of the conditioning response, which translates into an approximate threefold increase in the conditioning: test response ratio. Following a 15-min wash period, the conditioning: test response ratio returned to baseline levels. The initial slope of the EPSP also normally exhibited paired-pulse inhibition. However, in contrast to population spikes, paired-pulse inhibition of the evoked EPSPs was not affected by APV (Fig. 6*A*; $N = 4$). The effects of 10 μM APV on representative population spikes can be seen in Figure 6*B*. In summary, APV reversibly decreased the amplitude of the population spike and increased paired-pulse inhibition of the population spike, but not the EPSP.

APV reduces the disinhibitory effects of baclofen

Our results indicating that APV produced an apparent increase in paired-pulse inhibition prompted us to investigate whether or not APV could block the disinhibitory actions of baclofen. Figure 7*A* demonstrates the effect of 10 μM APV on the disinhibition produced by 5 μM baclofen. Here (as described for Fig. 3), baclofen induced a potentiation of the initial population spike, along with the occurrence of multiple spikes. Pre- and

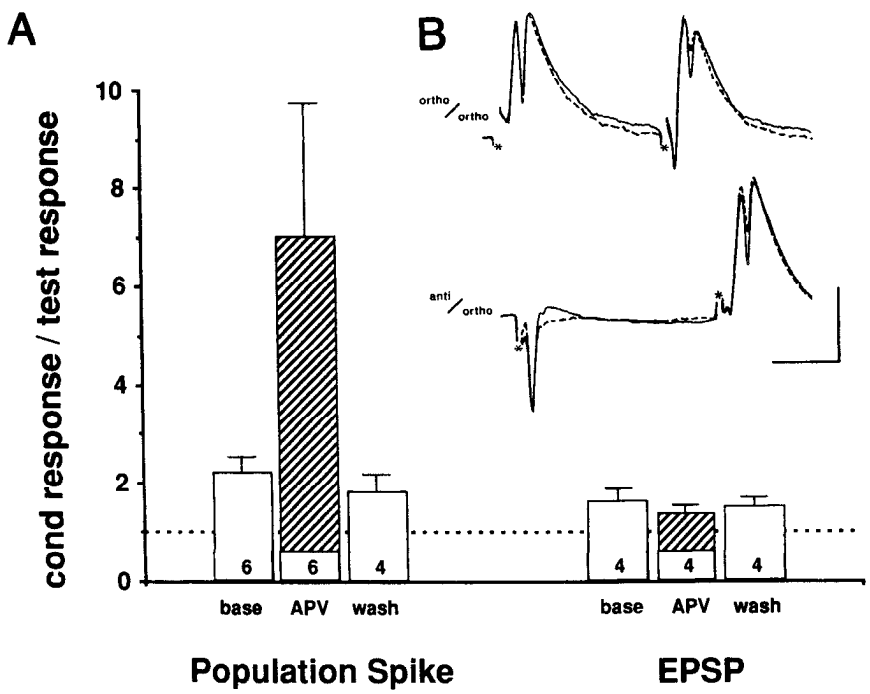

Figure 6. APV produces a net increase in paired-pulse inhibition of only the population spike. *A*, Bar graph displaying the conditioning response:test response ratio for both population spike and dendritically recorded EPSP in response to ortho/ortho stimulation. The ratio was calculated from amplitudes or initial negative slopes of individual paired-pulse responses, and these values were averaged. A value of 1.0 represents no difference between conditioning and test responses and, therefore, no paired-pulse inhibition. The greater the ratio, the greater the net inhibition. Ratios were calculated during baseline, during a 10-min bath application of 10 μM APV, and after a 15 min wash. The *numbers* within each bar denote the number of responses. APV produced an acute increase in paired-pulse inhibition of the population spike only. *B*, Representative traces recorded in response to paired-pulse stimulation both before (*solid lines*) and in the presence of (*broken lines*) 10 μM APV. The *upper pair* of traces was recorded in response to ortho/ortho stimulation (ISI, 25 msec), whereas the *lower pair* used anti/ortho stimulation (ISI, 30 msec). APV produced a net increase in paired-pulse inhibition. *Asterisks* denote stimulus artifacts. Calibration, 5 mV, 10 msec.

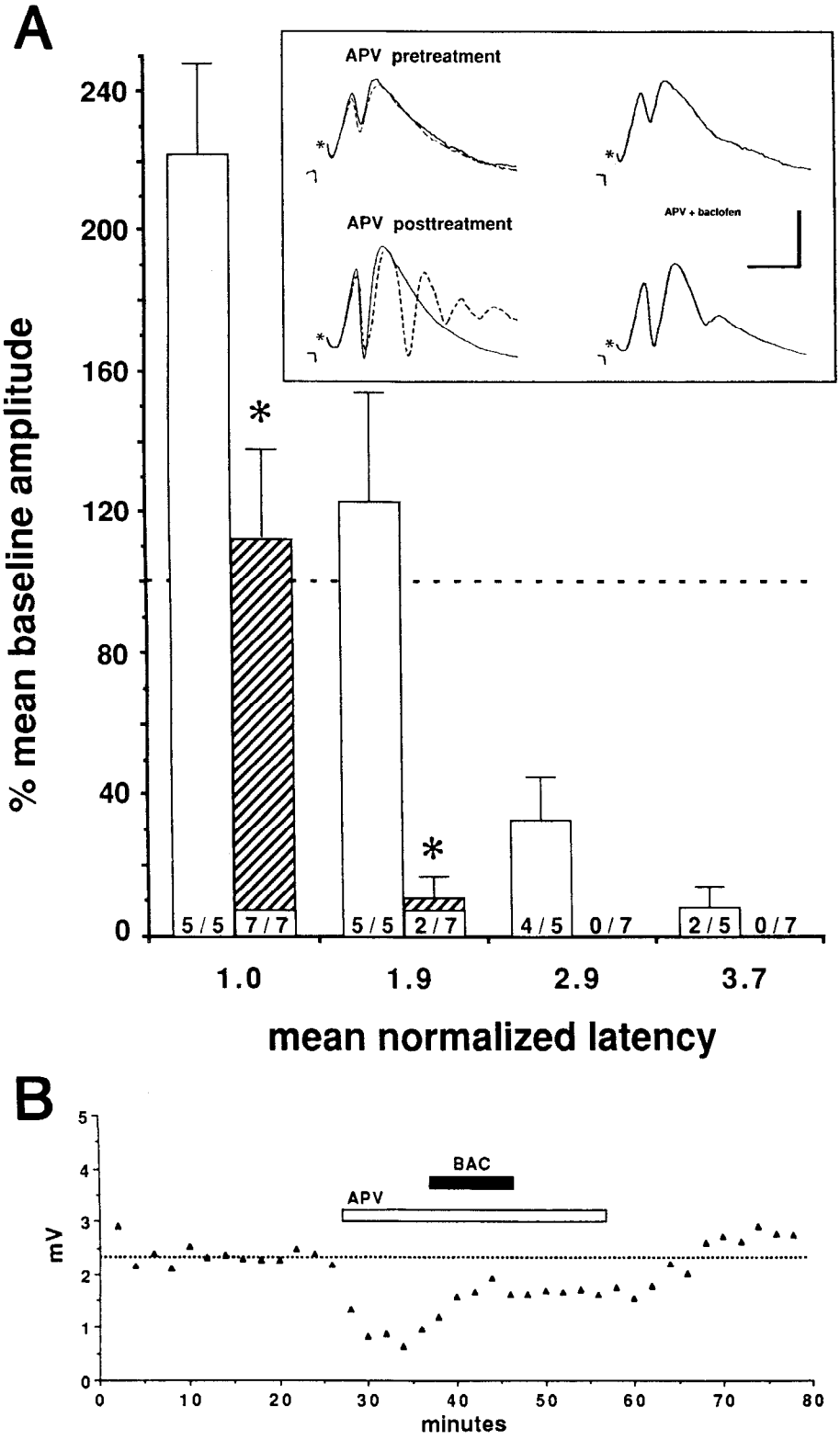

Figure 7. *A*, APV blocks the disinhibitory effects of baclofen. The *bar graph* depicts the effects of APV on the amplitude and occurrence of population spikes. The *ordinate* represents the percentage of the mean baseline amplitude of the population spike recorded after 10 min of 5 μM baclofen both in the absence (*open bars*) and in the presence (*hatched bars*) of 10 μM APV. For the *hatched bars*, APV was added 10 min prior to and during baclofen. The *abscissa* represents the mean latency to peak amplitude of each population spike, normalized to the first spike. As the graph demonstrates, APV decreases both the potentiation and the loss of inhibition seen with application of baclofen. The *numbers* within each bar are the ratio of the number of slices exhibiting the spike versus the number of slices studied. *Asterisks* denote significant difference compared to baclofen alone ($P < 0.05$, Student's t test). *Inset: Upper traces*, An example of the ability of APV pretreatment to block potentiation and multiple spiking even though there was no effect of APV on the initial spike in this slice. APV (10 μM) was applied 10 min before and during a 10-min application of 5 μM baclofen (*APV pretreatment*). Traces on the *left* were taken before (*solid line*) and in the presence of (*broken line*) 10 μM APV. The trace on the *right* was taken in the presence of baclofen plus APV. *Lower traces*, With APV posttreatment, the epileptiform activity was greatly reduced. APV (10 μM) was applied 10 min after baclofen (*APV posttreatment*). Traces on the *left* were taken before (*solid line*) and in the presence of (*broken line*) 5 μM baclofen. The trace on the *right* was taken in the presence of baclofen plus APV. *Asterisks* denote stimulus artifacts. Calibration, 5 mV, 5 msec for all traces. *B*, Time course of an experiment demonstrating the combined effects of APV and baclofen on the amplitude of the initial population spike. APV (10 μM) was bath applied 10 min before, during, and for 10 min after a 10-min application of baclofen (*BAC*; 5 μM). APV depressed the population spike and also blocked the potentiation usually seen with baclofen application. The response returned to baseline values following washout of both compounds.

cotreatment with APV prevented the potentiation of the initial population spike (112 ± 26% of baseline; two of seven responses potentiated) and prevented the appearance of almost all multiple spiking. In addition (data not shown), pre- and cotreatment with 10 μM APV could prevent the loss of paired-pulse inhibition produced by baclofen. The inset in Figure 7*A* shows traces taken from two different experiments. The top traces were chosen to demonstrate that, even when pretreatment with 10 μM APV did not depress the population spike, it could still completely block any epileptiform activity induced by the addition of baclofen. The bottom traces show an extreme example of the disinhibition produced by pretreatment with baclofen. If APV was subsequently added, the epileptiform activity could be significantly reduced (Fig. 7*A*, inset). However, posttreatment did not reverse the potentiation of the initial spike (not shown). Figure 7*B* is a representative time course demonstrating the blockade of ba-

clofen-induced potentiation of the initial population spike by 10 μM APV. APV depressed the population spike amplitude, yet baclofen, in the presence of APV, partially restored the amplitude. The ability of baclofen to produce a small enhancement of the population spike in the presence of APV indicates that APV is not directly inhibiting the action of baclofen at $GABA_B$ receptors. Nevertheless, no acute potentiation of the population spike above baseline amplitude was induced by baclofen. During the subsequent wash period, the population spike returned to baseline values, and no LLP was observed. This blockade was seen in three slices, where population spike amplitudes, measured after a 30 min washout of both compounds, were not significantly different from baseline values (107 ± 4.9%, Student's *t* test). These data suggest that the expression of baclofen-induced epileptiform activity and potentiation requires NMDA receptor activation. Continued activation of NMDA receptors is required for maintenance of epileptiform activity but not of baclofen-induced potentiation.

Discussion

Disinhibitory effects of baclofen in the dentate gyrus

We have demonstrated that baclofen produced a net disinhibitory effect in the dentate gyrus of the hippocampal slice, in contrast to its effects in other areas of the hippocampus. This disinhibitory effect was stereospecific, blocked by phaclofen, restricted to synaptically evoked responses, and concentration dependent. A concentration of 5 μM baclofen also produced an LLP of the evoked population spike. In addition, 5 μM baclofen produced a loss of paired-pulse inhibition and long-lasting epileptiform spiking within the granule cell layer. Both the potentiation of the population spike and the loss of inhibition were significantly reduced by *in vivo* pretreatment with PT, which suggests that these effects are mediated by a PT-sensitive G protein. No significant effects were observed on the dendritically recorded EPSP in the presence of baclofen. However, during the subsequent wash period, the EPSP slope increased significantly.

A concentration of 0.1 μM baclofen did not significantly affect the amplitude of the evoked population spike; however, it did decrease paired-pulse inhibition. As was mentioned in Materials and Methods, only responses that exhbited paired-pulse inhibition at a minimum of 20 msec ISI were used for all experiments. This was based on the fact that responses exhibiting paired-pulse inhibition only at ISIs less than 15 msec occasionally showed or developed second population spikes over time. Also, inhibition measured at very short ISIs (5–10 msec) may be confounded by the presence of early K^+ current activation (Hille, 1984) following elicitation of granule cell action potentials. At longer ISIs (20–30 msec), the inhibition measured should be mainly the result of $GABA_A$-mediated IPSPs (Rausche et al., 1989). The fact that 0.1 μM baclofen could decrease the degree of intrinsic inhibition without affecting the orthodromic response to a single stimulus suggests that the inhibitory (GABAergic) component to synaptic transmission in the dentate gyrus is more sensitive to the effects of baclofen than is the excitatory (glutamatergic) component. A decrease in inhibitory tone produced by 0.1 μM baclofen could account for the facilitation of LLP induction by isoproterenol. This could be accomplished by facilitating the depolarization of granule cells, and the subsequent facilitation of NMDA receptor activation, leading to LLP.

Mott et al. (1989) have previously demonstrated that, in the dentate gyrus, baclofen can produce epileptiform activity that is also associated with a decrease in paired-pulse inhibition. The mechanism underlying this effect appears to be an increased sensitivity of inhibitory GABAergic interneurons compared to granule cells to the direct actions of baclofen. The role of the interneuron is to inhibit multiple spike firing of hippocampal neurons (Andersen et al., 1964; Lubbers and Frotscher, 1987). Misgeld et al. (1989) have shown that baclofen selectively inhibits the activity of hilar interneurons directly via a $GABA_B$-mediated hyperpolarization. This hyperpolarization is presumably the result of activation of the same or a similar K^+ conductance responsible for depressing the activity of CA1 and CA3 pyramidal cells. Baclofen, by activating $GABA_B$ receptors located on GABAergic interneurons, could hyperpolarize and inhibit the firing of these neurons, leading to decreased IPSPs in the granule cells. This would result in the loss of inhibitory GABAergic tone and both feedforward and feedback inhibition.

Another possibility is that baclofen could be activating presynaptic $GABA_B$ autoreceptors located on GABAergic nerve terminals, resulting in an inhibition of GABA release (Harrison, 1990). However, presynaptic $GABA_B$ receptors appear to be insensitive to both PT and phaclofen (Colmers and Williams, 1988; Dutar and Nicoll, 1988b; Harrison, 1990), and we have found that baclofen's inhibitory effects in the dentate gyrus are mainly PT and phaclofen sensitive. We cannot rule out the presence of a PT-insensitive component; in fact, a small disinhibitory effect of baclofen was still seen in PT-treated slices. This may indicate the presence of a small PT-insensitive effect, or it may also be due to an incomplete ADP ribosylation of PT-sensitive G proteins. Overall, our data support the hypothesis that baclofen preferentially inhibits GABAergic interneurons, and that activation of $GABA_B$ receptors has a facilitatory action in the dentate gyrus.

Synergistic potentiation of β-adrenergic LLP by baclofen

At a concentration (0.1 μM) of either baclofen or isoproterenol alone that would produce no effects on single evoked population spikes, we observed a synergistic potentiation in the presence of both drugs. However, even at this low concentration, baclofen, but not isoproterenol, produced a subtle loss in paired-pulse inhibition.

Anatomical support for catecholaminergic/GABAergic interactions has been found by Milner and Bacon (1989). They demonstrated that both types of terminals synapse on the same perikarya or dendrites in the dentate gyrus. Also, a number of reports have proposed that concurrent activation of $GABA_B$ and β-adrenergic receptors can augment the intracellular increase in cAMP normally seen with β-receptor activation alone (Hill and Dolphin, 1984; Karbon et al., 1984; Karbon and Enna, 1985). With this in mind, we attempted to replicate these results electrophysiologically in the dentate gyrus. In hippocampal pyramidal cells, the electrophysiological effects of β-adrenergic agonists are mediated by cAMP (Madison and Nicoll, 1986). As mentioned previously, β-adrenergic receptor activation in the dentate gyrus, but not in fields CA1 or CA3, results in the induction of LLP, which is also associated with a persistent rise in cAMP (Stanton and Sarvey, 1985a,b). Our feeling was that if we could augment a β-adrenergic-induced rise in cAMP with baclofen, we could also facilitate the induction of LLP. However, at concentrations of baclofen used to produce synergistic effects on cAMP biochemically (≥10 μM; Karbon and Enna, 1985), we could already produce an LLP and disinhibition of evoked re-

sponses in the dentate gyrus. We had to reduce the concentration of baclofen approximately 100-fold in order to eliminate any effects on single evoked responses. Although baclofen and isoproterenol may synergistically increase cAMP levels at this concentration, we propose that baclofen increases the net excitability of granule cells by a $GABA_B$-mediated inhibition of inhibitory neurons. This leads to a facilitation of LLP induction by a mechanism of net disinhibition. This may be analogous to the facilitation of LTP by disinhibitory agents such as picrotoxin (Wigström and Gustafsson, 1983) and baclofen (Mott et al., 1990).

NMDA receptor activation mediates the initiation and maintenance of baclofen-induced disinhibition

We have demonstrated that 5 μM baclofen could produce an LLP of the population spike that was prevented by the NMDA receptor antagonist APV. NMDA receptors have been implicated in a number of synaptic processes, including LTP (Collingridge et al., 1983) and epileptiform activity (Dingledine et al., 1986). If, as mentioned above, NMDA receptor activation is facilitated during a loss of inhibition, then we expected to see an NMDA receptor-mediated component of the effects of baclofen. In fact, baclofen-induced potentiation appeared qualitatively similar to the induction of population-spike LLP by β-adrenergic agonists. LLP is also an NMDA receptor-dependent form of synaptic plasticity (Burgard et al., 1989; Stanton et al., 1989; Dahl and Sarvey, 1990). However, no epileptic activity is ever seen with isoproterenol or norepinephrine administration. Previous reports from our laboratory have demonstrated a significant NMDA receptor-mediated component of synaptic transmission in medial perforant path/granule cell synapses (Burgard et al., 1989; Dahl et al., 1990). This component is seen under the same conditions used in the present experiments ($[Mg^{2+}]_o$ = 1.3 mM). Given the relative ease of NMDA receptor activation here, we do not find it surprising that disinhibition can lead to an NMDA receptor-dependent potentiation of the population spike. LTP, β-adrenergic LLP, and baclofen-induced potentiation can all be prevented, but not reversed, by NMDA receptor antagonists. Whereas LTP and β-adrenergic LLP are evident as an LLP of both the population spike and EPSP, baclofen only produced potentiation of the population spike. We observed an increase in the EPSP only during washout of baclofen. Thus, though the population spike potentiation appeared similar to both LTP and LLP, it appeared not to originate from potentiation of the EPSP. Possibly this is an example of potentiation similar to EPSP-spike potentiation seen in the hippocampus following high-frequency stimulation, where the population spike is potentiated to an extent not accounted for by the degree of EPSP potentiation (Andersen et al., 1980). If this were the case, then a reduction in $GABA_B$-mediated inhibition at the cell soma as seen with baclofen could produce a population spike of greater amplitude in response to an EPSP that was unchanged. In fact, Chaves-Noriega et al. (1989) have demonstrated that a reduction in tonic inhibition may underlie EPSP-spike potentiation produced by tetanic stimulation in field CA1. Alternatively, the EPSP potentiation could be masked by an acute inhibitory action of baclofen, either on the release of excitatory neurotransmitter or on the granule cell dendrites, that counteracts the manifestation of potentiation, but not the consequences. This might explain the delayed onset of EPSP potentiation, where the EPSP becomes potentiated only during washout of baclofen.

We also found that, in addition to its depressant effects on the evoked population spike (Burgard et al., 1989), APV pretreatment could block the development of epileptiform activity produced by baclofen. In addition, posttreatment with APV could reduce the disinhibition produced by baclofen. These results suggest that not only the initiation, but also the maintenance of epileptiform activity is NMDA receptor dependent. APV also produced an apparent increase in paired-pulse inhibition of the population spike, but not the EPSP. This is referred to as an "apparent" increase in inhibition because there are two possible explanations: (1) APV directly increases paired-pulse inhibition, or (2) APV decreases a facilitatory component of paired-pulse inhibition, leading to a net increase in inhibition. The latter explanation is probably correct because the NMDA component of synaptic transmission in cultured hippocampal neurons displays a peak amplitude at approximately 73 msec after stimulation (Forsythe and Westbrook, 1988). Regardless of the mechanism, the net result is seen as an increase in inhibition. This increase in inhibition may underlie the ability of APV to block the initiation of epileptiform activity by baclofen, whereas the blockade of the maintenance is probably due to blockade of NMDA receptor-mediated synaptic activity in the presence of baclofen.

In conclusion, we have demonstrated that the disinhibitory actions of baclofen in the dentate gyrus are blocked by PT, APV, and phaclofen, and we have confirmed previous reports of these disinhibitory effects. In addition, we have shown that baclofen can facilitate the induction of isoproterenol-induced LLP. Baclofen can also produce an LLP of the evoked population spike that can be prevented by blockade of NMDA receptors. These findings suggest that reduced inhibition, in addition to facilitating the induction of synaptic plasticity, can itself induce plastic changes. These results also suggest that the physiological role of $GABA_B$ receptors in the dentate gyrus is to facilitate the transmission of impulses through the dentate gyrus.

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