Mechanisms Involved in Tetanus-Induced Potentiation of Fast IPSCs in Rat Hippocampal CA1 Neurons

T. SHEW, S. YIP, AND B. R. SASTRY
Neuroscience Research Laboratory, Department of Pharmacology and Therapeutics, The University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada

Shew, T., S. Yip, and B. R. Sastry. Mechanisms involved in tetanus-induced potentiation of fast IPSCs in rat hippocampal CA1 neurons. J Neurophysiol 83: 3388–3401, 2000. In the present study, possible mechanisms involved in the tetanus-induced potentiation of γ-amino-nobutyric acid-A (GABA-A) receptor-mediated inhibitory postsynaptic currents (IPSCs) were investigated using the whole cell voltage-clamp technique on CA1 neurons in rat hippocampal slices. Stimulation (100 Hz) of the stratum radiatum, while voltage-clamping the membrane potential of neurons, induces a long-term potentiation (LTP) of evoked fast IPSCs while increasing the number but not the amplitude of spontaneous IPSCs (sIPSCs). The potentiation of fast IPSCs was input specific. During the period of IPSC potentiation, postsynaptic responses produced by 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol hydrochloride and bacoafen, GABA-A and GABA-B agonists respectively, were not significantly different from control. CGP 36742, a GABA-B antagonist, blocked the induction of tetanus-induced potentiation of evoked and spontaneous IPSCs, while GTPγS, an activator of G proteins, substitution for GTP in the postsynaptic recording electrode did not occlude potentiation. Since GABA-B receptors work through G proteins, our results suggest that pre- but not postsynaptic GABA-B receptors are involved in the potentiation of fast IPSCs. A tetanus delivered when GABA-A responses were completely blocked by bicuculline suggests that GABA-A receptor activation during tetanus is not essential for the induction of potentiation. Rp-cAMPs, an antagonist of protein kinase A (PKA) activation, blocks the induction of potentiation of fast IPSCs. Forskolin, an activator of PKA, increases baseline evoked IPSCs as well as the number of sIPSCs, and a tetanic stimulation during this enhancement uncovers a long-term depression of the evoked IPSC. Sulphydryl alkylating agents, N-ethylmaleimide and p-chloromercuribenzoic acid, which have been found to presynaptically increase GABA release and have been suggested to have effects on proteins involved in transmitter release processes occurring in nerve terminals, occlude tetanus-induced potentiation of evoked and spontaneous IPSCs. Taken together our results suggest that LTP of IPSCs originates from a presynaptic site and that GABA-B receptor activation, cyclic AMP/PKA activation and sulphydryl-alkylation are involved. Plasticity of IPSCs as observed in this study would have significant implications for network behavior in the hippocampus.

**INTRODUCTION**

Even though normal function of the hippocampus is critically dependent on inhibition by GABAergic interneurons, plasticity of inhibitory synapses has received little attention when compared with studies on the plasticity of excitatory synapses. Only in recent years has attention been paid to the plasticity of GABAergic synaptic transmission. Tetanus-induced enhancements of GABAergic synaptic transmission were first described in the hippocampus (Morishita and Sastry 1991; Xie and Sastry 1991; Xie et al. 1995). Subsequently, plasticity of GABAergic inhibition was shown in deep cerebellar nuclei (Ouardouz and Sastry 1999), visual cortex (Komatsu 1994, 1996; Komatsu and Iwakiri 1993) and medulla (Glaum and Brooks 1996).

In the mammalian CNS, GABA activates both GABA-A and GABA-B receptors, activation of the former produces an increase in Cl⁻ conductance that generates the fast inhibitory postsynaptic current (Eccles et al. 1977) and the latter a G-protein-mediated increase in K⁺ conductance that results in the slow IPSC (Bowery et al. 1980).

There is evidence that GABA-B receptors exist both pre- and postsynaptically (Ault and Nadler 1982; Bowery et al. 1980; Olpe et al. 1982; Peet and McLennan 1986). In the hippocampus, the activation of presynaptic GABA-B receptors has been shown to decrease transmitter release (Ault and Nadler 1982; Bowery et al. 1980; Diesz and Prince 1989; Thompson and Gahwiler 1989) and activation of postsynaptic receptors causes a hyperpolarization of neurons (Alger 1984). These actions have been shown to modulate the induction of activity-dependent plasticity at excitatory synapses (Davies and Collingridge 1996; Davies et al. 1991). Although from previous studies we have shown that activation of GABA-B receptors is not associated with the maintenance of tetanus-induced potentiation of CA1 neuronal fast inhibitory postsynaptic potentials (IPSPs) (Xie et al. 1995), it is unclear if they participate in the induction of the potentiation.

Protein kinase A (PKA) can phosphorylate a variety of cellular substrates such as ion channels, receptors, and proteins on synaptic vesicles and presynaptic membranes of nerve terminals to modulate their function (Raymond et al. 1993). Forskolin binds to the catalytic subunit of adenylly cyclase, greatly increasing intracellular cAMP concentration, which can stimulate protein kinase A. The hippocampus contains forskolin binding sites (Gehlert et al. 1985), and in the CA1 area, adenylyl cyclase is more densely located in dendritic spines as well as in presynaptic terminals, than in somata (Mons et al. 1995). Modulation of excitatory synapses and long-term potentiation (LTP) of excitatory synaptic transmission by protein kinase activity has been intensely investigated (Malekna et al. 1986, 1987). Recently stimulation of PKA has been shown to potentiate hippocampal inhibitory synaptic transmission by a presynaptic mechanism of action (Capogna et al. 1995). It is,
however, unclear whether the induction of tetanus-induced plasticity of IPSCs requires activation of PKA.

It has been shown that activation of PKA inhibits GABA-B receptor-mediated effects, and GABA-B receptor activation inhibits forskolin-induced cAMP accumulation (Malcangio and Bowery 1993; Xi et al. 1997; Yoshimura et al. 1995). However, it has also been shown that GABA-B agonists increase intracellular cAMP and enhance neurotransmitter (noradrenaline, isoproterenol, β-adrenergic agonists) stimulated intracellular cAMP accumulation (Knight and Bowery 1996; Oset-Gasque et al. 1993; Scherer et al. 1989). In addition, GABA-B receptors have been shown to regulate GABA-A receptor function through G proteins linked to the adenylyl cyclase pathway (Barthel et al. 1996). Whether GABA-B and PKA pathways interact to modulate tetanus-induced potentiation of the GABA-A mediated IPSC is currently unknown.

Secretory machinery, cytoskeletal proteins and proteins involved in the interaction of the synaptic vesicle with the presynaptic membrane, have many putative sites for phosphorylation. Recently it has been suggested that activation of PKA directly facilitates the probability of exocytosis of individual vesicles in response to a constant Ca2+ signal (Trudeau et al. 1997). The same study concluded that PKA directly facilitates secretory machinery at a step downstream of Ca2+ influx and vesicle docking. In separate studies, AMP has been shown to enhance the phosphorylation of microtubule associated protein (MAP-2), synapsins and RAB 3A, proteins suggested to be involved in neurotransmitter release (Koszaka et al. 1991; Lonart and Sudhof 1998).

Many proteins have recently been cloned and suggested to be involved in neurotransmitter release. N-ethylmaleimide-sensitive factor (NSF), an ATPase whose hydrolysis of ATP, has been shown to be essential for cellular vesicular fusion and transmitter release (Whiteheart et al. 1994). N-ethylmaleimide (NEM), a sulfhydryl alkylating agent, has been shown to inhibit vesicle-membrane fusion in the Golgi apparatus, to decouple G proteins from their associated receptors (Kitamura and Nomura 1987; Shinoda et al. 1990) and more recently to enhance GABA release from presynaptic nerve terminals and block depolarization-induced suppression (DSI) of sIPSCs and of evoked IPSCs (Morishita et al. 1997). The effects of NEM on physiologically measured plasticity of inhibitory synaptic transmission is not well understood.

Previous studies from our laboratory have shown that in the presence of nL-2-amino-5-phosphonovaleric acid (APV) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a tetanic stimulation delivered to the stratum radiatum induced a posttetanic potentiation of hippocampal CA1 neuronal, GABA-A-mediated fast inhibitory postsynaptic potentials (IPSPs) (Xie et al. 1995). In this study, it was also concluded that the potentiation was at least partly localized to GABAergic synapses on CA1 neurons, although the contributions made by pre- and postsynaptic mechanisms were unknown. In the present study, we further characterize and investigate possible mechanisms involved in the tetanus-induced potentiation of fast IPSCs in rat hippocampal CA1 neurons with the use of whole cell patch-clamp recording techniques. Our results suggest that the potentiation arises from a presynaptic site of origin and that GABA-B receptor activation, cyclic AMP/PKA activation, and sulfhydryl-alkylation are involved in the tetanus-induced potentiation of fast IPSCs. Preliminary results from this study were presented at a scientific meeting (Shew and Sastry 1998).

METHODS
Slice preparation and recording solutions

Halothane was used to deeply anesthetize rats before decapitation. Transversely sectioned hippocampal slices (400 μM) were then obtained from 2-wk-old male Wistar rats as previously described (Xie et al. 1995). The CA3 region of the slices was dissected free to minimize the influence of spontaneous activity from CA3 neurons. The slices were then placed in an incubating chamber and allowed to recover for ≥1 h before being transferred and submerged in a constant-flow (2–2.5 ml/min) recording chamber (Pandanaobina and Sastry 1984). A single hippocampal slice was held between two nylon nets and then transferred into a recording chamber, which was superfused with oxygenated (95% O2–5%CO2) physiological medium (pH = 7.4). The physiological medium contained (in mM) 120 NaCl, 3 KCl, 1.8 NaH2PO4, 26 NaHCO3, 2 MgCl2, 2 CaCl2, and 10 g-glucose. All experiments were performed in the presence of 40 μM DL-2-amino-5-phosphonovaleric acid [APV, an N-methyl-D-aspartate (NMDA) antagonist] and 20 μM 6,7-dimethoxyxinoxaline-2,3-dione (DNQX) to pharmacologically isolate inhibitory responses from excitatory responses and to minimize the possibility of polysynaptic influences. All experiments were performed at room temperature (24–26°C). Since these studies were conducted at 24–26°C, it would be important to determine if similar changes occur at physiologically normal conditions.

For some studies on evoked IPSCs, patch electrodes with resistances of 4–6 MΩ were filled with patch solution containing (in mM) 135 K-glucosone, 10 HEPES, 10 KCl, 1 K3-bis-(o-aminophenoxy)-N,N,N′,N′-tetraacetic acid (KBAPTA), 5 Mg-ATP, 0.1 CaCl2, 10 Na2-phosphocreatine, and 0.4 Na3-GTP. Creatine phosphokinase was added to produce a final concentration of 50 U/ml (pH adjusted to 7.20–7.25 with KOH). For studies of evoked IPSCs and spontaneous IPSCs, the patch solution contained (in mM) 85 CH3O2SCs, 50 CsCl, 10 HEPES, 10 KCl, 1 BAPTA, 5 Mg-ATP, 0.1 CaCl2, 10 Na2-phosphocreatine, and 0.4 Na3-GTP and creatine phosphate 50 μM (pH adjusted to 7.20–7.25 with CsOH). ATP-regenerating patch solutions were used to maintain cytosolic levels of ATP and ensure that there was no rundown of GABA-B receptor-mediated responses.

All drugs were bath applied except for bicuculline methiodide. Bicuculline (100 μM) was applied locally through a glass micropipette with a tip diameter of ~40–50 μm. This micropipette was connected to a perfusing barrel and the drug was forced through by using overhead pressure. The flow of drug was calculated to be 0.3–0.5 ml/min. DNQX and NEM were made by dissolving the drug in dimethylsulfoxide. The final concentration of dimethylsulfoxide in the recording solution was 0.05%. Bicuculline methiodide, BAPTA, baclofen, and NEM were obtained from Sigma. APV, DNQX, and forskolin were purchased from Precision Biochemicals and 4,5,6,7-tetrahydrosoxazol(5,4-c)pyridin-3-ol hydrochloride (THIP) and Rp-cAMPs were from Research Biochemicals International. CGP 36742 was a gift from Ciba-Geigy.

Recording and analysis

CA1 pyramidal cell recordings were obtained using the blind, whole cell patch-clamp recording technique. Neurons were voltage-clamped at −70 mV for studies on sIPSCs and at cell resting membrane potential for studies only looking at evoked IPSCs. Acceptable neurons used for experiments had resting potentials between −58 and −65 mV immediately after break-in and input resistances >120 MΩ. Series resistances of neurons were between 10 and 30 MΩ and were compensated by ~70%, except in experiments on sIPSCs. If series
RESULTS

Tetanus-induced potentiation of evoked IPSCs

Using electrodes filled with either K-gluconate-based or CsCl-based patch solutions, bicusculine-sensitive GABA-A receptor-mediated fast IPSCs were recorded from hippocampal CA1 neurons with low-frequency stimulation (0.05 Hz) of the stratum radiatum. On tetanic stimulation (2 trains at 100 Hz for 1 s, 20-s interval) significant potentiations of evoked IPSCs and spontaneous IPSCs were observed in 60–70% of cells.

In neurons patched with gluconate-based patch solution, tetanic stimulation of the stratum radiatum induced a significant potentiation of the GABA-A receptor-mediated fast IPSC that developed gradually, reached its peak after ~7 min and maintained for ≤ 30 min posttetanus (Fig. 1A). This potentiation of the fast IPSC was reliably induced when the tetanus was delivered in both current- and voltage-clamp conditions. The magnitude, time of onset, and duration of potentiation were not significantly different in these cells so their data were pooled together. The average potentiation of IPSC amplitude was 125.0 ± 3.0% of control (n = 8, P < 0.05, ANOVA, Fig. 1A). The reversal potential of the IPSC was not significantly
changed during the potentiation (see also Xie et al. 1995).
When the peak amplitude of the control IPSC was scaled up to
the potentiated IPSC, there was no difference in the shape of
the IPSC, suggesting that the kinetics of the IPSC were not
changed during the potentiation (Fig. 1A).

In recordings of IPSCs from neurons filled with Cl\textsuperscript{-}-based
patch solution, a tetanic stimulation of the stratum radiatum
also produced a significant posttetanic potentiation. As in glu-
conate-filled cells, the potentiation occurred in neurons that
were either current- or voltage-clamped during the tetanic
stimulation. The potentiation of the IPSC, however, occurred
immediately with the first or second evoked response after the
tetanus. The average potentiation of the IPSC was 160.7 ±
2.7% of control and maintained for 30 min posttetanus (n = 7,
Fig. 1B, P < 0.05, ANOVA). The kinetics of the IPSC did not
appear to have changed, as there was no difference in the shape
of the pre- and posttetanus IPSC.

Tetanus-induced potentiation of evoked IPSCs does not
appear to be dependent on postsynaptic neuron membrane
potential changes induced during the tetanus since a potent-
iation could be obtained whether the postsynaptic mem-
brane potential was allowed to fluctuate during the tetanus
or not. Potentiation of evoked IPSCs could be induced
in neurons with either K-gluconate or CsCl as the major
patch solution constituent (others components of the patch
solution were identical and were of the similar concentra-
tions).

It is interesting that CA1 neurons filled with CsCl-based
patch solution displayed a larger and immediate potentiation
of the evoked IPSC while the potentiation of gluconate-filled
neurons was smaller and developed gradually peaking at 7–10
min posttetanus (Fig. 1). While these differences were not due
to changes in reversal potential, the exact reasons are unclear at
present.

FIG. 2. Tetanic stimulation (100 Hz) induced an increase in spontaneous IPSC (sIPSC) frequency. A: traces showing sIPSC
activity in control and 10 min posttetanus when the tetanus was delivered under current-clamp conditions. A1 and A2: sIPSC
amplitude histograms during control and posttetanus, respectively. Notice that sIPSC mean amplitude, standard deviation, and
coefficient of variation are not significantly altered when sIPSC frequency increases. A3: cumulative frequency curve for the data
in A1 and A2. B: sIPSC activity in a neuron when the tetanus was delivered under voltage-clamp conditions. B1 and B2: sIPSC
amplitude histograms that show that sIPSC amplitude, standard deviation, and coefficient of variation are not significantly altered
during this period of sIPSC potentiation. B3: cumulative frequency curve for the data in B1 and B2. Kolmogorov-Smirnov (K-S)
tests confirmed that there were no significant differences between the cumulative frequency curves. All neurons were recorded with
patch pipettes filled with CsCl-based solution were voltage-clamped at −70 mV. Events smaller than 10 pA in amplitude were not
included in the statistical analysis of these experiments.


Tetanus-induced potentiation of sIPSCs

When recording from CA1 neurons filled with Cl−-based patch solution, sIPSCs can be observed in the presence of APV and DNQX. sIPSCs were recorded continuously along with evoked IPSCs for 10–15 min before a tetanus was delivered. A tetanic stimulation delivered in either current- or voltage-clamp conditions produced a significant immediate increase in the number of sIPSCs (posttetanus 10 min C-clamp: 21.5 ± 15.0% of control; posttetanus 10 min V-clamp: 206.2 ± 12.5% of control, n = 8 and 6, respectively, P < 0.05, ANOVA with Duncan’s multiple comparisons test) but did not change the mean amplitude (C-clamp control: 21.7 ± 2.8 pA, C-clamp posttetanus: 22.5 ± 2.1 pA; V-clamp control: 25.6 ± 1.9 pA, V-clamp posttetanus: 27.1 ± 2.3 pA, P > 0.05, ANOVA with Duncan’s multiple comparisons test) or amplitude distribution of spontaneous IPSCs (Kolmogorov-Smirnov). The increase in sIPSC frequency sustained for 20 min. In Fig. 2 are typical examples of neurons that exhibited tetanus-induced potentiation of sIPSCs when the tetanus was delivered under and current- and voltage-clamp conditions. These results suggest that it is worthwhile to examine whether presynaptic mechanisms are involved in the potentiation.

As in evoked IPSCs, fluctuations in CA1 neuron membrane potential during the tetanus do not appear to significantly influence the potentiation of sIPSCs since in both cases the increase in the number of IPSCs was of similar magnitude. To minimize postsynaptic membrane potential fluctuations during the tetanus and possible postsynaptic modifications that may occur, in subsequent experiments, all neurons were voltage-clamped during the delivery of the tetanus.

Attempts were made to examine the effects of tetanus on miniature (mIPSC) amplitude and frequency; however, we were unable to effectively wash out TTX or STX in a reasonable amount of time so that a tetanus could be delivered. We tried to mimic the tetanus with high levels of K+ as well as block action-potential-driven spontaneous events by various methods that would allow faster recovery so that a tetanus could be delivered. These attempts, however, were also unsuccessful not effectively mimicking tetanus.

Input specificity of tetanus-induced potentiation of IPSPs

When IPSPs were evoked in CA1 neurons by two electrodes positioned in stratum radiatum such that the two inputs did not overlap, a tetanic stimulation of one input produced a significant potentiation of IPSPs induced by that, but not by the untetanized input, indicating input specificity of the potentiation (n = 5, Fig. 3, P < 0.05, ANOVA).

Postsynaptic responses to applied GABA-A and GABA-B agonists are not changed during tetanus-induced potentiation of IPSCs

To determine if the tetanus altered the postsynaptic sensitivity of GABA receptors to applied agonists, in neurons filled with gluconate-based patch solution, CA 1 neuron responses to the GABA-A agonist THIP (20 µM, for 1 min) were compared before and during the potentiation of the IPSC. During potentiation of the IPSC (posttetanus 7 min 126.0 ± 5.6% of control, n = 6, Fig. 4A1), the amplitude and shape of THIP-induced currents were not increased (96.2 ± 6.6% of control, n = 6, 6, Fig. 4A2, P > 0.05, Student’s t-test). Note that the potentiated IPSC was not altered by THIP (posttetanus 25 min 124.3 ± 4.0% of control, n = 6, Fig. 4A1). In other experiments, THIP was applied at twice the concentration and duration (40 µM, 2 min), as previously applied so that the drug-effect reached a plateau. In six of six neurons, neither the shape nor amplitude of the THIP-induced current (posttetanus THIP: 95.6 ± 6.2% of control, n = 6, P > 0.05, Student’s t-test, not shown) was significantly altered after the tetanic stimulation. These results suggest that the sensitivity of postsynaptic GABA-A receptors to applied agonists does not change during the tetanus-induced potentiation of the IPSC and that there was no change in the desensitization of THIP responses.

To test for changes in CA1 postsynaptic sensitivity to baclofen, the agent (50 µM, 1 min)-induced responses were compared before and during the potentiation of the IPSC (Fig. 4B1, n = 5). The baclofen-induced current was also not significantly altered (86.2 ± 8.2% of control, n = 5, Fig. 4B2, P > 0.05, Student’s t-test) during the potentiation of the IPSC. In addition, baclofen did not alter the potentiated IPSCs. It, therefore appears that potentiation of the fast IPSC is not secondary to tetanus-induced modifications of GABA-B receptors.

Tetanus-induced potentiation of evoked IPSCs in neurons containing BAPTA

Changes in postsynaptic Ca2+ have been shown to be important for the induction of LTP of excitatory synapses in CA1 neurons (Lynch et al. 1983; Malenka et al. 1988; Malinow et al. 1989). To determine if the potentiation of the IPSC was dependent on increases in postsynaptic Ca2+ concentration that may occur during and/or after the tetanus, experiments were performed in which the Ca2+ chelator, BAPTA (10 mM in the patch solution) was included in the Cl−-based patch solution. A tetanic stimulation delivered to the stratum radiatum, while neurons were current-clamped, produced a potentiation of the evoked IPSC. The onset of potentiation was immediate after the first or second response after tetanus and the potentiation in the IPSC was significantly larger than in neurons that did not contain BAPTA.
average potentiation: 202.2 ± 19.7% of control (n = 6, not shown) as compared with 160.7 ± 2.7% of control for non-BAPTA containing neurons, see Fig. 1B, P < 0.05, ANOVA] while the kinetics of responses were not altered. Spontaneous IPSCs number increased immediately (posttetanus 10 min: 234.0 ± 41.0%, n = 6, P < 0.05, ANOVA with Duncan’s multiple comparisons test) and maintained for 20 min while mean sIPSC amplitude remained unchanged (pretetanus: 27.8 ± 2.2 pA, posttetanus: 30.0 ± 2.0 pA, n = 6, P > 0.05, ANOVA with Duncan’s multiple comparisons test, not shown). These results suggest that postsynaptic Ca\(^{2+}\) is not essential for the potentiation and as suggested in earlier reports from this laboratory (Morishita and Sastry 1991; Xie at al. 1995) may even dampen the tetanus-induced potentiation of the IPSC.

It has been shown that elevations in postsynaptic Ca\(^{2+}\) concentrations suppress GABA-A-mediated responses through destabilizing the phosphorylation of the GABA-A receptor complex by the activation of several phosphatases (Chen et al. 1990; Morishita and Sastry 1996; Pitler and Alger 1992, 1994). Although further investigation into effects of postsynaptic Ca\(^{2+}\) levels on tetanus-induced potentiation was not the theme of this study and therefore not further pursued, our results are consistent with reports in literature.

Reports in literature show that increases in intracellular Ca\(^{2+}\) can lead to long-lasting suppression of GABA-A receptor function in central neurons (Chen and Wong 1995; Morishita and Sastry 1996; Stelzer and Shi 1994). Since APV was present throughout our experiments, Ca\(^{2+}\) entry into postsynaptic neurons during the tetanus is unlikely to be via NMDA channels. Tetanic stimulation of afferent fibers could, however, conceivably result in intracellular Ca\(^{2+}\) accumulation through other routes such as release from intracellular stores, activation of voltage-gated Ca\(^{2+}\) currents, etc.

**GABA-A receptor activation during the tetanus is not required for potentiation of IPSC**

To test whether activation of the fast IPSC during the tetanus was required for the potentiation of the IPSC to occur in gluconate-containing neurons, 100-Hz stimulations were delivered when the IPSC was blocked (0.13 ± 0.12% of control, n = 6, Fig. 5) by the GABA-A antagonist, bicuculline methiodide (100 μM, 2 min, locally applied). Bicuculline completely

---

**FIG. 4.** 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol hydrochloride (THIP) and baclofen responses are not altered during tetanus-induced potentiation of IPSCs. In separate experiments, THIP (A1, 20 μM, 1 min) and baclofen (B1, 50 μM, 1 min) were applied during control and during the potentiation of the evoked IPSC (A and B). Note that neither THIP nor baclofen induced lasting effects on the amplitude of the evoked IPSC since after washout of the drug the IPSC amplitude resembled those before drug application. A2: THIP-induced responses were not significantly altered in shape and amplitude during the period when the evoked IPSC was potentiated (n = 6). B2: baclofen-induced currents were also not significantly changed when the evoked IPSC was potentiated (n = 5). All neurons were recorded with K-gluconate-based patch solution and voltage-clamped between −58 and −63 mV.
abolished the fast IPSC within 1 min of application, revealing in two of six neurons a much smaller slow GABA-B-mediated IPSC. In control experiments, recovery of the fast IPSC occurred after a washout period of >25 min (n = 6). In test experiments, a tetanus was delivered when the fast IPSC had been completely abolished for 30 s. In slices that were tetanized, IPSCs recovered more quickly reaching pretetanus levels in <15 min and then became significantly greater than pretetanus levels (n = 6). Traces are averages of 2 IPSCs, they indicate faster recovery and a potentiation of IPSCs in tetanized as opposed to nontetanized neurons.

**GABA-B receptor activation during the tetanus is important for tetanus-induced potentiation of fast IPSCs**

Many reports in literature suggest the presence of presynaptic GABA-B receptors (autoreceptors) on GABAergic nerve terminals and their role in reducing GABA release (for review, see Misgeld et al. 1995). However, its functional role is still unclear. More recently, presynaptic GABA-B receptors have been suggested to be involved in the enhancement of transmitter release (Brenowitz et al. 1998; Glaum and Brooks 1996).

Previous studies from this laboratory (Xie et al. 1995) have shown that inhibition of GABA-B receptors does not effect already existing tetanus-induced potentiation of the fast IPSP, suggesting that GABA-B receptors are not involved in the maintenance of the potentiation of GABA-A-mediated synaptic transmission and that the increase in the observed fast IPSP was not secondary to changes in GABA-B-mediated responses.

In the present study, experiments were performed to determine whether GABA-B receptor activation is required for the induction of potentiation of the IPSC and sIPSCs. A tetanic stimulation was delivered to the stratum radiatum in the presence of the GABA-B antagonist CGP 36742 (500 μM, for 10 min before, during the tetanic stimulation and then for the remainder of the experiment, n = 6). While CGP 36742 did not affect the control response, only an initial transient increase in the evoked IPSC amplitude, which lasted for 1–3 min following the tetanus, could be induced in its presence (Fig. 6A, P > 0.05, ANOVA). Spontaneous IPSC number also increased transiently, but at 5 min after the delivery after the tetanus, they had returned to control levels (Fig. 6B, P > 0.05, ANOVA with Duncan’s multiple comparison’s test). Mean amplitude of sIPSCs did not change in the presence of CGP 36742 or posttetanus (pretetanus: 15.5 ± 1.6 pA, CGP 36742; 15.2 ± 1.3 pA, posttetanus: 16.0 ± 1.6 pA, P > 0.05, ANOVA with Duncan’s multiple comparison’s test) It was observed that in two of six neurons, CGP 36742 reduced the frequency of sIPSCs substantially but when results were pooled together this decrease was not significant. The kinetics for the IPSC and sIPSCs remained similar during the period of enhanced amplitude and after return to control levels. These observations suggest that activation of GABA-B receptors is an essential step in tetanus-induced potentiation of the fast IPSC.

Actions of GABA-B receptors are mediated via G proteins (Andrade et al. 1986; Dutar and Nicoll 1988; Thalmann 1988). It has been shown that when GTPγS, a nonhydrolyzable analogue of GTP that activates G proteins, was applied intracellularly, postsynaptic GABA-B-mediated responses could not be seen (Thalmann 1988). In the present study, in some neurons an equimolar substitution of GTPγS for GTP in gluconate patch solution was made. As expected these neurons had much more negative resting membrane potentials and lower input resistances, and bath-applied baclofen (50 μM, 1 min) elicited negligible responses (not shown). In these neurons, a tetanic stimulation induced a potentiation of evoked IPSCs that was seen with the first response after the 100-Hz stimulations (average potentiation:136.8 ± 3.0% of control, n = 6, Fig. 6C, P < 0.05, ANOVA) and lasted for 30 min. The kinetics of control and posttetanus IPSCs were not significantly different.

Postsynaptic GABA-B receptor-coupled K+ channels are known to be blocked by Cs+ (Gahwiler and Brown 1985; Jarolimek et al. 1995); in addition high concentrations of
intracellular Cl\(^-\) (such as concentrations used in this study) have been shown to greatly decrease postsynaptic GABA-B responses (Lenz et al. 1997). With high concentrations of Cs\(^+\) and Cl\(^-\) together in the postsynaptic electrode, one would expect postsynaptic GABA-B receptors to be nearly completely antagonized. GTP\(\gamma\)S, intracellularly, by activating G proteins occludes subsequent activation of postsynaptic GABA-B receptors. Our observation of tetanus-induced potentiation of the fast IPSC under both of the above-mentioned conditions strongly suggest that postsynaptic GABA-B receptors are not involved in the induction of potentiation of fast IPSCs. However, in experiments with CsCl in the recording electrode, tetanic stimulation delivered during the application of the GABA-B antagonist CGP 36742 blocked the potentiation of the fast IPSC. Taken together, these results suggest that presynaptic GABA-B receptors are involved in the induction of GABA-A-mediated potentiation.

**Dependence of the potentiation on PKA activity**

Several subunits of GABA-A receptors have putative phosphorylation sites for PKA. The involvement of these protein kinases in inhibitory synaptic transmission has only recently been studied (Capogna et al. 1995; Trudeau et al. 1997). The involvement of these enzymes in plasticity of GABAergic synapses is not well understood.

To determine if PKA activation is necessary for induction of tetanus-induced potentiation, a tetanus was delivered in the presence of the competitive PKA antagonist, Rp-cAMPs (20 \(\mu\)M, 10 min prior to the delivery of, during the tetanic stimulation and then for the remainder of the experiment). In the presence of Rp-cAMPs, no significant potentiation of the evoked IPSC was observed (posttetanus 5 min 102.0 ± 9.5% of control, posttetanus 20 min: 107.0 ± 13.3% of control, \(n = 6\), Fig. 7A, \(P > 0.05\), ANOVA). Tetanic stimulation failed to produce an increase in the number of sIPSCs (posttetanus 5 min: 92.5 ± 13.2% of control, \(n = 6\), Fig. 7B, \(P > 0.05\), ANOVA with Duncan’s multiple comparison’s test) and did not change the mean sIPSC amplitude or distribution (pretetanus: 17.3 ± 1.1 pA, Rp-cAMPs: 17.5 ± 1.1 pA, posttetanus: 17.2 ± 1.0 pA, \(P > 0.05\), ANOVA with Duncan’s multiple comparison’s test). Rp-cAMPs itself does not effect the control IPSC amplitude (Rp-cAMPs: 99.6 ± 2.7%, control, \(P > 0.05\), ANOVA with Duncan’s multiple comparison’s test) or the mean amplitude of sIPSCs.

In separate experiments, forskolin was used to stimulate PKA. In control experiments, bath applied forskolin (20 \(\mu\)M, 25 min) increased the amplitude of evoked IPSCs and the number but not the amplitude of sIPSCs, similar results are described by Capogna et al. (1995) (Fig. 8, A and B). These effects persisted even after 20 min of washout (evoked IPSC 20-min washout: 150.4 ± 13.6% of control, \(P < 0.05\), ANOVA, sIPSC frequency 20-min washout: 164.0 ± 8.6% of control, \(n = 7\), Fig. 8, A and B, \(P < 0.05\), ANOVA with Duncan’s multiple comparison’s test). To determine if a tetanic stimulation could induce a potentiation of the IPSC in addition...
to that produced by forskolin in different neurons, a tetanus was delivered during the period of increased GABAergic synaptic transmission 15 min after the beginning of the application of forskolin. In these experiments, no further increase in the evoked IPSC was observed, instead by 10 min posttetanus, tetanic stimulation did not alter the kinetics of evoked or spontaneous IPSCs nor did it change sIPSC amplitude.

Results from the cAMP antagonist suggest that PKA activation is needed for tetanus-induced potentiation of evoked and sIPSCs. If this is the case, PKA activation by forskolin would be expected to at least partially occlude potentiation induced tetanus. This was observed for sIPSCs but not observed for the evoked IPSC, instead it appeared that the tetanus caused a depotentiation of the evoked IPSC or induced a long-term depression of the IPSC.

Although our results suggest PKA activation is important in tetanus-induced potentiation of evoked IPSCs and sIPSCs, it is clear that further investigation is required to elucidate the exact mechanisms.

Sulfhydryl-alkylation is important for potentiation of IPSCs

NEM is a sulfhydryl alkylating agent that has been shown to block pertussis-toxin-sensitive GABA-B actions and increase the release of GABA from presynaptic terminals in the rat hippocampus by an unknown presynaptic mechanism (Morishita et al. 1997). To determine if the alkylation of sulfhydryl groups is important for tetanus-induced potentiation of fast IPSCs, the effects of NEM on tetanus-induced potentiation was investigated. Control studies showed that the NEM (250 μM, 11 min) caused significant increases in amplitude of the fast IPSC (P < 0.05, ANOVA) and the number, but not the mean amplitude (control: 25.2 ± 3.2 pA, NEM: 26.7 ± 2.6 pA, P > 0.05, ANOVA with Duncan’s multiple comparison’s test) of spontaneous IPSCs that returned to control levels after ~20 min of washout (Fig. 9, A and B). In separate experiments, a tetanus was delivered to the stratum radiatum during the 11th min of NEM application, during the peak of NEM-induced increase in inhibitory synaptic transmission. No further potentiation of the IPSC was observed, NEM occluded further potentiation of both evoked (Fig. 9A, P > 0.05, ANOVA) and sIPSCs without changing mean sIPSC amplitude (NEM: 26.7 ± 2.6 pA, posttetanus 27.1 ± 2.9 pA, Fig. 9B, P > 0.05, ANOVA with Duncan’s multiple comparison’s test).

To further test if the observed NEM effects were due to its effects on sulfhydryl groups, a different sulfhydryl alkylating agent p-chloromercuribenzoic acid (PCMB, 250 μM, 11 min) was tested. In control experiments, PCMB was also found to increase the amplitude of evoked IPSCs and the number but not the mean amplitude of sIPSCs, and these effects maintained for 30 min after washout (Fig. 9, C and D). PCMB then was applied for 11 min and a tetanus was delivered during the 11th min of drug application. PCMB was also found to occlude the potentiation of evoked and sIPSCs, with no significant increases in IPSC amplitude (Fig. 9C, P > 0.05, ANOVA) or sIPSC frequency (Fig. 9D, P > 0.05, ANOVA with Duncan’s multiple comparison’s test).

These results suggest that NEM modulates a component(s) of the pathway that leads to tetanus-induced potentiation of...
DISCUSSION

Plasticity of excitatory synapses has been extensively studied in laboratories around the world. However, plasticity of inhibitory synapses has not received such attention. In this paper we report that GABAergic synapses in the CA1 area of the hippocampus undergo plasticity. Previous studies from our laboratory have shown that a tetanic stimulation in the stratum radiatum induces a sustained potentiation of GABA-A receptor-mediated fast IPSPs recorded from rat hippocampal CA1 neurons (Morishita and Sastry 1991; Xie and Sastry 1991; Xie et al. 1995). This potentiation is likely localized to GABAergic synapses rather than other areas (see Xie et al. 1995), and our current results suggest that it is input specific. Postsynaptic neuronal protein kinase C (PKC) activation and Ca\(^{2+}\) accumulation are not necessary for, and may even dampen, the tetanus-induced potentiation of fast IPSPs (Xie and Sastry 1991; Xie et al. 1995). Major findings from the present study strongly suggest that the potentiation of GABA-A receptor-mediated synaptic transmission is due to modifications that occur presynaptically and involve GABA-B receptors and PKA.

GABA receptor involvement in tetanus-induced potentiation of IPSCs

Activation of GABA-A receptors during the tetanic stimulation appears to be unnecessary for the induction of a potentiation of the fast IPSCs. Moreover the potentiation could be induced if the tetanus was given while the CA1 neurons were voltage-clamped, indicating that alterations in postsynaptic neuronal membrane potentials were not needed to induce the potentiation. While blocking postsynaptic GABA-B actions by including Cs\(^+\) and Cl\(^-\) together (Lenz and Alger 1997) or occluding these effects by GTP\(_\gamma\)S, in the postsynaptic neurons, a potentiation of fast IPSCs could still be induced by high-frequency stimulation, suggesting that activation of postsynaptic GABA-B receptors is not needed for the induction of the potentiation. Interestingly, in neurons in which postsynaptic GABA-B actions were blocked, CGP 36742 still antagonized the induction of tetanus-induced potentiation, suggesting the involvement of presynaptic GABA-B receptors. The maintenance of tetanus-induced potentiation of fast IPSPs was not affected by blocking GABA-B receptors (Xie et al. 1995), suggesting that changes in GABA-B responses could not account for the observation of enhanced GABA-A IPSCs. Neither postsynaptic responsiveness to GABA-A receptor agonist, THIP, nor desensitization of GABA-A receptors to a prolonged
application of this agent was altered during the posttetanic sustained potentiation of the IPSC.

Many studies indicate the role of presynaptic GABA-B receptors in reducing GABA release from terminals (Baumann et al. 1990; Calabresi et al. 1991; Davies and Collingridge 1993; Davies et al. 1990; Diesz and Prince 1989; Olpe et al. 1994; Pittaluga et al. 1987). Presynaptic GABA-B receptors were also implicated in an enhancement of GABAergic synaptic transmission in different areas of the CNS (Brenowitz et al. 1998; Glaum and Brooks 1996). In our studies, it is unclear how activation of presynaptic GABA-B receptors during a tetanic stimulation can set up a long-lasting enhancement of GABAergic synaptic transmission. The fact that baclofen by itself does not induce a long-term change in fast IPSCs, however, suggests that the activation of presynaptic GABA-B receptors, if involved, must work in conjunction with other factors influenced by high-frequency stimulation. It is also possible that bath-applied baclofen simply does not accurately mimic the activation of presynaptic GABA-B receptors by endogenously released GABA during tetanic stimulation.

It is unclear why the potentiation of the IPSC was immediate and not gradual when GTPγS was included in the gluconate-patch solution. GTPγS is not specific to GABA-B related G proteins and as a result can upregulate other G-protein-linked receptor systems. Staley et al. (1995) showed that mechanisms modulating activity-dependent collapse of transmembrane Cl− currents are inhibited by PKC activation; it is conceivable that GTPγS would cause an activation of PKC. If this was the case, GTPγS would indirectly block the collapse of the transmembrane Cl− gradient during tetanus allowing for an immediate or early potentiation of IPSCs; this would be consistent with other findings in this study. However, the sustained potentiation of the IPSCs observed in our studies was not associated with a change in the reversal potential.

Involvement of PKA

There are several consensus sites for phosphorylation within major intracellular domains of GABA-A receptor subunits for cAMP-dependent protein kinase (Gehlert et al. 1985). There are conflicting reports on how PKA functionally affect GABA-A receptor actions. Studies have suggested that activation of presynaptic PKA enhances basal GABA release from GABAergic interneurons by independent mechanisms of action (Capogna et al. 1995). Our finding that Rp-cAMPs, a competitive antagonist of cAMP, blocks long-lasting potentiation of the evoked IPSC and sIPSCs without affecting sIPSC amplitude is consistent with findings by Capogna et al. (1995).
and Trudeau et al. (1997), who suggested that PKA activation enhances evoked and spontaneous GABA-A mediated synaptic transmission. In addition, adenyl cyclase activator, forskolin, which is known to activate PKA, enhanced GABA-A receptor-mediated evoked IPSCs as well as basal sIPSC frequency but not amplitude.

If PKA activation is involved in the tetanus-induced potentiation of IPSCs, it was felt that forskolin would occlude a tetanus-induced increase in IPSCs. In fact, a slowly developing depotentiation or a long-term depression (LTD) of evoked IPSCs was observed when tetanized in the presence of forskolin. Activity-mediated LTP and LTD at excitatory synapses have been shown to occur simultaneously (Bear and Malenka 1994), but factors that determine which phenomenon predominates under certain circumstances still need to be determined. It is possible that LTP and LTD at inhibitory synapses can also co-exist in a similar manner. In our laboratory, it has recently been shown that IPSCs in deep cerebellar nuclear cells undergo LTD (Morishita and Sastry 1996) or LTP depending on the induction parameters (Ouardouz and Sastry 1999). If a similar case exists in the hippocampus; a tetanus delivered when synapses were maximally potentiated could reveal a previously unseen depression.

It is unclear as to how tetanus can induce an enhancement of GABA release. Activation of presynaptic PKA can theoretically affect any part of the stimulation secretion-coupling pathway, from Ca^{2+} entry into the presynaptic terminal to transmitter release processes. Investigations to understand how GABA-B and PKA pathways interact in the mammalian CNS are currently being pursued by various groups (Barthel et al. 1996; Malcangio and Bowery 1993; Xi et al. 1997; Yoshimura et al. 1995). It has been suggested that GABA-B receptors are linked to the adenyl cyclase pathway via G proteins (Wojcik et al. 1989; Xi et al. 1997). By activating GABA-B receptors, one can modulate cAMP production and subsequently PKA activity (Bowery 1993; Oset-Gasque et al. 1993; Scherer et al. 1989; Wojcik et al. 1989; Xi et al. 1997). It has been suggested (Barthel et al. 1996; Xi et al. 1997) that through this pathway that GABA-B receptors can acutely suppress GABA-A currents. However, results in our study as well as those of others (Capogna et al. 1995; Trudeau et al. 1997) suggest that activation of PKA can lead to a long-lasting enhancement of GABA release and IPSCs. It is tempting to speculate that the long-term effect of presynaptic GABA-B receptor-mediated and tetanic stimulation-induced increase in PKA activity is to increase GABA release.

Several proteins recently identified as being associated with neuron cytoskeletal elements such as synapsin, GAP-43, dynamin, MAP-2, and RAB 3A, and proteins involved in the fusion of presynaptic vesicles with the presynaptic membrane such as α-SNAP, SNAP-25, and NSF, all having putative sites for protein kinase phosphorylation (for review, see Whatley and Harris 1996). Phosphorylation of any of these proteins could potentially lead to enhanced GABA release and IPSCs. Trudeau et al. (1997) suggested that PKA activation causes synaptic facilitation by directly elevating probability of exocytosis of individual vesicles at a step downstream from Ca^{2+} influx. Forskolin has been shown to enhance phosphorylation of synapsin and raphphilin, the effector protein of Rab3A, both proteins are essential for LTP at excitatory synapses (Lonart and Sudhof 1998).

**Sulphydryl-alkylating agents and tetanus-induced potentiation**

The sulphydryl-alkylating agent, NEM has been reported to enhance GABA release, potentiating evoked IPSCs and increasing the frequency of sIPSCs, from presynaptic terminals in the rat hippocampus via a presynaptic mechanism (Morishita et al. 1997). During NEM- and PCMB-induced enhancement of GABAergic IPSCs, a tetanus-induced potentiation of these synaptic transients was occluded. The simplest explanation for this observation is that tetanus- and sulphydryl-alkylating agent-induced enhancement of GABA-A receptor-mediated responses share common pathways. No changes in sIPSC kinetics, amplitudes, or amplitude distribution, which might indicate tetanus-induced postsynaptic modifications, were observed.

NEM has been shown to de-couple G protein receptors from their substrates in central neurons (Kitamura and Nomura 1987; Shinoda et al. 1990). If NEM and PCMB block presynaptic GABA-B actions in this manner and activation of these GABA-B receptors is some how important for induction of potentiation, as we have shown, then NEM and PCMB could block potentiation of IPSCs. In this case, the mechanism of how sulphydryl-alkylating agents occlude GABAergic plasticity may not be related to how they affect GABA release from presynaptic terminals.

NEM directly modulates NSF, an ATPase whose function is essential for vesicular fusion to presynaptic membranes and thus transmitter release (Whiteheart et al. 1994). If tetanus and NEM share similar pathways that affect GABAergic plasticity, it is possible tetanus modulates factors that directly affect proteins important in secretory machinery. It is also possible that tetanus can affect NSF indirectly through presynaptic GABA-B receptors and PKA.

It would be interesting to investigate if and how presynaptic GABA-B receptors and PKA interact and if they together regulate GABA-A-mediated synaptic plasticity. Does PKA modulate proteins important in presynaptic vesicular release and if so how do these proteins modulate potentiation of fast IPSCs?

In conclusion, based on the following observations, we suggest that the tetanus-induced potentiation of GABAergic IPSCs is presynaptic in origin. 1) With stimulation of two separate inputs that elicited fast IPSCs in the same neuron, only the pathway subjected to high-frequency stimulation showed a long-lasting potentiation; therefore the potentiation appears to be input specific. 2) Changes in postsynaptic membrane potential, during the tetanic stimulation, are not required to induce the potentiation. 3) Tetanic stimulation produced an increase in the frequency of spontaneous synaptic events without an increase in the mean sIPSC amplitude or a change in amplitude distribution of sIPSCs. 4) Presynaptic rather than postsynaptic GABA-B receptors appear to be needed for the induction of the tetanus-induced potentiation of evoked and sIPSCs. 5) NEM, which has been suggested to increase GABA release by a presynaptic mechanism (see Morishita et al. 1997), occludes tetanus-induced potentiation of evoked as well as sIPSCs.

While the potentiation of GABAergic IPSCs in CA1 neurons may involve an increase in GABA release at synapses, it is, however, possible that tetanic stimulation recruits and activates previously inactive presynaptic terminals and/or release sites as well as silent GABA-A receptor clusters. Another possibility is that the observed increase in evoked IPSCs might be due to an
increase in the safety factor for action potential propagation at axon branching into collaterals, leading to an increase in the number of detected sIPSCs in our samples and, therefore, in the size of evoked sIPSCs.

Significance of plasticity of GABAergic synaptic transmission

A great deal of information about activity-mediated plasticity at hippocampal excitatory synapses has accumulated in literature. It should, however, be realized that the hippocampus functions in complex network patterns and that inhibitory interneurons form numerous connections with, and regulate many functional aspects of, principal neurons (for review, see Freund and Buzsáki 1996). Moreover, interneurons have been shown to communicate with each other and in doing so, can strongly influence hippocampal function (Csicsvári et al. 1999; Freund and Buzsáki 1996; Hájos and Mody 1997). GABA is the major inhibitory transmitter and network behavior in the CNS is dependent on the balance between excitation and inhibition. Therefore potentiation of inhibition, as observed in our studies, could significantly modulate this balance and affect network function. The hippocampus has long been implicated as a site that is important in learning processes and the storage of memory (Bliss and Lomo 1973; Lynch et al. 1979).

Neurons in hippocampus can exhibit seizure activity (Stelzer et al. 1987) and can be greatly affected by neurodegenerative (Rapport et al. 1985; Wiley et al. 1998) and aging processes (Shefer 1977). The sustained potentiation of GABA-A receptor-mediated fast sIPSCs described in this study, thus would have important implications in developmental neurobiology, hippocampal physiology as it relates to learning and memory, and in neurological diseases and disorders. Our studies were conducted on 2-wk-old rats. Whether the observed plasticity exists in other age groups is unknown and should be determined.

We thank CIBA-Geigy for the generous gift of CGP 36742. This work was supported by Medical Research Council studentships to T. R. Shew and S. Yip and National Institute of Neurological Disorders and Stroke Grant NS-30959 to B. R. Sastry.

Address reprint requests to B. R. Sastry.

Received 4 November 1999; accepted in final form 29 February 2000.

REFERENCES


