Neuron, Vol. 10, 165-175, February, 1993, Copyright © 1993 by Cell Press

Local and Diffuse Synaptic Actions of GABA in the Hippocampus

J. S. Isaacson, J. M. Solís,* and R. A. Nicoll
Physiology Graduate Program
and the Departments of Pharmacology
and Physiology
University of California, San Francisco
San Francisco, California 94143-0450

Summary

In the CNS, γ-aminobutyric acid (GABA) acts as an inhibitory transmitter via ligand-gated $GABA_A$ receptor channels and G protein-coupled $GABA_B$ receptors. Both of these receptor types mediate inhibitory postsynaptic transmission in the hippocampus. In addition to these direct postsynaptic actions, $GABA_B$ receptor agonists inhibit excitatory transmission through presynaptic receptors on excitatory afferent terminals. However, a physiological role for the $GABA_B$ receptors on excitatory nerve endings has not been established. In this study, we have found a brief, heterosynaptic depression of excitatory synaptic transmission in the CA1 region of the hippocampal slice following short-lasting repetitive stimulation and determined that this inhibition is mediated by presynaptic $GABA_B$ receptors. The inhibition of GABA uptake greatly enhanced both the presynaptic action of GABA and the slow $GABA_B$-mediated inhibitory postsynaptic current. Transmitter uptake was also found to regulate the "spill-over" of GABA at conventional $GABA_A$ synapses. These results suggest that uptake mechanisms restrict the spatial range of both point-to-point synaptic transmission mediated by GABA and its action at a distance.

Introduction

Synaptic transmission is generally divided into two categories, fast and slow, based on the time course of the postsynaptic response (Hille, 1992; Kandel et al., 1991; Nicoll et al., 1990; Scheller and Hall, 1992). Fast transmission, on the time scale of a few milliseconds, is mediated by the transmitters acetylcholine, γ-aminobutyric acid (GABA), glutamate, and glycine and is characterized by ligand-gated ion channels that have a relatively low affinity for the transmitter. The actions of these transmitters are typically restricted to the synaptic region, and their clearance is accomplished by uptake or enzymatic degradation. In contrast with this point-to-point transmission, slow synaptic communication, largely studied in the peripheral nervous system and typified by peptidergic transmission (Jan and Jan, 1983), can last for seconds to minutes and is mediated by high affinity receptors coupled to G proteins. The transmitters mediating slow responses can act at diffusely distributed receptors and therefore may not be restricted to the synaptic region. While much attention has been given to the properties of the receptors mediating fast and slow transmission, little is known, especially in the CNS, about the lifetime of the transmitters giving rise to these responses and the distances over which they can act.

In addition to their postsynaptic actions, many neurotransmitters and their agonists have powerful presynaptic effects (Nicoll et al., 1990; Starke, 1981). In the CNS, receptors on nerve terminals are widespread, but in most cases the physiological role of these receptors is unclear, since it is not known whether synaptically released transmitters gain access to these sites. The best-characterized role for presynaptic receptors comes from studies on spinal presynaptic inhibition, in which axo-axonic synapses release GABA onto primary afferent fibers. This synaptically released GABA acts primarily through an action on $GABA_A$ receptors to inhibit transmitter release from the afferent terminals (Eccles et al., 1963; Stuart and Redman, 1992). It is generally agreed, however, that axo-axonic synapses on synaptic terminals are confined to the brain stem and spinal primary afferent fibers (Peters et al., 1991). Another way, in addition to axo-axonic synapses, in which neurotransmitters have direct access to presynaptic receptors is at terminals from which they themselves have been released (autoreceptors) (Langer, 1987; Starke et al., 1989). A third possibility is that released neurotransmitters act on distant synapses in a paracrine fashion (Fuxe and Agnati, 1991). Indeed, many instances have been found in which the neurotransmitter receptor distribution does not match the distribution of neurotransmitter (Herkenham, 1987). However, there is little physiological evidence in the CNS in support of the distant action of synaptically released neurotransmitters.

In the hippocampus, synaptically released GABA generates both fast ($GABA_A$ receptor-mediated) and slow ($GABA_B$ receptor-mediated) postsynaptic inhibition (Nicoll et al., 1990). Application of the $GABA_B$ agonist baclofen can block both excitatory and inhibitory synaptic transmission by activating presynaptic receptors (Bowery et al., 1980; Lanthorn and Cotman, 1981; Olpe et al., 1982; Dutar and Nicoll, 1988b; Inoue et al., 1985; Ault and Nadler, 1982; Harrison, 1990). While GABA regulates its own release locally via autoreceptors on inhibitory terminals (McCarren and Alger, 1985; Deisz and Prince, 1989; Davies et al., 1990), it is unclear whether GABA can diffuse to the presynaptic receptors on excitatory nerve terminals. We have designed experiments to address this issue as well as to explore the role of uptake in controlling the spatial domain of both the pre- and postsynaptic actions of GABA.

*Present address: Departamento de Investigacion, Hospital Ramòn y Cajal, Carretera de Colmenar Km 9, 28034 Madrid, Spain.

Results

We initially attempted to determine whether synaptically released GABA activates presynaptic $GABA_B$ receptors on excitatory terminals in the hippocampus by examining the actions of the selective $GABA_B$ receptor antagonist CGP 35348 on excitatory synaptic responses. However, the antagonist had no consistent effects on single synaptic responses evoked by stimulation in the dendritic region of CA1 pyramidal cells, nor did it affect responses evoked with a paired-pulse protocol similar to that used to study the autoinhibition of inhibitory synaptic responses (Davies et al., 1990). Nevertheless, a single conditioning stimulus may not have released enough GABA to produce the effect, or paired-pulse stimulation may have produced a presynaptic enhancement of synaptic strength that obscured the inhibition.

We then designed an experiment to study the action of GABA, released from a repetitively stimulated set of fibers, on a naive set of excitatory afferent inputs. As illustrated in Figure 1A, two stimulating electrodes that activated overlapping but independent excitatory fibers were placed on either side of the recorded cell. One electrode (S1) was used to deliver a brief tetanus (5 pulses, 50 Hz) that would activate both excitatory and inhibitory fibers in the hippocampal slice. The other electrode (S2) was used to evoke single excitatory postsynaptic currents (EPSCs). Whole-cell recording was used to record the synaptic events, and the pipette solution contained Cs^+ to minimize the postsynaptic contribution of the slow inhibitory postsynaptic current (IPSC). When the two stimulating electrodes were properly positioned, a brief tetanus to S1 reliably reduced the size of the EPSC evoked by electrode S2 (Figure 1B). To determine whether $GABA_B$ receptors were involved in this inhibition, the selective $GABA_B$ antagonist CGP 35348 (Olpe et al., 1990) was added to the solution superfusing the slice. Shortly after its addition, the antagonist abolished the inhibition of the test response, indicating that $GABA_B$ receptors mediated the depression of excitatory synaptic transmission.

The time course of the inhibition, examined by varying the interval between S1 and S2, is shown by the closed circles in Figure 2. The inhibition peaks at about 300 ms and lasts for more than 1 s. The time course of a typical $GABA_B$-mediated slow IPSC, evoked with a brief tetanus in a cell in the absence of Cs^+ in the recording pipette, is shown on the same graph. The time course of the postsynaptic $GABA_B$ response was quite constant, as shown by the average time course of the slow IPSC derived from recordings in 4 cells (Figure 2, diamonds). Although the time courses of the pre- and postsynaptic actions of GABA are similar, the inhibition of EPSCs reaches its maximum somewhat later than does the slow IPSC and lasts for a longer time. The inhibition of the EPSCs was entirely blocked by CGP 35348 (Figure 2, open circles).

The inhibition of excitatory transmission was also observed using field potential recording of excitatory postsynaptic potentials (EPSPs). In these experiments, the same experimental design was used as in Figure 1A, but the extracellular recording electrode was placed midway between the two stimulating electrodes in stratum radiatum. The stimulation protocol is shown schematically in Figure 3A. To establish firmly that the depression was indeed presynaptic, paired-pulse facilitation was monitored by delivering pairs of stimuli separated by 50 ms. If the inhibition is presynaptic, the degree of facilitation should increase, as with other manipulations that decrease transmitter release (Katz and Miledi, 1968; Mallert and Martin, 1968; Harris and Cotman, 1983; Muller and Lynch, 1989). Figure $3B_1$ shows that when preceded by a conditioning tetanus, the test EPSP is reduced (closed circles) compared with the test EPSP alone (open circles) and that this difference is abolished by application of CGP 35348. As shown in Figure $3B_2$, paired-pulse facilitation, expressed as a facilitation ra-

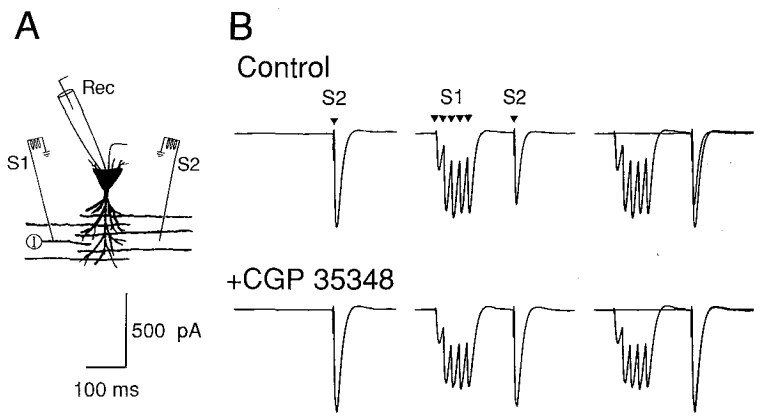

Figure 1. $GABA_B$ Receptors Mediate a Short-Lasting Heterosynaptic Depression of Excitatory Synaptic Transmission

(A) Schematic diagram illustrating the placement of stimulating and recording electrodes used to study heterosynaptic depression. One stimulating electrode (S1), which activates excitatory afferent fibers as well as inhibitory fibers from local interneurons (I), was used to deliver a brief conditioning tetanus (5 pulses, 50 Hz). This conditioning stimulus was delivered 200 ms before the stimulation of a seperate set of test inputs from electrode S2.

(B) Heterosynaptic depression in a voltage-clamped cell (V_h = −80 mV, temperature = 38°C) was blocked by CGP 35348. (Top) Test EPSCs alone (S2) and preceded by a conditioning tetanus (S1). Both traces are shown superimposed on the right. The conditioning tetanus caused a clear reduction in the amplitude of the test EPSC. (Bottom) This effect was blocked by the subsequent addition of CGP 35348 (500 μM) to the superfusing medium.

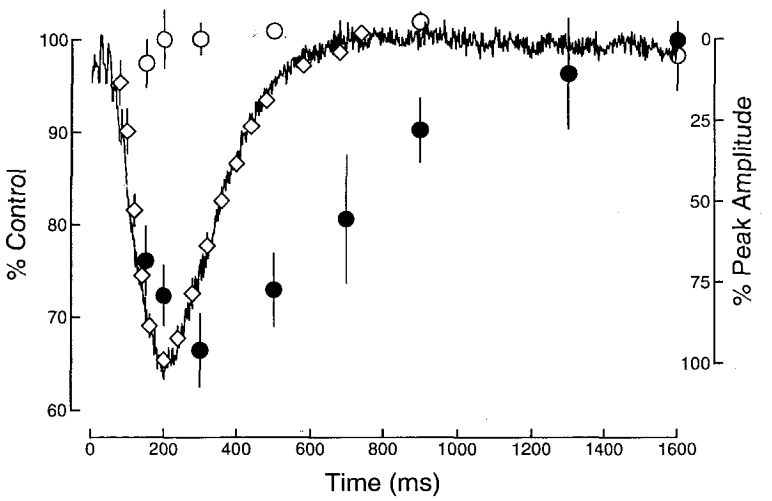

Figure 2. The Time Course of the Heterosynaptic Depression Is Similar to the Time Course of the Slow IPSC

The time course of heterosynaptic depression was studied by varying the interval between the conditioning tetanus (5 pulses, 50 Hz) and the test stimulus. The amount of depression is plotted as the amplitude of the test pulse when preceded by the conditioning tetanus relative to the amplitude of the test pulse alone (closed circles, n = 9 cells). In 4 of the cells, CGP 35348 was added to the superfusing solution. The $GABA_B$ antagonist completely blocked the depression at the 6 intervals tested (open circles). A monosynaptic slow $GABA_B$ IPSC was evoked with a brief tetanus (5 pulses, 50 Hz) in a cell recorded with K^+ in place of Cs^+ in the patch electrode and with 20 μM CNQX added to the superfusing solution. The trace is shown inverted for comparison with the time course of the heterosynaptic depression. The peak amplitude of the slow IPSC was 60 pA. Superimposed on the individual trace is the average time course of the slow IPSC derived from 4 cells (diamonds) plotted relative to their peak amplitude. The slow IPSCs were normalized with respect to their peak amplitudes before averaging.

tio, was also monitored in this experiment and increased during the inhibition. As with the inhibition, the change in the facilitation ratio was blocked by CGP 35348. Sample records from this experiment are shown in Figure 3C.

Although the $GABA_B$-mediated presynaptic inhibition was clearly present at 38°C (Figure 1), the inhibition was more robust at lower temperatures (30°C–32°C), facilitating characterization of the effect and suggesting that transmitter uptake mechanisms, which are strongly temperature sensitive (e.g., Iversen and Neal, 1968), might be involved. If the inhibition depends upon the spread of GABA over some distance, one might expect that GABA uptake would play an

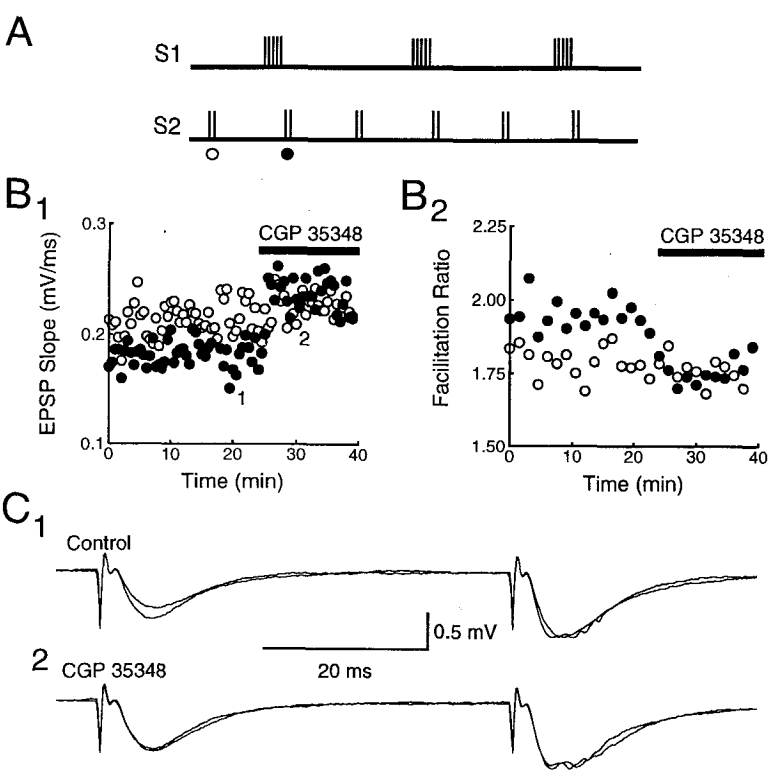

Figure 3. $GABA_B$ Receptor-Mediated Heterosynaptic Depression of the Field EPSP Is Accompanied by an Increase in Paired-Pulse Facilitation

(A) Stimulation protocol used to study heterosynaptic depression. Test EPSPs were evoked in one pathway (S2) with paired-pulse stimulation (interstimulus interval = 50 ms) every 15 s. Test responses were evoked with (closed circles) or without (open circles) a preceding conditioning tetanus delivered to an overlapping pathway (S1).

(B_1) Stimulation of the conditioning pathway caused a decrease in the slope of the test response. This inhibition was quickly reversed following the addition of CGP 35348 (500 μM) to the superfusing medium for the period marked by the bar. (B_2) The depression of the test response was accompanied by an increase in the amount of paired-pulse facilitation, calculated as the ratio of the slope of the second pulse to that of the first. This increase in facilitation ratio was completely abolished by the addition of CGP 35348 to the superfusing solution. Each point represents the average value for three consecutive responses.

(C) Representative field EPSPs preceded by the conditioning tetanus are superimposed upon the alternately evoked test EPSPs before (C_1) and after (C_2) the addition of CGP 35348. Traces are averages of 5–10 responses from the periods marked in (B_1).

important role in controlling this presynaptic action. We therefore examined the effect of the blockade of GABA uptake on this inhibition.

SKF-89976A, an N-(4,4-diphenyl-3-butenyl) derivative of nipecotic acid (Falch et al., 1990; Larsson et al., 1988), is a competitive blocker of the GABA transporter, but unlike other inhibitors, is not a substrate for the transporter. We first characterized its ability to block GABA uptake in hippocampal slices by comparing its action on responses with the somatic application of GABA and 4,5,6,7-tetrahydroisoxazolo[5,4-c] pyridine-3-ol (THIP), an analog of GABA that is not carried by the GABA transporter (Schousboe et al., 1985). Bath application of SKF-89976A greatly enhanced the GABA response, but had no effect on the response to THIP (n = 5) (Figure 4A$_1$). Thus, SKF-89976A selectively potentiates GABA responses and probably does so by affecting the GABA transporter. To rule out further a direct effect of SKF-89976A on $GABA_A$ receptors, the response to GABA in outside-out patches pulled from the somata of CA1 neurons was examined. SKF-89976A had no direct effect on GABA responses recorded in outside-out patches that had been removed from the slice (n = 4) (Figure 4B). In contrast, pentobarbital, which is known to alter the channel kinetics of the GABA receptor (Macdonald et al., 1989), prolonged GABA responses in outside-out patches (Figure 4B). These results indicate that the enhancement of GABA responses by SKF-89976A is not a direct effect on the receptors themselves, but rather results from the inhibition of the removal of GABA by the GABA transporter.

When the actions of SKF-89976A on the responses to GABA applied to the dendritic region and to the cell body region of the slice were compared, we found that the somatic responses were potentiated to a far greater extent than those evoked in the dendrites (n = 4) (Figure 4C). This result suggests that high affinity GABA uptake is less powerful in the dendritic region of the slice.

We next asked whether blocking GABA uptake affected the postsynaptic $GABA_B$ response generated from synaptically released transmitter. To address this question, we studied the effect of SKF-89976A on the monosynaptically evoked slow IPSC, which is $GABA_B$ receptor mediated. These IPSCs were isolated in the presence of D-2-amino-5-phosphonovaleric acid (APV), picrotoxin, bicuculline methiodide, and the non-N-methyl-D-aspartate (NMDA) receptor blocker 6-cyano-7-nitroquinoxaline-2.3-dione (CNQX). Under these conditions, SKF-89976A increased the peak amplitude of the IPSC by 66% ± 9% and prolonged its half-decay time by 116% ± 6% (n = 7). Figure 5A$_1$ shows a representative example of the effect of the uptake blocker on a slow IPSC evoked by a brief tetanus.

To examine whether inhibition of uptake affects the presynaptic inhibitory effect of GABA, we made use of the similar time course of the slow IPSC and of the inhibition of excitatory transmission (Figure 2). Thus, in the next series of field EPSP experiments, the conditioning stimulus consisted of fewer pulses than used previously (3 stimuli, 50 Hz), and the interval between the conditioning tetanus and test stimulus was set at 700–800 ms (Figure 5A$_2$). This long interval was chosen

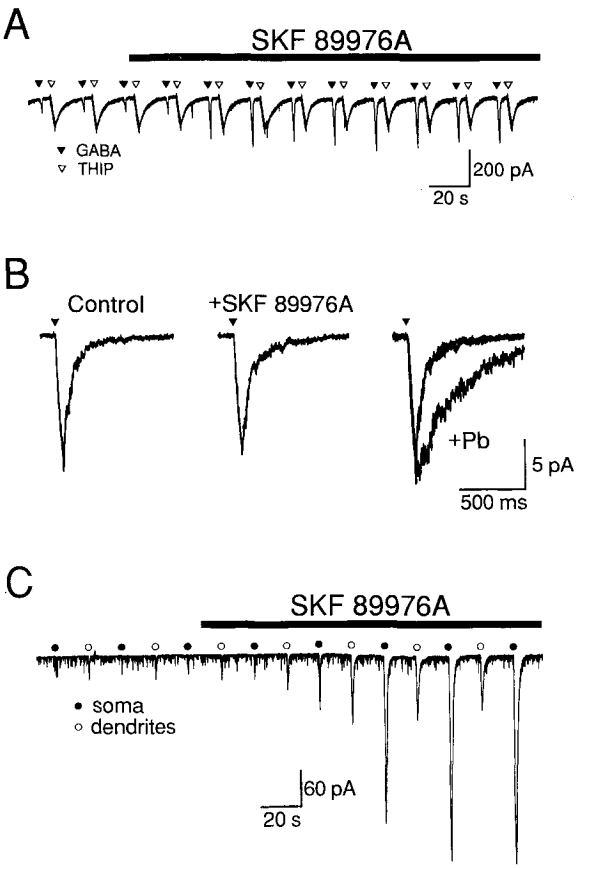

Figure 4. The GABA Uptake Inhibitor SKF-89976A Selectively Enhances the Responses to GABA Applied to Cells within the Hippocampal Slice

(A) Chart record of membrane current from a voltage-clamped CA1 pyramidal cell (V_h = −70 mV). Brief iontophoretic pulses of GABA (closed arrowheads, 100 nA/1s) and THIP (open arrowheads, 150 nA/2s) were alternately applied from separate electrodes positioned close to the cell soma. SKF-89976A (20 μM), added to the superfusing solution during the time indicated by the bar, greatly enhanced the GABA-induced current but had no effect on the responses to THIP.

(B) Responses to GABA (25 μM) in an outside-out patch taken from a CA1 cell (V_h = −70 mV). GABA was applied via puffer pipette (15 psi/50 ms) at the time indicated (closed arrowheads), and all records are the average of 10 applications. GABA evoked a macroscopic inward current that was unaffected by the addition of SKF-89976A (30 μM) to the superfusing solution but enhanced by the addition of 100 μM pentobarbital (Pb) to the superfusing solution. This record is shown superimposed over the responses obtained before and after treatment with SKF-89976A.

(C) Record of membrane current from a voltage-clamped CA1 cell (V_h = −70 mV). GABA was alternately applied to the soma (closed circles, 100 nA/1s) and apical dendrites (open circles, 70 nA/1s) via iontophoretic electrodes positioned in stratum pyramidale and stratum radiatum, respectively. SKF-89976A (20 μM) caused a marked increase in the amplitude of the responses to GABA applied at the soma, but had less of an effect on the responses to GABA generated in stratum radiatum. All recordings were made with CsCl-filled electrodes.

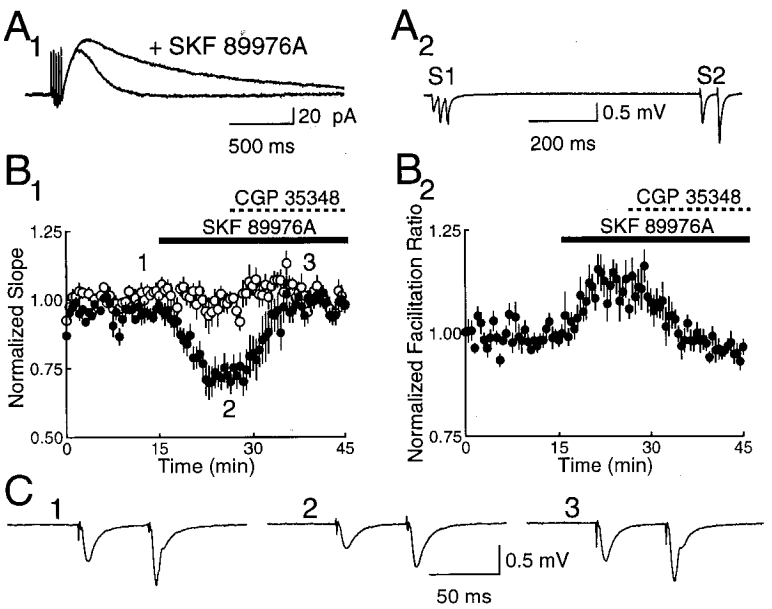

Figure 5. SKF-89976A Enhances Both the Pre- and Postsynaptic Actions Mediated by $GABA_B$ Receptors in the Slice

(A_1) A monosynaptic $GABA_B$-mediated IPSC from a voltage-clamped CA1 cell (V_h = −60 mV) evoked with a brief tetanus (5 pulses, 50 Hz) before and after the addition of SKF-89976A (25 μM) to the superfusing solution. The uptake inhibitor increased the peak amplitude of the slow IPSC and greatly prolonged its decay. (A_2) A representative trace of field EPSPs illustrating the protocol used to study the influence of GABA uptake on presynaptic inhibition. In this series of experiments, the conditioning tetanus (S1, 3 pulses at 50 Hz) was delivered 700–800 ms before the test stimuli (S2).

(B) SKF-89976A enhanced the inhibition of the field EPSP and increased paired-pulse facilitation. Control test stimuli (open circles) were evoked every 30 s, alternately accompanied by a preceding conditioning tetanus (closed circles). The field EPSP slope values were normalized to the average of the first 20 contol test stimuli. The plotted points represent the mean ± SEM from 6 slices. (B_1) Initially, at these long intervals the conditioning tetanus had little effect on the slope of the test stimulus. SKF-89976A (25 μM), added to the bath during the period marked by the bar, caused a 25% decrease in the slope of the test pulse evoked with a preceding conditioning tetanus. This inhibition of the field EPSP was rapidly blocked following the subsequent addition of CGP 35348 (500 μM) to the superfusing medium during the time indicated by the dashed line. The slope of the control test stimuli was not affected. (B_2) The facilitation ratios of the test stimuli preceded by the conditioning tetanus were normalized and averaged as in (B_1). SKF-89976A caused a marked increase in the facilitation ratio, which was completely reversed by CGP 35348. The facilitation ratio of the test EPSP evoked without the conditioning tetanus was not affected by the uptake inhibitor or receptor antagonist (data not shown).

(C_1–C_3) Representative traces of the test stimuli preceded by a conditioning tetanus from 1 experiment corresponding to the periods indicated in (B_1).

to reduce the degree of presynaptic inhibition in control conditions (Figure 2). Since SKF-89976A greatly prolonged the slow IPSC (Figure $5A_1$), it might be expected that the presynaptic inhibitory effect of GABA at this interval would be enhanced as well. As expected at this interval and with a briefer tetanus, a preceding conditioning tetanus (S1) had only a small effect on the test response (S2) (closed circles), as shown during the control period in Figure $5B_1$, which is the average of 6 separate experiments. In the presence of SKF-89976A, however, a marked depression of the test response occurred (Figures $5B_1$ and $5C_2$), which was associated with a clear increase in paired-pulse facilitation (Figure $5B_2$). The subsequent addition of CGP 35348 to the superfusing solution abolished the depression (Figures $5B_1$ and $5C_3$) and the change in paired-pulse facilitation (Figure $5B_2$). These results suggest that some "spill-over" of GABA onto the terminals of excitatory synapses occurs normally; this effect is greatly enhanced after blockade of GABA uptake.

In experiments in which an outside-out patch was placed on the surface of the slice as a sensor of GABA concentration, we obtained direct evidence that SKF-89976A augments transmitter spill-over. In control conditions, paired-pulse stimulation using high stimulus strengths often evoked brief channel activity in outside-out patches (Figure 6). In the presence of SKF-89976A, many more channels were activated, and the activity was greatly prolonged. Withdrawal of the patch from the surface of the slice (Figure 6) or addition of the $GABA_A$ antagonist bicuculline methiodide (data not shown) abolished the channel activity. Similar results were found using 3 other patches in different slices.

Models of fast synaptic transmission (Korn and Faber, 1987; Wathey et al., 1979), including that at $GABA_A$ synapses in the hippocampus (Busch and Sakman, 1990), postulate that transmitter diffuses from the synaptic cleft within a few milliseconds and that the decay of the synaptic current reflects the lifetime of the open channel. However, previous studies examining the role of GABA uptake in fast inhibitory transmission in the CNS have reported that uptake inhibitors prolong inhibitory synaptic potentials (Dingledine and Korn, 1985; Hablitz and Lebeda, 1985; Rekling et al., 1990), suggesting that uptake is required for terminating the synaptic action of GABA. To address this issue, we studied the actions of SKF-89976A on somatic, monosynaptic $GABA_A$ IPSCs evoked in the presence of 6,7-dinitroquinoxaline-2,3-dione (DNQX), APV, and the $GABA_B$ antagonist CGP 35348. Under normal conditions, IPSCs evoked with a weak stimulus had a time course quite similar to that evoked in

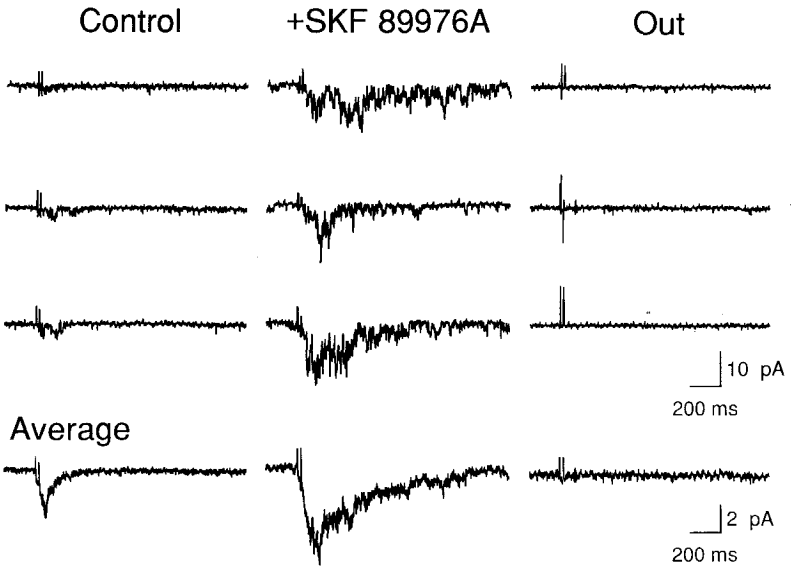

Figure 6. Stimulus-Evoked GABA Levels in the Slice Are Enhanced by SKF-89976A

A CA1 outside-out patch (V_h = −70 mV) was positioned at the surface of the slice, close to a bipolar stimulating electrode. (Left) Paired-pulse stimulation (20 ms interval) reproducibly elicited $GABA_A$ receptor channel activity in the patch. In this and the remaining panels, 3 representative responses are shown above the ensemble average of 10–15 consecutive episodes. (Middle) Shortly after its addition to the superfusing solution, SKF-89976A (20 μM) greatly increased the stimulus-evoked response. (Right) Stimulus-evoked channel activity in the patch was sharply reduced by moving the electrode 150 μm away from the slice. Channel activity could be restored by moving the electrode back to its original position (data not shown). A CsCl-containing electrode was used.

the same cell with a stronger stimulus (Figure 7). The inhibition of GABA uptake by SKF-89976A had little effect on the IPSC evoked with a weak stimulus, or on spontaneous or miniature IPSCs (data not shown). These findings are in agreement with models of fast synaptic transmission in which the diffusion of GABA from the synaptic cleft is fast relative to the time course of the synaptic current. When a strong stimulus was used, however, SKF-89976A greatly prolonged the late phase of the IPSC, which now required more than a second to decay completely, but had no effect on the amplitude or initial decay of the IPSC. The selective action of SKF-89976A on IPSCs evoked with strong versus weak stimuli was found in 7 slices. Thus, under normal conditions, GABA is largely confined to the synaptic region, even when large numbers of synapses are activated. Since IPSCs evoked with a weak stimulus (activating fewer synapses) are unaltered, such must also be the case at individual synapses when GABA uptake is inhibited. However, when many neighboring synapses are coactivated, as during a strong stimulus, and when uptake is inhibited, transmitter spill-over onto postsynaptic $GABA_A$ receptors can occur. These results indicate that for fast synaptic inhibition in the hippocampus, GABA removal by the GABA transporter becomes important in limiting the spatial domain of the transmitter when synchronous release occurs from a population of synapses.

Discussion

Presynaptic receptors for a number of transmitters including GABA, acetylcholine, adenosine, norepinephrine, and numerous peptides mediate the inhibition of excitatory synaptic transmission (Kandel et al., 1991; Nicoll et al., 1990). Although the presynaptic actions of these transmitters have been typically characterized by exogenously applied agonists, little is known about their physiological importance. Specifically, whether or not synaptically released transmitters gain access to the receptors at these sites has not been clearly established.

One hindrance to establishing a physiological role for presynaptic receptors is the general lack of anatomical evidence for axo–axonic synapses in the brain. How then, without a directly situated delivery site, do these neurotransmitters reach their receptors? Anatomical studies of cortical neurons have shown that GABA-releasing terminals form conventional synapses primarily onto the dendritic shafts and somata of target cells (Beaulieu and Somogyi, 1990; Kisvárday et al., 1990) and less frequently make synapses onto dendritic spines (Beaulieu and Somogyi, 1990; Fifková et al., 1992), the site at which excitatory afferent fibers terminate. It seems reasonable then that transmitter released from these conventional synapses could spill-over and act as a diffuse signal onto nearby (within micrometers) excitatory synaptic terminals.

In this study, we have provided evidence for this hypothesis by showing that repetitive stimulation of one bundle of afferent fibers in the stratum radiatum region of CA1 causes a marked, short-lasting, heterosynaptic depression of excitatory transmission in an independent, overlapping set of inputs. This inhibition is due to the activation of presynaptic $GABA_B$ receptors, since it is accompanied by an increase in paired-pulse facilitation and is blocked by the selective $GABA_B$ antagonist CGP 35348. In addition to the autoinhibition of GABAergic transmission (Davies et al., 1990), this result indicates that GABA, released by brief, repetitive stimulation of inhibitory afferent fibers, can act trans-synaptically to inhibit excitatory synaptic transmission (Figure 8). These results clearly provide a physiological role for neurotransmitter inhi-

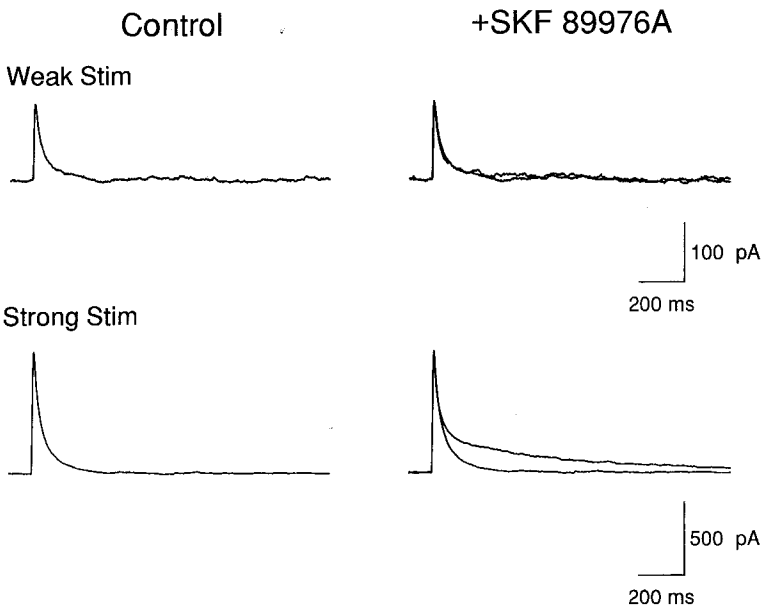

Figure 7. SKF-89976A Prolongs the Time Course of Large Monosynaptic $GABA_A$ IPSCs Evoked with Strong Stimulation, but Has Little Effect on Small Synaptic Currents Evoked with Weak Stimulation

Strong and weak monosynaptic IPSCs were recorded from a CA1 neuron in the presence of 500 μM CGP 35348 (V_h = −50 mV) and evoked by alternating the stimulus strength of a bipolar electrode placed in the stratum pyramidal from 14 V to 4 V. (Top) Weak stimulation evoked a small IPSC that appeared relatively unchanged after addition of SKF-89976A (20 μM) to the slice. The responses before and after treatment with the drug are shown superimposed to the right. (Bottom) In the same cell a strong stimulus evoked a much larger IPSC with a time course similar to that evoked by weak stimulation. SKF-89976A had no effect on the peak amplitude of the IPSC or its early time course, but markedly prolonged its decay. The IPSC recorded in the presence of the drug is superimposed over the control response. A cesium gluconate-containing electrode was used.

bition via presynaptic $GABA_B$ receptors on excitatory terminals in the hippocampus. A similar mechanism, due to transmitter spill-over from postsynaptic synapses, has been proposed to account for $GABA_A$ receptor-mediated presynaptic effects in the olfactory cortex (Pickles and Simmonds, 1976).

The time course of the $GABA_B$-mediated presynaptic inhibition was somewhat different from that of the $GABA_B$-mediated postsynaptic current. The presynaptic effect peaked later and lasted longer than the slow IPSC. A similar difference was found between the time course of the slow IPSP and that of the autoinhibition of IPSCs in the hippocampus (Davies et al., 1990). As suggested previously (Davies et al., 1990), the difference between pre- and postsynaptic $GABA_B$ responses may arise in a number of ways. Presynaptic receptors may differ from their postsynaptic counterparts in their affinity for transmitter or perhaps in their coupling to different G protein-mediated messenger systems. Alternatively, there may be more "spare receptors" on presynaptic terminals than on postsynaptic cells.

To act effectively at a distance, neurotransmitters require high affinity receptors. The affinity of receptors in brain slices cannot be determined, since potent uptake mechanisms interfere with studying the sensitivity of receptors to low concentrations of GABA. However, in cultured hippocampal cells, in which agonist concentrations are better controlled, presynaptic $GABA_B$ receptors were found to be highly sensitive to GABA (Yoon and Rothman, 1991). In these cells, a low concentration of GABA (1 μM) almost maximally attenuated excitatory synaptic currents, but had little direct effect on postsynaptic $GABA_A$ receptors. Therefore, a relatively low concentration of GABA in the dendritic region of CA1 would be sufficient to mediate the presynaptic inhibition of excitatory afferents without having a direct postsynaptic action on the receptors governing fast synaptic inhibition.

By using a dialysis probe in vivo, the concentration of GABA in the extracellular fluid of the hippocampus has been estimated to be 0.2–0.8 μM (Lerma et al., 1986; Tossman et al., 1986), raising the possiblity that ambient levels of GABA might tonically inhibit excitatory transmission. Our results do not support this possibility, since as has been reported previously (Davies et al., 1991), CGP 35348 has no consistent effect on normal excitatory transmission evoked at a low frequency. Ambient levels of GABA are therefore insufficient to activate presynaptic $GABA_B$ receptors tonically. Thus, either the concentrations measured with dialysis techniques do not reflect the concentrations at synaptic locations, or the sensitivity of the excitatory synapses in the slice to GABA is lower than that in culture.

Transmitter uptake into neurons and glial cells is a crucial factor in maintaining low ambient levels and limiting the action of a number of transmitters in the brain (Kandel et al., 1991). Transport processes with a high affinity for GABA (K_m values in the nanomolar to

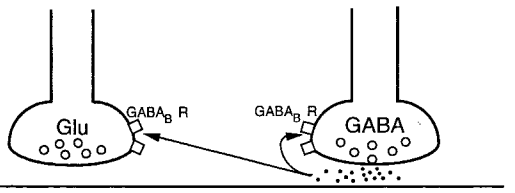

Figure 8. Simplified Diagram Illustrating the Presynaptic Actions of GABA in the Hippocampus

Synaptically released GABA can activate $GABA_B$ autoreceptors to inhibit its own release (Davies et al., 1990) and can also act via $GABA_B$ receptors, in a paracrine fashion, to inhibit transmitter release from neighboring excitatory nerve terminals.

low micromolar range for some systems) have been well characterized in the CNS (Hertz, 1979; Iversen and Kelly, 1975), and a family of genes encoding these transporters have been isolated (Guastella et al., 1990; Clark et al., 1992). We have found that SKF-89976A, a competitive inhibitor that is equally effective in blocking neuronal and glial GABA uptake (Falch et al., 1990; Larsson et al., 1988), dramatically enhanced the magnitude of the GABA-mediated inhibition of excitatory transmission, indicating that transmitter uptake actively regulates the diffuse inhibitory synaptic action of GABA.

In contrast with its action on GABA released transiently by synaptic stimulation, SKF-89976A, applied for 10–15 min, had no obvious effect on normal excitatory transmission evoked at a low frequency (0.1–0.033 Hz) or on the resting membrane potential, suggesting that blocking uptake does not raise ambient GABA to levels sufficient to activate either pre- or postsynaptic GABA receptors. The uptake blocker nipecotic acid, which elevates extracellular GABA via heteroexchange (Johnston et al., 1976; Szerb, 1982; Solis and Nicoll, 1992b), has been recently shown to inhibit evoked excitatory synaptic potentials in the neostriatum by a bicuculline-resistant action (Calabresi et al., 1991), most likely by increasing the ambient level of GABA.

Although the presynaptic inhibitory action of GABA presumably represents a diffuse signaling mechanism, the nature of the synaptic architecture mediating the postsynaptic slow IPSC is still unresolved. Recently, it has become clear that $GABA_A$- and $GABA_B$-mediated synaptic responses can be segregated at different locations on the same postsynaptic neuron (Otis and Mody, 1992; Solis and Nicoll, 1992b; Sugita et al., 1992; Williams and Lacaille, 1992). Furthermore, high levels of stimulation or other conditions favoring large amounts of transmitter release are often required to evoke a slow IPSC (Dutar and Nicoll, 1988a; Otis and Mody, 1992). If the receptors mediating postsynaptic $GABA_B$ responses are as sensitive to GABA as the presynaptic receptors, one possibility is that the slow IPSC represents the activation of diffusely distributed receptors. In support of this argument, we have found that the blockade of GABA uptake, which limits the diffuse presynaptic action of GABA, markedly enhanced the slow IPSC. Although generally accepted (but see Muller and Misgeld, 1989), this result also provides additional strong evidence that GABA is, in fact, the neurotransmitter mediating slow inhibitory transmission in the hippocampus (Dutar and Nicoll, 1988a; Newberry and Nicoll, 1985; Solis and Nicoll, 1992a; Soltesz et al., 1988). However, a clearer description of the synapses mediating this response requires a better understanding of the localization of the $GABA_B$ receptors on the postsynaptic cell.

Our results are in general agreement with a number of studies (Dingledine and Korn, 1985; Rekling et al., 1990; Thompson and Gähwiler, 1992) which have found that other GABA uptake inhibitors had little effect on the amplitude or initial decay of IPSPs, but greatly augmented their late decay. This result is seemingly inconsistent with models of fast synaptic transmission which suggest that transmitter diffuses from the cleft within a few milliseconds (Busch and Sakmann, 1990; Korn and Faber, 1987; Wathey et al., 1979). An important difference between the two approaches is that these models of synaptic transmission reconstruct the properties of an isolated, individual synapse, whereas physiological studies in brain slices of evoked synaptic responses usually reflect the properties of many neighboring synapses that are simultaneously active. Indeed, SKF-89976A had no effect on either miniature IPSCs or those evoked with weak stimulation, which activates fewer synapses, in contrast with its effects on IPSCs evoked by strong stimuli. A similar discrepancy between the actions of a GABA uptake inhibitor on evoked versus miniature IPSCs has recently been found in hippocampal slice cultures (Thompson and Gähwiler, 1992). These results suggest that GABA uptake is important for limiting the spread of GABA released by neighboring, coactivated synapses. In the presence of uptake inhibitors, the spill-over of GABA from coactivated boutons may reach $GABA_A$ receptors at neighboring synapses or those at an extrasynaptic location. The idea that SKF-89976A acts to promote the spill-over of transmitter is also supported by our measurements of evoked transmitter release in the slice with a GABA-sensing outside-out patch. The lateral diffusion of transmitter from one discrete postsynaptic domain to another one nearby has previously been shown to occur when neighboring glycinergic synapses on the Mauthner cell are coactivated (Faber and Korn, 1988). Similarly, at the neuromuscular junction, acetylcholinesterase inhibitors produce a "postsynaptic potentiation" by permitting the spread of transmitter to overlapping synaptic domains (Hartzell et al., 1975).

Why does spill-over occur onto presynaptic $GABA_B$ receptors but not onto neighboring $GABA_A$ synapses when uptake is intact? Two possibilities come to mind. First, as discussed above, the $GABA_B$ receptors may have a higher affinity than the $GABA_A$ receptors. Second, the extremely high density of inhibitory terminals on the somata and initial segment of CA1 pyramidal neurons (Somogyi et al., 1983) results in a high density of GABA transporters, as demonstrated by immunocytochemistry (Radian et al., 1990) and a more effective removal of GABA compared with dendritic sites, where inhibitory synapses are less concentrated (Figure 4C) (Rovira et al., 1984). These findings raise the possibility that the area over which a transmitter can act, and perhaps also the local concentration of transmitter, may vary in different regions depending on the local uptake processes.

Taken together, these results suggest that GABA released from inhibitory terminals can diffuse to neighboring presynaptic $GABA_B$ receptors and exert a pro-

found depression of excitatory transmitter release. GABA uptake, by modulating the effective spread of this diffuse inhibition, may therefore directly influence the strength of excitatory synaptic transmission in the hippocampus. In addition to its role in governing slow transmission, uptake has a strong influence on fast synaptic inhibition. It serves to localize fast $GABA_A$-mediated transmission by preventing its spillover to neighboring synapses. Thus, conditions that compromise uptake mechanisms augment diffuse transmitter actions at the expense of point-to-point synaptic transmission.

Experimental Procedures

Experiments were performed on guinea pig hippocampal slices (500 μm) prepared by standard methods (Zalutsky and Nicoll, 1990). Following a 1 hr recovery period, slices were placed in a recording chamber and superfused with a medium containing 125 mM NaCl, 2.5 mM KCl, 1.3 mM $MgCl_2$, 2.5 mM $CaCl_2$, 26 mM $NaHCO_3$, 1 mM NaH_2PO_4, and 10 mM glucose and equilibrated with 95% O_2 and 5% CO_2. A surgical cut was made between CA1 and CA3 to prevent epileptiform discharges. All experiments were performed at 30°C–32°C, unless otherwise indicated. For all experiments examining excitatory postsynaptic responses, the superfusing solution contained APV (25 μM) and picrotoxin (100 μM) to block NMDA and $GABA_A$ receptors, respectively. In whole-cell recordings of excitatory transmission, the peak amplitudes of EPSCs were used as the measure of synaptic strength. All inhibitory synaptic currents were recorded from slices in which NMDA receptors were blocked with APV (25 μM) and non-NMDA receptors were blocked with either CNQX (20 μM) or DNQX (20 μM). Monosynaptic fast IPSCs were evoked from a bipolar stimulating electrode placed in the cell layer, close (within 200 μm) to the site of recording, and CGP 35348 (500 μM) was included in the superfusing solution to block $GABA_B$ receptors. Excitatory synaptic responses and monosynaptic slow IPSCs were evoked from similar electrodes placed in stratum radiatum. Pure slow IPSCs were recorded in the the presence of picrotoxin (40 μM) and bicuculline methiodide (40 μM) to block $GABA_A$ receptors. In the experiments examining iontophoretic responses, tetrodotoxin (0.5–1 μM) was included to block Na^+-dependent action potentials. Currents were recorded with an Axopatch 1B amplifier from neurons in the CA1 pyramidal cell layer using the "blind" whole-cell recording technique (Blanton et al., 1989; Coleman and Miller, 1989). Field EPSPs were recorded in stratum radiatum with the same amplifier from low resistance glass pipettes filled with 2 M NaCl. To avoid contamination of the response by population spikes, the slope of the field EPSP was used to calculate synaptic strength. For voltage-clamp recordings, patch electrodes contained a cesium gluconate internal solution (117.5 mM cesium gluconate, 17.5 mM CsCl, 8 mM NaCl, 10 mM HEPES, 2 mM Mg-ATP, 0.2 mM GTP, 0.2–1 mM EGTA [pH 7.3]). In some experiments the cesium gluconate was replaced by CsCl. In other experiments, a K^+-based internal solution was used and contained 130 mM potassium gluconate, 4 mM NaCl, 10 mM HEPES, 1 mM EGTA, 4 mM Mg-ATP, and 1 mM GTP (pH 7.3). Iontophoretic electrodes were filled with either GABA (1 M in distilled H_2O or 50 mM in 150 mM NaCl [pH 4]) or the GABA analog THIP (50 mM in 150 mM NaCl [pH 4]). Responses evoked by iontophoresis were well below maximal, since increasing the ejecting current greatly enhanced the response. GABA (25 μM dissolved in the superfusion solution) was applied to outside-out patches by pressure ejection from a broken pipette. A stock solution of 2.5 mM SKF-89776A, dissolved in distilled H_2O, was diluted to the desired concentration in the superfusing medium. Illustrated traces are averages of 3–5 responses, except where indicated. Results are shown as mean ± SEM.

Acknowledgments

We thank Drs. D. Copenhagen, Z. Hall, S. Hestrin, and D. Perkel for their helpful comments on the manuscript. Computer programs for data acquisition and analysis were kindly provided by Drs. S. Hestrin and D. Perkel. SKF-89976A was a gift from Dr. W. Karbon, Nova Pharmaceutical Corporation, and CGP 35348 was generously provided by Drs. H. Schröter and L. Maitre, Ciba-Geigy. This research was supported by National Institutes of Health grants MH38256, MH00437, and NS24205 to R. A. N. and F05TW04396 to J. M. S. and by the Spanish Ministry of Education and Science. R. A. N. is a member of the Keck Center for Integrative Neuroscience and the Silvio Conte Center for Neuroscience Research.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 USC Section 1734 solely to indicate this fact.

Received September 29, 1992; revised December 1, 1992.

References

Ault, B., and Nadler, J. V. (1982). Baclofen selectively inhibits transmission at synapses made by axons of CA3 pyramidal cells in the hippocampal slice. J. Pharmacol. Exp. Ther. *223*, 291–297.

Beaulieu, C., and Somogyi, P. (1990). Targets and quantitative distribution of GABAergic synapses in the visual cortex of the cat. Eur. J. Neurosci. *2*, 296–303.

Blanton, M. G., Lo Turco, J. J., and Kriegstein, A. R. (1989). Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex. J. Neurosci. Meth. *30*, 203–210.

Bowery, N. G., Hill, D. R., Hudson, A. L., Doble, A., Middlemiss, D. N., Shaw, J., and Turnbull, M. (1980). (−)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. Nature *283*, 92–94.

Busch, C., and Sakmann, B. (1990). Synaptic transmission in hippocampal neurons: numerical reconstruction of quantal IPSCs. Cold Spring Harbor Symp. Quant. Biol. *55*, 69–80.

Calabresi, P., Mercuri, N. B., De Murtas, M., and Bernardi, G. (1991). Involvement of GABA systems in feedback regulation of glutamate- and GABA-mediated synaptic potentials in rat neostriatum. J. Physiol. *440*, 581–599.

Clark, J. A., Deutch, A. Y., Gallipoli, P. Z., and Amara, S. G. (1992). Functional expression and CNS distribution of a β-alanine-sensitive neuronal GABA transporter. Neuron *9*, 337–348.

Coleman, P. A., and Miller, R. F. (1989). Measurement of passive membrane parameters with whole-cell recording from neurons in the intact amphibian retina. J. Neurophysiol. *61*, 218–230.

Davies, C. H., Davies, S. N., and Collingridge, G. L. (1990). Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. J. Physiol. *424*, 513–531.

Davies, C. H., Starkey, S. J., Pozza, M. F., and Collingridge, G. L. (1991). $GABA_B$ autoreceptors regulate the induction of LTP. Nature *349*, 609–611.

Deisz, R. A., and Prince, D. A. (1989). Frequency-dependent depression of inhibition in guinea-pig neocortex in vitro by $GABA_B$ receptor feed-back on GABA release. J. Physiol. *412*, 513–541.

Dingledine, R., and Korn, S. J. (1985). γ-Aminobutyric acid uptake and the termination of inhibitory synaptic potentials in the rat hippocampal slice. J. Physiol. *366*, 387–409.

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

Dutar, P., and Nicoll, R. A. (1988b). Pre- and postsynaptic $GABA_B$ receptors in the hippocampus have different pharmacological properties. Neuron *1*, 585–591.

Eccles, J. C., Schmidt, R. F., and Willis, W. D. (1963). Pharmacological studies on presynaptic inhibition. J. Physiol. *168*, 500–530.

Faber, D. S., and Korn, H. (1988). Synergism at central synapses due to lateral diffusion of transmitter. Proc. Natl. Acad. Sci. USA *85*, 8708–8712.

Falch, E., Larsson, O. M., Schousboe, A., and Krogsgaard-Larsen, P. (1990). GABA-A agonists and GABA uptake inhibitors: structure-activity relationships. Drug Dev. Res. *21*, 169–188.

Fifková, E., Eason, H., and Schaner, P. (1992). Inhibitory contacts on dendritic spines of the dentate fascia. Brain Res. *577*, 331–336.

Fuxe, K., and Agnati, L. F., eds. (1991). Volume Transmission in the Brain: Novel Mechanisms for Neural Transmission (New York: Raven Press).

Guastella, J., Nelson, N., Nelson, H., Czyzyk, L., Keynan, S., Miedel, M. C., Davidson, N., Lester, H. A., and Kanner, B. I. (1990). Cloning and expression of a rat brain GABA transporter. Science *249*, 1303–1306.

Hablitz, J. J., and Lebeda, F. J. (1985). Role of uptake in γ-aminobutyric acid (GABA)-mediated responses in guinea pig hippocampal neurons. Cell. Mol. Neurobiol. *5*, 353–371.

Harris, E. W., and Cotman, C. W. (1983). Effects of acidic amino acid antagonists on paired-pulse potentiation at the lateral perforant path. Exp. Brain Res. *52*, 455–460.

Harrison, N. L. (1990). On the presynaptic action of baclofen at inhibitory synapses between cultured rat hippocampal neurones. J. Physiol. *422*, 433–446.

Hartzell, H. C., Kuffler, S. W., and Yoshikami, D. (1975). Postsynaptic potentiation: interaction between quanta of acetylcholine at the skeletal neuromuscular synapse. J. Physiol. *251*, 427–463.

Herkenham, M. (1987). Mismatches between neurotransmitter and receptor localizations in brain: observations and implications. Neuroscience *23*, 1–38.

Hertz, L. (1979). Functional interactions between neurons and astrocytes. I. Turnover and metabolism of putative amino acid transmitters. Prog. Neurobiol. *13*, 277–323.

Hille, B. (1992). G protein-coupled mechanisms and nervous signaling. Neuron *9*, 187–195.

Inoue, M., Matsuo, T., and Ogata, N. (1985). Characterization of pre- and postsynaptic actions of (−)-baclofen in the guinea-pig hippocampus in vitro. Br. J. Pharmacol. *84*, 843–851.

Iversen, L. L., and Kelly, J. S. (1975). Uptake and metabolism of gamma-aminobutyric acid by neurones and glial cells. Biochem. Pharmacol. *24*, 933–938.

Iversen, L. L., and Neal, M. J. (1968). The uptake of [^{3}H]GABA by slices of rat cerebral cortex. J. Neurochem. *15*, 1141–1149.

Jan, Y. N., and Jan, L. Y. (1983). A LHRH-like peptidergic neurotransmitter capable of "action at a distance" in autonomic ganglia. Trends Neurosci. *6*, 320–325.

Johnston, G. A. R., Stephanson, A. L., and Twitchin, B. (1976). Uptake and release of nipecotic acid by rat brain slices. J. Neurochem. *26*, 83–87.

Kandel, E. R., Schwartz, J. H., and Jessell, T. M. (1991). Principles of Neural Science. Third edition (New York: Elsevier).

Katz, B., and Miledi, R. (1968). The role of calcium in neuromuscular facilitation. J. Physiol. *195*, 481–492.

Kisvárday, Z. F., Gulyas, A., Beroukas, D., North, J. B., Chubb, I. W., and Somogyi, P. (1990). Synapses, axonal and dendritic patterns of GABA-immunoreactive neurons in the human cerebral cortex. Brain *113*, 793–812.

Korn, H., and Faber, D. (1987). Regulation and significance of probabilistic release mechanisms at central synapses. In Synaptic Function, G. Edelman, E. Gall, and W. M. Cowan, eds. (New York: Wiley), pp. 57–108.

Langer, S. Z. (1987). Presynaptic regulation of monoaminergic neurons. In Psychopharmacology: The Third Generation of Progress, H. Y. Meltzer, ed. (New York: Raven Press), pp. 151–157.

Lanthorn, T. H., and Cotman, C. W. (1981). Baclofen selectively inhibits excitatory synaptic transmission in the hippocampus. Brain Res. *225*, 171–178.

Larsson, O. M., Falch, E., Krogsgaard-Larsen, P., and Schousboe, A. (1988). Kinetic characterization of inhibition of γ-aminobutyric acid uptake into cultured neurons and astrocytes by 4,4-diphenyl-3-butenyl derivatives of nipecotic acid and guvacine. J. Neurochem. *50*, 818–823.

Lerma, J., Herranz, A. S., Herreras, O., Abraira, V., and Martin Del Rio, R. (1986). In vivo determination of extracellular concentration of amino acids in the rat hippocampus. A method based on brain dialysis and computerized analysis. Brain Res. *384*, 145–155.

Macdonald, R. L., Rogers, C. J., and Twyman, R. E. (1989). Barbiturate regulation of kinetic properties of the $GABA_A$ receptor channel of mouse spinal neurones in culture. J. Physiol. *417*, 483–500.

Mallert, A., and Martin, A. R. (1968). The relation between quantum content and facilitation at the neuromuscular junction of the frog. J. Physiol. *196*, 593–604.

McCarren, M., and Alger, B. E. (1985). Use-dependent depression of IPSPs in rat hippocampal pyramidal cells in vitro. J. Neurophysiol. *53*, 557–571.

Muller, D., and Lynch, G. (1989). Evidence that changes in presynaptic calcium currents are not responsible for long-term potentiation in the hippocampus. Brain Res. *479*, 290–299.

Muller, W., and Misgeld, U. (1989). Carbachol reduces I_K, baclofen, but not I_K GABA in guinea pig hippocampal slices. Neurosci. Lett. *102*, 229–234.

Newberry, N. R., and Nicoll, R. A. (1985). Comparison of the action of baclofen with gamma-aminobutyric acid on rat hippocampal pyramidal cells in vitro. J. Physiol. *360*, 161–185.

Nicoll, R. A., Malenka, R. M., and Kauer, J. A. (1990). Functional comparison of neurotransmitter receptor subtypes in mammalian central nervous system. Physiol. Rev. *70*, 513–565.

Olpe, H.-R., Baudry, M., Fagni, L., and Lynch, G. (1982). The blocking action of baclofen on excitatory synaptic transmission in the rat hippocampal slice. J. Neurosci. *2*, 698–703.

Olpe, H., Karlsson, G., Pozza, M. F., Brugger, F., Steinmann, M., Van Riezen, H., Fagg, G., Hall, R. G., Froestl, W., and Bittiger, H. (1990). CGP 35348: a centrally active blocker of $GABA_B$ receptors. Eur. J. Pharmacol. *187*, 27–38.

Otis, T. S., and Mody, I. (1992). Differential activation of $GABA_A$ and $GABA_B$ receptors by spontaneously released transmitter. J. Neurophys. *67*, 227–235.

Peters, A., Palay, S. L., and Webster, H. D. (1991). Synapses. In The Fine Structure of the Nervous System (New York: Oxford University Press), pp. 138–211.

Pickles, H. G., and Simmonds, M. A. (1976). Possible presynaptic inhibition in rat olfactory cortex. J. Physiol. *260*, 475–486.

Radian, R., Ottersen, O. P., Storm-Mathisen, J., Castel, M., and Kanner, B. I. (1990). Immunocytochemical localization of the GABA transporter in rat brain. J. Neurosci. *10*, 1319–1330.

Rekling, J. C., Jahnsen, H., and Laursen, A. M. (1990). The effect of two lipophilic γ-amino butyric acid uptake blockers in CA1 of the rat hippocampal slice. Br. J. Pharmacol. *99*, 103–106.

Rovira, C., Ben-Ari, Y., and Cherubini, E. (1984). Somatic and dendritic actions of γ-aminobutyric acid agonists and uptake blockers in the hippocampus *in vivo*. Neuroscience *12*, 543–555.

Scheller, R. H., and Hall, Z. W. (1992). Chemical messengers at synapses. In An Introduction to Molecular Neurobiology, Z. W. Hall, ed. (Sunderland, Massachusetts: Sinauer Associates). pp. 119–147.

Schousboe, A., Larsson, O. M., and Krogsgaard-Larsen, P. (1985). Lack of a high affinity uptake system for the GABA agonist THIP and isoguvacine in neurons and astrocytes cultured from mouse brain. Neurochem. Int. *7*, 505–508.

Solís, J. M., and Nicoll, R. A. (1992a). Pharmacolgical characterization of $GABA_B$-mediated responses in the CA1 region of the rat hippocampal slice. J. Neurosci. *12*, 3466–3472.

Solís, J. M., and Nicoll, R. A. (1992b). Postsynaptic action of endogenous GABA released by nipecotic acid in the hippocampus. Neurosci. Lett., *147*, 16–20.

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

Somogyi, P., Smith, A. D., Nunzi, M. G., Gorio, A., Takagi, H., and Wu, J. Y. (1983). Glutamate decarboxylase immunoreactivity in the hippocampus of the cat: distribution of immunoreactive synaptic terminals with special reference to the axon initial pyramidal neurons. J. Neurosci. *3*, 1450–1468.

Sugita, S., Johnson, S. W., and North, R. A. (1992). Synaptic inputs to $GABA_A$ and $GABA_B$ receptors originate from discrete afferent neurons. Neurosci. Lett. *134*, 207–211.

Starke, K. (1981). Presynaptic receptors. Annu. Rev. Pharmacol. Toxicol. *21*, 7–30.

Starke, K., Gothert, M., and Kilbinger, H. (1989). Modulation of neurotransmitter release by presynaptic autoreceptors. Physiol. Rev. *69*, 864–989.

Stuart, G. J., and Redman, S. J. (1992). The role of $GABA_A$ and $GABA_B$ receptors in presynaptic inhibition of 1a EPSPs in cat spinal motoneurones. J. Physiol. *447*, 675–692.

Szerb, J. C. (1982). Effect of nipecotic acid, a γ-aminobutyric acid transport inhibitor, on the turnover and release of γ-aminobutyric acid in rat cortical slices. J. Neurochem. *39*, 850–858.

Thompson, S. M., and Gähwiler, B. H. (1992). Effects of the GABA uptake inhibitor tiagabine on inhibitory synaptic potentials in rat hippocampal slice cultures. J. Neurophysiol. *67*, 1698–1701.

Tossman, U., Jonsson, G., and Ungerstedt, U. (1986). Regional distribution and extracellular levels of amino acids in rat central nervous system. Acta Physiol. Scand. *127*, 533–545.

Wathey, J. C., Nass, M. M., and Lester, H. A. (1979). Numerical reconstruction of the quantal event at nicotinic synapses. Biophys. J. *27*, 145–164.

Williams, S., and Lacaille, J.-C. (1992). $GABA_B$ receptor-mediated inhibitory postsynaptic potentials evoked by electrical stimulation and by glutamate stimulation of interneurons in stratum lacunosum-moleculare in hippocampal CA1 pyramidal cells in vitro. Synapse *11*, 249–258.

Yoon, K.-W., and Rothman, S. M. (1991). The modulation of rat hippocampal synaptic conductances by baclofen and gamma-aminobutyric acid. J. Physiol. *442*, 377–390.

Zalutsky, R. A., and Nicoll, R. A. (1990). Comparison of two forms of long-term potentiation in single hippocampal neurons. Science *248*, 1619–1624.