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GABA Spillover Activates Postsynaptic $GABA_B$ Receptors to Control Rhythmic Hippocampal Activity

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Summary

In the hippocampus, interneurons provide synaptic inhibition via the transmitter GABA, which can activate $GABA_A$ and $GABA_B$ receptors ($GABA_A$Rs and $GABA_B$Rs). Generally, however, GABA released by a single interneuron activates only $GABA_A$Rs on its targets, despite the abundance of $GABA_B$Rs. Here, I show that during hippocampal rhythmic activity, simultaneous release of GABA from several interneurons activates postsynaptic $GABA_B$Rs and that block of $GABA_B$Rs increases oscillation frequency. Furthermore, if GABA uptake is inhibited, even GABA released by a single interneuron is enough to activate $GABA_B$Rs. This occurs also on cells not directly contacted by that interneuron, indicating that GABA has to overcome uptake and exit the synaptic cleft to reach $GABA_B$Rs. Thus, activation of extrasynaptic $GABA_B$Rs by pooling of GABA is an important mechanism regulating hippocampal network activity.

Introduction

The majority of inhibitory neurons in the brain release the transmitter GABA, which can bind to two classes of receptors, the ionotropic $GABA_A$ receptors ($GABA_A$Rs) and the G protein–linked $GABA_B$ receptors ($GABA_B$Rs). $GABA_A$Rs are associated with chloride conductances (Allen et al., 1977), while activation of postsynaptic $GABA_B$Rs results in the opening of potassium conductances of the GIRK type (Gähwiler and Brown, 1985; Newberry and Nicoll, 1985; Lüscher et al., 1997) and inhibition of calcium conductances (Dolphin and Scott, 1987; Scholz and Miller, 1991). The recent development of dual recording techniques to monitor activities from synaptically connected neurons allows the detailed investigation of the influence exerted by single GABAergic neurons on their postsynaptic targets, and thus a better understanding of their physiological role. In the hippocampus, GABA release from single interneurons has been shown to modulate sodium spike frequency (Miles and Wong, 1984; Miles et al., 1996), to inhibit dendritic calcium spikes (Miles et al., 1996), to synchronize the activity of pyramidal cells, and to set the pace of oscillations (Cobb et al., 1995). All of these reported functions of GABAergic interneurons, however, are mediated by $GABA_A$Rs. Thus, the physiological mode of activation of postsynaptic $GABA_B$Rs and their role during normal hippocampal and neocortical activity remains speculative. In fact, despite the high expression of $GABA_B$Rs in the hippocampus (Kaupmann et al., 1997), $GABA_B$R activation is not or is rarely detected upon release of GABA from a single interneuron (Buhl et al., 1994; Ali et al., 1999; Thomson and Destexhe, 1999). Similarly, spontaneous inhibitory postsynaptic potentials (IPSCs) lack a $GABA_B$R-mediated component (Otis and Mody, 1992). Relatively strong extracellular stimulation is needed to activate postsynaptic $GABA_B$Rs (Dutar and Nicoll, 1988), implying that concomitant release of GABA by several interneurons may be necessary (Isaacson et al., 1993). Such simultaneous release may occur during hippocampal rhythmic activity, a phenomenon associated with spatial and cognitive tasks in animals and humans (O'Keefe and Recce, 1993; Soltesz and Deschenes, 1993; Skaggs et al., 1996; Csicsvari et al., 1998; Kahana et al., 1999).

In this paper, I examine the physiological conditions required for the activation of postsynaptic $GABA_B$Rs in the hippocampus and their role in the rhythmic activity of the network.

Results

Dual Recordings

Dual recordings from interneurons and pyramidal cells were performed in the CA3 region of hippocampal slice cultures at a temperature of 33°C–34°C. Putative presynaptic interneurons were held in the current clamp mode, and postsynaptic pyramidal cells were voltage clamped at −50 mV. In about 60% ($n = 35$) of the recordings, a short depolarization of the interneurons to trigger an action potential (AP) produced an outward current in the pyramidal cells. These IPSCs had a reversal potential of -72.1 ± 1 mV ($n = 9$), a slope conductance ranging from 0.5 to 6 nS (average of 3.1 ± 0.7 nS, $n = 9$; 3.2 ± 0.9 nS for oriens interneurons, $n = 5$; 2.8 ± 1 nS for radiatum interneurons, $n = 4$) and were $GABA_A$R mediated since they were completely blocked by the specific antagonist bicuculline (40 μM). When three APs in the interneuron were triggered with a 40–50 ms interval, the amplitude of the second and third IPSC was depressed to $73\% \pm 4\%$ and $61\% \pm 4\%$ of the first, respectively ($n = 17$; Figure 1A1; $72\% \pm 5\%$ and $60\% \pm 5\%$ for oriens interneurons, $n = 10$; $73\% \pm 6\%$ and $62\% \pm 5\%$ for radiatum interneurons, $n = 7$). The second and the third IPSCs were also abolished by bicuculline, indicating that these too were entirely $GABA_A$R mediated.

To determine whether GABA release from an interneuron apart from activating $GABA_A$Rs could also activate postsynaptic $GABA_B$Rs, longer pulses (270–450 ms) of current were injected into the interneuron to trigger high-frequency trains of APs (40–120 Hz). These trains produced an outward current in the pyramidal cells, which was also entirely $GABA_A$R mediated since it was abolished by bicuculline. Subsequent application of the specific $GABA_B$R antagonist CGP62349 (2 μM; Figures 1A1 and 1A2) had no significant effect ($p > 0.7$,

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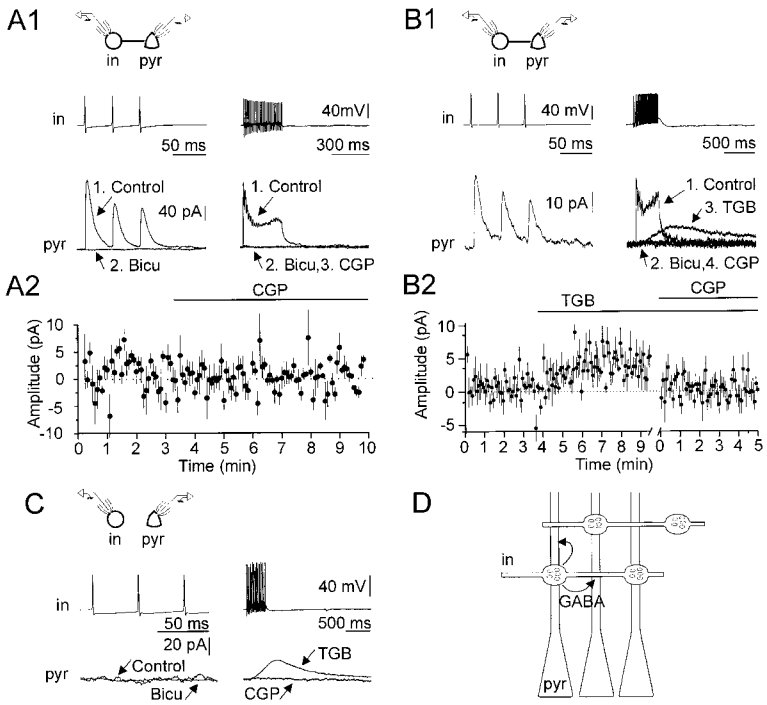

Figure 1. Activation of $GABA_BRs$ by Release of GABA from a Single Interneuron

(A1) Dual recording from a connected interneuron/pyramidal cell (in/pyr) pair. The interneuron was recorded in the current clamp mode and the pyramidal cell was voltage clamped at −50 mV. Three APs triggered in the interneuron by current injections at 40 ms intervals elicit three IPSCs in the pyramidal cell. A longer (300 ms) current injection in the interneuron to produce a train of APs (100 Hz) elicits an outward current in the pyramidal cell. Both types of responses are completely blocked by the $GABA_AR$ antagonist bicuculline (Bicu; 40 μM). Addition of the $GABA_BR$ antagonist CGP62349 (2 μM) has no further effect.

(A2) Summary graph of the time course of the amplitude of the response, after application of bicuculline, for eight experiments.

(B1) Similar experiment to the one illustrated in A1, with the difference that the GABA uptake-blocker tiagabine (TGB; 10 μM) was applied after perfusion of bicuculline. Under these conditions the AP train elicits a long-lasting outward current, which is abolished by CGP62349.

(B2) Summary graph of the time course of the amplitude of the response, after application of bicuculline, for six experiments.

(C) Dual recording from a nonconnected in/pyr cell pair. After application of tiagabine, a train of APs in the interneuron elicits an outward current that can be blocked by CGP62349. Current traces are the average of 10–40 sweeps; voltage traces are not averaged.

(D) Schematic diagram illustrating extrasynaptic $GABA_BR$ activation by diffusion of GABA on both postsynaptic and neighboring pyramidal cells.

measured 500–800 ms after onset of the AP train, n = 8 [3 oriens, 5 radiatum]), indicating that under these conditions GABA release from a single interneuron is incapable of activating postsynaptic $GABA_BRs$.

To test the possibility that $GABA_BRs$ are localized at some distance from the synapses and that GABA uptake prevents the access of GABA to these extrasynaptic receptors, a similar series of experiments was performed, but after blockade of the $GABA_AR$ with bicuculline, the GABA uptake blocker tiagabine (Braestrup et al., 1990; Rekling et al., 1990) was applied (Figures 1B1 and 1B2). In the presence of tiagabine (10 μM), a train of APs in the interneuron evoked a slow outward current in the pyramidal cell (5 ± 1 pA, $p < 0.02$, n = 6 [3 oriens, 3 radiatum]). This current was blocked by CGP62349, indicating that it was mediated by $GABA_BR$ activation. The presence of tiagabine did not significantly change the number of APs in a train (31.8 ± 5.8 before and 31.6 ± 5.9 during perfusion of tiagabine, $p > 0.75$; average frequency, 98.1 ± 10 Hz).

Since the half-maximally effective GABA concentration necessary for $GABA_BR$ activation is much lower than that for $GABA_AR$ activation (Sodickson and Bean, 1996), these results suggest that $GABA_BRs$ are located extrasynaptically and that their activation is controlled by GABA uptake mechanisms.

If GABA diffuses out of the synaptic cleft to activate postsynaptic $GABA_BRs$, it is possible that $GABA_BRs$ on a given cell are also activated by GABA released from terminals impinging on other cells. To directly test this possibility, dual recordings were obtained in which the interneuron did not contact the pyramidal cell via $GABA_AR$ synapses, as shown by the lack of a postsynaptic response to APs triggered in the interneuron and the absence of a bicuculline-sensitive component (Figure 1C). In two out of five experiments, a train of APs in the interneuron in the presence of tiagabine induced a clear $GABA_BR$-mediated outward current (Figure 1C). This result suggests that $GABA_BRs$ can be activated by spillover of GABA from terminals contacting other cells (Figure 1D).

Extracellular Stimulation

It is well established that with extracellular electrical stimulation, synaptic activation of $GABA_BRs$ on pyramidal cells occurs even though GABA uptake mechanisms are functional. This discrepancy with respect to the above results was addressed as illustrated in Figure 2A1. An extracellular stimulus was applied at increasing intensities and the amplitude of the $GABA_BR$-mediated response was plotted against the conductance of the preceding $GABA_AR$-mediated component (n = 6). It was apparent that for stimulation intensities resulting in $GABA_AR$-mediated components with a conductance of <6 nS, no $GABA_BR$-mediated current could be detected (Figure 2A2). Considering the conductance range of a unitary IPSC (0.5–6 nS; see above), this corresponds to an average input of 1–12 interneurons per pyramidal cell. Since the previous results indicate that postsynaptic $GABA_BRs$ can be activated even in the absence of a $GABA_AR$-dependent connection between interneurons and pyramidal cells, and that the average connectivity in this system is 60% (see above), these results suggest that to elicit detectable $GABA_BR$-mediated currents in the presence of functional GABA uptake a minimum of 2–20 interneurons must be stimulated. Thus, the results obtained with extracellular stimulation are consistent with those obtained with dual recordings.

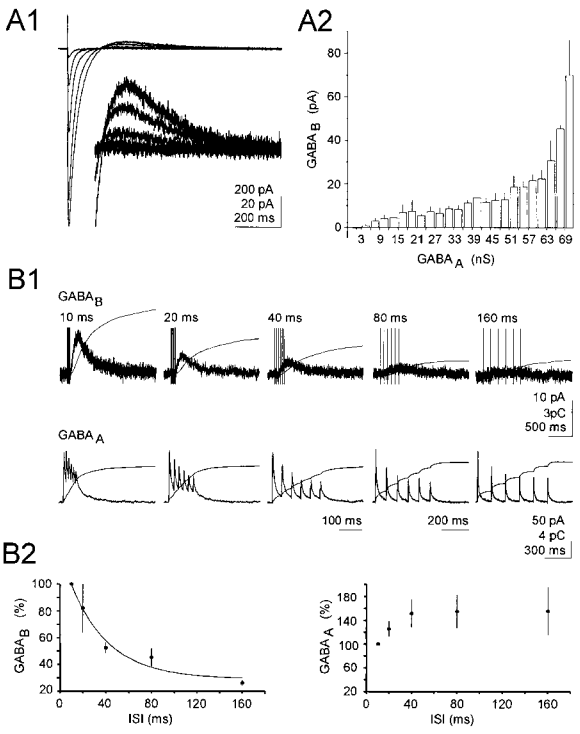

Figure 2. Nonlinear Relation between $GABA_A$R- and $GABA_B$R-Mediated IPSCs

(A1) Extacellularly evoked $GABA_A$R- (inward) and $GABA_B$R- (outward) mediated IPSCs in a pyramidal cell voltage clamped at −85 mV. Superimposed responses were elicited at different stimulation intensities. The inset illustrates the $GABA_B$R-mediated component at higher magnification (this component was abolished by CGP62349 [2 μM] at any stimulation intensity, n = 3).

(A2) The amplitudes of the $GABA_B$R-mediated component are plotted against the peak conductance of the $GABA_A$R-mediated component for six experiments (bin size, 3 nS).

(B1) The top current traces show pharmacologically isolated $GABA_B$R-mediated IPSCs evoked by six extracellular stimuli at increasing ISIs recorded from a pyramidal cell voltage clamped at −50 mV. The superimposed thin traces represent the time integral of the current traces (charge transfer). Note the decrease in charge with increasing ISI. The bottom current traces show pharmacologically isolated $GABA_A$R-mediated IPSCs evoked as described above; different pyramidal cell voltage clamped at −50 mV. Note that charge does not decrease with increasing ISI. Current traces are the average of 5–10 sweeps.

(B2) At left, a summary graph of the charge transfer through $GABA_B$R-mediated IPSCs is plotted against ISI and normalized for an ISI of 10 ms (n = 6). The curve shows a single exponential fit of the form $y = A + B\exp(-t/C)$ where C = 33 ms. At right, a summary graph of the charge transfer through $GABA_A$R-mediated IPSCs is plotted against ISI and normalized for an ISI of 10 ms (n = 3).

The plot in Figure 2A2 shows a supralinear increase in the $GABA_B$R-mediated response with increasing $GABA_A$R-mediated conductance, suggesting cooperativity between release sites for the activation of $GABA_B$Rs. Thus, a factor must be "pooled" to reach the necessary threshold concentration to elicit a $GABA_B$R-mediated response. An alternative way to reach the necessary threshold concentration is to "accumulate" the factor by repetitive activation of a given set of release sites. For this factor to be able to accumulate, however, the interval between the activation of release sites must be no longer than the time it takes for the factor to decay. I measured the charge transfer through $GABA_B$R-activated conductances elicited by six extracellular stimuli given at frequencies between 100 and 6.25 Hz (Figure 2B1). As shown in Figure 2B2, the charge transfer decayed with decreasing stimulation frequency with a time constant of about 33 ms. To ensure that the observed decrease in charge transfer was not due to decreased GABA release, $GABA_A$R-mediated IPSCs were evoked with the same stimulation protocol. As shown in Figure 2B2, the charge transfer through $GABA_A$Rs did not decrease but rather increased with increasing interstimulus interval (ISI), consistent with a recovery from the frequency-dependent depression characteristic of these synapses.

Rhythmic Activity

According to the results presented so far, activation of postsynaptic $GABA_B$Rs in the presence of a functional uptake system necessitates the simultaneous release of GABA from several interneurons. Under which physiological conditions is this requirement fulfilled? In the hippocampus in vivo, synchronous oscillation of the membrane potential of large neuronal populations can occur at various frequencies depending on the behavioral state. One type of oscillation, theta rhythm, can be mimicked in vitro by activation of muscarinic acetylcholine receptors (mAChRs) (Konopacki et al., 1987). In hippocampal slice cultures, rhythmic activity can be induced with very low (10–20 nM) concentration of the mAChR agonist methacholine (Fischer et al., 1999). This is important because at these concentrations GABA release, which is inhibited upon activation of presynaptic mAChRs (Pitler and Alger, 1992), is only affected to a minor degree (15% ± 4% reduction of $GABA_A$R-mediated monosynaptic IPSC, n = 6).

In pyramidal cells voltage clamped at −50 mV, the rhythmic activity induced by perfusion of methacholine (20 nM) was recorded as an EPSC–IPSC sequence, occurring at 5–15 Hz (average of 12 ± 2 Hz, n = 8; Figure 3A1). This activity was synchronous throughout the neuronal population as shown by the strong correlation with the signal recorded with a field electrode placed 150 μm from the patch pipette in the pyramidal cell layer (n = 4).

The number of GABAergic interneurons active during each cycle of the rhythmic activity was estimated either by comparing the conductance of the $GABA_A$R-mediated component of a cycle with the conductance of a unitary IPSC or by directly monitoring the spiking activity of interneurons. The histogram in Figure 3A2 illustrates the distribution of peak conductances of the IPSC component of the cycles for seven experiments. The average was 17.2 ± 2 nS, which compared to the unitary IPSC conductance (ranging from 0.5 to 6 nS) corresponds to the input of about 3–34 interneurons. Considering that the connectivity between interneurons and pyramidal cells was 60%, these results suggest that during any cycle an average of 5–57 interneurons was simultaneously active. A more direct estimate was achieved by recording the spiking activity of the interneurons in the

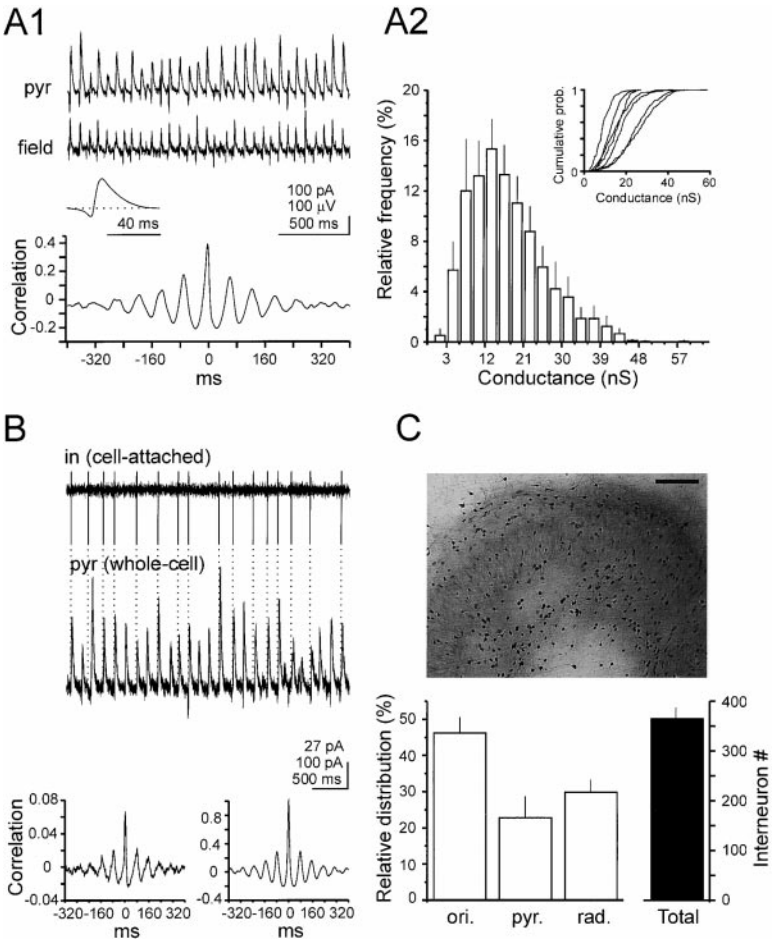

Figure 3. Activity of GABAergic Interneurons during Hippocampal Rhythmic Activity

(A1) Current and voltage traces are simultaneous voltage clamp (top trace) and field (bottom trace) recordings in the presence of 20 nM methacholine. The pyramidal cell was held at −54 mV. The inset (same cell) illustrates on an expanded time scale an EPSC–IPSC sequence averaged over several cycles. The cross-correlogram illustrates the synchrony between both recordings.

(A2) Summary graph of the peak conductance distributions of the IPSCs for seven experiments (bin size, 3 nS). The inset illustrates the cumulative distribution of the conductances for each experiment.

(B) Current traces show simultaneous cell-attached and whole-cell recordings from an interneuron and a pyramidal cell, respectively, in the presence of methacholine. Note that the spiking activity of the interneuron is phase locked with oscillations recorded in the pyramidal cell. The bottom panels show a cross-correlogram between both recordings (left) and an autocorrelogram from the pyramidal cell recording (right).

(C) The micrograph shows anti-GABA immunoreactivity in the CA3 region of a culture (bar = 250 μm). The histogram is a summary graph of the distribution of interneurons in the CA3 subregions and the total number of interneurons averaged over 11 cultures.

cell-attached mode, while monitoring the rhythmic activity in either field or pyramidal whole-cell recordings (Figure 3B). The average probability of firing for an interneuron during a cycle was 0.14 ± 0.04 (n = 9). Since the interneuron population in the CA3 region of a culture is 365 ± 71 (n = 11; Figure 3C; estimated with immunohistochemical staining of interneurons, see Experimental Procedures), this result indicates that for any cycle about 50 interneurons were spiking. This result is within the range estimated by comparing IPSC conductances and, thus, represents sufficient activity to stimulate postsynaptic $GABA_BRs$.

To find out whether $GABA_BRs$ are activated under such conditions, CGP62349 (2 μM) was perfused during methacholine-induced rhythmic activity. As shown in Figure 4, application of CGP62349 resulted in a pronounced increase in frequency of the oscillations (42% ± 13% increase, $p < 0.02$, n = 8), indicating that simultaneous release of GABA from several interneurons during rhythmic activity leads to $GABA_BR$ activation.

The increase in excitability upon block of $GABA_BRs$ could originate because of an action either on postsynaptic $GABA_BRs$, thereby depolarizing the pyramidal cells, or on presynaptic $GABA_BRs$ located on glutamatergic terminals, thereby increasing the release of glutamate. If GABA released during rhythmic activity activates postsynaptic $GABA_BRs$ to open a potassium conductance, perfusion of CGP62349 should produce an inward current in pyramidal cells. If, on the other hand, presynaptic $GABA_BRs$ are activated, thus inhibiting glutamate release, then CGP62349 should cause an increase in the amplitude of unitary EPSCs. Both possibilities were tested. As shown in Figure 5A1, after induction of rhythmic activity the pyramidal cell was voltage clamped at the reversal potential for IPSCs to reduce the large amplitude of the synaptic current fluctuations, and CGP62349 was bath applied. This resulted in the development of an inward current (8 ± 2 pA, $p < 0.01$, n = 4; Figure 5A2), indicating that postsynaptic $GABA_BRs$ were activated during rhythmic activity. On the other hand, the amplitude of unitary EPSCs between connected pyramidal cells was not significantly affected by perfusing CGP62349 during rhythmic activity ($p > 0.25$, n = 4), indicating that under these conditions presynaptic $GABA_BRs$ are not or are only weakly activated (Figures 5B1 and 5B2). As in the previous experiment, the postsynaptic pyramidal cell was voltage clamped at around −70 mV after induction of rhythmic activity, to decrease the amplitude of the large rhythmic IPSC, thereby facilitating the detection of unitary EPSCs. To ensure that CGP62349 did in fact increase the frequency of rhythmic activity, the holding potential of the pyramidal cell was then returned to −46 mV.

These results suggest that oscillation frequency is modulated by postsynaptic $GABA_BRs$. Since $GABA_BR$ activation is tightly controlled by GABA uptake mecha-

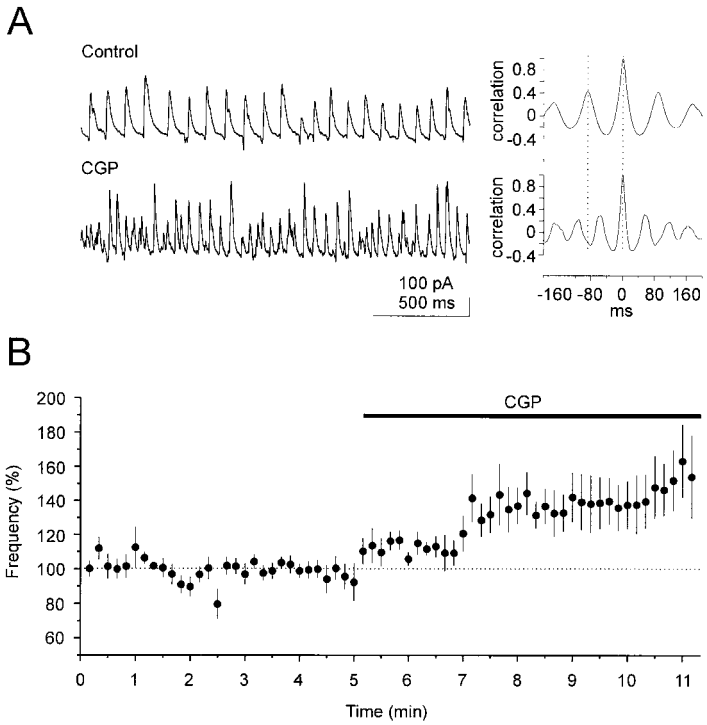

Figure 4. $GABA_B$Rs Modulate Hippocampal Rhythmic Activity

(A) Current traces from a pyramidal cell voltage clamped at −50 mV in the presence of methacholine. Inhibition of $GABA_B$Rs by CGP62349 leads to an increase of the frequency of the oscillations as illustrated in the autocorrelograms to the right of the recordings. (B) Summary graph for six to ten experiments.

nisms, it should be possible to decrease the oscillation frequency by reducing GABA uptake. This was tested as illustrated in Figure 6. After induction of rhythmic activity, bath application of a low concentration (0.5 μM) of tiagabine resulted in a marked decrease in the frequency (65% ± 7%, $p < 0.001$, $n = 4$). Because at this concentration tiagabine increased the charge transfer of evoked $GABA_A$R-mediated monosynaptic IPSCs by only 55% ± 21% ($n = 5$), as compared with the 312% ± 61% increase ($n = 4$) observed for $GABA_B$R-mediated monosynaptic IPSCs (data not shown), the reduction in the frequency of rhythmic activity most likely reflects increased $GABA_B$R activation rather than prolongation of $GABA_A$R-mediated IPSCs. Indeed, subsequent perfusion of CGP62349 brought oscillation frequency back to control levels, indicating that the decrease in frequency was mainly due to the enhanced activation of $GABA_B$Rs.

Discussion

Experimental approaches based on the simultaneous recording from identified pre- and postsynaptic neurons have been important in clarifying the role played by GABAergic interneurons in the modulation of the activity of their targets. While GABA can act on both $GABA_A$Rs and $GABA_B$Rs, synaptic transmission between a single hippocampal or neocortical interneuron and its targets generally appears to be mediated exclusively by $GABA_A$R activation (Miles and Wong, 1984; Otis and Mody, 1992; Buhl et al., 1994; Ali et al., 1999; Thomson and Destexhe, 1999). This is in contrast to other brain areas, such as the perigeniculate projection to thalamocortical cells, where a burst of APs in presynaptic GABAergic neurons was shown to invariably elicit both $GABA_A$R- and $GABA_B$R-mediated responses in postsynaptic relay neurons (Kim et al., 1997).

Thus, the conditions necessary for postsynaptic $GABA_B$R activation as well as the physiological importance of these receptors during normal cortical activity has remained elusive. This study identifies a functional role for postsynaptic $GABA_B$Rs activated by spontaneous network activity.

By recording from connected CA3 hippocampal interneuron to pyramidal cell pairs, I find that responses mediated by postsynaptic $GABA_B$Rs are detectable after inhibition of GABA uptake. The EC_{50} of GABA for $GABA_B$R activation is much lower than that for $GABA_A$R activation (Sodickson and Bean, 1996). Therefore, although a comparison between EC_{50} values is not entirely appropriate because equilibrium kinetics probably do not apply for synaptically released GABA, the data suggest that under physiological conditions $GABA_A$ and $GABA_B$Rs do not sense the same concentration of released GABA—in other words, they do not colocalize at the postsynaptic membrane. These results are most easily explained if one assumes that postsynaptic $GABA_B$Rs are located outside the synaptic cleft and that the diffusion of GABA to postsynaptic $GABA_B$Rs is under the tight control of GABA uptake mechanisms.

The data presented here also show that postsynaptic $GABA_B$Rs can be activated in the absence of a $GABA_A$R-dependent connection between the interneuron and the pyramidal cell, suggesting that $GABA_B$Rs located on a given neuron can be activated by GABA released from terminals impinging on other neurons. This is consistent with previous reports of evoked $GABA_B$R-mediated responses lacking a $GABA_A$R-mediated component (Solis et al., 1992; Thomson and Destexhe, 1999). Although one cannot exclude the possibility that a particular subset of interneurons contacts pyramidal cells via synapses expressing only $GABA_B$Rs, the present results, showing that any interneuron forming a $GABA_A$R-mediated con-

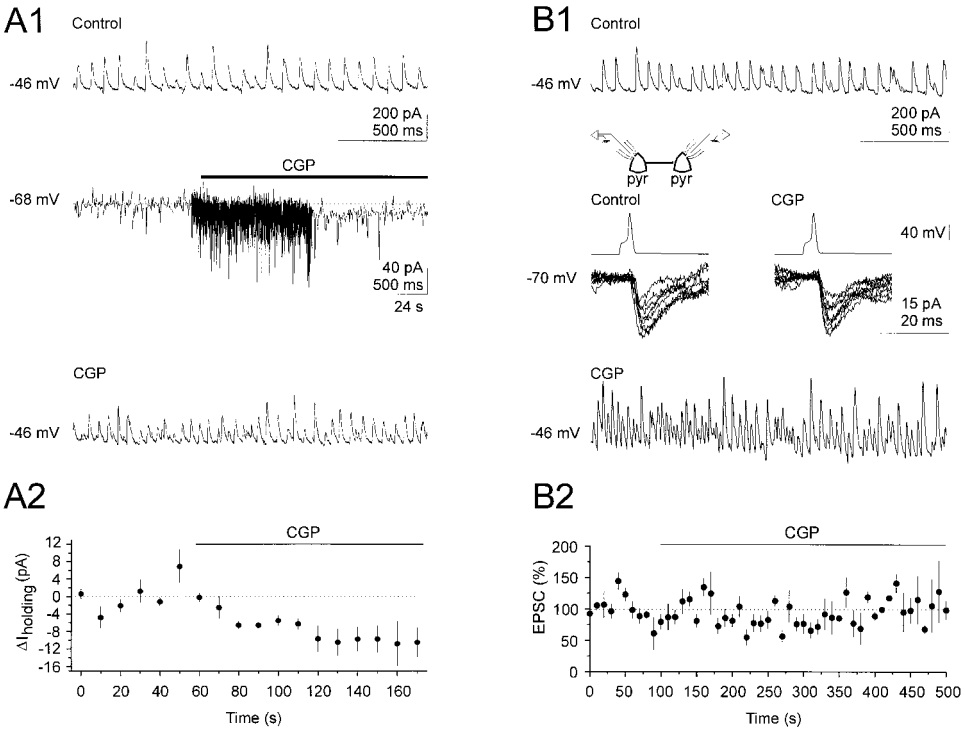

Figure 5. Postsynaptic $GABA_BRs$ Are Activated during Hippocampal Rhythmic Activity

(A1) The top trace shows a pyramidal cell voltage clamped at −46 mV in the presence of methacholine. In the middle trace, the pyramidal cell was voltage clamped at the reversal potential for IPSCs. Application of CGP62349 induces an inward current. Note that the central portion of the current trace is compressed to illustrate the development of the inward current over time. In the bottom trace, the holding potential was brought back to −46 mV. CGP62349 induced the expected increase in frequency of the oscillations.

(A2) Summary graph of the time course of the change in holding current for four experiments (each data point represents the average current over a 10 s period).

(B1) The top trace shows a pyramidal cell voltage clamped at −46 mV in the presence of methacholine. In the middle traces, before application of CGP62349 the cell was voltage clamped at −70 mV to facilitate the detection of unitary EPSCs evoked by triggering an AP in a connected presynaptic pyramidal cell recorded in current clamp mode. Perfusion of CGP62349 does not affect the amplitude of unitary EPSCs (eight superimposed consecutive sweeps in each condition). In the bottom trace, the holding potential of the postsynaptic cell was brought back to −46 mV. CGP62349 induced the expected increase in the oscillation frequency.

(B2) Summary graph of the time course of the unitary EPSC amplitudes for four experiments.

nection with a pyramidal cell can activate $GABA_BRs$ after inhibition of uptake, make this hypothesis unlikely.

The plot in Figure 2A2 displays a nonlinear relationship between $GABA_AR$ and $GABA_BR$-mediated responses upon extracellular stimulation. Unless some unknown factor is released upon higher stimulation intensities that would enhance $GABA_BR$-mediated responses, this result suggests a cooperative interaction between GABAergic release sites to produce $GABA_BR$-mediated outward currents. Two mechanisms have been proposed to account for this nonlinearity. According to the first proposal, GABA has to be released from several terminals before it can reach the necessary concentration to activate extrasynaptic $GABA_BRs$ (Thompson and Gähwiler, 1992). The nonlinearity may result from the necessity of GABA to first saturate the buffering capacity of transporters or other binding sites in order to accumulate extrasynaptically and/or from a cooperative activation of $GABA_BRs$ by GABA, as suggested by the heteromeric nature of $GABA_BRs$ (Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998; Kuner et al., 1999). The second proposal is based on the cooperative property of GIRK channel activation by G protein $\beta\gamma$ subunits ($G\beta\gamma$) (Ito et al., 1992; Krapivinsky et al., 1995). In this case, $GABA_BR$ activation could in principle progress linearly with an increase in the number of active GABAergic release sites, but the observed GIRK channel–mediated current would increase supralinearly (Destexhe and Sejnowski, 1995). In other words, either GABA has to be pooled extracellularly or $G\beta\gamma$ intracellularly. In the present study an experimental approach was used to distinguish between the two possibilities. For repetitive extracellular stimuli to result in the accumulation of either GABA or $G\beta\gamma$, the ISI must be shorter than the decay of GABA or $G\beta\gamma$ concentrations. Figure 2B2 shows that the charge transfer of $GABA_BR$-mediated responses to six extracellular stimuli is reduced with decreasing stimulation frequency with a time constant of about 33 ms. Availability of $G\beta\gamma$ is determined by the GTP hydrolysis rate of $G\alpha$ (Breitwieser and Szabo, 1988) and lasts several hundreds of milliseconds, even in the presence of regulators of G protein signaling, which accelerate GTPase activity (Doupnik et al., 1997; Saitoh et al., 1997). Therefore, the results presented here favor a model in which the cooperativity between release sites is mediated predominantly by pooling of GABA. The relatively

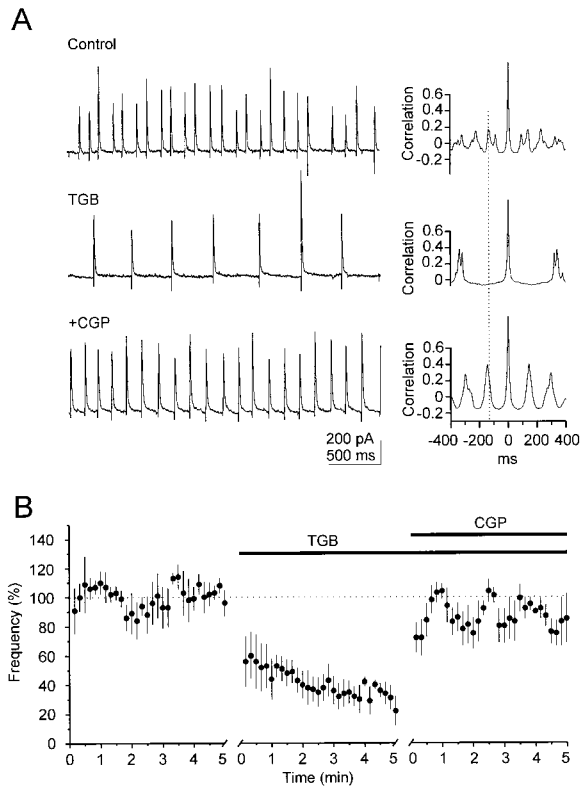

Figure 6. Inhibition of Uptake Reduces Oscillation Frequency by Increasing $GABA_BR$ Activation

(A) The top trace shows a pyramidal cell voltage clamped at −50 mV in the presence of methacholine. In the middle trace, application of a low concentration of tiagabine (TGB; 0.5 μM) reduces oscillation frequency. In the bottom trace, the effect of tiagabine was reversed upon application of CGP62349. The change in oscillation frequency is illustrated by the autocorrelograms on the right of the recordings. (B) Summary graph of four experiments.

rapid decrease in $GABA_BR$-mediated charge transfer upon prolongation of the ISI could be explained by pooling of Gβγ only if the cooperativity between Gβγ and GIRK channels is much higher than previously proposed (Ito et al., 1992), or if Gβγ would rapidly diffuse away from GIRK channels. This, however, does not seem to be the case, because the time course of decay of $GABA_BR$-mediated currents would then be much faster than that observed for synaptic (Otis et al., 1993) or pharmacological stimulation (Sodickson and Bean, 1996).

Thus, this study suggests that, in contrast to fast $GABA_AR$-mediated synaptic transmission where GABA acts locally at the site of release, $GABA_BRs$ are activated more diffusely, by pooling of GABA within the domain of a GABAergic axonal plexus formed by multiple neurons, and may be regarded as sensors of synchronous release of GABA from this population. It is conceivable that pooling of GABA may occur between release sites on a single axonal arbor if they are packed densely enough. This would explain why the activity of a single interneuron was reported to elicit a $GABA_BR$-mediated response in hippocampal pyramidal cells (Thomson and Destexhe, 1999). In contrast to my findings, they interpret the supralinear increase of $GABA_BR$-mediated responses upon repetitive release of GABA, based on modeling, as reflecting pooling of Gβγ rather than pooling of GABA. If, however, high GABA transporter concentrations were included in the model, nonlinearity could also be predicted with pooling of GABA. Furthermore, the model did not consider the possibility that more than one transmitter molecule may bind to $GABA_BRs$, such that $GABA_BRs$ would become nonlinear detectors of ambient GABA. Until precise data on the concentration of GABA transporters and the number of agonist binding sites on $GABA_BRs$ become available, one can not consider pooling of Gβγ as a major determinant of cooperativity between release sites generating $GABA_BR$-mediated responses.

Which physiological conditions promote simultaneous activity in neuronal populations? Several types of synchronous oscillatory activity in large neuronal populations have been described in the brain that are associated with specific behavioral states. One of these, the theta rhythm, has been extensively studied in vivo (O'Keefe and Recce, 1993; Soltesz and Deschenes, 1993; Skaggs et al., 1996; Csicsvari et al., 1998; Kahana et al., 1999), and protocols have been developed to mimic this activity in vitro by activation of muscarinic acetylcholine receptors (Konopacki et al., 1987; Fischer et al., 1999). In organotypic slice cultures, this rhythmic activity can be triggered by very low concentrations of muscarinic agonists (Fischer et al., 1999), an important consideration for the present study since higher concentrations strongly inhibit GABA release via presynaptic muscarinic acetylcholine receptors (Pitler and Alger, 1992), leading to epileptiform discharges (Williams and Kauer, 1997). Under these conditions of rhythmic population activity, 14% of the interneurons fired during each cycle. This corresponds to about 50 active interneurons, which according to the results with extracellular stimulation should be sufficient to activate postsynaptic $GABA_BRs$. Indeed, during rhythmic activity, block of $GABA_BRs$ resulted in an increase in the frequency of the oscillations as well as an inward current in the pyramidal cells, demonstrating their endogenous activation. Furthermore, the decrease in oscillation frequency after partial block of GABA uptake could be reversed by $GABA_BR$ antagonists. The fact that, under such conditions, the antagonist did not increase the frequency above control levels was probably due to the residual action of the GABA uptake inhibition on $GABA_AR$-mediated IPSCs.

From the experiments presented here, it appears that during rhythmic activity only postsynaptic $GABA_BRs$ were activated. Even after partial block of GABA uptake, thereby decreasing the frequency of rhythmic activity, no effect could be detected on the amplitude of unitary EPSCs upon perfusion of the $GABA_BR$ antagonist CGP62349 ($n = 4$; data not shown). In addition, perfusion of CGP62349 did not significantly increase the median and mean amplitudes of the inward component of the oscillation (14% ± 5% increase, $p > 0.13$, and 12 ± 9% increase, $p > 0.63$, respectively; $n = 10$). The lack of any detectable presynaptic $GABA_BR$-mediated action could be due to a more efficient GABA uptake system surrounding excitatory terminals or to a spatial segregation between GABAergic release sites active during

rhythmic activity and recurrent excitatory inputs. Alternatively, the increased oscillatory frequency upon perfusion of CGP62349 may shunt poorly voltage-clamped excitatory synapses, thereby masking a potential increase in EPSC amplitude (Spruston et al., 1993; Frerking et al., 1999). In this study no attempt was made to detect possible $GABA_BR$ activation on GABAergic terminals impinging on either pyramidal cells or interneurons. Endogenous inhibition of GABA release by presynaptic $GABA_BR$ activation may well influence rhythmic activity and will have to be addressed in the future.

From these results it appears that the GABAergic system in the hippocampus has two modes of operation: namely, point-to-point synaptic transmission, when interneurons act as individual units, and diffuse synaptic transmission, when interneurons act cooperatively during synchronous activity. When the diffuse mode comes into play, a new population of receptors, the extrasynaptic $GABA_BRs$, is recruited. Thus, one can speculate that specific behavioral states are associated with different levels of diffuse inhibitory synaptic transmission.

While it will be important to confirm $GABA_BR$ activation during rhythmic activity in vivo, the following extrapolation is of interest. A hippocampal interneuron in situ contacts between 1000 and 3000 pyramidal cells (Freund and Buzsaki, 1996). Considering that the population of interneurons represents ~10% of the entire hippocampal neuronal population, each target receives inputs from about 100–300 interneurons, which is very similar to the number estimated in hippocampal cultures (60% of 365 interneurons; see Results). During theta activity in the hippocampus in vivo, pyramidal cells receive a powerful phasic synaptic inhibition (Soltesz and Deschenes, 1993). In fact, up to 60% of interneurons have been reported to spike within a cycle (Csicsvari et al., 1998). This exceeds the estimated probability of firing in hippocampal cultures and thus makes $GABA_BR$ activation during theta rhythm even more likely in vivo. Accordingly, the $GABA_BR$ antagonist CGP51176 was found to significantly increase the frequency of the theta rhythm recorded in freely moving rats in a dose-dependent manner (Dr. H. R. Olpe, personal communication).

Oscillatory activity in the hippocampus has been related to several important cognitive functions in animals and humans (O'Keefe and Recce, 1993; Skaggs et al., 1996; Kahana et al., 1999). In many instances, the activity of GABAergic interneurons was found to be important in modulating the frequency and the degree of synchronicity among neurons through the activation of $GABA_ARs$ (Cobb et al., 1995; Jefferys et al., 1996). This study shows that simultaneous discharge of interneurons regulates oscillation frequency by recruiting postsynaptic $GABA_BRs$ on pyramidal cells.

Experimental Procedures

Hippocampal slice cultures were prepared from 5- to 6-day-old rats as described (Gähwiler, 1981). After 2–4 weeks in vitro, cultures were placed in a superfusion chamber for recording. The artificial cerebrospinal fluid (ACSF), equilibrated with 95% O_2 and 5% CO_2, contained (in mM): 119 NaCl, 2.5 KCl, 1 $NaHPO_4$, 2 $MgCl_2$, 2.8 $CaCl_2$, 26 $NaHCO_3$, and 11 glucose. Electrophysiological recordings were made at 33°C–34°C. Whole-cell recording electrodes were filled with a solution containing (in mM): 140 K gluconate, 10 KCl, 5 HEPES, 1.1 EGTA, 4 $MgCl_2$, 10 phosphocreatine, 0.5 Na_3GTP (in pyramidal cells only), and the pH was adjusted to 7.2 with KOH. The resistance of the pipettes ranged from 2 to 4 MΩ. Voltage measurements were corrected for the experimentally determined junction potential (10 mV). The reversal potential of unitary IPSCs was estimated by taking the intersection of a linear regression function of the IPSC amplitudes plotted against four different holding potentials between −50 and −80 mV with the 0 current axis. The reversal potential of rhythmic IPSCs was estimated online by changing the holding potential of the pyramidal cell until the deflection would change sign. Field recording electrodes were made with glass pipettes containing 3 M NaCl. Extracellular monopolar stimulation electrodes were made with similar glass electrodes containing ACSF or with steel electrodes. Interneurons located in the strata radiatum or oriens were visually identified using infrared DIC. Monosynaptic IPSCs were evoked in the presence of NBQX (12.5 μM) and CPP (50 μM). Bicuculline (40 μM) or CGP62349 (2 μM) were present to isolate $GABA_A$- or $GABA_BR$-mediated IPSCs, respectively. Data were digitized online via a digidata 1200 interface (Axon Instruments). EPSC/IPSC sequences during rhythmic activity were detected with a mini analysis program (Synaptosoft, Leonia, NJ), and the frequency was calculated for 10 s bins. The Student's t test was used for statistical comparisons. Average values are expressed as the mean ± SEM. Auto- and cross-correlograms were performed over 8–35 s data stretches. The number of interneurons in the CA3 region of cultures was assessed with anti-GABA immunostaining (Streit et al., 1989) (339 ± 21, n = 5) or acetylcholinesterase staining (Geneser-Jensen and Blackstad, 1971; Gähwiler, 1984) (386 ± 30, n = 6). The number of interneurons revealed was not significantly different for the two methods ($p > 0.25$). Drugs used were: methacholine (Sigma, Buchs, Switzerland); CPP, (−)-bicuculline methochloride, and NBQX (Tocris Cookson, Bristol, UK); tiagabine (a generous gift of NOVO Pharmaceuticals, Denmark); and CGP62349 (a generous gift of Novartis, Basel, Switzerland).

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