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Pharmacological Characterization of $GABA_B$-mediated Responses in the CA1 Region of the Rat Hippocampal Slice

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It is generally accepted that the bicuculline-resistant responses to GABA are mediated through the activation of $GABA_B$ receptors that mediate a slow IPSP. However, a number of reported observations are difficult to reconcile with this model. Specifically, $GABA_B$ antagonists only partially block bicuculline-resistant GABA responses, and both 4-aminopyridine (4-AP) and carbachol have been reported to block responses to the selective $GABA_B$ agonist baclofen, but not GABA itself. Thus, it has been argued that baclofen and GABA increase potassium conductance through separate receptor mechanisms. This suggestion is not easily reconcilable with the postulated physiological role of $GABA_B$ receptors in mediating the slow IPSP. We have addressed these discrepancies by using the new $GABA_B$ antagonists 2-hydroxy-saclofen (2-OH-SAC) and CGP 35348 in the presence of the GABA uptake inhibitor SKF 89976A.

The weak antagonism of 2-OH-SAC against the bicuculline-resistant GABA response was improved when the GABA uptake was inhibited with SKF 89976A, allowing for the application of lower GABA concentrations. Under these circumstances, 2-OH-SAC and CGP 35348 strongly antagonized GABA and baclofen responses, but did not have any effect on outward currents evoked by 5-HT. The slow IPSP evoked in the presence of glutamate antagonists was reversibly inhibited by CGP 35348 (IC_{50} = 14 μM), without affecting the fast IPSP. Carbachol (0.3–20 μM) had no effect on outward currents evoked by either baclofen or GABA. 4-AP (5 μM to 1 mM), despite causing a large increase in cell excitability, did not change baclofen responses. Higher concentrations of 4-AP (5 mM) induced inward current, and reduced both baclofen and GABA outward currents to a similar extent. The strong presynaptic inhibitory effect of baclofen and GABA on EPSPs was completely blocked by CGP 35348.

The fact that the post- and presynaptic effects of both baclofen and GABA have similar pharmacological characteristics suggests that both substances act through the same receptor mechanism. The present findings are entirely consistent with the proposal that the slow IPSP is generated by synaptically released GABA acting on $GABA_B$ receptors.

There is physiological evidence for the existence of both post- and presynaptic $GABA_B$ receptors in the hippocampus (Bowery et al., 1990). Much of this evidence is based on the actions of the selective $GABA_B$ agonist baclofen. While application of GABA in the presence of $GABA_A$ antagonists can mimic the increase in potassium conductance evoked by baclofen in hippocampal pyramidal cells (Newberry and Nicoll, 1984, 1985; Gähwiler and Brown, 1985; Inoue et al., 1985), a number of observations have suggested that differences may exist between these two responses. First, phaclofen can completely block the postsynaptic action of baclofen and therefore was called a $GABA_B$ antagonist. However phaclofen was much less effective in blocking the GABA response (Dutar and Nicoll, 1988a). The more potent $GABA_B$ antagonist 2-hydroxy-saclofen (2-OH-SAC) also has only a weak effect on the bicuculline-resistant GABA hyperpolarizations (Segal, 1990). The weak effect of $GABA_B$ antagonists on the GABA response, together with the finding that carbachol blocked baclofen, but not GABA, responses, led Müller and Misgeld (1989) to propose that different conductance mechanisms existed for these two responses. This supported an earlier similar proposal (Inoue et al., 1985; Ogata et al., 1987) based on the observation that 4-aminopyridine (4-AP) blocked the hyperpolarization evoked by baclofen, but not GABA. The suggestion that baclofen and GABA activate different receptor mechanisms is incompatible with the postulated physiological role of GABA as the transmitter in mediating the slow IPSP (Dutar and Nicoll, 1988a; Soltesz et al., 1988).

Fewer studies have been done with presynaptic $GABA_B$ receptors. While phaclofen has generally failed to affect the presynaptic action of baclofen (Dutar and Nicoll, 1988b; Harrison, 1989; Stirling et al., 1989; Wang and Dun, 1990), 2-OH-SAC has been effective (Curtis et al., 1988; Harrison et al., 1990). Moreover, it is not known whether GABA in the presence of $GABA_A$ antagonists mimics the presynaptic action of baclofen.

In this article, we have addressed these reported discrepancies and conclude that in the CA1 region, baclofen and GABA activate the same receptor mechanism at both postsynaptic or presynaptic sites and that GABA acting on $GABA_B$ receptors mediates the slow IPSP.

Materials and Methods

The methods used in this article are similar to those used in other studies from this laboratory (Nicoll and Alger, 1981). Rat hippocampal slices, 400 μm thick, were cut and placed in a holding chamber for at least 1

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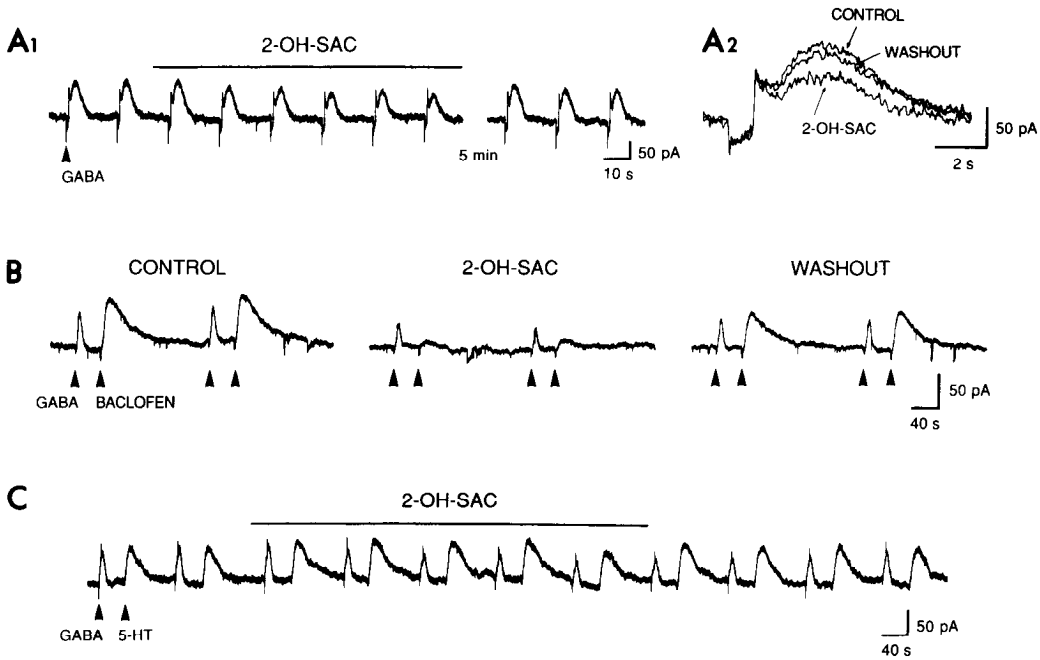

Figure 1. 2-OH-SAC antagonizes GABA and baclofen currents. Outward currents were evoked by iontophoresis of GABA (*A*), GABA and baclofen (*B*), or GABA and 5-HT (*C*). All three experiments were done in the presence of TTX (0.1 μM), bicuculline methiodide (40 μM), and picrotoxin (40 μM). The cells were voltage clamped at −70 mV. Solutions containing 500 μM 2-OH-SAC were perfused where indicated. Each experiment was carried out in a different cell. A_1, Outward currents evoked by GABA (80 nA × 1 sec) are partially and reversibly reduced by 2-OH-SAC. A_2, Averages (n = 5) of GABA responses obtained in the experiment depicted in *A1*. *B*, In another cell, currents evoked by baclofen (150 nA × 2 sec) are blocked by 2-OH-SAC, while responses to GABA (80 nA × 2 sec) are reduced. *C*, Bath application of 2-OH-SAC induces a small reduction in GABA (80 nA × 5 sec) currents but does not inhibit outward currents evoked by 5-HT (120 nA × 2 sec).

hr. A single slice was then transferred to the recording chamber and held between two nylon nets, submerged beneath a continuously superfusing medium that had been pregassed with 95% O_2 and 5% CO_2. The composition of the medium was (in mM) NaCl, 119; KCl, 2.5; $MgSO_4$, 1.3; $CaCl_2$, 2.5; NaH_2PO_4, 1.0; $NaHCO_3$, 26.2; glucose, 11. The temperature of the medium was maintained between 29°C and 31°C.

Drugs applied by addition to the superfusion medium included carbachol, picrotoxin, bicuculline methiodide, 4-AP, atropine, spiperone (all from Sigma), D,L-4-aminophosphonovalerate (APV), and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (both from CRB), 2-OH-SAC (Tocris Neuramin), tetrodotoxin (Calbiochem), (+)-baclofen and CGP 35348 (both gifts from Ciba-Giegy), and SKF 89976A (gift from Nova Pharmaceutical). GABA (1 mM, pH 5), serotonin (5-HT) (40 mM, pH 4), and baclofen (40 mM, pH 3) were also applied by ionotophoresis. In some experiments, GABA (5 mM in Ringer) and baclofen (5 mM in Ringer) were applied by pressure from a broken pipette.

Conventional intracellular recordings from CA1 pyramidal cells were obtained using glass micropipettes, filled with either 2 M potassium methylsulfate (resistance, 80–120 MΩ) or 3 M KCl (resistance, 25–70 MΩ). In some experiments, electrodes were filled with cesium chloride (3 M) to block the postsynaptic increase in K^+ conductance evoked by $GABA_B$ receptor activation. Excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) were induced using bipolar stimulating electrodes positioned in stratum radiatum to activate Schaffer collateral/commissural fibers and inhibitory fibers. When recording EPSPs, the cell was hyperpolarized to −75 to −85 mV to prevent contamination of EPSPs with action potentials. The synaptic events were recorded with an Axoclamp 2A (Axon Instruments) and stored and analyzed on a PC286 using pCLAMP software (Axon Instruments) and a Labmaster board. For voltage clamping, KCl-filled electrodes were used. The headstage voltage of the Axoclamp 2 was continuously monitored during the experiments, and the switching frequency was between 3 and 5 kHz, depending on the characteristics of the electrode employed.

Results

We first examined the effects of the $GABA_B$ antagonist 2-OH-SAC, which is reported to be approximately 10 times more potent than phaclofen (Kerr et al., 1988), on outward currents evoked by GABA in the presence of $GABA_A$ antagonists. At concentrations of 2-OH-SAC (0.5 mM) that completely blocked baclofen responses, GABA currents were reduced by approximately 35 ± 10% (n = 15) (Fig. 1). As with phaclofen, 2-OH-SAC essentially abolished the baclofen induced currents (n = 5) (Fig. 1*B*) and had no effect on 5-HT responses (Fig. 1*C*). Since 5-HT and baclofen responses converge onto the same conductance mechanism, 5-HT responses serve as a good control for any nonspecific effects of the antagonists.

One explanation proposed for the difference in sensitivity of the GABA and baclofen responses to $GABA_B$ antagonists is that uptake mechanisms exist for GABA but not for baclofen (Dutar and Nicoll, 1988a). To produce equivalent-sized responses, the local concentration of GABA is likely to be much higher than the concentration of baclofen. A weak competitive antagonist would be less effective against responses generated from the plateau of the dose–response curve. We have tested this possibility by applying the competitive GABA uptake blocker SKF 89976A (Yunger et al., 1984; Larsson et al., 1988), which allows the use of lower GABA concentrations and for a broader and more uniform distribution of GABA. GABA was applied by pressure on the surface of the slice in the presence of SKF 89976A, and the effects of 2-OH-SAC and CGP 35348, a new $GABA_B$ antagonist (Olpe et al., 1990), were compared. As expected, SKF 89976A enhanced the GABA response (Fig. 2). Under these conditions, CGP 35348 (1 mM) virtually abolished the outward current evoked by GABA (85.4 ± 8%) (n = 6). 2-OH-SAC was considerably more effective during blockade of GABA uptake but was clearly less active than CGP 35348 (Fig. 2). However, in the presence of the uptake blockers GABA responses were still less sensitive to CGP 35348 than were the

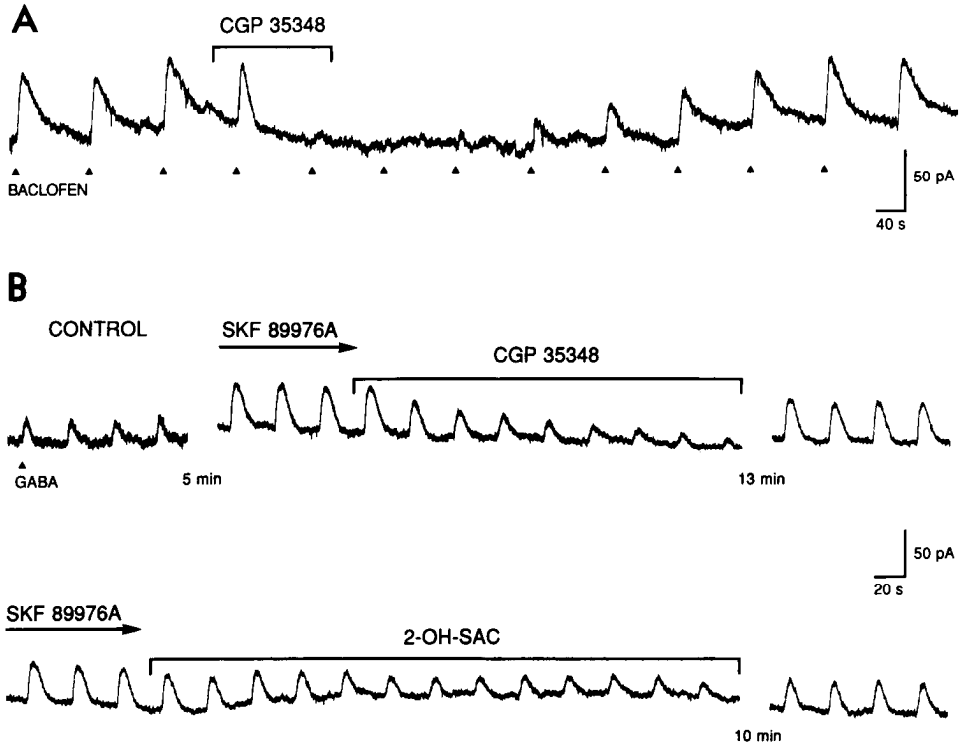

Figure 2. Antagonism of baclofen and GABA responses by CGP 35348. The experimental conditions are the same as those described in Figure 1. *A*, Outward currents evoked by pressure application of baclofen (250 msec) onto the dendrites are completely inhibited by 1 mM CGP 35348 in a reversible manner. Holding potential (V_H) = −62 mV. *B*, In another cell (V_H = −66 mV), the microejection of GABA (100 msec) on the surface of the slice at the level of stratum radiatum induces small responses that are greatly enhanced during the perfusion with 20 μM SKF 89976A (*top record*). These outward currents evoked by GABA are almost completely and reversibly blocked by 1 mM CGP 35348. The *bottom record* shows, in the same cell, that an equimolar concentration of 2-OH-SAC is less effective at inhibiting GABA responses.

baclofen responses. Whereas 3 μM CGP 35348 was threshold for antagonizing baclofen responses, 30 μM was threshold for antagonizing GABA responses.

In Figure 3, the action of GABA in the presence of $GABA_A$ antagonists is compared to the 5-HT response. SKF 89976A selectively enhanced the GABA response, and CGP 35348 selectively and reversibly blocked it. Even at a concentration of 1 mM, CGP 35348 had no effect on the 5-HT responses (n = 5). On the other hand, the 5-HT antagonist spiperone selectively abolished the 5-HT response. These results suggest that under appropriate conditions $GABA_B$ antagonists can effectively and specifically block bicuculline-resistant GABA currents.

We have compared in the presence of APV and CNQX the antagonist action of CGP 35348 on monosynaptically evoked IPSPs. In agreement with Olpe et al. (1990), CGP 35348 selectively and completely blocked the slow IPSP, while picrotoxin abolished the fast IPSP (Fig. 4). The IC_{50} for this inhibition was 14 μM (Fig. 5). No inhibitory response remained in the presence of both CGP 35348 and picrotoxin (n = 10).

Müller and Misgeld (1989) have reported in the CA3 region that carbachol blocks the action of baclofen but is without effect on GABA responses. We have therefore examined this finding carefully with concentrations of carbachol from 0.3 μM, which reduces the hyperpolarization that follows a burst of action potentials but causes no direct inward current (Madison et al., 1987), to 20 μM, which causes a direct inward current. Small baclofen responses that were considerably below the maximal effect were evoked by either bath (0.3 μM) or pressure application. We could not reliably antagonize the outward baclofen current (n = 12) (Fig. 6*A*). Carbachol also had no effect on the outward GABA currents evoked in the presence of $GABA_A$ antagonists (Fig. 6*B*) (n = 3).

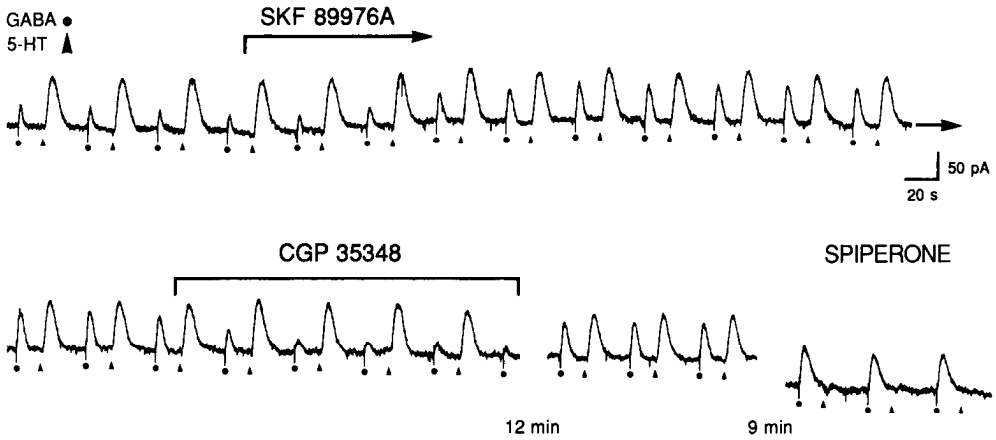

Figure 3. CGP 35348 specifically antagonizes GABA responses. Chart records depict outward currents induced by pressure ejection of GABA (100 msec) and iontophoresis of 5-HT (60 nA × 2 sec) on the surface of the slice at the level of stratum radiatum. Same experimental conditions as in previous experiments. V_H = −57 mV. As in Figure 2, SKF 89976A (20 μM) induces a large and specific increase in the GABA response. In this condition, after the GABA response stabilizes, 1 mM CGP 35348 was applied, which produced a near-complete blockade of GABA currents without affecting 5-HT responses. After the washout of the antagonist, GABA currents recovered. The perfusion with the 5-HT antagonist spiperone (100 μM) completely blocked 5-HT responses but did not reduce GABA currents.

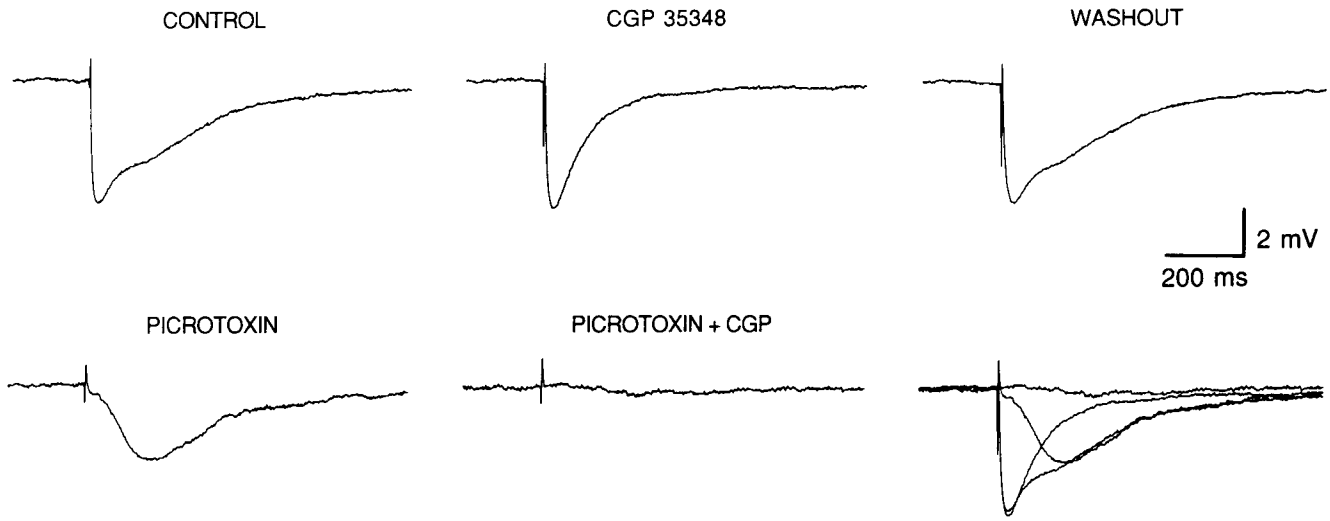

Figure 4. Pharmacological dissection of GABA-mediated IPSPs. In the presence of CNQX (20 μM) and APV (50 μM), monosynaptic IPSPs were evoked by orthodromic stimulation in stratum radiatum. Traces are averages of four to six consecutive sweeps obtained from a cell with a resting membrane potential of −60 mV recorded with a 2 M K methysulfate-filled electrode. Control response shows both fast and slow IPSPs. In the presence of 1 mM CGP 35348, the slow component is totally blocked, leaving the fast IPSP unchanged. After 19 min of CGP 35348 washout, the slow component returns to control amplitude. The perfusion with 100 μM picrotoxin inhibits the fast IPSP leaving intact the slow IPSP, which is subsequently blocked by reapplying CGP 35348. In this condition, no detectable potential is evoked. The records at the *bottom right* correspond to the superimposed traces shown in control, CGP 35348, picrotoxin, and picrotoxin plus CGP.

We have also examined the effects of 4-AP on the baclofen response, since it has been reported (Inoue et al., 1985) that very low concentrations (5 μM) can block the action of baclofen. Concentrations of 4-AP ranging from 5 μM to 5 mM were tested. In Figure 7*A*, the control baclofen outward currents are followed by a subthreshold depolarizing current pulse and an electrical stimulus to the excitatory afferents in stratum radiatum. In the presence of 4-AP (100 μM), the current pulse discharges the cell, presumably due to the block of the potassium current I_D (Storm, 1988), and enhances the excitatory postsynaptic potential triggering action potential discharge (Buckle and Haas, 1982). In the presence of 4-AP, considerable spontaneous synaptic activity occurred (note increased thickness of the trace and all-or-none discharges during the baclofen responses. Despite these clear effects of 4-AP on excitability, no change was detected in the size of the baclofen response at concentrations of 5 μM to 1 mM (n = 8) (Fig. 7*A*). At a concentration of 5 mM 4-AP, which induces an inward current, a clear depression of the baclofen response is observed (average depression = 59.7 ± 8.7%, n = 4), but this concentration also affects the GABA induced outward current to a similar extent (Fig. 7*B*) (average depression = 43.6 ± 10.9%, n = 4).

While it has been known that baclofen strongly reduces EPSPs (see Bowery et al., 1990), little is known about possible presynaptic actions of GABA. Here we report a presynaptic inhibitory action of GABA and compare the antagonistic action of CGP 35348 on this response and the baclofen response. To examine the presynaptic effects, EPSPs were recorded with cesium-filled microelectrodes, which blocked the postsynaptic potassium conductance increase normally evoked by baclofen and GABA. As reported by others, baclofen strongly reduced EPSPs and CGP 35348 (1 mM) completely reversed this inhibitory action of baclofen (n = 3) (Fig. 8*A*). GABA applied in the presence of SKF 89976A and $GABA_A$ antagonists also markedly depressed the EPSP, and this action was also completely blocked by CGP 35348 (n = 3) (Fig. 8*B*).

Discussion

In this article, we have considered the possibility that the GABA response evoked in the presence of $GABA_A$ antagonists has a different receptor mechanism from the baclofen response. This possibility was based on three observations. First, $GABA_B$ antagonists, as defined by their ability to antagonzie baclofen responses, only weakly affected GABA responses (Dutar and Nicoll, 1988a; Segal, 1990). Second, carbachol blocked baclofen

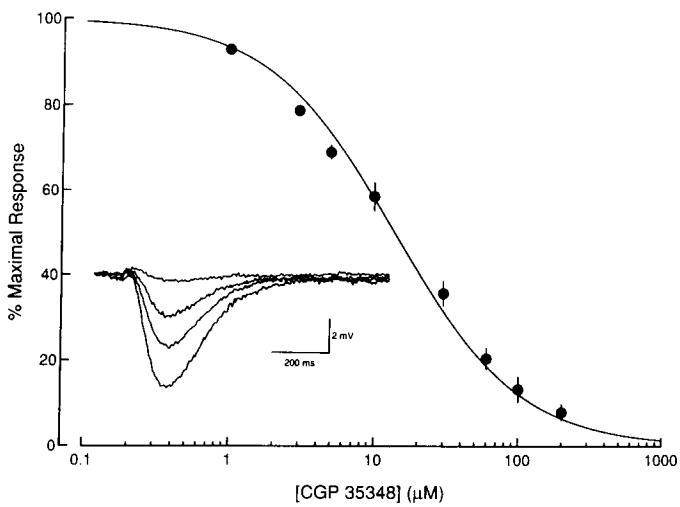

Figure 5. Concentration–effect curve of the CGP 35348 antagonism against the monosynaptically activated slow IPSPs. The experiments were carried out in the presence of CNQX (20 μM), APV (50 μM), bicuculline methiodide (40 μM), and picrotoxin (40 μM). The electrodes were filled with 3 M KCl. The antagonist concentration was increased sequentially. Points represent the mean ± SEM (n = 3–5 cells) of slow IPSP amplitudes measured at the peak. The data fit to a logistic function with p = 1 and IC_{50} = 14 μM. Records, from one representative experiment, are averaged (n = 5) slow IPSPs in the following conditions: control, 10, 30, and 200 μM CGP 35348.

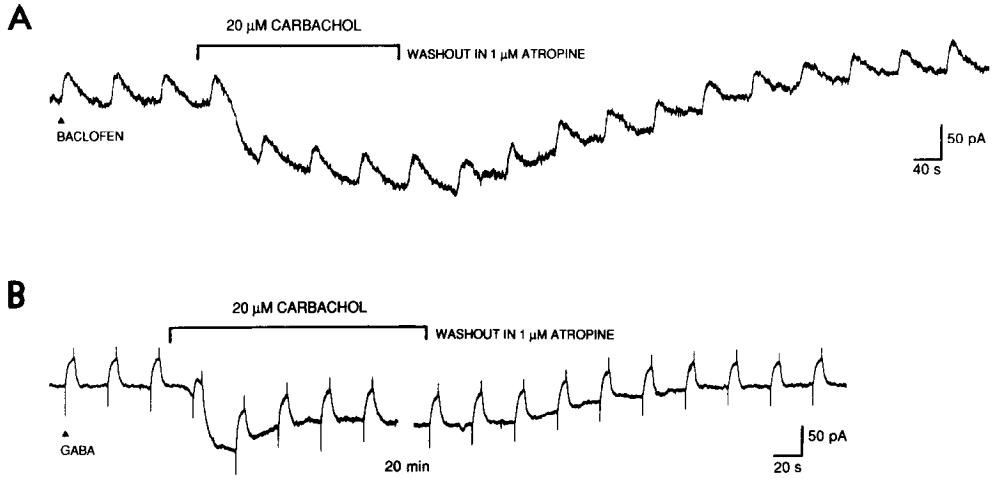

Figure 6. Carbachol fails to affect outward currents evoked by baclofen or GABA. In the presence of TTX (0.1 μM), bicuculline methiodide (40 μM), and picrotoxin (40 μM), carbachol evokes an inward current but fails to block the currents generated by pressure ejection of baclofen (140 msec) onto a cell held at −65 mV (*A*) or by iontophoresing GABA (5 sec × 50 nA) onto another cell held at −60 mV (*B*).

but not GABA responses (Müller and Misgeld, 1989). Third, 4-AP had a similar differential action (Inoue et al., 1985; Ogata et al., 1987).

Based on the present experiments, we have concluded that, while the bicuculline-resistant GABA response is less sensitive to $GABA_B$ antagonists, high concentrations of the $GABA_B$ antagonist CGP 35348 did block GABA responses as well as baclofen responses. This action of CGP 35348 was selective since responses to 5-HT were entirely unaffected. The finding that CGP 35348 can effectively block the bicuculline-resistant GABA response indicates that it is not necessary to postulate the existence of a third type of GABA receptor that is neither $GABA_A$ nor $GABA_B$. However, it is quite possible that multiple subtypes of $GABA_B$ receptor exist with varying sensitivity to the available $GABA_B$ antagonists. In agreement with the conclusion based on exogenous application of GABA that all of its actions can be accounted for by $GABA_A$ and $GABA_B$ antagonists is the finding that all IPSPs are blocked in the presence of $GABA_A$ and $GABA_B$ antagonists. Recently, it has been reported that glutamate-evoked IPSPs in stratum lacunosum moleculare are resistant to bicuculline and phaclofen (Williams and Lacaille, 1990). With electrically evoked monosynaptic IPSPs in stratum radiatum and oriens, we have found that the slow IPSP is entirely blocked by CGP 35348. The difference may be due to either the antagonist used or the site of stimulation.

It has been reported that the potassium conductance activated by GABA differs from that activated by baclofen, because 4-AP (Inoue et al., 1985; Ogata et al., 1987) and carbachol (Müller and Misgeld, 1989) blocked baclofen but not GABA responses. We have been unable to repeat the finding with 4-AP even with concentrations 100-fold higher than those previously used. These concentrations dramatically increased neuronal excitability and synaptic potentials. While 5 mM 4-AP did reduce baclofen responses in agreement with results in the dorsolateral septum (Stevens et al., 1985), GABA responses were also affected at this concentration. On spinal cord primary afferent fibers, even a

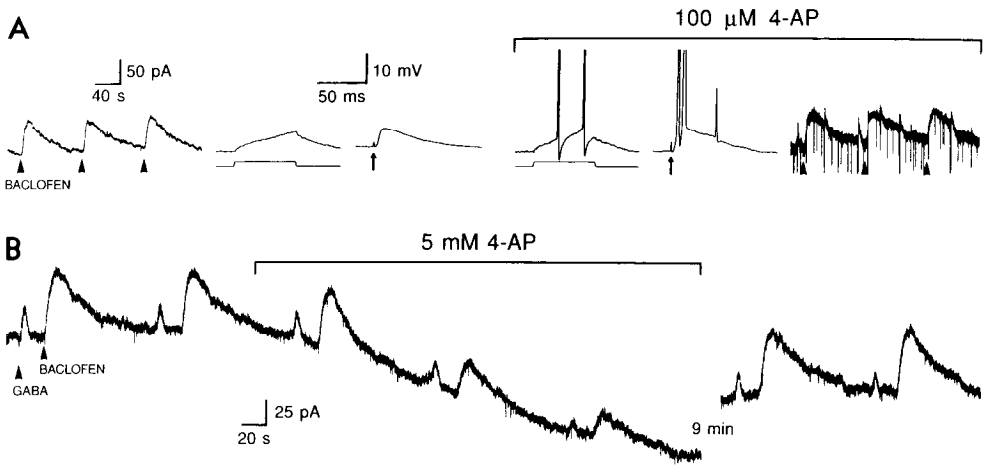

Figure 7. Effects of A-AP on baclofen and GABA responses. *A*, Records (from left to right) correspond to outward currents evoked by pressure ejection (*arrowheads*) of baclofen (50 msec × 30 psi) in a cell held at its resting membrane potential of −60 mV bathed in a normal Ringer's solution. In current clamp, 50 pA depolarizing pulse evokes membrane depolarization but not action potentials. Electrical stimulation (*arrows*) delivered in stratum radiatum evokes EPSPs (average of five sweeps). After 4 min perfusion with 100 μM A-AP, a 50 pA depolarizing pulse evokes two action potentials and the stimulation in radiatum now induces firing of two to three action potentials (record is average of four events). In voltage clamp, baclofen application induces responses similar to those evoked in control conditions. The increase in the noise of the trace and the fast inward currents correspond to an increase in spontaneous synaptic events that were blocked by TTX (not shown). *B*, In another cell held at −70 mV and bathed in TTX, bicuculline methiodide, picrotoxin, and SKF 89976A, the responses evoked by puffing GABA (300 msec) and baclofen (100 msec) were greatly reduced by the application of 5 mM A-AP, which also produces an inward current. After 9 min of A-AP washout, the GABA responses and holding current partially recover and baclofen responses regain their previous amplitude.

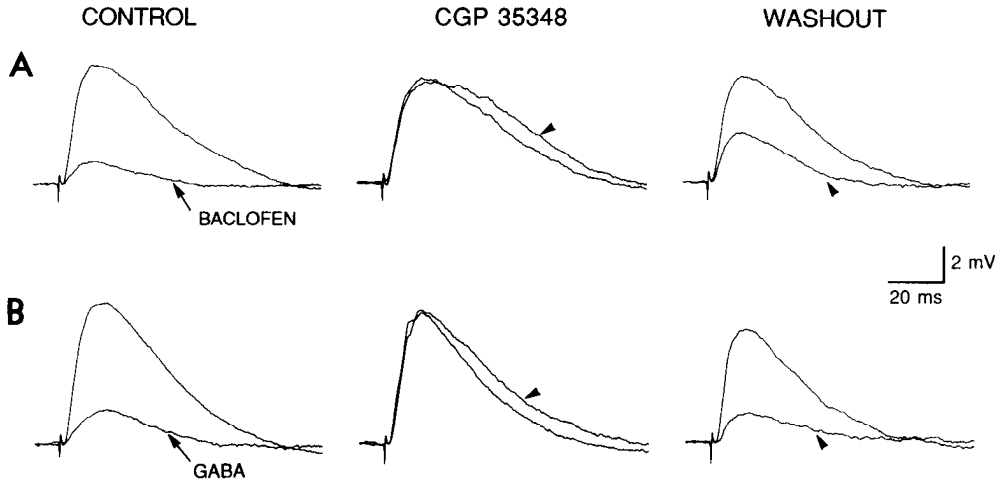

Figure 8. Presynaptic $GABA_B$ receptors activated by GABA or baclofen are inhibited by CGP 35348. The cell was recorded with a 3 M CsCl-filled electrode to block K^+-mediated slow IPSPs. The perfusion solution contained bicuculline methiodide, picrotoxin, and SKF 89976A. A surgical cut was made between CA1 and CA3 regions to prevent epileptiform bursting. The cell was hyperpolarized to −85 mV in order to prevent cell firing. Electrical stimuli were delivered through a bipolar electrode placed in stratum radiatum within 50 μm of the recorded cell. GABA (30 sec) or baclofen (1 sec) were applied by pressure from two independent micropipettes positioned in the same region of stratum radiatum. Each panel has two traces superimposed that are each averages of five sweeps. One of the pair corresponds to the potential recorded in the presence of either GABA or baclofen (*arrowheads* and *arrows*), and the other corresponds to the potentials recorded just prior to the application of the agonist. After 4 min of perfusion with CGP 35348 (1 mM) both baclofen (*A*) and GABA (*B*) are ineffective at reducing the EPSPs. Following washout of CGP 35348 (12 min), both agonists are again able to produce a great reduction in the EPSPs.

concentration of 5 mM 4-AP is ineffective in blocking the hyperpolarization induced by baclofen (Padjen and Mitsoglou, 1990). We also had difficulty demonstrating an interaction between baclofen and carbachol at concentrations ranging from 0.3 μM to 20 μM. The failure to observe clearly an interaction prevented us from testing for a pharmacological difference between GABA and baclofen responses. We have no obvious explanation for our negative results. However, in the article of Müller and Misgeld (1989) the effect of carbachol was compared to responses to bath-applied baclofen and responses to pressure-applied GABA. In a recent article from this laboratory, it was found that carbachol did not block the response to pressure-applied baclofen (Bijak et al., 1991).

We have also studied the pharmacological properties of the presynaptic $GABA_B$ response. Early studies, in general, failed to detect blockade of the presynaptic inhibitory action of baclofen by phaclofen (Dutar and Nicoll, 1988b; Harrison, 1989; Stirling et al., 1989; Wang and Dun, 1990). Curiously, while phaclofen was found to reduce the inhibitory action of baclofen on glutamate release from cerebellum granule cells and calcium currents of dorsal root ganglion cells, 2-OH-SAC was ineffective (Huston et al., 1990). In the hippocampus 2-OH-SAC (Harrison et al., 1990), as well as CGP 35348 (Davies et al., 1991; present results), can entirely prevent the baclofen-induced presynaptic inhibition. In addition, CGP 35348 can block the presynaptic inhibition evoked by baclofen and other new $GABA_B$ agonists in the striatum (Seabrook et al., 1991). GABA can mimic the action of baclofen, and this action is completely blocked by CGP 35348. Presumably the weak action of phaclofen on the presynaptic inhibition by baclofen could be due to the existence of spare receptors or, alternatively, as originally proposed (Dutar and Nicoll, 1988b), to a difference in $GABA_B$ receptors at pre- and postsynaptic sites. Another reported difference between postsynaptic and presynaptic $GABA_B$ receptors concerns their sensitivity to pertussis toxin. Previous studies found that pertussis toxin administered intraventricularly (Colmers and Williams, 1988; Dutar and Nicoll, 1988b; Colmers and Pittman, 1989; Gallagher et al., 1990) or directly applied to cultured neurons (Harrison, 1989) completely blocked the postsynaptic action of baclofen but failed to block the presynaptic action of baclofen. However, a more recent study has found that intrahippocampal pertussis toxin injections are effective (Stratton et al., 1989), suggesting that lack of access of toxin to the presynaptic G-proteins may account for the earlier negative results. Apart from the issue of pertussis toxin sensitivity, the lack of effect of Ba^{2+} on the presynaptic action suggests that a K^+ conductance is not involved (Lambert et al., 1991).

In summary, comparison of the pharmacological properties of the postsynaptic action of baclofen and GABA in the presence of $GABA_A$ antagonists favors a mechanism in which GABA and baclofen activate the same receptor mechanism. However, the quantitative difference between the sensitivity of the two responses raises the possibility of multiple subtypes of postsynaptic $GABA_B$ receptor. The present findings are entirely consistent with the proposal that synaptically released GABA, acting on $GABA_B$ receptors, mediates the slow IPSP. Thus, the actions of GABA and the inhibitory synaptic transmitter can be fully explained in terms of the $GABA_A$ and $GABA_B$ nomenclature. Furthermore, the presynaptic inhibitory action of baclofen, which is also mimicked by GABA, is effectively antagonized by the $GABA_B$ antagonist CGP 35348. The physiological role for the presynaptic $GABA_B$ receptors on excitatory synapses is unclear since there is no evidence that synaptically released GABA has access to these receptors. However, there is good evidence that $GABA_B$ receptors on the terminals of GABAergic synapses play an important role in autoinhibition of GABA release (Davies et al., 1990).

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