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# THE MODULATION OF RAT HIPPOCAMPAL SYNAPTIC CONDUCTANCES BY BACLOFEN AND γ-AMINOBUTYRIC ACID

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SUMMARY

1. We examined the effects of γ-aminobutyric acid (GABA) and baclofen on pre- and postsynaptic membrane conductances in dissociated rat hippocampal cells. Both GABA (5 μM with 10 μM-bicuculline) and baclofen (50 μM) caused small but significant increases in membrane conductance that were blocked by 2-hydroxy-saclofen (100 μM), a $GABA_B$ receptor antagonist. This increase in membrane conductance seems to be mediated by $GABA_B$ receptors.

2. At a low concentration of GABA (1 μM) which has a very small direct postsynaptic effect on $GABA_A$ receptors, no postsynaptic $GABA_B$ effect was detected. However, at this concentration, GABA near maximally attenuated both excitatory and inhibitory synaptic currents. This GABA effect on transmitter release was significantly attenuated by 2-hydroxysaclofen.

3. Baclofen was also more potent in attenuating the inhibitory synaptic conductance than increasing postsynaptic conductance. Concentrations below 1 μM diminished synaptic currents by greater than 50%. At these low baclofen concentrations 2-hydroxysaclofen significantly attenuated baclofen's reduction of synaptic currents.

4. The effects of GABA and baclofen on synaptic conductances were blocked by pretreating the cultures with pertussis toxin, suggesting that a GTP-associated protein, $G_i$ or $G_o$ is responsible for reducing transmitter release.

5. Despite the ability of GABA to diminish inhibitory synaptic currents through $GABA_B$ receptor activation, we observed no effect of 2-hydroxysaclofen on paired-pulse depression. Therefore, these presynaptic $GABA_B$ receptors may not be true 'autoreceptors'.

6. Our findings indicate that in culture, at least, the presynaptic $GABA_B$ effect responsible for synaptic modulation has a pharmacological profile similar to the postsynaptic $GABA_B$ effect. At present, it is unnecessary to postulate two different types of $GABA_B$ receptors.

## INTRODUCTION

The effect of GABA (γ-aminobutyric acid) in the central nervous system is mediated by at least two classes of receptors, $GABA_A$ and $GABA_B$ (Hill & Bowery,

1981). One effect of $GABA_B$-receptor activation is to diminish the release of many neurotransmitters (see Bowery, 1989 for review). The mechanism responsible for this action is controversial. $GABA_B$ receptor activation may directly reduce a calcium conductance (Dunlap & Fischbach, 1978; Bowery, 1989; Stirling, Cross, Robinson & Green, 1989). Alternatively it may increase a potassium conductance (Newberry & Nicoll, 1985; Gähwiler & Brown, 1985) and secondarily reduce calcium influx at the nerve terminal by limiting depolarization. Either of these effects could act presynaptically to reduce transmitter release.

In addition to their presynaptic effects the known $GABA_B$ agonists, GABA and baclofen, have a bicuculline-insensitive postsynaptic effect. They increase a potassium conductance resulting in postsynaptic hyperpolarization (Gähwiler & Brown, 1985; Newberry & Nicoll, 1985). This effect requires intact intracellular GTP-associated proteins (Neer & Clapham, 1988), $G_i$ or $G_o$ (Vandongen, Codina, Olate, Mattera, Joho, Birnbaumer & Brown, 1988), because ribosylation of either of these proteins by pertussis toxin (PTX) abolishes this effect (Dutar & Nicoll, 1988*a*, *b*). Baclofen-induced hyperpolarization is also readily blocked by phaclofen, a $GABA_B$ receptor antagonist (Dutar & Nicoll 1988*a*, *b*; Soltesz, Haby, Leresche & Crunelli, 1988).

Although the pre- and postsynaptic $GABA_B$ receptors share the same agonists, evidence available so far suggests that they have different pharmacological characteristics (Dutar & Nicoll, 1988*b*; Stirling *et al.* 1989; Harrison, 1990). First, the presynaptic $GABA_B$ effect was not affected by the $GABA_B$ antagonist phaclofen (Dutar & Nicoll, 1988*a*, *b*; Soltesz *et al.* 1988; Harrison, 1990). Second, the presynaptic $GABA_B$ effect did not seem to be dependent upon a G-protein (Dutar & Nicoll, 1988*b*; Harrison, 1990).

There are, however, several reports that suggest that the same receptor could be responsible for pre- and postsynaptic $GABA_B$ responses. Using a more potent $GABA_B$ receptor antagonist, 2-hydroxysaclofen (Kerr, Ong, Johnston, Abbenante & Prager, 1988), Curtis, Gynther, Beattie, Kerr & Prager (1988) demonstrated that they could limit baclofen's block of spinal cord excitation. Furthermore, baclofen inhibits some neuronal calcium fluxes in a G-protein-dependent manner (Kamatchi & Ticku, 1990).

To determine whether pre- and postsynaptic $GABA_B$ receptors are different in cultured hippocampal neurones, we studied the effect of GABA and some related compounds on evoked monosynaptic excitatory (Yamada, Dubinsky & Rothman, 1989) and inhibitory currents (Holland, Ferrendelli, Covey & Rothman, 1990). This has also allowed us to measure the sensitivity of presynaptic receptors to GABA, the natural agonist. During the course of our investigation, we discovered that pre- and postsynaptic GABA effects are blocked by 2-hydroxysaclofen. This finding led us to further investigate the pharmacology of $GABA_B$ receptors. Our results indicate that the pre- and postsynaptic $GABA_B$ receptors share a similar pharmacology and that there is no need to invoke more than one class of $GABA_B$ receptor.

## METHODS

We used cultures of postnatal rat hippocampal neurones after 5–12 days *in vitro* for all experiments; culture methods have been described elsewhere (Yamada *et al.* 1989). The tight-seal whole-cell recording technique (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) was used to

voltage clamp postsynaptic cells and stimulate presynaptic cells by direct current injection. Prior to an experiment, culture medium was exchanged with extracellular fluid containing (mM): NaCl, 140; KCl, 3; Na-HEPES, 10; glucose, 5·5; $CaCl_2$, 4; and $MgCl_2$, 4, with pH adjusted to 7·3. When studying excitatory connections, $MgCl_2$ was increased to 6 mM to decrease the likelihood of polysynaptic responses.

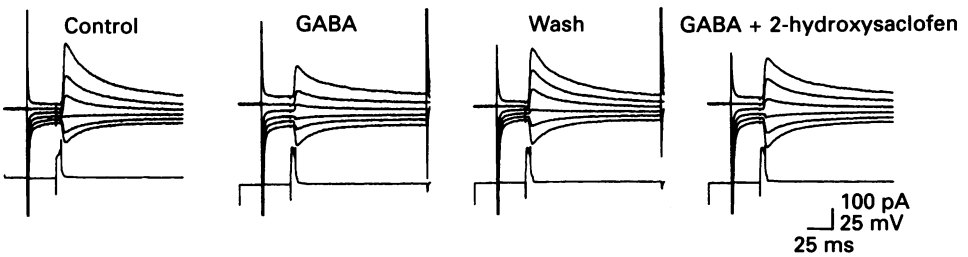


Fig. 1. Block of IPSCs by 1 mM-GABA and relief by 100 $\mu$M-2-hydroxysaclofen. The upper traces show current responses of the postsynaptic cell at −100, −90, −80, −70, −60 and −50 mV voltage commands. Drugs contained in the bath are noted above the traces. Each voltage step was repeated 3 times and the signal was averaged. The corresponding voltage traces of presynaptic action potentials are depicted in the lower tracings. The recording pipette solution contains 2 mM-chloride.

The electrodes were fabricated by pulling glass capillaries (1·2 mm outer diameter) in two steps. Series resistance was usually 10–15 MΩ. The intracellular solution contained (mM): potassium isethionate, 138; KCl, 2; Na-HEPES, 10; EGTA, 1·1; glucose, 4; and $Mg_2$-ATP, 2, with pH adjusted to 7·2. With these solutions, inhibitory postsynaptic currents (IPSCs) reversed at approximately −70 mV and excitatory postsynaptic currents (EPSCs) above 0 mV. In some experiments, the concentrations of potassium isethionate and KCl were changed to 70 mM. With this intracellular solution, the IPSCs reversed at −30 mV as predicted by the Nernst equation for a channel permeable to $Cl^-$. In other experiments, the recording pipette solution contained caesium chloride (CsCl; 130 mM) and tetraethylammonium chloride (TEA-Cl; 10 mM) to increase the membrane resistance by blocking outward potassium currents. With this solution IPSCs reversed at 0 mV.

In a typical experiment, voltage clamp and current clamp amplifiers were interfaced to a personal computer with commercially available software (pClamp). Each voltage or current trace was digitized at 1 kHz and repeated 3–5 times for averaging. Averages were stored on hard disc for off-line data analysis.

Within a microscopic field, we obtained synaptic pairs by simultaneously recording from two nearby neurones. The presynaptic neurones were maintained at around −60 mV with a continuous current injection. A depolarizing current of 5–10 ms duration was injected through a balanced bridge to initiate an action potential which usually overshot 0 mV. A pair of cells was considered monosynaptically connected if each action potential elicited a postsynaptic current with less than a 5 ms latency without any failures. Stimulus frequency was 0·2 Hz. In some experiments, the presynaptic neurones were repetitively stimulated to study the effect of a preceding synaptic current upon a subsequent one.

All drugs were added to the extracellular fluid from stock solution made up to 100 times desired concentration. The fluid in the culture dish (about 0·5 ml total volume) was bolus perfused with 5 ml of desired solution with a syringe and a suction pipette such that a constant fluid level was maintained.

We determined the slope conductance of the postsynaptic membrane by plotting the holding current for voltage steps over the range of −100 to −40 mV (Jack, Noble & Tsien, 1975) (Figs 1 and 2*A*). Holding current was the average current over a 10 ms segment that preceded the postsynaptic current by 10 ms. Least-squares linear regression was used to fit a line and calculate its slope. The resting membrane potential was the *X*-(voltage-) axis intercept or the zero current potential. A voltage step that activated voltage-dependent currents and caused gross deviation of the *I–V* curve from a linear relationship was discarded from the linear regression.

Synaptic conductance was estimated by determining the slope of the linear regression line by plotting peak synaptic currents against membrane potential after subtracting the holding current (Figs 1 and 2*B*). Usually within the range of −100 to −40 mV, holding currents and synaptic currents varied linearly with voltage. In determining the effect of GABA on inhibitory synaptic

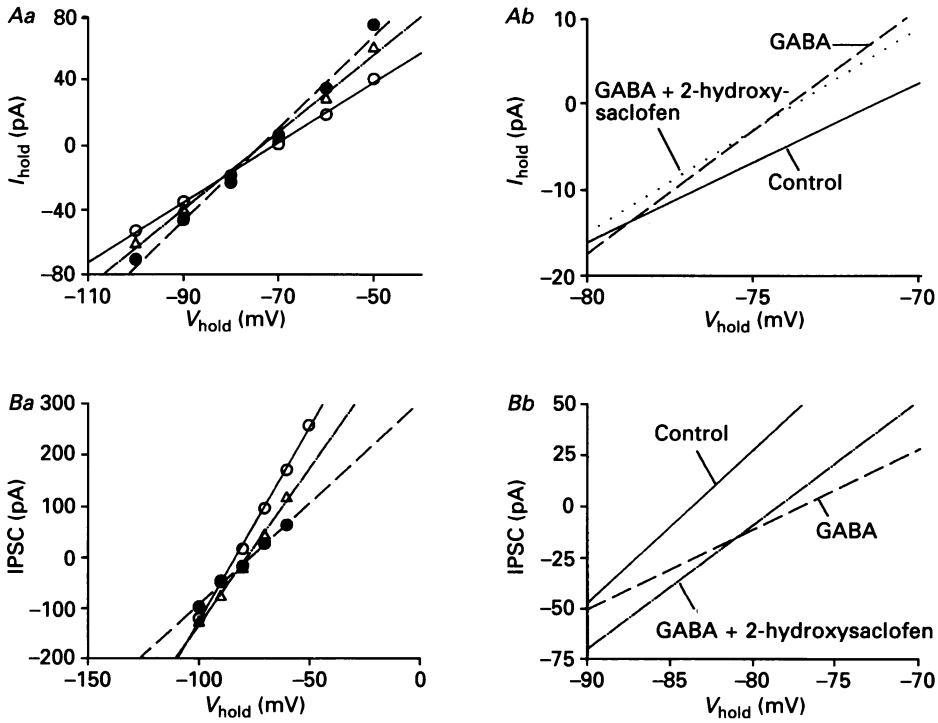


Fig. 2. Effect of GABA (1 μM) on membrane (*A*) and synaptic (*B*) conductance determined from the experiment in Fig. 1. *Aa*, the membrane conductance is estimated by plotting the holding current ($I_{hold}$) measurement (averaged over a 10 ms period 10 ms prior to the postsynaptic response) against voltage steps ($V_{hold}$). In this and most other cells, the relationship was linear over this range of voltage. The straight line is drawn by least-squares linear regression. The slope of this plot determines the conductance (nS) ○, control; ●, 1 μM-GABA; △, 1 μM-GABA + 100 μM-2-hydroxysaclofen. *Ab*, detail of the linear regression line near the resting membrane potential (*X*-intercept at 0 pA). Note that with 1 μM-GABA there is a slight hyperpolarization along with increase in membrane conductance. In this cell, there is a small attenuation of conductance change with 2-hydroxysaclofen. The effect of hyperpolarization is not significantly changed by 2-hydroxysaclofen. *Ba*, the synaptic conductance is determined by plotting the peak IPSC against the voltage command. Note that in the regression line for 1 μM-GABA, the data from −50 mV step was omitted due to its marked deviation from linearity. Symbols as in *Aa*. *Bb*, detail of the regression line around the reversal potential. With 2 mM-chloride in the recording pipette, there is a significant shift in the reversal potential of the IPSC. The shift in the reversal potential is not significantly different with 1 μM-GABA and 2-hydroxysaclofen but the synaptic conductance is almost equal to that of control.

transmission, we found that it was impossible to analyse drug effects by simply measuring the peak of the IPSC. When the recording pipette solution contained 2 mM-chloride, application of GABA in the bath caused a significant shift in the reversal potential of the IPSC (Fig. 2*B*). This is

predictable from the fact that opening of $GABA_A/Cl^-$ channels would cause a significant change in the intracellular chloride concentration (Huguenard & Alger, 1986; Deisz & Prince, 1989) especially when intracellular chloride ($[Cl^-]_i$) is low compared to $[Cl^-]_o$. The shift in reversal potential to more positive values (Figs 1 and 2*B*) suggests that there is an increase in $[Cl^-]_i$ that caused this shift. If we were to compare the effect of drug by only measuring synaptic currents at a holding potential of −70 mV, a shift of reversal potential from −80 to −75 mV would have underestimated the second current by 50%. A shift in the reversal potential over the range of the holding potential would have even reversed the direction of the current. Therefore, it was necessary for us to measure slope conductance changes rather than synaptic current.

Since drug effects on pre- and postsynaptic conductances depended somewhat on the size of the neurone and the extent of synaptic connections, which were highly variable, drug effects were expressed as a percentage change in either membrane or synaptic conductances recorded in the control extracellular fluid. All numerical data are expressed as means ± standard error of the mean. Significance was determined by two-tailed *t* test or paired *t* test. The sigmoid curves in Fig. 4 were constructed by probit analysis (Finney, 1971).

All chemicals were obtained from Sigma (St. Louis, MO, USA) except 2-hydroxysaclofen, which was supplied by Tocris Neuramin and (±) baclofen which was a gift from Ciba-Geigy.

## RESULTS

### *The postsynaptic $GABA_B$ effect*

Bath perfusion with 1 μM-GABA caused an increase in membrane slope conductance from 2·3 ± 0·3 to 3·2 ± 0·4 nS or 44·8 ± 24·1 % ($n = 4, P < 0·02$) (Figs 1, 2*A* and 3*A*). There was a slight hyperpolarization of the resting (zero current) membrane potential determined from the regression line (Fig. 2*A*). Perfusion of a $GABA_B$ receptor antagonist, 2-hydroxysaclofen (100 μM in this and all other experiments), had no significant effect on the change in membrane conductance with 1 μM-GABA (Fig. 3*A*) (41·5 ± 18·3 %, $P > 0·5$ compared to GABA alone). This suggests that at 1 μM, most of the effect on the postsynaptic membrane is mediated by GABA acting at $GABA_A$ receptors. A higher GABA concentration (5 μM) increased the membrane conductance from 2·5 ± 0·3 nS to 4·4 ± 0·5 nS or 105·6 ± 40·6 % ($n = 5$) (Fig. 3*A*). This increase in conductance was attenuated by 2-hydroxysaclofen to 40·8 ± 27·3 % ($P <$ 0·02 compared with GABA alone) suggesting that a $GABA_B$ effect is responsible for at least some of the increase in membrane conductance.

Baclofen (5 μM) had no significant effect on the postsynaptic membrane. However, at 50 μM, there was a small but significant increase (Fig. 3*A*) in the membrane conductance of 12·2 ± 5·1 % ($n = 4$, $P < 0·05$, paired *t* test) which was completely blocked by 2-hydroxysaclofen (100 μM) (change of −1·3 ± 2·2 %, $n = 4$, $P < 0·01$ compared to baclofen alone), again suggesting a direct $GABA_B$-mediated effect on these neurones.

Control perfusion with 2-hydroxysaclofen caused no significant change in the membrane conductance (−1·0 ± 1·3 %, $n = 5$) or the inhibitory synaptic conductance (−1·9 ± 4·7 %, $n = 5$, $P > 0·2$ paired *t* test).

We went on to perform a second set of experiments to determine whether $GABA_B$ receptor activation made a relatively greater contribution to the postsynaptic GABA response as the GABA concentration increased. We measured the change in membrane *potential* during GABA application while recording with pipettes containing either 70 mM-chloride or 2 mM-chloride (Fig. 3*B*). The former concentration should produce a chloride equilibrium (and $GABA_A$ reversal) potential of

−30 mV while the latter gives a chloride equilibrium potential of −100 mV. When we applied 1 μM-GABA and used the 2 mM-chloride solution, neurones hyperpolarized slightly (1·0±0·9 mV) from the average membrane potential of −68±2 mV ($n = 3$), while with the 70 mM-chloride solution neurones depolarized

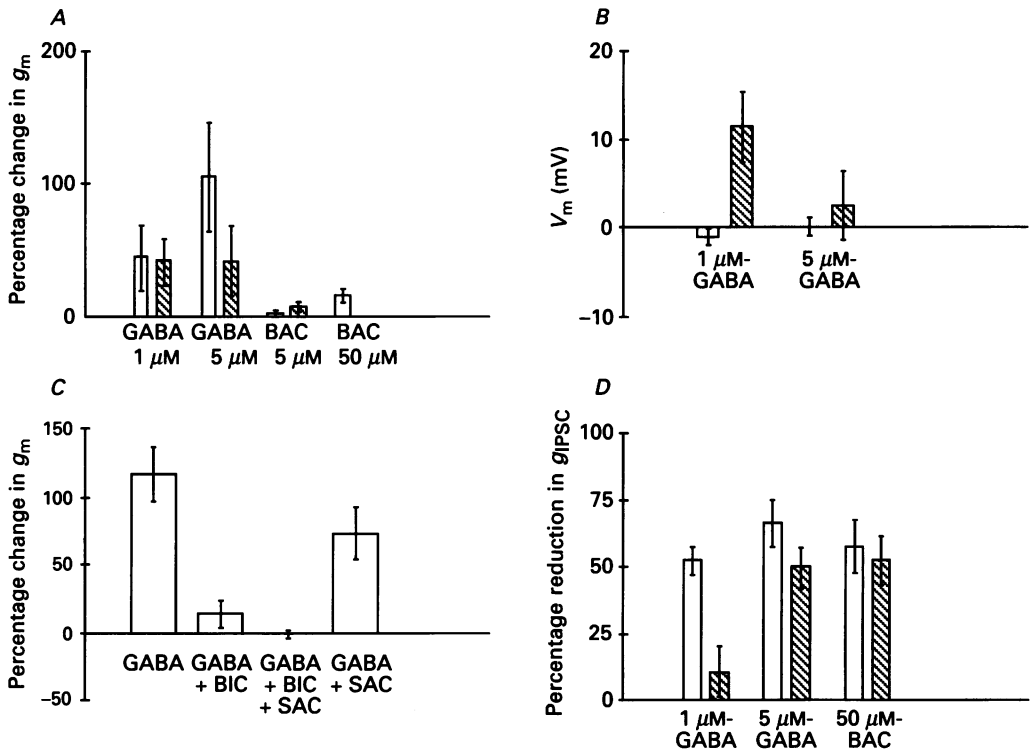


Fig. 3. *A*, change in membrane conductance ($g_m$) with GABA and baclofen (BAC) (□). The membrane conductance is increased by GABA in a dose-dependent manner. However, at 5 μM, some of the conductance increase is attenuated by 2-hydroxysaclofen (▨). Baclofen (5 μM) had a small effect on $g_m$, while at 50 μM it caused an increase in membrane conductance which was antagonized by 2-hydroxysaclofen and is not evident on the graph at this scale. *B*, shift in membrane potential ($V_m$) with 1 and 5 μM-GABA and different recording pipette fluids (□, 2 mM-chloride; ▨, 70 mM-chloride). In 1 μM-GABA, with a 70 mM-chloride solution, the membrane depolarized toward $E_{Cl}$. With 5 μM-GABA, this depolarization was attenuated by 77%. *C*, the effect of 5 μM-GABA on membrane conductance. With 10 μM-bicuculline (BIC) the effect of GABA was significantly decreased, while adding 2-hydroxysaclofen (SAC) to bicuculline abolished the GABA conductance entirely. The effect of (GABA + BIC) plus (GABA + SAC) approaches that of GABA alone. The effect of 5 μM-GABA + 10 μM-BIC on $g_m$ is comparable to that of 50 μM-baclofen (*A*) and the effect of 5 μM-GABA and 50 μM-baclofen on the synaptic conductance is also similar (*D*). *D*, the synaptic conductance is reduced by about the same degree with 1 and 5 μM-GABA. The effect of GABA at 1 μM, however, is readily blocked by 2-hydroxysaclofen (▨; control, □). The effects of 2-hydroxysaclofen on 50 μM-baclofen and 5 μM-GABA are comparable.

(11·4±3·9 mV) from −61±4 mV ($n = 7$) as expected from the chloride reversal potential. If this were a pure $GABA_A$ effect on the cell membrane, a higher concentration of GABA, 5 μM, should allow the membrane potential to more closely

approach the chloride reversal potential with the 70 mM intracellular chloride solution. On the contrary, with 5 $\mu$M-GABA, the depolarization was only $2{\cdot}6 \pm 3{\cdot}9$ mV from the resting potential of $61 \pm 4$ mV ($n = 5$, $P < 0{\cdot}05$ compared to 1 $\mu$M-GABA). This suggests that with 5 $\mu$M-GABA, both $GABA_B$ and $GABA_A$ receptors are

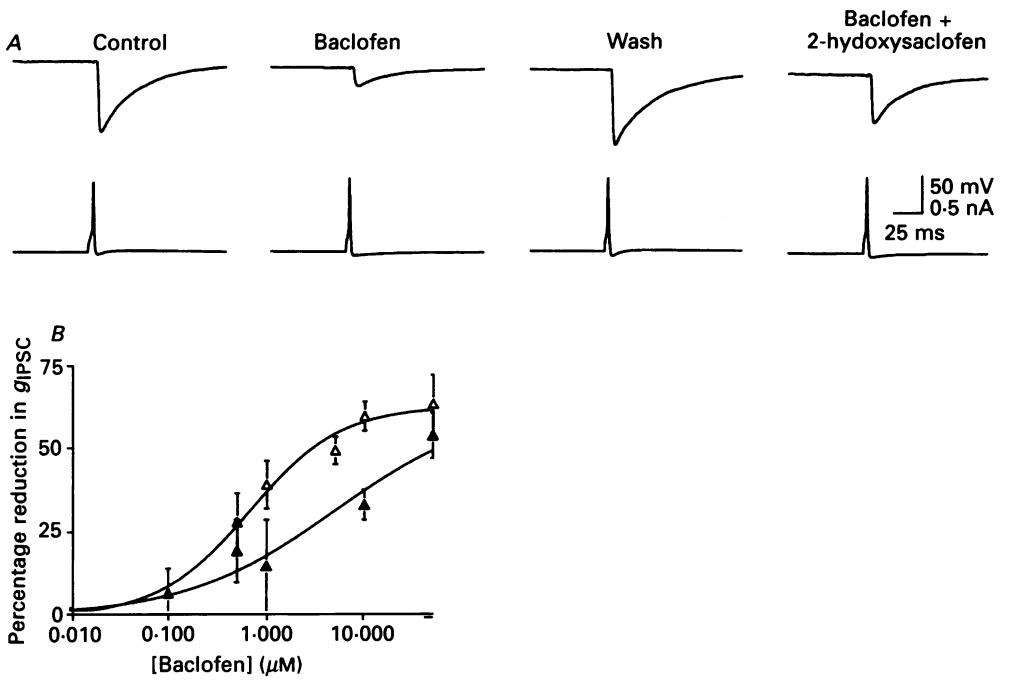


Fig. 4. *A*, current traces showing the effect of 10 $\mu$M-baclofen on an IPSC at the holding potential of $-60$ mV. The corresponding presynaptic action potentials are depicted in the lower traces. Baclofen (10 $\mu$M) reversibly depressed the peak IPSC amplitude and this effect was attenuated by 100 $\mu$M 2-hydroxysaclofen. In this experiment, the intracellular pipette solution contained 70 mM-chloride and the IPSC reversed at $-30$ mV. *B*, concentration–response curve constructed from the percentage reduction of synaptic conductance by baclofen (△) and baclofen + 2-hydroxysaclofen (▲). For the control curve, data points represent means ± S.E.M. of three to eight measurements. For the baclofen + 2-hydroxysaclofen curve the data points represent the means ± S.E.M. of four to five measurements. Above 10 $\mu$M-baclofen, 2-hydroxysaclofen had little effect on the IPSC.

activated. Outward current due to the opening of potassium channels linked to $GABA_B$ receptors (Newberry & Nicoll, 1985) reduces the depolarization produced by chloride channel opening.

In a different set of neurones the membrane conductance was increased by $137{\cdot}0 \pm 25{\cdot}7$ % ($n = 7$ in all groups) with 5 $\mu$M-GABA (Fig. 3*C*). Adding 10 $\mu$M-bicuculline reduced the increase of membrane conductance to $14{\cdot}4 \pm 9{\cdot}6$ %. Combining bicuculline and 2-hydroxysaclofen completely abolished the effect of 5 $\mu$M-GABA ($-0{\cdot}9 \pm 2{\cdot}7$ %, $n = 7$, $P < 0{\cdot}002$ compared with GABA + bicuculline). This suggests that the increase in membrane conductance seen with GABA + bicuculline is a $GABA_B$ effect. Finally, adding 2-hydroxysaclofen to 5 $\mu$M-GABA

again limited the increase in membrane conductance to 74·4±19·2%. All of the above observations are consistent with $GABA_B$ receptors responsible for a small component of postsynaptic GABA effects.

*The $GABA_B$ effect on PSCs*

While recording from monosynaptically connected neurones, we found that bath perfusion of 1 μM-GABA diminished the synaptic slope conductance by 52·8±5·3%

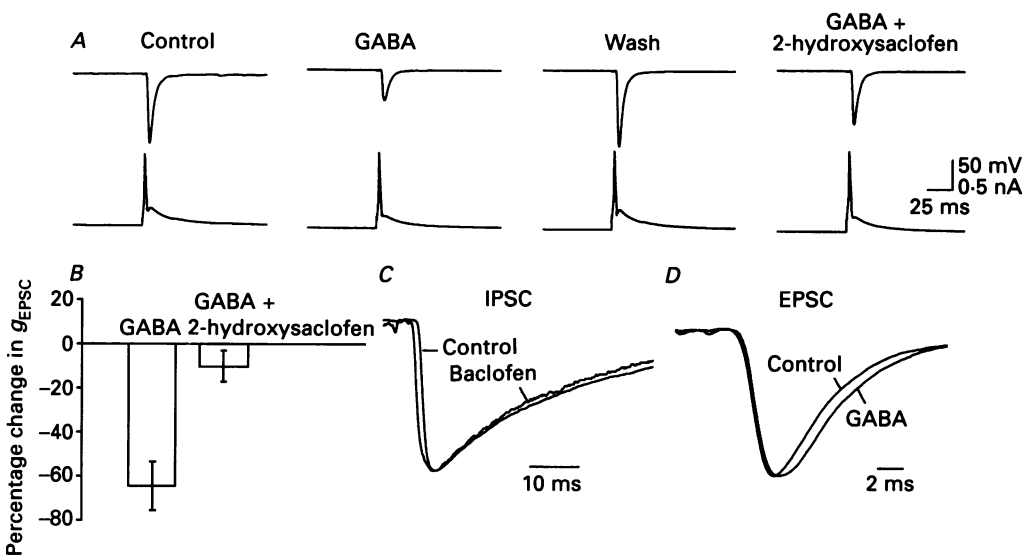


Fig. 5. *A* and *B*, the effect of 5 μM-GABA on excitatory synaptic conductance. In the presence of 10 μM-bicuculline, 5 μM-GABA reversibly reduces the excitatory synaptic conductance. This effect is attenuated by 100 μM-hydroxysaclofen. *C* and *D*, baclofen does not affect the time course of postsynaptic currents. *C*, the first two IPSCs in Fig. 4 have been approximately superimposed and the second IPSC has been scaled up to demonstrate that there is no significant change in rise time of the IPSCs. *D*, the first two EPSCs in *A* were superimposed. There is a slight broadening of the second EPSC with GABA but the rise time is unchanged.

($n = 4$, $P < 0·005$, paired $t$ test) (Figs 1 and 3*D*). This reduction was significantly attenuated by 2-hydroxysaclofen to 10·8±9·4% ($n = 4$, $P < 0·01$ compared with GABA alone). The attenuation of synaptic conductance by 5 μM-GABA (−66·7±8·4, $n = 5$) was not significantly different from 1 μM-GABA. However, 2-hydroxysaclofen at the same concentration was much less effective in reducing the 5 μM-GABA effect (Fig. 3*D*). This implies that 1 μM-GABA may be close to the saturating concentration for the presynaptic $GABA_B$ response. Baclofen at 50 μM reduced the synaptic conductance by 65·5±8·5% ($n = 5$) and this effect was not significantly changed by 2-hydroxysaclofen (54·9±6·9%, $n = 5$, $P > 0·05$ compared with baclofen alone). The reduction of synaptic conductance with 50 μM-baclofen was similar to that of 1 and 5 μM-GABA.

The inability of 2-hydroxysaclofen to relieve IPSC antagonism by 50 μM-baclofen could be due to the fact that the presynaptic baclofen effect was already maximal as

it was at 1 and 5 $\mu$M-GABA. It is also possible that at this high concentration, baclofen could activate presynaptic $GABA_A$ receptors to contribute to the synaptic modulation. In order to investigate this further, we compared the effect of varying concentrations of baclofen with and without 2-hydroxysaclofen on the inhibitory synaptic conductance (Fig. 4). The attenuation of the baclofen effect by 2-hydroxysaclofen was statistically significant (paired $t$ test) at 1 $\mu$M ($39{\cdot}9 \pm 6{\cdot}6\%$ to $15{\cdot}4 \pm 14{\cdot}0\%$, $n = 4$, $P < 0{\cdot}02$) and 10 $\mu$M ($66{\cdot}1 \pm 6{\cdot}4\%$ to $34{\cdot}1 \pm 4{\cdot}6\%$, $n = 5$, $P < 0{\cdot}001$). Therefore, the presynaptic effects of lower baclofen concentrations can be effectively antagonized by 2-hydroxysaclofen.

To rule out the possibility that the synaptic current reduction with GABA application was due to increased postsynaptic membrane conductance mediated by $GABA_A$ receptors, we examined the effect of 5 $\mu$M-GABA on EPSCs in the presence of bicuculline (10 $\mu$M; Fig. 5*A* and *B*). In our system, baclofen attenuates both EPSCs and IPSCs to a similar degree (Yoon & Rothman, 1991). In five excitatory pairs, 5 $\mu$M-GABA reduced the synaptic conductance by $64{\cdot}6 \pm 11{\cdot}4\%$ and this effect was attenuated to $9{\cdot}8 \pm 7{\cdot}2\%$ by 2-hydroxysaclofen ($P < 0{\cdot}001$). Thus, 2-hydroxysaclofen may be more potent in reversing the $GABA_B$ effect on excitatory synapses.

No significant change in the rise times of either IPSCs or EPSCs was seen with baclofen, arguing against any alteration of postsynaptic cable properties as an explanation for synaptic current reductions (Fig. 5*C* and *D*). Furthermore, to determine whether this $GABA_B$ effect could be secondary to an increase in postsynaptic potassium conductance, three postsynaptic cells were voltage clamped using a pipette solution containing CsCl and TEA-Cl to block potassium channels (Fig. 6*A* and *B*). Our standard intracellular solution was used for the presynaptic cells. In these experiments, 50 $\mu$M-baclofen still decreased the synaptic conductance by $74{\cdot}1 \pm 10{\cdot}9\%$ ($n = 3$) without any shift in the reversal potential.

To determine if the presynaptic $GABA_B$ effect seen in these experiments required G-protein-dependent processes known to be necessary for the postsynaptic $GABA_B$ effect (Dutar & Nicoll, 1988*b*), we studied the effect of pertussis toxin (PTX) on the reduction of synaptic conductance by GABA and baclofen (Fig. 6*C* and *D*). Overnight incubation with PTX (50 ng $ml^{-1}$) almost completely abolished the effect of baclofen at 1 and 5 $\mu$M on inhibitory synaptic conductance. In three pairs of neurones, 5 $\mu$M-baclofen changed the synaptic conductance by $0{\cdot}2 \pm 3{\cdot}4\%$ of control ($n = 3$) compared to the reduction of $50{\cdot}3 \pm 4{\cdot}1\%$ ($n = 8$, $P < 0{\cdot}001$) in Fig. 4. In two pairs of neurones in the presence of 10 $\mu$M-bicuculline 5 $\mu$M-GABA reduced the excitatory synaptic conductance by $18{\cdot}0 \pm 9{\cdot}2\%$ of control which was significantly different from the $64{\cdot}6 \pm 11{\cdot}3\%$ ($n = 5$, $P < 0{\cdot}005$) reduction without PTX (Fig. 5).

The pharmacology of the $GABA_B$ effect on synaptic currents therefore resembles the direct postsynaptic $GABA_B$ effect.

*The effect of repetitive stimulation on IPSCs*

The ability of exogenous GABA to suppress IPSCs suggested that the discharge of synaptic GABA might limit subsequent GABA release. We decided to investigate this possibility by examining whether paired presynaptic pulses produced a decrement of IPSCs mediated by $GABA_B$ receptors. In these experiments, to avoid any significant shift in the chloride gradient, the postsynaptic cells were voltage

clamped at −60 mV with 70 mM-chloride solution ($E_{Cl} = -30$ mV). The IPSCs were therefore outward. When an inhibitory presynaptic cell was stimulated twice in rapid succession, we saw a significant decline in the second IPSC (Fig. 7*A*). The maximum attenuation of the second IPSC peak was seen between 100 and 200 ms

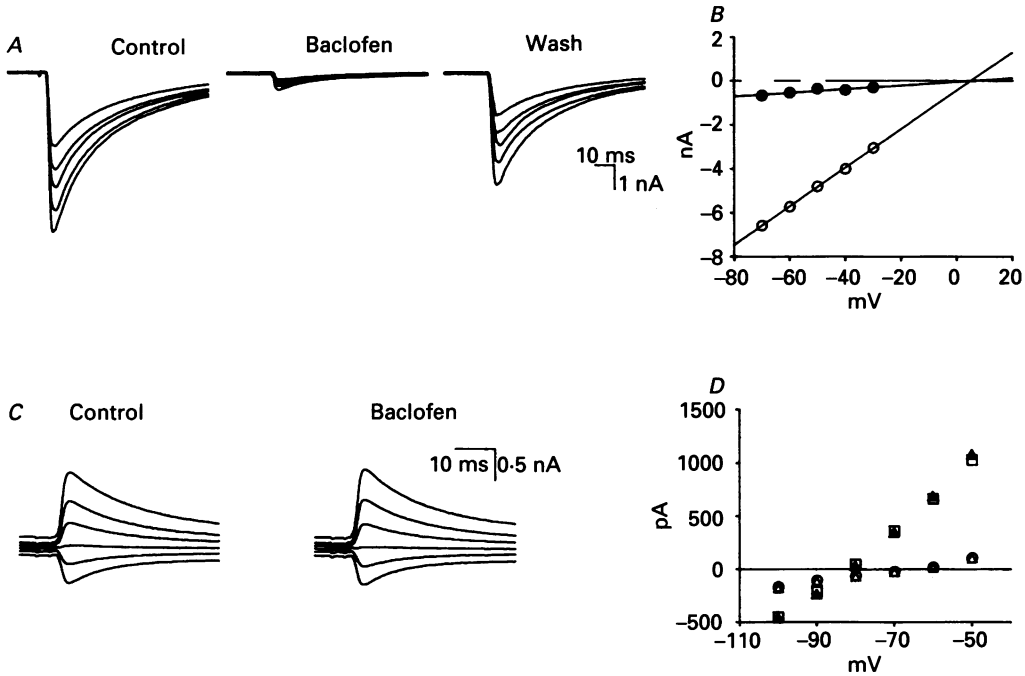


Fig. 6. *A*, the effect of 50 μM-baclofen on an IPSC pair. The postsynaptic cell was voltage clamped with a pipette containing 130 mM-CsCl and 10 mM-TEA-Cl. *B*, synaptic conductance from experiments in *A*. ○, control; ●, 50 μM-baclofen. The $E_{Cl}$ is approximately 0 mV and there is no shift in the reversal potential. *C*, the effect of 5 μM-baclofen on an IPSC pair following treatment with pertussis toxin (50 ng cc$^{-1}$, overnight). The recording solution contains 2 mM-chloride. *D*, *I–V* plot of the same pair. ○, control $g_m$; △, 5 μM-baclofen $g_m$; □, control $g_{IPSC}$; ▲, 5 μM-baclofen, $g_{IPSC}$. There is no significant change in membrane conductance or the synaptic conductance after baclofen application.

with complete recovery in 10 s. In four pairs of neurones with two successive stimulations at 100 ms intervals, the second IPSC was 77·4 ± 6·0 % of the initial IPSC.

The attenuation of the second IPSC was unaffected by 2-hydroxysaclofen (Fig. 7*B*). The average second IPSC peak at 100 ms interval was 83·5 ± 11·0 % of the initial IPSC in the same pair of neurones ($P > 0·2$, paired *t* test). This suggests that the attenuation of IPSCs with repetitive stimulation is not a $GABA_B$ effect. In addition, the failure of 2-hydroxysaclofen to noticeably alter IPSC decay rate indicates that there is little, if any, *postsynaptic* $GABA_B$ component to the IPSCs in our culture system. This is in contrast to the hippocampal microcultures recently described by Segal & Furshpan (1990).

DISCUSSION

We have shown that transmitter release by both GABAergic (inhibitory) and glutamatergic (excitatory) terminals is modulated by presynaptic $GABA_B$ agonists. This presynaptic effect is attenuated by 2-hydroxysaclofen and is near maximal at

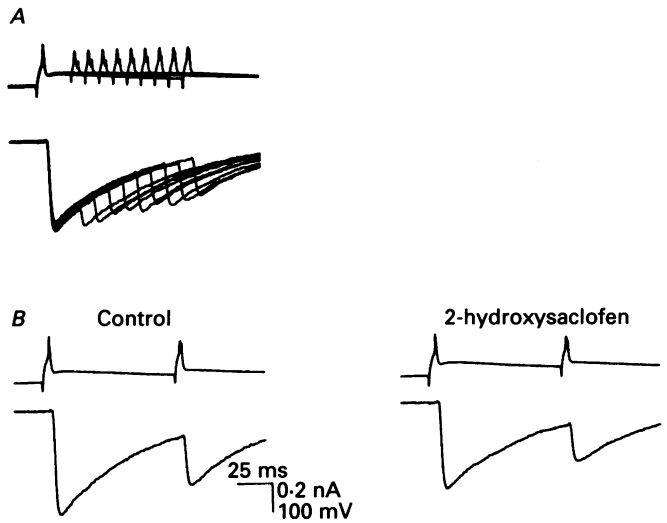


Fig. 7. Paired-pulse depression is not altered by 2-hydroxysaclofen. *A*, when the interval between paired IPSCs increases from 30 to 110 ms (in 10 ms increments), there is a progressive drop in the amplitude of the second IPSC. Nine sweeps with presynaptic action potentials (upper traces) and postsynaptic currents (lower traces) are superimposed. The interval between each sweep is 10 s. $E_{Cl}$ is $-30$ mV and the postsynaptic cell is voltage clamped at $-60$ mV. *B*, the ratio of the second IPSC amplitude to the first did not increase with addition of 2-hydroxysaclofen. The scale bars are for both *A* and *B*.

GABA concentrations much lower than the $K_D$ for the $GABA_A$ receptor (50 $\mu$M; Holland *et al.* 1990). These actions depend upon a GTP-associated protein, either $G_i$ or $G_o$. Our results strongly suggest that the pre- and postsynaptic $GABA_B$ receptors are similar, if not identical.

It is possible that synaptic conductances could be modulated by postsynaptic effects (Baumann, Wicki, Stierling & Waldmeier, 1990). For example an increase in the postsynaptic membrane potassium conductance could restrict propagation of synaptic currents from distant poorly clamped processes. We believe this is an unlikely mechanism for reduction in synaptic conductance for several reasons. First, there is no change in the rise time of synaptic currents in the presence of baclofen, which might occur if cable properties were altered. Second, there is no loss of baclofen efficacy when CsCl and TEA-Cl are injected into postsynaptic neurones. Third, in cultured hippocampal neurones, the direct postsynaptic effects produced by $GABA_B$ receptor stimulation are quite small. In fact Harrison, Lange & Barker (1988) and Harrison (1990) could detect virtually no direct effect of baclofen although he did not

do complete *I–V* plots. Fourth, other workers have convincingly demonstrated that $GABA_B$ receptor activation does limit transmitter release (Peng & Frank, 1989; Harrison, 1990).

At least three recent reports have suggested that the presynaptic $GABA_B$ receptor differs from the postsynaptic receptor (Dutar & Nicoll, 1988*b*; Stirling *et al.* 1989; Harrison, 1990). The basis of this conclusion is the failure of a known $GABA_B$ antagonist, phaclofen, to block baclofen reductions of synaptic currents or potentials. We suspect that inadequate antagonist concentrations were used in all three investigations. Our concentration–response curve for synaptic currents indicates that we needed a 100-fold excess of 2-hydroxysaclofen to significantly antagonize baclofen's depression of synaptic currents. As phaclofen is one-tenth as potent as 2-hydroxysaclofen (Kerr *et al.* 1988), the concentrations used in other physiology experiments were certainly too low to produce an effect. The seeming contradiction between phaclofen's efficacy in antagonizing pre- and postsynaptic baclofen responses may have nothing to do with the $GABA_B$ receptor *per se*. Rather, it may have a straightforward pharmacological explanation related to the manner in which $GABA_B$ receptors are coupled to ion channels by second-messenger systems (Nicoll, 1988). Our results would be obtained if low occupancy of presynaptic receptors still allowed near maximal block of transmitter release. Alternatively, if the density of presynaptic $GABA_B$ receptors was much higher than the density of postsynaptic receptors, $GABA_B$ antagonists would be less potent at the former site. We must also recognize that the assay of the *presynaptic* $GABA_B$ effect is actually a *postsynaptic* current change. There is little reason to expect that the two will be linearly related so that a small presynaptic action might ultimately translate into a large reduction in postsynaptic current. In support of our observations, Harrison and colleagues (Harrison, Lovinger, Lambert, Teyler, Prager, Ong & Kerr, 1990) have recently demonstrated a presynaptic block of $GABA_B$ receptors by 2-hydroxysaclofen.

The discrepancy between the present report and others on the effect of pertussis toxin on the $GABA_B$ effect is difficult to resolve. The high concentration of $GABA_B$ agonist could explain the persistence of presynaptic $GABA_B$ effect in some cases. In the slice experiments (Dutar & Nicoll, 1988*a*), insufficient toxin may have reached synaptic terminals. However, consistent with our observations there are several reports that show involvement of G-proteins in the $GABA_B$ effect on synaptic release (Kamatchi & Ticku, 1990). Finally, R. J. Miller and K. Scholz (unpublished observation) have also noted that the presynaptic effect of baclofen is blocked by pertussis toxin in cultured hippocampal neurones very similar to ours.

At least two issues are left unresolved by these experiments. First, the actual mechanism by which $GABA_B$ receptor activation inhibits transmitter release has not been determined. It is possible that potassium channels in or near synaptic terminals are opened with receptor activation. This would indirectly limit calcium influx and subsequent exocytosis of transmitter. If this is the case, then the actual pre- and postsynaptic effects of $GABA_B$ receptor activation are very similar. Alternatively, presynaptic $GABA_B$ receptors might be indirectly coupled to calcium channels, as has been described in dorsal root ganglion neurones (Dunlap & Fischbach, 1978; Dolphin & Scott, 1986). It is not inconceivable that both mechanisms could come into play simultaneously in synaptic terminals.

Second, we have not really determined the functional role of these presynaptic $GABA_B$ receptors. Clearly, low concentrations of GABA can block the synaptic release of GABA from inhibitory neurones, but it can also reduce release of transmitter, presumably glutamate, from excitatory neurones. It therefore seems unnecessary to postulate true 'autoinhibition' of GABA release.

Our inability to alter paired-pulse depression with 2-hydroxysaclofen further argues that these receptors are inaccessible to GABA feedback from the same terminal. While others have found evidence for presynaptic feedback on GABA release (Deisz & Prince, 1989; Davies, Davies & Collingridge, 1990), they do not establish the *exact* location of presynaptic $GABA_B$ receptors. When the technology for evaluating channels and receptors in nerve terminals improves, it will be extremely important to resolve whether the presynaptic $GABA_B$ receptors are actually within the cleft or adjacent to it.

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