Regulation of muscarinic acetylcholine receptor-mediated synaptic responses by $GABA_B$ receptors in the rat hippocampus

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1. Both $GABA_B$ and muscarinic acetylcholine receptors (mAChRs) influence hippocampal-dependent mnemonic processing. Here the possibility of a direct interaction between $GABA_B$ receptors and mAChR-mediated synaptic responses has been studied using intracellular recording in rat hippocampal slices.

2. The $GABA_B$ receptor agonist (−)-baclofen (5–10 μM) depressed an atropine-sensitive slow EPSP ($EPSP_M$) and occluded the $GABA_B$-receptor-mediated IPSP ($IPSP_B$) which preceded it. These inhibitory effects were accompanied by postsynaptic hyperpolarization (9 ± 2 mV) and a reduction in cell input resistance (12 ± 3 %).

3. The selective $GABA_B$ receptor antagonist CGP 55845A (1 μM) fully reversed the depressant effects of (−)-baclofen (5–10 μM) such that in the combined presence of (−)-baclofen and CGP 55845A the $EPSP_M$ was 134 ± 21 % of control.

4. (−)-Baclofen (5–10 μM) caused a small (28 ± 11 %) inhibition of carbachol-induced (3.0 μM) postsynaptic depolarizations and increases in input resistance.

5. CGP 55845A (1 μM) alone caused an increase in the amplitude of the $EPSP_M$ (253 ± 74 % of control) and blocked the $IPSP_B$ that preceded it.

6. In contrast, the selective GABA uptake inhibitor NNC 05–0711 (10 μM) increased the amplitude of the $IPSP_B$ by 141 ± 38 % and depressed the amplitude of the $EPSP_M$ by 58 ± 10 %. This inhibition was abolished by CGP 55845A (1 μM).

7. Taken together these data provide good evidence that synaptically released GABA activates $GABA_B$ receptors that inhibit mAChR-mediated EPSPs in hippocampal CA1 pyramidal neurones. The mechanism of inhibition may involve both pre- and postsynaptic elements.

One of the most widely characterized extrinsic inputs to the CA1 region of the rat hippocampus is the septo-hippocampal input (Dutar *et al.* 1995). This input comprises a heterogeneous population of afferents that mediate their effects through the release of various neurotransmitters including acetylcholine (ACh), γ-aminobutyric acid (GABA), 5-hydroxytryptamine (5-HT) and a variety of neuropeptides (Decker & McGaugh, 1991; Dutar *et al.* 1995). Of these transmitters, both cholinergic and GABAergic inputs have received most attention because of their critical involvement in mnemonic processing (Cole & Nicoll, 1983; Decker & McGaugh, 1991; Dutar *et al.* 1995). However, whilst both sets of fibres have been shown to increase hippocampal excitability (through activation of a muscarinic acetylcholine receptor (mAChR)-mediated slow excitatory postsynaptic potential and a reduction in spike frequency adaptation (Cole & Nicoll, 1983, 1984; Madison *et al.* 1987; Morton & Davies, 1997) and by $GABA_A$ receptor-mediated disinhibition of CA3 circuits (Tóth *et al.* 1997)) the possibility of direct interactions between GABAergic and cholinergic inputs has not been extensively investigated.

In this respect, the classical inhibitory role of GABA synapses might be expected to be appropriate for

providing negative regulatory control over the marked changes in excitability induced by mAChR activation. Certainly, the metabotropic nature of the $GABA_B$ receptor makes this receptor system a potential candidate for preventing the likely neurodegenerative and epileptogenic consequences of overactivation of mAChRs (Lothman *et al.* 1991; Wasterlain *et al.* 1993). Indeed, we have demonstrated recently that adenosine A_1 receptors, which share many of the same cellular effectors as $GABA_B$ receptors (Dutar & Nicoll, 1988*a*; Thompson *et al.* 1992), provide a strong inhibitory influence over mAChR-mediated synaptic depolarization and loss of spike frequency adaptation (Morton & Davies, 1997). However, there are only a few reports in the peripheral and central nervous systems of interactions between $GABA_B$ and mACh receptors (Brown & Higgins, 1979; Worley *et al.* 1987; Wichmann *et al.* 1987; Libri *et al.* 1998; Scanziani, 2000). As such, the aim of the present study is to extend these investigations by examining how pharmacological, and synaptic, activation of $GABA_B$ receptors modifies mAChR-mediated synaptic transmission in the hippocampus. Some of these data have appeared previously in abstract form (Morton *et al.* 1997).

METHODS

Female Wistar rats (2–4 weeks old) were killed by cervical dislocation and exsanguination followed by decapitation in accordance with UK Home Office guidelines. The brain was removed rapidly and transverse hippocampal slices prepared by hemisecting the whole brain minus the cerebellum and cutting 400 μm thick transverse slices containing hippocampal slices using a vibroslicer (Campden Instruments, Loughborough, UK). The CA3 region of each slice was then cut away to eliminate changes in network function that can occur due to epileptiform bursting in area CA3 when picrotoxin is applied to the slice. The resultant CA3-ectomized slices were placed on a nylon mesh at the interface of a warmed (32–34 °C), perfusing (1–2 ml min^{-1}) artificial cerebrospinal fluid and an oxygen-enriched (95% O_2–5% CO_2), humidified atmosphere. The standard perfusion medium comprised (mM): NaCl, 124; KCl, 3; $NaHCO_3$, 26; NaH_2PO_4, 1.25; $CaCl_2$, 2; $MgSO_4$, 1; D-glucose, 10; and was bubbled with 95% O_2–5% CO_2.

Following a 1 h equilibration period intracellular recordings were obtained from the CA1 pyramidal cell body region using 2 M potassium methylsulphate-filled microelectrodes (60–110 MΩ). (This recording configuration was chosen to limit run-down of G-protein-coupled receptor-mediated responses.) An Axoclamp-2B amplifier (Axon Instruments, Foster City, CA, USA) was used in discontinuous (3–5 kHz switching frequency) current-clamp mode. All impalements were made in control medium. Once stable recordings had been made for at least 10 min, all fast ionotropic glutamate receptor-mediated synaptic transmission was blocked using a combination of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor antagonist 6-nitro-7-sulphamoyl-benzo(*f*)quinoxaline-2,3-dione (NBQX; Tocris Cookson Ltd, Bristol, UK; 2–4 μM), and the *N*-methyl-D-aspartate (NMDA) receptor antagonists D-(E)-2-amino-4-methyl-5-phosphono-3-pentanoic acid (CGP 40116; Ciba-Geigy Ltd, Basle, Swizerland; 50 μM) or D-2-amino-5-phosphonopentanoate (AP5; Tocris Cookson Ltd, Bristol, UK; 50 μM). $GABA_A$ receptor-mediated synaptic transmission was abolished using the $GABA_A$ receptor antagonist picrotoxin (Sigma, St Louis, MO, USA; 50 μM).

Bipolar stimulating electrodes, made from 55 μm diameter insulated nickel–chromium wire (Advent Research Materials Ltd, Eynsham, UK), were positioned in the stratum oriens close to the recording electrode in the stratum pyramidale, to provide extracellular orthodromic activation of CA1 neurones. In every series of experiments stimuli comprised square-wave pulses (20–200 μs; 5–30 V) delivered at a fixed intensity every 5–10 min. Data were captured using pCLAMP6 software (Axon Instruments Inc.) and digitized records were stored on the hard disk of a PC for off-line analysis using Clampfit software.

During the period between stimuli the input resistance and extent of spike frequency adaptation of each neurone were measured routinely every 2 min using 300–600 ms long negative and positive current steps (± 0.15–0.40 nA), respectively. Input resistance was calculated from the voltage deflection in response to brief negative current injections. In all experiments in which mAChR-mediated EPSPs ($EPSP_M$s) were evoked, baseline recordings consisted of three or more successive $EPSP_M$s which had peak amplitudes that differed by no more than 15%. In responses in which action potentials were evoked peak amplitudes were measured following low-pass filtering of the response at frequencies that excluded action potentials but which maintained similar kinetic profiles of the rising and decay phases of the unfiltered $EPSP_M$. In experiments examining spike frequency adaptation, stimulation intensity was set to a level sub-threshold for evoking an $EPSP_M$. The extent of spike frequency adaptation was monitored 1 s prior to delivery of this stimulus and 2 s after by injecting identical 600 ms depolarizing current pulses through the recording electrode. Baseline responses were recorded in this manner for 15–20 min prior to drug applications to ensure stationarity of responses.

All drugs were applied by addition to the perfusion medium. To compare the $EPSP_M$s evoked in the presence and absence of a drug, DC was injected through the electrode to compensate for any drug-induced changes in membrane potential. Atropine and picrotoxin were purchased from Sigma. [1-(*S*)-3,4-dichlorophenyl)ethyl]amino-2-(*S*)-hydroxypropyl-*p*-benzyl-phosphonic acid (CGP 55845A) was obtained from Ciba-Geigy Ltd, Basle, Switzerland. Data are presented as means ± standard error of the mean (S.E.M.) and statistical significance was assessed using Student's paired or unpaired *t* tests performed on raw data with $P < 0.05$ being taken as indicating statistical significance. The *n* values refer to the number of times a particular experiment was performed, each in a different slice taken from a different rat.

RESULTS

Data were obtained from 42 stable intracellular recordings (1–6 h) from CA1 pyramidal neurones with overshooting action potentials, resting membrane potentials more negative than −55 mV and input resistance values of 30 MΩ or greater.

Characterization of cholinergic synaptic responses

Single shock stimulation in the stratum oriens evoked an EPSP that was followed by a biphasic IPSP ($n = 42$, data not shown). The combined application of NBQX (1–3 μM), AP5 (50 μM) or CGP 40116 (50 μM) and picrotoxin (50 μM) inhibited the fast EPSP and $GABA_A$ receptor-mediated IPSP ($IPSP_A$) leaving a slow $GABA_B$ receptor-mediated IPSP ($IPSP_B$). Increasing the stimulus intensity 2- to

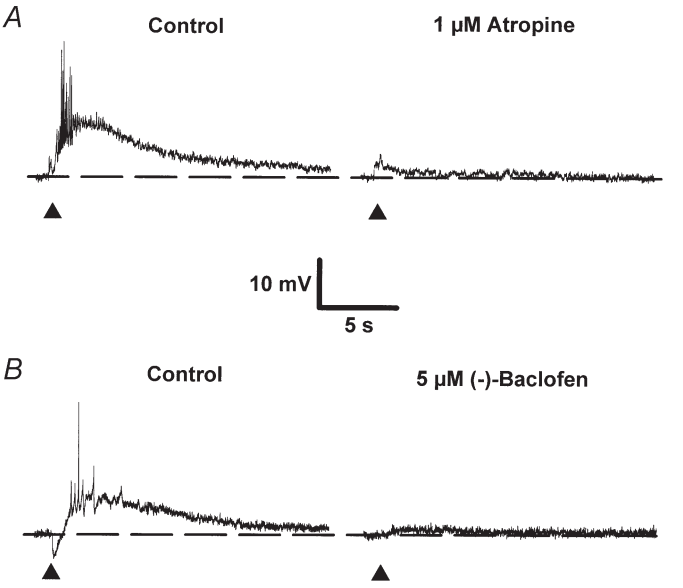

Figure 1. Effects of atropine and (–)-baclofen on the slow $EPSP_M$

A and *B*, single sweeps illustrating the effects of 1 μM atropine (*A*) and 5 μM (–)-baclofen (*B*) on slow EPSPs evoked in two separate neurones. The initial membrane potential of both cells was −64 mV. In *B* and in all subsequent figures, unless stated otherwise, traces are individual synaptic responses recorded intracellularly in response to a single stimulus delivered in the stratum oriens in the presence of 1–3 μM NBQX, 50 μM CGP 40116 and 50 μM picrotoxin. *A*, responses evoked in the additional presence of 1 μM CGP 55845A. Each sweep was taken at the same membrane potential achieved using DC injection to compensate for any drug-induced changes. Filled triangles mark the time of afferent stimulation. In all synaptic traces shown, action potentials are attenuated due to low sampling frequency.

10-fold and delivery of a single stimulus, or a short train of stimuli (2–10 stimuli at 20 Hz), evoked an $IPSP_B$ followed by a much slower EPSP in 34 neurones in which this was attempted (Figs 1*B*, 2, 5 and 6). This slow EPSP could be evoked reproducibly, in isolation, every 8–10 min. Its magnitude and duration could be increased by increasing the stimulus intensity or applying physostigmine ($n = 5$, data not shown) and reduced by applying atropine ($n = 4$; Fig. 1*A*). As such, this component of synaptic transmission will be referred to as an $EPSP_M$ to indicate its dependence on mAChR activation.

Effects of $GABA_B$ receptor activation on the $EPSP_M$

To establish whether $GABA_B$ receptor activation affected the $EPSP_M$ the effect of the selective $GABA_B$ receptor agonist (–)-baclofen was tested. At 5–10 μM (–)-baclofen caused a postsynaptic hyperpolarization (9 ± 2 mV) and a decrease in cell input resistance of 12 ± 3% ($n = 7$).

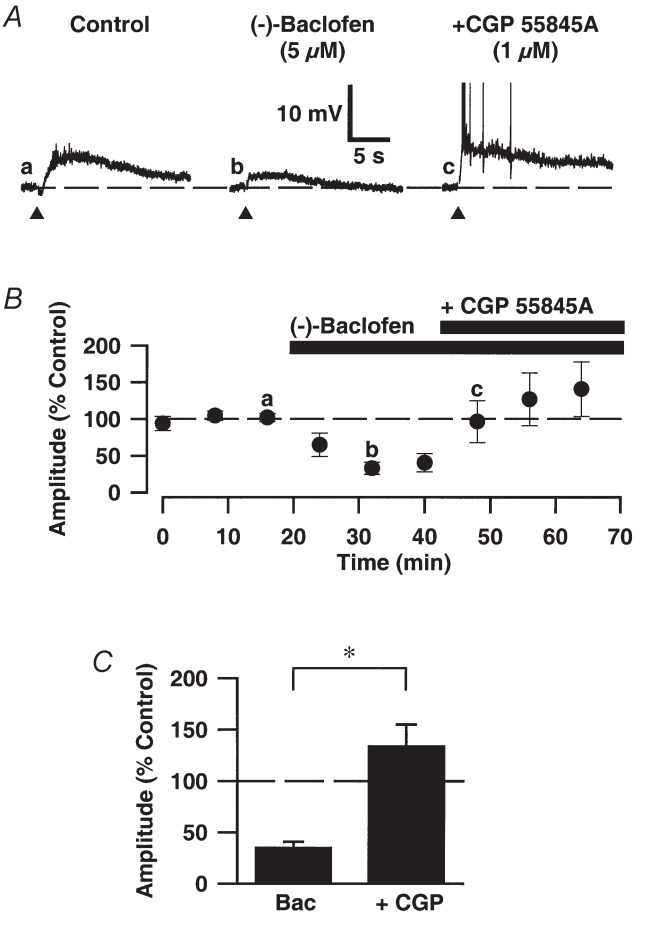

Figure 2. The (–)-baclofen-induced depression is reversed by CGP 55845A

A, synaptic traces of $EPSP_M$s recorded: *a*, in the presence of AMPA, NMDA and $GABA_A$ receptor antagonists (Control); *b*, in the additional presence of 5 μM (–)-baclofen; and *c*, subsequent co-application of 1 μM CGP 55845A. The membrane potential of the cell was −64 mV. The graph (*B*) shows a plot of the mean peak amplitudes of successive $EPSP_M$s, for pooled data, to illustrate the temporal profile of the depressant effect of (–)-baclofen on the $EPSP_M$ and its reversal by CGP 55845A. Amplitudes are expressed as a percentage of the mean amplitudes of the three $EPSP_M$s prior to application of (–)-baclofen. *C*, bar graph illustrating pooled data for the effects of 5–10 μM (–)-baclofen (Bac; $n = 7$) and 5–10 μM (–)-baclofen + 1 μM CGP 55845A (+ CGP; $n = 7$) on the $EPSP_M$. The amplitudes of $EPSP_M$s are expressed as a percentage of the mean value of the control $EPSP_M$s. Note that (–)-baclofen significantly depressed the $EPSP_M$ whilst responses following co-application of (–)-baclofen + CGP 55845A were not significantly different from control responses. Data are means ± S.E.M.; * statistically significant compared with (–)-baclofen group ($P < 0.05$). In this and subsequent figures, the bars above the graph (*B*) indicate the duration for which the drug was applied.

Comparison of $EPSP_M$s prior to (−)-baclofen application and following DC injection to compensate for this hyperpolarization revealed that (−)-baclofen had depressed both the $EPSP_M$ as well as the $IPSP_B$ preceding it (Fig. 1*B*). This effect was maintained for the period of the agonist application and was reversible on washout (not shown). In addition, the magnitude of the (−)-baclofen-induced depression of the $EPSP_M$ was not statistically different from its depression of pharmacologically isolated $IPSP_A$s and AMPA receptor-mediated EPSPs ($EPSP_A$s; not illustrated). Thus, 5 μM (−)-baclofen depressed the peak amplitudes of $EPSP_M$s, $EPSP_A$s and $IPSP_A$s to 30 ± 15 % ($n = 3$), 36 ± 16 % ($n = 4$) and 12 ± 3 % ($n = 5$) of control, respectively.

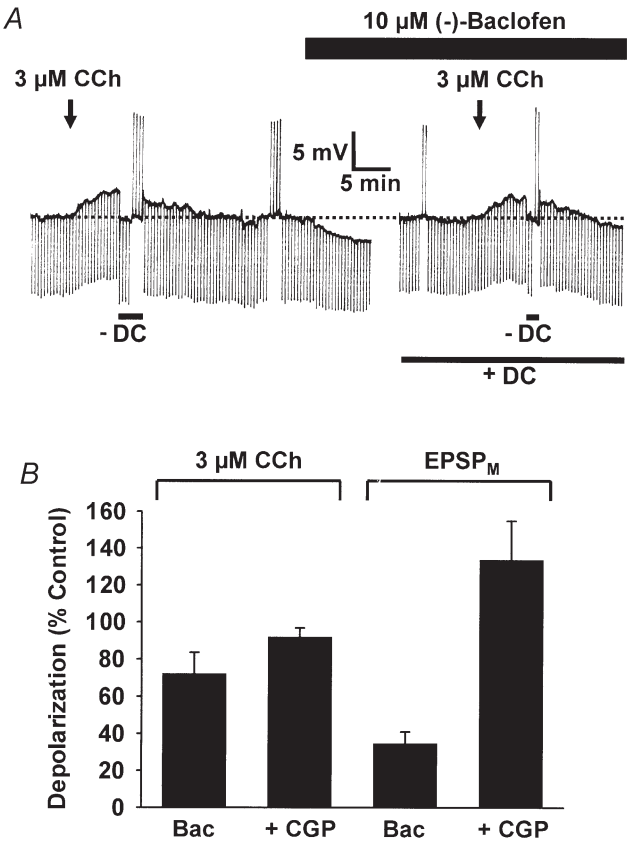

Figure 3. The effects of (–)-baclofen on postsynaptic responses evoked by carbachol

A, a chart record of membrane potential and input resistance (downward voltage deflections to −0.3 nA, 300 ms current steps) with the black arrows and the black bar indicating the period of carbachol (CCh, 3 μM) and (−)-baclofen (10 μM) applications, respectively. Constant DC injection (lower bar) was used in the presence of (−)-baclofen to restore the cell membrane potential to control levels. At the peak of the carbachol-induced depolarization DC injection was adjusted (−DC) to bring the membrane potential in line with that prior to the carbachol application. The transient upward deflections at various time points throughout the chart record are spike frequency adaptation responses to +0.3 nA, 300 ms current steps in the presence and absence of carbachol and (−)-baclofen. The initial membrane potential of this neurone was −64 mV. The bar graph in *B* shows the peak depolarization induced by carbachol, or evoked by the $EPSP_M$, in the presence of baclofen (Bac) or baclofen + CGP 55845A (+ CGP) plotted as a percentage of that evoked in its absence.

To confirm that the depressant action of (−)-baclofen on the $EPSP_M$ was mediated by $GABA_B$ receptor activation we tested next the ability of the selective $GABA_B$ receptor antagonist CGP 55845A to reverse this effect. In all cells tested ($n = 7$) CGP 55845A (1 μM) fully reversed all the effects of (−)-baclofen (barring the depression of the $IPSP_B$, which was directly inhibited by this antagonist). Thus, in the presence of 5–10 μM (−)-baclofen alone the peak amplitude of the $EPSP_M$ was 35 ± 10 % of control compared with 134 ± 21 % of control in the combined presence of 5–10 μM (−)-baclofen and 1 μM CGP 55845A ($n = 7$; Fig. 2).

Is the (–)-baclofen-induced depression of the $EPSP_M$ mediated pre- or postsynaptically?

Having established a depressant action of $GABA_B$ receptors on mAChR-mediated postsynaptic responses evoked by afferent stimulation, we addressed next whether these effects were mediated pre- or postsynaptically. To do this we tested the effect of (−)-baclofen on the postsynaptic depolarization and increase in cell input resistance evoked by brief bath applications of carbachol (3 μM for 30–60 s). A low concentration of carbachol was chosen to produce relatively small depolarizations so as to maximize the probability of observing an inhibitory influence of (−)-baclofen.

Repeated applications of carbachol caused consistent and reversible depolarizations (11 ± 4 mV; Fig. 3) that were associated with increases in cell input resistance (9 ± 1 %, $n = 4$); effects that were comparable to those associated with the $EPSP_M$ and that, like the $EPSP_M$, were abolished by atropine (1–5 μM, $n = 3$; not illustrated). Application of (−)-baclofen (10 μM) caused a hyperpolarization (11 ± 4 mV) that was associated with a small decrease in cell input resistance (13 ± 3 %). In addition, (−)-baclofen caused a 28 ± 11 % inhibition of carbachol-induced responses which was only partially reversed by CGP 55845A (1 μM; $n = 4$; Fig. 3*B*), possibly because of the gradual run-down of carbachol-induced responses on repeated applications. Irrespective of this, the depressant effect of (−)-baclofen was significantly smaller than its effect on the $EPSP_M$ ($P < 0.05$; Fig. 3*B*).

Effects of (–)-baclofen on the reduction in spike frequency adaptation evoked by synaptic activation of mAChRs

As shown previously, stimulation of cholinergic afferents at intensities sub-threshold for activating the $EPSP_M$ causes a reduction in spike frequency adaptation in response to depolarizing current steps delivered 2 s after

pathway stimulation (Morton & Davies, 1997; Fig. 4). Mechanistically, this mAChR-mediated effect arises from the inhibition of a distinct population of ion channels to that which generates the $EPSP_M$ (Madison *et al.* 1987). As such, a series of experiments were performed to examine whether $GABA_B$ receptor activation also affected this kind of cholinergic synaptic response. (−)-Baclofen (10 μM) completely occluded the $IPSP_B$ evoked by pathway stimulation (Fig. 4) but only partially inhibited the reduction in spike frequency adaptation evoked by afferent stimulation ($n = 5$; $P < 0.05$; Fig. 4*B*), even when applied at a concentration of 50 μM. Whilst this latter effect was small it was, nevertheless, fully reversed by subsequent application of CGP 55845A (1 μM; $n = 5$; Fig. 4*B*).

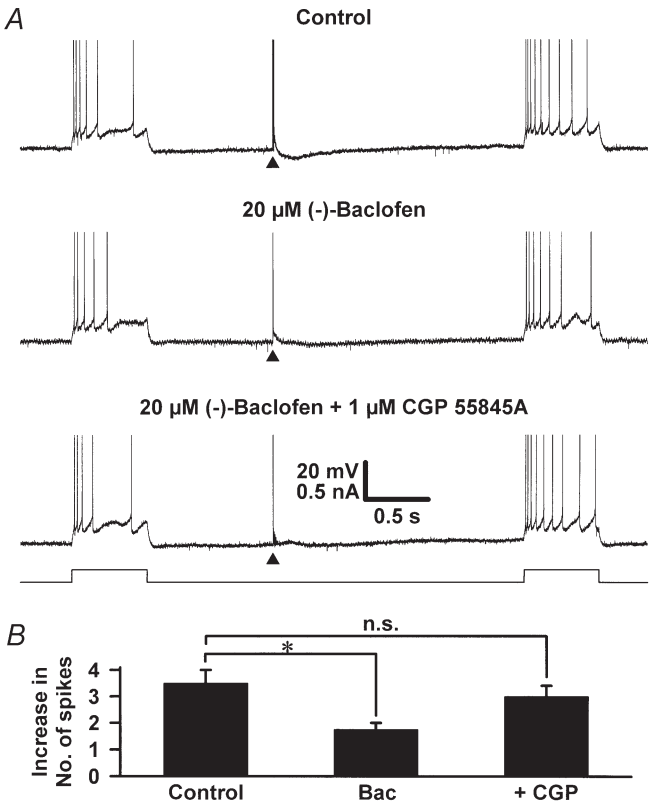

Figure 4. The effect of CGP 55845A on the (−)-baclofen-induced inhibition of stimulation-evoked reduction in spike frequency

A, continuous records of the membrane potential of a single cell in which a depolarizing current step (+0.2 nA, 600 ms) was delivered 1.0 s prior to, and 2.0 s after pathway stimulation. The stimulation was delivered at an intensity just suprathreshold for activating an $EPSP_M$ in control medium containing only the ionotropic glutamate and $GABA_A$ receptor antagonists (control), in the additional presence of 20 μM (−)-baclofen and in the additional combined presence of 20 μM (−)-baclofen and 1 μM CGP 55845A. Note that in control medium, pathway stimulation evoked an $IPSP_B$ and caused a reduction in spike frequency adaptation. (−)-Baclofen inhibited but did not abolish the stimulation-evoked reduction in spike frequency adaptation. It did, however, occlude the $IPSP_B$. *B*, pooled data for the difference in the number of action potentials fired during each 600 ms depolarizing step 1.0 s before and 2.0 s after a threshold stimulus in the absence and presence of 20 μM (−)-baclofen (Bac; $n = 5$) and in the additional presence of 1 μM CGP 55845A ($n = 5$). The initial membrane potential of the neurone was −65 mV and this was maintained using DC injection. Data are means ± S.E.M.; * significant ($P < 0.05$); n.s., not significant (compared with control).

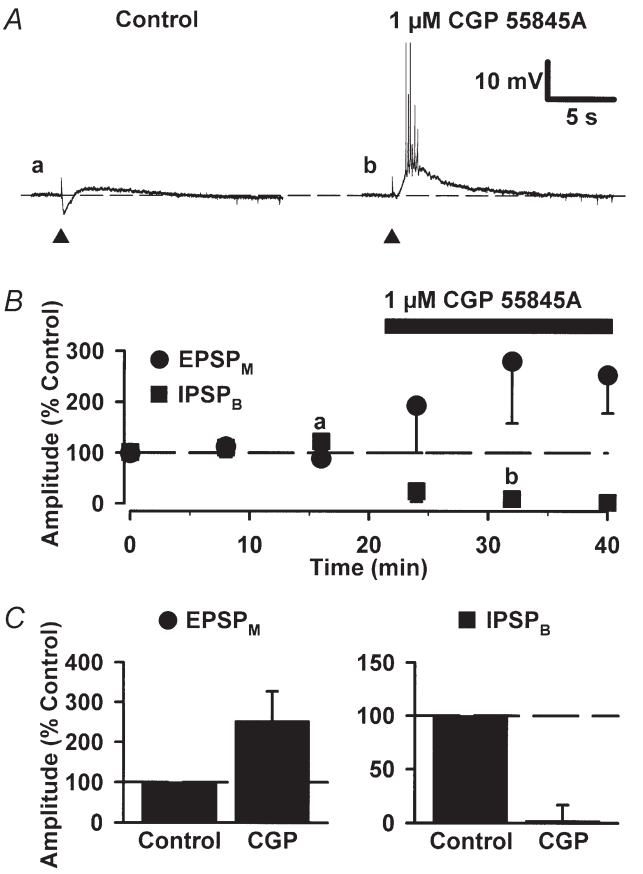

Figure 5. The effects of CGP 55845A alone on the $EPSP_M$ and $IPSP_B$

A, synaptic traces of representative $EPSP_M$s recorded in control (*a*) and in the presence of 1 μM CGP 55845A (*B*). Note that following stimulation, the neurone responds with a hyperpolarization ($IPSP_B$) followed by a small $EPSP_M$, and following the addition of CGP 55845A the $IPSP_B$ is inhibited and the $EPSP_M$ enhanced. The membrane potential of this neurone was maintained at −64 mV using DC injection. *B*, mean peak amplitude of successive $EPSP_M$s and $IPSP_B$s, for pooled data ($n = 3$), *versus* time illustrating the enhancement of the $EPSP_M$ and the depression of the $IPSP_B$ by CGP 55845A. The bar above the graph indicates the duration for which CGP 55845A was applied. *C*, bar graphs illustrating pooled data for the effect of 1 μM CGP 55845A on the amplitude of the $EPSP_M$ and $IPSP_B$ ($n = 3$).

Does endogenous GABA inhibit cholinergic synaptic responses?

It is well established that synaptically released GABA (1) activates a late $IPSP_B$ in CA1 pyramidal neurones (Dutar & Nicoll, 1988*B*) and (2) is responsible for activity-dependent depression of both GABA and glutamate receptor-mediated synaptic transmission (Thompson & Gähwiler, 1989; Davies *et al.* 1990; Nathan & Lambert, 1991; Davies & Collingridge, 1993; Isaacson *et al.* 1993). As such, we tested next whether CGP 55845A when applied alone affected the $EPSP_M$ to establish whether endogenously released GABA might inhibit $EPSP_M$s. CGP 55845A (1 μM) alone abolished the $IPSP_B$ preceding the $EPSP_M$ and caused a substantial but variable increase in the size of the $EPSP_M$. As such, the peak amplitude of the $EPSP_M$ in the presence of CGP 55845A was 253 ± 74 % of control ($n = 3$; Fig. 5). This result confirmed the small increase in peak amplitude recorded when CGP 55845A had been used to reverse the (−)-baclofen-induced depression of the $EPSP_M$ (Fig. 2); the larger effect observed in this current series of experiments stemming from the smaller starting amplitude of $EPSP_M$s and the non-linearity of $EPSP_M$ peak amplitude as this rises towards spiking threshold.

This CGP 55845A-induced increase in the $EPSP_M$ suggested that endogenous GABA released within the slice was capable of activating $GABA_B$ receptors which inhibit the $EPSP_M$. Therefore, the next series of experiments examined whether it was possible to potentiate the depression of the $EPSP_M$ that was induced by endogenous GABA within the slice. To do this, the effects of the selective GABA uptake inhibitor NNC 05–0711 were investigated (Suzdak *et al.* 1992). This compound extends the duration of pharmacologically isolated $IPSP_B$s from a mean duration of 623 ± 132 ms to 1830 ± 235 ms ($n = 4$), which still falls short of the mean latency to peak amplitude of isolated $EPSP_M$s, which was 2.6 ± 0.4 s ($n = 6$; Fig. 6). In addition, NNC 05–0711 (10 μM) increased the amplitude of the $IPSP_B$ to 241 ± 38 % of control and caused a 58 ± 10 % depression of the $EPSP_M$ ($n = 4$; Fig. 7). In the three cells in which recordings were

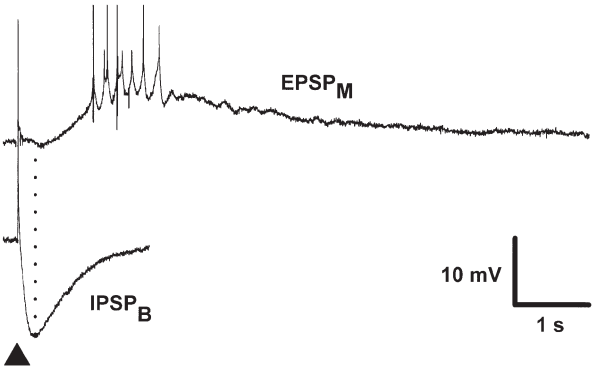

Figure 6. Kinetic comparison of the $IPSP_B$ with the $EPSP_M$

Traces are an $EPSP_M$ (top) recorded in the presence of GABA and glutamate receptor antagonists NBQX, CGP 40116, picrotoxin and CGP 55845A and an $IPSP_B$ (bottom) in the presence of NBQX, CGP 40116, picrotoxin and the GABA uptake inhibitor NNC 05–711. The membrane potentials of the neurones are −64 mV and −62 mV, respectively. Note that even in the presence of an inhibitor of GABA uptake, the isolated $IPSP_B$ peaks prior to the start of the depolarizing $EPSP_M$ and that the hyperpolarizing response is all but complete by the peak of the $EPSP_M$. The dotted line indicates the time of the peak of the isolated $IPSP_B$, which was evoked in the presence of the GABA uptake inhibitor.

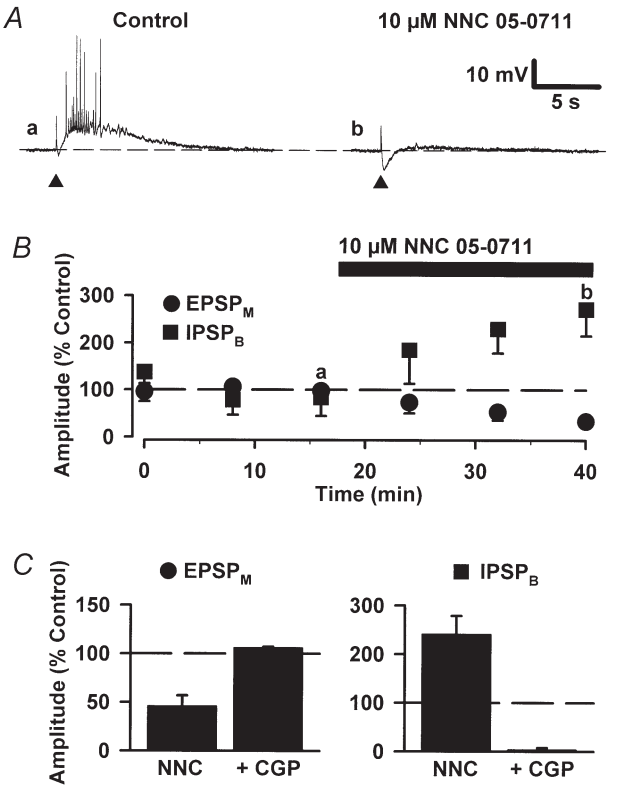

Figure 7. The effects of an inhibitor of GABA uptake on $EPSP_M$s and $IPSP_B$s

A, synaptic traces of representative $IPSP_B$/$EPSP_M$s recorded in control (*a*) and in the presence of NNC 05–0711 (10 μM) (*b*). The membrane potential of this neurone was −64 mV. *B*, mean peak amplitude of successive $EPSP_M$s and $IPSP_B$s, for pooled data ($n = 4$), *versus* time illustrating the depression of the $EPSP_M$ and the enhancement of the $IPSP_B$ by NNC 05–0711. The bar above the graph indicates the duration for which NNC 05–0711 was applied. *C*, bar graphs illustrating pooled data for the effects of 10 μM NNC 05–0711 (NNC; $n = 4$) and 10 μM NNC 05–0711 + 1 μM CGP 55845A (+CGP; $n = 3$) on the $EPSP_M$ and $IPSP_B$. Note that NNC 05–0711 depressed the $EPSP_M$ and that CGP 5845A reversed this effect.

maintained for long enough, subsequent application of CGP 55845A (1 μM) caused a complete reversal of the NNC 05–711-induced depression of the $EPSP_M$ (Fig. 7*C*).

DISCUSSION

Here we have demonstrated a $GABA_B$ receptor-mediated inhibition of mAChR-mediated synaptic responses in the hippocampal CA1 region. Potentially, this effect may be mediated either pre- or postsynaptically or both. Postsynaptic $GABA_B$ receptors classically cause membrane hyperpolarization (Newberry & Nicoll, 1984). However, this does not account for the baclofen-induced depression of the $EPSP_M$ since membrane hyperpolarization was routinely compensated for using DC injection. That said, the $GABA_B$ receptor-mediated reduction in input resistance could feasibly limit the extent of depolarization during the $EPSP_M$ by shunting membrane currents. This possibility, however, is unlikely since NNC 05–0711, which produces no tonic postsynaptic hyperpolarization or decrease in input resistance (but does enhance the $IPSP_B$), also inhibits the $EPSP_M$ via $GABA_B$ receptor activation. A caveat here, however, is that shunting from the $IPSP_B$ before the $EPSP_M$ should not preclude full $EPSP_M$ expression. In this respect, the $IPSP_B$, even during inhibited GABA uptake, will provide a shunt for up to 1 s with the peak conductance change occurring before the rising phase of the $EPSP_M$ (Fig. 6; Morton & Davies, 1997). A similar but opposite argument can be made for the facilitatory action of CGP 55845A on the $EPSP_M$.

If shunting were a major factor limiting mAChR-mediated depolarization, baclofen should produce a substantial inhibition of carbachol-induced depolarizations. Whilst baclofen caused a small inhibition, this was much smaller than its depressant effect on the $EPSP_M$. Based on these data, and the similarity of the cellular mechanisms underlying the $EPSP_M$ and carbachol-induced depolarizations, it is tempting to speculate that $GABA_B$ receptors act to inhibit ACh release. If true, baclofen might also be expected to inhibit stimulation-induced reductions in spike frequency adaptation afforded by synaptically released ACh. However, the effect of baclofen on this response was less clear than its effect on the $EPSP_M$. In particular, baclofen, even at concentrations (50 μM) that are maximal for activating pre- and postsynaptic $GABA_B$ receptors (Thompson & Gähwiler, 1992*a*), produced only a small reduction in spike frequency adaptation induced by pathway stimulation. However, this effect was statistically significant and antagonized by CGP 55845A, indicating that it was a true $GABA_B$ receptor-mediated response presumably reflecting, in part, the reduction in cell input resistance produced by postsynaptic $GABA_B$ receptor activation.

Interestingly, activation of adenosine A_1 receptors, which usually has similar effects to activation of $GABA_B$ receptors (Dutar & Nicoll, 1988a; Thompson *et al.* 1992; Thompson & Gähwiler, 1992*a*), abolished both $EPSP_M$s and stimulation-induced reduction in spike frequency adaptation (Morton & Davies, 1997). One possible explanation for the differences between the actions of these receptor systems is that presynaptic $GABA_B$ receptors, like galanin receptors, are restricted to a select population of cholinergic afferents whereas adenosine A_1 receptors are expressed universally. However, this scenario could only exist if release from a relatively small number of afferents is required to inhibit spike frequency adaptation as opposed to generate an $EPSP_M$. Two observations support this concept: (1) much greater intensities of pathway stimulation are required to evoke an $EPSP_M$ than are necessary to inhibit spike frequency adaptation (Cole & Nicoll, 1984; Morton & Davies, 1997) and (2) whilst acetylcholine modifies the activity of numerous ion channels (Benson *et al.* 1988; Colino & Halliwell, 1993; Fraser & MacVicar, 1996; Haj-Dahmane & Andrade, 1999) it is inhibition of a leak K^+ channel that is principally responsible for generation of the $EPSP_M$ (Madison *et al.* 1987), this action necessitating higher concentrations of ACh (and therefore potentially ACh release from more afferents) than are required to inhibit the Ca^{2+}-activated K^+ channel principally responsible for the loss of spike frequency adaptation (Madison *et al.* 1987).

Alternatively, it is possible that adenosine A_1 and $GABA_B$ receptors are co-expressed on all cholinergic terminals but couple to separate effector systems that exhibit different degrees of efficacy for inhibiting ACh release. In this respect, a limited capacity of $GABA_B$ receptors to inhibit ACh release may explain the relative lack of supporting neurochemical release data for this effect in the CNS (Wichmann *et al.* 1987; Taniyama *et al.* 1992; Ikarashi *et al.* 1999). That said, (1) $GABA_B$ receptors inhibit ACh release in the superior colliculus and (2) it is possible that activation of $GABA_B$ receptors may produce complex changes in the release of other neurotransmitters that may lead to an overall negative regulatory effect on the $EPSP_M$.

Leaving presynaptic mechanisms aside, non-electrophysiological postsynaptic interactions between $GABA_B$ receptors and mAChRs could conceivably contribute to the baclofen-induced depression of the $EPSP_M$. In particular, the small baclofen-induced inhibition of carbachol-evoked depolarizations may represent a postsynaptic interaction that reflects both shunting and additional receptor crosstalk interactions. In this respect, it is possible that $GABA_B$ receptors could inhibit mAChR-mediated responses postsynaptically via interactions with the G-protein(s)- or second messenger-activated signal transduction cascades responsible for the electrophysiological effects of these $G_{q/11}$-coupled mAChRs. Indeed, interactions between $G_{i/o}$- and $G_{q/11}$-coupled receptors, and specifically between $GABA_B$ and mACh receptors, have been described (Worley *et al.* 1987). However, the latter interaction was a mAChR-mediated

inhibition of $GABA_B$ receptor-mediated responses rather than the converse.

This aside, $GABA_B$ receptors promote the ability of a number of G-protein-coupled receptors (e.g. β-noradrenergic receptors) to inhibit the Ca^{2+}-activated K^+ channels responsible for spike frequency adaptation (Andrade *et al.* 1993). Such a scenario would oppose the proposed inhibitory influence of $GABA_B$ receptors on ACh release and, therefore, might account for the weak effect of baclofen on this phenomenon. However, this interaction has only been defined for G_s-coupled receptors and not $G_{q/11}$-coupled receptors to which the M_1 and/or M_3 mAChRs responsible for the $EPSP_M$ belong (Dutar & Nicoll, 1988*b*; Pitler & Alger, 1990; Segal & Fisher, 1992). That said, there are many complex examples of receptor crosstalk and, as such, the possibility that postsynaptic interactions between these two receptor systems that account, at least in part, for the inhibitory interactions described above cannot be dismissed. Should crosstalk exist, the differential effects of $GABA_B$ receptors on the $EPSP_M$ and mAChR-induced inhibition of spike frequency adaptation may result from the different molecular interactions governing each effect as well as the extent of saturation of each effector pathway.

Irrespective of this it is important to establish the physiological relevance of the inhibitory influence of $GABA_B$ receptors on the $EPSP_M$. Here synaptic activation of $GABA_B$ receptors has been shown to regulate the $EPSP_M$ since (1) CGP 55845A increases the amplitude of $EPSP_M$s beyond control levels and (2) NNC 05–0711 (Suzdak *et al.* 1992) inhibits the $EPSP_M$ (as well as increases the $IPSP_B$) in a CGP 55845A-sensitive manner. However, what is the source of the endogenously released GABA? Since neither CGP 55845A nor NNC 05–711 affect neuronal resting membrane properties, both compounds presumably modify the $EPSP_M$ by influencing the action of phasically released GABA (Thompson & Gähwiler, 1992*b*; Roepstorff & Lambert, 1994; Soltesz *et al.* 1995). This GABA could originate from: (i) release from hippocampal or septohippocampal GABAergic neurones or (ii) direct co-release from cholinergic nerve terminals (Frotscher *et al.* 1986; Kosaka *et al.* 1988; Beauleiu & Somogyi, 1991; Bayraktar *et al.* 1997). However, co-expression of GABA and ACh in septohippocampal as well as intrinsic hippocampal cholinergic afferents is controversial (Frotscher *et al.* 2000). As such, if GABA is released from non-cholinergic terminals it may operate through a diffusable, action-at-a-distance mechanism. Alternatively, closer structural interactions between GABAergic and cholinergic terminals, whether these be the cholinergic terminals forming synaptic specializations (Umbriaco, *et al.* 1995) or those mediating untargeted ACh release (Vizi & Kiss, 1998), may exist. In this respect, both diffuse and targeted GABA release can account for synaptically activated $GABA_B$ receptor-mediated events. Specifically, GABA spillover to extrasynaptic $GABA_B$ receptors, as a result of coincident activation of multiple GABAergic interneurones/release sites (Scanziani, 2000), is a favoured mechanism in the hippocampus. Indeed, this accounts for $GABA_B$ receptor-mediated inhibition of mAChR agonist-induced hippocampal rhythmic activity (Scanziani, 2000). However, more detailed physiological and anatomical analyses will be required to determine the source of the released GABA which modulates cholinergic synaptic transmission.

In conclusion, activation of $GABA_B$ receptors can control the level of activity at cholinergic synapses in the hippocampal CA1 region. As such, within the hippocampus there exists a reciprocal interaction between GABAergic and cholinergic systems whereby mAChRs inhibit GABAergic synaptic function (Freund & Buszáki, 1996; Manuel & Davies, 1998) and $GABA_B$ receptors inhibit cholinergic synaptic function. This control can be provided through phasic GABAergic inputs and, as such, differs from the control afforded by adenosine A_1 receptors (Morton & Davies, 1997). In particular, the high background levels of adenosine in cerebrospinal fluid, and its more generalized release during insults such as hypoxia, are consistent with the neuroprotective role of this purine. In contrast, the more dynamic regulation of cholinergic synaptic transmission by synaptically released GABA may be important in mnemonic processes for which a role for both receptor systems has been described (Cole & Nicoll, 1983; Decker & McGaugh, 1991; Mondadori *et al.* 1993; Dutar *et al.* 1995). However, $GABA_B$ receptors may also play a neuroprotective role through modulation of cholinergic activity. Indeed, activation of $GABA_B$ receptors inhibits mAChR-induced synchronous glutamatergic activity in both the rat piriform cortex (Libri *et al.* 1998) and hippocampus (Scanziani, 2000).

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