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PAIRED-PULSE DEPRESSION OF MONOSYNAPTIC GABA-MEDIATED INHIBITORY POSTSYNAPTIC RESPONSES IN RAT HIPPOCAMPUS

BY C. H. DAVIES, S. N. DAVIES AND G. L. COLLINGRIDGE

From the Department of Pharmacology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD

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SUMMARY

1. Intracellular recording techniques were used to characterize monosynaptic inhibitory postsynaptic potentials (IPSPs) and currents (IPSCs) in rat hippocampal slices and to study the mechanism of paired-pulse depression of these synaptic responses. This was achieved by stimulation in stratum radiatum close (< 0·5 mm) to an intracellularly recorded CA1 neurone after pharmacological blockade of all excitatory synaptic transmission.

2. Under these conditions, low-frequency stimulation (0·033 Hz) evoked a pure biphasic IPSP, which had a short and constant latency to onset. This IPSP was blocked by tetrodotoxin (1 μM) suggesting that it resulted from the electrical stimulation of the axons and/or cell bodies of a monosynaptic inhibitory pathway.

3. Picrotoxin (100 μM) abolished the early component of the biphasic IPSP/C. It left an intact, pure late IPSP/C (IPSP/C_B) which had a latency to onset of 29 ± 2 ms, latency to peak of 139 ± 4 ms, a duration of 723 ± 135 (range 390–1730) ms and a reversal potential of -93 ± 2 mV. The duration was highly dependent on the stimulus intensity whereas the latency to onset was largely independent of the stimulus intensity. The IPSP/C_B was reduced or abolished by 1 mM-phaclofen.

4. Phaclofen (1 mM) and 2-hydroxy-saclofen (0·1–1·0 mM) reversibly depressed (60–100%) the late component of the biphasic IPSP/C and, where maximally effective, left a pure, early IPSP/C (IPSP/C_A). The IPSP/C_A had a latency to onset of 3 ms or less, a latency to peak of 17 ± 1 ms, a duration of 225 ± 3 ms and a reversal potential of -75 ± 2 mV.

5. Two shocks of identical strength were applied in close succession to characterize, and to study the mechanisms underlying, frequency-dependent depression of inhibitory synaptic responses. Paired-pulse depression was seen for both phases of the biphasic IPSP/C and of the pure IPSP/C_B, recorded in the presence of picrotoxin. Paired-pulse depression was not accompanied by changes in the reversal potential of either component, indicating that it was caused by a reduction in the two synaptic conductances. Paired-pulse depression was greater when high stimulus intensities were employed.

6. Paired stimuli were applied at separation intervals of between 5 ms and 10 s to determine the temporal profile of frequency-dependent depression. Paired-pulse depression of both $IPSC_A$ and $IPSC_B$ was most pronounced at an interstimulus

interval of 100–125 ms and ceased to occur at intervals greater than 5–10 s. Linear summation of IPSCs was seen with intervals of 10 ms or less.

7. At rest, (−)-baclofen (5 μM) produced a small hyperpolarization (or outward current) associated with a slight increase in membrane conductance and abolished both components of the inhibitory synaptic response. Effects were stereoselective and reversible. A tenfold lower concentration of (−)-baclofen selectively depressed the synaptic response, causing depressions of 41 ± 3 and 54 ± 4% for the early and late components, respectively. This effect was reversed by 1 mM-phaclofen. In the presence of (−)-baclofen (0·5 μM), paired-pulse depression of IPSCs was less pronounced.

8. Phaclofen (1 mM) had little or no effect on paired-pulse depression. However, the more potent analogue, 2-hydroxy-saclofen (0·1–1·0 mM), reversibly reduced or abolished paired-pulse depression in a dose-dependent manner.

9. In summary, two kinetically distinct components of a monosynaptic γ-aminobutyric acid (GABA)-mediated inhibitory synaptic response have been isolated pharmacologically. Each component shows similar paired-pulse depression profiles. The mechanism underlying paired-pulse depression involves a reduction in inhibitory synaptic conductance and can be accounted for by GABA acting on presynaptic $GABA_B$ receptors to inhibit its own release.

INTRODUCTION

GABA is the major inhibitory neurotransmitter in the mammalian nervous system and has been the subject of extensive neurophysiological investigation. However, since most GABA-releasing neurones have short axons they are usually activated indirectly by stimulation of excitatory neurones. These excitatory neurones usually also innervate the cell from which recordings are obtained and consequently the resulting synaptic response is generally a composite of excitatory and inhibitory components. Therefore, accurate descriptions of the time course and reversal potential of GABA-mediated synaptic responses have been made rarely. Recently, however, we have shown that following pharmacological blockade of excitatory synaptic transmission it is possible to activate a pure monosynaptic IPSP in the CA1 region of hippocampal slices (Collingridge, Davies & Davies, 1988*a*). We have now utilized selective GABA antagonists to isolate and characterize pure $GABA_A$ and $GABA_B$ receptor-mediated synaptic responses.

A feature of GABA-mediated synaptic inhibition that is considered to be important in the expression of epileptiform activity is its ability to fatigue at frequencies of activation of above 0·1 Hz (Ben-Ari, Krnjević & Reinhardt, 1979; McCarren & Alger, 1985; Deisz & Prince, 1989; Thompson & Gähwiler, 1989*a*, *c*). Studies of the mechanisms underlying frequency-dependent fatigue have been complicated, not only by the temporal overlap of excitatory and inhibitory synaptic events, but by the likelihood of changes in the excitatory synapses that are used to drive the inhibitory neurones. We have therefore used activation of this monosynaptic inhibitory pathway to characterize and to study the mechanisms involved in fatigue of GABA-mediated synaptic responses.

Some of these results have appeared in abstract form (Davies, Davies & Collingridge, 1988; Davies & Collingridge, 1989*a*).

METHODS

Female albino rats (approximately 5 weeks old) were anaesthetized with halothane and decapitated. The brain was removed rapidly, both hippocampi were dissected out at room temperature, and transverse slices 400 μm thick were prepared using a McIlwain tissue chopper. The slices were placed on a nylon mesh at the interface of a warmed (30–32 °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.

Intracellular recordings were obtained from the CA1 pyramidal cell body region using 2 M-potassium methylsulphate-filled microelectrodes (40–80 MΩ). An Axoclamp-2 amplifier (Axon Instruments, Burlingame, CA, USA) was used in discontinuous (3–8 kHz switching frequency) current- or voltage-clamp mode. Digitized records were stored on magnetic discs for off-line analysis. Bipolar stimulating electrodes, made from 55 μm diameter insulated nickel–chromium wire, were positioned in stratum radiatum to provide orthodromic activation of CA1 neurones. Stimuli comprised square-wave pulses (0·1 ms) delivered at 0·033 Hz. As an indicator for healthy synaptic inhibition, experiments were only performed on slices where orthodromic stimulation in standard medium evoked a single population spike, recorded extracellularly, and impalements were obtained in normal medium, before addition of excitatory amino acid antagonists.

D-2-Amino-5-phosphonopentanoate (AP5; 40–80 μM), 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX; 20–40 μM), picrotoxin (0·1 mM), phaclofen (1 mM), 2-hydroxy-saclofen (0·1–1·0 mM) and tetrodotoxin (TTX; 1 μM) were administered via the perfusion medium.

Data are presented as means ± S.E.M. and statistical significance was assessed using paired *t* tests.

RESULTS

Data were obtained from sixty-two stable (1–7 h) intracellular recordings from CA1 neurones which had resting membrane potentials of -61 ± 3 mV and resting input resistances of 35 ± 1 MΩ.

Postsynaptic potentials in standard medium

Low-frequency (0·033 Hz) stimulation in stratum radiatum invariably elicited a stable multiphasic response which comprised a depolarization that was rapidly curtailed by a biphasic hyperpolarization, as reported many times previously. A combination of the excitatory amino acid antagonists 20 μM-CNQX and 40 μM-AP5 abolished the depolarizing component ($n = 62$), irrespective of the electrode placements and stimulation strength (up to 10× the threshold for evoking an excitatory postsynaptic potential (EPSP)). The effect, however, of the combined application of these antagonists on the biphasic hyperpolarization was more complex (Fig. 1). If the electrode separation was greater than ~ 0·5 mm this component was abolished. If, however, the separation distance was less than this the biphasic hyperpolarization was partly, or sometimes completely, unaffected. We have interpreted these observations as follows. With a large electrode separation the hyperpolarization is an IPSP that is entirely mediated via a polysynaptic pathway that involves CNQX- and AP5-sensitive receptors (Davies & Collingridge, 1989*b*).

When this is blocked an IPSP can be evoked by the direct stimulation of local interneurones providing the stimulating electrode is placed close enough to the cell under investigation (Collingridge *et al.* 1988*a*). This monosynaptic IPSP was blocked by tetrodotoxin ($n = 3$) (Fig. 1*B*), indicating that the cell body and/or axons, rather

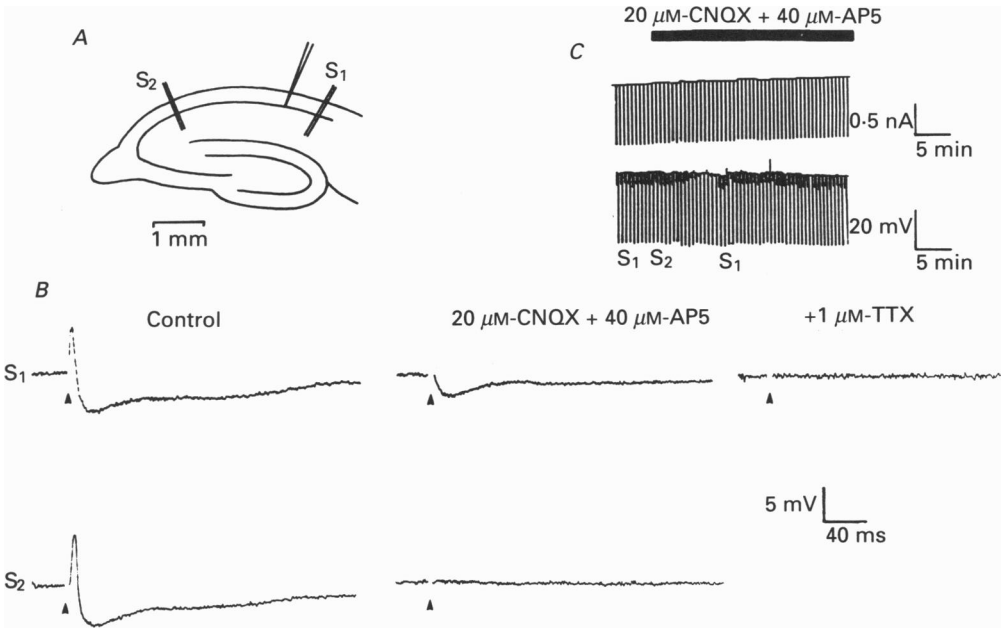

Fig. 1. Activation of monosynaptic IPSPs in area CA1 of a hippocampal slice. *A*, electrode placements in a hippocampal slice to elicit monosynaptic (S_1) as well as polysynaptic (S_1 and S_2) IPSPs. *B* shows intracellular recordings from a CA1 neurone in response to stimulation from S_1 and S_2. The combination of CNQX and AP5 blocked the EPSPs evoked from either site. The IPSP evoked from S_1, but not that from S_2, was partially preserved in the presence of the excitatory amino acid antagonists, but was blocked by TTX. *C* shows the time course of this effect and that CNQX and AP5 had no effect on passive membrane properties. The large deflections in the lower trace are voltage responses to constant current pulses; the smaller deflections are IPSPs evoked from S_1 (first four and last thirty-four responses) or S_2. It is the distance separating the stimulating and recording sites and not their relative positions in the CA1 subfield which is important for evoking a CNQX/AP5-resistant IPSP. In this and subsequent records traces are averages of three to five responses (unless indicated otherwise). The point of stimulation is marked by an arrow-head and artifacts are blanked for clarity. Unless otherwise stated each figure shows data from one representative cell.

than the terminals, of the interneurones (and possibly other GABAergic afferents) are being stimulated. All the following observations were made from monosynaptic inhibitory synaptic responses isolated in this manner.

Properties of the biphasic IPSP and its individual components

The biphasic IPSP had a short and constant latency to onset of 3 ms or less and a duration, that was highly dependent on stimulus strength, of 220–1730 ms. The two phases peaked in amplitude at latencies of 18 ± 1 and 143 ± 4 ms and their

respective reversal potentials, determined by interpolation, were -76 ± 1 and -92 ± 2 mV (data from a representative sample of thirteen cells) (Fig. 2). The size of the second phase relative to the first could be increased by either increasing the stimulus intensity or positioning the stimulating electrode further from the cell body layer.

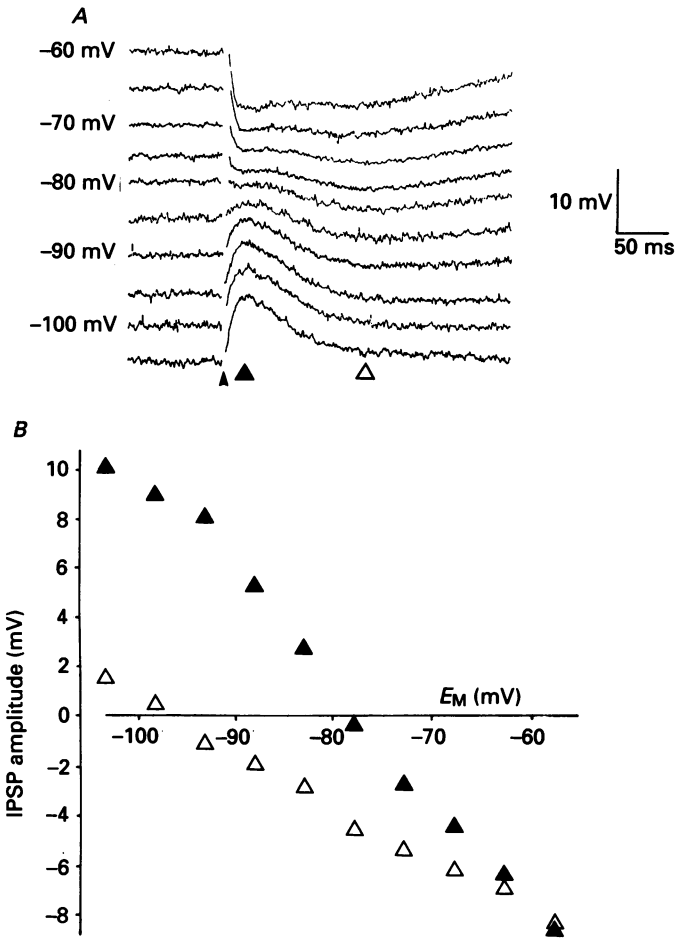

Fig. 2. Voltage dependence of a biphasic monosynaptic IPSP. *A* illustrates the IPSP evoked at a variety of membrane potentials. In *B*, the sizes of the two phases of this response, measured at latencies of 17 (▲) and 137 (△) ms, are plotted against membrane potential.

To define the properties of each component of the biphasic IPSP in isolation, a pharmacological approach was employed. Since the early and late phases of IPSPs elicited polysynaptically are believed to be mediated by $GABA_A$ and $GABA_B$ receptors (Newberry & Nicoll, 1985; Dutar & Nicoll, 1988*a*), picrotoxin (0·1 mM) and phaclofen (1 mM) were used to block these two components, respectively. Phaclofen reversibly blocked a late component ($n = 10$) (Fig. 3*A*) and picrotoxin blocked an early component ($n = 16$) (Fig. 3*B*). The combined application of phaclofen and

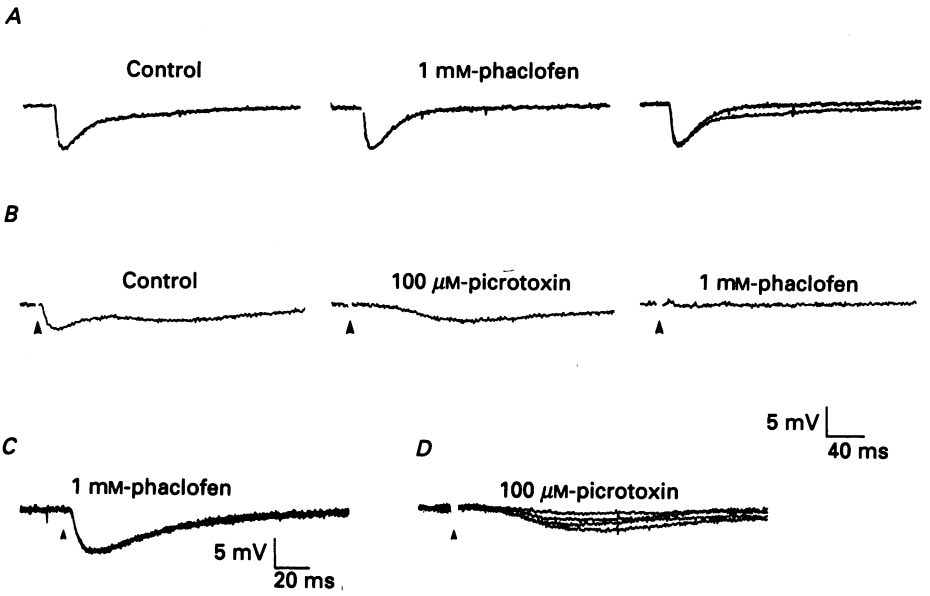

Fig. 3. Pharmacological separation of $IPSP_A$ and $IPSP_B$. *A*, phaclofen blocks a late component of the biphasic IPSP to leave a pure $IPSP_A$. Traces show (from left to right) control responses, the effect of 1 mM-phaclofen and a superimposition of these two traces. *B*, picrotoxin blocks an early component to leave a pure $IPSP_B$, which is blocked by phaclofen. *C* shows four consecutive single traces of $IPSP_A$ to illustrate its constant latency. *D* shows four responses of $IPSP_B$ evoked at different stimulus intensities (5, 6, 11, 22 V) to illustrate the stimulus-independent latency to onset. Traces in *A–D* are from four different cells.

TABLE 1. Properties of pharmacologically isolated inhibitory synaptic components

	Latency				
	to onset (ms)	to peak (ms)	Duration (ms)	Reversal potential (mV)	*n*
$IPSP/C_A$	< 3	17 ± 1	225 ± 3	−75 ± 2	5
$IPSP/C_B$	29 ± 2	139 ± 4	723 ± 135	−93 ± 2	9

$IPSP/C_A$ was isolated using 1 mM-phaclofen, and $IPSP/C_B$ was isolated using 0·1 mM-picrotoxin. The perfusate also contained 20 μM-CNQX and 40 μM-AP5 to block synaptic excitation. Data are presented as mean ± S.E.M.

picrotoxin abolished the entire IPSP ($n = 3$) (Fig. 3*B*). The picrotoxin-sensitive component will be referred to as $IPSP_A$ and the phaclofen-sensitive component as $IPSP_B$ (Deisz & Prince, 1989) and their properties are presented in Table 1. $IPSP_A$ had a constant latency to onset (Fig. 3*C*) consistent with it being a monosynaptic response. A notable feature of $IPSP_B$ was its long latency to onset, which was virtually independent of the stimulus strength employed (Fig. 3*D*).

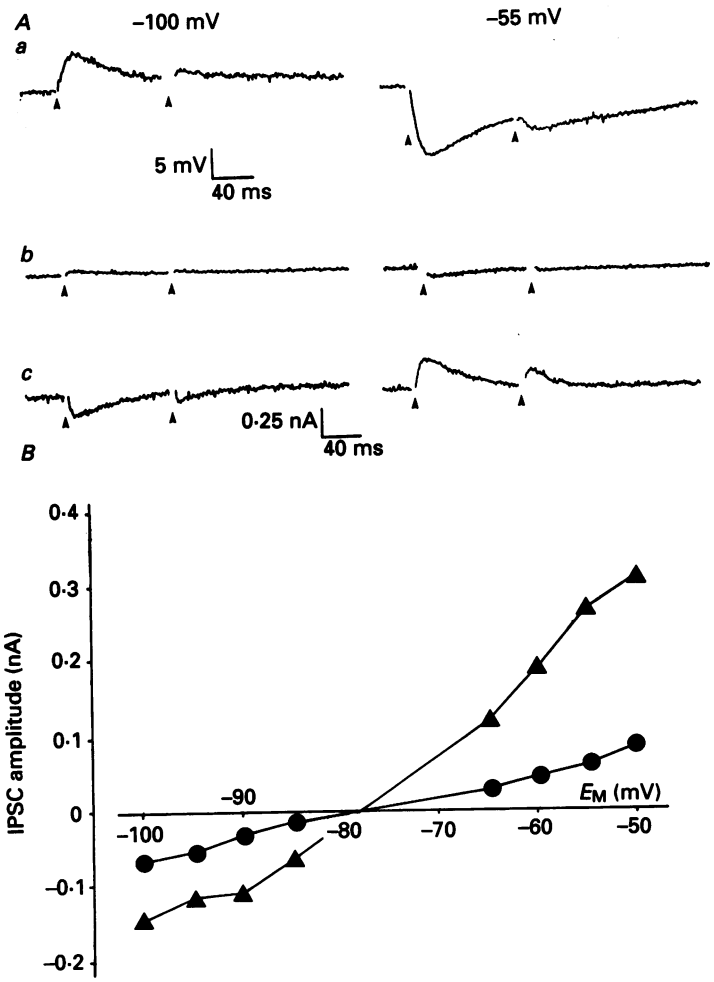

Fig. 4. Paired-pulse depression of monosynaptic IPSPs and IPSCs. *Aa* shows biphasic IPSPs evoked at two different membrane potentials by two shocks of identical strength delivered at an interval of 100 ms. *Ab* and *Ac* are the equivalent responses recorded under single-electrode voltage-clamp conditions (in *b* the voltage is plotted on the same gain as *a* to illustrate the performance of the clamp). *B* shows a plot of the peak amplitude of the first (▲) and second (●) of the pair of synaptic currents against voltage-clamped membrane potential. At its peak the response is essentially pure $IPSC_A$. Note the marked degree of paired-pulse depression at every membrane potential but no change in the reversal potential. The outward rectification was typical of all cells examined.

Paired-pulse depression of IPSPs and IPSCs

When two stimuli of identical strength were applied in close succession to the same input the second IPSP was markedly depressed ($n = 8$) (Fig. 4*Aa*). Qualitatively, similar observations were made under voltage-clamp conditions, used to minimize voltage changes associated with the synaptic responses ($n = 20$) (Fig. 4*Ab* and *c*).

The paired-pulse depression was not associated with any change in the reversal potential of the IPSC ($n = 7$) (Fig. 4*B*), demonstrating that the underlying mechanism involved a reduction in synaptic conductance rather than an alteration in ionic activities. The extent of depression was largely independent of membrane

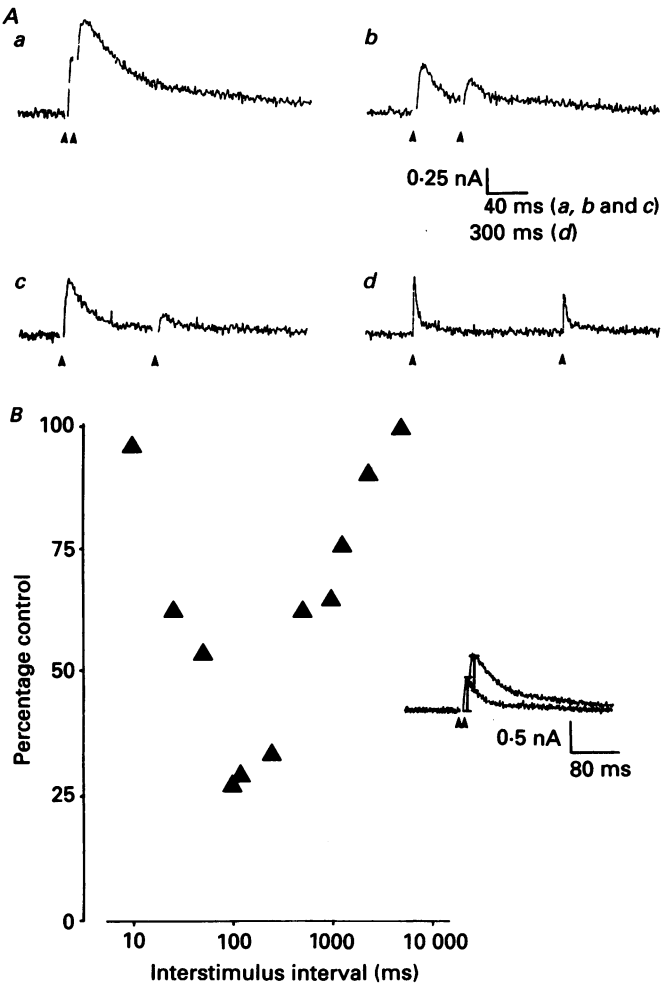

Fig. 5. Time course of paired-pulse depression of $IPSC_A$. *A*, pairs of biphasic IPSCs were evoked by two identical shocks delivered at intervals of between 10 ms and 5 s. *B* shows a plot of the peak amplitude of the second IPSC as a percentage of the first (i.e. percentage change in $IPSC_A$; see inset) against interstimulus interval.

potential (Fig. 4*B*). The apparent slight voltage dependence of the paired-pulse depression seen in Fig. 4*B* can be explained by the combined effect of imperfect clamp of the largest currents and outward rectification of the conductance.

To determine the time course of paired-pulse depression two shocks of identical strength were applied at separation intervals of between 5 ms and 10 s. To calculate the depression of $IPSC_A$, biphasic responses were evoked and measurements obtained at the peak of $IPSC_A$ (Fig. 5), a time when the response is uncontaminated by $IPSC_B$

(Table 1). With interstimulus intervals of 5 ms the second IPSC of the pair often failed to follow. At interstimulus intervals of 10 ms and 10 s there was no depression of the test response. At intermediate intervals, however, paired-pulse depression was observed. This was seen invariably with interstimulus intervals of between 25 ms and

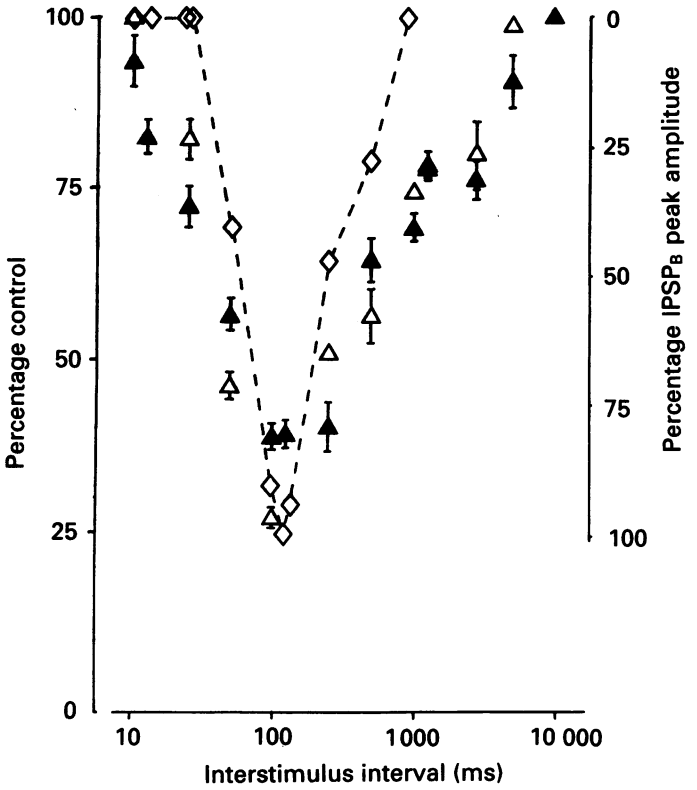

Fig. 6. Comparison of the time courses of $IPSP_B$, paired-pulse depression of $IPSC_A$ and paired-pulse depression of $IPSC_B$. The graph shows a plot of the peak amplitude of the second response as a percentage of the first for $IPSC_A$ (▲; $n = 20$) and $IPSC_B$ (△; $n = 3$) *versus* interstimulus interval. Error bars are shown where these are larger than the symbols. These profiles are superimposed upon a typical $IPSP_B$ plotted as the percentage of its peak amplitude (◇).

2·5 s; the maximum depression was observed at intervals of 100–125 ms. A representative experiment is illustrated in Fig. 5 and the pooled data from twenty cells are shown in Fig. 6. The extent of paired-pulse depression, but not its temporal profile, was dependent on the stimulus strength used; greater depression occurred when higher stimulus intensities were used ($n = 9$).

In order to study the depression of $IPSC_B$, $IPSC_A$ was blocked by 100 μM-picrotoxin ($n = 7$). Under these conditions, paired-pulse depression of $IPSC_B$ was observed (Fig. 7); it had a profile similar to that of $IPSC_A$ (cf. Fig. 6). The paired-pulse depression of $IPSC_B$ was independent of membrane potential and was not associated with any alteration in the extrapolated reversal potential of the response (Fig. 8). In these experiments there was no noticeable rectification of $IPSC_B$.

Actions of baclofen

At rest, (−)-baclofen (5 μM) caused a small (4–5 mV) dose-dependent hyperpolarization (or outward current) and an associated small (5–10%) increase in membrane conductance ($n = 6$) whereas (+)-baclofen was inactive ($n = 5$) (Fig. 9).

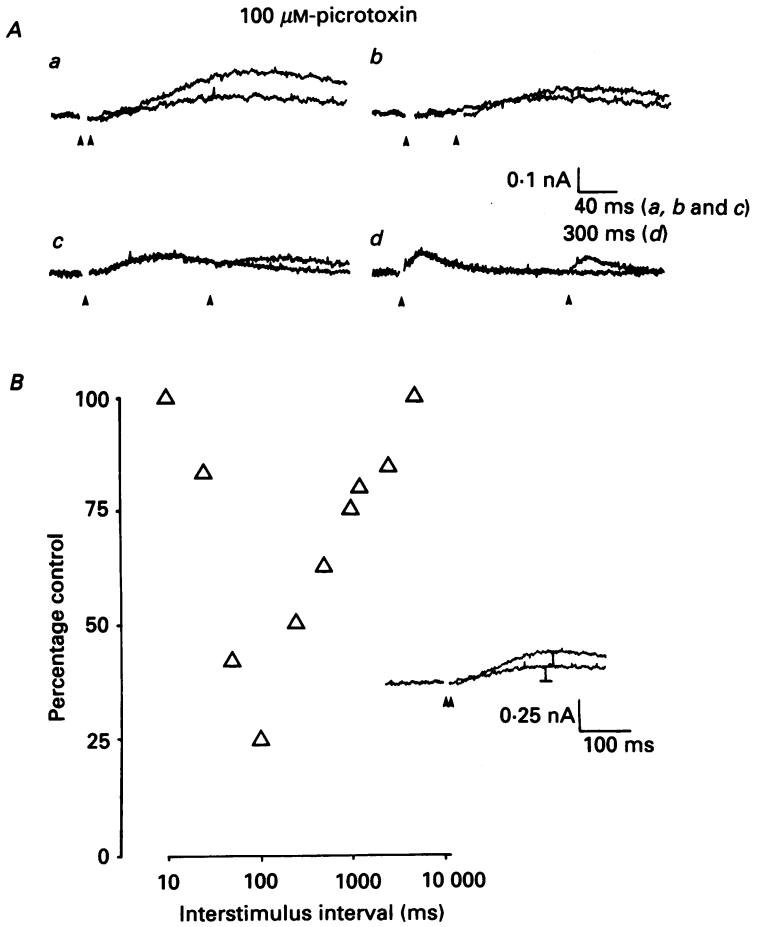

Fig. 7. Time course of paired-pulse depression of $IPSC_B$. Picrotoxin (100 μM) was used to block $IPSC_A$; the concentrations of AP5 and CNQX were 80 and 40 μM, respectively. Otherwise the protocol was the same as for Fig. 5. In *A*, the paired-pulse traces are superimposed upon equivalent single-shock responses.

Baclofen also blocked both phases of the IPSP in a stereoselective manner (Fig. 9). At 0·5 μM, (−)-baclofen depressed the IPSP/C (Fig. 10) without changing the passive membrane properties of the cell ($n = 5$). The late phase was depressed to a greater extent than the early phase; the percentage reductions were 54 ± 4 and 41 ± 3 ($n = 5$; $P < 0{\cdot}05$), respectively. The synaptic depressant effects of 0·5 μM-baclofen were completely reversed by 1 mM-phaclofen ($n = 3$) (Fig. 10).

Paired-pulse depression profiles were compared in the presence and absence of 0·5 μM-baclofen. As illustrated in Fig. 11, baclofen reduced the extent of paired-pulse

depression. To quantify the effects, the amplitudes of the first IPSCs in the presence of 0·5 μM-baclofen were matched to their control amplitudes by increasing the stimulus intensity. The percentage depressions, at an interstimulus interval of 100 ms, were 58 ± 3 for controls and 16 ± 4 in the presence of 0·5 μM-(−)-baclofen ($n = 5$; $P < 0{\cdot}01$).

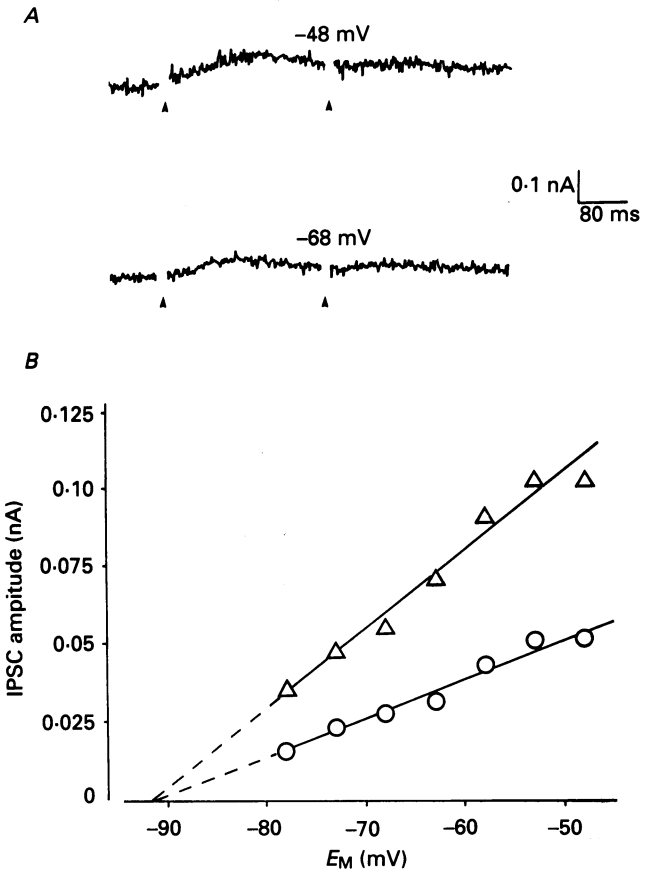

Fig. 8. Voltage dependence of paired-pulse depression of $IPSC_Bs$. *A* illustrates pure $IPSC_Bs$ (recorded in the same solution as the cell illustrated in Fig. 7). Two shocks of identical strength were applied at a separation interval of 250 ms. In *B* the peak amplitudes of the first (△) and second (○) IPSCs are plotted *versus* membrane potential. Note the marked degree of paired-pulse depression at every membrane potential but no change in the extrapolated reversal potential. The lines were fitted by linear regression. The linear *I–V* profile was typical of the cells studied.

Effects of $GABA_B$ antagonists on paired-pulse depression

The above experiments demonstrate that inhibitory neurones possess $GABA_B$ receptors and that activation of these receptors can inhibit transmitter release and modify the extent of paired-pulse depression. To determine whether these receptors are activated physiologically and are involved in paired-pulse depression, the effects of $GABA_B$ antagonists were examined. Phaclofen (1 mM) had little or no effect on

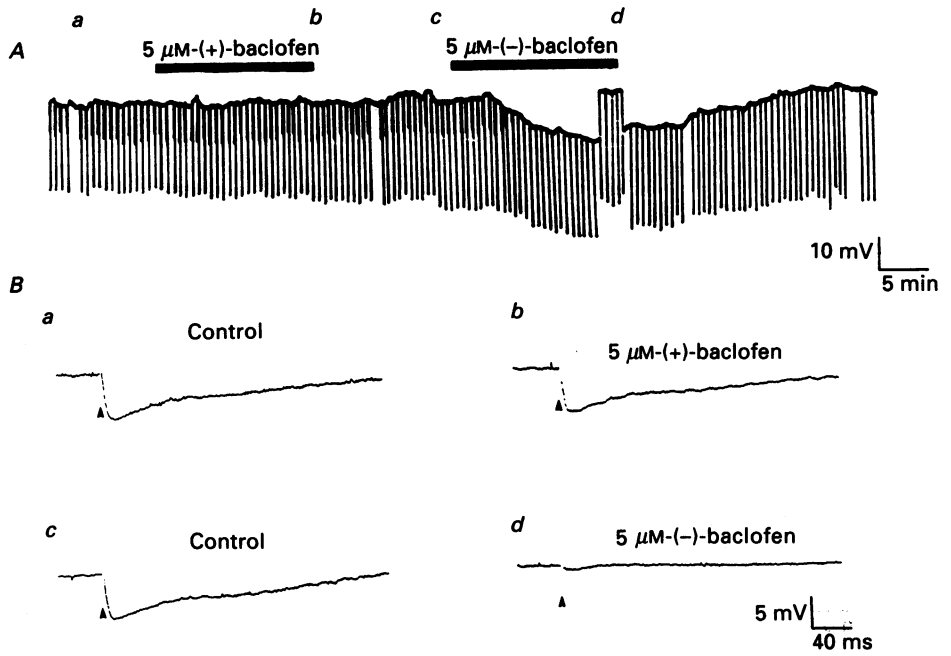

Fig. 9. Stereoselective actions of baclofen. *A*, effects of 5 μM-(+)-baclofen and 5 μM-(−)-baclofen on passive membrane properties. Large negative deflections are voltage responses in response to 0·5 nA constant current pulses and small deflections are IPSPs. The membrane potential was compensated by adjusting the current injection for the last five responses in the presence of (−)-baclofen. Note that the IPSPs are still blocked after the membrane potential has returned to control. *B* illustrates the monosynaptic IPSPs obtained at the times indicated in *A* (by *a–d*).

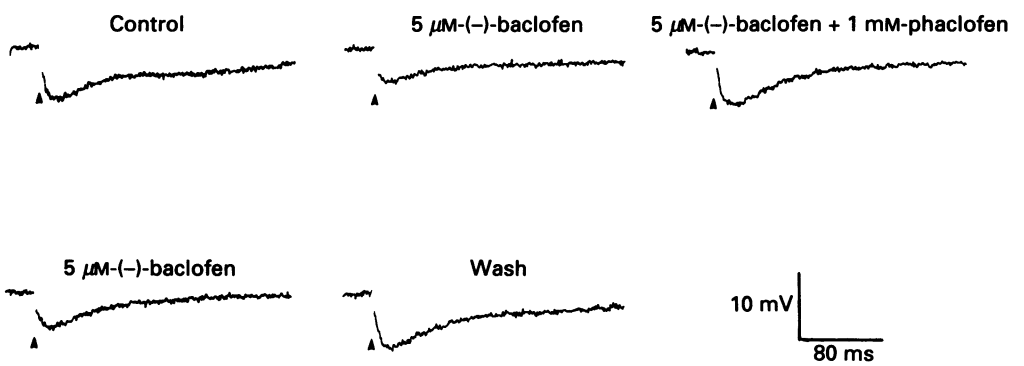

Fig. 10. The depression of monosynaptic IPSPs by baclofen is reversed by phaclofen. At 0·5 μM, (−)-baclofen depressed the IPSP by approximately 50% and was without effect on passive membrane properties.

paired-pulse depression ($n = 3$; not illustrated). We therefore tested the more potent analogue 2-hydroxy-saclofen (0·1–1·0 mM) ($n = 7$). This agent reversibly blocked $IPSC_B$ ($IC_{50} < 100\ \mu M$) and partially suppressed $IPSC_A$ (IC_{50} 300–400 μM). It reversibly suppressed paired-pulse depression in a dose-dependent manner (IC_{50}

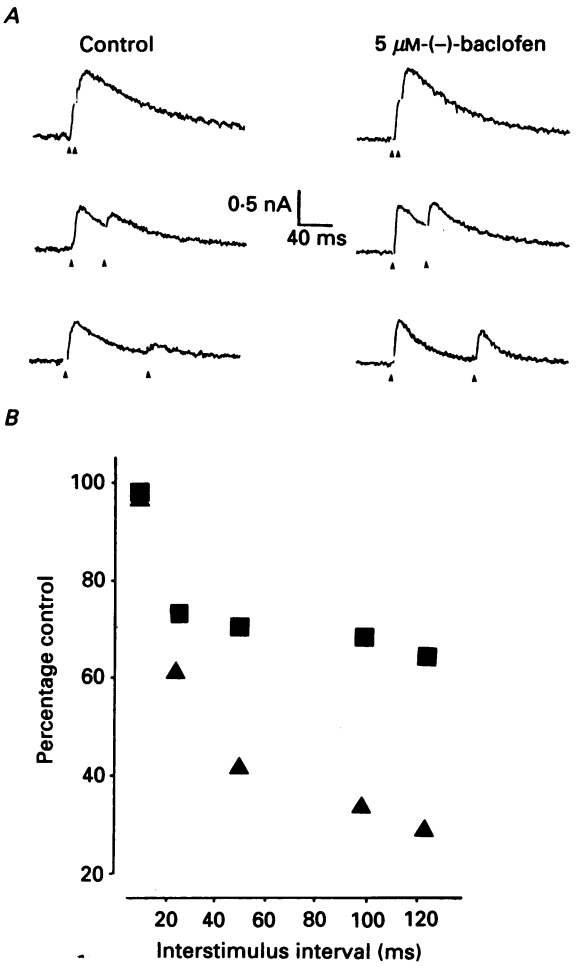

Fig. 11. Baclofen affects paired-pulse depression of monosynaptic IPSCs. *A*, pairs of biphasic IPSCs were evoked using two shocks of identical strength delivered at a range of separation intervals. In the presence of baclofen, the stimulus intensity was increased so as to match the amplitude of the first of the IPSCs in the pair (the change in the shape of the responses is due to the greater sensitivity of $IPSC_B$ to the actions of baclofen; see text). *B* shows a plot of the peak amplitude of the second IPSC as a percentage of the first (i.e. percentage change in $IPSC_A$) as a function of interstimulus interval, in the presence (■) and absence (▲) of 0·5 μM-(−)-baclofen. Note that paired-pulse depression is reduced by baclofen.

~ 200 μM). Figure 12*A* illustrates a cell where 2-hydroxy-saclofen (200 μM) partially reversed paired-pulse depression with only a slight effect on $IPSC_A$. Figure 12*B* shows a cell where 1 mM-2-hydroxy-saclofen abolished paired-pulse depression. This effect persisted when the size of the first IPSC in the presence of 2-hydroxy-saclofen

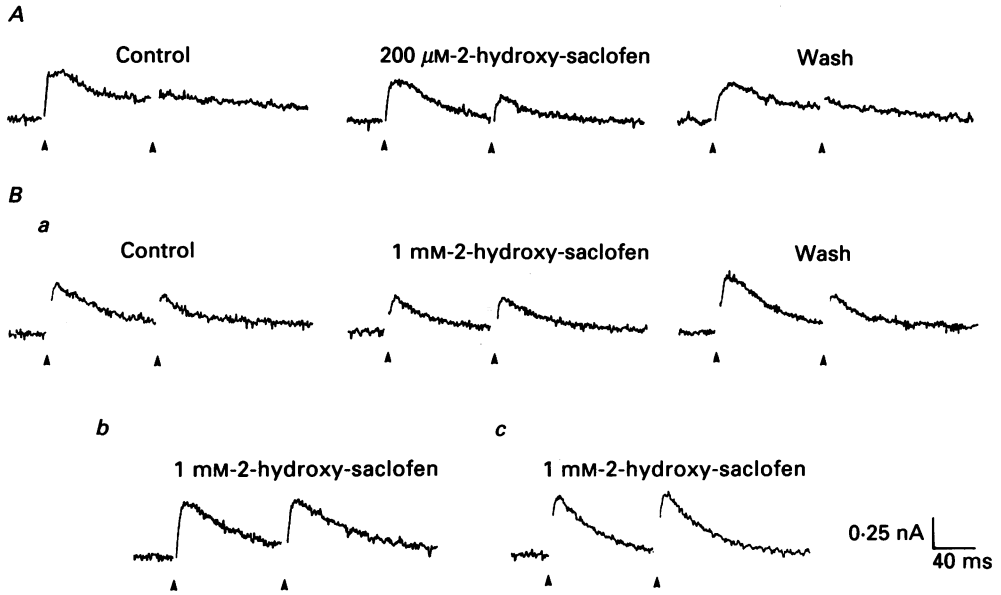

Fig. 12. 2-Hydroxy-saclofen reverses paired-pulse depression. *A* shows that 200 μM-2-hydroxy-saclofen reversibly depressed (but did not block) both $IPSC_B$ and paired-pulse depression of $IPSC_A$ with only a slight effect on $IPSC_A$. *B* shows that 1 mM-2-hydroxy-saclofen reversibly blocked $IPSC_B$ and eliminated paired-pulse depression (of $IPSC_A$). $IPSC_A$ was substantially depressed by this concentration of 2-hydroxy-saclofen. To compensate for this the size of the first IPSC of the pair in the presence of 2-hydroxy-saclofen was matched to the control by increasing the stimulus intensity (*b*) or by depolarizing the cell to −35 mV (from −50 mV) (*c*). Traces in *A* and *B* are from different cells.

was matched to its control size by either increasing the stimulus strength (Fig. 12*Bb*) or altering the membrane potential (Fig. 12*Bc*).

DISCUSSION

Monosynaptic GABA-mediated synaptic inhibition

Stimulation in stratum radiatum in standard physiological medium characteristically evokes a fast monosynaptic EPSP and a two-phase IPSP. The IPSP is generally considered to be mediated polysynaptically, via an excitatory amino acid released from Schaffer collateral–commissural fibres activating feed-forward interneurones and, if CA1 cells fire, by the activation of recurrent inhibition. As reported previously (Davies & Collingridge, 1989*b*), if the stimulating electrode is placed at a sufficient distance from the recording site then this is the case, since the IPSP is depressed together with fast EPSPs, by the non-NMDA type excitatory amino acid antagonist CNQX. Under these conditions, if high stimulus intensities are used *N*-methyl-D-aspartate (NMDA) receptors can also mediate the excitation of

feed-forward interneurones. For this reason, in the present experiments AP5 was always used in conjunction with CNQX.

However, when the stimulating electrode was placed close to the recording site the IPSP was, at least partially, conserved. The simplest explanation for this observation is that the feed-forward interneurones (and possibly axons of other inhibitory neurones) are stimulated directly. The short and constant latency of the biphasic IPSP supports the notion of monosynaptic activation of an inhibitory pathway and its sensitivity to tetrodotoxin suggests that it is cell bodies and/or axons, rather than terminals, that are activated by the stimulating electrode. The sensitivity of the IPSP to the combination of $GABA_A$ and $GABA_B$ antagonists indicates that under these conditions the response recorded is entirely GABA-mediated. The present experiments have utilized these methods for recording pure, monosynaptic GABA-mediated IPSPs and IPSCs for two purposes; firstly, to characterize pharmacologically isolated $GABA_A$ and $GABA_B$ receptor-mediated synaptic responses and secondly, to investigate the mechanism by which the inhibitory synaptic response fatigues during high-frequency stimulation.

Properties of the monosynaptic GABA receptor-mediated components

In previous studies in area CA1 of the hippocampus, and elsewhere in the brain, measurements of the time courses and *I–V* relationships of IPSPs (and IPSCs) have usually been complicated by superimposition of EPSPs and by the multiphasic nature of the GABA-mediated IPSPs (e.g. Alger & Nicoll, 1982; McCarren & Alger, 1985; Dutar & Nicoll, 1988*a*).

Several different approaches have been used to record pure $GABA_A$ receptor-mediated IPSPs and IPSCs. In the hippocampus, stimulation of pyramidal cell axons activates recurrent inhibitory neurones and can evoke a relatively pure $GABA_A$ receptor-mediated IPSP (Dingledine & Langmoen, 1980; McCarren & Alger, 1985). In addition, 'unitary' IPSPs and IPSCs may be recorded spontaneously (Alger & Nicoll, 1980; Collingridge, Gage & Robertston, 1984) or following stimulation of a synaptically coupled inhibitory cell (Segal & Barker, 1984; Barker & Harrison, 1988). The time course of $IPSC_A$ is similar to that reported in other studies where synchronous activation of inhibitory interneurones has been employed (Dingledine & Langmoen, 1980), but is much longer than that of 'unitary' IPSCs in CA1 neurones (Collingridge *et al.* 1984). The time course of the latter is believed to reflect channel kinetics; therefore additional factors, such as clearance of GABA from the synaptic cleft, must contribute to the decay of $GABA_A$ receptor-mediated IPSCs when inhibitory neurones are synchronously activated (Dingledine & Korn, 1985). The reversal potential of $IPSC_A$ is similar to that reported previously in studies using methylsulphate-containing electrodes (Newberry & Nicoll, 1985) and the outward rectification is consistent with previous studies (Collingridge *et al.* 1984; Barker & Harrison, 1988).

A striking feature of the $IPSP_B$ was its long latency to onset. This has been inferred but has not previously been directly observed. Under the present recording conditions it is most likely that the $IPSP_B$ is monosynaptic in origin. Its long latency to onset could be inherent in the receptor–channel coupling mechanism, which is believed to involve a pertussis toxin-sensitive G protein (Dutar & Nicoll, 1988*b*). The

reversal potential and linear I–V relationship of $IPSC_B$ is similar to that determined from measurements of multicomponent synaptic responses in various regions (e.g. Hablitz & Thalmann, 1987; Connors, Malenka & Silva, 1988).

A third, 'depolarizing' $GABA_A$ receptor-mediated component of the IPSP has been described following high intensity afferent stimulation in stratum radiatum (Perreault & Avoli, 1988). This potential, which may be observed between the 'hyperpolarizing' $IPSP_A$ and $IPSP_B$, was not seen in the present study, even when high stimulus intensities were employed.

Paired-pulse depression

The mechanisms underlying frequency-dependent depression of inhibitory synapses have been extensively investigated, usually by examining the effects of a high-frequency train on subsequent low-frequency responses. It is clear that depression of the inhibitory synaptic responses is due to shifts in E_{Cl} and E_K and a reduction in the inhibitory synaptic conductance(s) (McCarren & Alger, 1985; Deisz & Prince, 1989; Thompson & Gähwiler, 1989*a*, *b*). In an earlier study using paired-pulse depression in area CA1 of the hippocampus the major change was a decrease in conductance (McCarren & Alger, 1985). The present observation that both $IPSC_A$ and $IPSC_B$ showed similar paired-pulse depression profiles favours a presynaptic mechanism (Deisz & Prince, 1989; Thompson & Gähwiler, 1989*a*), although the possibility of a postsynaptic mechanism similarly affecting two distinct conductances cannot be entirely excluded.

Several recent studies have addressed the mechanism that might underlie a presumed alteration in GABA release. One suggestion has been that GABA effects a negative feedback on its own release via an action on presynaptic $GABA_B$ receptors, since baclofen (and GABA) depresses IPSPs in the hippocampus (Misgeld, Klee & Zeise, 1984; Peet & McLennan, 1986) and neocortex (Connors *et al.* 1988; Deisz & Prince, 1989). However, since baclofen can also inhibit EPSPs in many regions, including the hippocampus (Lanthorn & Cotman, 1981; Ault & Nadler, 1982; Olpe, Baudry, Fagni & Lynch, 1982), and can hyperpolarize inhibitory interneurones directly (Madison & Nicoll, 1988; Misgeld, Müller & Brunner, 1989), in most previous studies it was not possible to determine whether baclofen acted on the terminals or cell bodies of inhibitory neurones or the excitatory synapses that were used to drive them. Significantly, however, evidence from cultured hippocampal cells suggests that baclofen can act directly on (or near) the terminals of inhibitory neurones to inhibit IPSPs (Harrison, Lange & Barker, 1988). The present study extends this observation by showing that GABA-releasing terminals in stratum radiatum of CA1 possess $GABA_B$ receptors and that these presynaptic $GABA_B$ receptors are sensitive to phaclofen. Activation of these receptors by baclofen depresses transmitter release and modifies the extent of paired-pulse depression. We now provide two lines of evidence that activation of these presynaptic $GABA_B$ receptors on inhibitory neurones is responsible for the paired-pulse depression of the inhibitory conductances. Firstly, the temporal profiles of paired-pulse depression of $IPSC_A$ and $IPSC_B$ had several similarities to the time course of $IPSP_B$; they had latencies to onset of > 10 ms, latencies to peak of ~ 100 ms and durations of $> 0{\cdot}5$ s (cf. Fig. 6).

Secondly, the most potent currently available $GABA_B$ antagonist 2-hydroxy-saclofen (Kerr, Ong, Johnston, Abbenante & Prager, 1988), dose-dependently and reversibly suppressed paired-pulse depression of $IPSC_A$.

Differences in the pre- and postsynaptic effects of $GABA_B$ ligands

In the present study, baclofen was more potent on presynaptic (depression of IPSCs and modulation of paired-pulse depression of IPSCs) than postsynaptic (hyperpolarization) $GABA_B$ receptor-mediated events. Both populations of receptors were, unlike those on the excitatory terminals of Schaffer collateral–commissural fibres in this region (Dutar & Nicoll, 1988*b*), sensitive to phaclofen.

The differences that we observe between $GABA_B$ receptors on CA1 neurones and those on inhibitory terminals could be explained if the latter are (i) more abundant, (ii) have a higher affinity for agonists (GABA and baclofen), (iii) are coupled more efficiently to their effector mechanism or (iv) the effector mechanism is more efficient. Any of these differences could explain why (a) baclofen acted presynaptically at doses that had no perceptible postsynaptic effects, (b) phaclofen was able to depress $IPSP_B$ and to reverse the depression of $IPSP_A$ induced by low doses of baclofen but did not affect paired-pulse depression (because it is a weak $GABA_B$ antagonist), (c) 2-hydroxy-saclofen was more potent at depressing $IPSC_B$ compared to paired-pulse depression and (d) the time course of $IPSP_B$ did not exactly match that of paired-pulse depression of $IPSC_A$ and $IPSC_B$.

The present observation that $IPSP_B$ was slightly more sensitive than $IPSP_A$ to the actions of baclofen is in agreement with previous findings (Howe, Sutor & Zieglgänsberger, 1987; Connors *et al.* 1988). The explanation for this is probably that baclofen occludes postsynaptic $GABA_B$ receptors from the action of synaptically released GABA. This could also explain why paired-pulse depression is reduced by baclofen.

Physiological significance of paired-pulse depression

The property of fatigue of inhibitory synapses during high-frequency transmission confers upon neural networks susceptibility to epileptiform activity. It seems likely, therefore, that there is an important physiological reason for presynaptic $GABA_B$ receptors on inhibitory neurones. One such function may be to facilitate the synaptic activation of NMDA receptors during high-frequency transmission (Collingridge, Herron & Lester, 1988*c*). During low-frequency transmission in area CA1 the EPSP, elicited by stimulation of the Schaffer collateral–commissural pathway, is rapidly curtailed by the biphasic IPSP. The associated hyperpolarization takes the membrane into a region where NMDA channels are substantially blocked by extracellular Mg^{2+} (Mayer & Westbrook, 1985; Ascher & Nowak, 1988). In this manner functional synaptic inhibition largely prevents activation of NMDA receptors (Dingledine, Hynes & King, 1986; Collingridge, Herron & Lester, 1988*b*). Thus GABA, by depressing its own release during high-frequency transmission, could enable the expression of NMDA receptor-mediated components of the EPSP. This in turn can lead to plastic changes at the activated excitatory synapses (Collingridge, Kehl & McLennan, 1983).

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