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$GABA_B$ RECEPTOR-MEDIATED INHIBITION OF Ca^{2+} CURRENTS AND SYNAPTIC TRANSMISSION IN CULTURED RAT HIPPOCAMPAL NEURONES

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SUMMARY

1. The effects of activation of $GABA_B$ receptors on Ca^{2+} currents (I_{Ca}) were investigated by application of whole-cell patch-clamp techniques to pyramidal neurones and non-pyramidal interneurones from the rat hippocampus grown in cell culture.

2. (±)-Baclofen (10 μM) reduced I_{Ca} evoked in pyramidal neurones at 0 mV from a holding potential of −80 mV by 33±3%. Inhibition could be observed at the peak of I_{Ca} with significant inhibition still present after 200 ms at 0 mV. When Ba^{2+} was used as the charge carrier (I_{Ba}) baclofen inhibited 28±3% of the current at −20 mV from a holding potential of −80 mV. The $GABA_B$ receptor antagonist 2-OH-saclofen (50–200 μM) blocked the actions of baclofen.

3. The selective Ca^{2+} channel blocker, ω-conotoxin fraction GVIA (ω-CgTX), was used to characterize the Ca^{2+} currents inhibited by baclofen. ω-CgTX (5 μM) blocked 24±3% of I_{Ba}. Following block of the ω-CgTX-sensitive current, baclofen inhibited significantly less current than under control conditions.

4. Addition of the dihydropyridine Ca^{2+} channel antagonist nimodipine (1 μM) inhibited 18±5% of I_{Ca} at 0 mV from a holding potential of −80 mV and 44±9% from a holding potential of −40 mV. In addition, nimodipine partially occluded subsequent responses to application of baclofen.

5. In the presence of both 5 μM-ω-CgTX and 200 nM-nimodipine, responses to baclofen were almost completely blocked at depolarized holding potentials where the dihydropyridines are most effective.

6. Inclusion of 500 μM-guanosine 5′-*O*-(3-thiotriphosphate) (GTP-γ-S) in the patch pipette enhanced the response to a subsaturating concentration of baclofen and rendered the response irreversible. Subsequent addition of the adenosine receptor agonist 2-Cl-adenosine (2-CA) (1 μM; which also reduces I_{Ca} under control conditions) was without effect, suggesting that these two receptor–effector pathways converge.

7. The actions of baclofen on I_{Ca} were blocked by pre-treatment of the cultures with pertussis toxin (250 ng/ml).

8. Baclofen also inhibited I_{Ca} in non-pyramidal neurones from the hippocampus, but was slightly less effective.

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9. Baclofen reduced both excitatory- and inhibitory postsynaptic currents (EPSCs and IPSCs) recorded as a consequence of extracellular stimulation of presynaptic neurones. This action was blocked by 2-OH-saclofen (200 μM) and also by pre-treatment of the cultures with pertussis toxin.

10. The results establish that activation of $GABA_B$ receptors inhibits ω-CgTX-sensitive I_{Ca} in the soma of pyramidal neurones of the hippocampus through activation of G-proteins. In addition, some inhibition of dihydropyridine-sensitive currents was observed. The receptors and the intracellular signals mediating inhibition of I_{Ca} were indistinguishable from those mediating presynaptic inhibition of transmitter release.

INTRODUCTION

A major proportion of inhibitory synapses in the central nervous system utilize γ-aminobutyric acid (GABA) as a neurotransmitter (Nicoll, Malenka & Kauer, 1990). The fast actions of this neurotransmitter are mediated by the $GABA_A$ receptor, which is a ligand-gated ion channel. At many synapses, GABA also elicits a 'slow' inhibitory synaptic potential that has been attributed to activation of $GABA_B$-type receptors in some cases (Thalmann, 1988). $GABA_B$ receptors are not believed to be part of an ionophore complex. However, the $GABA_B$ receptor agonist baclofen has been shown to activate a K^+ current in several types of neurones. Both the K^+ current and the slow inhibitory synaptic potential require the activation of a pertussis toxin (PTX)-sensitive guanine nucleotide-binding protein (G-protein; for review see Nicoll *et al.* 1990).

In addition to activating K^+ currents, GABA induces presynaptic inhibition at many synapses. Classical forms of GABA-mediated presynaptic inhibition involve activation of presynaptic $GABA_A$ receptors, leading to an increase in the conductance of the presynaptic membrane (Dudel & Kuffler, 1961; Takeuchi & Takeuchi, 1966). However, in the central nervous system, it appears that $GABA_B$ receptors produce presynaptic inhibition of transmitter release by a fundamentally different mechanism, which is not well understood (Nicoll *et al.* 1990). One hypothesis states that activation of K^+ currents is responsible for inhibition of transmitter release by baclofen at central synapses (Gahwiler & Brown, 1985). In peripheral neurones, baclofen has been shown to inhibit Ca^{2+} currents (I_{Ca}) in the cell body (Dunlap & Fischbach, 1981; Dolphin & Scott, 1987). A similar action at the presynaptic terminal has also been proposed to account for presynaptic inhibition (cf. Dunlap & Fischbach, 1981; Holz, Kream, Spiegel & Dunlap, 1989; Miller, 1990).

The effects of baclofen on central synapses and on somatic K^+ currents have been studied extensively. In contrast, there is very little information about the actions of baclofen on I_{Ca} in central neurones. We have examined the effects of baclofen on I_{Ca} in two types of cultured hippocampal neurones, pyramidal neurones and non-pyramidal interneurones. In addition, excitatory and inhibitory monosynaptic connections between cells in culture have been studied to compare the role of pertussis toxin-sensitive guanine nucleotide-binding proteins in mediating the effects of baclofen on synaptic transmission and I_{Ca}.

METHODS

The techniques used for cell culture and for electrophysiology were essentially the same as described previously (Banker, 1980; Scholz & Miller, 1991). In addition, ω-conotoxin GVIA (ω-CgTX) was applied to cells through a puffer pipette at a pipette concentration of 5 μM. Dihydropyridine calcium channel ligands were dissolved at 5000-fold final concentrations in 95% ethanol, protected from light and stored at −20 °C until use. These were applied at final concentrations by bath perfusion.

For recordings of whole-cell currents (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) through Ca^{2+} channels the bath solution contained (in mM): tetraethylammonium chloride (TEACl), 140; $MgCl_2$, 1; $CaCl_2$, 5 (or $BaCl_2$, 2; with additional TEACl added to adjust osmolarity); HEPES, 10; glucose, 10 at pH 7·4; and the pipette solution contained (in mM): CsCl, 136; $MgCl_2$, 1; EGTA, 10; HEPES, 10; GTP, 1 and ATP, 3·6 at pH 7·15. For recording of postsynaptic currents the bath contained (in mM): NaCl, 140; KCl, 5; $CaCl_2$, 3; $MgCl_2$, 6; HEPES, 10, glucose 10, at pH 7·4; and the pipette contained (in mM): potassium acetate, 140; $MgCl_2$, 1; EGTA, 10; HEPES, 10 at a pH of 7·15.

In cultures prepared at 17 days gestation, 5–10% of neurones showed immunoreactivity for γ-aminobutyric acid (GABA). Recordings of synaptic currents revealed that predominantly excitatory synaptic potentials were present in these cells. Since all of the interneurones (except granule cells) in the hippocampus that have been described to date are inhibitory (Schwartzkrion, Scharfman & Sloviter, 1990), these results indicate that the cultures contained predominantly pyramidal cells. At this time in development, granule cells have not yet appeared in the hippocampus (Banker & Cowan, 1979) and are considerably smaller when they do. Cells demonstrating immunoreactivity for GABA were morphologically distinct from pyramidal neurones. In cultures prepared at 18 days gestation (just 1 day later) about 12–36% of the neurones showed immunoreactivity for GABA and about 50% of evoked synaptic potentials were inhibitory (K. P. Scholz & W. K. Scholz, unpublished observations). Therefore, for recordings of I_{Ca}, non-pyramidal neurones were identified initially by morphological criteria (small cell body, 8–12 μm with two to four discrete processes emanating from the cell body). Confirmation of the cell type was obtained following recording of I_{Ca}, which was smaller in these cells, and primarily by the inability to respond to A1 adenosine receptor agonists (Scholz & Miller, 1991; Yoon & Rothman, 1991).

Drugs

(±)-Baclofen and 2-OH-saclofen were obtained from Research Biochemicals (Natick, MA, USA). ω-CgTX GVIA was obtained from Calbiochem (Campbell, CA, USA). Dihydropyridines were gifts from Miles Research Laboratories.

RESULTS

Inhibition of Ca^{2+} channel currents in pyramidal neurones

Whole-cell voltage-clamp recordings of currents carried through Ca^{2+} channels (I_{Ca} or I_{Ba}) were obtained from hippocampal neurones in cell culture. Following identification of a cell as a pyramidal or non-pyramidal neurone (see Methods and Scholz & Miller, 1991), current commands were given every 10–20 s. Current responses of a pyramidal neurone evoked from a holding potential of −80 mV to test potentials ranging from −40 to +20 mV are shown in Fig. 1*A*. (±)-Baclofen (10 μM; henceforth referred to as baclofen) reduced the amplitude of I_{Ba} and slowed the activation kinetics at intermediate test potentials. With 2 mM-Ba^{2+} as the charge carrier, baclofen reduced the current measured 5 ms after the initiation of a depolarizing pulse from −80 to −20 mV by 28±3% (n = 10). In addition, the time-to-peak I_{Ba} shifted from 22±2 ms to 59±10 ms at −20 mV. When 5 μM-Ca^{2+} was used as the charge carrier, inhibition of I_{Ca} was still readily observed. For example, 10 μM-baclofen reduced I_{Ca} from a holding potential of −80 mV to a test potential

of 0 mV by 33±3% ($n = 8$).The shift in activation kinetics was much less evident when Ca^{2+} was used as the charge carrier, similar to that observed for inhibition of currents by adenosine receptor agonists in these same neurones (Scholz & Miller, 1991).

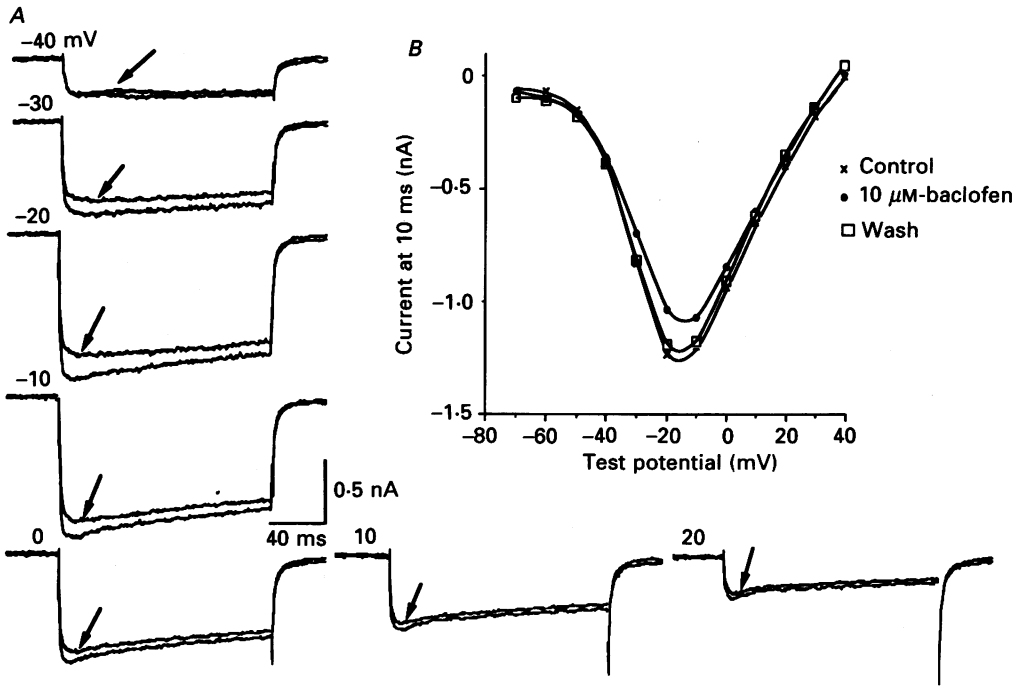

Fig. 1. Inhibition of I_{Ba} by baclofen. *A*, current responses to test potentials ranging from −40 to +20 mV from a holding potential of −80 mV in the absence and presence (arrows) of 10 μM-baclofen. *B*, inward current 10 ms into depolarizing pulses to different potentials plotted as a function of test potential for the traces shown in *A*. Charge carrier, 2 mM-Ba^{2+}.

A plot of current (measured at 10 ms) *versus* voltage for the results displayed in Fig. 1*A* is shown in Fig. 1*B*. The inhibition of I_{Ba} by baclofen was not accompanied by a shift in the voltage dependence of activation. Furthermore, there was no consistent shift in the apparent reversal potential of the current, indicating that the inhibition of I_{Ba} is not likely to be a result of activation of K^+ currents in poorly clamped regions of the cell. This conclusion is supported further by experiments described below.

Since the neurones used for these experiments have numerous processes arising from the soma, it is sometimes difficult to obtain good spatial control of the membrane potential. Indeed it is possible that the inhibition of I_{Ca} that is recorded in the soma could result from activation of K^+ currents in poorly clamped regions of the cell. However, the K^+ current that is activated by baclofen in pyramidal neurones is blocked by 2 mM-Ba^{2+} (Gahwiler & Brown, 1985). The results shown in Fig. 1 were obtained using 2 mM-Ba^{2+} as the charge carrier. Since the K^+ current that

is activated by baclofen is blocked under these conditions, these results indicate that baclofen is capable of inducing inhibition of current through Ca^{2+} channels in these cells. To address this issue further, we used selective Ca^{2+} channel antagonists to examine the effects of baclofen on Ca^{2+} channel currents more directly. In addition, these experiments provide a means of obtaining an initial assessment of the subtypes of Ca^{2+} channels that are inhibited by baclofen.

Effects of Ca^{2+} channel agonists and antagonists

Figure 2*A* shows inhibition of I_{Ba} in a pyramidal neurone in response to 10 μM-baclofen. Following recovery of this response, 5 μM-ω-conotoxin GVIA (ω-CgTX) was puffed onto the cell from a puffer pipette until block was complete. This concentration of ω-CgTX appeared to be maximally effective and the effects of ω-CgTX were not observed to recover appreciably during the course of any of the experiments described, as reported in other studies (cf. Plummer, Logothetis & Hess, 1989; Regan, Sah & Bean, 1991). ω-CgTX blocked only a portion of I_{Ca} (Fig. 2*B*; in seven cells, peak I_{Ba} was blocked by 212 ± 47 pA (24 ± 3 %) by ω-CgTX). In five of these cells, subsequent responses to baclofen were obtained and found to be reduced (Fig. 2*C*; 131 ± 61 pA (18 ± 3 %) inhibition by baclofen after ω-CgTX as compared to 244 ± 62 pA (25 ± 3 %) before toxin in the same five cells). Although the average of all the responses had a large variance, the response to baclofen decreased significantly in every cell tested. These results strongly support the conclusion that part of the baclofen-sensitive current is also blocked by ω-CgTX.

Similar types of experiments were conducted with the dihydropyridine Ca^{2+} channel antagonist nimodipine (Fig. 2*D–F*), which is believed to have a different selectivity than ω-CgTX (Plummer *et al.* 1989). Current responses were obtained at a test potential of -20 mV while the holding potential was alternated between -80 and -40 mV. The cell was held at each potential for 15 s prior to test pulse. Although 15 s is less time than is required to reach steady-state conditions (Plummer *et al.* 1989), this procedure was found to cause less run-down of I_{Ca} than does holding at a constant potential of -40 mV. Furthermore, we did not attempt to separate current components based upon voltage dependence of inactivation, but chose to use pharmacological tools to dissect current components. Since the effects of dihydropyridines are more evident at more depolarized holding potentials (Bean, 1984), we shall focus on the results obtained at the -40 mV holding potential. In addition to inhibiting current evoked from a holding potential of -80 mV (see above), baclofen inhibited I_{Ba} from a holding potential of -40 mV (Fig. 2*D*). Following recovery of the response, nimodipine (1 μM) was added to the bath leading to inhibition of part of the current (Fig. 2*E*). In four cells, 1 μM-nimodipine inhibited 164 ± 38 pA (44 ± 9 %) of Ca^{2+} channel current (pooled results from two experiments each with Ba^{2+} or Ca^{2+}) from a holding potential of -40 mV. Subsequent addition of baclofen (10 μM) in the continued presence of nimodipine led to a further inhibition of current (60 ± 12 pA; 29 ± 9 %). This compares with 119 ± 25 pA (38 ± 7 %) inhibition by baclofen in the same cells in the absence of nimodipine. These results also suggest that part of the current that is inhibited by baclofen may be nimodopine-sensitive I_{Ca}.

In further experiments, the ability of nimodipine to occlude the inhibition of I_{Ba}

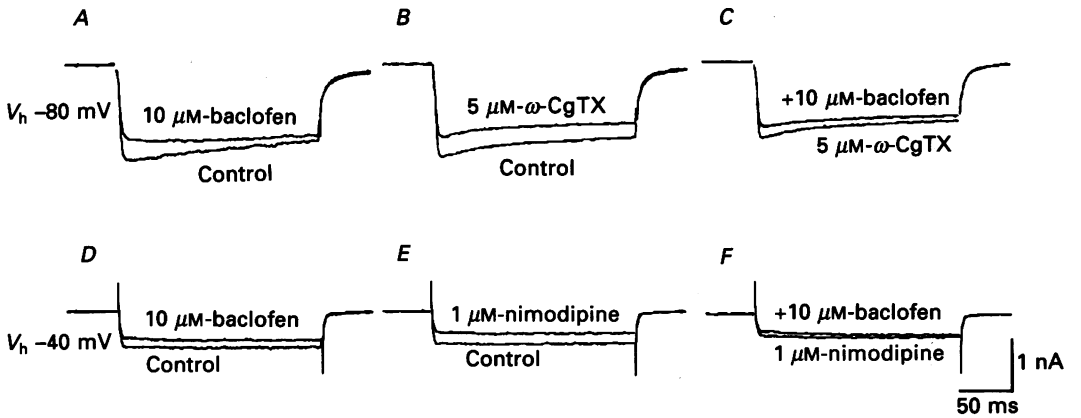

Fig. 2. ω-CgTX and nimodipine each partially occlude baclofen-sensitive current. *A*, response to baclofen applied through bath perfusion under control conditions. *B*, response to puffer application of 5 μM-ω-CgTX in the same cell after recovery from baclofen response. Toxin was applied until no further inhibition of current could be observed. *C*, response to 10 μM-baclofen after blockade of ω-CgTX-sensitive current. Charge carrier, 2 mM-Ba^{2+}. Holding potential V_h, −80 mV. Test potential, −20 mV. *D*, response to 10 μm-baclofen in another cell held at −40 mV and stepped to 0 mV (5 mM-Ca^{2+} charge carrier). *E*, response to bath application of 1 μM-nimodipine in the same cell after recovery from baclofen response. *F*, in the continued presence of 1 μM-nimodipine, application of 10 μM-baclofen yielded a smaller response.

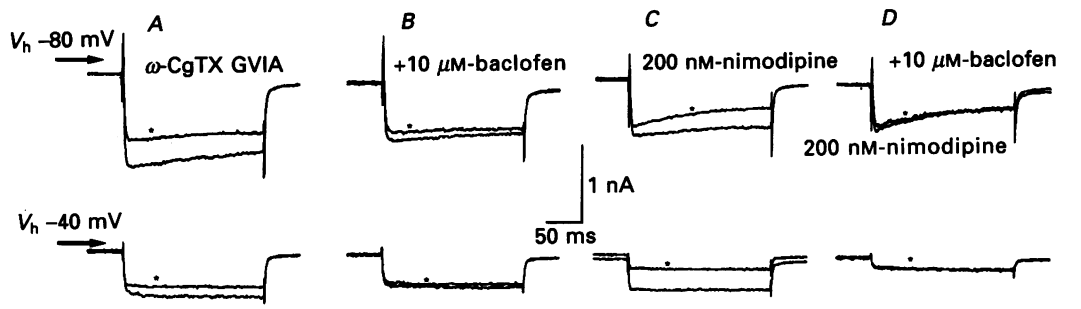

Fig. 3. ω-CgTX and nimodipine in combination block inhibition of I_{Ba} by baclofen. Cell held at alternating holding potentials of −80 and −40 mV for 15 s prior to test pulses to −20 mV. *A*, irreversible inhibition of I_{Ba} by 5 μM-ω-CgTX applied by puffer pipette until block was complete. *B*, following block of ω-CgTX-sensitive current, baclofen (applied by bath perfusion) was still able to inhibit a small portion of I_{Ba}. *C*, after recovery from the baclofen response, 200 nM-nimodipine was applied by bath perfusion and caused a further inhibition of I_{Ba}. *D*, in the continued presence of 200 nM-nimodipine, the effects of baclofen were almost completely abolished.

was assessed after the cells had been treated with ω-CgTX. As above, the cells were held at alternating holding potentials of −40 and −80 mV for 15 s prior to the test pulse. An example is shown in Fig. 3 ($n = 5$). As shown above, ω-CgTX blocked part of I_{Ca} irreversibly (Fig. 3*A*). Under these conditions, baclofen was still capable of inhibiting a small fraction of current (Figs 2*B* and 3*B*). Following recovery of the response to baclofen, addition of nimodipine (200 nM) inhibited additional current (Fig. 3*C*). This concentration of nimodipine was chosen deliberately to allow partial

recovery of the block at hyperpolarized holding potentials. Subsequent addition of baclofen, in the continued presence of nimodipine, had very little additional effect (Fig. 3*D*). The peak current evoked from a holding potential of −80 mV showed a small inhibition that is most probably a result of the relief of block by nimodipine at

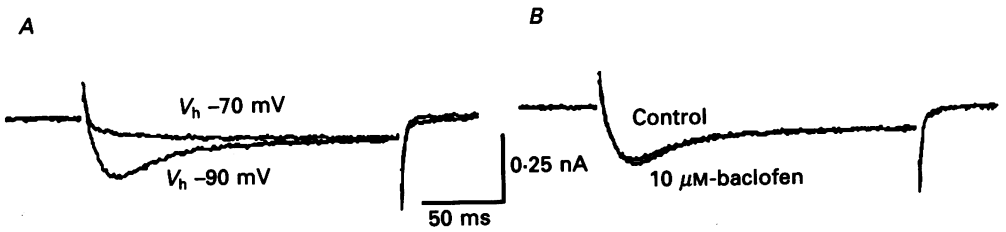

Fig. 4. Insensitivity of T-type Ca^{2+} current to baclofen. *A*, test pulses from −90 to −50 mV elicited T-type currents that were not observed during test pulses from −70 to −50 mV. *B*, application of 10 μM-baclofen had no detectable effect on T-type currents under the conditions used for these experiments. The cell shown here had a small increase in current but this was not a consistent finding.

hyperpolarized holding potentials. This finding also indicated that baclofen was still active although its effects on I_{Ba} were occluded by the pharmacological block of Ca^{2+} channels at more depolarized holding potentials. These results provide an initial indication that baclofen may inhibit both ω-CgTX-sensitive and dihydropyridine-sensitive high-threshold I_{Ca} (see Discussion).

Regardless of the type of Ca^{2+} channel that is inhibited by baclofen, the finding that the combination of ω-CgTX and nimodipine, both of which are selective for Ca^{2+} channels at these concentrations, completely occluded the effects of baclofen is very strong evidence that the inhibition of I_{Ca} is due to inhibition of Ca^{2+} channels by baclofen and is not simply a result of K^+ currents activated in dendritic processes. It is also interesting to note that a significant portion of I_{Ca} remained after application of both ω-CgTX and nimodipine (see also Regan *et al.* 1991; Mogul & Fox, 1991), although all of the inward current recorded under the conditions of these experiments was blocked by Cd^{2-} (not shown). It is apparent from the experiments described above that this remaining current is not strongly inhibited by baclofen.

In most cells that were in culture for less than about 10 days, a distinct low-threshold T-type I_{Ca} could be observed when depolarizing commands to −50 mV were elicited from a holding potential of −90 mV (Fig. 4*A*). These could be identified by their absence when commands were elicited to the same test potential from a holding potential of −70 mV (Fig. 4*A*). In contrast to high-threshold currents, the low-threshold I_{Ca} was found to be insensitive to 10 μM-baclofen (Fig. 4*B*; 2·7 ± 3·4% increase on average; $n = 4$). Since these experiments were conducted at room temperature, it is possible that inhibition of T-type current may exist at more physiological temperatures (cf. Schroeder, Fischbach & McCleskey, 1990).

Receptor pharmacology

Baclofen is considered to be a selective $GABA_B$ receptor agonist (Bowery, 1989). However, there are reports that certain molecules may inhibit I_{Ca} by actions other than binding to their intended receptor (Hockberger, Toselli, Swandulla & Lux,

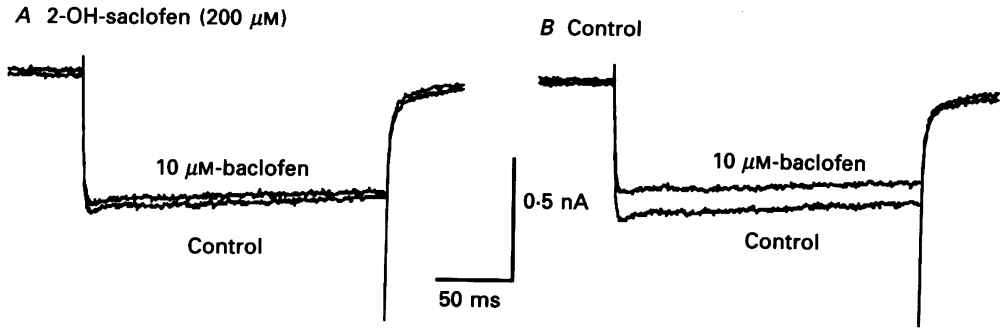

Fig. 5. Inhibition of I_{Ba} by baclofen was blocked by the $GABA_B$ receptor antagonist 2-OH-saclofen. *A*, response of a pyramidal cell to 10 μM-baclofen in the presence of 200 μM-2-OH-saclofen. 2-OH-saclofen alone had no effect. *B*, response of the same cell to 10 μM-baclofen under control conditions. V_h −80 mV, V_t −20 mV. Charger carrier, 2 mM-Ba^{2+}.

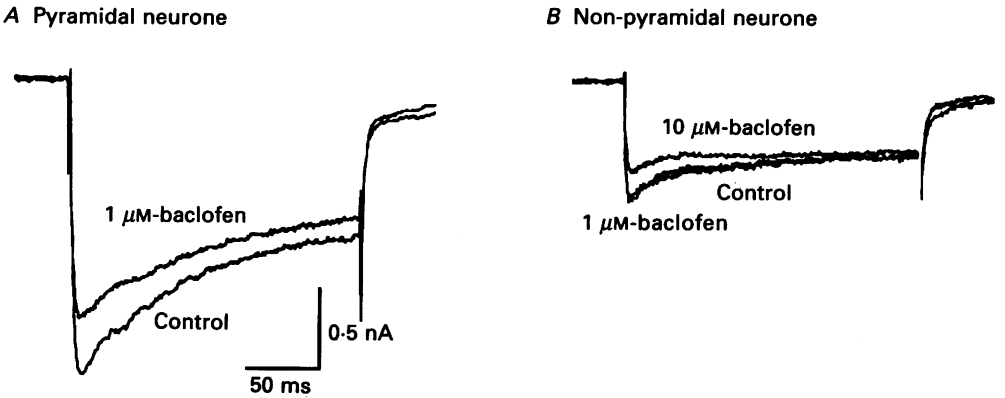

Fig. 6. Baclofen inhibits I_{Ca} in hippocampal interneurones, but to a lesser extent than in pyramidal neurones. *A*, inhibition of I_{Ca} in a pyramidal neurone by 1 μM-baclofen. *B*, in a hippocampal interneurone 1 μM-baclofen inhibited only a small fraction of I_{Ca}. Subsequent addition of 10 μM-baclofen further inhibited I_{Ca} (see text). V_h, −80 mV.

1989; Nam, Yousif & Hockberger, 1990), possibly by directly blocking Ca^{2+} channels. To test for such a possibility we used the selective $GABA_B$ receptor antagonist 2-OH-saclofen (Kerr, Ong, Johnston, Abbenante & Prager, 1989) to determine the role of $GABA_B$ receptors in the response to baclofen. Figure 5 demonstrates that 200 μM-2-OH-saclofen antagonized the actions of 10 μM-baclofen on I_{Ca}, indicating that the actions of baclofen are a consequence of binding to the $GABA_B$ receptor.

Inhibition of Ca^{2+} currents in hippocampal non-pyramidal neurones

Baclofen has been shown to produce presynaptic inhibition at both excitatory and inhibitory synaptic connections in the hippocampus (Lanthorn & Cotman, 1981; Harrison, Lange & Barker, 1988; Misgeld, Muller & Brunner, 1989; Davies, Davies & Collingridge, 1990). This implies that $GABA_B$ receptors may be found on both pyramidal neurones and non-pyramidal neurones. To determine whether baclofen inhibits I_{Ca} in interneurones, cultures were prepared from fetuses 1 to 2 days older

than usual (i.e. 18E or 19E). Such preparations were found to have significantly higher percentages of interneurones that showed GABA-like immunoreactivity (W. K. Scholz & K. P. Scholz, unpublished observations). During recordings of I_{Ca} inhibitory interneurones could be distinguished by virtue of their small currents,

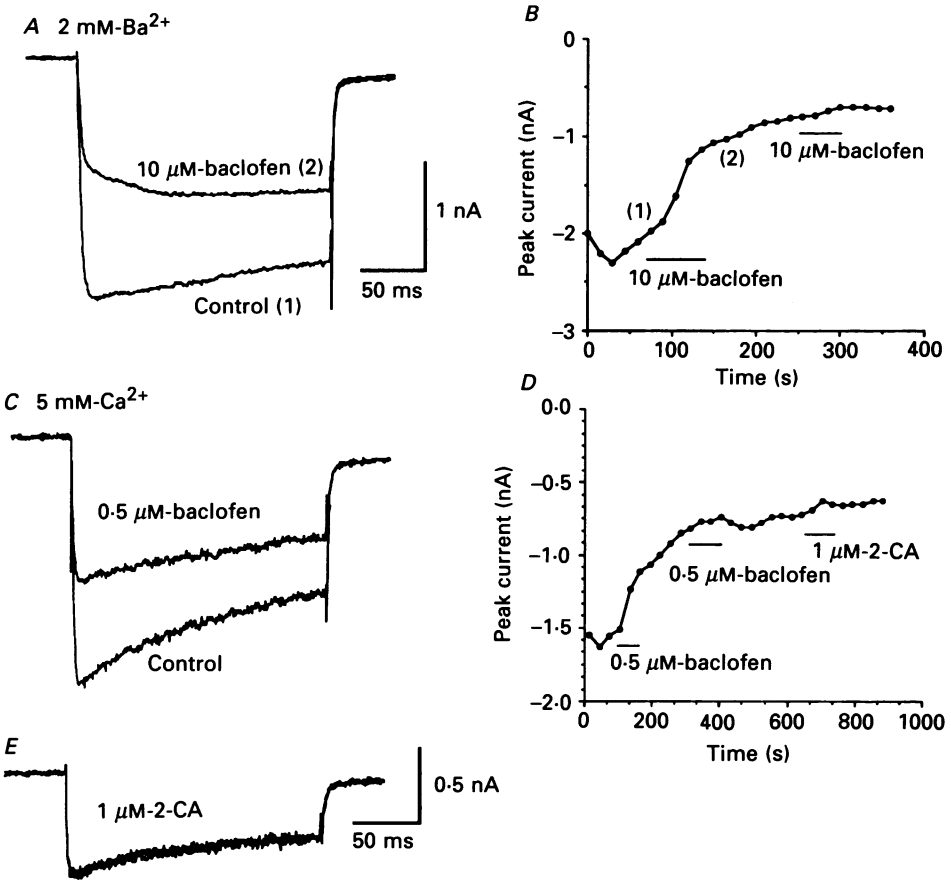

Fig. 7. Effects of intracellular perfusion of GTP-γ-S. *A*, I_{Ba} recorded in the presence of 500 μM-GTP-γ-S included in the patch pipette. Test pulses from a holding potential of -80 mV to a test potential of -20 mV. The effects of baclofen were enhanced by the presence of GTP-γ-S. *B*, current at 10 ms after initiation of the test pulse plotted as a function of experimental time. Numbers correspond to those in *A*. The effects of baclofen were irreversible in the presence of GTP-γ-S. *C*, Ca^{2+} current in another cell elicited by test pulses from -80 to 0 mV before and during application of 0·5 μM-baclofen with 500 μM-GTP-γ-S in the patch pipette. *D*, time course of currents from *C*. *E*, application of 2-Cl-adenosine (2-CA) after irreversible inhibition of I_{Ca} by baclofen yielded no further response; time course shown in *D*.

which showed comparatively little inactivation (Fig. 6), and by the inability of A1 adenosine receptor agonists to inhibit I_{Ca} (Scholz & Miller, 1991). In this same figure, it can be seen that 10 μM-baclofen inhibited I_{Ca} in non-pyramidal neurones. However, 1 μM-baclofen produced little inhibition of I_{Ca} in non-pyramidal neurones

(9±3%; $n = 5$), whereas in pyramidal neurones 1 μM (Fig. 6*A*) and concentrations as low as 0·5 μM (21±4% inhibition; $n = 7$) were quite effective. It is unclear whether this is due to a difference in receptor type, density or mode of coupling to Ca^{2+} channels. The interneurones provided more serious voltage-clamp difficulties

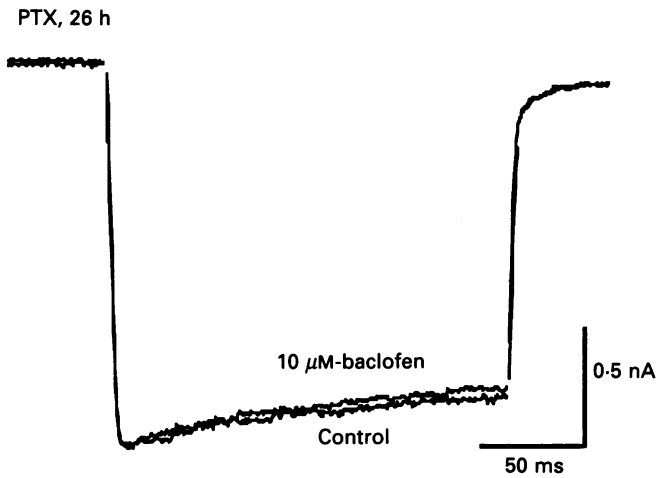

Fig. 8. Inhibition of currents by baclofen is blocked by pre-treatment of cultures with pertussis toxin. I_{Ba} evoked during test pulses to −20 mV from a holding potential of −80 mV in a cell that had been pre-treated with PTX (250 ng/ml) for 26 h. The effects of 10 μM-baclofen were almost completely abolished.

than did pyramidal neurones so that an extensive analysis of other properties of the Ca^{2+} currents was not attempted.

Role of guanine nucleotide-binding proteins

The inhibition of I_{Ca} by neurotransmitters in many cells has been shown to require a guanine nucleotide-binding protein (G-protein; Rosenthal, Hescheler, Trautwein & Schultz, 1988). We have tested the role of G-proteins in the inhibition of I_{Ca} by baclofen in pyramidal neurones by including GTP-γ-S in the patch pipette. Upon entry into whole-cell mode, I_{Ba} gradually declined in the presence of GTP-γ-S (500 μM; Fig. 7*B*). With GTP-γ-S in the cell, baclofen (10 μM) induced a large and irreversible inhibition of I_{Ba} (Fig. 7*A* and *B*; 51±3%; $n = 6$). Similar results were obtained with 5 mM-Ca^{2+} used as the charge carrier (Fig. 7*C* and *D*). In every cell, the time course of the inhibition of I_{Ca} appeared to proceed in two phases, a rapid phase followed by a smaller slower phase. The reasons for this are unclear.

Figure 7*D* and *E* shows that application of the adenosine receptor agonist 2-Cl-adenosine (2-CA), which has also been shown to inhibit I_{Ca} under control conditions in these cells (Scholz & Miller, 1991), was without effect when applied after baclofen (Fig. 7*E*; $n = 6$). This suggests that the two receptor–effector pathways may converge.

Figure 8 illustrates results obtained following pre-incubation of cultured cells with pertussis toxin (PTX; 250 ng/ml), which ADP-ribosylates and inactivates specific G-proteins (Ui, Katada, Murayama, Kurose, Yajima, Tamura, Nakamura & Nogimori,

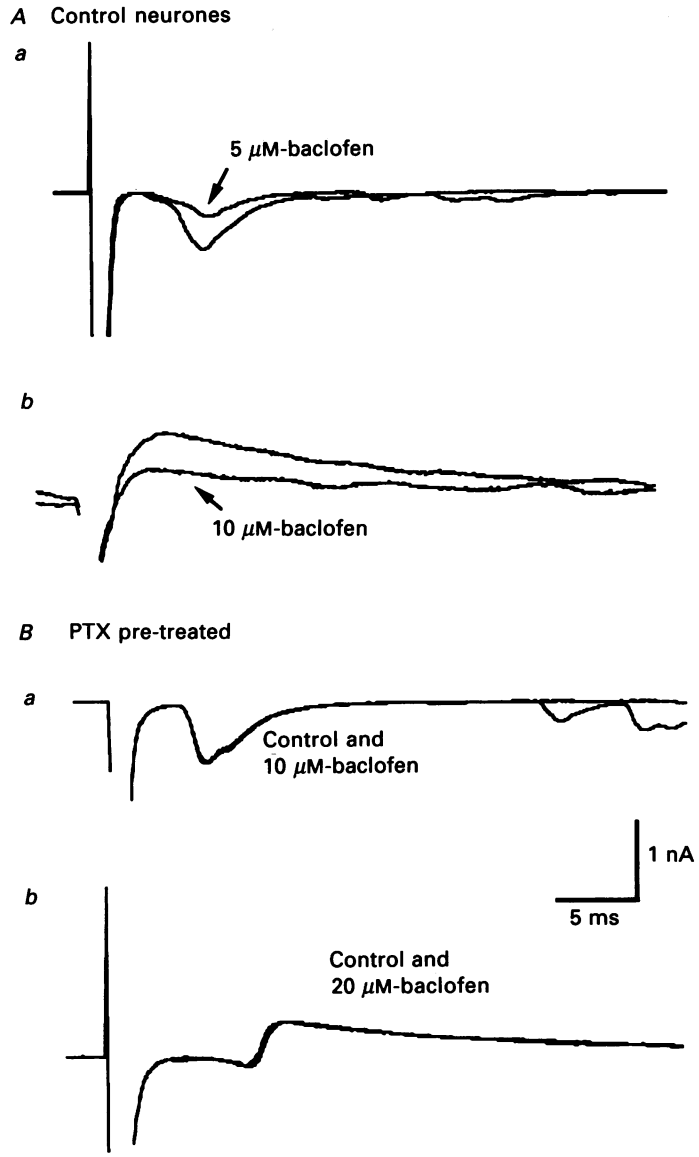

Fig. 9. Inhibition of evoked synaptic currents by baclofen and the effects of pre-treatment of cultures with PTX. *A*, monosynaptic excitatory postsynaptic currents (EPSCs) were inhibited by baclofen (*Aa*), this action was prevented by pre-treatment with PTX (250 ng/ml; *Ba*). Similarly, baclofen inhibited IPSCs (*Ab*); this action was also prevented by PTX (*Bb*).

1984). The inhibition of I_{Ca} by baclofen was almost entirely blocked by PTX (3 ± 1 % inhibition, $n = 5$).

Taken as a whole, these results support the conclusion that activation of the $GABA_B$ receptor in hippocampal pyramidal neurones inhibits I_{Ca} through the action of a PTX-sensitive G-protein.

Comparison with presynaptic inhibition

It has been reported that presynaptic inhibition of transmitter release at hippocampal synapses by baclofen may be insensitive to PTX (Dutar & Nicoll, 1988; Colmers & Pittman, 1989; Harrison, 1989). Since we found that inhibition of I_{Ca} was blocked by PTX, we re-examined this issue to test the possibility that the two actions of baclofen may have some aspects that are unrelated. Whole-cell recordings

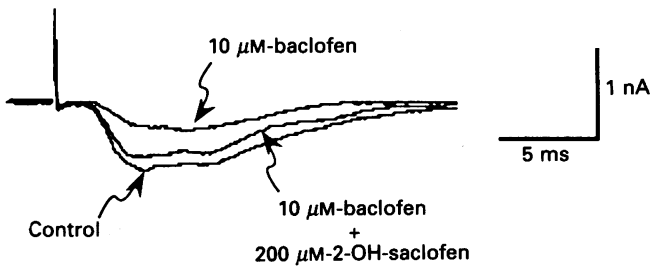

Fig. 10. 2-OH-saclofen antagonized the effects of baclofen on EPSCs. Monosynaptic EPSCs shown in the presence of 200 μM-2-OH-saclofen (control) and after addition of 10 μM-baclofen in the presence of 2-OH-saclofen and after washing out 2-OH-saclofen.

were obtained from postsynaptic neurones while an extracellular electrode was used to stimulate presynaptic neurones. Monosynaptic connections were studied and effects of changes in conductance of the postsynaptic dendrites could be assessed (for details of the technique see Scholz & Miller, 1991). Experiments in which baclofen produced a change in conductive properties of the postsynaptic cells were excluded from further analysis. In agreement with previous studies on hippocampal neurones in culture (Harrison, 1989; Forsythe & Clements, 1990), we found that in most cells, the primary locus of action of baclofen was presynaptic. Baclofen (10 μM) was found to inhibit both excitatory ($49 \pm 6\%$; $n = 6$) and inhibitory ($63 \pm 3\%$; $n = 4$) postsynaptic currents (EPSCs and IPSCs; Fig. 9*Aa* and *Ab*). However, when the cultures were pre-incubated with PTX, baclofen was ineffective at either type of synapse (Fig. 9*Ba* and *Bb*; $6 \pm 4\%$ inhibition of EPSCs; $n = 7$ and $2 \pm 4{\cdot}6\%$ inhibition of IPSCs; $n = 5$). These results support those reported by Stratton and colleagues (Stratton, Cole, Pritchett, Eccles, Worley & Baraban, 1989) who found that injection of PTX directly into the hippocampus (as opposed to injection into the third ventricle) blocked the actions of adenosine (and baclofen) on synaptic potentials in the hippocampus.

As shown for inhibition of I_{Ca} by baclofen, the $GABA_B$ receptor antagonist 2-OH-saclofen antagonized the actions of baclofen on synaptic transmission (Fig. 10).

DISCUSSION

Numerous reports have shown that baclofen activates a K^+ current in pyramidal neurones in slice preparations of the hippocampus (Nicoll *et al.* 1990). In addition, it is well established that baclofen produces presynaptic inhibition of both excitatory and inhibitory synaptic connections in the hippocampal slice preparation (Nicoll

et al. 1990) and of inhibitory synapses in culture (Harrison *et al.* 1988). While the inhibition of I_{Ca} in peripheral neurones by baclofen has been well documented (Dunlap & Fischbach, 1981; Dolphin & Scott, 1987) there is at least one report that concludes that baclofen does not inhibit sustained I_{Ca} in hippocampal pyramidal neurones (Gahwiler & Brown, 1985). Based on this finding, it was concluded that activation of a K^+ current may be the primary mechanism for presynaptic inhibition induced by baclofen in these cells. Since a number of advances have been made regarding the characterization, isolation and pharmacological properties of Ca^{2+} channels in recent years (Bean, 1989; Hess, 1990; Miller & Fox, 1990), we reinvestigated the ability of baclofen to inhibit I_{Ca} in pyramidal neurones grown in cell culture. The results demonstrate that baclofen can indeed inhibit I_{Ca} in pyramidal neurones, as well as in inhibitory interneurones from the hippocampus. Figures 2 and 3 demonstrate that the selective Ca^{2+} channel antagonists ω-CgTX and nimodipine, when applied together blocked virtually all of the actions of baclofen on I_{Ca}. This indicates that the inhibition of I_{Ca} that is observed is a consequence of direct inhibition of the activity of Ca^{2+} channels and not a result of the activation of other currents that may exist in poorly space-clamped regions of the cell.

Ca^{2+} channel subtypes inhibited by baclofen

The characterization of Ca^{2+} channel subtypes in peripheral neurones is well advanced (Bean, 1989; Hess, 1990). In contrast, there is a relative scarcity of information on the subtypes of Ca^{2+} channels present in central neurones. While single-channel studies indicate that multiple Ca^{2+} channel types exist in hippocampal neurones (Fisher, Gray & Johnston, 1990; Mogul & Fox, 1991), it is unclear whether the distinctions between whole-cell Ca^{2+} current subtypes that have been developed for peripheral neurones are appropriate for central neurones. We have chosen to use pharmacological antagonists of Ca^{2+} channels, alone and in combination, to characterize the Ca^{2+} current subtypes in cultured pyramidal neurones and to determine which are susceptible to inhibition by baclofen. The present understanding of the pharmacological and kinetic properties of Ca^{2+} current subtypes suggests that the current that we have termed ω-CgTX-sensitive current roughly corresponds to 'N'-type current described in peripheral neurones (Plummer *et al.* 1989; Regan *et al.* 1991). Regardless of this, there is good evidence that suggests that ω-CgTX is a selective blocker of a particular class of Ca^{2+} channels (Kasai, Aosaki & Fukuda, 1987; Plummer *et al.* 1989; Regan *et al.* 1991). Evidence from molecular studies of Ca^{2+} channels suggests that ω-CgTX-sensitive current may arise from the activity of a large family of different but related channels (Snutch, Leonard, Gilbert & Lester, 1990). Furthermore, it is likely that ω-CgTX does not block all channel types that contribute to whole-cell current that has previously been termed N-type current (see Hess, 1990).

The other major class of Ca^{2+} channels in peripheral neurones have been termed 'L'-type Ca^{2+} channels (Fox, Nowycky & Tsien, 1987). These channels are believed to be the primary target of dihydropyridine ligands (Tsien, Lipscombe, Madison, Bley & Fox, 1988; Plummer *et al.* 1989; Mogul & Fox, 1991). Although some evidence suggests that dihydropyridines may effect other Ca^{2+} channels (Takahashi, Wakamori & Akaike, 1989) such effects are usually seen at higher concentrations

than is needed to effect L-type current. Since it is unclear whether the classifications of high-threshold Ca^{2+} channels developed in peripheral neurones are appropriate for hippocampal pyramidal cells, we have chosen to label the different current components as ω-CgTX- and dihydropyridine-sensitive currents, as we feel that this provides the least ambiguous terminology.

ω-CgTX-sensitive Ca^{2+} channels are the targets for inhibition by a number of neurotransmitters in many cell types (cf. Kasai & Aosaki, 1989; Miller, 1990). In concord, we found that part of the Ca^{2+} current that was inhibited by baclofen was sensitive to ω-CgTX (Figs 2 and 3).

Several lines of evidence from our results suggested that baclofen might also inhibit a Ca^{2+} current or currents in addition to ω-CgTX-sensitive I_{Ca}. This is clear from the observation that baclofen inhibited more current on average than did concentrations of ω-CgTX that appeared to be maximal. This was most strikingly evident when the response to baclofen was observed while cells were loaded with GTP-γ-S. Under these conditions, baclofen inhibited over 44% of peak I_{Ca} in every cell tested, whereas the largest response to ω-CgTX that was observed was 33% inhibition. Experiments with the dihydropyridine Ca^{2+} channel antagonist nimodipine suggested that baclofen might inhibit dihydropyridine-sensitive current (Fig. 2). Furthermore, while ω-CgTX did not completely occlude the response to baclofen, the combination of ω-CgTX and nimodipine almost completely occluded the response (Fig. 3). These results indicate that baclofen may inhibit dihydropyridine-sensitive currents in pyramidal neurones.

The inhibition of dihydropyridine-sensitive currents by neurotransmitters in peripheral neurones has been the subject of much recent debate (Plummer *et al.* 1989; Bley & Tsien, 1990; Elmslie, Zhoue & Jones, 1990), For example, recent reports have concluded that norepinephrine inhibits ω-CgTX sensitive currents but not dihydropyridine-sensitive I_{Ca} in superior cervical ganglion neurones (Plummer *et al.* 1989). These conclusions were based on the use of the dihydropyridine agonist (+)−202-791 to prolong Ca^{2+} current tails that could then be attributed to dihydropyridine-sensitive currents. Such experiments are very difficult to perform in our cells due to space-clamp considerations. However, the lack of inhibition in peripheral neurones does not preclude such an action in central neurones. This is particularly evident given that the I_{Ca} of pyramidal neurones differs from that in peripheral neurones in a number of ways. First, ω-CgTX-sensitive current appears to contribute much less and dihydropyridine-sensitive current comparatively more current to the net I_{Ca} in the soma of pyramidal neurones (Figs 2 and 3 and Regan *et al.* 1991). Furthermore, in contrast to sympathetic neurones, following blockade of both of these classes of I_{Ca}, there is considerable high-threshold current remaining in pyramidal neurones (Fig. 3). Finally, as discussed above, baclofen inhibits more net I_{Ca} than does ω-CgTX. This is in contrast to reports in DRG and sympathetic neurones, in which ω-CgTX inhibits up to 90% of the total I_{Ca} whereas inhibitory neurotransmitters are generally less effective at inhibiting I_{Ca} (Plummer *et al.* 1989; Regan *et al.* 1991; Schroeder, Fischbach, Zheng & McCleskey, 1991).

Ca^{2+} current that was insensitive to either ω-CgTX or nimodipine

As mentioned above, the combination of ω-CgTX and nimodipine left a significant fraction of I_{Ca} remaining (see Fig. 3). Such results have been observed in other central neurones including other preparations of pyramidal neurones (Regan *et al.* 1991); Mogul & Fox, 1991). Our observations suggest that this residual Ca^{2+} current is not strongly inhibited by baclofen, although the small amount of inhibition present in Fig. 3*D* could represent inhibition of this current. It has been proposed that this current may represent the I_{Ca} that is present at the presynaptic terminals of neurones that release transmitter in a manner that is insensitive to ω-CgTX (Regan *et al.* 1991). Given that baclofen does not inhibit this current to a large extent, but is able to inhibit transmitter release, we feel that it is unlikely that this insensitive current is responsible for transmitter release at excitatory synapses between hippocampal pyramidal neurones. However, the degree of participation of ω-CgTX-sensitive Ca^{2+} channels in release of transmitter from hippocampal pyramidal neurones is still unclear (Jones, Kunze & Angelides, 1989; Horne & Kemp, 1989; Finch, Fisher & Jackson, 1990).

Role of G-proteins

Previous reports have shown that the K^+ current that is activated by baclofen in hippocampal pyramidal neurones requires the activity of a PTX-sensitive G-protein (Dutar & Nicoll, 1988). It is apparent that the inhibition of I_{Ca} also requires a G-protein (Fig. 8) and that this G-protein is inactivated by pre-treatment of the cultures with PTX (Fig. 9). Since some reports have found that presynaptic inhibition induced by baclofen in the hippocampus (Dutar & Nicoll, 1988; Colmers & Pittman, 1989), and in hippocampal cultures (Harrison, 1989) was insensitive to PTX, our findings with I_{Ca} raised the possibility that inhibition of I_{Ca} and presynaptic inhibition may be unrelated or act through different G-proteins or second messenger systems. However, in our preparation, the presynaptic inhibition induced by baclofen at both excitatory and inhibitory synapses was blocked by pre-treatment with pertussis toxin. It is unclear why our results differ. However, results similar to ours have been observed recently (K. Yoon & S. Rothman, personal communication) in hippocampal neurones in cell culture. Our results may underscore the importance of obtaining a positive control for the actions of PTX in the same cell in which an insensitive response has been observed.

It is possible that the actions of PTX in culture may be due to different G-proteins being present in cultured cells. However, no evidence for such an explanation was found by Al-Dahan & Thalmann (1990). Furthermore, Stratton *et al.* (1989) found that presynaptic inhibition in the hippocampal slice induced by adenosine was sensitive to PTX, although reports previous to theirs had concluded that it was not. Our results with adenosine receptor agonists supported the view that the actions of adenosine were sensitive to PTX (Scholz & Miller, 1991). Thus, it is possible that experiments with PTX in the slice may be difficult to perform, possibly resulting from the extremely small surface area of presynaptic terminals with regards to all other cell surfaces present. Thus we conclude that, with regards to the involvement

of PTX-sensitive G-proteins, our experiments have not detected a mechanistic difference between the effects of baclofen on I_{Ca} and synaptic transmitter release.

On the relationship between inhibition of I_{Ca} and inhibition of transmitter release

Our results establish the plausibility that inhibition of I_{Ca} is an important mechanism for the inhibition of transmitter release at excitatory and inhibitory synapses in the hippocampus. Unfortunately, since we have measured inhibition of I_{Ca} in the soma, we cannot conclude that this also occurs in the presynaptic terminal, where it could contribute to the inhibition of transmitter release. However, based on the experiments described in this paper, there does not appear to be a major mechanistic difference between the two phenomena. Our results support those of Finch *et al.* (1990), who found that ω-CgTX blocked part of the Ca^{2+} influx into presynaptic terminals of hippocampal neurones. Thus, there is evidence that inhibition of ω-CgTX-sensitive currents by baclofen may play an important role in presynaptic inhibition. An intriguing observation is that baclofen was clearly less effective at inhibiting I_{Ca} in inhibitory interneurones than in pyramidal neurones but was equally, if not slightly more effective at inhibiting IPSCs as compared to EPSCs. The possible significance of this result must await further experiments.

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REFERENCES

AL-DAHAN, M. I. & THALMANN, R. H. (1990). GABAb receptor binding in synaptic membranes that have (adult rat cerebrum) or lack (hippocampal culture) a postsynaptic conductance coupled to GABAb receptors. *Society for Neuroscience Abstracts* **16**, 1041.

BANKER, G. A. (1980). Trophic interactions between astroglial cells and hippocampal neurons in culture. *Science* **209**, 809–810.

BANKER, G. A. & COWAN, W. M. (1979). Further observations on hippocampal neurons in dispersed cell culture. *Journal of Comparative Neurology* **187**, 469–494.

BEAN, B. P. (1984). Nitrendipine block of cardiac calcium channels: high affinity binding to the inactivated state. *Proceedings of the National Academy of Sciences of the USA* **81**, 6388–6392.

BEAN, B. P. (1989). Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence. *Nature* **340**, 153–156.

BLEY, K. R. & TSIEN, R. W. (1990). Inhibition of Ca^{2+} and K^+ channels in sympathetic neurons by neuropeptides and other ganglionic transmitters. *Neuron* **2**, 379–391.

BOWERY, N. (1989). GABAb receptors and their significance in mammalian pharmacology. *Trends in Pharmacological Sciences* **10**, 401–407.

COLMERS, W. F. & PITTMAN, Q. J. (1989). Presynaptic inhibition by neuropeptide Y and baclofen in hippocampus: insensitivity to pertussis toxin treatment. *Brain Research* **498**, 99–105.

DAVIES, C. H., DAVIES, S. N. & COLLINGRIDGE, G. L. (1990). Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. *Journal of Physiology* **424**, 513–531.

DOLPHIN A. C. & SCOTT, R. H. (1987). Calcium channel currents and their inhibition by (−)-baclofen in rat sensory neurones: modulation by guanine nucleotides. *Journal of Physiology* **386**, 1–17.

DUDEL, J. & KUFFLER, S. W. (1961). Presynaptic inhibition at the crayfish neuromuscular junction. *Journal of Physiology* **155**, 543–562.

DUNLAP, K. & FISCHBACH, G. D. (1981). Neurotransmitters decrease the calcium conductance activated by depolarization of embryonic chick sensory neurones. *Journal of Physiology* **317**, 519–535.

DUTAR, P. & NICOLL, R. A. (1988). Pre- and post-synaptic GABAb receptors in the hippocampus have different pharmacological properties. *Neuron* **1**, 585–591.

ELMSLIE, K. S., ZHOU, W. & JONES, S. W. (1990). LHRH and GTP-γ-S modify calcium current activation in bullfrog sympathetic neurons. *Neuron* **5**, 75–80.

FINCH, D. M., FISHER, R. S. & JACKSON, M. B. (1990). Miniature excitatory synaptic currents in cultured hippocampal neurons. *Brain Research* **518**, 257–268.

FISHER, R. E., GRAY, R. & JOHNSTON, D. (1990). Properties and distribution of single voltage-gated calcium channels in adult hippocampal neurons. *Journal of Neurophysiology* **64**, 91–104.

FORSYTHE, I. D. & CLEMENTS, J. D. (1990). Presynaptic glutamate receptors depress excitatory monosynaptic transmission between mouse hippocampal neurones. *Journal of Physiology* **429**, 1–16.

FOX, A. P., NOWYCKY, M. C. & TSIEN, R. W. (1987). Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. *Journal of Physiology* **394**, 149–172.

GAHWILER, B. H. & BROWN, D. A. (1985). GABAb-receptor-activated K^+ current in voltage-clamped CA3 pyramidal cells in hippocampal cultures. *Proceedings of the National Academy of Sciences of the USA* **82**, 1558–1562.

HAMILL, O. P., MARTY, A, NEHER, E., SAKMANN, B. & SIGWORTH, F. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv* **391**, 85–100.

HARRISON, N. L. (1989). On the presynaptic action of baclofen at inhibitory synapses between cultured rat hippocampal neurones. *Journal of Physiology* **422**, 433–446.

HARRISON, N. L., LANGE, G. G. & BARKER, J. L. (1988). (−)-Baclofen activates presynaptic GABAb receptors on GABAergic inhibitory neurons from embryonic rat hippocampus. *Neuroscience Letters* **85**, 105–109.

HESS, P. (1990). Calcium channels in vertebrate cells. *Annual Review of Neuroscience* **13**, 337–356.

HOCKBERGER, P., TOSELLI, M., SWANDULLA, D. & LUX, H. D. (1989). A diacylglycerol analogue reduces neuronal calcium currents independently of protein kinase C activation. *Nature* **338**, 340–342.

HOLZ, G. G. IV, KREAM, R. M., SPIEGEL, A. & DUNLAP, K. (1989). G proteins couple alpha-adrenergic and GABAb receptors to inhibition of peptide secretion from peripheral sensory neurons. *Journal of Neuroscience* **9**, 657–666.

HORNE, A. L. & KEMP, J. A. (1989). Action of ω-conotoxin GvlA on rat hippocampal synaptic transmission *in vitro*. *Journal of Physiology* **410**, 14*P*.

JONES, O. T., KUNZE, D. L. & ANGELIDES, K. J. (1989). Localization and mobility of ω-conotoxin-sensitive Ca^{2+} channels in hippocampal CA1 neurons. *Science* **244**, 1189–1193.

KASAI, H. & AOSAKI, T. (1989). Modulation of Ca-channel current by an adenosine analog mediated by a GTP-binding protein in chick sensory neurons. *Pflügers Archiv* **414**, 145–149.

KASAI, H, AOSAKI, T. & FUKUDA, J. (1987). Presynaptic Ca-antagonist ω-conotoxin irreversibly blocks N-type Ca-channels in chick sensory neurons. *Neuroscience Research* **4**, 228–235.

KERR, D. I. B., ONG, J., JOHNSTON, G. A. R., ABBENANTE, J. & PRAGER, R. H. (1988). 2-hydroxysaclofen: an improved antagonist at central and peripheral GABAb receptors. *Neuroscience Letters* **92**, 92–96.

LANTHORN, T. H. & COTMAN, C. W. (1981). Baclofen selectively inhibits excitatory synaptic transmission in the hippocampus. *Brain Research* **225**, 171–178.

MILLER, R. J. (1990). Receptor-mediated regulation of calcium channels and neurotransmitter release. *FASEB Journal* **4**, 3291–3299.

MILLER, R. J. & FOX, A. P. (1990). Voltage-sensitive calcium channels. In *Intracellular Calcium Regulation* ed. BRONNER, F., pp. 97–138. Alan R. Liss, New York.

MISGELD, U., MULLER, W. & BRUNNER, H. (1989). Effects of (−)baclofen on inhibitory neurons in the guinea pig hippocampal slice. *Pflügers Archiv* **414**, 139–144.

MOGUL, D. J. & FOX, A. P. (1991). Evidence for four different types of Ca^{2+} channels in acutely isolated hippocampal CA3 neurones of the guinea-pig. *Journal of Physiology* **433**, 259–281.

NAM, S. C., YOUSIF, L. & HOCKBERGER, P. E. (1990). Adenosine antagonists fail to block the adenosine-induced reduction of calcium current in chick sensory neurons *Society for Neuroscience Abstracts* **16**, 357.

NICOLL, R. A., MALENKA, R. C. & KAUER, J. A. (1990). Functional comparison of neurotransmitter receptor subtypes in mammalian central nervous system. *Physiological Reviews* **70**, 513–565.

PLUMMER, M. R., LOGOTHETIS, D. E. & HESS, P. (1989). Elementary properties and pharmacological sensitivities of calcium channels in mammalian peripheral neurons. *Neuron* **2**, 1453–1463.

REGAN L. J., SAH, D. W. Y. & BEAN, B. P. (1991). Calcium channels in rat central and peripheral neurons: high-threshold current resistant to dihydropyridine blockers and ω-conotoxin. *Neuron* **6**, 269–280.

ROSENTHAL, W., HESCHELER, J., TRAUTWEIN, W. & SCHULTZ, G. (1988). Control of voltage-dependent Ca^{2+} channels by G-protein-coupled receptors. *FASEB Journal* **2**, 2784–2790.

SCHOLZ, K. P. & MILLER, R. J. (1991). Analysis of adenosine actions on Ca^{2+} currents and synaptic transmission in cultured rat hippocampal pyramidal neurones. *Journal of Physiology* **435**, 373–393.

SCHROEDER, J. E., FISCHBACH, P. S. & MCCLESKEY, E. W. (1990). T-type calcium channels: heterogeneous expression in rat sensory neurons and selective modulation by phorbol esters. *Journal of Neuroscience* **10**, 947–951.

SCHROEDER, J. E., FISCHBACH, P. S., ZHENG, D. & MCCLESKEY, E. W. (1991). Activation of μ opioid receptors inhibits transient high- and low-threshold Ca^{2+} currents, but spares a sustained current. *Neuron* **6**, 13–20.

SCHWARTZKROIN, P. A., SCHARFMAN, H. E. & SLOVITER, R. S. (1990). The hippocampal region as a model for the study of brain structure and function. In *Understanding the Brain through the Hippocampus*, ed. STORM-MATHISON, J., ZIMMER, J. & OTTERSON, O. P., pp. 269–286. Elsevier, Amsterdam.

SNUTCH, T. P., LEONARD, J. P., GILBERT, M. M. & LESTER, H. A. (1990). Rat brain expresses a heterogeneous family of calcium channels. *Proceedings of the National Academy of Sciences of the USA* **87**, 3391–3395.

STRATTON, K. R., COLE, A. J., PRITCHETT, J., ECCLES, C. U., WORLEY, P. F. & BARABAN, J. M. (1989). Intrahippocampal injection of pertussis toxin blocks adenosine suppression of synaptic responses. *Brain Research* **494**, 359–364.

TAKAHASHI, K., WAKAMORI, M. & AKAIKE, N. (1989). Hippocampal CA1 pyramidal cells of rats have four voltage-dependent calcium conductances. *Neuroscience Letters* **104**, 229–234.

TAKEUCHI, A. & TAKEUCHI, N. (1966). A study of the inhibitory action of gamma-aminobutyric acid on neuromuscular transmission in the crayfish. *Journal of Physiology* **183**, 418–432.

THALMANN, R. H. (1988). Evidence that guanosine triphosphate (GTP)-binding proteins control a synaptic response in brain: effect of pertussis toxin and GTP-γ-S on the late inhibitory postsynaptic potential of hippocampal CA3 neurons. *Journal of Neuroscience* **8**, 4589–4602.

TSIEN, R. W., LIPSCOMBE, D., MADISON, D. V., BLEY, K. R. & FOX, A. P. (1988). Multiple types of neuronal calcium channels and their selective modulation. *Trends in Neuroscience* **11**, 431–438.

UI, M., KATADA, T., MURAYAMA, T., KUROSE, T., YAJIMA, M., TAMURA, M., NAKAMURA, T. & NOGIMORI, K. (1984). Islet activating protein pertussis toxin: a specific uncoupler of receptor-mediated inhibition of adenylate cyclase. *Advances in Cyclic Nucleotide Research* **17**, 145–151.

YOON, K. & ROTHMAN, S. M. (1991). Adenosine inhibits excitatory but not inhibitory synaptic transmission in the hippocampus. *Journal of Neuroscience* (in the Press).