Br. J. Pharmac. (1986), **89**, 661–672

Effects of γ-aminobutyric acid and (−)-baclofen on calcium and potassium currents in cat dorsal root ganglion neurones *in vitro*

Brian Robertson[1] & W. Rowland Taylor.

Department of Physiology, John Curtin School of Medical Research, Australian National University, Canberra A.C.T., 2601, Australia

1 Calcium and potassium currents were examined in dialysed, isolated dorsal root ganglion neurones of the cat by use of a single electrode voltage clamp.

2 Two calcium currents were identified, a low threshold inactivating (transient) current and a higher threshold slowly inactivating (sustained) current. Both currents were blocked by 1 mM cadmium and replacement of calcium with manganese, which revealed an underlying nonspecific outward current. The sustained current disappeared with internal dialysis over a period of 20 to 30 min.

3 (−)-Baclofen (100 μM) and γ-aminobutyric acid (GABA, 100 μM) were found to reduce the peak amplitude of the sustained calcium current, an effect which became more pronounced with increasing concentrations of external magnesium (1–5 mM). In 5 mM external magnesium, 100 μM baclofen reduced the calcium current by 28%.

4 The voltage-activated delayed rectifier appeared to be the most prominent potassium current in these cells. We were unable to find any evidence for a significant contribution from calcium-activated potassium conductance or a transient potassium conductance under our recording conditions.

5 Baclofen and GABA at 100 μM had no consistent effect on the voltage-activated potassium current. Baclofen did not change the resting potassium conductance.

Introduction

The amino acid, γ-aminobutyric acid (GABA), is now believed to activate two different receptor types (Bowery *et al.*, 1983). $GABA_A$ receptors are bicuculline-sensitive and are coupled to chloride channels, whilst activation of $GABA_B$ receptors (bicuculline insensitive) has been implicated in the modulation of calcium and potassium conductances (e.g. Dunlap & Fischbach, 1981; Gahwiler & Brown, 1985). Baclofen (β-*p*-chlorophenyl-γ-aminobutyric acid), a synthetic analogue of GABA, specifically activates $GABA_B$ receptors (Bowery *et al.*, 1980). The physiological significance of $GABA_B$ receptors has yet to be determined. Systemic or oral administration of baclofen results in a reduction in skeletal muscle tone and reflex activity (Birkmayer, 1972) and electrophysiological investigations have revealed that baclofen reduces monosynaptic and polysynaptic excitation in the spinal cord (Pierau & Zimmermann, 1973; Fox *et al.*, 1978) and higher centres (Ault & Nadler, 1982; Inoue *et al.*, 1985). In particular, it has been proposed that baclofen has a presynaptic action on the terminals of primary afferent fibres, causing a reduction in excitatory transmitter release (e.g. Curtis *et al.*, 1981; Davies, 1981). Of relevance to this hypothesis is the finding that GABA (Dunlap & Fischbach, 1978) and baclofen (Dunlap, 1981; Desarmenien *et al.*, 1984) shorten the duration of calcium-dependent action potentials in dorsal root ganglion (DRG) cell bodies. These results are consistent with the notion that baclofen and GABA could reduce the release of excitatory transmitter from primary afferent terminals by reducing the calcium influx into the nerve terminal, although a depolarizing action of GABA (which is not seen with baclofen) on primary afferent terminals has also been suggested as a mechanism for presynaptic inhibition (Curtis, 1978; Curtis *et al.*, 1981). Dunlap & Fischbach (1981) found that GABA reduces the calcium current, without any action on the potassium currents in cultured chick DRG neurones. Dolphin & Scott (1986) have reported that baclofen

[1]Present address: Cellular Electrophysiology Laboratory, Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN.

decreases calcium and potassium currents in cultured rat DRG neurones. Deisz & Lux (1985) also reported that GABA and baclofen reduced the calcium current, although these authors did not examine the potassium currents. In contrast, Schlichter *et al.* (1984) suggest that the shortening of calcium spikes in rat DRG neurones by baclofen and GABA is due to a potentiation of the potassium conductance. Recent electrophysiological studies on hippocampal neurones also indicate that activation of $GABA_B$ receptors leads to an increase in potassium conductance (Newberry & Nicoll 1984; Gahwiler & Brown, 1985).

Here we have examined the effects of GABA and baclofen on calcium and potassium conductances in freshly isolated cat DRG neurones *in vitro*, using voltage clamp techniques and specific channel blockers to dissect out the various voltage-activated conductances. Cat neurones were used since these cells are large and exhibit strong calcium currents. The use of freshly isolated neurones avoids problems associated with changes in the receptor profile which can occur over time in cultured preparations (see e.g. Dunlap, 1981). In contrast to the results for central neurones and rat DRG neurones, we found a reduction in the Ca conductance with no change in the resting or voltage-activated K conductances.

Methods

Dorsal root ganglia (L_6, L_7 and S_1) with portions of central and peripheral nerve trunks were removed after dorsal laminectomy in adult cats (1.6–2.2 kg) anaesthetized with sodium pentobarbitone. Most of the connective tissue forming the capsule of the ganglion was removed with fine scissors and forceps. The cell membranes were then cleaned by enzyme treatment at 30°C. In the initial experiments each ganglion was exposed to 1% trypsin (Type IX, Sigma) in standard solution for 1 h; in later experiments, a combination of 0.5% collagenase (Type 1A, Sigma) and 0.5% trypsin was used. The latter treatment was found to be sufficient to permit good electrode sealing resistances while improving cell viability. The enzyme treatment was essential for dispersal of the neurones and for obtaining adequate sealing resistances ($> 20\ M\Omega$) between the suction electrode and the cell membrane. Enzymes were washed out of the preparation by perfusing with approximately 20 ml of standard solution before recording was attempted.

Ganglia were pinned to a Sylgard (Dow Corning) base in a recording bath fitted with a peltier temperature controller (20–22mC). Single neurones were then gently dispersed with a fine needle, under a binocular microscope (80 × magnification). Bath volume was 0.4 ml and the total dead space between a solution multiplexer and the bath was 0.5 ml. Solutions were continually perfused at a rate of 2 to 5 ml min^{-1}. The isolated neurone was separated from the ganglia thus ensuring that the exchange of solution around the cell was rapid and complete.

Solutions had the following compositions (mM):

External solutions

Standard solution: NaCl 140, $CaCl_2$ 5, N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid (HEPES) 5, glucose 10, KCl 2, pH 7.4 adjusted with NaOH.

Calcium current solution: Tris (hydroxymethyl) aminomethane chloride (Tris-Cl) 110, $CaCl_2$ 10, HEPES 5, glucose 10, CsCl 2, tetraethylammonium bromide (TEA-Br) 25, pH 7.4 adjusted with HCl.

Potassium current solution: As for the standard solution with 1 to 3 μM tetrodotoxin (TTX, Sankyo) or Na replaced with Tris and 1 mM $CdCl_2$ (or $CaCl_2$ replaced with $MnCl_2$).

H_2O_2 (0.001%) was sometimes included in solutions to increase O_2 tension and hopefully improve the viability of the neurones. $MgCl_2$ was included at concentrations of 1, 2.5, and 5 mM, $CaCl_2$ was reduced by a corresponding amount to maintain a constant divalent ion concentration.

Suction electrode internal solutions

Calcium currents: CsCl 140, ethyleneglycol-*bis*-(β-aminoethylether)-N, N-tetra-acetic acid (EGTA) 11, HEPES 10, pH 7.3 adjusted with CsOH.

Potassium currents: KCl 140, EGTA 11, HEPES 10, pH 7.3 or K-methylsulphate 140, EGTA 2, HEPES 10, pH 7.3 adjusted with KOH.

(−) Baclofen hydrochloride was a gift from Ciba-Geigy Switzerland. Bicuculline methiodide, picrotoxinin and GABA were purchased from Sigma.

Recording techniques

Suction electrode techniques were used, similar to those developed by Lee *et al.* (1980). Suction electrodes were made from Vitrex (Modulohm I/S Denmark) micro-haematocrit borosilicate glass by use of a David Kopf (model 700C) puller. Electrodes were scored against a glass coverslip and broken back to an outside diameter of 70 to 90 μm. The tips were then fire polished on a microforge to give an internal diameter of 10 to 20 μm. Suction electrode resistance was usually 0.5 to 1.0 MΩ when filled with the internal solution (see above). Micro-electrodes were held in a suction electrode holder (WPI) and mounted directly onto the headstage amplifier. Neither the electrode

nor the holder was shielded. The initial experiments were carried out using an Axon Instruments ASF 1 (maximum cycle rate 45 kHz) single electrode voltage clamp. Most of the experiments described here were done using the ASF 2 model, which allowed switching rates of up to 65 kHz with complete settling of the electrode voltage between current injection. The feedback gain was 100 nA mV^{-1} and phase lag was used to prevent ringing during the steps while maintaining optimal clamp speed.

When the tip of the suction electrode was pressed onto the cell membrane, steady suction would generally result in a 20 to 40 MΩ seal between the electrode and the cell membrane. The input resistance of the cells was 20 to 60 MΩ. Sometimes the membrane under the tip would break down spontaneously within a few seconds of obtaining the seal but usually it was necessary to apply a brief depolarizing current pulse (100 nA), to rupture the membrane. The cell soma was then isolated by breaking off the axon. An increase in cell input resistance was normally observed after the cell was isolated and the axon sealed over. Generally a length of axon remained attached to the cell body but this was never more than 100 μm and was usually less than 20 μm. In any case the remaining axon would be unlikely to have compromised the space clamp of the somatic membrane (see Brown *et al.*, 1981). Resting membrane potentials were approximately −40 to −60 mV with this technique, which compares favourably with potentials of −50 mV we obtained with conventional intracellular recording.

Current and voltage signals were digitized on line and the records were stored on magnetic disc. A 6809 (Motorola) based microprocessor controlled the data acquisition through a 10 bit analogue to digital converter, and also provided the command voltages to the voltage clamp via a digital to analogue converter. Equal and opposite pulses were applied and the resulting current traces were subsequently summed to remove contributions from linear leakage and capacitive components. No voltage-activated conductances were evident during the hyperpolarizing steps. Current and voltage signals were sampled at 160 μs per point for Ca^{2+} currents and 300 μs per point for K^+ currents. The current signal was low pass filtered at 2 kHz (4 pole Bessel), voltage was filtered at the output of the voltage clamp (30 kHz, single pole). Data were subsequently analysed on a PDP 11/44 computer. This involved subtracting the leakage and capacitive currents, and measurement of the peak amplitudes.

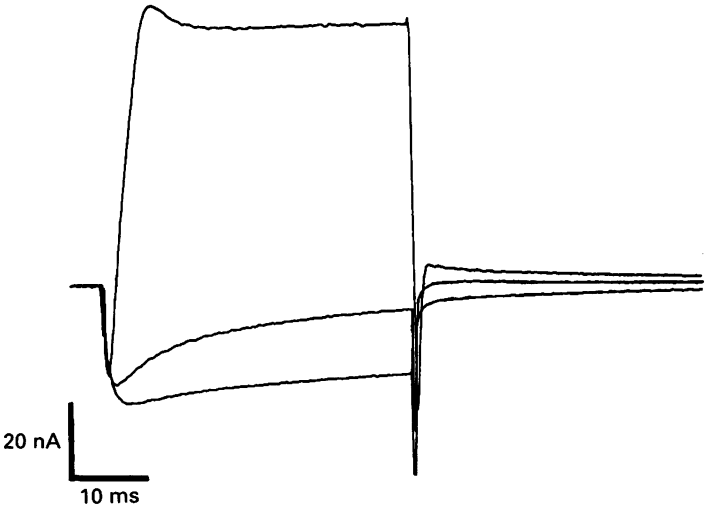

Figure 1 Separation of conductances in an isolated DRG neurone. Currents were activated by a +70 mV step from −50 mV. The top trace shows the currents recorded in standard solution. There is an initial rapid inward current followed by a large outward current. The external solution was then changed to a 'calcium current' solution. The middle trace was recorded during the exchange and the bottom trace upon completion. Potassium currents were completely suppressed by internal Cs^+ and external tetraethylammonium bromide. Cell 82G, clamp switching rate 64 kHz, diameter 65 μm, holding potential −50 mV.

Results

General observations

In standard solution, cells had resting potentials of −40 to −60 mV and would usually fire a spike of up to 100 mV in amplitude in response to a depolarizing current pulse. We do not believe that the enzyme treatment changed the electrophysiological properties of the neurones, because we found that the input resistance and active properties were similar to those of cells not treated with enzymes and examined with intracellular microelectrodes. A similar conclusion has been reached by other authors using enzyme pretreatment of mammalian DRG (Harper & Lawson 1985) and sympathetic neurones (Galvan & Sedlmeir 1984).

A major component of the action potential in normal solution is carried by Na^+ ions through TTX-sensitive channels, since 1 to 3 μM TTX blocked the action potential and fast inward current. In many cells, Ca spikes could be elicited even with only partial block of the potassium channels. Additionally, a small spike of up to 20 mV was observed in the presence of TTX and Ca channel blockers (1 mM $CdCl_2$, or substitution of $CaCl_2$ with 10 mM $MnCl_2$). This spike could only be blocked by substituting Tris for Na^+ ions. Therefore we suggest that some of these neurones possess a TTX-insensitive Na current. We have made no detailed study of either sodium current. Figure 1 illustrates voltage-activated currents elicited by a 70 mV depolarizing step from −50 mV in control solution, during solution change and after complete change over (< 3 min) to a 'calcium current' solution.

Calcium currents

Calcium currents were recorded by blocking sodium and potassium current using internal and external Ca current solutions described in the methods section. Ca^{2+} currents were not seen in all cells, but were present in approximately 80% of the neurones studied. Figure 2a shows a plot of peak inward Ca current (recorded from a holding potential of −50 mV) versus membrane potential. The I-V relationship in this figure shows two distinct components, a shoulder at negative potentials characteristic of the recently discovered 'transient' Ca current (Carbone & Lux, 1984) and a maximum at 10 to 20 mV typical of the well established 'sustained' Ca current (e.g. Fenwick *et al.*, 1982). The transient current was never greater than

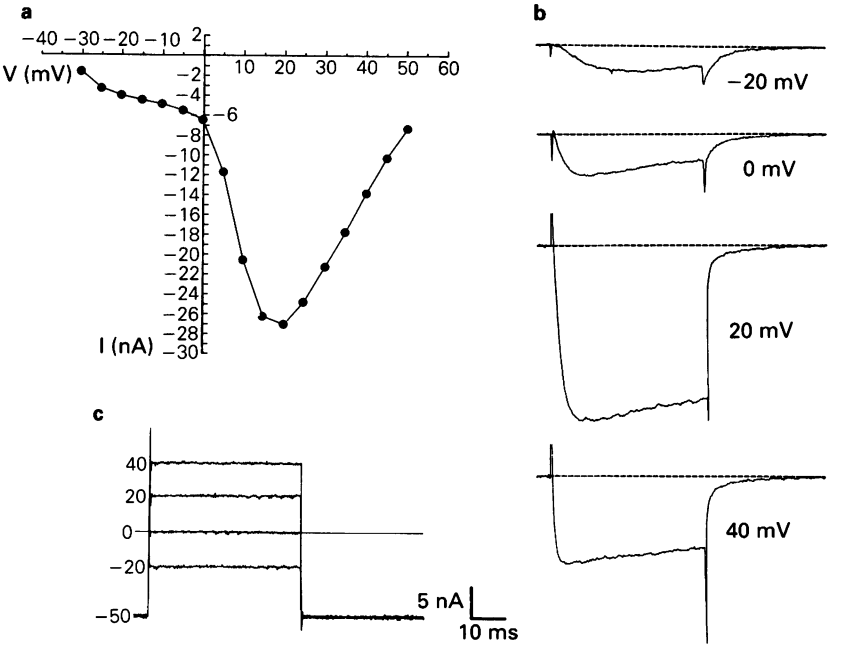

Figure 2 (a) Current-voltage plot of peak inward Ca current, note the initial shoulder at potentials less than 0 mV. (b) Sample current traces recorded at the potentials indicated. (c) Voltage traces corresponding to the current traces illustrated. Cell 65K, clamp switching rate 64 kHz, diameter 65 μm, holding potential −50 mV throughout.

15 nA at −5 mV, whereas the sustained current reached a peak of 20 to 50 nA at 10 to 20 mV. We did not observe the transient current in all neurones where the sustained current was present. The transient current showed significant inactivation during a 50 ms pulse from −90 to −20 mV, unlike the sustained current which did not inactivate appreciably during a similar pulse from −50 to +20 mV (e.g. Figures 2b and 5a, c). Since the transient current was present in very few cells (about 20%) the results focus on the sustained calcium current which we will refer to as I_{Ca}.

I_{Ca} was difficult to work with because it 'washes out' as the neurone is internally dialysed. Wash out is shown in Figure 3; the points represent the peak inward current recorded on stepping to +20 mV (from −50 mV) at 1 or 2 min intervals. The current amplitude was stable for 10 min after which a steady decline was observed for the next 20 min. In order to allow for the wash out, applications of baclofen were bracketed by control measurements and the control level was taken as the average of the peak inward current before and after baclofen application.

Since we were interested in measuring the effects of $GABA_B$ receptor activation on I_{Ca} we had to ensure that we had a pure calcium current. There were two possible sources of contamination of the Ca currents. Firstly, when Ca influx is blocked by Cd^{2+} (0.5–1 mM, Figure 4c) or by replacing Ca with Mn^{2+} (Figure 4a), a residual outward current (I_{ns}) can be seen. I_{ns} is similar to the nonspecific current previously observed by Byerly & Hagiwara (1982). The current becomes significant at positive potentials, showing voltage dependent activation (Figure 4b). This current had no effect on the measured peak I_{Ca} at potentials up to +30 mV as I_{Ca} activates much more rapidly than I_{ns} at these potentials. However, it undoubtedly contributes to the apparent inactivation of I_{Ca} during large depolarizing steps. A second possible source of contamination seen in some neurones (about 20% of the total) comes from a slowly decaying inward 'tail' current observed upon repolarization after I_{Ca} (see Figure 4d). This inward tail current is blocked by Ca channel blockers, and declined in parallel with I_{Ca}. The voltage sensitivity of the peak inward tail current followed that of the peak Ca current. It is probably not a slow calcium tail current as it was not present in every neurone where a Ca current was recorded. We believe this current is carried by chloride ions as it is

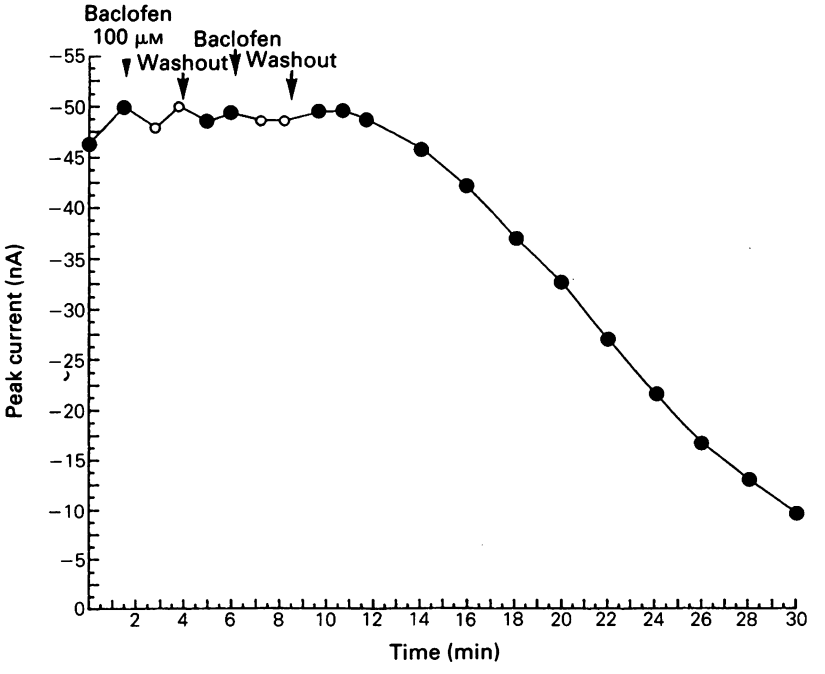

Figure 3 Illustration of the time course of the wash out of the calcium current in one cell. Each point is a single measurement obtained by stepping to +20 mV from −50 mV. Closed symbols control measurements, open symbols baclofen. Note that baclofen (100 μM) had little or no effect on the calcium current in this cell. Cell 77T, clamp switching rate 60 kHz, diameter 80 μm.

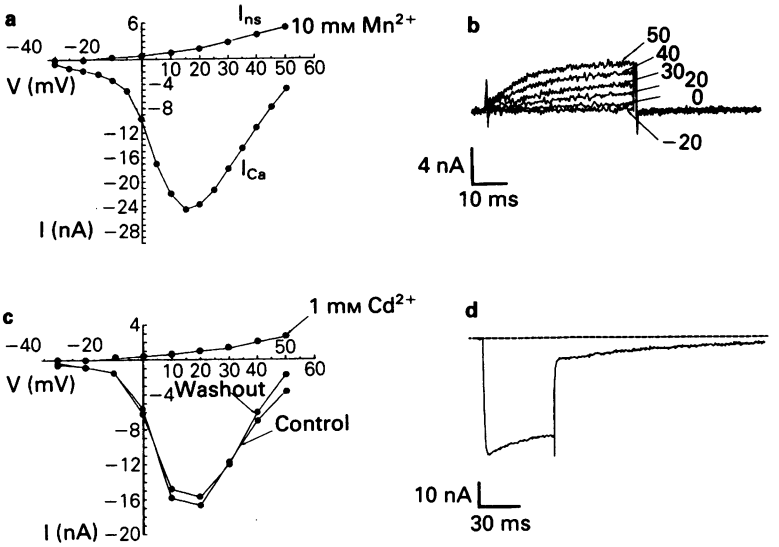

Figure 4 Possible sources of Ca current contamination. (a) Current-voltage plot before and after Ca^{2+} was replaced with Mn^{2+}. Block of the calcium current reveals the underlying nonspecific outward current, illustrated in (b). I_{ns} shows the voltage-dependent activation but is slower to activate than the calcium current at similar potentials (see Figure 2b). Clamp switching rate 60 kHz, cell 65E, diameter 60 μm. (c) Perfusion of 1 mM Cd^{2+} reversibly blocks I_{Ca}, and reveals I_{ns} in another cell. (d) The trace illustrates the size and duration of the slow inward tail that was recorded in one cell, upon repolarization after a step to +20 mV from −50 mV. Clamp switching rate 64 kHz, cell 79D, diameter 35 μm, holding potential −50 mV throughout.

recorded in solutions where Na and K currents were blocked, and the direction of the current is consistent with the null potential for chloride ions of ~0 mV in our recording situation. Since this Cl^- current would be an outward current at potentials positive to 0 mV, and therefore contaminate inward I_{Ca}, we discarded cells which showed a large slow inward component. This current appears similar to the Ca-activated chloride conductance recently reported by Owen *et al.* (1984) in cultured spinal neurones and by Mayer (1985) in cultured rat DRG. Cells showing large currents of this nature were discarded.

Effects of GABA and (−)-baclofen on Ca currents

(−)-Baclofen and GABA were applied at a concentration of 100 μM throughout. This concentration was chosen as it gave a reduction in the calcium current which could be measured reliably. When GABA was applied, we included 10 μM of bicuculline methiodide and picrotoxinin to block $GABA_A$-mediated chloride currents which can reach several nA in these cells. Even with these precautions, a transient increase in holding current was often observed, presumably due to residual activation of $GABA_A$ receptors. In contrast, no change in holding current was observed on application of baclofen, and we favoured the use of this agonist for this reason.

The effects of the agonists were assessed by measuring the change in the peak current during a step to +10 or 20 mV from a holding potential of −50 mV. Measurements were taken 1–2 min after changing solutions. The initial experiments were carried out without Mg ions in the external solution, and it was found that GABA and baclofen did not have a marked effect on either of the Ca currents (see Table 1).

The results from one such experiment are shown in Figure 5a and 5b and indicate that 100 μM baclofen has no obvious effect on either calcium current under these conditions. However, we found that both GABA and baclofen were able to reduce I_{Ca} in the presence of external Mg ions. Binding studies indicate that Mg^{2+} may potentiate binding of tritiated baclofen to crude synaptic membranes from rat brain (Bowery *et al.*, 1983). Our results indicate that 100 μM baclofen becomes more effective at reducing I_{Ca} as the external Mg concentration is raised. The results are shown in Figure 5c and 5d and Table 1. The effect shown in Figure 5d did not reverse completely. This was probably due to rundown of the calcium current as it took several minutes to record and store three full I-

Table 1 Depression of peak inward calcium current by 100 μM baclofen or GABA

Cell	Control	Peak current (nA) Baclofen or GABA (G)	% change
10 mM Ca, 0 mM Mg			
68D	9.9	9.6	−3.0
68F	29.7	28.3	−4.7
69C	19.1	17.4 (G)	−8.9
69G	9.1	9.1 (G)	0.0
70C	5.4	4.9 (G)	−9.2
		Mean ± s.e.	−5.2 ± 1.8
9 mM Ca, 1 mM Mg			
77A	6.6	6.0	−9.0
"	6.0	5.5	−8.3
77G	32.8	23.1	−29.6
77K	12.0	11.6	−3.3
"	11.0	10.8	−1.8
77N	24.2	21.3	−11.9
77T	49.2	49.0	−0.4
"	49.4	48.6	−1.6
		Mean ± s.e.	−8.2 ± 3.4
7.5 mM Ca, 2.5 mM Mg			
73D	13.5	5.5	−59.2
"	12.4	9.1	−26.6
"	13.1	10.4	−20.6
73F	14.2	10.9	−23.2
74A	30.0	27.1	−9.7
74F	42.2	39.4	−6.6
"	44.1	40.6	−7.9
"	42.0	38.0	−9.5
74H	29.9	25.6	−14.4
"	31.0	27.8	−10.3
"	25.6	19.6 (G)	−23.4
740	13.6	10.9	−19.8
"	11.4	9.1 (G)	−20.2
		Mean ± s.e.	−19.3 ± 3.8
5 mM Ca, 5 mM Mg			
79A	5.1	3.5	−31.4
"	4.7	2.9	−38.3
79B	30.8	12.4	−59.7
"	23.6	16.6	−29.7
79C	44.1	31.6	−28.3
79M	29.8	26.2	−12.1
82B	48.2	31.9	−33.8
87E	43.2	36.2	−16.2
"	42.2	37.0	−12.3
87M	9.8	7.8	−20.4
		Mean ± s.e.	−28.2 ± 4.5

In order to allow for the effects of rundown, control values represent the average of peak I_{Ca} before and after the application of agonist. Values in agonist are usaully the mean of two measures 1 min apart.

Vs. The effects of GABA and baclofen were rapidly and fully reversible (in absence of rundown), and the reduction appeared to be maintained as long as the agonist was present. There was no significant effect of baclofen on the rising phase of the calcium current.

In some neurones no effect of baclofen was observed in spite of a high concentration of Mg (e.g. Figure 3 and Table 1) and we attributed this to an absence of $GABA_B$ receptors on these cells. The variability in the responses to GABA and baclofen shown in Table 1 may also reflect differences in receptor density in the population of neurones studied.

Baclofen also reduced the amplitude of the slow inward chloride tail current by a proportion similar to that of the sustained Ca current. For example, in the cell shown in Figure 4d, baclofen reduced the slow current by 23% and the peak calcium current by 28%; the decrease was reversible for both currents. The suppression of the inward 'tail' current by baclofen is similar to the depression of this current by adenosine in rat DRG neurones (Dolphin *et al.*, 1986).

Effects of baclofen and GABA on potassium currents

Potassium currents were studied in isolation by blocking Na and Ca currents. A variety of potassium currents have been reported in mammalian neurones; these include the delayed rectifier $I_{K(V)}$, a Ca-activated K conductance $I_{K(Ca)}$ and a transient K current known as I_A. $I_{K(V)}$ was the most prominent K current in the cat DRG neurones examined in this study and was often as large as 200 nA at +40 mV. Figure 6 shows typical traces and current-voltage plots for $I_{K(V)}$ in two cells. We found no evidence for a significant I_A in these neurones; steps from −90 mV to 0 mV did not activate an outward current and no outward current was observed after a hyperpolarizing step to −120 mV from −50 mV. Both procedures should have revealed I_A if it were present (Adams *et al.*, 1982; Galvan & Sedlmeir, 1984; Belluzzi *et al.*, 1985). We could find no evidence in our experiments for the anomalous rectifier reported by Mayer & Westbrook (1983) in cultured mouse dorsal root ganglion neurones. It is very difficult to separate any $I_{K(Ca)}$ from $I_{K(V)}$ as both are activated over the same voltage range. We consider that contribution from a Ca-activated K conductance must be small, for the following reasons. Replacement of Ca with Mn or addition of 1 mM Cd^{2+} produced an increase in the total outward current, consistent with blocking a significant inward current and inconsistent with there being a large $I_{K(Ca)}$. The presence of a high concentration of internal EGTA (2 or 11 mM) might be expected to block a Ca-activated potassium current. It seems however that the free calcium near the cell membrane was not well buffered, since we were able to record a Ca-activated inward current (chloride current?) under the same conditions. Figure 6 and

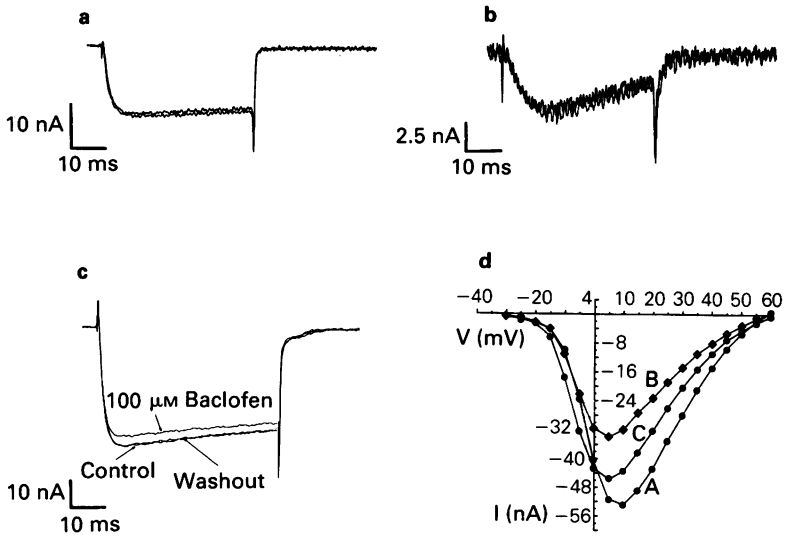

Figure 5 Baclofen (100 μM) had no effect on the sustained (a) or the transient (b) calcium current in this cell with no external Mg^{2+}. Traces show single responses before, and during baclofen application superimposed. Calcium currents were activated by a 60 mV step from −50 mV (a), and a 70 mV step from −90 mV (b). Cell 65U, clamp switching rate 61 kHz, diameter 45 μm. (c) Shows I_{Ca} in response to a 70 mV step from −50 mV, before, during and after baclofen application, with 2.5 mM external Mg^{2+}. There is a clear reversible reduction in the peak inward calcium current, which was consistent over three applications (see Table 1). Cell 74F, clamp switching rate 55 kHz, diameter 80 μm. (d) Current-voltage plot from another cell, 82B. The solid circles show the control (A) and wash out (C). The current shows some rundown. The peak currents recorded during exposure to 100 μM baclofen are illustrated by the diamonds (B) 5 mM Mg^{2+} present. Holding potential −50 mV.

Table 2 show the effect of 100 μM baclofen and GABA on activated K current. As can be seen there is no consistent effect of $GABA_B$ receptor activation on the K current with or without Mg ions. Similarly baclofen did not change the holding current, suggesting no effect on the resting K conductance.

Discussion and conclusions

DRG neurones *in vitro* provide a convenient preparation for the application of voltage clamp techniques to adult mammalian nerve cell bodies. They are readily accessible and when the soma and adjacent axon are isolated, allow for excellent space clamp. We used this preparation to examine the effect of $GABA_B$ receptor activation on voltage-activated conductances. Under our recording conditions, we observed six different conductances in these cells. The effects of TTX and Na replacement were consistent with there being two Na conductances, one blocked by TTX and the other insensitive to this toxin. Other workers have reported similar findings in sensory neurones (Yoshida *et al.*, 1978, Gallego, 1983). However, the TTX-insensitive current in cat neurones is not blocked by calcium channel blockers, unlike that reported by Bossu & Feltz (1984). Two inward Ca currents were also apparent, the low threshold rapidly inactivating Ca current (Nowycky *et al.*, 1984; Carbone & Lux 1984; Armstrong & Matteson, 1985; Fedulova *et al.*, 1985), and the higher threshold slowly inactivating current. Outward K currents were measured on depolarization to potentials greater than −20 mV, but no rigorous attempt was made to separate out the various components as has been done previously in mammalian neurones (e.g. Barrett & Barrett, 1976; Freschi, 1983). However, our results suggested that transient I_A and $I_{K(Ca)}$, if normally present, are very small. Finally a Ca-activated chloride current was observed as has been seen in egg cells (Barish, 1983) cultured spinal neurones (Owen *et al.*, 1984) and cultured neonatal rat DRG (Mayer, 1985).

Electrophysiological studies have shown that systemic and ionophoretic application of baclofen decrease excitatory neurotransmission in the spinal cord, both *in vivo* (Pierau & Zimmerman, 1973; Fox *et al.*, 1979; Curtis *et al.*, 1981; Davies, 1981) and *in vitro* (Ault & Evans, 1981). Intracellular recording from motoneurones showed changes in postsynaptic membrane properties only with high concentrations of baclofen (Fox *et al.*, 1979). However, Pierau & Zimmerman (1973) saw no changes in motoneurone

Table 2 Effect of 100 μM baclofen or GABA on the peak potassium currents

Cell	Control	*Peak current* (nA) *Baclofen or GABA* (G)	*% change*
5 mM Ca, 0 mM Mg			
63C	83.6	86.8	3.8
71D	62.8	65.2 (G)	3.8
71F	76.8	76.2 (G)	−0.8
'	60.5	58.3	−3.7
71J	130.6	127.7 (G)	−2.2
		Mean ± s.e.	0.2 ± 1.5
2.5 mM Ca, 2.5 mM Mg			
75E	164.0	151.0	−7.9
'	184.0	151.0	−17.9
'	174.5	158.0 (G)	−9.5
'	151.3	144.1 (G)	−4.8
75N	41.9	39.2	−6.4
'	40.9	43.0	5.1
76C	215.5	206.1	−4.3
'	208.1	191.9	−8.0
76G	208.5	167.6	−19.6
'	202.9	174.2	−14.1
76I	215.3	214.7	−0.3
		Mean ± s.e.	−8.0 ± 2.2
5 mM Ca, 5 mM Mg			
87Q	178.3	192.2	7.8
87W	205.5	209.4	1.9
87Z	55.3	49.8	−9.9
		Mean ± s.e.	−0.1 ± 5.2

The control values are mean measurements before and after the agonist.

properties with systemically applied baclofen, and suggested that the decrease in e.p.s.p. amplitude was due to an inhibitory action of baclofen on presynaptic primary afferent terminals. Curtis *et al.* (1981) and Curtis & Malik (1985) have shown that baclofen has no effect on transmitter release from excitatory spinal interneurones, cholinergic motor axon collaterals or from descending fibres in the dorsolateral funiculus (including the rubrospinal, long propriospinal and corticospinal tracts). The results of Glavinovic (1979) also suggest that there is no significant effect of baclofen on transmitter release from motoneurone terminals. These results may imply that there is a differential distribution of $GABA_B$ receptors between central and peripheral neurones, which might reflect different mechanisms for reducing release of transmitter from terminals.

Previous authors have reported that GABA and baclofen decrease the duration of Ca action potentials in cultured chick (Dunlap & Fischbach, 1978; Dunlap, 1981) and rat DRG neurones (McBurney, 1984; Schlichter *et al.*, 1984). Spike measurements are potentially ambiguous due to the presence of potassium currents and the extremely labile nature of the underlying Ca conductance. Their duration depends critically on the frequency of stimulation (e.g. Neering & McBurney, 1984) and on the membrane potential. In our experiments (unpublished observations) and those of others (e.g. Heyer & MacDonald, 1982), changes in membrane potential as small as 2 mV were sufficient to cause profound alterations in Ca spike duration. We therefore used voltage clamp techniques to examine Ca and K conductances more reliably. Our results indicate that baclofen and GABA significantly reduced the amplitude of the sustained Ca current only when Mg ions were present in the external solution. Our findings are similar to those of Dunlap & Fischbach (1981) and Deisz & Lux (1985) who also observed that GABA reduced I_{Ca} in cultured chick DRG neurones. Dolphin & Scott (1986) and Cottrell & Green (Physiological Society Proceedings, 1986) have found similar results in cultured mammalian DRG neurones. It is interesting that the effects of $GABA_B$ agonists as determined electrophysiologically, are potentiated by Mg ions. Bowery *et al.* (1983) have shown that baclofen binding to rat brain synaptic membranes is dependent on the concentrations of Ca^{2+} and Mg^{2+} in the incubation solution. However there are quantitative differences between the concentrations of Ca^{2+} and Mg^{2+} for maximal binding of baclofen and the reduction of calcium current observed here. This may be due to the different conditions under which the measurements were made or the source (i.e. central or peripheral nervous system) of the tissue studied. Sine & Steinbach (1986) have reported that replacing Ca^{2+} with Mg^{2+} alters the binding of acetylcholine to its receptors. Mg ions also appear to be important in modulating responses to excitatory amino acids (Ault *et al.*, 1980), but the mechanism of action here has been shown to be at the level of the channel rather than at the receptor (Nowak *et al.*, 1984).

The results of Holz *et al.* (1986), Dolphin *et al.* (1986) and Dolphin & Scott (1986) suggest that GTP binding proteins may be involved in $GABA_B$ receptor-mediated effects. The magnitude and variability of our results might be explained if important intracellular components (such as GTP binding proteins) were being removed from the cell with our recording procedure. Our preliminary experiments with intracellular electrodes also revealed a slight depression of calcium currents by GABA, but it is our experience that calcium currents are as labile with conventional intracellular techniques.

Activation of $GABA_B$ receptors in cat DRG neurones does not lead to a change in either the resting or the voltage activated potassium conductances as

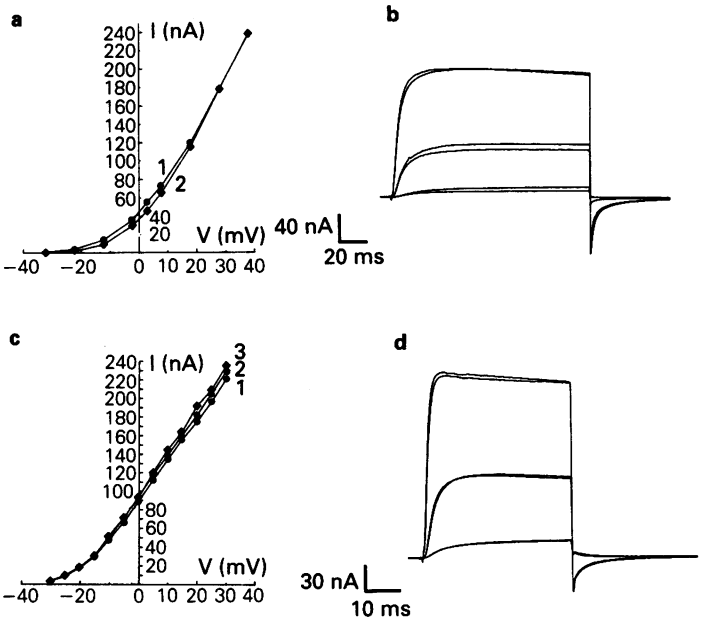

Figure 6 (a) Current-voltage plot of total potassium current, before (1) and during (2) exposure to 100 μM baclofen, with no external Mg^{2+}. Cell 62W, clamp switching rate 51 kHz. (b) Sample traces recorded at −10, 10 and 30 mV from the cell illustrated in (a), upper traces were recorded in control. (c), shows a plot of peak potassium currents recorded before (1), during (2) and after baclofen application (3), with 5 mM external Mg^{2+}. (d). Sample traces recorded at −20, 0 and 25 mV for cell 87R, illustrated in (c). The inward tail currents on the repolarization are probably due to K^+ accumulation in the perineuronal space (see e.g. Beluzzi *et al.*, 1985). Clamp switching rate 65 kHz, diameter 65 μm, holding potential −50 mV throughout.

has been suggested by Schlichter *et al.* (1984). Our results are therefore in contrast to those obtained in central neurones, where baclofen has been shown to hyperpolarize neurones (hippocampus: Newberry & Nicoll, 1984; 1985; dorsolateral septal nucleus: Gallagher *et al.*, 1984; substantia nigra: Pinnock, 1985), probably due to an increase in potassium conductance (Gahwiler & Brown, 1985). Gahwiler & Brown (1985) observed no effect of baclofen on inward currents in cultured hippocampal neurones.

Although we observed only a modest effect of baclofen and GABA on the peak inward Ca current in cat DRG neurones, it is possible that baclofen could still produce its pharmacological effects by reduction of the presynaptic Ca influx if one accepts the following arguments. Firstly, this study measured the effects of activation of non-synaptic $GABA_B$ receptors, and if $GABA_B$ synapses exist on the primary afferent terminals within the spinal cord then it is probable that the density of receptors is much higher at the synapses than on the cell bodies. A higher density of receptors could cause a larger reduction of the Ca current in the terminals than we noted in the somata, thus reducing the evoked release of excitatory transmitter (Katz & Miledi, 1965). Secondly, it appears that transmitter release is probably not directly proportional to Ca influx. Studies in accessible synapses such as the squid giant synapse have revealed that postsynaptic responses (proportional to the amount of transmitter released) vary with the second (Augustine & Eckert, 1984), or even the third power (Smith *et al.*, 1985) of presynaptic Ca current. A power function for transmitter release has also been suggested to account for the steep dependence of release on extracellular Ca concentration at the motor endplate (Jenkinson, 1957; Dodge & Rahamimoff, 1967; Katz & Miledi, 1970). If a third power relation holds at primary afferent terminals in the spinal cord, then a reduction of I_{Ca} of 20% would reduce the postsynaptic response to about half of normal.

We would like to thank Professor P.W. Gage for the use of facilities in the department during these experiments and for critical comments on the manuscript. We are grateful to Dr R.E.W. Fyffe who kindly provided us with the ganglia for these experiments. We would also like to thank Professors D.R. Curtis and A.R. Martin, and Drs R.E. Fyffe, A.J. Gibb, M.E. Krouse, S.J. Redman and D. van Helden for helpful comments on the manuscript. W.R.T. is supported by a Commonwealth Department of Education Scholarship, B.R. is supported by an Australian National University Scholarship.

References

ADAMS, P.R., BROWN, D.A. & CONSTANTI, A. (1982). M-currents and other potassium currents in bullfrog sympathetic neurones. *J. Physiol.*, **330**, 537–572.

ARMSTRONG, C.M. & MATTESON, D.R. (1985). Two distinct populations of Ca channels in a clonal cell line of pituitary cells. *Science.*, **227**, 65–67.

AUGUSTINE, G.S. & ECKERT, R. (1984). Divalent cations differentially support transmitter release at the squid giant synapse. *J. Physiol.* **346**, 257–271.

AULT, B., EVANS, R.H., FRANCIS, A.A., OAKES, D.J. & WATKINS, J.C. (1980). Selective depression of excitatory amino acid induced depolarizations by magnesium ions in isolated spinal cord preparations. *J. Physiol.*, **307**, 413–428.

AULT, B. & EVANS, R.H. (1981). The depressant action of baclofen on the isolated spinal cord of the neonatal rat. *Eur. J. Pharmac.*, **71**, 357–364.

AULT, B. & NADLER, J.V. (1982). Baclofen selectively inhibits transmission at synapses made by axons of CA3 pyramidal cells in the hippocampal slice. *J. Pharmac. exp. Ther.*, **223**, 291–297.

BARISH, M.E. (1983). A transient calcium-dependent chloride current in the immature Xenopus oocyte. *J. Physiol.*, **342**, 309–325.

BARRETT, E.F. & BARRETT, J.N. (1976). Separation of two voltage-sensitive potassium currents, and demonstration of a tetrodotoxin-resistant calcium current in frog motoneurones. *J. Physiol.*, **255**, 737–774.

BELLUZZI, O., SACCHI, O. & WANKE, E. (1985). A fast transient outward current in the rat sympathetic neurones studied under voltage-clamp conditions. *J. Physiol.*, **358**, 91–108.

BIRKMAYER, W. (ed). (1972). *Spasticity – A Topical Survey.* Vienna: Hans Huber Press.

BOSSU, J-L. & FELTZ, A. (1984). Patch-clamp study of the TTX-resistant sodium current in group C sensory neurones. *Neurosci. Lett.*, **51**, 241–246.

BOWERY, N.G., HILL, D.R. & HUDSON, A.L. (1983). Characteristics of $GABA_B$ receptor binding sites on rat whole brain synaptic membranes. *Br. J. Pharmac.*, **78**, 191–206.

BOWERY, N.G., HILL, D.R., HUDSON, A.L., MIDDLEMISS, D.N., SHAW, J. & TURNBULL, M. (1980). (−)Baclofen decreases neurotransmitter release in the mammalian CNS by action at a novel GABA receptor. *Nature*, **283**, 92–94.

BROWN, T.H., PERKEL, D.H., NORRIS, J.C. & PEACOCK, J.H. (1981). Electrotonic structure and specific membrane properties of mouse dorsal root ganglion neurones. *J. Neurophysiol.*, **45**, 1–15.

BYERLY, L. & HAGIWARA, S. (1982). Calcium currents in internally perfused nerve cell bodies of *Limnea stagnalis*. *J. Physiol.*, **322**, 503–528.

CARBONE, E. & LUX, H.D. (1984). A low voltage-activated calcium conductance in embryonic chick sensory neurones. *Biophys. J.*, **46**, 413–418.

CURTIS, D.R. (1978). Pre- and postsynaptic action of GABA in the mammalian spinal cord. *Advances in Pharmacology and Therapeutics*, volume 2, ed. Simon, P. pp. 281–298. Oxford: Pergamon Press.

CURTIS, D.R., LODGE, D., BORNSTIEN, J.C. & PEET, M.J. (1981). Selective effects of (−) baclofen on spinal synaptic transmission in the cat. *Exp. Brain Res.*, **42**, 158–170.

CURTIS, D.R. & MALIK, R. (1985). The differential effects of baclofen on segmental and descending excitation of spinal interneurones in the cat. *Exp. Brain Res.*, **58**, 333–337.

DAVIES, J. (1981). Selective depression of synaptic excitation in cat spinal neurones by baclofen: an iontophoretic study. *Br. J. Pharmac.*, **72**, 373–384.

DEISZ, R.A. & LUX, H.D. (1985). γ-Aminobutyric acid-induced depression of calcium currents of chick sensory neurons. *Neurosci. Lett.*, **56**, 205–210.

DESARMENIEN, M., FELTZ, P., OCCHIPINTI, G., SANTANGELO, F. & SCHLICHTER, R. (1984). Coexistence of $GABA_A$ and $GABA_B$ receptors on Aδ and C primary afferents. *Br. J. Pharmac.*, **81**, 327–333.

DODGE, F.A. & RAHAMIMOFF, R. (1967). Co-operative action of calcium ions in transmitter release at the neuromuscular junction. *J. Physiol.*, **193**, 419–432.

DOLPHIN, A.C., FORDA, S.R. & SCOTT, R.H. (1986). Calcium-dependent currents in cultured rat dorsal root ganglion neurones are inhibited by an adenosine analogue. *J. Physiol.*, **373**, 47–61.

DOLPHIN, A.C. & SCOTT, R.H. (1986). Inhibition of calcium currents in cultured rat dorsal root ganglion neurones by (−)-baclofen. *Br. J. Pharmac.*, **88**, 213–220.

DUNLAP, K. (1981). Two types of γ-aminobutyric acid receptor on embryonic sensory neurones. *Br. J. Pharmac.*, **74**, 579–585.

DUNLAP, D. & FISCHBACH, G.D. (1978). Neurotransmitters decrease the calcium component of sensory neurone action potentials. *Nature*, **276**, 837–839.

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

FEDULOVA, S.A., KOSTYUK, P.G. & VESELOVSKY, N.S. (1985). Two types of calcium channels in the somatic membrane of new-born rat dorsal root ganglion neurones. *J. Physiol.*, **359**, 431–446.

FENWICK, E.M., MARTY, A. & NEHER, E. (1982). Sodium and calcium channels in bovine chromaffin cells. *J. Physiol.*, **331**, 599–635.

FOX, S., KRNJEVIC, K. MORRIS, M.E., PUIL, E. & WERMAN,

R. (1978). Action of baclofen on mammalian synaptic transmission. *Neurosci.*, **3**, 495–515.

FRESCHI, J.E. (1983). Membrane currents of cultured rat sympathetic neurones under voltage clamp. *J. Neurophysiol.*, **50**, 1460–1478.

GAHWILER, B.H. & BROWN, D.A. (1985). $GABA_B$ receptor activated K^+ current in voltage clamped CA3 pyramidal cells in hippocampal cultures. *Proc. nat. Acad. Sci. U.S.A*, **82**, 1558–1562.

GALLEGO, R. (1983). The ionic basis of action potentials in petrosal ganglion cells of the cat. *J. Physiol.*, **242**, 591–602.

GALLAGHER, J.P., STEVENS, D.R. & SHINNICK-GALLAGHER, P. (1984). Actions of GABA and baclofen on neurones of the dorsolateral septal nucleus (DLSN) in vitro. *Neuropharmac.*, **23**, 7b, 825–826.

GALVAN, M. & SEDLMEIR, C. (1984). Outward currents in voltage-clamped rat sympathetic neurones. *J. Physiol.*, **356**, 115–133.

GLAVINOVIC, M.I. (1979). Effects of baclofen on junctional transmission in rat phrenic diaphragm preparation. *Neurosci.*, **4**, 2031–2035.

HARPER, A.A. & LAWSON, S.N. (1985). Electrical properties of rat dorsal root ganglion neurones with different peripheral nerve conduction velocities. *J. Physiol.*, **359**, 47–63.

HEYER, E.J. & MACDONALD, R.L. (1982). Calcium- and sodium- dependent action potentials of mouse spinal cord and dorsal root ganglion neurones in cell culture. *J. Neurophysiol.*, **47**, 641–655.

HOLZ, G.G., RANE, S.G. & DUNLAP, K. (1986). GTP-binding proteins mediate transmitter inhibition of voltage-dependent calcium channels. *Nature*, **319**, 670–672.

INOUE, M., MATSUO, T. & OGATA, N. (1985). Characterisation of pre- and postsynaptic actions of (−) baclofen in the guinea-pig hippocampus *in vitro*. *Br. J. Pharmac.*, **84**, 843–851.

JENKINSON, D.H. (1957). The nature of the antagonism between calcium and magnesium ions at the neuromuscular junction. *J. Physiol.*, **138**, 434–444.

KATZ, B. & MILEDI, R. (1965). The effect of calcium on acetylcholine release from motor nerve terminals. *Proc. R. Soc., B* **161**, 496–503.

KATZ, B. & MILEDI, R. (1970). Further study of the role of calcium in synaptic transmission. *J. Physiol.*, **207**, 789–801.

LEE, K.S., AKAIKE, N. & BROWN, A.M. (1980). The suction pipette method for internal perfusion and voltage clamp of small excitable cells. *J. Neurosci. Methods.*, **2**, 51–78.

MAYER, M.L. (1985). The calcium-activated chloride current generates the after-depolarization of rat sensory neurones in culture. *J. Physiol.*, **364**, 217–239.

MAYER, M.L. & WESTBROOK, G.L. (1983). A voltage-clamp analysis of inward (anomalous) rectification in mouse spinal sensory ganglion neurones. *J. Physiol.*, **340**, 19–45.

MCBURNEY, R.N. (1984). Membrane actions of GABA in cultured central neurones. In *Actions and Interactions of GABA and Benzodiazepines*, pp. 43–58, ed. N.G. Bowery. New York: Raven Press.

NEERING, I.R. & MCBURNEY, R.N. (1984). Role for microsomal Ca storage in mammalian neurones? *Nature*, **309**, 158–160.

NEWBERRY, N.R. & NICOLL, R.A. (1984). Direct hyperpolarizing action of baclofen on hippocampal pyramidal cells. *Nature*, **308**, 450–452.

NEWBERRY, N.R. & NICOLL, R.A. (1985). Comparison of the action of baclofen with GABA on rat hippocampal pyramiddal cells *in vitro*. *J. Physiol.*, **360**, 161–185.

NOWAK, L., BREGESTOVSKI, P., ASCHER, P., HERBET, A. & PROCHIANTZ, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. *Nature*, **307**, 462–465.

NOWYCKY, M., FOX, A.P. & TSIEN, R.W. (1984). Two components of calcium channel currents in chick dorsal root ganglion cells. *Biophys. J.*, **45**, 36a.

OWEN, D.G., SEGAL, M. & BARKER, J.L. (1984). A Ca-dependent Cl conductance in cultured mouse spinal neurones. *Nature*, **311**, 567–570.

PIERAU, F-K. & ZIMMERMANN, P. (1973). Action of a GABA-derivative on postsynaptic potentials and membrane properties of cat spinal motoneurones. *Brain Res.*, **54**, 376–380.

PINNOCK, R.D. (1984). Hyperpolarizing action of baclofen on neurones in the rat substantia nigra slice. *Brain Res.*, **322**, 337–340.

SCHLICHTER, R., DEMENEIX, B.A., DESARMENIEN, M., DESAULLES, E., LOEFFLER, J.P. & FELTZ, P. (1984). Properties of the GABA receptors located on spinal primary afferent neurones and hypophyseal neuroendocrine cells of the rat. *Neurosci. Lett.*, **47**, 257–263.

SINE, S. & STEINBACH, J.H. (1986). Acetylcholine receptor activation by a site selective ligand; nature of brief open and closed states in BC3H-1 cells. *J. Physiol.*, **370**, 357–379.

SMITH, S.J., AUGUSTINE, G.J. & CHARLTON, M.P. (1985). Transmission at voltage-clamped giant synapse of the squid: evidence for cooperativity of presynaptic calcium action. *Proc. natn. Acad. Sci. U.S.A.*, **82**, 622–625.

YOSHIDA, S., MATSUDA, Y. & SAMEJIMA, A. (1978). Tetrodotoxin-resistant sodium and calcium components of action potentials in dorsal root ganglion cells of the adult mouse. *J. Neurophysiol.*, **41**, 1096–1106.

(Received February 27, 1986.
Revised August 26, 1986.
Accepted August 29, 1986.)