Br. J. Pharmacol. (1988), **94**, 235–245

Actions of baclofen on components of the Ca-current in rat and mouse DRG neurones in culture

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1 Ca currents in rat and mouse sensory dorsal root ganglion (DRG) neurones were inhibited by concentrations of (−)-baclofen as low as 1 μM. The proportion of neurones responding to baclofen was low (<20%), except in young cultures of neonate rat DRG neurones (3 days in culture), where 86% of the neurones were responsive.

2 Three types of unitary Ca currents were observed in the rat DRG neurones, corresponding to the T-, N- and L-type currents of chick DRG neurones.

3 Baclofen produced two types of response on whole-cell currents of DRG neurones from both species. The first was on an early inactivating component of the Ca current. This early current was partially inactivated at a holding potential of −40 mV. It was also reduced during the second of a pair of depolarizing command pulses. The results suggest that this action of baclofen is due to an action on an N-type component of the current. The second response to baclofen persisted throughout the command step and was not reduced during the second of a pair of command pulses, indicating that this effect is due to an action on the L-type current.

4 Unitary or ensemble Ca currents recorded in cell-attached patches, on neurones previously shown to respond to baclofen in whole-cell clamp mode, were generally not inhibited by baclofen application external to the patch electrode. This indicates that a readily diffusible internal second messenger substance is probably not involved in coupling the $GABA_B$ receptor to the ion channels.

Introduction

Baclofen, a drug used widely for the treatment of spasticity, is believed to bind to a particular class of γ-aminobutyric acid (GABA) receptor, which has been termed the $GABA_B$ receptor (Bowery *et al.*, 1983). Baclofen is an agonist at these receptors, having similar effects to GABA. In chick and rat dorsal-root ganglion (DRG) neurones, baclofen and GABA can decrease the duration of action potentials artificially prolonged in the presence of K channel blockers (Dunlap, 1984; Désarmenien *et al.*, 1984). This effect was shown to be due to inhibition of a Ca current (Deisz & Lux, 1985; Dolphin & Scott, 1986). Such an action is important in DRG neurones because it provides a mechanism to explain the phenomenon of presynaptic inhibition of sensory input by reducing transmitter release at terminals within the spinal cord.

The effectiveness of baclofen has been reported to be variable in chick and rat DRG neurones. Some investigators have also reported a slowing of the onset of activation of the Ca current (Deisz & Lux, 1985; Dolphin & Scott, 1986). Three types of Ca channel have recently been described in chick DRG neurones (Nowycky *et al.*, 1985) which may serve different roles in neuronal function (Miller, 1987). The possibility that baclofen may preferentially act on one type of channel therefore arises. The exact mechanism by which $GABA_B$ receptors are linked to Ca channels is not yet known. Experiments of Holz *et al.* (1986), and Dolphin & Scott (1986; 1987) have indicated an involvement of G-proteins, although it is not known if the coupling is direct or via some type of second messenger.

We have studied the action of baclofen on Ca currents in rat and mouse DRG neurones. In particular, we have investigated the possibility of separate actions of baclofen on N- and L-like components of the whole cell currents and also, by making recordings of unitary currents, we have attempted to obtain evidence for the involvement of a second messenger system. Some of this work has been reported in preliminary form (Cottrell & Green, 1987).

Methods

Experiments were done at room temperature on mixed primary cultures of mouse spinal cord and DRG prepared according to the method of Ransom *et al.* (1977), and on pure DRG cultures prepared from 1–2 day old neonate rats by the method described by Forda & Kelly (1985). All cultures contained 7S-Nerve Growth Factor (7S-NGF) at a concentration of 40 ng ml^{-1}. It was necessary to maintain the mouse neurones in culture for a period of at least 2 weeks in order to be able to make stable reproducible recordings. We have found however that good stable currents may readily be recorded from rat neonate neurones after only 2–3 days in culture. In the mixed cultures of mouse neurones, DRG neurones were identified by their rounded appearance with few obvious processes. Ca currents were recorded by the whole-cell recording technique of Hamill *et al.* (1981) using an L/M EPC-7 headstage and amplifier manufactured by List-Electronic. Series resistance compensation was employed to minimize errors in the voltage-clamp due to the electrode tip resistance. Despite this precaution, some error in the clamp potential was apparent in recordings from some large neurones. However, since the deficiency in the space clamp was variable and was not correlated with the magnitude of the baclofen effect in different neurones, it can safely be assumed that baclofen was not having its action indirectly by affecting the space clamp. Patch pipettes had a resistance of 1–4 MΩ when filled with experimental solution. The standard physiological solution had the following composition in mM: 137 (or 47) tetraethylammonium chloride (TEA-Cl), 0 (or 90) NaCl, 10.6 glucose, 1.0 $MgCl_2$, 5.0 $BaCl_2$ or $CaCl_2$, 25 Cs HEPES (pH 7.4) (285 mosM) and 2 μM tetrodotoxin (TTX). In most experiments Ba was used as the charge carrier and TEA replaced all the Na in the physiological solution. When Ca was substituted for the Ba, or when 47 mM TEA was used, similar results were obtained. The standard recording pipette solution had the following composition in mM: 140 CsCl (or Cs acetate), 1 $MgCl_2$, 11 Cs-EGTA, 10 Cs-HEPES (pH 7.4). In all experiments on rat DRG neurones, Mg-ATP (2 mM) was added to the pipette solution to enhance stability of the currents. All drugs and test solution were applied by microperfusion from a pipette with a tip diameter of 3–5 μm positioned about 20 μm from the recorded cell. The drug pipette was kept out of the recording solution except when required and recovery relied on diffusion away from the recording site. The recording solution was periodically changed and 100% recovery to the control response was usually obtained. The optical isomers of baclofen and the racemic mixture were a gift from Ciba-Geigy.

Leakage currents were eliminated by digital subtraction of the response to hyperpolarizing steps of equal magnitude to the test pulses. The results were stored on tape for subsequent analysis on an IBM PC-AT via a 12-bit A/D converter, using a programme supplied by J. Dempster. To avoid possible problems of inactivation of the Ca currents, the interval between consecutive depolarizing pulses was always greater than 10 s except in experiments where twin pulses were used. Ca currents were usually evoked by commands to 0 mV or +10 mV from holding potentials of −70 to −90 mV. The step duration was varied between 30 and 80 ms. When longer pulses were given (80 ms), the holding potential was −70 mV, because the large step size required with a holding potential of −90 mV often caused instability in the recording towards the end of the hyperpolarizing step which was used for leakage cancellation. Blockade of opposing outward currents was shown to be complete by localised application of a physiological solution containing 5 mM EGTA and no Ba or Ca, and also a solution containing 10 mM Co, both of which effectively blocked the inward currents, without revealing any residual outward current.

Unitary Ca currents were recorded with a List Electronics L/M EPC-7 headstage and amplifier from cell-attached patches with a pipette containing 100 mM $BaCl_2$ and 10 mM CsHEPES, with 2 μM TTX added as a precaution to block Na channel activation. Unitary Ca currents were characterized in a physiological solution containing 150 mM aspartate, 5 mM $MgCl_2$, 20 μM K-EGTA, 10 mM K-HEPES (pH 7.4) and 2 μM TTX. For the experiments in which unitary currents and whole-cell currents were recorded from the same neurones, the physiological solution was that which was generally used for whole-cell currents and contained 5 mM Ba. Under these conditions it was found to be possible to record whole-cell currents from a neurone for a brief period (just long enough to test the effect of baclofen on the recorded currents), and then carefully withdraw the recording pipette. The neurone could then be used to record from cell-attached patches with fresh pipettes filled with the 100 mM Ba containing solution. This enabled unitary currents to be recorded from neurones which were known to respond to baclofen. Patches were voltage-clamped at a hyperpolarized potential and unitary Ca-currents were evoked by depolarizing step commands of 150 ms in most cases. Stimulation rate was 0.2 Hz. Recordings were stored on tape for subsequent analysis on an IBM PC-AT computer. With most sets of recordings there were sufficient nulls to enable them to be averaged and used for cancellation of leakage currents and most of the non-cancelled capacitance artifact. All data were filtered at 1 kHz prior to analysis.

Unitary current amplitudes were measured on a Nicolet 3091 digital oscilloscope. Selected events were measured which showed a clear plateau level and no significant inflection on the rising or falling phase.

Results

Mouse DRG neurones

Baclofen was generally used at concentrations from 10–100 μM and inhibitory effects were obtained in 30 out of 170 neurones on which it was tested. The responsiveness of mouse DRG neurones in both mixed and semi-pure cultures was low and in many plates no effect was seen. In those neurones, where an effect was seen, there was considerable variation in the magnitude of the inhibition observed. The degree of inhibition by baclofen was generally much greater towards the beginning of the command step. In 10 neurones the peak amplitude reduction in the presence of 100 μM (±)-baclofen was 24% (± 3.4%). A much larger reduction of the Ca current, of greater than 80% of the control, was seen in two cells, one of which recovered to 84% of control level, whilst the other was lost before recovery could be obtained. Effects were always rapid in onset with the maximum effect being attained within 10 to 20 s of positioning the application pipette near the recorded neurone. However, the exact time course could not be determined because currents were activated only once every 15 s. Recovery time was slower and was probably dependent on the rate of diffusion of the drug away from the recording site.

Rat DRG neurones

The proportion of rat DRG neurones that responded to baclofen was very much higher. In 2 to 4 day cultures, about 63% of 149 recorded neurones showed clear inhibitory effects of baclofen application. After 4 days in culture, the population of neurones affected by baclofen became progressively less. Only occasional neurones showed any effect of baclofen application after 6 days in culture and in some plates no responses were seen at all (see Table 1).

The percentage inhibition of the Ca current by baclofen in a number of cells is plotted in Figure 1 for intervals after the start of the step command of 30 ms and 80 ms. Responses were obtained with concentrations of baclofen down to 1 μM and were rather variable, with some neurones showing particularly large effects. As with the mouse neurones, the effect was often larger towards the start of the pulse. In many cases the percentage inhibition by the end

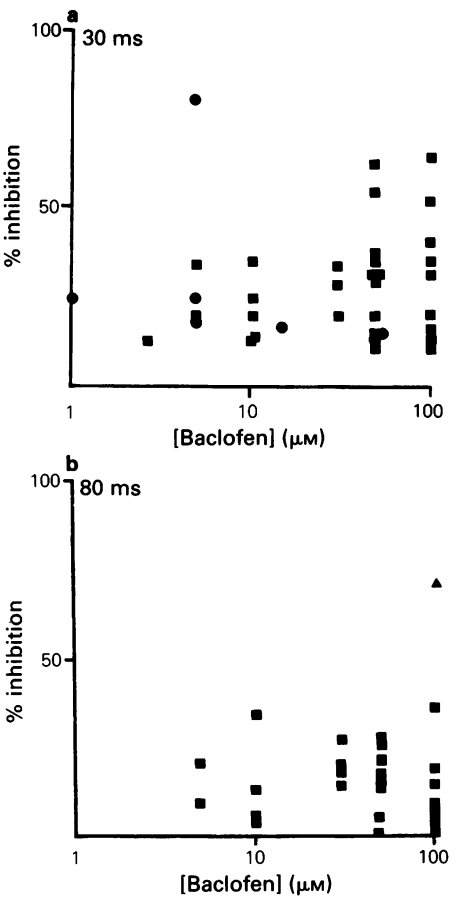

Figure 1 The percentage inhibition observed with the different concentrations of baclofen in a number of rat dorsal root ganglion (DRG) neurones at intervals of (a) 30 ms and (b) 80 ms after the start of the command step: (■) show applications of (−)-baclofen; (●) show applications of (±)-baclofen where the applied concentration has been halved since the (+)-isomer of baclofen is inactive; (▲) shows the percentage inhibition in one cell showing a large effect after 60 ms since this was the length of the step used in this case. Effects were rather variable between neurones and were generally larger towards the start of the command step.

of an 80 ms step was small. This was not always the case, however, with about 12% of all neurones which responded to baclofen showing an inhibitory effect that was prolonged throughout the 80 ms of the command step.

For the neurone illustrated in Figure 2a, a 46% reduction in the amplitude of the Ca current was seen in the presence of baclofen at 10 ms into the command step, but after 80 ms the current was only reduced by 4%. For the neurone in Figure 2b the

Table 1 The proportion of neurones responding to baclofen (various concentrations) in rat DRG cultures of different ages

Age of culture in days	*No effect*	*Reversible effect*	*% responding*
2	9	10	53
3	10	59	86
4	36	25	41
5	2	4	67
6	14	11	44
7	8	0	0
8	6	1	14
10	8	0	0
26	5	1	17
29	4	1	20
Total	102	112	52

inhibition (about 55%) was maintained throughout the duration of the 60 ms step. Activation of the current was not slowed in the presence of baclofen in this cell. This more prolonged action of baclofen on the Ca currents appears similar to large inhibitions reported to occur in cultures of rat DRG neurones which were over 4 weeks old (Dolphin & Scott, 1986). We have observed this type of response in comparatively few (12%) of the neurones that we have tested. The type of response obtained was not clearly related to the concentration of baclofen or the age of the culture over the range we have studied.

The preferential inhibition of the early part of the Ca current seen in many neurones might be explained by a reduction of an inactivating component of the current, such as the N-current, which has been described in chick DRG neurones by Nowycky *et al.* (1985). To investigate this possibility we have used twin command pulses and compared the extent of the inhibition by baclofen during the first and second pulses. A transient component of the current would still be partially inactivated during the second pulse and hence any effect of baclofen to inhibit this current will be larger during the first pulse than during the second. With commands to 0 mV or +10 mV, the amplitude of the current during the second pulse was usually slightly less than during the first pulse and the rate of activation was faster (also reported by Fenwick *et al.*, 1982, in chromaffin cells).

Figure 3 shows current recordings from two different neurones in which twin command pulses were

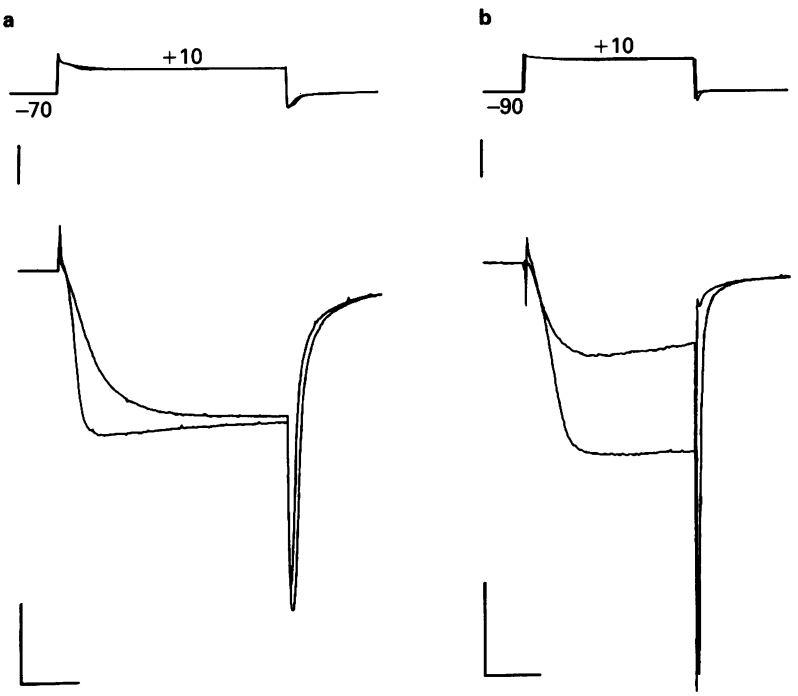

Figure 2 Different effects of baclofen application in rat DRG neurones. Upper traces show the voltage command. Lower traces show current. (a) Effect of 50 μM (−)-baclofen in a cell showing a transient inhibitory effect. (b) Prolonged effect of 100 μM (−)-baclofen in a different cell. Calibration 1 nA, 20 ms; 100 mV.

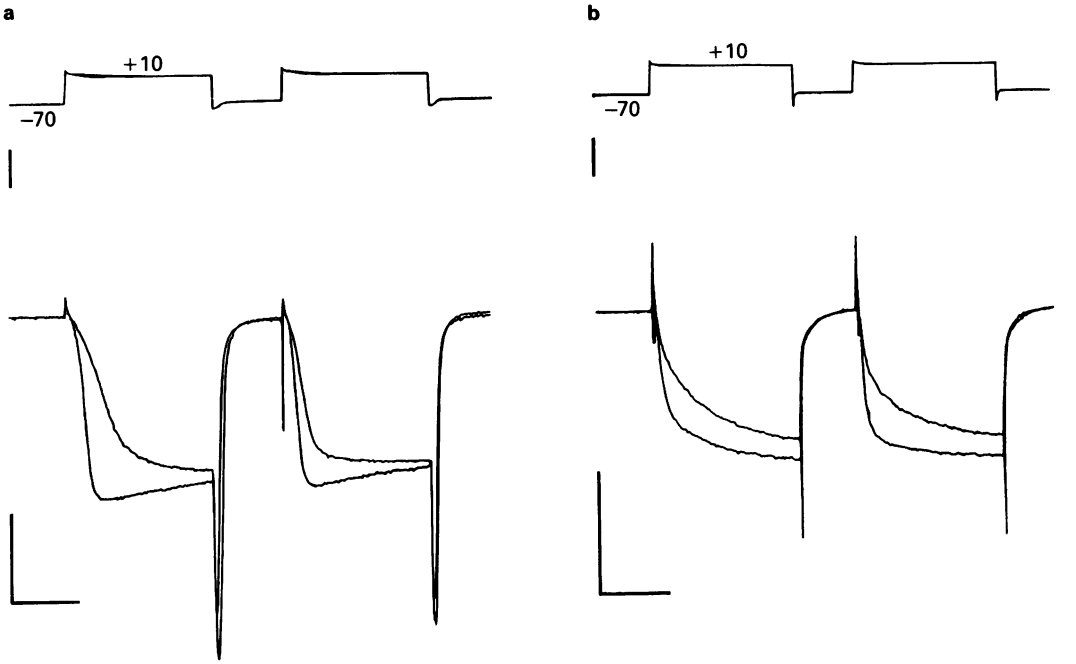

Figure 3 Responses to 100 μM (−)-baclofen application in 2 different rat DRG neurones during twin pulses. The effect of baclofen on the neurone in (a) appeared to be predominantly on an inactivating component of the current with a larger effect on the current during the first pulse. For the neurone in (b) the effect of baclofen was similar for each pulse. Upper trace shows command potential, lower traces show current. Calibration 1nA, 40ms; 100mV.

used. For the neurone in Figure 3a, which showed a preferential inhibitory effect of baclofen on the early part of the current, the inhibition seen during the first pulse was 27% while only a 15% reduction was seen during the second pulse (comparisons made from areas under the current traces). For the neurone in Figure 3b, a prolonged reduction in the amplitude of the current evoked by the first pulse was observed in the presence of baclofen. The percentage inhibition was 24% during the first pulse and 23% during the second pulse. Similar results to those shown in Figure 3a and b were seen in a further 21 and 5 neurones respectively.

Further evidence that the predominant inhibitory effect of baclofen is on an inactivating type of Ca channel was obtained by comparing the effect of baclofen on currents activated by steps to the same command potential from different holding potentials in 7 neurones. In each cell the magnitude of the inhibitory effect produced by baclofen was reduced by holding at more depolarized potential prior to the command step. Figure 4a and b shows currents activated by commands to +10mV from holding potentials of −70mV and −40mV respectively. Currents activated from −70mV were larger than those evoked from −40mV and the effect of baclofen was also larger, thus suggesting voltage-dependent inactivation of the baclofen-sensitive component. Double pulses, which were given in this neurone, also show a larger effect of baclofen during the first pulse in each case, again indicating voltage-dependent inactivation of the baclofen-sensitive component of the current.

Recordings from single Ca channels

Unitary inward currents through Ca channels were recorded from 85 cell-attached patches with pipettes containing 100mM Ba. For 40 of these the cells were exposed to a physiological solution containing a high concentration of K aspartate to nullify the neuronal resting potential and zero Ca or Ba. Under these conditions we observed 3 different types of unitary inward current which had properties similar to those already described in chick DRG neurones (Nowycky *et al.*, 1985). Figure 5 illustrates these channel types with recordings made from cell-attached patches on 3 different neurones with the physiological solution containing a high concentration of K aspartate. Three types of channel are distinguishable on grounds of conductance and the voltage-dependence of their activation and inactivation. Figure 5a shows activity of T-type channels

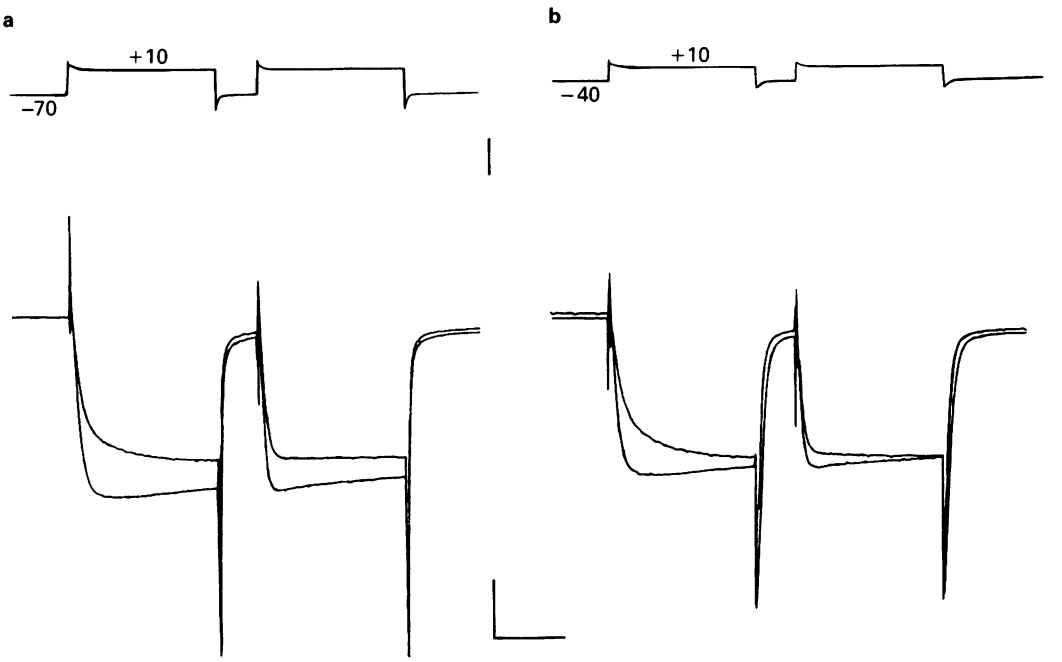

Figure 4 Responses to 50 μM (−)-baclofen application in the same rat DRG neurone with twin command pulses and different holding potentials. (a) Holding −70, step to +10 mV; (b) Holding −40 step to +10 mV. The effect of baclofen was larger when holding at −70 mV indicating that it is acting on a component of the Ca-current which is inactivated by holding at depolarized levels. There was also time-dependent inactivation of the baclofen effect as shown by the smaller effect on the second pulse in each case. Calibration 1 nA, 40 ms; 100 mV.

with a conductance of 8.2 pS during a depolarizing command from −100 to −40 mV. This channel is generally the only one active at this potential and hence has been termed the low-threshold Ca channel (Carbone & Lux, 1985). The ensemble average of T-type channels shows weak activation at this command potential but with more depolarizing command steps both activation and inactivation are accelerated. These channels were inactivated by holding at potentials less depolarized than −70 mV.

Unitary N-type Ca currents are illustrated in Figure 5b. These channels had a conductance of 13.3 pS and were partially inactivated by holding at −50 mV. Large depolarizing command steps were required for activation and with increasing depolarization the channels showed more pronounced inactivation during the command step.

The third type of channel which has been termed L-type was also observed and examples are shown in Figure 5c. These channels had a conductance of 23 pS and required steps to highly depolarized levels for activation. They were not inactivated by holding at −50 mV, neither did they show inactivation during the command step as is evident from the ensemble average current. Long openings were observed in the presence of 10 μM Bay K 8644 (a dihydropyridine) in 2 patches displaying this type of activity as illustrated in Figure 6, thus confirming that these channels correspond to L-type channels described in chick sensory neurones.

All 3 types of Ca channel could also be distinguished on similar criteria in recordings made with the physiological solution which was used for recording whole-cell currents, although in this solution the membrane potential was not fixed at zero mV (see Figure 6d).

Baclofen and single channel currents

The effect of baclofen applied over a whole cell has been tested on unitary current activity in cell-attached patches. Since baclofen was not applied to the area of membrane under the patch pipette, if an effect were to be seen then there must be involvement of a second messenger to relay the effect of receptor activation to the channels in the patch. In 5 neurones whole-cell currents were recorded prior to monitoring unitary currents from the same cell just long enough to determine whether baclofen inhibited the whole cell current; the effect of baclofen

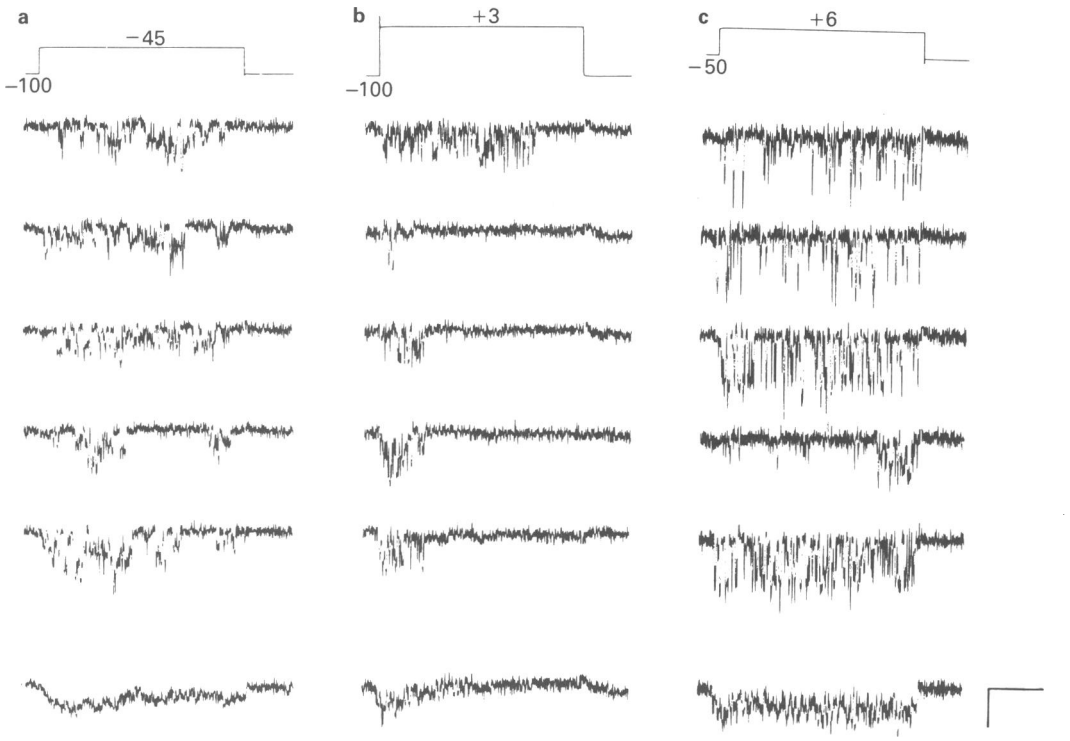

Figure 5 (a), (b) and (c) each show 5 consecutive active sweeps obtained from cell-attached patches on 3 different neurones with a physiological solution containing K aspartate and zero Ca. The remaining non-cancelled capacitance artifact following leakage subtraction was edited from all traces to improve clarity. The lowermost trace in each case shows the ensemble average of all the active sweeps recorded with the respective command protocol as shown by the uppermost traces. (a) T-type: this patch contained more than one T-type channel and in some traces multiple openings are apparent. (b) N-type: one trace contains apparently larger openings due to simultaneous opening of a number of very much smaller (at this command potential) T-type channels. There was some inconsistency of the capacitance artifacts following return to the holding potential on some individual traces and this has given rise to an apparent inward current on repolarization of the ensemble average trace. (c) L-type: high activity on these traces is due to there being more than one active channel on this patch since double openings were apparent on some traces. Calibration 1 pA; 50 ms individual traces, 0.5 pA, 50 ms ensemble average traces.

was mainly on the early part of the current in each case. Each cell was repatched after recovery with a pipette containing 100 mM Ba and unitary currents through Ca channels recorded. Ensemble averages of traces recorded before, during, and after removal of the drug pipette were compared with at least 30 traces being obtained under each condition. In only one patch, which had contained all 3 channel types, was there a clear reversible reduction of the ensemble average (Figure 7a). Each trace was averaged over 60 sweeps. In the presence of baclofen (10 μM), the integrated current under the average during the first 30 ms was reduced by 35%, while the whole cell current recorded from the same cell was reduced by 28%. Although T-type unitary currents were present in this patch they did not contribute significantly to the ensemble average at the command potential used in this experiment. In 4 other patches, which also contained N-type channel activity the ensemble average in the presence of baclofen (10 or 100 μM) was not reduced ($109 \pm 9.2\%$ of control value) (Figure 7b). We were also unable to detect any effect of baclofen on multiple Ca channel activity from four large patches on DRGs in 3 day old cultures without prior recording of whole-cell currents (Figure 7c). Most neurones (86%) at this age respond to baclofen and subsequent recording of whole-cell currents in one of these neurones did show a reduction of the Ca current by 24% with baclofen. The mean current amplitude over the first 30 ms in these patches in the presence of baclofen was 107% ($\pm 11.2\%$) of control.

It has recently been reported that inhibitory effects of noradrenaline on single Ca channel activity in

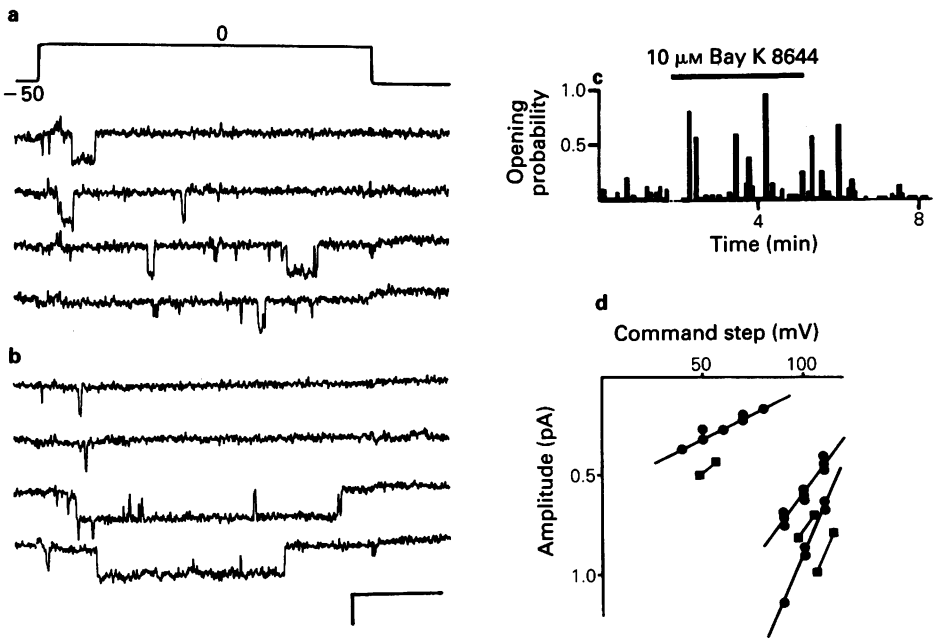

Figure 6 Effects of 10 μM Bay K 8644 on unitary currents recorded from a cell-attached patch in K aspartate/zero Ca physiological solution and 100 mM $BaCl_2$ in the recording pipette, with commands to 0 mV, from a holding potential of −50 mV. (a) Four consecutive active traces obtained immediately prior to application of Bay K 8644; (b) 4 consecutive active traces obtained immediately following positioning a blunt pipette containing 10 μM Bay K 8644 near the cell; (c) shows the opening probabilities for each trace before, during and after application of Bay K 8644 (bar denotes period of application). A brief discontinuity in the recordings occurred while positioning the drug pipette shown by the short dotted line on the time axis. (d) Graph showing amplitudes of unitary currents plotted against size of command step: (●) denote data obtained from a single cell-attached patch with the normal whole-cell recording physiological solution. Holding potential was 70 mV hyperpolarized from the neuronal membrane potential. Three sizes of unitary current were present with conductances of 5, 13.5 and 22.5 pS. (■) Indicate measurements from 3 different cell attached patches for which traces are shown in Figure 5, with the K aspartate physiological solution. The respective conductances measured for these patches, 8.2, 13.3 and 23, agree well with those measured in the standard high TEA physiological solution. The absolute holding potential was not known for patches recorded in TEA physiological solution. It must have been less than the −100 mV at which patches were mostly held in K aspartate solution since the points are displaced by 10–20 mV along the voltage axis. Calibration for (a) and (b): 1 pA, 40 ms.

cell-attached patches on rat DRG neurones may be obtained when using a zero Ca physiological solution (Ewald & Miller, 1987) and recording pipettes containing 100 mM Ba. In 3 day old neurones we have recorded multiple unitary Ca channel activity from 4 patches with a bathing solution containing zero Ca, EGTA, and 5 mM Mg added to enable baclofen to bind to the $GABA_B$ receptor (Bowery *et al.*, 1983). For 2 of these cells the physiological solution contained a high concentration of TEA-Cl (137 mM), while for the other 2 cells the physiological solution contained K aspartate (150 mM). The averaged Ca channel activity in these patches was unaffected by the presence of baclofen, the mean current integral (2–30 ms) being 103% (±2.9%) of control. It may therefore be that $GABA_B$ receptors are coupled to Ca channels by a different mechanism from noradrenaline, or possibly that a different Ca channel is involved.

Discussion

Our results confirm that baclofen has variable depressant actions on Ca currents in mouse and rat DRG neurones as has been previously described for chick DRG neurones (Deisz & Lux, 1985) and rat neurones (Dolphin & Scott, 1986; 1987). Inhibitory effects of baclofen on mouse DRG neurones were similar to those seen in the rat DRG neurones although the number of neurones in which responses were obtained was low in the mouse. This may be

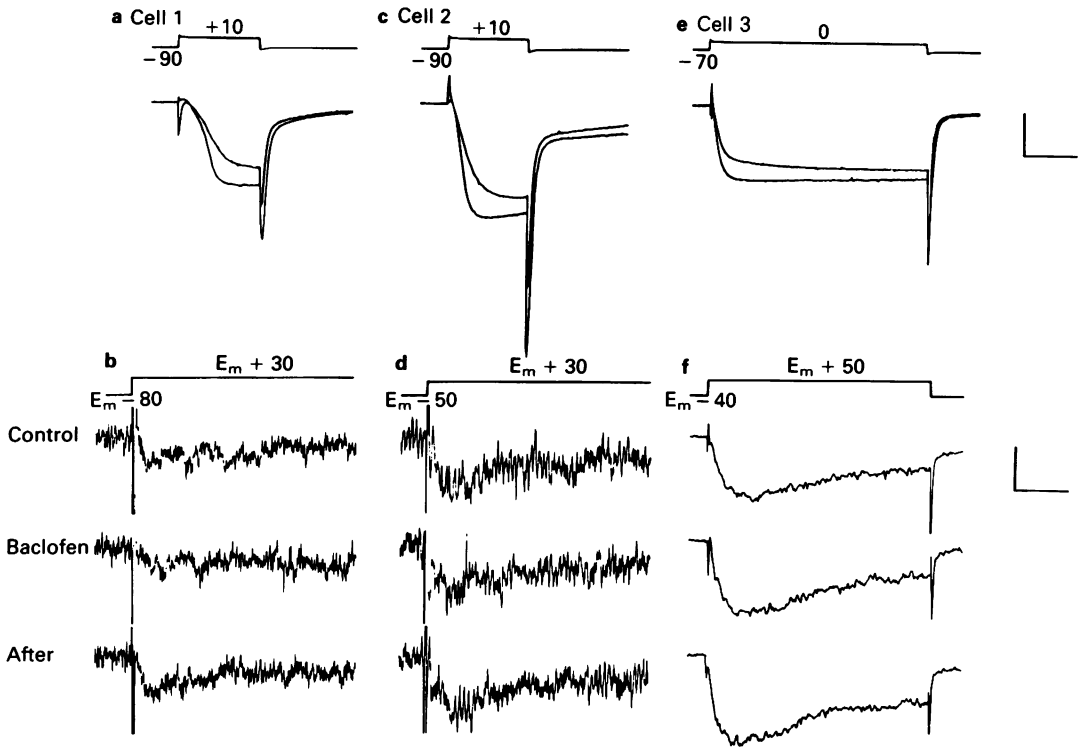

Figure 7 Traces in (a), (c) and (e) show transient inhibitory effects of baclofen on whole-cell currents in 3 different neurones; (b), (d) and (f) respectively show averaged unitary Ca current activity recorded from cell-attached patches on the same 3 cells before, during, and after application of baclofen. Whole-cell currents were recorded before the unitary currents except in cell 3 where they were done after. Each cell-attached patch contained active T-, N- and L-type channels. At the command potentials used in each case the major part of the inactivating component of the averaged unitary current record was due to opening of N-type channels. The cell-attached patch for which traces are shown in (f) was obtained using a larger recording pipette size than (a) or (b) and contains multiple Ca channels (N.B. different calibration for f). Only cell 1 showed a reversible effect of baclofen application on the averaged unitary current activity (60+ sweeps in each case). The current integral under the average trace (2–30 ms) was reduced to 65% while the whole-cell current was reduced to 72% by baclofen. Neither cell 2 nor cell 3 showed a clear effect of baclofen on the averaged unitary currents (30+ sweeps in each case) although the whole-cell currents were reduced to 76% and 74% respectively in the presence of baclofen. The integrated average unitary current activity (2–30 ms) was respectively 94% and 98% of control. The control value of the integrated average unitary current activity was taken as the mean value calculated from the traces before and after removal of the baclofen pipette. This was done since in some cases there was either an increasing or a decreasing trend in the level of activity (e.g. cell 3). Calibration: horizontal 20 ms, all traces; vertical 1 nA in (a), (c) and (e); 0.2 pA in (b) and (d); 1.4 pA in (f).

directly related to the age of the cultures in which recordings were made, because we have found a very high proportion of neurones showing responses to baclofen in young cultures prepared from neonatal rats. Days 3–4 were found to be the optimum time to observe the effects of baclofen with the rat neonatal cells, with the number of responding neurones decreasing over a period of a few days thereafter. Changes in the ionic currents present in the cell membrane of neonatal rat motoneurones have been reported by Walton & Fulton (1986). They observed that Ca spikes could only be elicited in animals 3–5 days old. Also, action potentials in non-mammalian neonatal neurones have been shown to have a more prominent Ca-dependent component than more mature cells (Spitzer, 1979). The difference may reflect variation in the numbers and distribution of Ca channels, or perhaps changes in the regulation of different types of Ca channels. Our results suggest one or a combination of the following: (i) The distribution of susceptible Ca channels becomes altered such that they are less accessible to voltage clamping

at the soma. (ii) The number of receptors on the perikarya decreases as the number of days in culture increases. (iii) The channels maintain the same distribution but the receptors to which they are coupled (either directly or indirectly) become distributed so that they only affect channels situated remotely from the perikarya. (iv) Some other control mechanism on the same channels becomes more important as the cells get older. In the cultured rat DRG neurones from which we have recorded, the magnitude and time course of the Ca currents were variable in different neurones and we have observed no correlation with their age in culture. Our results contrast with those of Dunlap (1984) on chick DRG neurones where 60% of neurones were found to respond to baclofen on day 3, while 90–100% of neurones responded to baclofen after 7 days in culture. The difference may be due to the different culture technique and in particular the age of the cells on preparation of the cultures, or it may be due to a species difference.

The preferential inhibitory effect of baclofen on the N component of the Ca current is of particular interest because of the probable connection with presynaptic inhibition of transmitter release. Uptake and release studies on the synaptosomal and cultured cells (Reynolds *et al.*, 1986) have implicated N-type Ca channels in the release process in sympathetic neurones. In DRG neurones in culture, however, there is evidence of a role for L-type channels in transmitter release also although this was not the case in adult spinal cord slices (Miller, 1987). This is consistent with release studies in brain slices which have shown inhibitory effects of baclofen on transmitter release (Bowery *et al.*, 1980). Currents through T-type channels may also be affected but this alone would not explain the observed time-dependent actions of baclofen since they are not clearly present in all neurones in which responses were obtained. Also T-type channels do not contribute significantly to the whole-cell current activated at $+10\,mV$ when Ba^{2+} are used as the charge carrier. Deisz & Lux (1985) have reported that there was no voltage-dependence for the effect of GABA on the amplitude of Ca currents in chick DRG neurones, and their results indicate a predominant effect on the non-inactivating current in these cells. Only the minority of neurones in our cultures showed an action of baclofen on the non-inactivating component (carried by L-type channels), although in some cases the distinction was not clear. Dolphin & Scott (1986) described large effects of baclofen on neurones in 4–8 week old rat DRG cultures accompanied by slowed activation such as we have seen in only a small proportion of neurones. We have not observed an elevated number of neurones showing effects on the non-inactivating current in older cultures nor have we observed an inverse correlation between the magnitude of Ca currents and the effectiveness of baclofen as described by Dolphin & Scott (1986). However, as the majority of our results have been obtained on young cultures, an effect of age cannot be ruled out. The actions of GTP analogues GTP-γ-S and GDP-β-S described by Dolphin & Scott (1987) were preferentially on a transit component of the Ca current, on which they produced inhibition and enhancement, respectively. Thus selective control of N- and L-type components is certainly possible as is suggested by our results.

Since baclofen generally had no effect on unitary currents in cell-attached patches, it appears unlikely that a readily diffusible second messenger is involved unless its action is effectively localized by catabolic enzymes. It has been reported that in rat and chick DRG neurones there is probably an involvement of G-proteins in coupling of both the $GABA_B$ and noradrenaline receptors to Ca channels (Dolphin & Scott, 1987; Holz *et al.*, 1986) and there is evidence that the coupling for the noradrenaline effect in chick DRG neurones does not involve a diffusible second messenger (Forscher *et al.*, 1986). The reversible effect on the Ca current in one patch reported here might be the result of some coupling across the rim of the patch involving a lipid soluble factor with limited access to the channels within the patch, which might therefore only rarely affect channel activity recorded in this way. It could also be, however, that only a fraction of the total Ca channels present on a neurone are sensitive to any second messenger and these channels might be located in restricted areas of the neuronal membrane. Further experiments are in progress to investigate these different possibilities by recording Ca channel activity in isolated patches.

The work was supported by a grant from the Scottish Hospitals Endowments Research Trust.

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(Received November 6, 1987
Accepted December 14, 1987)