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Modulation of divalent cation-activated chloride ion currents

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1 Voltage-sensitive calcium channel currents carried by Ca^{2+} (I_{Ca}) or Ba^{2+} (I_{Ba}) were followed by tail currents carried by Cl^- ions in approximately 45% of cultured dorsal root ganglion neurones.

2 Extracellular application of (−)-baclofen (100 μM) inhibited I_{Ba} and $I_{Cl(Ba)}$. Bay K 8644 (5 μM) potentiated both currents.

3 Intracellular GTP-γ-S increased the proportion of neurones in which $I_{Cl(Ba)}$ was recorded. In addition, the activation by GTP-γ-S of a pertussis toxin-sensitive GTP binding (G)-protein resulted in a steady increase in the Cl^- tail current with time, despite a concurrent reduction in I_{Ba}.

4 Extracellular application of 10 mM caffeine selectively reduced $I_{Cl(Ba)}$ without significant change in I_{Ba}. When Ca^{2+} was the charge carrier, caffeine had little effect on $I_{Cl(Ca)}$, and increased the inactivation of I_{Ca}.

5 We conclude that, in addition to being regulated by divalent cation entry through Ca^{2+} channels, the Cl^- current is also regulated by G-protein activation. The mechanism of activation of $I_{Cl(Ba)}$ may involve Ca^{2+} release from intracellular stores.

Introduction

In a proportion of dorsal root ganglion (DRG) neurones loaded with Cl^-, the firing of an action potential is followed by an after-depolarization (Dichter & Fischbach, 1977). Under whole cell voltage clamp, this depolarization corresponds to a slowly decaying inward Cl^- tail current ($I_{Cl(Ca)}$) which follows the inward Ca^{2+} current, and is presumably activated by entry of Ca^{2+} through voltage-dependent Ca^{2+} channels (Mayer, 1985; Dolphin *et al.*, 1986). Cl^- currents can also be induced by intracellular injection of Ca^{2+} and, to a lesser extent, Ba^{2+} and Sr^{2+} into *Xenopus* oocytes (Miledi & Parker, 1984). In spinal cord neurones Cl^- tail currents are inhibited by Ca^{2+} channel antagonists such as cobalt, cadmium and methoxyverapamil (D600) (Owen *et al.*, 1984). Similarly, 2-chloroadenosine, an analogue of the neuromodulator adenosine, inhibits both the Ca^{2+} channel current and the divalent cation-activated Cl^- tail current in DRG neurones (Dolphin *et al.*, 1986).

In the present study we have investigated further the relationship between the Ca^{2+} channel current, carried by either Ca^{2+} or Ba^{2+}, and the Cl^- tail current, to provide more information on the source of divalent cation which is able to activate the chloride current, and to determine whether Ba^{2+} can activate this current directly. Thus, we have examined the influence on the tail currents of agents which reduce or enhance calcium channel current amplitude, or affect intracellular divalent cation levels. (−)-Baclofen, a $GABA_B$-receptor agonist, has been shown to inhibit Ca^{2+} channel currents in cultured DRG neurones (Dolphin & Scott, 1986), and its effect has now been investigated on the associated Cl^- tail current. The effects of Bay K 8644, a 1,4-dihydropyridine calcium channel agonist (Brown *et al.*, 1984; Hess *et al.*, 1984) and caffeine, which causes release of intracellular Ca^{2+} (Fabiato & Fabiato, 1977), have also been examined.

In previous studies we have shown that persistent activation of a guanosine 5′-triphosphate (GTP) binding (G)-protein(s) by the GTP analogue GTP-γ-S results in preferential inhibition of the transient component of the Ca^{2+} channel current (Scott & Dolphin 1986; Dolphin & Scott 1987a; Dolphin *et al.*, 1988). In addition to mimicking responses to agonists such as baclofen, by inhibiting voltage-activated Ca^{2+} channel currents, GTP-γ-S has been shown to mimic responses to 5-hydroxytryptamine and acetylcholine in other systems (Evans & Marty,

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1986; Dascal *et al.*, 1986). In this case, the response is thought to involve release of intracellular Ca^{2+} and activation of Ca^{2+}-dependent currents including Cl^- currents. We have therefore investigated the action of intracellular GTP-γ-S on the divalent cation-activated Cl^- tail current in DRG neurones.

A preliminary communication of this work has been presented (Dolphin *et al.*, 1987).

Methods

DRG neurones were prepared from 3 day old rats and maintained in dissociated cell culture (Forda & Kelly, 1985) for 2–6 weeks. Recordings were made at room temperature using the whole cell clamp technique (Hamill *et al.*, 1981). Patch pipettes of 1–5 MΩ were used and cells were voltage-clamped using an Axoclamp-2 switching clamp amplifier operated at a sampling rate of 10 to 20 kHz. Inward Ba^{2+} and Ca^{2+} currents (I_{Ba}, I_{Ca}) and accompanying Cl^- tail currents ($I_{Cl(Ba)}$, $I_{Cl(Ca)}$) were evoked at a frequency of 0.03 Hz. The holding potential (V_H) was −80 mV unless otherwise stated. The amplitudes of I_{Ba} and I_{Ca} were measured at the peak of the maximum current (usually evoked at V_{clamp} 0 to +5 mV). In some cases sustained current was also measured, at the end of the voltage step command. $I_{Cl(Ca)}$ and $I_{Cl(Ba)}$ were measured 20 ms after the end of the voltage step command. All measurements were made following subtraction of the linear leakage and capacitance currents using a PDP 11/23 computer. Results are given as mean ± s.e. mean and statistical significance was determined by use of Student's *t* test, or paired *t* test where appropriate.

The standard recording medium contained (mM): NaCl 130, KCl 3.0, $MgCl_2$ 0.6, $NaHCO_3$ 1.0, HEPES 10, glucose 4.0, and either $CaCl_2$ 4.0 or $BaCl_2$ 2.5. Tetrodotoxin (2.5 μM) and tetraethylammonium bromide (25 mM) were also present. All recording media were adjusted to pH 7.4 with NaOH and to 320 mosmol by addition of sucrose. Electrodes were filled with a solution containing (mM): CsCl 140, EGTA 1.1, $MgCl_2$ 2.0, $CaCl_2$ 0.1, ATP 2 and HEPES 10. The pH was adjusted to 7.2 with CsOH and the osmolarity to 310 mosmol with sucrose. In some experiments the GTP analogue, guanosine 5'-0-3-thiotriphosphate (GTP-γ-S) or GDP analogue guanosine 5'-0-2-thiodiphosphate (GDP-β-S) (Boehringer Mannheim) were included in the patch solution at a concentration of 500 μM.

(−)-Baclofen (β-*p*-chlorophenyl GABA) (Ciba-Geigy), Bay K 8644 (Bayer) and caffeine (Sigma) were applied by low pressure ejection (less than 1 psi) from a micropipette (tip diameter approximately 10 μm) placed about 100 μm from the cell.

Results

In about 45% of DRG neurones loaded with intracellular CsCl, activation of I_{Ca} or I_{Ba} was followed by a slowly decaying inward tail current. Previous evidence strongly suggests that this current is carried by Cl^- (Mayer, 1985), and it is thus denoted $I_{Cl(Ca)}$ or

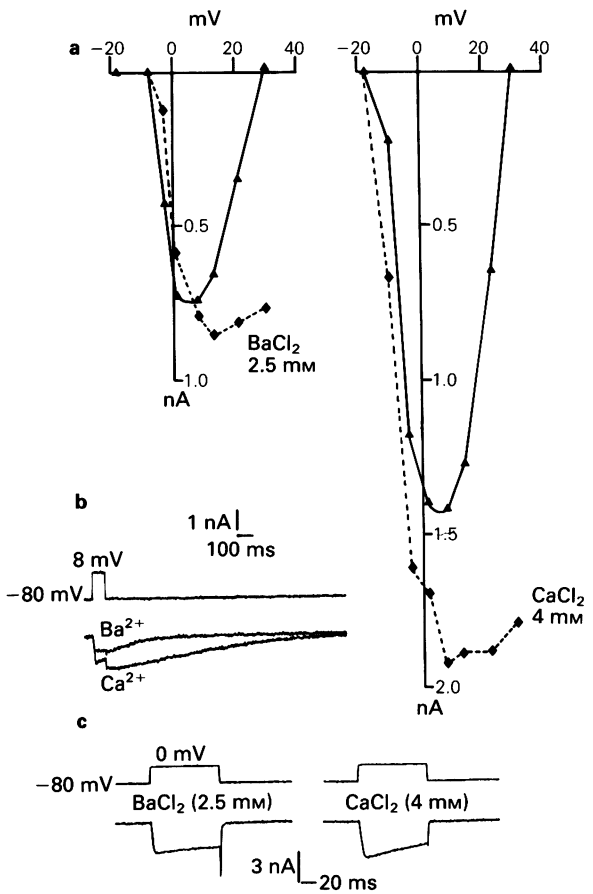

Figure 1 (a) Current-voltage relationships of the Ca^{2+} channel currents and Cl^- tail currents, following leakage subtraction. The peak Ca^{2+} channel currents (▲——▲), and Cl^- tail currents (◆ - - - ◆), measured 20 ms after the end of the 100 ms voltage step command, are illustrated. Recordings were made in Ba^{2+} medium before microperfusion of medium containing Ca^{2+}. (b) An example of voltage and current recordings from one cell, which shows the peak inward and Cl^- tail currents in Ba^{2+} followed (2 min later) by Ca^{2+} containing medium. Note the complex deactivation of the $I_{Cl(Ca)}$ tail current. (c) Recordings from a cell in which no tail current was activated. Greater inactivation of the calcium channel current is observed following application of Ca^{2+} medium, compared to Ba^{2+} medium.

$I_{Cl(Ba)}$. It was not present when Cl^- in the patch pipette solution was replaced by acetate or aspartate. The activation of $I_{Cl(Ba)}$ did not contaminate the maximum I_{Ba}, recorded at V_{clamp} 0 to +5 mV, because the reversal potential for Cl^- was about 0 mV. A conductance increase was associated with the Cl^- tail current of 15–20 nS for a 1 nA tail current. The replacement of Ba^{2+} by Ca^{2+} medium did not change the voltage-dependence of the Ca^{2+} channel current or the Cl^- tail current (Figure 1a, b). The peak Ca^{2+} channel currents were 2.0 ± 0.4 nA and 2.6 ± 0.3 nA ($n = 8$) in Ba^{2+} (2.5 mM) and Ca^{2+} (4 mM) media, respectively. These divalent cation concentrations were chosen to give approximately similar amplitude currents in order to compare the Cl^- tail current amplitudes. In Ba^{2+} medium the tail current was 1.22 ± 0.03 nA, and increased to 2.3 ± 0.6 nA ($n = 8$) in Ca^{2+} medium. In addition there was a 3–8 fold increase in the decay time of the Cl^- tail current when Ca^{2+} medium was substituted for Ba^{2+}. As discussed previously by Evans & Marty (1986), the decay of divalent cation activated Cl^- currents following a depolarizing voltage step is dependent both on the level of hyperpolarization and on the rate of return of the divalent cation concentration to its resting level. The deactivation of $I_{Cl(Ba)}$ could usually (e.g. Figures 1b and 3b) be described by a single exponential (unweighted least squares fit). More complex deactivation kinetics were observed for $I_{Cl(Ca)}$ following a 100 ms depolarizing voltage step to activate I_{Ca}, although single exponential kinetics were restored when the voltage step was reduced to 30 ms. These findings are in line with those previously described by Mayer (1985) (Figure 1b). In cells in which a tail current was absent in Ba^{2+} medium, the change to Ca^{2+} medium did not induce the appearance of $I_{Cl(Ca)}$ (Figure 1c).

During time-dependent current run-down, the reduction in I_{Ba} and $I_{Cl(Ba)}$ amplitudes occurred in parallel (Figure 2). Data from 5 cells, in which there

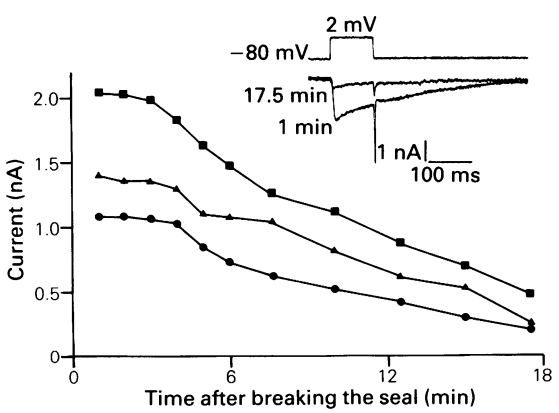

Figure 2 The time-dependent reductions of I_{Ba} and $I_{Cl(Ba)}$ in a cell which displays particularly rapid current rundown. Current amplitudes (following leakage subtraction) are plotted against time (in min) after breaking the membrane seal between the patch pipette and cell cytoplasm. The data illustrate the maximum I_{Ba} measured at the peak of the current (■); I_{Ba} measured at the end of the 100 ms voltage step command (▲) and $I_{Cl(Ba)}$ measured 20 ms after the end of the voltage step command (●).

Inset trace shows voltage and current records at 1 min and 17.5 min after breaking the seal.

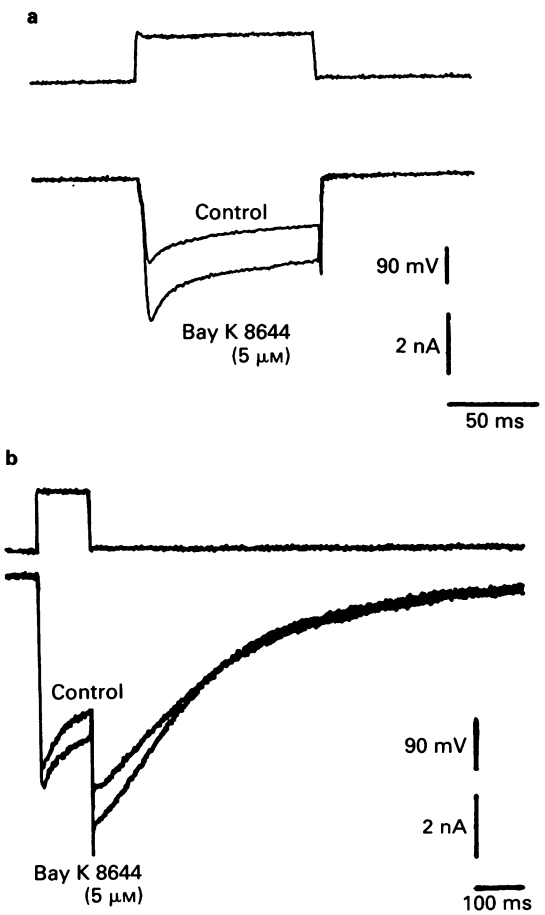

Figure 3 The effect of Bay K 8644 (5 μM) on I_{Ba} and $I_{Cl(Ba)}$. (a) Traces from a cell in which a tail current was initially absent show the enhancement of I_{Ba} by Bay K 8644, but no tail current is induced. (b) Bay K 8644 potentiates $I_{Cl(Ba)}$ as well as I_{Ba}. The maximum tail currents under control conditions and in the presence of Bay K 8644 are shown. Due to the hyperpolarizing shift in voltage dependence of I_{Ba} by Bay K 8644, the maximum I_{Ba} is not illustrated here although some increase due to Bay K 8644 is apparent.

was a particularly rapid current rundown, showed a decrease of $68 \pm 15\%$ for I_{Ba} compared with $57 \pm 13\%$ for $I_{Cl(Ba)}$, over a 15 min period. Inactivation of the transient component of I_{Ba} by depolarizing V_H from -80 mV to between -60 mV and -20 mV also reduced $I_{Cl(Ba)}$.

In the presence of 5 μM Bay K 8644, I_{Ba} was potentiated by approximately 25% and the amplitude of the Cl^- tail current was increased to a similar extent, from 2.0 ± 1.0 nA to 2.5 ± 1.1 nA ($n = 5$, $P < 0.05$) (Figure 3). There was no significant change in the time constant (τ) of deactivation of $I_{Cl(Ba)}$ in the presence of Bay K 8644. Values of 204 ± 34 ms and 194 ± 35 ms ($n = 5$) were obtained before and during Bay K 8644 application, respectively. The voltage-dependence of activation of I_{Ba} was hyperpolarized by Bay K 8644, the maximum I_{Ba} being activated at potentials 6–12 mV more negative in its presence, in agreement with previous findings (Hess *et al.*, 1984; Docherty & Brown, 1986). In contrast, Bay K 8644 did not change the voltage-dependence of $I_{Cl(Ba)}$; the maximum tail current in the presence of Bay K 8644 was observed on stepping back to V_H -80 mV from the same V_{clamp} as in the control, although this no longer activated the maximum I_{Ba}.

Application of (−)-baclofen (100 μM) gave results consistent with those previously found for 2-chloroadenosine (Dolphin *et al.*, 1986); I_{Ba} and $I_{Cl(Ba)}$ were inhibited to similar extents (Figure 4).

Under control conditions using Ba^{2+} as charge carrier, Cl^- tail currents were observed in 64/141 cells, and did not develop with time if not initially present. In the presence of intracellular GTP-γ-S, Cl^- tail currents were expressed in a greater proportion (11/12) of cells. Despite an inhibition of I_{Ba} by GTP-γ-S (Scott & Dolphin, 1986; Dolphin & Scott, 1987a), $I_{Cl(Ba)}$ developed and increased with time (Figure 5). The peak amplitude of I_{Ba} was reduced by

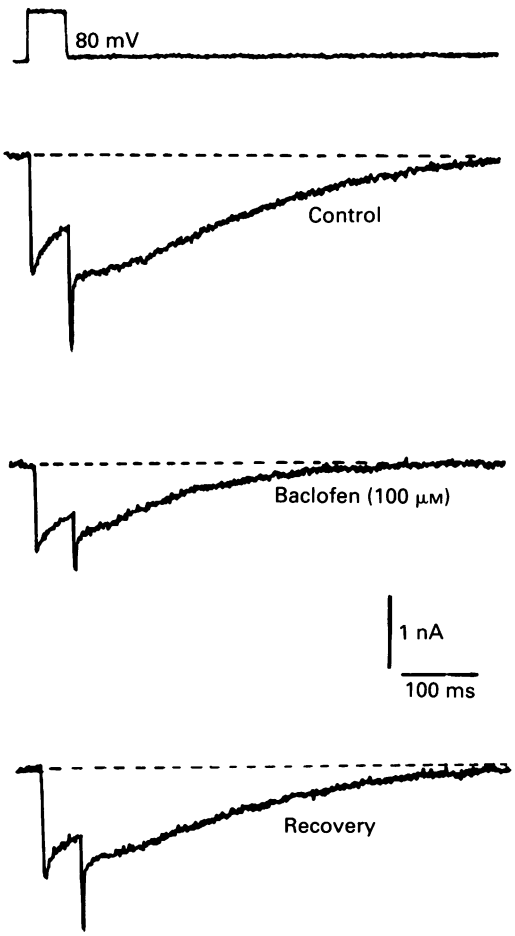

Figure 4 The inhibition of both I_{Ba} and $I_{Cl(Ba)}$ by (−)-baclofen (100 μM). Partial recovery of the currents was observed 2 min following removal of the (−)-baclofen containing pressure pipette.

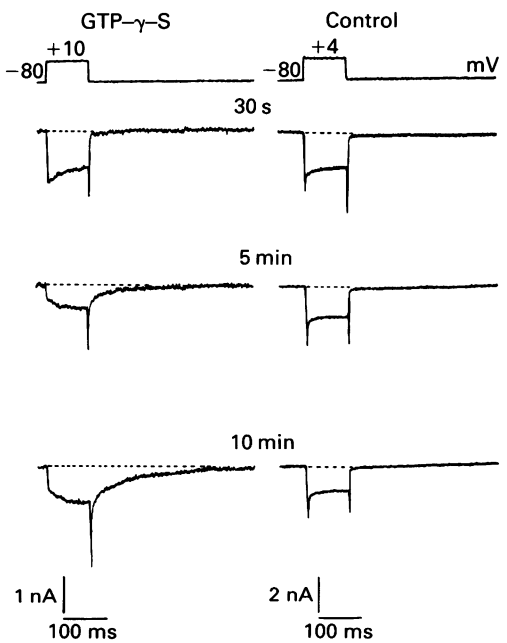

Figure 5 The effect of intracellular GTP-γ-S on the development and maintenance of $I_{Cl(Ba)}$. The traces show records from 2 cells in which no tail current was recorded initially. On the left, recordings were made using patch pipette medium containing 500 μM GTP-γ-S. The slowing of activation and reduction in amplitude of I_{Ba} occurred during the first 5 min of recording, whereas the tail current $I_{Cl(Ba)}$ continued to develop over 10 min. The control experiment is shown on the right. In the absence of intracellular GTP-γ-S, no tail current developed with time, even up to 25 min (not shown).

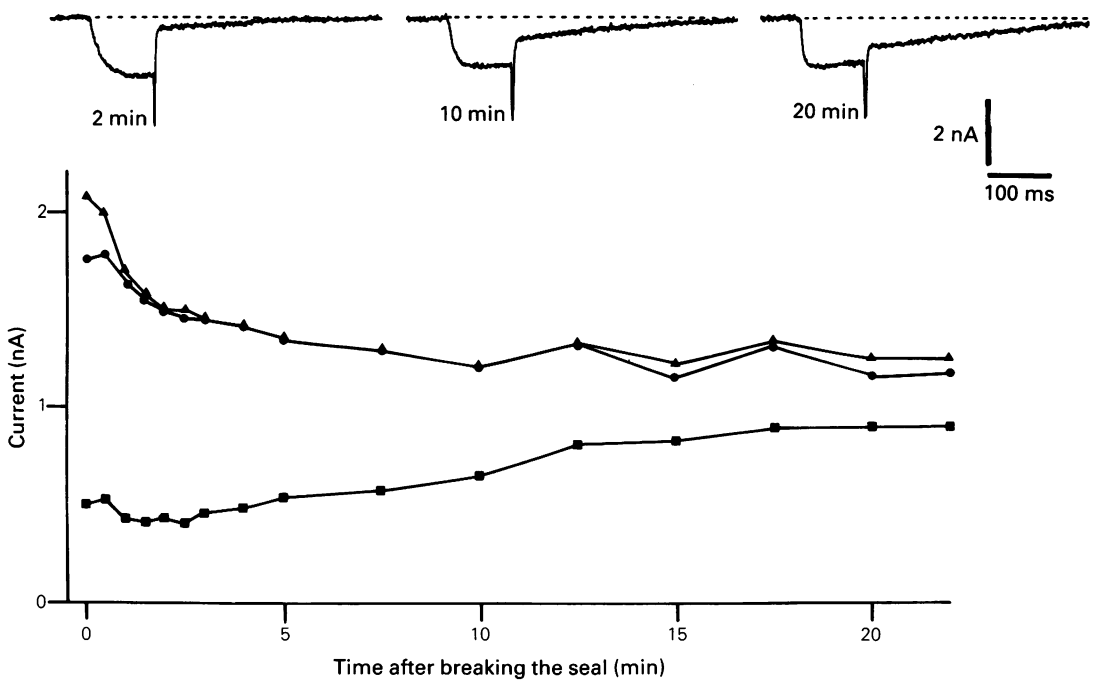

Figure 6 The effect of GTP-γ-S in a cell which initially displayed a Cl^- tail current. The top traces show enhancement of the Cl^- tail current by GTP-γ-S over 20 min. Current amplitudes (following leakage subtraction) measured in the same cell, are plotted against time in the lower graph. Illustrated are the maximum I_{Ba} measured at the peak of the current (▲), the steady state current measured at the end of the 100 ms voltage step command (●) and the Cl^- tail current amplitude, measured 20 ms following the voltage step (■).

GTP-γ-S from 2.3 ± 0.2 nA to 1.1 ± 0.1 nA (mean ± s.e.; $n = 7$; $P < 0.01$: paired t test) during the first 5 min of recording, whereas the amplitude of $I_{Cl(Ba)}$ increased from 0.3 ± 0.1 nA to 0.7 ± 0.1 nA (mean ± s.e.; $n = 7$; $P < 0.05$) over a longer period of up to 20 min (Figure 6). The τ of deactivation of the Cl^- tail current also increased from 91 ± 27 ms to 162 ± 35 ms ($n = 7$; $P < 0.05$) (Figure 7). When patch pipette solutions containing Cs acetate or aspartate were used, GTP-γ-S did not induce the development of tail currents. In preliminary experiments using Ca^{2+} medium $I_{Cl(Ca)}$ was present in 10/11 cells recorded with CsCl patch pipette solution containing 500 μM GTP-γ-S.

To investigate further the G-protein-mediated potentiation of $I_{Cl(Ba)}$, patch solution containing 500 μM GDP-β-S was used. This inhibits G protein activation by endogenous GTP. As previously described (Dolphin & Scott, 1987a), in the presence of internal GDP-β-S, I_{Ba} has a greater transient component than control I_{Ba}. $I_{Cl(Ba)}$ was expressed in 14/26 cells under these conditions, but no differences were observed between $I_{Cl(Ba)}$ recorded under control conditions and with internal GDP-β-S. Recordings of up to 25 min revealed that time-dependent changes in I_{Ba} were followed by a corresponding change in $I_{Cl(Ba)}$ in a manner similar to the run-down observed in control cells. Experiments were also conducted to determine which class of G-protein may be involved

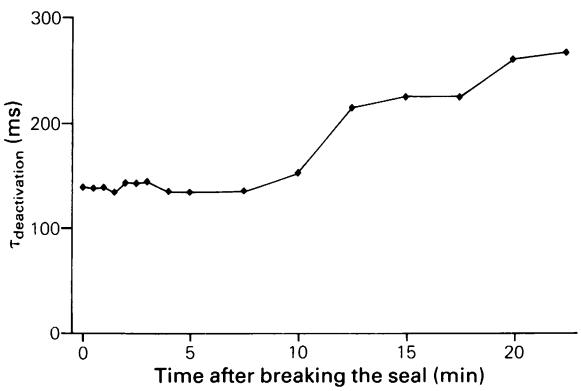

Figure 7 The effect of extracellular GTP-γ-S on the kinetics of deactivation of $I_{Cl(Ba)}$. The time constant of deactivation ($\tau_{deactivation}$) is plotted against time after breaking the seal. There is a progressive increase in $\tau_{deactivation}$ after 5 min recording in the presence of internal GTP-γ-S (500 μM).

in the effect of GTP-γ-S on Cl^- tail currents. DRG neurones in cell culture were treated with pertussis toxin (0.5 μg ml^{-1} culture medium at 37°) for 2–3 h before recordings were made. In the presence of intracellular GTP-γ-S, Cl^- tail currents were recorded in 6/15 pertussis toxin-treated cells, a much lower incidence than in untreated cells. In addition, intracellular GTP-γ-S did not induce development of, or potentiate the Cl^- tail current in these cells. A surprising result was that both I_{Ba} and the associated $I_{Cl(Ba)}$ were markedly reduced over 10 min (Figure 8), and almost abolished after 15 min. This rapid rundown of I_{Ba} only occurred in pertussis toxin-treated cells recorded with Cl^- containing patch pipette solution.

Extracellular application of 10 mM caffeine did not cause any significant change in I_{Ba}. The peak currents were 3.1 ± 0.5 nA and 3.0 ± 0.6 nA and the sustained currents were 2.1 ± 0.4 nA and 2.0 ± 0.5 nA ($n = 13$) for control I_{Ba}, and in the presence of caffeine respectively. In contrast, $I_{Cl(Ba)}$ was rapidly (within 30 s) reduced from 2.4 ± 0.7 nA to 0.7 ± 0.21 nA ($n = 8$; $P < 0.05$) and there was little recovery from the effect of caffeine (Figure 9a). Caffeine had no consistent effects on holding current at V_H −80 mV, although in a small proportion of cells (2/8), application of 10 mM caffeine caused a transient inward current. When Ca^{2+} was the charge carrier, $I_{Cl(Ca)}$ was expressed in 14/25 cells, and application of 10 mM caffeine had little effect on $I_{Cl(Ca)}$ (Figure 9b). However, caffeine increased the inactivation of I_{Ca}. Peak I_{Ca} was 1.8 ± 0.3 nA and 1.6 ± 0.3 nA ($n = 12$) before and during caffeine application respectively. The sustained I_{Ca} measured at the end of the pulse was 1.1 ± 0.2 nA for control and 0.5 ± 0.1 nA ($n = 12$; $P < 0.01$) in the presence of caffeine. $I_{Cl(Ca)}$ was 1.3 ± 0.3 nA and 1.0 ± 0.3 nA under the same conditions. Following removal of the caffeine pipette, peak I_{Ca} increased over 5 min to 2.3 ± 0.4 nA, and $I_{Cl(Ca)}$ also increased to 1.4 ± 0.4 nA ($n = 9$).

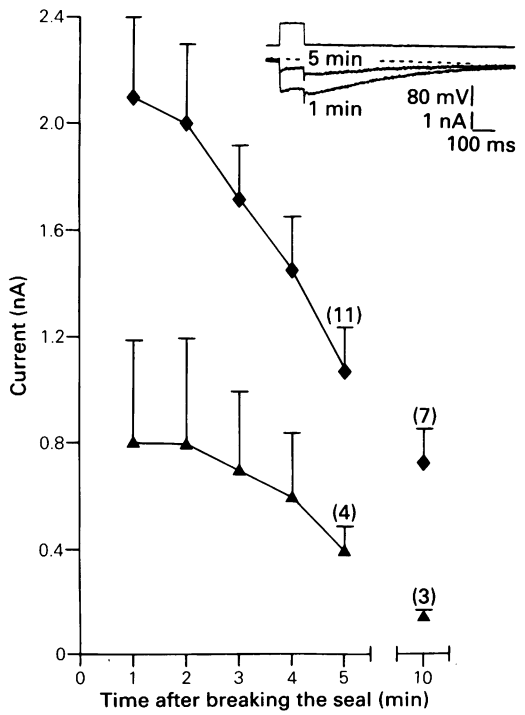

Figure 8 The inhibitory effect of pertussis toxin on the potentiation of $I_{Cl(Ba)}$ by intracellular GTP-γ-S. The current amplitude, for the peak I_{Ba} (◆) and Cl^- tail current $I_{Cl(Ba)}$ (▲) is plotted against time after breaking the seal. Eleven experiments were performed, and in 4 of these Cl^- tail currents were present. Not all cells were recorded for 10 min. Data are given as mean of numbers in parentheses; vertical lines indicate s.e. Inset trace shows currents activated at 1 and 5 min after breaking the seal.

Discussion

It has been proposed that Ca^{2+}-activated Cl^- channels are dependent for their activation on the elevation of intracellular Ca^{2+} which may occur by Ca^{2+} entry through voltage-gated calcium channels (Miledi, 1982; Barish, 1983) or other means. There is strong evidence for this which includes: (1) An increase in extracellular Ca^{2+} concentration potentiates the Cl^- tail currents which follow Ca^{2+} channel currents (Barish, 1983; Owen *et al.*, 1984) whilst a reduction in extracellular Ca^{2+} decreases the amplitude of $I_{Cl(Ca)}$ (Mayer, 1985). (2) Prolongation of the voltage-activated Ca^{2+} channel current enhances the Cl^- tail current (Mayer, 1985). (3) Organic and inorganic Ca^{2+} channel blockers reduce $I_{Cl(Ca)}$ (Owen *et al.*, 1984; Mayer, 1985). (4) The Ca^{2+} ionophore A23187 or direct injection of Ca^{2+} activates a Cl^- current (Miledi & Parker, 1984; Marty *et al.*, 1984; Byrne & Large, 1987).

We have previously observed in DRG neurones that the Cl^- tail current is also activated in Ba^{2+} containing medium (Dolphin *et al.*, 1986), although our present results show that application of Ca^{2+} medium results in a considerable increase of the current amplitude and duration. In contrast, data from previous work indicated that Ba^{2+} entry through voltage-gated Ca^{2+} channels did not activate $I_{Cl(Ba)}$ (Barish, 1983; Mayer, 1985). It is possible that, because of the poor ability of Ba^{2+} compared to Ca^{2+} to activate the divalent cation-dependent Cl^- conductance (Miledi & Parker, 1984), $I_{Cl(Ba)}$ was undetectable in previous studies. In the present study we have further examined the properties of $I_{Cl(Ba)}$ in comparison with $I_{Cl(Ca)}$.

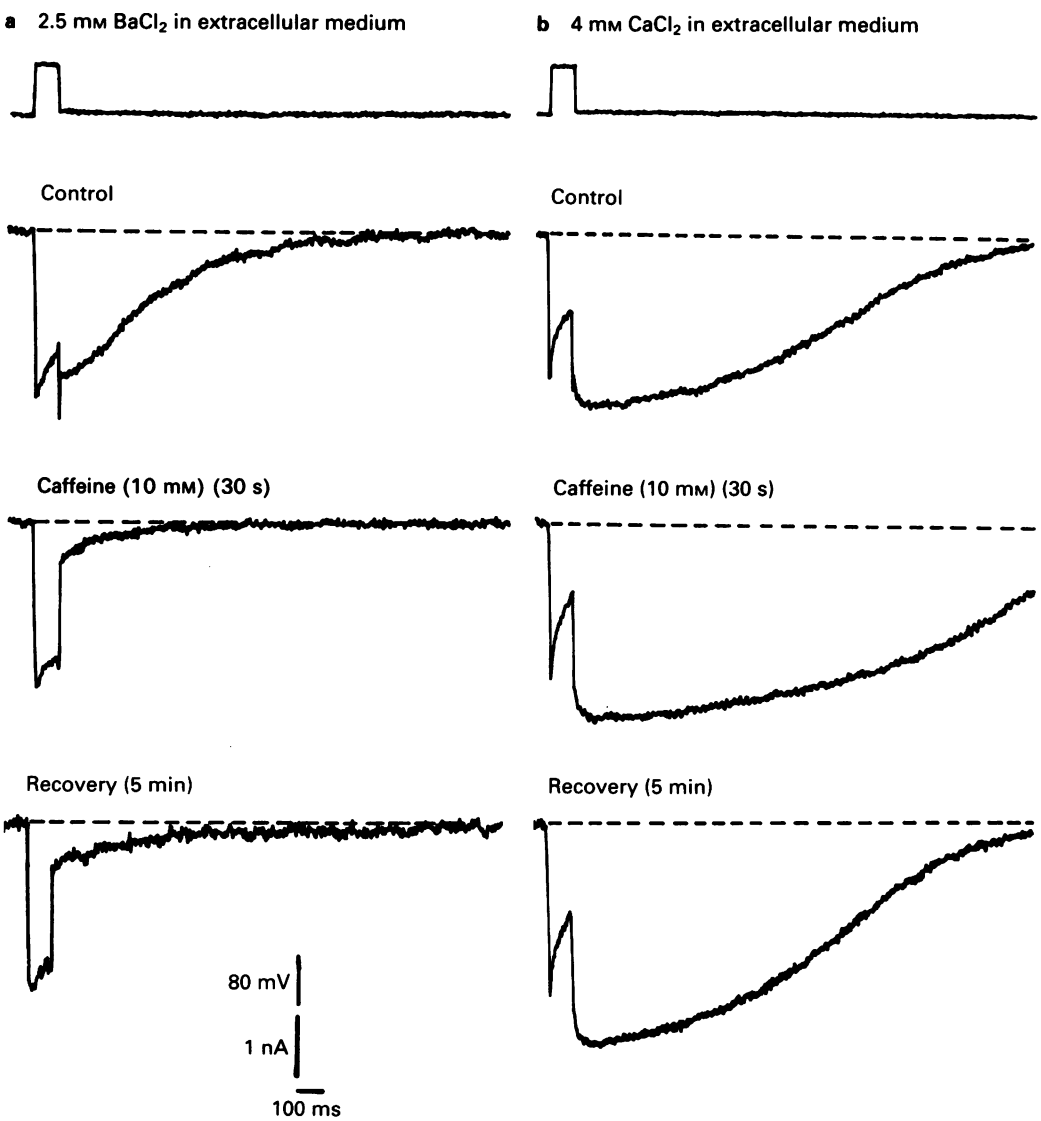

Figure 9 The action of caffeine on $I_{Cl(Ba)}$ and $I_{Cl(Ca)}$. (a) Caffeine (10 mM) selectively inhibited $I_{Cl(Ba)}$ without significant alteration of I_{Ba}. A small amount of recovery of the tail current was observed 5 min after removal of the caffeine pipette. (b) Caffeine inhibited neither I_{Ca} nor $I_{Cl(Ca)}$, but 5 min after removal of the caffeine pipette, both currents increased to greater than control levels. Although $I_{Cl(Ca)}$ was prolonged by caffeine in 4/14 cells, it was unchanged in 8 and shortened in 2 cells, so the increased duration observed in this trace appears not to be significant.

Currents were activated at V_{clamp} + 5 mV under control conditions and after 30 s application of caffeine from a pressure pipette. Note the greater inactivation of I_{Ca} in the presence of caffeine.

Results from experiments investigating the effects of current rundown, Bay K 8644 and (−)-baclofen on $I_{Cl(Ba)}$ support the idea that the size of the Cl^- current varies in parallel with the amplitude of I_{Ba} and may thus be dependent upon influx of Ba^{2+} through voltage-activated channels.

A number of studies have found that only a proportion of neurones express the divalent cation-dependent Cl^- tail current when loaded with Cl^- (Owen *et al.*, 1984; Mayer, 1985; Dolphin *et al.*, 1986). One possible factor contributing to this heterogeneity may be whether K^+ currents are sufficiently

attenuated to allow observation of Cl^- currents, since Owen *et al.* (1984) found that when recordings were made in the presence of intracellular CsCl and extracellular TEA, the Cl^- tail current was detected in a greater proportion of spinal cord neurones. The intracellular concentration of free Ca^{2+} is also important. Miledi & Parker (1984) showed that intracellular Ca^{2+} injection into *Xenopus* oocytes invariably elicited a Cl^- current even in cells in which depolarization was unable to elicit a response, and that intracellular EGTA injection markedly inhibited $I_{Cl(Ca)}$. It has been suggested that the relevant Cl^- channels are only present in a subpopulation of sensory neurones (Mayer, 1985). However, although we also found that approximately 45% of DRG neurones express the divalent cation-activated Cl^- conductance under control conditions, in the presence of intracellular GTP-γ-S $I_{Cl(Ba)}$ and $I_{Cl(Ca)}$ were recorded in over 90% of neurones. Furthermore, GTP-γ-S appeared to promote the development and maintenance particularly of $I_{Cl(Ba)}$. Thus, the observation of Cl^- tail currents in sensory neurones may be determined in part by their metabolic state, and in particular by levels of endogenous GTP, rather than by the presence or absence of divalent cation-activated Cl^- channels.

The inhibitory action of GTP-γ-S on the transient component of I_{Ba} occurred within 5 min of recording (Scott & Dolphin, 1986; Dolphin *et al.*, 1988). In contrast, potentiation of the Cl^- tail current by GTP-γ-S occurred over a much longer period, between 5 and 20 min. This suggests a different site of action for GTP-γ-S in the two responses and a mechanism for the enhancement of Cl^- tail currents not involving increased divalent cation influx through voltage-gated channels.

Nowycky *et al.* (1985) have demonstrated that three types of Ca^{2+} channel coexist in cultured DRG neurones. It has been suggested that the sustained non-inactivating Ca^{2+} conductance (L-type) is important for the activation of $I_{Cl(Ca)}$ (Mayer, 1985) although V_H −60 mV used in Mayers' study would not cause complete inactivation of the N and T channels. L type channels appear to be resistant to GTP-γ-S (Dolphin & Scott 1987a), but sensitive to Bay K 8644 (Nowycky *et al.*, 1985; Dolphin & Scott 1987b). It is possible that these findings may explain why the initial action of GTP-γ-S to reduce the transient component of I_{Ba} caused no inhibition of $I_{Cl(Ba)}$. However, in preliminary experiments, in which V_H was depolarized to −30 mV (which completely inactivates the transient Ca^{2+} channel currents), $I_{Cl(Ba)}$ was markedly depressed. This result suggests that Ba^{2+} passing through N and/or T type Ca^{2+} channels also contributes to the activation of $I_{Cl(Ba)}$.

In this study, intracellular GDP-β-S did not alter the characteristics of activation of $I_{Cl(Ba)}$. A study by Dascal *et al.* (1986) has shown that responses to 5-hydroxytryptamine (5-HT), involving G-protein regulated Ca^{2+}-dependent currents including Cl^- currents, were reduced by both GDP and GDP-β-S. An effect of GDP and its analogues on agonist-induced responses may be more easily detected than a direct action of GDP-β-S on the Cl^- current itself. In contrast caffeine did not affect $I_{Cl(Ca)}$, while increasing the (presumably Ca-dependent) inactivation of I_{Ca}. This reflects caffeine-induced disruption of Ca^{2+} sequestration in internal stores. Pertussis toxin pretreatment of DRG neurones did not prevent the expression of $I_{Cl(Ba)}$. However, the GTP-γ-S induced potentiation of this current was abolished, indicating that the G-protein upon which GTP-γ-S acts to produce this effect is probably either G_i or G_o. One observation for which we cannot account is that in pertussis toxin-treated cells loaded with CsCl both I_{Ba} and $I_{Cl(Ba)}$ were rapidly reduced within a 10 min period. In contrast, in pertussis toxin-treated cells recorded using Cs acetate containing pipettes, the rundown of I_{Ba} occurred with a time course similar to that in control cells and only the actions of GTP-γ-S and (−)-baclofen were inhibited by the toxin (Dolphin & Scott, 1987a).

In experiments using caffeine we found that its application rapidly inhibited $I_{Cl(Ba)}$ but did not significantly alter the amplitude of I_{Ba} itself. In DRGs, it has been found that caffeine causes a wave of Ca^{2+} release from intracellular stores and their consequent depletion of Ca^{2+} (Neering & McBurney, 1984). These authors also showed that Ca^{2+} stores subsequently refilled during electrical activity, and this depended on extracellular Ca^{2+}. However, in the present study, using Ba^{2+} medium, the Cl^- tail current showed only partial recovery 3–5 min after removal of caffeine, possibly because of the absence of external Ca^{2+} to replenish internal stores. It thus appears that release of intracellular Ca^{2+} may be essential for activation of the Cl^- tail current in Ba^{2+} medium. It is unlikely that these results are due to phosphodiesterase inhibition by caffeine, since isobutyl methylxanthine (0.1 mM), which does not cause release of intracellular calcium (Neering & McBurney, 1984), did not mimic the effect of caffeine. Our results support the idea that Ba^{2+} does not directly activate the Cl^- tail current to any great extent, but triggers release of intracellular Ca^{2+}. Barium influx into smooth muscle cells via voltage-dependent Ca^{2+} channels has been shown to induce release of Ca^{2+} from internal stores before contraction (Satoh *et al.*, 1987). In the same study Ba^{2+} was unable to refill depleted Ca^{2+} storage sites, in agreement with the small recovery of $I_{Cl(Ba)}$ observed following application of caffeine in our experiments.

Based on these and previous findings, there are several possible sites of action which may be postu-

lated to explain the ability of GTP-γ-S to promote the expression of Cl^- tail currents in DRGs. Firstly, GTP-γ-S may interact with the processes involved in the homeostasis of intracellular calcium. Since a major pool for rapid sequestration and release of calcium in neurones is likely to be the endoplasmic reticulum (for review see McBurney & Neering, 1987), it is possible that GTP-γ-S promotes the release of intracellular Ca^{2+} by activating a G-protein (G_p) associated with phospholipase C. Dascal *et al.* (1986) have suggested that this G-protein may mediate the potentiation by GTP-γ-S of Ca^{2+}-dependent K^+ and Cl^- currents in mRNA injected oocytes, since $InsP_3$ has been implicated as the second messenger in the regulation of these currents by acetylcholine and 5-HT. However, in DRG neurones G_p does not appear to be sensitive to pertussis toxin (G. Burgess, personal communication).

It is unlikely that GTP-γ-S has a direct action on the release of Ca^{2+} from internal stores. A study of Gill *et al.* (1986) has shown that GTP can directly activate Ca^{2+} release from endoplasmic reticulum. However, GTP-γ-S did not mimic GTP in this respect but rather inhibited the release of Ca^{2+}.

Another possibility with regard to Ca^{2+} homeostasis is that GTP-γ-S may slow the return of elevated cytoplasmic Ca^{2+} to its resting level, by interacting with either Na^+–Ca^{2+} exchange or with the Ca^{2+}-ATPase on the cytoplasmic membranes or on intracellular organelles.

An alternative site of action for GTP-γ-S is at the level of the Cl^- channel itself. The effect might be mediated either directly via an activated G-protein or indirectly via effects on second messengers such as cyclic AMP or diacylglycerol. Subsequent changes in the post-translational modification of the Cl^- channel might then alter its gating properties. The endogenous activator of protein kinase C, diacylglycerol, has been implicated in the regulation of ionic currents in several systems (Higashida & Brown, 1986; Malenka *et al.*, 1986). Further work using exogenous agents which activate or inhibit protein kinase C will be required to provide evidence for the involvement of this pathway in the modulation of divalent cation-activated Cl^- currents.

The physiological importance of calcium-activated chloride channels in DRGs will depend on whether they are present, together with calcium channels, at the primary afferent terminals. Here they could play a role in controlling the frequency of invasion of synaptic boutons by an action potential. The fact that $I_{Cl(Ca)}$ is enhanced by GTP-γ-S, may indicate that it can be regulated, independently of calcium channels, by neuromodulators.

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