Voltage-dependent modulation of rat sensory neurone calcium channel currents by G protein activation: effect of a dihydropyridine antagonist

¹R.H. Scott & ²A.C. Dolphin

Department of Pharmacology, St George's Hospital Medical School, London SW17 0RE

The ability of a depolarizing prepulse to increase the rate of activation of I_{Ba} has been examined in cultured sensory neurones of the rat. Both in control neurones and in the presence internally of the guanine nucleotide analogue, guanosine 5'-O-3-thiotriphosphate (GTP γ S) which markedly slows the rate of activation of I_{Ba} and reduces its amplitude, a depolarizing prepulse increased the rate of activation of I_{Ba} , but did not increase its amplitude measured at the end of the 100 ms voltage step. The calcium channel antagonist (–)-202-791, which we have previously shown to increase the amplitude of I_{Ba} in the presence of GTP γ S, did not occlude the response to a depolarizing prepulse, suggesting that the mechanism of action of (–)-202-791 is not to disrupt the interaction of the channels with activated G proteins.

Introduction It has been observed in several cell types that the effect of internal guanine nucleotide analogues on calcium currents is to slow the rate of activation and reduce the current amplitude (Scott & Dolphin, 1986; Lewis *et al.*, 1986). We have previously shown that calcium channel antagonist ligands, including the dihydropyridine (–)-202-791 (2,6-dimethyl-3-carbomethoxy-5-nitro-4-(2-trifluoromethyl-phenyl)-1,4-dihydropyridine) increase rather than decrease calcium channel currents (I_{Ba}) in the presence of internal guanine nucleotide analogues (Scott & Dolphin, 1987; Dolphin & Scott, 1989). Several explanations for this phenomenon are possible. Activated G protein may interact with the calcium channel ligand binding site such that either agonist binding or its efficacy is increased. It is also possible that the dihydropyridines reverse the effect of activated G proteins on the calcium channel, thus increasing current amplitude.

It has recently been suggested by Grassi & Lux (1989) that the kinetic effect of the neurotransmitter γ -aminobutyric acid (GABA) and the non-hydrolysable GTP analogue guanosine 5'-O-3-thiotriphosphate (GTP γ S) in slowing calcium current activation in chick dorsal root ganglion neurones (DRGs) is due to gradual recovery of the calcium channels from the voltage-dependent block conferred by their interaction with activated G proteins.

In the present study we have confirmed the voltage-dependence of the kinetic effect of GTP γ S on I_{Ba} in rat DRGs. We have further shown that the amplitude inhibition of I_{Ba} is not restored by a depolarizing prepulse, and that the agonist response to (–)-202-791 does not prevent a prepulse from reversing the kinetic effect of GTP γ S.

Methods Neonatal rat DRGs were dissociated and maintained in culture as previously described, (Dolphin *et al.*, 1986), except that they were plated on polyornithine-laminin coated coverslips and maintained in a serum-free defined medium (Bottenstein *et al.*, 1980; Winter, 1987). Calcium channel currents were recorded from cells between 1 day and 2 weeks in culture.

The whole-cell recording technique (Hamill *et al.*, 1981) was used to examine Ca^{2+} channel currents (I_{Ba}). Low resistance pipettes (2–4 M Ω) were used, and cells were voltage-clamped with an Axoclamp-2 switching amplifier, operated at a sampling rate of 25–30 kHz. The recording medium contained (mM): NaCl 130, KCl 3, MgCl₂ 0.6, NaHCO₃ 1.0, HEPES 10, glucose 4, BaCl₂ 2.5, tetra-ethylammonium bromide (TEA) 25,

and tetrodotoxin 0.0025. It was adjusted to pH 7.4 with NaOH and to 320 mosm with sucrose. Patch electrodes were filled with a solution containing (mM): Cs acetate 140, CaCl₂ 0.1, EGTA 10, MgCl₂ 2, ATP-Na 2 and HEPES 10. The pH was adjusted to 7.2 with Tris and the osmolality to 310 mosm with sucrose. In some experiments GTP γ S (100 or 500 μ M was included in the patch pipette). Initial current-voltage relationships were generated, and only cells showing adequate space clamp were used.

The maximum I_{Ba} was recorded by a 100 ms test depolarization from V_H –80 mV to –10 or 0 mV. Leak subtraction was carried out using hyperpolarizing steps to –130 mV performed immediately beforehand. When a prepulse was applied the prepulse current was not leak subtracted. Analysis was carried out by use of programmes supplied by Dr J. Dempster, Strathclyde University.

Results The maximum I_{Ba} examined over the first minute in a cell recorded with a pipette containing GTP γ S, generally showed a transient component, before the effect of the GTP analogue was manifest (Figure 1a, initial current). GTP γ S then slowed the activation of I_{Ba} , and reduced its amplitude over 2–5 min (Figure 1a, GTP γ S). As shown by Grassi & Lux (1989), a prepulse (pp) of 40 ms to +30 mV, terminating 5 ms before activation of the maximum inward current, reversed the GTP γ S-induced slowing of activation of I_{Ba} (Figure 1a, +pp; n = 7). However, in all cells in which the initial current was recorded, the prepulse did not restore the amplitude of the current to its initial level before the response to GTP γ S had taken effect. The initial current was 2.5 ± 0.2 nA (mean $\pm$ s.e.mean; n = 6); in the presence of GTP γ S it was reduced to 1.9 ± 0.2 nA and following the prepulse the amplitude remained at 1.9 ± 0.2 nA. In all cases I_{Ba} was measured at the end of the 100 ms step. Larger depolarizing prepulses to up to +100 mV did not increase the current amplitude at the end of the 100 ms step, although the initial peak amplitude was increased.

It is unlikely that the inability of the prepulse to reverse the inhibition by GTP γ S of the amplitude of I_{Ba} is due to current rundown, because none is observed in these cells over the first 5–10 min of recording (Dolphin *et al.*, 1986). In a proportion of control neurones, in the absence of GTP γ S, an identical prepulse also enhanced the initial rate of activation of the control I_{Ba} (5/8 control neurones; e.g. Figure 1b). Again the amplitude of I_{Ba} at the end of the 100 ms voltage step was not increased. In no case did the prepulse reduce the amplitude of the maximum I_{Ba} .

Since it is possible that the agonist response to (–)-202-791 observed in the presence of GTP γ S is due to a reversal of the

¹ Present address: Dept of Physiology, St George's Hospital Medical School, London SW17 0RE.

² Author for correspondence.

Figure 1 The effect of depolarizing prepulses on guanosine 5'-O-3-thiotriphosphate (GTP γ S)-induced inhibition of I_{Ba} . The maximum I_{Ba} was evoked by a 100 ms step from V_H -80 mV, to V_C 0 mV. Leak subtraction was performed using steps to -130 mV immediately beforehand. The effect was examined of a 40 ms prepulse to +30 mV, terminating 5 or 10 ms before activation of I_{Ba} . Currents are superimposed, and the prepulse current is not leak subtracted. (a) An initial maximum I_{Ba} was recorded immediately after establishing whole cell clamp using a pipette containing 100 μ M GTP γ S. After 5 min the effect of GTP γ S is maximal and a prepulse (+pp) restores the rate of activation of I_{Ba} to that of the initial current, although the amplitude remains reduced. (b) A control I_{Ba} was recorded in the absence of GTP γ S in the patch pipette. A prepulse to +40 mV increased the initial transient component of I_{Ba} (+pp), but did not affect the amplitude at the end of the 100 ms step. (c) I_{Ba} was recorded in the presence of 500 μ M GTP γ S with or without a prepulse (+pp) (left panel). (-)-202-791 (5 μ M) was then applied by low pressure ejection from a blunt micropipette placed 100 μ m from the cell. An increase in I_{Ba} was observed in the currents recorded both with and without a prepulse (right panel).

effect of activated G protein on I_{Ba} , we examined the ability of the prepulse protocol to reverse the kinetic effect of GTP γ S before and during application of 5 μ M (-)-202-791. In the cell shown in Figure 1c, the prepulse increased the control peak I_{Ba} in the presence of GTP γ S (Figure 1c, left panel). Application of (-)-202-791 to this cell then produced an agonist response, 30 s after the start of application, enhancing the amplitude of the current by 25%. However, the kinetic effect

of GTP γ S remained present and the activation of I_{Ba} was still increased to the same extent by a depolarizing prepulse (Figure 1c, right panel). Further application of (-)-202-791 for 2 min did not abolish this effect of GTP γ S, although I_{Ba} was further enhanced. This experiment was repeated in 5 other cells with identical results. The amplitude of I_{Ba} in the presence of GTP γ S was 2.0 ± 0.6 nA ($n = 6$), and the increase in amplitude of I_{Ba} due to (-)-202-791, both in the absence and in the presence of a prepulse, was 0.8 ± 0.1 nA.

Discussion In these experiments we have confirmed the finding of Grassi & Lux (1989) that the slowed activation of the calcium channel current by GTP γ S is reversed by a depolarizing prepulse. However, the amplitude of the current is not restored, when measured at the end of the 100 ms depolarizing voltage step. The inability of the prepulse to restore the current amplitude is not because of prepulse-induced inactivation, or current run-down, since neither phenomenon was observed in these experiments. The voltage-dependence of the effect of agonists on neuronal calcium currents was previously suggested by Bean (1989) who showed that noradrenaline no longer inhibited outward calcium channel currents activated by large depolarizing steps in frog DRGs. However we have observed that both the GABA $_B$ agonist baclofen and GTP γ S reduce the amplitude of the outward calcium channel currents in rat DRGs, although the rate of activation of the outward current in the presence of these agents is much faster than the rate of activation of the inward I_{Ba} (Dolphin & Scott, 1990). Taken together with the present result, this points to two effects of G protein activation by agonists or GTP γ S, a kinetic effect on activation of the calcium current, and an additional effect on the current amplitude. The finding that a prepulse also increased the rate of current activation in a proportion of control cells suggests that the current may be under partial tonic inhibition by GTP-activated G proteins.

The finding that (-)-202-791 remained an agonist in the presence of GTP γ S when I_{Ba} was recorded with a depolarizing prepulse, and that (-)-202-791 did not reverse the kinetic effect of GTP γ S, indicates that the mechanism of action of (-)-202-791 is unlikely to involve displacement of GTP γ S binding or prevention of interaction of activated G protein with the calcium channel.

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References

- BEAN, B.P. (1989). Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage-dependence. *Nature*, **340**, 153-155.
- BOTTENSTEIN, J.E., SKAPER, S.D., VARON, S.S. & SATO, G.H. (1980). Selective survival of neurons from chick embryo sensory ganglionic dissociates utilizing serumfree supplemented medium. *Exp. Cell Res.*, **125**, 183-190.
- 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.*, **386**, 1-17.
- DOLPHIN, A.C. & SCOTT, R.H. (1989). Interaction between calcium channel ligands and guanine nucleotides in cultured rat sensory and sympathetic neurones. *J. Physiol.*, **413**, 271-288.
- DOLPHIN, A.C. & SCOTT, R.H. (1990). Activation of calcium channel currents in rat sensory neurones by large depolarisations: effect of guanine nucleotides and (-)-baclofen. *Eur. J. Neurosci.*, **2**, 104-108.
- GRASSI, F. & LUX, H.D. (1989). Voltage-dependent GABA-induced modulation of calcium currents in chick sensory neurons. *Neurosci. Lett.*, **105**, 113-119.
- HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.*, **391**, 85-100.
- LEWIS, D.L., WEIGHT, F.F. & LUINI, A. (1986). A guanine nucleotide binding protein mediates the inhibition of voltage-dependent calcium current by somatostatin in a pituitary cell line. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 9035-9039.
- SCOTT, R.H. & DOLPHIN, A.C. (1986). Regulation of calcium currents by a GTP analogue: potentiation of (-)-baclofen mediated inhibition. *Neurosci. Lett.*, **56**, 59-64.
- SCOTT, R.H. & DOLPHIN, A.C. (1987). Activation of a G protein promotes agonist responses to calcium channel ligands. *Nature*, **330**, 760-762.
- WINTER, J. (1987). Characterization of capsaicin-sensitive neurones in adult rat dorsal root ganglion cultures. *Neurosci. Lett.*, **80**, 134-140.

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