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Modulation of calcium currents by G-proteins and adenosine receptors in myenteric neurones cultured from adult guinea-pig small intestine

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1 Whole-cell patch clamp methods were used to analyse voltage-dependent calcium currents in cultured myenteric neurones enzymatically isolated from adult guinea-pig small intestine.

2 Activation of G-proteins by intracellular administration of GTP-γ-S (100-200 μM in pipette) decreased the amplitude of high voltage activated Ca^{2+} current (I_{Ca}) by more than 50%. Residual I_{Ca} was activated more slowly and was non-inactivating during 500 ms test pulses when GTP-γ-S was included in the pipette solution.

3 Inclusion of 500 μM GDP-β-S in the patch pipettes increased the amplitude of I_{Ca} by over 30% without altering the voltage-dependency.

4 Extracellular application of 2-chloroadenosine suppressed I_{Ca} dose-dependently by reducing both transient and sustained components of the current.

5 Pretreatment of the neurones with cholera toxin or forskolin did not alter the actions of GTP-γ-S or GDP-β-S or 2-chloroadenosine.

6 The results suggest that high threshold calcium channels in myenteric neurones are influenced by G-proteins and that the inhibitory action of 2-chloroadenosine on I_{Ca} involves G-protein coupling of the adenosine receptors to the Ca^{2+} channel.

Keywords: Enteric nervous system; myenteric neurones; Ca^{2+} channels; intestine; G-proteins; adenosine; autonomic ganglia

Introduction

Myenteric neurones from guinea-pig small intestine express predominantly high voltage-activated Ca^{2+} channels in patch clamp studies with EGTA and CsCl included in the patch pipette (Baidan *et al.*, 1992a,b,c). Interaction of activated guanine nucleotide binging proteins (G-proteins) with this kind of channel and involvement of G-proteins in transduction of agonist signals have been demonstrated for the variety of neuronal types. Nevertheless, mechanisms of agonist actions and involvement of G-proteins relative to the Ca^{2+} channels in myenteric neurones has not been thoroughly investigated.

G-proteins in other systems regulate Ca^{2+} channels both through direct receptor coupling and indirect cytoplasmic second messenger pathways. Microelectrode results suggest that adenosine 3':5'-cyclic monophosphate (cyclic AMP) is a second messenger in signal transduction for slow synaptic excitation (slow e.p.s.p) in myenteric neurones (Nemeth *et al.*, 1986; Palmer *et al.*, 1986). Elevation of cyclic AMP is associated with suppression of Ca^{2+}-activated K^+ conductance and suppression of post spike hyperpolarizing after-potentials which, in turn, enhances excitability and permits repetitive spike discharge.

Adenosine acts at postsynaptic adenosine A_1 receptors on AH/type 2 myenteric neurones to inhibit both slow synaptic excitation and the actions of exogenously applied slow e.p.s.p. mimetics (Palmer *et al.*, 1987). It also acts presynaptically to suppress transmitter release in the enteric, as well as the central and peripheral nervous system (Williams, 1987; Christofi & Wood, 1989; 1993; Barajas-Lopez *et al.*, 1991; Christofi *et al.*, 1992). Accumulation of endogenously released adenosine in longitudinal muscle-myenteric plexus preparations from the intestine *in vitro* tonically suppresses synaptic transmission (Christofi & Wood, 1993).

Reduction of I_{Ca} is one of the possible mechanisms for suppression of synaptic transmission by adenosine. Voltage clamp studies have shown that adenosine decreases I_{Ca} in other neuronal types (Dolphin *et al.*, 1986; Ribeiro & Sebastiao, 1986). Strong evidence suggests that G-proteins are involved in coupling adenosine receptors to channels and second messenger enzyme systems (Scott & Dolphin, 1987; Gross *et al.*, 1989; Kasai & Aosaki, 1989; Scholtz & Miller, 1991). The aims of the present study were to examine the effects of GTP-γ-S and GDP-β-S on I_{Ca} in enteric neurones and relate the results to the action of an adenosine analogue. Results have been published in abstract form (Baidan *et al.*, 1992b).

Methods

Myenteric ganglia were enzymatically dissociated from longitudinal muscle-myenteric plexus preparations dissected from adult guinea-pig small intestine as described earlier (Xia *et al.*, 1991). Initiation of the cultures was based on established methodology for the adult guinea-pig (Hanai *et al.*, 1994). Dissociated ganglia, as individual ganglia or clusters with interconnecting fibre tracts, were collected in suction pipettes under microscopic control. Collections were made from a medium consisting of sterile Krebs solution with a 5% antibiotic-antimycotic mixture. The ganglia, with no visible smooth muscle present, were transferred into medium 199 supplemented with 15% L-glutamine, 10% heat-inactivated foetal calf serum, 33 mM glucose, 1% Penn-Strep solution (10,000 units penicillin and 10 mg streptomycin ml^{-1}), 0.1% fungisone, and 0.5% gentamicin. The ganglia were transferred onto 22 x 22 mm cover slips at the bottom of 33 mm plastic Petri dishes. Each dish contained 15-30 ganglia. The cultures were kept in a humidified incubator at 37°C, with 5% CO_2. Proliferation of non-neuronal cells was reduced by including cytosine arabinoside (10 μM) in the medium for 24 h, beginning 48 h after plating. The culture medium was changed three times each week. Medium 199 and all other reagents were obtained from Sigma Biochemicals, St. Louis, MO, U.S.A. The neurones were used in the patch clamp studies after 2-10 days in culture.

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Standard patch clamp techniques for studying whole-cell currents were used (Hamill *et al.*, 1981). Ionic currents were recorded and voltage clamp test pulses were applied with an Axopatch 200 amplifier and Labmaster interfaced to an IBM computer with pClamp programme (Axon Instruments, Foster City, CA, U.S.A.). Data were stored on disk for later analysis with pClamp software. Amplitude of I_{Ca} was determined as the change from baseline current at the holding potential. Data are given as means ± s.e. with n representing the number of neurones.

The bathing solution contained (in mM): NaCl 120, $CaCl_2$ 5, $MgCl_2$ 1.2, glucose 11.5, HEPES 10, tetrodotoxin 0.2 μM. The patch pipettes had resistances of 2 to 4 mΩ and were filled with (in mM): CsCl 115, $MgSO_4$ 1, Na_2ATP 1, creatine 5, glucose 20, EGTA 5, HEPES 10 and pH adjusted to 7.2 with NaOH. The experiments were done at room temperature (22–25°C).

Results

GTP-γ-S and GDP-β-S

Voltage activated inward I_{Ca} was evoked by 500 ms depolarizing voltage steps from a holding potential of −80 mV. The current was studied under conditions that effectively suppressed Na^+ and K^+ currents. Inward currents reached maximum with depolarizing steps to 0 mV. Peak amplitude of the current was 447 ± 54 pA (n = 21). They consisted of an early inactivating component and a sustained plateau (Figures 1,2). The currents were dependent upon Ca^{2+} in the bathing medium, were resistant to blockade by dihydropyridines and were suppressed by ω-conotoxin GVIA (Baidan *et al.*, 1992c).

With 200 μM GTP-γ-S in the patch pipette, peak I_{Ca} decreased by 41–78% for 59 neurones. The current progressively decreased and reached a steady level 3 to 5 min after rupture of the membrane patch (Figure 1). After 3 to 5 min, the current activated more slowy and displayed minimal inactivation during 500 ms test pulses.

Comparison of I_{Ca} evoked in the same neurones with and without GTP-γ-S in the pipette confirmed reduction of the current after infusion of the GTP analogue into the cell (Figure 2a,b). A family of control currents was first elicited with the protocol shown at the top of Figure 2. Currents evoked by steps to potentials ranging from −120 to 30 mV from a

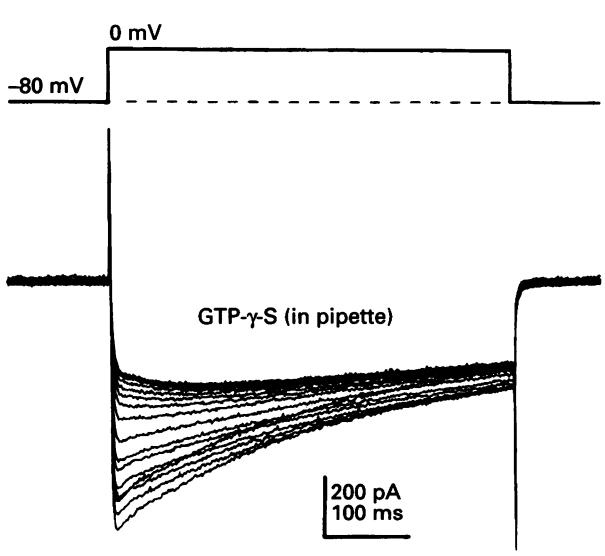

Figure 1 Time course of the decrease in I_{Ca} amplitude induced by internally applied GTP-γ-S (200 μM). Recordings were started 10 s after the rupture of the membrane. The currents were elicited by 500 ms depolarizing voltage steps to 0 mV from the holding potential of −80 mV with 15 s interpulse intervals.

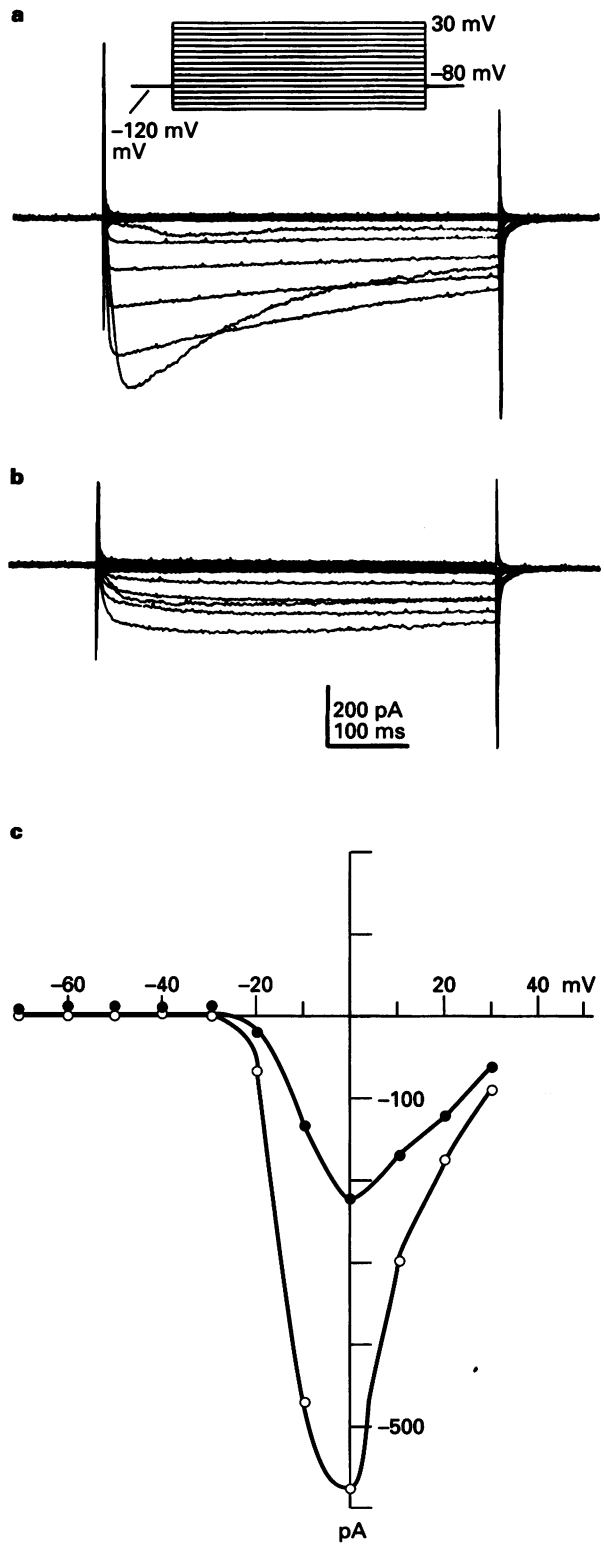

Figure 2 Calcium currents in a myenteric neurone under control conditions (a) and after inclusion of 200 μM GTP-γ-S in patch pipette (b). Currents were activated by 500 ms voltage steps in 10 mV increments from a holding potential of −80 mV. Recordings in (a) were started after control maximum current remained stable for 3 min. After that, the patch pipette was removed and all further recordings were made by means of a second patch pipette containing 200 μM GTP-γ-S after stabilization of the amplitude of maximum current. (c) Voltage-dependence of peak whole-cell I_{Ca} in a myenteric neurone under control conditions (○) and with GTP-γ-S in the patch pipette (○). Data are from (a) and (b).

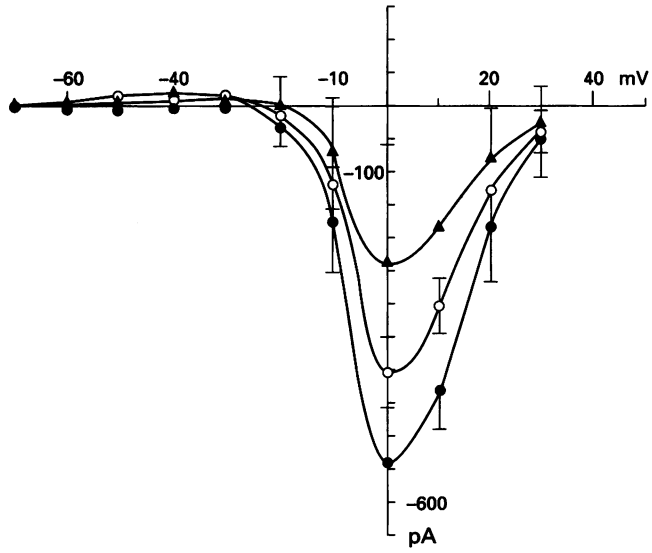

Figure 3 Current-voltage relationships for I_{Ca} in myenteric neurones under control conditions (○), $n=21$; in the presence of 200 μM GTP-γ-S (▲) $n=11$ and in the presence of 500 μM GDP-β-S in the patch pipettes (●), $n=7$. Each point indicates mean ± s.e.

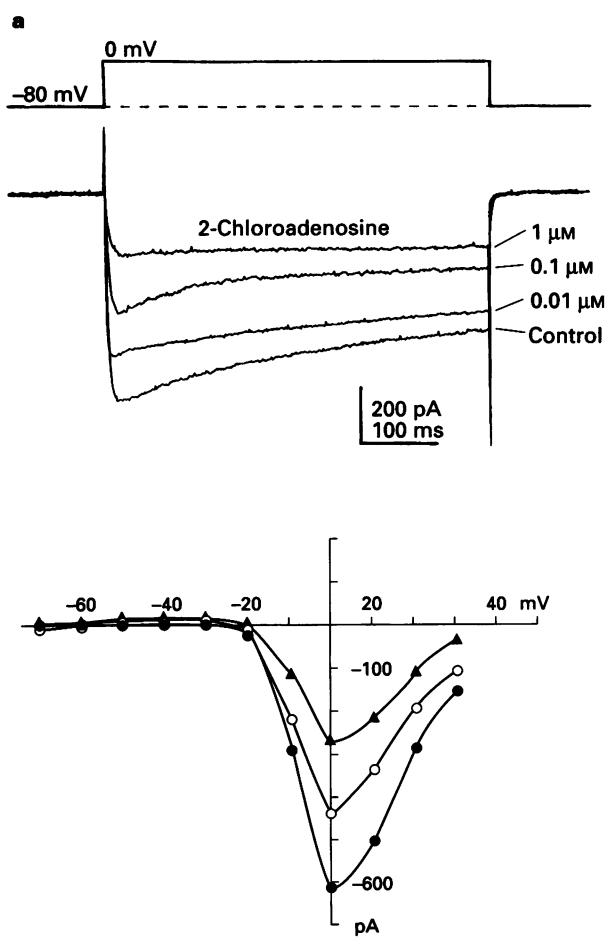

Figure 4 Dose-dependent inhibition of I_{Ca} by 2-chloroadenosine in myenteric neurones. (a) Superimposed current traces recorded in the absence (largst inward current) and in the presence of three different concentrations of 2-chloroadenosine. The currents were elicited by voltage steps to 0 mV from a holding potential of −80 mV. (b) Current-voltage relations for the same neurone before (●) and in the presence of 0.1 μM (○) and 1 μM (▲) 2-chloroadenosine.

holding potential of −80 mV were determined without GTP-γ-S in the pipette (Figure 2a). When stable currents were recorded for 3 min, the patch pipette was replaced with one containing 200 μM GTP-γ-S and the protocol repeated (Figure 2b). Current-voltage curves derived from the protocol executed with and without the GTP analogue in the pipette showed suppression of I_{Ca} at all test potentials more positive than −30 mV when GTP-γ-S was infused (Figure 2c). The position of the current-voltage curves on the voltage axis was unaltered by GTP-γ-S over the range of steps between −70 and 30 mV.

Effects of infusion of GDP-β-S from the patch pipettes were opposite to those found with GTP-γ-S (Figure 3). When GDP-β-S was administered intraneuronally, the amplitude of the maximum I_{Ca} was increased by 23–47% relative to control for

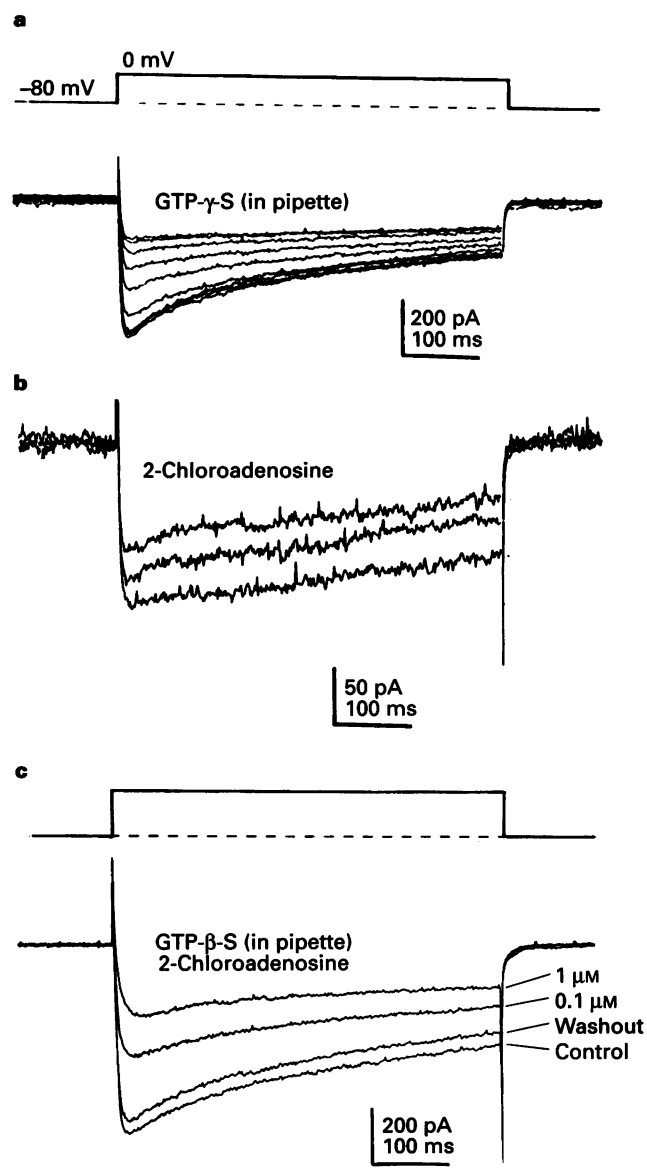

Figure 5 Effect of 2-chloroadenosine (1 μM) on I_{Ca} in myenteric neurones dialyzed with 200 μM GTP-γ-S or GDP-β-S. (a) Time course of GTP-β-S action. Recordings were started 10 s after rupture of patch membrane. Currents were elicited by stepping from −80 to 0 mV with interpulse intervals 15 s. (b) Application of 2-chloroadenosine (1 μM) after stabilization of the current resulted in further decline of the amplitude of I_{Ca}. Note the different current scales for (a) and (b). (c) Inhibition of I_{Ca} by 2-chloroadenosine (0.1 and 1 μM) after dialysis with GDP-β-S. Recordings were started 5 min after rupture of the patch and stabilization of the currents.

7 neurones. No significant changes in the kinetics of activation or inactivation of I_{Ca} were found in any of the neurones after introduction of the GDP analogue.

The analogue, 2-chloroadenosine (2-CA), was used instead of adenosine in our studies because it is known not to be taken up into the neurones and to act at receptors on the neuronal surface (Sturgill *et al.*, 1975; Londos & Wolff, 1977). Application of 2-CA in the bathing solution abolished the transient component and reduced the sustained component of I_{Ca}. Inhibition of I_{Ca} by 2-CA was similar to the suppression seen with GTP-γ-S in the pipette. The effects of 2-CA were dose-dependent over a concentration-range of 0.01 to 1 μM (Figure 4a). The threshold was about 1 nM and the IC_{50} was 210 nM. Current-voltage plots for I_{Ca} in the presence of 0.1 or 1 μM 2-CA revealed no significant shifts in the voltage-dependence of the current (Figure 4b). The inhibitory action of 2-CA was observed in all of 47 neurones tested and did not show any indications of desensitization to multiple applications at 3 min intervals.

When pipettes containing GTP-γ-S were used to record I_{Ca}, suppression of I_{Ca} progressed to a steady state over a time course of about 3 min (Figure 5a). Application of 1 μM 2-CA, after a steady state was reached with GTP-γ-S in the pipette, decreased the current beyond that already produced by GTP-γ-S (Figure 5b). This was found in 7 out of 10 neurones when GTP-γ-S was in the pipette.

Suppression of I_{Ca} by 0.1 or 1 μM 2-CA still occurred when the pipette contained 500 μM GDP-β-S in each of 11 neurones (Figure 5c). However, the degree to which 2-CA decreased the peak current was significantly less with GDP-β-S in the pipette. The mean reduction in I_{Ca} was $31 \pm 8\%$ ($n = 5$) of the decrease found in the absence of GDP-β-S in the pipette.

Forskolin and cholera toxin

Treatments known to elevate intraneuronal cyclic AMP in myenteric neurones (Xia *et al.*, 1992) did not affect I_{Ca} as recorded in the present study. Neither forskolin nor cholera toxin changed the peak current or the current-voltage relations when applied in the bathing solution of by diffusion from the patch pipette. Forskolin (0.1 – 10 μM) in the bathing solution did not alter the current in 11 neurones and inclusion of the same range of concentrations in the pipettes did not alter I_{Ca} in 5 cells (Figure 6). The presence of 10 μM forskolin in the bath did not alter the inhibitory action of 2-CA on I_{Ca}.

Dolphin (1991) reported that 10 μM forskolin in the bath increased the amplitude of Ca^{2+} currents in rat dorsal root ganglion neurones but only when GTP-γ-S was included in the pipette solution. We did not find this effect in the myenteric neurones.

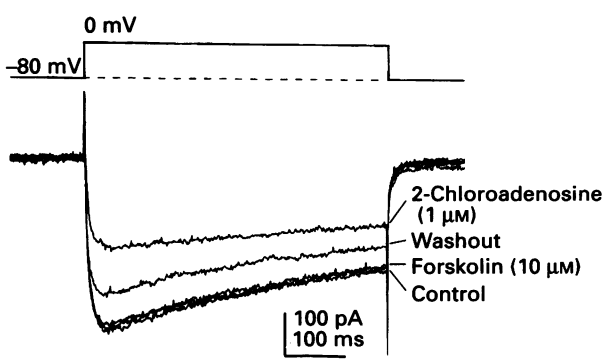

Figure 6 Lack of effect of forskolin (10 μM) on I_{Ca} in a myenteric neurone. The currents were evoked by depolarizing voltage steps to 0 mV from a holding potential of −80 mV. Superimposed current traces, recorded from the same neurone, in the absence (control) and 30 s and 1 min after application of forskolin and 15 s after application of 2-choloradenosine in the presence of forskolin.

Pretreatment of the cultures with cholera toxin (500 ng ml^{-1} for 16 h) did not alter I_{Ca} in 13 neurones. There were also no changes in the inhibitory effects of GTP-γ-S or 2-CA on I_{Ca} in 5 and 6 of the cholera-treated neurones, respectively.

Discussion

The importance of calcium currents for signal transduction and modulation of excitability in enteric neurones is firmly established by intracellular microelectrode studies (North, 1973; Grafe *et al.*, 1980; Hirst *et al.*, 1985a,b). Opening of voltage-activated Ca^{2+} channels during the action potential in AH/type 2 enteric neurones transiently elevates intraneuronal Ca^{2+}. This leads to increased Ca^{2+}-activated K^+ conductance and prolonged hyperpolarizing after-potentials that restrict the frequency of spike discharge. Steady influx of Ca^{2+} when the neurones are at rest keeps cytoplasmic concentrations elevated and a population of Ca^{2+}-activated K^+ channels open to produce a hyperpolarized state near E_K. Activation of receptors that mediate slow synaptic excitation in AH/type 2 neurones block opening of the Ca^{2+} channels during the spike and, thereby, suppresses hyperpolarizing after-potentials. Receptor activation also closes the channels responsible for influx of Ca^{2+} at rest. This, in turn, leads to closure of Ca^{2+}-activated K^+ channels and the slowly activating depolarization and increased input resistance characteristic of slow synaptic excitation as recorded with 'sharp microelectrodes'. Most of the evidence suggests that regulation of I_{Ca} and relations to other conductances are the basic mechanisms for modulation of excitability over a range from inexcitability with the membrane potential near E_K to hyper-excitability with repetitive spike discharge at frequencies approaching 100 Hz (Wood, 1989; 1994). Patch clamp recording of whole-cell I_{Ca} in the present study was another approach to a better understanding of the behaviour of Ca^{2+} in enteric neurones.

Results of the present study support the feasibilty of investigating I_{Ca} in myenteric neurones dissociated from adult guinea-pig small bowel. They suggest that G-proteins are involved in the modulation of the high-threshold calcium channels that were the focus of the study. This is consistent with reports that G-proteins influence behaviour of Ca^{2+} channels in other excitable cells (Scott *et al.*, 1991; Ohya & Sperelakis, 1991) and in neurones of the submucous plexus (Surprenant *et al.*, 1990).

The effects of GTP-γ-S on I_{Ca} may result from sequestration of a population of channels into a closed state complexed with activated G-proteins. Activation of G-proteins by GTP-γ-S in other cells is known to result in up- or down-regulation of Ca^{2+} channels. This was inhibitory in the myenteric neurones comparable to similar effects in other neuronal types including neurones of the guinea-pig submucous plexus (Surprenant *et al.*, 1990; Scott *et al.*, 1991). GTP-γ-S is a substitute for endogenous GTP at binding sites on G-proteins and because it is a poorly hydrolyzable substrate for GTPases, expected to activate G-protein coupled events irreversibly.

Failture to find effects of cholera toxin or forskolin suggests that modulation of I_{Ca} in myenteric neurones does not result from activation of G_s-proteins. Others also have found that experimental elevation of intraneuronal cyclic AMP has no effect on I_{Ca} (Bley & Tsien, 1990; Scott *et al.*, 1991) or produces small enhancement (Gray & Johnston, 1987). This differs from I_{Ca} in cardiac and smooth muscle and may be due to a higher level of basal activity of adenylyl cyclase resulting in a high level of channel phosphorylation in the normal state (Dolphin, 1990).

The increase in I_{Ca} evoked by infusion of GDP-β-S may be explained by ongoing regulation of the channels by endogenous GTP. GDP-β-S presumably competes with GTP at binding sites on the proteins and by displacing bound GTP from the sites reverses GTP-mediated tonic inhibition of I_{Ca}. Scott and Dolphin (1987) reported increases in I_{Ca} in dorsal root ganglion cells similar to those in myenteric neurones after

infusion of GDP-β-S. Adenosine is known to accumulate and suppress neuronal excitability in *in vitro* preparations of the guinea-pig myenteric plexus (Christofi & Wood, 1993). Whether this is reflected by GTP-mediated tonic inhibition in the myenteric cultures is uncertain.

The results obtained with 2-CA are consistent with the suggestion that one of several mechanisms by which adenosine suppresses synaptic transmission is through depression of I_{Ca}. Inhibition of I_{Ca} by 2-CA in the myenteric neurones was reminiscent of the action of adenosine in dorsal root ganglion cells from a variety of species (Dolphin *et al.*, 1986; Kasai & Aosaki, 1989; Gross *et al.*, 1989), in avian ciliary ganglia (Bennet *et al.*, 1992), in rat hippocampal neurones (Scholtz & Miller, 1991) and in rat cervical ganglion cells (Zhu & Ikeda, 1993). Similarity of effects of infusion of GTP-γ-S suggest that receptors for 2-CA are coupled by G-proteins to the Ca^{2+} channels. Nevertheless, our finding of further suppression of I_{Ca} by 2-CA after maximal suppression by infusion of GTP-γ-S may be indicative of a mechanism of action independent of G-protein coupling to the channels.

References

BAIDAN, L.V., ZHOLOS, A.V., SHUBA, M.F. & WOOD, J.D. (1992a). Patch-clamp recording in myenteric neurones of guinea-pig small intestine. *Am. J. Physiol.*, **262,** G1074–G1078.

BAIDAN, L.V., ZHOLOS, A.V. & WOOD, J.D. (1992b). Modulation of Ca^{2+} current by G-proteins and their involvement in the inhibitory effect of 2-chloroadenosine on isolated myenteric neurones. *Dig. Dis. Sci.*, **37,** 972, A10.

BAIDAN, L.V., ZHOLOS, A.V. & WOOD, J.D. (1992c). Properties of calcium currents determined by patch-clamp recording in myenteric neurones from adult guinea-pig small intestine. *Gastroenterology*, **102,** A420.

BARAJAS-LOPEZ, C., SURPRENANT, A. & NORTH, R.A. (1991). Adenosine A_1 and A_2 receptors mediated presynaptic inhibition and postsynaptic excitation in guinea-pig submucosal neurones. *J. Pharmacol. Exp. Ther.*, **258,** 490–495.

BENNETT, M.R., KERR, R. & KHURANA, G. (1992). Adenosine modulation of calcium currents in postganglionic neurones of avian cultured ciliary ganglia. *J. Pharmacol.*, **106,** 25–32.

BLEY, K.R. & TSIEN, R.W. (1990). Inhibition of Ca and K channels in sympathetic neurones by neuropeptides and other ganglionic transmitters. *Neuron*, **4,** 379–391.

CHRISTOFI, F.L., TACK, J. & WOOD, J.D. (1992). Suppression of nicotinic synaptic transmission by adenosine in myenteric ganglia of the guinea-pig gastric antrum. *Eur. J. Pharmacol.*, **216,** 17–22.

CHRISTOFI, F.L. & WOOD, J.D. (1989). Release of endogenous adenosine modulates the synaptic and electrical behaviour of myenteric neurones of the guinea-pig distal ileum. *J. Gastrointest. Motility*, **1,** 77A.

CHRISTOFI, F.L. & WOOD, J.D. (1993). Presynaptic inhibition by adenosine A_1 receptors on guinea-pig small intestinal myenteric neurones. *Gastroenterology*, **104,** 1420–1429.

DOLPHIN, A.C. (1990). G protein modulation of calcium current in neurones. *Annu. Rev. Physiol.*, **52,** 243–255.

DOLPHIN, A.C. (1991). Ca^{2+} channel currents in rat sensory neurones: interaction between guanine nucleotides, cyclic AMP and Ca^{2+} channel ligands. *J. Physiol.*, **432,** 23–43.

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.

GRAFE, P., MAYER, C.J. & WOOD, J.D. (1980). Synaptic modulation of calcium-dependent potassium conductance in myenteric neurones in the guinea-pig. *J. Physiol.*, **305,** 235–248.

GRAY, R. & JOHNSTON, D. (1987). Noradrenaline and adrenoreceptor agonists increase activity of voltage-dependent Ca channels in hippocampal neurones. *Nature*, **327,** 620–622.

GROSS, R.A., MACDONALD, R.L. & RYAN-JASTROW, T. (1989). 2-Chloroadenosine reduces the N-calcium current of cultured mouse sensory neurones in a pertussis toxin-sensitive manner. *J. Physiol.*, **411,** 585–595.

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

HANANI, M., XIA, Y. & WOOD, J.D. (1994). Myenteric ganglia from the adult guinea-pig small intestine in tissue culture. *Neurogastroenterol. Motility*, **6,** 103–118.

HIRST, G.D.S., JOHNSON, S.M. & VAN HELDEN, D.F. (1985a). The calcium current in a myenteric neurone of the guinea-pig ileum. *J. Physiol.*, **361,** 297–314.

HIRST, G.D.S., JOHNSON, S.M. & VAN HELDEN, D.F. (1985b). The slow calcium-dependent potassium current in a myenteric neurone of the guinea-pig ileum. *J. Physiol.*, **361,** 315–337.

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

LONDOS, C. & WOLFF, J. (1977). Two distinct adenosine-sensitive sites on adenylate cyclase. *Proc. Natl. Acad. Sci. U.S.A.*, **74,** 5482–5486.

NEMETH, P.R., PALMER, J.D., WOOD, J.D. & ZAFIROV, D.H. (1986). Effects of forskolin on electrical behaviour of myenteric neurones in guinea-pig small intestine. *J. Physiol.*, **376,** 439–450.

NORTH, R.A. (1973). The calcium-dependent slow after-hyperpolarization in myenteric plexus neurones with tetrodotoxin-resistant action potentials. *Br. J. Pharmacol.*, **49,** 709–711.

OHYA, Y. & SPERELAKIS, N. (1991). Involvement of a GTP-binding protein in stimulating action of angiotensin II on calcium channels in vascular smooth muscle cells. *Circ. Res.*, **68,** 763–771.

PALMER, J.M., WOOD, J.D. & ZAFIROV, D.H. (1986). Elevation of cyclic adenosine monophosphate mimics slow synaptic excitation in myenteric neurones of the guinea-pig. *J. Physiol.*, **376,** 451–460.

PALMER, J.M., WOOD, J.D. & ZAFIROV, D. (1987). Purinergic inhibition in the small intestinal myenteric plexus of the guinea-pig. *J. Physiol.*, **387,** 357–369.

RIBEIRO, J.A. & SEBASTIAO, A.M. (1986). Adenosine receptors and calcium: Basis for proposing a third (A_3) adenosine receptor. *Prog. Neurobiol.*, **26,** 179–209.

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

SCOTT, R.H. & DOLPHIN, A.C. (1987). Inhibition of calcium by an adenosine analogue 2-chloroadenosine. In *Topics and Perspectives in Adenosine Research.* ed. Gerlach, E. & Becker, B.F. pp. 549–558. Berlin, Heidelberg: Springer-Verlag.

SCOTT, R.H., PEARSON, H.A. & DOLPHIN, A.C. (1991). Aspects of vertebrate neuronal voltage-activated calcium currents and their regulation. *Prog. in Neurobiol.*, **36,** 485–520.

STURGILL, T.W., SCHRIER, M.B.K. & GILMAN, A.G. (1975). Stimulation of cyclic AMP accumulation by 2-chloroadenosine: Lack of incorporation of nucleoside into cyclic nucleotides. *J. Cyclic Nucleotide Res.*, **1,** 21–30.

SURPRENANT, A., SHEN, K.Z., NORTH, R.A. & TATSUMI, H. (1990). Inhibition of calcium currents by noradrenaline, somatostatin and opioids in guinea-pig submucosal neurones. *J. Physiol.*, **431,** 585–608.

WILLIAMS, M. (1987). Purine receptors in mammalian tissues: pharmacology and functional significance. *Annu. Rev. Pharmacol. Toxicol.*, **27,** 315–345.

WOOD, J.D. (1989). Electrical and synaptic behaviour of enteric neurones. In *Handbook of Physiology. The Gastrointestinal System. Motility and Circulation.* ed. Wood, J.D., pp. 465–517. Bethesda, MD: American Physiological Society.

WOOD, J.D. (1994). Physiology of the enteric nervous system. In *Physiology of the Gastrointestinal Tract,* 3rd edition. ed. Johnson, L.R. pp. 423–482. New York: Raven Press.

XIA, Y., BADIDAN, L.V., FERTEL, R.H. & WOOD, J.D. (1991). Determination of levels of cyclic AMP in the myenteric plexus of guinea-pig small intestine. *Eur. J. Pharmacol.*, **206,** 231–236.

ZHU, Y. & IKEDA, S.R. (1993). Adenosine modulates voltage-gated Ca^{2+} channels in adult rat sympathetic neurones. *J. Neurophysiol.*, **70,** 610–620.

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