J. Physiol. (1986), **376**, *pp.* 439–450
With 9 text-figures
Printed in Great Britain

EFFECTS OF FORSKOLIN ON ELECTRICAL BEHAVIOUR OF MYENTERIC NEURONES IN GUINEA-PIG SMALL INTESTINE

BY P. R. NEMETH*, J. M. PALMER, J. D. WOOD AND D. H. ZAFIROV†

From the Department of Physiology, College of Medicine, The Ohio State University, 4196 Graves Hall, 333 West 10th Avenue, Columbus, OH 43210-1238, U.S.A.

(*Received 11 September 1985*)

SUMMARY

1. The actions of forskolin on electrical behaviour of myenteric neurones were investigated with intracellular recording methods in guinea-pig small intestine.

2. The actions of forskolin were: membrane depolarization, increased input resistance, suppression of post-spike hyperpolarizing potentials and repetitive spike discharge. These effects occurred always in AH/Type 2 myenteric neurones and never in the cells classified as S/Type 1.

3. Reversal potentials for the depolarizing effects were near the estimated potassium equilibrium potential. Analyses based on the 'constant field equation' indicated that the permeability ratios of K^+ to other permeant ionic species were reduced by forskolin.

4. Pretreatment of the neurones with a phosphodiesterase inhibitor potentiated the effects of forskolin.

5. The results suggest that activation of adenylate cyclase by forskolin and subsequent elevation of intraneuronal adenosine 3′,5′-phosphate (cyclic AMP) mimic slow synaptic excitation in AH/Type 2 myenteric neurones. They support the possibility that cyclic AMP functions as a second messenger in signal transduction which appears to involve closure of calcium-dependent K^+ channels and other membrane changes that lead to depolarization and a dramatic increase in the excitability of the neurones.

INTRODUCTION

Forskolin is a diterpene of the lab dane family of hydrocarbons that has been isolated from the roots of *Coleus forskolii* (Bhat, Bajiva, Dornauer & de Souza, 1977). It is a specific activator of adenylate cyclase in a variety of broken cell preparations and whole tissues and is a useful tool for investigation of cellular responses that are dependent upon adenosine 3′,5′-phosphate (cyclic AMP) as a second messenger (Seamon & Daly, 1981). Activation of adenylate cyclase by forskolin results in large

* Present address: Procter and Gamble Company, Miami Valley Laboratories, Cincinnati, OH 45247, U.S.A.

† Present address: Physiological Institute, Bulgarian Academy of Sciences, Sofia, Bulgaria.

increases in the amount of cyclic AMP in a variety of cell types including neurones (Kilmer & Carlsen, 1984).

Slow synaptic excitation (slow excitatory post-synaptic potential (e.p.s.p.)) in enteric neurones is a prominent functional event that undoubtedly has important physiological significance in enteric nervous control of gastrointestinal function (reviewed by Wood, 1984). It has a slow onset and long persistence after transient activation of the synaptic inputs or application of putative neurotransmitters. These characteristics suggest that synthesis of a second messenger might be involved in signal transduction during the slow e.p.s.p. We used forskolin as a tool to investigate the possibility that cyclic AMP is the second messenger. The results showed that forskolin mimicked the electrophysiological changes that occur in AH/Type 2 myenteric neurones during the slow e.p.s.p. and provided evidence that cyclic AMP has a role in slow synaptic modulation of excitability. A preliminary report has been published (Nemeth, Zafirov & Wood, 1984).

METHODS

Segments of intestine were obtained 10–20 cm orad to the ileocaecal junction of adult guinea-pigs (250–600 g) that had been stunned by a blow to the head and exsanguinated. Flat sheet preparations of longitudinal muscle with myenteric plexus attached were prepared, mounted in a superfusion chamber and observed with an interference contrast microscope as described in a previous report (Wood & Mayer, 1978). Conventional intracellular recording methods with bridge circuitry for intracellular current injection through the micro-electrode were used (Wood & Mayer, 1978). Synaptic input to the ganglion cells was activated by electrical shocks (200 μs duration) applied to the ganglia or interganglionic connectives with electrodes made from Teflon-insulated Pt-wire (20 μm diameter) and a S-88 square-wave stimulator (Grass Instruments, Quincy, MA, U.S.A.).

The reversal potential for the forskolin effect was estimated by continuously recording current–voltage relationships with four different steps of hyperpolarizing current intensities as previously described (Grafe, Mayer & Wood, 1980). Current injection was controlled by a programmed pulse generator which delivered a sequence of four 100 ms rectangular pulses which increased with constant increments of strength at 100 ms intervals. The sequence of pulses was repeated at 1–2 s intervals, and the data also were used for preparation of current–voltage plots. Data were recorded on magnetic tape for later analysis and reproduction on a strip-chart recorder (Gould Instruments). Amplitudes of the action protentials in the illustrations are attenuated slightly due to the limited frequency response of the chart recorder.

The tissues were maintained at 37 °C and pH 7·4 in Krebs solution gassed with 95 % O_2–5 % CO_2. Composition of the Krebs solution in mM was NaCl, 120·9; KCl, 5·9; $MgCl_2$, 1·2; NaH_2PO_4, 1·2; $NaHCO_3$, 14·4; $CaCl_2$, 2·25; glucose, 11·5.

Forskolin and other substances were applied by addition to the superfusion solution or by microejection from fine-tipped pipettes (tip diameter 10–20 μm) with nitrogen pulses of controlled pressure and duration. Concentrations of chemical agents in the pipettes were three to four orders of magnitude higher than the lowest effective concentration applied in the superfusion solution. Concentrations attained at the neurone were impossible to determine when the microejection method was used because the tips of the pipettes were positioned 20–50 μm from the impaled neurone and the 1 ml volume of the tissue chamber was perfused at a rate of 10–14 ml/min. Relative changes in concentrations were achieved by varying the duration of the pressure ejection pulse. Amount of substance released from the pipette was a linear function of the pulse over the range of durations used. All substances in the pipettes were freshly prepared and dissolved in Krebs solution. Forskolin was dissolved in 95 % ethyl alcohol and final dilutions were in Krebs solution. Ethyl alcohol alone was added to the superfusion in control trials that ruled out possible actions of the carrier.

Drugs used were tetrodotoxin and 3-isobutyl-1-methylxanthine (Sigma Chemical Co.) and forskolin (Calbiochem-Behring).

RESULTS

Results were obtained from 139 neurones selected from 175 impalements of myenteric ganglion cells in preparations from 119 guinea-pigs. 129 of the neurones were classified as AH/Type 2 neurones and 10 were S/Type 1 neurones (Nishi & North, 1973; Hirst, Holman & Spence, 1974; Wood, 1984). Impalements were maintained for periods of 20 min to several hours. AH/Type 2 neurones selected for analysis

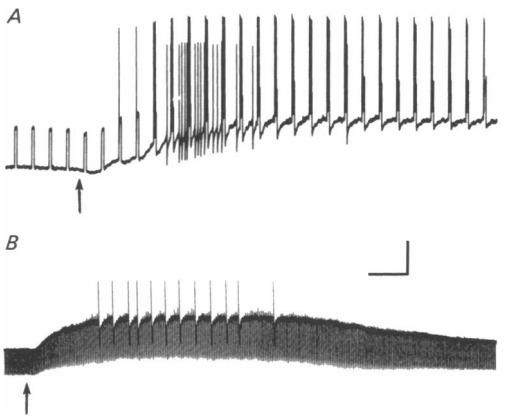

Fig. 1. The effects of forskolin on electrical behaviour of AH/Type 2 myenteric neurones. *A*, response to forskolin. The arrow indicates application of a 20 ms pulse of 0·5 mM-forskolin. Constant-current, depolarizing pulses were injected repetitively into the cell soma throughout the trace. Forskolin depolarized the cell and enhanced excitability as evidenced by spontaneous spike discharge and increased frequency of spike discharge during depolarizing current pulses. *B*, effects of forskolin after 10 min in the presence of (1 μM) TTX. The time at which 50 μM-forskolin entered the bath is indicated by the arrow. Forskolin was in the bath for 30 s. Constant-current hyperpolarizing pulses were injected into the cell repetitively and an increase in the width of the base line reflects an increase in the input resistance. Spike discharge at the offset of the hyperpolarizing current pulses reflects enhanced excitability produced by forskolin. Vertical calibration, 20 mV for *A* and 32 mV for *B*. Horizontal calibration, 2·5 s for *A* and 13·2 s for *B*.

maintained a relatively high membrane potential (−60 to −75 mV) and low input resistance (10–60 MΩ) for periods of at least 1 h without apparent oedematous changes occurring in the vicinity of the impaled neurones (Grafe *et al.* 1980). S/Type 1 neurones had relatively low resting membrane potentials (−30 to −45 mV) and high input resistances (60–180 MΩ) which were maintained for periods of at least 30 min. Neurones that demonstrated the occurrence of swelling after impalement, with concomitant changes in electrical properties and behaviour, were not used for the experiments (Nishi & North, 1973; Grafe *et al.* 1980).

Effects of forskolin on electrical behaviour

Application of forskolin either by focal microejection or by addition to the superfusion solution resulted in depolarization of the membrane potential, increased input resistance, reduction of post-spike hyperpolarizing potentials and augmented excitability in all of 129 AH/Type 2 myenteric neurones (Fig. 1). All of these effects

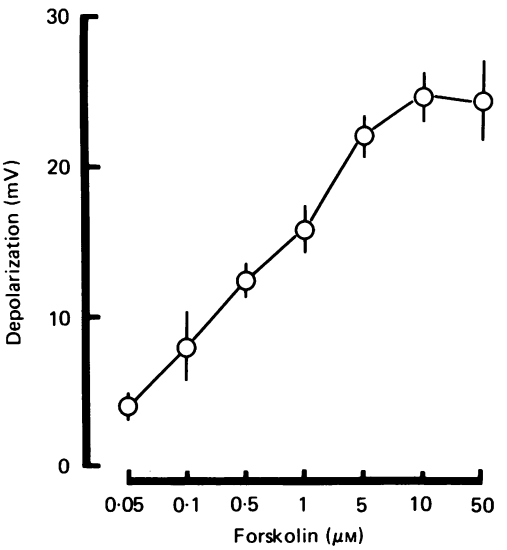

Fig. 2. Dose–response relation for membrane depolarization and concentration of forskolin applied in the superfusion solution. Data are means ±S.E. for twelve different AH/Type 2 myenteric neurones.

of the drug were reversed as it was washed from the tissue chamber. However, for applications of higher concentrations (> 1 μM) for periods greater than 1 min, wash times of 15 min or more were required for complete reversal. Forskolin did not significantly affect the electrical behaviour of 10 S/Type 1 myenteric neurones.

The membrane depolarization, which required several seconds to develop to peak, ranged from 5 to 26 mV depending upon the concentration. Peak amplitudes of the depolarizing responses were concentration dependent over a range of 0·05 to 10 μM in the superfusion solution (Fig. 2) as was the rate of rise of the depolarization. With application of the drug by pressure microejection, the magnitude of the depolarization increased with longer duration pressure pulses.

An increase in the input resistance was associated with the membrane depolarization. The increase in input resistance began with the onset of depolarization and progressively increased as the neurones depolarized. The relationship between input resistance and membrane potential was linear when the increase in input resistance was normalized to a percentage and plotted as a function of membrane potential (Fig. 3).

Use of the step-pulse method to estimate the reversal potential for the effect of forskolin showed that forskolin produced depolarization at membrane potentials more positive than −82 to −84 mV, that no potential change occurred with the membrane clamped between −82 and −84 mV and that forskolin hyperpolarized the membranes at membrane potentials more negative than −84 mV (Fig. 4).

Forskolin-induced increases in the input resistance were reflected by increased amplitudes of the electrotonic potentials produced by intraneuronal injection of constant-current hyperpolarizing pulses (Fig. 1*B*). The slopes of current–voltage curves, obtained by the step-pulse method at the time the depolarizing response to

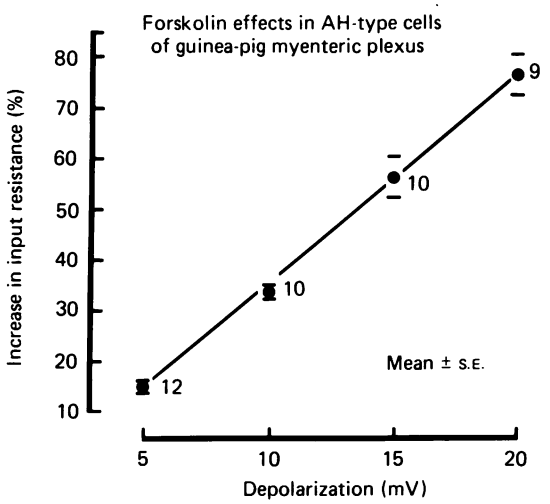

Fig. 3. Relation between amount of depolarization produced by various concentrations of forskolin and the corresponding increase in input resistance in the number of AH/Type 2 neurones indicated by each data point. Mean values ±S.E. are given.

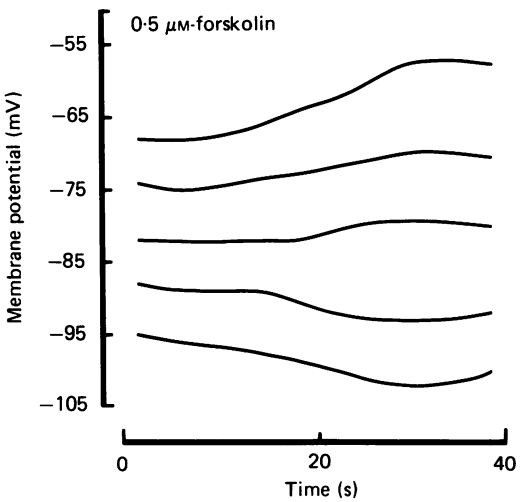

Fig. 4. Estimated reversal potential for effects of forskolin in an AH/Type 2 myenteric neurone. The depolarizing action of forskolin was reversed to hyperpolarization at current-clamped membrane potentials more negative than −82 mV. Top trace represents resting membrane potentials without injection of hyperpolarizing current. Successively lower traces represent membrane potentials produced by four steps of injected current from the step-pulse generator. The group of four stepped pulses was repeated at 1 s intervals. The traces are plots of the data over a 40 s time course.

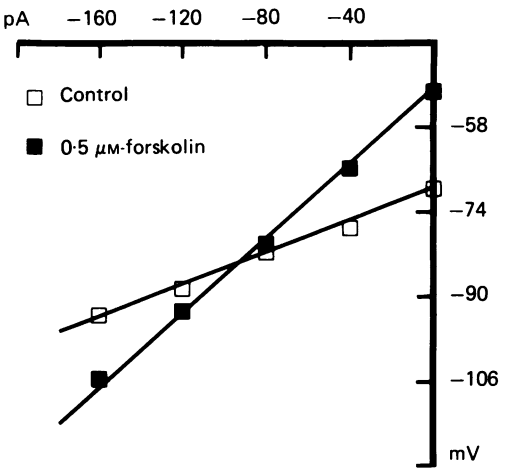

Fig. 5. Current–voltage relations in the presence and absence of forskolin applied in the superfusion solution. The vertical axis represents the actual membrane potential. The data points are changes in membrane potential to steps of hyperpolarizing current injected through the micro-electrode.

forskolin reached its maximum, were always greater than the slopes of equivalent plots in Krebs solution (Fig. 5). Current–voltage curves obtained in the presence of forskolin and in normal solutions always intersected at membrane potentials between −82 and −84 mV (Fig. 5). This is evidence that the reversal potential for the effect is within this range of membrane potentials.

Characteristic hyperpolarizing after-potentials accompanied the spikes in all of the cells classified as AH/Type 2. In each of these neurones, application of forskolin either reduced the amplitude and duration of the after-potentials or abolished them (Fig. 6). In about 10% of the cells, spontaneous discharge of spikes accompanied by hyperpolarizing after potentials occurred after wash-out of forskolin from the tissue. This was the case for the neurone in Fig. 6. Forskolin suppressed the after-potential for both spontaneous spikes and spikes that were evoked by intracellular injection of depolarizing current pulses. Reduction of the after-potentials was not due to rectification during the depolarizing response to forskolin because the same reduction still occurred when the drug-evoked depolarization was prevented by steady injection of hyperpolarizing current during the response.

Augmented excitability during the depolarizing responses to forskolin was reflected by a significant increase in the number of action potentials evoked by rectangular pulses of contant current injected into the neuronal somas (Fig. 1*A*). Enhanced excitability also was apparent in some cells as a train of action potentials that appeared at the crests of the forskolin-induced depolarization (Fig. 1*A*) and also in most cases as discharge of one or two action potentials at the offset of the hyperpolarizing current pulses that were injected into the cells to assess changes in input resistance (Fig. 1*B*). The augmented excitability resulted from membrane actions additional to depolarization because none of the indications of increased excitability occurred during steady depolarization of the membrane by injection of inward current before application of forskolin.

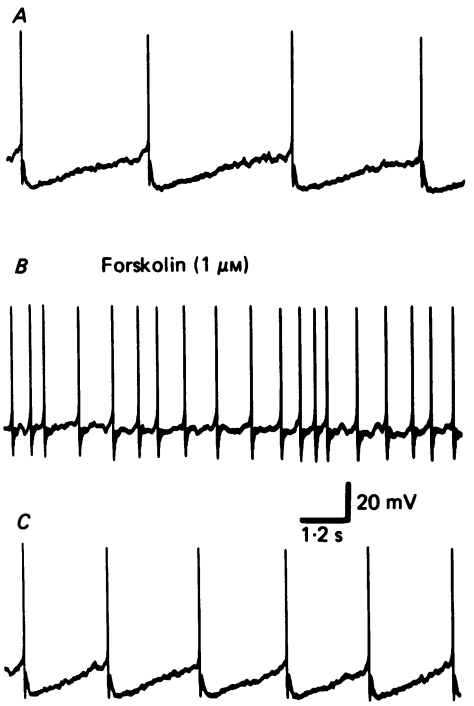

Fig. 6. Effects of forskolin on hyperpolarizing after-potentials in an AH/Type 2 myenteric neurone. *A*, spontaneously occurring spikes were followed by prolonged hyperpolarizing after-potentials in the absence of forskolin. *B*, application of 1 μM-forskolin in the superfusion solution increased the frequency of spontaneous spike discharge and abolished the after-potentials. *C*, return to control condition after wash. All records from the same neurone.

Relationship between external K^+ concentration, membrane potential and forskolin

The purpose of this series of experiments was to assess further the possibility that forskolin acted to decrease the resting permeability of the membranes for K^+. This was done by comparing the effects of variation of external K^+ concentration on the resting membrane potential in the presence and absence of forskolin. The data when plotted as the resting potential against the logarithm of external K^+ agreed well with theoretical curves plotted according to the 'constant field equation' (Fig. 7). At all points on the plots below 50 mM-K^+, the slopes of the curves in Krebs solution were much steeper than the slopes of the curves in solutions containing forskolin. This suggested that resting potassium conductance (G_K) was reduced in the presence of forskolin, and this could account for the increased input resistance that was observed in the presence of forskolin (Fig. 5).

Effects of tetrodotoxin and elevated Mg

The actions of forskolin were not the result of synaptic input from other neurones because blockade of axonal spike conduction with tetrodotoxin (TTX) or prevention of neurotransmitter release in solutions containing 16 mM-Mg^{2+} and 1·25 mM-Ca^2 did not prevent the membrane depolarization, increased input resistance or augmented

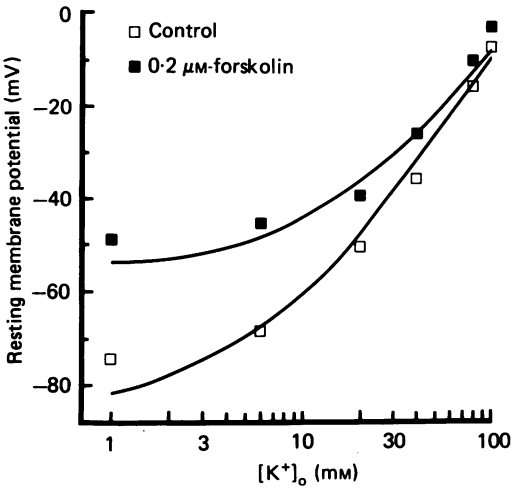

Fig. 7. Membrane potential of an AH/Type 2 myenteric neurone *versus* the logarithm of external K^+ concentration ($[K^+]_o$) in the presence and absence of forskolin. The 'constant field equation' was used to fit the continuous lines to the data points. The following ionic concentrations were inserted into the constant field equation (mM): intracellular $K^+ = 140$; intracellular $Na^+ = 10$; intracellular $Cl^- = 10$. The permeability ratios were: $P_K:P_{Na}:P_{Cl} = 50:2{\cdot}2:1$ in the presence of normal Krebs solution, and $P_K:P_{Na}:P_{Cl} = 15:2{\cdot}2:1$ in the presence of 0·2 μM-forskolin. These data suggest that potassium permeability was decreased by more than 3 × in the presence of forskolin.

excitability (Figs. 1*B* and 8). The action potential discharge that is apparent in Fig. 1*B* occurred at the offset of the hyperpolarizing current pulses and reflected the augmentation of excitability that resulted from treatment with forskolin. These somal spikes are generated by voltage-sensitive calcium channels that are insensitive to TTX (North, 1973; Hirst, Johnson & van Helden, 1985*a*); whereas, changes in sodium conductance, which are blocked by TTX, are responsible for the spikes in the processes of the neurones. We have already demonstrated that elevated Mg^{2+} and reduced Ca^{2+} act like forskolin to depolarize the cells, increase the input resistance and augment excitability (Grafe *et al.* 1980) and this action is apparent in Fig. 8*B*. It has been reported also that both fast and slow synaptic transmission in the guinea-pig myenteric plexus were suppressed by elevated Mg^{2+} and reduced Ca^{2+} within the time span of forskolin application (Fig. 8) in the present study (Wood & Mayer, 1979*a*, *b*).

Effects of 3-isobutyl-1-methylxanthine

If the actions of forskolin resulted from activation of adenylate cyclase and elevation of cyclic AMP, then treatment with 3-isobutyl-1-methylxanthine (IBMX) would be expected to potentiate the effects of forskolin. This should occur because IBMX suppresses the enzymatic action of the phosphodiesterase that converts cyclic AMP to an inactive metabolite.

Eleven of the AH/Type 2 neurones were pretreated with IBMX for 3–5 min prior to a second application of forskolin in the presence of IBMX. In each of these cells, the second application of the same amount of forskolin produced a greater degree

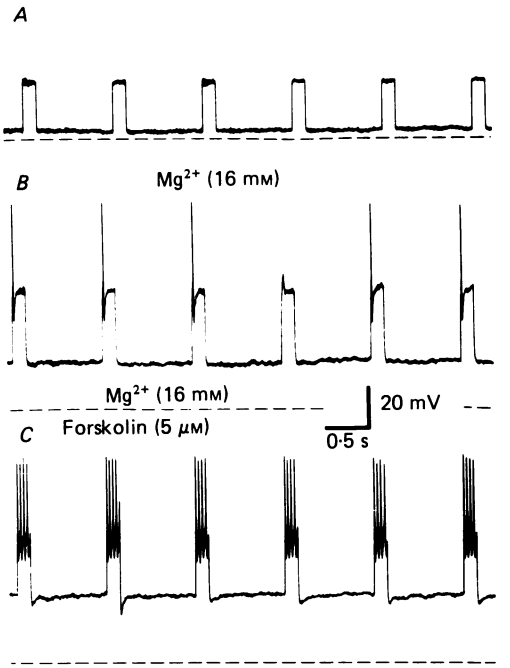

Fig. 8. Effects of forskolin during synaptic blockade by elevated Mg^{2+} and reduced Ca^{2+} in an AH/Type 2 myenteric neurone. *A*, control. *B*, after 4 min in the presence of elevated Mg^{2+}, the membrane potential depolarized and enhanced excitability was reflected by spike discharge evoked by depolarizing current pulses. *C*, application of 5 μM-forskolin in the presence of elevated Mg^{2+} resulted in further depolarization and enhancement of excitability. Constant-current pulses were injected throughout the records. Dashed lines are references for changes in membrane potential.

of depolarization, a larger increase in the input resistance and a greater augmentation of excitability, as evidenced by increased spike discharge (Fig. 9).

DISCUSSION

Slow synaptic excitation in myenteric neurones is characterized by depolarization, increased input resistance, suppression of hyperpolarizing after-potentials and augmented excitability (reviewed by: Wood, 1984). The actions of substance P (Katayama & North, 1978), 5-hydroxytryptamine (Wood & Mayer, 1979*a*), histamine (Nemeth, Ort & Wood, 1984), vasoactive intestinal peptide, bombesin and gastrin-releasing peptide (Zafirov, Palmer, Nemeth & Wood, 1985), cholecystokinin octapeptide and caerulein (Nemeth, Zafirov & Wood, 1985) and blockade of calcium entry (Grafe *et al.* 1980) simulate the electrical behaviour of AH/Type 2 myenteric neurones during slow synaptic excitation. The foregoing results show that application of forskolin also simulates the electrical events of slow synaptic excitation in these ganglion cells.

Grafe *et al.* (1980) reported evidence that the depolarization of the membrane potential and increased input resistance during the myenteric slow e.p.s.p. reflected

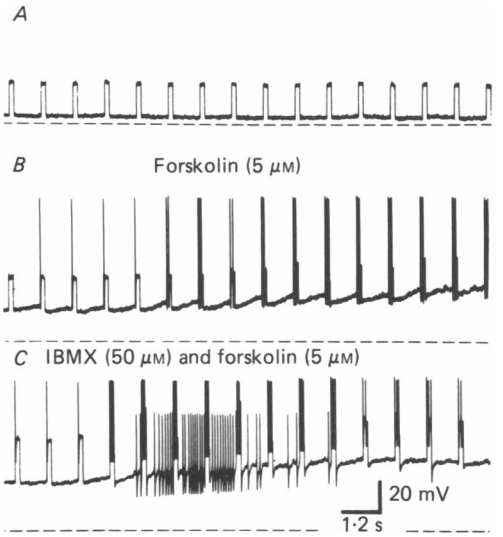

Fig. 9. Potentiation of forskolin effects by IBMX in an AH/Type 2 myenteric neurone. *A*, control. *B*, application of 5 μM-forskolin produced depolarization and enhancement of excitability. Record begins 20 s after forskolin entered bath. *C*, pretreatment for 8 min with IBMX potentiated the effects of forskolin. The effects recorded in *B* were reversed by washing prior to application of IBMX. Constant-current depolarizing pulses were injected repetitively throughout the records. Dashed lines are references for changes in membrane potential.

a decrease in calcium-dependent G_K produced by the transmitter(s) for the event. The reversal potential for the slow e.p.s.p. is near the estimated potassium equilibrium potential (Grafe *et al.* 1980), as is the reversal potential for the effects of substance P (Katayama, North & Williams, 1979), 5-hydroxytryptamine (Johnson, Katayama & North, 1980), histamine (Nemeth *et al.* 1984), vasoactive intestinal peptide, bombesin and gastrin-releasing peptide (Zafirov, *et al.* 1985) and cholecystokinin octapeptide and caerulein (Nemeth *et al.* 1985). Exposure to divalent cations that block calcium entry suppresses resting G_K in the AH/Type 2 neurones (Grafe *et al.* 1980). These cations also block calcium entry during the action potential and thereby suppress the increase in calcium-dependent G_K that accounts for post-spike hyperpolarizing potentials in these cells (North, 1973; Hirst & Spence, 1973).

In the present study, the reversal potential for the effects of forskolin was the same (−82 mV) as the reversal potential for the current in calcium-dependent K^+ channels that was determined recently with voltage-clamp methods by Hirst, Johnson & van Helden in guinea-pig myenteric neurones (1985*b*). Furthermore, the effects of forskolin on the relationship between external K^+ concentration and membrane potential were the same as for multivalent cations that block calcium entry, except forskolin produced a greater reduction in resting G_K (Grafe *et al.* 1980). Permeability ratios for K^+ were reduced by 2·5 times in elevated Mg^{2+} as compared with 3·3 times in forskolin (Fig. 7). This suggests that one of the actions of forskolin was to reduce resting G_K and that this accounts for the depolarizing action and the increased input resistance. It also indicates that forskolin is more effective than blockade of Ca^{2+} in

closing the K^+ channels. The suppression of hyperpolarizing after-potentials by forskolin could reflect a reduction of calcium entry during the rising phase of the spike and secondary failure of activation of the calcium-dependent G_K channels, or this could be the result of a direct action on the conformation of the K^+ channels.

Neither multivalent cations that block calcium entry (Grafe *et al.* 1980) nor forskolin, in the present study, altered significantly the membrane behaviour of S/Type 1 myenteric neurones. These results suggest that signal transduction in these neurones may not operate through calcium-dependent G_K channels and other mechanisms that are functional in the neurones with AH/Type 2 properties.

The augmentation of excitability and tendency to repetitive spike discharge that were produced by forskolin appear to involve mechanisms additional to the decreased G_K and membrane depolarization. Additional mechanisms were suggested by the observations that current clamping of the membrane potential at depolarized levels did not lead to enhanced excitability in the AH/Type 2 neurones. It may be that additional changes, such as phosphorylation of ionic channels in the membrane, are involved in the dramatic conversion from the low to high state of excitability.

Application of forskolin results in large increases in the amounts of cyclic AMP in virtually all cells that have been studied, including neurones (Kilmer & Carlsen, 1984), and this is assumed to be the case for the AH/Type 2 myenteric neurones in the present report. Our observation that pretreatment with a phosphodiesterase inhibitor potentiated the effects of forskolin is consistent with this action.

All of these results and those in the following paper (Palmer, Wood & Zafirov, 1986) support the hypothesis that cyclic AMP is involved in slow synaptic excitation of AH/Type 2 myenteric neurones. It appears that slow e.p.s.p.s produced by endogenously released neurotransmitters as well as the mimicry of the slow e.p.s.p. produced by a variety of putative gastrointestinal messenger substances are associated with activation of adenylate cyclase and second messenger function of newly synthesized cyclic AMP.

This research was supported by National Institutes of Health Grant AM26742.

REFERENCES

BHAT, S., BAJIVA, B. S., DORNAUER, H. & DE SOUZA, N. J. (1977). Structure and stereochemistry of new lab dane diterpenoids from *Coleus forskolii*. *Tetrahedron Letters* **19**, 1669–1672.

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

HIRST, G. D. S., HOLMAN, M. E. & SPENCE, I. (1974). Two types of neurones in the myenteric plexus of duodenum in the guinea-pig. *Journal of Physiology* **236**, 303–326.

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

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

HIRST, G. D. S. & SPENCE, I. (1973). Calcium action potentials in mammalian peripheral neurones. *Nature* **243**, 54–56.

JOHNSON, S. M., KATAYAMA, Y. & NORTH, R. A. (1980). Multiple actions of 5-hydroxytryptamine on myenteric neurones of the guinea-pig ileum. *Journal of Physiology* **304**, 459–470.

KATAYAMA, Y. & NORTH, R. A. (1978). Does substance P mediate slow synaptic excitation within the myenteric plexus? *Nature* **274**, 387–388.

KATAYAMA, Y., NORTH, R. A. & WILLIAMS, J. T. (1979). The action of substance P. on neurones of the myenteric plexus of the guinea-pig small intestine. *Proceedings of the Royal Society* **206**, 191–208.

KILMER, S. L. & CARLSEN, R. C. (1984). Forskolin activation of adenylate cyclase *in vitro* stimulates nerve regeneration. *Nature* **307**, 455–456.

NEMETH, P. R., ORT, C. A. & WOOD, J. D. (1984). Intracellular study of effects of histamine on electrical behaviour of myenteric neurones in guinea-pig small intestine. *Journal of Physiology* **355**, 411–425.

NEMETH, P. R., ZAFIROV, D. H. & WOOD, J. D. (1984). Forskolin mimics slow synaptic excitation in myenteric neurons. *European Journal of Pharmacology* **101**, 303–304.

NEMETH, P. R., ZAFIROV, D. H. & WOOD, J. D. (1985). Cholecystokinin-octapeptide and caerulein but not pentagastrin alter electrical behavior of myenteric neurons of guinea-pig ileum. *European Journal of Pharmacology* **116**, 263–269.

NISHI, S. & NORTH, R. A. (1973). Intracellular recording from the myenteric plexus of guinea-pig ileum. *Journal of Physiology* **231**, 471–491.

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

PALMER, J. M., WOOD, J. D. & ZAFIROV, D. H. (1986). Elevation of adenosine 3′,5′-phosphate mimics slow synaptic excitation in myenteric neurones of the guinea-pig. *Journal of Physiology* **376**, 451–460.

SEAMON, K. B. & DALY, J. W. (1981). Forskolin: a unique diterpene activator of cyclic AMP-generating systems. *Journal of Cyclic Nucleotide Research* **1**, 201–224.

WOOD, J. D. (1984). Enteric neurophysiology. *American Journal of Physiology* **10**, G585–598.

WOOD, J. D. & MAYER, C. J. (1978). Intracellular study of electrical activity of Auerbach's plexus in guinea-pig small intestine. *Pflügers Archiv* **338**, 247–256.

WOOD, J. D. & MAYER, C. J. (1979*a*). Serotonergic activation of tonic-type enteric neurons in guinea-pig small intestine. *Journal of Neurophysiology* **42**, 582–593.

WOOD, J. D. & MAYER, C. J. (1979*b*). Adrenergic inhibition of serotonin release from neurons in guinea-pig Auerbac's plexus. *Journal of Neurophysiology* **42**, 594–693.

ZAFIROV, D. H., PALMER, J. M., NEMETH, P. R. & WOOD, J. D. (1985). Bombesin, gastrin releasing peptide and vasoactive intestinal peptide excite myenteric neurons. *European Journal of Pharmacology* **115**, 103–107.