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SYNAPTIC MODULATION OF CALCIUM-DEPENDENT POTASSIUM CONDUCTANCE IN MYENTERIC NEURONES IN THE GUINEA-PIG

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SUMMARY

1. Ganglion cells of the myenteric plexus of the guinea-pig small intestine were studied with intracellular recording methods.

2. Electrical stimulation of the interganglionic connectives elicited slow synaptic excitation (slow e.p.s.p.) that was associated with an increase in the input resistance of the cell. The slow e.p.s.p.s continued for several seconds after termination of stimulation, and they occurred only in neurones in which prolonged hyperpolarizing after-potentials followed an action potential.

3. Superfusion of the neurones with solutions containing either 1–5 mM-Mn^{2+} or 16 mM-Mg^{2+} and 1 mM-Ca^{2+} mimicked the slow e.p.s.p. The common characteristics of Mn^{2+}, Mg^{2+} and the slow e.p.s.p. were: (*a*) depolarization of the membrane potential, (*b*) increased input resistance of the cell, (*c*) augmented excitability, (*d*) blockade of post-pike hyperpolarizing potentials and (*e*) reversal potential between -70 and -75 mV.

4. Analyses based on the 'constant field equation' indicated that the permeability ratios of K^+ to other permeant ionic species were reduced when Ca^{2+} influx was blocked by Mn^{2+} or Mg^{2+}.

5. The organic Ca antagonist D-600 did not affect the neurones.

6. The results suggest that slow synaptic modulation of excitability within the myenteric plexus involves a reduction of both resting G_K and post-spike G_K which is secondary to suppression of Ca^{2+} influx by the neurotransmitter for the slow e.p.s.p.

INTRODUCTION

One kind of ganglion cell in the myenteric plexus is a model for long-lasting modulation of neuronal excitability by neurotransmitter substances. This type of neurone has been referred to by various authors as a Type 2 neurone (Nishi & North, 1973), AH neurone (Hirst, Holman & Spence, 1974), and tonic type neurone (Wood & Mayer, 1978; Wood & Mayer, 1979*a*). Chemical modulation of excitability occurs at the soma of these cells. The soma in the absence of the chemical modulator shows low excitability characterized by a high resting membrane potential, low input resistance, inability to discharge repetitive spikes during intracellular injection of depolarizing current and prolonged post-spike hyperpolarizing potentials. Synaptic release of

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5-hydroxytryptamine (5-HT) (Wood & Mayer, 1979*b*) and perhaps paracrine release of substance P (Katayama & North, 1978; Grafe, Mayer & Wood, 1979) produce a dramatic increase in the excitability of the somal membranes of these cells. This stage of augmented excitability (slow e.p.s.p.) is associated with membrane depolarization, an increase in input resistance, an increase in the membrane time and space constants, reduction of the hyperpolarizing after-potentials and often intense spike discharge (Wood & Mayer, 1979*a*, *b*). One aspect of the functional significance of the slow e.p.s.p. is facilitation of transmission of spike information between processes at opposite poles of the multipolar soma. The slow e.p.s.p. imparts a gating function to the soma which regulates transmission of information between processes of the same cell and thereby controls the spread of excitation within the myenteric plexus along the bowel (Wood & Mayer, 1979*a*).

The synaptic mechanisms which modulate the somal membrane excitability seem to operate by reducing membrane conductance for potassium ions (G_K) in two Ca^{2+}-associated G_K systems (Wood & Mayer, 1979*a*; Wood, Mayer, Ninchoji & Erwin, 1979). One Ca^{2+}-associated G_K is triggered by influx of Ca^{2+} during the action potential and is manifest as prolonged hyperpolarizing after-potentials (North, 1973; Hirst & Spence, 1973). The second Ca^{2+}-associated G_K appears to be responsible for the high membrane conductance and high membrane potential that are characteristic of the low excitability state of the neurone. Evidence for the second system is presented in this report. We report that Ca^{2+}-antagonistic ions suppress resting G_K and mimic both the slow e.p.s.p. and the action of 5-HT, suggesting that the primary action of the neurotransmitter for the slow e.p.s.p. is to restrict the availability of Ca^{2+} for a Ca^{2+}-dependent process within the somal membrane.

METHODS

Segments of intestine were obtained from the first 10 cm of bowel caudal to the gastroduodenal junction of adult non-albino guinea-pigs (250–800 g) that had been stunned by a blow to the head and exsanguinated. Flat preparations of longitudinal muscle with myenteric plexus attached were prepared, mounted in a superfusion chamber and observed with an interference contrast microscope as detailed in another report (Wood & Mayer, 1978). Conventional intracellular recording methods were used. Impalements were achieved at low magnification without visualization of individual neurones. Glass micropipettes were filled with either 3 M-KCl or 4 M-K acetate and had resistance of 50–80 MΩ The results were the same with both kinds of electrodes. The preamplifier (W-P Instruments Co.) contained negative capacity compensation and bridge circuitry for injecting electrical current through the recording electrode and recording the resulting electrotonic potentials. Synaptic input to the ganglion cells was activated by electrical shocks (200 μsec duration) applied to the ganglia and interganglionic connectives with electrodes made from Teflon-insulated Pt-wire (20 μm diameter) and a Grass S-4 square-wave stimulator.

Step-pulse method for estimation of the reversal potential of the slow e.p.s.p.

The reversal potential for the slow e.p.s.p. and for the action of Ca-antagonistic ions was estimated by continuously recording current–voltage relationships with four different steps of hyperpolarizing current intensities. Current injection was controlled by a programmed pulse generator which delivered a sequence of four 100 msec rectangular pulses which increased with constant increments of strength at 100 msec intervals (Fig. 1*B*). Each series of pulses was preceded by a trigger pulse and repeated at intervals of 1–2 sec. Current monitor output, membrane potential, trigger pulses and digitally coded time signals were recorded on magnetic tape (Fig. 1*A*). Data analysis was performed off-line using an Ortec Model 4622A Amplitude Histogram Analyzer

set to sequential sample and a 4620A Memory Control. Trigger pulses from the tape were fed into a 4654 Delay-Duration-Control to activate the 4622A. Depending on the preset delay, the computer would analyse either membrane potential or one of the four current-clamped potentials. The 4620A Memory Control was interfaced with a 9821A Hewlett Packard calculator which controlled a 9862A Hewlett Packard plotter. The plots consisted of five superimposed traces, one

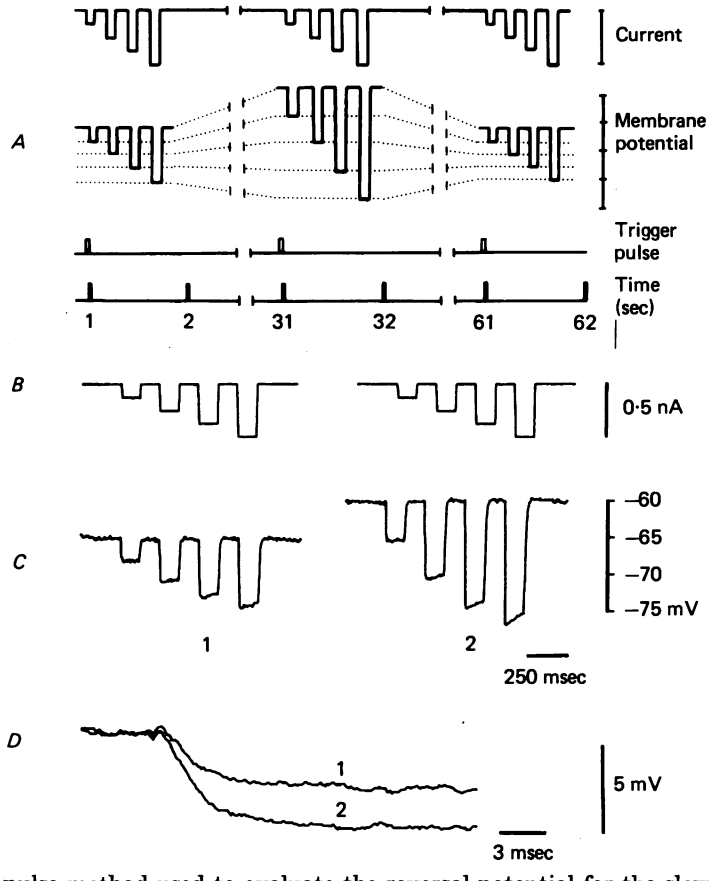

Fig. 1. Step-pulse method used to evaluate the reversal potential for the slow e.p.s.p. and the depolarizing action of Mg^{2+} and Mn^{2+}. *A*, timing sequence of the pulses. One sequence of four pulses is shown diagrammatically before, during and after a slow e.p.s.p. The pulse generator produces a trigger pulse that precedes the first pulse of each four-pulse sequence. These pulses are stored on one magnetic tape channel and are used to trigger the Amplitude Histogram Analyzer during analysis. A digital time code on an additional magnetic tape channel provided precise identification of the trigger pulses. *B*, a record of current injected through the electrode and *C*, corresponding current-clamped potentials before and during a stimulus-evoked slow e.p.s.p. *D*, fast time sweeps of current-clamped potentials for evaluation of amplifier bridge balance before and during slow e.p.s.p.

of which was amplitude of the e.p.s.p. without current control of the membrane potential, and four of which represented the amplitude of the e.p.s.p. produced during each of the four steps of injected current (refer to Figs. 3 and 6).

The pulse method of estimating the reversal potential was utilized because we were unable to control the membrane potential with continuous injection of hyperpolarizing current for the prolonged time periods of the slow e.p.s.p. This was probably due to inability of the high resist-

ance micro-electrodes to pass large currents over long time spans and to the delayed rectifying properties of the cell membranes especially when higher current intensities were used (note the occurrence of rectification in Fig. 1*C*). Because of the uncertainty of consistently evoking the same e.p.s.p. amplitudes with each stimulus, the method also had the advantage that each of the slow e.p.s.p.s served as its own control by switching between membrane potential and current-clamped potentials. Care was taken to maintain proper bridge balance during these experiments (Fig. 1*D*) and electrodes were tested for rectifying properties before and after impalement of each cell.

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 solution in mM was: NaCl, 120·9; KCl, 5·9; $MgCl_2$, 1·2; NaH_2PO_4, 1·2; $NaHCO_3$, 14·4; $CaCl_2$, 2·5; and glucose, 11·5. Normal osmolarity in the solutions with modified ionic composition was maintained by appropriate alteration in concentration of either glucose or NaCl. In experiments with Mn^{2+}, HEPES was substituted for the bicarbonate-phosphate buffer system, and the solutions were gassed with 100% O_2. In these experiments, the control solutions before and after application of Mn^{2+} were also HEPES buffered.

The liquid junction potentials of the micro-electrodes never varied by more than 4 mV in the various solutions and there were no consistently measurable changes in the resistance of the electrodes.

RESULTS

The results were obtained from forty-two neurones selected from 150 impalements of the so-called AH or Type 2 myenteric neurones (Nishi & North, 1973; Hirst *et al.* 1974) from eighty different animals. The selected neurones maintained a relatively high membrane potential (−60 to −70 mV) and low input resistance (10–60 MΩ) for periods of at least 1 hr, and no oedematous changes occurred in the vicinity of the impaled neurones. Nishi & North (1973) first described swelling of myenteric neurones following impalement. Vacuole-like formations, which encompassed one to four cell bodies, formed within a few minutes after about 80% of the impalements in the present study. This may reflect interganglionic osmotic disturbances associated with disruption of the periganglionic sheath. The occurrence of swelling was accompanied by decline of the membrane potential, increase in the input resistance, repetitive discharge throughout 100 or 200 msec depolarizing current pulses and loss of ability to elicit the slow e.p.s.p. These neurones were not used for the experiments.

Slow synaptic excitation

Fig. 2*A* illustrates the properties of the stimulus-evoked slow e.p.s.p. Electrical stimulation of presynaptic fibres elicited membrane depolarization and an associated increase in input resistance which outlasted the period of stimulation by several seconds. Hyperpolarizing after-potentials accompanied the spikes in these cells, and both the amplitude and duration of the after-potentials were reduced during the slow e.p.s.p. (Fig. 2*B*). Reduction in the after-potentials was not due to the depolarization during the slow e.p.s.p. because the same reduction still occurred when the synaptically evoked depolarization was prevented by steady injection of hyperpolarizing current during the slow e.p.s.p.

Utilization of the step-pulse method to estimate the reversal potential for the slow e.p.s.p. showed that the e.p.s.p. was a depolarizing potential at membrane potentials more positive than −70 mV, that no potential change occurred at a membrane potential between −70 and −75 mV and that the e.p.s.p. was a hyperpolarizing potential at membrane potentials more negative than −75 mV (Fig. 3*A*).

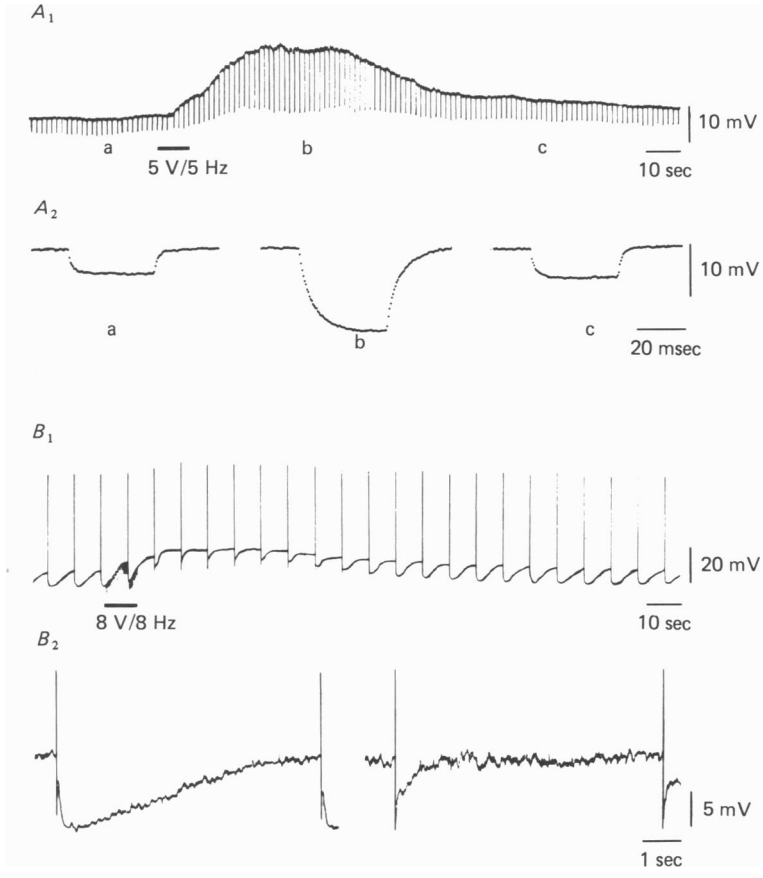

Fig. 2. Properties of slow synaptic excitation in a guinea-pig myenteric neurone. A_1, slow e.p.s.p. evoked by electrical stimulation of an interganglionic fibre tract. Onset and offset of stimulus are indicated by horizontal bar. Constant-current hyperpolarizing pulses injected repetitively revealed increased input resistance during the e.p.s.p. A_2, averaged constant-current hyperpolarizing pulses recorded on expanded time base before, during and after slow e.p.s.p. B_1, reduction of the amplitude and duration of hyperpolarizing after-potentials during slow e.p.s.p. Action potentials were triggered at 6 sec intervals by injection of depolarizing current pulses of sufficiently short duration that each pulse elicited only one spike. Onset and offset of fibre tract stimulation indicated by horizontal bar. B_2, hyperpolarizing after-potentials before and during slow e.p.s.p. recorded with an expanded time base.

The increase in input resistance during the slow e.p.s.p. was reflected on plots of the current–voltage relationship for the injected current pulses (Fig. 3*B*). The current–voltage plots were ohmic at low current strengths and had much greater slopes during the slow e.p.s.p. than during the resting state. The current–voltage curves for the e.p.s.p. and the resting state always intersected at a membrane potential between -70 and -75 mV (Fig. 3*B*). These observations indicated that the reversal potential for the slow e.p.s.p. was between -70 and -75 mV.

Effects of Ca antagonists on myenteric neurones

Superfusion of the AH/Type 2 neurones with solutions containing either 16 mM-Mg^{2+} and 1 mM-Ca^{2+} or 2 mM-Mn^{2+} mimicked the electrical changes that occurred during the stimulus-evoked slow e.p.s.p. (Figs. 4 and 5). The effects of elevated Mg^{2+} and Mn^{2+} were: (1) membrane depolarization of 10 to 30 mV, (2) a one- to five-fold

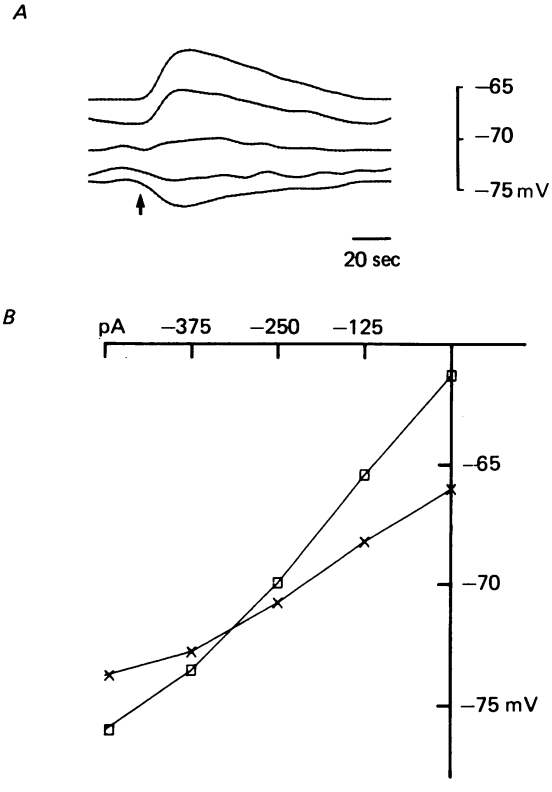

Fig. 3. Estimated reversal potential and current–voltage relationship for the slow e.p.s.p. in a guinea-pig myenteric neurone. *A*, depolarization of the slow e.p.s.p. was reversed to hyperpolarization at current-clamped membrane potentials more negative than −70 mV. Top trace represents the resting membrane potential without injection of hyperpolarizing current. Successively lower traces represent membrane potentials produced by the four steps of injected current from the step-pulse generator. The group of four stepped pulses was repeated at 1 sec intervals. The traces are plots of the data over a 2·7 min time course. *B*, current–voltage relationship for the four current pulses injected by the step-pulse generator before (×) and at the peak (□) of the slow e.p.s.p.

increase in input resistance, (3) reduction of post-spike hyperpolarizing potentials, (4) neurones that discharged a single spike only at the onset of 100 or 200 msec current pulses in Krebs solution would discharge throughout the depolarizing pulses, with a frequency dependent on the strength of the depolarizing current.

Elevation of Mg^{2+} to 16 mM in Krebs solution with 2·5 mM-Ca^{2+} also depolarized the neurones and increased the input resistance; however, these effects were always less pronounced than in solutions with 16 mM-Mg^{2+} and 1 mM-Ca^{2+}. Reduction of the

Ca^{2+} concentration to 1 mM did not affect the membrane potential and did not increase the input resistance. The effects of elevated Mg^{2+} and of Mn^{2+} were reversed 2–3 min after returning to superfusion with normal solution.

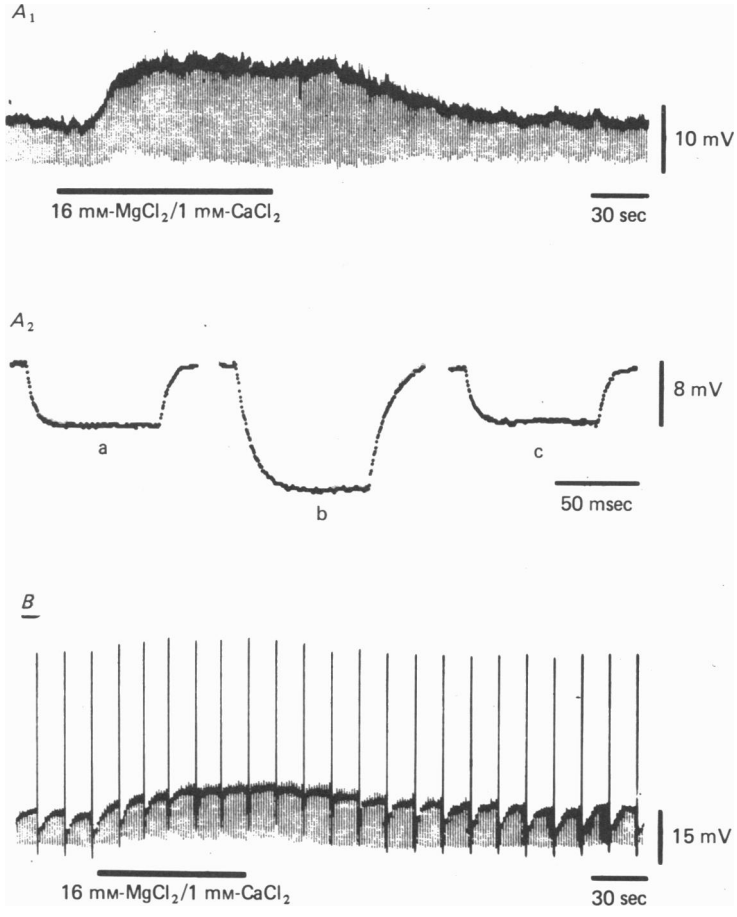

Fig. 4. Effects of elevated Mg^{2+} and reduced Ca^{2+} on resting membrane potential, input resistance and hyperpolarizing after-potentials in an AH/Type 2 myenteric neurone. A_1, superfusion with elevated Mg^{2+} depolarized the cell and increased the input resistance. A_2, expanded time base record of averaged electrotonic potentials produced by injection of constant-current, hyperpolarizing pulses before, during and after application of elevated Mg^{2+}. *B*, reduction of the amplitude and duration of hyperpolarizing after-potentials in the presence of elevated Mg^{2+} and reduced Ca^{2+}. Action potentials were triggered at 15 sec intervals by injection of depolarizing current pulses of sufficiently short duration that each pulse elicited only one spike. Constant-current hyperpolarizing pulses were injected repetitively throughout the experiment.

Utilization of the step-pulse method to estimate the reversal potential for the effects of elevated Mg^{2+} showed that high Mg^{2+} depolarized the membrane at membrane potentials more positive than -70 mV, that no potential change was produced by elevated Mg^{2+} at a membrane potential between -70 mV and -75 mV and that elevated Mg^{2+} hyperpolarized the membrane at membrane potentials more negative

than -75 mV (Fig. 6*A*). The slopes of current–voltage curves obtained from the step-pulse method in the presence of elevated Mg^{2+} were always greater than the slopes of equivalent plots in Krebs solution. Current–voltage curves obtained in the presence of elevated Mg^{2+} and in normal solution always intersected each other at a

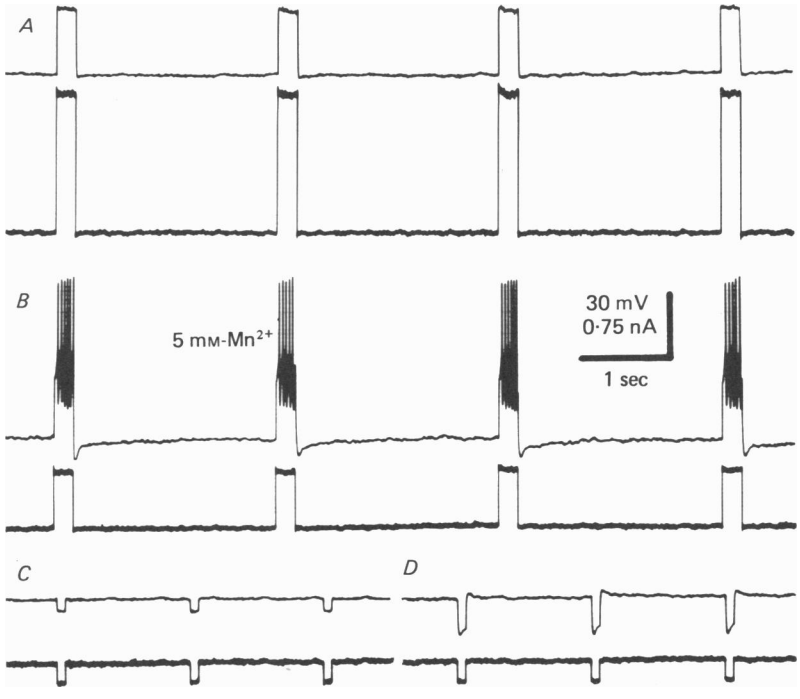

Fig. 5. Effects of Mn^{2+} on excitability of an AH/Type 2 myenteric neurone. *A*, large-amplitude depolarizing current pulses did not evoke spikes in Krebs solution. *B*, neurone discharged repetitively throughout depolarizing current pulses of reduced amplitude relative to *A* in the presence of Mn^{2+}. *C*, electrotonic potentials produced by injection of constant-current hyperpolarizing pulses in Krebs solution. *D*, increased amplitude of electrotonic potentials produced by same constant-current pulses in the presence of Mn^{2+} reflects increased input resistance. Upper trace is voltage, lower trace current.

membrane potential between -70 and -75 mV (Fig. 6*B*). These observations indicate that the reversal potential for the potential change produced by elevated Mg^{2+} was between -70 and -75 mV, which was the same as the reversal potential of the slow e.p.s.p.

We tested for effects of methysergide on the action of elevated Mg^{2+} because methysergide is an effective blocking agent for both the stimulus-evoked slow e.p.s.p. and the action of exogenously applied 5-HT (Wood & Mayer, 1979*b*). Methysergide, at a concentration which blocked the slow e.p.s.p. (30 μM), did not alter the effects of elevated Mg^{2+} in any of seven trials on five neurones. This suggested that the action of the Ca-antagonist ions was not on the serotonergic receptors.

The effects of 16 mM-Mg^{2+} and 1 mM-Ca^{2+} were examined in the presence of 1 μM-tetrodotoxin (TTX) to test the possibility that the action of Mg^{2+} was due to pre-synaptic suppression of tonic release of an inhibitory substance at synapses on the

neurone. Tetrodotoxin by itself did not change membrane conductance or membrane potentials and the effects of elevated Mg^{2+} were unchanged in the presence of TTX. This suggested a direct action of Mg^{2+} on the impaled neurone.

Solutions containing either 16 mM-Mg^{2+} and 1 mM-Ca^{2+} or 2 nM-Mn^{2+} were applied to seven different neurones that were classified as S or Type 1 neurones on the basis of the criteria of Hirst *et al.* (1974) and Nishi & North (1973). There was a suppressant

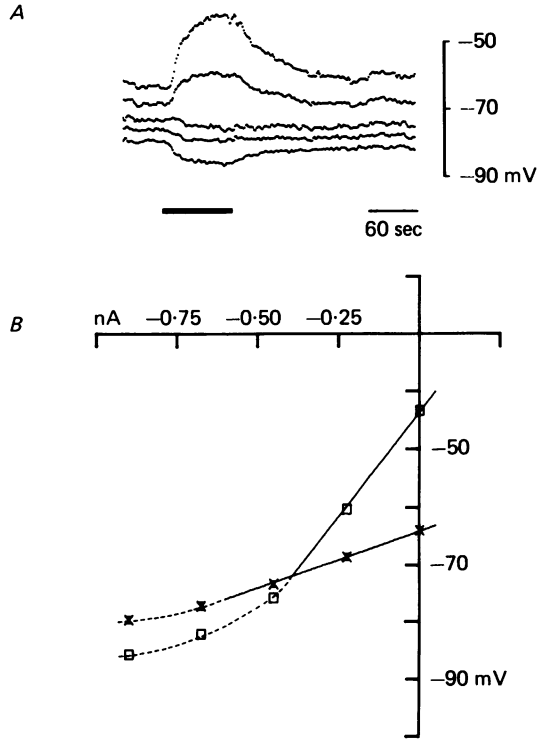

Fig. 6. Estimated reversal potential and current–voltage relationships for effects of elevated Mg^{2+} and reduced Ca^{2+} in an AH/Type 2 myenteric neurone. *A*, depolarizing action of Mg^{2+} was reversed to hyperpolarization at current-clamped membrane potentials more negative than −70 mV. Top trace represents resting membrane potentials without injection of hyperpolarizing current. Successively lower traces represent membrane potentials produced by the four steps of injected current from the step-pulse generator. The group of four stepped pulses was repeated at 1 sec intervals. The traces are plots of the data over a 4 min time course. *B*, current–voltage relationship for the four current pulses injected by the step pulse generator in the presence (□) and absence (×) of elevated Mg^{2+}. Dashed lines indicated non-ohmic portions of the current–voltage relationships.

effect of both elevated Mg^{2+} and Mn^{2+} on the discharge of action potentials during depolarizing current pulses in four of the neurones, and in the remainder of the cells there was no notable effect on excitability. The input resistance of S or Type 1 neurones was never increased in the presence of elevated Mg^{2+} or Mn^{2+} (Fig. 7). The fast cholinergic e.p.s.p.s in these neurones were always blocked reversibly in the presence of elevated Mg^{2+} or Mn^{2+}. These results suggest that the specific membrane

properties responsible for the responses to the Ca-antagonistic ions are present only in the AH/Type 2 myenteric neurones.

The organic Ca-antagonist, D-600 was tested on twelve different AH/Type 2 neurones. Exposure of the neurones to concentrations of D-600 as high as 100 μM for periods of up to 1 hr did not alter the membrane potential, the input resistance nor the hyperpolarizing after-potentials associated with the action potentials.

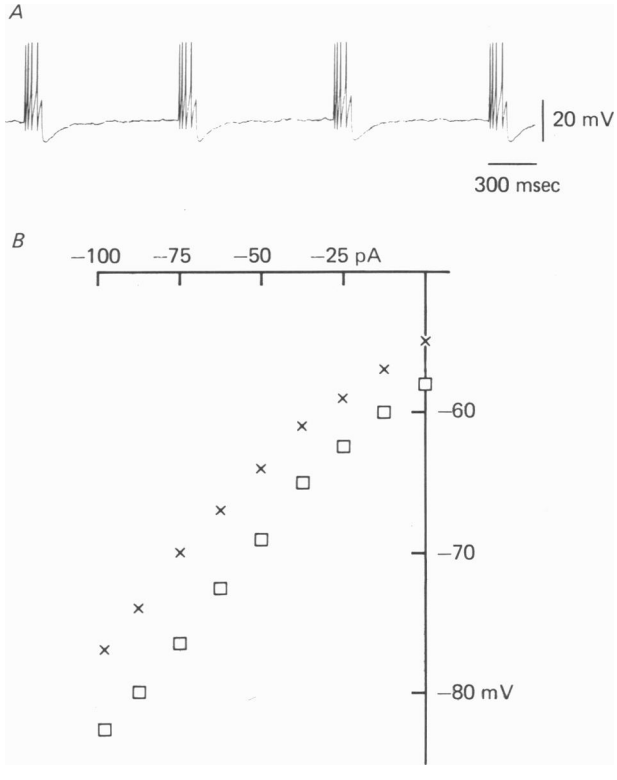

Fig. 7. Effects of elevated Mg^{2+} and reduced Ca^{2+} on an S/Type 1 myenteric neurone. *A*, repetitive discharge of the cell during injection of depolarizing current pulses in normal Krebs solution. Spike amplitude reduced by frequency response of chart recorder. *B*, current–voltage relationship for S/Type 1 neurone in Krebs solution (×) and in Krebs solution with elevated Mg^{2+} and reduced Ca^{2+} (□). Note hyperpolarization and effect of Mg^{2+} on input resistance.

Relationship between external K^+ concentration, membrane potential and elevated Mg^{2+}

The results described thus far suggested that the primary action of both the neurotransmitter for the slow e.p.s.p. and the Ca^{2+}-antagonistic ions, Mg^{2+} and Mn^{2+}, was to decrease the resting permeability of the membranes for K^+. This was studied further by comparing the effects of variation of the external K^+ concentration on the resting membrane potential in Krebs solution and Krebs solution containing 16 mM-Mg^{2+} and 1 mM-Ca^{2+}. These studies required that impalements be maintained for several hours, and a complete study was accomplished on three different neurones. These data when plotted as the resting potential against the logarithm of the external K^+

concentration agreed well with theoretical curves calculated on the basis of the 'constant field equation' (Fig. 8). At all points on the plots below 50 mM-K^+, the slope of the curve in Krebs solution was much steeper than the slope of the curve in solution containing 16 mM-Mg^{2+}. This suggested that resting G_K was reduced in the presence of elevated Mg^{2+}, and that this could account for the increased input resistance that was observed in the presence of elevated Mg^{2+} (Fig. 4).

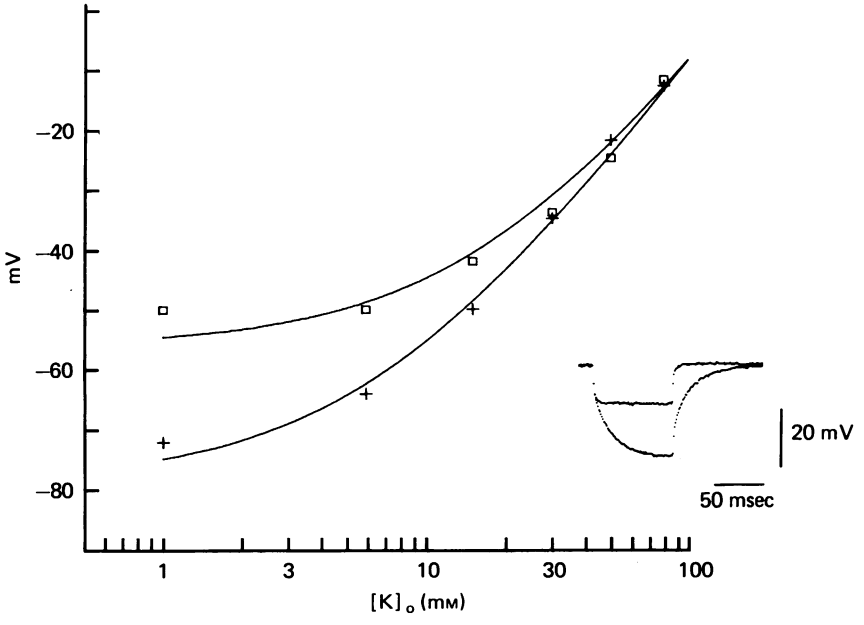

Fig. 8. Membrane potential of an AH/Type 2 myenteric neurone versus the logarithm of external K^+ concentration in the presence (□) and absence (×) of elevated Mg^{2+}. The 'constant field' equation was used to fit the continuous lines to the data points. The following ion concentrations were inserted into the constant field equation (mM), $[K]_i = 140$; $[Na]_i = 10$; $[Cl]_i = 10$. The permeability ratios used were: $P_K : P_{Na} : P_{Cl} = 50:2{\cdot}2:1$ in the presence of normal Krebs, and $P_K : P_{Na} : P_{Cl} = 20:2{\cdot}2:1$ in the presence of 16 mM-Mg^{2+}, 1 mM-Ca^{2+}. The values for P_K in elevated Mg^{2+} and in Krebs solution correspond to an approximate ratio of 5:2 between amplitude of electrotonic potentials produced by constant-current pulses in elevated Mg^{2+} and Krebs solution. The inset shows superimposed the averaged electrotonic potentials produced by injection of constant current hyperpolarizing pulses in Krebs solution (top trace) and in Krebs solution with elevated Mg^{2+} and reduced Ca^{2+} (bottom trace). Increased amplitude of bottom trace reflected increased input resistance in elevated Mg^{2+}.

DISCUSSION

Manganese, high magnesium concentrations and other divalent cations have been used widely as experimental tools which impede transmembrane movement of Ca^{2+} and block Ca^{2+}-dependent processes (Rubin, 1970; Reuter, 1973). Manganese and elevated Mg^{2+} were utilized for this purpose in this study because we suspected that the low excitability state of the AH/Type 2 myenteric neurones was related to Ca^{2+}-associated G_K which was suppressed during slow synaptic excitation of the neurone (Wood *et al.* 1979; Wood & Mayer, 1979*a*, *b*). The results of the present study are

consistent with this hypothesis because both Mn^{2+} and elevated Mg^{2+} mimicked the stimulus-evoked slow e.p.s.p. The common characteristics of the slow e.p.s.p. and the effects of Mn^{2+} and Mg^{2+} were: (1) depolarization of the membrane potential, (2) increased input resistance, (3) augmented excitability, (4) blockade of prolonged post-spike hyperpolarizing potentials and (5) reversal potential between -70 mV and -75 mV.

The basic membrane mechanisms for slow synaptic excitation and the effects of Mg^{2+} and Mn^{2+} on these mechanisms within the myenteric plexus appear to be unique to the AH/Type 2 neurones because the divalent cations either had no effect or suppressed excitability in other types of myenteric neurones; in the latter the effects were similar to the generally reported depressant action of extracellularly applied Mg^{2+} and Mn^{2+} on neurones within the central nervous system where the action is thought to be membrane stabilization synergistic with Ca^{2+} (Erulkar, Dambach & Mender, 1974; Kelly, Krnjević & Somjen, 1969).

Our observations of blockade of prolonged post-spike hyperpolarizing potentials during Mn^{2+} or Mg^{2+} application confirm the results of North (1973) and Hirst & Spence (1973), which indicated that an influx of Ca^{2+} during an action potential increases G_K to produce the hyperpolarizing after-potentials. Our results additionally suggest that the high resting membrane potential of the AH/Type 2 myenteric neurones is related to a Ca^{2+}-dependent G_K. The evidence for this is that treatments which are known to block Ca^{2+} movements across excitable membranes produced membrane depolarization associated with increased membrane resistance. The reversal potential for the depolarization was near the equilibrium potential for K^+ suggesting that both the depolarizing effect of Ca^{2+} antagonism and the associated increase in membrane resistance can be accounted for by a decrease in G_K. The observation that the slopes of curves relating membrane potential to extracellular K^+ concentration was reduced in the presence of high Mg^{2+} is also evidence for Ca^{2+}-dependence of resting G_K in these neurones.

With respect to Ca^{2+}-associated G_K systems, the AH/Type 2 myenteric neurones are similar to a variety of neurones and other cells in which the resting membrane permeability for K^+ appears to be a direct function of the concentration of free Ca^{2+} within the cytoplasm (Krnjević & Lisiewicz, 1972; Meech, 1974*a*; Krnjević, Puil & Werman, 1976; Isenberg, 1975). In AH/Type 2 neurones and many vertebrate and invertebrate neurones, the intracellular concentration of Ca^{2+} appears to be increased transiently by Ca^{2+} influx during the action potential, and this leads to increased G_K which is reflected as post-spike hyperpolarization (Jansen & Nicholls, 1973; Meech, 1974*b*; Barrett & Barrett, 1976; Krnjević, Lamour, MacDonald & Nistri, 1978). Our results suggest that the neurotransmitter for the slow e.p.s.p. in AH/Type 2 neurones functions to block both the Ca^{2+} channels that are opened during an action potential and the channels that carry a steady influx of Ca^{2+} in the resting state. This does not necessarily imply blockade of two different Ca^{2+} channels. It might be that only a single Ca^{2+} channel is involved and, because of voltage dependence of the channel, more channels are opened during the depolarizing phase of the spike.

The observations suggest that the mechanism of long-term synaptic augmentation of excitability of the AH/Type 2 neurones involves neurotransmitter suppression of Ca^{2+}-dependent G_K. The widespread occurrence of Ca^{2+}-dependent G_K systems in

other neurones raises the question of whether synaptic modulation of Ca^{2+}-dependent G_K might be of generalized functional significance for long-term modulation of neuronal excitability. The recent observations that 5-HT increases Ca^{2+} influx in sensory neurones of *Aplysia* (Klein & Kandell, 1978) and in chick dorsal root ganglion cells suggest that this may be true (Dunlap & Fischbach, 1978).

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