Short communications

The calcium-dependent slow after-hyperpolarization in myenteric plexus neurones with tetrodotoxin-resistant action potentials

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Intracellular recordings were made from neurones of the myenteric plexus of the guinea-pig ileum. The slow after-hyperpolarization which followed an action potential in some neurones was abolished by Mn^{++}, La^{+++} and by solutions which contained no Ca^{++}. In these neurones, the action potential and the slow after-hyperpolarization persisted in Na^{+}-free solutions or in the presence of tetrodotoxin (2 μM). The findings suggest that an inward Ca^{++} current during the action potential is essential for the slow after-hyperpolarization.

The output of acetylcholine from the myenteric plexus of the guinea-pig ileum is often measured in pharmacological investigations of the factors which affect transmitter release. However, little is known of the properties of the individual cells which comprise the plexus. Hirst, Holman, Prosser & Spence (1972) recently found that some myenteric plexus neurones in the guinea-pig duodenum showed a hyperpolarization which followed an action potential evoked by passing a depolarizing current across the cell membrane. Nishi & North (1973) independently reported this slow after-hyperpolarization in some cells of the myenteric plexus of the guinea-pig ileum and they showed that it was due to a long-lasting increase in K^{+} conductance. Nishi & North (1973) further showed that the slow after-hyperpolarization was reversibly abolished when Ca^{++} was removed from the solution perfusing the tissue; they surmised that Ca^{++} entered the cell during the action potential and that this led to the increased K^{+} conductance. In cat spinal motoneurones, intracellular injection of Ca^{++} leads to an increase in K^{+} conductance (Krnjević & Lisiewicz, 1972).

Methods.—Intracellular recordings were made from neurones of myenteric plexus of the guinea-pig ileum in the manner previously described (Nishi & North, 1973). Action potentials were elicited by focal stimulation of a cell process at a distance of up to 100 μm from the impaled soma (indirect stimulation) or by passing a depolarizing current pulse (up to 1 nA) through the recording micro-electrode (direct stimulation). The tissue was perfused with a solution of the following composition (mM): NaCl, 118; KCl, 4·7; $CaCl_2$, 2·5; $MgCl_2$, 1·2; $NaHCO_3$, 25; NaH_2PO_4, 1·2; glucose, 11·1; bubbled with 5% CO_2 and 95% O_2. After successful impalement of a cell, this solution was changed to one containing tetrodotoxin (TTX), $MnCl_2$ or $LaCl_3$, or to one of a different ionic composition. To avoid precipitation in solutions containing $MnCl_2$ or $LaCl_3$, $NaHCO_3$ was replaced by an equimolar amount of Tris buffer (tris-(hydroxymethyl)-amino-methane) at pH 7·4 bubbled with 100% O_2. In Na^{+}-free solutions, NaCl, $NaHCO_3$ and NaH_2PO_4 were completely replaced by equimolar amounts of Tris buffer at pH 7·4, bubbled with 100% O_2. In Ca^{++}-free solutions, $CaCl_2$ was replaced by 5 mM $MgCl_2$ and the NaCl concentration was reduced to 116 mM. Stable intracellular recordings were made for at least 25 min from each cell.

Results.—About one quarter of the excitable cells impaled showed a slow after-hyperpolarization following a single action potential (see Fig. 6A; Nishi & North, 1973). Whenever the slow after-hyperpolarization followed a spike induced by indirect stimulation, it also followed an action potential due to direct stimulation. The slow after-hyperpolarization never followed potential changes which were not associated with an action potential. Synaptic responses could not be evoked in cells which showed a slow after-hyperpolarization, but were frequently observed in the other excitable cells of the plexus.

The exclusion of Ca^{++} from the perfusing solution, or the addition of $MnCl_2$ (1 mM) or $LaCl_3$ (1 mM), resulted in the disappearance of the slow after-hyperpolarization. Under these conditions, the action potential persisted but membrane resistance usually fell and there was often a depolarization of up to 15 mV which led to a reduction of the spike amplitude. In ganglion cells which did not show a slow

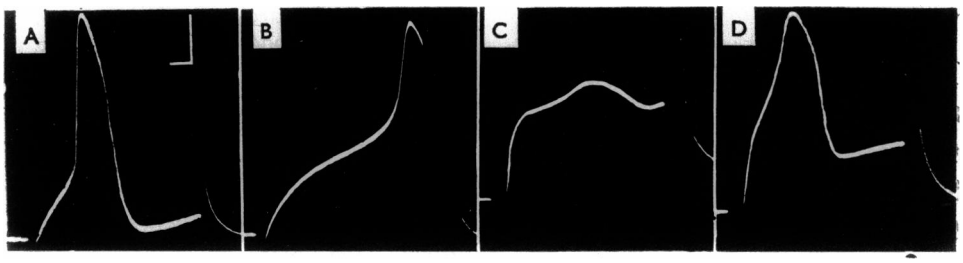

FIG. 1. The effects of tetrodotoxin (TTX) and $MnCl_2$ on the action potential of a single myenteric plexus neurone which showed a slow after-hyperpolarization. A rectangular depolarizing current pulse of 15 ms duration was passed through the recording micro-electrode; the records show the changes in intracellular potential which this produced. A: Control; this was taken after 1·5 h of stable intracellular recording, during which time the slow after-hyperpolarization had been reversibly abolished by $MnCl_2$ (1 mM). (Membrane potential, −55 mV; current pulse, 1 nA.) B: Record taken 6·5 min after changing to a solution containing TTX (400 nM). (Membrane potential −55 mV; current pulse, 1 nA.) The threshold depolarization for spike initiation is increased and the maximum rate of rise of the spike is reduced. C: Record taken 3·5 min after changing to a solution containing TTX (400 nM) and $MnCl_2$ (1 mM). (The cell became depolarized to −40 mV; a current pulse of 3 nA failed to initiate a spike.) D: Record taken 3·5 min after changing back to a solution containing only TTX (400 nM). (The cell had partially repolarized (membrane potential −45 mV); the same current pulse as in C (3 nA) now initiated a spike.) Calibrations: horizontal, 2 ms; vertical, 20 mV.

after-hyperpolarization, TTX (200 nM) abolished the action potentials elicited by direct and indirect stimulation. In each ganglion cell which showed a slow after-hyperpolarization, the action potential evoked by indirect stimulation was reversibly abolished by TTX, whereas an action potential could still be elicited by direct stimulation. In such cells, the spike which remained in the presence of TTX (2 μM) had a higher threshold and a lower rate of rise than the control spike (maximum rates of rise: control, 118 ± 9 V/s ($n=11$); after TTX, 43 ± 7 V/s ($n=11$)). In the presence of TTX, the addition of $MnCl_2$ (1 mM) or $LaCl_3$ (1 mM), or the substitution of a Ca^{++}-free solution, caused a fall in membrane resistance and a depolarization similar to that observed in the absence of TTX; however, the TTX-resistant spike was reversibly abolished by these agents (Figure 1). The TTX-resistant spike was followed by the slow after-hyperpolarization. In four cells which showed a slow after-hyperpolarization, perfusion of the tissue for up to 30 min with a Na^+-free solution had effects similar to those of TTX.

Discussion.—Evidence has accumulated that Ca^{++} can act as a charge carrier across various neuronal membranes (Reuter, 1973). In some myenteric plexus neurones, which show a slow after-hyperpolarization, the Ca^{++} current seems to be sufficient to allow spike generation in the presence of TTX or in Na^+-free solutions. These TTX-resistant spikes are blocked by an absence of Ca^{++} and by Mn^{++} and La^{+++}, which are known to prevent Ca^{++} fluxes across excitable membranes. These findings confirm the Ca^{++}-dependence of the slow after-hyperpolarization and further support the hypothesis that the slow after-hyperpolarization involves an inward Ca^{++} current during the somatic action potential.

The myenteric plexus of the guinea-pig ileum is anatomically (Gabella, 1972) and electrophysiologically (Nishi & North, 1973) complex; the properties of some of the neurones which are reported here render them unique amongst all the mammalian nerve cells which have so far been investigated with intracellular microelectrodes.

Note added in proof—

Calcium action potentials have been reported recently for some neurones of the guinea-pig duodenum. HIRST, G. D. S. & SPENCE, I. (1973). *Nature, New Biol.*, **243**, 54–56.

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