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Quinine blocks a calcium-activated potassium conductance in mammalian enteric neurones

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Quinine (100 μM) abolished the slow calcium-dependent afterhyperpolarization which occurs after an action potential in some neurones of the guinea-pig myenteric and submucous plexus. This occurred without any effect on the amplitude or time course of the action potential itself, or on the faster calcium-independent afterhyperpolarization. Tetraethylammonium did not reduce the slow afterhyperpolarization. Quinine also abolished the hyperpolarization which was evoked by intracellular injection of calcium ions.

Introduction A proportion of the neurones in the guinea-pig myenteric and submucous plexuses are unusual among autonomic neurones in that their action potential is followed by a long-lasting (several seconds) afterhyperpolarization (Nishi & North, 1973; Hirst *et al.*, 1974; Surprenant, 1984). This prolonged afterhyperpolarization is due to an increased membrane conductance to potassium ions; it is dependent upon the entry of extracellular calcium, because it is blocked by cobalt, lanthanum and manganese ions, or by superfusion with calcium-free solution (Nishi & North, 1973; Hirst *et al.*, 1974; Morita *et al.*, 1982). These findings suggest that a calcium-activated potassium conductance ($g_{K,Ca}$) underlies the afterhyperpolarization.

In some non-neuronal tissues, such as red blood cells and pancreatic β–cells, quinine has been reported to inhibit specifically a $g_{K,Ca}$ (Atwater *et al.*, 1979; Burgess *et al.*, 1981). A less selective suppression of potassium currents occurs in a variety of other tissues (e.g. *Helix* neurones, Walden & Speckmann, 1981). In the present experiments the effect of quinine was examined on the prolonged afterhyperpolarization (AH) recorded from guinea-pig enteric neurones in order to determine whether it selectively affected $g_{K,Ca}$ in differentiated mammalian nerve cells.

Methods Dissection, superfusion *in vitro*, and intracellular recording from guinea-pig enteric ganglia were described previously (Nishi & North, 1973; Surprenant, 1984). Ganglia were superfused with a heated (37°C) Krebs solution of the following composition (mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 25, and glucose 11; gassed with 95% $_2$/5% CO_2. In some experiments, two microelectrodes were inserted independently into one myenteric neurone; one was used to record membrane potential and the other to inject calcium ions. Calcium ions were injected by passing hyperpolarizing current pulse of 1–3 nA for 30–200 ms, from the electrode containing calcium chloride (10–20 mM). Drugs were applied by changing the superfusion solution to one which differed only in its content of drug. Substances used were quinine bromide (Sigma), tetrodotoxin (TTX, Sigma) and tetraethylammonium bromide (TEA, Sigma).

Results The effects of quinine on the intracellularly recorded action potentials were examined in a total of 16 AH neurones from the myenteric plexus and 8 AH neurones of the submucous plexus. AH neurones had a prolonged (4–12 s) afterhyperpolarization following the action potential, calcium action potentials in the presence of TTX, and lacked fast nicotinic excitatory synaptic potentials (Nishi & North, 1973; Hirst *et al.*, 1974; Surprenant, 1984).

Quinine (100–150 μM) partially or completely abolished the prolonged afterhyperpolarization in all AH cells tested within 30–90 s (Figure 1). The amplitude and time course of the action potential itself and the initial afterhyperpolarization which followed the action potential, were not affected. In the myenteric ganglia, the effects of quinine were generally slow to reverse, often requiring 20–60 min of washing; in the submucous plexus full recovery occurred within 5–10 min. Quinine (100–150 μM) also completely blocked the prolonged afterhyperpolarization that followed the TTX-resistant action potentials. Higher concentrations of quinine (300–500 μM), or longer periods of exposure (> 5–10 min) to lower concentrations, caused an increase in duration of the action potential; however, the increase in the duration of the action potential occurred later in the superfusion period than the blockade of the prolonged afterhyperpolarization.

Calcium ions were injected intracellularly into myenteric neurones after the prolonged afterhyperpolarization had been abolished by superfusing with

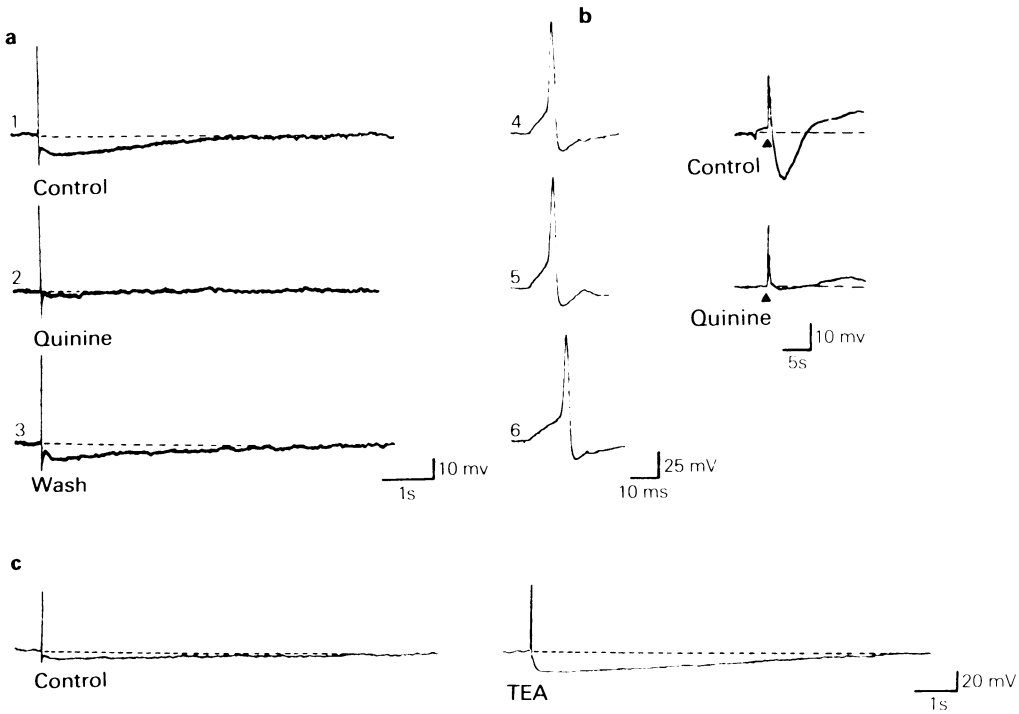

Figure 1 Quinine blocks the prolonged afterhyperpolarization following the directly evoked action potential as well as the hyperpolarization evoked by intracellular injection of calcium. (a) Intracellular recordings obtained in one neurone when one action potential was evoked by direct depolarization of the membrane before (1), during (2) and after (3) the addition of quinine (100 μM) to the superfusing solution. Recovery occurred after 50 min of washout in this neurone. Note that the undershoot of the action potential was unaffected by quinine when the prolonged afterhyperpolarization was abolished. Traces 4–6 show action potentials in 1–3 at fast sweep speed. Quinine had no effect on the amplitude or time course of the directly evoked action potential. (b) Hyperpolarization induced by the intracellular injection of calcium ions (3 nA for 100 ms) recorded in one myenteric neurone before and during superfusion with quinine 150 μM. (c) The prolonged afterhyperpolarization following one directly evoked action potential before (left) and during (right) superfusion of tetraethylammonium (TEA, 5 mM).

a calcium-free, high magnesium (12 mM) solution. Intracellular calcium injection evoked a hyperpolarization, the amplitude and time constant of decay of which were similar to those of the spike afterhyperpolarization; this hyperpolarization may be presumed to result from potassium conductance increase because it reversed in polarity at −92 mV, which was the same as the reversal potential of spike-evoked prolonged afterhyperpolarization. Quinine (100 μM) abolished the hyperpolarization evoked by calcium injection (Figure 1b).

Tetraethylammonium (TEA) (1–5 mM), which is known to block a variety of K^+ channels (LaTorre & Miller, 1983), caused an increase in the duration of the action potential and in the amplitude and duration of the prolonged afterhyperpolarization. The effect on the afterhyperpolarization is difficult to interpret because of probable changes in the amount of calcium entering the cell during the action potential. Lower concentrations of TEA (50–200 μM), which had minimal or no effect on the directly evoked action potential, did not alter the prolonged afterhyperpolarization ($n = 5$).

Discussion The present study indicates that quinine can selectively inhibit the potassium current which underlies the prolonged afterhyperpolarization in neurones of the enteric plexuses; it appears to do so by preventing the action of intracellular calcium ions. This quinine-sensitive potassium conductance appears not to be the calcium-activated 'big K' channel common to neural, muscle and endocrine cells so far examined in patch clamp studies (Latorre & Miller, 1983; Petersen & Maruyama, 1984) because it is relatively independent of membrane potential in the range of −60 to −110 mV (Morita *et al.*, 1982); the 'big K' channel requires very high calcium concentra-

tions for its activation at such negative potentials. It also appears to differ from the calcium-activated potassium conductance described in sympathetic and hippocampal neurones, which is very sensitive to TEA (Adams *et al.*, 1981; Brown & Griffith, 1983). Quinine-sensitive potassium conductance increases resulting from elevation of intracellular calcium ion concentration have previously been described in liver (Burgess *et al.*, 1981), pancreatic β-cells (Atwater *et al.*, 1979), photoreceptors (Hanani & Shaw, 1977) and erythrocytes (Reichstein & Rothstein, 1981). The present study shows that this quinine-sensitive conductance may be distinct from that which is responsible for action potential repolarization, or the resting membrane potential.

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References

ADAMS, P.R., CONSTANTI, A., BROWN, D.A. & CLARK, R.B. (1982). Intracellular Ca^{2+} activates a fast voltage-sensitive K^+ current in vertebrate sympathetic neurones. *Nature*, **296,** 746–749.

ATWATER, I., DAWSON, M., RIBALET, B. & ROJAS, E. (1979). Potassium permeability activated by intracellular calcium ion concentration in the pancreatic β-cell. *J. Physiol.*, **288,** 575–588

BROWN, D.A. & GRIFFITH, W.H. (1983). Calcium activated outward current in voltage clamped hippocampal neurones of the guinea-pig. *J. Physiol.*, **337,** 287–301.

BURGESS, G.M., CLARET, M. & JENKINSON, D.H. (1981). Effect of quinine and apamin on the calcium-dependent potassium permeability of mammalian hepatocytes and red cells. *J. Physiol.*, **317,** 67–90.

HANANI, M. & SHAW, C. (1977). A potassium contribution to the response of the barnacle photoreceptor. *J. Physiol.*, **270,** 151–163.

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

LATORRE, R. & MILLER, C. (1983). Conduction and selectivity in potassium channels. *J. Membr. Biol.*, **71,** 11–30.

MORITA, K., NORTH, R.A. & TOKIMASA, T. (1982). The calcium-activated potassium conductance in guinea-pig myenteric neurones. *J. Physiol.*, **329,** 341–345.

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

PETERSEN, O.H. & MARUYAMA, Y. (1984). Calcium-activated potassium channels and their role in secretion. *Nature*, **307,** 693–696.

REICHSTEIN, E. & ROTHSTEIN, A. (1981). Effects of quinine on Ca^{++} induced K^+ efflux from human red blood cells. *J. Membr. Biol.* **59,** 57–63.

SURPRENANT, A.M. (1984). Two types of neurones lacking synaptic input in the submucous plexus of guinea-pig small intestine. *J. Physiol.*, **351,** 363–378.

WALDEN, J. & SPECKMANN, E.J. (1981). Effects of quinine on membrane potential and membrane currents in identified neurones of *Helix pomatia*. *Neurosci. Letts*, **27,** 139–143.

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