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CLONIDINE ACTIVATES MEMBRANE POTASSIUM CONDUCTANCE IN MYENTERIC NEURONES

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1 Intracellular recordings were made from neurones in the myenteric plexus of the ileum removed from guinea-pigs. The effects of clonidine and adrenaline on membrane potential and resistance were observed.

2 Clonidine (100 pM–30 nM) caused a concentration-dependent membrane hyperpolarization associated with a fall in neurone input resistance.

3 The amplitude of the clonidine hyperpolarization, but not the conductance increase, was greater in cells with lower resting potentials and smaller in more polarized neurones. In a given cell, membrane hyperpolarization decreased and membrane depolarization increased the clonidine effect.

4 Low potassium solutions enhanced and high potassium solutions reduced the hyperpolarizing action of clonidine but did not significantly change the conductance increase caused by clonidine.

5 The concentration-effect curve for clonidine was displaced to the left when the extracellular calcium concentration was reduced. Conversely, clonidine was almost ineffective in elevated calcium concentrations. This was true for both the hyperpolarization and the conductance increase.

6 It is suggested that clonidine activates a potassium conductance by causing an elevation in the free intracellular calcium concentration.

7 Clonidine reversibly depressed the amplitude of the nicotinic fast excitatory postsynaptic potential and the noncholinergic slow excitatory postsynaptic potential.

8 All the effects of clonidine were shared by adrenaline and the actions of both were reversed or prevented by phentolamine (100 nM – 1 μM).

Introduction

The sympathetic fibres which reach the intestine terminate largely in the myenteric plexus, where they make extensive arborizations around ganglion cells (Jacobowitz, 1965; Costa & Furness, 1973). Stimulation of the fibres inhibits peristalsis (Finklemann, 1930). Although this may be due in part to a hyperpolarization of smooth muscle cells (Bennett, Burnstock & Holman, 1966), it is probably due mostly to direct inhibitory effects on the myenteric plexus. An inhibition of cholinergic synaptic transmission between myenteric neurones has been shown to occur during periarterial nerve stimulation (Hirst & McKirdy, 1974). Application of exogenous noradrenaline also inhibits intraganglionic cholinergic synaptic transmission in the myenteric plexus (Nishi & North, 1973a).

There is a large body of literature describing the reduction by noradrenaline of the amount of acetylcholine released by electrical field stimulation of the entire myenteric plexus (*inter alia* Schaumann, 1958; Paton & Vizi, 1969; Kosterlitz, Lydon & Watt, 1970). This amount of acetylcholine presumably includes that released at intraganglionic synapses, but is largely that released at the ill-defined neuromuscular junction. Later studies have indicated that this effect is due to α_2-receptor activation (Werner, Starke & Schumann, 1972; Drew, 1978a; Wikberg, 1978; Andréjak, Pommier, Mouillé & Schmitt, 1980). The present paper describes a further study of the actions of a α-adrenoceptor agonists, in particular clonidine, on the membrane properties of single myenteric neurones.

Methods

Adult guinea-pigs were stunned and bled. The ileum was removed rapidly and placed in a Krebs solution of the following composition (mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25, NaH_2PO_4 1.2, glucose 11, gassed with 5% CO_2/95% O_2. Intracellular recordings were made

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from myenteric neurones by the method described by Nishi & North (1973b). Microelectrodes containing potassium chloride (2M) were used (d.c. resistance 50–80 MΩ). Intracellular potentials were filmed from an oscilloscope and displayed on a pen recorder (frequency response d.c. to 1 kHz). Fibres within a myenteric ganglion were stimulated by passing short (100–500 μs) current pulses through an extracellular stimulating electrode, the tip of which (diameter 10–20 μm) was moved to various positions on the ganglion surface. A single pulse applied to such an electrode led to the appearance of a fast excitatory postsynaptic potential (e.p.s.p.) in Type 1 cells (for definition of cell types see Nishi & North, 1973b). In Type 2 cells (but sometimes also in Type 1 cells), such a pulse caused an action potential in a cell process which propagated to the soma. Repeated pulses (up to 10 Hz for 3 s) led to the appearance of slow e.p.s.ps and occasionally slow i.p.s.ps in both types of cell. Further details of the techniques of evoking these slow potentials, as well as their ionic mechanisms, have been described (Johnson, Katayama & North, 1980). The tissue was superfused (2–4 ml/min) throughout the experiment with Krebs solution which was heated so that the temperature at the recording site was 35–37°C. Drugs were applied by changing the perfusing solution to one that differed only in its content of the drug. In a few experiments, acetylcholine was applied to the soma membrane by electrophoresis (see North, Henderson,

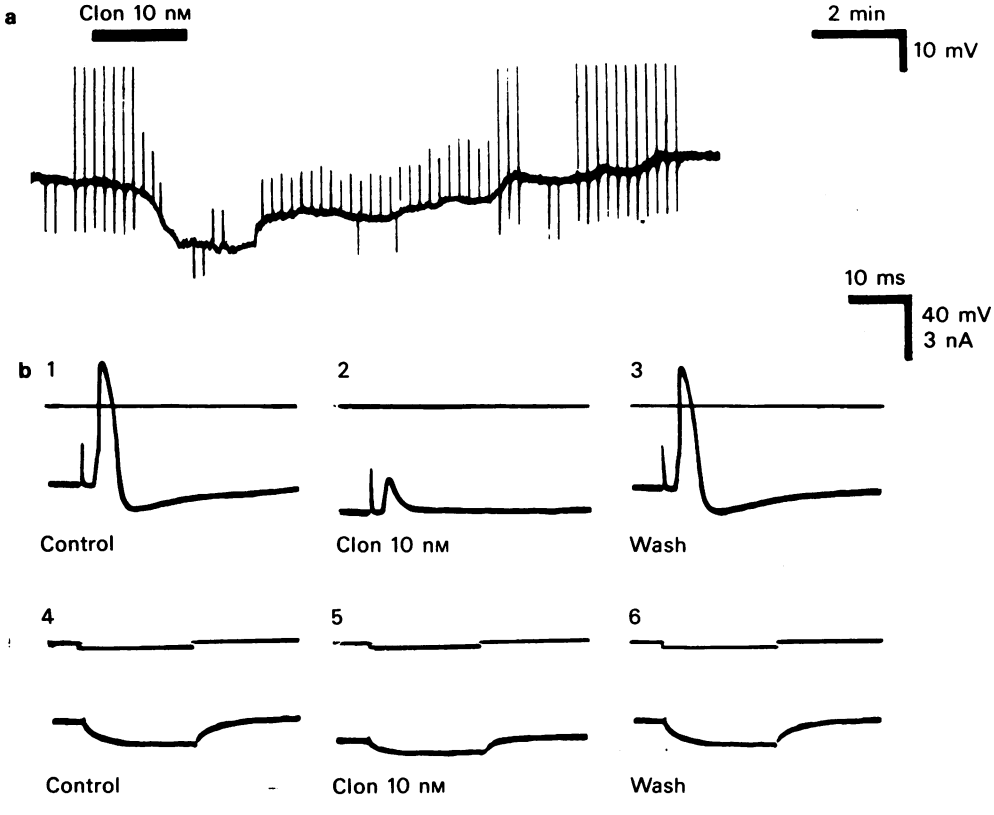

Figure 1 (a) Pen recording of membrane potential of neurones in myenteric plexus of guinea-pig ileum. Large upward deflections are action potentials evoked by focal stimulation of a cell process. Full amplitude and time course of action potentials are shown in (b). Small upward deflections are all-or-nothing depolarizations caused by action current entering the soma from the action potential in the proximal part of the cell process (see b, 2). Downward deflections are action potential after-hyperpolarizations, or hyperpolarizing electrotonic potentials caused by passing a constant current across the soma membrane. The perfusing solution contained clonidine (Clon, 10 nM) during the time shown by the bar. The membrane hyperpolarized, the action potential fractionated and the input resistance was reduced. (b) Oscilloscope photographs of membrane potential (lower traces) and transmembrane current (upper traces). (1) Control. The delay between the stimulus artifact and the action potential is due to propagation along the cell process. (2) During perfusion with clonidine (10 nM). The membrane is hyperpolarized and the propagating action potential fails to invade the soma. (3) Washout. (4) Control electrotonic potential. (5) During clonidine (10 nM). (6) Washout.

Katayama & Johnson, 1980). Figures in this paper indicate the time at which the experimenter changed the superfusing solution from one to another; there was a delay of 40–80 s before the changed solution reached the tissue due to its passage through a pump and heat exchanger. Solutions which contained high or low potassium concentrations, or low calcium concentrations, were maintained iso-osmotic by adjusting the NaCl concentration. Calcium-free solutions were iso-osmotic either because of increased NaCl or $MgCl_2$; some contained ethylene glycol-bis [β-aminoethyl ether]N,N′-tetra acetic acid (EGTA) (1 mM). Drugs used were clonidine hydrochloride (Boehringer), adrenaline bitartrate (Sigma), and phentolamine hydrochloride (Sigma).

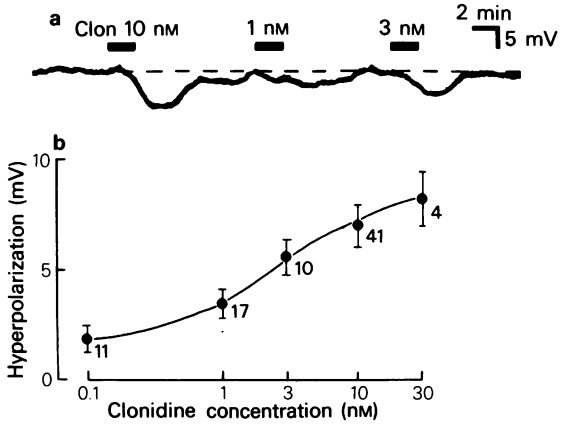

Figure 2 Concentration-dependence of clonidine-induced hyperpolarization of guinea-pig myenteric plexus neurones. (a) Membrane potential of a myenteric neurone. Clonidine (Clon, 10, 1 and 3 nM) was applied during the periods indicated by the bars. (b) The amplitude of the membrane hyperpolarization at different clonidine concentrations. The points are the means (bars indicated s.e.mean) of the number of observations indicated.

Results

These results are based on recordings from 98 cells in tissue samples removed from 55 animals.

Effect of clonidine on membrane potential and resistance

The cell membrane was almost always hyperpolarized by clonidine (10 nM) (Figure 1). Only a small proportion of cells (12%) ($n = 41$) were unaffected, and these cells generally had high resting potentials and low resistances. The mean hyperpolarization caused by clonidine (10 nM) was 6.9 ± 1.0 mV (mean ± s.e.mean, $n = 41$). The hyperpolarization reached its maximum level within 2–3 min and the resting potential was recovered within 5–10 min after washing with drug-free solution. The membrane hyperpolarization was always accompanied by a fall in input resistance. Clonidine (10 nM) reduced input resistance to $71 \pm 2.7\%$ ($n = 22$) of its control value. For a given clonidine concentration, the fall in input resistance was greater in cells which showed larger hyperpolarizations.

The magnitude of the clonidine hyperpolarization was related to the concentration applied within the range tested (100 pM–30 nM) (Figure 2). These concentrations embrace the range at which [^{3}H]-clonidine binds to a high affinity saturable site in guinea-pig homogenized ileum (Tanaka & Starke, 1979).

The effect of changing the membrane potential on the clonidine hyperpolarization During the recordings from many neurones, it was noticed that the higher the resting membrane potential the smaller was the clonidine hyperpolarization. These findings are summarized in Figure 3b. Extrapolation from this graph indicates that clonidine would not cause any hyperpolarization at a potential of −84 mV. The conductance change caused by clonidine (10 nM) (ratio of control input resistance to input resistance at peak clonidine hyperpolarization) was not significantly correlated with resting membrane potential ($r = -0.0043$, $n = 22$). In a given neurone, the amplitude of the clonidine hyperpolarization was made greater if it was elicited at a time when the cell was depolarized by passing a small inward current across the membrane and was made smaller by membrane hyperpolarization (Figure 3a). Because of the difficulties in passing large d.c. currents through the recording electrode for long periods of time, it has not yet been possible to demonstrate clear reversal of the clonidine effect.

Effect of extracellular potassium concentration on the clonidine hyperpolarization The membrane potential decreased by 5–20 mV, and membrane resistance fell when the perfusing solution was changed to one which contained 10 to 20 mM potassium. Perfusion with an elevated potassium solution reduced the amplitude of the clonidine hyperpolarization (Figure 4a). The clonidine effect was restored to normal when the potassium concentration of the perfusing solution was reduced to its normal value. When the perfusing solution contained a reduced potassium concentration (1 to 2 mM) the membrane hyperpolarized (5–20 mV) within 5–10 min. In most cells (76%) ($n = 17$), the clonidine hyperpolarization was increased in amplitude in low potassium solutions. The effects of changing the extracellular potas-

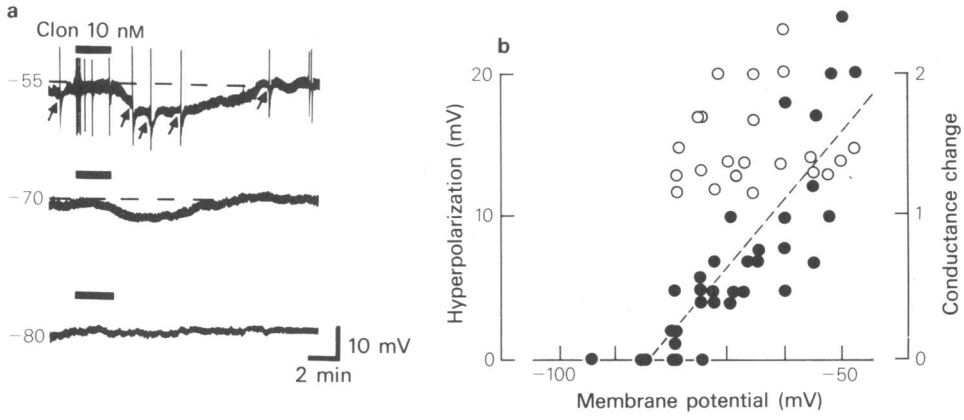

Figure 3 Effect of resting membrane potential on the amplitude of the clonidine-induced hyperpolarization of myenteric neurones in guinea-pig ileum. (a) Clonidine (10 nM) was applied during the periods indicated by the bars. The middle recording shows the effect of clonidine at the resting potential level (−70 mV). The top recording was made during the passage of a constant inward current which depolarized the neurone to −55 mV. The cell discharged occasional spontaneous action potentials. Arrows indicate times at which an action potential was induced by passing a brief depolarizing current pulse. The bottom recording was made when the neurone was hyperpolarized to −80 mV by passage of a constant outward current. (b) Each filled point (●) correlates the amplitude of the hyperpolarization caused by clonidine (10 nM) (left ordinate scale) with the resting membrane potential of the neurone. The broken line is the least squares regression ($y = -0.46x + 38.8$, $r = -0.843$, $P < 0.001$) from which the membrane potential at which clonidine produced no effect is −84 mV. Each open point (○) correlates the clonidine-induced conductance change (ratio of control input resistance to input resistance at peak clonidine hyperpolarization, right ordinate scale) with the resting membrane potential of the neurone ($r = -0.0043$, no significant correlation). Resting membrane potentials were determined by sudden withdrawal of the electrode from the neurone.

sium concentration on the amplitude of the clonidine response are summarized in Figure 4b. The effect of changing the potassium concentration on the amplitude of the clonidine hyperpolarization was probably mediated by a change in driving force, because the clonidine-induced conductance change was not different among the three potassium concentrations at which reliable conductance measurements were obtained (Figure 4b).

The effect of extracellular calcium concentration on the clonidine hyperpolarization An increase in the calcium concentration of the perfusing solution to 5 mM usually caused no change in membrane potential or input resistance, but occasionally a small hyperpolarization. In 5 mM calcium, the amplitude of the clonidine hyperpolarization was much reduced (Figure 5a). A reduction in the extracellular calcium concentration to 1.2 mM caused either no change in membrane potential and input resistance or a depolarization (up to 10 mV, but usually less than 5 mV). In low calcium solutions, the amplitude of the clonidine hyperpolarization was increased (Figure 5a). Complete removal of calcium from the perfusing solution usually decreased the membrane potential and increased input resistance. This was occasionally preceded by a transient hyperpolarization. Several neurones fired action potentials spontaneously in a calcium-free solution. Both the amplitude and duration of the clonidine hyperpolarization were increased in calcium-free solutions. The change in membrane potential which sometimes occurred in different calcium concentrations may of course contribute in part to the change in amplitude of the clonidine hyperpolarization (e.g. Figure 5). However, in some cells a change in calcium concentration caused no change in resting potential or resistance. Furthermore, the membrane conductance change induced by clonidine (ratio of control input resistance to input resistance at peak clonidine hyperpolarization) was itself changed in different calcium concentrations (Figure 6). Clonidine hyperpolarizations could be repeatedly elicited for at least 1 h after changing to a calcium-free solution (e.g. Figure 9a). Similar results were obtained when the calcium-free perfusing solution also contained EGTA (1 mM).

Effect of clonidine on synaptic potentials

Fast e.p.s.p. The cholinergic fast e.p.s.p. was depressed or abolished by clonidine (10 nM) (Figure 7). This action occurred rapidly and reversed when clonidine perfusion was discontinued. The de-

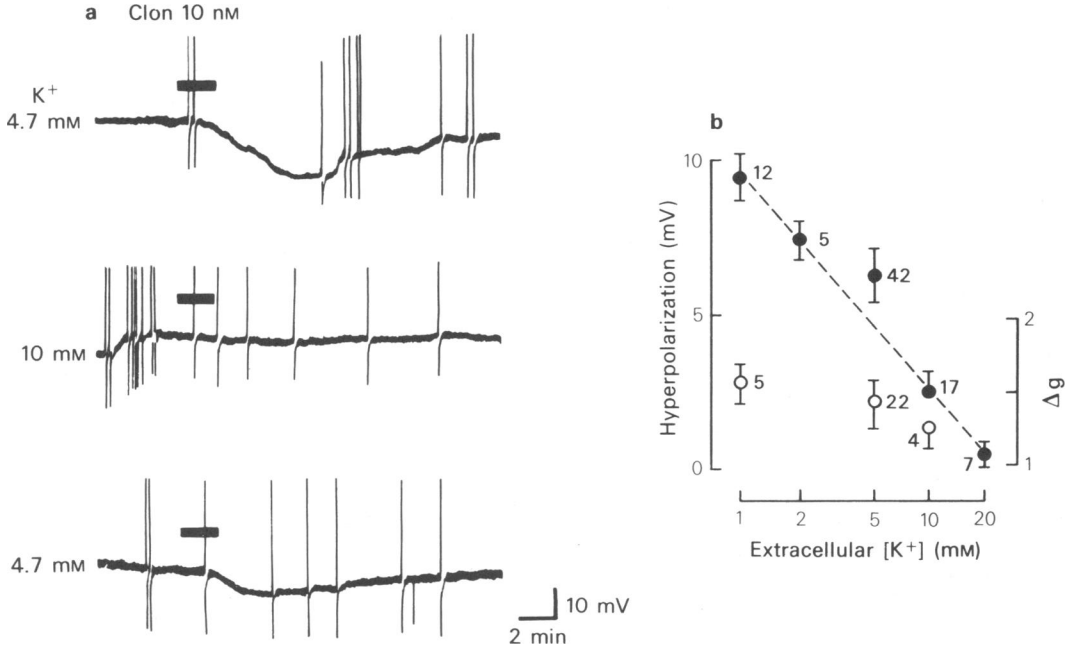

Figure 4 (a) Membrane potential of a spontaneously firing neurone. Clonidine (Clon, 10 nM) was applied during the periods shown by the bars. Top trace, control effect 4.7 mM potassium. Middle trace, effect of clonidine during perfusion with 10 mM potassium solution. Bottom trace, effect of clonidine after returning to 4.7 mM potassium. (b) Left ordinate scale, (●): amplitude of hyperpolarization caused by clonidine (10 nM) in solutions of different potassium ion concentration. Line is fitted by eye. Right ordinate scale, (○): clonidine-induced conductance change (ratio of control input resistance to input resistance at peak clonidine hyperpolarization). These values are not different (one-way AOV, $F = 2.02, \nu_1 = 2, \nu_2 = 26$).

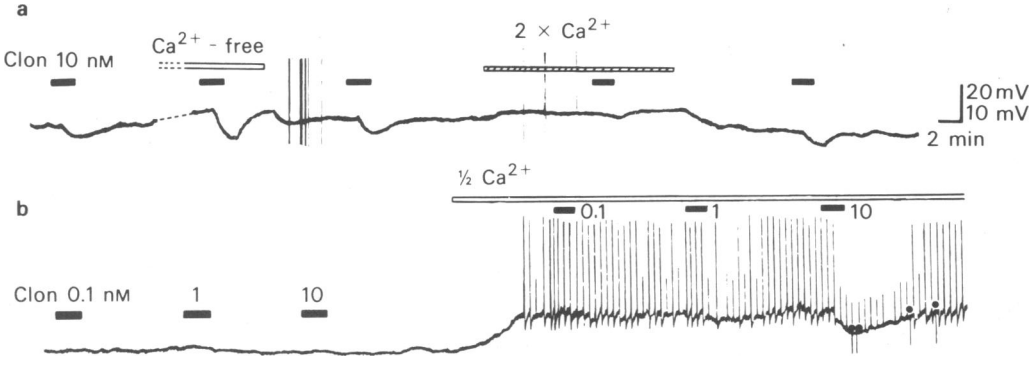

Figure 5 Membrane potential of two myenteric neurones of guinea-pig ileum. (a) Clonidine (Clon, 10 nM) was applied during the periods indicated by the solid bars. The open bar indicates perfusion with a calcium-free solution (break in trace is 30 min, during which calcium-free solution was applied). Hatched bar indicates perfusion with a high calcium (5 mM) solution. The effect of clonidine was enhanced in the calcium-free solution and reduced in the high calcium solution. (b) Clonidine was applied (solid bars) in increasing concentrations, but was without effect. Low calcium (1.26 mM) solution perfused the tissue during the period shown by the open bar. In low calcium solution 1 nM clonidine caused a slight hyperpolarization and 10 nM a large hyperpolarization associated with a fall in input resistance. Action potentials were evoked by focal stimulation of a cell process (see Figure 1 legend) and failed to invade the soma during the clonidine hyperpolarization. Dots indicate hyperpolarizing electrotonic potentials caused by passing a fixed outward current pulse.

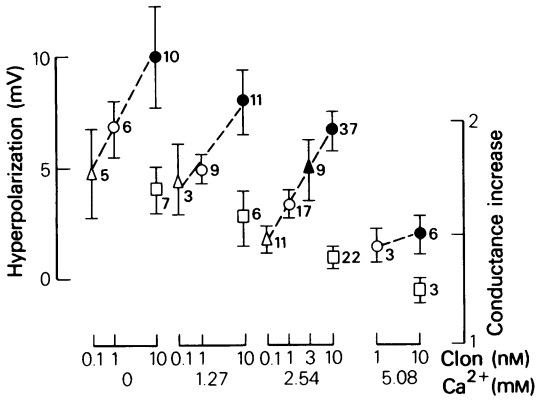

Figure 6 Calcium dependence of clonidine-induced hyperpolarization of myenteric neurones. Clonidine (Clon, 0.1, 1, 3 or 10 nM) was applied to myenteric neurones at each of four extracellular calcium concentrations. The mean hyperpolarizations are indicated (bars are s.e.mean) for the number of neurones indicated. Lines are least-squares regression. The effect of clonidine was dependent on its concentration (see also Figure 1) and inversely dependent on the calcium ion concentration. □: Indicate clonidine-induced conductance change (ratio of control input resistance to input resistance at peak clonidine hyperpolarization). The least-squares regression of conductance change (y) on calcium concentration (x) is $y = -0.021x + 1.7$ ($r = 0.367$, $\nu = 36$, $P < 0.05$), and one-way analysis of variance indicates that calcium has a significant effect on the conductance change ($F = 3.04$, $\nu_1 = 3$, $\nu_2 = 34$, $P < 0.05$.

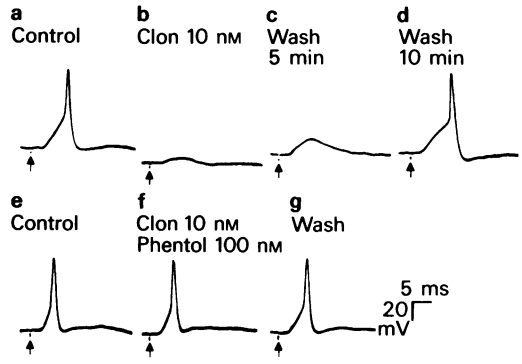

Figure 7 Depression of the fast e.p.s.p. by clonidine in guinea-pig myenteric neurone. Membrane potential of two neurones. Presynaptic fibres were stimulated with a single pulse (arrow). (a) Control. The e.p.s.p. gives rise to an action potential. (b) In the presence of clonidine (Clon, 10 nM) the membrane is hyperpolarized and the e.p.s.p. amplitude is greatly reduced. (c) Five min after ending clonidine perfusion, the membrane potential has returned to its original level, but the e.p.s.p. is still somewhat depressed. (d) After 10 min washing. (e) Control e.p.s.p. and action potential. (f) In the presence of clonidine (10 nM) and phentolamine (Phentol, 100 nM). (g) After washout of both drugs.

pression of the fast e.p.s.p. was observed in neurones which were hyperpolarized by clonidine ($n = 5$) and also in other cells ($n = 2$) which showed no change in membrane potential. Clonidine (10 nM), which completely abolished the fast e.p.s.p., did not significantly depress the amplitude of the depolarizing response to electrophoretic application of acetylcholine (ACh) onto the cell soma. The small changes which were observed in the amplitude of the ACh potential could be attributed to the combined effects of the hyperpolarization and fall in input resistance caused by the clonidine.

Slow e.p.s.p. This non-cholinergic synaptic potential was depressed or abolished by clonidine (10 nM) (Figure 8). This depression occurred within a few min of starting clonidine application but sometimes passed off during the application. This occurred even though the clonidine hyperpolarization persisted, thus establishing that the depression of the slow e.p.s.p. was not simply a result of membrane hyperpolarization (Johnson *et al.*, 1980). The duration of the slow e.p.s.p. was shortened even when clonidine was applied to the preparation after the slow e.p.s.p. had been initiated. This finding implies that clonidine may be terminating the intracellular mechanism which is responsible for the slow e.p.s.p. rather than acting presynaptically to inhibit transmitter release.

Effects of α-receptor antagonists on clonidine effects

The clonidine-induced hyperpolarization, inhibition of spontaneous firing and the depression of the fast and slow e.p.s.ps were not observed when the perfusion solution contained phenoxybenzamine (1 μM) or phentolamine (100 nM – 1 μM). Propranolol (1 μM) had no effect on any action of clonidine. None of these agents had any effect of its own on membrane potential or resistance. The blockade of the clonidine effects by phentolamine reversed within a few min of perfusion with normal Krebs solution (Figure 9). Prazosin (1 μM) was without effect on the clonidine hyperpolarization.

Effect of of adrenaline

Adrenaline (10 nM – 1 μM) also hyperpolarized myenteric neurones ($n = 7$), and this was associated with a fall in membrane resistance. The adrenaline hyperpolarization was also observed in calcium-free solutions, and was prevented by phentolamine (1 μM).

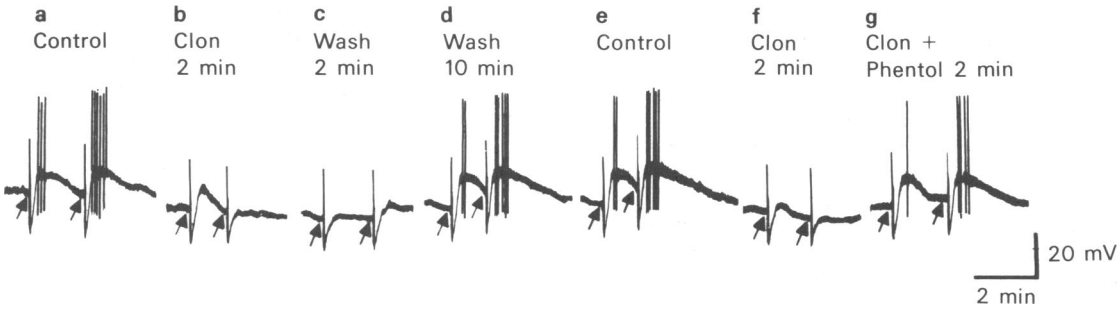

Figure 8 Membrane potential of one myenteric neurone of guinea-pig ileum. Each recording shows the effect of two stimuli applied to the presynaptic nerves (arrows). Each stimulus was 30 pulses at 10 Hz. (a) Control. Each stimulus evoked a slow e.p.s.p. The stimulus also excited a cell process which propagated to the soma; the repetitive action potentials resulted in a summated after-hyperpolarization and this precedes the slow e.p.s.p. (b) After 2 min perfusion with clonidine (Clon, 10 nM). The slow e.p.s.ps are much reduced, particularly the second one. (c) After 2 min washing. The slow e.p.s.ps remain almost completely abolished. (d) After 10 min washing. (e) Control slow e.p.s.ps evoked later in the same neurone. (f) After 2 min perfusion with clonidine (10 nM). (g) Two min after changing to a solution containing clonidine (10 nM) and phentolamine (1 μM).

Discussion

Effects on membrane properties

Other vertebrate autonomic neurones are hyperpolarized by α-receptor agonists (Lundberg, 1952; de Groat & Volle, 1966; Libet & Kobayashi, 1974; Hirst & Silinsky, 1975; Koketsu & Nakamura, 1976; Brown & Caulfield, 1979; Horne & McAfee, 1980) but in most of these studies the ionic mechanisms of the effects have not been reported. The present results provide three pieces of evidence which indicate that clonidine and noradrenaline hyperpolarize myenteric neurones by increasing the resting potas-

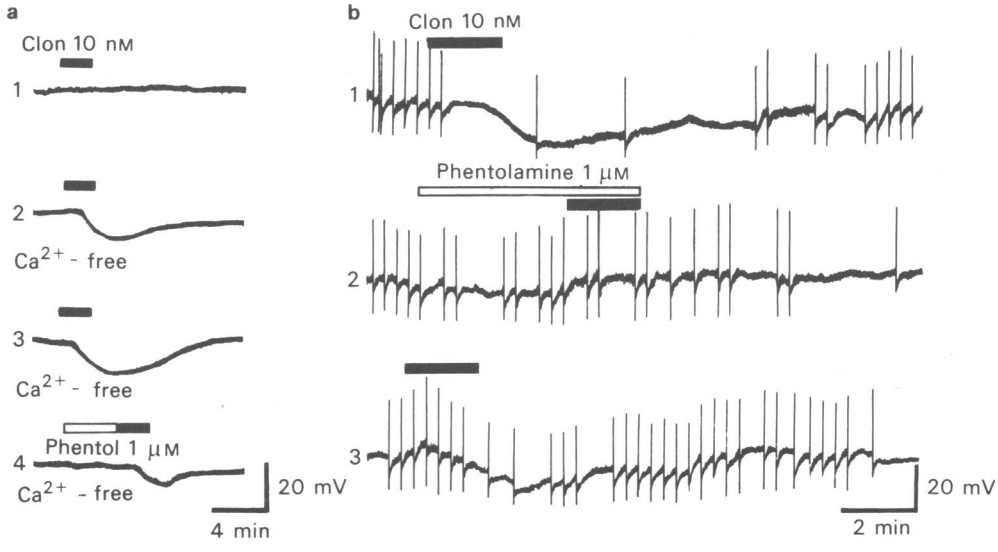

Figure 9 Antagonism of clonidine-induced hyperpolarization by phentolamine in guinea-pig ileum: membrane potentials of two myenteric neurones. Clonidine (10 nM) and phentolamine (1 μM) were applied to the neurones during the periods indicated by the solid and open bars. (a) (1) Control, calcium, 2.54 mM. Clonidine had no effect. (2) Ten min after changing to calcium-free solution. (3) Thirty min after changing to calcium-free solution. (4) Forty min after changing to calcium-free solution. When clonidine was applied immediately after perfusion with phentolamine, its effect was much reduced. (b) (1) Control. (2) Clonidine was ineffective when applied in the presence of phentolamine. (3) After washout of phentolamine, clonidine again hyperpolarized the neurone. Action potentials were evoked by depolarizing current pulses.

sium conductance. These are the associated fall in input resistance, the potential dependence of the hyperpolarization and the effects of changing the extracellular potassium concentration. α-Agonists have been shown to increase the membrane potassium conductance in smooth muscle cells (Bülbring & Tomita, 1969) and hepatocytes (Egashira, 1980).

We found that the clonidine effect was made larger by removal of calcium ions and reduced by increasing the extracellular calcium ion concentration. Similar effects of calcium ion have been reported for the inhibitory action of clonidine on transmitter release (Drew, 1978b; Magnan, Regoli, Quirion, Lemaire, St. Pierre & Rioux, 1979). Brown & Caulfield (1979), using extracellular recording, also found that the clonidine hyperpolarization of rat sympathetic ganglion cells showed a similar inverse calcium-dependence.

A possible explanation of the results is that the α-agonists cause an elevation of the intracellular calcium concentration, which leads to an activation of the potassium conductance. A similar proposal was made to explain the findings on hepatocytes (Egashira, 1980) and smooth muscle (Bülbring & Tomita, 1977). However, in those tissues it was considered that the α-agonists in some way enhanced an inward movement of calcium. This is not likely to be true for the myenteric neurones because large hyperpolarizations could be observed after up to 1 h in solutions with no added calcium and EGTA. For this reason we favour the hypothesis that a direct action of α-agonists may be to elevate intracellular calcium by, perhaps, inhibiting binding to an intracellular storage site. The enhancement of the clonidine effect in low or very low extracellular calcium concentrations could have three possible explanations. The first is a direct interference with clonidine receptor binding; however, elevation of the calcium concentration tends to increase the amount of high affinity binding of clonidine to brain homogenates (Rouot, U'Prichard & Snyder, 1980). The second is that the intracellular calcium binding site which is affected by the α-agonist is only one of several and is of high affinity and low capacity. In this case, when extracellular calcium is very low, and intracellular calcium is also low, binding to the clonidine-sensitive site is an important determinant of the free intracellular calcium concentration. When extracellular calcium is high, and intracellular calcium also rises, the clonidine-sensitive site may become saturated, and the control of intracellular calcium would be largely taken over by other lower affinity buffering systems such as mitochondria. The third factor which would of course contribute in some cells to the enhancement of the clonidine *hyperpolarization* in low calcium solutions is the membrane depolarization and resistance increase which removal of calcium sometimes caused. This is not likely to be a significant factor because the clonidine-induced *conductance* change was itself inversely calcium-dependent, and myenteric neurones have linear (ohmic) membrane properties over the potential range in question (Nishi & North, 1973b).

Inhibition of voltage-dependent calcium conductance by noradrenaline has been described in sympathetic ganglion cells (Horn & McAfee, 1980) and in cultured dorsal horn neurones (Mudge, Leeman & Fischbach, 1979). No particular study was made of the action potential configuration in the present study. However, the prolonged after-hyperpolarization of Type 2 cells is a measure of the calcium which enters the neurone during the action potential (North, 1973; North & Nishi, 1976). This was not reduced by clonidine, arguing against a direct blockade of voltage-dependent calcium entry.

Effects on synaptic transmission

Both the fast and slow e.p.s.ps were reversibly depressed by clonidine and adrenaline. The depression of the fast e.p.s.p. extends the findings of Nishi & North (1973a) and North *et al.* (1980). Wood & Mayer (1979) also observed a depression of the fast and slow e.p.s.ps by noradrenaline. It was previously shown that noradrenaline does not depress the amplitude of the depolarizing response to electrophoretic application of ACh (North *et al.*, 1980), which is the transmitter mediating the fast e.p.s.p. (Nishi & North, 1973b; Hirst, Holman & Spence, 1974). Hirst & McKirdy (1975) have shown that a similar reduction in the fast e.p.s.p. can be evoked by repetitive stimulation of the periarterial nerves to the ileum. In the present experiments, both the synaptic termination on the impaled neurone and the point of stimulation of the presynaptic fibres were exposed to the drugs. Therefore, we cannot distinguish unequivocally between a hyperpolarization of presynaptic fibres causing a block of spike initiation, and a reduction of the amount of acetylcholine released by each action potential invading the terminals. One should not overlook the intriguing possibility that the postulated intracellular site to which clonidine may inhibit binding could be a site essential for promoting transmitter release. In such a case a common mechanism may both inhibit transmitter release and hyperpolarize the membrane.

The mechanism of depression of the slow e.p.s.p. may be presynaptic, postsynaptic or both. A direct presynaptic effect is difficult to show because of doubts relating to the identity of the transmitter. Some evidence favours a postsynaptic effect, namely, the finding that clonidine reduced the slow e.p.s.p. even when applied after the commencement of the synaptic depolarization. The slow e.p.s.p. is gener-

ated by potassium conductance inactivation (Johnson *et al.*, 1980) mediated perhaps by a reduction in the intracellular calcium level (Grafe, Mayer & Wood, 1980); if clonidine activates the potassium conductance by elevating intracellular calcium (see above) then such a direct postsynaptic interaction would be reasonable.

Physiological significance

All the actions of clonidine and adrenaline were antagonized by phentolamine and unaffected by prazosin. This finding, together with the selectivity of the agonist clonidine, suggests that the effects are mediated by an α_2-receptor. Both pharmacological (Drew, 1978a; Wikberg, 1978; Andrejak *et al.*, 1980) and biochemical (Tanaka & Starke, 1979) evidence indicates the presence of α_2-receptors on myenteric neurones. Previously, the only consequence of their activation described was a reduction in the amount of acetylcholine released by electrical field stimulation. It is possible that the reduction in transmitter release occurs because fewer myenteric neurones are excited in the presence of clonidine, or that a hyperpolarization of nerve processes prevents action potential propagation into varicosities, or because clonidine directly inhibits calcium binding to an intracellular release site.

Noradrenaline released from the sympathetic nerves, and perhaps adrenaline in the systemic circulation, will inhibit enteric neuronal activity by two distinct mechanisms. First, the membrane hyperpolarization would greatly reduce the excitability of neurones, especially if they were tonically depolarized by the slow e.p.s.p. Second, release of ACh, and perhaps other excitatory transmitters, is reduced by the catecholamines both at intraganglionic synapses and at the nerve-muscle junction. The relative contributions of these two actions to the sympathetically mediated inhibition of peristalsis remains to be determined.

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