J. Physiol. (1985), **364**, *pp.* 265–280
With 9 text-figures
Printed in Great Britain

ON THE POTASSIUM CONDUCTANCE INCREASED BY OPIOIDS IN RAT LOCUS COERULEUS NEURONES

BY R. A. NORTH AND J. T. WILLIAMS

From the Neuropharmacology Laboratory, 56-245, Massachusetts Institute of Technology, Cambridge, MA 02139, U.S.A.

(*Received 4 December 1984*)

SUMMARY

1. Intracellular recordings were made from locus coeruleus neurones in slices cut from rat pons and superfused *in vitro*. Membrane currents were recorded with a single-electrode switch-clamp amplifier.

2. Opioids, enkephalin analogues or morphine, caused a concentration-dependent potassium current, which had a maximum value of about 300 pA at −60 mV.

3. The opioid-sensitive potassium conductance was independent of membrane potential between −60 and −130 mV, but became less as the membrane potential was changed from −60 to −30 mV.

4. The opioid outward current was reduced by quinine (100 μM–1 mM) and barium (30 μM–2 mM), but not by 4-aminopyridine (100 μM–1 mM) or tetraethylammonium (10 mM).

5. A potassium current with similar properties flowed for several seconds after a burst of action potentials; this appeared to result from calcium entering the neurone during the action potentials.

6. The α_2-adrenoceptor agonists noradrenaline and clonidine caused a concentration-dependent potassium conductance increase which had the same maximum value as that caused by opioids in the same neurones. Experiments in which an opioid and an α_2-adrenoceptor agonist were superfused together indicated that the same potassium conductance is increased by both agonists.

INTRODUCTION

Opioids inhibit the firing of nerve cells in various regions of the mammalian nervous system; this effect appears to account for many of the pharmacological actions of exogenously administered opiate drugs and may also represent a more physiological action of endogenous opioid peptides (for review see Duggan & North, 1983). It has been found that the inhibition of firing results from a membrane hyperpolarization in neurones of the rat (Williams, Egan & North, 1982) and guinea-pig (Pepper & Henderson, 1980) locus coeruleus (l.c.), the rat substantia gelatinosa (Yoshimura & North, 1983) and guinea-pig myenteric plexus (Morita & North, 1981*a*); evidence from voltage recordings suggested that the membrane hyperpolarization results from an increased potassium conductance of the cell membrane. The particular subtype

of opioid receptor which is linked to the increase in potassium conductance has been characterized as a μ-receptor in the rat l.c. (Williams & North, 1984). Agonists selective for μ- and δ-receptors also increase a potassium conductance of dorsal root ganglion cells from neonatal mice (Werz & Macdonald, 1983).

Noradrenaline also increases membrane potassium conductance of various neurones (rat l.c.: Egan, Henderson, North & Williams, 1983; rat substantia gelatinosa: North & Yoshimura, 1984; guinea-pig enteric neurones: Morita & North, 1981*b*; North & Surprenant, 1985). The receptor subtype which mediates this effect has been characterized as the α_2-adrenoceptor (Williams, Henderson & North, 1985).

In the present experiments, the voltage-clamp technique was used to measure membrane currents in order to investigate three further aspects of the hyperpolarizing action of opioids and α_2-adrenoceptor agonists on locus coeruleus neurones. First, it was asked whether the change in membrane potassium conductance could account quantitatively for the hyperpolarization observed or whether some other ion conductances might be involved? Secondly, what were the particular properties of the potassium conductance increased by these agonists? Thirdly, was the same potassium conductance increased by opioids and α_2-adrenoceptor agonists? It was considered that answers to these questions were an important prerequisite both to the further study of the process by which receptor occupancy is coupled to the potassium conductance increase, and to an understanding of the role of endogenous opioids which might act upon l.c. neurones during the course of normal brain function.

METHODS

Slices of rat pons that contained the locus coeruleus were prepared as described previously (Williams, North, Shefner, Nishi & Egan, 1984). Briefly, slices (300 μm in thickness) containing the caudal part of the l.c. were cut on a vibratome and completely submerged in physiological saline solution which flowed at 1·5 ml/min. The composition of the solution was (mM): NaCl, 147; KCl, 2·5; NaH_2PO_4, 1·2; $MgCl_2$, 1·2; $CaCl_2$, 2·4; $NaHCO_3$, 25; glucose, 11 equilibrated with 95% O_2–5% CO_2 at 37 °C. Drugs were added by changing the superfusing solution to one which differed only in its content of the drug; some drugs were also administered by application of pressure to a pipette (tip diameter 10–30 μm) which had its tip positioned beneath the surface of the solution but above the tissue slice.

Membrane voltages and currents were recorded with micro-electrodes containing potassium chloride (3 M, d.c. resistance 35–70 MΩ), using a single-electrode voltage-clamp amplifier (Dagan 8100 or Finkel Axoclamp II). Correct operation of the switch clamp was monitored continuously by displaying the voltage at the amplifier head stage. Switching from current passing to voltage recording was at a frequency of 3–10 kHz. Fast responses were often averaged (Dagan 4800 signal averager) and recordings were plotted directly on a chart recorder. Voltage commands were depolarizing rectangular steps (2–5 s) or depolarizing ramps (100 mV/50 s). Full details of these methods have been published (Williams *et al.* 1984).

Some results are presented as mean values ± S.E. of mean, and values were compared using Student's *t* test. The drugs which were used (and their sources) were: quinine bromide (Sigma), 4-aminopyridine (Sigma), tetraethylammonium bromide (Sigma), apamin (Sigma, Serva) and forskolin (islet activating protein, Calbiochem).

RESULTS

Opioid-activated potassium currents

L.c. neurones fired spontaneously in the present experimental conditions, with action potentials arising at a threshold potential of about −55 mV (Williams *et al.*

1984). Spontaneous firing was prevented by sufficient constant current (50–150 pA) to hold the membrane potential at −60 mV. Superfusion with opioid agonists produced an outward current, the amplitude of which was dependent on the concentration applied (Fig. 1). The effective concentration range varied with the

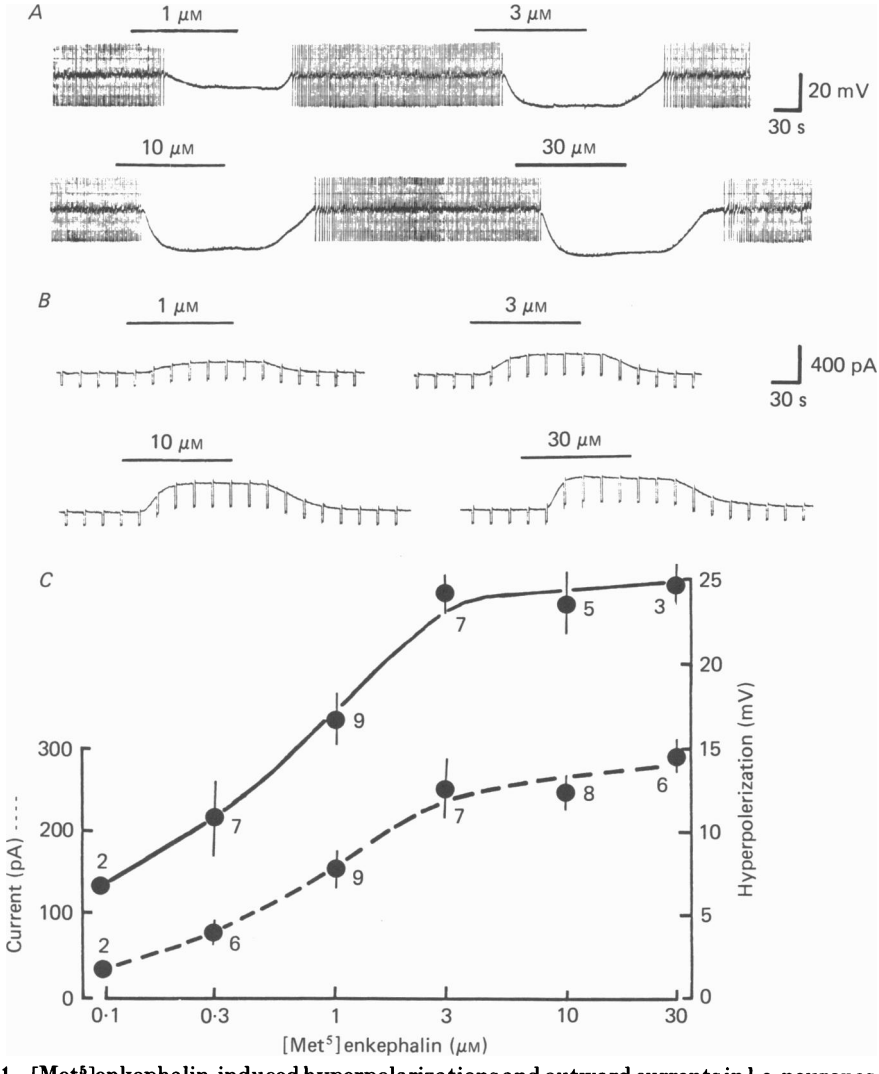

Fig. 1. [Met⁵]enkephalin-induced hyperpolarizations and outward currents in l.c. neurones. *A*, voltage recording of a spontaneously active l.c. neurone. In this and other chart recordings the full amplitude of the action potential is not shown. The slice was superfused with solutions containing [Met⁵]enkephalin during the periods indicated by the bars. *B*, current recording of a cell voltage clamped at −60 mV. Downward deflexions are the currents resulting from 2 s hyperpolarizing step commands to −80 mV. [Met⁵]enkephalin caused an outward current, the amplitude of which was dependent on the concentration applied. This was accompanied by an increase in membrane conductance. *C*, amplitude of outward current (dashed line) and hyperpolarization (continuous line) as a function of [Met⁵]enkephalin concentration. Outward currents were measured at −60 mV. Vertical bars are S.E. of mean for number of neurones indicated.

agonist used: [Met^5]enkephalin, 100 nM–30 μM; [Leu^5]enkephalin, 100 nM–100 μM; [D-Ala^2,D-Leu^5]enkephalin (DADLE), 10 nM–10 μM; normorphine, 100 nM–30 μM; [D-Ala^2, MePhe^4, Gly^5]enkephalin-ol (FK33824), 10 nM–10 μM. The maximum outward current induced by all the agonists tested was similar; in the case of [Met^5]enkephalin (at -60 mV) this was 317 ± 15 pA ($n = 17$) (range was 190–440 pA among neurones).

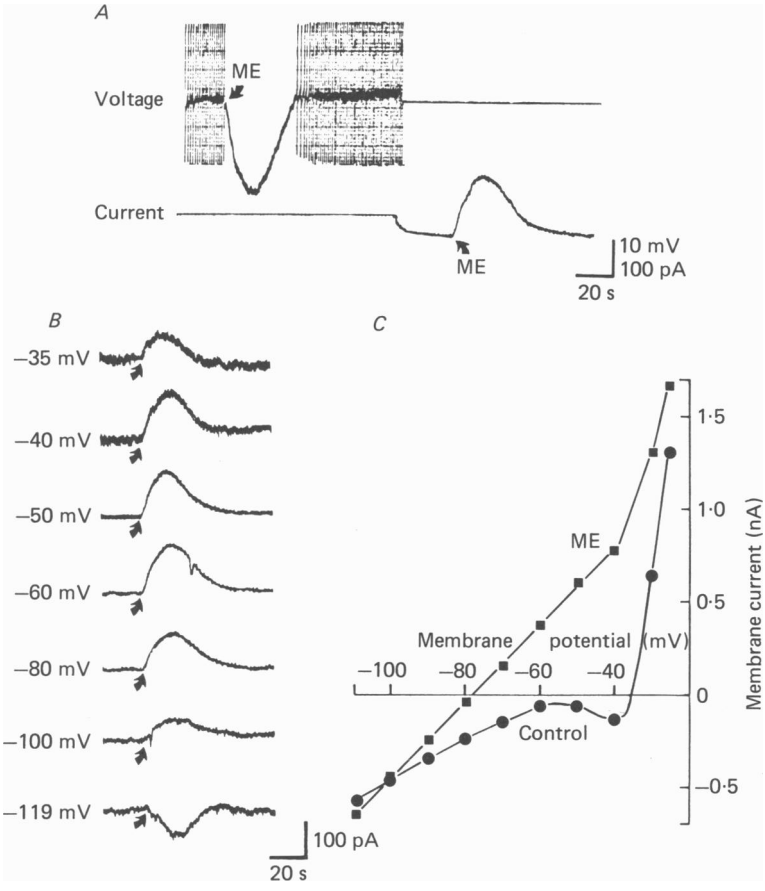

Fig. 2. Voltage dependence of [Met^5]enkephalin-induced outward currents. *A*, upper trace is voltage recording and lower trace is current recording. [Met^5]enkephalin was applied twice by pressure ejection (arrows, ME); between the applications the recording mode was changed from current clamp to voltage clamp (holding potential -60 mV). Note the similarity in time course of current and voltage response. *B*, opioid-induced currents at different membrane potentials. [Met^5]enkephalin was applied by pressure ejection (arrows); potentials indicated beside each trace (mV). [Met^5]enkephalin-induced current became inward at potentials more negative than -110 mV. *C*, steady-state current–voltage relation constructed from a slow (2 mV/s) depolarizing ramp starting at a holding potential of -110 mV. ●, control. ■, during superfusion with [Met^5]enkephalin (10 μM).

Voltage dependence. Pressure application of [Met^5]enkephalin (typically 20–100 kPa, 10–20 ms) produced an outward current which had a duration of 20–40 s. The time course of the outward current matched that of the membrane hyperpolarization produced by a similar application of [Met^5]enkephalin when the cell was not voltage-clamped (Fig. 2*A*). The outward current induced by [Met^5]enkephalin

was reproducible with repeated applications for periods over 3 h. The [Met^5]enkephalin current was studied at a series of holding potentials (Fig. 2*C*). The outward current was largest at about −50 mV and as the holding potential was moved from −60 to −110 mV the amplitude of the enkephalin current decreased linearly. The reversal potential determined in this way was −110 mV (Figs. 2 and 3). The [Met^5]enkephalin current–voltage relation deviated from linearity between −60 and −50 mV and the current declined sharply at potentials less negative than −50 mV (Figs. 2 and 3).

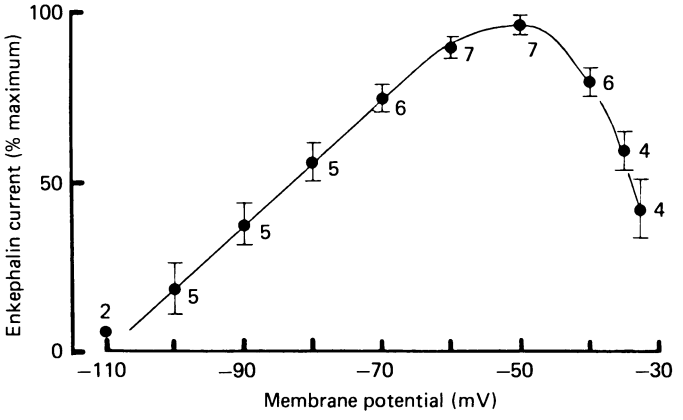

Fig. 3. Voltage dependence of the [Met^5]enkephalin-induced current. [Met^5]enkephalin was applied by pressure ejection and the response was measured at different holding potentials. Ordinate is the current expressed as a percentage of the maximum current observed in that neurone (which was either at −50 or −60 mV). Vertical bars represent S.E. of mean.

The same results were obtained by constructing steady-state current–voltage plots before and during superfusion with [Met^5]enkephalin. The slope conductance was increased in the potential range from −60 to −110 mV (Fig. 2*C*). At potentials less negative than about −50 mV the steady-state current–voltage curve turned sharply outward, and the additional outward current produced by [Met^5] enkephalin also decreased markedly.

Potassium-channel blockers. Quinine (50 μM–1 mM) produced up to 90 % decrease in the hyperpolarization or outward current induced by opioids (Figs. 4 and 5*C*). The rate of onset of the inhibition was dependent on the concentration of quinine applied; steady-state blockade required 15–20 min superfusion with quinine (100 μM). This action of quinine reversed very slowly and usually not completely even after 3 h. Higher concentrations of quinine (300 μM–1 mM) decreased the rate of rise and increased the duration of the action potential. In tetrodotoxin (TTX, 1 μM), step depolarizations from −45 to −25 mV produced an inward current (due to calcium entry) which was followed after 5–7 ms by a calcium-activated outward current (Williams *et al.* 1984; Fig. 5). Quinine (1 mM) decreased this outward current (Fig. 5). The time course with which this calcium-activated outward current was depressed corresponded to that of the depression of the opioid current. Similarly, the

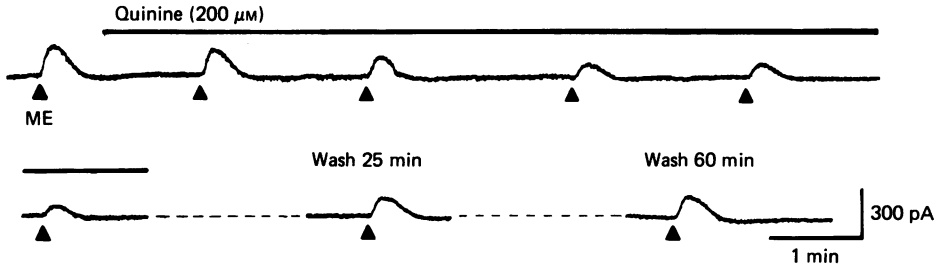

Fig. 4. Quinine reduces the [Met^5]enkephalin-induced current. [Met^5]enkephalin was applied by pressure ejection (▲). Superfusion with quinine (200 μM) (thick line) reduced the outward current to about 30 % of control over a period of 5–10 min, without causing any current of its own. Left part of lower trace is continuous with upper trace. Middle and right part of lower trace show recovery of the [Met^5]enkephalin-induced current 25 and 60 min after washing out the quinine. Holding potential −60 mV.

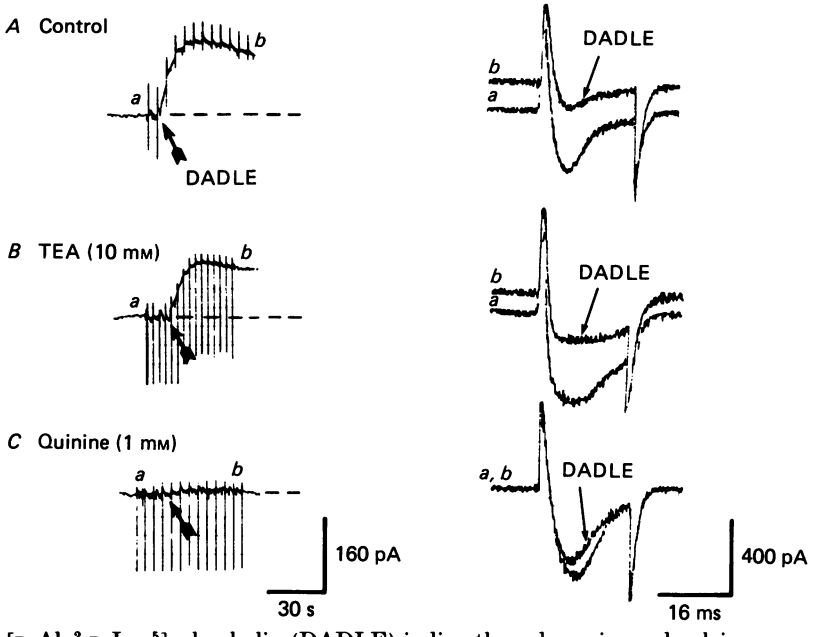

Fig. 5. [D-Ala^2,D-Leu^5]enkephalin (DADLE) indirectly reduces inward calcium current. Records are membrane currents from one neurone in the presence of TTX (1 μM). The holding potential was −45 mV, and at intervals of 3 s the potential was stepped to −30 mV for 20 ms. Left side are pen recordings; downward deflexions are inward calcium currents induced by the depolarizing command pulses, but these were too rapid to be reproduced completely by the pen recorder. Arrows indicate the times of application of one pressure pulse of DADLE. Right side shows membrane current responses to the depolarizing step command. Four responses were averaged before application of DADLE (*a*) and during the presence of DADLE (*b*). *A*, control. DADLE caused an outward current of about 150 pA (left) and this is associated with an apparent decrease in the inward calcium current. *B*, in TEA (10 mM). The outward current induced by DADLE was only slightly reduced. The inward current induced by the step depolarization is increased by TEA (because of suppression of opposing outward current). During the steady outward current caused by DADLE, the inward calcium current was reduced. *C*, in quinine (1 mM) the outward current induced by DADLE is almost totally abolished (left). There is now almost no effect of DADLE on the inward calcium current which flows during the step depolarization.

recovery from the quinine depression of the calcium-activated outward current was slow (greater than 2 h) and sometimes incomplete.

Barium, at concentrations as low as 30 μM, decreased the opioid hyperpolarization. This concentration of barium increased the frequency of spontaneous action potentials and decreased the amplitude of the after-hyperpolarization following the spike, but had no effect on action potential wave form. Higher concentrations (300 μM–2 mM) increased the input resistance, and also increased the duration and amplitude of the action potential and the after-hyperpolarization which followed the action potential. These higher concentrations of barium also reduced the opioid-activated potassium conductance (or hyperpolarization) in all cells tested. However, the maximum reduction observed was about 50 %. On the other hand, substitution of barium (4 mM) for calcium (rather than simple addition of barium to the control solution) totally blocked the outward current produced by [Met^5]enkephalin (Fig. 6).

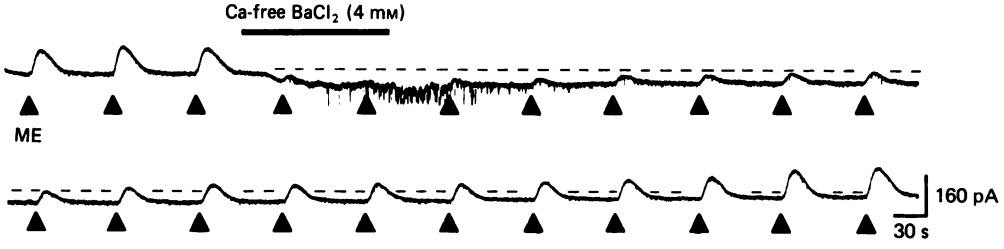

Fig. 6. Barium blocks [Met^5]enkephalin-induced current. [Met^5]enkephalin was applied by pressure ejection (▲). Superfusion with calcium-free solution which contained $BaCl_2$ (4 mM) (bar) produced an inward current and rapidly abolished the [Met^5]enkephalin-induced outward current. Holding potential −60 mV.

Rubidium is known not to permeate certain types of potassium channels (Adrian, 1964; Findlay, 1984). When the recording electrode was filled with RbCl (2 M) and the extracellular potassium was completely replaced with RbCl, there was an increase in the amplitude of the action potential (by 3–10 mV), the duration of the action potential (from 1·5 to 3 ms) and the amplitude of the after-hyperpolarization (by 5–15 mV). The amplitude of the hyperpolarization induced by DADLE (3 μM) was about 50 % of that observed in normal solutions. This effect of rubidium was only observed when a RbCl-filled electrode was used, and not observed when rubidium was simply substituted for extracellular potassium during recordings with a KCl-filled electrode.

Intracellular application of caesium by diffusion from a recording electrode (filled with CsCl (1 M)) resulted in a broadening of the action potential, an increase in apparent input resistance and a decline in slope of the steady-state current–voltage plots (Williams *et al.* 1984). These actions were attributed to a decrease in potassium currents (Williams *et al.* 1984). Following prolonged impalements with a CsCl-filled electrode, the increase in potassium conductance by opiates declined to 10–50 % of its value recorded with KCl-filled electrodes. The increase in potassium conductance caused by opiates was never totally abolished even after recording for more than 3 h.

Tetraethylammonium (TEA, 10–25 mM) had the largest effect of the blockers tested on the duration of the action potential, increasing the duration up to several hundred

milliseconds. The hyperpolarization or current induced by opioids was only slightly decreased by TEA (10 mM) (Fig. 5). Increasing the concentration of TEA to 25 mM decreased the hyperpolarization by up to 50% of control in about one-half of the neurones tested, and was without effect in the remainder.

4-aminopyridine (4-AP) (100 μM–1 mM) greatly reduces or abolishes the transient outward current ($I_{K,A}$) which flows following a step depolarization from voltages more negative than −70 mV (Williams *et al.* 1984). 4-AP had no effect on the outward current produced by opioids, and opioids did not change this transient outward current.

The bee venom apamin is known to inhibit a calcium-dependent potassium permeability of hepatocytes, as well as preventing the α-adrenoceptor-mediated hyperpolarization of smooth muscle (Banks, Brown, Burgess, Burnstock, Claret, Cocks & Jenkinson, 1979). Apamin (up to 1 μM) had no effect on the outward current resulting from superfusion with [Met^5]enkephalin or noradrenaline. The samples of apamin which were tested on l.c. neurones were effective in blocking the relaxation of guinea-pig isolated taenia coli produced by electrical stimulation of intramural nerves (the method used was similar to that described by Banks *et al.* 1979).

Forskolin is a diterpene which activates adenylate cyclase in broken or intact cells (Seamon, Padgett & Daly, 1981). There is evidence that opioid receptors are negatively coupled to adenylate cyclase in various systems (see West & Miller, 1983). If forskolin were able to reverse or prevent the opioid hyperpolarization, this might indicate that a reduction in cyclic adenosine 3′,5-monophosphate was intermediate between receptor occupancy and potassium conductance increase. However, superfusion with forskolin (up to 1 μM) had no effect on the hyperpolarization or outward current caused by [Met^5]enkephalin.

The role of calcium ions. The voltage independence of the potassium conductance and the results of the experiments with quinine described above suggested that the opioid conductance might be one which is sensitive to the intracellular calcium concentration (Burgess, Claret & Jenkinson, 1981; Cherubini, North & Surprenant, 1984). L.c. neurones have a prominent inward calcium current at potentials close to rest, and this holds open a calcium-activated potassium conductance (Williams *et al.* 1984); therefore, it was possible that the entry of calcium was necessary for the opioid conductance increase to occur. Two procedures were used to decrease inward calcium currents: the use of calcium-free solutions, and solutions of normal (2·5 mM) calcium content to which were added $CoCl_2$ (500 μM–2 mM) and $MgCl_2$ (2 mM). Each of these solutions blocked calcium action potentials and synaptic potentials within 5 min (Williams *et al.* 1984; Egan *et al.* 1983); however, these solutions resulted in marked changes in the properties of the neurones during exposures longer than 5 min. Calcium-free solutions caused a membrane depolarization, increased frequency of action potentials, and a blockade of the after-hyperpolarization (Fig. 7). Solutions containing cobalt and high magnesium concentration decreased spontaneous activity, the amplitudes of the action potential and the after-hyperpolarization. During the first 20 min of exposure to these solutions, the hyperpolarization produced by opioids persisted (Fig. 7). With longer exposures (30–60 min), the membrane potential decreased and the action potential amplitude was further depressed. The hyperpolarization induced by opioids was also depressed during this time and was

eventually abolished. The persistence of opioid actions during the first 20 min of perfusion with the solutions described indicates that inward movement of calcium across the plasma membrane is probably not a prerequisite for the opioid hyperpolarization.

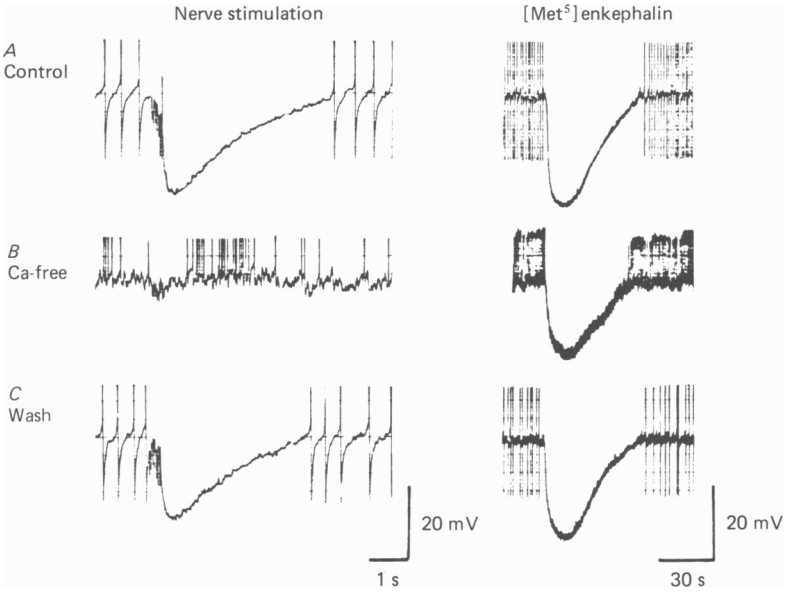

Fig. 7. [Met⁵]enkephalin-induced hyperpolarization persists after blockade of synaptic transmission by calcium-free solution. Left side shows responses of a l.c. neurone to focal electrical stimulation (6 pulses, 20 Hz) to the slice surface; the hyperpolarization is an inhibitory post-synaptic potential mediated by noradrenaline (see Egan *et al.* 1983). Right side shows hyperpolarizations of same neurone evoked by pressure application of [Met⁵]enkephalin (note different time scales). *A*, control. *B*, after 15 min in calcium-free solution. Synaptic potential and action potential after-hyperpolarizations were completely blocked, but opioid response persisted. *C*, wash-out.

The possible role of intracellular calcium was investigated by applying the calcium chelating agent [ethylenebis(oxyethylenenitrilo)]tetraacetic acid (EGTA) inside the cell. Recording electrodes were filled with a mixture of KCl (1 M) and EGTA (1 mM), buffered to pH 7. [Met⁵]enkephalin hyperpolarized neurones soon after impalement, but the amplitude of the hyperpolarizations decreased with time. These experiments might be taken as evidence that the opioid potassium conductance is ultimately dependent on the availability of free calcium within the cell. There is little confidence in this interpretation because the membrane properties changed drastically (depolarization, action potential inactivation) as a result of EGTA leakage, with the same time course as the reduction and eventual loss of the opioid hyperpolarizations.

Opioids decreased calcium entry into l.c. neurones whether this was measured as a decrease in amplitude and duration of the calcium action potential (North & Williams, 1983), or as a decrease in inward current under voltage clamp (Fig. 5). However, the inhibition of calcium entry was always associated with an increase in potassium conductance. Opioids did not reduce calcium currents after the increase in potassium conductance was largely blocked by intracellular caesium, quinine

(Fig. 5) or extracellular barium. When barium (4 mM) was substituted for calcium (2·5 mM), a cobalt-sensitive inward current could still be recorded when the membrane potential was stepped from −60 to −40 mV. This current (I_{Ba}) was unaffected by opioids in concentrations which caused a maximal outward current before addition of barium.

Comparison of opioid currents with those caused by α_2-adrenoceptor agonists

Effects of α_2-adrenoceptor agonists. α_2-adrenoceptor agonists increase a potassium conductance in l.c. neurones (Williams *et al.* 1985). This conductance has a voltage dependence similar to the opioid conductance, being essentially voltage-independent between −110 and −50 mV, but decreasing in amplitude with further depolarization. It has been reported previously (Williams *et al.* 1985) that the current activated by α_2-adrenoceptor agonists is also depressed by barium; in the present study we found that noradrenaline currents were reduced by intra- and extracellular rubidium, and blocked by quinine. Outward currents induced by either noradrenaline or [Met^5]enkephalin were equally sensitive to these blocking drugs.

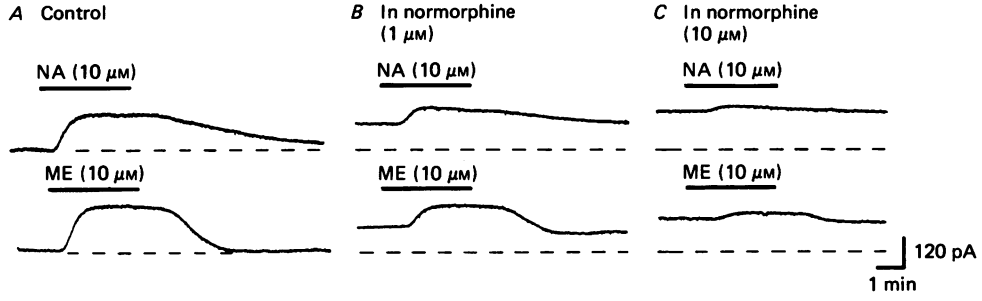

Fig. 8. Interactions between noradrenaline and normorphine (top traces) and between [Met^5]enkephalin and normorphine (bottom traces). Current recordings from a neurone voltage clamped at −60 mV. This experiment was carried out in the presence of desmethylimipramine (1 μM) to prevent noradrenaline uptake (see Egan *et al.* 1983). *A*, control. Currents induced by noradrenaline (NA; 10 μM) and [Met^5]enkephalin (ME; 10 μM). *B*, normorphine (1 μM) was superfused for 5 min prior to the record shown; this caused a steady outward current. The superfusing solution was then changed to one which contained both normorphine and noradrenaline (top trace) or normorphine and [Met^5]enkephalin (bottom trace). *C*, a higher concentration of normorphine (10 μM) caused a larger steady outward current, and further reduced the effect of noradrenaline or [Met^5]enkephalin. The outward current caused by noradrenaline or [Met^5]enkephalin alone (*A*) was not different from the total currents when these agonists were applied in the presence of high normorphine concentrations.

Interaction between opioids and α_2-adrenoceptor agonists. The similarities between the effects of the α_2-agonists and the opioids suggested that both may increase the same ion conductance. In this case, one would expect the maximum conductance change produced by agonists at the two receptors to be the same, and any combination of agonists should produce currents which never exceed this maximum. Therefore, a series of experiments were conducted in which membrane currents were measured at a holding potential of −60 mV and various agonists were applied by superfusion. In some experiments, normorphine was applied continuously for

10–20 min, during which time the outward current remained steady; for 2 min during this period the solution was changed to one which contained both normorphine and either [Met⁵]enkephalin or noradrenaline, and the additional outward current measured after it reached its steady state (Fig. 8). The current induced by [Met⁵]enkephalin or noradrenaline in the presence of normorphine was smaller than

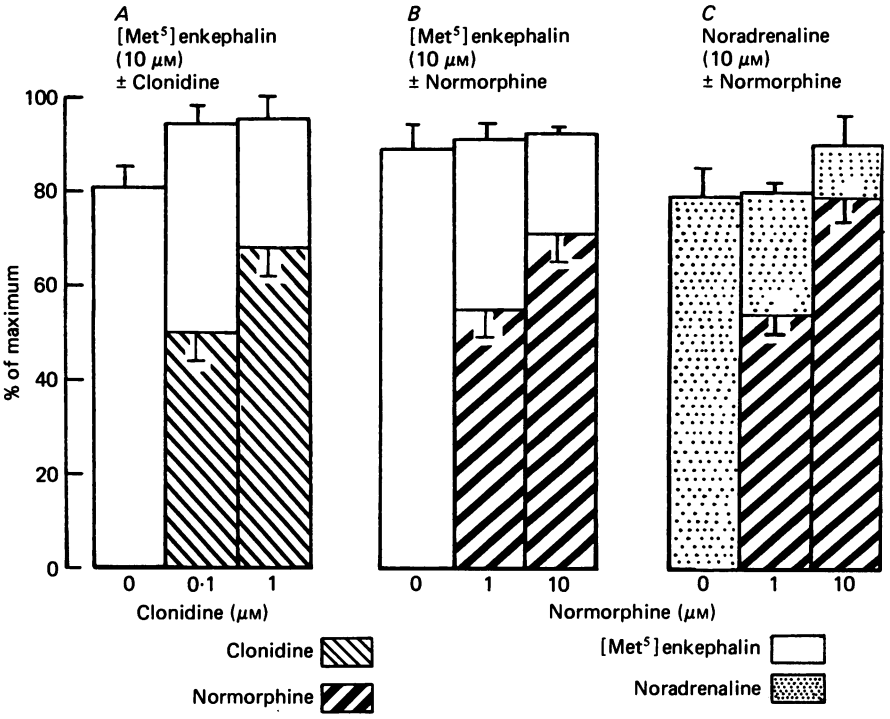

Fig. 9. Interaction of the outward currents induced by opioids with those induced by α_2-adrenoceptor agonists. In each neurone, the maximum current observed in response to the highest concentration of any agonist (either alone or in combination) was ascribed the value 100%. This current was 279 ± 28 pA ($n = 13$). The ordinate represents the current induced by the various agonists alone or in combination relative to this value, *for that particular neurone*. *A*, interaction between [Met⁵]enkephalin and clonidine. Outward current caused by clonidine is shown in hatched columns; that caused by [Met⁵]enkephalin is shown by open columns. Increasing the clonidine concentration was accompanied by a decrease in the [Met⁵]enkephalin current, but the total cell current did not increase. *B*, similar experiments in which [Met⁵]enkephalin was added alone or during superfusion with two concentrations or normorphine. *C*, noradrenaline was superfused either alone or during superfusion with two concentrations of normorphine. When a maximum outward current is caused by α_2-adrenoceptor agonists, occupancy of μ-receptors will cause no further outward current, and vice versa.

that caused by the same concentration in the absence of normorphine (Figs. 8 and 9). The converse experiments were also carried out, in which the current caused by a brief application of [Met⁵]enkephalin was compared before and during a more prolonged application of clonidine; the response to [Met⁵]enkephalin was depressed in amplitude in the presence of clonidine. Higher concentrations of clonidine, which caused larger steady outward currents, caused greater depression of [Met⁵]enkephalin

current relative to its control value. The maximum current induced by the simultaneous application of any combination of opiate or α_2-adrenoceptor agonist was 279 ± 28 pA ($n = 13$), which was not different from that observed with application of the highest concentrations of [Met^5]enkephalin alone (317 ± 15 pA, $n = 17$, $P < 0.001$) (Fig. 9).

DISCUSSION

The type of potassium conductance affected

Two main sets of observations provide information regarding the type of potassium conductance increased by opioids and α_2-adrenoceptor agonists. The first is the voltage sensitivity of the conductance, and the second is the effects of various drugs. The opioid conductance change was essentially independent of membrane voltage in the range of -60 to -130 mV. The reduction in opioid conductance which occurred with depolarization to potentials less negative than -50 mV may come about from a genuine voltage sensitivity of the conductance, or because there is already a large outward current at such potentials (Williams *et al.* 1984). One such outward current is dependent on the calcium entry which occurs at voltages less than -60 mV. The calcium-activated outward current causes an upward bend in the steady-state current–voltage plot, which is reduced by agents which block calcium entry (Williams *et al.* 1984). At the same potentials (-45 to -30 mV) at which this outward current occurred, the opioid-induced current declined (Fig. 3). This might indicate that the opioid-activated current and the potassium current activated by depolarization into this voltage range are one and the same; on the other hand, the opioid-induced current may be less simply because potassium ion accumulation reduces the driving force, or even because the large conductance increase affects the space clamp and restricts the detection of any component of the opioid-induced current which is generated at a distance from the recording site. The opioid-sensitive conductance in l.c. neurones is remarkably similar in its voltage dependence to the potassium conductance increase activated in myenteric plexus and nodose ganglion cells by the calcium entry during a burst of action potentials (Morita, North & Tokimasa, 1982; Higashi, Morita & North, 1984).

The outward tail current which followed either a burst of action potentials or a depolarizing step command, which in both cases requires extracellular calcium, was reduced in amplitude by opioids; this action occurred only in concentrations which already resulted in a steady outward current. The magnitude of the decrease in this outward tail current was dependent on the concentration of normorphine applied, but as the concentration increased so did the steady-state opioid current. Quinine or barium reduced or blocked the outward tail current in the same concentrations in which they reduced the steady-state opioid current. When these results are taken together, one might conclude that opioids increase a potassium conductance which can also be increased by calcium entering the neurone as a result of membrane depolarization.

The second set of findings which help to define the potassium conductance comes from experiments with channel blockers of more or less selectivity. Quinine prevents the increase of potassium conductance which results from a rise in intracellular

calcium (Burgess *et al.* 1981; Hanani & Shaw, 1977). Barium is well known to block potassium conductances but the concentrations are usually in the millimolar range. The present finding that barium at 100 μM and quinine at 100 μM significantly reduce the opioid-induced current, without prolonging the action potential, is paralleled by effects of these substances on the prolonged potassium conductance increase following one or a brief burst of action potentials in myenteric neurones (North & Tokimasa, 1984; Cherubini *et al.* 1984). Apamin, however, does not reduce the prolonged hyperpolarization which follows the myenteric neurone action potential (E. Cherubini, S. Mihara & R. A. North, unpublished observations); nor did it block opioid hyperpolarizations in l.c. neurones. Thus, there are several similarities between the opioid conductance increase in l.c. neurones and the potassium conductance increase which follows a burst of action potentials in myenteric neurones (voltage independence, barium and quinine sensitivity, apamin insensitivity), but there is one important difference. Forskolin had no effect on the opioid conductance increase, although it does block the prolonged action potential after-hyperpolarization in myenteric neurones, even at concentrations of 20–100 nM (A. Surprenant & R. A. North, unpublished observations; see also Nemeth, Zafirov & Wood, 1984). The lack of sensitivity to apamin and the sensitivity to quinine are reminiscent of the calcium-dependent potassium permeability of red blood cells (Banks *et al.* 1979; Burgess *et al.* 1981).

The insensitivity to TEA of the opioid current indicates that it is likely to be different from the calcium-activated potassium conductance ascribed to channels of large unit conductance ('maxi' channels; LaTorre & Miller, 1983). Those channels are blocked by low concentrations (1–5 mM) of extracellularly applied TEA (Adams, Constanti, Brown & Clark, 1982; LaTorre & Miller, 1983). In the present study, concentrations of TEA (10 mM) which prolonged the action potential did not affect opioid currents, implying that the 'maxi' channel is not involved. Two further reasons why the 'maxi' channel is unlikely to underlie the opioid current are the strong voltage sensitivity of that channel wherever it has been observed (Marty, 1983; LaTorre & Miller, 1983), and the observation that opioid currents persist, although of reduced amplitude, after substitution of intracellular and extracellular rubidium for potassium (see Petersen & Maruyama, 1984).

The similarities between opioid and α_2-adrenoceptor actions

No differences were observed between opioid currents and α_2-adrenoceptor currents in respect of voltage dependence or sensitivity to any of the blockers. Both groups of agonists caused the same peak conductance increase; and when the conductance was maximally increased by an agonist of one group, agonists of the second group had no effect. Interaction between the agonists at the receptor level is extremely unlikely, because it has been shown that antagonist molecules discriminate between the two receptors with high resolution (Williams *et al.* 1985; Williams & North, 1984). These results therefore imply that separate cell surface receptors control the same potassium ion conductance.

Physiological significance of the results

The firing of l.c. neurones is particularly sensitive to inhibition by exogenous opioids and α_2-agonists when it is recorded *in vivo* (Aghajanian & van der Maalen, 1982; Bird & Kuhar, 1977; Cedarbaum & Aghajanian, 1977; Korf, Bunney & Aghajanian, 1974). Both spontaneous firing and the excitations evoked by noxious stimuli to the skin of the animal are strongly depressed. The present results allow one to suggest the underlying reasons for this. In the first place, there is a persistent inward calcium current in the potential range near threshold for action potential generation (−55 mV); this current is responsible for the ongoing spontaneous activity recorded in l.c. neurones in the slice preparation (Williams *et al.* 1984). The current is inactivated at potentials more negative than about −70 mV, but increases progressively with depolarization. Any outward current induced by opioids not only hyperpolarizes the membrane directly, but the hyperpolarization moves the membrane to a potential at which the inward calcium current is decreased, thus adding to the hyperpolarization.

The second factor which will contribute to the inhibition of firing of the l.c. neurones is the modification of synaptic input. When the cell is hyperpolarized beyond −65 mV, sudden depolarization will activate a transient outward current ($I_{K,A}$) (Williams *et al.* 1984). Noxious stimuli to the skin would be expected to evoke excitatory post-synaptic potentials (e.p.s.p.s) in the l.c. neurones. However, the transient current ($I_{K,A}$) activates within 5 ms and inactivates with a time constant of 130 ms; therefore it directly opposes inward synaptic currents. As a result of this, the e.p.s.p. amplitude does not increase with membrane hyperpolarization, and its duration is reduced (see Fig. 1 of Egan *et al.* 1983). The hyperpolarizations induced by opioids or α_2-adrenoceptor agonists will not only reduce the membrane excitability by bringing the membrane potential further from the threshold for action potential generation, but also reduce the effectiveness of excitatory synaptic input by moving the membrane into the potential range of $I_{K,A}$. Such an inhibitory effect of $I_{K,A}$ on synaptic potentials was analysed by Daut (1973).

Opioids of the dynorphin type can directly reduce calcium conductance (Macdonald & Werz, 1983, 1984; Cherubini & North, 1985); however, in the rat l.c. the only effect of the κ-agonists so far observed is a potassium conductance increase which could be ascribed to an action on μ-receptors (Williams & North, 1984). However, the present results and earlier studies (North & Williams, 1983; Werz & Macdonald, 1983), show that the potassium conductance increase produced by the opioids will have additional effects on cell function by virtue of the reduction in calcium entry which it secondarily brings about. This might be expected to have manifold effects on cell metabolism, ion conductances and transmitter release.

This work was supported by U.S. Department of Health and Human Services grants AM32979, DA03160 and DA03161.

REFERENCES

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

ADRIAN, R. H. (1964). The rubidium and potassium permeability of frog muscle membrane. *Journal of Physiology* **175**, 134–159.

AGHAJANIAN, G. K. & VAN DER MAALEN, C. P. (1982). α_2-adrenoceptor mediated hyperpolarization of locus coeruleus neurons: intracellular studies *in vivo*. *Science* **215**, 1394–1396.

BANKS, B. E. C., BROWN, C., BURGESS, G. M., BURNSTOCK, G., CLARET, M., COCKS, T. M. & JENKINSON, D. H. (1979). Apamin blocks certain neurotransmitter-induced increases in potassium permeability. *Nature* **282**, 415–417.

BIRD, S. J. & KUHAR, M. J. (1977). Iontophoretic application of opiates to the locus coeruleus. *Brain Research* **122**, 523–533.

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. *Journal of Physiology* **317**, 67–90.

CEDARBAUM, J. M. & AGHAJANIAN, G. K. (1977). Catecholamine receptors of locus coeruleus neurones: pharmacological characterization. *European Journal of Pharmacology* **44**, 375–385.

CHERUBINI, E. & NORTH, R. A. (1985). μ and κ opioids inhibit transmitter release by different mechanisms. *Proceedings of the National Academy of Sciences of the U.S.A.* (in the Press).

CHERUBINI, E., NORTH, R. A. & SURPRENANT, A. (1984). Quinine blocks a calcium activated potassium conductance in mammalian enteric neurones. *British Journal of Pharmacology* **83**, 3–6.

DAUT, J. (1973). Modulation of the excitatory synaptic response by fast transient K current in snail neurones. *Nature* **246**, 193–196.

DUGGAN, A. W. & NORTH, R. A. (1983). Electrophysiology of opioids. *Pharmacological Reviews* **35**, 219–281.

EGAN, T. M., HENDERSON, G., NORTH, R. A. & WILLIAMS, J. T. (1983). Noradrenaline mediated synaptic inhibition in locus coeruleus neurones. *Journal of Physiology* **345**, 477–488.

FINDLAY, I. (1984). A patch-clamp study of potassium channels and whole cell currents of the mouse lacrimal gland. *Journal of Physiology* **350**, 179–196.

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

HIGASHI, H., MORITA, K. & NORTH, R. A. (1984). Calcium-dependent after-potentials in visceral afferent neurones of the rabbit. *Journal of Physiology* **355**, 479–492.

KORF, J., BUNNEY, B. S. & AGHAJANIAN, G. K. (1974). Noradrenergic neurons: morphine inhibition of spontaneous activity. *European Journal of Pharmacology* **25**, 165–167.

LATORRE, R. & MILLER, C. (1983). Conduction and selectivity in potassium channels. *Journal of Membrane Biology* **71**, 11–30.

MACDONALD, R. L. & WERZ, M. A. (1983). Dynorphin decreases calcium conductance of mouse cultured dorsal root ganglion neurons. *Neuroscience Abstracts* **9**, 1129.

MACDONALD, R. L. & WERZ, M. A. (1984). Dynorphin reduces voltage-dependent calcium conductance of sensory neurones: a voltage clamp study. *Neuroscience Abstracts* **110**, 1108.

MARTY, A. (1983). Ca-dependent K channels with large unitary conductance. *Trends in Neuroscience* **6**, 262–265.

MORITA, K. & NORTH, R. A. (1981*a*). Opiate activation of potassium conductance of myenteric neurons: inhibition by calcium ions. *Brain Research* **242**, 145–150.

MORITA, K. & NORTH, R. A. (1981*b*). Clonidine activates potassium conductance in myenteric neurones. *British Journal of Pharmacology* **74**, 419–428.

MORITA, K., NORTH, R. A. & TOKIMASA, T. (1982). The calcium-activated potassium conductance in guinea-pig myenteric neurones. *Journal of Physiology* **329**, 341–354.

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

NORTH, R. A. & SURPRENANT, A. (1985). Inhibitory synaptic potentials resulting from α_2-adrenoceptor activation in guinea-pig submucous plexus neurones. *Journal of Physiology* **358**, 17–33.

NORTH, R. A. & TOKIMASA, T. (1984). The time course of muscarinic depolarization of guinea-pig myenteric neurones. *British Journal of Pharmacology* **82**, 85–91.

NORTH, R. A. & WILLIAMS, J. T. (1983). Opiate activation of potassium conductance inhibits calcium action potentials in rat locus coeruleus neurones. *British Journal of Pharmacology* **80**, 225–228.

NORTH, R. A. & YOSHIMURA, M. (1984). The actions of noradrenaline on neurones of the rat substantia gelatinosa *in vitro*. *Journal of Physiology* **349**, 43–55.

PEPPER, C. & HENDERSON, G. (1980). Opiates and opioid peptides hyperpolarize locus coeruleus neurones *in vitro*. *Science* **209**, 394–396.

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

SEAMON, K. B., PADGETT, W. & DALY, J. W. (1981). Forskolin: unique diterpene activator of adenylate cyclase in neuroblastoma glioma hybrid cells. *Proceedings of the National Academy of Sciences of the U.S.A.* **79**, 690–694.

WERZ, M. A. & MACDONALD, R. L. (1983). Opioid peptides selective for mu- and delta-opiate receptors reduce calcium-dependent action potential duration by increasing potassium conductance. *Neuroscience Letters* **42**, 173–178.

WEST, R. E. & MILLER, R. J. (1983). Opiates, second messengers and cell response. *British Medical Bulletin* **39**, 53–58.

WILLIAMS, J. T., EGAN, T. M. & NORTH, R. A. (1982). Enkephalin opens potassium channels in mammalian central neurones. *Nature* **299**, 74–76.

WILLIAMS, J. T., HENDERSON, G. & NORTH, R. A. (1985). Characterization of α_2-adrenoceptors which increase potassium conductance in rat locus coeruleus neurones. *Neuroscience* **14**, 95–102.

WILLIAMS, J. T. & NORTH, R. A. (1984). Opiate-receptor interactions on single locus coeruleus neurones. *Molecular Pharmacology* **26**, 489–497.

WILLIAMS, J. T., NORTH, R. A., SHEFNER, S. A., NISHI, S. & EGAN, T. M. (1984). Membrane properties of rat locus coeruleus neurones. *Neuroscience* **13**, 137–156.

YOSHIMURA, M. & NORTH, R. A. (1983). Substantia gelatinosa neurones *in vitro* hyperpolarized by enkephalin. *Nature* **305**, 529–530.