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FINE TUNING OF NEURONAL ELECTRICAL ACTIVITY: MODULATION OF SEVERAL ION CHANNELS BY INTRACELLULAR MESSENGERS IN A SINGLE IDENTIFIED NERVE CELL

BY DAVID P. LOTSHAW, EDWIN S. LEVITAN AND IRWIN B. LEVITAN

Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254, USA

SUMMARY

The identified neurone R15 in the abdominal ganglion of the marine mollusc, *Aplysia californica*, exhibits a rhythmic bursting pattern of electrical activity. This pattern, which is generated endogenously by the interaction of several voltage- and time-dependent ion currents in R15's membrane, is subject to long-term modulation by synaptic stimulation and application of several neurotransmitters. At micromolar concentrations the transmitter serotonin causes neurone R15 to hyperpolarize, as a result of the activation of an anomalously rectifying potassium conductance. Furthermore under some conditions serotonin can excite R15, as a result of the activation of a voltage-dependent calcium current. Both of these effects of serotonin are mediated by the intracellular second messenger cyclic AMP. In addition, serotonin can modulate a chloride current by a cyclic-AMP-dependent mechanism. In contrast to the *activation* of the voltage-dependent calcium current by serotonin/cyclic AMP, a cyclic GMP analogue alters the bursting pattern by *inhibiting* this current. The results indicate that a single neurotransmitter, acting *via* a single intracellular messenger, can modulate several classes of ion channels in a single nerve cell. Furthermore a single class of ion channel, that is responsible for a voltage-dependent calcium current, may be the target for modulation by at least two different intracellular messengers. These findings emphasize the intricacy of the regulatory pathways which contribute to fine tuning of neuronal electrical activity.

INTRODUCTION

Long ago in a faraway land, Levitan, Harmar & Adams (1979) contributed a chapter to an earlier volume in this series, in which they described preliminary attempts to elucidate molecular mechanisms involved in the modulation of the activity of an identified nerve cell. This cell, neurone R15 in the abdominal ganglion of *Aplysia*, has fascinated electrophysiologists for many years because of its endogenous rhythmic pattern of electrical activity (Strumwasser, 1965; Frazier *et al.* 1967). When R15 is observed in the isolated abdominal ganglion in a Petri dish, its membrane potential cycles through alternating periods of action potential 'bursts' and interburst hyperpolarizations (e.g. Figs 1A, 9A). The ionic mechanism of this

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rhythmicity has been widely studied, and has been the subject of considerable controversy (Kramer & Zucker, 1985*a*,*b*; Adams, 1985; Adams & Levitan, 1985; for a comprehensive review see Adams & Benson, 1985).

But R15 is more than just another pretty endogenous oscillator – this endogenous activity is subject to modulation, both short-term and long-term, by a variety of hormones (Mayeri *et al.* 1985), neurotransmitters (Ascher, 1972; Drummond, Benson & Levitan, 1980) and synaptic stimuli (Parnas & Strumwasser, 1974; Tremblay, Woodson, Schlapfer & Barondes, 1976; Adams, Parnas & Levitan, 1980). The search for molecular mechanisms of these modulatory phenomena in our laboratory over the last 10 years has produced some answers but many more new questions. This chapter will review briefly the well-established answers, and present some recent findings which illustrate the exquisite complexity of the regulation of R15's activity.

SEROTONIN ACTIVATES AN ANOMALOUSLY RECTIFYING POTASSIUM CURRENT BY A CYCLIC-AMP-DEPENDENT MECHANISM

The second-messenger-mediated modulatory effect which we have studied most thoroughly and understand best is the activation of a specific potassium conductance by serotonin. When serotonin is applied to the medium bathing an abdominal ganglion, the interburst hyperpolarization is enhanced (see for example Fig. 1B), and R15 hyperpolarizes and eventually stops bursting (Drummond *et al.* 1980). Furthermore, this effect is mimicked by the bath application or intracellular injection of cyclic AMP analogues (Treistman & Levitan, 1976*a*; Drummond *et al.* 1980). In addition, activation of adenylate cyclase within R15, by the intracellular injection of the GTP analogue guanylylimidodiphosphate, causes R15 to hyperpolarize (Treistman & Levitan, 1976*b*), and the effect of serotonin is inhibited by intracellular injection of the GDP analogue GDPβS (Lemos & Levitan, 1984), a blocker of adenylate cyclase. An extensive series of biochemical, pharmacological and electrophysiological experiments of this type (summarized in Levitan & Benson, 1981) have led to the conclusion that the hyperpolarization produced by serotonin is mediated by cyclic AMP.

We have studied the ionic mechanism underlying the hyperpolarization of R15 by serotonin and cyclic AMP using voltage clamp techniques. The membrane potential is swept between -120 and -40 mV at a rate of $4\,\text{mV}\,\text{s}^{-1}$, and the total membrane current is measured. This rate of change of the voltage is slow compared to the time constant of the cell's membrane but rapid compared to the response to serotonin. Alternatively, the voltage can be pulsed to hyperpolarized potentials from a more positive holding potential, and the steady-state current measured after 2 s or more. In both cases a plot of the membrane potential *versus* the total membrane current yields a steady-state current–voltage (I–V) relationship, the slope of which is the conductance of the cell membrane (Fig. 2). Note that in this neurone there is an inwardly or anomalously rectifying region in the hyperpolarized range of membrane potential, and a negative resistance region between about -60 and -40 mV.

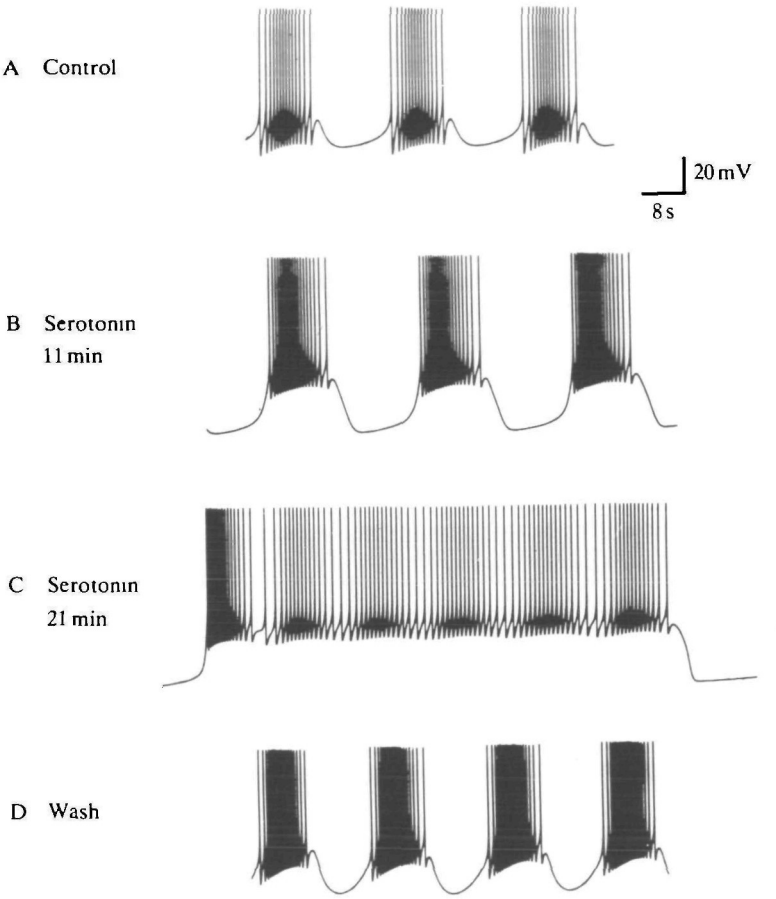

Fig. 1. Effect of 50 μmol l^{-1} serotonin on bursting activity in R15. (A) Control. (B),(C) Serotonin first enhances the interburst hyperpolarization (B), as it does at lower concentration, then increases the number of action potentials per burst (C). (D) Wash.

If the abdominal ganglion is perfused with 1–10 μmol l^{-1} serotonin, there is an increase in conductance which reverses direction (compared to the resting conductance) at around −80 mV (Fig. 2), which is the potassium equilibrium potential (E_K) in this cell (Benson & Levitan, 1983). This reversal at E_K suggests that serotonin increases a potassium conductance. To test this further the external potassium concentration was changed and the cell's response to serotonin was examined. The results (Benson & Levitan, 1983) indicate that the current elicited by serotonin reverses at the theoretical E_K for each external potassium concentration tested. This is not true for any other external ion. Furthermore, although the serotonin-evoked current is not blocked by some potassium channel blockers, such as tetraethylammonium, it is blocked by caesium, rubidium and barium (Benson & Levitan, 1983). These pharmacological findings, together with the pronounced inward rectification, indicate that the serotonin-evoked current is an anomalously rectifying potassium conductance.

THE SEROTONIN RESPONSE IS MEDIATED BY CYCLIC-AMP-DEPENDENT PROTEIN PHOSPHORYLATION

Protein kinase inhibitor (PKI) is a 10 000 Da protein which binds with high affinity to the active catalytic subunit of cyclic-AMP-dependent protein kinase and inhibits its activity (Walsh *et al.* 1971). We have found that PKI purified to homogeneity from rabbit skeletal muscle is a potent inhibitor of cyclic-AMP-dependent protein kinase from *Aplysia* (Adams & Levitan, 1982). When PKI is pressure-injected, *via* a microelectrode, directly into neurone R15, the increase in potassium conductance normally elicited by serotonin (Fig. 2) is completely blocked (Adams & Levitan, 1982). Furthermore, the cell's response to cyclic AMP analogues is also blocked by PKI (J. R. Lemos & W. B. Adams, unpublished observations). The blocking of the neurotransmitter effect appears to be produced specifically by PKI, and is selective for the serotonin-induced, cyclic-AMP-mediated increase in potassium conductance (Adams & Levitan, 1982). Therefore cyclic-AMP-dependent protein phosphorylation is a necessary step in the regulation of electrical activity in neurone R15.

IDENTIFICATION OF PHYSIOLOGICALLY RELEVANT PHOSPHOPROTEINS

Having implicated protein phosphorylation in the control of potassium conductance in R15, it is important to identify the phosphoproteins which may be involved in this regulation. Previous attempts to measure protein phosphorylation in individual nerve cells have involved incubating ganglia with ^{32}P-labelled inorganic phosphate, followed by isolation of individual nerve cell somata and analysis of radioactive phosphoproteins (Levitan, Madsen & Barondes, 1974; Paris, Castellucci, Kandel & Schwartz, 1981; Jennings *et al.* 1982; Neary & Alkon, 1983). Although

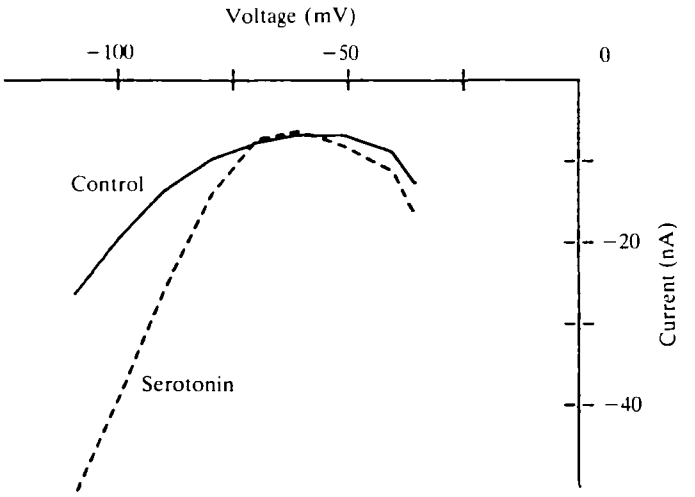

Fig. 2. Effect of 5 μmol l^{-1} serotonin on steady-state subthreshold currents in R15. Currents were evoked with 2-s pulses from a holding potential of −45 mV. Dotted line shows currents after 20 min exposure to serotonin, solid line shows control.

this approach has provided useful information, it suffers from several disadvantages. First, the cell body can never be isolated totally free of glia and portions of neighbouring cell bodies, which will contribute to the labelling pattern, and secondly, the neuropile processes of the cell, which are the sites at which most or perhaps all synaptic contacts occur (Frazier *et al.* 1967), are not sampled by this procedure. To circumvent these problems we have developed methods to inject [γ-^{32}P]ATP directly into R15 (Lemos, Novak-Hofer & Levitan, 1982) in amounts sufficient to label phosphoproteins. It is important to remember that the physiological properties of the cell are monitored with intracellular microelectrodes throughout the labelling period, so changes in the phosphoprotein labelling pattern may be related to changes in membrane conductance.

We have found that, in a control R15 perfused with normal *Aplysia* medium for 50 min after injection with [γ-^{32}P]ATP, more than 70 phosphoproteins can be detected (Lemos, Novak-Hofer & Levitan, 1984, 1985). It is important to note that most of these are not major proteins in neurone R15 (or the abdominal ganglion) and thus this phosphorylation pattern does not merely reflect labelling of major substrates in the cell. Concomitant with the potassium conductance increase, serotonin alters the amount of ^{32}P incorporated into at least a dozen proteins in neurone R15 (Lemos *et al.* 1984, 1985). The phosphorylation of several of these appears to be very closely correlated with the activation of the anomalously rectifying potassium conductance by serotonin/cyclic AMP (Lemos *et al.* 1985), and thus they may be potassium channel regulatory components. However these experiments were carried out before we became aware that serotonin can also modulate several other ion channels in neurone R15 (see below), and it will be necessary to re-evaluate the phosphorylation data in the light of these more recent findings.

MULTIPLE RESPONSES TO SEROTONIN IN A SINGLE NERVE CELL

We have developed techniques for maintaining isolated *Aplysia* neurones in cell culture (Dagan & Levitan, 1981), and have succeeded in inducing neurone R15 to survive and extend neurites in culture. Among the advantages of this approach is the possibility of studying the properties of the cell free of synaptic interference. However, cultured R15s rarely exhibit vigorous bursting, and thus we have chosen to use both the culture and *in situ* systems to investigate modulation of R15's activity.

Both *in situ* in the abdominal ganglion and in culture, R15 responds similarly to stimulation by neurotransmitters. In culture the resting cell exhibits a biphasic response to serotonin consisting of an initial depolarization and increased spiking, followed by prolonged hyperpolarization and silence. *In situ* the depolarization is also observed, but only when higher serotonin concentrations are employed (Fig. 1C). It has previously been demonstrated that 5 μmol l^{-1} serotonin can hyperpolarize and silence R15 (Drummond *et al.* 1980) whereas 500 μmol l^{-1} serotonin can depolarize R15 (Tremblay *et al.* 1976). We have recently found that 20–50 μmol l^{-1} serotonin first enhances the interburst interval (Fig. 1B), and then later

enhances bursting *in situ* (Fig. 1C). Often these concentrations convert R15's bursting activity to constant spiking or beating activity.

We have reported previously that cultured R15s exhibit the anomalously rectifying potassium current, and that serotonin and forskolin, an activator of adenylate cyclase, increase the conductance of this current (Lotshaw & Levitan, 1985) as they do *in situ*. However it is now apparent that serotonin and forskolin modify other ionic currents as well, both in culture and *in situ*. These currents include a hyperpolarization-activated chloride current previously described in *Aplysia* neurones (Chesnoy-Marchais, 1983) and a voltage-dependent calcium current.

SEROTONIN AND FORSKOLIN MODULATE A CHLORIDE CURRENT

The hyperpolarization-activated chloride current is observed in cultured R15 neurones only when chloride-containing electrodes are employed for voltage clamping. This is consistent with the results of Chesnoy-Marchais (1983) that indicate this current is regulated by the intracellular chloride concentration. This current has not been described previously for R15, possibly because of the large size of R15 in mature animals (200–300 μm in diameter, as compared with 70–150 μm for our cultured R15s). In the larger cells it may be difficult to raise intracellular chloride concentration sufficiently to generate a discernible current. This chloride current appears as a slowly increasing inward current in response to hyperpolarizing voltage-clamp pulses as shown in Fig. 3A. The chloride current can be separated from the anomalously rectifying potassium current kinetically and by ion substitutions or addition of pharmacological blockers. The anomalously rectifying potassium current exhibits instantaneous kinetics in R15 and can be measured as the current which immediately follows the capacitive transient during a hyperpolarizing voltage pulse (Fig. 3A). The chloride current can be measured as the additional, time-dependent component. The current response to a hyperpolarizing pulse in the absence of the chloride current is shown in Fig. 3B. This is the same cell in voltage clamp using potassium acetate electrodes in place of potassium chloride electrodes, after allowing the cell to recover from the chloride load. Note that the instantaneous current remains, but that the time-dependent component has disappeared.

Serotonin treatment reduces the magnitude of the chloride current, from 8·25 to 6 nA in this cell (Fig. 3A). The chloride current returns to control levels within 15 min following removal of serotonin. A similar effect is seen in response to forskolin treatment. This combination of serotonin effects on the chloride current and the anomalously rectifying potassium current modify the steady-state, current–voltage relationship as shown in Fig. 4A. Steady-state I–V curves were obtained by applying voltage ramps at a rate of $4\,mV\,s^{-1}$. The decreased conductance of the chloride current in response to serotonin shifts the intersection of the control and serotonin I–V curves to the left of the potassium equilibrium potential, E_K (compare with Fig. 2). In the absence of this chloride current (using acetate electrodes) the same cell shows the intersection of the control and serotonin I–V curves farther to the right, near E_K (Fig. 4B). Intersection of the control and serotonin I–V curves at E_K

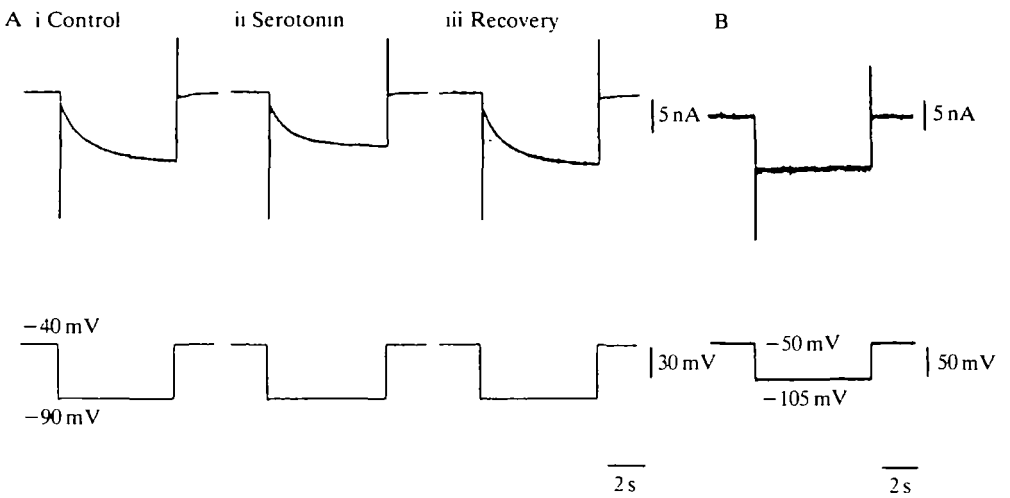

Fig. 3. Effect of serotonin on the hyperpolarization-activated chloride current in a voltage-clamped R15 in cell culture. (A) With KCl electrodes, a slowly increasing inward current is seen in response to a voltage pulse from $V_H = -40$ mV to -90 mV. Serotonin decreases this time-dependent current, and the serotonin effect reverses upon washing. There is little or no effect of serotonin on the instantaneous current because the pulse potential (-90 mV) is very close to E_K. (B) Slowly increasing chloride current is absent when the same cell is allowed to recover and is voltage clamped using potassium acetate electrodes. Only the instantaneous, anomalously rectifying potassium current is apparent. Voltage pulse to -105 mV from $V_H = -50$ mV. V_H, holding potential; E_K, potassium equilibrium potential.

is expected if the anomalously rectifying potassium current is the only serotonin-modulated current active in that voltage range.

At present the function of the rectifying chloride current and its modulation by serotonin can only be speculated upon. Presumably, this current functions to maintain the intracellular chloride concentration below certain limits. If the serotonin-induced increase in the anomalously rectifying potassium current functions primarily to inhibit cell activity, then simultaneously decreasing the chloride current would effectively increase the length constant of the cell and inhibit distant regions of the cell.

SEROTONIN AND FORSKOLIN MODULATE A VOLTAGE-DEPENDENT INWARD CURRENT

The excitatory response to serotonin seen in neurone R15 has not been characterized in detail for the neurone *in situ*, although high concentrations of serotonin have been reported to induce depolarization in R15 (Tremblay *et al.* 1976), as have cyclic AMP analogues and phosphodiesterase inhibitors (Treistman & Levitan, 1976*a*). In addition, the phosphodiesterase inhibitor isobutylmethylxanthine was reported to cause a sodium-dependent conductance increase of the negative slope resistance region of the steady-state I–V curve (Drake & Treistman, 1981). A similar voltage-dependent increase in sodium conductance in response to cyclic AMP

injection has also been reported (Connor & Hockberger, 1984) in several *Aplysia* neurones including R15.

We have found that the excitatory effect of serotonin (Fig. 1C) is manifested in the steady-state I–V curve as a voltage-dependent increase in net inward current, over the negative slope resistance range of membrane voltage. This effect can be seen *in situ* to a limited extent at 5 μmol l^{-1} serotonin (Fig. 2), and is more pronounced at 20–50 μmol l^{-1} (Fig. 5). In the cultured cell, 1 μmol l^{-1} serotonin is sufficient to evoke a large increase in inward current (Fig. 6). In the *in situ* experiment described here the high concentration of serotonin elicits increases in both the anomalously rectifying potassium current and the voltage-dependent inward current (Fig. 5). In

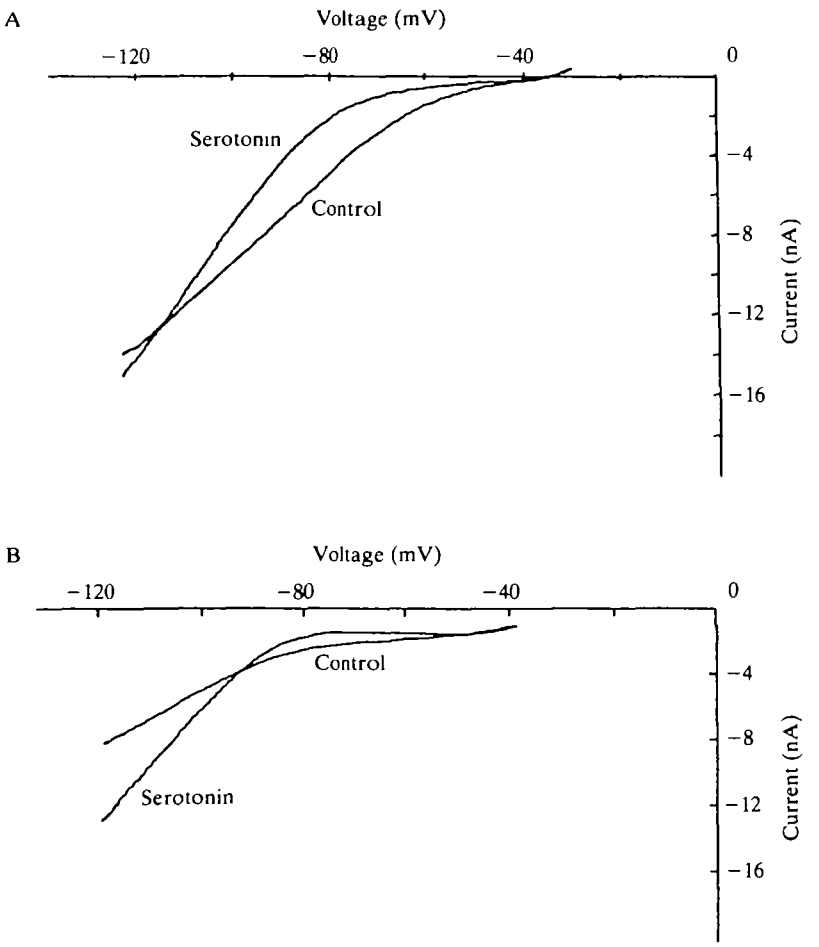

Fig. 4. Effects of 1 μmol l^{-1} serotonin on the steady-state, current–voltage relationship of a cultured R15 in the presence of the hyperpolarization-activated chloride current (A) and in the absence of detectable hyperpolarization-activated chloride current (B). Current–voltage curves were obtained by applying a voltage ramp (4 mV s^{-1}) under voltage clamp. Same cell as in Fig. 3. Potassium chloride electrodes were used to observe the chloride current in (A), potassium acetate electrodes were used for (B).

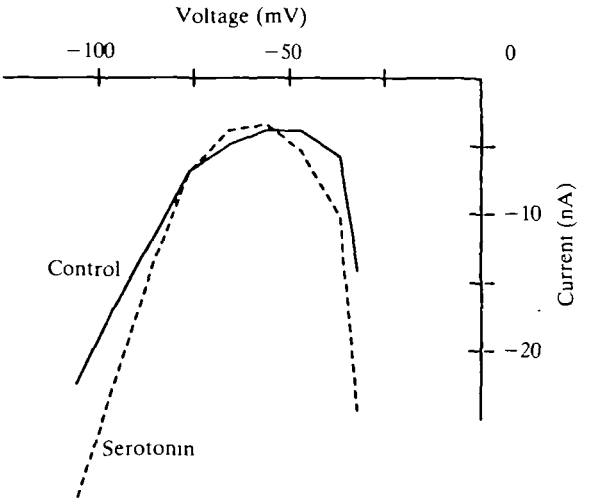

Fig. 5. Effects of $20\,\mu mol\,l^{-1}$ serotonin on steady-state, subthreshold currents in R15. Currents were evoked with 2-s pulses from a holding potential of −36 mV. Dotted line shows currents after 23 min exposure to serotonin, solid line shows control.

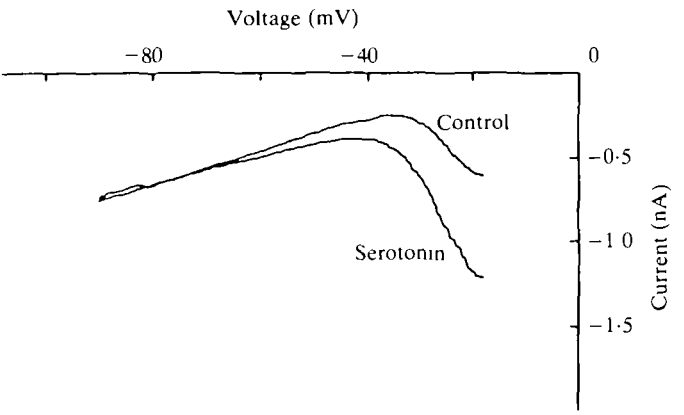

Fig. 6. Effect of serotonin on the negative slope resistance region of the steady-state current–voltage relationship in a cultured R15. Membrane current–voltage curve was obtained by applying a voltage ramp as in Fig. 4. External potassium concentration was reduced from the normal $10\,mmol\,l^{-1}$ to $2\,mmol\,l^{-1}$ and $50\,mmol\,l^{-1}$ tetraethylammonium was added to the external solution. The cell was voltage clamped using potassium acetate electrodes.

the cultured cell the anomalously rectifying potassium current was blocked by reducing external potassium, and potassium acetate electrodes were employed to minimize the chloride current, thus isolating the inward current (Fig. 6).

THE VOLTAGE-DEPENDENT INWARD CURRENT MODULATED BY SEROTONIN IS A CALCIUM CURRENT

The voltage-dependent inward current in R15 has been the object of intensive study. It is reduced by removing sodium or calcium, but the effects of low sodium

appear to be indirect. Gorman, Hermann & Thomas (1982) have demonstrated that this current is insensitive to removal of sodium when internal calcium is buffered by EGTA, and potassium channels are blocked with tetraethylammonium and 4-aminopyridine. It has also been shown that the inward current is accompanied by calcium influx into both the soma and well-clamped regions of the axon (Lewis, Evans & Wilson, 1984). These workers have also demonstrated that the inward current is blocked by the calcium channel blockers lanthanum and manganese (Gorman *et al.* 1982; Lewis, 1984). Finally, evoking the inward current by depolarization often produces an outward calcium-dependent tail current (Lewis, 1984; Adams & Levitan, 1985; Kramer & Zucker, 1985*b*). These data taken together show that the voltage-dependent inward current is largely a calcium current, although it can be affected by removal of extracellular sodium.

The increase in net inward current seen with serotonin (Figs 5–8) could be due to an increase in the level of voltage-dependent inward current, or a decrease in opposing outward current. We have found that conditions which block the inward current, such as external manganese, cadmium or low sodium, inhibit the effect of serotonin. The serotonin effect remains at least partially intact in low sodium when the cell is iontophoretically loaded with caesium and EGTA. Potassium channel blockers, such as external tetraethylammonium and 4-aminopyridine, or internal caesium, do not interfere with modulation of the current by serotonin. Likewise, the modulatory effect remains intact when the chloride currents are reversed by lowering external chloride. Finally, the serotonin-evoked increase in inward current is accompanied by an increase in the slow outward calcium-dependent tail current (Fig. 7A). These data, together with those described below, suggest that serotonin increases the subthreshold calcium current. We have also found that internal EGTA does not interfere with the modulation of the current, indicating that the increase seen is not due to a change in the internal level of calcium. Rather, serotonin appears to modulate directly the channels responsible for the current.

The serotonin- or forskolin-induced increase in the negative slope resistance region of the steady-state I–V curve in cultured R15 (Fig. 6) also appears to reflect an increase in the calcium current (Fig. 8). In this experiment calcium current was isolated by loading the cell with caesium (using caesium acetate electrodes) and adding 50 mmol l^{-1} tetraethylammonium and 5 mmol l^{-1} 4-aminopyridine to the bath to block potassium currents. Sodium currents were blocked by Tris substitution for external sodium. Under these conditions, serotonin still increases the inward current seen during depolarizing voltage-clamp pulses. The inward current evoked by depolarization under these conditions, and the effect of serotonin, are blocked when 10 mmol l^{-1} cobalt is substituted for calcium (Fig. 8).

The serotonin-evoked increase in inward current in R15 *in situ* persists when the external magnesium concentration is raised to 230 mmol l^{-1}. This high concentration is known to decrease dramatically membrane excitability and synaptic transmitter release, and thus it is unlikely that the effect is synaptic. This is shown most directly by the fact that serotonin enhances the inward current in entirely isolated neurone

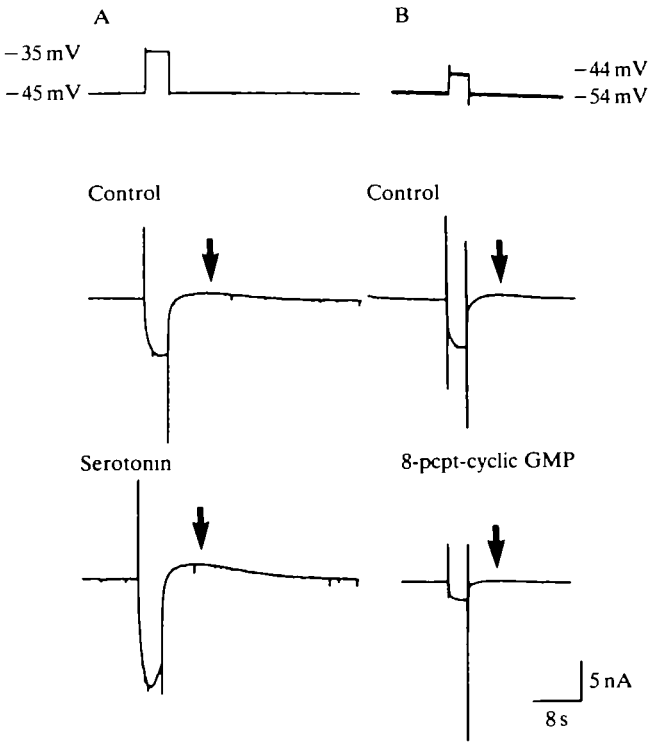

Fig. 7. The effects of serotonin and 8-parachlorophenylthio-cyclic GMP (8-pcpt-cyclic GMP) on slow inward current and the calcium-dependent outward tail current. (A) Top shows a 10-mV depolarizing voltage pulse from −45 mV; middle shows evoked current in control; bottom shows evoked current after 20 min exposure to 20 μmol l^{-1} serotonin. (B) Top shows a 10-mV depolarizing voltage pulse from −54 mV; middle shows evoked current in control; bottom shows evoked current after 20 min exposure to 2·5 mmol l^{-1} 8-pcpt-cyclic GMP. Arrows indicate calcium-dependent outward tail current. Note the opposite changes in currents evoked by serotonin and 8-pcpt-cyclic GMP both during and after the pulse.

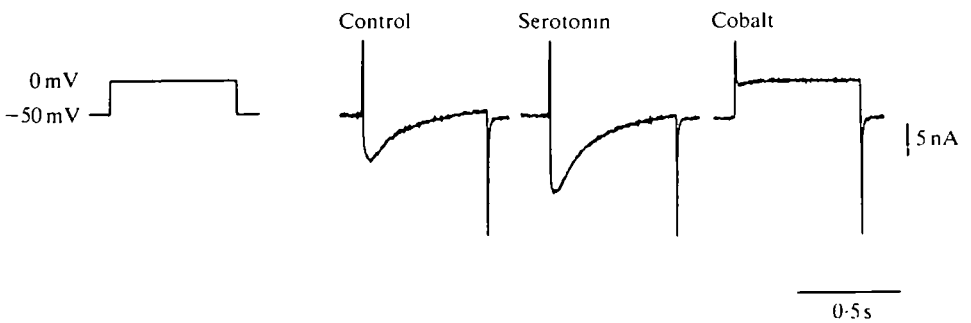

Fig. 8. Effect of serotonin on the calcium current, recorded from a cultured R15 neurone under voltage clamp. Calcium current was isolated as described in the text. Records show the current responses to a 0·6-s depolarizing voltage pulse from $V_H = -50$ mV to 0 mV before and after treatment with serotonin. The final trace shows the current response when cobalt is substituted for calcium. V_H, holding potential.

R15 in culture (Figs 6, 8). The increase in inward current is also seen with external application of forskolin (together with a phosphodiesterase inhibitor), suggesting that, as in the case of the anomalously rectifying potassium current and the chloride current, modulation of the voltage-dependent inward current by serotonin is mediated by cyclic AMP.

A CYCLIC GMP ANALOGUE MODULATES THE INWARD CURRENT

The results presented thus far demonstrate that serotonin, working through a single second messenger, cyclic AMP, can produce multiple and even opposing effects on the activity of the identified neurone R15. It is also known that dopamine and synaptic activity produce long-lasting alterations in R15's electrical activity that differ from the actions of cyclic AMP (Adams *et al.* 1980; Lewis *et al.* 1984). The possibility therefore exists that other second messengers may affect membrane ion channels in R15. One such second messenger is cyclic GMP.

Levitan & Norman (1980) reported that bath application of the membrane permeable analogue 8-parachlorophenylthio-cyclic GMP (8-pcpt-cyclic GMP) increases the number of action potentials within a burst in R15 in a dose-dependent manner. We have recently found that this effect (Fig. 9C) is preceded or accompanied by a decrease in action potential frequency in a burst (Fig. 9B). No increase in interburst hyperpolarization is seen, in contrast to the effects of serotonin and cyclic AMP (e.g. Fig. 1B). The effects of 8-pcpt-cyclic GMP appear and reverse slowly and are not mimicked by bath application of cyclic GMP itself. These results suggest that 8-pcpt-cyclic GMP acts intracellularly and not on an extracellular membrane surface receptor. Furthermore the observations described below suggest that 8-pcpt-cyclic GMP acts on subthreshold ion currents which are required for maintenance of bursting activity.

To investigate the mechanism of the cyclic GMP response, voltage-clamp experiments were performed. Fig. 10 shows the effect of bath application of 8-pcpt-cyclic GMP on the steady-state I–V relationship of R15. As indicated earlier, two currents dominate this steady-state I–V plot – the anomalously rectifying potassium current accounts for the inward current at voltages more negative than −75 mV, while a voltage-dependent calcium current contributes to the inward current in the negative slope region above −60 mV. Clearly the anomalously rectifying potassium current is not affected by 8-pcpt-cyclic GMP (Fig. 10), indicating that the analogue is not cross reacting with cyclic-AMP-dependent protein kinase or activating a cyclic-GMP-dependent phosphodiesterase. The inward current at the more depolarized potentials, however, is approximately halved. Preliminary work indicates that this effect persists under conditions which greatly reduce excitability and synaptic release, namely in the presence of 230 mmol l^{-1} external magnesium. Thus it seems probable that the cyclic GMP analogue is acting directly on R15's subthreshold currents.

We have not determined unequivocally whether the decrease in inward current seen is due to activation of an outward current or inactivation of the inward calcium current. However the latter seems more likely. Increasing the inward current with depolarization or serotonin (Fig. 7A) *increases* the slow outward calcium-dependent tail current, whereas 8-pcpt-cyclic GMP *decreases* both the depolarization-evoked slow inward current and the slow outward tail (Fig. 7B). These results are consistent with the hypothesis that the cyclic GMP analogue decreases subthreshold calcium current. The possibility therefore exists that cyclic AMP and cyclic GMP modulate one ion channel but in opposing directions. Interestingly, dopamine has also been found to decrease subthreshold calcium current in R15. Future work will be aimed at determining whether cyclic AMP, cyclic GMP and dopamine all modulate the same ion channels, and whether these effects are due to activation of protein kinases.

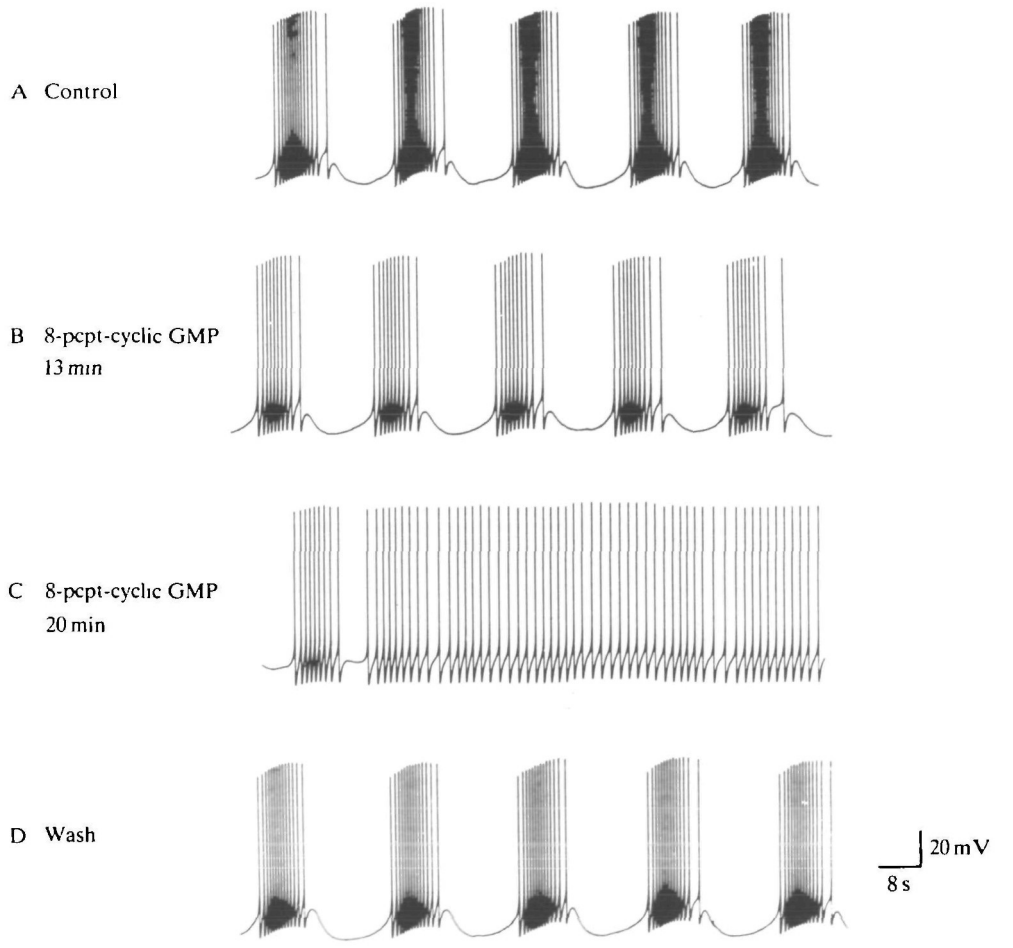

Fig. 9. Effects of 0·8 mmol l^{-1} 8-parachlorophenylthio-cyclic GMP (8-pcpt-cyclic GMP) on bursting activity in R15. (A) Control. (B),(C) 8-pcpt-cyclic GMP. Spike frequency decreases (B), then the number of action potentials per burst increases dramatically (C). Interburst interval is not enhanced. (D) Wash.

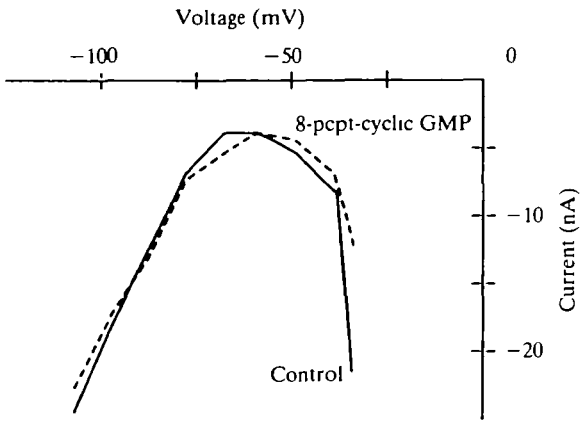

Fig. 10. Effect of 8-parachlorophenylthio-cyclic GMP (8-pcpt-cyclic GMP) on steady-state, subthreshold currents in R15 (same cell as Fig. 5). Currents were evoked with 2-s pulses from a holding potential of −38 mV. Dotted line shows currents after 20 min exposure to 1·7 mmol l^{-1} 8-pcpt-cyclic GMP, solid line shows control. Note the large decrease in voltage-dependent inward current, with no effect on the anomalously rectifying K^+ current.

CONCLUSIONS

About five years ago, most workers in the field of neuromodulation would have predicted that only one or a very few ion channels would turn out to be regulated by protein phosphorylation. However, it now is evident that many different classes of channels can be modulated in this way, and that the channels regulated differ from cell to cell (for a recent review see Levitan, 1985). Indeed, in some cells several ion channels can be regulated by a single intracellular messenger, cyclic AMP. The most thoroughly documented case is in neurosecretory bag cell neurones from *Aplysia*, in which L. K. Kaczmarek and his collaborators have found that at least three distinct potassium channels are inhibited by cyclic-AMP-dependent protein phosphorylation (see chapter by Kaczmarek, this volume). All of these actions of cyclic AMP result in an increase in excitability of the bag cell neurones. The present results demonstrate that cyclic AMP can also regulate several different classes of ion channels in the identified neurone R15, but in this case some of the actions of cyclic AMP may result in inhibition of the cell, whereas others will result in excitation. It is of particular interest that the same neurotransmitter, serotonin, can produce both excitation and inhibition, and that cyclic GMP can inhibit one of the ion currents that cyclic AMP activates. These findings make it clear that there are a series of regulatory pathways in nerve cells, pathways which interact in complex ways. The task for the future is to unravel the details of the individual regulatory mechanisms, with the goal of understanding how they work together to produce subtle modulation of the neurone's overall electrical activity.

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