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DOES CYCLIC 3′,5′-ADENOSINE MONOPHOSPHATE ACT AS SECOND MESSENGER IN A VOLTAGE-DEPENDENT RESPONSE TO 5-HYDROXYTRYPTAMINE IN *Aplysia*?

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1 The possibility that cyclic adenosine 3′,5′-monophosphate (cyclic AMP) mediates a voltage-dependent inward current elicited by 5-hydroxytryptamine (5-HT) in RB and LB cells of the abdominal ganglion of *Aplysia* was tested.

2 Intracellular injection of cyclic AMP elicited an inward current with a similar time course, potential dependence and ionic sensitivity as the response to 5-HT.

3 Intracellular injection of guanylyl imidodiphosphate (GMP-PNP), which activates adenylate cyclase, neither mimicked nor enhanced the 5-HT-evoked current. On the contrary, it reduced the current.

4 The phosphodiesterase inhibitors, Ro20-1724, isobutyl methylxanthine (IBMX) and theophylline, each antagonized the voltage-dependent response to 5-HT. To varying degrees they each induced an inward current.

5 The adenylate cyclase antagonist, dithiobisnitrobenzoic acic (DTNB), had no effect on the response to 5-HT when applied either intracellularly or extracellularly. Intracellular injection of the phosphodiesterase activator imidazole also had no effect.

6 Tubocurarine and neostigmine did not reduce the voltage-dependent inward current evoked by 5-HT; methysergide elicited an inward current.

7 Although the observation that cyclic AMP and 5-HT can evoke similar voltage-dependent inward currents in RB and LB neurones of *Aplysia* might suggest a second messenger role for the cyclic nucleotide, the pharmacological data are inconsistent with this hypothesis.

Introduction

In the identified cell clusters of LB and RB neurones of *Aplysia californica* (nomenclature of Frazier, Kandel, Kupfermann, Waziri & Coggeshall, 1967), 5-hydroxytryptamine (5-HT) evokes a voltage-dependent inward current (Pellmar & Carpenter, 1980). In the same neurones intracellular injection of cyclic 3′,5′-adenosine monophosphate (cyclic AMP) appears to mimic the actions of the neurotransmitter (Pellmar, 1981a,b; Pellmar & Carpenter, 1981). This observation suggests the possibility that the response to 5-HT is mediated by an intracellular increase of cyclic AMP. If the mechanism of a neurotransmitter-mediated response involves cyclic nucleotides, the following criteria should be fulfilled (Beam & Greengard, 1976). (1) The neurotransmitter should activate adenylate cyclase to increase cyclic AMP levels in the responsive neurones. (2) Intracellular application of cyclic AMP should mimic the electrophysiological actions of the neurotransmitter. (3) Agents known to alter cyclic nucleotide metabolism should affect the electrophysiological response in a manner consistent with their metabolic effect. For example, phosphodiesterase inhibitors enhance the production of cyclic AMP induced by 5-HT (Cedar & Schwartz, 1972) and would be expected to potentiate the electrophysiological response to this amine. (4) Pharmacological blocking agents which alter the current evoked by the transmitter should affect similarly the biochemically measured increase in cyclic AMP.

In this study, the possibility that the response to 5-HT in RB and LB neurones is mediated by cyclic AMP was evaluated with respect to the above criteria. Some of these criteria have been satisfied. In whole abdominal ganglion preparations, a 5-HT-sensitive adenylate cyclase is present (Levitan, Madsen & Barondes, 1974) and though 5-HT can increase cyclic AMP levels (Cedar & Schwartz, 1972; Levitan *et al.*, 1974) this effect has not yet been demonstrated specifically for LB and RB cells. Intracellular injection of cyclic AMP produces an elec-

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trophysiological response with a similar time course, voltage-dependence and ionic sensitivity to that elicited by 5-HT in LB and RB cells (Pellmar, 1981a). Other criteria, however, have not been satisfied. This raises the question as to whether cyclic AMP does, in fact, mediate the response to 5-HT.

Methods

Methods are essentially the same as those described previously (Pellmar & Carpenter, 1980; Pellmar, 1981a). The abdominal ganglion of *Aplysia californica* was dissected and pinned out on to a layer of Sylgard in a plexiglass chamber. Seawater solutions were perfused continuously at a rate of approximately 2 ml per min. The temperature of the bathing solution was maintained at 23° ± 1°C. The connective tissue over the left and right caudal quarters of the ganglion was removed. A cell of the LB or RB cluster was impaled with a 1–2 megohm electrode filled with 1.5 M KCl. Cells were voltage-clamped using the single electrode method (Wilson & Goldner, 1974). Only electrodes which showed no rectification at a switching frequency of 3000 Hz were used. Switched voltage and injected current were continuously monitored to ensure constant electrode characteristics throughout the experiment. After removal of the electrode from the cell, it was again tested for rectification and d.c. offset. Experiments in which large changes occurred were discarded.

5-HT (serotonin creatinine sulphate, saturated) was applied by iontophoresis through a microelectrode positioned extracellularly. Pulses of positive current 200–1000 nA for 200–1000 ms were used to eject the transmitter. 5-HT, in concentrations from 0.1 μM to 1000 μM, was applied extracellularly to obtain a dose-response curve. Increasing concentrations of 5-HT were perfused sequentially at a fast rate (approximately 10 ml/min) without intervening wash. Sequential application was possible because the response to 5-HT did not appear to desensitize with prolonged exposure. 5-HT induced current was measured after a 4 min exposure to each concentration. Only one cell per ganglion was used for determining a dose-response relationship. Cyclic AMP (1 mM in deionized water with 3 mg/ml fast green, pH 7.4) was injected intracellularly with pressure pulses of 10–50 psi for 20–100 ms. Fast green (FCF, disodium salt of *p,p'* dibenzyldiethyldiamino- *p'* hydroxytriphenylcarbinol-trisulphonic acid anhydride) was used to allow visual verification that cyclic AMP was entering the cell. Control injections of fast green alone had no obvious adverse effects on the cell but often produced a fast transient current.

Drugs applied extracellularly were added to artificial seawater (ASW) that consisted of (mM): NaCl 480, KCl 10, $CaCl_2$ 10, $MgCl_2$ 20, $MgSO_4$ 30, $NaHCO_3$ 2. Isobutyl methylxanthine (IBMX) and (±)-4- [3-butoxy-4-methoxybenzyl] -2-imidazolidinone (Ro20-1724) were dissolved in ethanol and dispersed in ASW; the final ethanol concentration was less than 1%. Control solutions with 1% ethanol alone produced no effect. The pH of all solutions was adjusted to 7.8 by addition of HCl or NaOH. Drugs were applied intracellularly through a second microelectrode. NaF was applied intracellularly either by diffusion from the pipette tip or by pulses of negative current. Other drugs were dissolved either in 0.5 M KCl or in deionized water and injected with pressure pulses. Fast green (3 mg/ml) was included often to allow visual verification of a successful injection of drug solution. A more quantitative measure of the amount of pressure-injected dithiobisnitrobenzoic acid (DTNB) or imidazole was obtained using the following method: the chloride equilibrium potential (E_{Cl}) was estimated by the reversal potential of the response to iontophoretically applied glutamate (M.J. McCreery, personal communication). The change in intracellular chloride concentration required to produce the observed shift in E_{Cl} after injection was calculated. Assuming the ratio of concentrations of drug and chloride injected was the same as the ratio of concentrations in the pipette, intracellular drug concentration could be determined.

The following drugs were used: 5-hydroxytryptamine (serotonin) creatinine sulphate (5-HT) (Sigma); adenosine 3',5'-cyclic monophosphoric acid, sodium salt (cyclic AMP) (Sigma); sodium fluoride (NaF) (Sigma); 5' guanylyl-imidodiphosphate, tetrasodium salt (GMP-PNP) (ICN); 3-isobutyl-l-methyl-xanthine (IBMX) (Sigma); theophylline (Sigma); (±)-4-[3-butoxy-4-methoxybenzyl]-2-imidazolidinone (Ro20-1724) (Hoffmann-La Roche); 5,5'-dithiobis-(2-nitrobenzoic acid (DTNB) (Sigma); cyclic AMP antiserum rabbit (New England Nuclear); methysergide maleate (Sandoz); (+)-tubocurarine chloride (tubocurarine) (Sigma); and neostigmine methyl sulphate (Sigma).

Results

5-Hydroxytryptamine and cyclic AMP evoke voltage-dependent currents

It has been reported previously that 5-HT and cyclic AMP elicit voltage-dependent currents in LB and RB cells (Pellmar, 1981a,b; Pellmar & Carpenter, 1980). Figure 1 illustrates that in the same cell, the voltage-dependence of the currents induced by cyclic

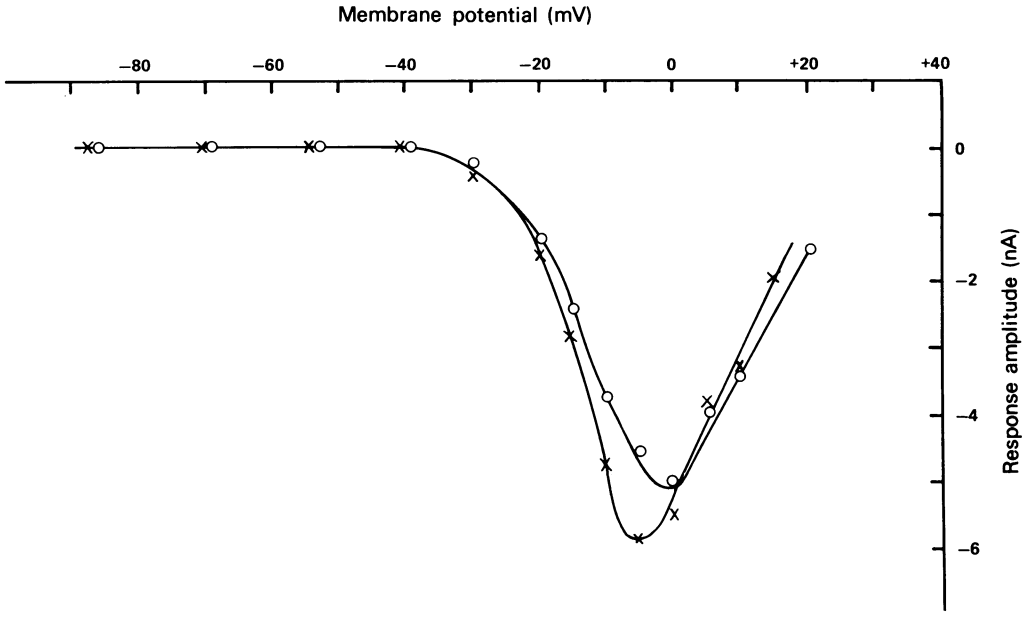

Figure 1 The effect of membrane potential on the amplitude of the slow voltage-dependent inward currents evoked by 5-hydroxytryptamine (5-HT) (×) and cyclic AMP (○) in the same cell. 5-HT was applied iontophoretically (500 nA, 800 ms); cyclic AMP was injected intracellularly (20 psi, 20 ms). Neither agent evoked the current at membrane potentials more hyperpolarized than − 40 mV; the response amplitude was maximal between − 5 and 0 mV.

AMP and 5-HT are similar. Both agents evoke a current which is absent at potentials more hyperpolarized than about − 40 mV and has a maximum amplitude near 0 mV. The currents are unaffected by changes in chloride or potassium concentration, persist in the absence of sodium and are blocked by 2 mM cadmium. Because of these similarities, the ionic mechanisms of the evoked currents appear to be identical (Pellmar, 1981a). This observation suggests the possibility that cyclic AMP mediated the response to 5-HT.

Effects of agents that alter cyclic nucleotide metabolism

In order to test the second messenger hypothesis, several agents that alter nucleotide metabolism were evaluated on the response to 5-HT. If cyclic AMP mediated the response, these agents should have affected the current in a predictable manner.

Agents that increase cell cyclic AMP levels: Sodium fluoride Because sodium fluoride activates adenylate cyclase (Robison, Schmidt & Sutherland, 1970; Cedar & Schwartz, 1972; Perkins, 1973), one might expect it to mimic the actions of cyclic AMP. As previously found (Pellmar & Carpenter, 1981), fluoride injections evoke an inward current at depolarized potentials. In the present study, a reduction in the response to 5-HT was found to accompany the inward current induced by fluoride. Two explanations of this antagonism are possible: (1) fluoride directly antagonizes the 5-HT-induced current. (2) Fluoride induces an inward current by affecting the same channels as 5-HT and therefore fewer channels remain available for 5-HT activation.

GMP-PNP Guanine nucleotides are important for the physiological interaction of the neurotransmitter or hormone receptor with adenylate cyclase (Lad, Welton & Rodbell, 1977). Guanylyl imidodiphosphate (GMP-PNP) activates adenylate cyclase in *Aplysia* (Treistman & Levitan, 1976) as well as in other systems (Londos, Lin, Welton, Lad & Rodbell, 1977). Addition of GMP-PNP to neurones showing a voltage-dependent response to 5-HT would be expected either to mimic or to enhance the transmitter response. Figure 2 illustrates the actions of GMP-PNP injections in LB and RB cells. The voltage-dependent response to 5-HT was decreased greatly after a large injection of GMP-PNP (qualitatively defined as one which turned the cell completely green; 20 psi, 200–600 ms) (6 cells). Many of the neurones used in this study had, in addition to the voltage-dependent response, a fast sodium-dependent response to 5-HT which could be re-

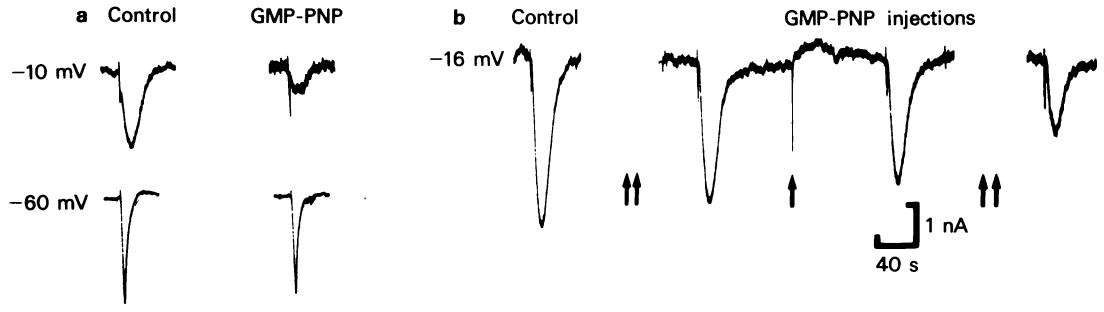

Figure 2 The effect of guanylyl imidodiphosphate (GMP-PNP) in LB and RB cells of *Aplysia*. (a) Voltage-dependent current elicited by 5-hydroxytryptamine (5-HT) recorded at −10 mV was reduced by GMP-PNP injection (20 psi, 200 ms). A fast sodium response to 5-HT in the same cell recorded at −60 mV was unaffected by same injection. (b) Small injections of GMP-PNP (22 psi, 20 ms) occurred at the arrows (6 pulses at first set of double arrows, 5 pulses at second set of double arrows, 1 at single arrow). A gradual attenuation of the response to 5-HT at −16 mV resulted. GMP-PNP injection (at single arrow) itself did not induce an inward current.

corded at hyperpolarized potentials. The fast sodium response was not significantly affected by the GMP-PNP injection (4 cells), indicating that the decrease was not a generalized reduction in cell responsiveness. Small, successive injections of GMP-PNP (22 psi, 50 ms) (Figure 2b) caused a gradual reduction in the amplitude of the voltage-dependent response to 5-HT and never by themselves evoked a slow inward current.

Phosphodiesterase inhibitors Three agents (Ro20-1724, IBMX, and theophylline) were used to inhibit the breakdown of cyclic AMP by phosphodiesterase. By prolonging the lifetime of the cyclic AMP, these

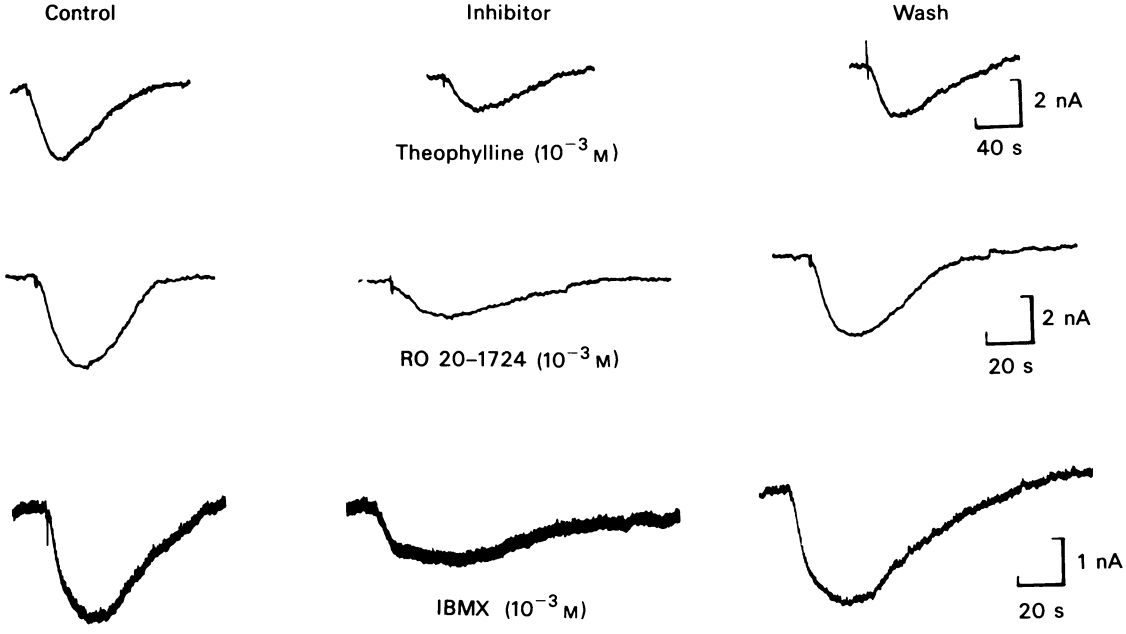

Figure 3 The antagonistic effect of phosphodiesterase inhibitors on the voltage-dependent inward current evoked by 5-hydroxytryptamine (5-HT). Theophylline, Ro20-1724 and IBMX (each 1 mM) applied to three different cells. The parameters for 5-HT iontophoresis in the cells treated with theophylline, Ro20-1724 and IBMX are 900 nA, 400 ms; 1000 nA, 800 ms; and 1000 nA, 500 ms respectively. In all traces, membrane potential was voltage-clamped to −10 mV. The actions of the inhibitors were usually reversible on washing.

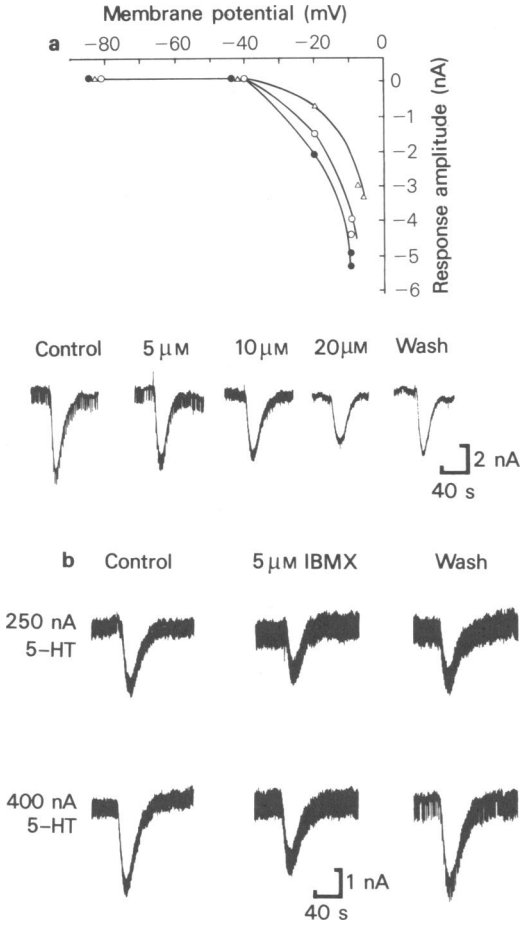

Figure 4 The effects of low concentrations of IBMX on the voltage-dependent response to 5-hydroxytryptamine (5-HT) in *Aplysia* neurones. (a) Plot of the response amplitude versus membrane potential illustrates the actions of 20 μM IBMX (△) and wash (○) at several membrane voltages; (●) control. Sample traces show a progressive attenuation of the response to 5-HT at − 10 mV in same cell with increasing concentrations of IBMX. (b) Two iontophoretic currents of 5-HT (250 nA, 800 ms and 400 nA, 800 ms) elicit responses of different amplitudes in the same cell (but different from that in a). Both responses were reduced reversibly by 5 μM IBMX. All responses obtained with the membrane voltage of the cell clamped to − 10 mV.

agents were expected to potentiate the electrophysiological response mediated by a cyclic nucleotide. Figure 3 illustrates the effects of 1 mM concentration of each phosphodiesterase inhibitor on the voltage-dependent response to 5-HT. Invariably the responses to 5-HT were reduced (9 cells Ro20-1724, 4 cells theophylline, 3 cells IBMX). It was found previously (Pellmar & Carpenter, 1981) that at this concentration Ro20-1724 and, less often, IBMX induced an inward current, although theophylline rarely produced this effect. To test whether the blocking action might be due to maximal current being induced by IBMX through the same channels affected by 5-HT and thereby preventing a further increase in current by 5-HT, lower concentrations of IBMX were tested in 6 cells. Typical results with IBMX are illustrated in Figure 4. Concentrations as low as 5 μM were capable of reducing the 5-HT evoked current. As the concentration of IBMX was increased, the amplitude of the 5-HT response was depressed progressively. The effects of IBMX were not always completely reversible. These low concentrations of IBMX (5 to 20 μM) were observed occasionally to elicit small inward currents. The threshold concentration for the induction of current and for the reduction of the 5-HT response was not always identical. In some cells, the responses to 5-HT were reduced at concentrations of IBMX that did not induce an inward current. The absence of potentiation by the phosphodiesterase inhibitors does not appear to be due to a maximally activated 5-HT response, since, as illustrated in Figure 4b, two iontophoretic responses to 5-HT of different amplitudes were both reduced by IBMX.

Dithiobisnitrobenzoic acid (DTNB) DTNB inhibits the formation of cyclic AMP by antagonism of adenylate cyclase (Ferrendelli, Johnson, Chang & Needleman, 1973). In cerebral cortical tissue, DTNB 0.2 mM produced a 50% inhibition of adenylate cyclase activity; the effects of DTNB were immediate; no incubation time was necessary (Ferrendelli *et al.*, 1973). DTNB was applied to LB and RB neurones of *Aplysia* both extra- and intracellularly in the present investigation. An extracellular concentration of 1 mM had no apparent effect on the voltage-dependent inward current elicited by 5-HT (3 cells). DTNB was applied intracellularly by pressure injection from a pipette containing DTNB 10 mM and KCl 0.5 M. Figure 5 illustrates an experiment in which approximately 1.39 mM DTNB was injected into an LB cell. In this cell, glutamate evoked a chloride-dependent response. Due to the leak of chloride from the recording electrode, the glutamate response reversal potential (E_{Cl}) was − 34 mV before DTNB injection. Figure 5c illustrates the shift in the response amplitude-membrane potential curve to glutamate following DTNB injection. E_{Cl} shifted to − 24 mV. This 10 mV shift in E_{Cl} corresponds to an increased intracellular chloride concentration of 69.5 mM. If chloride and DTNB were injected proportionately, the intracellular concentration of DTNB would be approximately 1.39 mM. The intracellular administration of DTNB had no apparent

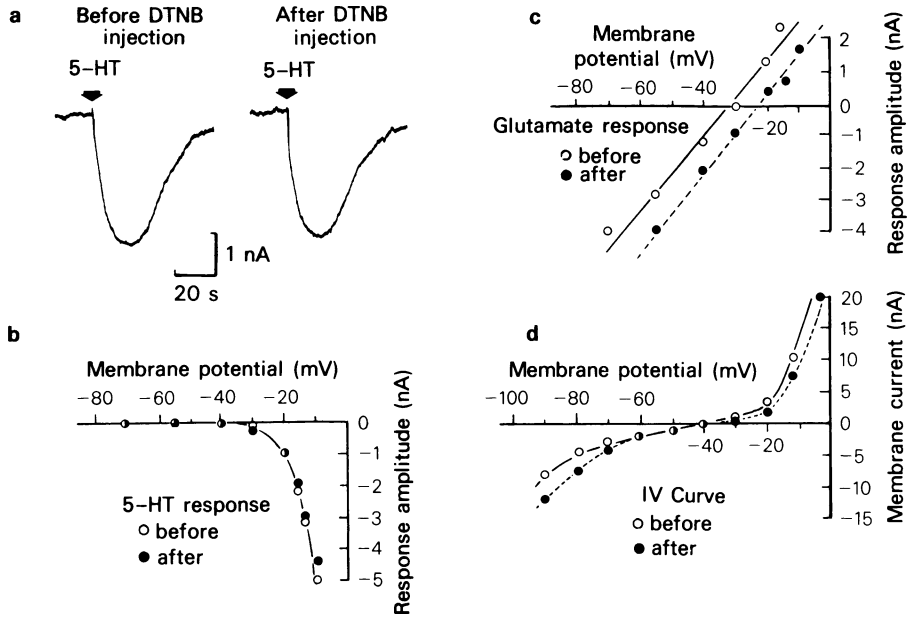

Figure 5 The effects of dithiobisnitrobenzoic acid (DTNB) injection on the voltage-dependent response to 5-hydroxytryptamine (5-HT) and to glutamate in *Aplysia* neurones. (a) The response to iontophoretic application of 5-HT in a cell in which membrane potential was clamped to −14 mV was unaffected by injection of DTNB (1.39 mM). (b) Graph of response amplitude versus membrane potential for the same cell as in (a), illustrating the absence of any effect at all potentials tested of DTNB on the 5-HT response. (c) The response amplitude versus membrane potential curve for response to glutamate in same cell. Glutamate increased chloride conductance in this cell. Concurrent injection of KCl with DTNB caused a shift in reversal potential (E_{Cl}). This shift was used to calculate concentration of DTNB injected. (d) Current-voltage relationship of same cell before and after injection of DTNB. The conductance increased at hyperpolarized potentials and delayed rectification was slightly reduced.

effect on the voltage-dependent response to 5-HT (Figure 5a,b). The current-voltage relationship of the cell (Figure 5d) was slightly altered; there was an increase in conductance at potentials < -70 mV and a small decrease in delayed rectification. This effect need not be due to DTNB; it might be a consequence of injection of K and/or Cl or due to injury from the pressure pulse. Control injections of the KCl and fast green carrier solution produced similar changes in the current-voltage relationship of other cells. Similar results with DTNB were obtained in two additional cells with estimated intracellular concentrations of 1.85 and 1.63 mM.

Agents that decrease cell cyclic AMP: Imidazole Imidazole has been reported to block some hormonal and transmitter actions that are mediated by cyclic AMP (e.g. Kukovetz & Poch, 1967; Nakano, Oliver & Ishii, 1970; Takagi, Takayanagi & Tsuchida, 1972). At concentrations ranging from 5 to 50 mM, imidazole activates phosphodiesterase (Butcher & Sutherland, 1962); yet at concentrations greater than about 20 mM, imidazole can also inhibit the high affinity form of the enzyme (Chasin & Harris, 1976). As with DTNB, imidazole was applied intracellularly by pressure injection and quantified by using the shift in reversal potential of the chloride-dependent response to glutamate. Intracellular imidazole concentrations estimated at 13.6, 15.0 and 16.6 mM had no apparent immediate or delayed effects on the response to 5-HT (3 cells) (data not shown).

Cyclic AMP antibody A commercially available antibody to cyclic AMP (New England Nuclear), was diluted by half with deionized water containing 6 mg/ml fast green. This solution was injected into two neurones which responded to 5-HT with a slow voltage-dependent inward current. One might expect the antibody to bind to cyclic AMP and prevent a response mediated by the cyclic nucleotide. No change in the 5-HT evoked current was observed. However, this result may be inconclusive since it is possible that the antibody does not bind to the cyclic AMP at rates sufficient to prevent or reduce the response and/or that the cyclic AMP is compartmentalized and therefore inaccessible to the antibody.

The sensitivity of the 5-hydroxytryptamine receptor as indicated by electrophysiological and biochemical methods

A second messenger mechanism involving AMP in the response to 5-HT would require that the 5-HT receptor mediating both the electrophysiological response and the biochemically measured increase in cyclic AMP should be identical. The receptor mediating the voltage-dependent inward current was tested for its sensitivity to 5-HT and various antagonists and compared with biochemical data in the literature (Cedar & Schwartz, 1972; Levitan *et al.*, 1974).

Dose-response curve for 5-hydroxytryptamine In order to construct dose-response curves for 5-HT, fast perfusion techniques were used. This was possible because the voltage-dependent response to 5-HT did not appear to desensitize with time. Cells were voltage-clamped to a potential near -10 mV, increasing concentrations of transmitter were superfused over the ganglion and the current elicited was measured. Five cells tested fell into two classes which were not obviously distinguishable by anatomical or electrophysiological properties. In three cells the concentration of 5-HT required to produce a half maximal induction of current was approximately 3 μM. In two cells, the half maximal concentration was 0.3 μM. Since only one cell was used per ganglion, differences among the cells would reflect biological differences among animals or among cells in one animal. Cedar & Schwartz (1972) found that the concentration of 5-HT required to stimulate cyclic AMP synthesis half maximally was 6 μM. The dose-response curve which they published was very similar to that of the three cells with current elicited half maximally at a concentration of 3 μM.

Effect of tubocurarine and methysergide Cedar & Schwartz (1972) found that tubocurarine 140 μM and neostigmine 300 μM decreased the 5-HT induced formation of cyclic AMP by 50% and 41% respectively, and that methysergide 100 μg/ml (approx. 0.3 mM) had no effect on cyclic AMP levels in the presence of absence of 5-HT in abdominal ganglion of *Aplysia*. In contrast, Levitan *et al.* (1974) found that methysergide 1 mM blocked the cyclic AMP increase to 5-HT in the same preparation.

These three agents were tested on the electrophysiological response to 5-HT in LB and RB cells. Methysergide (100 μg/ml) rapidly induced an inward current at depolarized potentials. Neither neostigmine (500 μM) (3 cells) nor tubocurarine (500 μM) (3 cells) elicited a current by themselves and had no apparent effect on the 5-HT-induced current.

Cedar & Schwartz (1972) suggested that the actions of neostigmine and tubocurarine might be non-specific. Gerschenfeld & Paupardin-Tritsch (1974) reported that tubocurarine blocks the fast, sodium-dependent excitatory response to 5-HT and that neostigmine blocks the chloride response. In the present experiments the fast sodium response was reduce by approximately 25% with 500 μM neostigmine and by approximately 75% with 500 μM curare. Thus, it is possible that in the study by Cedar & Schwartz (1972) the sodium response is a prerequisite for the increase in cyclic AMP possibly because membrane depolarization is necessary.

Discussion

The observation that intracellular injection of cyclic AMP can mimic the electrophysiological action of 5-HT in evoking a slow voltage-dependent inward current suggests the possibility that intracellular cyclic AMP is a second messenger in the 5-HT response. Yet inconsistencies with this mechanism raise doubts about such a conclusion.

Many pharmacological agents that alter cyclic AMP levels do not affect the electrophysiological response to 5-HT in the expected manner. GMP-PNP, for example, should either mimic or enhance the 5-HT response. It did neither. In fact sufficiently large injections decreased the 5-HT-induced current. This agent has been shown to increase cellular levels of cyclic AMP (Treistman & Levitan, 1976) and to evoke (with some variability) membrane hyperpolarization in R15 and other abdominal ganglion cells of *Aplysia* (Treistman & Levitan, 1976; Treistman & Drake, 1979). As with any applied drug, compartmentalization and a non-specific action might explain the absence of an expected result.

Imidazole is effective in many systems in reducing cyclic AMP-mediated actions of hormonal and transmitter agents (Kukovetz & Poch, 1967; Nakano *et al.*, 1970; Takagi *et al.*, 1972). Chasin & Harris (1976) found that in homogenates of rat brain, imidazole activated only the low affinity phosphodiesterase and at concentrations greater than 20 mM, it inhibited the higher affinity form of the enzyme. They suggested that the potentiating effect of imidazole might occur through some alternative mechanism. In *Aplysia*, the ability of imidazole to activate phosphodiesterase has not been tested. In a report by Juel (1981), imidazole 3 mM antagonized the actions of dibutyryl cyclic AMP which increased rate of transmitter mobilization in *Helix*. In the experiments described here the intracellular injection of concentrations of approximately 12 mM had no effect on the voltage-dependent response to 5-HT.

The biochemical effects of DTNB in *Aplysia* have

not been reported. In the mammalian central nervous system it inhibits adenylate cyclase (Ferrendelli *et al.*, 1973). In the present experiments, DTNB had no effect when applied either intracellularly or extracellularly on the current evoked by 5-HT. It is, of course, possible that DTNB is not effective in neurones of *Aplysia*; alternatively, though effective, it may not have reached the appropriate site.

The phosphodiesterase inhibitors, Ro20-1724, IBMX, and theophylline, did not produce the expected potentiation of the response to 5-HT. Instead they reduced the induced current. The absence of any potentiation is not due to there being a maximal current elicited by 5-HT since the action of phosphodiesterase inhibitors was independent of the amplitude of the 5-HT response (Figure 4). Theophylline is effective as a phosphodiesterase inhibitor in *Aplysia* abdominal ganglion and enhances the 5-HT evoked increase in cyclic AMP (Cedar & Schwartz, 1972). Suprisingly, phosphodiesterase inhibitors also antagonize the electrophysiological response to intracellularly injected cyclic AMP (Pellmar, 1981b). This might suggest that, in addition to acting on the enzyme, the agents interfere with some common step in the electrophysiological mechanism. One possibility is that phosphodiesterase inhibitors, by inducing an inward current, reduce the number of channels available for 5-HT and cyclic AMP actions. However, low concentrations of the phosphodiesterase inhibitors that do not induce an inward current, still reduce the transmitter response. Another possibility is that 5-HT is activating an adenosine receptor and methylxanthines are antagonists (e.g. Phillis, Kostopoulos, Edstrom & Ellis, 1979; Hartzell, 1979). This is unlikely since adenosine and 5'AMP are not effective in eliciting the voltage-dependent current; in addition, IBMX antagonism of 5-HT appears to be non-competitive (unpublished observations). A third possibility is that the phosphodiesterase inhibitors increase cyclic guanosine monophosphate (cyclic GMP) which could counteract a cyclic AMP response. However, injections of cyclic GMP do not have any effect on the response to 5-HT (unpublished observations).

In terms of the second messenger hypothesis it would be expected that 5-HT would increase the cyclic AMP content of the cells in which this amine induces a voltage-dependent inward current. 5-HT increases the cyclic AMP content in whole abdominal ganglion, the giant cell R2 and the burst-firing neurone R15 (Cedar & Schwartz, 1972; Levitan *et al.*, 1974). Preliminary experiments indicate that the cyclic AMP content does not increase in RB and LB neurones exposed to 100 μM 5-HT for 5 min, although the radioimmunoassay was sufficiently sensitive to detect the expected rise in cyclic AMP levels in R15 in the same ganglion. This observation is not conclusive evidence that 5-HT does not increase cyclic AMP in RB and LB cells, since the increase may (1) occur in axons which were not assayed; (2) be too small to be detected, although sufficient to evoke a current; or (3) be compartmentalized.

If cyclic AMP mediates the response to 5-HT, one would expect the biochemical and electrophysiological receptor pharmacology to be identical. In 3 of 5 cells, the dose-response curve for 5-HT-induced current appeared similar to that for the 5-HT-induced increase in cyclic AMP in abdominal ganglion (Cedar & Schwartz, 1972). However, the biochemical and electrophysiological responses to 5-HT were not equally sensitive to receptor antagonists. Although Cedar & Schwartz (1972) found that tubocurarine and neostigmine reduce cyclic AMP synthesis, in the present study these pharmacological agents had no effect on the voltage-dependent current evoked by 5-HT. In the present study, methysergide induced an inward current but biochemically it has been reported to decrease (Levitan *et al.*, 1974) or to have no effect (Cedar & Schwartz, 1972) on the cyclic AMP content of the abdominal ganglion. This might suggest that two receptors are mediating the two responses to 5-HT. Different receptors to the same transmitter for electrophysiological and biochemical events have been demonstrated in other systems (MacDermot, Higashida, Wilson, Matsuzawa, Minna & Nirenberg, 1979; Brown, Caulfield & Kirby, 1979).

In other molluscan neurones there is strong evidence that 5-HT can induce an electrophysiological response through a cyclic AMP mechanism. In R15 of *Aplysia*, 5-HT and cyclic AMP analogues induce similar hyperpolarizations (Drummond, Benson & Levitan, 1980) which can be activated by GMP-PNP injection (Treistman & Levitan, 1976; Treistman & Drake, 1979; Drummond *et al.*, 1980) and enhanced by phosphodiesterase inhibitors (Drummond *et al.*, 1980). 5-HT has been shown to cause an increase in cyclic AMP content of R15 (Cedar & Schwartz, 1972; Levitan *et al.*, 1974). In addition, in R15 the pharmacology of the 5-HT receptor mediating the hyperpolarization is very similar to that of the 5-HT receptor linked to adenylate cyclase (Drummond *et al.*, 1980). An inward current induced by 5-HT in *Helix* neurones also has been reported to involve cyclic AMP (Deterre, Paupardin-Tritsch, Bockaert & Gerschenfeld, 1981); the 5-HT-induced current is mimicked by intracellular injection of cyclic AMP and enhanced by phosphodiesterase inhibitors.

In summary, the observation that cyclic AMP and 5-HT can evoke similar voltage-dependent inward currents in LB and RB neurones of abdominal ganglion of *Aplysia*, might suggest that the cyclic nucleotide acts as the intracellular second messenger. However, pharmacological agents that alter cyclic

AMP metabolism do not have the effects expected if cyclic AMP mediates the response to 5-HT. In addition, the receptor pharmacology for the 5-HT-induced increase in ganglionic cyclic AMP appears to be different from that for the 5-HT-induced current. Although alternative explanations are possible for many of the observations, the results do not support a role for cyclic AMP as intracellular second messenger in the voltage-dependent response to 5-HT.

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