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Correlation of ^{125}I-LSD Autoradiographic Labeling With Serotonin Voltage Clamp Responses in *Aplysia* Neurons

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ABSTRACT Autoradiographic receptor binding studies using ^{125}I-LSD (2-[^{125}I]lysergic acid diethyamide) revealed intense labelling on the soma of a symmetrically located pair of cells in the abdominal ganglion of *Aplysia californica*. This binding was blocked by micromolar concentrations of serotonin and lower concentrations of the serotonergic antagonists, cyproheptadine and mianserin (Kadan and Hartig, 1988). Electrophysiological investigation of responses to serotonin of neurons in the left upper quadrant, where one of the labeled neurons is located, revealed a range of serotonin responses. Cells L3 and L6 have a K^+ conductance increase in response to serotonin that is not blocked by cyproheptadine or mianserin. Cells L2 and L4 have a biphasic response to serotonin: a Na^+ conductance increase, which can be blocked by cyproheptadine and mianserin, followed by a voltage dependent Ca^{2+} conductance which is blocked by Co^{2+} but not the serotonergic antagonists. Cell L1, and its symmetrical pair, R1, have in addition to the Na^+ and Ca^{2+} responses observed in L2 and L4, a Cl^- conductance increase blocked by LSD, cyproheptadine and mianserin. LSD had little effect on the other responses. We conclude that the symmetrically located cells L1 and R1 have a Cl^- channel linked to a cyproheptadine- and mianserin-sensitive serotonin receptor that is selectively labelled by ^{125}I-LSD. This receptor has many properties in common with the mammalian serotonin 1C receptor.

INTRODUCTION

5-Hydroxytryptamine or serotonin has been demonstrated to be a neurotransmitter and neuromodulator in a variety of vertebrate (Aghajanian, 1981; Vandermaelen, 1985) and invertebrate (Gelperin, 1981; Gerschenfeld et al., 1981) nervous systems. The effects of serotonin are complex, and at least five different types of serotonin receptors have been identified in mammalian neurons (Peroutka, 1988). As in other animals, serotonin is present throughout the nervous system of the marine mollusk, *Aplysia* (Carpenter et al., 1971); a number of identified neurons contain high concentrations of this putative transmitter (Brownstein et al., 1975; Jahan-Parwar et al., 1987; Longley and Longley, 1986). Receptors for serotonin have been reported on neurons (Gerschenfeld and Stefani, 1965), heart (Koester et al., 1973) and gill (Kebabian et al., 1979). Electrophysiological studies of molluscan neurons have revealed a complex pattern of responses to serotonin which include three excitatory and three inhibitory mechanisms (Gerschenfeld and Paupardin-Tritsch, 1974a,b) and a voltage and calcium dependent response (Pellmar and Carpenter, 1980). These responses can be distinguished under voltage clamp conditions in terms of their time course, ionic selectivity, and whether they are mediated by an increase or decrease of membrane conductance.

In addition, serotonin can function to activate adenylate cyclase and stimulate synthesis of cyclic AMP (Cedar and Schwartz, 1972; Kebabian et al., 1979; Weiss and Drummand, 1981). It is unclear exactly which, if any, of the electrical responses is generated by this mechanism, but currents carried by both potassium and calcium ions have been implicated (Adams and Levitan, 1982; Levitan and Levitan, 1988) as well as a conductance decrease response to potassium (Deterre et al., 1982). Various agonists and antagonists can be used to distinguish among some of the response types. The serotonergic antagonists cyproheptadine and mianserin, both of which have proven very useful in the pharmacological characterization of vertebrate serotonin receptor types, have not yet been studied using electrophysiological techniques in terms of their effects

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on the spectrum of serotonin responses in *Aplysia* neurons.

Lysergic acid diethylamide (LSD) has been long known to be an antagonist of serotonin receptors, and tritiated LSD has been used to map the presence of such receptors in the mammalian CNS (Whitaker and Seeman, 1978). Detection of serotonin receptors has been greatly facilitated by the development of the high specific activity radioligand ^{125}I-LSD (Engel et al., 1984; Kadan et al., 1984). The high energy emissions of the ^{125}I-isotope make this compound especially useful for autoradiographic studies (Altar et al., 1986; Kadan and Hartig, 1988; Yagaloff and Hartig, 1985). In *Aplysia*, studies with ^{125}I-LSD have revealed serotonin binding sites located on both the neuropil and soma of various populations of cells in the central ganglia (Kadan and Hartig, 1988). This labelling is blocked by 10^{-6} M serotonin and 10^{-7} M cyproheptadine and mianserin.

Kadan and Hartig (1988) reported that ^{125}I-LSD binds to the neuropil and to two large, symmetrical neuronal cell bodies on the rostral edge of the *Aplysia* abdominal ganglion. These neurons are most likely cells L1 and R1, the only large symmetrical neurons in this position (Frazier et al., 1967). In the present study, we have taken advantage of the autoradiographic localization of these neurons, focusing on the five major neurons which constitute the abdominal ganglion left upper quadrant (L1, L2-4, and L6). We have characterized the serotonergic response pattern and pharmacology of each of these five neurons in order to determine which of the serotonin receptor–ionophore complexes correlates best with the pharmacological profile and location of the ^{125}I-LSD autoradiographic labeling previously reported.

MATERIALS AND METHODS

Autoradiography

Sectioning and labeling procedures were carried out as described previously (Kadan and Hartig, 1988). Briefly, ganglia were mounted for cryostat sectioning in O.C.T. (Tissue-Tek II embedding medium), and rapidly frozen by bringing the specimen holder into contact with Dry Ice. Sectioning was performed at −14°C using a Hacker model 5030 cryostat; 10-μm sections were thaw mounted onto gelatin-subbed glass slides and air dried before use.

Sections were incubated with 0.3 nM ^{125}I-LSD for twenty minutes at 37°C on a slide warmer, washed for 40 min in ice-cold distilled H_2O and dried under a gentle stream of cold air. Autoradiograms were obtained by exposing slides to either ultrafilm 3H (LKB) or Kodak Min R for 1 and 5 days, respectively. All ^{125}I-LSD binding experiments were carried out in an assay buffer consisting of 0.1% Na ascorbate, 5 mM $MgSO_4$, 10 μM pargyline, and 300 μM dopamine in 5 mM Tris–Cl, pH 7.6.

Electrophysiology

Abdominal ganglia were removed from 150 to 200 g *Aplysia* and were incubated in artificial seawater (ASW) containing 0.7 M sucrose (to shrink the cell and facilitate removal of the connective tissue capsule) for 30 min as previously described (Slater et al., 1984; Salanki et al., 1989). All experiments were performed at 23°C. Neuron cell bodies were exposed by desheathing using fine forceps and scissors to remove the connective tissue. Ganglia were washed with normal ASW for at least 30 min before starting recordings. Neurons were impaled by two glass microelectrodes (5–10 MΩ, filled with 2 M K acetate) connected to a Dagan 8500 voltage clamp unit, and were voltage clamped as previously described (Slater et al., 1984; Salanki et al., 1989). Voltage and current records were displayed on an oscilloscope and stored on tape.

Drugs were applied by a rapid local perfusion method described by Slater et al. (1984) which has been demonstrated to produce a concentration jump within 100 ms. This procedure has advantages over iontophoresis in that it generates large currents which can be easily distinguished and separated both by voltage dependence and rates of desensitization. The experiments consisted of study of 2 R1s, 4L1s, 3L2s, 4L3s, 3L4s, and 5L6s.

Materials

^{125}I-LSD was synthesized by the method of Hartig et al. (1985). Drugs were obtained from the following sources: acetylcholine bromide and 5-hydroxygramine (Aldrich); 5-hydroxytryptamine creatinine sulphate, neostigmine bromide and d-tubo curarine chloride (Sigma); cyproheptadine and mianserin (Merck, Sharp and Dohme Research Laboratories); cobalt acetate (Fisher); tryptamine HCl (Calbiochem). Nonradioactive d-LSD was provided by the National Institute on Drug Abuse.

RESULTS

Binding of ^{125}I-LSD to *Aplysia* ganglia

Figure 1 shows a histological cross section of the abdominal ganglion of *Aplysia* (A) and an autoradiograph from an adjacent section showing the binding of ^{125}I-LSD. This figure is derived from data of the previously reported study of Kadan and Hartig (1988). There is significant labeling in the neuropil (np), the central region of the ganglion that does not contain nerve cell bodies but does contain numerous processes and synaptic contacts. In addition, there is clear and dense labeling on a single nerve cell body in the upper left quadrant of the ganglion. The neuron which appears to correspond to that labeled cell is indicated by the arrow in Figure 1A. This large neuron is part of the upper left quadrant identified cells of the L1–6 group. By virtue of its location, it appears to be cell L1. Although not illustrated in this particular section, a second labeled neuron was found in a symmetrical position in the upper right quadrant. This cell was tentatively identified as cell R1 on the basis of location, size and pigmentation. The electrophysiologic investigations below were done to identify the cells definitively and to characterize their responses to serotonin.

Identification of cells

Cells in the upper left quadrant of the abdominal ganglion could be identified by position, color, action potential firing pattern, synaptic input and response to acetylcholine. We use the nomenclature established by Frazier et al. (1967). Cells L1, L2, L3, L4, and L6 were originally described as having H (hyperpolarizing) responses to acetylcholine. As described by Kehoe (1972), the acetylcholine (ACh) response of L2, L3, L4, and L6 could be resolved into a biphasic hyperpolarization un-

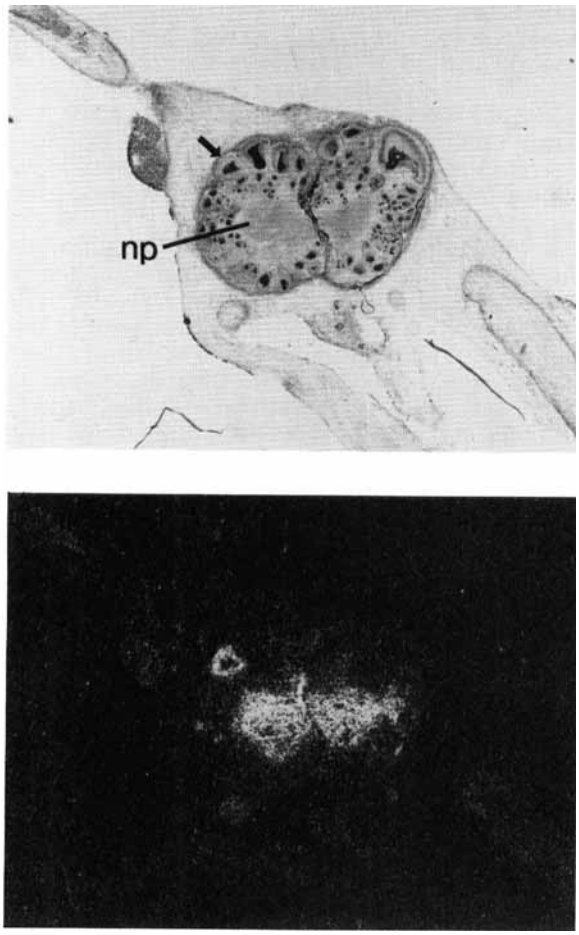

Fig. 1. Distribution of ^{125}I-LSD binding to a tissue section from an abdominal ganglion. A: Hematoxylin-stained tissue section from the abdominal ganglion. Nuclei of individual neuronal soma appear as darkly stained amorphous structures surrounding the central neuropil (np). The arrow in the upper left quadrant points to a single neuronal cell body (L1), which appears to be stained more lightly throughout the cytoplasm than adjacent neurons. B: Autoradiogram of ^{125}I-LSD total binding. A high level of labeling is observed throughout the neuropil of both hemiganglia. In addition, a single large neuronal soma in the upper left quadrant is labeled by ^{125}I-LSD. Note that the labeled soma corresponds precisely in shape and position with the neuron indicated by an arrow in A. A,B: Two immediately adjacent 10-μm tissue sections.

der current clamp conditions. Under voltage clamp, the current consisted of an initial rapidly rising, rapidly desensitizing current with equilibrium potential close to the calculated equilibrium potential for Cl^- (−60 mV), followed by a slowly rising, slowly desensitizing current that tended to zero near the calculated K^+ equilibrium potential (−85 mV) (right-hand records in Fig. 2). This is similar to the acetylcholine response of the medial cells of the pleural ganglion (Kehoe, 1972; Salanki et al., 1989).

The acetylcholine response of L1 and R1 consisted of a single component, a rapidly rising, rapidly desensitizing current reversing close to the Cl^- equilibrium potential (left records in Fig. 2). This difference in the acetylcholine response, together with the soma size, location, and color, permitted an unambiguous identification of L1 and R1.

Serotonin responses of cells L3 and L6

Despite the similarities of their acetylcholine responses, the serotonin response of cells L2, L3, L4, and L6 fell into two categories. Cells L3 and L6 responded to 50 μM serotonin with a slowly rising outward current due to a conductance increase that showed little desensitization (Fig. 3A). This current declined to near zero close to the K^+ equilibrium potential but did not reverse sign even at −120 mV. This response has some similarities to the B type of response described by Gerschenfeld and Paupardin-Tritsch (1974a), although we could not block it with 5-methoxygramine. Subsequent experiments using voltage ramps to generate current-voltage curves demonstrated an increase of the slope of the inwardly rectifying portion of the current–voltage curve similar to that described by Adams and Benson (1985).

Application of 20 μM cyproheptadine or mianserin for 10 min prior to application of 50 μM serotonin plus drug did not block the K^+ response. This is illustrated for cyproheptadine in Figure 3B.

Serotonin responses of cells L2 and L4

Cells L2 and L4 gave a biphasic response to 50 μM serotonin consisting of an inward current that was rapidly rising and desensitizing and associated with a conductance increase, followed by a sustained, voltage dependent inward current apparently due to a conductance decrease (Fig. 4). The extrapolated equilibrium potential for the fast phase was close to 0 mV, indicating that this response was the A type described by Gerschenfeld and Paupardin-Tritsch (1974a). This was further confirmed by demonstrating that it was blocked by 10–30 μM tryptamine.

The slow response was voltage dependent in a similar fashion to the serotonin response described by Pellmar and Carpenter (1980) and was blocked by Co^{2+} ion. The apparent conductance decrease in response to the 10 mV hyperpolarizing command pulses applied from the holding potential can be accounted for by assuming that the voltage step was sufficient to close a significant number of the steeply voltage dependent channels, resulting in a decrease of the membrane current in response to the command steps compared with the control. Cyproheptadine or mianserin (20 μM) pretreatment for ten minutes blocked only the Na^+ conductance (lower records).

Serotonin responses of cells L1 and R1

L1 and R1 gave a triphasic response to serotonin (Fig. 5). The initial response was a very fast, inward current identical to that seen in cells L2 and L4. There was also the late, voltage dependent inward current characteristic of L2 and L4. In addition to the Na^+ conductance and the voltage dependent conductance, an outward current due to a conductance increase was interposed between these two. This current had an equilibrium potential close to that for Cl^-, and was partially blocked by 100 μM neostigmine (not illustrated). Thus, it appears similar to the C serotonin response described by Gerschenfeld and Paupardin-Tritsch (1974a). The current voltage responses of the three serotonin responses of a representative L1 cell are shown in Figure 6.

Cyproheptadine and mianserin (20 μM) both blocked the Na^+ and Cl^- responses but did not affect the voltage

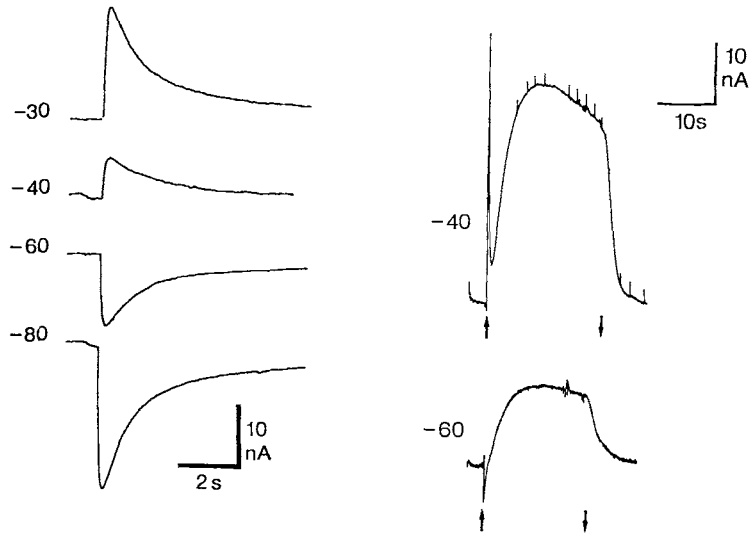

Fig. 2. Acetylcholine responses of L1 and L3. The records on the left are responses to a brief microperfusion of acetylcholine (5×10^{-5} M) onto L1. The numbers at the left of each panel are the holding potential in mV. The R1 acetylcholine response was similar. Both appear to be pure Cl^- conductances. On the right, responses of L3 to acetylcholine (5×10^{-3} M, applied between arrows). The response is clearly biphasic at both −40 and −60 mV, with the early fast component reversing near to −60 mV (the Cl^- equilibrium potential) and the late outward current smaller at −60. This is a K^+ conductance. Acetylcholine responses of L2, L4, and L6 were similar. Note slower time scale of L3 recording.

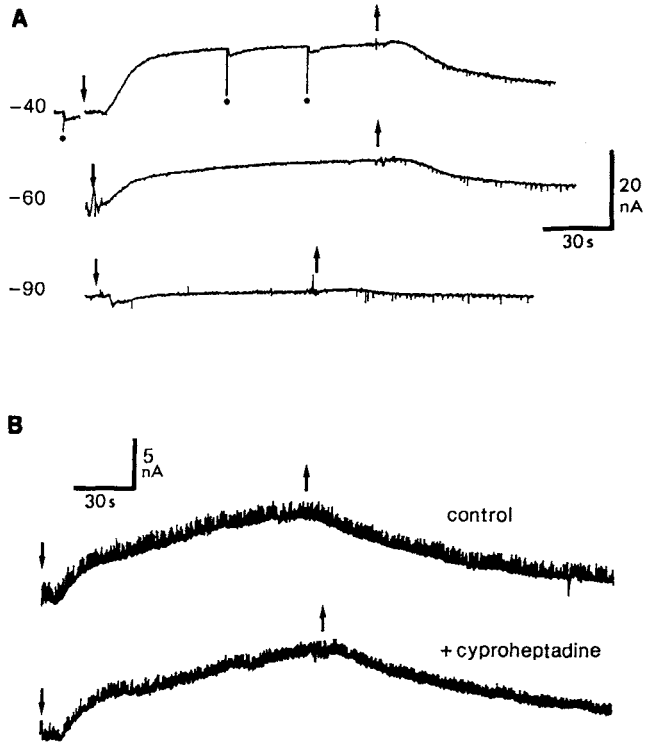

Fig. 3. Serotonin responses of cells L3 and L6. A: Responses of L6 at three holding potentials (in mV at left of each record). Serotonin (50 μM) was applied by microperfusion between arrows. Asterisks (*) below the −40 mV record indicate current in response to 10 mV hyperpolarizing voltage command steps. The response is an outward current associated with a conductance increase which is abolished at −90 mV and is therefore identified as a conductance increase to K^+. B: Recordings from L3 and the lack of effect of cyproheptadine on the L3/L6 serotonin response. Upper record: Control application of 50 μM serotonin. Lower record: Application of 50 μM serotonin + 20 μM cyproheptadine following 10 min pretreatment with 20 μM cyproheptadine. Holding potential −40 mV. Mianserin also had no effect on the L3/L6 serotonin response.

dependent response. This is shown in Figure 7, where a holding potential of −40 mV was used to show the Na^+ and Cl^- components of the response but to minimize the voltage dependent Ca^{2+} component. The Cl^- response was also blocked by d-tubocurarine (100 μM), in agreement with the previous observations of Gerschenfeld and Paupardin-Tritsch (1974a). LSD (500 nM) blocked the Cl^- response of L1 with little if any effect on the early Na^+ component. The late voltage and calcium dependent current was not affected by LSD. This is shown in Figure 8.

DISCUSSION

The results presented in this study suggest that ^{125}I-LSD binds to a subset of serotonin receptors that are coupled to a Cl^- channel on the somata of cells L1 and R1 and is widely distributed in the neuropil. These two cells are anatomically symmetrical, are unusual for neurons with cell bodies in the abdominal ganglion in having their axons in the connective nerves and might be expected to have similar pharmacological properties. Although we have not surveyed other regions of the abdominal ganglion as thoroughly as the left upper quadrant, we have not seen this serotonin induced Cl^- current in any other cells (e.g., L2–6, RB or RC neurons). Thus, for the cell bodies studied the localization and pharmacological similarity between the ^{125}I-LSD binding site and the serotonin evoked electrophysiological response in these cells is precise.

There are multiple responses that can be elicited by serotonin in both mammalian (Peroutka, 1988) and invertebrate (Gerschenfeld and Paupardin-Tritsch, 1974a) neurons. LSD and its derivatives have been found to have actions at several of these responses, although with differing efficacy (Peroutka, 1988). In *Aplysia*, Gerschenfeld and Paupardin-Tritsch (1974a) reported that LSD blocks the A (fast Na^+), B (K^+), and C (Cl^-) responses without affecting the A′ (slow Na^+) or α and β responses, due to conductance decreases. In ver-

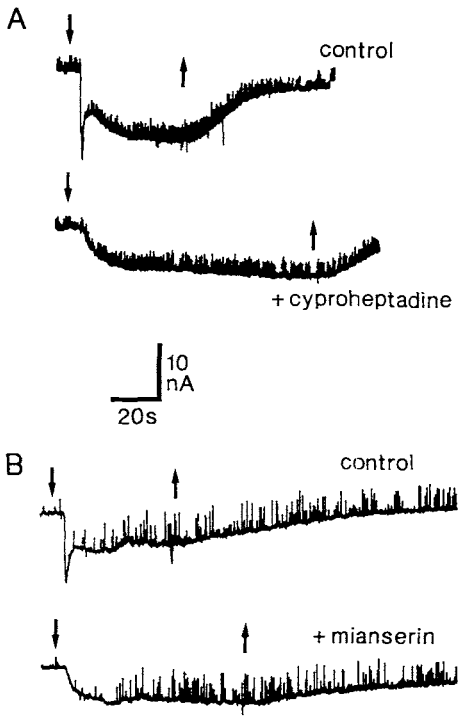

Fig. 4. Effect of cyproheptadine and mianserin on the L2/L4 serotonin responses. Records were obtained from two experiments on cell L4. The upper traces (A,B) show the control responses to 50 μM serotonin, applied between the arrows, obtained at a holding potential of −35 mV. A,B: The lower traces show effects of application of 50 μM serotonin 10 min after perfusion of 20 μM cyproheptadine or mianserin, respectively. Both blocked the Na^+ but not the Ca^{2+} current.

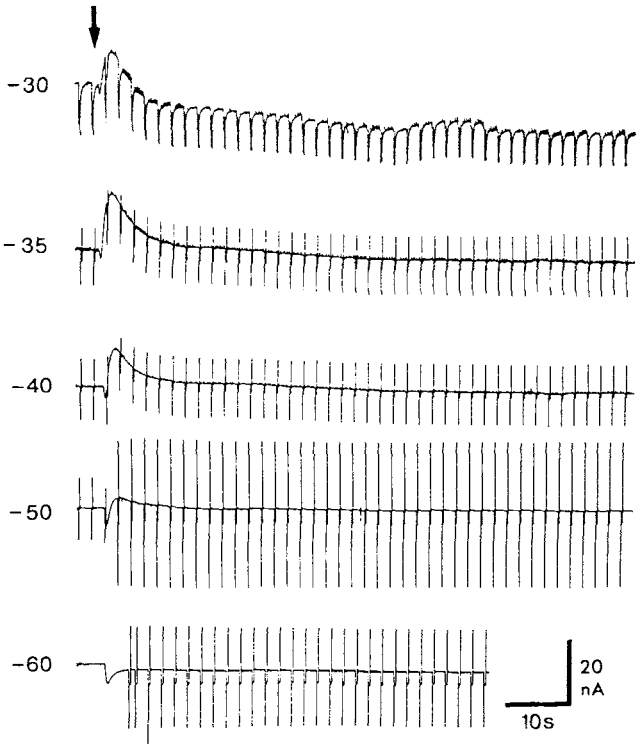

Fig. 5. Serotonin responses of L1 and R1, in this case a recording from cell R1. Holding potentials are indicated at the left of each record. Serotonin was applied at the arrow. Conductance monitoring pulses indicate the current in response to 10 mV hyperpolarizing command steps. Note the very early, rapid inward and late slow sustained inward currents as in cells L2 and L4. There is an additional outward current due to a conductance increase interposed between the early inward and late inward currents.

tebrate systems LSD also binds to more than one serotonin receptor (Peroutka and Snyder, 1979; Seeman et al., 1980).

We have confirmed the report of Gerschenfeld and Paupardin-Tritsch (1974a) that the Cl^- response is very sensitive to LSD, but find the A responses considerably less sensitive. We also have confirmed the observation that D-tubocurarine and neostigmine block the serotonin induced Cl^- current. These compounds do not antagonize the autoradiographic labelling of L1 and R1 by ^{125}I-LSD (Kadan and Hartig, 1988). This apparent discrepancy may be accounted for by proposing ^{125}I-LSD binds to the agonist recognition site of the receptor–channel complex. Cyproheptadine, mianserin, and LSD presumably act at the serotonin recognition site, blocking both autoradiographic labeling and the electrophysiological response. D-Tubocurarine and neostigmine may act as channel blocking drugs at the Cl^- channel and block only the electrophysiological response. There is evidence that high concentrations of carbamate anticholinesterases antagonize the Na^+ acetylcholine channel (Gage and McBurney, 1975), although there is no equivalent information for the acetylcholine Cl^- response. D-Tubocurarine also antagonists the acetylcholine Cl^- response and may act as a general Cl^- channel blocker at the effective concentration of 100 μM (Carpenter et al., 1977). It appears that cyproheptadine and mianserin are specific and potent antagonists of both the serotonin Na^+ and Cl^- responses and most probably act at the agonist recognition site.

Kadan and Hartig (1988) provided evidence that guanine nucleotides modulate the serotonin affinity at the LSD-sensitive site, suggesting that a G protein is involved in transduction at this receptor-ionophore complex. G proteins have been implicated in serotonin stimulation of cyclic AMP production in the mammalian central nervous system (Nelson et al., 1980) as well as in *Aplysia* (Drummond et al., 1980). However, the serotonin-evoked ionic responses in which cyclic AMP has been specifically implicated are a potassium conductance decrease (Deterre et al., 1986) and a potassium conductance increase (Drummond et al., 1980). The identification of the LSD-sensitive site as a serotonin receptor coupled to a Cl^- channel supports the conclusion that a G protein is essential in the receptor–ionophore coupling for Cl-dependent responses on some neurons. While not all G protein responses are coupled to cyclic AMP, the previous studies linking serotonin stimulation of cyclic AMP to G proteins raise the possibility, which deserves further study, that cyclic AMP may also play a role in some Cl-dependent responses in *Aplysia*.

There are several striking similarities between the *Aplysia* serotonin Cl^- receptor and the mammalian serotonin 1C receptor which has recently been cloned and expressed in *Xenopus* oocytes (Gunderson et al., 1983; Julius et al., 1988; Lübbert et al., 1987a,b). Activation of this receptor in oocytes induces a Cl^- conductance increase response. Activation is dependent on a G protein, and LSD, mianserin, and cyproheptadine all bind to the receptor with high affinity. The activation of

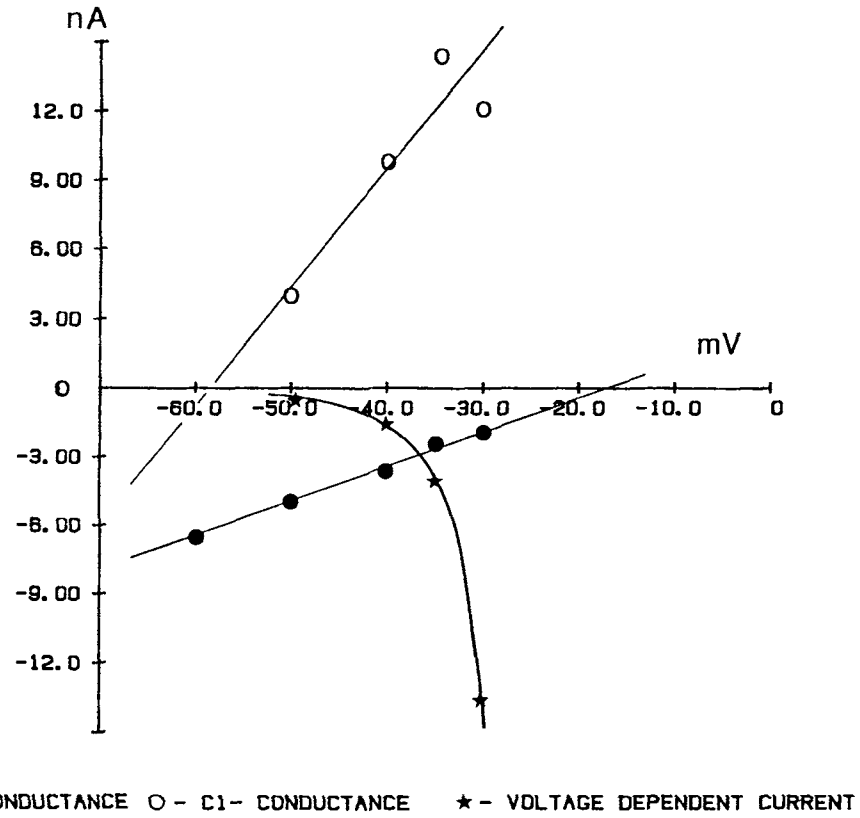

Fig. 6. Current-voltage relation for the three currents shown in Figure 5. (●) early inward (Na) current, (○) fast outward (Cl) current and stars the slow inward (Ca) current.

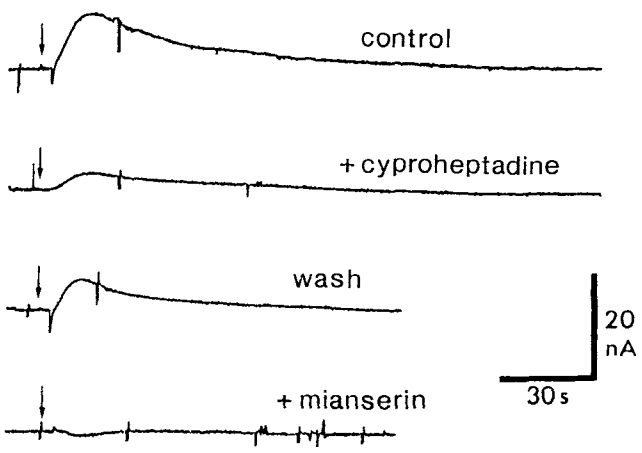

Fig. 7. Effect of cyproheptadine and mianserin on the L1/R1 serotonin responses. The control response is from R1 clamped at a holding potential of −40 mV with application of 50 μM serotonin, applied at the arrow. This holding potential was chosen to minimize the slow inward current in order to study the blockade of the fast inward and outward currents. Pretreatment (10 min) with 20 μM cyproheptadine reversibly blocked the rapid inward and reduced the later outward currents in response to application of 50 μM serotonin + 20 μM cyproheptadine. Serotonin (50 μM) applied after 30 min ASW wash shows full recovery of the rapid inward and partial recovery of outward current. Application of 50 μM serotonin + 20 μM mianserin following 10 min pretreatment with 20 μM mianserin resulted in total blockade of both components. This blockade was also almost totally reversible with prolonged washing.

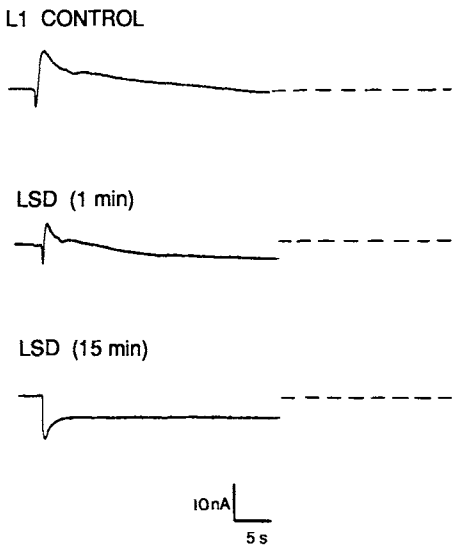

Fig. 8. Effect of LSD on the L1/R1 serotonin response. This recording comes from an experiment on L1 recorded at a holding potential of −40 mV. In the control there is an obvious rapid inward current followed by the relatively rapid outward current which reverses at the Cl^- equilibrium potential. The voltage-dependent Ca^{2+} current is not clearly apparent because of the holding potential, but some trace of it is indicated by the irregularities on the falling phase of the Cl^- component. Following a 1-min application of 500 nM LSD, there is a clear reduction in the Cl^- component of the response to 50 μM serotonin plus 500 nM LSD. After 15-min LSD application, the Cl^- component in response to serotonin plus LSD is gone, but the fast Na^+ remains, and a prolonged inward current that had been obscured becomes apparent.

the receptor is thought to be associated with synthesis of inositol phosphates which lead to release of Ca^{2+} from intracellular stores. The Cl^- channel is thought to be activated secondary to the elevated intracellular Ca^{2+} concentration. We have no information on possible Ca^{2+} dependency of the *Aplysia* response.

It is of interest to note that the *Aplysia* Cl^- conductance increase has a relatively slow time course compared to acetylcholine Cl^- responses. The time to peak of the serotonin Cl^- response is in the range 2–10 s in our recordings, whereas the acetylcholine Cl^- response (which is presumably a directly linked receptor-ionophore unit) rises to a peak in 300–800 ms and shows rapid desensitization (see Fig. 2). Thus far, the agonist responses most convincingly demonstrated to be mediated by G proteins in *Aplysia* are the slow time course K^+ acetylcholine, dopamine, and histamine responses (Sasaki and Sato, 1987). The demonstration that guanine nucleotides and their analogues alter the binding of ^{125}I-LSD in the abdominal ganglion suggests that in cells L1 and R1, one type of serotonin receptor is coupled to Cl^- channels via a G protein mechanism whereas the acetylcholine receptors are directly coupled to Cl^- channels. The Cl^- channels may have similar properties. Further electrophysiological studies will be needed to confirm this point.

REFERENCES

Adams, W.B., and Benson, J.A. (1985) The generation and modulation of endogenous rhythmicity in the *Aplysia* bursting pacemaker neurone R15. Prog. Biophys. Mol. Biol., 46:1–49.

Adams, W.B., and Levitan, I.B. (1982) Intracellular injection of protein kinase inhibitor blocks the serotonin-induced increase in K^+ conductance in *Aplysia* neuron R15. Proc. Natl. Acad. Sci. USA, 79:3877–3880.

Aghajanian, G.K. (1981) The modulatory role of serotonin at multiple receptors in brain. In: Serotonin Neurotransmission and Behavior. B.L. Jacobs and A. Gelperin, eds. MIT Press, Cambridge, pp. 156–188.

Altar, C.A., Boyar, W.C., and Marien, M.R. (1986) ^{125}I-LSD autoradiography confirms the preferential localization of caudate–putamen S_2 receptors to the caudal (peripallidal) region. Brain Res., 372:130–136.

Brownstein, M.J., Saavedra, J.M., Axelrod, J., Zeman, G.H., and Carpenter, D.O. (1975) Coexistence of several putative neurotransmitters in single identified neurons of *Aplysia*. *Proc. Natl. Acad. Sci. (USA)*, 71:4662–4665.

Carpenter, D., Breese, G., Schanberg, S., and Kopin, I. (1971) Serotonin and dopamine: Distribution and accumulation in *Aplysia* nervous and non-nervous tissues. Int. J. Neurosci., 2:49–56.

Carpenter, D.O., Swann, J.W., and Yarowsky, P.J. (1977) Effect of curare on responses to different putative neurotransmitters in *Aplysia* neurons. J. Neurobiol., 8:119–132.

Cedar, H., and Schwartz, J. (1972) cAMP in the nervous system of *Aplysia californica*. II. Effect of serotonin and dopamine. J. Gen. Physiol., 60:570–581.

Deterre, P., Paupardin-Tritsch, D., Bockaert, J. and Gerschenfeld, H.M. (1982) cAMP-mediated decrease in K^+ conductance evoked by serotonin and dopamine in the same neuron: A biochemical and physiological single cell study. Proc. Natl. Acad. Sci. USA 79:7934–7938.

Deterre, P., Paupardin-Tritsch, D., and Bockaert, J. (1986) Serotonin- and dopamine-sensitive adenylate cyclase in molluscan nervous system. Biochemical and electrophysiological analysis of the pharmacological properties and the GTP-dependence. Mol. Brain Res., 1:101–109.

Drummond, A.H., Bucher, F., and Levitan, I.B. (1980) D-[^{3}H]Lysergic acid diethylamide binding to serotonin receptors in the molluscan nervous system. J. Biol. Chem., 255:6679–6686.

Engel, G., Muller-Schweinitzer, E., and Palacios, J.M. (1984) 2-^{125}I-LSD, a new ligand for the characterization and localization of 5-HT_2 receptors. Naunyn-Schmiedbergs Arch. Pharmacol., 325:328–336.

Frazier, W.T., Kandel, E.R., Kupfermann, I., and Coggeshall, R.E. (1967) Morphological and functional properties of identified neurons in the abdominal ganglion of *Aplysia californica*. J. Neurophysiol., 30:1228.

Gape, P.W., and McBurney, R.N. (1975) Effects of membrane potential, temperature and neostigmine on the conductance change caused by a quantum of acetylcholine at the toad neuromuscular junction. J. Physiol. (Lond.), 244:385–407.

Gelperin, A. (1981) Synaptic modulation by identified serotonin neurons. In: Serotonin Neurotransmission and Behavior. B.L. Jacobs and A. Gelperin, eds. MIT Press, Cambridge, pp. 288–306.

Gerschenfeld, H.M., and Stefani, E. (1965) 5-Hydroxytryptamine receptors and synaptic transmission in molluscan neurones. Nature 205:1216–1218.

Gerschenfeld, H.M., and Paupardin-Tritsch, D. (1974a) Ionic mechanisms and receptor properties underlying the responses of molluscan neurons to 5-hydroxytryptamine. J. Physiol. (Lond.), 243:427–456.

Gershenfeld, H.M., and Paupardin-Tritsch, D. (1974b) On the transmitter function of 5-HT at excitatory and inhibitory monosynaptic junctions. J. Physiol. (Lond.), 243:457–481.

Gerschenfeld, H.M., Paupardin-Tritsch, D., and Deterre, P. (1981) Neuronal responses to serotonin: A second view. In: Serotonin Neurotransmission and Behavior. B.L. Jacobs and A. Gelperin, eds. MIT Press, Cambridge, pp. 105–130.

Gunderson, C.B., Miledi, R., Parker, F.R.S., and Parker, J. (1983) Serotonin receptors induced by exogenous messenger RNA in *Xenopus* oocytes. Proc. R. Soc. Lond. B, 219:103–109.

Hartig, P.R., Krohn, A.M., and Hirrschman, S.A. (1985) Microchemical synthesis of the serotonin receptor ligand, ^{125}I-LSD. Anal. Biochem., 144:441–446.

Jahan-Parwar, B., S.-Rozsa, K., Salanki, J., Evans, M.L., and Carpenter, D.O. (1987) *In vivo* labeling of serotonin containing neurons by 5,7-dihydroxytryptamine in *Aplysia*. Brain Res., 426:173–178.

Julius, D., MacDermott, A.B., Axel, R., and Jessell, T.M. (1988) Molecular characterization of a functional cDNA encoding the serotonin 1C receptor. Science, 241:558–564.

Kadan, M.J., and Hartig, P.R. (1988) Autoradiographic localization and characterization of [^{125}I]-lysergic acid diethylamide binding to serotonin receptors in *Aplysia*. Neuroscience 24:1089–1102.

Kadan, M.J., Krohn, A.M., Evans, M.J., Waltz, R.L., and Hartig, P.R. (1984) Characterization of ^{125}I-LSD binding to serotonin receptors in rat frontal cortex. J. Neurochem., 43:601–606.

Kazuhiko, S., and Sato, M. (1987) A single GTP-binding protein regulates K^+-channels coupled with dopamine, histamine and acetylcholine receptors. Nature, 325:259–262.

Kebabian, P.R., Kebabian, J.W., and Carpenter, D.O. (1979) Regulation of cyclic AMP in heart and gill of *Aplysia* by the putative neurotransmitters dopamine and serotonin. Life Sci., 24:1757–1764.

Kehoe, J.S. (1972) Three acetylcholine receptors on *Aplysia* neurones. J. Physiol. (Lond.), 225:115–146.

Koester, J., Mayeri, E., Liebeswar, G., and Kandel, E.R. (1973) Cellular regulation of homeostasis: neuronal control of the circulation in *Aplysia*. Fed. Proc. 32:2179–2187.

Levitan, E.S., and Levitan, I.B. (1988) Serotonin acting via cyclic AMP enhances both the hyperpolarizing and depolarizing phases of bursting pacemaker activity in the *Aplysia* neuron R15. J. Neurosci., 8:1152–1161.

Longley, R.D., and Longley, A.J. (1986) Serotonin immunoreactivity of neurons in the gastropod *Aplysia california*. J. Neurobiol., 17:339–358.

Lübbert, H., Hoffman, B.J., Snutch, T.P., van Dyke, T., Levine, A.J., Hartig, P.R., Lester, H.A., and Davidson, N. (1987a) DNA cloning of a serotonin 5-HT 1C receptor by electrophysiological assays of mRNA-injected *Xenopus* oocytes. Proc. Natl. Acad. Sci. USA, 84:4332–4336.

Lübbert, H., Snutch, T.P., Dascal, N., Lester, H.A. and Davidson, N. (1987b) Rat brain 5-HT 1c receptors are encoded by a 5–6 kbase mRNA size class and are functionally expressed in injected *Xenopus* oocytes. J. Neurosci., 7:1159–1165.

Nelson, D.L., Herbert, A., Enjalbert, A., Bockaert, J., and Hamon, M. (1980) Serotonin-sensitive adenylate cyclase and [^{3}H]serotonin binding sites in the CNS of the rat. 1. Kinetic parameters and pharmcological properties. Biochem. Pharmacol., 29:2445–2453.

Pellmar, T.C., and Carpenter, D.O. (1980) Serotonin induces a voltage-sensitive calcium current in neurons of *Aplysia californica*. J. Neurophysiol., 44:423–439.

Peroutka, S.J. (1988) 5-Hydroxytryptamine receptor subtypes. Annu. Rev. Neurosci., 11:45–60.

Peroutka, S.J., and Snyder, S.H. (1979) Multiple serotonin receptors: Differential binding of [H3]5-hydroxytryptamine, [H3]lysergic acid diethylamide and [H3]spiroperidol. Mol. Pharmacol., 16:687–699.

Salanki, J., Evans, M.L., and Carpenter, D.O. (1989) Desensitization kinetics of a K^+ acetylcholine response in *Aplysia*. Brain Res., 495:298–308.

Sasaki, K., and Sato, M. (1987) A single GTP-binding protein regulates K^+-channels coupled with dopamine, histamine and acetylcholine receptors. Nature 325:259–262.

Seeman, P., Westman, K., Coscina, D., and Warsh, J.J. (1980) Serotonin receptors in hippocampus and frontal cortex. Eur. J. Pharmacol., 66:179–191.

Slater, N.T., Hall, A.F., and Carpenter, D.O. (1984) Kinetic properties of cholinergic desensitization in *Aplysia* neurons. Proc. R. Soc. Lond. B, 223:63–78.

Vandermaelen, C.P. (1985) Serotonin. In: Neurotransmitter Actions in the Vertebrate Nervous System. M.A. Rogawski and J.L. Barker, eds. Plenum Press, New York, pp. 201–240.

Weiss, S., and Drummand, G.I. (1981) Dopamine- and serotonin-sensitive adenylate cyclase in the gill of *Aplysia californica*. Mol. Pharmacol., 20:592–597.

Whitaker, P.M., and Seeman, P. (1978) Selective labeling of serotonin receptors by d-[^{3}H]lysergic acid diethylamide in calf caudate. Proc. Natl. Acad. Sci. USA, 75:5783–5787.

Yagaloff, K.A., and Hartig, P.R. (1985) ^{125}I-LSD binds to a novel serotonergic site on rat choroid plexus epithelial cells. J. Neurosci., 5:3178–3183.