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SEROTONIN-INDUCED MODULATION OF EXCITABILITY IN AN IDENTIFIED *HELISOMA TRIVOLVIS* NEURON

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Summary

Exogenous serotonin (5-hydroxytryptamine; 5-HT) induced inhibitory effects in *Helisoma trivolvis* buccal neuron 5 (B5), which included a hyperpolarization of the membrane potential, a reduction in input resistance and a decrease in the stimulus-evoked spiking rate. The reversal potential for the 5-HT-induced hyperpolarization was –88.7 mV, suggesting the involvement of an enhanced K^+ conductance. Input/output curves measuring the number of action potentials evoked by current injection demonstrated a 5-HT-induced reduction in B5 neuronal excitability that was dose-dependent with a 50 % inhibitory concentration (IC_{50}) of 1.1 μmol l^{-1}. These inhibitory effects on neuron B5 were in striking contrast to the excitatory effects of this neurotransmitter on the buccal radular tensor motoneuron B19. In this neuron, 5-HT elicited a sustained depolarization and enhanced spiking activity. Previous cell culture studies have demonstrated that 5-HT also inhibits B19 growth cone motility and neurite outgrowth, but has no effect on the extension of neuritic processes from neuron B5. These effects, and the divergent effects of 5-HT on the outgrowth of neurons B5 and B19, probably result from differential effects of 5-HT on neuronal membrane potential and excitability. Modulation of the levels of spiking activity, translated into changes in neuronal Ca^{2+} levels, would cause alterations in growth cone motility. Previous studies have shown that the effects of 5-HT on B19 membrane potential and neurite extension are cyclic-AMP-dependent. We have used specific activators and inhibitors of signal transduction mechanisms to demonstrate that the 5-HT-induced reductions in B5 neuronal excitability are cyclic-AMP-independent and may involve the lipoxygenase pathway of arachidonic acid metabolism. Our results support the hypothesis that modulatory signals and signal transduction pathways regulating the plasticity of mature neural circuits may also modulate the differentiation of the neurons comprising those networks during development.

Key words: serotonin, neuromodulation, excitability, arachidonic acid, cyclic AMP, *Helisoma trivolvis*.

Introduction

Modulation of neuronal excitability is an important mechanism underlying cellular and behavioral plasticity (Kaczmarek and Levitan, 1987). The biogenic amine serotonin (5-hydroxytryptamine; 5-HT) modulates a wide range of biophysical properties of neurons. The specific neuronal effects of serotonin are dependent on the receptor subtype involved and the signal transduction pathways activated. Thus, for example, variable modulatory responses to serotonin in crab stomatogastric neurons are mediated by distinct 5-HT receptors (Zhang and Harris-Warrick, 1994). Serotonin also elicits differential effects on excitability in dorsal horn neurons of the frog spinal cord (Tan and Miletic, 1990). In addition, multiple serotonergic mechanisms are thought to contribute to diverse modulatory actions ranging from synaptic plasticity associated with molluscan learning (Pieroni and Byrne, 1992) to pre-pulse inhibition of startle responses in rats (Sipes and Geyer, 1994). Differential responses of neuronal growth cones to 5-HT have also been demonstrated at regenerating tips of *Helisoma trivolvis* neurons in cell culture (Haydon *et al.* 1984) and *in vivo* (Murrain *et al.* 1990). In the present study, we have addressed the question of whether 5-HT induces differential effects on the excitability of specific neurons from *Helisoma trivolvis* and whether such modulation might account for the varied effects of 5-HT on neuritic differentiation.

Serotonin has been implicated in the modulation of neuronal development, especially arborization, in several invertebrate nervous systems (Budnik *et al.* 1989; Goldberg and Kater, 1989; Diefenbach *et al.* 1995; Oland *et al.* 1995). Such findings have resulted in the hypothesis that neurotransmitters involved in the modulation of adult behavior may also be crucial regulators of neuronal differentiation during development and regeneration of the nervous system (Haydon and Drapeau, 1995; Zoran and Poyer, 1996). The buccal nervous system in *Helisoma trivolvis* has played a significant role in our

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understanding of how neurotransmitters regulate growth cone motility and neurite extension (Kater and Mills, 1991; Davis *et al.* 1992). Haydon *et al.* (1984) demonstrated that serotonin had pronounced and reversible inhibitory effects on neurite extension of neuron B19, but had no effect on the growth cone of another buccal neuron, B5. Subsequently, it was shown that serotonin depletion with 5,7-dihydroxytryptamine during embryogenesis resulted in abnormal differentiation and synaptic coupling of B19 (Goldberg and Kater, 1989). In addition, serotonin caused disruption of axonal regeneration of neuron B19, but not B5, following the crushing of a nerve within the adult buccal nervous system (Murrain *et al.* 1990).

Cultured *Helisoma trivolvis* buccal neurons provide an experimental system in which the mechanisms underlying the modulatory effects of serotonin can be readily investigated. A series of studies have determined that inhibition of B19 neurite outgrowth by 5-HT is probably a result of the sustained depolarization associated with a 5-HT-activated, cyclic-AMP-dependent Na^+ current (Price and Goldberg, 1993). Action potentials elicited by this depolarization caused Ca^{2+} influxes that, in turn, mediated the cessation of growth cone motility (Cohan *et al.* 1987; McCobb and Kater, 1988), perhaps through activation of the Ca^{2+}-binding protein calmodulin (Polak *et al.* 1991). Treatment with analogs of cyclic AMP mimicked the effect of 5-HT on B19 neurite outgrowth (Mattson *et al.* 1988).

The present study examines the effects of serotonin on *Helisoma trivolvis* buccal neuron B5 in order to determine whether the differential effects of 5-HT on neurite outgrowth of buccal neurons might stem from activation of disparate membrane biophysical properties (e.g. ion conductances and signaling mechanisms). Our results demonstrate a robust inhibitory effect of serotonin on the resting membrane potential and membrane excitability of neuron B5 following exogenous exposure to 5-HT. We have characterized these inhibitory effects both electrophysiologically and pharmacologically and have investigated the role of cyclic-AMP-dependent mechanisms in the mediation of this serotonin-induced neuromodulation.

Materials and methods

Experiments were conducted on laboratory stocks of albino (red) pond snails, *Helisoma trivolvis* (Say), which were maintained in 75 l aquaria at 26 °C. Aquaria were kept on a controlled photoperiod of 12 h:12 h L:D. Animals were fed lettuce and trout chow daily.

Reduced ganglia preparations

Snails were deshelled and pinned to a Sylgard-coated dissecting dish. For studies of semi-intact (reduced) ganglion preparations, a midline incision was made in the dorsal body wall. Removal of the buccal ganglia was carried out by severing the cerebrobuccal connectives and the heterobuccal, ventrobuccal and posterior buccal nerves. In addition, the esophagus was cut from its site of connection to the buccal musculature. When ganglia were pinned dorsal-side-up, neuronal cell bodies, including that of B5, could be readily identified. In some studies, the central ring and buccal ganglia were removed together leaving the cerebral–buccal connectives intact. Prior to electrophysiological recording, dissected buccal ganglia were stored briefly in defined medium (DM) consisting of Leibowitz-15 (L-15, formula no. 82-5154EC Gibco Laboratories) containing *Helisoma trivolvis* salts (in mmol l^{-1}): 40.0 NaCl, 1.7 KCl, 4.1 $CaCl_2$, 1.5 $MgCl_2$ and 10.0 Hepes; pH 7.5.

Neuronal cultures

For studies of neurons isolated into cell culture, excised buccal ganglia were placed into 0.2 % trypsin (Sigma) in DM for 20 min to digest and weaken the ganglion sheath. Ganglia were pinned to a Sylgard-coated dish containing 2 ml of high-osmolarity DM (in mmol l^{-1}): 56.0 NaCl, 2.4 KCl, 5.7 $CaCl_2$, 2.1 $MgCl_2$ and 14.0 Hepes; pH 7.5). Esophageal nerve trunks, containing the axons of B5 neurons, were crushed using fine forceps. The ganglion sheath was cut along the dorsal surface, next to the soma of B5, using an electrolytically sharpened microknife. Pressure applied to the ganglion forced the cell body of B5 through the incision, and the neuron was collected into a fire-polished, non-adhesive micropipette using negative pressure produced by a microsyringe (Gilmont). The pipette was made non-adhesive by pretreatment with hemolymph collected from snails using sterile procedures. Neurons were then transferred into a non-adhesive, 35 mm culture dish (no. 1008, Falcon) containing 2 ml of DM. Culture dishes were made non-adhesive by pretreatment with a 0.5 % solution of bovine serum albumin (BSA). The neurons were maintained in these culture conditions until transferred to recording chambers for electrophysiological study.

Electrophysiological recordings

Electrophysiological properties of neurons were studied using intracellular recording techniques. Intracellular microelectrodes (borosilicate glass; Frederick Hear & Co.) were filled with 1.5 mol l^{-1} potassium chloride (or 1.5 mol l^{-1} potassium acetate) and possessed tip resistances ranging from 8 to 20 MΩ. Current-clamp recordings of neuronal membrane potentials were amplified using a bridge-balanced electrometer (Getting Instrumental Inc.), and recordings were viewed on a storage oscilloscope (Tektronix). Neuronal input resistance and excitability were measured by injecting constant-amplitude current pulses generated by a Grass S44 stimulator. Permanent records were obtained using a strip chart recorder (Gould).

Analysis of reduced ganglion preparations

The modulatory effects of serotonin on the biophysical properties of B5 were determined by eliciting bursts of action potentials using depolarizing current injection pulses. Preparations were pinned onto a Sylgard-coated glass Petri dish containing 2 ml of high-Ca^{2+} DM: L-15 containing (in mmol l^{-1}) 39.90 NaCl, 1.7 KCl, 41.0 $CaCl_2$, 1.5 $MgCl_2$ and 10.0 Hepes; pH 7.5). Increased levels of Ca^{2+} were used to

reduce the general feeding motor pattern activity of the preparation. B5 neurons were then penetrated using glass microelectrodes, and depolarizing current injection pulses (0.2–1.8 nA for 3 s) were applied every 20 s. Constant perfusion of the recording chamber (3 ml min^{-1}) was maintained throughout the experiment using a peristaltic pump (Pharmacia). Serotonin creatine sulfate (5-HT; Sigma) in high-Ca^{2+} DM was perfused through the recording chamber for 2 min. The numbers of stimulus-evoked action potentials were recorded before, during and after bath perfusion with serotonin. Experiments using DM (with normal Ca^{2+} levels) were also performed in the same manner. To determine the effects of serotonin on neuronal excitability, experiments were conducted in which the membrane potential was manipulated by current injection to a relatively constant basal level. Changes in neuronal input resistance were measured by injecting negative current pulses sufficient to elicit 10–60 mV changes in membrane potential.

Simultaneous electrophysiological recordings were made to investigate the effects of serotonergic cerebral neuron C1 on buccal neuron B5. Activity was evoked in B5 using a stimulating current pulse (0.2–1.8 nA for 3 s at 5 Hz) sufficient to elicit approximately 4–5 action potentials. A current pulse (6 s in duration) was then injected into neuron C1 to elicit neuronal spiking. The identity of cerebral and buccal neurons was confirmed by Lucifer Yellow injections and fluorescence microscopy (data not shown).

Analysis of neuronal cell cultures

The effects of exogenous serotonin on neuron B5, in the absence of all synaptic inputs, were examined using neurons isolated into cell culture as described above. Cells were transferred from the non-adhesive culture dish into an adhesive (0.1 % poly-L-lysine-coated) dish containing DM. Cells were penetrated using a microelectrode as described above and current pulses were injected to elicit bursts of action potentials before, during and after bath perfusion with 50 μmol l^{-1} 5-HT. Experiments were also performed in which membrane voltage was manipulated by current injection into the soma to compensate for the effects of 5-HT on membrane potential. Dose-dependent effects of serotonin were examined both on isolated neurons and on reduced ganglion preparations.

Analysis of intracellular mechanisms involved in serotonin-induced modulation

Current–voltage relationships were determined by injecting isolated B5 neurons with a range of negative current pulses in the presence or absence of 5-HT. Reversal potentials for the 5-HT-induced effects on B5 membrane potential were estimated in the presence of extracellular K^+ concentrations of 0.85, 1.7 and 3.4 mmol l^{-1}. Serotonin was perfused in DM containing the appropriate extracellular K^+ concentration. The effects of microapplication of 5-HT to localized regions of isolated B5 neurons were studied using a Picospritzer II microinjection system (General Valve Corp.). Micropuffs (4 ms pulses at 300 kPa) of 50 μmol l^{-1} 5-HT in DM were delivered specifically, using a fine glass pipette to somata, to axons and to fine neuritic regions of neuron B5. Puffs were visualized under the microscope by addition of Fast Green (Sigma) to the 5-HT pipette solution. The micropuffs were rapidly washed away from other regions of the neuron by a constant perfusion of DM flowing across the culture dish.

Pharmacological studies were performed to examine the intracellular mechanisms underlying serotonin-induced modulation of B5. The role of cyclic-AMP-dependent mechanisms was examined using activators or inhibitors of this second messenger pathway. Experiments included bath perfusion with an activator of adenylyl cyclase, forskolin (Sigma; at a concentration of 30 μmol l^{-1} in dimethyl sulfoxide, DMSO) and microinjection of an inhibitor of adenylyl cyclase, SQ22536 (CalBiochem; at a concentration of 100 μmol l^{-1} in distilled water). Neurons were injected with the activator or the inhibitor using glass pipettes connected to a pressure-injection system. Pipette tips were broken by fine contact with the bottom of the dish just prior to injection. Following cell penetration, several pulses of 5–10 ms duration (at 150 kPa) were applied until the neuronal soma began to swell. We estimate that microinjection led to an intracellular inhibitor concentration of between 100 and 1000 nmol l^{-1}. Sp-cAMP (adenosine-3′,5′-cyclic monophosphothioate, Sp-isomer; CalBiochem), a specific activator of cyclic-AMP-dependent protein kinase, and Rp-cAMP (adenosine-3′,5′-cyclic monophosphothioate, Rp-isomer; CalBiochem), an inhibitor of cyclic-AMP-dependent protein kinase, were injected as described above. The number of stimulus-evoked action potentials was averaged for current injection pulses applied as described above. 10 min after the injection of inhibitors, 50 μmol l^{-1} 5-HT was perfused through the recording chamber and its effects on B5 were assessed. The role of arachidonic acid in this serotonin-induced modulation was also examined using bath perfusion experiments with 50 μmol l^{-1} arachidonic acid (Sigma). In addition, the effects of the phospholipase A2 inhibitor BPB (4-bromophenacylbromide; Sigma; 50 μmol l^{-1} in 0.1 % DMSO) and the lipoxygenase pathway inhibitor NDGA (nordihydroguaiaretic acid; Sigma; 50 μmol l^{-1} in 0.1 % DMSO) were investigated. Following exposure to these inhibitors, serotonin was perfused through the recording chamber and its effects on B5 spiking frequency were assessed. Solutions for both the cyclic AMP pathway and the arachidonic acid pathway investigations were freshly made each day and stored at 4 °C until experimentation.

Data analysis

In many experiments, data were normalized to correct for variations in excitability between neurons. Statistical analyses were performed using StatView 4.1 (Abacus Concepts, Inc.). Comparisons of the effects of 5-HT on the electrophysiological properties of B5 during and after perfusion were made using paired Student's *t*-tests. Data from studies using pathway activators and inhibitors were calculated as a percentage of the effect of 5-HT on untreated and sham-treated controls not exposed to pharmacological agents. One-way analysis of

variance (ANOVA) followed by Fisher's paired least significant difference (PLSD) test was used for between-group comparisons. Data are presented as means ± S.D. or S.E.M. as indicated.

Results

Modulation of B5 neuronal properties by serotonin

The electrophysiological effects of serotonin on buccal neuron B5 were studied using current-clamp recordings (Fig. 1A). Bath perfusion of 5-HT at a concentration of 50 μmol l^{-1} across buccal ganglia preparations caused a significant reduction in somata input resistance (Student's *t*-test; $P<0.005$) from 73.3±15.8 MΩ before treatment to 44.3±17.2 MΩ (mean ± S.D.; $N=14$) during treatment. The effect of 5-HT was reversible, with input resistance returning to 95 % of pre-control levels following approximately 3 min of washing with DM. The resting membrane potential of B5 was −55.5±11.1 mV prior to 5-HT exposure and −60.2±9.9 mV (mean ± S.D., $N=14$) in the presence of 5-HT. These values were not statistically different. Associated with the 5-HT-induced reduction in input resistance was a pronounced decrease in the number of action potentials elicited by depolarizing current pulse injections. Neurons were injected with a continuous series of current pulses (3 s in duration) of sufficient strength in each cell to evoke a burst of 3–6 action potentials. As illustrated in Fig. 1A, stimulus-evoked bursts of action potentials in B5 displayed spike frequency adaptation such that spikes were only present during the initial phase of the depolarizing current pulse. In the presence of 50 μmol l^{-1} 5-HT, the number of action potentials within these bursts was significantly reduced (Student's *t*-test; $P<0.001$, $N=11$) to 10 % of pretreatment levels (Fig. 1B). After perfusion with DM for 3 min, there was a significant reversal of this effect compared with the levels in the presence of serotonin (Student's *t*-test; $P<0.001$).

In similar experiments, the membrane potential of B5 was manipulated using sustained depolarizing current injection to compensate for 5-HT-induced hyperpolarization, thereby returning the membrane potential to pretreatment levels while still in the presence of serotonin. Under these conditions (Fig. 1A, I+5-HT), significant 5-HT-induced reductions in stimulus-evoked spike frequency were still observed (Student's *t*-test; $P<0.02$, $N=6$) and were largely reversible, so that washing in DM led to a significant recovery of spiking frequency (Student's *t*-test; $P<0.05$). Taken together, these results show that exposure of buccal ganglia to serotonin caused a significant and reversible reduction in the input resistance and stimulus-evoked spiking of neuron B5. The

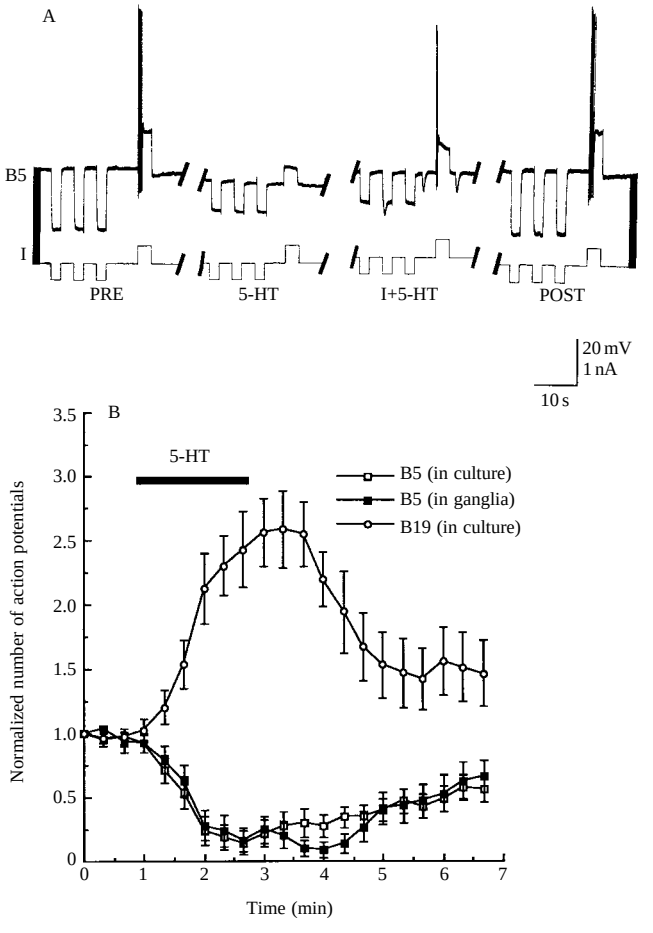

Fig. 1. Effects of serotonin (5-HT) on electrophysiological properties of neuron B5 in buccal ganglia preparations and in cell culture. (A) The modulatory effects of 5-HT at a concentration of 50 μmol l^{-1} on neuron B5 in a buccal ganglion preparation are shown. An intracellular recording of neuronal membrane potential (B5; upper trace) and associated current injection pulses (I; lower trace) show that 5-HT caused a membrane hyperpolarization of approximately 10 mV. Hyperpolarizing current injection pulses (2 s pulses) demonstrated a reduction in B5 input resistance during 5-HT perfusion (5-HT). Depolarizing current injections were applied to measure the effects of 5-HT on the number of action potentials elicited by stimulation (3 s pulses). Note that the depolarizing pulse given prior to 5-HT perfusion (PRE) evoked a burst of action potentials that accommodated after approximately 1 s of spiking. During 5-HT perfusion (5-HT), spiking was no longer elicited by depolarizing current pulses. In addition, a sustained reduction in evoked spiking was observed even following injection of current to restore the membrane potential to the initial resting values (I+5-HT). The 5-HT-induced hyperpolarization, the reduction in input resistance and the decrease in the stimulus-evoked spiking were all reversed following a 10 min wash in DM (POST). Note that inhibitory synaptic potentials were often recorded in B5, especially during serotonin perfusion (I+5-HT). (B) Bath perfusion of B5 neurons in ganglia preparations and in cell culture with serotonin (bar represents the duration of 5-HT perfusion) caused a reduction in the normalized number of action potentials elicited by depolarizing current injection pulses delivered every 20 s (in ganglia, $N=11$; in culture, $N=16$). The effects of 50 μmol l^{-1} 5-HT on these B5 neurons were indistinguishable. In contrast, the same stimulus parameters and perfusion protocols used with acutely isolated neuron B19 produced a pronounced increase in the number of action potentials elicited by constant-amplitude current injection pulses ($N=11$). Values for all preparations were normalized to initial values by dividing the number of evoked spikes at each time point by the number of spikes measured in that preparation at the first time point. Data points represent means ± S.E.M.

inhibitory actions of serotonin on this molluscan esophageal effector neuron are in contrast to the potent excitatory effects of serotonin on radular motoneurons in the buccal ganglia. Radular tensor motoneuron B19 responds to 5-HT exposure with a maintained depolarization and an increased frequency of spiking (Granzow and Kater, 1977; Price and Goldberg, 1993). Serotonin at a concentration of 50 μmol l^{-1} elicited a significant increase (Student's *t*-test; $P<0.01$; $N=5$) in stimulus-evoked spiking in neuron B19. This neuron displayed no spike frequency adaptation during treatment with depolarizing current pulses, and the maximal effect of 5-HT constituted a 250 % rise in the number of action potentials elicited relative to pretreatment levels (Fig. 1B).

Stimulation of giant serotonergic cell cerebral neuron 1 (C1) activates the feeding motor program in cerebral–buccal ganglia preparations of *Helisoma trivolvis* and increases spiking activity in neuron B19 (Granzow and Kater, 1977). These effects on B19, elicited by C1 stimulation, are similar to those induced by exogenous application of 5-HT. Stimulation of neuron C1 in the present study, however, produced effects that were opposite to those induced in neuron B5 by exposure to exogenous 5-HT. Following activation of a maintained burst of spiking in C1, no detectable change in membrane potential in neuron B5 was observed and there was an increase in stimulus-evoked spiking (Fig. 2A). The mean number of action potentials elicited by a constant-amplitude current pulse (3 s in duration) increased significantly from 4.1±0.4 to 7.7±1.6 (mean ± S.D.; $N=9$) during C1 activation (Student's *t*-test; $P<0.04$; Fig. 2B). Subthreshold depolarizations and hyperpolarizations of C1 had no effect on B5 spike frequency and, in one preparation, a burst of only three action potentials in neuron C1 enhanced B5-stimulus-evoked spiking. Stimulation of C1, although modulating stimulus-evoked spiking, never directly elicited B5 activity.

One explanation for the contrary effects of C1 stimulation and 5-HT perfusion on B5 is that this neuron may be subject to polysynaptic modulation in ganglionic preparations. In fact, at least one inhibitory synaptic input was consistently present during 5-HT treatment (Fig. 1A). To determine the direct effects of serotonin on neuron B5, in isolation from the complex synaptic and chemical environment of the nervous system, individual neurons were acutely isolated into cell culture (1–2 h in DM). In isolation from all synaptic inputs (Fig. 3A), bath perfusion wih 5-HT at a concentration of 50 μmol l^{-1} caused a reduction in stimulus-evoked spiking (Student's *t*-test; $P<0.0001$, $N=16$) similar to the effects of exogenous 5-HT exposure in buccal ganglia (Fig. 1B). However, unlike the effects on B5 in ganglia, 5-HT elicited a significant hyperpolarization of membrane potential from −65.5±9.1 mV to −92.3±24.4 mV (mean ± S.D., $P<0.02$, $N=8$) in isolated B5 neurons. This hyperpolarization was accompanied by a significant decrease in soma input resistance ($P<0.005$; $N=8$) from 50.4±9.6 MΩ prior to exposure to 32.4±10.5 MΩ in the presence of 5-HT. The pronounced reduction in B5 stimulus-evoked spiking was also seen during repolarization of the membrane potential with maintained depolarizing current injection ($P<0.02$; $N=8$; Fig. 3A).

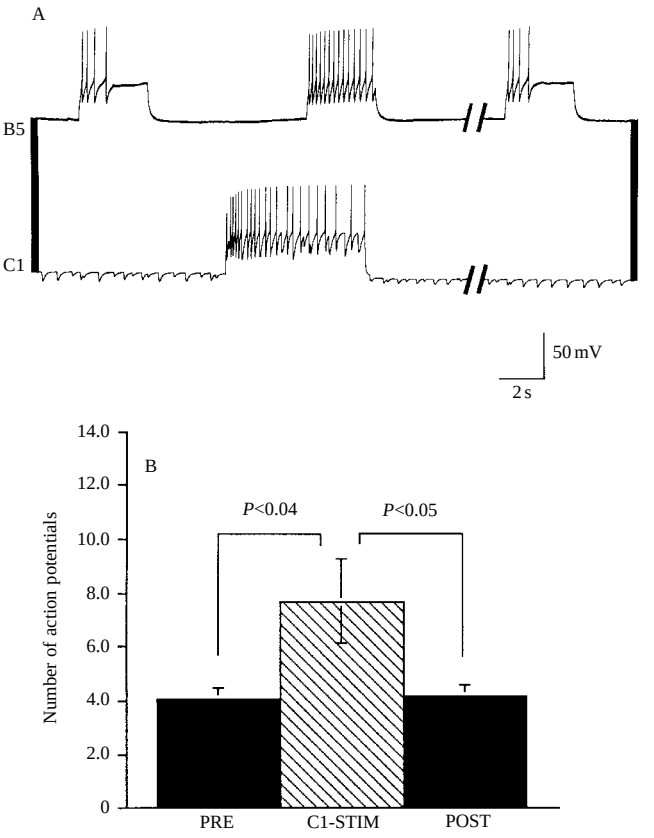

Fig. 2. Effect of stimulation of serotonergic neuron C1 on stimulus-evoked spiking of neuron B5. (A) Simultaneous intracellular recordings from buccal neuron 5 (B5; upper trace) and serotonergic cerebral neuron 1 (C1; lower trace) in a reduced cerebral/buccal ganglia preparation. Spiking in neuron B5 was activated by a series of depolarizing current injection pulses (3 s in duration) of sufficient strength to elicit an initial train of action potentials displaying spike frequency adaptation. This pulse amplitude was then kept constant throughout the experiment. Upon stimulation of neuron C1 (6 s depolarizing current injection pulse) and activation of spiking, an increased frequency of stimulus-evoked spiking was recorded in neuron B5. This elevated spiking frequency returned to pre-stimulation levels within 1 min of cessation of activity in C1 (last section of recording). Note the characteristic inhibitory synaptic potentials present in the C1 recording. (B) The mean number of action potentials elicited by stimulation of B5 was measured before (PRE), during (C1-STIM) and 1 min after (POST) C1 stimulation. The modulatory effects of C1 on B5-evoked spiking involved a significant increase in the number of action potentials elicited by depolarizing current injection ($N=9$). Values are means ± S.E.M.

The number of action potentials elicited by depolarizing current pulses varied with current level, although 5-HT caused a reduction in evoked spike number at all current-injection levels (Fig. 4A). This indicated that at least part of the 5-HT inhibitory effect on B5 involves a reduction in neuronal excitability. Input/output curves, such as that shown in Fig. 4A, were generated for B5 neurons in buccal ganglia and

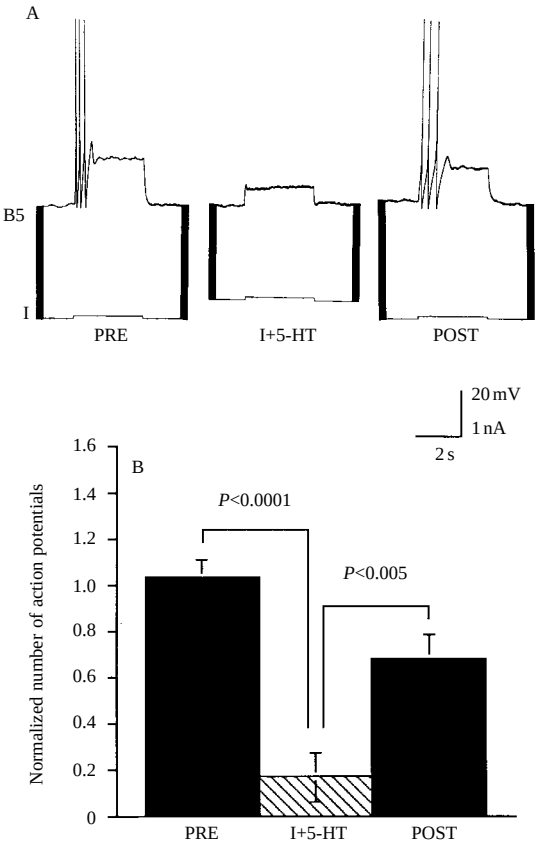

Fig. 3. Effects of serotonin on neuronal excitability of acutely isolated B5 neurons in cell culture. (A) Intracellular recordings of neuronal membrane potential (B5; upper trace) illustrate that injection of depolarizing current pulses (I; lower trace) elicited a burst of action potentials in B5 (1–2 h after isolation in cell culture). Stimulus-evoked bursts of action potentials exhibited spike frequency adaptation in synaptic isolation. Inhibitory effects of 5-HT on stimulus-evoked spiking were present following manipulation of neuronal membrane potential to the resting values present prior to 5-HT-induced hyperpolarization (bottom trace; I+5-HT). Stimulus-evoked spiking was activated by depolarizing pulses of 3 s duration. The amplitude of the current pulse (sufficient to elicit a burst of several action potentials) was kept constant throughout the experiment. The effects of 5-HT were reversed after washing in DM for 2 min (POST). (B) The mean number of action potentials evoked by current injection before 5-HT treatment (PRE), during exposure to 50 μmol l^{-1} 5-HT in the presence of repolarizing current injection (I+5-HT) and after removal of 5-HT by washing in DM for 2 min (POST). Values for all preparations were normalized to initial values by dividing the number of evoked spikes at each time point by the number of spikes measured in that preparation at the first time point. A significant reduction in the number of action potentials elicited was induced by perfusion of B5 with 5-HT (N=8). In addition, a significant recovery of evoked spiking occurred after washing for 2 min in DM. Values are means ± S.E.M.

in acute cell culture. The maximum numbers of spikes elicited for individual neurons were determined and these were used to demonstrate the dose-dependence of the 5-HT-induced modulation of B5 soma excitability. A decrease in the maximal number of spikes elicited was found at all concentrations of 5-HT above 0.01 μmol l^{-1} in both buccal ganglia preparations (N=6–9 cells per concentration; IC_{50}=10 μmol l^{-1}; data not shown) and isolated neuron cultures (N=5–10 cells per concentration; IC_{50}=1.1 μmol l^{-1}; Fig. 4B). Therefore, the effects of 5-HT on neuronal excitability of B5, in the absence of any potential polysynaptic effects, represent a dose-dependent modulation identical to that observed in neurons treated exogenously within the semi-intact nervous system.

To determine whether these were detectable regional differences in the responses of B5 to serotonin, somata of newly isolated neurons (N=7) were penetrated with microelectrodes, and micropuffs of 5-HT at a concentration of 50 μmol l^{-1} in DM were delivered by a glass pipette connected to a pressure-injection system. Applied puffs were visualized by the addition of Fast Green to the 5-HT pipette solution. Brief, focal applications (4 ms in duration) of 5-HT were delivered to the somata, to the original axonal stump and to the fine dendrite-like processes that branch from the axon hillock region of B5. Constant perfusion of the recording chamber with DM quickly washed the solution away from adjacent regions of the neuron. Consistent 5-HT-induced hyperpolarizations of membrane potential and reductions in stimulus-evoked spiking in B5 were elicited at each site of 5-HT application. Thus, at a gross level of resolution, no evidence for 5-HT receptors mediating different (i.e. excitatory rather than inhibitory) effects were detected, and 5-HT receptors mediating inhibitory effects were widely distributed on the surface of the B5 neuron.

Ionic basis of the serotonin-induced hyperpolarization

Experiments were performed on isolated B5 neurons to determine the ionic conductance underlying the serotonin-induced membrane hyperpolarization. The long recovery time for the effect of serotonin on membrane potential (i.e. 6–10 min) required the use of a less conventional technique for measurement of the reversal potential for the 5-HT-induced hyperpolarization. Acutely isolated neurons were bath-perfused with 50 μmol l^{-1} 5-HT, and membrane potential was calculated at a series of injected current steps before and after treatment (modified from Bahls, 1990). The mean reversal potential for the 5-HT-induced change in membrane potential was −88.7±11.6 mV (mean ± S.D., N=7) in normal external K^+ concentration ($[K^+]_o$; 1.7 mmol l^{-1}; Fig. 5A). This value approximated closely the −88 mV reversal potential for K^+ predicted by the Nernst equation for *Helisoma trivolvis* neurons. By manipulating the extracellular K^+ concentration to either one-half or twice the normal concentration, the mean reversal potential was shifted to −111.6±5.8 mV (N=6) in half-concentration $[K^+]_o$ and −71.9±4.9 mV (N=6) in double-concentration $[K^+]_o$. These experimental values were also similar to the reversal potentials of −106 mV and −70 mV, respectively, predicted by the Nernst equation for K^+ (Fig. 5B).

Second messenger involvement in 5-HT-induced modulation

Since previous studies have established a role for cyclic-

AMP-dependent mechanisms in the mediation of 5-HT cellular effects in some other molluscan neurons, activators and inhibitors of the cyclic AMP second messenger pathway were used to determine the role of this signal transduction cascade in serotonin-induced modulation of B5 neuronal excitability. In these experiments, the effects of pharmacological agents were compared with those of 50 μmol l^{-1} serotonin on stimulus-induced spiking. The mean 5-HT-induced reduction in the number of action potentials elicited by a 3 s depolarizing

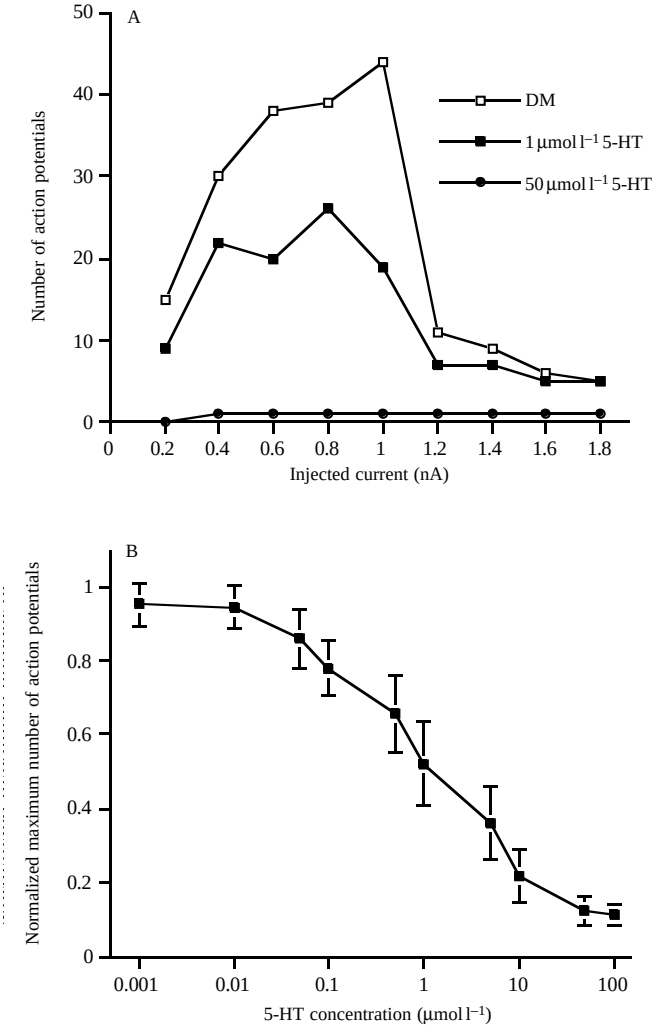

Fig. 4. Dose-dependence of the serotonin-induced reduction in B5 neuronal excitability. (A) Input/output curves illustrate the dose-dependent effect of 5-HT on the number of action potentials elicited by depolarizing current injection of B5. These curves show the number of spikes elicited over a range of current injection steps (3 s duration; 30 s between pulses) in the absence of 5-HT (DM only) or in the presence of 1 or 50 μmol l^{-1} 5-HT. Decreases in evoked spike number with increasing 5-HT concentration were present at all current injection levels. From these input/output curves, the maximal number of action potentials evoked over a range of current pulses was determined. For example, the maximum number of spikes evoked in this neuron was 43 in DM at 1 nA of injected current; however, its maximal spiking response was only 25 spikes in 1 μmol l^{-1} 5-HT at 0.8 nA. (B) A dose–response curve depicting the inhibitory effects of serotonin on maximal stimulus-evoked spiking as determined from input/output curves. The concentration of 5-HT at 50 % inhibition (IC_{50}) of maximal spiking was 1.1 μmol l^{-1}. This IC_{50} was estimated from analysis of acutely isolated B5 neurons. Values are means ± S.E.M. (*N*=5–10 neurons per point).

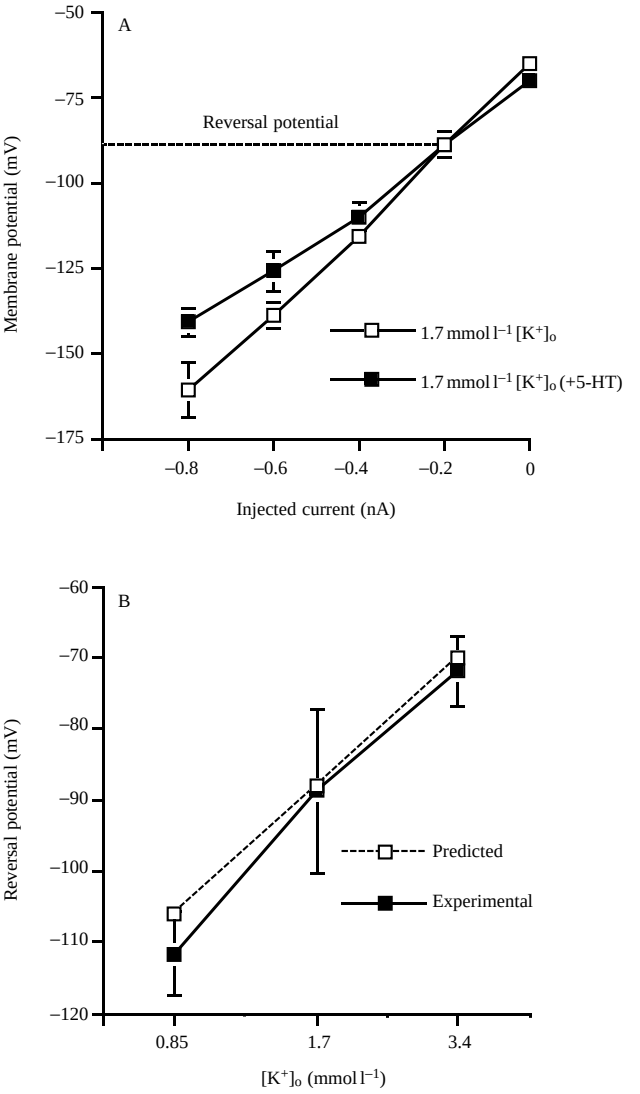

Fig. 5. Serotonin-induced modulation of membrane potential in isolated B5 neurons involved an increased K^+ conductance. (A) Reversal potential for the 5-HT-induced hyperpolarization of B5 (indicated by the broken line) was extrapolated from current–voltage (*I*/*V*) relationships. Membrane potentials were measured at a series of hyperpolarizing current steps injected before and during exposure to a 5-HT concentration of 50 μmol l^{-1}. Multiple measurements (2–3 per cell) were made for individual neurons, and the *I*/*V* values presented represent means (± S.D.) averaged across all neurons tested in normal saline K^+ concentrations (*N*=7). (B) Manipulation of the extracellular K^+ concentration to half (*N*=6) and twice (*N*=6) normal values changed the reversal potential for the 5-HT-induced hyperpolarization in a manner predicted by the Nernst equation for B5 assuming external K^+ concentrations of 0.85, 1.7 and 3.4 mmol l^{-1}. Mean experimental (open squares) and predicted (filled squares) reversal potential values (± S.D.) are plotted.

current pulse injected into isolated neuron B5 was 92.5±6.6 % (mean ± S.E.M. *N*=15). When B5 was exposed to forskolin (30 μmol l^{-1}), an activator of adenylyl cyclase, an opposite effect was detected. Forskolin caused a 6.1±1.2 mV depolarization of B5 that was accompanied by a 91.2 % increase in B5 spiking (Fig. 6A). This effect of forskolin was significantly different from that elicited by 5-HT (Fisher's PLSD; $P<0.005$, *N*=11). Injection of B5 with a specific activator of cyclic-AMP-dependent protein kinase, Sp-cAMP (100 μmol l^{-1}), caused an 8.1±1.9 mV (mean ± S.D., *N*=6) membrane depolarization and a 153±68.6 % increase in evoked spiking (Fig. 6A). This effect was again significantly different (Fisher's PLSD; $P<0.02$, *N*=6) from the inhibitory response of B5 to 5-HT (Fig. 6A). Taken together with the effect of forskolin, activation of the cyclic AMP pathway produced responses in B5 in the opposite direction to those induced by 5-HT.

Injection of B5 neurons (*N*=5) with SQ22536, an inhibitor of adenylyl cyclase, failed to disrupt 5-HT-induced inhibitory responses (Fig. 6B); that is, when serotonin (50 μmol l^{-1}) was perfused across SQ22536-injected neurons, a reduction in stimulus-evoked spiking was detected that was indistinguishable from the effect of 5-HT on sham-injected controls (*N*=6). In addition, injection of neurons with Rp-cAMP (100 μmol l^{-1}), a specific inhibitor of protein kinase A, was ineffective in antagonizing the response of B5 5-HT perfusion (*N*=11; Fig. 6B). The effect of 5-HT on these inhibitor-injected neurons was not significantly different from those obtained with untreated (*N*=15) and sham-injected (*N*=6) control neurons.

Previous reports have implicated lipoxygenase metabolites of arachidonic acid in the mediation of the inhibitory effects of FMRFamide on neuron B5 (Bahls *et al.* 1992) and sensory neurons of *Aplysia californica* (Piomelli *et al.* 1987). Experiments using arachidonic acid pathway activators and inhibitors were performed and again compared with the inhibitory effects of serotonin on B5 stimulus-induced spiking. Exposure to arachidonic acid (50 μmol l^{-1}) had no significant effect on the resting membrane potential of B5 (2.9±1.7 mV depolarization; *N*=8) or the evoked spike frequency (10.1± 20.5 % change; *N*=8). However, following treatment of neuron B5 with 50 μmol l^{-1} BPB, a phospholipase A2 inhibitor, the effect of 50 μmol l^{-1} 5-HT on stimulus-evoked spiking was significantly reduced (Fisher's PLSD; $P<0.05$; *N*=5; Fig. 6B). In addition, inhibition of the lipoxygenase pathway of arachidonic acid metabolism by perfusion with 50 μmol l^{-1} NDGA also disrupted this 5-HT-induced effect (Fisher's PLSD; $P<0.04$; *N*=6; Fig. 6B). These results, together with the previous pharmacological findings, suggest that serotonin-induced modulation of neuronal spiking in B5 is a cyclic-

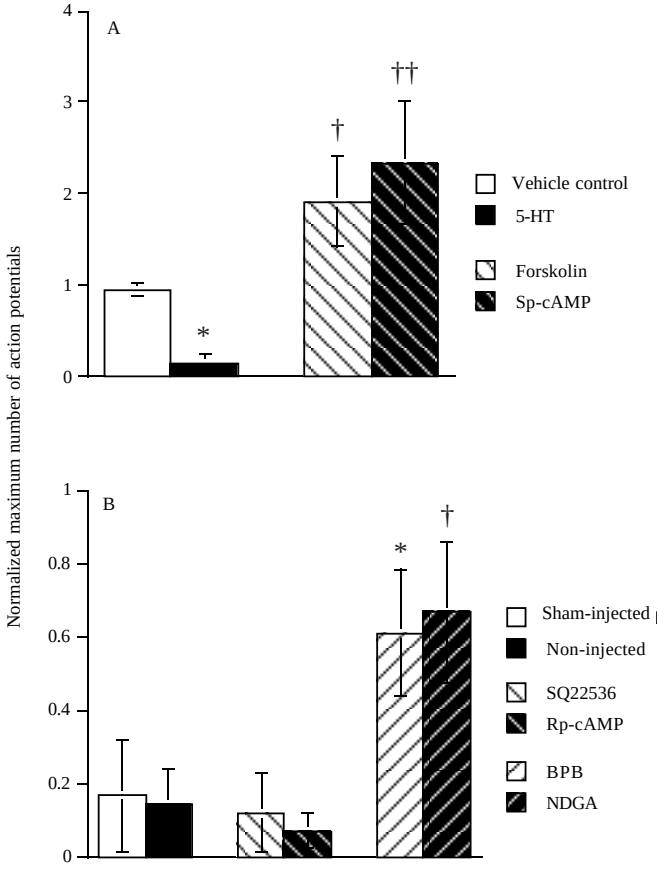

Fig. 6. Serotonin-induced changes in neuronal excitability were cyclic-AMP-independent and were disrupted by antagonists of the arachidonic acid signaling pathway. (A) Activators of the cyclic AMP signal transduction pathway did not mimic the effects of 5-HT on B5 neuronal excitability. Perfusion of B5 with forskolin (30 μmol l^{-1}), an adenylyl cyclase activator, caused a significant increase (*N*=11, $P<0.005$; †) in stimulus-evoked spiking compared with parallel controls perfused with vehicle in DM (*N*=10). This excitatory effect of forskolin was in the opposite direction to the significant reduction in evoked spiking elicited by 50 μmol l^{-1} 5-HT (*N*=15, $P<0.001$; *). Injection of B5 with 100 μmol l^{-1} Sp-cAMP, an activator of cyclic-AMP-dependent protein kinase, also resulted in a significant increase (*N*=6, $P<0.02$; ††) in evoked spiking compared with vehicle-injected controls (*N*=5). Histograms represent the maximal number of spikes calculated from input/output curves using depolarizing current pulses. All values were normalized to basal levels (maximal spiking) prior to 5-HT treatment. Error bars represent ±1 S.E.M. (B) Inhibitors of the arachidonic acid, but not of the cyclic AMP, signal transduction pathway disrupted serotonin-induced modulation of B5 spiking. Effects of 5-HT (50 μmol l^{-1}) on B5 stimulus-evoked spiking were not altered by injection of SQ22536 (100 μmol l^{-1}), an inhibitor of adenylyl cyclase (*N*=5). In addition, injection of the cyclic-AMP-dependent protein kinase inhibitor Rp-cAMP (100 μmol l^{-1}; *N*=11) did not significantly affect the 5-HT-induced response. In contrast, exposure of B5 to the phospholipase A2 inhibitor BPB (50 μmol l^{-1}) caused a significant diminution of the 5-HT effect (*N*=5) compared with untreated (*N*=15) or sham-injected (*N*=6; $P<0.085$; *) control neurons exposed to 5-HT. Similarly, treatment with the lipoxygenase metabolic pathway inhibitor NDGA (50 μmol l^{-1}) caused significant suppression of the 5-HT response (*N*=6; $P<0.04$; †) compared with sham-injected controls. Histograms represent the maximal number of spikes calculated from input/output curves using depolarizing current pulses. All values were normalized to basal levels (maximal spiking) prior to 5-HT treatment. Error bars respresent ±1 S.E.M. Fisher's PLSD (paired least significant difference) test was used as a *post-hoc* comparison after analysis of variance (ANOVA).

AMP-independent effect that might be mediated by lipoxygenase metabolites of arachidonic acid.

Discussion

A body of evidence has emerged that supports the hypothesis that neurotransmitters function as important regulators of neuronal differentiation. Regenerating *Helisoma trivolvis* neurons in buccal ganglia and in cell culture have been used to elucidate mechanisms through which neurotransmitters such as serotonin might modulate the development and connectivity of a neuron (Haydon *et al.* 1987; Mattson *et al.* 1988; Goldberg and Kater, 1989; Diefenbach *et al.* 1995). For example, growth cone motility and neurite extension of neuron B19, but not of neuron B5, are reversibly inhibited by exposure to 5-HT both *in vitro* (Haydon *et al.* 1984) and *in vivo* (Murrain *et al.* 1990). Studies of the cellular mechanisms governing 5-HT-induced cessation of B19 neurite outgrowth have determined that 5-HT elicits a sustained depolarization and voltage-dependent influx of Ca^{2+} (Cohan *et al.* 1987; McCobb and Kater, 1988). In the present study, we have demonstrated that exposure of neuron B5 to 5-HT, both in reduced preparations and in cultured neurons, elicits a hyperpolarization of the resting membrane potential that is accompanied by a significant reduction in neuronal input resistance. This result is consistent with previous findings that elevations in intracellular Ca^{2+} levels do not occur in B5 following exposure to 5-HT (Murrain *et al.* 1990), even though electrical stimulation of neuronal activity triggers inhibition of B5 growth cone advance (Cohan and Kater, 1986). In mass-dissociated cultures of *Helisoma trivolvis* neurons (i.e. large populations of unidentified cells isolated from ganglia), approximately 50 % of the neurons displayed a 5-HT-induced increase in internal Ca^{2+} concentration (Goldberg *et al.* 1992). Thus, the differential effects of 5-HT on B5 and B19 are probably representative of a systemic dichotomy in serotonin-mediated neuromodulation.

Serotonin, although a potential modulator of neuronal development, is also an important regulator of behavioral plasticity. It is involved in modulating the neural circuits underlying molluscan gill-withdrawal reflexes (Byrne *et al.* 1986), leech swimming (Nusbaum and Kristan, 1986; Hashemzadeh-Gargari and Friesen, 1989) and feeding (Lent *et al.* 1989), and crustacean stomatogastric function (Beltz *et al.* 1984; Katz and Harris-Warrick, 1990). At the cellular level, serotonin influences the activity of a variety of neuronal ion channels (Klein *et al.* 1982; Kirk *et al.* 1988; Taussig *et al.* 1989; Blumenfeld *et al.* 1990; Baxter and Byrne, 1990). In *Helisoma trivolvis*, serotonin has pronounced modulatory effects on feeding motor programs. Stimulation of serotonergic cerebral neuron 1 (C1) activates action potential bursting in neurons such as motoneuron B19, located within the buccal ganglia (Granzow and Kater, 1977). This sustained enhancement of B19 spiking is mimicked by exogenous serotonin perfusion of the buccal ganglia (Granzow and Kater, 1977) and isolated neurons in cell culture (Price and Goldberg, 1993). Exposure to 5-HT also enhances B19-evoked contractions of its radular tensor muscle targets (Zoran *et al.* 1989). Both exogenous serotonin application (Paupardin-Tritsch and Gerschenfeld, 1973) and stimulation of serotonergic metacerebral cells (Rosen *et al.* 1983) modulate similar feeding motor patterns in the marine mollusc *Aplysia californica*.

The depolarization induced by 5-HT in *Helisoma trivolvis* neuron B19 is due to a sustained Na^+ current (Price and Goldberg, 1993). The present results indicate that serotonin enhances a K^+ conductance in neuron B5. Experimental manipulations of extracellular K^+ concentration and analyses of shifts in reversal potential suggest that the serotonin-induced hyperpolarization was the product of an increased K^+ conductance; however, a contribution from a Cl^- conductance, albeit minor, cannot be eliminated. Similarly, octopamine (Bahls, 1990) and FMRFamide (Murphy *et al.* 1985*b*) have been shown to cause slow hyperpolarizations of *Helisoma trivolvis* neuron B5, and both of these modulatory effects are mediated by an increased K^+ conductance. Studies in *Aplysia californica* have also shown that serotonin modulates neuronal activity in some neurons by modifying K^+ currents. These serotonin-modified *Aplysia californica* K^+ currents include the S-type K^+ current (Siegelbaum *et al.* 1982), a voltage-dependent K^+ current (Baxter and Byrne, 1990) and an inward rectifying current (Benson and Levitan, 1983). In *Hirudo medicinalis*, serotonin enhances an early transient K^+ current and suppresses a delayed voltage-dependent K^+ current (Acosta-Urquidi *et al.* 1989).

The reductions in stimulus-evoked spiking associated with exposure of B5 to serotonin both in ganglion preparations and in cell culture were, at least in part, due to a depression of neuronal excitability. At relatively constant membrane potentials, manipulated by maintained current injection, 5-HT caused a reduction in the maximal number of action potentials elicited by a complete range of current-injection pulses capable of evoking spikes. These results are in striking contrast to the effects of 5-HT on B19 stimulus-evoked spiking (Fig. 1B). Serotonin elicits excitatory effects on some *Aplysia californica* neurons (Klein and Kandel, 1978; Braha *et al.* 1990; Mercer *et al.* 1991), while having inhibitory effects on others (Drummond *et al.* 1980). These differences in 5-HT-induced modulation of membrane potential and excitability between neurons suggest that the equally disparate effects of 5-HT on neurite outgrowth might reside in the activation of membrane conductances with opposing cellular consequences.

Interestingly, stimulation of serotonergic cerebral neuron C1, which has axonal projections (and probably synaptic contacts) throughout the buccal ganglion (Murphy *et al.* 1985*a*), increased the spiking frequency of B5 in cerebrobuccal ganglia preparations. This increased excitability was contrary to our results using exogenous serotonin perfusion of buccal ganglia. Several lines of evidence suggest that the excitatory effects of C1 on B5 were not direct, but rather resulted from 5-HT activation of an undefined polysynaptic modulatory pathway. First, serotonin elicited inhibitory effects over a range

of concentrations from 0.01 to 100 μmol l^{-1} without any indication of excitatory effects. Second, application of micropuffs of serotonin to specific regions of B5 indicated no gross regional distribution of a serotonin receptor subtype mediating excitatory effects. Third, 5-HT effects were recapitulated in cultured neurons isolated from confounding synaptic inputs, ruling out the possibility that inhibitory effects elicited by exogenous perfusion of ganglia were due to activation of intermediate modulatory neurons. However, very little activity in C1 was required to cause a significant change in B5 excitability in some preparations. This observation is difficult to explain simply by recruitment of an intermediate neuron. Thus, these differences in the effects of 5-HT perfusion and C1 stimulation remain paradoxical.

There are many possible signal transduction pathways that could mediate serotonin-induced effects. However, studies of the 5-HT-induced depolarization of neuron B19 indicated that activation of the underlying Na^+ current was cyclic-AMP-dependent (Price and Goldberg, 1993). Similarly, excitatory and inhibitory effects in *Aplysia californica* neurons are in many cases mediated by cyclic-AMP-dependent second messenger cascades (Drummond *et al.* 1980; Adams and Levitan, 1982; Siegelbaum *et al.* 1982; Pollock *et al.* 1985; Hochner and Kandel, 1992). However, some effects of serotonin on *Aplysia californica* sensory neurons are cyclic-AMP-independent (Baxter and Byrne, 1990). Injection of the cyclic AMP agonist Sp-cAMP and activation of adenylyl cyclase using forskolin failed to elicit membrane hyperpolarizations or reductions in B5 stimulus-evoked spiking. On the contrary, both treatments elicited depolarization of B5 membrane potential and enhanced neuronal spiking activity. This result correlates with previous studies of the cyclic-AMP-dependence of neurite outgrowth inhibition. Treatment with either forskolin or dibutyryl cyclic AMP suppressed neurite elongation and growth cone motility of both B5 and B19 neurons (Mattson *et al.* 1988). In the present studies, treatment of B5 with the adenylyl cyclase inhibitor SQ22536 or the specific protein kinase A inhibitor Rp-cAMP failed to block the above-mentioned effects of 5-HT on this neuron. Therefore, these results indicate that the inhibitory effects of 5-HT on neuron B5 are not mediated through changes in intracellular cyclic AMP levels, although further research is required to determine the possible role of inhibitory GTP-binding proteins in the regulation of a cyclic-AMP-dependent cascade.

Arachidonic acid pathways have been implicated in the mediation of neuronal plasticity in both *Aplysia californica* (Piomelli *et al.* 1987) and *Helisoma trivolvis* (Bahls *et al.* 1992). Those studies have shown that the inhibitory neuropeptide FMRFamide activates a K^+ conductance which is probably governed by the lipoxygenase pathway of arachidonic acid metabolism. The present study demonstrates that the subcellular mechanisms underlying serotonin-induced reductions in B5 neuronal excitability may also be mediated by an arachidonic acid metabolic pathway similar to that implicated in FMRFamide-induced hyperpolarizations. Application of the phospholipase A2 inhibitor BPB disrupted the effects of 5-HT on stimulus-evoked spiking. In addition, the response to serotonin in the presence of the lipoxygenase metabolic pathway inhibitor NDGA was significantly reduced. Previous studies on neuron B5 had shown that perfusion with NDGA blocks the modulation of a K^+ conductance by FMRFamide (Bahls *et al.* 1992). These results are similar to those found in *Aplysia californica*, where modulation of the S-type K^+ channel by FMRFamide was blocked by NDGA (Piomelli *et al.* 1987). Perfusion of B5 in culture with arachidonic acid produced highly variable effects. Equally varied responses of B5 to arachidonic acid have been reported previously (Bahls *et al.* 1992). Consequently, further study is required to confirm a role for arachidonic acid metabolites in the serotonin-induced inhibition of neuron B5 excitability.

The differential effects of 5-HT on B5 and B19 might be mediated by different receptor subtypes. Multiple modulatory responses to 5-HT in crab stomatogastric neurons (Zhang and Harris-Warrick, 1994), frog spinal neurons (Tan and Miletic, 1990) and a wide range of other cells are thought to be mediated by distinct 5-HT receptors. Differences in 5-HT affinity, as indicated by its dose–response characteristics for modulation of neurite outgrowth of cultured embryonic neurons (Goldberg *et al.* 1991), ciliary activity in intact embryos (Goldberg *et al.* 1994), Na^+ currents of neuron B19 (Price and Goldberg, 1993) and stimulus-evoked spiking of neuron B5 (Fig. 4B), suggest the existence of different 5-HT receptor subtypes in *Helisoma trivolvis.* Thus, the difference in effects of 5-HT on B5 and B19 might be governed by differences in 5-HT receptor expression by these neurons and, consequently, in the signal transduction pathways they activate. However, the use of classic pharmacological agents to characterize 5-HT receptors is perplexing. This is primarily due to the fact that vertebrate agonists and antagonists have nonspecific effects on invertebrate neurons. For example, in *Aplysia californica* sensory neurons, the 5-HT antagonist cyproheptadine blocks 5-HT-induced facilitation of synaptic transmission, but does not block 5-HT-induced neuronal excitability (Mercer *et al.* 1991). In *Helisoma trivolvis* neuron B19, cyproheptadine inhibits the 5-HT-dependent Na^+ current, but also inhibits voltage-gated Na^+ and K^+ conductances (Price and Goldberg, 1993). This raises the question of whether these agents act at specific 5-HT receptors or directly on ion channels themselves.

In summary, serotonin induced a membrane hyperpolarization in neuron B5 that is thought to be mediated by an increased K^+ conductance. In addition, serotonin caused a reduction in B5 neuronal excitability. These inhibitory effects contrast with the excitatory effects of 5-HT on buccal neuron B19. We present evidence that the effects of 5-HT on B5 neuronal excitability are activated by a receptor linked to a cyclic-AMP-independent signal transduction pathway. Thus, 5-HT regulates differentially electrophysiological properties of neurons within the buccal nervous system of *Helisoma trivolvis* through the activation of distinct signal transduction pathways. These differential effects of 5-HT on neurons B5 and B19 may

account for previously reported divergent effects on neurite outgrowth and indicate that distinct modulatory pathways governing transmitter-mediated neuronal plasticity in the adult nervous system might also regulate the differentiation of those systems during development and regeneration.

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