Serotonin Reduces Inhibition via 5-HT_{1A} Receptors in Area CA1 of Rat Hippocampal Slices *in vitro*

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We studied the effects of serotonin (5-HT) on intrinsic and synaptic responses of hippocampal CA1 cells. The effects were partially mimicked by the 5-HT_{1A} receptor agonist, 8-OH-DPAT, and prevented by the 5-HT_{1A} receptor antagonist, NAN-190. Polysynaptic fast and slow inhibitory postsynaptic potentials (IPSPs) were reduced in amplitude by 60–70% following application of both 5-HT and 8-OH-DPAT. Monosynaptic fast IPSPs were reduced by 60% and slow IPSPs by 90% following application of both drugs. Since there is a temporal overlap of fast and slow IPSPs, the reductions in fast IPSPs could have arisen indirectly from the larger effect of 5-HT on slow IPSPs. To overcome this problem we blocked the slow IPSPs with new, potent GABA-B antagonists, but still observed a similar reduction in the fast IPSP with 5-HT and 8-OH-DPAT. However, the reductions in the fast IPSPs could also have arisen from the 5-HT–induced total conductance increases. Using single-electrode voltage clamp and intracellular K^+ channel blockers we still observed similar changes. 5-HT and 8-OH-DPAT had no effect upon GABA-A–mediated currents evoked by iontophoretic GABA application to the dendrites or the soma of CA1 pyramidal cells. Putative inhibitory interneurons were hyperpolarized by 5-HT and their evoked EPSPs strongly reduced by 5-HT and 8-OH-DPAT. Our data indicate that 5-HT modulates fast and slow synaptic inhibition of principal cells using presynaptic mechanisms involving the inhibition of inhibitory interneurons.

[Key words: hippocampus, CA1, serotonin, 8-OH-DPAT, inhibition, interneurons]

Serotonergic inputs to the hippocampus from the Raphe nuclei are involved in the modulation of hippocampal "theta" oscillations (Assaf and Miller, 1978; Vanderwolf, 1988). These inputs to the hippocampus are also thought to be important for functional spatial learning behavior (Richter Levin et al., 1993).

A number of *in vitro* and *in vivo* studies suggest that serotonin has differential excitatory and inhibitory effects on hippocampal neurons. These effects seem to be mediated by a number of different serotonin (5-HT) receptor subtypes. Serotonin directly hyperpolarizes pyramidal cells through the activation of 5-HT_{1A} receptors (Andrade and Nicholl, 1987; Colino and Halliwell, 1987). The effect results from opening of Ca^{2+} independent K^+ channels and underlies the decrease in unit firing observed *in vivo* (Richter Levin and Segal, 1990). However, inconsistent with an inhibitory effect of 5-HT are increased population spike amplitudes recorded *in vivo* from the dentate gyrus (Klancnik et al., 1989) and transient increases in both population spikes (Beck et al., 1985) and field EPSPs in area CA1 (Ropert, 1988). These 5-HT–mediated increases in excitability may use two separate mechanisms. Suppression of the Ca^{2+}-dependent K^+ current (I_{AHP}) will lead to increased cell firing, while a decrease in the voltage-dependent K^+ conductance, I_m, leads to an overall depolarization of the cell (Colino and Halliwell, 1987). These changes may be mediated via 5-HT_4 receptors, a novel 5-HT receptor or 5-HT_{1C} receptors (Chaput et al., 1990; Andrade and Chaput, 1991; Beck, 1992).

A number of different effects of serotonin on synaptic potentials in the hippocampus have been reported. Decreases in excitatory postsynaptic potentials (EPSPs) (Jahnsen, 1980; Segal, 1980; Ropert, 1988) and slow inhibitory postsynaptic potentials (IPSPs) have been observed with 5-HT (Segal, 1990; Oleskevich and Lacaille, 1992; Ghadimi et al., 1994), but the only described effect on fast IPSPs was their transient increase mediated via 5-HT_3 receptors (Ropert and Guy, 1991). *In vivo* 5-HT decreases feed-forward inhibition of the dentate gyrus, and consistent with this result was an increased excitability following activation of 5-HT release (Richter Levin and Segal, 1990).

Here, using intracellular recordings from CA1 cells in slices from ventral hippocampus, we aimed to clarify the effects of 5-HT on synaptic inhibition in area CA1.

Materials and Methods

Slice preparation. Thick horizontal slices (400 μm) from adult female Wistar rats (200–250 gm) containing the ventral hippocampus, entorhinal, perirhinal, and temporal cortices (as previously described; Dreier and Heinemann, 1991) were cut using a Vibroslice (Campden Instruments, Loughborough, UK). After transfer to a standard interface chamber maintained at 34°C, the slices were perfused at a rate of 1.5–1.8 ml/min with artificial cerebrospinal fluid, ACSF, containing in mM: NaCl, 124; $NaHCO_3$, 26; KCl, 3; NaH_2PO_4, 1.25; $CaCl_2$, 1.6; $MgSO_4$, 1.8; glucose, 10, saturated with 95% O_2–5% CO_2, pH 7.4.

Electrophysiological recordings. Electrodes for impalement contained 2–3 M K^+- or Cs^+-acetate and in some recordings 50 mM QX314 was also included. Recordings were made using a Neurodata IR 183 amplifier (Neurodata Instruments Corp., New York) and a SEC10L (npi Instruments, Tamm, Germany). We only accepted cells with membrane potentials more negative than −50 mV and overshooting action potentials. Signals were filtered at 3 kHz, sampled, and collected using both a CED 1401 and an ITC-16 (Cambridge Electronic Design, Cambridge, UK) with an IBM compatible computer and also a chart recorder

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(Linearcorder, MkVII WR3101, Graphtec Corp., Japan). Resting membrane potentials of the cells were estimated by subtraction of the tip potential following withdrawal from the cell.

Bridge mode recordings. Input resistances of the cells were determined from responses to negative current pulses (−0.1 to −0.3 nA, 50–100 msec duration). Fast and slow afterhyperpolarizations following one to nine action potentials were evoked with positive current injection (+0.1 to +1.0 nA, 100–300 msec). We used 700 msec positive current injections (+0.3 to +1.0 nA) to evoke a train of action potentials.

Compound polysynaptic potentials were evoked following electrical stimulation (0.05–0.1 msec duration, 1–12 V) every 10–20 sec via a bipolar insulated stimulating electrode to the Schaffer collateral pathway. Monosynaptic inhibitory postsynaptic potentials were evoked by supramaximal stimulation from a close (< 0.2 mm) position in the presence of CNQX or NBQX (10–20 μM) and APV (30 μM) (Davies et al., 1990).

Voltage clamp recordings. Electrodes filled with K^+- or Cs^+-acetate and QX314 with resistances ≤ 65 MΩ were used for voltage clamp experiments. The presence of QX314 led to a blockade of action potentials in these cells 15–20 min after impalement. After voltage clamp at the resting membrane potential of the cell, the gain (5–9 nA/mV), capacitance compensation, and switching frequency (8–25 kHz) were optimized. The continuously monitored head stage voltage always decayed fully before the next current injection during a 1/4 duty cycle. Clamp efficiency estimated from the difference between the clamped and unclamped IPSP ranged from 70–90%.

Stimulus-evoked monosynaptic GABA-A–mediated fast IPSCs were studied at different holding potentials as were currents evoked by GABA application to both the cell dendrites and soma. GABA was applied from double barrelled theta glass pipettes (Science Products, Frankfurt, Germany), 1–1.5 μm tip diameter, containing 0.5–0.7 M GABA pH 4.2, resistance 30–60 MΩ, using positive ejection currents of 12–20 nA, duration 3–5 sec (Neurophore BH-2). To avoid GABA receptor desensitization, applications were made once every 30–60 sec.

Drugs applied. The following drugs were bath applied; bicuculline methiodide (SIGMA, Deisenhofen, Germany), 2–5 μM, (±)-2-amino-5-phosphonopentanoic acid (APV) (Research Biochemicals, Natick, MA), 30 μM, 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) (Tocris Neuramin, Bristol, UK), 20 μM, 6-Nitro-7-sulphamoylbenzo(f)quinoxaline-2,3-dione (NBQX) (a gift from Novo Nordisk), 10 μM, 1-(2-Methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine hydrobromide (NAN-190), 10–50 μM, CGP35348 and CGP55845A (both gifts from CIBA-GEIGY, Basle, Switzerland) at 100 and 2–5 μM, respectively. 5-Hydroxytryptamine creatine sulphate complex (5-HT) (Sigma), 50–100 μM and 1–20 μM to assess membrane and synaptic properties, respectively, and ±8-hydroxy-2-(di-n-propylamino)tetralin HBr (8-OH-DPAT) (Research Biochemicals, Natick, MA), 1–20 μM.

5-HT and 8-OH-DPAT dissolved in ACSF were also applied to the surface of the slice using a 10–20 μm tip diameter glass pipette. Concentration in the pipette was 0.5 mM and a blue or green food dye (McCormick, Baltimore, MD) was also included to monitor spread of the applied drug. Control experiments only with ACSF and the blue or green food dye were without any effects. The area of drug application was approximately 100 μm in diameter and placed either in the dendritic area or close to the soma. In a few cells we compared bath applications with drop applications where the effects from the latter corresponded to ≤ 20 μM.

Data analysis and statistical treatments. All data were analyzed offline using Sigavg (CED, UK). Measurements of the fast and slow AHPs were made relative to baseline potential at 30–50 msec and 400–600 msec after the end of the current pulse.

Amplitudes of evoked synaptic potentials and currents were measured at different membrane potentials at a constant latency from an average of three to eight sweeps. Reversal potentials of evoked synaptic potentials and responses to exogenously applied GABA were calculated for each cell using a linear regression (Sigmaplot, Jandel, Corte Madera, USA and AXUM, Axum, Seattle, WA). Fast inhibitory postsynaptic potentials (IPSP) conductances were estimated from the change in amplitude of the synaptic potential and the total input resistance of the cell (Ginsborg, 1973). Conductances of fast inhibitory post synaptic currents (IPSCs) were calculated directly from the change in amplitude of the current at different holding potentials.

Means ± standard error of the mean were calculated using Sigmaplot and AXUM. Student t tests (Sigmaplot) were used to compare the two groups.

Results

In this study we made stable recordings from 156 CA1 cells, each held for 0.5–5 hr. One hundred thirty cells were recorded in bridge mode, using electrodes containing K^+-acetate. Mean action potential amplitude was 87.8 ± 0.9 mV (n = 102). In 30 cells we also included QX314, but this had no significant effect (p = 0.49, t test) upon the mean input resistance of 48.9 ± 4.6 MΩ. Single-electrode voltage-clamp recordings were made from 26 cells using electrodes filled with 2 M Cs^+-acetate and QX314. In these cells the input resistance was increased to 68.4 ± 5.5 MΩ ($p < 0.05$, t test). Mean resting membrane potential was −64.7 ± 0.5 mV (n = 148; in eight cells impaled with Cs^+-filled electrodes the resting membrane potential was depolarized to −50 mV and these cells were not included in the mean value).

Effects of 5-HT and 8-OH-DPAT on intrinsic membrane properties

In 20 cells impaled with K^+-containing electrodes we investigated the amplitude of the 5-HT–induced hyperpolarization (Fig. 1*A*). 5-HT applied at concentrations between 50 and 100 μM hyperpolarized 18 of these cells (mean value was −7.2 ± 0.64 mV), but in 2 there was no response. In six cells a slow depolarization followed the slow hyperpolarization. The 5-HT–induced hyperpolarization was associated with a decrease in input resistance from 49.8 ± 6.5 MΩ to 36.4 ± 4 MΩ (n = 18, $p <$ 0.05, paired t test). In all subsequent cells where we applied 5-HT we either brought the membrane potential back to control levels by constant current injection or we blocked K^+ currents intracellularly and used discontinuous voltage clamp.

8-OH-DPAT applications did not result in large hyperpolarization of the cells, as shown in Figure 1*B*. In 10 out of 18 cells a small hyperpolarization of 1.03 ± 0.1 mV was seen, but in the remainder there was no change. Neither was there any significant change in input resistance (48.7 ± 7 MΩ to 46.0 ± 6 MΩ, n = 10).

5-HT decreased the slow afterhyperpolarization (sAHP) (see Fig. 1*C*) following a short train of action potentials (Segal et al., 1989), but this was not observed following 8-OH-DPAT.

Effects of 5-HT and 8-OH-DPAT on synaptic events

In all studies on synaptic potentials we used concentrations of 5-HT and 8-OH-DPAT between 1–20 μM.

EPSP-IPSP sequences were evoked following Schaffer collateral stimulation. Occasionally, an increase in the EPSPs (by approximately 15–20%) was observed, but the most prominent effect was a reduction in both the fast and slow IPSPs. Mean values of the fast IPSPs changed from −2.7 ± 0.3 mV to −1.0 ± 0.2 mV (n = 10) and the slow IPSPs from −2.4 ± 0.3 mV to −0.7 ± 0.2 mV (n = 10), respectively ($p < 0.05$, paired t tests). The IPSPs were measured at their peaks, 23.4 ± 1.3 msec, 125.8 ± 4.9 msec, respectively (n = 10), and at the resting membrane potential of the cell. A comparison of the percentage reductions by 5-HT between the fast and slow IPSPs showed that both were affected to a similar extent (p = 0.58, t test).

Similar reversible reductions in IPSPs were also seen following 8-OH-DPAT, but there was no effect upon EPSPs with the concentrations we used. Mean values of IPSPs were changed from −2.8 ± 0.3 mV to −1.1 ± 0.2 mV and from −2.5 ± 0.4 mV to −0.9 ± 0.3 mV (n = 11) for fast and slow IPSPs, respectively.

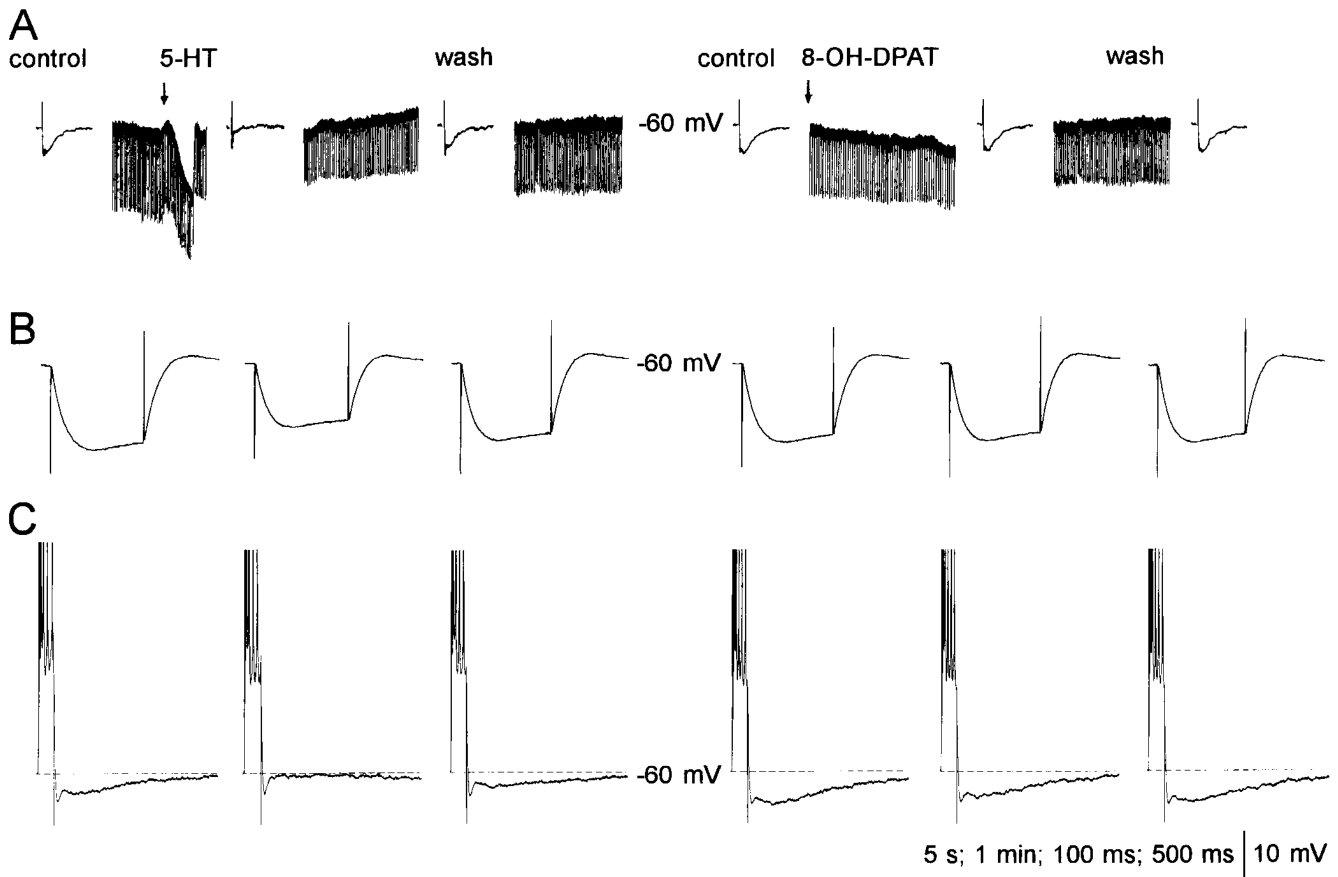

Figure 1. Effects of 5-HT and 8-OH-DPAT on the membrane potential (*A*) the input resistance (*B*), and the slow AHP (*A* and *C*), of the same pyramidal cell. In *A*, the downward deflections represent the changes in membrane potential during negative current pulses; following 5-HT there was a large hyperpolarization that we brought back to the control potential by constant positive current injection. Note also the small depolarization following the 5-HT application that we sometimes saw 3–5 min after the application. Following application of 8-OH-DPAT we occasionally observed a small slow hyperpolarization, as in this cell. 5-HT application was associated with a decrease in the input resistance, as shown in *B*, but this was not the case following 8-OH-DPAT. In *C*, we show that the slow AHP was reduced following 5-HT; however, the fast AHP was unchanged (n = 17, p = 0.36, paired t test). Mean values of the slow AHP changed from −3.3 ± 0.3 mV to −0.8 ± 0.1 mV (n = 17, $p < 0.05$, paired t test). 8-OH-DPAT had no effect upon either the fast or the slow AHP.

Monosynaptic inhibitory potentials

To avoid complications from the effects of 5-HT upon EPSPs and also to look at the direct effects of 5-HT on inhibitory interneurons, we elicited monosynaptic IPSPs in the absence of excitatory synaptic transmission (Davies et al., 1990) (Fig. 2). The fast and slow IPSPs were pharmacologically identified as GABA-A and GABA-B receptor-mediated events by their sensitivity to bicuculline (n = 2) and CGP55845A and CGP35348 (n = 16) (Karlsson et al., 1992; Davies et al., 1993), respectively. Following the application of both 5-HT and 8-OH-DPAT, reductions in the peak amplitudes of the monosynaptic fast and slow IPSPs were approximately 60 and 80%, respectively (details in legend to Fig. 2). The reduction in the slow IPSP was significantly larger than the reduction of the fast IPSP ($p < 0.05$, t test); however, there was no change in the reversal potentials of either inhibitory potential following 5-HT or 8-OH-DPAT (see Fig. 2).

In CA1 pyramidal cells, both types of monosynaptic IPSPs overlap in their times of onset (Davies et al., 1993); therefore, the decreases in the fast IPSPs could have arisen from the larger effects of 5-HT and 8-OH-DPAT on the slow IPSP. To remove this possibility we isolated the fast IPSP from the slow using CGP35348 or CGP55485A. Under these conditions fast IPSPs were still reduced by 5-HT and 8-OH-DPAT (see legend to Fig. 3).

Monosynaptic inhibitory currents

To be sure that the effects of 5-HT were at a synaptic level rather than a result of changes in total conductance of the membrane, we used single-electrode voltage clamp and blocked K^+ channels with intracellular Cs^+ and QX314. The blockade of 5-HT–induced hyperpolarizations by this method was not always immediate, but in all the cells used for analysis the total conductance of the membrane was unchanged following the application of 5-HT. With this technique fast IPSCs were still significantly reduced by both 5-HT and 8-OH-DPAT (Fig. 3), and to a similar extent as observed in the bridge mode recordings, p = 0.4, t test. The reversal potentials of the remaining currents were also unchanged (see Fig. 3).

Effect of a 5-HT_{1A} antagonist

The similar effects of 8-OH-DPAT and 5-HT applications on fast inhibition suggested that this action of 5-HT was mediated by 1A receptors. To test this further, we first applied the 5-HT_{1A} receptor antagonist, NAN-190, before application of 5-HT. In three cells, NAN-190 alone had no significant effect upon monosynaptic fast IPSPs (p = 0.9, paired t test). Neither was there any significant change in fast IPSPs when both 5-HT and NAN-190 were applied together (mean values were −6.8 ± 1.1 mV

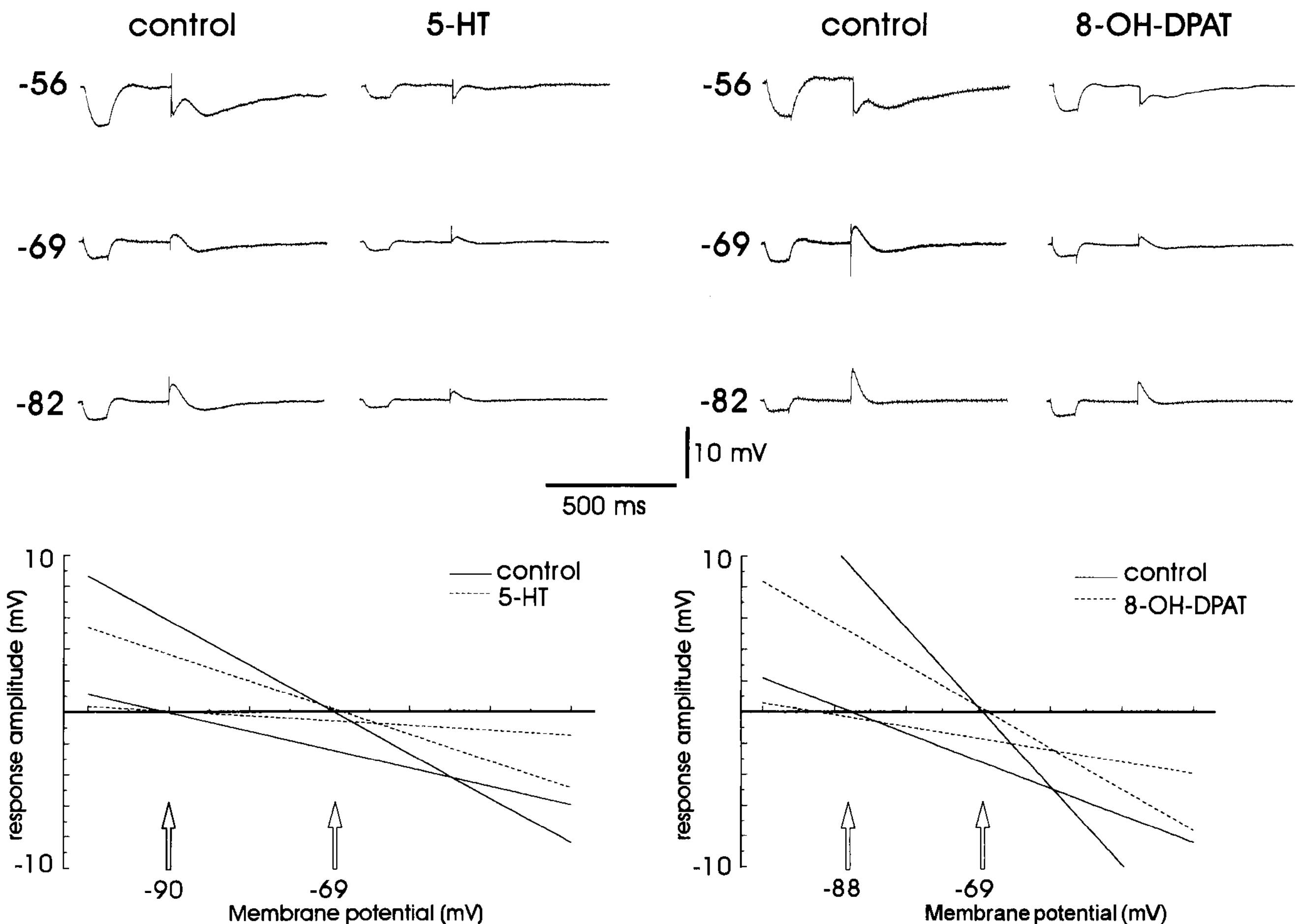

Figure 2. Monosynaptic IPSPs (close stimulation site in the presence of NBQX and APV) were evoked from the same cell before and after the application of both 5-HT and 8-OH-DPAT. A 100 msec current prepulse, 0.18 nA, was given prior to the evoked synaptic potential in order to monitor bridge balance and also to show the change in total membrane conductance following the application of 5-HT. This was not significant following the application of 8-OH-DPAT. Measured at their peaks of 10.8 ± 0.7 msec and 133 ± 4.7 msec, respectively, and close to resting membrane potentials fast and slow IPSPs were reduced from −5.1 ± 0.4 mV to −2.1 ± 0.4 mV and from −3.8 ± 0.6 mV to −0.4 ± 0.2 mV, respectively (n = 10) by 5-HT, $p < 0.05$, paired t tests. Similar results were also obtained following the application of 8-OH-DPAT. Mean values were −4.6 ± 0.4 mV to −2.4 ± 0.3 mV and from − 2.1 ± 0.5 mV to −0.75 ± 0.1 mV (n = 11) $p < 0.05$, paired t tests, fast and slow IPSPs, respectively. For the cell shown, the change in amplitude of the fast and slow IPSPs was plotted against membrane potential. Regression lines show no significant effect, by either drug (*dotted lines*), on the reversal potentials of both IPSPs, (p = 0.35, t tests). We have omitted the individual points for clarity. However, mean reversal potential values were −65.1 ± 1.0 mV and −91.4 ± 1.8 mV for the fast and slow IPSPs, respectively, before the application of 5-HT and −63 ± 0.9 mV and −94 ± 2.2 mV afterwards. Reversal potentials were also unchanged by 8-OH-DPAT; mean values were −65.6 ± 1.3 mV and −91 ± 1.6 mV for fast and slow IPSPs, respectively. In this cell we applied 5-HT and waited for a 20 min recovery period before applying 8-OH-DPAT. Often, a full recovery of the IPSP after 5-HT was not seen, as was the case in this cell. However, slightly increasing the stimulus intensity could return IPSPs to control levels. In this cell, we used 5V in the 5-HT experiment and 7 V when applying 8-OH-DPAT.

compared with −7.2 ± 1.3 mV, p = 0.8, paired t test, in five cells held at −59 ± 2.4 mV using constant current injection). In two cells we observed a reversible increase in excitability following 5-HT when 1A receptors were blocked (Beck, 1992). In the other cells, the electrodes contained QX314, and this prevented the excitability and allowed quantitative analysis of the IPSPs.

Responses of cells to exogenously applied GABA

The reductions in inhibition mediated by 5-HT could be due to a presynaptic effect at the inhibitory interneurons or to a postsynaptic effect at the GABA-A receptor complex. To test this, we applied GABA iontophoretically to the soma and dendrites of CA1 cells using the same voltage clamp conditions as described above.

GABA application to the cell soma resulted in mixed biphasic responses (Fig. 6) that were blocked by bicuculline (n = 2) and also differentiated on the basis of their reversal potentials (Alger and Nicoll, 1982; Xie and Smart, 1993). Application of GABA to the dendrites resulted in a predominant inward current (see Fig. 5). 5-HT and 8-OH- DPAT, whether applied to the soma or the dendrites, had no effect upon either somatic (Fig. 4) or dendritic (Fig. 5) responses of the cells to GABA. However, as shown in Figure 5, in cells where there was no change in the GABA responses we still observed typical reductions in the stimulus evoked IPSCs by 5-HT and 8-OH-DPAT.

Responses of putative inhibitory interneurons

Ten cells possessed electrophysiological characteristics that were very different to the pyramidal cells. A number of properties have been described as signatures of inhibitory interneurons present within the cell body layer of CA1 (Lacaille, 1991; Buhl

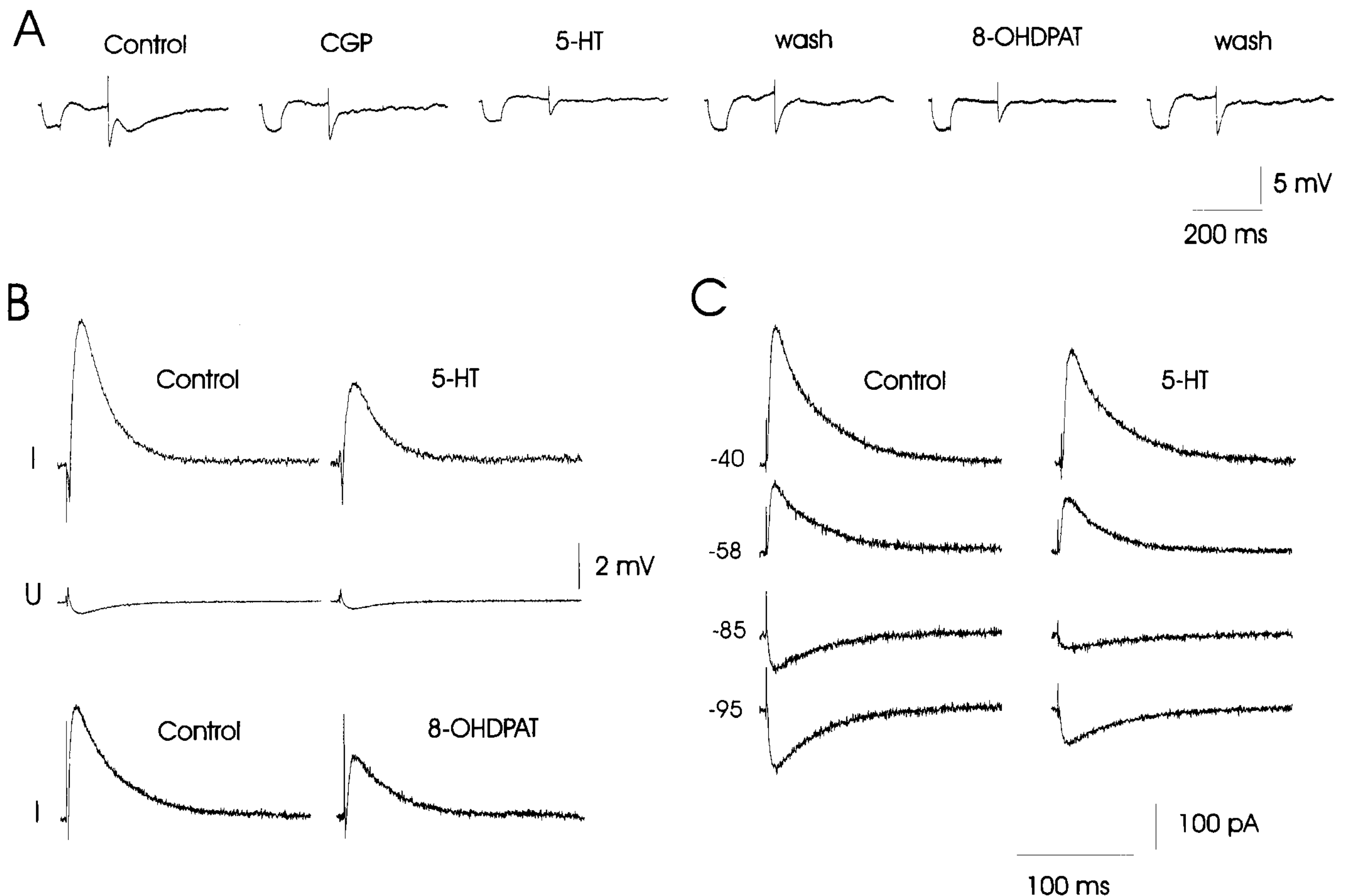

Figure 3. Fast monosynaptic IPSPs were isolated from the slow IPSP by the blockade of the latter using CGP55485A (*CGP*) (*upper traces, A*). The amplitudes of the isolated fast IPSPs, measured at their peak, 8.3 ± 0.5 msec and close to resting membrane potential were still significantly reduced ($p < 0.05$, paired t tests) following the application of 5-HT and 8-OH-DPAT. Mean values changed from −5.7 ± 0.4 mV to −3.3 ± 0.5 mV ($n = 8$) and from −5.6 ± 0.6 mV to −3.3 ± 0.7 mV ($n = 7$) for 5-HT and 8-OH-DPAT, respectively. There was no effect on the reversal potentials of the isolated fast IPSPs. Mean values were −65.0 ± 1.1 mV before and −62.9 ± 0.8 mV after 5-HT and −63.2 ± 1.0 mV before and −64.1 ± 0.9 mV after 8-OH-DPAT ($p > 0.36$, t test). Mean values of estimated conductance changes during the fast IPSP were calculated (Ginsborg, 1973) and changed from 21.1 ± 5.9 nS to 9.6 ± 2.3 nS ($n = 7$, $p < 0.05$ paired t test) after 5-HT. Fast monosynaptic IPSCs (close stimulation, NBQX and APV in the bath, Cs^+-acetate and QX314 in the electrode) were reduced following application of 5-HT and 8-OH-DPAT. *B,* The cell was voltage clamped at −52 mV, and following 5-HT application the fast IPSCs were clearly reduced in this cell. Mean values of the peak IPSCs, measured at 6.6 ± 0.4 msec ($n = 8$) at holding potentials ranging from −55 to −40 mV, were reduced from 325 ± 49 pA to 159 ± 43 pA ($n = 8$) by 5-HT. Recovery was observed in five cells. Below the current traces for this cell we show the corresponding voltage deflections. We compared the amplitudes of these voltage changes with the amplitude of the unclamped IPSP to calculate a clamp efficiency of 90% in this cell. In another cell (*lower trace in B*), held at −52 mV, evoked IPSCs were also reduced by 8-OH-DPAT. There was no change in the current required to hold the cell at −52 mV. Recovery was observed after a few minutes. *C,* The reversal potentials of monosynaptic fast IPSCs were unchanged following the application of 5-HT. This is shown for the same cell at different holding potentials before and after the application of 5-HT. The mean reversal potential for the fast IPSC was −76.5 ± 1.1 mV ($n = 13$). In this cell, no slow outward current was seen. Changes in the amplitude of the fast IPSCs with holding potential allowed the direct calculation of the IPSC conductances. Mean values changed from 14.4 ± 1.9 nS to 8.8 ± 1.6 nS after the application of 5-HT ($n = 12$), with a recovery to 12.5 ± 2.2 nS in seven cells. These values and their reductions following 5-HT were not significantly different ($p > 0.2$, t tests) to indirectly estimated conductances of isolated monosynaptic IPSPs.

et al., 1994a,b) and our 10 cells possessed these (Fig. 6*A*). They had faster action potentials (width at half amplitude ≤ 0.5 msec) and also fired at higher frequencies and with less accommodation than seen in pyramidal cells. Accommodation ranged from 0 to 33% when the second action potential was compared with the last during a 700 msec current pulse. Following a short train of action potentials only a fast AHP was observed. A high incidence of spontaneous synaptic excitatory activity was also present. EPSPs, evoked by Schaffer collateral stimulation, only required very low stimulus intensities (1–2 V) in these cells compared with pyramidal cells (8–12 V).

5-HT hyperpolarized the putative interneurons by between 4–10 mV, but unlike its effects upon pyramidal cells, 5-HT did not induce a later depolarization (see above) (Fig. 6*B*). The fast AHPs evoked by a short train of action potentials were also not affected by 5-HT. As for the pyramidal cells, we did not observe any changes in these parameters with 8-OH-DPAT.

EPSPs evoked in the putative interneurons by Schaffer collateral stimulation, measured at their peak (7.8 ± 0.2 msec), were reduced by 5-HT and 8-OH-DPAT (Fig. 6*C*) from 10.5 ± 2.3 mV to 1.3 ± 0.5 mV ($n = 4$, $p < 0.05$, paired t test) and from 8.6 ± 2.5 mV to 0.5 ± 0.5 mV ($n = 4$, $p < 0.05$, paired t test), respectively. In all cases where 5-HT induced a hyperpolarization in these cells this was overcome by the injection of constant positive current before recording stimulus evoked EPSPs.

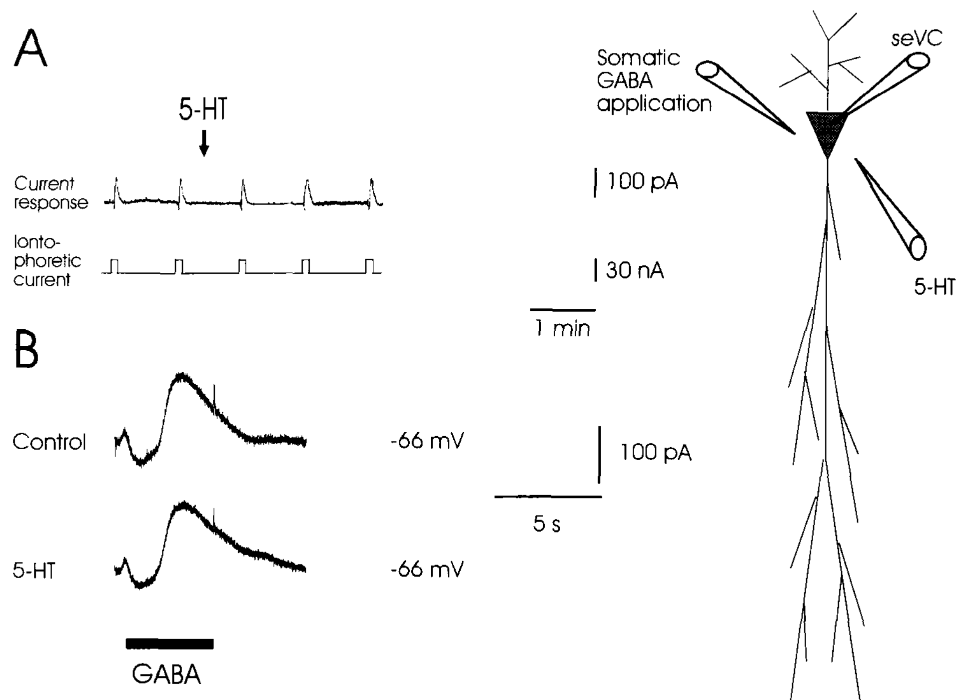

Figure 4. Effects of 5-HT on the responses of a pyramidal cell to iontophoretically applied GABA. When applied to the soma of the cell, GABA elicited a mixed biphasic current responses. In three cells the somatic responses reversed at -73.3 ± 5.4 mV, while the dendritic responses reversed at -49.3 ± 6.7 mV ($p < 0.05$, paired t test). Neither of these responses were affected by 5-HT. Mean somatic GABA-induced conductances were unchanged at 12.2 ± 3.3 nS compared with 12.0 ± 3.4 nS following the application of 5-HT to the soma ($p = 0.69$, paired t test) and also unchanged in one cell following 8-OH-DPAT. Note also on a slow time scale the lack of any outward current during the application of 5-HT.

Discussion

In area CA1, 5-HT decreased both fast and slow synaptic inhibition of the pyramidal cells. These effects were mimicked by the 5-HT_{1A} agonist 8-OH-DPAT and blocked by the 1A antagonist NAN-190. We suggest that the mechanism was presynaptic since 5-HT and 8-OH-DPAT strongly inhibited putative inhibitory interneurons and had no effect upon exogenous GABA applications to pyramidal cells.

Effects on intrinsic properties

5-HT has large effects upon membrane properties (Jahnsen, 1980; Segal, 1980; Colino and Halliwell, 1987). 5-HT decreased the slow hyperpolarization (sAHP) in pyramidal cells and also hyperpolarized and later depolarized these cells with corresponding decreases and increases in input resistance. The 5-HT–induced increase in total cell conductance (seen as the hyperpolarization and decreased input resistance) was an important consideration during our analysis of inhibition, since in itself it could apparently reduce the amplitude of the synaptic events (Ginsborg, 1973). We minimized this by using a single-electrode voltage clamp where the outward current corresponding to the hyperpolarization was blocked by intracellular Cs^+ and QX314 (Andrade, 1991) but where the intracellular milieu was otherwise unchanged.

Effects on synaptic inhibition in pyramidal cells

Polysynaptic fast and slow IPSPs were reduced to the same extent by 5-HT and 8-OH-DPAT. This strongly suggested that the effects of 5-HT on both types of inhibition were mediated by 5-HT_{1A} receptors. The effects of 5-HT were complicated by the increase in EPSPs. The mechanism underlying the increased EPSPs is still not clear. A decrease in fast inhibition seems unlikely, since even in slices incubated with the GABA-A antagonist picrotoxin, the 5-HT–induced increases in the EPSPs were still observed (Segal, 1990). More recent studies have attributed the increased EPSPs to an action of serotonin at 5-HT_{1C} receptors (Beck, 1992), a result that is also consistent with our lack of effect on EPSPs by 8-OH-DPAT.

To overcome these complicating effects of the EPSPs we recorded monosynaptic IPSPs where excitation was completely blocked. Again, both fast and slow IPSPs were reduced by 5-HT. We confirmed that these effects of 5-HT were via 5-HT_{1A} receptors not only because the effects were mimicked by the agonist 8-OH-DPAT, but because they were also prevented by the 5-HT_{1A} receptor antagonist NAN-190. The results contrast with previous work where 5-HT was thought to decrease only slow inhibition (Segal, 1990; Oleskevich and Lacaille, 1992) via an unknown receptor but possibly via a G-protein–mediated action on the postsynaptic K^+ conductance activated by GABA-B receptors (Andrade et al., 1986). Our results demonstrate that 5-HT acting through 5-HT_{1A} receptors decreases both fast and slow inhibition.

We observed a greater effect of 5-HT on monosynaptic slow IPSPs compared with fast IPSPs, although both were significantly reduced. Monosynaptic IPSPs recorded in the pyramidal cell reflect the direct activation of the inhibitory interneurons (Davies et al., 1990). The effects upon the slow IPSP are likely to be due to an effect of 5-HT upon those interneurons thought to mediate slow, GABA-Bergic inhibition present within stratum lacunosum moleculare (SLM) of CA1 (Lacaille and Schwartzkroin, 1988a,b; Williams and Lacaille, 1992). Indeed, these interneurons possess a Ca^{2+} current that is sensitive to 5-HT (Fraser and MacVicar, 1991) and there is anatomical evidence showing a major 5-HT-ergic innervation into SLM (Moore and Halaris, 1975; Freund et al., 1990). Nevertheless, the reduction of the fast IPSPs by 5-HT and also 8-OH-DPAT requires explanation. It could be argued that our initial results with 5-HT on the synaptic potentials were simply due to a postsynaptic 5-HT–induced conductance increase. Clearly, this cannot explain the effects of 8-OH-DPAT. In addition, the use of a single-electrode voltage clamp, where K^+ channels were also blocked intracellularly, minimized the possibility that the reduction in fast IPSCs

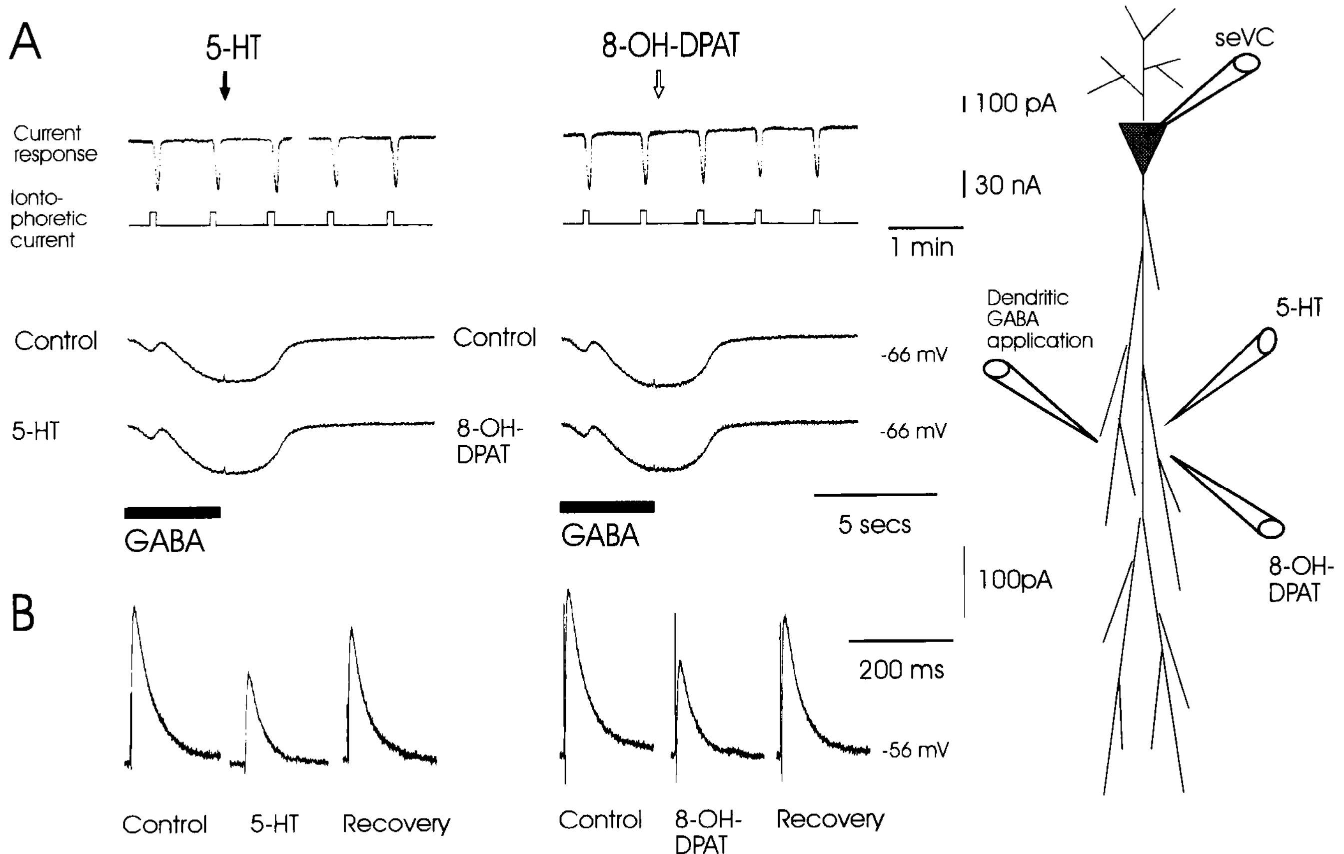

Figure 5. Effects of 5-HT and 8-OH-DPAT on the responses of a cell to GABA applied to its dendrites. GABA elicited a mainly dendritic, inward response that reversed at a mean value of -47.4 ± 5.1 mV ($n = 4$). There was no change in the GABA-induced currents following the application of either 5-HT or 8-OH-DPAT by a drop application placed in the dendritic tree. Mean conductance change by GABA in the dendrites was 10.1 $\pm$ 3.9 nS compared with 9.9 $\pm$ 3.8 nS ($n = 7$) following 5-HT ($p = 0.69$, paired t test). Similarly, there was no change in the dendritic GABA response of three cells by 8-OH-DPAT. In *A*, the responses of the cells to the GABA applications, as well as the iontophoretic current, are shown on a slow time scale. Note the absence of any outward current following the application of 5-HT and 8-OH-DPAT. In *B*, averages of the responses of the cell to dendritic GABA applications before and after the drug treatments are shown on a faster time scale. *C* shows stimulus evoked IPSCs from the same cell, recorded approximately 30 min after the GABA applications. IPSCs were reversibly reduced following the application of both 5-HT and 8-OH-DPAT.

by 5-HT was just due to an increased conductance shunt. Our results therefore strongly suggest an additional effect of 5-HT, acting through 5-HT_{1A} receptors, to reduce fast inhibition. We can speculate that axo-axonic inhibitory cells that mediate somatically located, fast GABA-A inhibition and that extend a characteristic tuft of apical dendrites into SLM (Buhl et al., 1994b) are sensitive to 5-HT. The diversity of inhibitory interneurons (Buhl et al., 1994a), some of which may be less sensitive to 5-HT, may explain our incomplete reduction of fast inhibition. In addition, with the present techniques we cannot comment on the possible contributions from dendritic inhibition.

Therefore, it seems that the known anatomy showing a strong serotonergic innervation in SLM is consistent with our electrophysiological results. A differential effect of 5-HT on these two types of inhibition was only seen when the interneurons were stimulated directly. In contrast, under polysynaptic conditions where excitation of the inhibitory interneurons was intact, 5-HT blocked both fast and slow inhibition to the same extent. This suggest that in addition to its effects on different types of interneurons 5-HT can also act globally to reduce inhibition simply by reducing the synaptic excitation of interneurons.

Effects on putative inhibitory interneurons

All putative inhibitory interneurons were located in the CA1 pyramidal layer and selected on the basis of their electrophysiological parameters. In all ways 5-HT was inhibitory in these cells. They were hyperpolarized but never depolarized, as we had seen in pyramidal cells. Their lack of a sAHP meant that reduction of this component by 5-HT could not increase their excitability as is the case in pyramidal cells. The dramatic reduction in stimulus evoked EPSPs in these cells was also an additional dominant inhibitory effect of 5-HT and also 8-OH-DPAT. Since these cells were all impaled with K^+-containing electrodes, we cannot exclude the possibility that these effects were due partially to a postsynaptic conductance increase. However, the large effects on the EPSPs in these interneurons following 5-HT and 8-OH-DPAT contrasts with the much smaller reductions seen in EPSPs evoked from pyramidal cells at higher concentrations (Schmitz et al., unpublished observations).

All our results from interneurons are consistent with the reductions by 5-HT and 8-OH-DPAT of the fast IPSP/Cs evoked from principal cells. However, the mechanisms by which 5-HT inhibits inhibitory interneurons to produce this effect are still not clear. The 5-HT–induced hyperpolarizations point to a 5-HT–mediated increase in an intrinsic K^+ conductance. The decrease in evoked excitatory transmission by both 5-HT and 8-OH-DPAT also suggests that activation of 5-HT_{1A} receptors can reduce glutamate release from axon terminals onto the in-

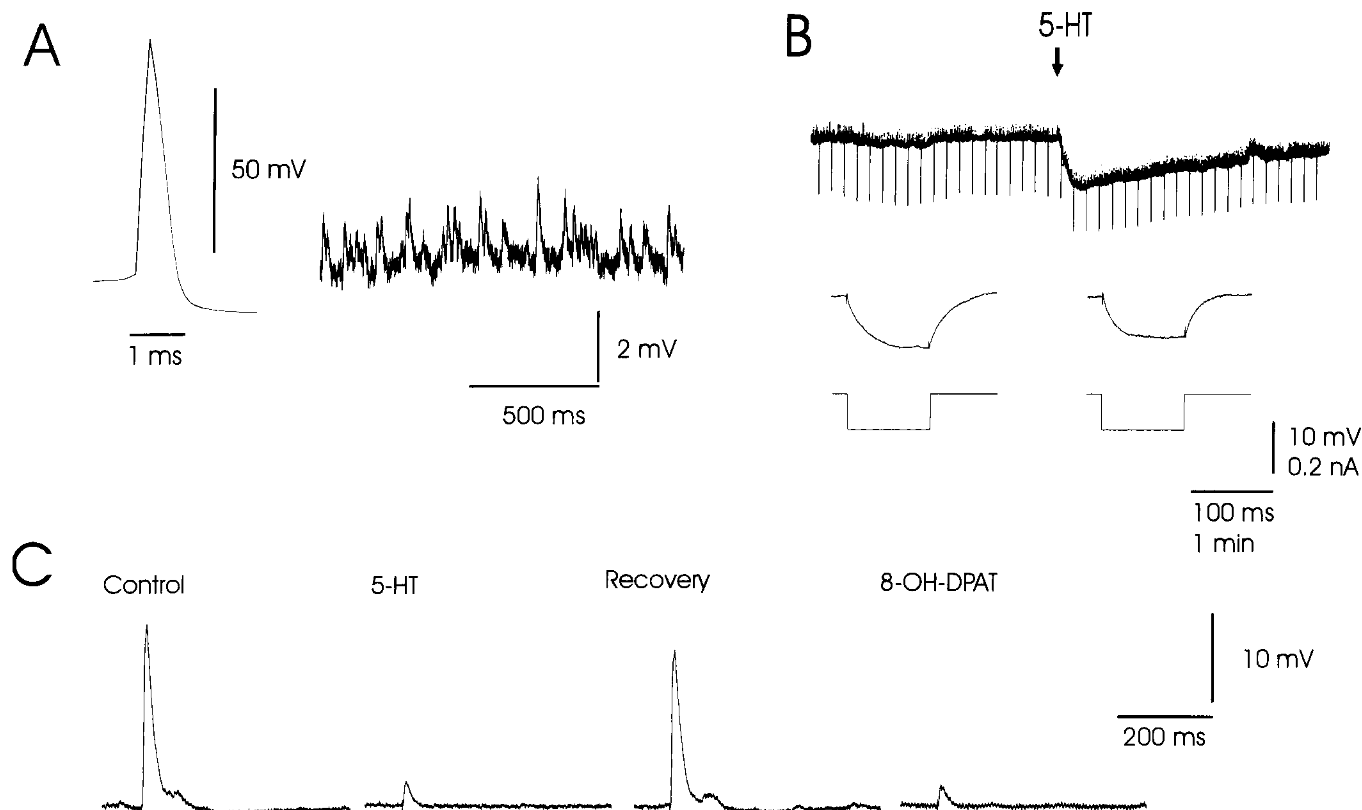

Figure 6. Responses of a putative inhibitory interneuron to 5-HT and 8-OH-DPAT. *A*, Characteristics of a typical putative inhibitory interneuron recorded in the cell body layer of CA1. The width of the action potential at half height was ≤ 0.5 msec and note also the sharp onset of the spike AHP. A large amount of spontaneous synaptic activity was also observed in this and other cells. *B*, Following the application of 5-HT to this cell a large hyperpolarization was observed that was also associated with a reduced input resistance. *C*, The response of the cell in *A* to low-intensity Schaffer collateral stimulation (1.7 V). A clear EPSP was seen at this holding potential of −81 mV. The EPSPs were strongly reduced by both 5-HT and 8-OH-DPAT (both at concentrations of 10 μM and after only 5 min of bath application). Note also the presence of the spontaneous EPSPs within the averaged traces (six sweeps). These recordings were made after a second application of 5-HT to this cell when the membrane potential during the 5-HT–induced hyperpolarization was returned to control levels by injection of constant positive current.

terneurons or negatively modulate the postsynaptic glutamate receptors. However, additional mechanisms might include reduction of GABA release directly from the axon terminals of the interneurons.

Our recordings from putative inhibitory interneurons suggested that, at least for the pyramidal cell, 5-HT was acting presynaptically to reduce inhibition. In support of this, GABA-Aergic currents elicited by GABA application directly to the CA1 pyramidal cells were completely unaffected by either 5-HT or 8-OH-DPAT.

Physiological relevance

We have described the effects of 5-HT on the modulation of inhibition of the principal output cells of the hippocampus. We are aware that studies *in vitro* may not accurately reflect the situation *in vivo*. We could only mimic the actions of 5-HT with bath and drop applications, whereas *in vivo* the release of 5-HT will depend upon the activity within the Raphe nuclei. In addition, the concentrations of 5-HT used may be very different from those encountered *in vivo*. However, previous studies have shown that full equilibration of applied drugs within slices takes at least 1 hr (Müller et al., 1988). We recorded after only 10–15 min of wash, in and together with 5-HT uptake and oxidation mechanisms, the nominal concentrations of 5-HT were likely considerably more than actually "seen" by the neurons.

However our *in vitro* results show that 5-HT can excite pyramidal cells by reducing both fast and slow synaptic inhibition and also via slow depolarizations and reductions in the sAHP. Its sole inhibitory effect on the pyramidal cells was a hyperpolarization. In contrast, 5-HT only inhibited presumed inhibitory interneurons both by hyperpolarization and reduction of their synaptic excitation.

We suggest that 5-HT via disinhibition of CA1 pyramidal cells can alter the balance between excitation and inhibition within area CA1. Such contributions by 5-HT may underlie its modulation of rhythmical slow activity and its requirement during spatial learning.

References

Alger BE, Nicoll RA (1982) Pharmacological evidence for two kinds of GABA receptor on rat hippocampal pyramidal cells studied *in vitro*. J Physiol (Lond) 328:125–141.

Andrade R (1991) Blockade of neurotransmitter-activated K^+ conductance by QX-314 in the rat hippocampus. Eur J Pharmacol 199:259–262.

Andrade R, Chaput Y (1991) 5-HT4-like receptors mediate the slow excitatory response to serotonin in the rat hippocampus. J Pharmacol Exp Ther 257:930–937.

Andrade R, Nicoll RA (1987) Pharmacologically distinct actions of serotonin on single pyramidal neurons of the rat hippocampus recorded *in vitro*. J Physiol (Lond) 394:99–124.

Andrade R, Malenka R, Nicoll R (1986) A G-protein couples serotonin

and GABA-B receptors to the same channel in hippocampus. Science 234:1261–1265.
Assaf SY, Miller JJ (1978) The role of a Raphe serotonin system in the control of septal unit activity and hippocampal desynchronisation. Neuroscience 3:539–550.
Beck SG (1992) 5-Hydroxytryptamine increases excitability of CA1 hippocampal pyramidal cells. Synapse 10:334–340.
Beck SG, Clarke WP, Goldfarb J (1985) Spiperone differentiates mutiple 5-HT responses in rat hippocampal slices *in vitro*. Eur J Pharmacol 116:195–197.
Buhl EH, Halasy K, Somogyi P (1994a) Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites (see comments). Nature 368:823–828.
Buhl EH, Han ZS, Lorinczi Z, Stezhka VV, Karnup SV, Somogyi P (1994b) Physiological properties of anatomically identified axo-axonic cells in the rat hippocampus. J Neurophysiol 71:1289–1307.
Chaput Y, Araneda RC, Andrade R (1990) Pharmacological and functional analysis of a novel serotonin receptor in the rat hippocampus. Eur J Pharmacol 182:441–456.
Colino A, Halliwell JV (1987) Differential modulation of three separate K^+ conductances in hippocampal CA1 neurons by serotonin. Nature 328:73–77.
Davies CH, Davies SN, Collingridge GL (1990) Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. J Physiol (Lond) 424:513–531.
Davies CH, Pozza MF, Collingridge GL (1993) CGP 55845A: a potent antagonist of GABA-B receptors in the CA1 region of rat hippocampus. Neuropharmacology 32:1071–1073.
Dreier JP, Heinemann U (1991) Regional and time dependent variations of low Magnesium induced epileptiform activity in rat temporal cortex. Exp Brain Res 87:581–596.
Fraser DD, MacVicar BA (1991) Low-threshold transient calcium current in rat hippocampal lacunosum-moleculare interneurons: kinetics and modulation by neurotransmitters. J Neuroscience 11:2812–2820.
Freund TF, Gulyas AI, Acsady L, Gorcs T, Toth K (1990) Serotonergic control of the hippocampus via local inhibitory interneurons. Proc Natl Acad Sci USA 87:8501–8505.
Ghadimi BM, Jarolimek W, Misgeld U (1994) Effects of serotonin on hilar neurons and granule cell inhibition in the guinea pig hippocampal slice. Brain Res 633:27–32.
Ginsborg BL (1973) Electrical changes in the membrane in junctional transmission. Biochem Biophys Acta 300:289–317.
Jahnsen H (1980) The action of 5-hydroxytryptamine on neuronal membranes and synaptic transmission in area CA1 of the hippocampus *in vitro*. Brain Res 197:83–94.
Karlsson G, Kolb C, Hausdorf A, Portet C, Schmutz M, Olpe HR (1992) GABA-B receptors in various *in vitro* and *in vivo* models of epilepsy: a study with the GABA-B receptor blocker CGP 35348. Neuroscience 47:63–68.
Klancnik JM, Baimbridge KG, Philips AG (1989) Increased population spike amplitude in the dentate gyrus following systemic administration of 5-hydroxytryptophan or 8-hydroxy-2-(di-n-propylamino)tetralin. Brain Res 505:145–148.
Lacaille JC (1991) Postsynaptic potentials mediated by excitatory and inhibitory amino acids in interneurones of stratum pyramidale of the CA1 region of rat hippocampal slices *in vitro*. J Neurophysiol 66: 1441–1454.
Lacaille JC, Schwartzkroin PA (1988a) Stratum lacunosum moleculare interneurons of hippocampal CA1 region II Intrasomatic and intradendritic recordings of local circuit synaptic interactions. J Neurosci 8:1411–1424.
Lacaille JC, Schwartzkroin PA (1988b) Stratum lacunosum-moleculare interneurones of hippocampal CA1 region. I Intracellular response characteristics, synaptic responses, and morphology. J Neurosci 8:1400–1410.
Moore RY, Halaris AE (1975) Hippocampal innervation by serotonin neurons of the midbrain raphe in the rat. J Comp Neurol 164:171–183.
Müller W, Misgeld U, Heinemann U (1988) Carbachol effects on hippocampal neurones *in vitro*: dependence on the rate of rise of carbachol tissue concentration. Exp Brain Res 72:287–298.
Oleskevich S, Lacaille JC (1992) Reduction of GABAB inhibitory postsynaptic potentials by serotonin via pre- and postsynaptic mechanisms in CA3 pyramidal cells of rat hippocampus *in vitro*. Synapse 12:173–188.
Richter Levin G, Greenberger V, Segal M (1993) Regional specificity of raphe graft-induced recovery of behavioral functions impaired by combined serotonergic/cholinergic lesions. Exp Neurol 121:256–260.
Richter Levin G, Segal M (1990) Effects of serotonin releasers on dentate granule cell excitability in the rat. Exp Brain Res 82:199–207.
Ropert N (1988) Inhibitory action of serotonin in CA1 hippocampal neurons *in vitro*. Neuroscience 26:69–81.
Ropert N, Guy N (1991) Serotonin facilitates GABAergic transmission in the CA1 region of rat hippocampus *in vitro*. J Physiol (Lond) 441: 121–136.
Segal M (1980) The action of serotonin in the rat hippocampal slice preparation. J Physiol (Lond) 303:423–439.
Segal M (1990) Serotonin attenuates a slow inhibitory postsynaptic potential in rat hippocampal neurons. Neuroscience 36:631–641.
Segal M, Azmitia EC, Whitaker-Azmitia PM (1989) Physiological effects of selective 5-HT_{1a} and 5-HT_{1b} ligands in rat hippocampus: comparison to 5-HT. Brain Res 502:67–74.
Vanderwolf CH (1988) Cerebral activity and behavior: control by central cholinergic and serotonergic systems. Int Rev Neurobiol 30:225–340.
Williams S, Lacaille JC (1992) GABA-B receptor-mediated inhibitory postsynaptic potentials evoked by electrical stimulation and by glutamate stimulation of interneurons in stratum lacunosum moleculare in hippocampal CA1 pyramidal cells *in vitro*. Synapse 11:249–258.
Xie X, Smart TG (1993) Properties of GABA-mediated synaptic potentials induced by zinc in adult rat hippocampal pyramidal neurones. J Physiol (Lond) 460:503–523.