Hippocampal Interneurons Are Excited Via Serotonin-Gated Ion Channels

LORI L. McMAHON AND JULIE A. KAUSER

Department of Neurobiology, Duke University Medical Center, Durham, North Carolina 27710

McMahon, Lori L. and Julie A. Kauer. Hippocampal interneurons are excited via serotonin-gated ion channels. J. Neurophysiol. 78: 2493 ± 2502, 1997. Serotonergic neurons of the median raphe nucleus heavily innervate hippocampal GABAergic interneurons located in stratum radiatum of area CA1, suggesting that this strong subcortical projection may modulate interneuron excitability. Using whole cell patch-clamp recording from interneurons in brain slices, we tested the effects of serotonin (5-HT) on the physiological properties of these interneurons. Serotonin produces a rapid inward current that persists when synaptic transmission is blocked by tetrodotoxin and cobalt, and is unaffected by ionotropic glutamate and γ-aminobutyric acid (GABA) receptor antagonists. The 5-HT-induced current was independent of G-protein activation. Pharmacological evidence indicates that 5-HT directly excites these interneurons through activation of 5-HT3 receptors. At membrane potentials negative to −55 mV, the current-voltage (I-V) relationship of the 5-HT current displays a region of negative slope conductance. Therefore the response of interneurons to 5-HT strongly depends on membrane potential and increases greatly as cells are depolarized. Removal of extracellular calcium, but not magnesium, increases the amplitude of 5-HT-induced currents and removes the region of negative slope conductance, thereby linearizing the I-V relationship. The axons of 5-HT-responsive interneurons ramify widely within CA1; some of these interneurons also project to and arborize extensively in the dentate gyrus. The organization of these inhibitory connections strongly suggests that these cells regulate excitability of both CA1 pyramidal cells and dentate granule cells. As our results indicate that 5-HT may mediate fast excitatory synaptic transmission onto these interneurons, serotonergic inputs can simultaneously modulate the output of both hippocampus and dentate gyrus.

INTRODUCTION

GABAergic interneurons in the hippocampus, through synapses on dendritic and somatic sites, powerfully inhibit excitatory pyramidal cells. Through extensive axon arbors, a single interneuron forms over 1,000 synapses onto multiple pyramidal cells and can make up to 12 synaptic contacts with a single postsynaptic cell (Buhl et al. 1994). The activity of an individual interneuron can phase lock spontaneous firing and membrane potential oscillations of multiple pyramidal cells (Cobb et al. 1995), implying that a single interneuron synchronously depresses the activity of the hundreds of pyramidal cells it innervates (Freund and Buzsaki 1996).

Because of the importance of interneurons in controlling pyramidal cell output, it is crucial to understand how interneuron excitability is regulated. Most observations on this point are indirect, derived from recording GABAergic inhibitory postsynaptic potentials (IPSPs) from CA1 pyramidal cells. Biogenic amines modulate GABAergic neurotransmission in hippocampus, but little is known about direct effects on interneurons (Bergles et al. 1996; Doze et al. 1991; Fraser and MacVicar 1991). We have focused on serotonin (5-HT), which increases IPSP frequency in pyramidal cells, suggesting that GABAergic interneurons are excited by this neurotransmitter (Ropert and Guy 1991). The 5-HT-induced increase in IPSP frequency is abolished when synaptic transmission is blocked, and also by antagonists of 5-HT3 receptors. These data are consistent with excitation of inhibitory interneurons present within the hippocampal slice preparation via 5-HT3 receptors (Ropert and Guy 1991). Unlike all other 5-HT receptors, the 5-HT3 receptor subtype is a cation-selective ligand-gated ion channel, thought to mediate fast synaptic transmission (Derkach et al. 1989; Maricq et al. 1991; Sugita et al. 1992). Although direct functional evidence for 5-HT3 receptors on hippocampal interneurons is lacking, 5-HT3 receptors are present on basket cells in dentate gyrus (Kawa 1994). However, in situ hybridization studies localized mRNA for 5-HT3 receptors to interneurons within the stratum radiatum and at the border between strata radiatum and lacunosum in the hippocampus (SR interneurons) (Morales et al. 1996; Tecott et al. 1993). Moreover, serotonergic afferents originating in the brain stem median raphe nucleus strongly innervate SR interneurons, forming as many as 40 synaptic contacts onto a single cell (Acsady et al. 1993; Freund et al. 1990). Because the 5-HT3 receptor is thought to mediate fast synaptic transmission analogous to that of ligand-gated ion channels for glutamate, serotonin may mediate fast synaptic transmission onto these interneurons by activating 5-HT3 receptors. If the heavy serotonergic innervation of SR interneurons leads to 5-HT3 receptor activation, this pathway may represent one of the most important mechanisms by which subcortical regions control hippocampal output.

We used whole cell patch-clamp recording from visually identified SR interneurons to determine directly whether these cells are excited by serotonin. We find that application of 5-HT potently excites SR interneurons via postsynaptic 5-HT3 receptors. Analysis of axon arbors shows that one class of interneuron with 5-HT responses innervates not only the local region in hippocampus, but also the dentate gyrus. These data suggest that serotonergic innervation of hippocampal interneurons strongly excites these cells, which release γ-aminobutyric acid (GABA) both in hippocampus and in dentate gyrus. By exciting hippocampal interneurons via 5-HT-gated ion channels, serotonergic afferents can exert potent control over hippocampal excitability.
METHODS

Preparation of brain slices

Preparation of brain slices and interneuron recording followed previously described techniques (McMahon and Kauer 1997). In brief, 16- to 22-day-old Sprague-Dawley rats were anesthetized with halothane and quickly decapitated. The brain was rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 119 NaCl, 2.5 KCl, 1.3 MgSO4, 2.5 CaCl2, 1.0 NaH2PO4, 26.0 NaHCO3, and 11 glucose; the solution was continuously gassed with 95% O2-5% CO2 (pH 7.4, osmolality 285 mosm). A vibratome was used to cut 300- to 400-μm thick coronal slices through the dorsal hippocampus. Slices were allowed to rest at least 1 h in an interface chamber at room temperature. Slices were used for experiments within 5 h after cutting.

Electrophysiology

Whole cell voltage- and current-clamp recordings were obtained from CA1 hippocampal interneurons located in s. radiatum or at the s. radiatum/lacunosum border, using techniques previously described (McMahon and Kauer 1997). ACSF warmed to 28–30°C was constantly perfused at 2–4 ml/min while the slice was held submerged between two nets. Water-immersion optics were used to locate recorded interneurons, most often located within 100 μm of the surface of the slice. Patch pipettes had resistances of 2–4 MΩ when filled with the following solution (in mM): 100 cesium gluconate, 5 MgCl2, 40 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 0.6 ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA), and 2 Na2-ATP (265–270 mosm; pH 7.3 with CsOH). In experiments recorded under current-clamp conditions, cesium gluconate was replaced with potassium gluconate. In many experiments, 300 μM guanosine-5’-O-(3-thiotriphosphate) (GTPγS) was added to the pipette solution to irreversibly activate G-proteins, and thus to isolate the 5-HT response.

An Axopatch 200 in voltage-clamp mode or an Axoclamp 2A amplifier (Axon Instruments) in either the continuous, single-electrode voltage-clamp or bridge mode was used to amplify recorded signals, and the output was monitored on an oscilloscope. Holding potential in voltage-clamp recordings was −85 mV, unless otherwise specified. In current-clamp, interneurons were recorded at the resting membrane potential (−62 to −75 mV). Data were filtered at 3 kHz and displayed on a Gould chart recorder and stored on videotape for off-line analysis (Vetter 200 PCM analog data recorder). Interneuron input resistances ranged from 175 to 700 MΩ; series resistance ranged from 15 to 30 MΩ. Reported holding potentials have been corrected for the liquid junction potential (−15 mV). Responses were measured by hand from chart records; data are expressed as means ± SE.

5-HT and other 5-HT receptor agonists (30–300 μM) were pressure ejected for 3–6 s using a Picospritzer, with at least a 2-min interval between applications. The drug-containing pipette was positioned −50–100 μm from the recorded interneuron cell soma. One method that successfully reduced problems with desensitization (see below) was to apply a small amount of negative pressure through a 1-ml syringe positioned between the drug pipette and the Picospritzer between each application of 5-HT. In some experiments, a U-tube, fabricated from narrow-bore silicone tubing (0.02 × 0.037 in.), was used for application of 100–300 μM 5-HT or 50 μM N-methyl-d-aspartate (NMDA) (Bormann 1989; Fenwick et al. 1982). Phenol red or Fast Green was added to the drug-containing solution to monitor leakage from the pipette between drug applications and to monitor the region of drug application. The peak amplitude of 5-HT-mediated responses was variable from cell to cell, perhaps as a result of varying depths of the recorded cell within the slice contributing to differences in drug access to the cell surface. Receptor desensitization was apparently a significant problem: the slightest leak in the local application system could prevent any response to applied 5-HT, whereas using the same application apparatus with glycine or NMDA elicited responses in the same cell. We do not report the absolute numbers of interneurons that responded to 5-HT, because we believe this would give a spuriously low value. Once a given U-tube or Picospritzer pipette was effective, nearly every SR interneuron tested had a 5-HT response.

After obtaining at least two control responses to 5-HT, antagonists were added directly to the bath and allowed to equilibrate for at least 10 min before reapplying agonist. In most experiments, 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10–20 μM), (+)-2-amino-3-phosphonopentanoic acid (APV; 50–100 μM), picrotoxin (50–100 μM), tetrodotoxin (TTX; 0.5–1.0 μM), and CoCl2 (100–300 μM) were added to the extracellular solution to block α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), NMDA, and GABAA receptors and voltage-activated sodium and calcium channels, respectively. GABAA responses were blocked by intracellular Cs+ present in all voltage-clamp experiments.

Studies using reduced divalent cations

ACSF was prepared that was nominally free of divalent cations by omitting CaCl2 and MgSO4, and adding of 0.5–5.0 mM EGTA (EGTA-ACSF). 5-HT responses were observed in normal ACSF, then EGTA-ACSF was bath applied for 5 min, and 5-HT responses were retested. In most cells, exposure to EGTA-ACSF for >10 min produced increases in holding current and decreased input resistance, making it impossible to obtain an entire current-voltage (I-V) relationship. We have only reported data from interneurons during the initial exposure to divalent cation-free solution during which holding current and input resistance were stable.

In other experiments, slices were prepared and stored submerged in ACSF either without added Mg2+, or without added Ca2+, for at least two hours before use. Either 5-HT or NMDA was locally applied to interneurons in the same Mg2+/Ca2+-free ACSF; in some cases responses were retested after returning to normal ACSF.

Morphological characterization

In all recordings, biocytin (0.4%) was included in the pipette solution to allow post hoc identification and characterization of interneuron axon and dendrites. Immediately after recording ended, the slice was fixed in 4% paraformaldehyde for at least 24 h. Slices were immersed in a 30% sucrose solution before resectioning at 305 mosm. A vibrotome was used to cut 300- to 400-μm coronal slices through the dorsal hippocampus. Slices were allowed to rest at least 1 h in an interface chamber at room temperature. Slices were then preincubated for 2 h in 3% normal goat serum with Triton X-100 and were then incubated overnight with avidin–horseradish peroxidase (HRP; Vectastain Elite ABC Kit), and the reaction product was enhanced with a solution containing 1% nickel ammonium sulfate and cobalt chloride, a step that was essential for visualization of interneuron axon. The slices were mounted on gelatin-coated slides, dehydrated, and coverslipped for camera lucida reconstruction.

Calbindin staining

After a 1-h rest period, freshly cut hippocampal slices were fixed overnight in 4% paraformaldehyde. Fixed slices were incubated in 0.2 M glycine followed by 30% sucrose and serially resectioned at 30 μm. Slices were then preincubated for 2 h in 3% normal goat serum with Triton X-100 and were then incubated overnight in rabbit anti-calbindin antibody (1:500) at 4°C, followed by a 1-h incubation in rhodamine-conjugated donkey anti-rabbit secondary
antibody (1:100) for visualization. Antibodies were obtained from Swant (Switzerland).

Materials

Drugs used in this study include 5-hydroxytryptamine hydrochloride (serotonin), picrotoxin, cobalt chloride, and biocytin (Sigma); TTX (Calbiochem); 2-methyl-5-hydroxytryptamine (2-methyl-serotonin), mCPBG [1-[(m-chlorophenyl)-biguanide hydrochloride], DNQX, and (±)-APV (RBI); and GTPßS (Boehringer Mannheim). All drugs except DNQX were prepared as stock solutions in water and frozen (–20°C) in aliquots; DNQX was prepared as a 10-mM stock in equimolar NaOH. Immediately before beginning experiments, drugs were thawed and diluted to the appropriate concentrations.

RESULTS

5-HT excites interneurons through a direct postsynaptic mechanism

To test whether 5-HT excites interneurons, 5-HT was applied locally over the cell body region of SR interneurons. In the majority of recordings from >145 interneurons, 5-HT produced a rapid excitatory inward current, with peak amplitudes ranging from –15 to –600 pA at a holding potential of –85 mV (Fig. 1A, left panel). To ensure that the 5-HT–mediated inward current was the result of its direct action at postsynaptic 5-HT receptors on the recorded interneuron, rather than an indirect action on nearby cells, 5-HT was applied in the presence of AMPA, NMDA, and GABA_A receptor antagonists. Although glutamate and GABA antagonists silenced spontaneous excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs, seen in Fig. 1A, left panel, as rapid downward deflections), they had no effect on the 5-HT–mediated inward current (Fig. 1A, middle panel). The addition of TTX and cobalt (which block voltage-activated sodium and calcium channels, respectively) also had no effect on the 5-HT–mediated response (Fig. 1A, right panel). These data demonstrate that 5-HT exerts a direct postsynaptic action on SR interneurons, presumably mediated by functional serotonin receptors.

Figure 1B shows a voltage-clamp and current-clamp response in the same cell to 5-HT application. 5-HT could depolarize the cell as much as 40 mV from rest, producing strong action-potential firing (n = 6; Fig. 1B). 5-HT responses could also be elicited by bath perfusion of 5-HT (100 μM; n = 8). Bath perfusion, in which the drug reaches the neuron slowly, probably causes increased receptor desensitization as compared with rapid pressure ejection of 5-HT near the recorded cell. Thus these responses were generally smaller than responses in the same cell elicited by 5-HT applied locally (Fig. 1C; n = 4). In subsequent experiments, 5-HT was always locally applied with reproducible 5-HT responses elicited every 2–4 min (peak amplitude varied by <20% from trial to trial).

We occasionally observed an additional, slower inward current in interneurons voltage clamped to –85 mV. This current was not seen in experiments using GTPγS in the recording pipette (see next page), suggesting that it is likely mediated by G protein–coupled 5-HT receptors (Andrade and Nicoll 1987; Fraser and MacVicar 1991) and may be the result of activation of 5-HT_2 receptors (Shen and Andrade 1996).

5-HT excites interneurons through activation of 5-HT– gated ion channels

Interneurons in s. radiatum/s. lacunosum express high levels of mRNA for the 5-HT_1 receptor (Tecott et al. 1993), which is a cation-selective ligand-gated ion channel (Derkach et al. 1989), in contrast to all other known 5-HT receptors (for review see Jackson and Yakel 1995; Peters et al. 1992; Roth 1994). We therefore tested whether the 5-HT–induced inward current is mediated by 5-HT_3 channels. We found that the 5-HT_3 receptor mediates inward currents in interneurons based on the following observations: 1) it does not require intracellular GTP, 2) its pharmacological properties are consistent with this receptor subtype, and 3) its I–V relationship is consistent with that of the 5-HT_3 receptor channel.
First we examined the dependence of the response on intracellular GTP. Whereas responses to neurotransmitter receptors coupled to G-proteins “wash out” during prolonged whole cell recording when GTP is omitted from the pipette solution, the activity of ligand-gated ion channels is independent of intracellular GTP. 5-HT-mediated inward currents in SR interneurons remained unchanged for the duration of the whole cell recording (up to 2 h) without GTP in the pipette solution, suggesting that the responses do not require G-proteins (Fig. 2A). Furthermore, responses to repeated applications of 5-HT were unaffected when the pipette solution included GTPγS (300 μM), which irreversibly activates G-proteins, preventing further action of neurotransmitters at G-protein-coupled receptors (Fig. 2B). Together, these data strongly suggest that the 5-HT-mediated response is independent of G-proteins, and are consistent with activation of a ligand-gated ion channel. In subsequent experiments, GTPγS was generally included in the patch pipette, to isolate the ligand-gated ion channel response to 5-HT in these cells.

We next used agonists and antagonists of the 5-HT3 receptor to characterize the interneuron response. Inward currents similar to those elicited by 5-HT were produced during bath or local pressure ejection of either of two 5-HT3 receptor agonists [2-methyl-serotonin (2-Me-5-HT; 30–100 μM; n = 4) and 1-(m-chlorophenyl)-biguanide (mCPBG; 30–100 μM; n = 3); data not shown]. Responses to these agonists were relatively small (−5 to −35 pA at Vhold = −85 mV), perhaps due to desensitization during bath application.

We next used three chemically distinct 5-HT3 antagonists and found that they all blocked the 5-HT response in SR interneurons. The highly selective 5-HT3 receptor antagonist, ICS 205-930 (10 nM) (Richardson et al. 1985), potently and completely blocked the 5-HT-mediated response (Fig. 3A, n = 5). The response to 5-HT partially recovered after a prolonged wash of the antagonist (1.5 h). A second 5-HT3 receptor antagonist, metoclopramide (30 μM), also reversibly blocked the response to 5-HT (Fig. 3B, n = 4). D-Tubocurarine blocks several structurally related ligand-gated ion channels, including the nicotinic acetylcholine receptor (Jenkinson 1960), the GABA_A receptor (Labeleda et al. 1982; Wotring and Yoon 1995), and the 5-HT3 receptor (Higashi and Nishi 1982; Peters and Usherwood 1983), but it has no effect at other 5-HT receptor subtypes. We found that d-tubocurarine (1 μM) potently and reversibly blocked the 5-HT-induced inward current at 10 μM and completely blocked it at 30 μM (n = 4). The response recovered after a 45-min wash out of the antagonist. C: d-tubocurarine, an antagonist at several ligand-gated ion channels including the 5-HT3 receptor, reversibly blocked the 5-HT-mediated inward current at 1 μM, with a partial block at 500 nM (n = 3). The response recovered after washing for 30 min. Scale bar: 50 pA, 20 s.

5-HT3-mediated I-V relationship is nonlinear

We predicted that the I-V relationship of the 5-HT3-mediated response should pass through 0 mV if the response is mediated by the 5-HT3 receptor, consistent with a channel
permeable to monovalent cations. We found that the 5-HT response indeed reversed polarity between 0 mV and +10 mV (Fig. 4B; n = 9). An unusual feature of the I-V relationship is a region of negative slope conductance at holding potentials negative to −55 mV; as the membrane potential is held at more negative holding potentials, 5-HT elicits smaller inward currents (Fig. 4). The 5-HT current at −85 mV was 65.2 ± 6.9% (mean ± SE) of that at −45 mV (n = 48), whereas the 5-HT current at −15 mV was 39.8 ± 3.8% of that at −45 mV (n = 23).

We considered the possibility that the region of negative slope conductance at negative holding potentials results from enhanced receptor desensitization, because 5-HT3 responses in cultured neurons desensitize more rapidly at negative holding potentials (Yakel et al. 1988). The kinetics of our responses do not support this interpretation, however, since the rise time and decay time at −85 mV are not any faster than that at −45 mV, as would be expected if desensitization were responsible (Fig. 5; n = 4/4).

**Extracellular Ca^{2+} modulates the 5-HT3 response**

The shape of the 5-HT-mediated I-V relationship we observe is a characteristic of the I-V relationship for the NMDA subtype of glutamate receptor. The negative slope conductance region in the NMDA receptor I-V plot is caused by the voltage-dependent block of the channel by Mg^{2+}; in the absence of extracellular Mg^{2+}, the NMDA-mediated I-V relation is linear (Mayer et al. 1984; Nowak et al. 1984). Because of this similarity in the voltage dependence of 5-HT3 and NMDA currents, we tested whether the 5-HT3-mediated responses in interneurons are similarly sensitive to extracellular divalent cations. At a holding potential of −85 mV, 5-HT responses in divalent cation-free extracellular solution were greatly enhanced, with peak amplitudes 2- to 20-fold larger than control responses from the same cell in normal extracellular solution (2.5 mM Ca^{2+} and 1.3 mM Mg^{2+}; data not shown; n = 10).

To compare the 5-HT3 receptor directly with the NMDA channel, we measured the voltage dependence of interneuron responses to NMDA and 5-HT with and without extracellular Mg^{2+}. As reported in many other cell types, removal of Mg^{2+} caused the I-V curve for NMDA to become linear (Fig. 6A, right panel; n = 4). In contrast, the removal of Mg^{2+} alone had no effect on the 5-HT I-V curve (Fig. 6A, left panel; n = 7). These data suggest that the 5-HT3 receptor is functionally different from the NMDA receptor, retaining a region of negative slope conductance even when enough Mg^{2+} has been washed from the slice to permit the NMDA channel to open at negative voltages.

We next tested the effects of removing extracellular Ca^{2+} on the I-V relationship to 5-HT (Fig. 6B). Slices exposed to 0-Ca^{2+} ACSF for 2 h before the experiment had a linear response to 5-HT, with responses as large as 1.5 nA at −85 mV (n = 7). None of these slices showed the characteristic region of negative slope conductance. Subsequent washing in normal ACSF (containing 2.5 mM Cu^{2+}) reduced the size of the currents evoked by 5-HT at all potentials and restored the region of negative slope resistance in all cells where it was tested (n = 4).

**Interneuron morphology**

We successfully labeled 90 5-HT3-responsive SR interneurons with biocytin. Approximately 87% of the labeled interneurons were morphologically similar to one another. Cell somas were 20–40 μm in diameter and were multipolar with two to six primary dendrites. The dendritic arbor of these neurons was fairly simple, with few secondary dendrites. In six cells, small spiny processes were observed. The dendrites are contained within s. radiatum in 51 of 90 of the labeled cells and extend in all directions for up to 400 μm. In 39 cells, 1 or 2 dendrites projected into s. oriens; in 14 cells, dendrites were observed to project up to or cross the hippocampal fissure. Axon was recovered in 80% of cells (72 of 90). The axons of these interneurons were quite complex, ramifying extensively within s. radiatum and more modestly within s. oriens (Fig. 7, left neuron; 60/72). Of these cells, 15% (9 of 60) had axon that crossed the hippocampal fissure and arborized throughout the molecular layer and hilar region of the dentate gyrus. The axons appear to be confined to the...
FIG. 6. External divalent cations modulate 5-HT–induced inward currents. A, left panel: plot of the I-V relationship of the 5-HT response in 0 Mg2+ (n = 7) and normal artificial cerebrospinal fluid (ACSF; n = 9). Responses in the absence/presence of Mg2+ were obtained from different neurons. Right panel: I-V relationship of the N-methyl-D-aspartate (NMDA) response in 0 Mg2+ and normal ACSF (1.3 mM Mg2+; n = 4). Data plotted in this graph were obtained from the same 4 neurons in the absence/presence of Mg2+. In both panels, responses at −85 and −15 mV were normalized to the response obtained at −45 mV. Slices were incubated in Mg2+-free ACSF for 2–5 h before recording. B, left panel: plot of the I-V relationship of the 5-HT response in 0 Ca2+ (n = 4) and normal ACSF (2.5 mM Ca2+; n = 4). Responses in the absence/presence of Ca2+ were obtained from the same neurons. Responses at −85 and −15 mV were normalized to the response at −45 mV. Slices were incubated in Ca2+-free solutions for 2–5 h before recording. Right panel: Individual 5-HT responses at different holding potentials in 0 Ca2+ or normal ACSF taken from a representative experiment. Scale bar: 250 pA, 20 s.

inner one-half of the molecular layer. In an additional 12% of these cells (7 of 60), the axon stopped at the fissure, likely a result of the axon being cut during slicing.

An additional 13% of labeled cells (12 of 72) had multipolar dendritic trees similar to those of the cells described above, but the axon densely innervated the pyramidal cell layer, suggesting...
that these interneurons exert their inhibitory control over the cell somata of pyramidal cells (Fig. 7, right neuron). The axons of these cells did not extend into the dentate gyrus.

The calcium-binding protein, calbindin, is concentrated in interneurons located in the stratum of hippocampus, and these neurons are selectively innervated by axons from the median raphe nucleus (Freund 1990). We stained our slices for calbindin and find that typical SR interneurons observed in live slices with Hoffman optics and recorded from in the experiments described above very closely resemble the interneurons that stain for calbindin.

**Discussion**

**CA1 hippocampal interneurons express functional 5-HT₃ receptors**

We have demonstrated that CA1 SR interneurons are excited by 5-HT via direct activation of postsynaptic 5-HT₃ receptors. The 5-HT responses persist during blockade of fast glutamatergic and GABAergic synaptic transmission, indicating that 5-HT receptors are present on interneurons themselves. The responses are independent of G-protein activation and are effectively blocked by three distinct 5-HT₃ receptor antagonists. This 5-HT receptor subtype may be preferentially expressed by GABAergic interneurons and not by glutamatergic pyramidal cells, because functional responses are recorded from GABAergic interneurons in both hippocampus and dentate gyrus but not from principle neurons (Kawa 1994; Ropert and Guy 1991).

Unlike the majority of 5-HT release sites, which are nonsynaptic, electron microscopy shows true synaptic sites associated with the presynaptic boutons of median raphe afferents innervating SR interneurons (Acsady et al. 1993). We speculate that 5-HT₃ receptors mediate fast synaptic transmission at these synapses, similar to that mediated by ionotropic AMPA and NMDA receptors for glutamate. Fast excitatory transmission mediated by 5-HT₃ receptors has been reported in the rat lateral amygdala nucleus (Sugita et al. 1992).

In a subset of hippocampal interneurons we also observed 5-HT responses probably mediated by G-protein–coupled 5-HT receptors, because they were abolished when GTPγS was included in the internal solution. Both inward and outward currents were observed in different cells, but more work will be required to characterize these 5-HT responses.

The agonist/antagonist profile of the 5-HT–mediated response in SR interneurons is consistent with 5-HT₃ receptor–mediated responses described in other preparations. The majority of studies examining the properties of these receptors have come from experiments involving neuroblastoma cell lines, acutely dissociated or cultured neurons (Furukawa et al. 1992; Jones and Suprenant 1994; Peters et al. 1988; Shao et al. 1991; van Hooft et al. 1994; Yakel and Jackson 1988; Yakel et al. 1990; Yang et al. 1992) or 5-HT₃ receptors expressed in oocytes (Mariqc et al. 1991; Yakel et al. 1993). Functional native 5-HT₃ receptors in brain slices have also been reported on basket cells in the dentate gyrus (Kawa 1994), on nucleus tractus solitarius (Glaum et al. 1992), and in amygdala (Sugita et al. 1992). The 5-HT₃ responses on SR interneurons are similar in pharmacology to 5-HT₃ responses in basket cells.

Although the basic pharmacological characteristics of all reported 5-HT₃ receptor–mediated responses are similar, there remains considerable variability in the reported biophysical properties of the channel. For example, different single-channel conductances (Jones and Suprenant 1994; Yang et al. 1992), cation permeability ratios (Furukawa et al. 1992; Lambert et al. 1989; Neijt et al. 1989; Yakel et al. 1990; Yang 1990; Yang et al. 1992), and the shapes of the I-V relationship (Kawa 1994; Mariqc et al. 1991; Peters et al. 1988; Yakel and Jackson 1988; Yakel et al. 1993; Yang et al. 1992) have been reported in different preparations. These data may suggest that multiple 5-HT₃ receptor subtypes or posttranscriptional modifications exist.

**I-V relationship**

The most striking difference in the 5-HT₃ receptor–mediated response between preparations is the presence or absence of a negative slope conductance region observed in the I-V relationship (Kawa 1994; Mariqc et al. 1991; Peters et al. 1988; Yakel and Jackson 1988; Yakel et al. 1993; Yang et al. 1992). This characteristic of the I-V curve was first observed in a clone encoding a 5-HT₃ receptor isolated from a mouse neuroblastoma cell line and expressed in oocytes (Mariqc et al. 1991). However, further examination of the functional characteristics of the same clone, also expressed in oocytes, yielded an entirely linear I-V curve (Yakel et al. 1993). The reason for the discrepancy is unclear. However, consistent with the original study, we have observed a negative slope conductance region in the 5-HT₃–mediated I-V relationship resulting from activation of native receptors expressed in hippocampal interneurons. Similar findings have been reported in basket cells in slices of dentate gyrus (Kawa 1994). Although increased desensitization with hyperpolarization has been described for the 5-HT₃ channel (Yakel et al. 1993; Yang et al. 1992), this is unlikely to account for the observed region of negative slope conductance because voltage-dependent differences in decay kinetics of our responses are not large.

Removal of extracellular divalent cations strongly increases the 5-HT response at negative membrane potentials. This effect of divalent cations has previously been observed in dentate basket cells, superior cervical ganglion cells, and in oocytes expressing a 5-HT₃ receptor clone (Kawa 1994; Mariqc et al. 1991; Yang et al. 1992). The simplest interpretation is that the 5-HT₃ channel is blocked by divalent cations in a voltage-dependent manner similar to that of the NMDA channel (Mayer et al. 1984; Nowak et al. 1984). Previous studies in other preparations have demonstrated that both Ca²⁺ and Mg²⁺ can modulate the channel (Kawa 1994; Peters et al. 1988). Under our recording conditions, at nominally 0 Mg²⁺, 2.5 mM Ca²⁺, NMDA receptor channels are unblocked, but the 5-HT₃ channel remains blocked. In contrast, removal of extracellular Ca²⁺ (with 1.3 mM Mg²⁺ remaining) results in a linear I-V relationship. Adding back extracellular Ca²⁺ restores the region of negative slope conductance. These results indicate that physiological levels of Mg²⁺ have little effect on the channel, and agree with previous studies showing that significant block by physiological levels of Ca²⁺ occurs at negative membrane potentials (Kawa 1994; Mariqc et al. 1991; Peters et al. 1988; Yang 1990).
5-HT$_3$ receptor as a potential coincidence detector

The strikingly nonlinear I-V curve has important implications for the way interneurons respond to 5-HT. When an interneuron is hyperpolarized below $-55$ mV (for example, when GABA is released onto the cell), serotonergic inputs from the raphe will have little effect, because the channel will pass very little current. In contrast, when the interneuron is depolarized by another excitatory input arriving simultaneously, the same serotonergic input will elicit a powerful excitatory response. The 5-HT$_3$ receptor will therefore act as a “coincidence detector,” strongly depolarizing the interneuron when serotonergic input arrives coincident with other excitatory inputs, but having little effect when the cell is hyperpolarized. SR interneurons are selectively innervated by septal afferents thought to release GABA (Freund and Antal 1988). We therefore speculate that GABAergic septal input will minimize the effect of 5-HT afferents onto the same population of SR interneurons, and hypothesize that the membrane potential of SR interneurons could gate information flow from the median raphe into the hippocampus.

For the Ca$^{2+}$-permeable NMDA receptor, coincidence detection is essential for the induction of long-term potentiation (LTP), a form of synaptic plasticity that may be important in learning and memory (for review see Bliss and Collingridge 1993). Intracellular Ca$^{2+}$ triggers LTP, and the relevant Ca$^{2+}$ enters the cell only when the NMDA channel has bound glutamate and the neuron is simultaneously depolarized. To determine whether the 5-HT$_3$ receptor may play a similar role in interneurons, it will be important to determine the Ca$^{2+}$ permeability of the channel in our system. There is some disagreement as to the Ca$^{2+}$ permeability of 5-HT$_3$ receptors in different cell types. In sympathetic ganglion neurons, in neuroblastoma cells, and in HEK 293 cells transfected with mRNA for the 5-HT$_3$ channel, Ca$^{2+}$ permeability is high (Hargreaves et al. 1994; Yang 1990; Yang et al. 1992), whereas in other studies using cultured hippocampal neurons and neuroblastoma cells, Ca$^{2+}$ permeability cannot be detected (Gilon and Yakel 1995; Yakel et al. 1990). Our data indicate that Ca$^{2+}$ is a very effective channel blocker, but we have not yet tested whether Ca$^{2+}$ is also a permeant ion. Even a relatively small Ca$^{2+}$ permeability could lead to relevant physiological responses, because Ca$^{2+}$ is a ubiquitous intracellular messenger.

Effects on the local circuit

Ropert and Guy (1991) demonstrated that GABAergic IPSPs recorded from CA1 pyramidal cells increase in frequency during bath application of 5-HT. Excitation of SR interneurons via 5-HT$_3$ receptors probably causes this increase, particularly because it was blocked by a 5-HT$_3$ receptor antagonist. Our anatomic data are consistent with this interpretation, because these interneurons appear to form hundreds of synaptic contacts either with apical dendrites or cell bodies of CA1 pyramidal cells. The axons of s. oriens interneurons target primarily either the cell body layer (s. pyramidale), or s. lacunosum (McBain et al. 1994), where they inhibit pyramidal cell responses to entorhinal cortical afferents (Maccarferri and McBain 1995). SR interneurons similarly target either pyramidal cell bodies or layers that contain pyramidal cell dendrites; however, the dendritic layers the SR interneurons preferentially innervate are s. radiatum and s. oriens, where Schaffer collaterals from CA3 pyramidal cells form excitatory synapses. The extensive axonal arborization observed in the biocytin-filled 5-HT$^+$-responsive cells suggests that these neurons are well suited to synchronize electrical activity in hundreds of pyramidal cells. It is also possible that the SR interneurons provide GABAergic inputs to neighboring interneurons, because IPSP frequency increases when 5-HT is bath applied (unpublished observations).

A percentage of labeled SR interneurons project axonal branches across the hippocampal fissure to ramify within the dentate molecular layer and the hilar region. In addition to their innervation of the dentate gyrus, this group of cells always projected axons to s. radiatum of the hippocampus. Because in some of our interneurons a portion of the axon may have been transected during slice preparation, potentially many of the interneurons that we observed innervating s. radiatum also project to the dentate region. The term “interneuron” is somewhat misleading, because at least some of these cells actually project to a distinct brain region. These interneurons thus have the unusual potential to simultane-
erosely control the excitability of CA1 pyramidal cells and of dentate granule cells.

Interneurons in s. radiatum/s. lacunousum have previously been observed to project across the hippocampal fissure (Lacaille and Schwartzkroin 1988), and our extensive axon fills permit a more exact definition of the potential targets of these axons. SR interneurons strongly project to both the inner third of the molecular layer and to the dendritic region of CA1 (Fig. 8). The major excitatory pathways projecting to the inner one-third of the dentate molecular layer originate in proximal CA3 pyramidal cells (in the region termed CA3c): CA3c neurons also project to CA1 via Schaffer collaterals. One role of SR interneurons may therefore be to regulate information flow from CA3 into both CA1 and dentate gyrus. The other major excitatory afferents to the distal dendrites of both CA1 pyramidal cells and dentate granule cells originates in entorhinal cortex. We speculate that by selectively inhibiting dendritic sites of excitatory input from the CA3 region to CA1 and dentate gyrus, SR interneurons may mute intrahippocampal signals from CA3 while permitting information flow from cortical afferents. If this scenario is correct, serotonergic input may act to open the “gate” for cortical inputs, while damping hippocampal reverberations from the CA3 region.

We thank S. Douglas for performing the biocytin and calbindin staining procedures. We also thank Drs. Lawrence Katz, Donald Lo, Felix Schweizer, and John Williams for helpful comments on this manuscript. This work was supported by National Institute of Neurological Disorders and Stroke Grants NS-09734 to L. L. McMahon and NS-30500 to J. A. Kauer.

Received 28 April 1997; accepted in final form 18 July 1997.

REFERENCES


Morales, M., Battenberg, E., de Leca, L., and Bloom, F. E. The type 3 serotonin receptor is expressed in a subpopulation of GABAergic neurons in the rat neocortex and hippocampus. Brain Res. 731: 199–202, 1996.


