Serotonin Reduces Polysynaptic Inhibition via 5-HT₁₅ Receptors in the Superficial Entorhinal Cortex

DIETMAR SCHMITZ,¹ TENGIS GLOVELI,¹ RUTH M. EMPSON,² AND UWE HEINEMANN¹
¹Department of Neurophysiology, Institute of Physiology at the Charité, Humboldt University Berlin, 10117 Berlin, Germany; and ²University Department of Pharmacology, Oxford University, Oxford OX1 3QT, United Kingdom

Schmitz, Dietmar, Tengis Gloveli, Ruth M. Empson, and Uwe Heinemann. Serotonin reduces polysynaptic inhibition via 5-HT₁₅ receptors in the superficial entorhinal cortex. J. Neurophysiol. 80: 1116–1121, 1998. The superficial cells of the entorhinal cortex (EC), main input to the hippocampus, receive a serotonergic input from the raphe nuclei and express 5-hydroxytryptamine creatine sulfate complex (5-HT) receptors at high density. With the use of intracellular recordings, we investigated the effects of serotonin on synaptic inhibition of layer II and III neurons of the EC. Serotonin reduced both polysynaptic fast and slow inhibitory postsynaptic potentials (IPSPs) in projection neurons of the superficial EC. Polysynaptic fast and slow IPSPs were depressed by serotonin in a dose-dependent manner (0.1–100 μM). Serotonin in a concentration of 1 μM reduced the amplitudes of polysynaptic fast and slow IPSPs by ~40 and 50%, respectively. To identify the subtype of the 5-HT–receptor mediating the effects on polysynaptic IPSPs, we applied various 5-HT–receptor agonists and antagonists. Although the serotonin agonists for the 5-HT₁₅,₂,₃ receptors were ineffective, the effects were mimicked by the 5-HT₁₅–receptor agonists (8-OH-DPAT, 5-CT) and prevented by the 5-HT₁₅–receptor antagonist NAN-190. To look at the direct effects of 5-HT on inhibitory interneurons, we elicited monosynaptic IPSPs in the absence of excitatory synaptic transmission. In contrast to the polysynaptic IPSPs, monosynaptic IPSPs were not significantly affected by serotonin. Recordings from putative inhibitory interneurons revealed that their excitatory postsynaptic potentials (EPSPs) were reversibly reduced by serotonin. We conclude that serotonin suppresses polysynaptic inhibition in projection neurons of layers II and III of the EC by depression of EPSPs on inhibitory interneurons via 5-HT₁₅ receptors.

INTRODUCTION

The superficial cells of the entorhinal cortex (EC) form the two branches of the perforant path, the main input to the hippocampus (Steward and Scoville 1976). Layer II cells project to the dentate gyrus and CA3 region, whereas layer III cells project to CA1 and the subiculum (Steward and Scoville 1976).

It has been reported that serotonergic fibers entering the EC and 5-HT receptors are expressed at high density in the superficial layers (Bobillier et al. 1975; Mengod et al. 1996; Pazos and Palacios 1985; Pazos et al. 1985). So far, there is only little known about the functional role of serotonin in the EC. However, changes in the serotonergic system are associated with characteristic lesions in the EC in Alzheimer’s and Korsakow’s disease (Beal et al. 1989; Crino et al. 1989; Langlais et al. 1987; Tejani-Butt et al. 1995).

We previously showed that serotonin can effectively decreases excitatory postsynaptic potentials (EPSPs) in principal cells in the EC (Schmitz et al. 1995a, 1997). However, it has been reported that most of the superficial EC neurons are under strong inhibitory control (Gloveli et al. 1997a–c; Jones 1993−1995). Moreover, the balance between synaptic excitation and inhibition seems to play a crucial role within this area for epileptogenesis (Heinemann 1987; Jones and Heinemann 1988) and ‘‘information transfer’’ to the hippocampus (Gloveli et al. 1997b; Jones 1993).

We therefore recorded from EC layers II and III projection and local circuit cells to clarify the effects and mechanisms by which serotonin affects inhibitory synaptic transmission in the superficial EC.

METHODS

Slice preparation

Horizontal slices containing the hippocampus, entorhinal, perirhinal, and temporal cortex were prepared from adult Wistar rats (180–250 g). In brief, the animals were deeply anesthetized with ether, decapitated, and the brain removed. Tissue blocks containing the temporal cortex and hippocampus were mounted on a Vibratome (Campden Instruments, Loughborough, UK) in a chamber filled with cold (~4°C) artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 26 NaHCO₃, 3 KCl, 1.25 NaH₂PO₄, 1.6 CaCl₂, 1.8 MgSO₄, and 10 glucose, saturated with 95% O₂−5% CO₂, pH 7.4. Horizontal slices were cut at 400-μm thickness and transferred to an interface chamber where they were maintained at 35°C and perfused with ACSF at a rate of 1.5–1.8 ml/min. The slices were allowed to rest for ≈1 h after the preparation before recording.

Electrophysiological recordings

Intracellular electrodes were pulled from borosilicate glass (1.2 mm OD) and filled with 2 M K⁺-acetate. Electrode resistance was 35–120 MΩ. Intracellular recordings were performed with a Neurodata IR 183 (Neurodata Instruments, New York, NY) or a SECIOL (NPI Instruments, Tamm, Germany) amplifier. Cells were impaled and then allowed to rest for 5–10 min before recording. Only cells with resting potentials more negative than −50 mV were accepted. Resting membrane potentials of the cells were corrected after the recording by subtraction of the remaining tip potential after withdrawal of the pipette. Compound polysynaptic potentials were evoked by electrical stimulation (0.05-ms duration, 1–30 V) at 0.1–0.05 Hz via a bipolar insulated stimulation electrode placed in the lateral EC. The signals were filtered at 3 kHz, digitized at

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Drugs and solutions

Bicuculline methiodide (5 μM) and 5-HT (0.1–100 μM) were both purchased from Sigma (Deisenhofen, Germany). (-)-2-Amino-5-phosphonopentanoic acid (APV; 30 μM), 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine hydrobromide (NAN-190; 50 μM), ±8-hydroxy-2-(di-n-propylamino) tetralin HBr (8-OH-DPAT; 10 μM), CGS-12066 maleate (10 μM), 2-methyl-5-hydroxytriptamine maleate (2-methyl-5-HT; 50 μM), α-methyl-5-hydroxytriptamine maleate (α-methyl-5-HT; 50 μM), 5-carboxamido-tryptamine maleate (5-CT; 1 μM), and ritanserin (10 μM) were all from Research Biochemicals (Natwick, MA). 1,2,3,4-Tetrahydro-6-nitro-2,3-dioxo-benzol[f]quinoxaline-7-sulfonamide (NBQX; 10 μM) was a gift from Novo Nordisk (Bagsvaerd, Denmark), and CGP55845A (2 μM) was a gift from Ciba-Geigy (Basel, Switzerland).

Data analysis and statistics

Data were analyzed off-line with SIGAVG (CED, Cambridge, UK) or Wintida (HEKA, Lambrecht, Germany) software. Amplitudes of evoked synaptic potentials were measured from averaged (3–10) sweeps. Data are expressed as means ± SE. Because the mean data values are rounded to one number after the comma, the percentage change is in some occasions slightly different. Drug effects were analyzed with Student’s t-test (Sigmaplot, Jandel, Corte Madera, USA) for paired data, and an error probability of P < 0.05 was regarded as significant.

RESULTS

We performed intracellular recordings from projection and local circuit neurons of layers II and III of the medial EC. We could identify different projection and local circuit-cells on the basis of the previously reported morphological and electrophysiological characteristics (Alonso and Klink 1993; Gloveli et al. 1997a; Jones 1994).

Serotonin reduces IPSP independent of the effects on membrane properties

IPSP—polysynaptic inhibitory postsynaptic potential (IPSP) sequences in projection neurons of the superficial EC were evoked by electrical stimulation of the lateral EC. In a first series of experiments we tested serotonin in a concentration range of 0.1–100 μM on polysynaptic IPSPs. Both polysynaptic fast and slow IPSPs (fIPSPs and sIPSPs, respectively) were depressed by serotonin in a dose-dependent manner. At a concentration of 0.1 μM the polysynaptic fIPSP and sIPSPs were reduced from −3.0 ± 0.6 to 2.6 ± 0.3 mV (12% decrease; n = 4, P < 0.05) and −2.5 ± 0.5 to −2.1 ± 0.3 mV (17% decrease; n = 4, P < 0.05), respectively. At a concentration of 1 μM the fIPSPs were reduced from −2.7 ± 0.6 to −1.7 ± 0.5 mV (38% decrease; n = 13, P < 0.01) and the sIPSPs from −2.0 ± 0.5 to −1.0 ± 0.3 mV (52% decrease; n = 16, P < 0.01). After application of 10 μM, fIPSPs were depressed from −3.7 ± 0.2 to −1.4 ± 0.5 mV (62% decrease; n = 9, P < 0.01) and the sIPSPs from −2.5 ± 0.4 to −0.8 ± 0.2 mV (68% decrease; n = 10, P < 0.01). The highest concentration tested was 100 μM. At this concentration, serotonin reduced the fIPSP amplitude from −3.0 ± 0.9 to −0.8 ± 0.4 mV (74% decrease; n = 7, P < 0.01), whereas the sIPSPs were diminished from −3.4 ± 0.8 to −0.9 ± 0.4 mV (74% decrease; n = 6, P < 0.01; Fig. 1, A and B). We did observe in approximately one-third of the cells (6/20 neurons tested) that serotonin induced a small hyperpolarization (1–3 mV) that reversed at approximately −95 mV and was blocked by K+—channel blockers (not shown). However, in most cases (7/9 of the cells) the effects of serotonin on IPSPs were independent of any effects of 5-HT on intrinsic properties of the cell, suggesting that the effects of 5-HT on synaptic transmission were independent of the properties of the postsynaptic cell.

Effects of 5-HT receptor agonists and antagonists on polysynaptic IPSPs

To identify the subtype of the 5-HT receptor mediating the depression of polysynaptic IPSPs, we applied various specific agonists: 8-OH-DPAT and 5-CT (both agonists on the 5-HT1A and 5-HT7 receptor), CGS-12066 (5-HT1B), α-methyl-5-HT (5-HT1C), and 2-methyl-5-HT (5-HT1D). However, only 8-OH-DPAT and 5-CT mimicked the effect of 5-HT on polysynaptic IPSPs (Fig. 2, A–C). 5-CT reduced the polysynaptic fIPSPs from −2.5 ± 0.5 to −1.0 ± 0.4 mV (62% decrease; n = 7, P < 0.01) and the polysynaptic sIPSPs from −2.4 ± 0.5 to −0.7 ± 0.2 mV (70% decrease; n = 7, P < 0.01). In seven cells 8-OH-DPAT reduced the polysynaptic fIPSPs from −4.0 ± 1.1 to −1.5 ± 0.7 mV (70% decrease; P < 0.01) and the sIPSPs from −3.0 ± 0.6 to −1.0 ± 0.4 mV (67% decrease; P < 0.01). In contrast, all other specific agonistic drugs (CGS-12066, α-methyl-5-HT, 2-methyl-5-HT) were ineffective to mimick the serotonin effect on polysynaptic IPSPs (not shown). This result suggests that 5-HT1A and/or 5-HT7 receptors are involved in the effect of serotonin. The 5-HT7 receptor is a recently identified 5-HT−receptor subtype (Lovénberg et al. 1993; Ruat et al. 1993) that has a closely related agonist profile as the 5-HT1A receptor. However, the 5-HT7 receptor antagonist ritanserin could not antagonize the effect of serotonin on polysynaptic IPSPs (n = 5, P = 0.3). On the other hand, the 5-HT1A receptor antagonist NAN-190 antagonized the effect of serotonin on polysynaptic IPSPs (fIPSPs: −2.6 ± 0.5 to −2.5 ± 0.3 mV, n = 5, P = 0.7; sIPSPs: −2.1 ± 0.6 to −2.2 ± 1.0 mV, n = 5, P = 0.6). Thus the depression of the polysynaptic IPSPs appears to be mediated by 5-HT1A receptors.

Effects of serotonin on monosynaptic IPSPs

To look at the direct effects of 5-HT on inhibitory interneurons, we elicited monosynaptic IPSPs in the absence of excitatory synaptic potentials by local stimulation in the presence of NBQX and APV (Fig. 3). Under these conditions, a fIPSP or a sIPSP sequence could be recorded. The fIPSP and sIPSPs were pharmacologically identified as γ-aminobutyric acid-A (GABA_A) and GABA_B receptor-mediated events by their sensitivity to bicuculline and CGP55845A (n = 4 for each). However, serotonin (10 μM) had no significant effect on monosynaptic IPSPs in the
FIG. 1. Effects of serotonin on synaptic potentials in the superficial entorhinal cortex (EC). A: effects of serotonin (1 μM) on an excitatory postsynaptic potential (EPSP)-polysynaptic fast inhibitory post-synaptic potential (fIPSP)-slow IPSP (sIPSP) sequence in a layer III projection neuron (type 2) of the medial EC (Gloveli et al. 1997a). Serotonin suppressed all components of the postsynaptic potentials (top trace). There was no hyperpolarization and no change in the total conductance of the cell (as shown by the voltage response to a current step, before the synaptically evoked PSP). B: statistical diagrams pooled data from the effects of serotonin in different concentrations on polysynaptic fIPSPs (□) and sIPSPs (■). * Significant decreases in IPSP-amplitudes (see RESULTS).

DISCUSSION

Our data show that serotonin induces changes of synaptic inhibition of EC layer II and III cells. Because these neurons of the EC form the main input to the hippocampus proper (Steward and Scoville 1976), the modulation by serotonin will markedly alter the information flow to the hippocampus.

Effects of serotonin on IPSPs in the EC

In our experiments polysynaptic fIPSP and sIPSPs were reduced by serotonin at low concentrations (~40 and 50% reduction at 1 μM for fIPSP and sIPSPs, respectively). A reduction of polysynaptic fIPSP (Schmitz et al. 1995b) and sIPSPs (Schmitz et al. 1995b; Segal 1990) was also reported for hippocampal area CA1. In area CA3 and in the dentate gyrus a modulation of GABA_A responses by serotonin was reported (Ghadimi et al. 1994; Oleskevich and Lacaille 1992), and more recently a reduction of spontaneous GABA_A-mediated IPSPs via 5-HT_1A receptors was described in granule cells of the area dentata (Bijak and Misgeld 1997). Interestingly, activation of 5-HT_1A receptors can reverse this effect (Bijak and Misgeld 1997). However, when IPSPs were pharmacologically isolated and monosynaptically isolated in the superficial EC they were much more resistant to serotonin and were only occasionally and then not often homogenously affected by serotonin. This lack of affection of monosynaptic GABA-mediated transmission in the EC was also described for the noradrenergic and dopaminergic system (Pralong and Jones 1993; Pralong and Magistretti 1995). This is in direct contrast to the hippocampus, where both the monosynaptic fIPSP and sIPSPs are reduced by serotonin via a presynaptic mechanism (Ghadimi et al. 1994;
5-HT REDUCES POLYSYNAPTIC INHIBITION IN THE EC

Oleskevich and Lacaille 1992; Schmitz et al. 1995b; Segal 1990). Thus the suppression of polysynaptic f IPSP and sIPSPs in the EC is most likely caused by a reduced feed-forward or feed-back glutamate-mediated excitation of inhibitory interneurons. Indeed, this suggestion is supported by recordings from local circuit cells, presumably inhibitory interneurons, where the EPSPs were reversibly reduced by serotonin (see Fig. 4). Thus it is likely that the effects of 5-HT on polysynaptic IPSPs are mediated by a suppression of excitatory potentials at inhibitory interneurons. We recently showed that EPSPs in principal cells of the superficial EC are reduced by a presynaptic mechanism (Schmitz et al. 1998), and it is conceivable that this is the mechanism of EPSP reduction in putative interneurons as well.

The increase in total cell conductance induced by 5-HT is important for the interpretation of the depressant effects on synaptic responses because a general membrane conductance increase can reduce the amplitude of synaptic events (Ginsborg 1973). However, at least four arguments exclude such a postsynaptic shunting effect. First, only about one-third of neurons of the superficial medial EC were slightly hyperpolarized by serotonin, and in the other two-thirds of neurons we could observe a significant reduction in synaptic responses in the absence of any intrinsic conductance changes (e.g., Fig. 1A). Second, when increases in total cell conductance were seen, they were almost always outlived by the reduction of synaptic responses. Third, the concentration of serotonin necessary to affect synaptic potentials was lower than that which directly

FIG. 2. Effects of the 5-HT1A-receptor agonists 5-CT (1 µM) and 8-OH-DPAT (10 µM) on polysynaptic f IPSP and sIPSPs. A: both drugs, 5-CT and 8-OH-DPAT, strongly reduced polysynaptic f IPSPs in a layer II-stellate cell. B: polysynaptic sIPSPs in 2 different cells of layer III of the medial EC were suppressed by 5-CT (B1) and 8-OH-DPAT (B2). C: statistical diagrams pooled data from the effects of 5-CT and 8-OH-DPAT on polysynaptic f IPSP and sIPSPs. * Significant decreases in IPSP-amplitudes (see RESULTS). Control value is 100% in both diagrams.

FIG. 3. Effects of serotonin on mono- synaptic IPSPs. After the application of the glutamate-receptor antagonists NBQX and APV, all components of the PSPs were blocked (not shown; same cell as in Fig. 1; layer III-type 2 neuron) but we were able to evoke monosynaptic f IPSP and sIPSPs by a close stimulation position. In contrast to the polysynaptic IPSPs (see Fig. 1), the monosynaptic IPSPs were not affected by serotonin (10 µM).
FIG. 4. Effects of serotonin on synaptically evoked EPSPs in a putative inhibitory interneuron of the superficial EC. A–C: electrophysiological characteristics of a putative inhibitory interneuron recorded on the border of layer II and III of the medial EC. A: neuron fired at high frequency (up to 400 Hz) and generated by lower current injection groups of action potentials, which were interrupted by membrane potential oscillations (top trace). B: width of the action potential at half height was <250 μs; note also the sharp onset and the amplitude of the spike afterhyperpolarization (same cell, but the action potential was elicited by a short depolarizing pulse). C: synaptic response of the same cell to lateral EC stimulation. Often more than 1 action potential could be triggered on the top of the EPSPs. Note also the after-spike depolarization. D: after the application of 5-HT (10 μM) the EPSPs were reduced and recovered completely after washout. Note the differences in time and amplitude scales.

The 5-HT$_{1A,1B,2C,3}$-receptor subtypes show a high density of serotonin receptors in the EC (Pazos et al. 1985, 1990; Pazos and Palacios 1985). Therefore we tested the most commonly used selective agonists and antagonists of these receptor subtypes. The 5-HT$_{1A}$-receptor agonists 8-OH-DPAT and 5-CT could both mimic the effect of serotonin on polysynaptic IPSPs, whereas the specific agonists for 5-HT$_{1B,2C,3}$ receptors were ineffective. Because 8-OH-DPAT as well as 5-CT binds with high affinity to 5-HT$_{1A}$ and 5-HT$_{7}$ receptors and the limbic structures express significant levels of 5-HT$_{7}$ receptors (Lovenberg et al. 1993; Ruat et al. 1993), we tested both 5-HT$_{1A}$ and 5-HT$_{7}$-receptor antagonists. However, the effect of serotonin on polysynaptic IPSPs was only antagonized by the 5-HT$_{1A}$-receptor antagonist. These results suggest that activation of the 5-HT$_{1A}$ receptor is most likely responsible for the depression of polysynaptic IPSPs.

In summary, we conclude that serotonin suppresses polysynaptic inhibition in layers II and III of the EC via 5-HT$_{1A}$ receptors and depression of EPSPs on putative inhibitory interneurons. This modulatory action might affect the balance between excitation and inhibition within cortical networks and will alter the “information flow” from the EC to the hippocampus.

We thank Dr. A. Draguhn for helpful discussions and for reading the manuscript.

This work was supported by the Bundesministerium für Bildung und Forschung, a Royal Society Exchange Program Fellowship to R. M. Empson, and Deutsche Forschungsgemeinschaft Grant INK21/A1–1.

Address for reprint requests: D. Schmitz, Dept. of Neurophysiology, Institute of Physiology at the Charité, Humboldt University Berlin, Tucholskystr. 2, 10117 Berlin, Germany.

Received 4 March 1998; accepted in final form 20 May 1998.

REFERENCES


