INTRODUCTION

The activity of hippocampal principal, pyramidal neurons remains under control of GABAergic interneurons, whose axons form inhibitory feed-forward or feed-back connections with pyramidal cells (1, 2). Based on electrophysiological, morphological and neurochemical criteria at least 16 types of GABAergic interneurons could be distinguished in the CA1 area of the hippocampus (3). In response to the injection of depolarizing current pulses many of these cells express fast spiking activity pattern (“fast spiking” interneurons), whose function was attributed to the control of rhythmic activity of large populations of principal cells (4).

The effects of the activation of serotonin-7 (5-HT7) receptors were investigated in the CA1 area pyramidal cells and stratum radiatum fast spiking GABAergic interneurons of rat hippocampal slices. To activate 5-HT7 receptors, 5-carboxamidotryptamine (5-CT), a nonselective 5-HT1A/5-HT7 agonist, was applied in the presence of N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide (WAY 100635), a selective 5-HT1A receptor antagonist. The activation of 5-HT7 receptors resulted in a dose-dependent increase in the mean frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) recorded from pyramidal neurons while the mean amplitude of sIPSCs remained unaltered. A nonselective glutamate receptor antagonist, kynurenic acid, and voltage-gated sodium channel blocker, tetrodotoxin (TTX), attenuated but did not prevent the 5-HT7 receptor-mediated increase of sIPSCs frequency in pyramidal cells. 5-CT application did not influence the excitability of stratum radiatum interneurons but it dose-dependently increased the mean frequency of spontaneous excitatory postsynaptic currents (sEPSCs) recorded from interneurons while the mean amplitude of sEPSCs remained unaltered. These data suggest that the activation of 5-HT7 receptors results in an enhancement of the GABAergic transmission in the hippocampal CA1 area via two mechanisms. The first one involves an enhancement of excitatory glutamatergic input to GABAergic interneurons and is likely to be mediated by presynaptic 5-HT7 receptors. The second effect, most likely related to the activation of 5-HT7 receptors located on interneurons, results in an enhancement of the release of GABA.

Key words: 5-carboxamidotryptamine, hippocampal slice, GABA, fast spiking interneurons, serotonin

5-HT7 RECEPTORS MODULATE GABAERGIC TRANSMISSION IN RAT HIPPOCAMPAL CA1 AREA

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5-HT7 receptors are involved in serotonergic and GABAergic interactions in certain brain structures. The activation of 5-HT7 receptors affects 5-HT release by modulating GABAergic transmission in raphe nuclei (26). It is suggested that in the raphe 5-HT7 receptors are not localized directly on 5-HT cells but rather on GABAergic and/or glutamatergic neurons (27, 28). The activation of 5-HT7 receptors enhances the excitability of GABAergic neurons in globus pallidus neurons (29). On the other hand 5-HT7 receptors decrease GABA-dependent currents in neurons of the hypothalamic suprachiasmatic nucleus (30).

Serotonergic fibers form multiple synaptic contacts with hippocampal interneurons (20) and these cells readily react to serotonin. The application of 5-HT results in a severalfold increase in the frequency of inhibitory postynaptic currents (IPSCs) in hippocampal CA1 neurons and it was concluded this effect is mediated by presynaptic, ionotropic 5-HT1A receptors (21-23). Also the activation of metabotropic 5-HT2 receptors exerts an excitatory effect on GABAergic transmission in the CA1 area of the hippocampus (24). In the dentate gyrus the enhancement of GABAergic transmission occurs due to activation of 5-HT1A receptors, while 5-HT1A receptors exert an opposite effect (25).

5-HT7 receptors are involved in serotonergic and GABAergic interactions in certain brain structures. The activation of 5-HT7 receptors affects 5-HT release by modulating GABAergic transmission in raphe nuclei (26). It is suggested that in the raphe 5-HT7 receptors are not localized directly on 5-HT cells but rather on GABAergic and/or glutamatergic neurons (27, 28). The activation of 5-HT7 receptors enhances the excitability of GABAergic neurons in globus pallidus neurons (29). On the other hand 5-HT7 receptors decrease GABA-dependent currents in neurons of the hypothalamic suprachiasmatic nucleus (30).
study we aimed at establishing whether the mechanism of the serotoninergic modulation of hippocampal activity in the CA1 area involves local inhibitory circuitry through 5-HT7 receptor activation.

**MATERIALS AND METHODS**

**Animals and slice preparation**

Experimental procedures were approved by the Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences and were carried out in accordance with the “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) and national law. Male Wistar rats, weighing approx. 120 g at the beginning of the experiment, were housed in groups under a controlled light/dark cycle (light on: 7:00–19:00) and had free access to standard food and tap water. Rats were anesthetized with isoflurane and then decapitated. Their brains were quickly removed and placed in cold (0°C) artificial cerebrospinal fluid (aCSF) containing in mM: 130 NaCl, 5 KCl, 2.5 CaCl2, 1.3 MgSO4, 1.25 KH2PO4, 26 NaHCO3, 10 D-glucose, bubbled with 95% O2-5% CO2.

**Whole-cell recording**

After at least 3 h of preincubation, a single slice was placed in the recording chamber superfused at 2.5 ml/min with warm (32±0.5°C), modified aCSF of the following composition (in mM): 132 NaCl, 2 KCl, 1.25 KH2PO4, 26 NaHCO3, 1.3 MgSO4, 2.5 CaCl2, 10 D-glucose, bubbled with 95% O2-5% CO2. Their brains were quickly removed and placed in cold (0°C) artificial cerebrospinal fluid (aCSF) containing in mM: 130 NaCl, 2 KCl, 1.25 KH2PO4, 26 NaHCO3, 1.3 MgSO4, 2.5 CaCl2, 10 D-glucose, bubbled with 95% O2-5% CO2.

**Reagents**

To selectively activate 5-HT7 receptors, 5-carboxamidotryptamine (5-CT, Tocris), an agonist of 5-HT7 receptors, was applied for 15 min in the presence of N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexancarboxamide (WAY 100635, Sigma Aldrich), a selective 5-HT7 receptor antagonist, which was introduced at least 20 min earlier (19, 31). In part of the experiments 2 µM kynurenic acid (Sigma Aldrich) and 200 nM tetrodotoxin (TTX, Sigma Aldrich) or 10 µM bicuculline methiodide (Sigma Aldrich) was added to the aCSF to block glutamate receptors, voltage dependent Na+ channels, respectively.

**RESULTS**

**Effect of 5-HT7 receptor activation on sIPSCs recorded from pyramidal neurons**

Whole-cell recordings were obtained from 65 CA1 pyramidal neurons (Fig. 1A). Basic membrane properties of these cells are summarized in Table 1. Spontaneous IPSCs were recorded at the holding potential of 0 mV as outward currents (Fig. 2A, 32, 33). In control conditions, in the presence of 2 µM WAY 100635, before addition of 5-CT to the aCSF, the mean frequency of sIPSCs was 0.91±0.3 Hz. The application of 50 nM of 5-CT did not change the mean frequency of sIPSCs (Fig. 2B). However, 5-CT at higher concentrations (100–500 nM) resulted in a dose-dependent increase in the frequency of sIPSCs (P<0.05) (Fig. 2B). The activation of 5-HT7 receptors did not significantly affect the mean amplitude of sIPSCs (Fig. 2C), which in control conditions was 40.4±3.88 pA.

To test the involvement of the excitatory synaptic input to interneurons in 5-CT-mediated effects, in a separate set of slices, kynurenic acid (2 µM WAY 100635, before addition of 5-CT to the aCSF), the mean frequency of sIPSCs was 0.91±0.3 Hz. The activation of 5-HT7 receptors 5-CT still induced an increase in sIPSCs frequency in pyramidal cells, however, the effect was significantly weaker (Fig. 3). 5-HT7 receptor-mediated effect of a similar magnitude was observed in the presence of kynurenic acid and, moreover, after addition of 200 nM TTX (n=10 cells) to aCSF to block action potentials. Simultaneous application of kynurenic acid and TTX did not influence either the mean amplitude (39.5±1.54 pA) or the mean frequency (0.9±0.12 Hz) of sIPSCs (P>0.05).

Effect of 5-HT7 receptor activation on sEPSCs recorded from GABAergic interneurons

Activation of 5-HT7 receptors raises the excitability of hippocampal pyramidal cells (17, 19). To test whether the same holds true for CA1 fast spiking interneurons in a set of 10 cells a series of depolarizing current pulses was applied before and after addition of 5-CT (in the presence of WAY 100635) after blockade of glutamatergic and GABAergic transmission by kynurenic acid and bicuculline, respectively. As illustrated in Fig. 4 the activation of 5-HT7 receptors did not influence the mean number of spikes generated by interneurons for a given current.

Spontaneous EPSCs were recorded at the holding potential of -76 mV as inward currents (Fig. 3A) from 58 fast spiking interneurons (Fig. 1B). Basic membrane properties of these cells are summarized in Table 1. The mean frequency of sEPSCs recorded in standard aCSF was (1.43±0.18 Hz). 5-CT (100–500 nM), administered in the presence of 2 µM WAY 100635, dose-dependently increased the frequency of sEPSCs (P<0.05) (Fig. 5B), without changing the mean amplitude of sEPSCs (Fig. 5C).

Statistical analysis

Statistical analysis of was carried out using Student’s t test. Throughout the text data are presented as means ±SEM.
The main finding of the present study is that the activation of 5-HT7 receptors results in an increase in the mean sIPSCs frequency, but not the mean sIPSCs amplitude, in hippocampal CA1 pyramidal cells. Increased frequency of spontaneous postsynaptic currents reflects either enhanced firing of presynaptic cells or increased neurotransmitter release probability and/or changes in the number of neurotransmitter release sites. Thus, several mechanisms may potentially account for the 5-HT7 receptor-dependent enhancement of the release of GABA. We excluded the possibility that 5-HT7 receptor activation directly raises the excitability of stratum radiatum fast-spiking interneurons, an effect which occurs in hippocampal principal, excitatory cells (17-19). Alternatively, enhanced release of GABA may be an indirect consequence of increased glutamatergic input to GABAergic cells due to the activation of 5-HT7 receptors located on glutamatergic neurons. Immunohistochemical studies demonstrated 5-HT7 receptors on cell bodies of pyramidal neurons in the CA1 area (34). Glutamatergic synapses excite hippocampal GABAergic interneurons via AMPA and NMDA receptors (35-37).

Table 1. Basic membrane properties of recorded neurons.

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<tr>
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<th>pyramidal neurons</th>
<th>interneurons</th>
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<tr>
<td>Resting membrane potential Vm (mV)</td>
<td>-66±3</td>
<td>-71±4</td>
</tr>
<tr>
<td>Input resistance Rm (MΩ)</td>
<td>76±23</td>
<td>180±28</td>
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<tr>
<td>n</td>
<td>61</td>
<td>58</td>
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Fig. 2. The influence of 5-CT on spontaneous IPSCs recorded from pyramidal neurons. (A) Representative examples of sIPSCs (individual events marked by black dots) recorded from pyramidal neuron before (upper trace) and after (lower trace) application of 150 nM 5-CT. (B) Dose-response curve for the effect of 5-CT on the mean frequency of sIPSCs (±S.E.M.). The solid line represents fit of the data with the Hill equation. (C) The application of 5-CT does not influence the amplitude of sIPSCs. Shown are means ±S.E.M.
Indeed, after blockade of glutamatergic transmission by a nonselective antagonist, kynurenic acid, the magnitude of 5-HT7 receptor-induced effect on frequency of sIPSCs recorded from pyramidal cells was significantly weaker. Thus, the data suggest that 5-HT7 receptor-induced increase in GABA release is partially due to increased glutamate release from excitatory terminals located on interneurons.

The second mechanism underlying the stimulatory effect of 5-HT7 receptor activation on sIPSCs frequency seems to involve 5-HT7 receptors localized on GABAergic cells. Several authors suggested that 5-HT7 receptors are expressed on GABAergic interneurons in the dorsal raphe nucleus (40). Also Roberts et al. (26) and Glass et al. (41) have proposed, on the basis of a series of functional studies, that 5-HT7 receptors in the DRN are localized, at least in part, on GABAergic cells. However, to our knowledge, there are no data which confirm the occurrence of these receptors on cell bodies of hippocampal interneurons. An increase in interneuron firing rate cannot account for the observed effect as we demonstrated no 5-CT-induced change in interneuron excitability and, moreover, in the presence of kynurenic acid and additionally TTX, a sodium channel blocker, activation of 5-HT7 receptors still induced an increase in sIPSCs frequency. Thus the data suggest that 5-HT7 receptors modulate the release of GABA from axon terminals. Belenky and Pickard (42) found 5-HT7 receptor-labeled GABAergic terminals in the SCN. Further studies, employing immunochemical methods, are necessary to verify the occurrence of 5-HT7 receptors on hippocampal GABAergic interneurons.

The data obtained in the course of the present study indicate that serotonin, acting through 5-HT7 receptors, exerts a complex modulatory influence over glutamate- and GABA-mediated synaptic transmission. This study provides new information regarding the role of 5-HT7 receptors in the mechanisms which allow serotonin to simultaneously remodel neuronal activity in a functionally appropriate manner in a wide variety of cell types.

Fig. 3. The influence of 5-CT (applied in the presence of WAY 100635) on the mean frequency of spontaneous IPSCs recorded from pyramidal neurons in control aCSF (labeled: 5-CT), in the presence of kynurenic acid (labeled: 5-CT+KYN) and in the presence of kynurenic acid and tetrodotoxin (labeled: 5-CT+KYN+TTX). * P<0.05 vs. baseline frequency; # P<0.05 vs. 5-CT.

Fig. 4. The activation of 5-HT7 receptors does not influence the excitability of CA1 fast-spiking interneurons. Shown are mean (±S.E.M.) numbers of spikes generated by depolarizing current steps lasting 500 ms.

Fig. 5. The influence of 5-CT on spontaneous EPSCs recorded from interneurons. (A) Examples of sEPSCs (marked by black dots) recorded from a representative interneuron before (upper trace) and after (lower trace) application of 150 nM 5-CT. (B) Dose-response curve for the effect of 5-CT on the mean frequency of sEPSCs (±S.E.M.). The solid line represents fit of the data with the Hill equation. (C) The application of 5-CT does not influence the mean amplitude of sEPSCs.

* P<0.05 vs. baseline frequency; # P<0.05 vs. 5-CT.
and excitatory as well as inhibitory circuits in the hippocampus. The identification of this novel 5-HT<sub>7</sub> receptor-mediated effect is of importance for elucidating the physiological significance of these receptors. We have previously shown using extracellular recording in rat ex vivo hippocampal slices that repetitive corticosterone administration to rats, used as a model of non-adaptive stress, resulted in an enhancement of the effect related to the activation of 5-HT<sub>7</sub> receptors without changes in binding properties of the receptors (43). Treatment with a tricyclic antidepressant, imipramine, counteracted the corticosterone-induced increase in the reactivity of the hippocampal circuitry to the activation of 5-HT<sub>7</sub> receptors. We reasoned that the relevance of the effect of imipramine might be related to the dampening of corticosterone-induced excessive excitatory effect of serotonin acting on hippocampal principal neurons via 5-HT<sub>5</sub> receptors. In addition the activation of 5-HT<sub>7</sub> receptor inhibits the 5-HT transporter (44). Present data suggest that the adaptive effects of pro depressive and antidepressant treatments on the function of the hippocampus might, potentially, also involve 5-HT<sub>7</sub> receptor-dependent changes in excitatory and inhibitory transmission. The modulatory influence of 5-HT<sub>7</sub> receptors on GABAergic transmission in the hippocampus may also be relevant for developing new treatments of diseases related with abnormalities of GABAergic inhibition (45).

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Conflict of interests: None declared.

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