5-Hydroxytryptamine$_7$ Receptor Activation Decreases Slow Afterhyperpolarization Amplitude in CA3 Hippocampal Pyramidal Cells$^1$

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ABSTRACT
The 5-hydroxytryptamine$_7$ (5-HT$_7$) receptor was originally defined by molecular biology techniques. The 5-HT$_7$ receptor protein and mRNA are found in brain areas, such as the CA3 subfield of the hippocampus, that are involved in various neuropsychiatric disease states. No functional response has previously been attributed to activation of the 5-HT$_7$ receptor in any of these brain areas. Calcium spike-induced slow afterhyperpolarizations (sAHP) were recorded from CA3 hippocampal pyramidal cells using intracellular recording techniques in a brain slice preparation maintained in vitro. A concentration-dependent inhibition of the sAHP amplitude was obtained when 5-HT$_7$ was used as the agonist. To identify whether the 5-HT$_7$ receptor was one of the receptors mediating the inhibition of the sAHP amplitude, 5-HT agonists and antagonists were tested in the presence of WAY-100635 and GR-113808 to block 5-HT$_{1A}$ and 5-HT$_4$ receptor activation, respectively. The rank order potency of the agonists was 5-carboxyamidotryptamine (5-CT) $>$ 5-HT $>$ 5-methoxytryptamine (5-MeOT). Other agonists with high affinity at 5-HT$_2$, 5-HT$_3$, 5-HT$_{1B}$, 5-HT$_{1D}$, or 5-HT$_6$ receptors did not produce any response when tested at 10 $\mu$M. Ritanserin, mesulergine, and SB-269770 were competitive antagonists of the 5-CT inhibition of sAHP amplitude, with affinity ($pA_2$) values of 6.8, 7.9, and 8.8, respectively. Methiothepin was also an effective antagonist but was insurmountable. Other antagonists with affinity for the 5-HT$_2$, 5-HT$_3$, or 5-HT$_6$ receptor had no effect. Based on the rank order potency of the agonists and antagonists, one of the receptors that mediates the decrease in sAHP amplitude in CA3 hippocampal pyramidal cells was concluded to be the 5-HT$_7$ receptor.

The 5-hydroxytryptamine$_7$ (5-HT$_7$) receptor was originally identified solely by molecular biology techniques (reviewed in Eglen et al., 1997; Terrón 1998; Vanhoenacker et al., 2000). In situ hybridization and receptor binding assays in transfected cells, peripheral tissue or brain tissue revealed a distinct pharmacological profile for this receptor with a rank order affinity of 5-carboxyamidotryptamine (5-CT) $>$ 5-HT $>$ 5-methoxytryptamine (5-MeOT) $>$ methiothepin $>$ ritanserin $>$ 8-hydroxydipropylaminotetralin (8-OH-DPAT) $>$ clozapine $>$ spiperone [reviewed in Eglen et al. (1997), Terrón (1998), and Vanhoenacker et al. (2000)]. The highest density of binding or in situ hybridization was located in the thalamus and CA3 subfield of the hippocampus (Ruat et al., 1993; Tsou et al., 1994; Gustafson et al., 1996; Vizuete et al., 1997; Heidmann et al., 1998). Due to the distinct pharmacological profile, regional distribution, and low sequence homology with other 5-HT receptors, this receptor was identified as the 5-HT$_7$ receptor.

When transfected into different cell lines, the novel 5-HT$_7$ receptor was found to stimulate adenylyl cyclase activity [reviewed in Eglen et al. (1997), Terrón (1998), and Vanhoenacker et al. (2000)]. The rank order potency of agonists agreed with the rank order affinity from binding, i.e., 5-CT $>$ 5-HT $>$ 5-MeOT $>$ 8-OH-DPAT. Antagonists include methiothepin ($pK_B = 8.1$–$8.5$), mesulergine ($pK_B = 6.7$–$8.0$), ritanserin ($pK_B = 6.4$–$7.4$), clozapine ($pK_B = 6.7$–$7.6$), and spiperone ($pK_B = 7.0$–$7.2$). None of these antagonists are selective, however. Two selective antagonists for the 5-HT$_7$ receptor have been developed, i.e., SB-258719 [$pK_B$ of 7.2 (Thomas et al., 1998)] and SB-269770 [$pK_B$ of 8.8 (Lovell et al., 2000)].

Even though the CA3 subfield of the hippocampus contains a high density of 5-HT$_7$ receptor binding sites and mRNA, the physiological consequences of 5-HT$_7$ receptor activation are

**ABBREVIATIONS**: 5-HT, 5-hydroxytryptamine; GR-113808, [1-{2-methylsulfonylamino}ethyl]-4-piperidinyl)methyl-1-methyl-1H-indole-3-carboxylate; 5-CT, 5-carboxyamidotryptamine; 5-MeOT, 5-methoxytryptamine; ACSF, artificial cerebrospinal fluid; sAHP, slow afterhyperpolarization; 8-OH-DPAT, 8-hydroxydipropylaminotetralin; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; WAY-100635, N-(2-(4-methoxyphenyl)-1-piperazinyl)ethyl)-(2-pyridinyl)cyclohexane carboxamide; SB-269770, (R)-3-(2-(4-methylpiperidin-1-yl)-ethyl)pyrrolidine-1-sulfonylethanol.
unknown. In the CA1 and CA3 subfields of the hippocampus, adenylyl cyclase activity modulates the amplitude of the sAHP that is elicited by a train of action potentials or a calcium spike (Madison and Nicoll, 1986b; Pedarzani et al., 1998). The ion channel underlying the sAHP is a calcium-activated potassium channel (reviewed in Sah, 1996). The hallmarks of the sAHP that differentiate it from other calcium-activated potassium channels include a very long time course and its modulation by the activation of neurotransmitter receptors (Sah, 1996). The sAHP amplitude is decreased by activation of β-adrenergic (Madison and Nicoll, 1986a), muscarinic (Cole and Nicoll, 1984), 5-HT(1A) (Torres et al., 1994), and metabotropic glutamatergic (Pedarzani and Storm, 1996) receptors. In this study we report that the sAHP amplitude is decreased by 5-HT through the activation of the 5-HT(7) receptor.

Materials and Methods

Procedures for preparation of hippocampal slices and intracellular electrophysiological recording are as previously described for our laboratory (Beck et al., 1992; Birnstiel and Beck, 1995). Rats (100–200 g, Harlan Sprague-Dawley) were decapitated, and the brain rapidly removed and rinsed in ice-cold artificial cerebrospinal fluid (ACSF) containing 124 mM NaCl, 3 mM KCl, 1.25 mM NaH2PO4, 2 mM MgSO4, 2.5 mM CaCl2, 10 mM dextrose, and 28 mM NaHCO3. The hippocampus was dissected free and starting at the dorsal/septal tip sections (500–600 μm) were cut on a vibratome. Slices were placed in a holding vial containing room temperature ACSF bubbled with 95% O2, 5% CO2 at 32°C at a flow rate of 2 to 3 ml/min.

Standard intracellular recordings were made by pulling electrodes from borosilicate capillary tubing (1.2-mm o.d., 0.69-mm i.d.; Sutter Instruments, Novato, CA) on a Brown and Flaming electrode puller (Sutter Instruments, Novato, CA) to obtain resistances of 60 to 100 MΩ (2 M potassium methyl sulfate, 10 mM KCl). Pyramidal cells in area CA3 were impaled by briefly (10–50 ms) increasing the capacityM, and2 the recording electrode. The impaled neuron was hyperpolarized to facilitate sealing of the cell. Electrical signals were amplified using an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA), stored on disk for later analysis and recorded on-line on a Gould series 3200 chart recorder (Gould, Inc., Valley View, OH). Data were collected on-line with pCLAMP software (Axon Instruments). Only neurons with a resting membrane potential < –55 mV, input resistance > 30 MΩ, and action potential overshoot of at least 10 mV were used for the experiments.

The sAHP was elicited by a calcium spike. Tetrodotoxin (1 μM) and tetraethylammonium (5 mM) were included in the ACSF. A current pulse (70–100 ms) of sufficient intensity (0.8–2 nA) was used to elicit the calcium spike. Each sAHP used for data analysis was the average of three to five sAHPs elicited 30 s apart.

Drugs were added to the ACSF in known concentrations. A stock solution was made (usually 10 mM) and diluted on the day of the experiment to obtain the desired concentrations in the ACSF.

For data analysis the magnitude of the sAHP amplitude was measured during the administration of agonist and normalized by directly comparing it to the magnitude of the sAHP in the absence of agonist. The percentage inhibition for each concentration of agonist was fit to a logistic equation \( E = \frac{E_{\max}(1 + (EC_{50}/A)^n)}{1 + (EC_{50}/A)^n} \), where \( E \) is the response elicited by the concentration of drug (A), \( N \) is the slope, and \( EC_{50} \) is the concentration of drug needed to elicit a response 50% of maximum. Estimates of \( E_{\max} \), \( EC_{50} \), and slope were obtained for each cell and were used for statistical analyses.

Chemicals for making the stock buffer and 5-HT were obtained from Sigma Chemical Co. (St. Louis, MO). The agents 5-CT, 5-MeOT, ICS-205-930, metergoline, ritanserin, mesulergine, 1-(2,5-di-methoxy-4-iodophenyl)-2-amino propane (DOI), clozapine, ketanserin, methiothepin, 2-methyl 5-HT, sumatriptan, and α-methyl 5-HT were obtained from Research Biochemicals International (Natick, MA). WAY-100635 (N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridinyl)cyclohexanecarboxamide) was a generous gift from Wyeth Ayerst Research (Princeton, NJ). GR-113808 [(1-[2-(methylsulfonylamino)ethyl]-4-piperidinyl)methyle-1-methyl-1H-indole-3-carboxylate] was generously donated by Glaxo Group Research (Greenford, UK). SB-269770 (R)-3-(2-((4-methylpiperidin-1-yl)ethyl)pyrroolidine-1-sulfonyl)phenol) was generously provided by SmithKline Beecham (Essex, UK).

Results

The magnitude of the sAHP elicited by a calcium spike in CA3 hippocampal pyramidal cells ranged from 9 to 25 mV, with a mean of 18.9 ± 0.4 mV, \( n = 79 \). The values for the sAHP amplitude were normally distributed.

To isolate any response mediated by 5-HT(7) receptor activation, other postsynaptic 5-HT receptor-mediated responses present in CA3 pyramidal cells were blocked by the addition of selective antagonists in the ACSF. Activation of a postsynaptic 5-HT(1A) receptor by 5-HT leads to a very pronounced hyperpolarization of the membrane potential (Beck et al., 1992), averaging 17 mV. This hyperpolarization shunts or attenuates the magnitude of the sAHP, making it difficult to measure a 5-HT-induced inhibition of the sAHP. In previous experiments (Birnstiel and Beck 1995), spiperone, an antagonist at 5-HT(1A), 5-HT(2A), and 5-HT(2C) receptors, was added to the ACSF to block the 5-HT(1A) hyperpolarization. However, in the presence of spiperone (10 μM), 5-HT did not inhibit the sAHP (Fig. 1); a concentration of 5-HT greater than 300 μM was needed to obtain any inhibition of the sAHP \( (N = 3) \). When the selective 5-HT(1A) receptor antagonist WAY-100635 [0.1–1.0 μM, 100 and 1000 times the reported \( K_p \) at the 5-HT(1A) receptor (Corradetti et al., 1996)] was added to the ACSF instead of spiperone to block the hyperpolarization, a pronounced inhibition of the sAHP amplitude by 10 μM 5-HT was needed to obtain any significant degree of inhibition of the sAHP amplitude. The resting membrane potential for each of the traces was –67 mV for control, –68 mV for 10 μM, –66 mV for 30 μM, –70 mV for 100 μM, and –66 mV for 350 μM.

![Fig. 1. Spiperone blocks the 5-HT inhibition of sAHP amplitude.](image-url)
was seen, i.e., 60.3 ± 4.4% (N = 26) inhibition compared with the control sAHP without 5-HT. Some of the inhibition of the sAHP by 5-HT could be blocked by the selective 5-HT4 receptor antagonist GR-113808 (0.1 and 1.0 μM, 100 and 1000 times the reported Kᵣ for GR-113808 at the 5-HT₄ receptor (Torres et al., 1994)], i.e., 54.9 ± 5% inhibition by 10 μM 5-HT and 38.2% ± 7% inhibition by 5-HT in the presence of GR-113808 (paired t-test = 2.72, P < .01, df = 14). Therefore, due to the presence of both a 5-HT₁₆ and 5-HT₄ postsynaptic receptor-mediated response in CA3 pyramidal cells, the selective 5-HT₁₆ receptor antagonist WAY-100635 (0.1–1 μM) and the selective 5-HT₄ receptor antagonist GR-113808 (0.1 μM) were included in the ACSF for all of the experiments described below.

The inhibition of the sAHP by 5-HT was concentration-dependent and reversible. Figure 2 contains sAHPs recorded from a CA3 pyramidal cell. The sAHPs were collected at the beginning of the experiment (control) and during the perfusion of the slice by the concentration of 5-HT shown above each trace. Following the administration of 5-HT, the drug was removed from the ACSF and the sAHP amplitude returned to baseline values (recovery). To save space not all of the “recovery” sAHP values are shown. In Fig. 2B, the control sAHP and the sAHP recorded in the presence of 15 μM 5-HT are superimposed. For each concentration tested, the magnitude of the sAHP recorded in the presence of 5-HT was normalized as a percentage of the sAHP collected just before each administration of 5-HT. The normalized percentage inhibition was plotted against the concentration of 5-HT and fit to a logistic equation (Fig. 2C). The form of the logistic equation is \( E = E_{\text{max}} / (1 + (EC_{50}/[A])^n) \), where \( E \) is equal to the response magnitude at a given concentration of ligand, \( A \), \( E_{\text{max}} \) is the maximum inhibition that can be elicited by the ligand, \( EC_{50} \) is the concentration of \( A \) that elicits a response 50% of maximum, and \( n \) is the slope.

Agonists known to have activity at the 5-HT₄ receptor in other bioassays, i.e., 5-CT and 5-MeOT, were tested and compared with 5-HT. WAY-100635 (0.1–1 μM) and GR-113808 (0.1 μM) were included in the buffer to block 5-HT₁₆ and 5-HT₄ receptors. Figure 3 contains sAHPs recorded from a CA3 hippocampal pyramidal cell before, during and after the administration of 5-CT. Even though recovery sAHPs were obtained following each 5-CT concentration tested, the only recovery sAHP shown is the one collected following the removal of 100 nM 5-CT from the ACSF. A similar concentration-dependent inhibition of the sAHP amplitude was obtained following 5-MeOT administration. Table 1 is a summary of the mean ± S.E. for the \( EC_{50} \), \( E_{\text{max}} \), and slope values obtained for all the cells tested with 5-HT, 5-CT, and 5-MeOT. The \( EC_{50} \) values were significantly different between the agonists, with 5-CT demonstrating the greatest potency. The \( E_{\text{max}} \) and slope values were not significantly different between the three agonists.

Figure 4 is a summary graph of the mean ± S.E. values for the inhibition of the sAHP at each of the tested concentrations of the agonists 5-HT (triangles), 5-CT (squares), and 5-MeOT (circles). The rank order potency of the agonists for the inhibition of the sAHP in the presence of WAY-100635 and GR-113808 was 5-CT > 5-HT > 5-MeOT (Fig. 4; Table 1). Other agonists known to have high or fairly selective affinity at other 5-HT receptors were tested at a concentration of 10

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**Fig. 2.** Concentration-dependent inhibition of the sAHP by 5-HT. The sAHP traces are the average of five sAHPs. A, following a calcium spike, the sAHP magnitude was 25 mV. WAY-100635 (0.1–1 μM) and GR-113808 (0.1 μM) were included in the buffer to block 5-HT₁₆ and 5-HT₄ receptors. In the presence of increasing concentrations of 5-HT, the magnitude of the sAHP response was reduced. In between each concentration of 5-HT the response was allowed to return to control amplitude before the next concentration of 5-HT was administered, i.e., recovery sAHPs. Not all recovery traces are shown. The resting membrane potential for each of the traces was −64 mV for control, −63 mV for 1 μM, −62 mV for 3 μM, −64 mV for recovery, −64 mV for 1 μM, −66 mV for recovery, −64 mV for 10 μM, −61 mV for 15 μM, and −66 mV for recovery. B, the control response and 15 μM response are superimposed to demonstrate that 5-HT maximally inhibited the magnitude of the sAHP. C, the magnitude of the sAHP amplitude was measured during the administration of 5-HT and normalized by directly comparing it to the magnitude of the sAHP in the absence of 5-HT. The percentage inhibition at each concentration of 5-HT was fit to a logistic equation as described under Materials and Methods.

**Fig. 3.** 5-CT decreases the amplitude of the sAHP evoked by a calcium spike in a concentration-dependent manner. WAY-100635 (1.0 μM) and GR-113808 (0.1 μM) were included in the buffer to block 5-HT₁₆ and 5-HT₄ receptors. The sAHP traces are the average of five sAHPs. The concentration of 5-CT that was bath-administered is shown above each trace. In between each concentration of 5-CT the sAHP was allowed to return to baseline values, but only the recovery sAHP after the highest concentration of 5-CT is shown. The bottom right hand composite trace contains the superimposed traces obtained during control and in the presence of 100 nM 5-CT. The resting membrane potential for each trace was −72 mV for control, −72 mV for 1 nM, −72 mV for 3 nM, −72 mV for 10 nM, −70 mV for 30 nM, −68 mV for 100 nM, and −69 mV for recovery.
Agonists effective at reducing the amplitude of sAHP in CA3 hippocampal pyramidal cells

Values are mean ± S.E., and the number of cells is shown in parentheses. WAY-100635 (0.1–10 μM) and GR-113808 (0.1 μM) were included in the stock buffer.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>EC_{50}^a</th>
<th>E_{max}</th>
<th>Slope</th>
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<tbody>
<tr>
<td>5-HT (8)</td>
<td>5.3 ± 0.11</td>
<td>58.2 ± 4.7</td>
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<td>5-CT (7)</td>
<td>7.5 ± 0.08</td>
<td>71.9 ± 7.5</td>
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<tr>
<td>5-MeOT (7)</td>
<td>4.8 ± 0.12</td>
<td>60.2 ± 6.7</td>
<td>1.9 ± 0.3</td>
</tr>
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</table>

^a Significant P = 174, P < .001. All comparisons were significantly different.

μM, and the magnitude of the inhibition of the sAHP by each of these ligands is presented in Table 2. None of the agonists with affinity for the 5-HT_{1A}, 5-HT_{2}, 5-HT_{1B}, 5-HT_{1D}, or 5-HT_{6} receptors produced any degree of inhibition of the sAHP at the concentration of 10 μM.

Antagonists known to have affinity for the 5-HT_{7} receptor were tested for their ability to block the inhibition of the sAHP amplitude by 5-CT. The agonist used for these experiments was 5-CT because of its high affinity for the 5-HT_{7} receptor and poor affinity for the 5-HT_{4} receptor. Ritalserin, methiothepin, and mesulergine have previously been shown to be effective antagonists at the 5-HT_{7} receptor [reviewed in Eglen et al. (1997), Terrón (1998), and Vanhoenacker et al. (2000)]. The concentrations of the tested antagonists were chosen to be at least 10- to 100-fold greater than the reported K_{D} of the antagonist for the 5-HT_{7} receptor. In preliminary experiments, the equilibration time for methiothepin and mesulergine was determined to be at least 30 min. The equilibration time for the lipophilic antagonist ritalserin took over 1 h. In some cases ritanserin was added to the stock buffer at the beginning of the experiment and therefore was constantly present. WAY-100635 (1–30 μM) and GR-113808 (0.1 μM) were included in the stock buffer to block 5-HT_{1A} and 5-HT_{4} receptors, respectively. Due to the larger concentrations of 5-CT used in the antagonist experiments, the concentration of WAY-100635 was also increased to prevent 5-CT activation of 5-HT_{1A} receptors.

Figure 5 contains sAHP traces taken from one cell before, during 5-CT perfusion, and during the perfusion of 5-CT in the presence of mesulergine (Fig. 5A). The graph in Fig. 5B depicts summary concentration-response information for all of the cells tested with 5-CT alone and those tested with 5-CT alone and in the presence of mesulergine. Due to the length of the experiment, instead of a complete concentration-response curve, just two concentrations of 5-CT were used for the collection of control data, i.e., 30 and 100 nM 5-CT. These

Table 2

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Inhibition</th>
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<tr>
<td>DOI, 10 μM (3)</td>
<td>-2 ± 2</td>
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<tr>
<td>2-Methyl 5-HT, 10 μM (3)</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>α-Methyl 5-HT, 10 μM (3)</td>
<td>-4 ± 3</td>
</tr>
<tr>
<td>Sumatriptan, 10 μM (3)</td>
<td>-5 ± 3</td>
</tr>
<tr>
<td>Clozapine, 10 μM (3)</td>
<td>-4 ± 3</td>
</tr>
</tbody>
</table>

DOI, 10 μM (3) | -2 ± 2 |
2-Methyl 5-HT, 10 μM (3) | 0 ± 1 |
α-Methyl 5-HT, 10 μM (3) | -4 ± 3 |
Sumatriptan, 10 μM (3) | -5 ± 3 |
Clozapine, 10 μM (3) | -4 ± 3 |

Fig. 5. Mesulergine blocks the inhibition of the sAHP amplitude by 5-CT. WAY-100635 (0.1–30 μM) and GR-113808 (0.1 μM) were included in the buffer to block 5-HT_{1A} and 5-HT_{4} receptors. A, sAHPs recorded from a CA3 hippocampal pyramidal cell before and during the administration of 5-CT alone, and 5-CT in the presence of mesulergine (0.1 μM). Each trace is the average of five sAHPs. The concentration of 5-CT that was bath-administered is shown above each trace. The resting membrane potential for each trace was -62 mV for control, -62 mV for 30 nM, -63 mV for 100 nM, -62 mV for wash, -64 mV for 100 nM 5-CT with mesulergine, and -60 mV for 1 μM 5-CT with mesulergine. B, summary graph of all of the data for the cells tested with mesulergine. The open squares are the control responses to 5-CT (from Fig. 4), and the filled squares are the data obtained in the experiments designed to test mesulergine. The two filled square boxes that lie near the control 5-CT (open squares) concentration-obtained in the experiments designed to test mesulergine. The two filled square boxes that lie near the control 5-CT (open squares) concentration-
data points were contiguous with the sum of the measured data for all of the cells tested with 5-CT alone. Mesulergine was added to the perfusion buffer, and after 30 min data for the construction of a concentration-response curve for 5-CT was collected. As can be seen, mesulergine blocked the inhibition by 5-CT and a concentration of 1 μM 5-CT was needed to obtain an inhibition of approximately 50%. Mesulergine (0.1 μM) acted as a competitive antagonist (N = 7), i.e., the $E_{\text{max}}$ values were not statistically significant from 5-CT alone (Table 3). The apparent affinity ($pA_2$) of 7.9 was obtained using the formula $pA_2 = -\log(\text{concentration of antagonist}/[\text{EC}_{50} \text{ with antagonist}/[\text{control EC}_{50}] - 1))$. The value used for [control EC$_{50}$] was the EC$_{50}$ from the summed 5-CT alone data, i.e., $-\log 7.5$.

Methiothepin (0.1 μM) acted as a noncompetitive antagonist by significantly reducing the $E_{\text{max}}$ of 5-CT from 72% to 48% (Fig. 6A, n = 8). The values obtained for $E_{\text{max}}$ are provided in Table 3. There was a significant difference between the $E_{\text{max}}$ for cells tested with methiothepin compared with 5-CT alone (Table 3).

Ritanserin (1 μM) was a competitive antagonist (n = 4). Figure 6B depicts the summary data for all of the cells tested in the presence of 5-CT alone or in the presence of 5-CT with ritanserin. The calculated values of $E_{\text{max}}$-EC$_{50}$, and slope for 5-CT in the presence of ritanserin are summarized in Table 3. The calculated $pA_2$ for ritanserin was 6.8.

The selective 5-HT$_7$ receptor antagonist recently developed by SmithKline Beecham (Lovell et al., 2000), i.e., SB-269770, was also a competitive antagonist (Fig. 7). Figure 7A contains sAHPs recorded before and during the administration of 30 and 100 nM 5-CT and the sAHPs recorded during the administration of 5-CT in the presence of 0.1 μM SB-269770. Figure 7B contains the summary graphs for all of the cells tested with 5-CT alone or tested with 5-CT in the presence of SB-269770 (N = 3). The affinity of SB-269770 was very high, i.e., $pA_2$ of 8.8.

Other antagonists known to have affinity at other 5-HT receptors were tested for their ability to block the inhibitory effect of 5-CT on the calcium-induced sAHP. In the presence of the 5-HT$_2$/5-HT$_3$/5-HT$_4$, receptor antagonist clonazepam (10 μM) or the 5-HT$_3$, antagonist ICS-205,930 (100 nM) no differences were found in the percentage inhibition of the sAHP amplitude by 100 nM 5-CT, i.e., 52% ± 7% inhibition in control (N = 5), 43% ± 16 with clonazepam (N = 3), and 45% ± 5% inhibition with ICS-205,930 (N = 2). Unexpectedly, clonazepam did not have a large effect as an agonist or antagonist. As an antagonist at a concentration of 10 μM, it reduced the maximal inhibition by only 30%.

### Table 3

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>$E_{\text{max}}$ $^a$</th>
<th>$E_{\text{max}}$ $^b$</th>
<th>Slope</th>
<th>$pA_2$</th>
<th>% Inhibition</th>
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<td>None 5-CT (7)</td>
<td>7.53 ± 0.1</td>
<td>72 ± 7.5</td>
<td>1.6 ± 0.4</td>
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<tr>
<td>Ritanserin, 1.0 μM (4)</td>
<td>6.61 ± 0.1</td>
<td>53 ± 5.7</td>
<td>1.5 ± 0.22</td>
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<tr>
<td>Methiothepin, 0.1 μM (8)</td>
<td>7.35 ± 0.2</td>
<td>48 ± 4.4</td>
<td>2.3 ± 0.33</td>
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<tr>
<td>Mesulergine, 0.1 μM (7)</td>
<td>6.57 ± 0.2</td>
<td>54 ± 4.5</td>
<td>1.5 ± 0.20</td>
<td>7.9</td>
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<tr>
<td>SB-269770, 0.1 μM (3)</td>
<td>5.68 ± 0.1</td>
<td>76 ± 6.3</td>
<td>1.1 ± 0.04</td>
<td>8.8</td>
<td></td>
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$^a$ Significantly different ANOVA, F = 8.615, P < 0.001; 5-CT different from ritanserin, mesulergine and SB-269770, P < 0.05, by the Student-Newman-Keuls method.

$^b$ Significantly different ANOVA, F = 5.43, P = 0.003; 5-CT and SB-269770 different from methiothepin, P < 0.05, by the Student-Newman-Keuls method.

**Discussion**

Using intracellular recording techniques in area CA3 of a hippocampal slice preparation maintained in vitro, 5-HT reduced the amplitude of the sAHP in area CA3 hippocampal pyramidal cells in a reversible, concentration-dependent manner. The inhibition of the sAHP amplitude was through activation of the 5-HT$_7$ receptor.

The mainstay of pharmacology for the characterization of a neurotransmitter receptor is to compare the rank order potency of agonists and absolute affinity of antagonists. The 5-HT$_7$ receptor was originally identified solely by molecular biology techniques. Affinity measurements using receptor binding assays in transfected cells, peripheral tissue, or brain tissue revealed a distinct pharmacological profile for this receptor with a rank order affinity of 5-HT$_7$ > 5-HT$_3$ > 5-HT$_6$ > methiothepin > ritanserin > 8-OH-DPAT > clozapine > spiperone [reviewed in Eglen et al. (1997), Terrón (1998), and Vanhoenacker et al. (2000)]. The highest density of binding or in situ hybridization was located in the thalamus and CA3 subfield of the hippocampus (Ruat et al., 1993; Tsou et al., 1994; Gustafson et al., 1996; Vizuete et al., 1997; Heidmann et al., 1998).

Various bioassays have been used to obtain rank order affinity of agonists and antagonists for the 5-HT$_7$ receptor. Activation of the 5-HT$_7$ receptor in transfected cell lines leads to stimulation of adenylyl cyclase [reviewed in Eglen et al. (1997), Terrón (1998), and Vanhoenacker et al. (2000)]. In the periphery 5-HT$_7$ receptor activation mediates constriction or dilation of vasculature smooth muscle, hypotension, tachycardia, and phase shift in circadian rhythms [reviewed in Eglen et al. (1997), Terrón (1998), and Vanhoenacker et al. (2000)]. The rank order potency of agonists in these bioassays agreed with the rank order affinity from binding, i.e., 5-CT > 5-HT$_3$ > 5-MeOT > 8-OH-DPAT. The rank order potency of the competitive antagonists at the 5-HT$_7$ receptor is methiothepin (p$K_b$ = 8.1–8.5) > mesulergine (p$K_b$ = 6.7–8.0) > ritanserin (p$K_b$ = 6.4–7.4) > clozapine (p$K_b$ = 6.7–7.6) > spiperone (p$K_b$ = 7.0–7.2).

In this study, the rank order potency of the tested agonists for the inhibition of the sAHP amplitude in CA3 hippocampal pyramidal cells was 5-CT > 5-HT$_3$ > 5-MeOT. Agonists known to have affinity at other 5-HT receptors is negligible. Therefore, 5-CT is currently the most selective agonist available for the characterization of the 5-HT$_7$ receptor and was the agonist used in the experiments to determine the ability of antagonists to block 5-HT$_7$ receptor-mediated inhibition of the sAHP.

Receptor characterization is dependent on rank order affinities of antagonists and their absolute affinity. Initially,
spiperone (10 μM) was used as an antagonist to block the 5-HT1A-mediated hyperpolarization. However, in the presence of spiperone, 5-HT did not reduce the sAHP amplitude. Spiperone has affinity for both the 5-HT1A as well as the 5-HT7 receptor [reviewed in Eglen et al. (1997) and Terrón (1998)]. When the selective 5-HT1A receptor antagonist WAY-100635 was used at a concentration that was 100 to 1000 times its reported Ki to block 5-HT1A receptor activation instead of spiperone, the inhibition of the sAHP by 5-HT was apparent.

Ritanserin and mesulergine acted as competitive antagonists to the inhibition of the sAHP by 5-CT with apparent pA2.
values of 6.8 and 7.9, respectively. An apparent affinity value could not be obtained for methiothepin, because it was a noncompetitive antagonist. In previous studies using biosays to measure 5-HT\textsubscript{7} receptor-mediated actions, methiothepin has been reported to be an insurmountable antagonist (McLean and Coupar, 1996; Terron, 1997; Terron and Falcon-Neri, 1999). The selective 5-HT\textsubscript{7} receptor antagonist SB-269770 was the most effective antagonist with a pA\textsubscript{2} value of 8.8. The rank order potency, i.e., SB-269770 > mesulergine > ritanserin, and the absolute affinity values we obtained for the antagonists effective at blocking the 5-CT inhibition of the sAHP amplitude agree well with previous reports for these antagonists acting at the 5-HT\textsubscript{7} receptor (Thomas et al., 1998) [reviewed in Eglen et al. (1997), Terron (1998), and Vanhoenacker et al. (2000)].

One surprising result was the small effect of clozapine as an agonist or antagonist. At a concentration of 10 \mu M it was ineffective at producing any significant block of the 5-CT-mediated inhibition of the sAHP. Although many studies report that clozapine is an effective competitive antagonist (Leung et al., 1996; Hirst et al., 1997; Kitazawa et al., 1998), other studies have found that clozapine is relatively inactive at 5-HT\textsubscript{7} receptors either as an antagonist, i.e., with micro-molar affinity (De Vries et al., 1997; Villalon et al., 1997), or as a partial agonist (Terron, 1996; Villalon et al., 1997).

Very little is known regarding the biological significance of 5-HT\textsubscript{7} receptor activation in the central nervous system. An increasing body of evidence is becoming available to support the theory that the 5-HT\textsubscript{7} receptor plays a role in psychiatric disease states. The regional distribution of the 5-HT\textsubscript{7} receptor includes limbic areas, and the binding profile includes high affinity to antidepressants, antipsychotics, and hallucinogens [reviewed in Eglen et al. (1997), Terron (1998), and Vanhoenacker et al. (2000)]. Regulation of the 5-HT\textsubscript{7} receptor-efector pathway may underly the clinical efficacy of certain classes of antidepressant drugs and/or the etiology of neuropsychiatric disease states such as depression. Chronic treatment with the serotonin selective reuptake inhibitor fluoxetine decreases the 5-HT\textsubscript{7} receptor number in hypothalamus (Sleight et al., 1995; Mullins et al., 1999) and enhances 5-HT\textsubscript{7} receptor-mediated stimulation of adenyl cyclase activity in frontal cortical astrocytes (Shimizu et al., 1996). Removal of the stress hormone corticosterone by adrenalectomy or treatment with the synthetic stress hormone dexamethasone increases and decreases the 5-HT\textsubscript{7} receptor number, respectively (Le Corre et al., 1997; Shimizu et al., 1997).

A high density of the 5-HT\textsubscript{7} receptor within the CA3 subfield of the hippocampus positions this receptor to be of primary importance in the regulation of the neural circuitry underlying brain wave activity. The 5-HT-hippocampal-CA3 circuit is a primary regulator of electroencephalogram activity and sleep patterns (Vertes et al., 1994; Kinney et al., 1995; Maru et al., 1979; Varga et al., 1998), and disrupted circadian rhythms and/or sleep patterns are often seen in individuals with psychiatric disorders (reviewed in Thase, 1998). One of the primary factors that regulates the frequency of theta brain wave activity that originates in the CA3 subfield of the hippocampus is the sAHP. Increasing the amplitude of the sAHP decreases theta frequency, whereas decreasing the amplitude of the sAHP increases theta frequency (Traub et al., 1992). Our findings that 5-HT\textsubscript{7} receptor activation decreases the sAHP amplitude provides an important link in understanding how the 5-HT-hippocampal-CA3 circuit regulates theta activity.

Our results provide important new information on the physiological function of the 5-HT\textsubscript{7} receptor. Based on the agreement of the rank order potencies of agonists and antagonists, and the pA\textsubscript{2} values for the antagonists with the known values for the 5-HT\textsubscript{7} receptor in other bioassay systems, the conclusion is that one of the receptors mediating the decrease in sAHP amplitude in CA3 hippocampal pyramidal cells is the 5-HT\textsubscript{7} receptor. The 5-HT\textsubscript{7} receptor-mediated inhibition of the sAHP amplitude in CA3 hippocampal pyramidal cells should be an important bioassay for use in the development of new compounds that are selective for the 5-HT\textsubscript{7} receptor. The identification of the 5-HT\textsubscript{7} receptor-mediated response allows for further investigations into the regulation of this receptor by drugs used in the treatment of psychoactive disease states and by chronic stress and how this receptor may regulate hippocampal function.

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