Inhibition of Perforant Path Input to the CA1 Region by Serotonin and Noradrenaline

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Otmakhova, Nonna A., Jennifer Lewey, Brent Asrican, and John E. Lisman. Inhibition of perforant path input to the CA1 region by serotonin and noradrenaline. J Neurophysiol 94: 1413–1422, 2005. First published May 11, 2005; doi:10.1152/jn.00217.2005. Bath-applied monoamines—dopamine (DA), serotonin (5-HT), and noradrenaline (NE)—strongly suppress the perforant path (PP) input to CA1 hippocampal region with very little effect on the Schaffer collaterals (SC) input. The effect of DA action on PP field excitatory postsynaptic potential (fEPSP) has been characterized in detail, but relatively little is known about the NE and 5-HT effects. Here we show that the maximal inhibition of the PP fEPSP by NE is ~55%, whereas 5-HT inhibition is weaker (~35%). The half-maximal inhibitory concentration of both 5-HT and NE is ~1 μM. Neither NE nor 5-HT affected paired-pulse facilitation, suggesting that the effect is not presynaptic. This is in contrast to DA, which does have a presynaptic effect. The NE effect was blocked by α1 antagonists, whereas the α1 antagonist corynanthine and β-antagonist propranolol were ineffective. The effect of 5-HT was mimicked by the agonist, 5-carboxamidotryptamine maleate (5-CT), and not affected by adrenergic and dopaminergic antagonists. To determine the 5-HT receptors involved, we tested a number of 5-HT antagonists, but none produced a complete suppression of the 5-HT effect. Of these, only the 5-HT7 and 5-HT2 antagonists produced weak but significant inhibition of 5-HT effect. We conclude that NE inhibits the PP fEPSP through postsynaptic action on α2-adrenoceptors and that 5-HT1, 5-HT2, and some other receptor may be involved in 5-HT action in PP.

INTRODUCTION

The CA1 hippocampal region is strongly controlled by monoamine neuromodulators, serotonin (5-HT), noradrenaline (NE), and dopamine (DA) (reviewed in Otmakhova and Lisman 1996). DA axons arrive from all three major midbrain sources (Gasbarri et al. 1989, 1991, 1996); NE axons come from the locus coeruleus (Lopes da Silva et al. 1990; Swanson et al. 1987); and 5-HT axons come from the dorsal raphe nuclei, unlike other hippocampal regions that are innervated by the median raphe nucleus (Azmitia and Segal 1978; Barnes and Sharp 1999; Lopes da Silva et al. 1990; Morin and Meyer-Bernstein 1999; Swanson et al. 1987). Monoamines can affect both the excitability of CA1 cells and their synaptic plasticity. For instance, NE, DA, and 5-HT increase the excitability of pyramidal cells through β, D1, and 5-HT4 receptors, all of which are positively coupled to cAMP-dependent mechanisms (Andrade 1998; Madison and Nicoll 1986; Malenka and Nicoll 1986; Pedarzani and Storm 1995). On the other hand, 5-HT2 receptors decrease their excitability (Andrade 1998). Inhibitory interneurons are excited by α-adrenergic (Bergles et al. 1996) and 5-HT3 serotonergic action (McMahon and Kauer 1997; Shen and Andrade 1998). DA and NE may also affect activity-dependent synaptic plasticity, facilitating long-term potentiation (LTP) (Frey et al. 1993; Huang and Kandel 1995; Katsuki et al. 1997; Otmakhova and Lisman 1996; Otmakhova et al. 2000; Swanson-Park et al. 1999; Thomas et al. 1996) and inhibiting depotentiation in the CA3→CA1 synapses (Otmakhova and Lisman 1998). Both effects seem to be mediated by cAMP-dependent mechanisms.

Recent work points to a third type of action of monoamines in CA1, the modulation of synaptic conductances (Otmakhova and Lisman 1999, 2000). Monoamines strongly suppress the baseline synaptic transmission of the perforant path (PP) input that comes directly from cortex. The other major input to CA1 comes from CA3 through the Schaffer collaterals (SC) and is much less strongly affected by all three monoamines. The PP is important for hippocampal function because it is the main source of specific sensory information for the CA1 region (McNaughton et al. 1989; Vinogradova 1984, 2001). A heightened level of the PP activity is required for performance of learned behavior in monkeys (Sybirska et al. 2000). Furthermore, selective inhibition of the PP input to CA1 may interfere with the “comparator function” of CA1 in which novelty is computed based on a comparison of PP and SC inputs. Therefore abnormalities in the monoamine system could potentially underlie the deficits in hippocampal novelty detection that have been implicated in schizophrenia (Gray 1998; Lisman and Otmakhova 2001). We have already described in detail dopaminergic action in the PP (Otmakhova and Lisman 1999). The goal of this paper is to characterize the 5-HT and NE effects. In particular we sought to determine whether the site of action is pre- or postsynaptic and to analyze the receptor subtypes involved.

METHODS

Transverse slices (400 μm thick) from the dorsal hippocampus of 28- to 45-day-old Long-Evans rats were used in this study. Rats were deeply anesthetized by isoflurane inhalation to avoid possible stress or pain during subsequent decapitation. The animal’s brain was immediately removed in cold (0 ± 4°C) artificial cerebrospinal fluid (ACSF), and five to seven slices were prepared from each hemisphere using a Vibratome Series 1000S. Parts of the dentate gyrus and the CA3 field were cut from the slices as shown in Fig. 1A. For recording, slices were placed on a nylon net and superfused (on both sides) with ACSF at a flow rate of 1.5–2.5 ml/min (constant during the experiment). ACSF contained (in mM) 120 NaCl, 26 NaHCO3, 1 NaH2PO4, 2.5 KCl, 2.5 CaCl2, 1.3 MgSO4, and 10 d-glucose and was oxygen-
interneurons (Desmond et al. 1994; Dolleman-Van Der Weel and Witter 1996). Following previous researchers and to simplify the description, we term these inputs the PP input. Data acquisition and initial on-line analysis were done using a PC through a Digidata 1200 interface (Axon Instruments, Foster City, CA) using a custom-made AXOBASIC program. We alternated the stimulation between PP and SC inputs; each input was stimulated every 20 s.

All drugs were purchased at Sigma-RBI (Natick, MA) or Tocris. 5-HT stock solutions in 0.02% ascorbic acid were freshly prepared daily and diluted before application (1,000–10,000 times) in ACSF. Water insoluble drugs were initially dissolved in DMSO and then sonicated in ACSF immediately before each experiment. The final concentration of DMSO during perfusion did not exceed 0.05–0.1%. At this concentration, DMSO does not affect the field excitatory postsynaptic potential (fEPSP) in the CA1 region (Otmakhova and Lisman 1998, 1999; Otmakhova et al. 2000). Previously performed model experiments with methylene blue (50 μM) (Otmakhova and Lisman 1996) showed that the dye reached the recording chamber within 12–15 s after the start of the perfusion and was washed out within 1.5–2.5 min after dye flow was switched off.

For statistical analysis, responses were collected and averaged in 1- and 5-min periods. The amplitudes (mV) of the fEPSP and the fiber volley were measured. Data were normalized relative to baseline. The effects of drugs on the baseline were estimated in each slice relative to baseline and analyzed for the whole experimental series using two-tailed paired t-test for means (Microsoft Excel). Concentration–response curve fitting was done in Microcal ORIGIN. Antagonist potency was estimated by comparing the monoamine effect in the presence or absence of the antagonist on the same slices because within-slice comparison is more sensitive at detecting drug effects than between-slice comparison (Otmakhova and Lisman 1998). The whole 25-min period of application and washout was analyzed in two-factor ANOVA for repeated measurements, followed by posthoc paired t-test for each 5-min interval (Microsoft Excel). Considered factors were drug (presence or absence of the antagonist; df = 1), time (since the start of amine application in 5-min bins, df = 4), and drug × time interaction (df = 4). As a standard requirement, an a priori value of 0.05 was established before all experiments. Figures show means and SE.

RESULTS

In all our experiments, the PP and the SC inputs were measured alternately in the same slice (Fig. 1A). The fEPSP evoked by the PP stimulation appeared as a negative deflection of potential at the PP recording electrode and as a positive deflection at the SC electrode. Conversely, stimulation of the SC caused a negative deflection of potential at the SC recording electrode and a positive deflection at the PP electrode. This indicates that the two electrodes stimulated separate populations of axons that selectively made synaptic connections in the two strata. Further support for the selective stimulation of the PP and SC synapses was the finding that monoamines selectively affected the PP response (Otmakhova and Lisman 2000). Indeed, because the effects on the SC were minor, we will restrict our description to the PP pathway.

NE and 5-HT effects on the PP fEPSP

In this study as before (Otmakhova and Lisman 2000), application of 5-HT (20 μM, n = 13) and NE (20 μM, n = 9; Fig. 1B and C) caused a suppression of the PP fEPSP. The effect was evident in the first 1–2 min of application and reached a maximum by 5 min (Fig. 1C). The reversal of this effect followed a similar time-course (Fig. 1C) and was on
average ~5 min slower than the reversal of the DA effect (Otmakhova and Lisman 1999, 2000). Neither monoamine affected the fiber volley, the indicator of axonal excitability. At 20 μM concentration, the NE-induced suppression was consistently stronger than suppression caused by 5-HT (Fig. 1, B and C). It should be noted that the action of 5-HT was the most variable of the three monoamines.

To investigate the concentration-dependence of the 5-HT and NE effects in PP (Fig. 1, D and E), monoamines were applied to the slice at five different concentrations (0.5, 1, 5, 20, and 100 μM), and the maximal fEPSP amplitude suppression was measured (between 10 and 15 min of application). Monoamine was applied for 15 min with a 30-min period of washout between applications. No more than three applications of different concentrations were used per slice. After averaging (n = 4–9 for each concentration), concentration–response curves were fitted on logarithmic scale (with SE as weights). The 5-HT effect saturated at 35.9 ± 1.3%. Half-maximal inhibition (IC50) occurred at 0.98 ± 0.03 μM. The maximal inhibition for NE was much stronger (~55.5 ± 3.9%; Fig. 1, C and F), but the IC50 was similar to 5-HT (1.2 ± 0.25 μM). Compared with DA-induced inhibition of the PP fEPSP (45 ± 2%) (Otmakhova and Lisman 1999), the maximal effect of NE was the largest, whereas that of 5-HT was the smallest of the three monoamines (Fig. 1G). However, the half-maximal inhibition was achieved by a smaller concentration of NE and 5-HT compared with DA (~3 μM; Fig. 1F).

Monoamines are easily oxidized in solutions; we were concerned that oxidation products might be responsible for the effect in slices. To control for this, 5-HT (10 μM, n = 4) and NE (10 μM, n = 3) were applied in the presence of a very high concentration of antioxidant, ascorbic acid (400 μM). The inhibition of the PP fEPSP observed in these experiments (46.7 ± 2.6 and 58.2 ± 3.4%, respectively) was in the upper range of the distribution of the response in regular ACSF, indicating that the effect is not mediated by breakdown products of 5-HT or NE.

**Site of the action of NE and 5-HT**

The best current input-specific test of presynaptic localization of a substance’s effect is the change in probability of release as monitored by the speed of irreversible NMDA blockade during synaptic stimulation (Hessler et al. 1993). However, this test is flawed if the tested substance can affect the behavior of NMDA channels. This is known to happen for NE (Raman et al. 1996) and 5-HT (Arvanov et al. 1999). A simpler and more widely used test that generally reflects presynaptic action and allows input-specific testing is paired-pulse facilitation (PPF). Under our experimental conditions (1.3 mM Mg2+ in ACSF), this test should be largely NMDA-independent, although other postsynaptic effects cannot be excluded and care should be taken with interpretation of results. We previously concluded on the basis of PPF changes that DA-induced suppression of the PP fEPSP was at least partially presynaptic (Otmakhova and Lisman 1999). Here we tested whether it was the same for 5-HT and NE effects. The PPF experiments were performed in the presence of picrotoxin (50 μM) to avoid the interference of GABA_A inhibition. Stimuli were applied every 30 s in pairs of pulses with an interpulse delay of 50 ms. 5-HT (10 μM) or NE (10 μM) were perfused for 10 min after 15 min of stable baseline to insure the maximal suppression of the PP fEPSP. To control for the possible effect of decreased fEPSP amplitude on the PPF, the power of stimulation was increased to return fEPSP amplitude to the baseline level. Pairs of the increased fEPSP were recorded for an additional 5 min. PPF was calculated during baseline, monoamine application, and increased stimulation using the following formula: PPF = 100% × second/first.

Figure 2 compares the effect of 5-HT and NE of PPF with previously obtained DA data (Otmakhova and Lisman 1999). It shows that the decrease in the PP fEPSP amplitude by 5-HT or NE was not associated with significant changes in PPF (Fig. 2). For 5-HT, PPF was 141.5 ± 5% in control, 151.4 ± 5.1% during application, and 149.7 ± 6.7% during a stronger stimulation period (P > 0.15, n = 7). For NE, it was 140.6 ± 11.2, 136 ± 10.1, and 127.4 ± 5.8%, respectively (P > 0.2, n = 6; Fig. 2). To compare, PPF increase by DA was highly significant: 140.2 ± 2, 170.3 ± 4, and 163.4 ± 6% (P < 0.01, n = 6). Therefore a presynaptic site of action for 5-HT or NE appears unlikely.

**Receptor mechanisms of NE action in PP**

To study the receptors mediating 5-HT and NE action, we used the experimental design shown efficient in the study of DA (Otmakhova and Lisman 1999). NE was always applied at 10 μM. In each slice, we first applied the NE alone (control) for 15 min. After 30–40 min of washout, we applied the antagonist and later NE again in the presence of antagonist. The effects of two applications (Fig. 3) were compared using two-factor ANOVA. This comparison was valid because we ascertained that repeated NE application produced reproducible effects (P > 0.5, n = 4).

NE could in principle act through any of the three basic types of adrenergic receptors, each of which involves different
specific antagonist, yohimbine, largely blocked the NE effect. D: another α2-specific antagonist, efaroxan, completely blocked the NE effect in PP.

Receptors involved in 5-HT action in PP

Our previous experiments (Otmakhova and Lisman 2000) showed that clozapine inhibited the 5-HT effect on the PP fEPSP by 26.2 ± 1.7%. Clozapine is not a selective drug; aside from dopaminergic, adrenergic, and muscarinic receptors (Jackson and Mohell 1996), clozapine has been shown to inhibit multiple 5-HT receptors: 5-HT2, 5-HT3 (Hedlund et al. 1999; Larkman and Kelly 1997; Thomas et al. 1998), 5-HT2A, 5-HT2C, 5-HT1A, and 5-HT1D (Jackson and Mohell 1996; Richelson and Souder 2000). Therefore we had to test multiple 5-HT receptors one by one. In all these experiments, 5-HT was applied at saturating 10 μM concentration. Because there were too many possibilities to check, in most cases we used only one concentration of antagonist. This was chosen in the upper range of doses shown to be effective in published experiments. We used this strategy because it worked well in DA and NE experiments before; however, its value may be decreased if some of the antagonists are not very specific or are partial agonists on same or other receptors. As with DA and NE, 5-HT was initially applied alone, and then after 30–40 min of washout, 5-HT was applied to the same slice in the presence of the antagonist. As with other monoamines, there was no change in the 5-HT effect with repeated applications 30–40 min apart (P > 0.55, n = 8).

Because the most abundant 5-HT receptor in CA1 region is G1/G2-coupled 5-HT1A receptor (Kia et al. 1996; Swanson et al. 1987; Wright et al. 1995), we started by examining the effect of the selective 5-HT1A-receptor antagonist, WAY 100635. The reported effective doses of this drug were unusually low (nM) but we tested it at several concentrations (10 nM, 100 nM, 500 nM, and 1 μM). It was ineffective at all concentrations (F = 2.5, P > 0.2, n = 4). We also checked whether the selective antagonist of the 5-HT1B receptor GR 56662 (5 μM) inhibited the action of 5-HT. The result was also negative (F = 3.2, P > 0.09, n = 4; Table 1).

As the initial approach to the analysis of 5-HT2 receptors, we tested the effect of the 5-HT2 subtype-selective antagonist ketanserin (5 μM), which has relatively high affinity to the 5-HT2A and 5-HT2C receptors (Barnes and Sharp 1999). Ketanserin inhibited the effect of 5-HT weakly (17 ± 4.3%) but significantly (F = 6.9, P < 0.02, n = 4; Table 1). These results suggest that some subtype of 5-HT2 receptors might participate in the 5-HT effect, but in a minor way.

The 5-HT3 antagonist tropisetron (30 μM) unexpectedly had an effect on its own: it significantly increased the baseline synaptic response (by 18.4 ± 2.3%, P < 0.01). The baseline usually stabilized in first 10 min of antagonist application and did not change after that. Although tropisetron may also work on 5-HT4 receptors, this effect is probably not attributable to 5-HT4 antagonistic action because a more selective 5-HT4 antagonist did not have this effect. However, partial agonistic action on 5-HT4 receptors may be involved in this effect. What
TABLE 1. Action of serotonergic drugs on the PP fEPSP

<table>
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<tr>
<th>Drug</th>
<th>Receptor Action</th>
<th>Serotonin Agonists</th>
<th>Effect on the PP</th>
<th>Significance</th>
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<tbody>
<tr>
<td>Serotonin</td>
<td>5-HT$_{1a}$</td>
<td>Suppression of the PP fEPSP, 35%</td>
<td>$P &lt; 0.001$</td>
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<tr>
<td>5-CT</td>
<td>5-HT$_{1B}$,5-7</td>
<td>Suppression of the PP fEPSP, 40%</td>
<td>$P &lt; 0.001$</td>
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<th>Serotonin Antagonists Against Serotonin</th>
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<tr>
<td>Clozapine*</td>
<td>D1, D2, $\alpha_1$, $\alpha_2$, $\mu$, 5-HT$_{1A,1D,2A,2C,6,7}$</td>
<td>Inhibition of serotonin effect, 26%</td>
</tr>
<tr>
<td>WAY 100,635</td>
<td>5-HT$_{1A}$</td>
<td>No effect</td>
</tr>
<tr>
<td>GR 55662</td>
<td>5-HT$_{1B}$</td>
<td>No effect</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>5-HT$_{2A,2C,1D,1A}$</td>
<td>Inhibition of serotonin effect, 17%</td>
</tr>
<tr>
<td>Tropisetron</td>
<td>5-HT$_{3A}$</td>
<td>Increase of the PP fEPSP, 18%</td>
</tr>
<tr>
<td>SDZ 205-557</td>
<td>5-HT$_{3A}$</td>
<td>No blockade of serotonin effect in the PP</td>
</tr>
<tr>
<td>SB-258585</td>
<td>5-HT$_{3B}$</td>
<td>Inhibition of serotonin effect, 26%</td>
</tr>
<tr>
<td>SB-269970</td>
<td>5-HT$_{3C}$</td>
<td>Facilitation of serotonin effect</td>
</tr>
<tr>
<td>Ketanserin + SB-269970</td>
<td>5-HT$<em>{2} + 5-HT</em>{3}$</td>
<td>Inhibition of serotonin effect, 15%</td>
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<th>Catecholamine Antagonists Against Serotonin</th>
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<tbody>
<tr>
<td>SCH 23390 + Ectolipride</td>
<td>D1 + D2</td>
<td>No effect</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>$\alpha_2$, 5-HT$_{3B}$</td>
<td>Inhibition of serotonin effect, 14%</td>
</tr>
<tr>
<td>Propranolol</td>
<td>$\beta$, 5-HT$_{1A}$</td>
<td>No effect</td>
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*Clozapine data were taken from (Otmakhova and Lisman 2000); the references for the drug action are in RESULTS.

is more important, tropisetron had no effect on 5-HT–induced suppression of the PP fEPSP ($F = 0.07$, $P > 0.7$, $n = 4$; Table 1).

As a test for the role of 5-HT$_4$ receptors, we tried 5-HT$_4$–selective antagonist SDZ 205–557. Paradoxically, SDZ 205–557 (10 $\mu$M) significantly increased the effect of 5-HT (26.2 ± 4%; $F = 11.4$, $P < 0.002$, $n = 4$; Table 1). SDZ 205–557 is also active against 5-HT$_3$ receptor (~30 times lower affinity). However, because we did not observe any effects of more selective 5-HT$_3$ antagonist tropisetron, the SDZ 205–557 effects were probably 5-HT$_4$–dependent. This suggests that normally 5-HT$_4$ receptor inhibits the 5-HT action on the PP.

There were no selective antagonists for 5-HT$_3$ receptors available. Therefore we could not investigate these receptors directly. 5-HT$_6$ receptors could be a functionally attractive target because of their sensitivity to neuroleptics, antidepres- sants, and LSD (Branchek and Blackburn 2000). We have tested a 5-HT$_6$ antagonist, SB-258585 (5 $\mu$M). There were no significant effects of this antagonist on either baseline or 5-HT–induced suppression of PP fEPSP ($F = 3.4$, $P > 0.07$, $n = 6$, Table 1). However, this was not surprising because 5-HT$_6$ receptor immunoreactivity was shown to be concentrated in stratum oriens and s. radiatum of CA1 region, not in s. lacunosum-moleculare (Gerard et al. 1997). To check on 5-HT$_3$ receptor action, we used the 5-HT$_{1C}$–selective antagonist SB-269970 (5 $\mu$M). SB-269970 significantly inhibited the 5-HT–induced suppression of PP fEPSP ($F = 4.78$, $P < 0.04$, $n = 5$; Table 1). The average effect of SB-269970 was quite strong (~37% inhibition); however, there was a large dispersion of data between slices, ranging from 100% to 5% inhibition of 5-HT action. These results suggest that 5-HT$_3$ receptor participates in 5-HT action, but that some additional receptor is also involved.

Because only two antagonists (5-HT$_2$ and 5-HT$_7$) showed significant inhibition of serotonin effect in PP, we decided to check whether their combined effect would be more substantial. Before the second 5-HT application, we applied the combination of ketanserin and SB-269970 (5 $\mu$M each). The effect of combined antagonist on the second 5-HT application was significant ($F = 17.1$, $P < 0.001$, $n = 6$) but not larger than the effects of each antagonist alone: the 5-HT effect in PP was inhibited by only 15% (Table 1).

There remained the possibility of nonspecific action of 5-HT on DA and NE receptors. DA strongly inhibits the PP input acting through D1 and D2 type of receptors (Otmaikova and Lisman 1999) and, as established above, NE inhibits the PP through the $\alpha_2$ type of receptors. If 5-HT inhibits the PP by acting on DA or NE receptors then the antagonists of D1, D2, or $\alpha_2$ type of receptors should block its action. We performed experiments to check these possibilities. Applying 5-HT in the presence of a mixture of D1 antagonist SCH 23390 (5 $\mu$M) and D2 antagonist ectolipride (5 $\mu$M) did not inhibit the 5-HT effect ($F = 3.37$, $P > 0.08$, $n = 4$; Table 1). Therefore 5-HT evidently does not act through DA receptors.

To check whether 5-HT might act through NE receptors we applied the $\alpha_2$ antagonist, yohimbine (5 $\mu$M). However, yohimbine only weakly inhibited the serotonin action (14.4 ± 4.8%; $F = 4.77$, $P < 0.04$, $n = 4$; Table 1). This result suggested that 5-HT could not act primarily through $\alpha_2$-adrenoceptors, because NE effect was almost completely blocked by yohimbine (Fig. 3C). The small effect we see need not necessarily be due to $\alpha_2$-adrenoceptors because yohimbine is also known to block some 5-HT$_2$ receptors (Marcoli et al. 1997; Sanden et al. 2000). We also studied whether $\beta$-adrenergic receptors might participate in 5-HT action, using the $\beta$-adrenergic antagonist, propranolol (1 $\mu$M). This drug did not affect the NE action, but we considered a test prudent because propranolol is also known to antagonize 5-HT$_1$ type of receptors. Propranolol did not affect the 5-HT–induced suppression of the PP synaptic input ($F = 3.2$, $P < 0.09$, $n = 5$). Therefore the 5-HT effect on the PP was not mediated by the nonspecific action on $\beta$-adrenergic receptors. This experiment also provides an additional confirmation that 5-HT in PP did not act through 5-HT$_{1C}$–dependent mechanism (Table 1).

As a final test of the specificity of 5-HT action in PP, we investigated whether it might be mimicked by 5-HT agonist, 5-CT. 5-CT is an effective agonist for 5-HT$_{1A,1D,2A,2C,6,7}$; 5-HT$_6$ and 5-HT$_7$ receptors (Alexander and Peters 1999;
Hoyer et al. 1994; Kebabian and Neumeyer 1994). To each slice (n = 6), we first applied 5-HT (10 μM) and then 5-CT (0.3 μM) for 15 min each with 40-min washout between applications. The results were compared in two-factor ANOVA. We found that 5-CT strongly inhibited the PP fEPSP (P < 0.001) to slightly higher degree than 5-HT, although this tendency did not reach significance (P < 0.09, 2-tailed). The washout of 5-CT was slower than 5-HT (Fig. 4). These results together with previous data indicate that 5-HT action was indeed 5-HT receptor-specific.

We have to conclude that 5-HT–induced suppression of the PP fEPSP is 5-HT receptor-specific, although full receptor mechanism of this action may be still unclear. A relatively strong effect of 5-HT7 antagonist (SB-269970) and weak but significant effect of 5-HT2 antagonists (ketanserin, yohimbine) suggests participation of these two receptor types. In support of this conclusion, 5-HT2 and 5-HT7 receptors are activated by 5-CT and inhibited by clozapine (Table 1). However, it remains possible that additional receptors might be involved for which antagonists are not yet available.

**DISCUSSION**

The PP input provides direct cortical input to CA1. Because this input influences the cell firing and has been implicated in synaptic plasticity and consolidation of memory (Colbert and Levy 1993; Dvorak-Carbone and Schuman 1999; Remondes and Schuman 2002, 2004), it is important to understand the mechanisms that modulate this input. Previous work has shown that the PP fEPSPs are strongly reduced by the three monoamines, DA, NA, and 5-HT (Fig. 1), whereas the SC inputs are nearly unaffected (Otmakhova and Lisman 2000). It has been unclear whether these monoamine effects have similar sites of action. Interestingly, the results revealed a profound difference from previously obtained DA data. DA appeared to act at least partially through inhibition of presynaptic release of glutamate as was indicated by a significant increase in PPF (Otmakhova and Lisman 1999). In contrast, neither NE nor 5-HT affected PPF (Fig. 2), suggesting a postsynaptic site of action.

We have also characterized the receptors involved in serotonergic and adrenergic action, complementing the previous work on dopamine (Otmakhova and Lisman 1999). We have found that NE suppresses the PP fEPSP through α2-specific action. The effect was almost completely blocked by the α2-antagonists, but was not affected by either α1 or β antagonists (Fig. 3). The concentration of adrenergic fibers (Oleskevich et al. 1989) and α adrenoceptors (Swanson et al. 1987) in CA1 s. lacunosum-moleculare has long been recognized. More recent publications show that it is the α5 type (and α2C subtype) of α adrenoceptors that is localized to this region both in rodent (Dossin et al. 2000; Holmberg et al. 2003) and in human brain (Gonzalez et al. 1994; Pazos et al. 1985). Therefore our conclusion is consistent with histological data on α2 receptor localization.

Our extensive efforts to characterize the receptors mediating 5-HT action strongly restricted the range of possibilities but still did not yield a complete picture. We were never able to completely inhibit the effect of 5-HT, the strongest inhibition being ~37%. The partially effective antagonists implicate 5-HT7 and 5-HT2 receptors (Table 1). Consistent with this, histological data suggest that 5-HT5 (Swanson et al. 1987) and 5-HT2 receptors (Neumaier et al. 2001) might be selectively enriched in s. lacunosum-moleculare compared with other CA1 layers, which would explain our physiological effects. However, the lack of complete blockade of 5-HT effect by 5-HT7 and 5-HT2 antagonists and even by clozapine (the antagonist for multiple 5-HT receptors) suggests a more complicated picture of 5-HT targets in the PP.

One possibility is that the 5-HT effect depends mostly on the receptors known to be present in the hippocampus but for which we do not yet have effective antagonists (like 5-HT1F or 5-HT2). The immunostaining for 5-HT5B receptors appears relatively high in s. lacunosum-moleculare (Oliver et al. 2000). These receptors are still pharmacologically inaccessible since no selective ligands are commercially available. A new group of trace amine receptors (TAR) with a high homology to serotonin receptors has been recently described. TAR may also be activated by 5-HT, DA, and NE and, when expressed, couple to Gq protein and cAMP synthesis (Berry 2004; Borowsky et al. 2001; Bunzow et al. 2001). The details of TAR localization are not yet available, but the trace amines (agonists of TAR) are present in the hippocampus (Berry 2004).

It might also be that we observed some form of cooperation between two or more serotonin receptor subtypes, where each subtype inhibition alone does not have substantial effect, and two or more receptors should be inhibited simultaneously. A clustering (heterodimerization) of different subtypes of G protein–coupled receptors was recently described (Franco et al. 2000; George et al. 2000; Hebert and Bouvier 1998; Rocheville et al. 2000a,b) but the full functional significance of such
dimerization is not yet known. It might be that the striking similarity of the distribution of 5-HT\textsubscript{7} and 5-HT\textsubscript{5b} protein in the hippocampus and relatively high level of both in the s. lacunosum-moleculare (Brownfield et al. 1998) indicates a possible interaction of these two receptors in the control over PP synaptic transmission. This might be a cause for variability of the effect of 5-HT\textsubscript{3a} antagonist, SB-269970 (and clozapine) more reasonable because 5-CT (and, of cause, 5-HT) activates both 5-HT\textsubscript{1a} and 5-HT\textsubscript{3a} receptors while SB-269970 (and clozapine) do not inhibit the 5-HT\textsubscript{3a} type (Table 1). Another possibility is the interaction between 5-HT\textsubscript{7} and 5-HT\textsubscript{1a} receptors as described for ventral pallidum (Bengtson et al. 2004). In this particular case, 5-HT effect was mimicked by 5-HT\textsubscript{1a}/5-HT\textsubscript{7} agonists and was not blocked by either 5-HT\textsubscript{7} or 5-HT\textsubscript{1a} antagonist alone. This last work is of particular interest, because in this case, 5-HT acted through hyperpolarization-activated nonspecific cationic channels (Ih channels) through cAMP-dependent mechanisms (Bengtson et al. 2004). We have recently shown a strong Ih-dependence of the PP EPSP (Otmakhova and Lisman 2004) according to which increase in the resting Ih current might cause the decrease in EPSP size. It will therefore be of interest to determine whether 5-HT (and other monoamines) suppression of PP EPSP may be mediated by Ih current. Studies to understand the mechanisms underlying monoamine modulation are now needed, and we have begun such efforts in our laboratory.

**Functional significance of monoaminergic control of the PP**

NE and 5-HT participate in control of mood and mood disorders—aggression (Chiavegatto et al. 2001; Gurvits et al. 2000; Kavoussi et al. 1997; Maj et al. 2000; Oquendo and Mann 2000), depression, stress, and anxiety (Brody et al. 1999; Grath et al. 1999; Lopez et al. 1999; Mayberg et al. 2000; Svensson 2000). NE, especially its α\textsubscript{2} receptors are important in the maintenance of selective attention and the decrease of distractibility (Coulle 1994). Hippocampal NE and 5-HT turnover is increased by stress in mice (Belzung et al. 2001), consistent with a role in such behaviors. However, the main focus of research on hippocampal NE has been on its release during novelty signal and its effect on hippocampal excitability (Kichigina et al. 1997).

NE and 5-HT also play some role in schizophrenia psychopathogenesis (Syvalath 1994; Carlsson 1995; Elkashef et al. 1995; Oades et al. 1996; Frederick and Meador-Woodruff 1999). The increase in DA and NE levels by introduction of amphetamines could induce psychosis (Curran and Travill 1997; Leruelle and Abi-Dargham 1999; Yui et al. 1997). Amphetamine psychosis recurrence correlates with blood levels of NE and metabolites (Yui et al. 1997). The NE agonist ephedrine can produce hallucinations (Prokop 1968). NE contribution to the causes of psychosis is suggested by the therapeutic action of neuroleptic drugs having a potent α-adrenergic binding abilities correlating with their clinical efficacy (Cohen et al. 1988). Similarly to NE and DA, substantial changes in serotonin function are associated with dramatic distortions in cognitive processes, including hallucinations. Specific examples include schizophrenia (Iqbal and van Praag 1995; Kapur and Remington 1996; Meltzner 1995), Lewy body dementia (Perry et al., delusional state of depression (Benedetti et al. 1999), indolamine hallucinations (Aghajanian and Marek 1999; Egan et al. 1998; Iqbal and van Praag 1995; Perry et al. 1990; Schifano et al. 1998).

Several lines of investigations suggest that the hippocampus might be a critical site for hallucinogenesis (Gloor 1997). Hallucinations were observed in cases of hippocampal dysfunction with glioma in the hippocampus (Kan et al. 1989), temporal lobe impairments in Alzheimer’s disease (Lopez et al. 2001), and in temporal lobe epilepsy (Conlon et al. 1990; Ferguson et al. 1969; Hyde and Weinberger 1997; Maier et al. 2000; Sachdev 1998; Stevens and Lonsbury-Martin 1985). The role of the hippocampus in auditory hallucinations has been shown in schizophrenic patients (Silbersweig et al. 1995; Woodruff et al. 1997). Some authors specifically stress the role of the CA1 region in epileptic psychosis (Suckling et al. 2000) noting that degree of psychosis paradoxically correlates with the degree of preservation of CA1; evidently the presence of the dysfunctional CA1 is required for psychosis. The CA1 controls the hippocampal output to the cortex and receives two cortical inputs—the PP and SC inputs. Interestingly, all hallucinogenic mechanisms (NMDA antagonism or increases in monoamine function) would specifically target the same CA1 input, the PP (Otmakhova and Lisman 1999, 2000; Otmakhova et al. 2002). A deeper understanding of receptor and ionic mechanisms controlling the PP may be helpful in the development of effective antipsychotic drugs.

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**PERFORANT PATH INHIBITION BY NORADRENALINE AND SEROTONIN**


