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Short communication

5-HT_{1A} receptor-mediated inhibition of long-term potentiation in rat visual cortex

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Abstract

We investigated the effect of 8-hydroxy-2-(*N*,*N*-dipropylamino)tetralin (8-OH-DPAT), a 5-HT_{1A} receptor agonist, on the induction of long-term potentiation in rat visual cortex slices. Perfusion of 8-OH-DPAT (0.1–10 μM) did not affect layer II/III field potentials evoked by test stimulation of layer IV, but significantly reduced long-term potentiation induced by tetanic stimulation. The inhibitory effect of 8-OH-DPAT was blocked by the 5-HT_1 receptor antagonist, pindolol (10 μM), but not by the 5-$HT_{2,7}$ receptor antagonist, ritanserin (100 μM), nor by the 5-$HT_{3,4}$ receptor antagonist, MDL72222 (100 μM). These results suggest that the rat visual cortex long-term potentiation is inhibited by 5-HT_{1A} receptor stimulation.

Keywords: Long-term potentiation; Visual cortex; 5-HT (5-hydroxytryptamine, serotonin); 5-HT_{1A} receptor; 8-OH-DPAT (8-hydroxy-2-(*N*,*N*-dipropylamino)tetralin); Field potential

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) functions as a neurotransmitter or neuromodulator in the central nervous system. Serotonergic neurons are located mainly in the dorsal and medial raphe nuclei in the mesencephalon, and send neural projections to many brain regions, including the amygdala, hippocampus, septum, basal ganglia and cerebral cortex (Smith and Sweet, 1978). 5-HT contributes to many physiological functions in the central nervous system, including the control of blood pressure, body temperature, sleep, pain, anxiety and depression (Smith and Sweet, 1978; Iversen, 1984; Curzon, 1988). There is also evidence that 5-HT is involved in learning and memory (McEntee and Crook, 1991; Cassel and Jeltsch, 1995). 5-HT receptors are currently classified into seven families, with fourteen subtypes, based on their pharmacological properties and primary amino acid sequence (Humphrey et al., 1993; Hoyer and Martin, 1997). The 5-HT_{1A} receptor is one of the best characterized 5-HT receptor subtypes.

Long-term potentiation of synaptic transmission is a form of activity-dependent synaptic plasticity that may underlie learning and memory (Bliss and Collingrige, 1993). The effect of 5-HT on long-term potentiation has been studied mainly in the hippocampus. 5-HT was first supposed to facilitate hippocampal long-term potentiation (Bliss et al., 1983; Klancnik and Phillips, 1991), but has recently been found to inhibit it (Corradetti et al., 1992; Villani and Johnston, 1993; Staubli and Otaky, 1994; Staubli and Xu, 1995). Furthermore, there is evidence that the 5-HT_{1A} receptor mediates the inhibitory effect of 5-HT on hippocampal long-term potentiation (Sakai and Tanaka, 1993). However, it was unknown whether the 5-HT_{1A} receptor contributes to the modulation of synaptic plasticity in other brain regions. In the present study, therefore, we investigated the role of 5-HT_{1A} receptor in the induction of long-term potentiation in the rat primary visual cortex, a brain region that has been demonstrated to express 5-HT_{1A} receptors (Wright et al., 1995).

2. Materials and methods

Whole brain was quickly isolated from male Wistar rats (3–5 weeks old; Nihon SLC, Shizuoka, Japan) and placed in ice-cold artificial cerebrospinal fluid consisting of (in

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mM): 124 NaCl, 5 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 26 $NaHCO_3$, 10 glucose, bubbled with 95% O_2/5% CO_2. The brain was trimmed to an occipital brain block containing the primary visual cortex, and then cut into slices of 400 μm thickness with a Vibratome (DTK-1500; Dosaka, Kyoto, Japan). The slices were allowed to recover for more than 40 min in an incubation chamber containing artificial cerebrospinal fluid that was oxygenated (95% O_2/5% CO_2) and maintained at 34°C. Each slice was transferred into a submersion chamber (2 ml), where it was continuously perfused with warmed (34°C) and oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid at a rate of 2 ml/min.

A bipolar tungsten electrode was placed on layer IV, and single-pulse test stimulation (0.05 ms duration) was applied at intervals of 30 s. A glass capillary microelectrode filled with 0.9% NaCl (tip resistance: 2–3 MΩ) was placed on layer II/III for extracellular recording, and the evoked potential was recorded as shown in Fig. 1A. The stimulus intensity was adjusted to evoke a synaptic potential of about 50% of the maximum amplitude. To induce long-term potentiation, tetanic stimulation (100 Hz for 1 s, twice at an interval of 30 s) was applied at the same intensity through the same electrode as used for test stimulation. Drugs were delivered by perfusion.

(±)-8-Hydroxy-2-*N*,*N*-dipropylaminotetralin (8-OH-DPAT) and pindolol were purchased from Sigma Chemical (St. Louis, MO, USA). 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX), MDL72222 and ritanserin were purchased from Research Biochemicals (Natick, MA, USA). Other chemicals were purchased from Wako Pure Chemicals Industries (Osaka, Japan).

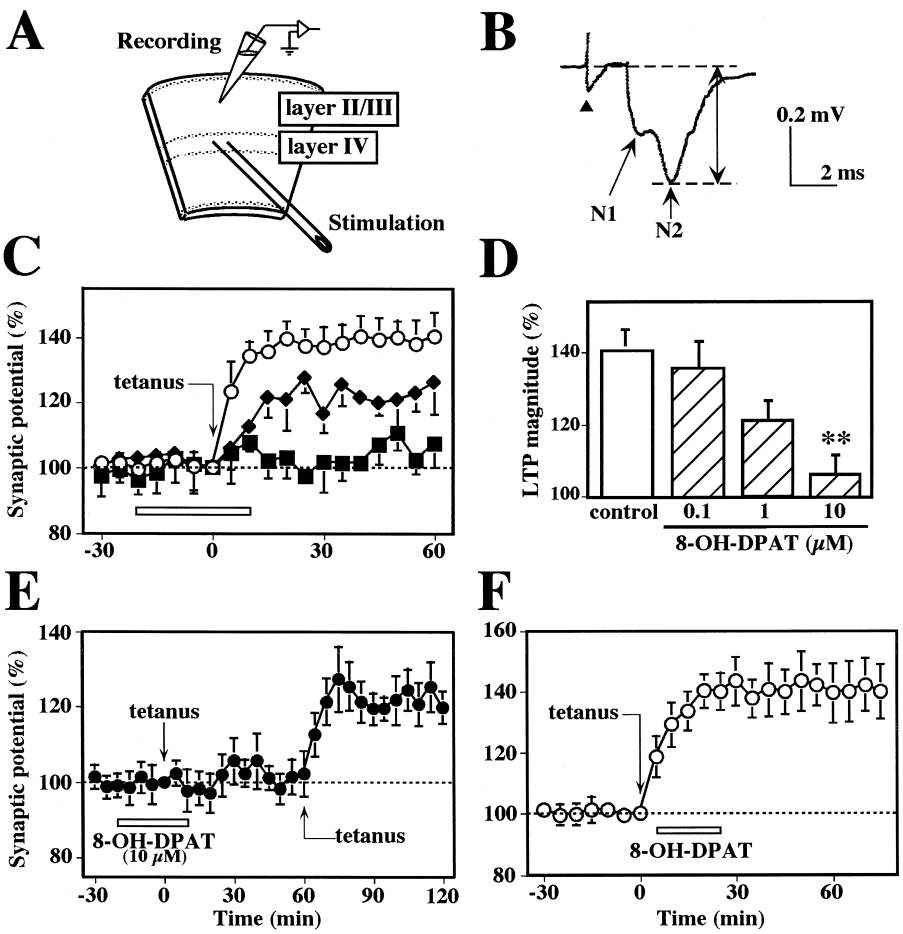

Fig. 1. The effect of 8-OH-DPAT on the induction of long-term potentiation in rat visual cortex slices. (A) Schematic illustration of visual cortex slice showing stimulating and recording sites. (B) Sample record of evoked potentials. Test stimulation was delivered at the time indicated by an arrowhead. The amplitude of the synaptic potential (N2) was defined as the voltage difference between baseline and peak. (C) Time-course of long-term potentiation induced by tetanus in the absence (○) or presence of 8-OH-DPAT (♦, 1 μM; ■, 10 μM). 8-OH-DPAT was applied by perfusion during the time indicated by a horizontal bar. (D) Concentration-dependent effect of 8-OH-DPAT on the magnitude of long-term potentiation. The average of the percentage amplitude of synaptic potentials 30–60 min after tetanus was calculated to compare the magnitude of potentiation in each group. Asterisks indicate a significant difference from the control group (without 8-OH-DPAT): $** P < 0.01$, Tukey's test following analysis of variance. (E) Reversibility of the effect of 8-OH-DPAT. Tetanus was first applied in the presence of 10 μM 8-OH-DPAT, and the same tetanus was applied again after washout of 8-OH-DPAT. (F) No effect of 8-OH-DPAT (10 μM) applied 5–25 min after tetanus. All data are presented as the means ± S.E.M. of five separate observations.

3. Results

As shown in Fig. 1B, layer II/III field potential evoked by test stimulation of layer IV often consisted of two negative-going waves, the fast component (N1) with about 2.0-ms peak latency and the late component (N2) with about 3.5-ms peak latency. When the perfusing medium was changed from normal artificial cerebrospinal fluid to Ca^{2+}-free fluid, N2 disappeared completely and N1 remained unchanged, indicating that N1 and N2 represent non-synaptic and synaptic potentials, respectively. In addition, N2 was completely abolished by the glutamate receptor antagonist, CNQX (10 μM). Since it was difficult to accurately measure the rising slope of N2 due to possible contamination with N1, the amplitude of N2 was employed as a measure of excitatory synaptic potentials.

In control slices, tetanic stimulation (100 Hz for 1 s, twice at an interval of 30 s) induced robust long-term potentiation of synaptic potentials. The 5-HT_{1A} receptor agonist, 8-OH-DPAT (0.1–10 μM), had no effect on the baseline synaptic potential. When tetanic stimulation was applied in the presence of 8-OH-DPAT, the induction of long-term potentiation was significantly inhibited (Fig. 1C). The inhibitory effect of 8-OH-DPAT was concentration-dependent (Fig. 1D). To examine if the inhibitory effect of 8-OH-DPAT on the induction of long-term potentiation was reversible, the second tetanic stimulation was applied after washout of 8-OH-DPAT (Fig. 1E). The first tetanic stimulation in the presence of 10 μM 8-OH-DPAT failed to induce long-term potentiation, but the second tetanic stimulation after washout of 8-OH-DPAT did so. To test if 8-OH-DPAT is required simultaneously with tetanic stimulation to exert the inhibitory effect on long-term potentiation, 8-OH-DPAT was added 5 min after tetanic stimulation (Fig. 1F). Perfusion of 10 μM 8-OH-DPAT after tetanic stimulation did not inhibit the induction of long-term potentiation.

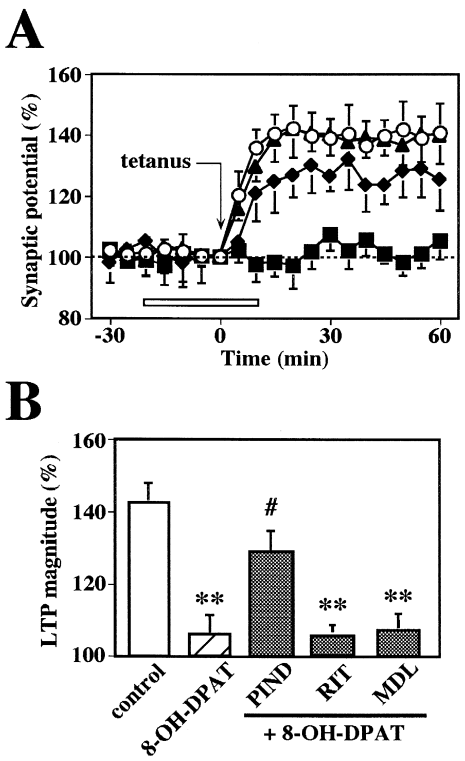

Fig. 2. The effects of 5-HT receptor antagonists on long-term potentiation-inhibiting effect of 8-OH-DPAT. (A) Time-course of long-term potentiation induced by tetanus in the absence (○) or presence of 10 μM 8-OH-DPAT (■), 10 μM pindolol (▲) or 10 μM 8-OH-DPAT + 10 μM pindolol (◆). (B) Comparison of the effects of pindolol (PIND, 10 μM), ritanserin (RIT, 100 μM) and MDL72222 (MDL, 100 μM) on 8-OH-DPAT-induced inhibition of long-term potentiation. All data are the means ± S.E.M. of five separate observations. $**P < 0.01$ vs. control, $\#P < 0.05$ vs. 8-OH-DPAT alone; Tukey's test following analysis of variance.

To confirm that the inhibiting effect of 8-OH-DPAT is 5-HT_{1A} receptor mediated, the effects of several 5-HT receptor antagonists were investigated. The 5-HT_1 receptor antagonist, pindolol (10 μM), when added together with 8-OH-DPAT (10 μM), significantly blocked the inhibitory effect of 8-OH-DPAT (Fig. 2A and B). Pindolol alone did not affect the baseline synaptic potential or the induction of long-term potentiation (Fig. 2A). Unlike pindolol, the 5-$HT_{2,7}$ receptor antagonist, ritanserin (100 M), and the 5-$HT_{3,4}$ receptor antagonist, MDL72222 (100 μM), failed to block the inhibitory effect of 8-OH-DPAT (Fig. 2B).

4. Discussion

We found that the 5-HT_{1A} receptor agonist, 8-OH-DPAT, inhibited the induction of long-term potentiation in the rat visual cortex in vitro. Since long-term potentiation was normally induced after washout of 8-OH-DPAT, the inhibitory effect of 8-OH-DPAT is not caused by irreversible tissue damage. The inhibitory effect of 8-OH-DPAT was blocked by the presence of the 5-HT_1 receptor antagonist, pindolol, supporting the assumption that the effect of 8-OH-DPAT results from 5-HT_{1A} receptor stimulation. Since 8-OH-DPAT has also been reported to work as a 5-HT_7 receptor agonist (Plassat et al., 1993; Boess and Martin, 1994), we checked whether the 5-HT_7 receptor mediates the effect of 8-OH-DPAT. However, the 5-$HT_{2,7}$ receptor antagonist, ritanserin, failed to block the inhibitory effect of 8-OH-DPAT, even at a concentration (100 μM) high enough to block the 5-HT_7 receptor-mediated effect of 8-OH-DPAT (Boess and Martin, 1994). Thus the effect of 8-OH-DPAT observed here is unlikely to result from 5-HT_7 receptor stimulation. We conclude that the induction of visual cortex long-term potentiation is inhibited by 5-HT_{1A} receptor stimulation.

Sakai and Tanaka (1993) have reported that 8-OH-DPAT at concentrations of 100 nM and 1 μM reduced the population spike in slices of rat hippocampal dentate gyrus. However, in our present study, 8-OH-DPAT had no effect

on baseline synaptic potentials even at a higher concentration (10 μM). The impact of 5-HT_{1A} receptor stimulation on normal synaptic transmission may be different between the hippocampus and visual cortex. Furthermore, Sakai and Tanaka (1993) have reported that the induction of long-term potentiation in the dentate gyrus is not affected by 8-OH-DPAT at 1 or 10 nM, but is significantly enhanced by the 5-HT_{1A} receptor antagonist, NAN-190. In our present study, the induction of long-term potentiation in visual cortex slices was not affected by the 5-HT_1 receptor antagonist pindolol alone. Endogenous 5-HT may contribute to long-term potentiation in hippocampal slices, but not under our experimental conditions.

Although cellular mechanisms by which 5-HT_{1A} receptor stimulation leads to the inhibition of visual cortex long-term potentiation are unknown, several possibilities can be argued. It has been reported that 5-HT_{1A} receptor stimulation leads to a decrease in glutamate release in several brain regions (Dijk et al., 1995; Matsuyama et al., 1996; Maura and Raiteri, 1996). However, since 8-OH-DPAT caused no change in baseline synaptic potential, it is unlikely that 5-HT_{1A} receptor stimulation would suppress glutamate release under our experimental conditions. It is also unlikely that 5-HT_{1A} receptor stimulation leads to a change in membrane excitability of postsynaptic cells. Considering that 8-OH-DPAT was effective to inhibit the induction of long-term potentiation only when applied during tetanic stimulation, the activation of 5-HT_{1A} receptors probably modulates activity-dependent events that occur during or immediately after tetanic stimulation. It has been reported that 5-HT_{1A} receptors activate K^+ channels in neurons (Okuhara and Beck, 1994; Ehrengruber et al., 1997). Although it is unknown whether or not 5-HT_{1A} receptors are associated with K^+ channels in our preparations, this mechanism may explain the activity-dependent effect of 8-OH-DPAT. 5-HT_{1A} receptor-mediated activation of K^+ channels would not significantly alter synaptic transmission under resting conditions, but could largely inhibit depolarization of the postsynaptic neuron during tetanic stimulation. Further investigations are underway to elucidate the cellular mechanism.

It is not fully understood what physiological function is associated with visual cortex long-term potentiation. Specific 5-HT_{1A} receptor agonists may be a useful tools for studying its physiological relevance. For example, it would be interesting to investigate the effect of 5-HT_{1A} receptor agonists on visual functions.

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