Enhancement of serotonin₁A receptor function following repeated electroconvulsive shock in young rat hippocampal neurons in vitro

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Abstract

Effects of repeated electroconvulsive shock (ECS) treatment on 5-hydroxytryptamine (5-HT) response were investigated to elucidate the ECS-induced changes, which may be related to antidepressant effects, using electrophysiological methods with hippocampal slices in vitro. ECS was applied to Wistar rats once daily for 14 d from 3 wk of age (ECS group). Control animals did not receive ECS (control group). Twenty-four hours after the final ECS treatment, hippocampal slices were prepared for intracellular recording analysis. Application of 5-HT (0.1–30 µM) caused a dose-dependent hyperpolarization in hippocampal CA1 neurons. 5-HT-induced hyperpolarization in the ECS group was significantly greater than that in the control group. Furthermore, 8-OH-DPAT [8-hydroxy-2-(di-n-propylamino)tetralin], a 5-HT₁A receptor agonist, also induced significantly larger hyperpolarization in the ECS group than in the control group. These results suggest that repeated ECS treatment enhances function of the 5-HT₁A receptor for 5-HT. This supports the hypothesis that enhanced 5-HT₁A receptor function, at least in part, contributes to the effectiveness of ECS treatment for depression directly and/or indirectly.

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Introduction

Serotonin (5-hydroxytryptamine; 5-HT), is believed to play an important role in regulating affective disorders (Murphy, 1990). 5-HT-modulating drugs such as tricyclic antidepressants, which inhibit monoamine re-uptake to synaptic terminals, are effective treatments for depression (Baldessarini, 1989). Recently, selective 5-HT re-uptake inhibitors (SSRI) have been developed and used for treatment of depression (Brely and Moret, 1993; Hyttel, 1994). However, some patients exhibit depression refractory to all known antidepressants. Electroconvulsive shock (ECS) therapy is an effective treatment for such drug-resistant depression (Avery and Winokur, 1977; Strober et al., 1998). ECS treatments have been reported to increase 5-HT₁A receptor binding in the hippocampus and cerebral cortex of rats (Hayakawa et al., 1993; Nowak and Dulinski, 1991). However, the physiological relevance of increasing 5-HT₁A receptor binding remains unclear.

Therefore, we performed electrophysiological studies to determine whether serotoninergic responses increase after repeated treatment with ECS using intracellular recording techniques with hippocampal slices. This experiment was approved by the Ethical committee of Hiroshima University for animal experiments.

Method

Animals

Male Wistar rats aged 3 wk old at the start of ECS treatment were used. All animals were kept in shoebox-type cages in an air-conditioned room (room temperature 23 ± 2 °C, humidity 55 ± 5%, at the Animal Facility in Hiroshima University School of Medicine) until preparation of hippocampal slices. This experiment was approved by the Ethical Committee of Hiroshima University for animal experiments.

ECS treatment

ECS, 100 V at 60 Hz for 1 s, was applied to the animals through ear-clip electrodes once daily for 14 d. Animals in the ECS-untreated (control) group were treated the same
as the ECS group except they did not receive ECS. Ear-clip pressure points were locally anaesthetized using 8\% lidcaine spray. After each ECS treatment, the animals behaved normally within a few minutes. Hippocampal slices for electrophysiological analysis were prepared 24 h after the final ECS treatment.

**Electrophysiological recording**

When preparing hippocampal slices, the weight of animals ranged between 130 and 200 g. After decapitation, the brain was removed and placed in ice-cold artificial cerebrospinal fluid (ACSF). Hippocampal slices (450 \( \mu \)m) were made with a microslicer (DTK-1000, Dosaka EM, Kyoto, Japan). Slices were incubated for 1 h at 34\( ^\circ \)C and then kept at room temperature until use. Single slices were transferred to the recording chamber into which ACSF was perfused continuously at a rate of 2–3 ml/min. Composition of ACSF was as follows (in m\( \text{mol}\)): NaCl, 113; KCl, 3; NaH\( _2\)PO\( _4\), 1; CaCl\( _2\), 2; MgCl\( _2\), 1; NaHCO\( _3\), 25; \( \delta \)-glucose, 11 (bubbled with 95\% O\( _2 \) + 5\% CO\( _2 \)). The microelectrode for intracellular recording of the neurons in the pyramidal cell layer of the CA1 region of the hippocampus was filled with 3 mKCl (electrical resistance, 40–100 M\( \Omega \)). The signal was amplified with a microelectrode amplifier (MEZ-8201, Nihon Kohden, Tokyo, Japan) and then displayed on a memory oscilloscope (VC-11, Nihon Kohden). Data were recorded onto digital audio tapes (DT101, TEAC, Tokyo, Japan) for further analysis.

**Drugs**

5-HT and 8-OH-DPAT [8-hydroxy-2-(di-n-propylamino)-tetralin] were obtained from Research Biochemical International (Natick, MA, USA).

**Statistics**

Drug effects are represented as mean \( \pm \) S.E.M. ANOVA was used for comparison between control and ECS-treated groups.

**Results**

**Effects of 5-HT on CA1 neurons**

The effects of 5-HT and 8-OH-DPAT were examined in 32 and 35 CA1 neurons obtained from 25 and 31 slices in 18 ECS-untreated and 17 ECS-treated animals, respectively. The mean resting membrane potentials of the neurons were \(-61.2 \pm 1.8\) mV \((n = 32)\) and \(-59.4 \pm 1.5\) mV \((n = 35)\) in ECS-untreated and ECS-treated animals, respectively. Application of 5-HT at concentrations of 1–30 \( \mu\)M to the bath caused hyperpolarization and inhibition of spontaneous firing in the CA1 neurons in a concentration-dependent manner (Figure 1). Concentration–response curves are shown in Figure 2A. Hyperpolarization induced by 5-HT was potentiated in CA1 neurons from ECS-treated animal slices. The mean hyperpolarization induced by 30 \( \mu\)M 5-HT was \(7.4 \pm 0.6\) mV in ECS-untreated animals \((n = 4)\), while it was \(10.0 \pm 0.8\) mV in ECS-treated animals \((n = 4)\) (Figure 2A). Although the lower concentration of 5-HT (3 \( \mu\)M) induced only slight hyperpolarization of \(3.3 \pm 0.6\) mV \((n = 4)\) in the control animals, much greater hyperpolarization was obtained in ECS-treated animals \((10.0 \pm 0.8\) mV; \(n = 4)\) (Figure 1). The concentration–response curve of 5-HT-induced hyperpolarization was shifted to the upper and left in ECS-treated animals (Figure 2A). While the EC\(_{50}\) and maximal response was 3.2 \( \mu\)M and 8.2 mV in control, 1.2 \( \mu\)M and 10.6 mV were obtained respectively in ECS-treated animals.

**Effects of 8-OH-DPAT on CA1 neurons**

A 5-HT\(_{1A}\) receptor agonist, 8-OH-DPAT, also induced hyperpolarization at concentrations of 1–30 \( \mu\)M in a
concentration-dependent manner. Hyperpolarization induced by 8-OH-DPAT was also augmented in the CA1 neurons of the ECS-treated animals, compared to the ECS-untreated animals. Hyperpolarization induced by 8-OH-DPAT at 30 µM in the ECS-treated animals was increased to 7.3 ± 1.3 mV (n = 3) from 3.0 ± 0.1 mV (n = 3) in the ECS-untreated animals (Figure 2B). The concentration–response relationship shifted to the upper and left in the ECS-treated animals, compared with the ECS-untreated animals. While the EC₅₀ and maximal response of the 8-OH-DPAT-induced response was 4.0 µM and 3.1 mV in control, 2.4 µM and 8.2 mV were obtained respectively in ECS-treated animals.

Discussion

Bath application of 5-HT induced hyperpolarization of CA1 neurons in a concentration-dependent manner in both ECS-treated and ECS-untreated animals. This 5-HT-induced hyperpolarization was probably caused by the opening of K⁺ channels resulting from activation of 5-HT₁A receptors, shown previously by Andrade and Nicoll (1987), since 8-OH-DPAT, a 5-HT₁A receptor agonist, also induced hyperpolarization of membrane potential in a concentration-dependent manner. Furthermore, the present results show that 5-HT-induced hyperpolarizations of the hippocampal CA1 neurons are augmented in slices prepared from animals subjected to daily ECS treatment for 14 d, compared to ECS-untreated animals. These findings are in agreement with previous in vivo data, reported by De Montigny (1984), that 5-HT-induced inhibition of neuronal activity of the CA1 region through 5-HT₁A receptor activation is elevated in ECS-treated rats. This sensitization of 5-HT₁A receptors by ECS treatment in vivo and in vitro studies may at least partly explain the effectiveness of ECS treatment for depressive diseases, since activation of 5-HT₁A receptors is known to decrease the elevated numbers of 5-HT₂A receptors which are observed in the depressive state (Wieland et al., 1993; Yates et al., 1990); the 5-HT₁A agonist, tandospirone is also effective in treating animal models of depression (Wieland and Lucki, 1990). Augmentation of 5-HT-induced hyperpolarization may be because of an increase in the number of 5-HT₁A receptors. However, repeated ECS treatment-induced increases in 5-HT₁A receptor binding and mRNA have only been observed in the dentate gyrus of the hippocampus, not in the CA1 region (Burnet et al., 1995; Hayakawa et al., 1994). Therefore, augmentation of 5-HT₁A-induced hyperpolarization in CA1 neurons may be due to facilitation of 5-HT₁A receptor–effector coupling related to Gi protein and/or a second messenger system, which causes activation of the K⁺ channels to efflux K⁺. It has been speculated that activation of neurons by ECS induces...
gene expression by quantitatively enhancing effector enzymes involved in the 5-HT$_{1A}$ receptor function. Augmentation effects of 8-OH-DPAT were greater than those of 5-HT by ECS. This difference may have resulted from the combined action (for example, hyperpolarization via 5-HT$_{1A}$ receptors and depolarization via 5-HT$_{2A}$ and/or 5-HT$_{4}$ receptors) of 5-HT via multiple receptor subtypes, especially at higher concentrations.

In conclusion, ECS treatment-induced sensitization of 5-HT$_{1A}$ receptor-mediated responses may directly and/or indirectly contribute, at least in part, to the therapeutic effectiveness of ECS treatments for refractory depression.

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