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ラット扁桃体基底外側部におけるセロトニン基礎遊離量に及ぼすグルココルチコイド受容体拮抗薬 mifepristone の影響

清水典史、原　千高

第一薬科大学　薬物治療学分野、〒815-8511　福岡県福岡市南区玉川町 22-1

Effects of mifepristone, a glucocorticoid receptor antagonist, on basal release of serotonin in the basolateral amygdala in rats

Norifumi SHIMIZU and Chiaki HARA

Department of Advanced pharmacology, College of Pharmacy, Daiichi University, 22-1 Tamagawa-cho, Minami-ku, Fukuoka 815-8511, Japan

Tel: +81-92-541-0161,　E-mail: shimizu@daiichi-cps.ac.jp

Abstract

Based on the “serotonin hypothesis”, the agents which could increase the serotonin transmission in the brain are thought to be effective for the palliation of depression. Present study, we investigated the effect of chronic or acute treatment with mifepristone, a glucocorticoid receptor antagonist, on the basal release of serotonin in the basolateral amygdala (BLA) in rats. The basal release of serotonin in the BLA was significantly increased after chronic treatment with mifepristone for 14 days. On the other hand, the basal release of serotonin in the BLA was not affected by acute treatment with mifepristone. These results suggest that mifepristone may have a therapeutic potential for depression by novel mechanisms.

Keywords: depression, glucocorticoid receptor, mifepristone, serotonin, microdialysis, basolateral amygdala

1. Introduction

There are many studies on the effect of antidepressants in paradigms designed to mimic symptom of human depression (Cryan et al., 2002). Since it is well known that hypothalamic-pituitary-adrenal axis is involved in the pathophysiology of depression in human (Dinan, 1994; Reus and Miner, 1985), chronic treatment with corticosterone, a major glucocorticoid in rodents, has been used as an animal model of depression. For example, chronic corticosterone injections increased the immobility time on the forced swim test (Gregus et al., 2005; Johnson et al., 2006). In addition, chronic treatment with corticosterone has been shown to suppress hippocampal neurogenesis (Mayer et al., 2006), which might contribute to the hippocampal volume reductions observed in depression (Sheline at al., 1996). However, few studies have examined the pharmacological mechanisms by which chronic treatment with corticosterone causes the depressive-like symptoms.

The effects of corticosterone are mediated by two types of intracellular receptor molecules: glucocorticoidreceptors (GRs) and mineralocorticoidreceptors (MRs). Recently, treatment with GR antagonist mifepristone have shown to relieve the symptoms of depression in clinical studies (Belanoff et al., 2002; DeBattista et al., 2006; Flores et al., 2006). In animal models of depression, treatment with mifepristone reverses the increased immobility time in the rat forced swim test in a maternal separation model (Aisa et al., 2007). Moreover, mifepristone has been reported to normalize the reduction in neurogenesis caused by chronic corticosterone injection or chronic stress (Mayer et al., 2006; Oomen et al., 2007). These results suggest that mifepristone may be effective for the remission from depression.

It is well known that the central serotonin systems in the brain play a crucial role in depression. The mechanism of major effective therapeutic agents, such as tricyclic antidepressant (TCA) and selective serotonin reuptake inhibitor (SSRI), involve the enhancement of serotonergic neurotransmission in the brain. However, the relationship between the central serotonin system and GR has remained unclear.

Therefore, we investigated the effect of mifepristone on the basal release of serotonin in the basolateral amygdala (BLA) in rats.

2. Materials and methods

2.1. Animals

Eight-week-old male Wistar rats were used in the present study. Rats were housed

under controlled temperature (24 ± 1°C) and light-dark cycle (on 7:00-19:00). Food and water were available *ad libitum*.

2.2. Chronic mifepristone treatment

Rats were treated with mifepristone, 20 mg/kg, i.p., or saline (control group) twice a day (9:00 and 19:00) for 14 days. On the day of the final treatment, surgery for implantation of guide cannula was performed.

2.3. Acute mifepristone treatment

On the next day of the surgery for implantation of guide cannula, rats were treated with mifepristone, 20 mg/kg, i.p., or saline (control group) at 9:00. Immediately after treatment, basal release of serotonin was measured.

2.4. In vivo microdialysis

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic apparatus. A guide cannula (one site per animal) for microdialysis probe was implanted just above the basolateral nucleus of the amygdala (BLA; AP: -2.56 mm, ML: 5.0 mm, DV: 5.6 mm, from the bregma and skull). The cannula was fixed in place with acrylic resin dental cement.

On the next day of the implantation of a guide cannula, microdialysis probes were inserted through the guide cannula into the BLA under brief anesthesia and perfused with artificial cerebrospinal fluid (CSF) solution at constant flow rate of 1 μl/min (Fig. 1). A stabilization period of 120 min was established before the onset of experiments. The perfused dialysates were collected in the sample pool of an automated sample injector and the serotonin contents in the dialysates were measured every 20 min (20 μl) by HPLC-ECD system connected to the sample injector.

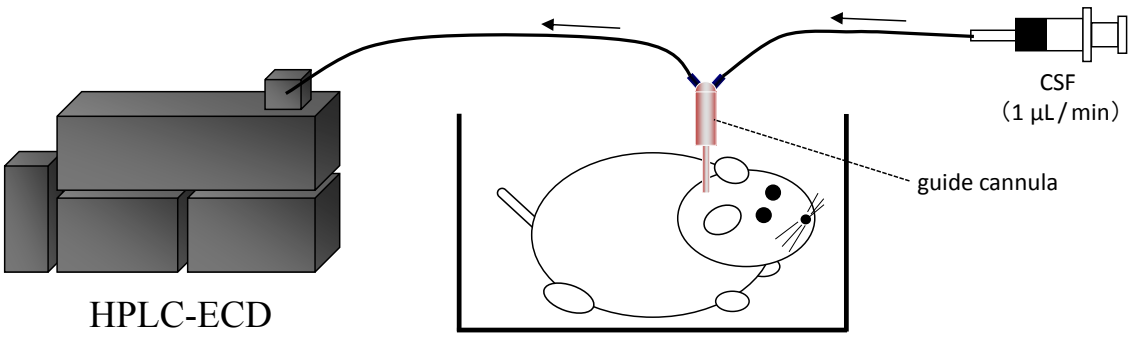

Fig. 1　Schematic illustration of the in vivo microdialysis method.

2.5. Statistical analysis

Data are presented as mean with standard error of the mean (S.E.M.). Two-way ANOVA was used to determine the significance of differences between the mifepristone-treated group and the saline-treated group.

3. Results

3.1. Basal release of serotonin in the BLA in chronically mifepristone-treated rats

Two-way ANOVA analysis showed that the chronic mifepristone treatment caused a slight but significant increase in the basal extracellular level of serotonin in the BLA [$p < 0.05$] (Fig. 2A). The serotonin contents in the dialysates on average for 240 min were 1.34 ± 0.05 pg in the chronically saline-treated group and 2.68 ± 0.22 pg in the chronically mifepristone-treated group, respectively. The total amount of serotonin in the dialysates for 240 min were 16.08 ± 3.30 pg in the chronically saline-treated group and 32.10 ± 8.50 pg in the chronically mifepristone-treated group, respectively (Fig. 2B).

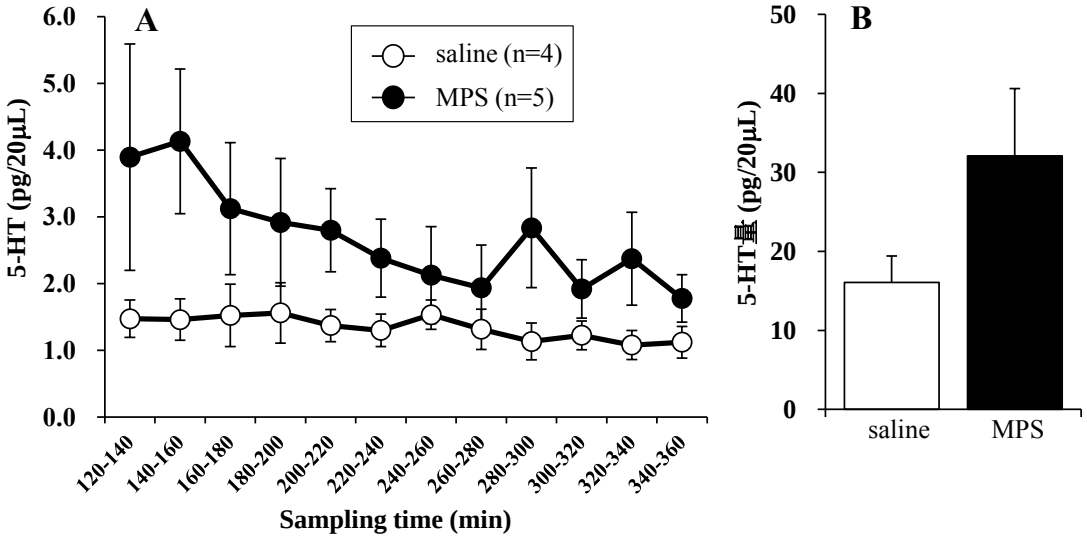

Fig. 2 Effects of chronic treatment with mifepristone (MPS) on basal release of serotonin in the basolateral amygdala. Rats were chronically treated with saline (○) or mifepristone 20mg/kg (●) twice a day for 14 days. A: The serotonin contents in the dialysates every 20 min. B: The total amount of serotonin in the dialysates for 240 min. Data are presented as mean with standard error of the mean (S.E.M.) of 4-5 rats.

3.2. Basal release of serotonin in the BLA in acutely mifepristone-treated rats

The basal extracellular level of serotonin in the BLA was not affected by acute treatment with mifepristone [p = 0.67] (Fig. 3). The serotonin contents in the dialysates on average for 240 min were 1.63 ± 0.10 pg in the chronically saline-treated group and 1.97 ± 0.20 pg in the chronically mifepristone-treated group, respectively. The total amount of serotonin in the dialysates for 240 min were 19.60 ± 6.00 pg in the chronically saline-treated group and 23.59 ± 5.90 pg in the chronically mifepristone-treated group, respectively.

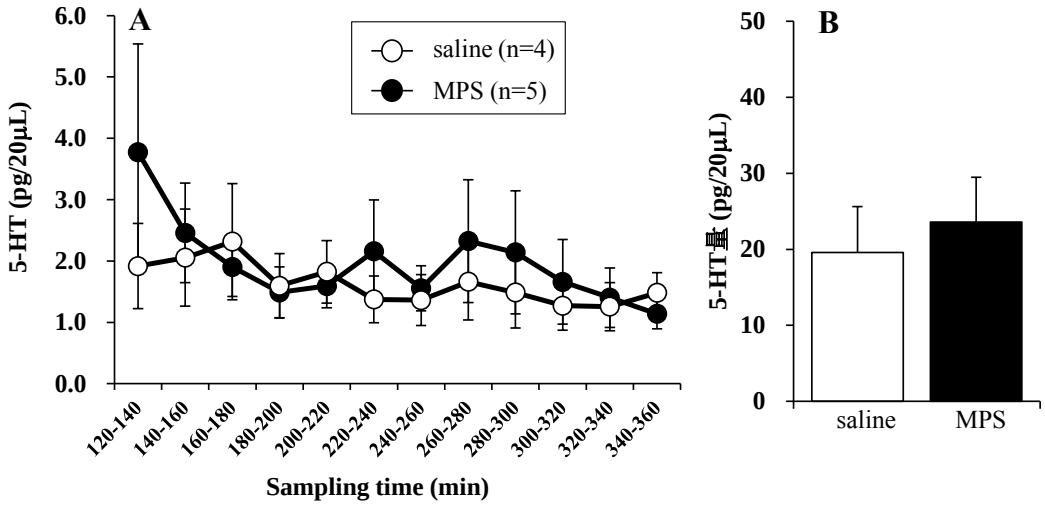

Fig. 3 Effects of acute treatment with mifepristone (MPS) on basal release of serotonin in the basolateral amygdala. Rats were acutely treated with saline (○) or mifepristone20mg/kg (●) just before measurement of serotonin release. A: The serotonin contents in the dialysates every 20 min. B: The total amount of serotonin in the dialysates for 240 min. Data are presented as mean with standard error of the mean (S.E.M.) of 4-5 rats.

4. Discussion

The present study showed that the basal release of serotonin in the basolateral amygdala (BLA) was increased after 14 days treatment with mifepristone in rats. BLA receives the serotonergic projections from dorsal raphe nucleus, a largest serotonergic nucleus in the brain (Ma et al., 1991). Thus, basal release of serotonin in the BLA has been thought to reflect the serotonergic neural activity in the brain.

Clinical treatment with mifepristone has been reported to be effective in antagonizing the depressive- like behavior of psychotic depression (Belanoff et al., 2002; DeBattista et al., 2006; Flores et al., 2006). In animal studies, treatment with mifepristone reverses the increased immobility time in the rat forced swim test in a maternal separation model (Aisa et al., 2007). In addition, mifepristone normalize the chronic stress- and corticosterone-induced reduction of hippocampal neurogenesis (Mayer et al., 2006; Oomen et al., 2007), which may contribute to the hippocampal volume reductions observed in depression (Henn and Vollmayr, 2004; Jacobs et al., 2000). Thus, present result led to the hypothesis that the antagonizing the depressive-like behaviour after mifepristone treatment was mediated, at least in part, by increase in the serotonin release in the brain.

Though the mechanisms by which chronic treatment with mifepristone increases the basal release of serotonin in the BLA are unclear, quantitative alteration in particular functional proteins may be involved. GR is members of the steroid hormone receptor superfamily which recognizes specific DNA elements in the regulatory regions of genes: glucocorticoid response elements (GREs) and, accordingly, acts as transcription factors in the nucleus of the cell to change mRNA and protein synthesis of target genes (Fink, 2007). Thus, chronic GR blockade by mifepristone may causes the alteration in the expression of particular functional proteins that play an important role in the regulation of the basal release of serotonin in the BLA. This hypothesis also explains our result that basal release of serotonin in the BLA was not affected by acute treatment with mifepristone. Namely, acute treatment with mifepristone may not be enough to change the expression of particular functional proteins adequately.

Based on the “serotonin hypothesis”, the agents which could increase the serotonin transmission in the brain, such as tricyclic antidepressants (TCA) and selective serotonin reuptake inhibitors (SSRI), are now commonly used as pharmacotherapies for depression (Schatzberg, 2003). Our data demonstrated that chronic treatment mifepristone also increase the serotonin transmission in the BLA. Moreover, the mechanism by which increases the serotonin transmission may differ from TCA and SSRI. Thus, mifepristone may have a therapeutic potential for depression by a novel mechanisms.

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