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Chronic coadministration of carbamazepine together with imipramine produces antidepressant-like effects in an ACTH-induced animal model of treatment-resistant depression: involvement of 5-HT_{2A} receptors?

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Abstract

The use of carbamazepine has been reported to be an effective treatment for severe depression. We have already shown that the antidepressant-like effects of tricyclic antidepressants in the rat forced swim test (FST) are blocked by chronic treatment with adrenocorticotropic hormone (ACTH). In the present study, we examined the effect of the chronic administration of carbamazepine on the FST and the wet-dog shakes induced by (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5-HT_{2A} receptor agonist, in ACTH-treated rats. Chronic administration of carbamazepine did not affect the duration of immobility in saline-treated and ACTH-treated rats. The reduction of immobility, induced by chronic administration of imipramine, was blocked by treatment with ACTH. When carbamazepine was administered concurrently with imipramine, we observed a significant decrease in immobility in rats treated with ACTH. Chronic ACTH treatment increased the number of the wet-dog shakes induced by DOI. This effect of ACTH was significantly increased by the coadministration of carbamazepine and imipramine. These results suggest that the use of carbamazepine together with tricyclic antidepressants had the effect of reducing immobility time in the FST in a tricyclic antidepressant-treatment-resistant depressive model induced by chronic

ACTH treatment.

Keywords

imipramine, carbamazepine, ACTH, 5-HT_{2A} receptor, forced swim test, wet-dog shakes, treatment-resistant

Introduction

We previously reported that the reduction in the duration of immobility in the forced swim test (FST) caused by the chronic administration of imipramine was inhibited by chronic treatment with adrenocorticotropic hormone (ACTH) (Kitamura et al., 2002a). Furthermore, chronic coadministration of lithium, an agent which potentiates the actions of antidepressants in patients with depression, including those with treatment-resistant depression (de Montigny et el., 1981), significantly decreased the duration of immobility, even when given concurrently with ACTH (Kitamura et al., 2002a). Namely, we reported that ACTH-treated rats served a valuable animal model of tricyclic antidepressant-resistant depressive conditions.

The Serotonin (5-HT) receptor subtypes, particularly the 5-HT_{2A} receptor, have been postulated to play an important role in the pathogenesis of depression (Arango et al., 1990; Arora and Meltzer, 1989; Biegon et al., 1987; Mann et al., 1986; Pandey et al., 2002). However, psychoendocrinological studies have focused on the regulation of the hypothalamic-pituitary-adrenal (HPA) axis in patients with depression (Carroll et al., 1976). Information on the mechanism whereby steroid

hormones regulate 5-HT function will impact on our understanding of hypercortisolism, a condition typical of affective disorders and treatment-resistant depression (Christie et al., 1986). We previously reported chronic administration of corticosterone or ACTH increased the number of wet-dog shakes induced by (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5-HT_{2A} receptor agonist (Takao et al., 1997, Kitamura et al., 2002b; Kawakami et al., 2005). The DOI-induced wet-dog shakes is mediated by 5-HT_{2A} receptor function. This animal model of activation of the HPA axis may shed light on the mechanism governing the 5-HT_{2A} receptor's up-regulation, a condition associated with the pathophysiology of depression. Namely, a model of the hyperfunction of 5-HT_{2A} receptors induced by chronic treatment with ACTH may be useful for examining the mechanism of action of antidepressant drugs for tricyclic antidepressant-treatment-resistant depression.

Carbamazepine is an antiepileptic and mood-stabilizing drug. It has also been shown to be effective in the treatment of several forms of affective disorders, such as treatment-resistant depression (Calabrese and Delucchi, 1989; Kramlinger and Post, 1989). Although previous reports have evaluated the effect of carbamazepine in naive animals, few attempts have been made to examine its effects in a model of abnormal activation of the HPA axis.

The purpose of the present study was to examine the effect of carbamazepine and coadministration of imipramine and carbamazepine on the duration of immobility in the FST and 5-HT_{2A} receptor-mediated behavioral responses induced by DOI in ACTH-treated rats.

Materials and Methods

Animals

Male Wistar rats (Charles River, Japan) weighing 180-230 g, were utilized in this study. The rats were kept under a constant light-dark cycle (lights on, 07.00 - 19.00 h), with access to standard laboratory food and tap water in an air-conditioned room (23 ± 1 °C with approximately 60% humidity). All experiments were conducted according to the guidelines for Animal Experimentation at Okayama University Medical School. Every effort was made to minimize the number of animals used and their suffering.

Drugs

The following drugs were used in the study: carbamazepine (Sigma-Aldrich, St. Louis, MO, USA), imipramine hydrochloride (Wako Pure Chemical.,

Osaka, Japan), DOI ((±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (Sigma-Aldrich, St. Louis, MO, USA) and ACTH-(1-24)-Zinc (Cortrosyn-Z : Daiichi Seiyaku Co., Tokyo, Japan). Carbamazepine was suspended in a 0.5% methylcellulose solution. Imipramine and DOI were dissolved in saline. The drugs were freshly prepared and injected in a volume of 2 ml/kg body weight. Rats were administered drugs once daily (09.00 to 10.00 h). Control rats received an equivalent volume of the 0.5% methylcellulose solution or saline for the same treatment period. Six to 8 animals were used for each experimental group.

Behavioral studies

Measurement of immobility

To measure immobility, rats were individually placed in plastic cylinders (height 37 cm, diameter 15.5 cm) containing 20 cm of water at 25 °C, as described by Porsolt et al. (1978). Two swim sessions were conducted in the initial 13 min pre-test; a 6 min test followed 24 h later. The total period of immobility during the 6 min testing period was recorded using the TARGET series/7M analysis program (Neuroscience Inc., Tokyo, Japan).

Measurement of DOI-induced wet-dog shakes

Rats were placed in individual clear polycarbonate home cages (35×30×17 cm) and treated with DOI (1 mg/kg, s.c.). The DOI-induced wet-dog shakes were counted over the first 30 min commencing immediately following the injection. Wet-dog shakes were identified as a paroxysmal shudder of the head, neck, and trunk. The observers were blinded to the drug administrations.

Experiment 1: Effects of chronic administration of imipramine or carbamazepine, and chronic coadministration of imipramine and carbamazepine on the duration of immobility in saline-treated rats

We chronically administered imipramine (10 mg/kg, i.p.), carbamazepine (3-30 mg/kg, i.p.), or both imipramine (10 mg/kg, i.p.) and carbamazepine (30 mg/kg, i.p.) to normal rats once daily for 15 days. The final treatment with imipramine or carbamazepine on day 15 was given 30 min before the observation.

Experiment 2: Effects of chronic administration of imipramine or carbamazepine, and chronic coadministration of imipramine and carbamazepine on the duration of immobility in ACTH-treated rats

We chronically administered imipramine (10 mg/kg, i.p.), carbamazepine (30 mg/kg, i.p.), or both imipramine (10 mg/kg, i.p.) and carbamazepine (30 mg/kg, i.p.) to ACTH (100 μg/day, s.c.)-treated rats. These treatment combinations were given once daily for 14 days. On the 15th day, doses of imipramine and carbamazepine were given without ACTH. The immobility was observed 30 min after the administration of imipramine and carbamazepine.

Experiment 3: Effects of chronic administration of imipramine or carbamazepine, and chronic coadministration of imipramine and carbamazepine on DOI-induced wet-dog shakes in saline-treated rats

We chronically administered imipramine (10 mg/kg, i.p.), carbamazepine (3-30 mg/kg, i.p.), or both imipramine (10 mg/kg, i.p.) and carbamazepine (30 mg/kg, i.p.) to normal rats once daily for 14 days. The number of DOI (1 mg/kg, s.c.)-induced wet-dog shakes was recorded 1 day after the final

administration of imipramine and carbamazepine.

Experiment 4: Effects of chronic administration of imipramine or carbamazepine, and chronic coadministration of imipramine and carbamazepine on DOI-induced wet-dog shakes in ACTH-treated rats

We chronically administered imipramine (10 mg/kg, i.p.), carbamazepine (30 mg/kg, i.p.), or both imipramine (10 mg/kg, i.p.) and carbamazepine (30 mg/kg, i.p.) to ACTH (100 μg/day, s.c.)-treated rats. These treatment combinations were given once daily for 14 days. The number of DOI (1 mg/kg, s.c.)-induced wet-dog shakes was recorded 1 day after the final administration of ACTH, imipramine, and carbamazepine.

Statistics

Values are expressed as the mean ± S.E.M. Data were analyzed with a one-way analysis of variance (ANOVA); the group means were compared using Dunnett's test or Tukey's test for multiple comparisons. Probability values of less than 0.05 were considered to show a significant difference.

Results

Experiment 1: Effects of chronic administration of imipramine or carbamazepine, and chronic coadministration of imipramine and carbamazepine on the duration of immobility in saline-treated rats

The administration of carbamazepine (3 – 30 mg/kg, i.p.) for a period of 15 days had no effect on the duration of immobility in the FST [$F(3,20)=0.75$, $P=0.53$] (Fig. 1A). The administration of imipramine (10 mg/kg, i.p.) for 15 days significantly decreased the duration of immobility in the FST [$F(2,15)=5.42$, $P<0.05$] (Fig 1B). The effect of imipramine on the duration of immobility did not change with the coadministration of carbamazepine (Fig. 1B).

Experiment 2: Effects of chronic administration of imipramine or carbamazepine, and chronic coadministration of imipramine and carbamazepine on the duration of immobility in ACTH-treated rats

The administration of carbamazepine (30 mg/kg, i.p.) for 15 days had no

effect on the duration of immobility in the FST in ACTH-treated rats [$F(2,15)=1.41$, $P=0.28$] (Fig 2A). The administration of imipramine (10 mg/kg, i.p.) decreased the duration of immobility (Fig. 1B), an effect blocked by 14 days of treatment with ACTH (Fig. 2B). Coadministration of imipramine (10 mg/kg, i.p.) and carbamazepine (30 mg/kg, i.p.) significantly decreased the duration of immobility [$F(3,28)=4.76$, $P<0.05$] (Fig. 2B).

Experiment 3: Effects of chronic administration of imipramine or carbamazepine, and chronic coadministration of imipramine and carbamazepine on DOI-induced wet-dog shakes in saline-treated rats

The administration of carbamazepine (3 – 30 mg/kg, i.p.) for 14 days had no effect on the number of DOI-induced wet-dog shakes [$F(3,20)=2.14$, $P=0.13$] (Fig. 3A). The administration of imipramine (10 mg/kg, i.p.) for 14 days significantly decreased the number of DOI-induced wet-dog shakes [$F(2,15)=4.09$, $P<0.05$] (Fig 3B). The effect of imipramine on the number of DOI-induced wet-dog shakes did not change with the coadministration of carbamazepine (Fig. 3B).

Experiment 4: Effects of chronic administration of imipramine or

carbamazepine, and chronic coadministration of imipramine and carbamazepine on DOI-induced wet-dog shakes in ACTH-treated rats

The number of DOI-induced wet-dog shakes was increased in ACTH-treated rats compared to saline-treated rats [$F(2,15)=7.82$, $P<0.05$] (Fig. 4A). This increase was not affected by the administration of carbamazepine (30 mg/kg, i.p.) for 14 days (Fig. 4A). However, the number of DOI-induced wet-dog shakes was increased by the coadministration of imipramine (10 mg/kg, i.p.) and carbamazepine (30 mg/kg, i.p.) compared to that in ACTH-treated rats [$F(3,28)=27.42$, $P<0.05$] (Fig. 4B).

Discussion

It is the results of the present study suggest that the chronic coadministration of imipramine and carbamazepine and carbamazepine caused a significant decrease in immobility in the FST in rats treated with ACTH for 14 days. We previously reported that chronic treatment with ACTH inhibited the immobility-decreasing effects of imipramine (Kitamura et al., 2002a). The current findings confirmed the results of repeated imipramine administration in ACTH-treated rats. Clinically, the use of

lithium has been shown to be effective in the treatment of several forms of affective disorders, such as treatment-resistant depression. Using lithium together with tricyclic antidepressants may be a promising way to improve the efficacy of the treatment of resistant depression. It was reported that the inhibition of the immobility-decreasing effect of imipramine was reversed by coadministration of lithium in ACTH-treated rats in the FST (Kitamura et al., 2002a). Furthermore, we reported that repeated electroconvulsive stimuli decreased the duration of immobility in the FST in chronic ACTH-treated rats (Li et al., 2006). Electroconvulsive stimuli therapy is considered to be the most effective biological treatment for depression, especially severe intractable depression (Fink et al., 1990). These findings suggest that the chronic coadministration of lithium and imipramine, and repeated electroconvulsive stimuli produce an antidepressant-like effect in an animal model of the abnormal activation of the HPA axis induced by ACTH. The results of the present study suggest that the chronic coadministration of imipramine and carbamazepine caused a significant decrease in immobility in the FST in rats treated with ACTH for 14 days. The combination of carbamazepine and imipramine (a tricyclic antidepressant) may be an effective treatment for tricyclic antidepressant-resistant depression the same as the augmentation strategy for treatment-resistant depression using lithium.

The chronic administration of ACTH, a treatment that up-regulates activation of the HPA axis, increases the density of 5-HT_{2A} receptors in the forebrain neocortex and the number of wet-dog shakes induced by DOI, a 5-HT_{2A} receptor agonist (Kuroda et al., 1992; Kitamura et al., 2002b, 2007). We recognized that the expression levels of 5-HT_{2A} receptor mRNA increase with the chronic administration of ACTH (Kitamura et al., in press). Furthermore, the enhancing effect on DOI-induced wet-dog shakes is blocked by the coadministration of lithium and imipramine, but not by imipramine alone (Kitamura et al., 2002b). Thus, it is proposed that the 5-HT_{2A} receptor's hyperfunction is related to the animal model for tricyclic antidepressant-treatment-resistant depressive conditions induced by chronic ACTH treatment. Furthermore, inhibition of the 5-HT_{2A} receptor's function may contribute to the therapeutic treatment of tricyclic antidepressant-resistant depression. In this study, chronic administration of carbamazepine did not affect the number of DOI-induced wet-dog shakes of naive rats. The increasing effect of ACTH was not influenced by the chronic administration of carbamazepine. The chronic coadministration of imipramine and carbamazepine significantly increased the number of DOI-induced wet-dog shakes in rats treated with ACTH. A previous report suggested that the chronic administration of carbamazepine increased 5-HT_2

receptor-mediated head twitching behavior after an injection of carbidopa followed by 5-hydroxytryptophan. On the other hand, the chronic administration of carbamazepine did not alter the binding of the 5-HT_2 receptor in the rat frontal cortex (Elphick et al., 1990). The effect of carbamazepine on the 5-HT_2 receptor's function is conflicting. Previously, we reported that repeated electroconvulsive stimuli significantly increased the number of DOI-induced wet-dog shakes in both saline and ACTH-treated rats (Li et al., 2006), as in the present study. This result, indicating that the coadministration of imipramine and carbamazepine, and electroconvulsive stimuli action resulted in a disparity between the FST and DOI-induced wet-dog shakes, did not support the hypothesis that the 5-HT_{2A} receptor's hyperfunction is involved in the animal model for tricyclic antidepressant-treatment-resistant depressive conditions induced by chronic ACTH treatment (Kitamura et al., 2002b). Namely, it may mean that inhibition of the 5-HT_{2A} receptor's function is not a common mechanism behind the effect of antidepressants. It is reported that stimulation of the β–adrenergic receptor with isoproterenol increased cyclic adenosine monophosphate levels and this effect was significantly inhibited by carbamazepine. (Montezinho et al., 2006). The density of β–adrenergic receptors decreased after chronic treatment of rats with tricyclic

antidepressant drugs (Kellar, 1987). Therefore, further studies are needed to determine whether or not β–adrenergic receptors are involved in the antidepressant-like action of carbamazepine in ACTH-treated rats.

In summary, chronic administration of carbamazepine did not change the duration of immobility in the FST in saline-treated and ACTH-treated rats. Chronic coadministration of imipramine and carbamazepine decreased the duration of immobility in the FST in ACTH-treated rats. Furthermore, the chronic coadministration of imipramine and carbamazepine did not improve the 5-HT_{2A} receptor's hyperfunction induced by chronic ACTH treatment.

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Legends

Fig. 1. Effects of chronic administration of carbamazepine (A), or both imipramine and carbamazepine (B) on the duration of immobility in the FST in rats.

Imipramine (IMI) and carbamazepine (CBZ) were administered to rats once daily for 15 days. Control (cont) rats were treated with a 0.5% methylcellulose solution (2 ml/kg, i.p.) or saline (2 ml/kg, i.p.) once daily for 15 days. The immobility time was measured the day following the final treatment. The final administrations of imipramine and carbamazepine were given 30 min before the measurement. Values are expressed as the means ± S.E.M. for six animals. Data were analyzed by one-way ANOVA, followed by Dunnett's test (A) and Tukey's test (B). * $P<0.05$, significant difference from the control value.

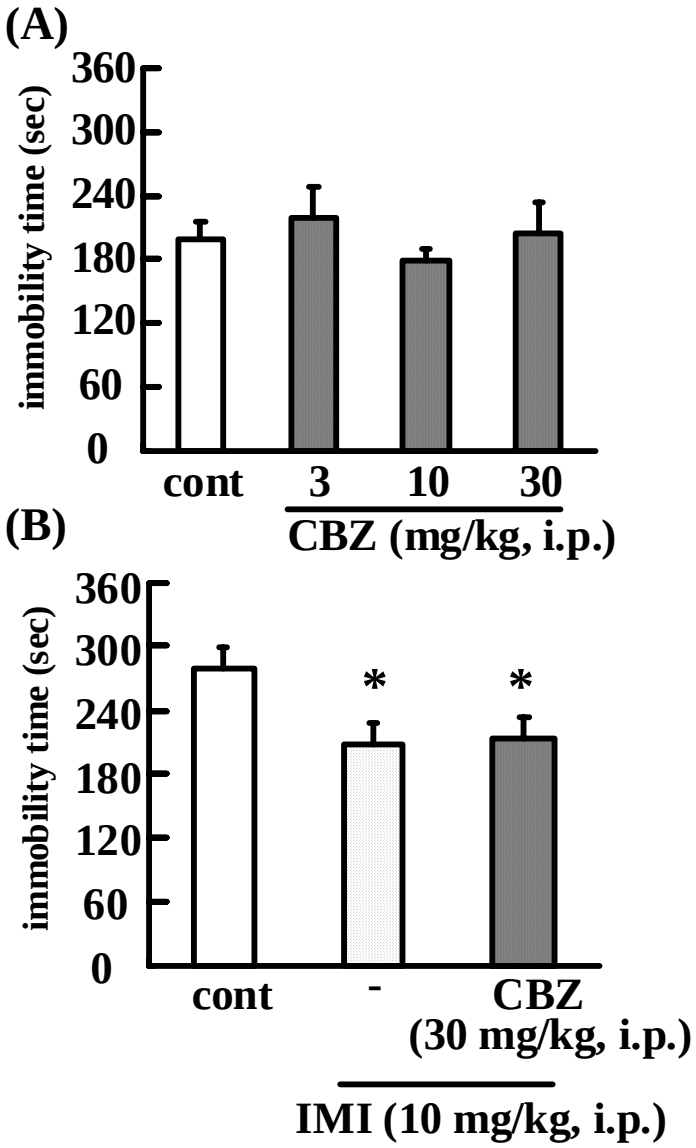
Fig. 2. Effects of chronic administration of carbamazepine (A), or both imipramine and carbamazepine (B) on the duration of immobility in the FST for ACTH-treated rats. ACTH (100 μg/day, s.c.), imipramine (IMI: 10 mg/kg, i.p.) and carbamazepine (CBZ: 30 mg/kg, i.p.) were administered to rats once daily for 14 days. Control (cont) rats were treated with a 0.5%

methylcellulose solution (2 ml/kg, i.p.) or saline (0.2 ml/rat, s.c. and 2 ml/kg, i.p.) once daily for 14 days. The immobility time was measured the day following the final treatment with ACTH. Imipramine and carbamazepine were administered 30 min before the measurement. Values are expressed as the means ± S.E.M. for 6-8 animals. Data were analyzed by one-way ANOVA, followed by Tukey's test. ** $P<0.01$, significant difference from the control value, # $P<0.05$, significant difference from the ACTH treatment.

Fig. 3. Effects of chronic administration of carbamazepine (A), or both imipramine and carbamazepine (B) on DOI-induced wet-dog shakes in rats. Imipramine (IMI) and carbamazepine (CBZ) were administered to rats once daily for 14 days. Control (cont) rats were treated with a 0.5% methylcellulose solution (2 ml/kg, i.p.) or saline (2 ml/kg, i.p.) once daily for 14 days. The number of DOI-induced wet-dog shakes were counted on the day following the final treatment. Values are expressed as the means ± S.E.M. for eight animals. Data were analyzed by one-way ANOVA, followed by Dunnett's test (A) and Tukey's test (B). * $P<0.05$, significant difference from the control value.

Fig. 4. Effects of chronic administration of carbamazepine (A), or both

imipramine and carbamazepine (B) on DOI-induced wet-dog shakes in ACTH-treated rats. ACTH (100 μg/day, s.c.), imipramine (IMI: 10 mg/kg, i.p.) and carbamazepine (CBZ: 30 mg/kg, i.p.) were administered to rats once daily for 14 days. Control (cont) rats were treated with a 0.5% methylcellulose solution (2 ml/kg, i.p.) or saline (0.2 ml/rat, s.c. and 2 ml/kg, i.p.) once daily for 14 days. The number of DOI-induced wet-dog shakes were counted the day following the final treatment. Values are expressed as the means ± S.E.M. for 6-8 animals. Data were analyzed by one-way ANOVA, followed by Tukey's test. * $P<0.05$, ** $P<0.01$, significant difference from the control value, # # $P<0.01$, significant difference from the ACTH treatment.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IMI (10 mg/kg, i.p.)

Fig. 1

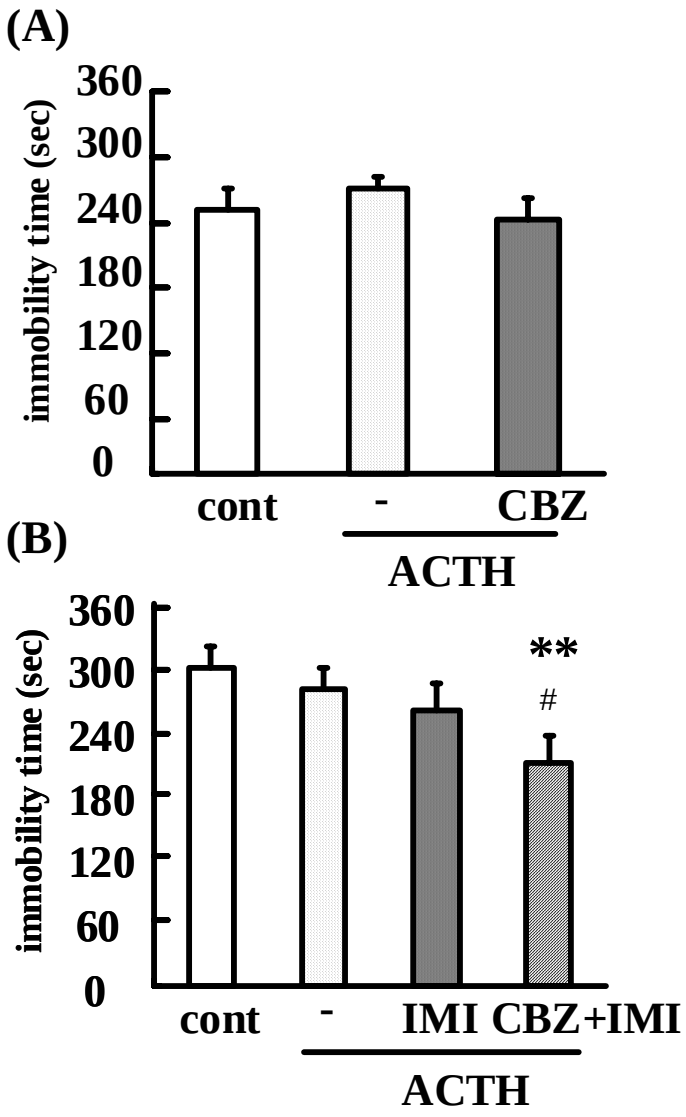
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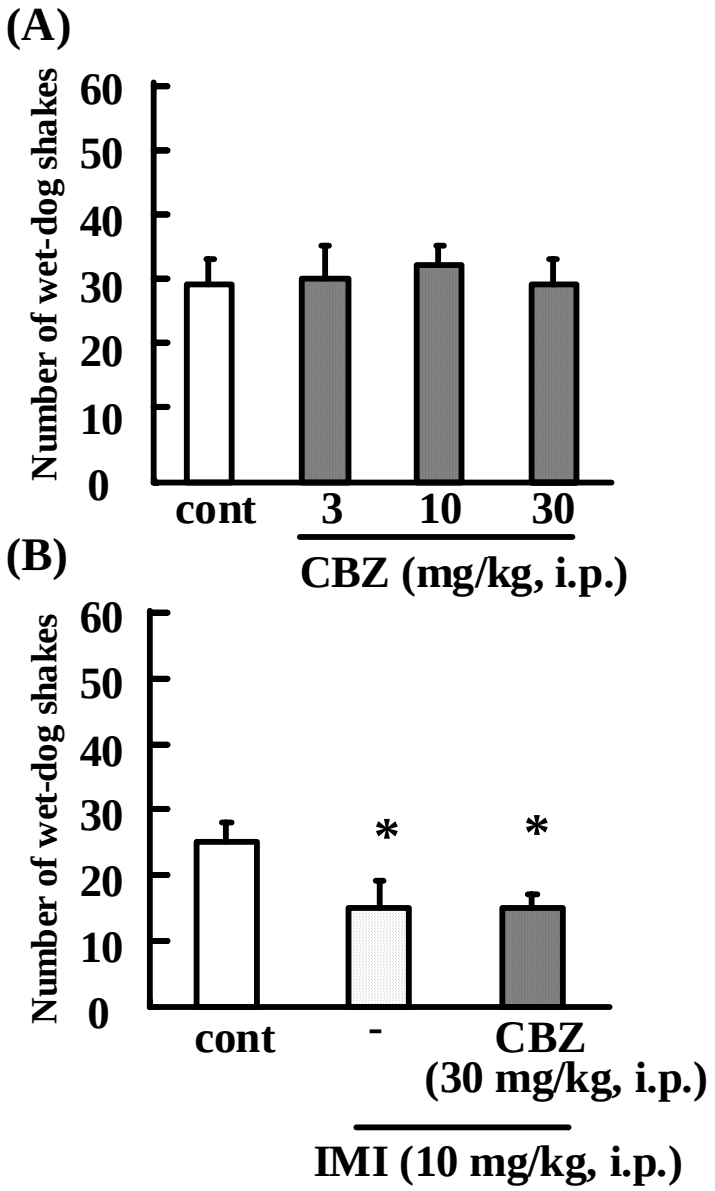
Fig. 2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0
cont
3
10
30
CBZ (mg/kg, i.p.)
(B)
Number of wet-dog shakes
60
50
40
30
20
10
0
*
*
cont
-
CBZ
(30 mg/kg, i.p.)
IMI (10 mg/kg, i.p.)

Fig. 3

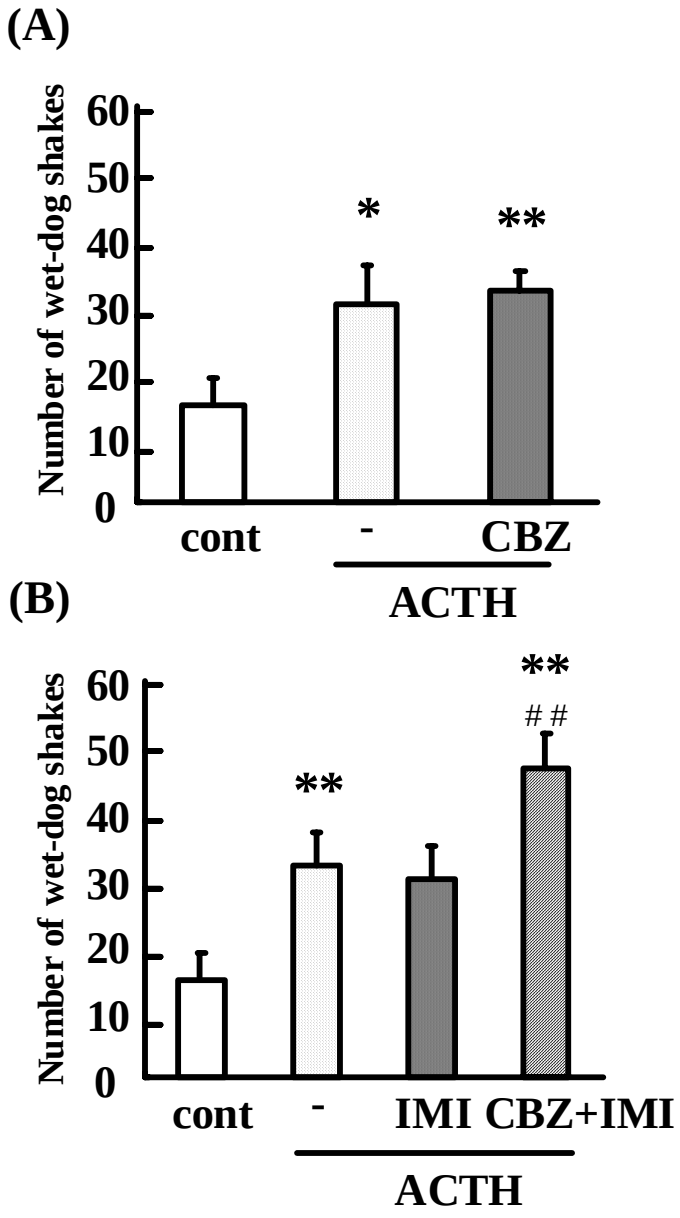
(A)
Number of wet-dog shakes
60
50
40
30
20
10
0
*
**
cont
-
CBZ
ACTH
(B)
Number of wet-dog shakes
60
50
40
30
20
10
0
**
**
# #
cont
-
IMI
CBZ+IMI
ACTH

Fig. 4