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Effects of rolipram, a phosphodiesterase 4 inhibitor, in combination with imipramine on depressive behavior, CRE-binding activity and BDNF level in learned helplessness rats

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Abstract

The brain cAMP regulating system and its downstream elements play a pivotal role in the therapeutic effects of antidepressants. We previously reported the increase in activities of phosphodiesterase 4, a major phosphodiesterase isozyme hydrolyzing cAMP, in the frontal cortex and hippocampus of learned helplessness rats, an animal model for depression. The present study was undertaken to examine the combination of effects of rolipram, a phosphodiesterase 4 inhibitor, with imipramine, a typical tricyclic antidepressant, on depressive behavior in learned helplessness rats. Concurrently, cAMP-response element (CRE)-binding activity and brain-derived neurotrophic factor (BDNF) levels related to the therapeutic effects of antidepressants were determined. Repeated administration of imipramine (1.25–10 mg/kg, i.p.) or rolipram (1.25 mg/kg, i.p.) reduced the number of escape failures in learned helplessness rats. Imipramine could not completely ameliorate the escape behavior to a level similar to that of non-stressed rats even at 10 mg/kg. However, repeated coadministration of rolipram with imipramine (1.25 and 2.5 mg/kg, respectively) almost completely eliminated the escape failures in learned helplessness rats. The reduction of CRE-binding activities and BDNF levels in the frontal cortex or hippocampus in learned helplessness rats were ameliorated by treatment with imipramine or rolipram alone. CRE-binding activities and/or BDNF levels of the frontal cortex and hippocampus were significantly increased by treatment with a combination of rolipram and imipramine compared to those in imipramine-treated rats. These results indicated that coadministration of phosphodiesterase type 4 inhibitors with antidepressants may be more effective for depression therapy and suggest that elevation of the cAMP signal transduction pathway is involved in the antidepressive effects.

Keywords: Phosphodiesterase 4; Rolipram; Antidepressant; Learned helplessness; cAMP response element; Brain-derived neurotrophic factor

1. Introduction

The brain cAMP signal transduction pathway has been shown to be deeply involved in the therapeutic action of antidepressants (D'Sa and Duman, 2002; Manji et al., 2003). Animal studies have demonstrated that chronic antidepressant or electroconvulsive shock treatment potentiates the downstream region of the cAMP system including specific transcription factors, such as the cAMP response element binding protein (CREB), and neurotrophic factors, such as the brain-derived neurotrophic factor (BDNF), which regulate neuroprotection or neuroplasticity (Frechilla et al., 1998; Nibuya et al., 1996). Some unique studies have reported that over-expression of CREB in the hippocampus or infusion of BDNF into the midbrain produces an antidepressant-like effect in animal depressive models (Chen et al., 2001; Siuciak et al., 1997). Although these target therapies might be difficult to apply in actual clinical trials due to their limited treatment capabilities, the findings suggest that the induction of CREB or BDNF induced by activation of the cAMP signaling pathway plays a pivotal role in depression therapy.

Intracellular cAMP is generated by adenylyl cyclase, which is regulated by G-protein coupled membrane neuro-

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transmitter receptors, including subtypes of dopamine, serotonin, and noradrenergic receptors. On the other hand, cAMP is degraded by cyclic nucleotide phosphodiesterase. Phosphodiesterase constitutes a group of at least 11 families of enzymes that hydrolyze cAMP and cGMP (Francis et al., 2001). The cAMP-specific phosphodiesterase, designated phosphodiesterase 4, is the major phosphodiesterase isozyme hydrolyzing cAMP in the mammalian brain and plays a pivotal role in regulating neuronal cAMP level and antidepressant effects (Fujimaki et al., 2000; Houslay, 2001; Takahashi et al., 1999). Chronic, but not acute, treatment with antidepressant has been found to upregulate the cAMP second messenger system at several levels, including enhanced coupling of the stimulatory GTP-binding protein (Gs) to adenylyl cyclase, increase levels of cAMP-dependent protein kinase, and increase the expression and function of CREB (Duman, 1998). Also, chronic antidepressant treatment has been reported to increase the expression of phosphodiesterase 4 in the frontal cortex and nucleus accumbens using non-stressed rats (Takahashi et al., 1999). The upregulation of phosphodiesterase 4 by treatment with antidepressants is a compensatory response to an increased cAMP level and might cause attenuation of the antidepressant effects. In addition, we suggested that hypofunction of the cAMP-dependent signal transduction system observed in learned helplessness rats, an animal model for depression, might be involved in an increase in cortical and hippocampal phosphodiesterase 4 activity (Itoh et al., 2003).

Rolipram is a well-documented isozyme-selective inhibitor of phosphodiesterase 4 and readily passes the blood–brain barrier (Krause and Kuhne, 1988). In the present study, we investigated the combination effects of rolipram, a phosphodiesterase 4 inhibitor, with imipramine on depressive behavior in learned helplessness rats. Alterations in CRE-binding activity and BDNF level related to the therapeutic effects of antidepressants were also determined in the frontal cortex, striatum and hippocampus.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (7–8 weeks old, Clea Japan, Tokyo, Japan) were used in the experiments. Five animals were housed in an aluminum cage ($W \times D \times H$; 400×500×200 mm) under standard conditions: maintained at a temperature of 23±2 °C, relative humidity of 55±15% and ventilation frequency over 10 times/h with 100% fresh air under a 12-h light/12-h dark schedule (lights turned on at 08:00 h and turned off at 20:00 h). They were allowed free access to water and food (solid chow; CA-1, Clea Japan).

All animal experiments and procedures were approved and conducted in accordance with the Animal Care and Use Committee of Shionogi Research Laboratories, Osaka, Japan.

2.2. Apparatus

A shock pre-treatment Plexiglas chamber (200×160×210 mm) and automated two-way shuttle-boxes (400×160×210 mm; Neuroscience, Tokyo, Japan) were used. The floors consisted of 4.5 mm diameter stainless-steel grids spaced 11 mm apart center to center. The shuttle-boxes were divided into two equal compartments by a partition with a central opening (70×70 mm). The exposures to inescapable electric footshocks and the behavioral escape tests took place in a sound-attenuating chest, containing an exhaust fan that masked extraneous noise.

2.3. Learned helplessness paradigm

Learned helplessness rats were produced and their escape behavior assessed by a previously described method (Itoh et al., 2003). Each rat was placed in a shock pre-treatment Plexiglas chamber and exposed to 60 inescapable electric footshocks (intensity 1.0 mA, duration 15 s) at variable interstimulation intervals of 20–90 s (mean=45 s) once a day for 3 days (Days 1–3). A shock generator-scrambler (Neuroscience) was used to deliver electric shocks to the grid floor. Non-stressed rats were placed in identical chambers for 60 min without receiving electrical footshocks. The exposures to inescapable footshocks were performed in the morning, starting at 08:00 h.

The rats were tested for escape performance in the automated two-way shuttle-boxes at 1, 5 and 7 days after the third day of exposure to inescapable footshocks (Days 4, 8 and 10, respectively). The animals were individually placed in the shuttle-box, allowed to habituate to the environment for 5 min and then subjected to testing. A testing session consisted of 30 trials (variable intertrial interval of 7.5–22.5 s, mean=15 s). In each trial, a tone signal (80–90 dB) was first presented for a maximum of 3 s with a light signal. The rats could avoid the electric shock by moving to the other side of the shuttle-box (avoidance response); signals terminated on the response. However, if no avoidance response occurred during the 3-s period, an electric shock (0.8 mA) was applied to the rats through the grid floor for a maximum of 3 s with the tone and light signal. Rats could escape the shock by moving to the other side of the box (escape response); the signals and the shock terminated on the response. If no escape response occurred, the shock and signals terminated automatically. A non-crossing response during the shock delivery was referred to as an escape failure.

2.4. Plasma corticosterone level

Separate groups of animals were used for measurement of plasma corticosterone levels in learned helplessness and

non-stressed rats. The animals were sacrificed 2 h after the escape test and blood samples were collected. These samples were kept on ice and centrifuged immediately at 2,000×*g* for 15 min at 4 °C. The obtained plasma was kept at −80°C until analysis. Corticosterone levels were measured using commercially available radio immunoassay kits (ICN Biomedicals, Costa Mesa, CA, USA).

2.5. Drug administration

Imipramine hydrochloride (Sigma, St. Louis, MO, USA) and rolipram (Tocris Cookson, Bristol, UK) were dissolved in 0.2% dimethylsulfoxide solution (vehicle). These drugs or vehicle solutions were intraperitoneally administered at a volume of 2 ml/kg either repeatedly or acutely. The repeated treatment was performed as follows: in the evening (17:00–18:00 h) on Day 1 (the first day of exposure to inescapable footshocks); in the morning (08:00–09:00 h) and evening (twice a day) on Days 2–9; 60 min before the escape test on Day 10. Acutely treated rats received the vehicle on Days 1–9 and the drugs on Day 10 according to the same schedule. The dose levels of these drugs were determined by referring to previous studies (Fujimaki et al., 2000; Nakagawa et al., 1999; O'Donnell, 1993) and expressed as the salt.

2.6. Preparation of nuclear extracts

Nuclear protein extracts were prepared from brain tissues according to methods previously described (Frechilla et al., 1998; Itoh et al., 2003) with minor modifications. Namely, tissues were homogenized in 10 volumes of ice-cold buffer containing 50 mM Tris–HCl (pH 6.8), 0.25 M sucrose, 25 mM NaCl, 4 mM $MgCl_2$, 1 mM EGTA, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, and 5 μg/ml aprotinin and centrifuged at 11,000×*g* for 20 min at 4 °C. The pellet was incubated in the same Tris buffer containing 1% of Nonidet P-40 for 10 min at 4 °C and then vigorously mixed for 15 s. The nuclear fraction was precipitated by centrifugation at 11,000×*g* for 20 min at 4 °C and suspended in 5 volumes of ice-cold high-salt HEPES buffer containing 50 mM HEPES (pH 7.9), 0.75 M NaCl, 0.75 mM $MgCl_2$, 0.5 mM EGTA, 12.5% glycerol, 5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin and 5 μg/ml aprotinin. The mixture was kept for 30 min at 4 °C with continuous agitation. The supernatants of the nuclear extracts were prepared by centrifugation at 15,000×*g* for 15 min at 4 °C and stored in aliquots at −80 °C until assay.

2.7. Electrophoretic mobility shift assay (EMSA)

cAMP-response element (CRE) consensus oligonucleotide (5′ -AGAGATTGCCTGACGTCAGAGAGCTAG-3′) was ^{32}P-end-labeled by incubation for 10 min at 37 °C with [γ-^{32}P]ATP (New England Nuclear, Boston, MA, USA, specific activity 10 mCi/ml) using T4 polynucleotide kinase (Promega, Madison, WI, USA) and purified by G-25 spin column chromatography.

Nuclear protein extracts (10 μg) were preincubated for 15 min at room temperature in 10 μl of reaction buffer containing 10 mM Tris–HCl (pH 7.5), 4% glycerol, 1 mM $MgCl_2$, 0.5 mM EDTA, 50 mM NaCl, 0.5 mM dithiothreitol, and 0.5 μg of poly[dI–dC]. Approximately 100,000 cpm of the ^{32}P-end-labeled CRE consensus oligonucleotide was then added, and the incubation was continued at room temperature for 30 min. The DNA–protein complexes were electrophoresed for 30 min at 350 V in 5% polyacrylamide gels. After gel electrophoresis, the gel was dried and exposed to Bio-imaging plate. The relative density of the detected bands was measured and analyzed with the Bio-imaging analyzer system (BAS-2000, Fuji Photo Film, Tokyo, Japan).

2.8. BDNF measurement by enzyme-linked immunosorbent assay (ELISA)

For extraction of the neurotrophic factor, the brain tissues were homogenized in 10 volumes of ice-cold lysis buffer containing 20 mM Tris (pH 8.0), 137 mM NaCl, 1% Nonidet P-40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 1 μg/ml leupeptin, and 0.5 mM sodium vanadate. Homogenates were sonicated and centrifuged at 10,000×*g* for 30 min. The supernatant was collected and stored at −80 °C until assay.

Quantification of endogenous BDNF was performed using a two-site enzyme immunoassay kit (Promega) according to the manufacturer's specifications. Briefly, 96-well immunoplates (NUNC, Denmark) were coated with anti-BDNF monoclonal antibody diluted in carbonate buffer (pH 9.7; 1:1,000) and incubated at 4 °C for 18 h. The plates were then treated with blocking buffer for 60 min at room temperature. The samples and BDNF standards (0–500 pg/ml) were applied to the coated wells in duplicates at room temperature under conditions of shaking for 120 min, followed by washing with washing buffer containing 20 mM Tris–HCl (pH 7.5), 150 mM NaCl, 0.05% v/v Tween-20. The plates were incubated with antihuman BDNF polyclonal antibody at room temperature for 60 min with mild shaking. After washing, the plates were incubated in peroxidase substrate and tetramethyl benzine solution to produce a color reaction. The reaction was stopped with 1 M phosphoric acid and the absorbance of samples was measured at 450 nm using an Emax automated microplate reader (Thermo Electron, Finland). BDNF concentrations were determined from the regression line for the BDNF standards and expressed as pg of BDNF/mg of total proteins.

2.9. Statistics

The results are expressed as mean±S.E.M. values. Statistical analyses were carried out using the SAS system

(Version 6.12, SAS Institute, Tokyo, Japan) for Microsoft Windows. The statistical significance of differences for experiments on groups of two was determined using the unpaired Student's *t*-test. Experiments on groups of three or more were subjected to Dunnett's test following one-way analysis of variance (ANOVA). The *P* values of less than 0.05 were considered to be statistically significant.

3. Results

3.1. Escape failures in rats subjected to inescapable footshocks

Compared with non-stressed rats that had not been exposed to inescapable shocks, learned helplessness rats (stress-treated) showed a significantly higher number of escape failures when tested for their escape performance abilities at 1, 5 and 7 days after the third day of exposure to inescapable shocks (Days 4, 8 and 10, respectively). Exposure to inescapable electric footshocks for 3 days induced a subsequent increase in escape failures (Fig. 1).

3.2. Plasma corticosterone level

The plasma corticosterone level of the learned helplessness rats was significantly higher than that of the non-stressed rats (Table 1). This effect persisted for at least 7 days after the exposure to inescapable footshocks.

3.3. Effect on learned helplessness behavior

Repeated administration of imipramine (0.625–10 mg/kg, i.p.) for 10 days reduced the number of escape failures in learned helplessness rats (Fig. 2). Significant effects were observed at doses from 1.25 to 10 mg/kg of imipramine

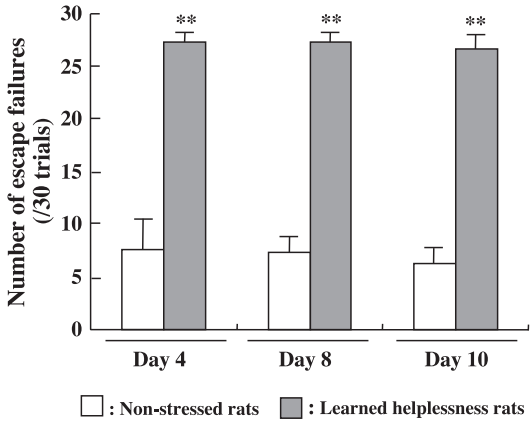

Fig. 1. Number of escape failures in rats subjected to inescapable electric footshocks (1.0 mA, 15 s×60 times×3 days). Rats received 30 escape tests on 1, 5 and 7 days after the inescapable shocks (Days 4, 8 and 10, respectively). Values are means±S.E.M. of the total number of escape failures with 30 trials of 7–8 rats. **P<0.01 versus each non-stressed control rats (Student's *t*-test).

Table 1
Plasma corticosterone levels in learned helplessness and non-stressed rats

	Corticosterone (ng/ml)		
	Day 4	Day 8	Day 10
Non-stressed rats	41.0±6.8	44.5±9.7	64.6±11.8
Learned helplessness rats	138.7±13.2[b]	126.7±20.8[b]	120.9±14.7[a]

Mean value±S.E.M. of six rats for each day group. Each plasma sample was collected 2 h after the escape test at 1, 5 and 7 days after the third day of exposure to inescapable footshocks (Days 4, 8 and 10, respectively).
[a] P<0.01 versus each non-stressed rats (Student's *t*-test).
[b] P<0.05 versus each non-stressed rats (Student's *t*-test).

(mean number of escape failures±S.E.M.: vehicle=27.5±0.7; imipramine 0.625 mg/kg=24.5±3.3; 1.25 mg/kg=17.7±2.5; 2.5 mg/kg=17.2±2.5; 5 mg/kg=12.5±1.4; 10 mg/kg=13.7±2.2). However, none of the doses of imipramine could completely ameliorate the escape behavior to a level similar to that of the non-stressed rat group (7.3±1.3, P<0.05; Student's *t*-test).

Fig. 3 shows the effects of imipramine, rolipram and their combinations on the escape failures in learned helplessness rats. A significant difference was found in the number of escape failures between non-stressed rats and vehicle-treated helplessness rats (mean number of escape failures±S.E.M.: non-stressed=8.9±1.7; vehicle=25.9±1.4). Chronic treatment with imipramine (2.5 mg/kg) or rolipram (1.25 mg/kg) for 10 days significantly ameliorated the increased escape failures induced by the inescapable shocks (imipramine=18.9±1.5; rolipram=17.1±2.0). Furthermore, chronic coadministration of imipramine with rolipram (2.5 and 1.25 mg/kg, respectively) markedly reduced the increased escape failures (8.3±1.4) and this antidepressive effect was

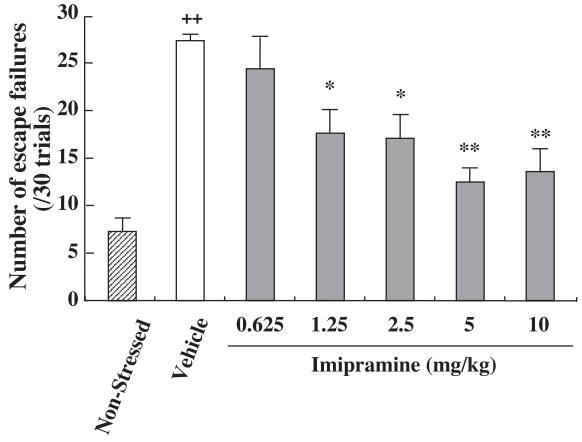

Fig. 2. Effect of imipramine on the number of escape failures in learned helplessness rats. Chronic treatment with imipramine (0.625, 1.25, 2.5, 5 or 10 mg/kg) was given intraperitoneally for 10 days: the evening (17:00–18:00 h) of Day 1 (the first day of exposure to inescapable footshocks); the morning (08:00–09:00 h) and the evening of Days 2–9; 60 min before the escape test on Day 10. Values are the number of escape failures±S.E.M. during 30-trial escape test of six rats. *P<0.05 and **P<0.01 versus vehicle-treated group (Dunnett's test). ^{++}P<0.01 versus non-stressed group (Student's *t*-test).

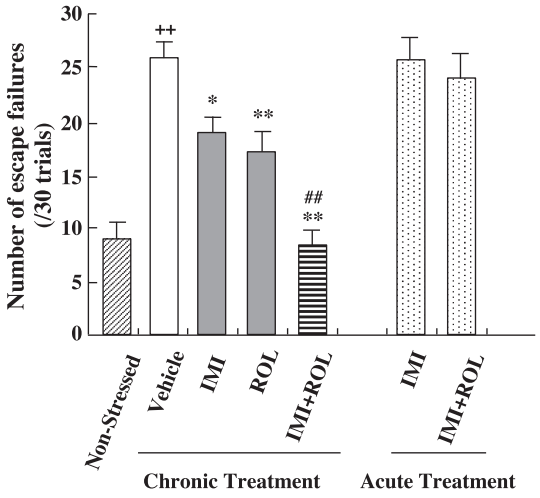

Fig. 3. Effect of imipramine and rolipram treatment on the number of escape failures in learned helplessness rats. In chronic treatment groups (n=11–12), imipramine (IMI, 2.5 mg/kg), rolipram (ROL, 1.25 mg/kg) or coadministration of IMI 2.5 mg/kg with ROL 1.25 mg/kg (IMI+ROL) was given intraperitoneally for 10 days: the evening (17:00–18:00 h) of Day 1 (the first day of exposure to inescapable footshocks); the morning (08:00–09:00 h) and the evening of Days 2–9; 60 min before the escape test on Day 10. In acute treatment groups (n=6), IMI or IMI+ROL was given as a single dose 60 min before the escape test on Day 10. Values are the number of escape failures±S.E.M. during 30-trial escape test. *P<0.05 and **P<0.01 versus vehicle-treated group (Dunnett's test). $^{\#\#}P$<0.01 versus chronic IMI-treated group (Student's t-test). ^{++}P<0.01 versus non-stressed group (n=12, Student's t-test).

significantly superior to that by imipramine treatment alone. On the other hand, there was no effect on inescapable shock-induced increased escape failures by a single treatment with imipramine (25.7±2.1) or coadministration of imipramine with rolipram (24.0±2.4).

3.4. Effect on CRE-binding activity in learned helplessness rats

CRE-binding activities in the frontal cortex and hippocampus in the vehicle-treated learned helplessness rats were about 70% lower than those in the non-stressed rats (mean percentage of non-stressed rats±S.E.M.: frontal cortex=69.5±8.1%; hippocampus=68.3±5.7%). Imipramine (2.5 mg/kg) or rolipram (1.25 mg/kg) significantly increased the CRE-binding activity in the hippocampus (imipramine=110.9±12.3%; rolipram=116.2±7.9%) compared with that of the vehicle-treated rats. Moreover, the levels of CRE-binding activity in the frontal cortex and hippocampus by the coadministration of their combination were greatly increased (frontal cortex=213.2±35.2%; hippocampus=194.3±10.4%) and significantly higher than those by the treatment with imipramine alone. By contrast, in the striatum, none of the drug treatments significantly influenced the CRE-binding activity compared with vehicle-treated learned helplessness rats. Only the CRE-binding activity in chronic coadministration of imipramine with the rolipram treatment group was significantly higher than that in the group treated with imipramine alone. In addition, in the acute treatment groups, all the CRE-binding activities in the frontal cortex and hippocampus were not significantly increased, although there was a tendency for an upregulation by each single administration (Fig. 4).

3.5. Effect on BDNF levels in learned helplessness rats

The BDNF level in the frontal cortex of the chronic vehicle-treated learned helplessness rats was significantly lower than that of the non-stressed rats (45.3±6.9 and 70.5±6.4 pg/mg protein, respectively). In the hippocampus, but not in the striatum, the BDNF levels were also lower in the vehicle-treated learned helplessness rats than in the non-stressed rats (121.0±17.0 and 177.1±14.9

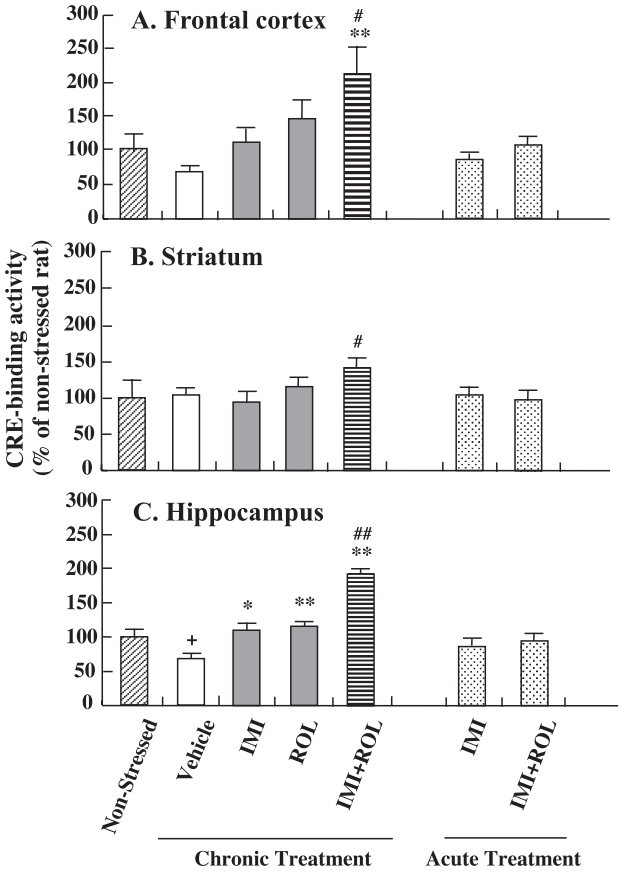

Fig. 4. Effect of imipramine and rolipram treatment on the levels of CRE binding activity in the frontal cortex (A), striatum (B) and hippocampus (C) from learned helplessness rats. Imipramine (IMI, 2.5 mg/kg), rolipram (ROL, 1.25 mg/kg) or coadministration of IMI 2.5 mg/kg with ROL 1.25 mg/kg (IMI+ROL) was given intraperitoneally in repeated or single administration according to the schedule in the Fig. 3 legend. Each of the regions was collected 2 h after the escape test, and the levels of CRE-binding activity were determined by electrophoretic mobility shift assay. The results are expressed as the percent of non-stressed rats and are the means±S.E.M. of six experiments. *P<0.05 and **P<0.01 versus each vehicle-treated group (Dunnett's test). $^{\#}P$<0.05 and $^{\#\#}P$<0.01 versus each chronic IMI-treated group (Student's t-test). ^{+}P<0.05 versus each non-stressed group (Student's t-test).

pg/mg protein, respectively). Chronic treatment of imipramine (2.5 mg/kg) or rolipram (1.25 mg/kg) almost compensated for the decrease of the BDNF level noted in the frontal cortex and hippocampus in the learned helplessness rats, although no significant influence was detected. In addition, the BDNF levels were significantly increased in the frontal cortex (93.0±12.3 pg/mg protein) and hippocampus (267.6±22.3 pg/mg protein) by coadministration of imipramine with rolipram. This treatment had a significantly greater increasing effect on the BDNF level in the hippocampus than treatment with imipramine alone. On the other hand, acute treatment with a single dose of imipramine or coadministration of imipramine with rolipram did not influence all the BDNF levels studied (Fig. 5).

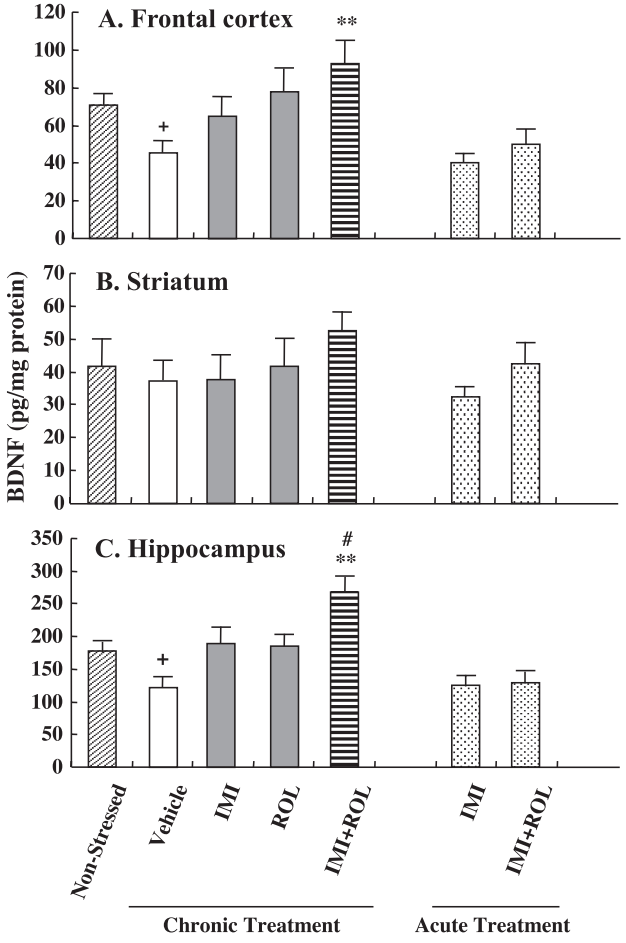

Fig. 5. Changes in the levels of brain-derived neurotrophic factor (BDNF) in the frontal cortex (A), striatum (B) and hippocampus (C) from learned helplessness rats treated chronically or acutely with imipramine and rolipram. Imipramine (IMI, 2.5 mg/kg), rolipram (ROL, 1.25 mg/kg) or coadministration of IMI 2.5 mg/kg with ROL 1.25 mg/kg (IMI+ROL) was given intraperitoneally in repeated or single administration according to the schedule in the Fig. 3 legend. Each of the regions was collected 2 h after the escape test, and the levels of BDNF were determined by enzyme-linked immunosorbent assay. Values are means±S.E.M. of six experiments. $^{**}P<0.01$ versus each vehicle-treated group (Dunnett's test). $^{\#}P<0.05$ versus each chronic IMI-treated group (Student's t-test). $^{+}P<0.05$ versus each non-stressed group (Student's t-test).

4. Discussion

In the present study, we clearly demonstrated that the coadministration of imipramine with rolipram, a phosphodiesterase 4 inhibitor, was more effective against depressive behavior than either drug alone in learned helplessness rats. Furthermore, upregulation of CRE-binding activity and BDNF levels in the frontal cortex and hippocampus were coincident with improvement of the depressive behavior in learned helplessness rats.

The learned helplessness model has a high overall validity for depression and has been widely used for assessment of antidepressive effects (Ferguson et al., 2000; Tordera et al., 2002; Willner, 1986). In the present study, the exposure of rats to inescapable and uncontrollable stress produced a learned helplessness behavior with elevation of the plasma corticosterone level. In learned helplessness rats, repeated administration of imipramine or rolipram significantly attenuated the increased escape failures. However, neither imipramine nor rolipram could completely ameliorate the escape behavior to a level similar to that of non-stressed rats at any dose. In the present study, imipramine produced antidepressive effects at dose ranging from 1.25 to 10 mg/kg. However, high doses of imipramine caused loss of body weight during the drug treatment period. Tricyclic antidepressants are well known to possess anticholinergic activity, which is responsible for many of the side effects of these drugs (Blackwell, 1981). On the other hand, a dose of 1.25 mg/kg of rolipram was chosen because a dose of 0.3 to 3 mg/kg of rolipram have been reported to show sufficient antidepressive action in previous work (O'Donnell, 1993). An excess dose (10 mg/kg) of rolipram has also been reported to have some adverse effects, including a reduction of food intake and weight loss (Fujimaki et al., 2000; Nibuya et al., 1996). Therefore, the dose of imipramine and rolipram was set at 2.5 and 1.25 mg/kg, respectively, in the combination treatment study.

Repeated administration of rolipram (1.25 mg/kg, i.p. for 10 days) in combination with imipramine (2.5 mg/kg) ameliorated the escape failures in learned helplessness rats, although 5 or 10 mg/kg of imipramine or 1.25 mg/kg of rolipram could not reduce the number of escape failures to a level similar to that of non-stressed rats. Rolipram, a selective phosphodiesterase 4 inhibitor, has been suggested to show its antidepressive effects through accumulation of cAMP and activation of the cAMP signaling pathway downstream (Fleischhacker et al., 1992; Fujimaki et al., 2000; Nibuya et al., 1996). Chronic administration with desipramine (a main metabolite of imipramine and a norepinephrine reuptake inhibitor) has been reported to increase the expression of phosphodiesterase 4 in rat brain (Takahashi et al., 1999; Ye et al., 2000). Recent studies have suggested that chronic, but not acute, treatment with the antidepressants desipramine or fluoxetine upregulates the high-affinity rolipram binding sites on phosphodiesterase 4 in the rat hippocampus and cerebral cortex (Zhao et al.,

2003). These findings indicate that induction of phosphodiesterase 4 is increased by upregulation of the cAMP level. Therefore, repeated administration of imipramine exhibits an antidepressive effect but might induce the upregulation of phosphodiesterase 4, which suppresses the cAMP signal transduction downstream. We have previously reported an increase in phosphodiesterase 4 activity in learned helplessness rats (Itoh et al., 2003). Therefore, rolipram might ameliorate the augmentation of the cAMP degradation system induced by imipramine. Thus, rolipram might potentiate the antidepressive effect of imipramine in learned helplessness rats.

Cyclic AMP is a major second messenger in the brain signal transduction system and has been reported to be involved in depression, long-term potentiation and long-term memory (Sunahara et al., 1996; Wong et al., 1999). cAMP-dependent protein kinase (PKA) and CREB activated by cAMP modulate the expression of BDNF, which mediates neuronal survival and synaptic plasticity in the cAMP-signaling pathway downstream. Clinical postmortem studies have shown that CREB levels in the temporal cortex of depressive patients are decreased and can be significantly increased by antidepressant treatment when compared with those in untreated patients (Dowlatshahi et al., 1998). Also, chronic treatment with different classes of antidepressants leads to significant homologous changes in gene expressions, including upregulation of CREB, and has been related to neurogenesis and synaptogenesis in the rat hippocampus (Drigues et al., 2003). These findings suggest that the cAMP signaling pathway plays a pivotal role in pathophysiological depressive behavior and the action of antidepressants. In the learned helplessness rats, the reduction of CRE-binding activity and BDNF levels were observed in the frontal cortex and hippocampus, but not in the striatum. Coadministration of imipramine with rolipram upregulated these downstream targets of the cAMP signaling pathway in both brain regions, with the effect in the hippocampus being somewhat greater. The implications for the hippocampus have been reported for both the pathophysiology and treatment of depression and other stress-related psychiatric disorders (Bremner et al., 2000; Vermetten et al., 2003). Malberg and Duman (2003) reported a decrease of hippocampal cell proliferation by inescapable stress and a reversal of the downregulation by antidepressant treatment using the learned helplessness model. The frontal cortex has also been implicated in depressive disorders and the BDNF protein levels in this region were increased after consecutive administration of antidepressants and electroconvulsive shock treatment (Altar et al., 2003; Drevets et al., 1997). Thus, alterations in the cAMP-mediated signal transduction pathway of the hippocampus and frontal cortex might contribute to the pathology and treatment of depression.

In our study, acute treatment with imipramine or coadministration of imipramine with rolipram had no significant influence on the depressive behavior or CRE-binding activity and BDNF levels. Chronic administration of the antidepressants was found to be required for the display of their antidepressive effects in most previous studies. However, some investigators have reported that acute treatment with electroconvulsive shock or 6-(3-dimethylaminopropionyl)forskolin (NKH477), an activator of cAMP production system, upregulates the CRE modulator or BDNF and its receptor, trkB mRNA using non-stressed rats (Fitzgerald et al., 1996; Morinobu et al., 1999). Thus, phosphodiesterase 4 might play an important role in the therapeutic effects and the upregulation of the cAMP system including specific transcription factors, such as BDNF by traditional antidepressant drugs. Further experiments are required to investigate this issue by assessing the time-course of antidepressive effects and the induction of the cAMP-mediated signal pathway function in the learned helplessness rats or other stress-treated models.

Haracz et al. (1988) reported that learned helplessness rats showed impaired feedback regulation in the hypothalamic–pituitary–adrenal (HPA) axis. Increases in plasma corticosterone levels in these rats suggest that prior exposures to inescapable footshocks sensitize the rats to acute stress associated with the escape test session in this study. Conti et al. (2004) showed that chronic desipramine treatment suppresses swim stress-induced elevations in mice plasma corticosterone levels through inducible cAMP early repressor, an isoform of CRE modulator. Therefore, coadministration of imipramine with rolipram might reduce corticosterone levels in the learned helplessness rats and be able to suppress the sensitization to acute stress. Further studies are required to clarify this view.

In conclusion, these results suggest that coadministration of antidepressants with rolipram, a phosphodiesterase 4 inhibitor, is more effective than treatment with either drug alone and that the activation of the cAMP signal transduction pathway, such as CRE-binding activity or BDNF, is involved in the antidepressive effects. For depression therapy, phosphodiesterase 4 inhibitor may be a more useful drug together with a monoamine transporter inhibitor.

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