Administration of a cAMP Phosphodiesterase 4 Inhibitor Enhances Antidepressant-Induction of BDNF mRNA in Rat Hippocampus

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The influence of two selective phosphodiesterase 4 (PDE4) inhibitors, rolipram and Ro 20–1724, on the induction of BDNF mRNA by antidepressant treatment was examined. Coadministration of rolipram or Ro 20–1724 with an antidepressant (either desipramine or Org 4428) for 21 d resulted in a significant induction of BDNF mRNA in hippocampus relative to administration of vehicle. Coadministration of a PDE4 inhibitor with an antidepressant for 7 or 14 d also increased levels of BDNF mRNA. In contrast, acute coadministration did not influence levels of BDNF mRNA. In situ hybridization analysis demonstrated that the induction of BDNF mRNA in response to the repeated coadministration paradigm occurs in the dentate gyrus granule and CA1 and CA3 pyramidal cell layers of hippocampus. These findings demonstrate that coadministration shortens the time required for the upregulation of BDNF mRNA, supporting the possibility that this treatment may provide an effective therapy for major depression.
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Although numerous preclinical studies have been undertaken to identify a common action of antidepressant drugs, the mechanism(s) which underlies the therapeutic action of these treatments remains unknown. One likely target of antidepressant administration is the cAMP signal transduction system, because chronic, but not acute, antidepressant treatment up-regulates this intracellular cascade at several levels (Nestler et al. 1989; Ozawa and Rasenick 1991; Nibuya et al. 1996; Duman et al. 1997). On the other hand, the

therapeutic response to antidepressants requires several weeks of treatment, suggesting that long-term neuronal adaptations mediated by changes in gene expression may be important to the therapeutic action of antidepressant treatment (Heninger and Charney 1987; Duman et al. 1995, 1997). Based on these findings, it is postulated that neural plasticity induced by activation of the cAMP signal transduction cascade may be involved in the therapeutic action of antidepressant drugs.

Brain-derived neurotrophic factor (BDNF) is reported to play an important role in the survival of mature neurons as well as damaged neurons in the central nervous system (Ghosh et al. 1994; Mamonous et al. 1994). Recent studies revealed that the activation of the cAMP system, as well as β-adrenergic receptors that couple to this system, is closely involved in the regulation of BDNF mRNA expression (Zafra et al. 1990, 1992; Condorelli et al. 1994; Nibuya et al. 1996; Bishop et al. 1997). We have reported that chronic administration (21 days) of several different types of antidepressant in-

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creases the expression of BDNF mRNA in rat frontal cortex and hippocampus (Nibuya et al. 1995, 1996). In addition, local infusion of BDNF into midbrain has an antidepressant like effect in behavioral models of depression (Siuciak et al. 1997). Conversely, levels of BDNF mRNA are reported to be decreased by acute as well as chronic restraint stress in rat hippocampus (Smith et al. 1995). Taken together, these findings support the possibility that activation of the cAMP system and increased expression of BDNF contribute to the neural adaptations that underlie the action of antidepressant treatment.

This model suggests that agents which activate the β-adrenergic receptor and cAMP intracellular cascade would potentially be effective antidepressant agents. One approach is to use an inhibitor of phosphodiesterase (PDE), the enzymes responsible for the breakdown of cAMP. Although there are many different types of PDE, cAMP PDE type 4 may be one relevant target (Beavo et al. 1994; Conti et al. 1995; Conti and Jin in press). This is based on reports that PDE4 inhibitors, such as rolipram, have antidepressant effects in behavioral models of depression (Wachtel and Schneider 1986; Griebel et al. 1991; O'Donnell 1993) and clinical trials demonstrating that rolipram has antidepressant efficacy in depressed patients (Bobon et al. 1988; Fleischhacker et al. 1992). In spite of these reports, the use of rolipram for the treatment of depression has been limited by its side effects.

The present study was undertaken to examine the influence of acute or chronic coadministration of two different PDE4 inhibitors with an antidepressant (desipramine or Org 4428) on the expression of BDNF mRNA. Desipramine as well as Org 4428 is a reuptake inhibitor with higher affinity for norepinephrine transporter. This work extends a preliminary report on the influence of rolipram on the expression of BDNF mRNA in rat hippocampus (Nibuya et al. 1996).

MATERIALS AND METHODS

Animals and treatment paradigms. Male Sprague-Dawley rats (200 g; Japan Charles River, Japan) were group housed and maintained on a 12-h light cycle with food and water freely available. For the studies of coadministration of a PDE4 inhibitor and an antidepressant, groups of rats were administered desipramine (15 mg/kg), Org 4428 (20 mg/kg), rolipram (1.25 mg/kg), Ro 20–1724 (12.5 mg/kg), or vehicle (1% Tween 80) once daily for 1, 7, 14, or 21 d via intraperitoneal injection and sacrificed by decapitation 2 h after the last treatment. All animals use procedures were approved by the Yamagata University Animal Care Committee.

The brains were removed and immediately frozen for in situ hybridization, or sections of hippocampus were dissected for RNA extraction and Northern blot analysis as described below. The same animals were used for in situ and Northern blot experiments. The drugs used for these studies were obtained from the following sources: rolipram was a generous gift of Solvay-Meiji Pharmaceutical Company (Tokyo, Japan), Ro 20–1724 was a generous gift of Hoffmann-LaRoche INC (Nutley, NJ), desipramine was purchased from Sigma, and Org 4428 was a generous gift of Organon Pharmaceutical Company (Oss, The Netherlands).

Northern Blot Analysis

Total RNA was isolated from sections of hippocampus by the guanidine isothiocyanate/cesium chloride centrifugation method, as previously described (Morinobu et al. 1995; Nibuya et al. 1995). The rat BDNF cDNA clone was provided by Regeneron (Tarrytown, NY). The BDNF cDNA, subcloned into pBluescript SK-(Stratagene, La Jolla, CA), was linearlized with Bpu1102 I (Takara Shuzo CO., LTD, Otsu, Japan) and uniformly radiolabeled riboprobes corresponding to the antisense strand (bp 507–833) were generated with T7 RNA polymerase and ^{32}P-UTP (Dupont NEN, Boston, MA) as previously described (Nibuya et al. 1995). Briefly, 20 μg of total RNA were electrophoresed on a 1% agarose gel and the RNA was transferred to nitrocellulose filters. The resulting filters were then incubated with the ^{32}P-labeled BDNF riboprobes for 18 h at 65°C and washed twice in 2 × SSC (SSC: 0.15 M NaCl, 0.015 M sodium citrate at pH 7.0), 0.1% sodium dodecyl sulfate (SDS) at 65°C for 15 min, and then in 0.2 × SSC, 0.1 % SDS at 65°C for 15 min. Levels of total RNA for each lane were determined by reprobing the nitrocellulose filters with a ^{32}P-labeled cyclophilin cDNA probe that was radiolabeled using a random prime kit (Amersham, Beckinghamshire, England) as previously described (Morinobu et al. 1995). The levels of BDNF mRNA were divided by cyclophilin mRNA to account for differences in the amount of RNA per lane. The results are expressed as percent of sham.

In Situ Hybridization Analysis

Analysis of BDNF mRNA by in situ hybridization was conducted as previously described (Nibuya et al. 1995, 1996). Briefly, coronal sections of frozen brain (18 μm) were cut on a cryostat and stored at −70°C. Tissue sections were thaw mounted on RNase-free Probon slides (Fisher Biotech, Pittsburgh, PA), fixed, and dried. The ^{35}S-radiolabeled BDNF riboprobes were hybridized with the brain sections (2 × 10^6 CPM per section) for 18 h at 55°C in hybridization buffer (50% formamide, 0.6 M
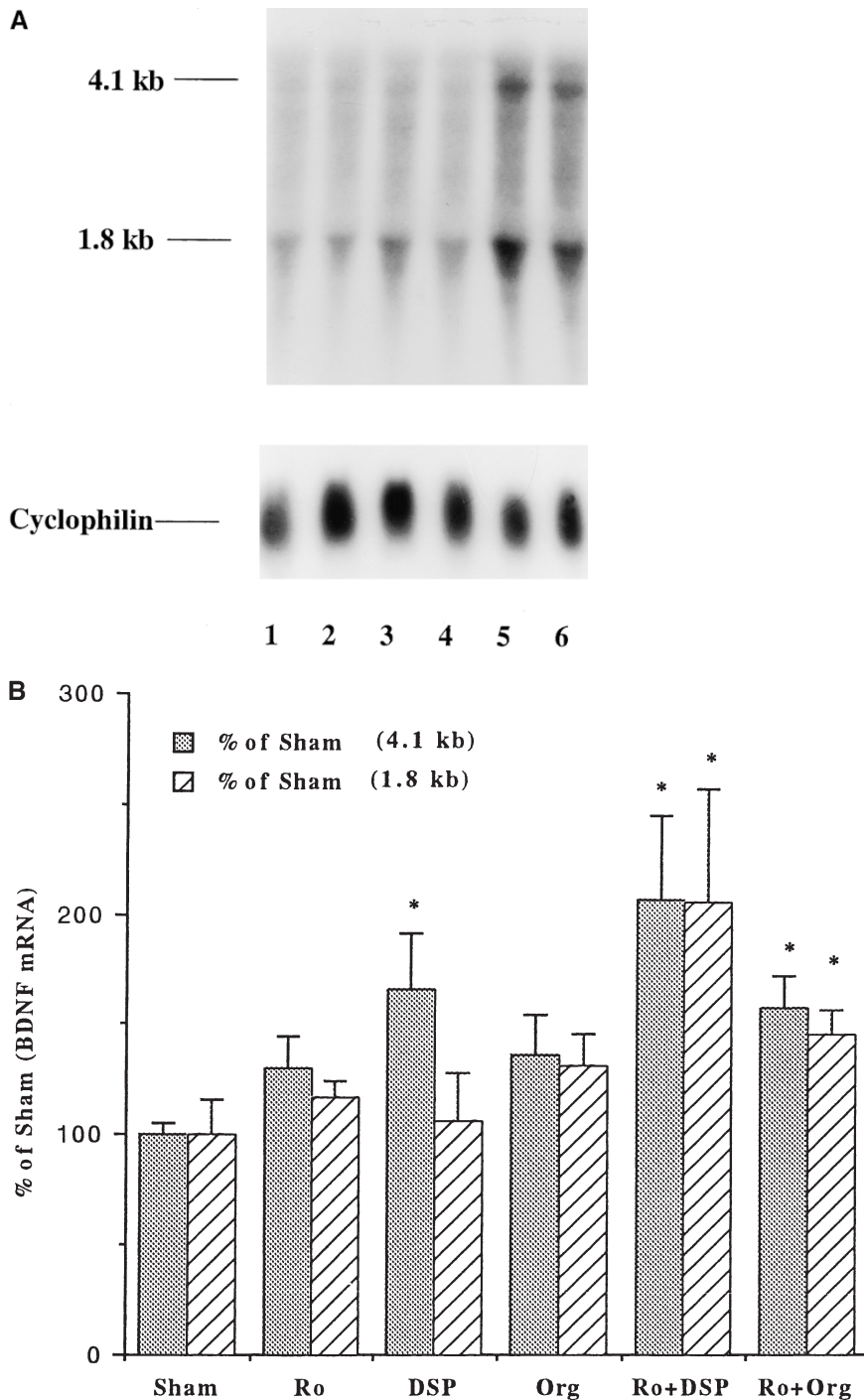
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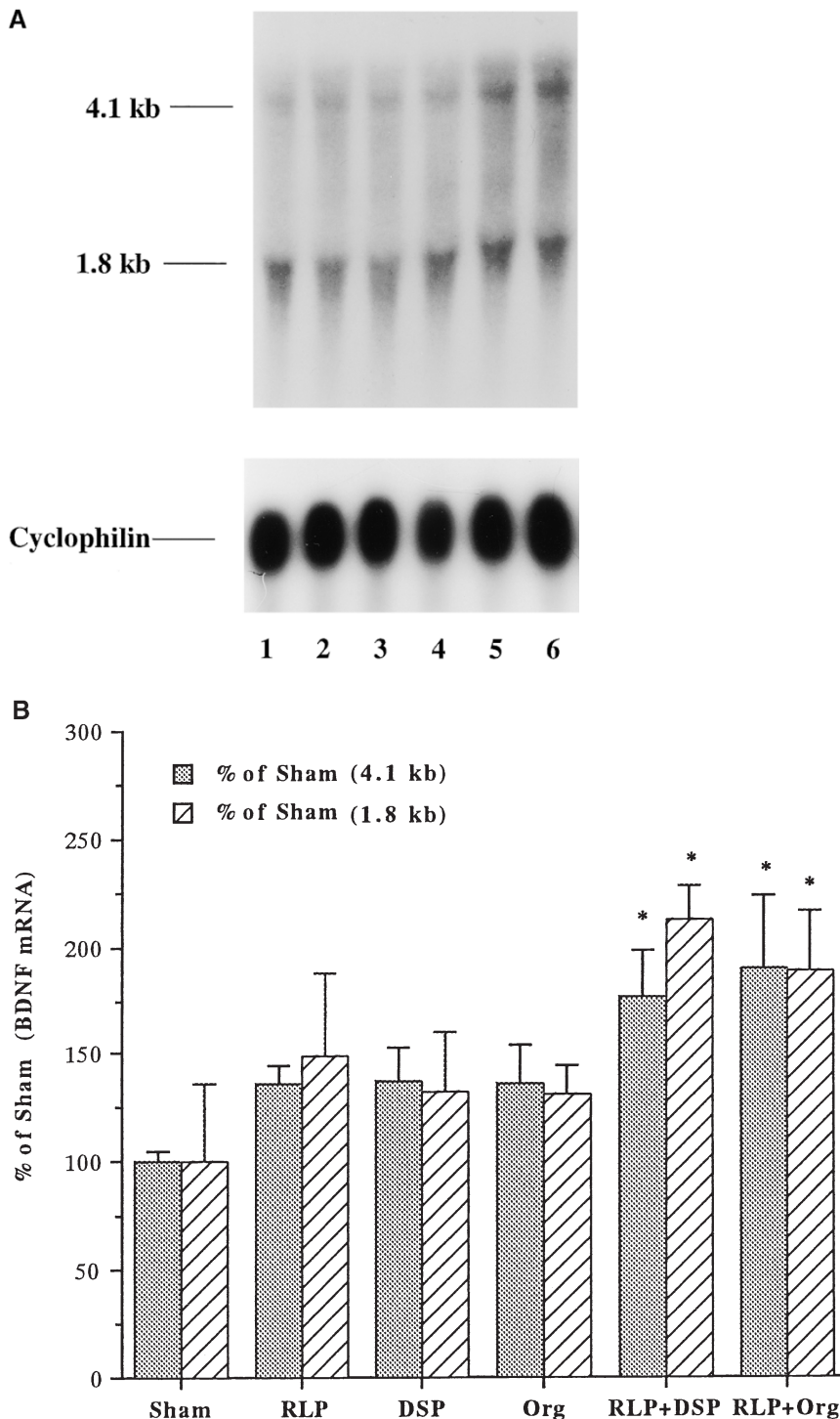
Figure 1. Influence of chronic coadministration (21 days) of rolipram (RLP) with desipramine (DSP) or Org 4428 (Org) on BDNF mRNA in rat hippocampus. Levels of BDNF were measured 2 h after the last injection by Northern blot analysis. **(A)** Representative Northern blots for chronic coadministration: 1, sham (vehicle); 2, RLP; 3, DSP; 4, Org; 5, RLP + DSP; and 6, RLP + Org. **(B)** Quantitation of BDNF mRNA. Levels of mRNA were quantified by densitometry and standardized for the amount of mRNA in each lane by reprobing the filters with radiolabeled cyclophilin. Data are presented as percentage of sham (vehicle treatment) and are the mean ± S.E.M. of 5 to 6 rats for each treatment group. *$p < .05$ compared with vehicle control (ANOVA and Fisher's test).

NaCl, 10 mM Tris, 1x Denhardt's solution, 2 mM EDTA, 10 mM DTT, 10% dextran sulfate, 200 μg/ml salmon sperm DNA, and 250 μg/ml tRNA). The sections were washed in 2 × SSC at room temperature, and then treated with 20 μg/ml RNase A for 30 min in 0.5 M NaCl, 10 mM Tris, and 1 mM EDTA at room temperature. The sections were then washed twice in 0.2 × SSC at 55°C, 30 min per wash. The sections were dried, exposed to Hyperfilm (Amersham, Arlington Heights, IL).

Data Analysis

Levels of BDNF and cyclophilin mRNA were determined by outlining the band on Northern blots or the regions of interest in situ hybridization sections and then quantified on a Macintosh-based ATTO Image analysis program, Version 3.01 (Tokyo, Japan). The results were then subjected to statistical analysis. Experiments containing groups of three or more were sub-

Figure 2. Influence of chronic coadministration (21 days) of Ro 20–1724 (Ro) with desipramine (DSP) or Org 4428 (Org) on BDNF mRNA in rat hippocampus. Levels of BDNF were measured 2 h after the last injection by Northern blot analysis. **(A)** Representative Northern blots for chronic coadministration: 1, sham (vehicle); 2, Ro; 3, DSP; 4, Org; 5, Ro + DSP; and 6, Ro + Org. **(B)** Quantitation of BDNF mRNA. Levels of mRNA were quantified by densitometry and standardized for the amount of mRNA in each lane by reprobing the filters with radiolabeled cyclophilin. Data are presented as percentage of sham (vehicle treatment) and are the mean ± S.E.M. of 5 to 6 rats for each treatment group. *$p < .05$ compared with vehicle control (ANOVA and Fisher's test).

jected to ANOVA, with a significance level of $p < .05$, and Fisher's post hoc test. Experiments containing groups of two rats were subjected to the Student's t-test, with a significance level of $p < .05$.

RESULTS

The influence of chronic coadministration (21 days) of a PDE4 inhibitor (rolipram or Ro 20–1724) and an antide-
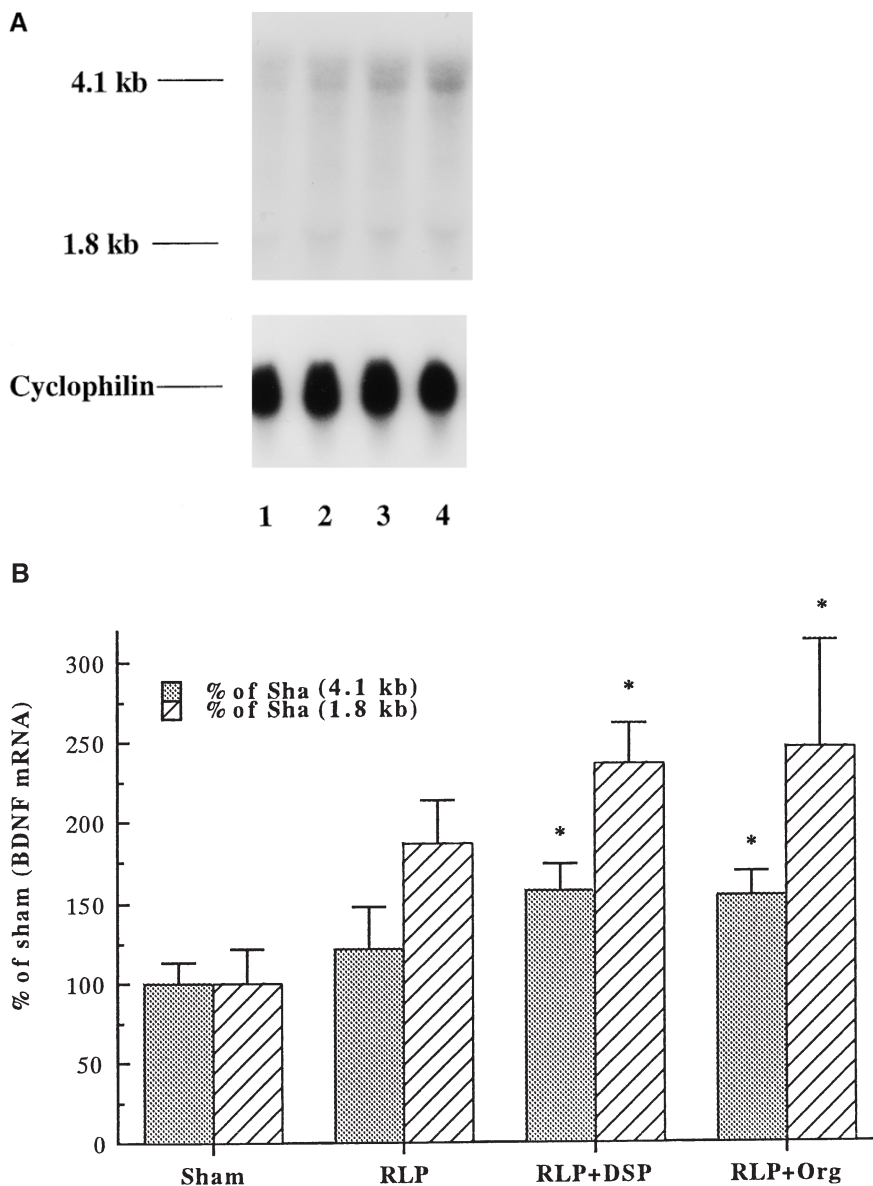
pressant (desipramine or Org 4428) on BDNF mRNA was examined by Northern blot analysis. These studies demonstrate that all four different combinations tested, including rolipram + desipramine, rolipram + Org 4428, Ro 20–1724 + desipramine, and Ro 20–1724 + Org 4428, significantly increased the expression of BDNF mRNA in hippocampus relative to chronic administration of vehicle (Figures 1 and 2). Levels of both major forms of BDNF mRNA (4.1 and 1.8 kb) by the coadministration paradigm were significantly higher

Table 1. Influence of Acute Treatment with a PDE4 Inhibitor and/or an Antidepressant on BDNF mRNA in Rat Hippocampus by Northern Blot Analysis.

Treatment	BDNF mRNA	
	4.1 kb	**1.8 kb**
Sham	100.0 ± 10.8	100.0 ± 5.1
Rolipram	96.4 ± 15.8	103.2 ± 8.3
Ro 20–1724	104.7 ± 1.4	102.7 ± 17.6
Desipramine	129.7 ± 21.5	96.5 ± 15.3
Org 4428	132.7 ± 35.9	117.5 ± 11.7
Rolipram + Desipramine	136.7 ± 14.3	110.5 ± 18.3
Rolipram + Org 4428	131.7 ± 34.9	113.2 ± 23.7
Ro 20–1724 + Desipramine	116.7 ± 14.1	101.3 ± 1.7
Ro 20–1724 + Org 4428	98.6 ± 13.4	99.7 ± 5.5

than those by the vehicle treatment. In contrast, statistical analyses indicated that BDNF induction by all four different combinations for 21 d was not greater than that by the two different single antidepressant treatment (Figures 1 and 2). Two BDNF mRNA bands, 4.1 kb and 1.8 kb, result from alternative polyadenylation sites within the 3' untranslated region (Timmusk et al. 1993). In addition, chronic administration of either a PDE4 inhibitor or an antidepressant alone did not significantly increase the expression of BDNF mRNA in the hippocampus, although there was a tendency for an upregulation by the individual treatments (Figures 1 and 2).

The time course for upregulation of BDNF mRNA by the coadministration paradigm was examined. We first examined the influence of acute treatments. The PDE4 inhibitor and antidepressants were administered and

Figure 3. Influence of coadministration of rolipram (RLP) with desipramine (DSP) or Org 4428 (Org) for 7 d on BDNF mRNA in rat hippocampus. Levels of BDNF were measured 2 h after the last injection by Northern blot analysis. **(A)** Representative Northern blots for chronic coadministration: 1, sham (vehicle); 2, RLP; 3, RLP + DSP; and 4, RLP + Org. **(B)** Quantitation of BDNF mRNA. Levels of mRNA were quantified by densitometry and standardized for the amount of mRNA in each lane by reprobing the filters with radiolabeled cyclophilin. Data are presented as percentage of sham (vehicle treatment) and are the mean $\pm$ S.E.M. of 4 to 5 rats for each treatment group. $*p < .05$ compared with vehicle control (ANOVA and Fisher's test).

two hours later levels of BDNF mRNA were determined. Acute coadministration of a PDE inhibitor and an antidepressant did not significantly influence levels of BDNF mRNA in hippocampus (Table 1). This included the four different combinations tested in the chronic study. In addition, acute administration of either a PDE4 inhibitor or an antidepressant alone did not significantly influence levels of BDNF mRNA (Table 1). We next determined the influence of the coadministration paradigm for longer treatment times. Statistical analyses indicated that the BDNF induction (both 4.1 and 1.8 kb transcript) by coadministration of rolipram with either desipramine or Org 4428 for either 7 or 14 d was significantly greater than that by the vehicle treatment (Figures 3 and 4). In addition, the BDNF induction by coadministration of rolipram with Org 4428 for 14 d was significantly higher than that by desipramine or Org 4428 (Figure 4). In contrast, administration of rolipram alone for 7 or 14 d or either antidepressant alone for 14 d did not significantly influence BDNF levels. Because antidepressant treatment alone for 14 d had no significant effect on BDNF mRNA, the influence of 7 d antidepressant treatment alone was not determined.

To elucidate the hippocampal cell layers where the expression of BDNF is regulated, we used in situ hybridization. Because we observed a significant upregulation of BDNF by Northern blot analysis after coadministration of rolipram with either desipramine or Org 4428 for 7 days, this treatment paradigm was used.
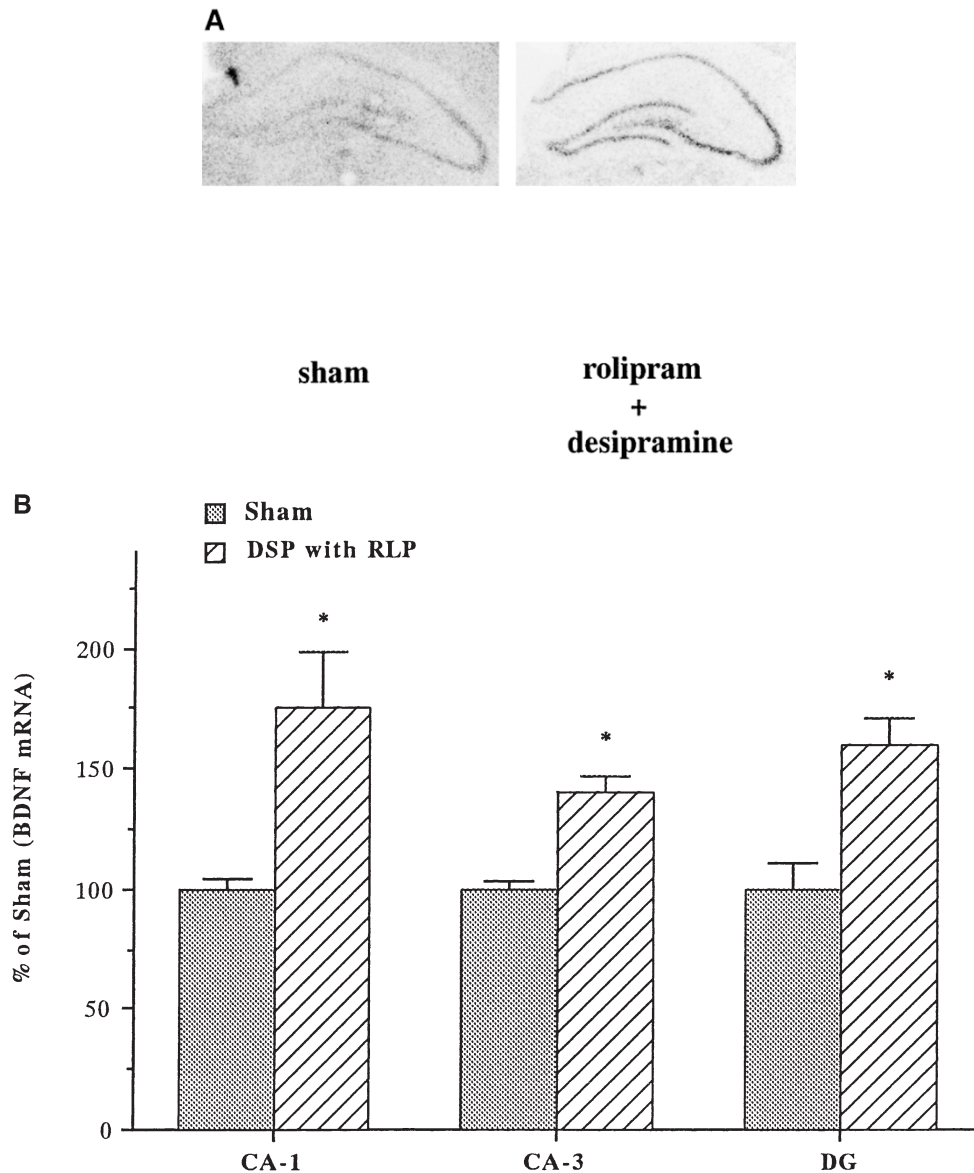
Coadministration of rolipram and either desipramine or Org 4428 significantly increased levels of BDNF mRNA in the major subfields of hippocampus, including the CA1 and CA3 pyramidal cell layers and the dentate gyrus granule cell layer (Figures 5 and 6). In contrast, acute coadministration of rolipram with either desipramine or Org 4428 did not significantly influence levels of BDNF mRNA in these subfields of hippocampus (data not shown).

DISCUSSION

This study demonstrates that chronic, but not acute, coadministration of a PDE4 inhibitor with an antidepressant significantly increases the expression of BDNF mRNA in rat hippocampus. The PDE4 inhibitors and antidepressant drugs tested influence the cAMP signal transduction system via different mechanisms: the PDE4 inhibitor blocks the breakdown of cAMP and the antidepressant increases norepinephrine levels, thereby activating the β-adrenergic receptors coupled to this second messenger pathway. In this way, repeated coadministration of a PDE4 inhibitor with an antidepressant could potentiate cAMP-mediated signal transduction and subsequently produce the marked induction of BDNF mRNA expression. This explanation is supported by previous studies demonstrating that activation of β-adrenergic receptors and the cAMP signal
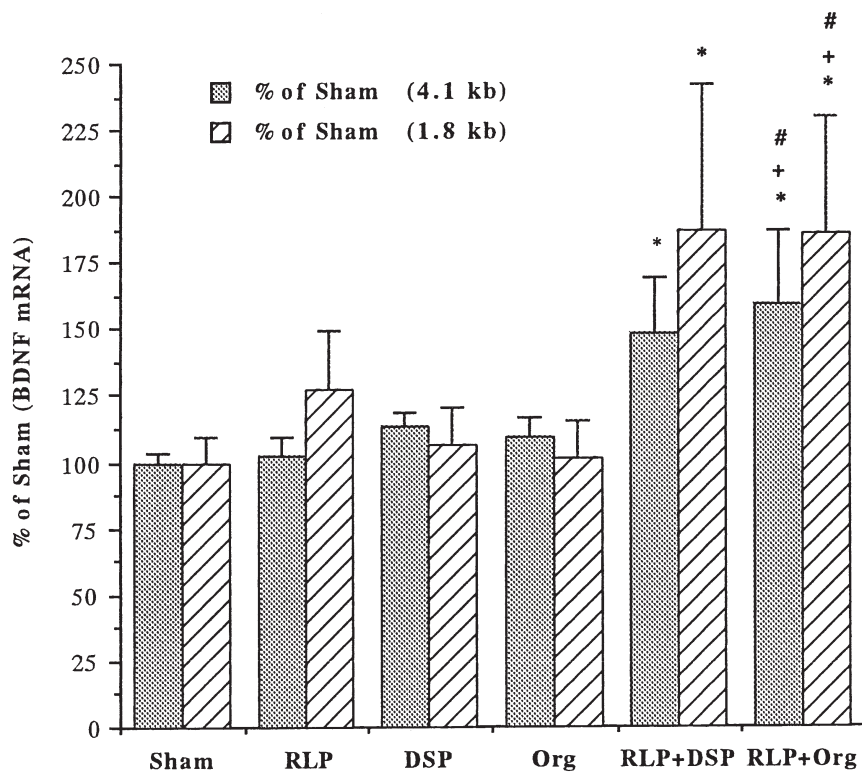
Figure 4. Influence of coadministration of rolipram (RLP) with desipramine (DSP) or Org 4428 (Org) for 14 d on BDNF mRNA in rat hippocampus. Levels of BDNF were measured 2 h after the last injection by Northern blot analysis. Levels of mRNA were quantified by densitometry and standardized for the amount of mRNA in each lane by reprobing the filters with radiolabeled cyclophilin. Data are presented as percentage of sham (vehicle treatment) and are the mean ± S.E.M. of 5 to 7 rats for each treatment group. *$p <$ .05 compared with vehicle control, $^{+}p <$ .05 compared with desipramine treatment, and #$p <$ .05 compared with Org 4428 treatment (ANOVA and Fisher's test).
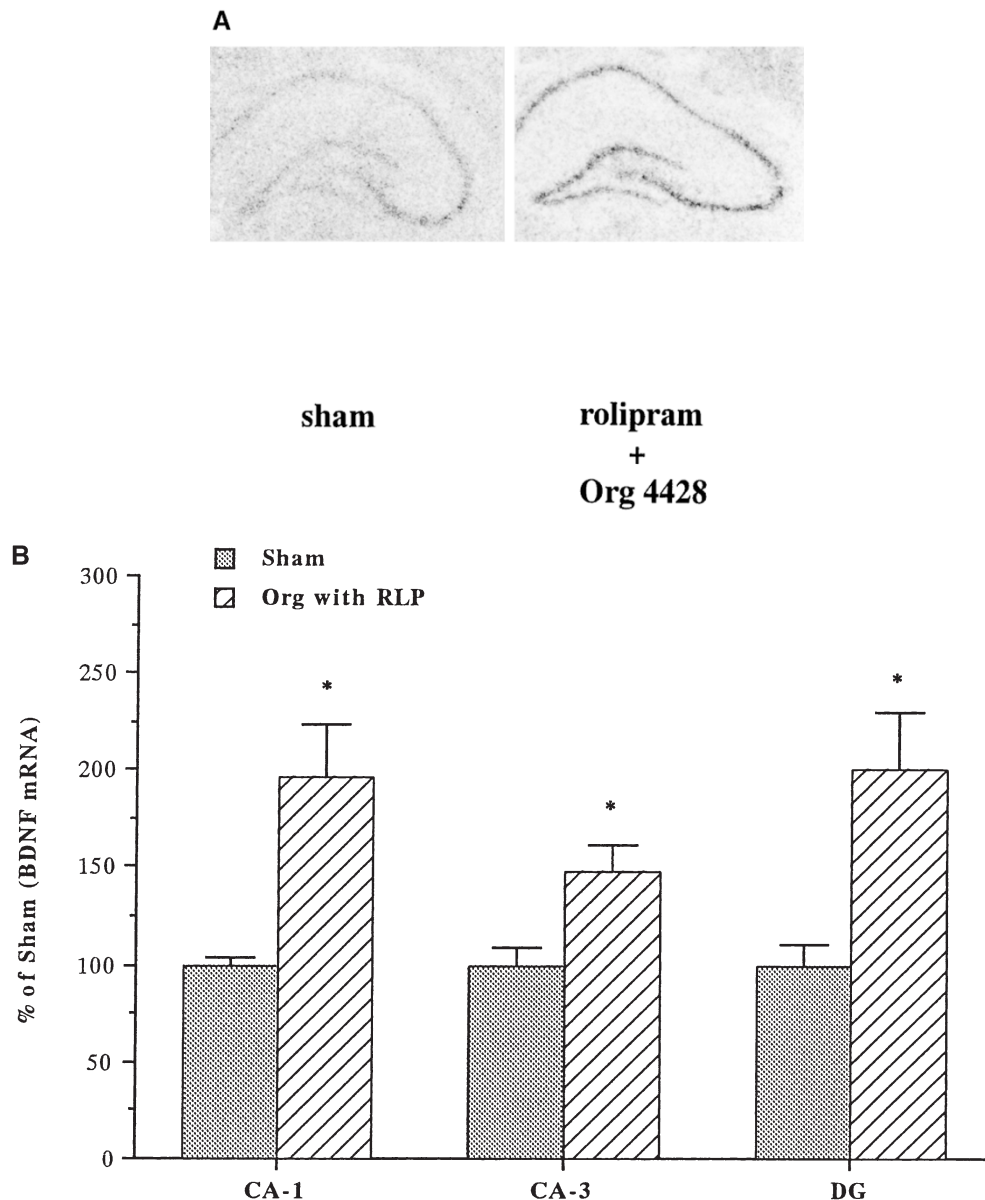
Figure 5.   Influence of coadministration of rolipram with desipramine for 7 d on BDNF mRNA in CA1 and CA3 pyramidal cell layers, and dentate gyrus (DG) granule cell layers. **(A)** Representative in situ hybridization autoradiogram for coadministration, left: sham (vehicle), right: rolipram + desipramine. **(B)** Quantitation of BDNF mRNA. Levels of BDNF mRNA were determined 2 h after the last injection by quantitative densitometry on in situ hybridization autoradiograms. Data are presented as percentage of sham (vehicle treatment) and are the mean ± S.E.M. of 4 to 5 rats for each treatment group. *$p < .05$ compared with vehicle control (the Student's t-test).

transduction cascade increase BDNF mRNA levels in primary cultures of brain and in cultured cell lines (Zafra et al. 1992; Condorelli et al. 1994; Bishop et al. 1997). On the other hand, it is also plausible that a metabolic interaction between rolipram and antidepressant drugs may increase plasma levels of the antidepressant and subsequently lead a greater upregulation of the BDNF mRNA expression. Although there has been no report examining metabolic interaction between these drugs, we can not rule out the possibility that the signif-

icant induction of BDNF mRNA observed in this study may reflect increased plasma levels of antidepressants.

Local infusion of BDNF into cerebral cortex is reported to prevent the damage of 5-HT axons induced by neurotoxin treatment and to promote the sprouting of these neurons (Mamonous et al. 1994). BDNF is also reported to potentiate 5-HT-mediated signal transduction in rat brain and 5-HT-related behaviors (Martin-Iverson et al. 1994; Siuciak et al. 1994). In addition, Smith and co-workers (1995), and Ueyama and col-

Figure 6. Influence of coadministration of rolipram with Org 4428 for 7 d on BDNF mRNA in CA1 and CA3 pyramidal cell layers, and dentate gyrus granule cell layers. **(A)** Representative in situ hybridization autoradiogram for coadministration, left: sham (vehicle), right: rolipram + Org 4428. **(B)** Levels of BDNF mRNA were determined 2 h after the last injection by quantitative densitometry on in situ hybridization autoradiograms. Data are presented as percentage of sham (vehicle treatment) and are the mean ± S.E.M. of 4 to 5 rats for each treatment group. *$p < .05$ compared with vehicle control (the Student's *t*-test).

leagues (1997) have demonstrated that chronic stress down-regulates the expression of BDNF mRNA in rat hippocampus, raising the possibility that decreased expression of BDNF might contribute to the atrophy and degeneration of neurons in response to chronic stress (see Duman et al. 1997). However, Kuroda and McEwen (1998) failed to observe a similar effect in response to restraint stress, suggesting that down-regulation of BDNF may not be a common action of all types of stress. The results of this study suggest that coadmin-

istration of a PDE4 inhibitor could potentiate neurotrophic effects induced by antidepressant treatment and thereby facilitate antidepressant efficacy. However, further studies will be required to determine the exact neurotrophic effects of a PDE4 inhibitor when coadministered with an antidepressant.

In a previous study, we demonstrated that expression of BDNF mRNA is significantly increased by coadministration of rolipram with imipramine for 7 days, but not by either drug alone for the same time (Nibuya

et al. 1996). The current study confirms and extends this work. While 10 mg/kg of rolipram was administered in the previous study, 1.25 mg/kg of rolipram was used in the present study. Preclinical studies undertaken to examine the antidepressant efficacy of rolipram demonstrate that a dose of 0.3 to 1.25 mg/kg of rolipram is sufficient to exert an antidepressant effect in rats (Przegalinski and Bigajska 1983; O'Donnell 1993). In contrast, the higher dose of rolipram (10 mg/kg) used in the previous study has some adverse effects, including decreased food intake and weight loss. In our previous report we also found that chronic administration of rolipram (2 mg/kg, 14 days) resulted in a significant upregulation of BDNF mRNA (Nibuya et al. 1996). However, in the present study, chronic administration of rolipram at a lower dose (1.25 mg/kg) for 14 or 21 d did not significantly influence levels of BDNF mRNA, although there was a tendency for increased expression. It is possible that the slightly higher dose of rolipram used in the previous study was sufficient to induce the significant increase in BDNF mRNA expression relative to the result observed in the present study. However, based on previous preclinical studies, the dose of rolipram in the present study is more consistent with the doses required to achieve antidepressant actions, without unwanted side effects.

Several clinical studies have demonstrated that rolipram has antidepressant efficacy that is equivalent to that of a tricyclic antidepressant (Bertolino et al. 1988; Bobon et al. 1988; Eckmann et al. 1988; Hebenstreit et al. 1989; Scott et al. 1991). Moreover, a recent case report has demonstrated that coadministration of papaverine, nonselective PDE inhibitor, and venlafaxine is effective in the treatment of refractory major depression (Malison et al. 1997). These studies support the hypothesis that a PDE4 inhibitor augments the therapeutic efficacy of antidepressants. In addition, forskolin, a plant disterpene that directly activates adenylyl cyclase, is reported to have antidepressant efficacy in depressed patients (Bersudsky et al. 1996). Taken together, this work supports the hypothesis that activation of the cAMP signal transduction cascade and increased expression of BDNF may be a common target of antidepressant treatment (Duman et al. 1997).

In summary, the results of the present study indicate that repeated coadministration of a PDE4 inhibitor potentiates the induction of BDNF mRNA in response to repeated administration of an antidepressant. The coadministration paradigm shortens the time required for significant induction of BDNF mRNA in response to antidepressant administration. The results suggest that coadministration of a PDE4 inhibitor with an antidepressant may be an efficacious pharmacotherapy for major depression. Further studies to determine the efficacy and safety of repeated coadministration are required to elucidate the potential clinical usefulness of this combination therapy.

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