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Rapid communication

Chronic [D-Ala^2, D-Leu^5]enkephalin treatment increases the nerve growth factor in adult mouse brain

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Abstract

The δ opioid peptide [D-Ala^2, D-Leu^5]enkephalin (DADLE) has been shown to enhance the survival of dopaminergic neurons. Here, we found that chronic treatment with DADLE caused a significant increase in nerve growth factor (NGF) in the hippocampus and the midbrain of adult albino Swiss (CD-1) mice, but not in the striatum or frontal cortex. Glia-derived neurotrophic factor (GDNF) was not significantly affected. Thus, the neuroprotective action of DADLE may be mediated in part by NGF.

Keywords: DADLE ([D-Ala^2, D-Leu^5]enkephalin); NGF (Nerve growth factor); GDNF (Glia-derived neurotrophic factor)

The δ opioid peptide [D-Ala^2, D-Leu^5]enkephalin (DADLE), a stable analog of δ opioid pentapeptide leucine–enkephalin (tyrosine–glycine–glycine–phenylalanine–leucine), not only prolongs the survival of peripheral organs but also protects against neuronal deterioration in the central nervous system (Su, 2000). For example, DADLE blocked the methamphetamine-induced degeneration of dopaminergic terminals in the brain of albino Swiss (CD-1, or "Crl:CD-1(ICR) BR") mice (Tsao et al., 1998) and enhanced the survival of grafted dopaminergic neurons in rat brain (Borlongan et al., 2000). The mechanisms underlying the protective actions of DADLE are not totally clarified. DADLE apparently acts as an antioxidant (Tsao et al.,1998) and as an antiapoptotic agent, blocking the translocation of cytosolic Bax to mitochondria and inhibiting Bax polymerization (Tsao and Su, 2001). However, other mechanisms may be involved in the neuroprotective action of DADLE.

Neurotrophic factors, including nerve growth factor (NGF), are known to play important roles in the development and survival of neurons (Sofroniew et al., 2001). Opioids have been known to affect neuronal development. Thus, there are reports that opioid agonists and antagonists regulate NGF levels in the neonatal rodent brain. For example, the opioid antagonist naltrexone increased the level of NGF in the striatum of neonatal rats (Mitsuo and Schwartz, 1993). Opioid receptor agonists, such as methadone and buprenorphine, were reported to reduce the level of NGF in the striatum of newborn rats (Wu et al., 2001). However, to our knowledge, the effect of opioids on NGF levels in the brain of adult animals has never been reported.

Thus, because DADLE is neuroprotective in adult animals (Tsao et al., 1998) and because neurotrophic factors are neuroprotective in the brain, we examined whether DADLE affects the level of neurotrophic factors including NGF and glia-derived neurotrophic factor (GDNF) in adult CD-1 mice.

CD-1 mice (50 g; about 12 weeks old from Charles River Labs, USA) received a once-daily injection (i.p.) of DADLE (16 mg/kg; Multiple Peptides, CA, USA) for 8 days. Mice were killed 24 h after the last injection of DADLE and the brains were removed, dissected, and frozen at −80 °C until use in the Enzyme-Linked ImmunoSorbant Assay (ELISA) to determine the immunoreactive concentrations of NGF and GDNF. On assay days, brain tissues were homogenized in lysis buffer (137 mM NaCl, 20 mM Tris–HCl, 1% NP-40, 10% glycerol, 1 mM PMSF [phenylmethylsulfonyl fluoride], 10 μg/ml

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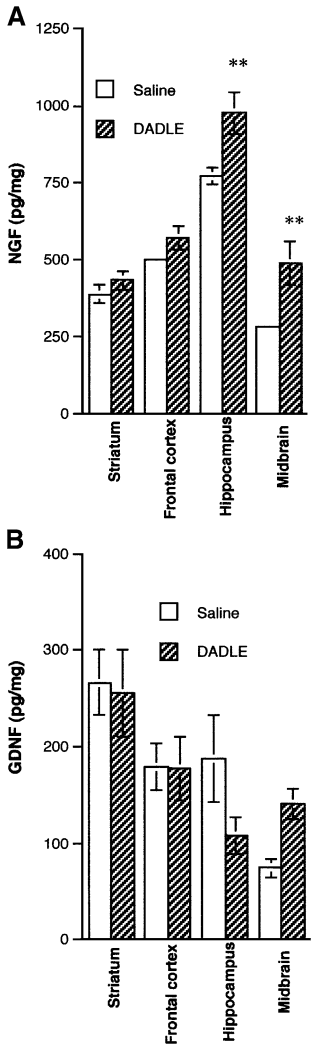

Fig. 1. Effects of chronic DADLE treatment on the content of immunoreactive NGF and GDNF in adult CD-1 mouse brain. Mice were treated with DADLE once daily (16 mg/kg; i.p.) for 8 days before assaying for NGF and GDNF by ELISA. The NGF and GDNF contents are expressed as pg per mg of total protein in the lysate sample. Data represent means ± S.E.M. (A) NGF contents in different brain regions. $N=5$ for the saline group; $N=6$ for the DADLE group. $^{**}P<0.01$ compared to the saline group. Note: S.E.M. for the saline bars in the frontal cortex and midbrain are too small to show. (B) GDNF contents in different brain regions. Note: $P=0.057$ for hippocampus; $P=0.09$ for midbrain. $N=4-6$ per group.

aprotinin, 1 μg/ml leupeptin, 0.5 mM sodium orthovanadate; pH 8.0) and the resultant lysates were acidified to pH 2.0 for 30 min. The sample was then neutralized to pH 7.4 and used for ELISA. Sandwich-style ELISAs were performed with the NGF Emax ImmunoAssay System kit (Promega, Madison, WI, USA) for NGF, or the GDNF assay (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. Detailed procedures are described elsewhere (Wang et al., 2001). NGF and GDNF concentrations are expressed as pg NGF or GDNF per mg of total protein in each sample. One-way analysis of variance (ANOVA) followed by Fisher's post hoc test was used for statistical analysis with the significance level set at $P<0.05$. Treatment of animals was in accordance with NIH guidelines.

The chronic treatment of adult CD-1 mice with DADLE caused a moderate but significant increase in NGF in the hippocampus (26% compared to controls; Fig. 1A). In the midbrain, NGF was strongly increased (73%, $P<0.01$; Fig. 1A) in DADLE-treated animals. The NGF level in the striatum or frontal cortex was not affected by the DADLE treatment (Fig. 1A). GDNF levels in all brain areas were not significantly altered by the DADLE treatment (Fig. 1B).

Thus, we have demonstrated that chronic DADLE causes region-specific NGF increases in the adult mouse brain. Our results suggest that the neuroprotective property of DADLE may be related to NGF. Specifically, the increase in NGF caused by DADLE in the midbrain, where a number of dopaminergic cell body exist (e.g., substantia nigra), may have a bearing on the ability of DADLE to block the dopaminergic terminal degeneration caused by methamphetamine in mice (Tsao et al., 1998) or its ability to block the loss of tyrosine hydroxylase-immunoreactive neurons in the substantia nigra in 6-hydroxydopamine-treated Parkinsonian rats (Borlongan et al., 2000). At present, we do not know if the NGF-inducing effect of DADLE involves opioid receptors. Nevertheless, the increase in NGF induced by DADLE in the adult rodent brain may have significant clinical implications for the therapeutic use of DADLE in treating neurodegenerative diseases.

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