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[D-Ala^2, D-Leu^5]enkephalin blocks the methamphetamine-induced c-*fos* mRNA increase in mouse striatum

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Abstract

The administration of methamphetamine caused an increase of c-*fos* mRNA in the striatum of the mouse. A systemic injection of the δ-opioid receptor agonist, [D-Ala^2, D-Leu^5]enkephalin (DADLE), attenuated the c-*fos* mRNA increase induced by methamphetamine. These results suggest that endogenous δ-opioid peptides might counteract certain genomic influences exerted by psychostimulants such as methamphetamine.

Keywords: DADLE ([D-Ala^2, D-Leu^5]enkephalin); c-*fos* mRNA; Methamphetamine; Opioid

[D-Ala^2, D-Leu^5]enkephalin (DADLE) is a δ-opioid receptor agonist which possesses relatively high affinity for δ-opioid receptor. DADLE can cause analgesia as well as an antiepileptic effect in animals. It was also reported that infusion of DADLE induces hibernation in summer-active ground squirrels (Oeltgen et al., 1988) and enhances the ex corpora preservation of organ (Oeltgen et al., 1996). Further, a pretreatment of DADLE protects the myocardium against the ischemia-reperfusion damage in isolated rabbit hearts (Bolling et al., 1998). These results suggest that DADLE might, via unknown mechanisms, possess a tissue protective property. As the survival of tissues depends largely on the oxidative state of tissue, we speculated that DADLE may also protect against certain types of central nervous system damage. Indeed, we showed that DADLE protects against prolonged striatal dopaminergic terminal damage induced by multiple administrations of methamphetamine (Tsao et al., 1998).

Methamphetamine is a well-known psychostimulant of abuse which induces severe long-lasting neurotoxic effect at high doses. Although the cellular and molecular sequelae involved in the neurotoxic action of methamphetamine are not completely understood, they are known to involve free radical formation (Cadet et al., 1994) as well as the activation of immediate early genes (e.g., c-*fos* and Zif268) (Nguyen et al., 1992; Hirata et al., 1998) and of certain transcription factors (Sheng et al., 1996). Because DADLE attenuates the methamphetamine-induced striatal dopaminergic terminal damage (Tsao et al., 1998), we were interested in examining a possibility that DADLE might also affect the expression of genes known to be influenced by methamphetamine. This study examined the effect of DADLE on methamphetamine-induced increase in the mRNA of an immediate early gene, c-*fos*.

Male CD-1 mice (Charles River) weighting 25–30 g were used. All animal care and use procedures were according to the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the local Animal Care and Use Committee of the National Institute on Drug Abuse. Mice received 25 mg/kg methamphetamine or saline via the intraperitoneal route. DADLE (20 mg/kg) or saline was injected intraperitoneally 30 min before the methamphetamine injection. Animal brains were rapidly removed after methamphetamine injection (0.5, 1, 3, and 8 h) and the total RNA was extracted from the mouse striatum according to a published method (Nguyen et al., 1992). For Northern blot analysis, total RNA (10 μg/lane) was electrophoresed and transblotted directly onto a nylon membrane (Hybond N, Amersham). Oligonucleotide probe (40 mer, Calbiochem, San Diego) was used to detect the c-*fos* mRNA. The probe was labeled with [^{32}P]dCTP using 3′-terminal deoxynucleotidyl transferase (Amersham, Piscataway) and hybridized to the membrane. Analyses of resulting bands

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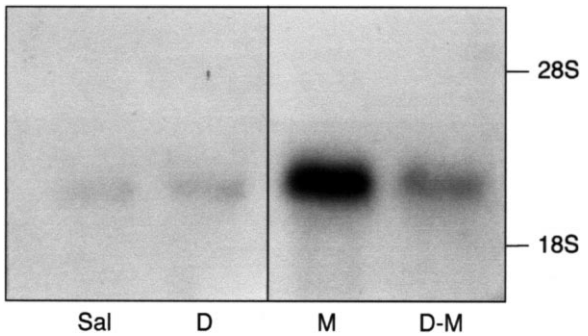

Fig. 1. Representative Northern blot analysis of c-*fos* mRNA in the striatum of mice after methamphetamine injection with and without the [D-Ala^2, D-Leu^5]enkephalin (DADLE) pretreatment. DADLE (20 mg/kg, i.p.) or saline was injected 30 min prior to the methamphetamine injection (25 mg/kg, i.p.). Each band represents the c-*fos* mRNA level 1 h after saline or drug administration. Sal, saline + saline; D, DADLE + saline; M, saline + methamphetamine; D–M, DADLE + methamphetamine. The positions of the major ribosomal bands (18 S and 28 S) are indicated. Results from one animal per treatment group are presented. The experiment was repeated in three or four animals per group with similar results. See text for values and statistical analyses.

were quantified using a Macintosh computer-based image analysis system (Image, NIH). Densitometrically determined intensities of c-*fos* mRNA were normalized to 18 S rRNA. One way analysis of variance followed by Fisher's PLSD as a post hoc test was used for statistical analyses with the significance level set at $p < 0.05$.

Methamphetamine (25 mg/kg) injection induced increases in c-*fos* mRNA in a time-dependent manner. One hour after the methamphetamine administration, the c-*fos* mRNA level increased considerably in the striatum to $861.3 \pm 94\%$ (mean $\pm$ S.E.M.; $N = 3$) of that of the control level (control as 100% with an interassay variation range of 2%; $N = 4$) ($p = 0.0001$). The increase of c-*fos* mRNA induced by methamphetamine subsided to the basal level 8 h after the methamphetamine administration. DADLE (20 mg/kg) did not change the c-*fos* mRNA level by itself ($105.3 \pm 4\%$ [$N = 3$] of control). The pretreatment with DADLE, however, markedly attenuated the methamphetamine-induced increase in c-*fos* mRNA ($256.3 \pm 36\%$ [$N = 4$] of control; $p < 0.0001$ when compared with methamphetamine alone; see Fig. 1).

These results indicate that DADLE attenuates the induction of the immediate early gene by toxic doses of methamphetamine in mice striatum and suggest that DADLE can counteract the genomic influences exerted by methamphetamine-like psychostimulants. The activation of c-*fos* has been suggested to be related to the neurotoxicity caused by methamphetamine. For example, Hirata et al. (1998) demonstrated that induction of c-*fos* mRNA by methamphetamine was significantly attenuated in the striatum of CuZn superoxide dismutase transgenic mice which also exhibit a high degree of resistance to the neurotoxic effect of methamphetamine. Thus, the attenuation of c-*fos* mRNA expression by DADLE may contribute to the DADLE protection against methamphetamine-induced neurotoxicity.

The exact mechanism underlying the antagonistic action of DADLE in the increase of c-*fos* mRNA caused by methamphetamine is unknown. However, since free radicals such as superoxide anions are implicated in the increase of c-*fos* mRNA caused by methamphetamine (Cadet et al., 1994; Hirata et al., 1998) and since DADLE has been shown by us to act directly to sequester superoxide anion and hydroxyl free radical formations in vitro (Tsao et al., 1998), it is tempting to speculate that DADLE might attenuate the methamphetamine-induced increase of c-*fos* mRNA via a mechanism involving the attenuation of free radical formation. As such, our results indicate a potential use of DADLE in attenuating psychostimulant-induced genomic influences and suggest that endogenous δ-opioid peptides may possess as yet to be fully recognized actions against cellular damage, even at the genomic level.

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