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European Journal of Pharmacology 372 (1999) R5–R7

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Reversal by [D-Ala^2,D-Leu^5]enkephalin of the dopamine transporter loss caused by methamphetamine

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Received 6 April 1999; accepted 7 April 1999

Abstract

A single administration of 40 mg/kg (i.p.) of methamphetamine caused a loss of dopamine transporter in the striatum of albino Swiss (CD-1) mouse for at least 3 weeks. The administration of a single dose of [D-Ala^2,D-Leu^5]enkephalin (DADLE) (18 mg/kg, i.p.), given at day 14 after the administration of methamphetamine, caused a significant, transient restoration of dopamine transporter level in the striatum. These results suggest that δ-opioid peptide DADLE is able to reverse the neuronal damage caused by methamphetamine.

Keywords: DADLE ([D-Ala^2,D-Leu^5]enkephalin); Methamphetamine; Neurotoxicity

[D-Ala^2,D-Leu^5]Enkephalin (DADLE) is a stable analog of endogenous δ-opioid leucine enkephalin. DADLE can induce hibernation in summer-active ground squirrels (Oeltgen et al., 1988) and to enhance the functional recovery of isolated hearts against ischemic insult (Bolling et al., 1998). Further, in mouse brain, the long-term loss of dopamine transporter and the increase of an immediate early gene c-*fos* caused by methamphetamine were shown to be blocked by a pretreatment of DADLE (Tsao et al., 1998; Hayashi et al., 1999). These results indicate that DADLE possesses tissue protective properties in the periphery as well as in the brain. Those studies, however, did not examine whether DADLE may reverse an existing damage caused by previous insults. The purpose of this study was therefore to investigate whether DADLE might reverse the dopaminergic terminal damage, as indicated by the dopamine transporter loss (Wilson et al., 1996), in the brain of albino Swiss (CD-1, or 'Crl:CD-1 (ICR) BR' from Charles River Labs, USA) mice previously treated with methamphetamine.

Male CD-1 mice received a high dose of methamphetamine (40 mg/kg, i.p.). On day 14 after the methamphetamine administration, animals received an injection of DADLE (Multiple Peptide systems, CA; 18 mg/kg, i.p.). Animals were killed by decapitation on days 15, 16, and 21, respectively, post-methamphetamine administration and their brains removed and processed for the autoradiographic examination of dopamine transporter level according to a published procedure (Tsao et al., 1998) by using a dopamine transporter marker 3β-(4-[^{125}I]iodophenyl)-tropane-2β-carboxylic acid isopropyl ester ([^{125}I]RTI-121 (Boja et al., 1995)). A Macintosh computer-based analysis system (Image, NIH) was used for the quantification of [^{125}I]RTI-121 binding to three regions of the brain: medial striatum, lateral striatum, and the nucleus accumbens. Data were first analyzed by two-way analysis of variance (ANOVA) with time and drug treatment considered as factors and then subjected to a stringent post-hoc analysis using the Scheffé's test. The significance level was set at $P < 0.05$.

There were significant main overall effects of drug and time ($F = 45.126$, $P = 0.0001$). A high dose of methamphetamine (40 mg/kg, i.p.) caused a long-term loss of dopamine transporter in the striatum. The dopamine transporter levels post-methamphetamine injection on days 15, 16, and 21, respectively, were significantly lower than those of the corresponding saline + saline controls or the saline + DADLE controls in both aspects of striatum (P's < 0.0011 for medial striatum; P's < 0.0001 for lateral striatum; Fig. 1A,B).

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00240-X

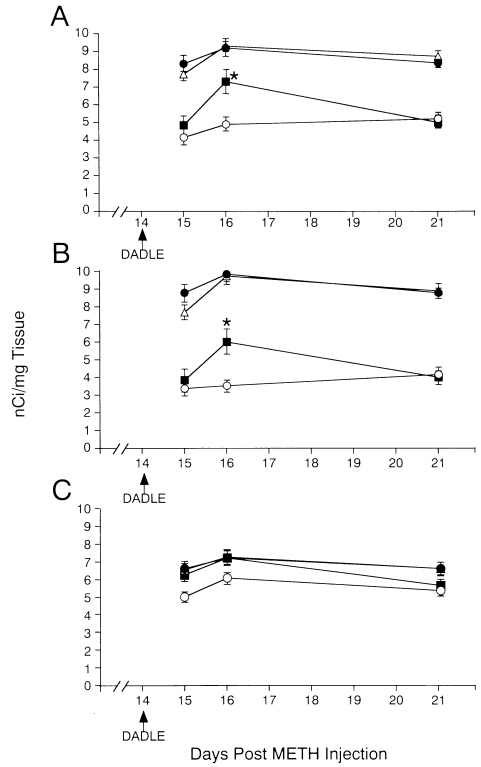

Fig. 1. Reversal by DADLE of the dopamine transporter loss induced by methamphetamine. Albino Swiss (CD-1) mice received a single injection of methamphetamine (40 mg/kg, i.p.). DADLE (18 mg/kg, i.p.) was given on day 14 post-methamphetamine administration. Animals were killed on days 15, 16, and 21, respectively, after methamphetamine administration and brains processed for the autoradiographic examination of dopamine transporter using [^{125}I]RTI-121. Specific binding of the radioligand is shown on *Y*-axis (mean ± S.E.M.). *X*-axis represents days post-methamphetamine injection. (A) Medial striatum; (B) lateral striatum; (C) nucleus accumbens. Closed circles, saline + saline ($N = 6$); open triangles, saline + DADLE ($N = 6$); closed squares, methamphetamine + DADLE ($N = 9$); open circles, methamphetamine + saline ($N = 9$). Note: Although not marked, all values of the methamphetamine + saline groups in (A) and (B) are significantly different from those of either saline + saline or saline + DADLE group on corresponding days (P's < 0.0011). $^{*}P < 0.0146$ for medial striatum, $^{*}P < 0.024$ for lateral striatum, for day 16 when compared to the methamphetamine + saline group.

Within the same treatment group, however, no significant difference was found between any two time points for all curves in Fig. 1, except for the methamphetamine + DADLE group. It has to be mentioned that although the DADLE treatment alone (i.e., saline + DADLE, Fig. 1A–C) tended to increase the dopamine transporter level, the differences between any two time points in this group of animals in all three areas examined did not reach statistical significance (e.g., $P = 0.6386$ between days 15 and 16 in the saline + DADLE group in the lateral striatum, Fig. 1B). The methamphetamine + DADLE group of animals exhibited a significantly higher level of dopamine transporter in both aspects of striatum on day 16 when compared to day 15 ($P = 0.0057$ for medial striatum; $P = 0.0115$ for lateral striatum; Fig. 1A,B). The dopamine transporter level of the methamphetamine + DADLE group in the medial striatum on day 16 was in fact restored to a level not significantly different from that of controls ($P = 0.3464$; Fig. 1A) and the level was significantly higher than that of the methamphetamine + saline group ($P = 0.0146$). In the lateral striatum, the dopamine transporter level on day 16 in the methamphetamine + DADLE group was also significantly higher than the methamphetamine + saline group ($P = 0.0240$), although it was not restored to the level of either control group (P's < 0.0002 when compared to controls). The level of dopamine transporter returned to the control level on day 21 in both aspects of striatum. In the nucleus accumbens, no significant difference could be found between any two data points (P's = 0.0670 to 0.9999; Fig. 1C).

Thus, our results show for the first time that a single administration of moderately high dose of methamphetamine is able to cause a long-term loss of dopamine transporter. The results also show that the δ-opioid peptide DADLE is able to reverse the loss of dopamine transporter in the striatal dopaminergic terminals caused by methamphetamine. Since the dopaminergic terminal damage caused by methamphetamine involves free radicals (Cadet et al., 1994; Fleckenstein et al., 1997) and since DADLE is able to sequester the free radical-induced damage in synaptosomal membrane preparation (Tsao et al., 1998), it is tempting to speculate that this action of DADLE may involve the restoration of the integrity of the membrane of dopaminergic terminals. The endogenous δ-opioid peptide may therefore play certain as yet unrecognized important physiological roles, such as neuroprotection, in the brain.

Acknowledgements

The partial support of the Basic Neurobiology and Biological Systems Research Branch, Division of Basic Research, NIDA, NIH is appreciated.

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