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Short communication

Methamphetamine alters prodynorphin gene expression and dynorphin A levels in rat hypothalamus.

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Abstract

Chronic administration of morphine or cocaine affects opioid gene expression. To better understand the possible existence of common neuronal pathways shared by different classes of drugs of abuse, we studied the effects of methamphetamine on the gene expression of the opioid precursor prodynorphin and on the levels of peptide dynorphin A in the rat brain. Acute (6 mg/kg, intraperitoneally, i.p.) and chronic (6 mg/kg, i.p. for 15 days) methamphetamine markedly raised prodynorphin mRNA levels in the hypothalamus, whereas no effect was observed in the hippocampus. Dynorphin A levels increased after chronic treatment in the hypothalamus and in the striatum, whereas no significant changes were detected after acute treatment. These results indicate that methamphetamine affects prodynorphin gene expression in the hypothalamus, which may be an important site (also for its relevant neuroendocrine correlates) for opioidergic mechanisms activated by addictive drugs.

Keywords: Prodynorphin; Gene expression; Methamphetamine; Brain; (Rat); Hypothalamus

1. Introduction

It is well known that repeated administration of drugs of abuse leads to the development of tolerance to some of their pharmacological effects (for reviews see Koob, 1992; Nestler et al., 1993; Hyman, 1996). These phenomena have long been investigated in the brain, where neurochemical and functional changes occur in the neurotransmitter systems after chronic exposure to addictive drugs (Beitner-Johnson and Nestler, 1991; Cox, 1993; Nestler et al., 1993). Several studies reported the involvement of the endogenous opioid system in the mechanisms underlying the effects of chronic exposure to opiates and cocaine (Uhl et al., 1988; Mocchetti et al., 1989; Cox, 1993; Daunais et al., 1993; Romualdi et al., 1991, 1995, 1996). Opioid peptides and their receptors may play a role in the positive reward system of the brain. The hypothesis is therefore proposed that endogenous opioid pathways might contribute to the acute and long-term effects of both opiate and stimulant drugs (Cox, 1993; Nestler et al., 1993; Hyman, 1996). Among such stimulants, amphetamines represent one of the major groups of psychoactive drugs that produce long-term biochemical changes when chronically administered. The effects of acute and chronic injection of amphetamines mainly involve the striatal pathways, and a relevant body of literature indicates that both acute and chronic amphetamine or methamphetamine increase dynorphin immunoreactivity and mRNA levels in the striatum (Hanson et al., 1988; Smith and McGinty, 1994; Jaber et al., 1995; Wang and McGinty, 1995).

Therefore in the light of these observations and in order to investigate the possibility that different classes of drugs of abuse use common neuronal pathways in the development of biochemical changes following chronic exposure, we investigated the effects of the acute and chronic administration of methamphetamine on the expression of the gene for the endogenous opioid precursor prodynorphin in rat brain. Levels of prodynorphin mRNA, as well as those of the opioid peptide dynorphin A (immunoreactive-dynorphin A), were measured in the hypothalamus, hippocampus and striatum after the rats were subjected to

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acute or chronic (15 days) intraperitoneal (i.p.) administration of methamphetamine.

2. Materials and methods

Male Sprague–Dawley rats (Charles River, Italy) weighing 200–250 g were used. Four groups of twelve animals were used in each experiment. Methamphetamine hydrochloride, or saline (0.2 ml/100 g), was administered i.p. to the rats at the dose of 6 mg/kg acutely or for 15 days.

Two hours after the acute treatment or the last injection of the chronic treatment the rats were killed, their brains were rapidly removed and the hypothalamus, hippocampus, striatum were dissected and frozen on dry-ice. Tissue from six of twelve rats from each group was processed for total RNA extraction and tissue from the other six was used for peptide extraction.

Total RNA was prepared according to the method of Chomczynski and Sacchi (1987) and quantitated by measurement of absorbance at 260 nm (1 OD/ml = 25 μg RNA/ml). The ratio OD260/OD280 > 1.8 provided an estimate of the purity of the total RNA.

Blots were hybridized with the probe BaPst, the restriction enzyme *BamHI* to PstI fragment (1 kb) of the rat genomic DNA complementary to the prodynorphin mRNA (Civelli et al., 1985). BaPst was labelled by the random priming method, using α-[^{32}P]dCTP to a specific activity of 7–9 × 10^5 cpm/ng.

A cDNA fragment recognizing β-actin mRNA (clone pHFβA-1, containing the full-length cDNA insert for human cytoplasmatic β-actin) was used as internal standard to hybridize the same blots (Gunning et al., 1983).

Total RNA from each tissue (20 μg) was electrophoresed through a 1% agarose gel containing 2.2 M formaldehyde, transferred by overnight capillary blotting to nylon membranes and then UV crosslinked and hybridized in an oven. After prehybridization for 3–6 h, blots were hybridized for 24 h at 42°C in a solution of 6 × SSC (sodium citrate solution), 1 × Denhardt's solution, 100 μg/ml denatured salmon sperm DNA, 0.1% SDS (Sodium Dodecyl Sulfate), 50% formamide, 10 mM Tris and 10% dextran sulfate, containing the probe at the concentration of 2–4 × 10^6 cpm/ml. X-ray films (Amersham β-max) were exposed to the hybridized blot backed by an intensifying screen (Dupont Cronex) at −70°C for 3–6 days. Blots were hybridized serially twice with probes directed against prodynorphin and β-actin mRNA. For β-actin mRNA hybridization, blots were prehybridized and hybridized as described elsewhere (Romualdi et al., 1996). Total RNA from treated animals was compared to RNA from control rats. The optical density of autoradiographic bands produced by prodynorphin and β-actin hybridization was determined using a Video Densitometer system (MDL 620). The ratios of prodynorphin mRNA/β-actin mRNA hybridization for treated or control animals were analyzed and then expressed as percentages of control (100%) for each experiment.

The opioid peptide dynorphin A (referred to as immunoreactive-dynorphin A content) was measured as previously described (Romualdi et al., 1995). A specific antiserum ('Lucia', kindly supplied by Prof. B.M. Cox) was used, diluted to give about 30% binding of ^{125}I-dynorphin A-(1–13) added (÷ 5000 cpm). In a typical assay, the IC_{50} was 10 fmol/assay tube (intra- and interassay coefficients of variation were 5% and 7%). Radioimmunoassays were carried out in 0.15 M sodium phosphate buffer (pH 7.4).

Data were statistically analyzed by analysis of variance (One-way ANOVA), followed by Newman–Keuls test.

3. Results

Under the conditions described, we detected hybridization to prodynorphin mRNA (size ca. 2.3–2.4 kb) with our cDNA probe. Methamphetamine was able to increase prodynorphin mRNA levels in the hypothalamus significantly after acute (214 ± 20% of control, $P < 0.01$ by Newman–Keuls test) and chronic treatment for 15 days (164 ± 18% of control, $P < 0.05$). Prodynorphin/β-actin mRNA optical density ratios are shown in Fig. 1. Prodynorphin gene expression was unaffected by acute or chronic methamphetamine administration in the hippocampus (92 ± 5% and 85 ± 5% of control, respectively; Fig. 1). In the striatum, acute and chronic methamphetamine did not change prodynorphin gene expression, under our experimental conditions (96 ± 5% and 106 ± 5% of control, respectively; Fig. 1). Internal control experiments were performed to confirm and validate these data. First of all, UV analysis of the total RNA (ribosomal 18S and 28S bands)

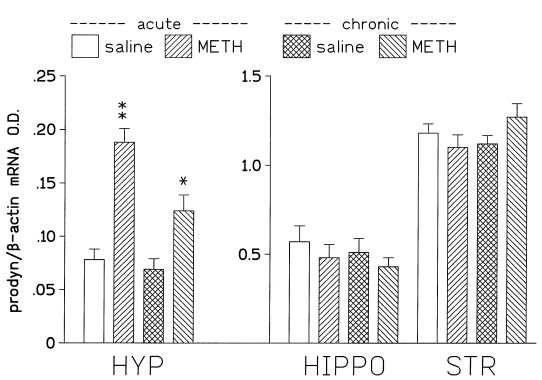

Fig. 1. Changes in prodynorphin mRNA levels in rat hypothalamus (HYP), hippocampus (HIPPO) and striatum (STR) after acute and chronic treatment with saline or methamphetamine (METH). Values represent the means ± S.E.M. ($n = 10–12$) of prodynorphin/β-actin mRNA optical density ratios for each tissue. Relative abundance of prodynorphin mRNA in autoradiograms from Northern analysis was quantitated by densitometry (* $P < 0.05$ and ** $P < 0.01$ vs. control, by Newman–Keuls test).

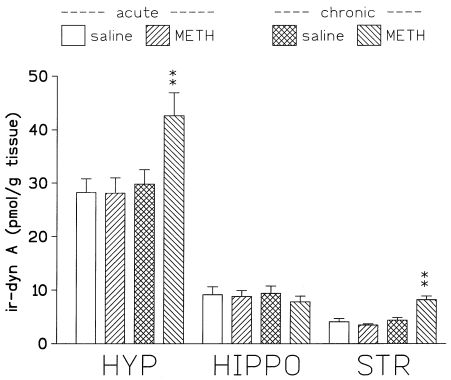

Fig. 2. Immunoreactive dynorphin A levels (ir-dyn A) in hypothalamus (HYP), hippocampus (HIPPO) and striatum (STR) after acute and chronic treatment with saline or methamphetamine (METH). Values represent the means ± S.E.M. ($n = 10–12$) of ir-dyn A pmol/g tissue (** $P < 0.01$ vs. control, by Newman–Keuls test).

after electrophoresis showed no differences in the intensity of single bands for control and treated rats, indicating that there were no differences between treatments in the amount of total RNA loaded in each lane. Furthermore, each blot was then serially hybridized with the cDNA probe to recognize β-actin mRNA. No differences in β-actin mRNA levels were found in either control or methamphetamine-treated animals in all the tissues examined.

In the hypothalamus, immunoreactive-dynorphin A levels increased after chronic treatment with methamphetamine (42.6 ± 4.3 vs. 27.9 ± 2.5 pmol/g tissue; $P <$ 0.01 by Newman–Keuls test, Fig. 2) but remained unchanged following acute administration (28.2 ± 2.9 vs. 29.7 ± 3.0 pmol/g tissue, Fig. 2).

In the hippocampus no changes were detected after either acute or chronic treatment with methamphetamine (8.8 ± 1.3 vs. 8.9 ± 1.1 and 7.8 ± 1.1 vs. 9.3 ± 1.4 pmol/g tissue, respectively; Fig. 2). In the striatum immunoreactive-dynorphin A levels were unaffected after a single injection (3.5 ± 0.2 vs. 3.9 ± 0.7 pmol/g tissue, Fig. 2), but increased significantly after chronic administration (8.2 ± 0.7 vs. 4.3 ± 0.5 pmol/g tissue; $P < 0.01$, Fig. 2).

4. Discussion

These results indicate that chronic exposure to methamphetamine affects prodynorphin gene expression, in a tissue-specific manner, in rat brain. In the hypothalamus, prodynorphin mRNA levels increased markedly, both after acute and chronic treatments, whereas no significant changes were observed in the rat hippocampus and striatum. Immunoreactive-dynorphin A levels in the rat hypothalamus were unaffected after acute methamphetamine treatment but increased after administration of methamphetamine for 15 days. At the same time, peptide levels remained unchanged in the hippocampus after acute and chronic treatments, whereas the 15-day methamphetamine treatment elicited a significant increase in the striatum.

Opiate agonists, acting on μ, k and δ opioid receptors, inhibit the biosynthesis of components of the endogenous opioid system in, namely, proenkephalin, proopiomelanocortin and prodynorphin, the rat brain (Uhl et al., 1988; Mocchetti et al., 1989; Romualdi et al., 1991). The hypothesis was put forward that alterations in the expression of genes coding for components of the endogenous opioid system might be involved in the mechanisms underlying the induction of tolerance to some pharmacological effects of opiate drugs. It has recently been shown that chronic cocaine produces tissue-specific effects on the opioid system (Daunais et al., 1993; Romualdi et al., 1996). In particular, we observed an inhibition of prodynorphin mRNA levels in the hypothalamus, whereas chronic exposure to cocaine increased prodynorphin biosynthesis in the striatum. These findings suggest that different mechanisms in these areas lead to the phenomena induced by chronic cocaine.

Methamphetamine, as well as opiates and cocaine, is also able to affect the endogenous opioid system. This is the first evidence that acute and chronic methamphetamine induces a marked stimulation of prodynorphin mRNA levels in the hypothalamus. This effect is the opposite of that observed after chronic treatment with opiates or cocaine (Romualdi et al., 1991, 1996). This different direction of changes in prodynorphin mRNA levels in the hypothalamus following chronic methamphetamine or opiates and cocaine might indicate that prodynorphin expression, which primarily occurs in the magnocellular neurons, is not always influenced in the same way by addictive drugs. The hypothalamus is an important site because of its neuroendocrine function, and dynorphin may participate in neuronal mechanisms involved in endocrine responses activated by addictive drugs. In this regard, some evidence of neuroendocrine correlates of psychostimulant treatment has been reported (Cabrera et al., 1994). The increase in immunoreactive-dynorphin A levels after chronic methamphetamine treatment might represent the functional consequence of the sustained stimulation of biosynthesis in this area and strengthen its possible role in the effects elicited by addictive drugs. Other authors also suggested the crucial role of the hypothalamus in the development of behavioral sensitization to amphetamines (Cole et al., 1990).

Under our experimental conditions, no changes in the prodynorphin system were detected in the hippocampus after acute and chronic methamphetamine treatments. In this regard, it is useful to remember that cocaine, which acts differently from opiates, failed to produce effects on prodynorphin gene expression in this area (Romualdi et al., 1996). Therefore, it is possible to speculate that opioids do not participate in methamphetamine- and cocaine-induced effects, whereas they are involved in opiate-exerted actions in this area (Romualdi et al., 1991).

As regards the striatum, at the 2-h time point we were not able to detect an increase in prodynorphin mRNA levels after acute and chronic exposure to metham-

phetamine. Several authors showed a significant stimulation of prodynorphin gene expression in the striatum (Hanson et al., 1988; Smith and McGinty, 1994; Jaber et al., 1995; Wang and McGinty, 1995) between 3 and 6 h, or later (Bronstein et al., 1996). In this study, the use of tissue homogenates for Northern analysis and the 2-h time point might possibly explain the apparently conflicting results in this structure. The increase in immunoreactive-dynorphin A levels after chronic methamphetamine might suggest an increase in the rate of enzymatic cleavage of the precursor.

Finally, these results are in agreement with those of Schad et al. (1995), who reported a reduction in the neurochemical effects of amphetamine elicited by naloxone, further supporting the involvement of opioids in the action of amphetamine.

In conclusion, these results suggest that not only opiates and cocaine, but also methamphetamine affect prodynorphin gene expression in rat brain in a tissue-specific manner. Different classes of addictive drugs might, at least partly, share common neuronal pathways involving endogenous opioids in the mechanisms triggered by their chronic administration (Koob, 1992).

Opioidergic pathways appear to be involved in the alterations induced by chronic exposure to methamphetamine, at least as far as the hypothalamus is concerned.

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