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ScienceDirect
Pharmacology, Biochemistry and Behavior 87 (2007) 232–240
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Changes in Proenkephalin mRNA expression in forebrain areas after amphetamine-induced behavioural sensitization

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Received 2 June 2006; received in revised form 17 April 2007; accepted 26 April 2007
Available online 1 May 2007

Abstract

Acute and repeated psychostimulant administration induces a long-lasting enhanced behavioural response to a subsequent drug challenge, known as behavioural sensitization. This phenomenon involves persistent neurophysiological adaptations, which may lead to drug addiction. Brain dopaminergic pathways have been implicated as the main neurobiological substrates of behavioural sensitization, although other neurotransmitters and neuromodulators may also participate. In order to investigate a possible involvement of opioid systems in amphetamine (AMPH) behavioural sensitization, we studied the AMPH-induced changes in Proenkephalin (Pro-Enk) mRNA expression in forebrain areas in both drug-naïve and AMPH-sensitized rats. Male Sprague–Dawley rats were sensitized to AMPH by means of a single AMPH (1 mg/kg s.c.) injection and the same dose was injected 7 days later to assess the expression of sensitization. Pro-Enk mRNA levels were evaluated by in situ hybridization in coronal brain sections. AMPH injection induced an increase in Pro-Enk mRNA expression in the nucleus accumbens and the medial–posterior caudate-putamen in drug-naïve rats. Challenge with AMPH to rats injected 1 week earlier with AMPH induced motor sensitization and increased and decreased Pro-Enk mRNA expression in the prefrontal cortex and the anterior medial caudate-putamen, respectively. Our results suggest that alterations in cortical and striatal enkephalinergic systems could contribute to the expression of AMPH behavioural sensitization.

Keywords: Amphetamine; Motor activity; Behavioural sensitization; Proenkephalin mRNA; Enkephalins; Opioid peptides

1. Introduction

Acute and repeated administration of psychostimulant drugs like amphetamine (AMPH) and cocaine induces a progressive and persistent hypersensitivity to their psychomotor activating effects to a subsequent drug challenge (Panayi et al., 2005; Post, 1980; Robinson et al., 1982; Robinson and Berridge, 1993; Vanderschuren et al., 1999; Wolf, 1998). This phenomenon, known as behavioural sensitization, has been suggested to involve persistent neurophysiological adaptations which may lead to drug addiction (Robinson and Berridge, 1993, 2001; Vezina, 2004; Wise and Bozarth, 1987). Brain dopaminergic (DAergic) pathways have been implicated as the main neurobiological substrates of behavioural sensitization (Kalivas, 1995; Wolf, 1998). The induction of AMPH behavioural sensitization involves the somatodendritic release of dopamine (DA) in the ventral tegmental area (VTA), as well as the release of glutamate from afferent terminals in the VTA projecting from the prefrontal cortex (pfc) (Cador et al., 1999; Kalivas, 1995). In contrast, the expression of this process implicates the release of DA from terminals in the nucleus accumbens (NAcc) and the caudate-putamen (CP) (Robinson and Becker, 1982; Robinson et al., 1988). Several neurotransmitters and neuromodulators have been shown to participate in the development and expression of AMPH behavioural sensitization besides DA and glutamate, including γ-aminobutiric acid (GABA) (Zhou et al.,

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doi:10.1016/j.pbb.2007.04.019

2004), acetylcholine (Ach) (Florin et al., 1992) and opioid peptides (Gonzalez-Nicolini et al., 2003; Magendzo and Bustos, 2003).

Interactions between DAergic and opioidergic systems in the brain have been revealed by anatomical and neurochemical studies (Mansour et al., 1988, 1995), particularly at the level of the mesolimbic and nigrostriatal pathways (Herz, 1995; Urwyler and Tabakoff, 1981). For instance, morphine and other mu (μ) opioid receptor agonists increase the extracellular DA levels in the NAcc (Devine et al., 1993; Di Chiara and Imperato, 1988; Urwyler and Tabakoff, 1981). Mu agonists induce locomotor activity when injected in the VTA and the NAcc (Cunningham and Kelley, 1992; Daugé et al., 1988; Druhan et al., 1993), and these effects are similar to those produced by AMPH (Druhan et al., 1993). In addition, delta (δ) opioid receptor agonists, injected in the NAcc and the striatum, induce motor activation (Cunningham and Kelley, 1992; Daugé et al., 1988), suggesting that opioid receptors may have a role in AMPH actions. Accordingly, naloxone, a non-selective opioid receptor antagonist, decreases the DA release and locomotor activity induced by AMPH (Feigenbaum and Howard, 1997; Hooks et al., 1992; Jones and Holtzman, 1994; Schad et al., 1995). Selective μ and δ opioid receptor antagonists decrease the stimulatory effect of acute AMPH on vertical activity when injected in the striatum (Gonzalez-Nicolini et al., 2003). Besides its locomotor stimulatory effect, acute AMPH increases the expression of Proenkephalin (Pro-Enk) mRNA in both the NAcc and the striatum (Gonzalez-Nicolini and McGinty, 2002; Gonzalez-Nicolini et al., 2003; Mao and Wang, 2003; Tzaferis and McGinty, 2001; Wang and McGinty, 1995, 1996a,b; Zhou et al., 2004), suggesting that the acute effects of the drug may also involve alterations in enkephalin biosynthesis.

Opioid peptides and their receptors have also been suggested to participate in the long-term behavioural effects of AMPH. The repeated administration of AMPH decreases and increases μ opioid receptor mRNA levels in the shell region of the NAcc and the striatum, respectively, and induces μ and δ opioid receptor mRNA expression in the VTA of sensitized rats 2 days after AMPH withdrawal (Magendzo and Bustos, 2003; Vecchiola et al., 1999). Naltrexone, a non-selective opioid receptor antagonist, reduces the rearing but not the locomotion induced by AMPH in sensitized rats (Balcells-Olivero and Vezina, 1997), suggesting that opioid peptides may be specifically involved in the rearing component of AMPH behavioural sensitization. The effects produced by the repeated administration of AMPH on Pro-Enk mRNA expression are less clear. Opposed effects have been reported in the NAcc and the striatum in response to AMPH (Wang and McGinty, 1995). Thus, the role of Pro-Enk mRNA expression in AMPH behavioural sensitization remains to be determined. The aim of this work was to investigate the changes in enkephalin biosynthesis associated to AMPH-induced behavioural sensitization in several forebrain areas (pfc, NAcc and CP) involved in the long-term effects of the drug. Behavioural sensitization to AMPH was induced by a single low dose of AMPH as previously reported (Panayi et al., 2005). We have shown that Pro-Enk mRNA expression was increased in the core and shell regions of the NAcc and the medial-posterior region of the CP (mpCP) after a single administration of AMPH to drug-naïve rats. In contrast, Pro-Enk mRNA levels in the pfc and the anterior–medial portion of the CP (amCP) were respectively increased and decreased after a challenge injection of AMPH to rats previously administered with AMPH. Our results indicate that AMPH induces changes in enkephalinergic transmission that could be linked to the neuroadaptations underlying behavioural sensitization.

2. Methods

2.1. Drugs

D-amphetamine sulphate (Sigma) was dissolved in saline (SAL) (1 mg/ml) and injected subcutaneously (s.c.) at a dose of 1 mg/kg.

2.2. Animals

Male Sprague–Dawley rats weighing 250–275 g were housed four per cage (plexiglass, 33.5×43.5×19.0 cm) in a temperature controlled (22 °C) colony room under a 12-h light–dark cycle (lights on at 7:00 h), with ad libitum access to food and water. Rats were habituated to these conditions for 1 week prior to their use. All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23, revised 1996), as well as with the project's commission approval of the Instituto Nacional de Psiquiatría Ramón de la Fuente.

2.3. Experimental design

Behavioural sensitization to AMPH was induced by a single low dose of AMPH as previously described (Panayi et al., 2005). Four groups of 6 rats were made: 1) SAL–SAL, 2) SAL–AMPH (drug-naïve group), 3) AMPH–SAL and 4) AMPH–AMPH. On day 1, rats of the SAL–SAL and SAL–AMPH groups were injected with saline, whereas the AMPH–SAL and AMPH–AMPH rats were injected with AMPH (1 mg/kg s.c.). Immediately after injection, rats of these four groups were individually transferred to test cages (identical dimensions; without litter) and their motor activity was assessed (see below) during 120 min after which they were returned to their home cages. Seven days later (day 8), SAL–SAL and AMPH–SAL rats received saline, whereas SAL–AMPH and AMPH–AMPH rats received AMPH (1 mg/kg s.c.). After injection, their motor activity was assessed in the test cages as at day 1. Animals were sacrificed at day 8, three h after AMPH or SAL injection, as previously reported (Gonzalez-Nicolini et al., 2003; Tzaferis and McGinty, 2001; Wang and McGinty, 1995; Zhou et al., 2004). Each rat was decapitated and its brain was immediately removed and frozen. The brains were stored at −70 °C, coronal brain sections (20 μ) were obtained in a cryostat and processed for in situ hybridization.

2.4. Behavioural assessment

Behavioural testing was performed between 10:00 and 12:00 h. Motor activity was evaluated using the behavioural scale of MacLennan and Maier (1983), which provides an estimate of increasing behavioural intensity from exploratory behaviours to stereotypy. Rats were rated for 30 s periods every 10 min for 2 h by an investigator unaware of the drug treatment: 0, inactive; 1, intermittent locomotor activity; 2, continuous locomotor activity; 3, intermittent stereotypy (stereotyped sniffing or repetitive head and limb movements) with locomotion over a wide area; 4, continuous stereotypy with locomotion over a wide area; 5, continuous stereotypy in a restricted area; 6, pronounced, continuous stereotypy expressed in a restricted area. These scores represent the intensity of the behavioural response to AMPH. Mean behavioural scores were calculated for each rat for a 2 h test session.

2.5. In situ hybridization

In situ hybridization was performed as previously described (de Gortari et al., 2000). Brain sections mounted in gelatin coated slides were fixed by immersion in 4% paraformaldehyde in phosphate–saline buffer (PBS) for 20 min, rinsed three times with PBS (5 min each) and treated with 0.25% acetic anhydride in 0.1 M triethanolamine, 4x standard saline citrate (SSC) buffer (pH 8.0) for 10 min. After dehydration in increasing concentrations of ethanol, slices were delipidated in chloroform for 20 min and air-dried. All solutions were made with autoclaved 0.05% diethylpyrocarbonate-treated water.

Pro-Enk probe used was a 130–145 fragment of the rat Pro-Enk cDNA (Howells et al., 1984). Pro-Enk probe was labeled at the 3′ end using α [^{35}S] dATP (1000 Ci/mmol, Amersham) and desoxynucleotidyl transferase (25 U/μl, Roche Applied Science) to a specific activity of 3.25×10^8 cpm/μg. Sections were preincubated 1 h at 42 °C and then incubated overnight in hybridization buffer containing 4x SSC, 50% deionized formamide, 2.5x Denhard's solution (100x = 0.5% Ficoll, 0.5% polyvinyl pyrrolidone, 0.5% bovine albumin), 100 μg/ml sheared single-stranded salmon sperm DNA (Sigma), 10% dextran sulphate, 10 mM dithiothreitol (DTT), 100 mM Na phosphate buffer (pH 7.4), 250 μg/ml yeast tRNA (Sigma) and ^{35}S-labeled oligonucleotide probe to get 250,000 cpm/section. After incubation sections were washed during 30 min in 4x SSC and 0.02% sodium dodecyl sulphate (SDS) at 42 °C,

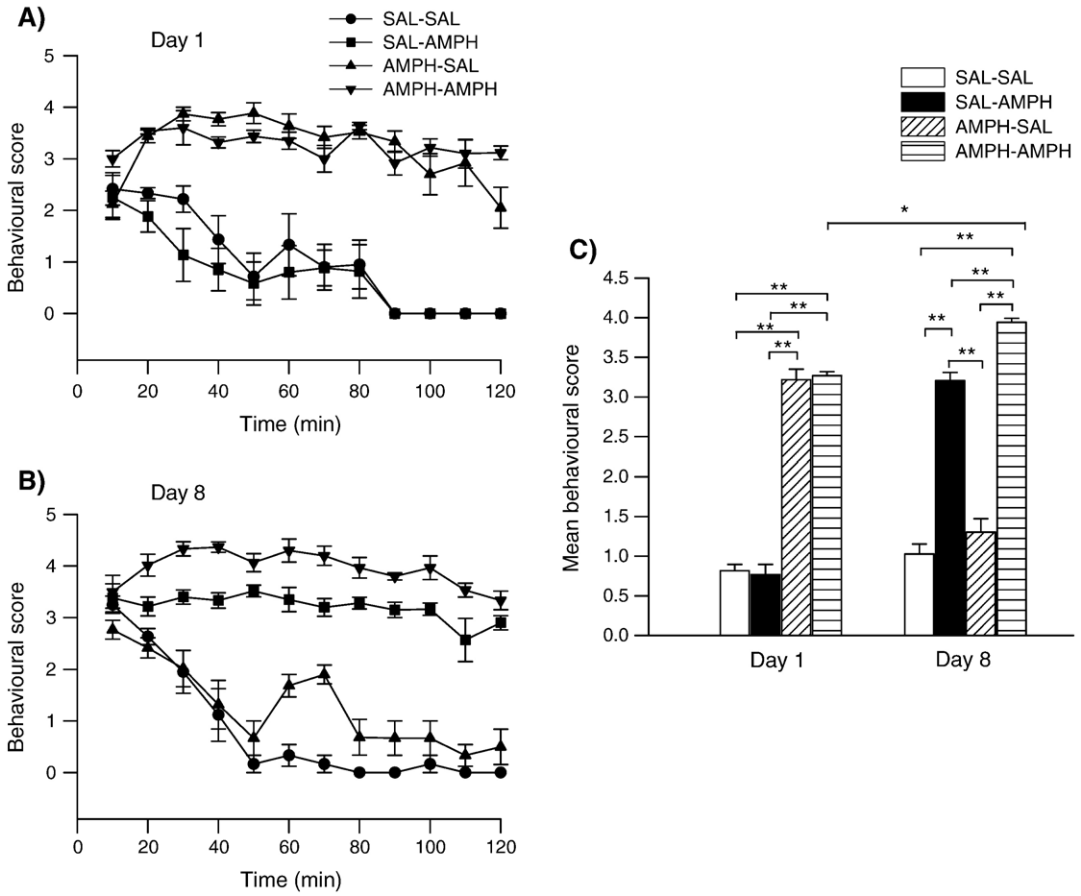

Fig. 1. Behavioural acute and sensitized responses to amphetamine. Rats received SAL (SAL–SAL and SAL–AMPH groups) or AMPH (1 mg/kg s.c.) (AMPH–SAL and AMPH–AMPH groups) on day 1 and behavioural scores were recorded (A). Seven days later, rats were challenged with SAL (SAL–SAL and AMPH–SAL groups) or with the same dose of AMPH (SAL–AMPH and AMPH–AMPH groups) (B). Mean behavioural scores were calculated for the 2 h test session for all rat groups (C). Data represent the mean±SEM of 6 animals in each group. ** $p<0.0001$; * $p<0.0006$.

followed by 30 min in 2x SSC and 0.02% SDS at 42 °C, 30 min in 1x SSC and 0.02% SDS at 42 °C and 10 min in 0.5x SSC at room temperature. Sections were dehydrated in increasing concentrations of ethanol and 95% ethanol containing 0.3 M ammonium acetate and air-dried. Slices were exposed to βmax Hyperfilm (Amersham) for 4 weeks at room temperature in the dark. Non-specific Pro-Enk hybridization was tested with an excess of 100 times the oligonucleotide concentration and the signal was substracted from total hybridization.

2.6. Film autoradiogram quantitation

Autoradiograms were quantitated by densitometry using an image analysis system (Image-Pro Plus Software, Media Cybernetics). Brain structures were identified according to Paxinos and Watson (1998). Since the distribution of opioids and their receptors has been reported to be differential along the CP (Mansour et al., 1988; Tempel and Zukin, 1987), we were interested in assessing if AMPH would differentially affect Pro-Enk mRNA expression in distinct portions of this brain region. Analyses of optical densities were performed in whole areas at two levels of the CP. Coordinates are given in millimeters with reference to bregma: A) anterior–medial CP (amCP): from 2.2 to 0.7; B) medial–posterior CP (mpCP): from 0.48 to −0.3. Five to fifteen density measurements from five different sections were collected per brain structure from each animal.

2.7. Statistical analysis

Behavioural data were analyzed by a repeated measures analysis of variance (ANOVA), with day as within-subjects factor and treatment as between-subjects factor. Sensitization was assessed by comparison of mean behavioural scores of AMPH–AMPH rats at days 1 and 8 (paired Student's *t*-test) and by comparison of mean behavioural scores of AMPH–AMPH and SAL–AMPH rats at the challenge day (day 8) (unpaired

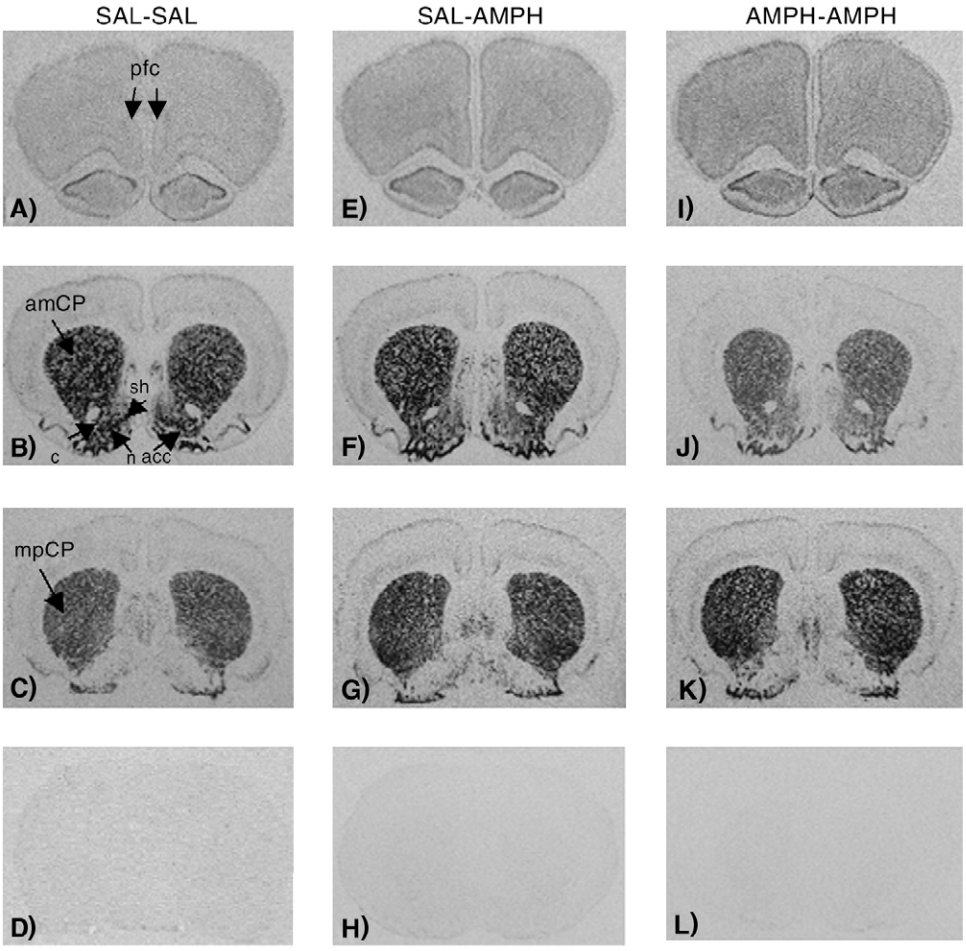

Fig. 2. Pro-Enk mRNA in situ hybridization in the rat brain in response to AMPH. Rats were pretreated with saline (SAL) or amphetamine (AMPH) (1 mg/kg s.c.) on day 1 and challenged with saline or the same dose of AMPH on day 8. Animals used for behavioural experiments were sacrificed 3 h after the amphetamine or saline challenge on day 8. Pro-Enk mRNA levels were quantitated by in situ hybridization in coronal brain sections. Rostro-caudal views of the hybridization signals of SAL–SAL (A,B,C,D), SAL–AMPH (E,F,G,H) and AMPH–AMPH (I,J,K,L) groups of animals are shown for the prefrontal (pfc) cortex (A,E,I), the nucleus accumbens (n.acc) (B,F,J), the amCP (B,F,J) and the mpCP (C,G,K). Non-specific hybridization signals are shown for the n.acc and the amCP (D,H,L). c = core region of the n.acc; sh = shell region of the n.acc.; amCP = anterior–medial portion of the CP; mpCP = medial-posterior portion of the CP; pfc = prefrontal cortex.

Student's *t*-test). One-way ANOVA was applied to in situ hybridization data and differences between groups were analyzed using the post-hoc Tukey-HSD test. Results were considered to be significant at $p<0.05$. Data are expressed as the mean±SEM of 6 animals in each group.

3. Results

3.1. Behavioural effects of amphetamine

The administration of AMPH (1 mg/kg s.c.) on day 1 increased behavioural scores in AMPH–SAL and AMPH–AMPH rats in comparison with animals injected with SAL (SAL–SAL and SAL–AMPH groups) (Fig. 1 A). When administered at day 8, AMPH increased behavioural scores in drug-naïve rats (SAL–AMPH group) or AMPH-pre-treated rats (AMPH–AMPH group), in comparison with animals injected with SAL (SAL–SAL and AMPH–SAL groups) (Fig. 1B). Statistical analysis of mean behavioural rating scores revealed significant main effects of treatment [$F(3,20)=171.602$, $p<0.0001$] and day [$F(1,20)=17.832$, $p<0.0001$], as well as a significant interaction between these factors [$F(3,20)=124.094$, $p<0.0001$]. Similar increases in behavioural scores were produced by a single injection of AMPH on day 1 in AMPH–SAL and AMPH–AMPH rats in comparison with SAL–SAL or SAL–AMPH rats: 1) AMPH–SAL versus SAL–SAL (3.9-fold increase, $t_{10}=15.29$, $p<0.0001$) and versus SAL–AMPH rats (4.2-fold increase, $t_{10}=13.17$, $p<0.0001$); 2) AMPH–AMPH versus SAL–SAL (4.0-fold increase, $t_{10}=25.75$, $p<0.0001$) and versus SAL–AMPH rats (4.3-fold increase, $t_{10}=18.14$, $p<0.0001$) (Fig. 1C). Sensitization was confirmed by significant higher behavioural scores on day 8 compared to day 1 in AMPH–AMPH rats ($t_5=7.55$, $p<0.0006$) and by significant higher scores in AMPH–AMPH rats compared with SAL–AMPH (drug-naïve) rats on day 8 ($t_{10}=6.37$, $p<0.0001$) (Fig. 1C). No significant differences were observed when comparing the behavioural responses elicited by a single injection of AMPH administered on day 1 (AMPH–SAL and AMPH–AMPH groups) or on day 8 (SAL–AMPH group) (Fig. 1C).

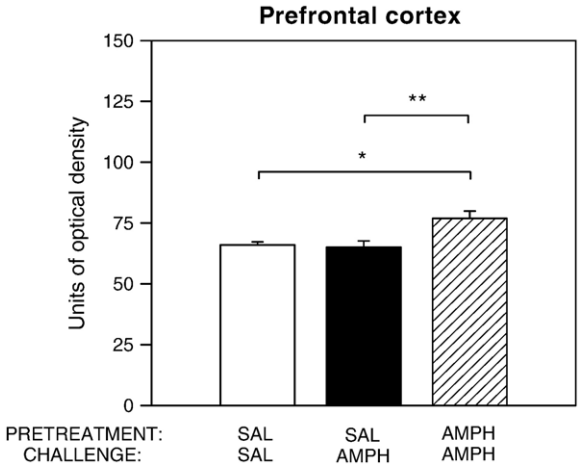

Fig. 3. Effect of amphetamine on Pro-Enk mRNA expression in the rat prefrontal cortex. Animal treatment and Pro-Enk mRNA in situ hybridization were performed as described in Fig. 2. Data are expressed as units of optical density and are the mean±SEM ($n=6$). ** $p<0.01$; * $p<0.05$.

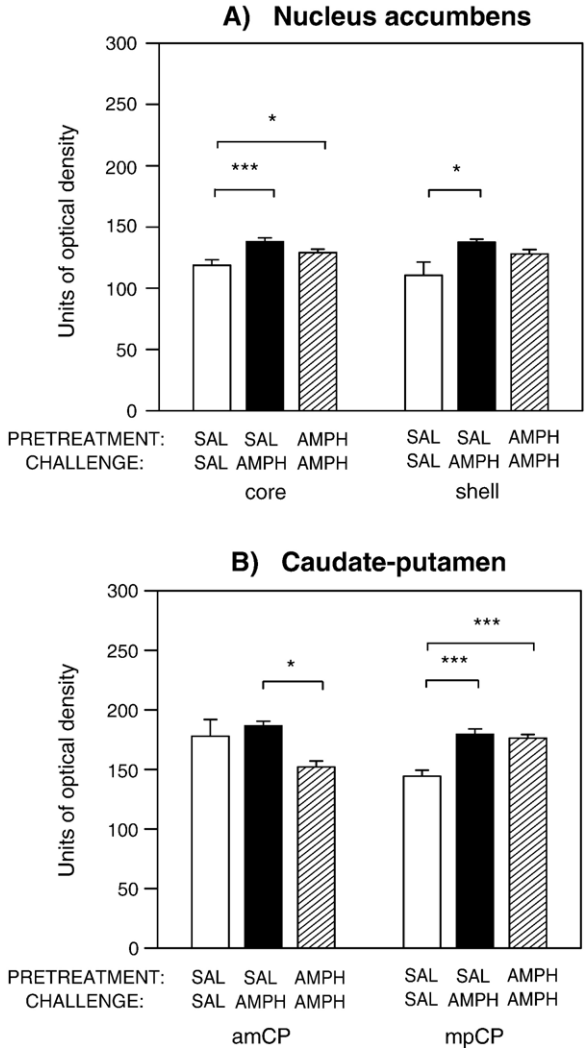

Fig. 4. Effect of amphetamine on Pro-Enk mRNA expression in the rat nucleus accumbens (A) and caudate-putamen (B). Animal treatment and Pro-Enk mRNA in situ hybridization were performed as described in Fig. 2. Data are expressed as units of optical density and are the mean±SEM ($n=6$). *** $p<0.001$; ** $p<0.01$; * $p<0.05$.

3.2. Amphetamine-induced changes in Pro-Enk mRNA expression

Behavioural scores recorded on day 8 indicate that there are no significant differences between the SAL–SAL and AMPH–SAL groups (Fig. 1 C), suggesting that the acute stimulating effect of AMPH does not persist 7 days after the first injection. Thus, Pro-Enk mRNA expression was only studied in the SAL–SAL, SAL–AMPH and AMPH–AMPH groups of rats.

Pro-Enk mRNA levels measured in the SAL–SAL group of animals were similar to those previously reported (Gonzalez-Nicolini et al., 2003; Tzaferis and McGinty, 2001; Wang and McGinty, 1995; Zhou et al., 2004). Highest Pro-Enk mRNA

levels were detected in the amCP and mpCP (Figs. 2B and C, 4B), followed by the NAcc core and shell regions (Figs. 2B, 4A) and the pfc (Figs. 2A, 3). Non-specific hybridization signal is shown in the SAL–SAL (Fig. 2D), SAL–AMPH (Fig. 2H) and AMPH–AMPH (Fig. 2L) groups at the level of the NAcc and the amCP.

One-way ANOVA revealed significant treatment effects in the pfc [$F(2,15)=7.211$, $p<0.006$], the core [$F(2,15)=14.359$, $p<0.0001$] and shell [$F(2,15)=4.665$, $p<0.027$] regions of the NAcc, and in the amCP [$F(2,15)=4.881$, $p<0.023$] and the mpCP [$F(2,15)=27.314$, $p<0.0001$]. Three h after the administration of a single low dose of AMPH (1 mg/kg s.c.), Pro-Enk mRNA levels were significantly increased by 16 and 24% in the core and shell regions of the NAcc (Figs. 2F and 4A), respectively, and by 24% in the mpCP (Figs. 2G and 4B), when compared to the SAL–SAL group of animals. In contrast, Pro-Enk mRNA levels were not changed in the pfc (Figs. 2E and 3) and the amCP (Figs. 2F and 4B) after acute AMPH administration.

A challenge with AMPH (1 mg/kg s.c.) to rats injected 1 week earlier with the same dose of AMPH significantly increased Pro-Enk mRNA levels by 17% in the pfc (Figs. 2I and 3), 9% in the core of the NAcc (Figs. 2J and 4A) and 22% in the mpCP (Figs. 2K and 4B), when compared to the SAL–SAL group. In contrast, no effect was observed in the shell of the NAcc and the amCP (Figs. 2J, 4A and B). After AMPH challenge, significant differences in Pro-Enk mRNA expression were found between the drug-naïve and the AMPH-pre-treated groups in the pfc (Fig. 3) and the amCP (Fig. 4B). The AMPH challenge to AMPH-sensitized rats induced a significant 18% increase and 18% decrease in Pro-Enk mRNA levels in the pfc and the amCP, respectively (Figs. 3 and 4B). No significant differences were found after AMPH challenge between the drug-naïve and the AMPH-sensitized rats in the NAcc or the mpCP (Fig. 4A and B).

4. Discussion

The DAergic mesolimbic system plays a key role in the mechanisms of brain positive reinforcement and reward elicited by drugs of abuse (Koob, 1992; Wise and Bozarth, 1982, 1987). The neurophysiological adaptations occurring during behavioural sensitization may be involved in the mechanisms underlying drug addiction processes (Robinson and Berridge, 1993, 2001; Vezina, 2004; Wise and Bozarth, 1987). Although DA plays a major role in AMPH-induced behavioural sensitization, the neurochemical and behavioural alterations induced by the drug result from a complex process that involves interactions between several neurotransmitters and neuromodulators, including opioid peptides (Magendzo and Bustos, 2003).

We have shown that a single dose of 1 mg/kg of AMPH increased motor activity as well as Pro-Enk mRNA expression in the core and shell regions of the NAcc and the mpCP. The observed changes in Pro-Enk mRNA expression may reflect an AMPH-induced increase in enkephalin biosynthesis. Alternatively, these changes may be due to a drug-induced decrease in mRNA degradation processes, which would lead to different mRNA stabilities. Alterations in enkephalin biosynthesis in these brain regions could lead to changes in endogenous opioid peptide levels in intracellular pools available for release. Moreover, AMPH could affect the release of endogenous opioid peptides, thereby altering their interaction with opioid receptors. The increases in Pro-Enk mRNA expression observed in this study in the core and shell regions of the NAcc and the mpCP suggest that AMPH might enhance endogenous enkephalin levels from enkephalinergic neurons in these areas and that these opioids would participate in the acute AMPH-induced motor activation, presumably through interaction with accumbal and striatal μ and δ opioid receptors. Accordingly, δ receptor agonists injected in the NAcc and the striatum have been shown to induce motor activation (Cunningham and Kelley, 1992; Daugé et al., 1988). Both selective μ and δ opioid receptor antagonists decrease the stimulatory effect of acute AMPH on vertical activity when injected in the striatum (Gonzalez-Nicolini et al., 2003). In addition, the intracisternal administration of naltrindole (a selective δ opioid receptor antagonist), as well as the s.c. injection of naloxone reduce the locomotor activity and DA release induced by AMPH (Jones and Holtzman, 1992; Jones et al., 1993; Schad et al., 1996). In agreement with our results, increases in Pro-Enk mRNA expression in the NAcc and the CP have also been found in other studies in response to high AMPH doses (2.5–5 mg/kg) (Gonzalez-Nicolini and McGinty, 2002; Gonzalez-Nicolini et al., 2003; Mao and Wang, 2003; Tzaferis and McGinty, 2001; Wang and McGinty, 1995, 1996a,b; Zhou et al., 2004).

We have shown that a challenge of AMPH 7 days after the administration of the same dose of psychostimulant induced behavioural sensitization, in agreement with previous studies (Colussi-Mas et al., 2005; Panayi et al., 2005; Robinson et al., 1982; Vanderschuren et al., 1999). After the AMPH challenge to sensitized rats, Pro-Enk mRNA levels were significantly increased in the core of the NAcc, the mpCP and the pfc, and were decreased in the amCP. These effects could reflect AMPH-induced changes in transcription and/or mRNA stability. The increases in Pro-Enk mRNA levels in the core of the NAcc and the mpCP were similar to those attained by a single injection of AMPH to drug-naïve rats. These findings are in agreement with previous results showing that a challenge dose of AMPH (1 mg/kg) administered 10 days after the repeated administration of AMPH at a dose of 5 mg/kg for 5 days did not alter Pro-Enk mRNA levels in the NAcc in comparison with Pro-Enk mRNA levels attained after the acute AMPH administration (Wang and McGinty, 1995). In addition, we have also observed that the AMPH challenge to AMPH-sensitized rats significantly increased and decreased Pro-Enk mRNA expression in the pfc and the amCP, respectively, whereas no changes were found in these regions after acute AMPH administration. In contrast to our results, Hurd and Herkenham (1992) and Wang and McGinty (1995) reported no change in Pro-Enk mRNA levels in the striatum of AMPH-sensitized rats in comparison with acute AMPH-treated animals. This discrepancy may be attributed to the different AMPH doses and experimental designs used in both studies (in particular, habituation of animals to the

test cage before drug administration), as well as to the different areas studied in each brain structure. In this work, animals were not habituated to the test cage because various studies have reported that when administered in a novel environment, low doses of AMPH induce c-fos mRNA expression in Preproenkephalin mRNA-containing neurons in the CP and the subthalamic nucleus, whereas when administered at home, AMPH does not produce these effects (Uslaner et al., 2001, 2003). Overall, our findings suggest that changes in Pro-Enk mRNA expression in the NAcc and the mpCP could be relevant in both the acute and sensitized AMPH responses, while enkephalins in the pfc and the amCP could contribute more specifically to the expression of AMPH behavioural sensitization. The contribution of an AMPH associated environment effect on Pro-Enk mRNA expression observed in the AMPH–AMPH group can not be excluded, since in our study Pro-Enk mRNA levels were not evaluated in the AMPH–SAL group of rats. This issue remains to be determined in further studies.

DAergic transmission in the pfc and the CP has been suggested to play a critical role in behavioural sensitization to psychostimulants (Bank and Gratton, 1995; Beyer and Steketee, 1999; Robinson and Becker, 1982). A single exposure to AMPH has been reported to induce a long-lasting motor sensitization and to increase DA release from the medial prefrontal cortex (mpfc) (Vanderschuren et al., 1999). In addition, studies showing an enhancement of c-fos immunoreactive cells in the mpfc of AMPH-sensitized rats compared to controls (Hedou et al., 2002) further support a role of this brain region in sensitization. On the other hand, the role of cortical opioid peptides in the development and the expression of behavioural sensitization to AMPH has not been previously studied. Our present data show that Pro-Enk mRNA expression in the pfc is unchanged after a single AMPH injection, while it is increased after a challenge of AMPH to AMPH-sensitized rats, suggesting a possible implication of enkephalins in the pfc only in the sensitized response to AMPH. Modulation of neural activity by opioid peptides in the pfc appears to be complex. Mu receptor agonists have been shown to increase DA release from the pfc, whereas δ agonists have no effect (Heijna et al., 1990; Weatherspoon et al., 1996; Wood and Rao, 1991). Stimulation of μ opioid receptors hyperpolarizes GABAergic interneurons, while that of δ receptors hyperpolarizes glutamatergic neurons (Tanaka and North, 1994). In addition, Met-enkephalin inhibits the presynaptic release of GABA and glutamate and this effect is mimicked by a δ receptor agonist and inhibited by a selective δ receptor antagonist, suggesting that these effects occur via δ receptors (Tanaka and North, 1994). The increase in Pro-Enk mRNA expression in the pfc in sensitized animals observed in this study could be interpreted as an activation of enkephalinergic transmission by AMPH. If so, stimulation of endogenous enkephalin release by AMPH might also be suspected. In these conditions, released enkephalins could modulate DAergic activity through interactions with δ opioid receptors. An indirect modulation of DAergic transmission via δ receptors on interneurons (i.e., GABAergic and/or glutamatergic) could be suggested. However, this hypothesis remains to be confirmed.

Acute and repeated administration of AMPH induces motor sensitization and increases DA release from the CP (Patrick et al., 1991; Vanderschuren et al., 1999). Several neurotransmitters and neuromodulators, including opioid peptides, regulate the DAergic activity of the nigrostriatal pathway. Both μ and δ receptor agonists stimulate DA synthesis and release from the striatum (Rawls and McGinty, 2000; Urwyler and Tabakoff, 1981). Naloxone and naltrindole reduce the AMPH-induced release of DA from the striatum (Hooks et al., 1992; Schad et al., 1995, 1996) and naloxone also decreases the locomotor activity induced by AMPH (Hooks et al., 1992; Schad et al., 1995), suggesting a major role of opioid receptors in AMPH actions. On the other hand, AMPH and DA D1 agonists have been reported to stimulate the release of Ach from the striatum, suggesting that this effect is mediated by released DA in response to AMPH (Damsma et al., 1991; Guix et al., 1992). In addition, muscarinic agonists stimulate, whereas antagonists inhibit the psychostimulant-induced increase in Pro-Enk mRNA expression (Lucas and Harlan, 1995; Wang and McGinty, 1996b). These findings suggest that Ach released by AMPH may modulate the expression of Pro-Enk mRNA in the striatum, which could occur by activation of muscarinic receptors localized in striatopallidal enkephalin-containing neurons (Gonzalez-Nicolini et al., 2003; Wang and McGinty, 1997). Besides DA and Ach, glutamate also exerts a modulatory control of Pro-Enk mRNA expression in the striatum. Blockade of N-methyl-D-aspartate (NMDA) and kainate/α-amino-3-hydroxy-5-methylisoxazole-4-propioinic acid (AMPA) receptors inhibits the AMPH-induced increase in Pro-Enk mRNA expression (Liste et al., 1999; Mao and Wang, 2003). AMPH increases glutamate release in the striatum (Rawls and McGinty, 2000) and a tonic stimulation of cholinergic interneurons by glutamate has been proposed to occur through activation of NMDA receptors (Knauber et al., 1999). Thus, Pro-Enk mRNA expression might be modulated by the glutamate-induced release of Ach. Since striatopallidal neurons receive δ opioid receptor-bearing glutamate projections from the cortex and thalamus and δ receptor-bearing DAergic projections from the substantia nigra pars compacta (Gonzalez-Nicolini et al., 2003), Pro-Enk mRNA expression could also be regulated by opioid peptides. Therefore, regulation of Pro-Enk mRNA expression in the striatum may be the result of multiple neurotransmitter interactions. Since in the present study a challenge of AMPH decreased Pro-Enk mRNA expression in the amCP of sensitized animals, an AMPH-induced reduction in enkephalinergic transmission could be proposed. On the other hand, the physiological relevance of the distinct AMPH-induced responses observed in the amCP and mpCP is unknown, but could be related to the differential distribution of opioids and their receptors (Mansour et al., 1988; Tempel and Zukin, 1987), as well as that of other neuropeptides and neurotransmitters in this brain structure.

In conclusion, we have shown that AMPH challenge induces similar changes in Pro-Enk mRNA expression in the NAcc and the mpCP in drug-naïve and AMPH-sensitized rats. In contrast, Pro-Enk mRNA expression is selectively modified in the pfc and the amCP in AMPH-sensitized rats. We suggest that increased and decreased enkephalinergic transmission in the pfc

and the amCP, respectively, could contribute to the expression of AMPH behavioural sensitization. The mechanisms underlying these processes remain to be determined. Interaction of opioid peptides with several neurotransmitter systems deserves further research.

Acknowledgments

We thank J.M. Pérez-Luna and S.R. Mejía for technical assistance. This work was supported by Consejo Nacional de Ciencia y Tecnología (CONACyT 34359-N) and Acuerdo de Cooperación Científica México-Francia (ANUIES-ECOS M01S02).

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