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Research Report

Contingency does not contribute to the effects of cocaine self-administration on prodynorphin and proenkephalin gene expression in the rat forebrain

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ABSTRACT

Neuroadaptations in the brain opioid systems produced by chronic exposure to drugs of abuse may contribute to the drug dependence and addiction. Although regulation of the gene expression of the opioid propeptides proenkephalin (PENK) and prodynorphin (PDYN) by psychostimulants has previously been described, little attention has been paid to dissociating effects of pharmacological actions of the drugs from those produced by motivational processes driving active drug intake in self-administration paradigms. In the present study, effects of response-dependent (contingent) and response-independent (noncontingent) cocaine administration on the PENK and PDYN gene expression in the rat forebrain have been directly compared using the "yoked" self-administration procedure. The i.v. cocaine treatment lasted for 5 weeks, and rats were sacrificed 24 h after the last self-administration session. In situ hybridization analysis revealed that levels of the PDYN mRNA were significantly increased in the caudate/putamen, to the same extent in rats self-administering cocaine as in animals receiving noncontingent injections of the drug at the same frequency and dosage. No changes in the expression of the PDYN gene were detected in the nucleus accumbens or in the central nucleus of amygdala. Levels of the PENK mRNA remained unaltered in all the above-mentioned forebrain regions of rats receiving contingent or noncontingent cocaine injections. The obtained data indicate that up-regulation of the PDYN gene expression in the caudate/putamen results from direct pharmacological actions of cocaine rather than from the motivational and cognitive processes underlying active self-administration of the drug.

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1. Introduction

Endogenous opioid peptides are considered to play important roles in the actions of drugs of abuse. Some addictive drugs, e.g., alcohol, cannabinoids and nicotine, directly and acutely influence the activity of the endogenous opioid systems either by producing opioid peptide release or opioid-like intracellular actions (Berrendero et al., 2002, 2005; Fattore et al., 2004; Oswald and Wand, 2004). On the other hand, many of these drugs, including the psychostimulants cocaine and amphetamines, when administered repeatedly, produce adaptive changes in the opioid systems such as alterations in the expression of the opioid peptide precursors and opioid receptors densities in the brain (e.g., Kreek, 1996; Nestler, 2004; Oswald and Wand, 2004; Turchan et al., 1997, 1998, 1999; Unterwald, 2001). Taking into account that opioid peptides are mediators of the sense of well-being and reward, and that they interact with the motivation- and reward-related dopaminergic system (Di Chiara and Imperato, 1988; Koob and Le Moal, 2001; Mansour et al., 1995; Skoubis et al., 2005), the above-mentioned changes may be responsible, at least in part, for affective disturbances underlying drug abuse, withdrawal symptoms and addiction.

In neurochemical studies of addiction, it is important to employ animal models that dissociate the consequences of the pharmacological action of addictive substances from those that result from the motivational and cognitive processes underlying active self-administration of these drugs. To this end, the effects of a given abusive substance are tested on animals which actively self-administer the drug (contingent drug administration) and on animals receiving passive drug injections (noncontingent drug administration). To directly compare effects of contingent and noncontingent drug administration, the so-called yoked self-administration procedure is employed, in which both groups of animals receive the drug simultaneously at the same dose, administration rate, and in the same experimental environment (Jacobs et al., 2003; Kuzmin and Johansson, 1999; Mierzejewski et al., 2003; Stefanski et al., 1999). Using this paradigm, it has been demonstrated in multiple studies that some neurochemical changes produced by addictive substances do indeed markedly differ depending on whether the drugs are self-administered or noncontingently injected to animals (reviewed by Jacobs et al., 2003).

Although the influence of various drugs of abuse on the activity of endogenous opioid systems was assessed in a large number of studies, only very few of them made a clear distinction between the effects of active and passive drug intake. One such attempt was made by Crespo et al. (2001), who studied expression of the opioid peptide precursor proenkephalin (PENK) in the rat brain during extinction of cocaine self-administration. These authors observed a sustained down-regulation of the PENK mRNA levels in the central amygdaloid nucleus and ventromedial hypothalamic nucleus that occurred selectively in animals which had actively self-administered cocaine, but not those receiving noncontingent injections of the drug. On the other hand, up-regulation of the PENK gene expression was found in several forebrain regions in both cocaine-treated groups (Crespo et al., 2001). These data provided the first indication that some changes in an opioid propeptide expression might be related to the process of active cocaine intake. However, they contradicted the results of most earlier studies on the impact of noncontingent cocaine administration on the PENK gene expression (Arroyo et al., 2000; Branch et al., 1992; Daunais and McGinty, 1994, 1995; Daunais et al., 1995; Hurd et al., 1992; Mathieu-Kia and Besson, 1998), and thus require corroboration. On the other hand, expression of another opioid peptide precursor prodynorphin (PDYN) has been consistently shown to be elevated in the striatum as a result of either contingent or noncontingent cocaine administration (Adams et al., 2000, 2003; Daunais and McGinty, 1994, 1995; Daunais et al., 1993, 1995, Fegergren et al., 2003; Hurd et al., 1992; Hurd and Herkenham, 1992a,b; Mathieu-Kia and Besson, 1998; Steiner and Gerfen, 1993; Turchan et al., 1998). Nevertheless, effects of the drug self-administration vs. passive injections have never been directly compared. Therefore, it remains unclear if the increases in PDYN expression are due solely to pharmacological actions of cocaine, or whether they are also associated, to some extent, with the motivational process which drives active cocaine intake.

To assess behavioral, as distinct from pharmacological, factors in determining neuroadaptations in the opioid peptide systems, the current study directly contrasts the effects of contingent and noncontingent cocaine administration on the PENK and PDYN mRNA levels in the rat forebrain using a "yoked" self-administration procedure.

2. Results

2.1. *"Yoked" self-administration procedure: contingent vs. noncontingent cocaine administration*

Self-administration sessions were conducted in operant chambers equipped with two nose-poke operanda (holes). Responding on the hole defined as "active" resulted in cocaine delivery to the rat, whereas responding on the other, "inactive" hole, had no consequences.

Fig. 1 shows the average number of active and inactive hole responses for rats actively self-administering cocaine and receiving "yoked" injections of either cocaine or saline. The number of responses required to produce each injection was increased over days, reaching a final value of 5 (fixed-ratio 5 schedule of drug injection; FR-5) by the 15th session of training.

For the data from the cocaine self-administration group (left panel), a two-factor ANOVA for repeated measures revealed significant effects between active and inactive hole responding [$F(1,12) = 102$, $P < 0.001$] over the 25 sessions [$F(24,288) = 27$, $P < 0.001$]. In addition, there was an overall significant interaction between nose-poke responding and sessions [$F(24,288) = 26.7$, $P < 0.001$]. Post hoc analysis revealed that a significant preference for the active hole occurred on sessions 6–25 ($P < 0.01$).

For the data from the "yoked" cocaine group (middle panel), a two-factor ANOVA for repeated measures indicated a significant effect of sessions [$F(24,288) = 7.2$, $P < 0.001$]. The