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Short communication

Immediate withdrawal from chronic "binge" cocaine administration increases μ-opioid receptor mRNA levels in rat frontal cortex

Alexis Bailey*, Vadim Yuferov, Jacob Bendor, Stefan D. Schlussman, Yan Zhou, Ann Ho, Mary Jeanne Kreek

Laboratory of the Biology of Addictive Diseases, The Rockefeller University, New York, NY 10021, USA

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Abstract

An increase in preprodynorphin (ppdyn) mRNA was detected in the caudate putamen of chronically cocaine-treated and 3-h withdrawn rats. An increase in μ-opioid receptor (MOP) mRNA levels was observed in the frontal cortex of 3-h withdrawn rats. Naloxone had no effect on the increase of MOP or ppdyn mRNA levels. The results indicate that the opioid system is altered during early withdrawal from chronic cocaine administration.

Theme: Neural basis of behavior
Topic: Drugs of abuse: cocaine

Keywords: μ-Opioid receptor; "Binge" cocaine administration; Frontal cortex; Naloxone; Withdrawal

There is a large body of evidence indicating that opioid systems interact with the dopamine system at the anatomical, neurochemical, and behavioral level [15,16,38]. A consistent finding has been the increase of preprodynorphin (ppdyn) mRNA, which encodes for the κ opioid receptor selective endogenous peptide dynorphin, in the caudate putamen of rats following acute, subacute, and chronic cocaine treatment [6,7,13,27–31,43]. Preproenkephalin (ppenk) mRNA (which encodes for the δ opioid receptor selective endogenous peptide enkephalin) changes have been reported following acute and subacute cocaine treatment [30] and during withdrawal [24], but not after chronic treatment in rats [3]. Moreover, an increase in μ-(MOP) and κ-opioid receptor density has been reported in the nucleus accumbens, the caudate putamen and the cingulate cortex of chronic cocaine-treated rats [39,40].

Although the acute and chronic effects of cocaine and its interaction with the opioid system have been studied in depth, a limited number of studies have been carried out on the neurobiology underlying cocaine withdrawal (i.e., the cessation of chronic cocaine treatment). The persistent psychological changes observed following chronic cocaine self-administration in humans [15–17] imply the presence of alterations in neuronal systems. To test the hypothesis that the opioid systems are altered in animals withdrawn from cocaine, we used the solution hybridization-RNase protection assay to determine whether there are any changes in ppenk, ppdyn, and MOP mRNA levels in the nucleus accumbens, caudate putamen, and frontal cortex of rats chronically treated with cocaine and sacrificed 1 h (chronic "binge" cocaine group) or 3 h (early withdrawal group) following chronic "binge" cocaine administration.

Moreover, systemic administration of opioid antagonists decreased cocaine-induced conditioned place preference [11,12,18], depressed cocaine self-administration, and delayed cocaine initiation on the following day of self-administration [14]. As cocaine administration results in changes in opioid peptide and receptor mRNA levels in the brain, we hypothesize that blockade of the MOP by

* Corresponding author. Fax: +1 212 327 8574.
E-mail address: baileya@rockefeller.edu (A. Bailey).

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administration of naloxone may affect the mRNA response to cocaine. To test that hypothesis, we examined, by solution hybridization-RNase protection assay, whether a single dose of naloxone (1 mg/kg) would produce any changes in ppenk, ppdyn, and MOP mRNA levels in the nucleus accumbens, caudate putamen, and frontal cortex of rats chronically treated with cocaine and of rats withdrawn for 3 h from chronic "binge" cocaine administration.

Male Fischer rats (Charles River Laboratories, Kingston, NY), 210–240 g, were housed individually in a temperature-controlled room with a 12-h light/dark schedule. Food and water were available ad libitum. Following 7 days of acclimation, intraperitoneal (i.p.) injections of either saline (1 ml/kg) or cocaine (15 mg/kg in 1 ml/kg saline) were administered. Three daily i.p. injections (1 h apart) were administered for a total daily cocaine dose of 45 mg/kg, with the first injection 30 min after the start of the light cycle. The rats received 14 days of either saline or cocaine injection. On day 14, 30 min after the third injection of saline or cocaine, a fourth i.p. injection of either saline (1 ml/kg) or naloxone (1 mg/kg) was administered.

Animals were sacrificed 1 h or 3 h after the last cocaine injection. Brains were immediately removed and the nucleus accumbens, caudate putamen, and frontal cortex were dissected on ice. Brain regions from each animal were homogenized in guanidine thiocyanite and RNA was extracted by the acid phenol and chloroform method [5]. The study was conducted in conformity with an animal protocol approved by the Animal Care and Use Committee of The Laboratory Animal Research Center of The Rockefeller University. Solution hybridization-RNase protection assays of ppenk, ppdyn, and MOP mRNA were performed as previously described [3,45]. Comparison of quantitative measures of mRNA levels from withdrawn and chronic saline- and cocaine-treated animals was carried out using two-way analysis of variance (ANOVA), drug condition (saline, cocaine) × time point (1 h, 3 h after last cocaine administration). The effect of naloxone on mRNA levels after chronic "binge" cocaine administration was examined by two-way analysis of variance (ANOVA), drug condition (saline, cocaine) × antagonist (saline, naloxone).

No significant change in ppenk mRNA levels was detected in any brain region studied from 3 h withdrawn and chronically "binge" cocaine-treated rats compared to saline controls (Tables 1, 2, Fig. 1). Two-way ANOVA, however, for ppdyn mRNA levels in the caudate putamen showed a significant main effect for cocaine treatment [$F(1,26) = 11.05$, $P < 0.003$] (Table 2). No change in ppdyn mRNA levels was observed in the nucleus accumbens of chronic "binge" cocaine-treated and early cocaine-withdrawn rats (Table 1). No change in MOP mRNA levels was observed in the nucleus accumbens or caudate putamen of chronically "binge" cocaine-treated or early cocaine-withdrawn rats (Tables 1, 2). Two-way ANOVA showed no effect of naloxone (1 mg/kg) on any of the mRNA levels in any brain regions studied (Tables 1, 2 and Fig. 1). There was, however, a significant increase in the levels of MOP mRNA in the frontal cortex of 3-h cocaine-withdrawn rats [$F(1,27) = 6.31$, $P < 0.02$] (Fig. 1).

The 3-h period after the cessation of cocaine was chosen as a time point for early withdrawal because during that time period the extracellular levels of dopamine in the striatum have been shown to return to basal levels [20] and a decrease in locomotor activity was also observed at that time point [22]. The 3-h withdrawal period is used as a model for the "crash" phase reported to occur 12–48 h after the termination of cocaine "binge" in humans [10].

The absence of change in ppenk mRNA levels in any region of chronically "binge" cocaine-treated and 3-h withdrawn rats studied is in agreement with several studies [3,21,32]. These results suggest that the activity of ppenk mRNA-containing neurons remains unaltered both during chronic "binge" cocaine administration and in early withdrawal.

The increase in ppdyn mRNA levels in the caudate putamen of chronically cocaine-treated and 3-h withdrawn rats and the lack of change in ppdyn mRNA in the nucleus accumbens are in agreement with earlier studies [6–8,21,27,28,30,33,43]. This increase in ppdyn mRNA might play a role in decreasing dopamine release. Indeed, dynorphin (1–17) was shown to block the cocaine-induced rise in dopamine levels [47].

This is the first study to investigate MOP mRNA levels at an early withdrawal time point. The lack of change in MOP mRNA levels in the nucleus accumbens, caudate putamen, and frontal cortex of chronic "binge" cocaine-

Table 1
Levels of preproenkephalin, preprodynorphin, and MOP mRNA in the nucleus accumbens of rats chronically treated with cocaine for 14 days and in 3-h withdrawn rats, with and without naloxone challenge

mRNA/total RNA	Treatment	Saline	Cocaine	Saline + naloxone	Cocaine + naloxone
Preproenkephalin (attomole/μg)	Chronic	10.3 ± 1.08	9.3 ± 0.89	11.0 ± 2.31	12.7 ± 1.80
	Early withdrawal	9.9 ± 0.74	11.0 ± 1.20	10.2 ± 1.10	9.3 ± 0.45
Preprodynorphin (attomole/μg)	Chronic	8.9 ± 0.88	7.2 ± 0.88	9.3 ± 0.93	10.8 ± 1.32
	Early withdrawal	8.3 ± 0.54	8.5 ± 0.93	7.5 ± 0.30	7.3 ± 0.45
MOP (attomole/μg)	Chronic	12.9 ± 1.77	8.6 ± 1.23	9.9 ± 1.15	10.2 ± 1.13
	Early withdrawal	11.0 ± 1.08	12.9 ± 2.71	11.8 ± 1.89	8.7 ± 0.66

The mean levels of preproenkephalin, preprodynorphin, and MOP mRNA/total RNA (attomole/μg) ± standard error of the mean (n = 7–8) in the nucleus accumbens of rats chronically treated with cocaine for 14 days (chronic group) and in 3-h withdrawn rats (early withdrawal group), with and without naloxone challenge.