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## Involvement of dynorphin and kappa opioid receptor in yohimbine-induced reinstatement of heroin seeking in rats

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### Abstract

Although kappa opioid receptor (KOP-r) antagonists are known to reduce reinstatement of cocaine, alcohol and nicotine seeking induced by a variety of stressors, the role of KOP-r in yohimbine-induced reinstatement of heroin seeking has not been investigated. Yohimbine, used as a stressor, increases the hypothalamic-pituitary-adrenal (HPA) hormones, causes anxiety and induces heroin craving in humans. The present experiments were undertaken to assess the effects of yohimbine on reinstatement of heroin seeking and associated changes in preprodynorphin (ppDyn) expression and HPA hormonal levels; and to determine whether these effects could be reduced by pretreatment with the selective KOP-r antagonist nor-binaltorphimine (nor-BNI). After heroin self-administration for 12 days (3h/day, 0.05 mg/kg/infusion, i.v.) and extinction for 8 days, reinstatement included the first baseline test after vehicle injection, the second test of yohimbine-induced reinstatement (1.25 mg/kg, i.p.), pretreatment with vehicle or nor-BNI (20 mg/kg, i.p.), the third baseline test after vehicle injection, and the final test of yohimbine-induced reinstatement. Immediately after the last test, several mesolimbic regions and plasma were collected for analyses of ppDyn and KOP-r mRNA levels and HPA hormones. Yohimbine-induced reinstatement was fully blocked by nor-BNI pretreatment. Furthermore, yohimbine elevated plasma HPA hormones, and this increase was blunted by nor-BNI. Finally, rats pretreated with yohimbine displayed increased ppDyn mRNA levels in the nucleus accumbens shell and central nucleus of the amygdala. These data suggest that the stress responsive ppDyn/KOP-r system is a critical component of the neural circuitry underlying the effect of yohimbine stress on heroin seeking behavior and HPA activity.

### Keywords

dynorphin; kappa opioid receptor; nucleus accumbens shell; central nucleus of the amygdala; heroin self-administration; yohimbine-induced reinstatement

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One well-documented neurobiological change in response to chronic exposure to opiates, psychostimulants, alcohol and nicotine is the increased activity of the dynorphin (Dyn) and kappa opioid receptor (KOP-r) systems within brain circuits involved in motivated behavior [Shippenberg et al., 2007]. KOP-r agonists or natural Dyn peptide dose-dependently reduce

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basal and drug-induced dopamine release in the striatum of rodents [Spanagel et al., 1992; Zhang et al., 2004]. Activation of KOP-r produces depressive-like behaviors in rodents [Todtenkopf et al., 2004], whereas KOP-r blockade can result in antidepressant-like effects [Newton et al., 2002]. KOP-r antagonists exhibit anxiolytic activity in rodents [Knoll and Carlezon, 2010]. In recent years, KOP-r antagonists have been extensively investigated for their effects on the addictive properties of cocaine. KOP-r agonists can, under some circumstances, potentiate the reinforcing effects of cocaine [McLaughlin et al., 2006], whereas KOP-r antagonists can prevent stress-induced reinstatement of cocaine-seeking behavior [Beardsley et al., 2005; Aldrich et al., 2009]. In addition, a selective KOP-r antagonist nor-binaltorphimine (nor-BNI) attenuates drug self-administration in rats dependent on alcohol [Walker and Koob, 2008] or cocaine [Wee et al., 2009], and block nicotine withdrawal [Jackson et al., 2010].

Many stress-related peptides, and their receptors, are involved in drug cravings in humans [Sinha et al., 2006; Koob and Kreek, 2007] and in reinstatement of lever pressing in animals triggered by exposure to various stressors [Leri et al., 2002; Shaham et al., 2003; Zhou et al., 2008]. Although the ability of KOP-r antagonists to prevent stress-induced reinstatement of cocaine, alcohol and nicotine seeking behavior has been established, the effects of such compounds on reinstatement of heroin seeking have not been explored. Our hypothesis of the research described here is that a hyper-responsivity within the mesolimbic Dyn/KOP-r system is one cause of heroin seeking behavior. Therefore, the first aim of this study was to investigate the effect of the KOP-r antagonist nor-BNI on reinstatement of heroin seeking induced by yohimbine (Yoh). Laboratory studies in humans found that Yoh increased hypothalamic-pituitary-adrenal (HPA) activity, as well as subjective anxiety in normal subjects [Rosen et al., 1999] and drug craving in abstinent opiate addicts [Stine et al., 2002]. Yoh enhances central noradrenergic activity by acting as an antagonist at alpha-2 adrenergic autoreceptors and reinstates methamphetamine, cocaine, heroin, alcohol and food seeking [see recent reviews by See and Waters, 2011; Sinha et al., 2011]. The second aim was to explore alterations in Dyn activity, as well as plasma HPA hormonal levels, associated with Yoh-induced reinstatement. Therefore, male Sprague-Dawley rats were trained to self-administer heroin intravenously, followed by a period of withdrawal/extinction, and then tested for Yoh-induced reinstatement of heroin seeking. Following the behavioral test, specific brain regions were collected, and preprodynorphin (ppDyn) mRNA levels were quantified in the nucleus accumbens (NAc) core and shell, caudate-putamen (CPu), central nucleus of the amygdala (CeA), and medial/basolateral amygdala (Me/BLA). To allow for the correct interpretation of neurochemical data obtained in the Yoh-treated rats after heroin self-administration, alterations of ppDyn mRNA and HPA hormonal levels were also analyzed in heroin naïve rats treated with Yoh. Detailed methods are provided in the Supporting Information (SI), along with the plasma HPA hormone levels.

***Behavior.*** Over the 12 sessions of heroin self-administration, there was an increase in responding on the active lever (Table S1A), and this was subsequently reduced by 8 days of extinction. Fig. 1 represents responding on the active and inactive levers during the reinstatement phase of the experiment in animals treated with Yoh (1.25 mg/kg, i.p.) alone in Reinstatement test I (R I) and with either 0 or 20 mg/kg nor-BNI prior to the second Yoh-induced Reinstatement test II (R II) (see Fig. S1 for a detailed timeline). The ANOVA revealed significant Group  $\times$  Test  $\times$  Lever interaction [ $F(3,48)=3.31$ ,  $p<0.05$ ], Group  $\times$  Test interaction [ $F(3,48)=3.56$ ,  $p<0.05$ ], and Test  $\times$  Lever interaction [ $F(3,48)=9.36$ ,  $p<0.01$ ]. There were also significant main effects of Test [ $F(3,48)=21.1$ ,  $p<0.01$ ] and of Lever [ $F(1,16)=19.9$ ,  $p<0.01$ ]. In the group that did not receive nor-BNI, Yoh enhanced responding on the active and inactive levers in both the R I and R II tests (Fig. 1A), but the increase was significant only on the active lever ( $p<0.01$  for both the R I and R II, Newman-Keuls post-hoc tests). In the group pre-treated with nor-BNI prior to the R II test, however, Yoh

produced significant reinstatement of responding only on the active lever in the RI test ( $p < 0.01$ ) (Fig. 1B). The inhibiting effect of nor-BNI on Yoh-induced reinstatement was further revealed by a significant difference ( $p < 0.01$ ) in active lever responding between groups on the R II test (compare Fig. 1A and 1B).

*Neurochemistry.* Levels of ppDyn mRNA were measured in the NAc core and shell, CPu, CeA, Me/BLA and lateral hypothalamus (LH) in rats that: (1) self-administered saline and then received vehicle (saline) prior to both R I and R II sessions; (2) self-administered heroin and received saline prior to both R I and R II; (3) self-administered heroin and received Yoh prior to both R I and R II, and (4) self-administered heroin, received Yoh prior to both R I and R II, and nor-BNI pretreatment before R II. In the NAc shell (Fig. 2A), one-way ANOVA revealed a significant effect of Group [ $F(3,29)=5.28$ ,  $p < 0.01$ ], and Newman-Keuls *post hoc* tests revealed that, compared to saline self-administration rats, there were significant increases in the ppDyn mRNA levels in the rats treated with either Yoh alone ( $p < 0.05$ ) or Yoh and nor-BNI ( $p < 0.05$ ) after heroin self-administration. No significant group differences were found in the NAc core (Fig. 2A). In the CeA (Fig. 2B), one-way ANOVA revealed a significant effect of Group [ $F(3,28)=3.09$ ,  $p < 0.05$ ] and the ppDyn mRNA levels were increased in the rats treated with either Yoh alone [ $df(1,28)=5.71$ ,  $p < 0.05$ , Planned comparison] or Yoh and nor-BNI [ $df(1,28)=5.33$ ,  $p < 0.05$ , Planned comparison] after heroin self-administration. No significant group differences were found in the Me/BLA (Fig. 2B). Finally, no significant group differences were found in the CPu and LH (see Table S2A in the SI). Analysis of KOP-r mRNA levels in the same groups and regions did not identify significant group differences in the NAc shell or core or Me/BLA (see Table S2B in the SI). In a separate experiment, levels of ppDyn mRNA were also compared in heroin naïve rats after Yoh (1.25 mg/kg) or saline treatment. As shown in Table S3 in the SI, there were no significant changes in ppDyn mRNA levels in the NAc shell or core, CeA or CPu.

KOP-r activation modulates drug-induced dopamine release in the NAc and CPu [Spanagel et al., 1992] and may potentiate or attenuate the reinforcing action of drugs depending on the stage of drug exposure or stress [McLaughlin et al., 2006; Maiya et al., 2009]. KOP-r antagonists have been investigated for their effects on the reinforcing action of drugs of abuse, depression and stress [Aldrich and McLaughlin, 2009; Knoll and Carlezon, 2010]. The present study provides initial evidence for the involvement of the ppDyn/KOP-r systems in heroin seeking. In fact, in rats trained to self-administer heroin, Yoh-induced reinstatement was blocked by pre-treatment with nor-BNI, and in heroin self-administration rats treated with Yoh, there were increases in ppDyn mRNA levels in the NAc shell and CeA.

In humans, heroin dependence is often characterized by negative affective states, including anhedonia (an inability to experience pleasure from rewarding stimuli), anxiety and disrupted stress responses during drug withdrawal [Koob and Kreek 2007]. Stress can potentiate negative affective states in heroin abstinent people and trigger craving and relapse [Sinha et al., 2011]. A relative hyper-responsivity of the Dyn system may represent one critical neuro-adaptation in response to stress during heroin withdrawal, and could be important for craving in humans. The present study utilized a drug-seeking behavioral model in which rats that underwent extinction after self-administering heroin displayed reinstatement of operant responding induced by Yoh. In the heroin-withdrawn rats, ppDyn mRNA levels were not different from the control (saline self-administration rats not treated with Yoh). But, in response to Yoh, the heroin self-administration rats displayed increased ppDyn mRNA level in the NAc shell and CeA, two brain regions known to play an important role in drug seeking and anxiety. Consistent with these results, Yoh has been found to potently induce neuronal activation in the NAc shell and CeA [Cippitelli et al., 2010]. Increased Dyn activity might be expected as a result of enhanced ppDyn gene

expression, and thus this heightened Dyn tone may be one factor involved in reinstatement of heroin seeking caused by Yoh. Although interesting, the ppDyn result of the current study should be interpreted with caution. The heroin naïve rats treated with Yoh (Table S3) did not control for a possible interaction between acquisition of operant behavior and Yoh (Fig. 2), and such experiment could be conducted in animals trained to self-administer sucrose and then treated with Yoh.

The behavioral objective of these experiments was to investigate the potential of KOP-r receptor blockade to reduce heroin seeking when reinstatement was induced by Yoh. Although the main effects of Yoh on behaviours motivated by drugs of abuse are related to noradrenergic and HPA axis activation, Yoh-induced reinstatement of cocaine and alcohol seeking also has a considerable serotonergic component [see recent review by Sinha et al., 2011]. Nor-BNI (20 mg/kg, i.p.), a systemically active and selective KOP-r antagonist, significantly blocked Yoh-induced reinstatement. Consistent with our finding, KOP-r antagonists have been found to reduce drug seeking induced by a variety of stressors, like foot-shock or forced swim, but not by drug priming with drugs of abuse [Beardsley et al., 2005; Aldrich et al., 2009]. It is unlikely that this resulted from a suppression of general activity because the well-established anxiolytic profile of the compound is based on performance in tests where reductions in anxiety are indicated by enhanced motor activity [Knoll and Carlezon 2010]. Therefore, these results suggest that Dyn activation of KOP-r receptors in response to Yoh plays a critical role in modulating the effects of Yoh stress on reinstatement of heroin seeking. To our knowledge, this is the first demonstration of KOP-r involvement in heroin seeking behavior.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

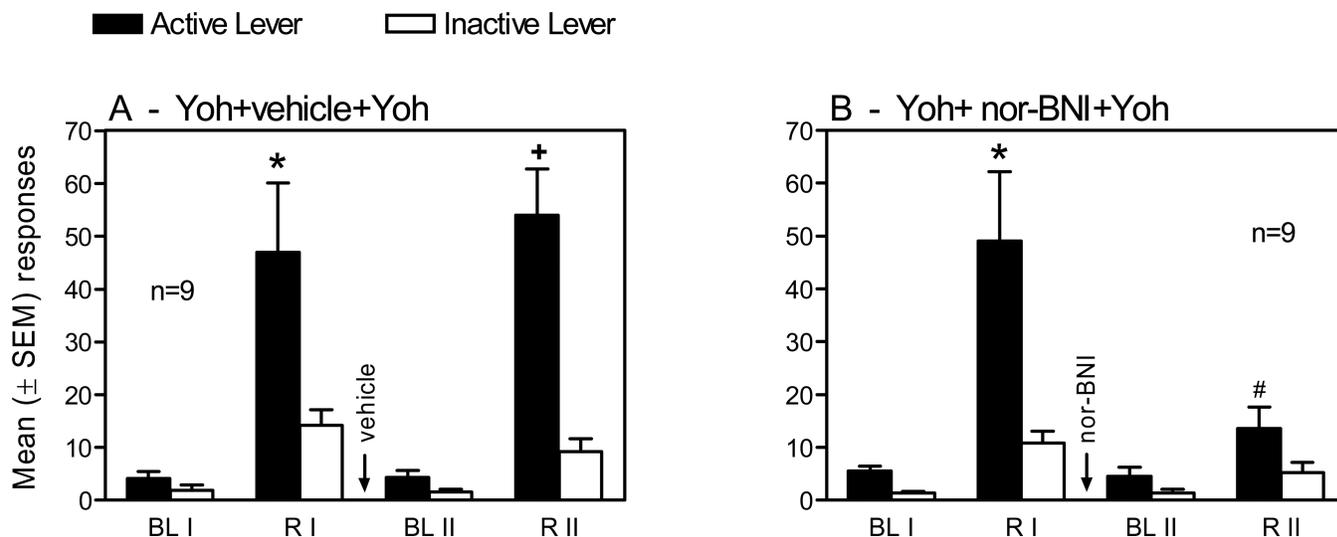
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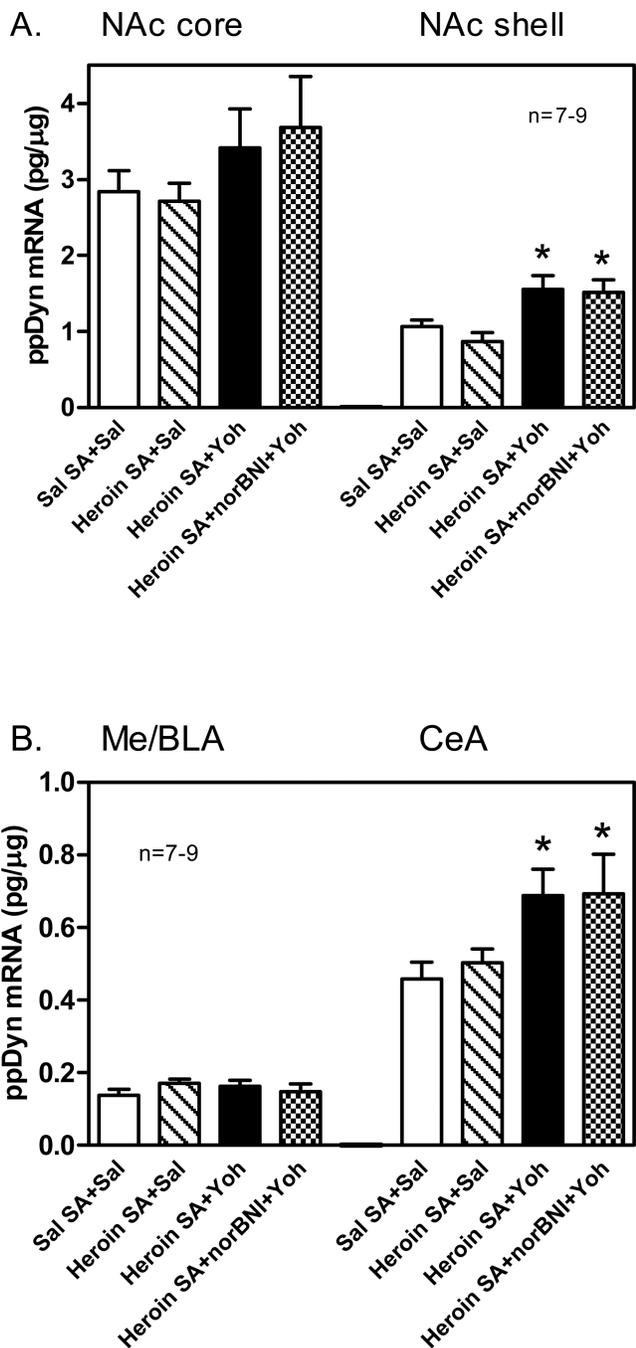
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**Fig 1.**

Mean ( $\pm$ SEM) responses on the active and inactive levers during 3 hours after exposure to yohimbine (Yoh) in reinstatement tests following extinction in heroin self-administering (SA) Sprague-Dawley rats. Twenty min prior to the reinstatement tests I and II (R I and R II), rats were injected with Yoh (1.25 mg/kg, i.p.). Before each reinstatement test, a separate 3-hour extinction served as baselines I and II (BL I and BL II). Two days before Yoh-induced R II, rats were pretreated with an injection of nor-BNI (20 mg/kg, i.p.) or vehicle (saline). See Fig. S1 in SI section for a detailed timeline. \*  $p < 0.01$  vs. active lever in BL I and inactive lever in R I; +  $p < 0.01$  vs. active lever in BL II and inactive lever in R II; #  $p < 0.01$  vs. active lever in R I and active lever in R II in the vehicle-pretreated Yoh group.



**Fig 2.** Preprodynorphin (ppDyn) mRNA levels in nucleus accumbens (NAc) core and shell (**A**), medial/basolateral amygdala (Me/BLA) and central nucleus of amygdala (CeA) (**B**) in saline (Sal) or heroin self-administering (SA) Sprague-Dawley rats after yohimbine (Yoh, 1.25 mg/kg, i.p.) with KOP-r antagonist nor-BNI pretreatment (20 mg/kg, i.p.). Animals were sacrificed 10 min after R II test, and tissues processed as described in the SI. Significant differences are indicated: \* $p < 0.05$  vs. Sal SA+Sal group.