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Short Communication

A therapeutic combination of metyrapone and oxazepam increases brain levels of GABA-active neurosteroids and decreases cocaine self-administration in male rats

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HIGHLIGHTS

- Combination of metyrapone and oxazepam (MET/OX) decreases drug self-administration.
- MET/OX treatment increases neurosteroid levels in rat brain regions.
- Modifying neurosteroidogenesis may be a useful mechanism to treat addiction.

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ABSTRACT

In rodents, the behavioral and neurochemical effects resulting from the pharmacological blockade of the hypothalamo–pituitary–adrenal (HPA) axis are unclear. Metyrapone, a corticosterone synthesis inhibitor, has been demonstrated to reduce cocaine-related behaviors, especially in a low-dose combination with oxazepam, a benzodiazepine. Although this combination therapy (MET/OX) also reduces drug-taking and drug-seeking behaviors in both rodents and cocaine-dependent humans, these effects are not correlated with plasma glucocorticoid levels. In this brief report, we present data demonstrating that this MET/OX combination enhances brain levels of the GABA-active steroid metabolites, tetrahydrodeoxycorticosterone (THDOC) and allopregnanolone. Male rats, trained to self-administer cocaine or that received yoked-saline infusions, were pretreated with MET/OX, at doses that reduced cocaine-motivated responding, or vehicle. Allopregnanolone and THDOC were measured using liquid chromatography–mass spectroscopy (LC–MS/MS) in the prefrontal cortex and amygdala in the brains from these rats. THDOC levels were enhanced following MET/OX pretreatment in both brain regions, regardless of cocaine self-administration experience. However, allopregnanolone was selectively enhanced in the rats that self-administered cocaine, but not in rats in the yoked-saline group. Thus, the MET/OX combination increased neurosteroid content in brain regions important for drug addiction. These neurosteroids have been shown to reduce cocaine-related behaviors and may contribute to the behavioral effects of MET/OX combination therapy.

1. Introduction

Early preclinical research into the interaction of stress with cocaine addiction suggested that decreasing the activation of the HPA axis may be an effective mechanism for reducing cocaine-related behaviors [1,2]. Pretreatment with glucocorticoid synthesis inhibitors, such as metyrapone, decreased cocaine-taking and -seeking by rats [3,4]. Previous research indicated that the combination of metyrapone and a benzodiazepine, oxazepam (MET/OX) significantly decreased drug-seeking and -taking behaviors in rodents and cocaine-dependent humans [5–8]. However, these effects may actually occur independently of adrenally derived corticosterone since metyrapone has been shown to produce similar efficacy against cocaine self-administration in adrenalectomized rats [9]. Recent converging evidence also suggests that there

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is no correlation between plasma corticosteroid levels and the cocaine-related responses to MET/OX [5,9]. These data suggest that a modulation of HPA axis activity may not be necessary for metyrapone's effects on cocaine-related behaviors.

Other literature indicates that metyrapone, by inhibiting 11β-hydroxylase, may shift the steroidogenic pathway toward the formation of GABA-active neurosteroids, such as tetrahydrodeoxycorticosterone (THDOC) and allopregnanolone [10,11]. Via this enhancement of neurosteroid synthesis, metyrapone may potentiate GABA-mediated anxiolytic and anticonvulsant effects [12,13]. Allopregnanolone has been reported to decrease several drug-related behaviors, including cue-, cocaine- and stress-induced cocaine-seeking, drug-induced methamphetamine-seeking and escalated cocaine self-administration [14,15]. Therefore, we hypothesized that the administration of the MET/OX combination would increase neurosteroid levels while decreasing cocaine self-administration.

2. Experimental procedures

2.1. Cocaine self-administration

Male Wistar rats (60–90 days old, maintained at 330 g body weight, $N = 24$) were implanted with jugular catheters and divided into yoked pairs. The first rat was trained to intravenously self-administer cocaine (0.25 mg/kg/infusion) under a fixed-ratio 4 (FR4) schedule of reinforcement during daily 2-h sessions. When the self-administration rat earned a cocaine infusion, a second rat received a yoked infusion of saline of the same duration, volume and temporal presentation as the cocaine infusion. Responses by the yoked-saline rat resulted in no programmed consequences. Following stable cocaine self-administration, all rats were then divided into two groups that received either vehicle (5% Emulphor in saline) or MET/OX (50 mg/kg, metyrapone and 10 mg/kg, oxazepam, IP) 30 min prior to the behavioral session. This dose combination and pretreatment time has been demonstrated to decrease cocaine and nicotine self-administration, as well as cocaine- and methamphetamine-seeking behaviors in rats [5–7]. All rats were treated three times, separated by at least two stable cocaine self-administration sessions. The first two MET/OX or vehicle pretreatment sessions were 120 min long. The duration of the final pretreatment session was only 30 min so that we could measure the hypothesized increase in neurosteroid levels at a time of maximum behavioral effect. Rats were removed from the final test session after 30 min and immediately sacrificed. Brains were removed from the skull and placed on wet ice and quickly and bluntly dissected into several regions of interest. This experiment focused on the prefrontal cortex and amygdala as these brain regions are thought to be involved in the behavioral effects of cocaine and are sensitive to neurosteroid manipulations [16,17]. The brain tissue samples were frozen at −80 °C until analysis. All procedures were approved by the LSUHSC-S Institutional Animal Care and Use Committee and were carried out in accordance with the NIH "Principles of laboratory animal care" (NIH publication no. 85–23).

2.2. Neurosteroid analysis

Rat brain sections were homogenized in 50:50 acetonitrile:water using MagNA Lyser (Roche). All samples, standards and QC samples were extracted with ethanol containing 1 mg/ml hydroxylamine and an internal standard (1 ng/ml pregnenolone-$^{13}C_2$-d_2). These extracts were incubated at 60 °C for 2 h to form the hydroxylamine derivatives of allopregnanolone, tetrahydrodeoxycorticosterone and pregnenolone-$^{13}C_2$-d_2. The derivatized extracts were dried under nitrogen and reconstituted in 70:30 methanol:water for UPLC–MS/MS analysis. All MS/MS data were acquired on an Orbitrap Velos mass spectrometer (Thermo Scientific, San Jose, CA) equipped with Waters' Acquity UPLC (Waters Corporation, Milford, MA) operating in the high resolution ElectroSpray Ionization positive ion mode. Allopregnanolone and THDOC were separated from the matrix on a Waters' BEH C_{18} 2.1 mm × 50 mm 1.7 μm column operating at 40 °C using water with 0.1% formic acid as mobile phase A and acetonitrile with 0.1% formic acid as mobile phase B (MPB). The analytes were eluted off the column using a gradient flow starting at 20% MPB and ending at 70% MPB over 6 min at a flow rate of 0.6 ml/min. Allopregnanolone and THDOC extracted from brain tissue samples were quantified against a standard calibration curve prepared in blank brain homogenate using a standard addition method (to account for endogenous level of allopregnanolone and THDOC in the blank brain) and normalized to the weight of each brain region. Mass spectrometric data acquisition and analysis used Xcalibur software package (Thermo Scientific). All quality control samples were within ±20% of theoretical concentration for all analytical runs.

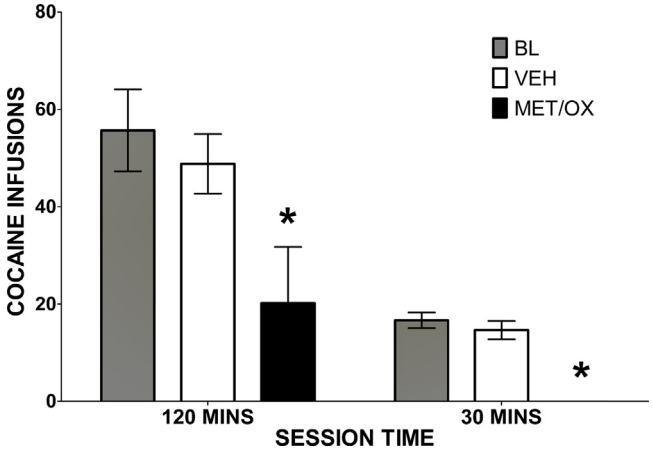

Fig. 1. MET/OX pretreatment attenuated cocaine self-administration in rats. Two distinct test sessions are presented: 120 min (left) and 30 min prior to sacrifice for neurosteroid analysis (right). Following stable baseline (BL) cocaine self-administration, vehicle (VEH) or MET/OX (50 and 10 mg/kg, IP, respectively) was administered 30 min prior to the start of both test sessions. MET/OX pretreatment resulted in a significant decrease in the number of cocaine infusions in both test sessions (*, $p < 0.05$ compared to corresponding vehicle pretreatment, $N = 6$).

3. Results

As expected, the administration of the MET/OX combination resulted in a significant reduction in cocaine self-administration compared to rats pretreated with vehicle when cocaine-reinforced responding was analyzed either following 120 min or 30 min self-administration sessions ($F(2,23) = 7.45$, $p < 0.01$ and $F(2,23) = 36.32$, $p < 0.01$, respectively, repeated measures ANOVA). In fact, MET/OX pretreatment completely suppressed all cocaine-directed responses during the 30-min test session in which rats were sacrificed for neurosteroid analysis. Importantly, the administration of the vehicle had no effect on cocaine self-administration when compared to baseline self-administration sessions with no pretreatment (Fig. 1). As hypothesized, pretreatment with MET/OX also corresponded to increased allopregnanolone content in the amygdala and prefrontal cortex when compared to rats pretreated with vehicle [$F(3,39) = 67.0$, $p < 0.01$]. This increase in allopregnanolone was specific to cocaine self-administration as this increase was not seen in yoked-saline control rats ($t = 8.0$, $p < 0.01$; Fig. 2a). MET/OX pretreatment also increased THDOC levels in the prefrontal cortex and amygdala [$F(3,39) = 269.0$, $p < 0.01$]. In