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Research report

# Role of GABA-active neurosteroids in the efficacy of metyrapone against cocaine addiction

Christopher D. Schmoutz*, Glenn F. Guerin, Nicholas E. Goeders

*Department of Pharmacology, Toxicology & Neuroscience, Louisiana State University Health Sciences Center, 1501 Kings Highway, Box 33932, Shreveport, LA 71130, USA*

## HIGHLIGHTS

- The rapid actions and pharmacological mechanisms of metyrapone were investigated in a rat model of cocaine addiction.
- Metyrapone decreased cocaine self-administration in a dose-dependent manner.
- Bicuculline, a $GABA_A$ receptor antagonist, partially attenuates metyrapone's effects on cocaine self-administration.
- Finasteride, a 5alpha-reductase inhibitor, affected cocaine self-administration as well.
- These results suggest that metyrapone may act via GABA-active steroid metabolites to decrease cocaine self-administration.

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## ABSTRACT

Previous research has demonstrated a complicated role for stress and HPA axis activation in potentiating various cocaine-related behaviors in preclinical models of drug dependence. However, the investigation of several antiglucocorticoid therapies has yielded equivocal results in reducing cocaine-related behaviors, possibly because of varying mechanisms of actions. Specifically, research suggests that metyrapone (a corticosterone synthesis inhibitor) may reduce cocaine self-administration in rats via a nongenomic, extra-adrenal mechanism without altering plasma corticosterone. In the current experiments, male rats were trained to self-administer cocaine infusions and food pellets in a multiple, alternating schedule of reinforcement. Metyrapone pretreatment dose-dependently decreased cocaine self-administration as demonstrated previously. Pharmacological inhibition of neurosteroid production by finasteride had significant effects on cocaine self-administration, regardless of metyrapone pretreatment. However, metyrapone's effects on cocaine self-administration were significantly attenuated with bicuculline pretreatment, suggesting a role for GABA-active neurosteroids in cocaine-reinforced behaviors. In vitro binding data also confirmed that metyrapone does not selectively bind to GABA-related proteins. The results of these experiments support the hypothesis that metyrapone may increase neurosteroidogenesis to produce effects on cocaine-related behaviors.



## 1. Introduction

Preclinical research into the interactions of stress with cocaine addiction reveals a complicated and time-dependent mechanism (as reviewed most recently in [1–3]). Physical stressors such as footshock or restraint have been demonstrated to sensitize rats to the behavioral and neurochemical effects of cocaine [4–9]. For example, rats subjected to noncontingent electric footshock acquire stable cocaine self-administration, responding at lower doses than non-stressed rats [5]. Similarly, pharmacological modulators of HPA axis activation also decrease cocaine-related behaviors, most notably corticotropin-releasing hormone (CRH) receptor antagonists and benzodiazepines [10–14]. Our laboratory has demonstrated the efficacy of several benzodiazepines, including alprazolam and oxazepam (OX) against stimulant-related behaviors, suggesting an important role of the $GABA_A$ receptor in modulating addiction-related processes [15–22].

Numerous studies have also demonstrated the efficacy of metyrapone (MET, a glucocorticoid biosynthesis inhibitor) in reducing the behavioral effects of cocaine in rodents. Early research indicated that pretreatment with MET reduced the psychomotor stimulant, reinforcing and discriminative stimulus effects of cocaine [23–28]. Subsequently, the combination of metyrapone and oxazepam (MET/OX) was demonstrated to decrease cocaine,

* Corresponding author. Tel.: +1 318 675 7862; fax: +1 318 675 7857.
*E-mail address:* cschm1@lsuhsc.edu (C.D. Schmoutz).

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methamphetamine and nicotine-taking and nicotine-seeking behaviors in rats [18,29,30]. Furthermore, this MET/OX combination is effective in reducing craving and decreasing urinary cocaine metabolites in cocaine-dependent human subjects [31]. However, the mechanism(s) by which MET reduces cocaine's behavioral effects is unknown at this time, especially since the reductions in cocaine self-administration and cocaine use were not correlated with changes in plasma glucocorticoid hormones, suggesting that the suppression of adrenal glucocorticoid synthesis is not a prerequisite for metyrapone's behavioral effects [24,29,31–33]. For example, MET, especially when combined with a sub-effective dose of oxazepam, decreases cocaine self-administration in rats without significantly reducing plasma corticosterone or altering the pharmacokinetics of these drugs [29]. Indeed, 11beta-hydroxylase inhibition is the canonical mechanism of action for metyrapone, which typically results in significant decreases in circulating glucocorticoids [34,35]. In addition, the effects of metyrapone on cocaine-related behaviors are quite rapid, occurring within a 30-min pretreatment window, suggesting a fast action of MET not likely associated with glucocorticoid receptor-mediated transcriptional changes. The most recent observations suggest a role for metyrapone outside the adrenal gland as well [33]. The characteristics of this mechanism have suggested that neurosteroids affecting $GABA_A$ receptors may be involved in the actions of metyrapone and the MET/OX combination [36].

The selective inhibition of 11beta-hydroxylase by metyrapone results in a specific pathway shift in the mammalian steroidogenic pathway (Fig. 4). Metyrapone administration has been demonstrated to increase 11-deoxycorticosterone (11-DOC) levels in humans and rodents via this mechanism [37–44]. This increased 11-DOC is transformed into dihydrodeoxycorticosterone (DHDOC) and tetrahydrodeoxycorticosterone (THDOC) by the successive activities of 5alpha-reductase and 3alpha-hydroxysteroid dehydrogenase [36,41,45–52]. A few studies also suggest that MET may also increase progesterone-derived metabolites and precursors such as dihydroprogesterone and tetrahydroprogesterone (allopregnanolone) [37,38,40,41,53].

Neurosteroids, such as allopregnanolone and THDOC, are endogenous steroid metabolites that function as positive allosteric modulators of the $GABA_A$ receptor and do not activate nuclear receptors [54–56]. The prototypical neurosteroid allopregnanolone increases the frequency of $GABA_A$-associated channel openings, resulting in prolonged inhibitory postsynaptic potentials and decreased neuronal excitation [57,58]. Enhancing endogenous neurosteroidogenesis produces behavioral effects similar to exogenous benzodiazepine administration: anticonvulsant, anxiolytic and anti-stress effects are predominant [59,60]. For example, stimulation of the translocator protein of 18 kDa (TSPO; formerly known as the peripheral benzodiazepine receptor) enhances allopregnanolone biosynthesis, resulting in significant clinical anxiolysis without dependence-associated withdrawal symptoms [61–63].

Thus, metyrapone influences GABA-mediated behaviors through this indirect mechanism of enhanced neurosteroidogenesis. For example, MET enhances the anticonvulsant effects of several GABA-active drugs, including midazolam and etifoxine [64,65]. Metyrapone also potentiates the anxiolytic and antidepressant effects of ethanol and allopregnanolone [66–68]. Most recently, Kaminski and Rogawski [36] demonstrated that MET increases THDOC levels in mice, thereby increasing protection against the 6-Hz seizure test, a model of induced epileptic convulsions that is especially sensitive to GABAergic drugs. This protective effect was reduced by finasteride, an inhibitor of 5alpha-reductase, the rate-limiting enzyme in THDOC biosynthesis, indicating the necessity of neurosteroidogenesis for the anticonvulsant efficacy of MET [36].

Preclinical evidence suggests that these neurosteroids may affect cocaine-related behaviors. In male rats, GABA-active neurosteroids decrease the cue-induced reinstatement of cocaine-seeking behavior without affecting ongoing cocaine self-administration [69]. In female rats, allopregnanolone decreases cocaine- and stress-induced reinstatement responding [70,71]. Neurosteroids may also interfere with cocaine-induced associative learning, having important implications for cue-associated reinstatement [72,73]. The latest published research indicates that extrasynaptic $GABA_A$ receptors may be especially important in modulating tonic and reward-related dopamine within the mesolimbic circuit [74–78], which supports a role for neurosteroids in these effects since extrasynaptic $GABA_A$ receptors are a sensitive target for allopregnanolone and THDOC [75,77,79,80].

Collectively, these data support the investigation of inhibitory neurosteroids as a potential mechanism of action for metyrapone's efficacy in reducing cocaine-related behavioral effects. We hypothesized that GABAergic neurosteroids are critical for the actions of MET on cocaine self-administration and tested this by preventing neurosteroid biosynthesis and the activation of the $GABA_A$ receptor. Pretreatment with finasteride partially increased baseline self-administration behavior without significant effect on metyrapone's reduction in cocaine self-administration. Also, the GABAergic receptor antagonist, bicuculline, partially blocked the effects of MET on cocaine self-administration. In vitro binding data suggest that metyrapone does not directly bind with high affinity to GABA-related proteins. Taken together, these data demonstrate the importance of neurosteroids in the efficacy of metyrapone against cocaine-related behavioral effects.

## 2. Methods

### 2.1. Animals and housing conditions

Male Wistar rats (~330 g) were housed in individual cages in a temperature- and humidity-controlled animal care facility on a reversed 12-h light–dark cycle for the duration of the experiments. Rats were maintained at 85% of their free-feeding body weights by presentation of food pellets during the behavioral sessions and by supplemental post-session feeding with free access to water. Previous research has demonstrated increased drug consumption and behavioral motivation for drug-seeking in food-deprived rats [81,82]. Rats were implanted with chronic indwelling jugular catheters under pentobarbital anesthesia (50 mg/kg, IP) with methylatropine nitrate pretreatment (10 mg/kg, IP) according to previously reported procedures (e.g., [17,29]). During experimental sessions, the implanted cannula was attached to Tygon tubing threaded through a stainless-steel spring leash attached to a fluid swivel (Instech) suspended above the experimental chamber. Additional tubing connected the swivel to a 20-ml syringe in a motor-driven pump (Razel Scientific Instruments, Stamford, CT, USA) located outside the experimental chamber. When an experimental session was completed, the catheters were flushed with a solution of streptokinase (0.0067 mg/0.1 ml) and timentin (6.7 mg/0.1 ml) and a small piece of Tygon tubing, sealed on one end, was placed on the cannula to preserve catheter patency. All procedures were approved by the LSUHSC 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. Cocaine and food self-administration

Experimental chambers (Med-Associates) within sound-attenuating enclosures were equipped with two retractable responses levers, a stimulus light above each lever and a food