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Metyrapone blunts stress-induced hyperthermia and increased locomotor activity independently of glucocorticoids and neurosteroids

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Summary Metyrapone, a cytochrome P_{450} inhibitor used to inhibit corticosterone synthesis, triggers biological markers of stress and also reduces stress-induced anxiety-like behaviors. To address these controversial effects, 6 separate investigations were carried out. In a first set of investigations, abdominal temperature (T_{abd}), spontaneous locomotor activity (A_S) and electroencephalogram (EEG) were recorded in freely moving rats treated with either saline or 150 mg kg^{-1} metyrapone. An increase in T_{abd} and A_S occurred in saline rats, while, metyrapone rats exhibited an immediate decrease, both variables returning to basal values 5 h later. Concomitantly, the EEG spectral power increased in the gamma and beta 2 bands and decreased in the alpha frequency band, and the EMG spectral power increased. This finding suggests that metyrapone depressed stress-induced physiological response while arousing the animal. In a second step, restraint stress was applied 5 h after injection. Metyrapone significantly blunted the stress-induced T_{abd} and A_S rise, without affecting the brain c-fos mRNA increase. Corticosterone (5 and 40 mg kg^{-1}) injected concomitantly to metyrapone failed to reverse the observed metyrapone-induced effects in T_{abd} and A_S. Finasteride (50 mg kg^{-1}), which blocks neurosteroid production, was also unable to block these effects. In conclusion, metyrapone acutely reduced stress-induced physiological response in freely behaving rats independently from glucocorticoids and neurosteroids.

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1. Introduction

Metyrapone is a cytochrome P_{450} inhibitor (Williamson and O'Donnell, 1969) that blocks the 11β-hydroxylation of deoxycorticosterone (DOC) into corticosterone in the adrenal cortex (Jenkins et al., 1958). It has been extensively used in rodents to study the role of glucocorticoids in stress processes (Mousa et al., 1981; Haleem et al., 1988; Calvo et al., 1998; Mikics et al., 2005). However, metyrapone administration leads to apparently contradictory findings.

Metyrapone limits stress-induced behaviors. Metyrapone reduces immobility time when administrated one (Healy et al., 1999) or 3 h (Baez and Volosin, 1994) prior to forced swim test. Its administration 3 h before inescapable foot-shock exposure also decreases the percentage of inactive trials during the test (Baez et al., 1996). These behavioral changes occurring during stress exposure are likely to reflect a decrease in stress-induced anxiety. The subsequent anxiety-like behaviors are also reduced 24 h after stressor exposure. Metyrapone administration 3 h prior restraint exposure increases the time spent in open arms during an elevated-plus-maze test carried out 1 day after (Calvo et al., 1998; Calvo and Volosin, 2001).

Conversely, as stated by Rotllant et al. (2002), "metyrapone can act as a stressor". Metyrapone increases c-fos mRNA expression in the hypothalamic paraventricular nucleus (PVN) (Herman et al., 1992) and Fos-like immunostaining throughout the brain (Rotllant et al., 2002). Metyrapone also activates the hypothalamo-pituitary-adrenocorticotrope (HPA) axis. The heteronuclear corticotropin-releasing factor (CRF) mRNA transcription is enhanced in the PVN (Herman et al., 1992) and vasopressin and CRF concentrations are increased in the pituitary portal blood (Conte-Devolx et al., 1992). Plasma ACTH concentration rises (Conte-Devolx et al., 1992; Herman et al., 1992; Rotllant and Armario, 2005) as well as that of 11-desoxycortisol (Conte-Devolx et al., 1992) and deoxycorticosterone (DOC, Krugers et al., 2000).

In order to examine the apparent controversial effects of metyrapone administration (anxiolysis concomitant to brain activation), a set of 6 investigations was carried out in freely moving rats. The first experimental step aimed at analyzing the immediate and delayed reactions to the injection of metyrapone through measuring two physiological variables. Abdominal temperature (T_{abd}) was taken to approach anxiolytic properties of metyrapone through the stress-induced hyperthermia paradigm (Bouwknecht et al., 2007; Vinkers et al., 2009). Spontaneous locomotor activity (A_S) was recorded because it increases after social conflict (Sgoifo et al., 2002) and saline injection (Marinelli et al., 1997). Brain activation was assessed by recording the electroencephalogram (EEG). Arousal is reflected by fast β2 (19–30 Hz) and γ (γ1: 30–35 Hz and γ2: 35–50 Hz) frequency bands (Maloney et al., 1997). Variations in locomotor activity are associated with variations in the θ (4–8 Hz) band (Oddie and Bland, 1998). It increases with locomotion speed (Slawinska and Kasicki, 1998), but disappears when the animal is immobile (Whishaw and Vanderwolf, 1971). The second experimental step aimed at analyzing the effects of stress by applying a 60-min restraint after the extinction of the immediate response to metyrapone administration. The effects of metyrapone on stress-induced physiological activation were addressed using the time course of T_{abd} and A_S. The cerebral effects were assessed using brain c-fos mRNA expression (Chan et al., 1993). Metabolic effects of metyrapone administration were quantified in the blood using glycaemia, which increases after metyrapone (Werner, 1988; Rotllant et al., 2002) and stress (Armario et al., 1990), triglyceride concentration, which decreases after stress (Ricart-Jané et al., 2002), and lactate concentration, a marker of anaerobic metabolism. The role of the inhibition of glucocorticoid synthesis in the effects observed after metyrapone administration was evaluated through corticosterone supplementation. The place of the increased DOC production (Krugers et al., 2000) was analyzed by blocking the 5-α reductase using finasteride (Lephart et al., 1996). In fact, the transformation of DOC into tetrahydro-DOC (THDOC) by 3- and 5-α reductases (Raven et al., 1996; Rupprecht et al., 1998) acts as a positive modulator of $GABA_A$ receptor (Reddy, 2006).

2. Methods

2.1. Animals

The investigation was conducted in 250 male OFA Sprague–Dawley rats (Charles River Laboratories, L'arbresle, France) weighing 175–200 g upon arrival at the laboratory. Animals were housed at constant temperature (23 ± 2 °C) and relative humidity (50 ± 10%), and in a 12 h–12 h light-dark cycle (light on at 0800 h). The rats were accustomed to laboratory conditions during 10 days before surgery and were allowed 10 days to recover from the surgical operation. They were weighed 5 days a week to reduce handling stress (Briese and de Quijada, 1970). Experimental procedures were approved by the institutional ethics committee for animal care and performed in accordance with the principles of animal care (NIH publication no. 86-23, revised 1985) and the European Community Council Directive (86/609 EEC).

2.2. Drugs

Metyrapone was purchased from Interchim (Montluçon, France) for investigations no. 1, 3 and 6 (Fig. 1) and from Sigma–Aldrich (St-Quentin Fallavier, France) for investigations no. 2, 4 and 5 (Fig. 1). The substance was dissolved in 1 ml sterile saline (SAL) and injected IP. The chosen dosage was 150 mg kg^{-1}, in order to block the stress-induced increase in blood corticosterone (Haleem et al., 1988). Corticosterone (CORT, Sigma–Aldrich) and finasteride (FIN, Interchim) were dissolved in 300 μL sesame oil (VEH) and injected SC. CORT was used either at a physiological (5 mg kg^{-1}) or at a pharmacological dosage (40 mg kg^{-1}). The latter dosages were chosen as they mimic the stress-induced blood corticosterone concentrations observed respectively in naive (Baez et al., 1996; Calvo and Volosin, 2001) and metyrapone-treated rats (Krugers et al., 1998). Finasteride was used at 50 mg kg^{-1}, a dosage that blocks completely the 5-α reductase (Lephart et al., 1996).

2.3. Variables

2.3.1. T_{abd} and A_S

T_{abd} and A_S were assessed with a TA10TA-F40 implantable radiotransmitter and the telemetric signal was acquired using a DataQuest system running on ART-gold software 3.1