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Single administration of metyrapone modifies sleep–wake patterns in the rat

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ABSTRACT

Metyrapone is a glucocorticoid synthesis inhibitor largely used to study glucocorticoid involvement in stress and memory processes. Metyrapone also acts as a stressor and therefore might modify sleep/wake patterns. However, its effects on rat sleep are unknown. We equipped 8 rats for telemetric assessment of EEG and EMG. They received first a saline injection and 2 days later a 150 mg/kg metyrapone injection. Metyrapone provoked immediately a waking effect together with a 3-h decrease in slow-wave sleep (SWS) and a 5-h decrease in rapid eye movement sleep (REM sleep). Thereafter, the rats exhibited homeostatic compensation through SWS and REM sleep rebounds recovering totally the sleep debt. The finding that metyrapone modified sleep patterns is important to consider for stress and memory studies using metyrapone.

1. Introduction

Metyrapone is a cytochrome P_{450} inhibitor (Williamson and O'Donnell, 1969) that blocks the 11-β-hydroxylation of deoxycorticosterone into corticosterone in the adrenal cortex (Jenkins et al., 1958). It has been extensively used to study the role of glucocorticoids on stress (Baez and Volosin, 1994; Calvo et al., 1998), memory (Roozendaal et al., 1996) and their interactions. For instance, metyrapone reduces stress-induced immobility behaviours not only during stressor exposure but also during a second exposure on the following day (Baez and Volosin, 1994). Its initial anxiolytic effect crosses its effects on emotional memory (Tiba et al., 2008). However, sleep and wake states also intervene on the cross-talk between stress and memory. Indeed, a short-lasting stress is accompanied by a waking effect, which is immediately followed by a sleep rebound that depends on blood glucocorticoid level (Marinesco et al., 1999). Furthermore, sleep appears to be a crucial factor for declarative and emotional memory consolidation (Diekelmann and Born, 2010; Wagner and Born, 2008). The effect of metyrapone on sleep–wake states are important to be considered since metyrapone modifies glucocorticoid production and induces a brain activation (Campeau et al., 1997) suggested by the increase in cfos mRNA (Herman et al., 1992) and protein (Rotllant et al., 2002) observed shortly after its administration.

However, data dealing with metyrapone effects on sleep–wake patterns remain conflicting. In healthy humans, metyrapone enhances wakefulness (Jahn et al., 2003; Neylan et al., 2003; Otte et al., 2007), reduces slow-wave sleep (SWS) duration (Jahn et al., 2003; Neylan et al., 2003), although inconstantly (Gillin et al., 1974), and have inconsistent effects on rapid eye movement sleep (REM sleep) duration. It slightly decreases REM sleep duration (Gillin et al., 1974) or increases it during the first part of the night (Jahn et al., 2003) or even respects it (Neylan et al., 2003; Otte et al., 2007). In the rat, metyrapone effects on sleep–wake stages are almost unknown: metyrapone reduces the anaesthetic action of pentobarbital (Burade et al., 1996).

Our aim was, therefore, to characterise in the rat the short-term action of metyrapone on sleep–wake stages in order to determine whether metyrapone induces a waking effect in relation to its biological brain activating effect and whether it would be followed by a sleep rebound.

2. Materials and methods

2.1. Animals

The investigation was performed in 8 male OFA Sprague–Dawley rats (Charles River Laboratories, L'arbresle, France) weighing 175–200 g upon arrival at the laboratory. They were housed in an environment-controlled room: ambient temperature (22 ± 1 °C) and relative humidity (50 ± 10%), 12 h–12 h dark-light cycle conditions (lights on at 08:00 a.m.). All experimental procedures were reviewed and approved by the institutional ethics committee for animal care and performed in accordance with the principles of animal care (NIH publication n°80–23, revised 1996) and the 24th of November 1986 European Community Council Directive (86/609 EEC).

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2.2. Drug

Metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone) was purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). It was injected i.p. at the dose of 150 mg/kg dissolved in 1 ml sterile saline. Vehicle injection consisted in 1 ml sterile saline. This dose was chosen because it was effective to block the stress-induced rise in corticosterone whereas 50 or 100 mg/kg failed to reach this objective (Haleem et al., 1988).

2.3. Surgery

The rats were allowed 7 days to accustom to laboratory conditions before surgery. They were then surgically implanted with the TL10M3-F50-EEE telemetric recorder. The transmitter was inserted in an interscapular subcutaneous tissue pouch under deep anaesthesia (pentobarbital sodium, 60 mg/kg, i.p.). The electrodes for electroencephalogram (EEG) were placed in 5 holes drilled in the calvarium: one pair of electrodes at 2 mm anterior and ±3 mm lateral to the bregma; and one pair at 4 mm posterior and ±2 mm lateral to the central suture. The reference electrode was placed 10 mm posterior to the bregma. Electrodes were maintained by stainless screws and anchored with dental cement (Dentalon Plus, Heraeus Kulzer, Dormagen, Germany). The electromyogram (EMG) electrodes were then placed inside the dorsal neck muscle. The rats received antibiotic (Extencilline®, Sanofi-Aventis, 60,000 IU per rat, i.p.) and anti-inflammatory treatments (Ketofen 3 mg/kg, i.m., Merial, Lyon, France) and were allowed 10 days to recover. Afterwards, each animal received alternatively saline and metyrapone injections. Each animal is consequently its own control to take into account the inter-individual variability of sleep structure. In the control situation, they received 1 ml of sterile saline i.p. at 10:00 a.m. Metyrapone was injected 2 days later at the same time of day.

2.4. Recordings and analyses

The recording system consisted in a telemetric device TL10M3-F50-EEE allowing EEG and EMG assessment, a RPC-1 receiver plates and a Data Exchange matrix connected to a computer (Data Sciences International, Saint-Paul, MN, USA). The signal was recorded at a sampling rate of 500 Hz using ART-gold software 3.1 (Data Sciences International). The analysis of sleep recordings was conducted continuously during 24 h (from 3 h before injection to 21 h after injection), a duration chosen to account for the short half-life of metyrapone (Nagamine et al., 1997). The EEG and EMG traces were first transformed from ART Raw file format into Somnologica file format (Medcare, Reykjavik, Iceland) using Neuroscore (V1.1.1, Data Sciences International). The EEG was filtered at 0.5–49.9 Hz and the EMG at 15–49.9 Hz.

Sleep analysis was performed visually in 10-s epochs in a blind-to-treatment manner using the Somnologica software. The number and duration of wakefulness, SWS and REM sleep episodes was quantified. Latencies to SWS and REM sleep were considered as the delay between the injection and the appearance of the first 1-min bout of either SWS or REM sleep.

An EEG power spectral analysis was then performed using a Fast Fourier Transform algorithm (256 points and 50% overlap) using the Somnologica 3 software (Medcare). EEG spectrum was then divided into 5 adjacent bands (delta: 0.5–3.99 Hz; theta: 4–7.99 Hz, alpha: 8–11.9 Hz, sigma: 12–13.9 Hz and beta: 14–24.9 Hz). The relative power of each band was expressed in percent of the total power spectrum. The EEG power spectrum was computed for each Treatment period and for each sleep–wake state by averaging 10-s artefact free spectra.

The influence of metyrapone and saline on sleep–wake patterns was analysed during the 24-h time course using 1-hour time epochs. Baseline period (Baseline) represented the 3 h preceding the injection of either saline or metyrapone, i.e., the last hour of the dark period and the first 2 h of the light period. The Treatment period (Treatment) was constituted by the 22 h following injection, i.e., the10-h light periods preceding and the 11-h dark period following light turn-off.

Differences in duration of each sleep–wake stage were calculated in each rat for both treatments. Two periods were determined before and after a crossing point (Cp). The Cp was visually defined as the time at which reversal of initial drug effects occurred, namely 5 h, 6 h or 7 h post-injection for SWS, wake and REM sleep, respectively. The first period (i.e. Injection to Cp) was assumed to represent the early effect of metyrapone whereas the second period (i.e., from Cp to the end of analysis) corresponded to the recovery. Differences observed between metyrapone and saline in the duration of each sleep and wake

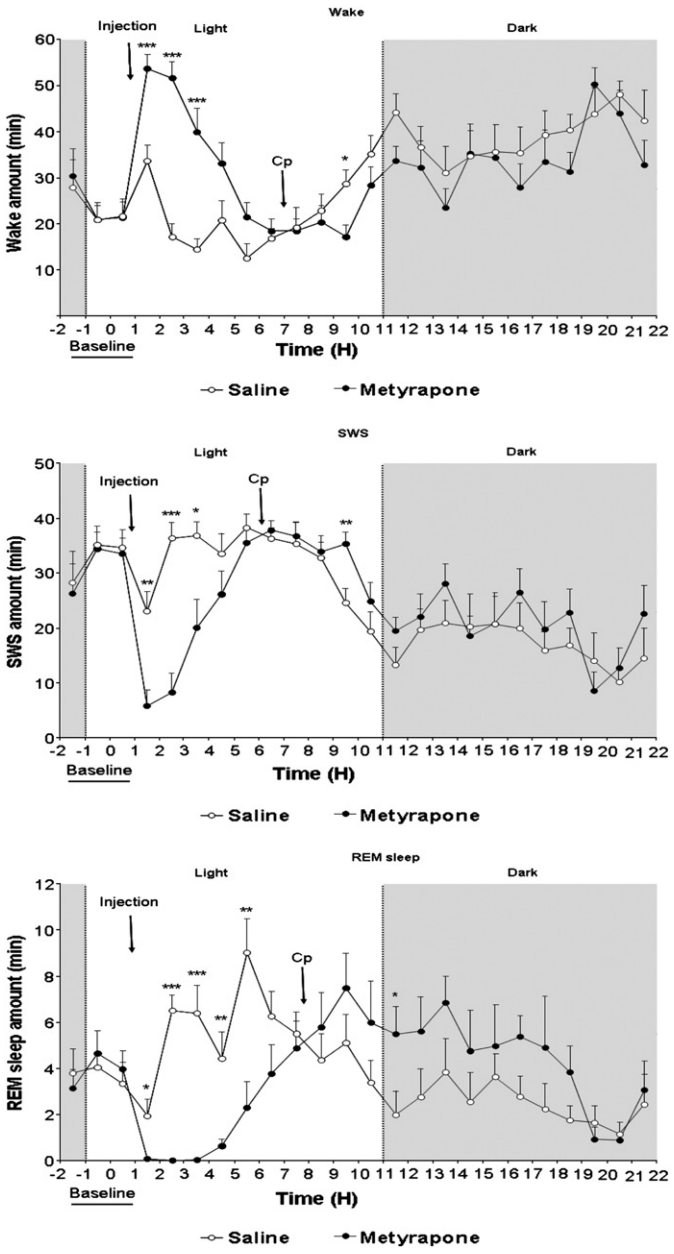

Fig. 1. Influence of metyrapone on the duration of wakefulness, SWS and REM sleep. Dark periods are highlighted in grey. The arrow corresponds to the time of metyrapone (150 mg/kg) and saline injections. Comparison between drugs is done by univariate ANOVA. Statistical significance is indicated as $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$. Values are expressed as means ± S.E.M. ($n=8$).