Psychoneuroendocrinology (2010) **35**, 1100—1112

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/psyneuen

Mifepristone decreases depression-like behavior and modulates neuroendocrine and central hypothalamic—pituitary—adrenocortical axis responsiveness to stress

Aynara C. Wulsin [a,1], **James P. Herman** [a,b,2], **Matia B. Solomon** [a,*]

[a] *Department of Psychiatry, University of Cincinnati, College of Medicine, Cincinnati, OH 45267, United States*
[b] *Neuroscience Program, University of Cincinnati, College of Medicine, Cincinnati, OH 45267, United States*

Received 4 August 2009; received in revised form 16 January 2010; accepted 19 January 2010

KEYWORDS
Glucocorticoids;
Forced swim test;
Depression;
Medial prefrontal cortex;
RU486;
HPA axis;
c-Fos;
Immunohistochemistry;
Neuronal activation

Summary Glucocorticoid dyshomeostasis is observed in a proportion of depressed individuals. As a result, glucocorticoid receptor (GR) antagonists are currently being tested as potential anti-depressants. The current study was designed to test the efficacy of mifepristone, a GR antagonist, in mitigating behavioral, neuroendocrine and central nervous system (CNS) responses to an acute stressor. Adult male rats were treated for 5 days with mifepristone (10 mg/kg) and then exposed to the forced swim test (FST). Treatment with mifepristone decreased immobility and increased swimming (but not climbing) behavior in the FST, consistent with anti-depressant action. In addition, mifepristone dampened the ACTH response to FST exposure. In the CNS, mifepristone increased c-Fos expression in all subdivisions of the medial prefrontal cortex (mPFC) and decreased neuronal activity in some subdivisions of the hippocampus including the CA2, CA3, and hilus region of the dentate gyrus in animals exposed to FST. In contrast, mifepristone increased neuronal activity in the ventral subiculum (output region of the hippocampus) and decreased c-Fos expression in the central amygdala (CeA) in animals exposed to FST. These data suggest that anti-depressant efficacy and perhaps HPA dampening properties of RU486 are related to alterations in key limbic circuits mediating CNS stress responses, resulting in enhanced stress inhibition (via the mPFC and ventral subiculum) as well as decreased stress excitation (central amygdala). Overall the data suggest that drugs targeting the glucocorticoid receptor may ameliorate stress dysfunction associated with depressive illness.

* Corresponding author at: University of Cincinnati, Genome Research Institute, 2170 E. Galbraith Rd. Room 216, Cincinnati, OH 45237-0506, United States. Tel.: +1 513 558 3025; fax: +1 513 558 9104.
E-mail addresses: aynara.chavez@gmail.com (A.C. Wulsin), james.herman@uc.edu (J.P. Herman), matia.solomon@uc.edu (M.B. Solomon).
[1] Address: University of Cincinnati, Genome Research Institute, 2170 E. Galbraith Rd. Bldg E. Room 216, Cincinnati, OH 45237-0506, United States. Tel.: +1 513 558 3025; fax: +1 513 558 9104.
[2] Address: University of Cincinnati, Genome Research Institute, 2170 E. Galbraith Rd. Bldg A. Room 145, Cincinnati, OH 45237-0506, United States. Tel.: +1 513 558 4813; fax: +1 513 558 9104.

0306-4530/$ — see front matter
doi:10.1016/j.psyneuen.2010.01.011

1. Introduction

Dysregulation of the hypothalamic pituitary adrenal (HPA) axis, manifested by elevation in circulating glucocorticoids (hypercortisolemia), is associated with affective disorders including major depression (Gold and Chrousos, 1999). It is hypothesized that hypercortisolemia is caused by disruptions in glucocorticoid-mediated negative feedback. The glucocorticoid-mediated negative feedback protects against prolonged activation of this axis, thereby preventing many of the adverse psychological and physiological consequences of glucocorticoid hypersecretion. Notably, decreased hypercortisolemia is commonly observed with successful anti-depressant treatment (Delbende et al., 1991; Reul et al., 1994; Pariante, 2003) implicating aberrant glucocorticoid signaling in depressive pathology. As such, the glucocorticoid receptor (GR) is currently a target for development of new anti-depressant therapies (Thomson and Craighead, 2008).

Mifepristone is a potent GR and progesterone receptor antagonist that is known to have anti-depressant effects following relatively brief treatment in humans (4–8 days) (Belanoff et al., 2001). The use of mifepristone in individuals with elevated glucocorticoids as a result of chronic stress or in the case of Cushing's syndrome may be particularly advantageous as preliminary blockade of GR can serve as a barrier against the deleterious effects of excess glucocorticoid production. An additional advantage of GR antagonists as discussed in Belanoff et al. (2001) and Thomson and Craighead (2008) is that prolonged antagonism of GR may actually lead to an up-regulation in GR in critical hypothalamic and limbic structures, thereby enhancing the GR negative feedback control of the HPA axis. Behavioral testing in rodents corroborates the efficacy of mifepristone or other GR antagonists (ORG34116) to ameliorate depression-like behaviors in the forced swim test (FST) (de Kloet et al., 1988; Bachmann et al., 2005). The FST, described originally by (Porsolt et al., 1977) is among the most widely used model for assessing pharmacological anti-depressant efficacy. An effective anti-depressant decreases immobility and increases active behaviors such as swimming and/or climbing in the FST (Cryan et al., 2005a). Previous studies have found that mifepristone (10 mg/kg, s.c.) administered 60 min prior to FST exposure, reverses the stress-induced increase in immobility normally observed in both male and female rats exposed to maternal separation in early life (Aisa et al., 2007, 2008). In addition, 4-day treatment of mifepristone normalizes the stress-induced reductions in hippocampal neurogenesis (Oomen et al., 2007). More importantly, this study demonstrated that mifepristone alone has no effect on neurogenesis, suggesting that the normalizing effect of the drug is evident during conditions of elevated glucocorticoids. Collectively, these findings suggest that mifepristone prevents stress-induced decreases in neuroplasticity as well as stress-induced increases in depression-like behaviors.

To our knowledge, there are no published studies that have extensively examined the effects of mifepristone on stress-induced neuronal activation in depression-related and stress-sensitive brain regions. Thus, the aim of the present study was to assess the neuroendocrine and central impact of mifepristone treatment in rats exposed to an acute stressor. This was accomplished by measuring adrenocorticotropin hormone (ACTH) and corticosterone concentrations as well as Fos induction following FST exposure. Based on the reported effects of anti-depressants on specific neural regions in depressed patients (Arce et al., 2008; Ishizaki et al., 2008; Kukolja et al., 2008) we predicted that the anti-depressant-like effect of mifepristone would be associated with alterations in forebrain regions responsible for regulation of stress responding.

2. Materials and methods

2.1. Subjects

Male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN; 250–300 G) were housed individually in standard rat shoebox cages and acclimated to the laboratory conditions for 1 week before initiation of the experiment. Rats were maintained in a temperature- and humidity-controlled room (lights on 06:00 to 18:00) with food and water available *ad libitum*. All experimental procedures and protocols were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Animals and approved by the University of Cincinnati Institutional Animal Care and Use Committee.

2.2. Drug treatment

In Experiment 1, 26 animals were matched by body weight and were divided into three groups, and administered a single daily injection of mifepristone ($n = 9$) (Sigma–Aldrich 10 mg/kg s.c. dissolved in propylene glycol), imipramine ($n = 9$) (Sigma–Aldrich10 mg/kg i.p. dissolved in saline) or vehicle ($n = 8$) (propylene glycol s.c.) for 5 days. Imipramine is known to have significant anti-depressant effects in the FST and was included as a positive control (Morley-Fletcher et al., 2004). On the fifth day, 1 h after the last injection, animals were exposed to the FST as described below. In Experiment 2, 36 animals were matched by body weight and divided into two groups and received mifepristone (10 mg/kg in propylene glycol) ($n = 20$) or the vehicle control, propylene glycol ($n = 16$) as described in Experiment 1. On the fifth day, 1 h after last injection, animals were exposed to the FST ($n = 10$ for mifepristone; $n = 10$ for vehicle). The remaining rats mifepristone ($n = 10$) and vehicle ($n = 6$) served as non-stressed controls. The mifepristone dose was selected based on successful anti-depressant effects previously observed in rats (Aisa et al., 2007, 2008).

2.3. Behavior

Forced swim test. The FST test was chosen as a way to assess the effects of mifepristone on depression-like behavior and as an acute stressor to activate the HPA axis. The modified FST was conducted according to the methodology described by Cryan et al. (2005b) which differs from the classical Porsolt FST (Porsolt et al., 1977), in that animals are only exposed to the FST once for 10 min. The behavioral apparatus was a Plexiglas cylinder 45 cm high and 20 cm in diameter filled with 31 cm of water (30–33 °C). Rats were placed in the cylinder for 10 min and the session was videotaped. Scoring was done by two independent observers blind to the treatment conditions. The behavior was scored every 5 s based on the criteria listed below. The total counts of each behavior during the 10 min