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Effect of a brief stress on progesterone plasma levels in pregnant and non-pregnant guinea pigs

Catherine L. Michel and Xavier Bonnet*

CEBC CNRS, UPR 1934, Villiers en Bois 79360 Beauvoir sur Niort, France

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Abstract

Steroid hormones are essential for vertebrate reproduction. They are involved in the regulation of major female reproductive events like ovulation, embryo implantation, or gestation. For instance, progesterone promotes foeto-maternal exchanges whereas glucocorticoids stimulate the mobilization of the required energy resources. However, glucocorticoids are key effectors of the stress response; chronic elevations of these hormones can exert negative effects on reproduction. Yet, little is known about the effects of a brief exposure to a stressor on the respective plasma concentrations of sex steroids and glucocorticoids, notably during pregnancy. We examined the impact of a brief stress (handling + 1.5 hours in a bag) on progesterone and cortisol plasma concentrations in pregnant and non-pregnant female guinea pigs. Analyses revealed that: 1) pregnant females exhibited higher baseline progesterone and cortisol concentrations compared to non-pregnant females, as expected; 2) cortisol concentrations increased rapidly following manipulation, revealing a typical hormonal stress response; and, 3) progesterone concentrations decreased on average by 50.9% following the brief stress period, both in pregnant and non-pregnant individuals. These experimental results show for the first time a drastic and rapid impact on progesterone concentration caused by a brief stress.

Keywords

Cortisol; gestation; mammals; reproduction

Introduction

Steroid hormones play major roles in reproduction (Mulac-Jericevic & Conneely, 2004; Akison & Robker, 2012), notably during gestation (Simpson & MacDonald, 1981). For instance the elevation of progesterone plasma concentration is essential for the establishment and maintenance of pregnancy (Soules, 1989; Baird et al., 1997; Magiakou et al., 1997; Ferin, 1999; Ark, 2001). This key hormone plays

*) Corresponding author; e-mail: bonnet@cebc.cnrs.fr

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multiple roles such as promoting foeto-maternal trophic and respiratory relationships, down regulation of the maternal immune system, or favouring angiogenesis (Clark et al., 2002; Ark et al., 2007). Glucocorticoids are also important endocrine factors involved in the gestation process. An increase in plasma cortisol levels along with increasing corticosteroid-binding globulin (CBG) levels stimulate maternal metabolism and the mobilization of maternal resources to sustain embryo requirements (Nolten & Rueckert, 1981; Laudat et al., 1987). However, the elevation of plasma glucocorticoid levels is considered a double-edged process because when abundantly released under stressful conditions these hormones can perturb reproduction (Breuner et al., 2008; Liu et al., 2012). Indeed, abundant release of glucocorticoids caused by a stimulation of the hypothalamic-pituitary adrenal axis (HPA) can perturb the hypothalamic-pituitary gonadic axis; for instance via the inhibition of the secretion of luteinizing hormone, and consequently can negatively impact the ovarian secretion of essential sex steroid hormones (Chatterton, 1990; Magiakou et al., 1997).

Although chronic marked elevations of stress hormones can be deleterious for organisms in general and for reproductive functions notably (Charmandari et al., 2005; Breuner et al., 2008), momentarily stress-induced cortisol elevation might be beneficial for gravid females. Indeed, a rapid elevation of stress hormones (e.g., stress response) is considered as an adaptive process that allows individuals to respond appropriately to environmental challenges (Charmandari et al., 2005). Thus, distinguishing chronic *versus* brief stress is important. Yet, there are few studies in which possible effects of acute stressors on glucocorticoid concentrations in pregnant females have been investigated (Ohkawa et al., 1991; Sugino et al., 1994). Although cortisol and progesterone are routinely assayed on captive and wild animal for both practical (e.g., diagnostic) and fundamental (life history trade-off studies) purposes (Charmandari et al., 2005; Kalz et al., 2006), previous studies typically compared distinct groups of individuals maintained under contrasted conditions during prolonged periods (Wiebold et al., 1986; Joachim et al., 2003). Consequently, simultaneous responses of sex and stress steroids in pregnant (or non-pregnant) females briefly exposed to a stressor remain poorly documented. However, brief exposure to a stressor is the most common situation encountered by free ranging and captive animals; handling, sudden noise, or the detection of a predator represent such brief stressors, for instance.

In the current study we monitored plasma concentration levels of progesterone and cortisol following exposure to a brief stressor (i.e., handling and isolation in a calico bag) in both pregnant and non-pregnant female guinea pigs. In this rodent species, high levels of progesterone are maintained throughout pregnancy (as in humans; Challis et al., 1971). In addition, in this precocial species, neonates are almost independent at birth; thus, gestation represents the most important phase in terms of reproductive investment for the females. Assaying simultaneously progesterone and cortisol concentrations allowed us to examine possible synergetic and/or antagonistic effects between these two steroids. In this framework we addressed

the following hypotheses: 1) Plasma levels of progesterone and cortisol of pregnant females should be higher than those of non-pregnant females. 2) Because cortisol could be involved in the regulation of progesterone secretion, the respective plasma concentrations of these two hormones should be correlated. 3) A brief stress should influence the plasma levels of these two hormones.

Materials and methods

Study species

Domestic guinea pigs (*Cavia aperea* f. *porcellus*) reproduce throughout the year; pregnancy lasts 68 days on average and entails a strong investment. Indeed females give birth to very large and well-developed offspring relative to their own size (Künkele, 2000). Maternal mass can increase by more than 50% during gestation. The Guinea pig is a widely used model to study human physiology; and results regarding various facets of their reproductive biology have been used to assess broad questions that pertain to other mammal species (Bähr & Wolf, 2012). For instance, the effects of prenatal stress on reproductive parameters, including sexual steroid concentrations have been recently examined in Guinea pigs (Schöpper et al., 2012a, b).

Experimental design and blood sampling

The experiment was performed on 38 adult female guinea pigs. Through natural colour markings each individual was identifiable. All females were maintained in similar housing conditions. Each female was kept in an individual box (60 cm × 50 cm × 35 cm) with a shelter (20 cm × 15 cm × 15 cm); in agreement with animal welfare authority regulations (N°A79-001, N°79-157). Food (commercial pellets, and fresh vegetables) and water were available *ad libitum*. Prior to the experiment, all females had successfully weaned one or two litters, demonstrating that they were potentially fertile. During three weeks, two males were placed in alternation with each female (each female thus encountered two males); the females were then maintained alone. Most of the females, $N = 24$, became pregnant; 14 females did not undergo gestation. Such a proportion of pregnant females (63%) has been previously observed in experiments with similar design, and was thus not unexpected (Trillmich et al., 2000). The fact that several females did not become pregnant was possibly related to a mismatch between current female reproductive cycle and the timing of the female/male encounters; indeed these 14 females became pregnant in further experiments.

We blood sampled all the females (pregnant and non-pregnant) twenty days after having separated the males from the females; thus 5 to 4 weeks before the first parturition (the mean gestation length is 68 days in this species). We selected this time period of gestation because it broadly corresponds to the phase where maximal plasma progesterone levels are recorded (Challis et al., 1971). Since hormones underlay a daily rhythm, the light regime (light on-light off) in the housing facilities

was standardised (12 h/12 h; 8:00-20:00 h). Samples were taken at 14:00 h from the main marginal ear vein using the tip of a sterile needle (0.9 × 40 mm) to make a small cut, and using two heparinised capillaries to collect approximately 200 μl of blood before haemostasis (individuals did not react (i.e. jumping, screaming) to blood sampling). For baseline cortisol and baseline progesterone levels, all guinea pigs were sampled within 3 minutes after removal from their box. Each individual was then kept in a calico bag to generate a restraint stress (Gärtner et al., 1980; Grandin, 1997). Blood samples were immediately centrifuged (3′, 10 000 g) and the plasma was collected and stored at −20°C until assays. Overall the stressor was represented by handling, blood sampling, and 1.5 h restrained in a calico bag. During this period in the bag animals had no access to food or water. All the bags were placed in the same room; we took care not to put the bags too close from each other (they were separated by approximately 50 cm). We note that it was still possible for guinea pigs to smell and hear conspecifics in these housing conditions. After 1.5 hours in the bag, we took a second blood sample to assess stress-induced hormone levels, following the same method. The guinea pigs were then returned to their box.

Hormonal assays

Hormonal assays were performed at the Centre d'Etudes Biologiques de Chizé. Plasma concentrations of progesterone and cortisol were measured by radioimmunoassay (RIA) on 50 μl of plasma after extraction with 0.5 ml of diethyl ether (mean extraction efficiency was 0.98 ± 0.10) (Bonnet et al., 2001, 2013). For progesterone, cross-reactivity of the Sigma antibody with other steroids was low (percentage of cross-reactivity at B/Bo: desoxycorticosterone, 6.6%, 5α-dihydroprogesterone, 3.9%, 5β-dihydroprogesterone, 2.6%, 6β-dihydroxyprogesterone, 2.1%). The sensitivity of the assay was 7.8 pg/tube. Inter- and intra-assay variations were, respectively, 12% and 6%. For cortisol, cross reactivity level was 9% with 1-desoxycorticosterone and less than 0.1% with other steroids. Sensitivity was 0.4 ng/ml; inter and intra assay variations were 10.0% and 7.1%.

Analyses

Hormonal data did not deviate significantly from normal distribution (Shapiro-Wilk tests; $P > 0.05$). Comparisons between groups (gravid *versus* non-gravid females) were performed using ANOVAs. Changes in hormone levels induced by handling + restraint procedure were analysed with ANOVAs for repeated measures over time (i.e., comparing individual changes from baseline to stress levels of progesterone and cortisol). We use R7.1.0 (R Development Core Team, 2008) software to perform the statistical analyses.

Results

Baseline cortisol and progesterone plasma levels

As expected, gestation entailed a marked increase of plasma cortisol and progesterone levels. Mean baseline cortisol levels were markedly more elevated in pregnant females compared to non-pregnant females (257.5 ± 94.0 ng/ml, $N = 24$ *versus* 128.6 ± 54.6 ng/ml, $N = 14$; same design ANOVA, reproductive status as the factor; $F_{1,36} = 20.15$, $P < 0.001$). Similarly, pregnant females exhibited elevated mean baseline levels of progesterone (mean value with standard deviation: 279.3 ± 109.2 ng/ml, $N = 24$); a mean value twenty times greater compared to non-pregnant females (13.2 ± 5.4 ng/ml, $N = 12$; ANOVA with baseline levels of progesterone as the dependent variable and reproductive status as the factor; $F_{1,36} = 82.10$, $P < 0.001$; fig. 1).

In pregnant females cortisol and progesterone baseline plasma levels were negatively correlated ($r = -0.44$, $F_{1,22} = 5.31$, $P = 0.031$; fig. 2). A similar, albeit non-significant, trend was observed in non-pregnant females ($r = -0.34$, $F_{1,12} = 1.55$, $P = 0.238$; fig. 2).

Brief stress period, cortisol and progesterone plasma levels

In pregnant females, following handling and isolation in a calico bag, cortisol plasma levels rose to a high mean value of 314.4 ± 104.8 ng/ml (ANOVA with baseline and stress plasma values as repeated measures, effect of time: $F_{1,23} = 9.82$,

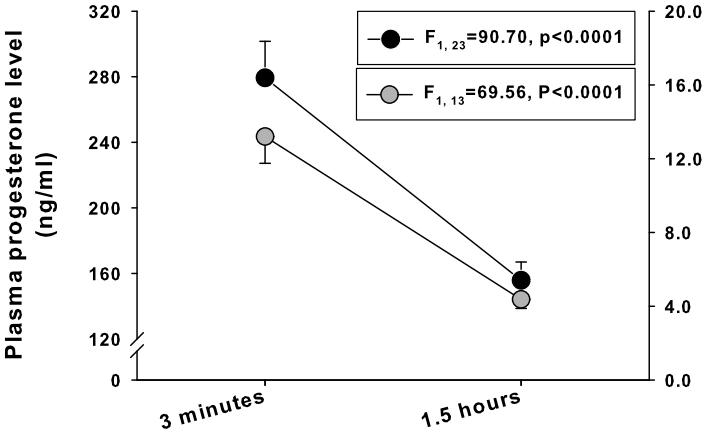

Figure 1. Changes in plasma progesterone levels following a brief stress period in pregnant ($N = 24$) and non-pregnant ($N = 14$) female guinea pigs (for pregnant females from 279.3 ± 22.3 ng/ml to 155.8 ± 11.2 ng/ml and for non-pregnant females from 13.2 ± 5.42 ng/ml to 4.3 ± 0.5 ng/ml; the ANOVA results are indicated in the figure). Black circles and left Y-axis refer to pregnant females; grey circles and right Y-axis refer to non-pregnant females. One hour and a half elapsed between the two blood-samples corresponding to pre- *versus* post-stress period. Mean values are presented with SE.

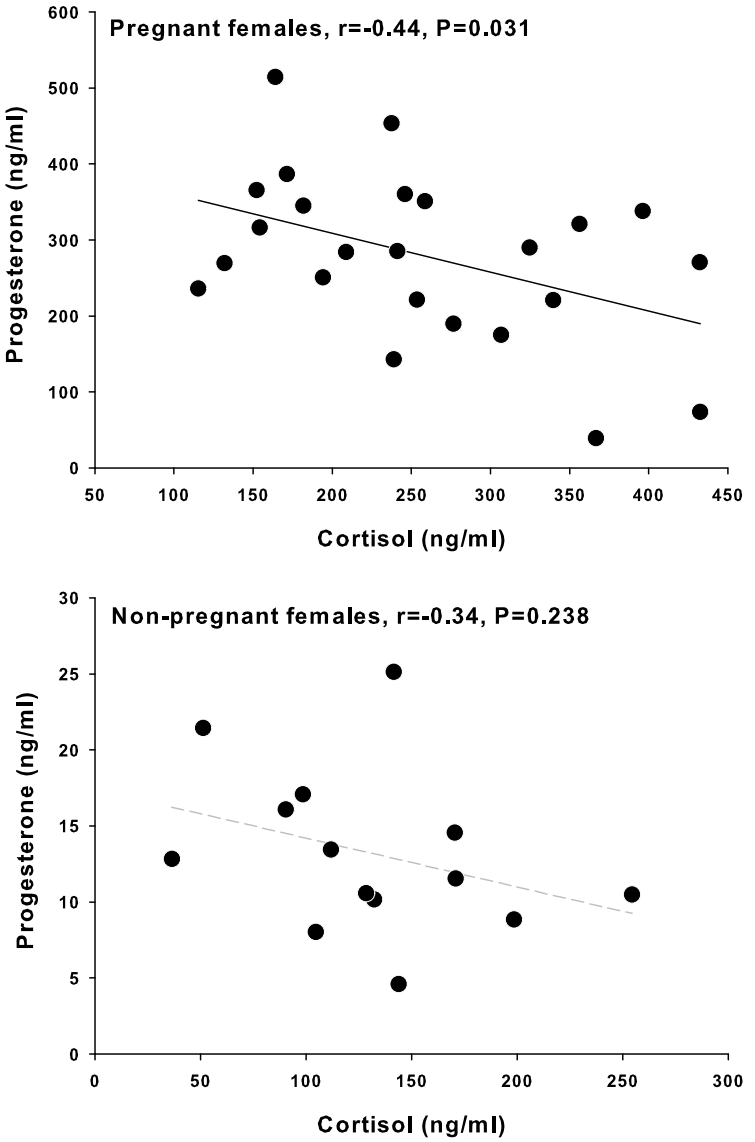

Figure 2. Relationships between basal plasma cortisol and progesterone levels in pregnant (top graph) and non-pregnant (lower graph) female guinea pigs. The black line indicates a significant negative correlation; the gray dashed line indicates a non-significant negative trend. The statistical results are also indicated within each figure.

$P < 0.001$). Similarly, in non-pregnant females the brief stress period provoked a strong increase in cortisol plasma concentration (mean value: 206.3 ± 73.8 ng/ml; same design ANOVA: $F_{1,13} = 32.32$, $P < 0.001$).

Following the brief stress period, progesterone levels decreased drastically, both in pregnant and non-pregnant females (fig. 1; for pregnant females from 279.3 ±

109.2 ng/ml to 155.8 ± 54.7 ng/ml, $F_{1,23} = 90.70$, $P < 0.0001$; for non-pregnant females from 13.2 ± 5.4 ng/ml to 4.4 ± 1.8 ng/ml, $F_{1,13} = 69.56$, $P < 0.0001$).

In both groups of females, stress-levels of cortisol and progesterone were not correlated ($P > 0.30$). The increase of plasma cortisol was not correlated with the decrease of plasma progesterone.

Discussion

To our knowledge this study is the first to report such a considerable decrease of progesterone plasma levels caused by a brief stress, both in pregnant and non-pregnant females. Previous studies reporting a negative impact of stress on sex steroid concentrations compared distinct groups of individuals, often maintained under prolonged contrasted situations, and thus they did not examine short term responses in individuals that were suddenly and briefly exposed to a stressor (Sugino et al., 1994; Tilbrook et al., 2000; Schöpper et al., 2011). In other words, the current study assessed possible impact of a brief stress on progesterone concentrations whereas most previous studies examined the impact of chronic stress. Further, heifers briefly exposed to acute restraint stress (2 h) during early pregnancy displayed increasing plasma cortisol levels but this stress response was not associated to any significant change in progesterone levels (Szenci et al., 2011). Overall, the results reported in this study provide new insights into possible impact of brief stress on plasma concentration of progesterone.

The exact kinetic of the stress-induced progesterone level collapse was not explored in the current study. Therefore the 1.5 hours timing used between sampling may have not provided the maximal response level: progesterone concentrations were possibly lower before or after. However, the negative impact of stress on progesterone levels was likely rapid, occurring before 1.5 hours, possibly following a typical "S" shape curve (Wingfield & Romero, 2001). Disregarding these speculations, we observed a considerable (−50.9 ± 14.8% on average, all females pooled) decrease of plasma concentrations, both in pregnant (−42.3 ± 10.0%) and non-pregnant (−65.7 ± 8.8%) females. We do not know the underlying regulations for this collapse of progesterone concentration. Basal plasmatic progesterone and cortisol concentrations were negatively correlated however, indicating a possible source of regulation between these two steroids (Challis et al., 1971) as demonstrated in rats (Kalil et al., 2013). Glucocorticoids can affect gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) secretions in rodents (Ahima & Harlan, 1992; Kononen et al., 1992; Chandran et al., 1994; Tilbrook et al., 2000). Under stressing conditions, the reproductive process can be inhibited at different levels; for instance hypothalamic neural circuits secrete corticotropin releasing hormone (CRH) which suppresses the secretion of GnRH by stimulating the pro-opio-melano-cortin (POMC) peptide secretion neurons (Hülse & Coleman, 1983; Chrousos et al., 1998; Dobson & Smith, 2000). The strong effects we observed were rapid however, and were possibly mediated by different mechanisms (Thomas

et al., 1991; Samuel et al., 1994; Dobson & Smith, 2000). In addition the rapid stress induced increase in cortisol did not correlate with the progesterone decrease, further investigations are required.

A practical outcome of our study is that even a short stress period should be taken into account to interpret field and experimental results based on sex steroid plasma concentrations. For instance, studies investigating progesterone levels in rodents (possibly in other mammals) should be careful with respect to stress (even moderate and/or brief). Transporting individuals from their usual living environment (e.g., from their cage, wild animals) to another place such as an experimental room is a typical handling stress, and progesterone levels may well be strongly and quickly influenced. This technical problem should also be investigated in veterinarian and stock breeding studies (Szenci et al., 2011), and also using non-invasive methods such as faecal or hair hormone assays (Bauer et al., 2008; Palme, 2012).

Guinea pigs are likely appropriate models to explore the impact of stressors on progesterone concentration during reproduction. However, further investigations on other species are needed. Indeed, the diversity of mammalian reproductive strategies is associated with contrasted underlying reproductive physiologies, notably regarding the roles of progesterone (Bradshaw & Bradshaw, 2011).

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