



# Relationship between alteration of the peptide hormone levels and depression during the gestational period

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## Abstract

**Background:** Stress is considered to be a major factor in the development of depressive illness. Pregnancy is also a kind of physical stress, and the rate of pregnancy-induced depression is increasing. This study analyzed alterations in the levels of peptide hormones in the gestational period in order to evaluate the prevalence of a depressed state during pregnancy.

**Methods:** Specific pathogen-free C57BL/6j gestation female mice were used for the experiments. In order to investigate the prevalence of a depressed state in the gestational period, we conducted forced swim test (FST) and open field test (OFT) and measured the levels of plasma adrenocorticotropic hormone (ACTH),  $\beta$ -endorphin ( $\beta$ -End), corticosterone and dopamine (DA) in the gestational period. In addition, we measured the expression of prohormone convertase 2 (PC2) in the pituitary gland.

**Results:** In the FST, the akinesia time (floating time) during the gestational period was the longest on gestational day (gd) 6, then gradually decreased toward parturition. On the other hand, in the OFT, the motor activity during the gestational period increased gradually to gd 17 after being the lowest on gd 6. The plasma levels of ACTH and corticosterone fell gradually to gd 18 after a peak on gd 6. In contrast, the plasma levels of  $\beta$ -End and DA and the expression of PC2 in the pituitary gland increased throughout the gestational period, peaking by gd 18.

**Conclusions:** These observations suggest that the levels of ACTH and corticosterone change in parallel with those of  $\beta$ -End and DA during the gestational period and function to regulate a depressed state.

**Keywords:** Depression,  $\beta$ -Endorphin, adrenocorticotropic hormone, dopamine, forced swim test, open field test

## Introduction

Adrenocorticotropic hormone (ACTH) is a peptide hormone of the hypothalamic-pituitary-adrenal axis (HPA axis) organs that is induced by various stressors following the stimulation of corticotropin-releasing hormone (CRH). The plasma concentrations of ACTH and CRH are maintained at high levels during pregnancy. The maternal secretion of pituitary ACTH and the subsequent plasma ACTH levels rise during pregnancy, while remaining within the normal limits, paralleling the rise in the plasma cortisol levels [1,2]. This rise in the maternal ACTH levels is due to circulating unbound placental CRH [3]. The ACTH concentration in amniotic fluid increases during pregnancy, peaking at the beginning of the third trimester, then exhibiting a decline [4].

On the other hand, depression is a common mental disorder that manifests as a depressed mood with loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy and poor concentration. The prevalence of depressive symptoms during the gestational period has increased in recent years. Stress is considered to be a major factor in the development of depressive illness [5]. Many of the physiological and behavioral responses associated with

stress are induced by CRH. CRH has also been found to play a role in depressive illness [6].

Therefore, in this study, we evaluated the incidence of depressive symptoms during the gestational period in mice and investigated the association between the HPA axis and depressive symptoms.

## Materials and methods

### Animals

Pregnant C57BL/6j mice (SLC, Hamamatsu, Japan) were housed individually on gestational day (gd) 0 to 18 of pregnancy. Pregnancy was determined by the observation of a vaginal plug. The plug date was considered to be gd 0 of gestation. The mice were allowed *ad libitum* access to food and water, and the 12-hour light/12-hour dark cycle, temperature (23°C) and humidity (55%) were kept constant. The animals were subjected to experiments according to the animal care regulations of Suzuka University of Medical Science. The mice were divided into 18 groups (n=6).

### Forced swim test (FST, floating test)

The mice were introduced to a transparent pool (20x35x15 cm<sup>3</sup>)

filled with warm water (30°C, height 9.5 cm) and subjected to forced swimming for six minutes. A video camera recorded the experiment for six minutes. Then, we observed the behavior of the animals and measured the duration of complete immobility of the entire body for four minutes during the second half of the experiment.

### Open field test (OFT)

The open field area (50x50x40 cm<sup>3</sup>) was made of plastic. The motor activity of the mice was measured over a 15-minute period using a video-tracking system (Smart2, Bio Research Center, Nagoya, Japan).

### Analysis of the levels of peptide hormones, corticosterone and dopamine using an enzyme-linked immunosorbent assay (ELISA)

Blood samples were obtained from the heart on each day of pregnancy, and the plasma samples were fractionated. The plasma levels of ACTH,  $\beta$ -endorphin ( $\beta$ -End), corticosterone and dopamine (DA) were determined using a commercial ELISA kits (ACTH and  $\beta$ -End; Phenix Pharmaceuticals Inc., CA; corticosterone; Assaypro, MO; DA; Abnova, Taipei, Taiwan) according to the manufacturer's instructions.

### Preparation and staining of the pituitary samples

The pituitary specimens were fixed in phosphate-buffered paraformaldehyde (4%), embedded in frozen Tissue-Tek, OCT compound and cut into 5-mm-thick sections. The sections of the pituitary gland were washed in PBS and then subsequently incubated overnight at 4°C with rabbit anti-prohormone convertase 2 (PC2; 1:100) polyclonal antibodies (Santa Cruz Biotechnology Inc., Santa Cruz, CA), in order to determine the expression of PC2. The sections were then washed in PBS and incubated at room temperature for two hours with FITC-conjugated anti-mouse immunoglobulin (1:30; Dako Cytomation, Glostrup, Denmark). The expression levels of PC2 were evaluated immunohistochemically using a fluorescent microscope.

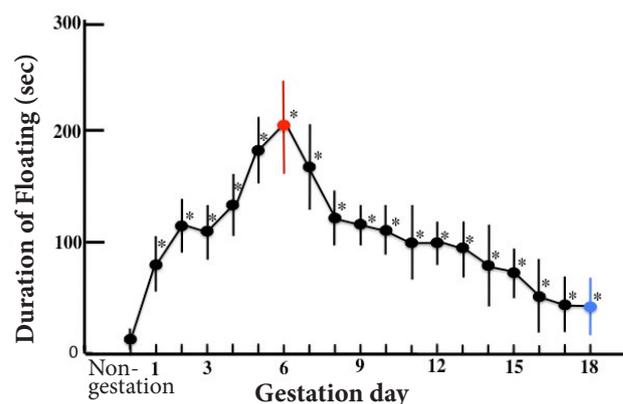
### Statistical analysis

All data are presented as the mean $\pm$ SD of results derived from six animals. The results for the two animal groups were analyzed with either Student's *t*-test or ANOVA using a computer software package. Differences were considered to be significant for  $p < 0.05$ .

## Results

### Effects of pregnancy on the mouse behavior during the FST

The FST is an examination that measures a depressed state. After being placed into the vessel containing water, the mice initially swim intensely to escape from the water, but then gradually give up and exhibit akinesia (immobility). In **Figure 1**, the duration of akinesia in the gestational period was the



**Figure 1.** Effects of pregnancy on the duration of akinesia (floating) in the FST. The values are presented as the means  $\pm$ SD derived from the results of six animals. \* $P < 0.05$  in comparison to the non-pregnant mice.

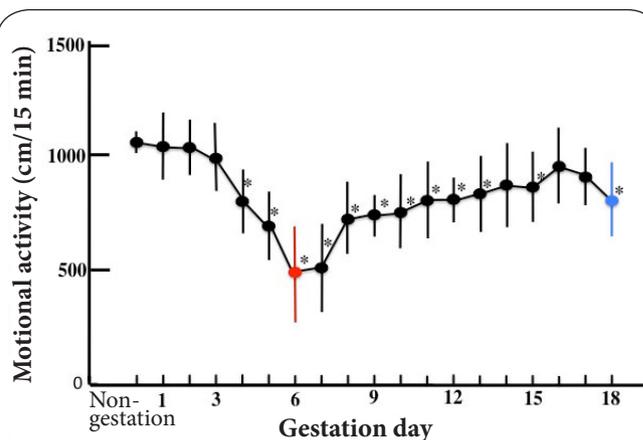
longest on gestational day (gd) 6, then decreased gradually toward parturition.

### Effects of pregnancy on the mouse behavior in the OFT

It has been reported that a depressed state decreases the motor activity [7]. As shown in **Figure 2**, the motor activity during the gestational period gradually increased to gd 17 after reaching the lowest level on gd 6.

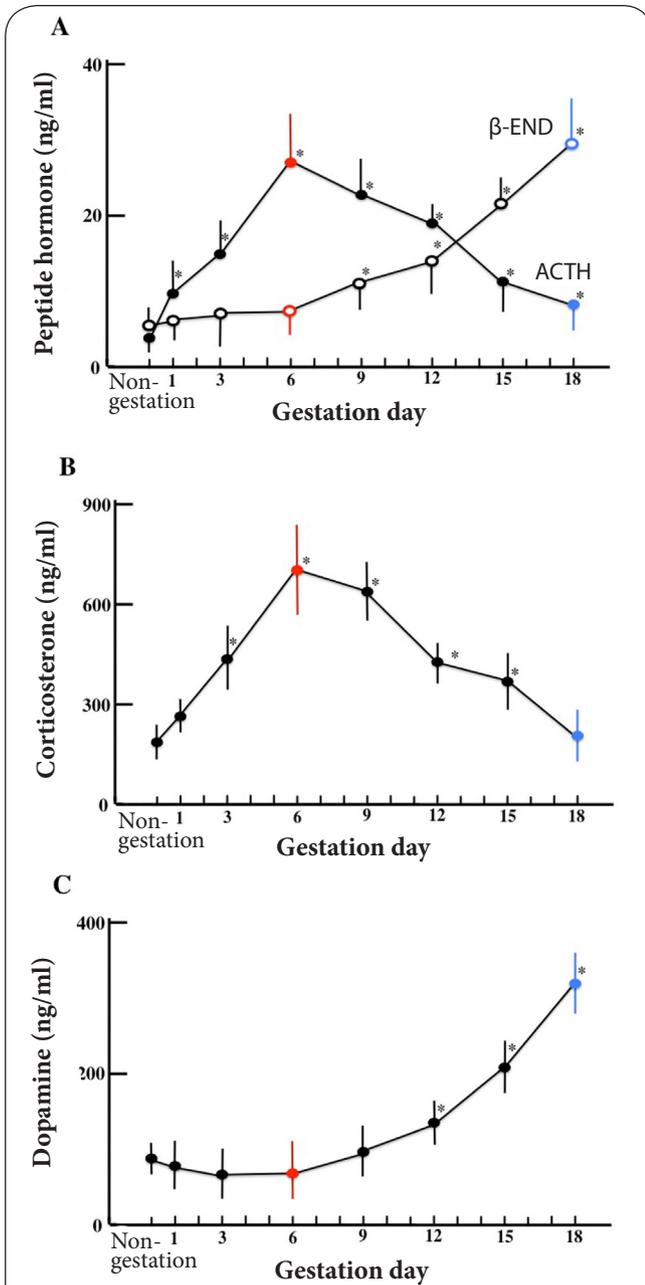
### Effects of pregnancy on the plasma levels of ACTH, $\beta$ -End, corticosterone and DA

We measured the plasma levels of ACTH,  $\beta$ -End, corticosterone and DA in the gestational period. The levels of ACTH (**Figure 3A**) and corticosterone (**Figure 3B**) fell gradually to gd 18 after



**Figure 2.** Effects of pregnancy on the duration of asthenia in the OFT. The values are presented as the means $\pm$ SD derived from the results of six animals. \* $P < 0.05$  in comparison to the non-pregnant mice.

exhibiting a peak on gd 6. In contrast, the levels of  $\beta$ -End (Figure 3A) and DA (Figure 3C) increased throughout the gestational period, peaking by gd 18.

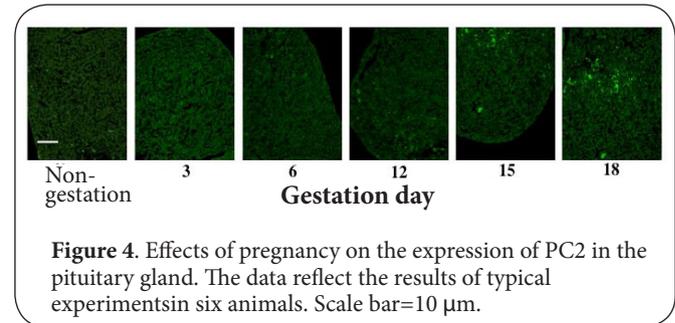


**Figure 3.** Effects of pregnancy on the plasma levels of ACTH,  $\beta$ -End (A), corticosterone (B), and DA (C). The values are presented as the means $\pm$ SD derived from the results of six animals. \*P<0.05 in comparison to the non-pregnant mice.

### Effects of pregnancy on the expression of PC2 in the pituitary gland

The production of the peptide hormones, ACTH,  $\alpha$ -MSH,

and  $\beta$ -End requires the proteolytic processing of proopiomelanocortin, which is hypothesized to utilize dual cysteine and subtilisin-like protease pathways, including the secretory vesicle cathepsin L pathway and PC pathway. In this study, the expression of PC2 increased gradually to gd 18, with a peak on gd 18 (Figure 4).



**Figure 4.** Effects of pregnancy on the expression of PC2 in the pituitary gland. The data reflect the results of typical experiments in six animals. Scale bar=10  $\mu$ m.

### Discussion

The present work demonstrated that the duration of akinesia on the FST was the longest at gd 6 and that a reduced level of motor activity was observed during the OFT. Furthermore, the levels of ACTH and corticosterone peaked on gd 6, while the levels of  $\beta$ -End and DA peaked on gd 18.

Females are more susceptible to depression during pregnancy [8]. Pregnancy has been explored from the immunological point of view, since it can be considered a semi-allograft situation [9]. In this context, locally produced embryonic and endometrial CRH plays a role in both the aseptic inflammatory process of implantation and the antirejection process that protects the fetus from the maternal immune system [10]. Early in pregnancy, the implantation sites in the rat endometrium contain 3.5-fold higher concentrations of CRH compared to that observed in the inter-implantation regions. The increase in CRH stimulates the secretion of ACTH from the pituitary gland. In addition, the increase in the plasma ACTH levels occurs in parallel to the rise in the total corticosterone levels. In this study, the ACTH and corticosterone concentrations in the plasma increased during pregnancy, peaking at gd 6, and exhibiting a decline. Dysregulation of the HPA axis characterized by glucocorticoid negative feedback resistance is frequently observed in human depressives [11]. Additionally, dysfunction of the dopaminergic and serotonergic systems in the prefrontal cortex (PFC) is thought to be involved in the development of a depressive state [11]. In rats, chronic stress induces a behaviorally depressive state, concomitant with dysregulation of the HPA axis and reductions in dopaminergic transmissions in the PFC [12]. From these reports, the presence of hypokinesia at the time of a psychical stress led to deviations in the dopaminergic neuron transmission due to the deficit of dopamine. Moreover, the cortisol level is adjusted based on the dopaminergic neuron transmission,

and the increase in the cortisol level at the time of a psychical stress causes hypokinesia. Therefore, when a pregnant mouse is exposed to psychological stress, in this study, the akinesia time was longest on gd 6, suggesting that malfunction of the dopaminergic system due to excess corticosterone was the cause. By gd 6, the ACTH/corticosterone system was considered to have contributed to the behavior strongly as opposed to the dopaminergic system. In these results, we propose that the ACTH/corticosterone system is concerned with the suppression of behavior under pregnancy and that the dopaminergic system is concerned with the activity of a behavior. Since it occurred during the gestational period, alterations of female hormones may be implicated; this issue is currently under examination. It is still not clear why this phenomenon occurred at that specific time (gd 6). In addition, in this study, we have measured corticosterone levels. In humans, the activity of cortisol is the highest of all glucocorticoids. However, in mice, the level of cortisol is very low, and corticosterone takes the place of cortisol. Therefore, we measured the corticosterone levels in the mice.

On the other hand, unlike ACTH,  $\beta$ -End increased gradually during the gestational period, exhibiting a peak by gd 18. The neuronal network is modulated by opioids at the level of DA neurons and afferent structures, typically by the activation of opioid receptor enhancing reward- and motivation-related processes [13,14]. Therefore, we suggest that the  $\beta$ -End levels could be related to the activation of the dopaminergic system and that the activation of behaviors could be attributed to the  $\beta$ -End levels. Although the plasma levels of  $\beta$ -End increased at the end of pregnancy, the results indicated that this phenomenon is based on the increase in PC2, which cleaves  $\beta$ -End from POMC in the pituitary gland (Figure 4). There is another cause of an increase in the  $\beta$ -End level, in addition to the high expression of PC2 in the pituitary gland. The levels of macrophages/monocytes, granulocytes, and lymphocytes increase during the gestational period [15,16], with the levels of leukocytes in particular increasing at the end of pregnancy [17]. Thus, macrophages/monocytes, granulocytes and lymphocytes all secrete  $\beta$ -End. In addition, POMC, PC1, and PC2 are present in leukocytes, and POMC and its product,  $\beta$ -End, are apparently stored and released from secretory granules, similar to that observed in the decreased pathway in the pituitary gland [18]. However, the molecular mechanisms by which PC2 increases during the late phase of pregnancy are unknown. Thus, two hormones, cortisol (corticosterone) and  $\beta$ -End, act at specific gestational ages, and they may have a role in maintaining the pregnancy.

In this study, on gd 6, the akinesia time was long, indicating a depressed state. Moreover, on gd 18,  $\beta$ -End was highly secreted and the gestation mice became active. According to the concept of Darwinian fitness, a spiritless state, which is one of the symptoms of depression that prevents the consumption of energy required for an immune response. In addition, the spiritless state also allows the mother to recover

by not moving and remaining still [19].

Therefore, a depressed state in a gestational age may be advantageous for maintaining a state of graviditas, and thereby improving the survival of the fetus. Our results therefore suggest that negative influences on the condition of the fetus can be avoided by suppressing the behavior of the mother's body on the most active day (gd 6) of embryonic differentiation. Conversely, this phenomenon is convenient for uplifting the mood during the intrapartum period (gd 18) and preparing for parturition. Furthermore, the level of IL-10 was highest on gd 6 (data not shown). Therefore, during the differentiation period, it is difficult to induce aggression of the immunological response to the fetus. Therefore, our results suggest that the peptide hormone-catecholamine-immune system protects the mother's body and the fetus from many risks during the gestational period.

#### List of abbreviations

FST: Forced swim test  
OFT: Open field test  
ACTH: Adrenocorticotrophic hormone  
 $\beta$ -End:  $\beta$ -Endorphin  
DA: Dopamine  
PC2: Prohormone convertase 2  
gd: Gestational day

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

Authors' contributions	KH	YY	HK
Research concept and design	✓	--	✓
Collection and/or assembly of data	✓	✓	--
Data analysis and interpretation	✓	✓	--
Writing the article	✓	--	--
Critical revision of the article	✓	--	✓
Final approval of article	✓	✓	✓
Statistical analysis	✓	--	--

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