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Low Levels of Corticotropin-Releasing Hormone during Early Pregnancy Are Associated with Precocious Maturation of the Human Fetus

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Abstract

Elevation in placental corticotropin-releasing hormone (pCRH) during the last trimester of pregnancy has been associated with an increased risk for preterm delivery. Less is known about the consequences for the human fetus exposed to high levels of pCRH early in pregnancy. pCRH levels were measured in 138 pregnant women at least once at 15, 20 and 25 weeks of gestation. At 25 weeks of gestation, fetal heart rate (FHR) responses to a startling vibroacoustic stimulus (VAS) were recorded as an index of maturity. pCRH levels at 15 weeks of gestation, but at no later point, predicted FHR responses to the VAS. Fetuses exposed to the lowest concentrations of pCRH at 15 weeks of gestation exhibited a distinguishable response to the VAS, whereas fetuses exposed to higher levels of pCRH did not respond. The findings suggest that exposure to low levels of pCRH early in gestation may be optimal and associated with a response pattern indicating greater maturity.

Keywords

Corticotropin-releasing hormone; Heart rate, fetal; Brain development, fetal; Fetal programming; Vibroacoustic stimulus

Introduction

Programming refers to the action of a factor during a sensitive developmental period that affects the organization and maturation of specific organs. One key assumption of the programming hypothesis is that biological systems undergoing rapid developmental changes are especially vulnerable to organizing and disorganizing influences [Nathanielsz et al., 2003; Seckl and Meaney, 2004]. Elevations in stress-sensitive hormones, such as placental corticotropin-releasing hormone (pCRH), are believed to have the potential to 'program' the developmental trajectory of the fetus [Sirianni et al., 2005].

Corticotropin-releasing hormone (CRH), a 41-aminoacid neuropeptide, is synthesized primarily in the paraventricular nucleus of the hypothalamus and has a major role in regulating pituitary-adrenal function and the physiological response to stress [Vale et al., 1981; Chrousos, 1992]. During the course of human pregnancy, the major stress hormone CRH is synthesized by syncytial cells in the placenta and reaches elevated levels in maternal circulation observed

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only in the hypothalamic portal system during physiological stress [Lowry, 1993]. pCRH is identical to hypothalamic CRH in structure, immuno-reactivity and bioactivity [Sasaki et al., 1988; Petraglia et al., 1989]. In contrast, however, to the inhibitory influence on the promoter region of the CRH gene in the hypothalamus, increased levels of glucocorticoids stimulate CRH gene expression in the placenta by interacting with proteins that bind to the cAMP response site of the CRH promoter [Cheng et al., 2000]. This positive feedback loop results in an amplification of stress physiology (i.e. simultaneous elevation of cortisol and pCRH) that has serious consequences for birth outcomes [McLean et al., 1995; Wadhwa et al., 2004; Sandman et al., 2006] and fetal development [Sandman et al., 1999a].

Compelling evidence from the western spadefoot toad indicates that CRH controls the developmental trajectory or metamorphosis of the tadpole. If the CRH response is blocked during stressful circumstances, the rate of development is arrested and the tadpole's survival is compromised. In order to survive, the tadpole must accelerate its development, but survival under these circumstances is associated with compromised adaptive functioning [Crespi and Denver, 2005; Kuzawa, 2005; Pike, 2005]. These findings are consistent with the effects of pCRH on human birth outcomes because it has been reported that increased levels of this stress peptide, especially during late gestation, accelerate the onset of labor and delivery [McLean et al., 1995; Wadhwa et al., 1998, 2004; Sandman et al., 2006] and the consequences of preterm birth for human development include impaired motor, cognitive and emotional functioning [Peterson et al., 2000; Anderson and Doyle, 2003].

To our knowledge, only 3 published studies have examined the developmental consequences of human fetal exposure to varying levels of pCRH [Sandman et al., 1999a; Davis et al., 2005; Ellman et al., 2008]. Fetuses of women with elevated pCRH during the third trimester were less responsive to the presence of a novel stimulus [Sandman et al., 1999a], presented with delayed neonatal maturation [Ellman et al., 2008] and displayed more fearful temperaments at 8 weeks postpartum [Davis et al., 2005]. Virtually nothing is known about how exposure in early pregnancy to varying levels of pCRH affects fetal development.

Fetal heart rate (FHR) monitoring has been used for more than 30 years to assess fetal well-being [Vlastos et al., 2007]. Measures of human FHR responses are accepted indicators of fetal maturity [Kisilevsky et al., 1992, 1999; Goome et al., 1993], reflecting the maturation and integrity of neural pathways through the cerebral cortex, midbrain, brainstem, vagus nerve and the cardiac conduction system [Van Leeuwen et al., 1999]. Several studies have shown that the fetus is capable of detecting and responding to a variety of ex utero stimuli, such as vibroacoustic stimuli (VAS) or buzzers [Leader et al., 1982; Shalev et al., 1989; Zimmer et al., 1993], clicks, and tones [Grimwade et al., 1971] by the 24th week of gestation [Crade and Lovett, 1988]. These responses to VAS are not reliable until after 26 weeks of gestation [Gagnon et al., 1987; Devoe et al., 1989; Shaw and Paul, 1990]. A VAS was used in the present study because it has been shown that a combined tactile and auditory stimulation elicits a greater FHR acceleration than an auditory stimulus alone [Kisilevsky and Muir, 1991].

Investigating how and when elevated levels of pCRH affect fetal development is of interest because there is evidence that fetal maturity predicts the rate of infant development [DiPietro et al., 2002, 2007; Werner et al., 2007]. The present study examined the influence of exposure to prenatal pCRH concentrations on FHR responsivity to an ex utero startle stimulation.

Method

Participants

As part of a larger study, 138 mother-fetus dyads were recruited prior to their 16th week of gestation and they provided written, informed consent. All pregnancies were singleton

intrauterine pregnancies in English-speaking women at least 18 years of age. Participants reported not using tobacco, alcohol or other drugs during pregnancy and did not present with uterine or cervical abnormalities. Women were excluded if they had any condition potentially associated with disregulated neuroendocrine function, such as endocrine, hepatic or renal disorders, or if they used corticosteroid medications. The mean maternal age at delivery was 28.3 ± 5.0 years. This was the first child (primiparous) for 54% of the sample and fetal sex was 49% female. The sample consisted of 43% non-Hispanic white, 33% Hispanic and 8% Asian women. The precise gestational age (GA) at each testing period was determined by last menstrual period and confirmed by an ultrasound scan conducted before 20 weeks' gestation.

pCRH Concentration

Maternal blood samples were collected 3 times during the course of pregnancy for analysis of pCRH concentration. Assessments took place at GAs of 13–17 weeks (mean 15.1 ± 0.8), 17–22 weeks (mean 19.6 ± 0.9) and 24–28 weeks (mean 25.4 ± 0.8). pCRH levels were available for 127 women at the first assessment, for 131 women at the second assessment and for 136 women at the third assessment. A 20-ml blood sample was withdrawn by antecubital venipuncture into siliconized EDTA vacutainers and immediately chilled to 6°C after 500 KIU/ml aprotinin (Sigma Chemical Company, St. Louis, Mo., USA) was added. Samples were centrifuged at 2,000 *g* (15 min) and the plasma was decanted into polypropylene tubes. Plasma samples were then stored at −70°C until assayed. CRH levels (pg/ml) were determined from extracted samples by radioimmunoassay (Bachem Peninsula Laboratories LLC, San Carlos, Calif.) as previously described [Sandman et al., 2006]. Intra- and inter-assay coefficients of variance ranged from 9.3% to 21% respectively.

Fetal Monitoring

Immediately following blood collection during the third prenatal visit, the fetal monitoring procedure was administered. The vibroacoustic stimulator was placed on the mother's abdomen above the fetal head, as determined by ultrasonography. During testing, all mothers reclined in a semi-Fowler's position (5- to 10-degree tilt) on a standard, padded examination table. Participants listened to pure-tone music presented through headphones to mask extraneous noise and the auditory component of the stimulation.

Fetal monitoring began with a 15-min baseline (resting) recording of the FHR, followed by a 1-second administration of a VAS on the mother's abdomen. The fetal monitoring period concluded with 5 min of FHR recording to assess the post-VAS startle response.

The instruments used in this study were designed to quantify FHR variation due to ex utero stimulation [Sandman et al., 1997]. Transabdominal transducers were attached to measure the FHR. Transducers were positioned until a robust signal was reliably detected. All fetal information and uterine contractions were quantified by a Toitu MT-430 ultrasound fetal monitor. The Toitu monitor measured Doppler frequency shifts in a weak ultrasound beam projected onto the fetus by an ultrasonic head and extrapolated the FHR from fetal movement and uterine contractions. Output from the fetal monitor was connected to Active II, a data acquisition system (Biosemi Instrumentation) with a 2 kHz sampling resolution. These data were transferred electronically to an offline server for data analysis. Integrity of the data was assured by visual inspection with customized software that included a viewer for examination of each tracing to scan for artifacts. If needed, an interpolation routine was applied with a 1-second resolution to each tracing to estimate missing data due to artifacts. Each tracing was examined by a trained observer and a judgment was made about the validity of interpolation. If a segment of the data resulted in invalid interpolations, that section of the data was omitted from the analyses.

All analyses were performed using FHR data at 1-second resolution. The average FHR at a GA of 25 weeks is shown in figure 1. ΔFHR scores were calculated by subtracting the average baseline FHR (120 s immediately preceding the startling VAS) from every 1-second post-startle stimulus for a 30-second interval for each subject (fig. 2).

Plan of Analysis

Paired t tests, comparing the average baseline FHR to that 30 s after VAS, at 1-second intervals, were applied to determine whether presentation of the VAS resulted in a significant change in FHR. A repeated measures ANOVA was computed to test changes in pCRH concentrations over the 3 assessments (15, 19 and 25 weeks' gestation). Bivariate correlations were performed to test the association between pCRH concentrations at the 3 different assessments and the average baseline FHR at a GA of 25 weeks.

Hierarchical linear modeling (HLM) procedures were used to analyze differences in trajectories of ΔFHR response to the VAS over a 30-second interval and to determine the influence of exposure to pCRH on these trajectories. The quadratic growth model of ΔFHR was a better fit for the data as compared to the linear model [$\chi^2(3) = 249.12$, $p < 0.001$]. The quadratic solution in HLM produces 3 coefficients for comparison: (1) the mean level differences or the intercepts at each interval tested, (2) the instantaneous rate of change (linear slope) at any time point of interest, and (3) the overall acceleration (shape) of the curve. The model was controlled for exact GA at FHR assessment. Potential covariates (fetal sex, parity, maternal race/ethnicity and maternal age) were screened for inclusion in the model. None of the proposed covariates significantly reduced the deviance or variance components (all $p > 0.10$), and were subsequently not included in final analyses.

A separate analysis was performed during the baseline period to determine whether early fetal exposure to pCRH was specifically associated with the FHR response to the VAS and not a result of random variation. A random time point during the resting baseline period was selected and defined as the phantom VAS. The average FHR for 60 s preceding the phantom VAS was calculated. ΔFHR scores were calculated for the succeeding 30 s by subtracting the 60-second average from every 1-second measurement subsequent to the phantom VAS being applied for each subject. HLM procedures were used to analyze the trajectories of ΔFHR following the phantom VAS and to determine the influence of exposure to pCRH on these trajectories.

Results

FHR Response to VAS

FHR after VAS did not differ significantly from the average baseline FHR ($p = 0.13$–0.96), indicating that, on average, the 25-week-old human fetuses were not responding to the VAS, which was consistent with previous reports [Gagnon et al., 1987; Devoe et al., 1989; Shaw and Paul, 1990].

Change of pCRH over Gestation

A repeated-measures ANOVA revealed the expected significant increase in pCRH concentrations over the course of gestation ($F_{1.1, 128.9} = 104.3$, $p < 0.001$) with mean concentrations of 19.7 ± 9.7 pg/ml at a GA of 15 weeks, 28.9 ± 20.5 pg/ml at a GA of 20 weeks and 76.0 ± 64.9 pg/ml at a GA of 25 weeks (fig. 3).

Influence of pCRH on Basal FHR

Bivariate correlations indicated that pCRH levels were not correlated with average baseline FHR for the 120 s preceding the application of the VAS at 15 weeks' gestation ($r_{127} = 0.13$, p

= 0.15), 20 weeks' gestation (r_{131} = 0.11, p = 0.23) or 25 weeks' gestation (r_{136} = 0.08, p = 0.35).

Influence of pCRH on FHR Responsiveness to the VAS

Significantly larger FHR responses to the VAS were detected in fetuses exposed to low levels of pCRH at a GA of 15 weeks but not at 20 or 25 weeks. Figure 4 displays the mean level differences in FHR that were found as a result of exposure to pCRH at 15 weeks' gestation (fig. 4 displays groups based on the highest and lowest quartiles of pCRH). Differences emerged between 6 and 10 s after VAS (βs = −0.16 to −0.15, p < 0.10), became significant at 12 s after VAS and remained significant until 30 s after VAS (βs = −0.25 to −0.168, p < 0.05). No differences in FHR responses to the VAS could be observed based on pCRH concentrations at GAs of 20 (p < 0.19) and 25 (p < 0.13) weeks. There were no differences in the overall shape of the curve (acceleration) or the instantaneous rates of change at any time point of the response pattern as a result of early exposures to pCRH at 15, 20 or 25 weeks of gestation (βs = −0.01 to 0.00, not significant).

Thus, concentrations of pCRH at a GA of 15 weeks, but not at 20 or 25 weeks, influenced FHR response to the startling stimulus. Only fetuses exposed to the lowest levels of pCRH early in pregnancy exhibited significant FHR responses to the startling stimulation.

Influence of pCRH on FHR Changes following the Phantom VAS

No significant differences in the intercepts (βs = −0.001 to −0.003, not significant), instantaneous rates of change at any time point (slope; βs = 0.00, not significant), or curvatures (βs = −0.00, not significant) of FHR following the phantom VAS during the baseline period were observed as a function of pCRH exposure at 15 weeks' gestation (fig. 5). Accordingly, it can be concluded that the FHR increase observed in fetuses exposed to lower concentrations of pCRH at 15 weeks' gestation reflects a response to the VAS and is not the result of random variation in FHR.

Discussion

The findings of the current study indicate that fetal exposure to pCRH early in pregnancy is associated with fetal responsiveness to a startling stimulus 10 weeks later. The influence of pCRH on fetal responsiveness to the startling stimulus followed a clear temporal pattern. Only levels of pCRH at a GA of 15 weeks, but not at 20 or 25 weeks, were associated with fetal response patterns at 25 weeks. The significant influence of early exposure to pCRH on human fetal responses has not been observed previously. The pattern observed in this study of FHR in fetuses exposed to low levels of pCRH has been linked to relatively mature responses [Kisilevsky et al., 1992]. Typically, reliable FHR responses to VAS are not observed before 26 weeks of gestation [Gagnon et al., 1987; Devoe et al., 1989; Shaw and Paul, 1990]; it therefore is reasonable to conclude that a trajectory of accelerated human development is associated with exposure to low concentrations of pCRH early in pregnancy.

The current findings are consistent with previous results that suggested pCRH plays a key role in the maturation of the fetal hypothalamic-pituitary-adrenal axis and may direct fetal nervous system development [Sandman et al., 1999a, b, 2003; Davis et al., 2005]. Interestingly, in the present study, pCRH concentrations at early (15 weeks) but not at later stages in gestation (20 and 25 weeks) predicted FHR responsiveness to the VAS. This timing effect is different from that reported for the effects of pCRH on birth outcomes, because elevated pCRH levels late in gestation (from about 30 to 35 weeks' gestation) are associated with an increased risk for preterm delivery [Wadhwa et al., 2004; Sandman et al., 2006]. Also, pCRH concentrations after midgestation, but not before, were associated with neonatal maturation [Ellman et al.,

2008] and infant temperaments [Davis et al., 2005]. It is not surprising that consequences of exposure to pCRH vary with timing of exposure, because different biological systems mature at different rates and at different times during gestation. The systems that are most vulnerable to programming influences are those that are undergoing rapid developmental changes [Nathanielsz et al., 2003; Seckl and Meaney, 2004]. In general, it can be concluded that exposure to elevated concentrations of pCRH, either early or late in gestation, is associated with possible health risks that include immature nervous system development, preterm birth, neonatal immaturity and a more difficult infant temperament.

A general principle in brain development is that maturation occurs in the form of a cascade of events and that each action may impact on following but not preceding ones [Stern et al., 2005]. The consequence of this cascade is that the earlier an event occurs (i.e. potential organizational influences), the larger its influence on subsequent brain development. This is consistent with our current findings in which early but not later exposure to low levels of pCRH influenced or programmed markers of fetal neurological maturation.

Fetal behavior is an indicator of the fetal nervous system's developmental stage [Krasnegor et al., 1998], and the fetal startle response in particular has been linked to brainstem functioning [Hanson, 1991]. During fetal life, neurons profilerate, migrate and aggregate, providing the 'hardware' for the developing brain. Neural proliferation before birth has been estimated at an average rate of 250,000 cells/min [Cowan, 1979]. Between the gestational ages of 8 and 16 weeks, migrating neurons form the subplate zone and await connections from afferent neurons originating in the thalamus, basal forebrain and brainstem [Kostovic et al., 2002]. Because of this rapid developmental trajectory, the human fetal brain is particularly vulnerable both to organizing and disorganizing influences at these early stages of gestation. Based on our results, it is reasonable to assume that low pCRH concentrations during this critical period of rapid development in early gestation are associated with optimal or organizing effects on brainstem development. This possibility is supported by the findings that only the subgroup exposed to low pCRH concentrations at 15 weeks' gestation exhibited a robust response to the VAS. These data suggesting that low concentrations of pCRH are associated with optimal development are consistent with our previous work demonstrating that lower levels of CRH predicted the development of easier temperament [Davis et al., 2005]. The absence of a fetal response to the VAS in those fetuses exposed to higher pCRH concentrations may reflect delayed brainstem maturation. Such disorganizing effects of high pCRH concentrations may be similar to those in which fetuses exposed to alcohol do not exhibit a normal startle response [Little et al., 2002]. In alcohol-exposed fetuses, decreased FHR responsiveness is attributed to aberrations in the migration of glial and neuronal cells and brainstem abnormalities [Peiffer et al., 1979]. Our results add to the literature suggesting that there are critical periods during development when environmental events can regulate the trajectory of brain development. The windows of plasticity close early during human development and are determined by fetal and infant ontogenetic development [Wells, 2007].

There is evidence to suggest that the normal trajectory of pCRH production over the course of gestation may be increased both by physiological and psychosocial stress. A series of in vitro studies have shown that pCRH is released from cultured human placental cells in a dose-response manner in reaction to all the major biological effectors of stress, including cortisol, catecholamines, and proinflammatory cytokines [Petraglia et al., 1997]. We recently reported that, in vivo, a maternal stress signal (elevated cortisol very early in pregnancy) was associated with a highly significant and precocious rise in pCRH later in pregnancy and this effect was associated with risk for preterm delivery [Sandman et al., 2006]. Other in vivo studies have found significant correlations among maternal pituitary-adrenal stress hormones (ACTH, cortisol) and pCRH levels [Goland et al., 1992; Chan et al., 1993; Wadhwa et al., 1997; Hobel et al., 1999a]. Moreover, maternal psychosocial stress is significantly correlated with maternal

pituitary-adrenal hormone levels (ACTH, cortisol) [Wadhwa et al., 1996] that are known to stimulate pCRH secretion. Some [Hobel et al., 1999b; Erickson et al., 2001], but not all studies [Petraglia et al., 2001], have also reported direct associations between maternal psychosocial stress and pCRH function. Thus, there are both in vitro and in vivo findings indicating that the placenta detects and responds to a variety of maternal physiological and psychological stress signals supporting the conclusion that levels of pCRH reflect fetal exposure to maternal stress. This conclusion is supported by findings from several studies that reported an impact of prenatal maternal psychosocial state on human fetal behavior [Van den Bergh et al., 1989; Groome et al., 1995; Sjostrom et al., 1997; Monk et al., 2000; DiPietro et al., 2002; Monk et al., 2003].

These influences of pCRH on the human fetus are consistent with the effects of stress-mediated CRH response in the western spadefoot toad. In both the toad and the human fetus, the trajectory of development is influenced by the levels of circulating CRH. Exposure to CRH late in development accelerates metamorphosis in the toad and exposure of the human fetus to CRH early in gestation delays nervous system maturation.

Here, we are the first to prospectively report programming influences on the human fetus. In addition to discovering the influence of pCRH on fetal maturity, the current study indicated that the timing of exposure was instrumental in determining the eventual developmental consequences. Exposure to elevated pCRH levels during the early second trimester but not later in gestation was associated with delayed maturity in the human fetus at 25 weeks' gestation.

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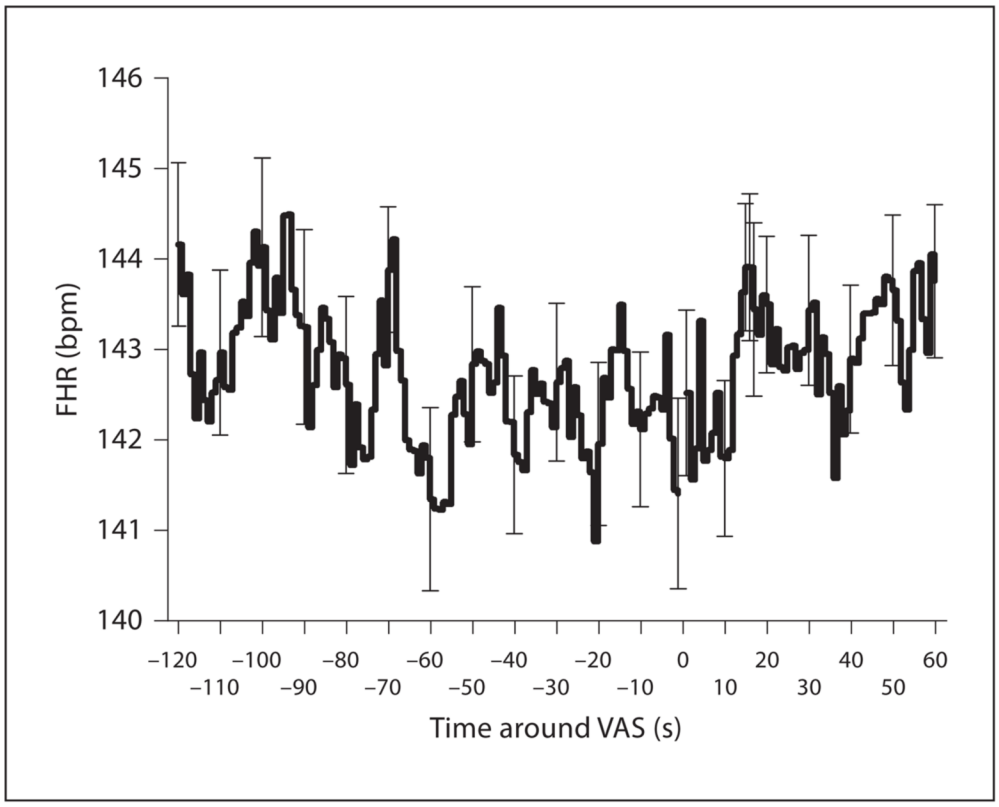

Fig. 1.
Average baseline and recovery FHR surrounding the startling VAS.

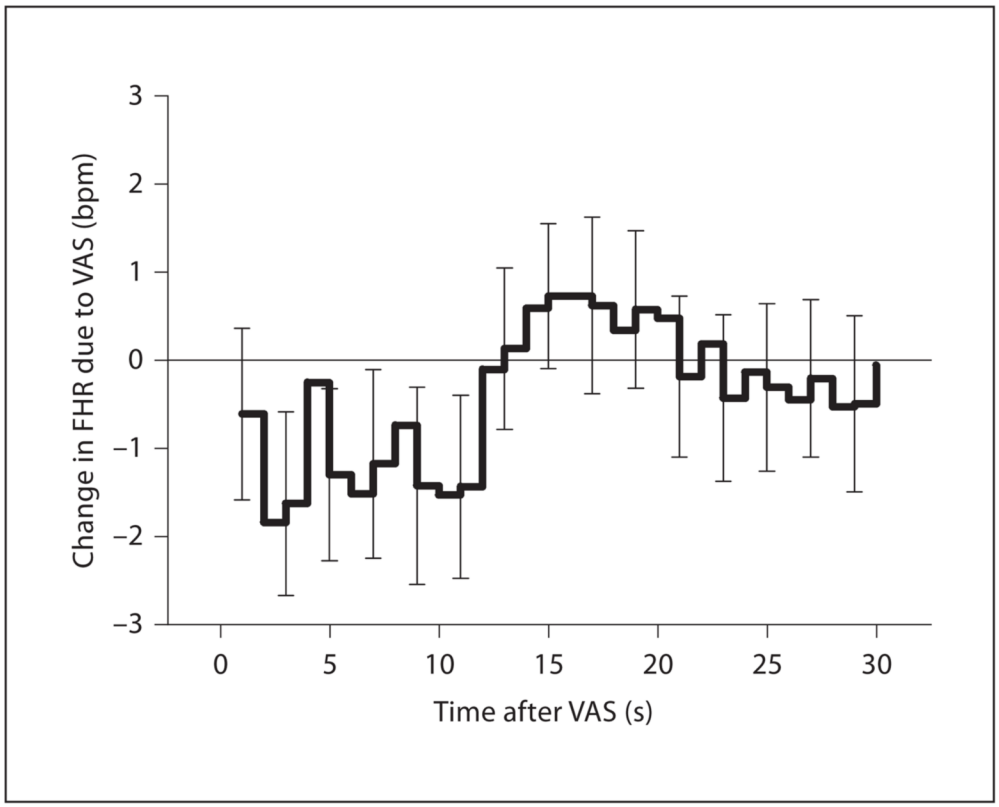

Fig. 2.
Change in FHR over the 30 s after the VAS had been applied.

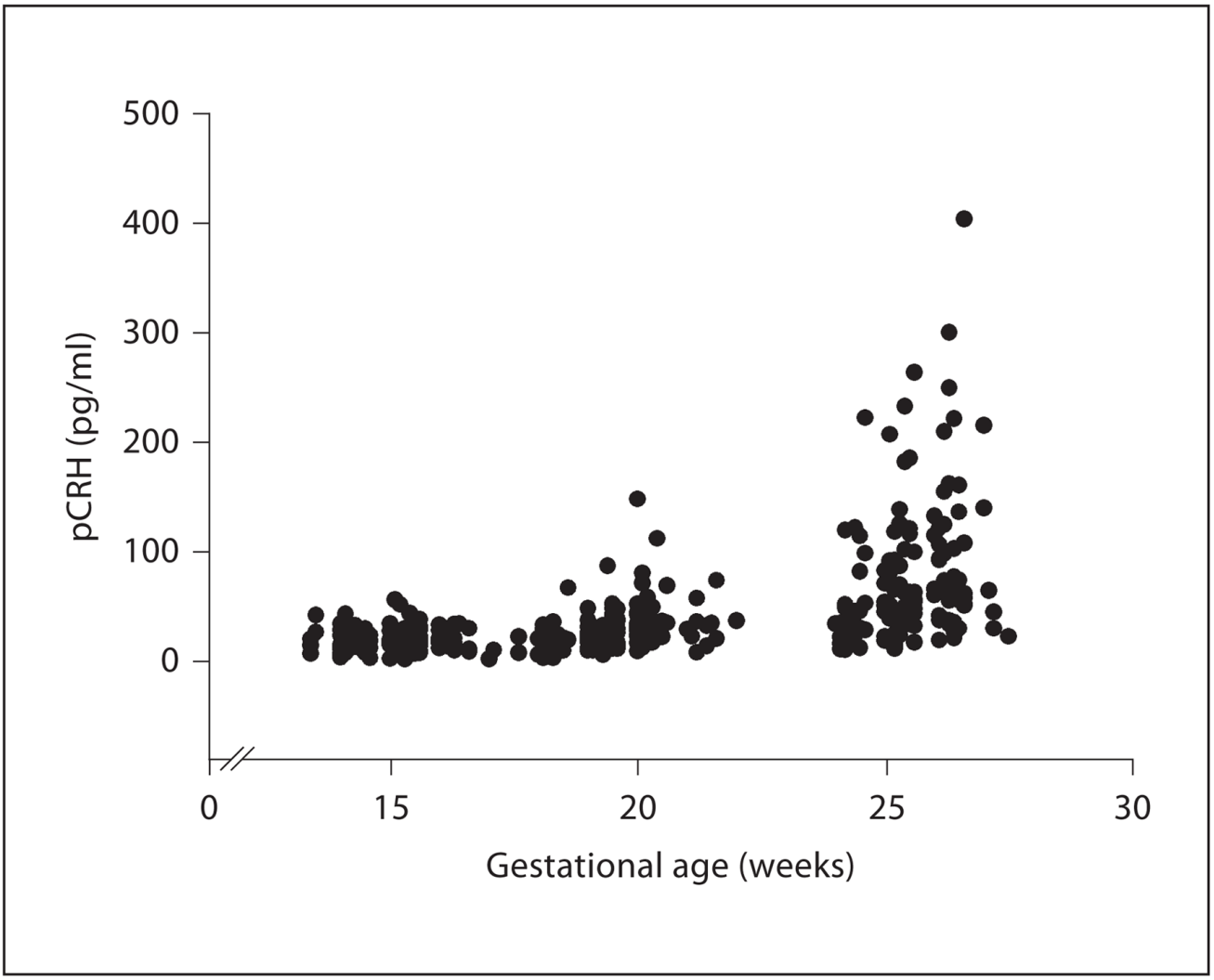

Fig. 3.
Increase in pCRH concentration over gestation.

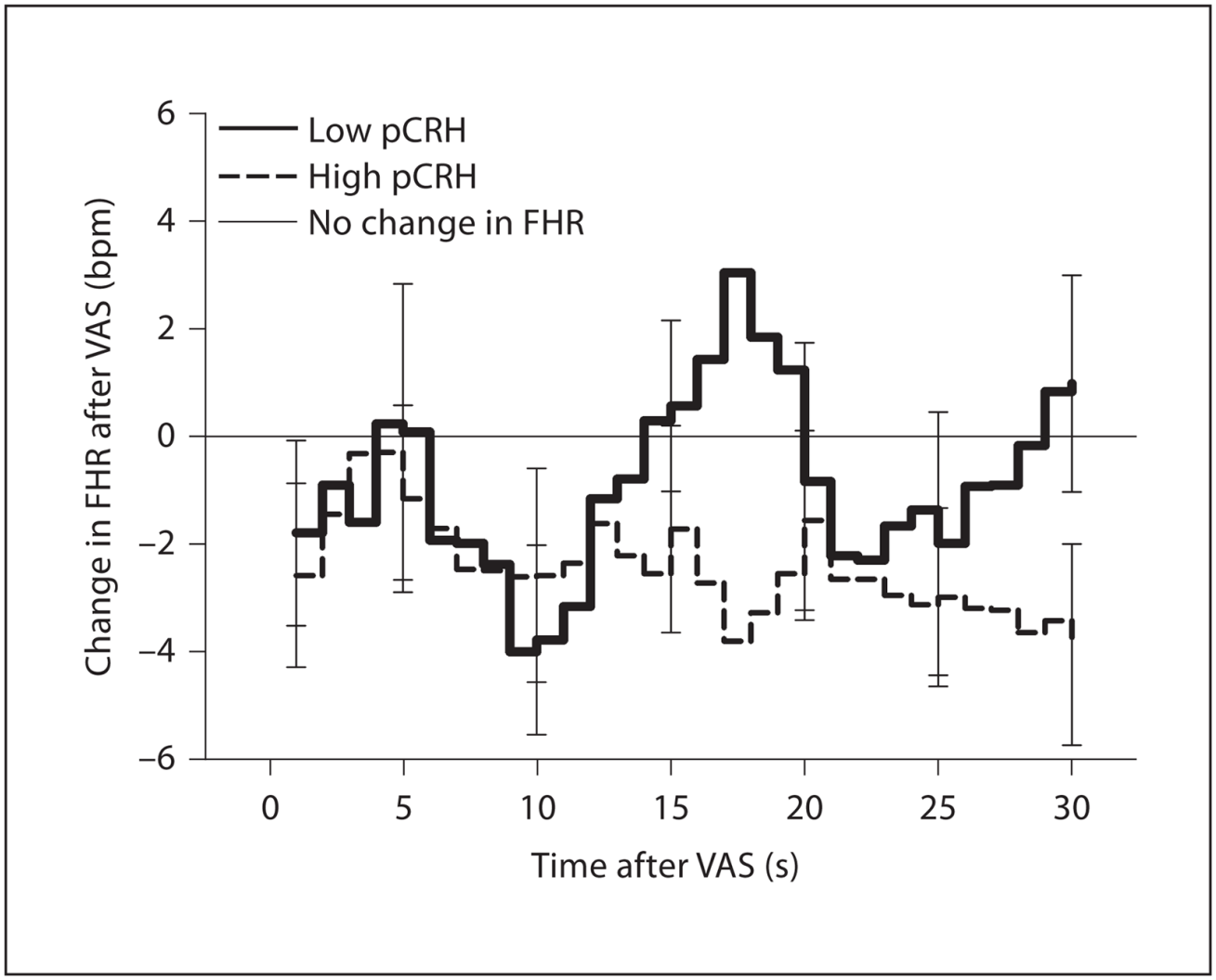

Fig. 4.
FHR response to the startle VAS based on pCRH levels at 15 weeks' gestation.

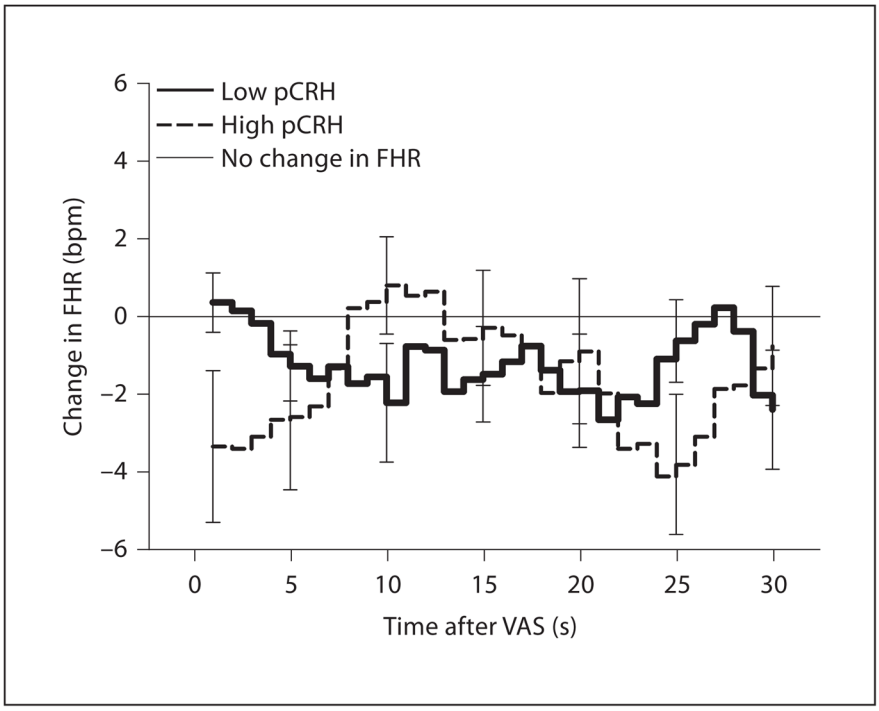

Fig. 5.
Changes in FHR at a randomly selected interval during the baseline period based on pCRH levels at 15 weeks' gestation.