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Psychobiological Influences of Stress and HPA Regulation on the Human Fetus and Infant Birth Outcomes[a]

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There are critical periods during development when profound changes in brain organization can occur.[1] These critical periods relate to developmental periods when the brain is undergoing transformations such as cell migration or receptor development.[2] Because many of these events transpire *in utero*, the fetal nervous system is especially vulnerable to a variety of influences, including subtle environmental (uterine) stress. Pregnancy provides a unique opportunity to study fetal exposure to certain neurochemical influences because of the maternal influence on the fetal environment. Evidence indicates that fetal exposure to abnormal levels of peptides can have profound influences on growth and development. For instance, post implantation mouse embryos exposed *in vitro* to vasoactive intestinal peptide (VIP) increase growth fourfold, increase somite number, increase embryonic volume, and increase DNA and protein content.[3-5] Central nervous system (CNS) studies[4] of embryonic and fetal mRNA indicate that the influence of VIP is entirely extraembryonic (i.e. maternal).

It is established that fetal exposure to either maternal or exogenous peptides produces changes in the brain and behavior that are greater and last longer than alterations in neonates or adults with similar exposure.[6-10] In addition to the "visible" effects described above (i.e. growth), some maternal peptides have influences on the fetus that are subtle, but have significant consequences for development.[9,11] Substantial evidence documents the influence on brain/behavior development of peptides from the hypothalamic-pituitary-adrenal (HPA) axis, including adrenocorticotropic hormone (ACTH) and β-endorphin (βE) (see Refs. 2, 12, and

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13 for reviews). For instance, at maturity, rats exposed as fetuses to βE had "permanent" decreases (subsensitivity) in the density of striatal, dopamine (D2) receptors (reduced plasticity), opiate receptor density, and changes in βE levels in discrete areas of the brain.[8,11,14,15] Perinatal exposure to ACTH and its analogues permanently alters behavior and growth.[16,17]

These peptides of the HPA axis that influence neurodevelopment are activated by stressors such as shock, restraint, surgery, illness, and pharmacological treatment in animals,[18] and by physical exertion in humans.[19] Psychological or subtle stress factors including sustained attention[19-22] and anticipation of a stressful event also can stimulate the HPA axis.[19,22] These findings raise the possibility that stress can influence birth outcome and fetal development by stimulation of maternal neuropeptide activity.

Growing evidence supports the direct and permanent influences of maternal stress on fetal CNS development.[6] Infant rats from mothers stressed by restraint during the third trimester had "permanently" decreased density of μ receptors in the brain.[7] Insel and collegues speculated that cells with opiate processes are especially sensitive to (i.e. eliminated by) prenatal stress. Prenatal stress increases βE levels in the hypothalamus of neonatal rats at 10 days of age, and the effects were proportional to the duration (in days) of prenatal stress.[23]

Exposure of pregnant primates to a variety of social, environmental, and neurochemical stressors such as removal from cage, unpredictable noise exposure, and administration of ACTH resulted in offspring with (i) enhanced behavioral reactivity to stressors later in life,[24] (ii) lower levels of motor behavior,[25] (iii) compromised neuromotor responses and shorter attention span,[26] and (iv) irritable temperament.[27] Prenatal stress also was related to increased HPA axis reactivity and decreased immune function in offspring.[28] Prenatal stress (noise) in baboons resulted in infants with lower birth weight that persisted for one month; however, other effects such as delayed neuromotor development, distractibility, and less activity were long-lasting.[25]

A small number of studies have examined the role of prenatal psychosocial state on human fetal behavior. Early studies reported an association between maternal anxiety, hyperactive fetuses, and fetal tachycardia.[29,30] Ultrasound examination of 28 acutely panic-striken women between 18 and 36 weeks of gestation indicated that all fetuses showed intense hyperkinesia lasting between 2 and 8 hours, with numerous, disorderded, and vigorous movements.[31] Pregnant women listening to favorite music resulted in significant decreases in fetal breathing and increases in fetal body movements.[32] Mild psychological stress during pregnancy resulted in a sudden fall in fetal heart rate (FHR) followed by overswing recovery.[33,34] Procedures that reduce maternal anxiety are associated with reduced fetal activity.[35] A larger literature (see Refs. 36 through 42) has suggested that maternal stress and anxiety influence birth outcome.

The evidence supports the possibility that maternal stress, by activating peptides from the HPA axis, influences fetal behavior and birth outcome. Our program of research is designed to examine the relationships among stress, neuropeptides of the HPA axis, and measures of fetal development and infant birth outcomes.

STRESS INFLUENCES ON INFANT BIRTH WEIGHT AND GESTATIONAL AGE

A sample of 90 adult, English-speaking, predominantly white, married, upper-middle-class, employed women with a singleton intrauterine pregnancy was adminis-

tered questionnaires to obtain measures of prenatal psychosocial stress, sociodemographic factors, and health practices (smoking, alcohol, and substance use) using standardized measures of stress (e.g., the Schedule of Recent Life Events, the Hopkins Symptom Checklist, Perceived Stress Scale, and questions specific to the experience of pregnancy). Complete demographic information, obstetric history, antepartum risk, and birth outcomes were obtained for each subject. (See Wadhwa *et al.*[43] for a complete description of the sample and the results.)

Independent of biomedical risk, life event stress and pregnancy anxiety significantly predicted infant birthweight and gestational age, respectively, at birth. Each unit increase of maternal life event stress during the third trimester (from a possible sample range of 14.7 units of life event stress) is associated with a 55.03 gram decrease in infant birthweight and with a 1.32 times increase in the likelihood of occurrence of low birthweight (<2500 g). Each unit increase of maternal pregnancy anxiety during the third trimester (from a possible sample range of 5 units of pregnancy anxiety) was associated with a three-day decrease in gestational age at birth. The magnitude of association among prenatal stress, birthweight, and gestational age at birth in the present sample may be a conservative estimate of the effect on size in the general population, because levels of prenatal stress may be lower in this relatively affluent group than in socioeconomically disadvantaged samples. Nevertheless, these results precisely define the influence of psychosocial stress on critical birth outcome measures and suggest that increased stress and anxiety during pregnancy result in low birth weight and preterm birth.

STRESS INFLUENCES ON HPA ACTIVATION DURING PREGNANCY

Among the many physiological changes during pregnancy, there is a gradual increase in plasma βE, ACTH, and cortisol.[44] Although, as reviewed above, stress activates the HPA axis, the effects of stress on the HPA axis against this background of change during pregnancy are not known. To examine this question, neuroendocrine data were obtained from maternal plasma of 54 women at 28 weeks' gestation and compared with the psychosocial data described above. Two composite indices of stress were generated: "perceived stress" and "pregnancy anxiety." Measures of social support during pregnancy were also collected.

The function of the relationship between maternal βE and ACTH during pregnancy is not known, but these peptides are commonly corelcased.[19–21,43,45] Scatterplot analysis confirmed the relatively high degree of correspondence between ACTH and βE, but a significant number of women failed to exhibit the usual corelease pattern. A disregulation, or pituitary tone, index was developed to describe the βE-ACTH corelease pattern by the equation below.

$$\text{D.I.} = \text{absolute value } (\beta\text{E-ACTH})/\beta\text{E}) \times 100$$

Perceived stress was positively associated with levels of ACTH ($r = 0.44$, $p < 0.001$) and negatively associated with the disregulation index ($r = -0.38$, $p < 0.01$), indicating that as levels of distress (a combination of pregnancy stress, chronic stress, hassles, and strain) increased, concentration of plasma ACTH levels increased. General social support was negatively associated with maternal levels of βE ($r = -0.27$, $p < 0.05$), ACTH ($r = -0.48$, $p < 0.001$), and cortisol ($r = -0.31$, $p < 0.05$), and was positively associated with the disregulation index ($r = 0.27$, $p < 0.05$). This indicates that subjects reporting greater social support during their pregnancy had lower levels of βE, ACTH, and cortisol. Pregnancy-related social support also was negatively associated with levels of ACTH ($r = -0.48$, $p < 0.0011$) and cortisol ($r = -0.29$, $p < 0.05$) and was positively associated with the disregulation index ($r = 0.37$, $p < 0.01$), indicating that availability of social support during pregnancy was related to lower levels of ACTH and cortisol.

These results provide evidence that stress during the background physiological activity of pregnancy stimulates the HPA axis. The major contribution of this study is the assessment of possible physiological mechanisms (i.e., HPA axis) by which psychosocial stress influences birth outcomes. The findings support the possibility that the effects of stress on birth outcome may be mediated by activation of the maternal HPA axis.

THIRD-TRIMESTER βE/ACTH DISREGULATION PREDICTS USE OF ANESTHESIA AT VAGINAL DELIVERY

Disregulation of βE/ACTH release (DI index) is associated with response to stress, as described above, and may predict adaptation during pregnancy and delivery. Significant elevation of plasma maternal βE at delivery probably reflects the stress of birth and provides alleviation of pain associated with childbirth. The significance of elevated levels of corelcased pituitary peptides βE and ACTH in the third trimester of pregnancy for the experience of pain is unknown. We investigated the possibility that peptides released *during the third trimester* prepared the mother for the pain and stress of delivery in a sample of 76 women, 58 of whom were delivered vaginally.

Third-trimester levels of maternal βE and ACTH were significantly ($r = 0.48$, $p < 0.001$) related; however, the significant relationship between third-trimester βE and ACTH was apparent only in women ($n = 24$) who did not receive conduction anesthesia at vaginal delivery ($r = 0.81$, $p < 0.001$; FIG. 1). The corelease relationship between βE and ACTH was uncoupled during the third trimester in women who received conduction anesthesia ($n = 34$) at vaginal delivery ($r = 0.28$, $p =$ ns).

The use of analgesia during vaginal delivery was significantly related to larger absolute values (magnitude) of the DI ($F_{1,56} = 4.76$, $p < 0.03$). These findings suggest that disregulation or uncoupling of the relationship between βE and ACTH during the third trimester prospectively predicted maternal utilization of conduction anesthesia during vaginal delivery. Changes in the release pattern of βE and ACTH during the third trimester in women receiving conduction anesthesia may alter opiate receptor sensitivity. If opiate receptors become subsensitive, then the dramatic elevation of βE at term may become less effective in response to pain, requiring the use of anesthesia.

Third-trimester disregulation of βE/ACTH release could result from activity of PC1 and PC2 enzymes triggered by environmental factors such as stress.[32] Uncoupling of βE and ACTH in women who use conduction anesthesia at birth may be a marker of conditions such as prenatal anxiety and stress known to relate to increased pain at birth.[34,35] Release of maternal peptides during labor and delivery may carry information important for fetal and infant development. Use of anesthesia during delivery can inhibit signaling of activities involved in neuropeptide release[16] and influence neurodevelopment.

INFLUENCE OF HPA PEPTIDES ON THE HUMAN FETUS

Consensus is growing that birth outcomes may not be the most sensitive index of fetal development, especially fetal CNS function. Alternatively, precise analysis of fetal *behavior* provides critical information about CNS development. For instance, Nijhuis (1984) suggested that "A problem with fetal monitoring methods so far is that their objective has been detection of serious fetal illness ('fetal distress') . . . rather than the confirmation of fetal optimality. Since normal functioning of the central nervous system (CNS) is so important to the quality of life after birth, assessment of the normality/abnormality of CNS functioning before birth would seem to be a promising means of approaching this sort of fetal evaluation."[47] Evidence[48,49] supports the view that measures reflecting fetal CNS development are more useful than "dramatic," rare or nonlinear outcomes (*e.g.*, prematurity) for predicting neurological and behavioral maturation. Fetal heart rate (FHR) is among the most common and sensitive measures of fetal development.

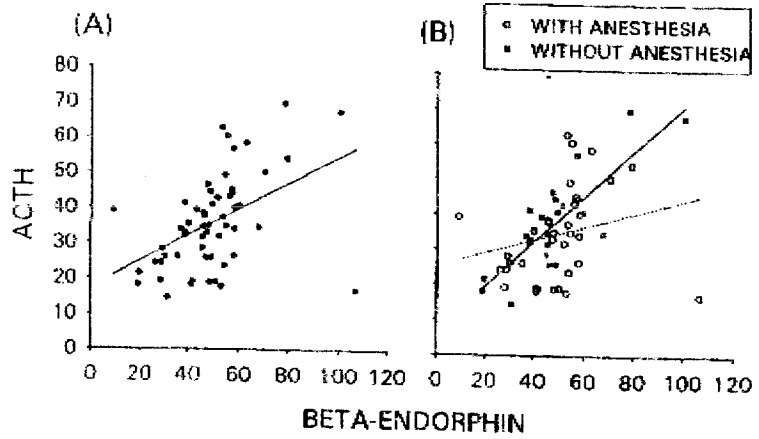

FIGURE 1. Panel A: Illustrates significant relationship between maternal plasma βE and ACTH during the third trimester in all women delivering vaginally ($n = 58$). Panel B: Relationship between maternal plasma βE and ACTH in women delivering vaginally who received conduction anesthesia ($n = 34$, *solid squares*) and those who did not ($n = 24$, *open dots*). The relationship between βE and ACTH was significant only in women who did not receive anesthesia at vaginal delivery.

Early in gestation, the fetal heart has its own resting rate with minimal variability. As the fetus matures, variability is modified by control from the sympathetic (SNS) and parasympathetic nervous system (PNS). PNS control (vagal tone) is reflected by FHR decelerations and is related to maturation of the CNS,[48] approaching adult levels by the third trimester. Indeed, release of PNS inhibition by cholinergic blocking agents results in a return to high resting HR.[50] Because the fetus has limited capacity to vary stroke volume, its primary response to increased demands for O_2 is increased HR.[51] Thus FHR carries important information about fetal health *and* CNS development.

Resting (or nonstress) evaluation of FHR is an important and common practice for determining fetal well-being. The importance of stress testing (or arousal) in the fetus to provoke HR change was illustrated by Sontag and Wallace[52] and has become standard practice in many clinical settings. The purpose of stress studies is to arouse the fetus as reflected by FHR acceleration, increased FHR variability, and increased fetal movement. This valuable clinical test has been used to determine fetal viability including growth retardation,[53] acidosis,[54] and CNS development.[55] Fetal arousal to external stimulation has been observed by 22 weeks[56] and can be elicited reliably by 30 weeks in normal development,[53] perhaps reflecting myelinization and neural organization in the diencephalic and mesencephalic areas of the brain.

Measures of fetal habituation to *ex utero* stimuli offer the opportunity to examine CNS activity associated with higher processes such as learning. Habituation measures the response decrement resulting from repeated exposure to a familiar stimulus[57,58] rather than simple arousal. Properly tested, habituation is a reflection of higher central nervous system integrity.[59] It requires that the organism detect and respond to information and then systematically "ignore" and cease responding to subsequent, identical information. The fetus compares contemporary information with the past by forming a representation (or a memory) of the stimulus.[57-59] Several studies have found that 25-week-old fetuses habituate to external stimulation.[54,56,60,61] Few studies were designed to distinguish between habituation (which is a process of the central nervous system) and receptor fatigue, a peripheral process (for exceptions see Refs. 62 and 63). Conclusions from the small number of FHR studies with dishabituation procedures suggest that this measure of behavior is a very sensitive index of fetal CNS maturity. We examined (i) whether the fetus displays habituation and not receptor fatigue; (ii) the effects of the HPA axis on habituation; and (iii) the effects of HPA on a critical measure of fetal hypoxia, the S/D ratio from Doppler measures of uteroplacental flow.

Fetal Heart Rate Habituation as an Index of CNS Development

Transabdominal transducers for measuring FHR were attached in 95 women during weeks 30 through 32. Resting FHR was measured for 10 min, and the baseline was calculated during the last minute. A series of 15 vibroacoustic stimuli (S1) were presented on mother's abdomen over the fetal head for two seconds with pseudorandom intervals between stimuli of 20 to 45 sec. On the 16th stimulus, a novel (dishabituating) stimulus (change in db) was presented. Trials 17 through 31 repeated the S1 series. On trials 32 through 41, the S1 was repeated, but stimulation was applied to the mother's thigh as a control. During the presentation, the mother listened to pure tone music presented through headphones that masked the auditory stimulus. Change in HR after stimulation was the primary variable of interest.

As illustrated in FIGURE 2, presentation of a single novel stimulus (S2, trial 16) altered the rate of FHR habituation. The rate of habituation to the first S1 is a classic habituation curve. The influence of the novel stimulus (S2) interrupts the rate of habituation for the second series of S1. The slope of the last four stimuli in the first iterative series (the four before the S2) was significantly ($F_{1,82} = 9.21$, $p < 0.003$) different from first four stimuli in the second iterative series of S1.

The procedures in this study ensure that habituation is the result of CNS activity and not stimulus-specific receptor fatigue. These findings validate the use of these procedures as sensitive measures of fetal behavior and CNS activity. Thus, despite the noisy uterine environment (85 db ambient), these findings provide compelling evidence that the fetus detects and responds to *ex utero* stimuli.

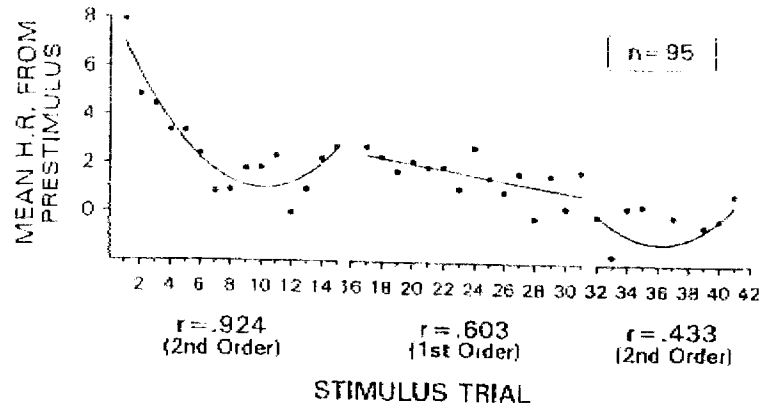

FIGURE 2. Change in FHR (maximum subtracted from prestimulus) transabdominal vibroacoustic stimulation for the first series of SI (trials 1–15), the series of SI after the dishabituating stimulus (trials 17–31). Trials 32 through 41 were control responses collected from stimulation applied to the mother's thigh.

Change in βE during the Third Trimester Predicts Fetal Dishabituation

Blood was collected from the mother at the time of the FHR series and another sample (a control value) was collected six weeks postpartum. The six-week sample provides a baseline unaffected by pregnancy so that peptide change during pregnancy can be estimated. As presented in FIGURE 3, increased maternal βE during the third trimester was significantly associated with change in the fetal habituation slope after a novel stimulus. The direction of this preliminary association indicates that increased maternal βE is related to a greater change of slope from the last four SIs of the first series and the first four SIs in the second series. As we have initially interpreted this result, a larger change in slope is evidence of susceptibility to the dishabituating stimulus or to novelty. Although these findings may not necessarily reflect learning, they do indicate sensitivity to *ex utero* information. Thus, these findings indicate that increases in maternal βE during the third trimester exert a significant influence on fetal CNS activity and increase fetal reactivity.

Change in βE during the Third Trimester Predicts Uteroplacental Flow

Doppler flow velocimetry was assessed by ultrasound during the same session that FHR was measured and peptides were determined from maternal plasma (between weeks 30 and 32 of gestation). Measures of systolic to diastolic (S/D) waveform ratio at the umbilical artery were made. Significant relationships between βE and measures of uteroplacental circulation (S/D ratio of umbilical artery) were discovered. Both the DI (FIG. 4, $p < 0.002$) and increases in βE during the third trimester (FIG. 5, $p < 0.05$) were associated with increased S/D. (Absolute levels of βE were not significant). These findings are consistent with parallel literature indicating the βE is a very sensitive marker of compromised O_2 in fetuses, infants, and adults.[64] However, this is the first evidence that maternal plasma level of βE is associated with *in vivo* uteroplacental flow. These results suggest that compromised

flow in the human fetus may be linked to peptides that are coupled with maternal stress.

It is interesting that increases in βE are associated with increased fetal sensitivity to environmental information and to decreased uteroplacental flow. Subsequent analyses indicated that βE exerted independent influences on these parameters. Thus, increased βE apparently is associated with an adaptive response of the fetus (sensitivity to external information) and with decreased flow, ostensibly a maladaptive response expected to compromise CNS activity. Exactly how increases in βE influence these two fetal parameters is not clear. It is possible that the profile of maternal peptides transduces information to the fetus about the external environment. Increases in βE may be a reflection that the mother is experiencing the environment as stressful and is receiving minimal support. This signal to the fetus may result in two responses. The fetus may develop its own adaptive abilities, including increased sensitivity to the environment. This possibility is consistent with literature indicating that some developmental stress actually enhances later development. Alternatively, the message conveyed by increased maternal βE may result in compromised uteroplacental flow, resulting in hypoxia of varying degrees. This possibility is consistent with a large body of literature indicating that a high level of βE is a marker of birth complications.[44,64-66]

CONCLUSIONS

The results of the initial series of studies from our program indicate that maternal stress activation during the third trimester influences the fetus and infant

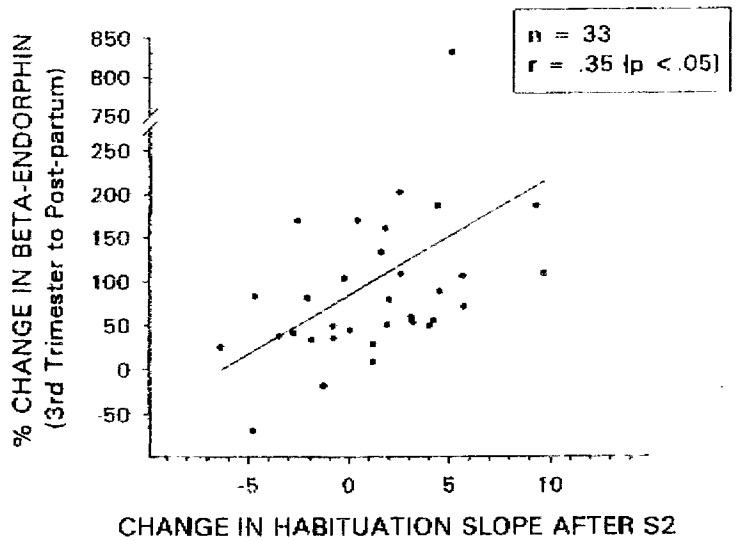

FIGURE 3. The relationship between the change in FHR habituation (difference between first and second series of SI) and the change in maternal βE between baseline and the third trimester.

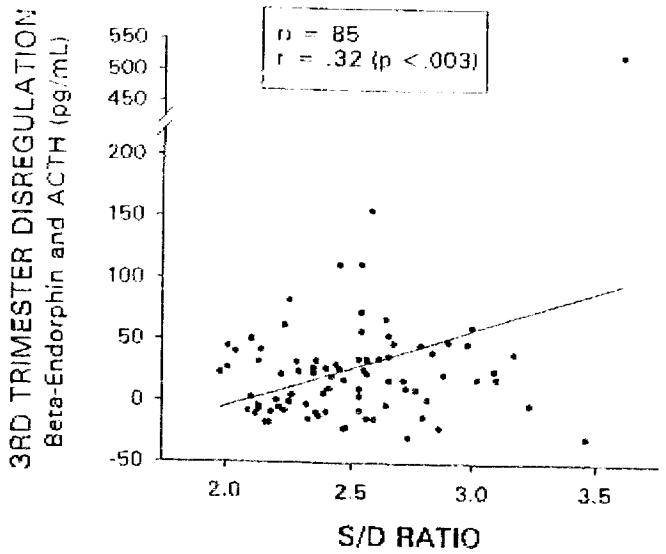

FIGURE 4. Illustration that increased maternal DI (increase in βE relative to ACTH) is associated with elevated S/D (compromised uteroplacental flow).

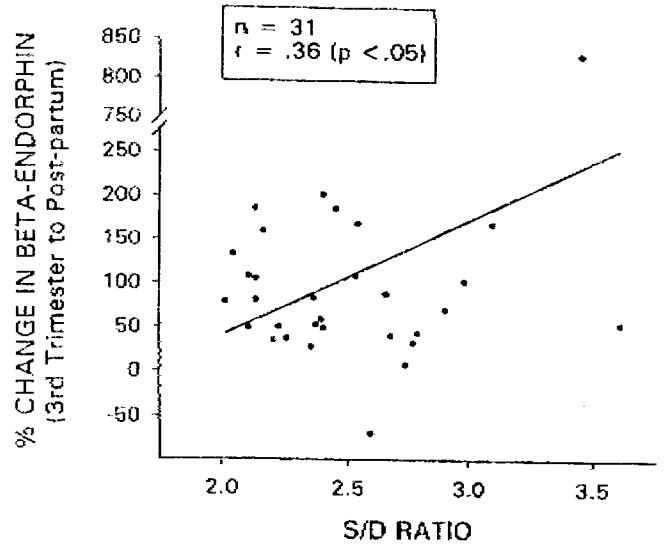

FIGURE 5. Compromised uteroplacental flow (elevated S/D) is associated with increased maternal βE during the third trimester.

birth outcomes. Moreover, the influences of stress on the fetus and birth outcomes are mediated by activation of the HPA axis. Our findings in human subjects are consistent with animal studies indicating that stress and HPA activation can influence behavior and brain mechanisms permanently.[7,9,24,27,28]

This series of studies precisely defines the influence of psychosocial stress on critical birth outcome measures and suggests that increased stress and anxiety during pregnancy result in low birth weight and prematurity. They provide evidence that stress during pregnancy stimulates the HPA axis and may be a primary physiological mechanism by which stress influences birth outcomes. These findings suggest that disregulation or uncoupling of the relationship between βE and ACTH during the third trimester and changes during the third trimester from baseline are more sensitive measures of peptide effects on the fetus than absolute measures of peptide concentration. The disregulation index (DI) prospectively predicted maternal utilization of conduction anesthesia during vaginal delivery and supported the possibility that maternal opiate receptor subsensitivity obviated putative analgesic benefits of elevated βE at vaginal delivery. The DI and change of βE from baseline were sensitive indices of FHR habituation (environmental reactivity) and uteroplacental flow. Relative elevation of βE was associated with increased fetal reactivity and uteroplacental flow parameters consistent with hypoxia. The overall pattern of results supports earlier speculations[13] that BE may be an endogenous teratogen and a final common pathway for the effects on the fetus of hypoxia and other stress-related complications of pregnancy.

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Blockade of VIP during Neonatal Development Induces Neuronal Damage and Increases VIP and VIP Receptors in Brain

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INTRODUCTION

Vasoactive intestinal peptide (VIP) is a 28-amino-acid peptide that is involved in diverse regulatory functions, including vasodilation, gastric secretion, and glycogenolysis.[1] In the central nervous system (CNS), VIP exhibits neurotransmitter and neuromodulator functions, and recent work has highlighted an important role for VIP in the regulation of CNS development. In CNS primary culture experiments, subnanomolar concentrations of VIP were shown to stimulate neuronal survival and astrocyte mitogenesis and to induce the secretion of trophic factors by astrocytes.[2–5] In the micromolar concentration range, VIP treatment was shown to stimulate neuronal mitosis, neurite extension, and neuronal survival in sympathetic and neuroblastoma cultures.[6–8] In addition, cultured whole embryo studies have demonstrated that a four-hour exposure to VIP resulted in a dramatic increase in growth.

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