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Permalink

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Journal

Developmental Neuroscience, 25(1)

ISSN

0378-5866

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Publication Date

2003

DOI

10.1159/000071467

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Maternal Hypothalamic-Pituitary-Adrenal Disregulation during the Third Trimester Influences Human Fetal Responses

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Key Words

Pro-opiomelanocortin · Hypothalamic-pituitary-adrenal axis · Stress · Opiates · Fetal heart rate · Habituation · Pregnancy

Abstract

Maternal peptides from the hypothalamic-pituitary-adrenal (HPA) axis rise during human pregnancy. The effects of circulating maternal adrenocorticotropin (ACTH) and β -endorphin (BE) on human fetal behavior was determined in 135 women during their 32nd week of gestation. Fetal behavior was measured by assessing heart rate habituation to a series of repeated vibroacoustic stimuli. Individual differences in habituation were determined by computing the number of consecutive responses above the standard deviation during a control period. There was no significant relation between levels of ACTH, BE and the rate of fetal heart rate habituation. However, an index of HPA disregulation (uncoupling of ACTH and BE) was related significantly to fetal behavior. Fetal exposure to high levels of maternal BE relative to ACTH was associated with significantly lower rates of habituation. Results indicate that maternal stress and

stress-related peptides influence fetal response patterns. It is possible that this influence persists over the life span.

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During the course of normal human pregnancy, the fetus is exposed to gradual elevations in pro-opiomelanocortin (POMC) peptides synthesized by, and released from, the maternal hypothalamic-pituitary-adrenal (HPA) axis. At term, the POMC peptides adrenocorticotropin (ACTH) and β -endorphin (BE) are two- to tenfold higher than nonpregnancy baselines [Hobel et al., 1999; Chan et al., 1993; Chan and Smith, 1992; Goland et al., 1986, 1988; Raisanen, 1988; Yen, 1994; Wolfe et al., 1988]. The purpose for the rise in POMC peptides during pregnancy is not known. Endogenous opioids, such as BE, may modulate the pain of childbirth [Varrassi et al., 1989; Sandman et al., 1995], but their influence on the human fetus has not been investigated.

Although the purpose for the escalating POMC peptide levels during pregnancy is not known, there is substantial evidence that the maternal HPA axis is a conduit for the effects of environmental stress on the fetus [Wadhwa et

al., 1996, 2002; Welberg and Seckl, 2001]. Stressful events experienced in utero imprint on the fetus a pattern of activity resulting in 'programs' that contribute to the course of development [Welberg and Seckl, 2001]. These programs alter the set point for release of critical neurochemical messengers and influence health and the subsequent behavioral and physiological repertoire of the individual [Barker, 1998]. For instance, a growing animal literature [Braastad, 1998; Day et al., 1998; Frye and Bayon, 1999; Insel et al., 1990; McEwen and Stellar, 1993; Sanchez et al., 1993; Sandman and Yessian, 1986; Reyes and Coe, 1997; Schneider et al., 1999] and new studies in humans [Peterson et al., 2000; Sandman et al., 1999a; van Os and Selton, 1998; Makheseed et al., 1999; Ponirakis et al., 1998; Sjostrom et al., 1997] indicate that prenatal exposure to stress and stress-related peptides result in changes in the nervous system and in behavior. It is well-established that the fetal HPA axis is especially susceptible to programming by exposure to stress and alterations in the maternal HPA axis [Matthews, 2002; Francis et al., 1999; Insel et al., 1990; Weinstock, 1997; Vallee et al., 1997; Fride et al., 1995; Sanchez et al., 1993; Sandman and Yessian, 1986].

An extensive literature [reviewed in Sandman and Kastin, 1981, 1990; Strand, 1999] supports the conclusion that although ACTH and BE arise from a common precursor, they have different influences on the brain and behavior. Within physiological limits, in both developing and adult organisms, ACTH exerts enhancing influences on neural and behavioral efficiency in contrast to the reciprocal and largely depressing influences of BE [Sandman and Kastin, 1990]. For instance, ACTH fragments administered concomitantly with opiates attenuate tolerance and dependence [Hendrie, 1988; Krivoy et al., 1977; Szekely et al., 1979]. Similar examples of reciprocity are reported for generation of characteristic waveforms ('elip-togenic' spiking) in the electroencephalogram of the nucleus gigantocellularis following periaqueductal gray stimulation [Sandman and Kastin, 1981], social behavior [Beckwith et al., 1977; Panskepp et al., 1978] and learning [Sandman et al., 1972, 1973; Beckwith et al., 1977; McGivern et al., 1979]. Moreover, we have reported [Sandman et al., 1995] that the pattern of POMC expression and not plasma concentration during human pregnancy predicts the use of anesthesia at delivery. Thus, the balance (or ratio) of circulating ACTH and BE is an important consideration in determining the influences of the HPA axis during pregnancy and on the human fetus.

The primary aim of the present study is to examine the influence of maternal POMC fragments, ACTH and BE,

on the human fetus. Fetal responses are assessed by measures of fetal habituation that reveal neurological integrity and maturity [Hepper, 1997; Doherty and Hepper, 2000; Sandman et al., 1997, 1999b]. Convincing evidence indicates that the fetus detects and responds to a variety of ex utero stimuli [Leader, 1994; Leader et al., 1982; Shalev et al., 1989; Zimmer et al., 1993; Grimwade et al., 1971; DeCasper and Fifer, 1980; Fifer and Moon, 1989; Groome et al., 1999; Lecanuet et al., 1988]. Fetal arousal to external stimulation is observed by 22 weeks [Leader et al., 1988] and by 25–28 weeks of gestation, fetuses habituate to external stimulation [Doherty and Hepper, 2000; Leader et al., 1982; Leader and Baille, 1988; Madison et al., 1986a, 1986b; Shalev et al., 1989, 1990; Kisilevsky et al., 1992; Shahidullah and Hepper, 1994]. Faster fetal habituation [Shalev et al., 1989; Doherty and Hepper, 2000] and increased sensitivity to external stimulation [Kisilevsky et al., 2000] are associated with advancing gestational age and neurological development.

Methods

Subjects

A sample of 135 women with a singleton pregnancy was recruited from a prenatal clinic associated with the University of California, Irvine, Calif., USA. The mean age was 26.0 ± 5.25 years, 41.5% of the sample were Hispanic and 57.8% of the sample were non-Hispanic white. Forty-four percent of the women were primiparous and 56% had at least one previous child. About one half of the fetuses (49.2%) were female.

Procedures

Maternal plasma samples were collected at 32.5 ± 1.49 weeks of gestation for analysis of ACTH and BE concentration. Immediately after the collection of plasma, a test was administered to assess fetal heart rate (FHR) responses to a series of vibroacoustic stimuli (VAS). Blood for measuring endocrine values was also collected from 93 of the women between 8–10 weeks postpartum.

Fetal Response Procedure

Subjects were placed in a low semi-Fowler's position (5–10 degree tilt) on a standard, padded examination table. The subject was fitted with headphones and listened to pure tone music during the entire evaluation to mask extraneous noise and prevent her from hearing the tones during the VAS challenge test. Electrodes from a fetal monitor [either a Hewlett-Packard FHR (Model 8030), or a Sonicaid System 8000] were applied to the abdomen and positioned for measuring a reliable FHR signal [Sandman et al., 1997]. The transducers were connected for amplification of the signal. Continuous records of FHR were collected for off-line analysis.

A series of 15 VAS (63 dB, 300 Hz) was presented on the mother's abdomen over the fetal head (determined by ultrasonography). Stimuli of 2 s were administered in a pseudorandom sequence that was identical for each fetus. A subsequent series of 10 VAS in a different pseudorandom arrangement was presented to the maternal thigh.

This procedure controlled for possible maternal responses to the stimulus that might contribute to fetal responses.

The FHR response was estimated by autocorrelation over 3-second epochs providing the beat-to-beat rate. The average FHR from the 5 seconds before each stimulus was computed and used as the prestimulus value for each trial. The prestimulus baseline FHR was subtracted from the poststimulus maximum FHR acceleration to provide measures of FHR change (Δ) for each stimulus. The prestimulus Δ value collected before each trial (as opposed to calculating a change score for each trial from the initial FHR baseline) controls for inevitably changing FHR related to the arousing effects of successive VAS presentations [Kisilevsky and Muir, 1991; Sandman et al., 1997]. We have shown that FHR with this method survives rigorous dishabituation control procedures [Sandman et al., 1997].

Method of Determining FHR Response

First, for each fetus, the mean and standard deviation during the control period (stimulation to mother's thigh) were calculated for the maximum post-stimulation FHR. For each fetus, the standard deviation value set the parameters for determining the presence of a response during the experimental (stimulation to the fetus) trials (i.e. each fetus served as his/her own control). Second, the maximum post-stimulation FHR acceleration was determined for each experimental trial. Third, beginning with the first presentation of a VAS, the maximum FHR for each experimental trial was compared with the standard deviation during the control period. The first post-stimulation trial in which maximum FHR acceleration exceeded the control standard deviation was defined as the first response [Lecanuet et al., 1986, 1988]. Fourth, after the first response had been detected, the number of *consecutive* responses was determined in which the post-stimulation FHR met the response criterion. This number defined the rate of habituation (the larger number of responses reflected slower habituation). Every fetus had at least one post-stimulation trial that exceeded the control series by more than one standard deviation.

Neuroendocrine Measures

Blood samples (20 ml/draw) were withdrawn by antecubital venipuncture into EDTA (purple top) vacutainers and chilled on ice immediately. Samples were centrifuged at 2,000 g (15 min) and the plasma decanted into polypropylene tubes containing 500 KIU/ml aprotinin (Sigma Chemical Co.; St. Louis, Mo., USA) and stored at -70°C until assayed.

Plasma ACTH Assay. Plasma levels of ACTH were measured by radioimmunoassay (Nichols Institute Diagnostics, San Juan Capistrano, Calif., USA). The antiserum has $<0.001\%$ cross-reactivity with BE and ACTH fragments. Duplicate samples (200 μl /assay tube) were incubated with ACTH ^{125}I -antibody solution and an avidin-coated bead overnight at room temperature. After washing, the bead with bound radiolabelled antibody complex was quantified using an ICN Biomedical Isoflex Gamma Counter. The ACTH assay has a minimal detectable dose of 1.0 pg/ml (95% confidence) with intra-assay coefficient of variance (CV) of 3.0% at 35 pg/ml and CV = 7.8% (inter-assay) at 36 pg/ml.

Plasma BE Assay. Plasma levels of BE were determined by a solid phase two-site immunoradiometric assay (Nichols Institute Diagnostics). The antiserum has 1.6% cross-reactivity with β -lipotropin at 500 pg/ml and has $<0.01\%$ cross-reactivity with related opiates at 5 $\mu\text{g}/\text{ml}$. The allegro BE immunoassay system has a minimum detect-

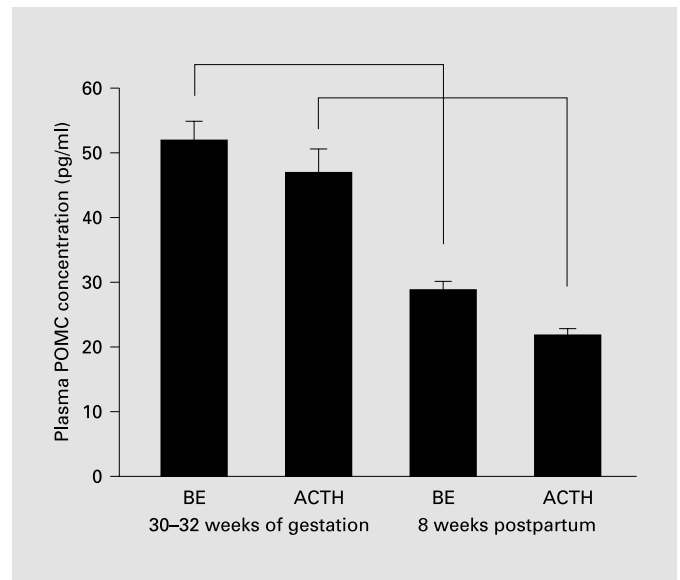


Fig. 1. Maternal levels of BE and ACTH during the early third trimester of pregnancy are significantly elevated compared with levels determined in the same women 8 weeks postpartum (a nonpregnancy control period). * $p < 0.001$.

able dose of 14 pg/ml (95% confidence limit) with a CV = 4.1% (intra-assay) and CV = 9.0% (inter-assay) at the highest concentrations expected in the present study.

POMC Disregulation. A disregulation, or pituitary tone, index (DI) [Sandman et al., 1995] was created to quantify the BE-ACTH corelease pattern by the equation below:

$$\text{DI} = [(\text{BE} - \text{ACTH}) / \text{BE}] \times 100$$

A high DI indicates that the plasma BE is higher than ACTH. Index values close to zero indicate no difference in levels of BE and ACTH, and negative scores reflect higher ACTH concentrations relative to BE.

Results

Relations between BE and ACTH

As expected, levels of BE ($t = 8.25$, d.f. = 92, $p < 0.001$) and ACTH ($t = 6.40$, d.f. = 92, $p < 0.001$) each were elevated significantly in weeks 30–32 of pregnancy compared with concentrations determined at 8–10 weeks postpartum (fig. 1). The coupling or correlation between BE and ACTH was calculated for a subgroup of women ($n = 93$) who had endocrine values for the late third trimester and during the postpartum period. The correlation between BE and ACTH during the early third trimester of pregnancy was highly statistically significant ($r = 0.92$, d.f. = 92, $p < 0.001$; fig. 2a). The correlation remained sig-

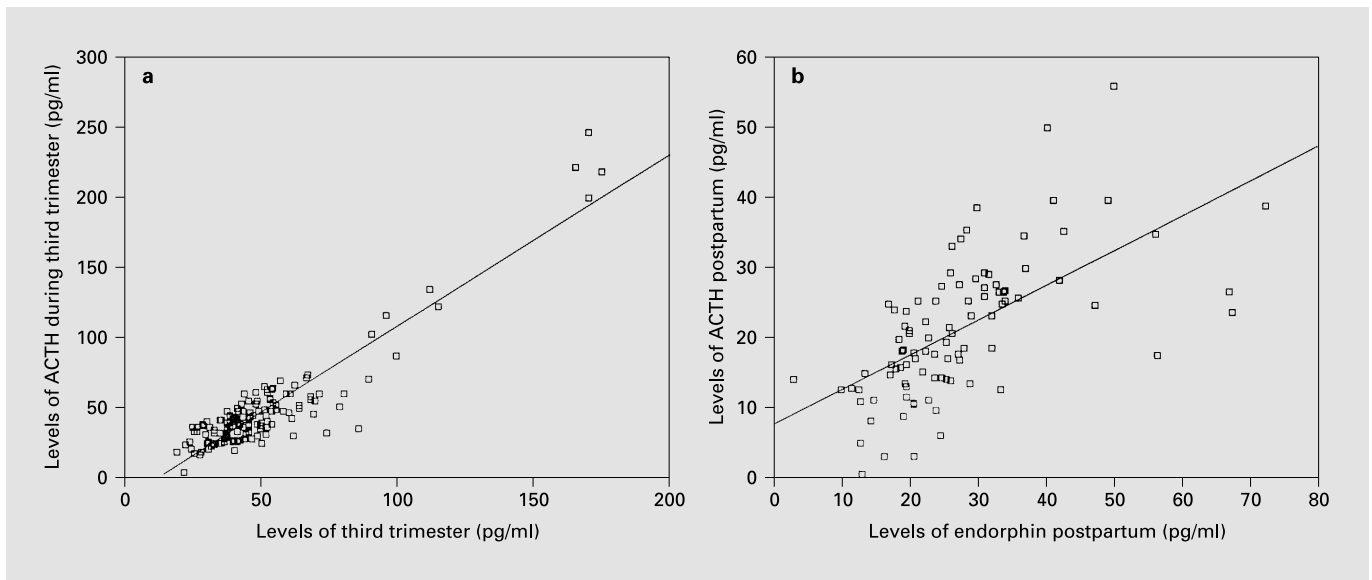


Fig. 2. The correlation between maternal BE and ACTH is highly significant during the early third trimester. The correlation remains significant during the postpartum period in the same women but accounts for less variance. **a** $r = 0.92$, $p < 0.001$. **b** $r = 0.62$.

nificant ($r = 0.62$, $d.f. = 92$, $p < 0.001$) in the women during the postpartum assessment (fig. 2b), but it accounted for considerably less variance (84 vs. 35%).

FHR Responses and Endocrine Disregulation

Regression analysis indicated that there was no significant relation between the number of trials to habituation and the endocrine levels either for ACTH ($R = 0.094$, $F = 1.19$, $p < n.s.$) or BE ($R = 0.015$, $F = 0.03$, $p < n.s.$). The index of endocrine disregulation was significantly related to FHR ($R = 0.17$, $F = 4.05$, $p < 0.05$). However, there was evidence that the relation is nonlinear (i.e. a cubic solution accounted for slightly more variance). Thus, concentrations of ACTH and BE were compared in groups of fetuses divided into quartiles based on the rate of habituation (i.e. the number of consecutive trials in which FHR acceleration exceeds individual response variability). The average number of trials to habituate for each group is presented in table 1. Group one comprises the rapid habituators (mean = 1.31 trials), groups two and three include subjects in the middle quartiles of habituation rates, and the slow habituators (mean = 11.29 trials) are in group four. Differences in the DI among the four groups were statistically significant ($F_{3, 131} = 4.10$, $p < 0.045$) by one-way analysis of variance (ANOVA). The group with the most rapid rate of habituation had the smallest DI, indi-

Table 1. Number of consecutive stimulation trials in which FHR exceeded control values by one standard deviation

Group	n	Average number of trials	Standard deviation
1 (rapid habituation)	43	1.31 (0–2)	0.51
2	32	3.46 (3–4)	0.51
3	26	5.19 (5–6)	0.40
4 (slow habituation)	34	11.29 (7–15)	3.39
Total	135	5.08	4.24

Figures in parentheses indicate ranges.

cating either higher relative levels of ACTH or a pattern of coreleased ACTH and BE. Conversely, the fetuses who were very slow or failed to habituate had the largest DI, indicating excessive BE (fig. 3). Tests of simple effects indicate that differences in the DI are significant only between the fast and slow habituators (least significant difference test, $p < 0.02$). The pattern of means indicates that higher levels of BE relative to ACTH are associated with significantly slower rates of FHR response habituation to challenge stimulation.

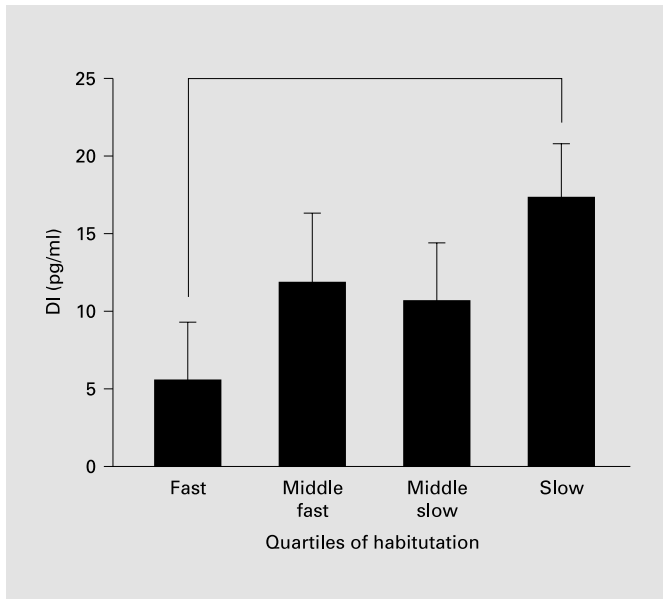


Fig. 3. Slow fetal habituation to repeated stimulation significantly associated with uncoupling (DI) of the relation between maternal BE and ACTH during the early third trimester of pregnancy. Elevated DI reflects greater concentrations of BE relative to ACTH. * $p < 0.02$.

Regression analysis indicated that there was no significant relation between the trial on which the first response occurred (or the number of prereponse trials) and the individual endocrine values for ACTH ($R = 0.03$), BE ($R = 0.03$) or the DI ($R = 0.04$). There were no significant differences among the four groups in the trial in which the first response was detected based on the rate of habituation (mean range: 1.02–1.14 trials; $F_{3, 131} = 0.76$, $p = 0.52$; one-way ANOVA). Bivariate correlations indicated that the relation between first trial to respond and the rate of habituation was not significant ($r = -0.11$, $p = 0.19$). It is important to acknowledge that all but 12 fetuses responded in the first trial.

The interaction of fetal sex with rate of habituation was tested with a two-way ANOVA [2 (fetal sex) \times 4 (quartile of habituation rate)]. The sex of the fetus was marginally ($F_{1, 127} = 3.41$, $p < 0.07$) related to the DI. Maternal levels of BE were higher relative to ACTH in women carrying female fetuses. However, there was no interaction between fetal sex and rate of habituation on the DI ($F_{3, 127} = 2.00$, $p = 0.12$).

Discussion

Results from this study indicate that maternal expression of the POMC molecule during the third trimester of pregnancy influences the response of the human fetus to external stimulation. Specifically, fetal exposure to relatively high levels of the maternal opioid fragment of POMC was associated with significantly lower rates of FHR habituation to repeated ex utero stimulation. In contrast, fetuses in maternal environments either with elevated ACTH or with balanced expression of POMC exhibited the most rapid rate of habituation.

Habituation measures the response decrement resulting from repeated exposure to a familiar stimulus [MacKintosh, 1987; Thompson and Spencer, 1966; Tighe and Leaton, 1976]. It requires that the organism initially detect and respond to information and then systematically ignore and cease responding to subsequent, identical information. To accomplish this, the fetus must compare the current stimulus with its most recent experiences. If the current stimulus is similar to the fetal memory of recent events, it loses its ability to provoke a response and the fetus will habituate to the stimulus [MacKintosh, 1987; Sokolov, 1963; Wagner, 1981]. Fast habituation is evidence that the organism rapidly extracts, and accurately 'recalls,' relevant information from its environment. Slower habituation, or prolonged responding to the stimulus, suggests that relevant information is not recognized and that 'recall' is imperfect. In our study, fetuses exposed to elevated BE require more trials to habituate than those exposed to coreleased POMC fragments or those exposed to elevated ACTH. These findings suggest that memory and its central nervous system substrates are influenced by prenatal exposure to fragments from the POMC molecule.

The ability of the fetus to detect novelty and habituate to repeated stimulation coincides with maturation of the parahippocampal region [Berger et al., 1993; Berger and Alvarez, 1994; Kostovic et al., 1990; Ben-Barak and Dudai, 1979; Crain et al., 1973; Milner et al., 1983] and development in this area of the cholinergic system [Bronstein et al., 1974; Campbell et al., 1969], peptides, calcium-binding protein, phosphoproteins, and monoamines [Berger et al., 1993; Berger and Alvarez, 1994]. Moreover, there is evidence across several species supporting the hippocampus as the neural basis of novelty preferences in mature nervous systems [Nelson, 1995; Bachevalier et al., 1993; McKee and Squire, 1993] and independent evidence supporting the role of the opioid system in hippocampal function [Vaccharino and Kastin,

2000]. Opiates decrease spontaneous firing rate, block formalin-induced excitation of CA1 hippocampal interneurons [Zheng and Khanna, 1999] and modulate long-term potentiation in mossy fibers of the CA3 region of the hippocampus [Jin and Chavkin, 1999]. These findings suggest plausible mechanisms for the developmental effects of POMC fragments, especially the endogenous opioids on the human fetus.

Opiate receptors are present by the end of the first trimester in the human spinal cord, proliferate corresponding to neurogenesis in the dorsal horn by the beginning of the second trimester and resemble adult distribution by week 24 of gestation [Ray and Wadhwa, 1999]. Opiates introduced to the maternal circulation, such as morphine, easily cross the human placenta and may result in fetal distress. Opiate antagonists (e.g. naloxone) are effective treatments for fetal distress but they do not alter the maternal-to-fetal transfer rate of morphine. Thus, the antagonism of fetal distress by naloxone is explained by interactions with the fetal opiate receptors and not by the rate of transfer from mother to fetus [Vaccarino and Kastin, 2000; Kopecky et al., 1999]. Prenatal exposure to opiate antagonists in rats results in acceleration in both physical and behavioral maturation [McLaughlin et al., 1997b], including increased body weights and wet weights of brain, heart, kidney, lungs, liver and muscle groups [McLaughlin et al., 1997a]. These animals have long-term alteration in the density of the μ receptor and insensitivity to opiate treatment as adults [Zagon et al., 1998].

These findings suggest that prenatal exposure to POMC fragments can exert programming effects directly on the well-developed fetal opiate receptor system. Previous studies indicate that prenatal exposure to elevated endogenous opiates results in depressed performance of learning tasks, decreased exploration and elevated pain thresholds in adult animals exposed prenatally to elevated levels of opiates [Sandman and Kastin, 1981; Zadina et al., 1987], 'permanent' downregulation of dopamine receptors [Sandman and Yessian, 1986], reduced numbers of opioid receptors in the brain [Insel et al., 1990], increased opioid levels in the hypothalamus [Sanchez et al., 1993], and reduced benzodiazepine inhibitory activity [Fride et al., 1985]. Prenatal exposure to morphine results in altered seizure thresholds [Veliskova et al., 1999], increased grooming and pinning, altered sensitivity of endogenous opiate receptors [Niesink et al., 1999] and lower incidence of respiratory distress (probably related to increased lung maturation) [Gewolb et al., 1999].

It is critical to acknowledge that our findings relate to the pattern of expression of the POMC molecule (DI)

rather than to levels of POMC products. Moreover, it is important to recognize that advancing pregnancy generally increases the coupling of POMC molecules (fig. 2). When dysregulation occurs, it could result from several possible sources including, but not limited to, mutations altering the three-dimensional conformation of the POMC molecule [Rosenblatt and Dickerson, 1997], lesions of the medial basal hypothalamus that disturb corticotropin-releasing hormone (CRH) and disrupt the corelease of BE and ACTH [Barna et al., 1992] and stress-induced increases in circulating levels of CRH, derived from both brain and peripheral sources [Sasaki et al., 1987] that stimulate primarily BE [Hendrie, 1989; Laatikainen, 1991] in sufficient concentrations to produce analgesia in animals [Hargreaves et al., 1990].

The most likely source of dysregulation during pregnancy, however, is the pattern and order of proteolytic processing of the POMC prohormone. A family of subtilisin-like enzymes called prohormone convertases (PCs) are responsible for converting the biologically inactive POMC molecule into the active peptide fragments ACTH and BE [Seidah and Chretien, 1992; Seidah et al., 1991]. Cleavage of the POMC precursor by two PCs (PC1 and PC2) is tightly controlled, occurs in a specific order [Mains and Eipper, 1999] and usually results in highly coupled expression of ACTH and BE [Strand, 1999]. Dysregulation can occur because of unequal distribution of PC1 and PC2. Although there is no evidence that PCs are differentially expressed in maternal tissue during pregnancy, there is evidence that PC2 is prominently expressed in fetal tissue. Inferential evidence suggests that PC2 may be dominant in the placenta because melanocyte-stimulating hormone (a PC2 fragment) rather than ACTH (a PC1 fragment) appears to be the prominent placental POMC product [Chan and Smith, 1992; Ciesla, 1998]. Moreover, both convertases are expressed by mid-gestation in the fetal brain but PC2, which cleaves BE from POMC, is much more broadly distributed than PC1 [Zheng et al., 1994]. It is reasonable to assume that the fetus will develop opiate receptors and respond to maternal POMC fragments because of the early programming by, and exposure to, PC2-liberated compounds including BE. The differences in the distribution of PC1 and PC2 that are evident prenatally begin to disappear as organisms reach adulthood [Zheng et al., 1994], which explains that in normal circumstances the concentrations of ACTH and BE are highly correlated in adults [Bertagna, 1994].

Although the gene encoding POMC (chromosome 2) has been detected in the human placenta [Chen et al.,

1986] and peptides from POMC are synthesized in trophoblasts [Liotta et al., 1977; Liotta and Krieger, 1980], the probable source of the circulating levels of maternal BE and ACTH is the pituitary gland [Ochedalski et al., 2001; McLean and Smith, 2001; Smith, 1998]. Placental CRH is proposed to exercise paracrine control on the local production of POMC peptides [Petraglia et al., 1987; Frim et al., 1988]; however, as described above, processing of POMC in the placenta is uncertain. In addition to the fact that placental POMC products are melanocyte-stimulating hormone (rather than ACTH) [Ciesla, 1998] and an acetylated form of BE (neither detected by our assays) [Chan and Smith, 1992], there is an observation that human pregnancy is the only condition in which intact POMC is produced and released physiologically

[Raffin-Sanson et al., 1999]. These observations eliminate the placenta as the source of maternal ACTH and BE and strongly suggest that they are of maternal pituitary origin. Because circulating maternal POMC fragments reflect stress-sensitive HPA and not placental activity, the developing human fetus is susceptible to maternal patterns of response to internal and external events.

Acknowledgement

This study was supported by research (HD28413) awards from the National Institutes of Child Health and Human Development and National Institute of Neurological Diseases and Stroke (NS41298).

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