Quantitative assessment of the ontogeny of met-enkephalin, norepinephrine and epinephrine in the human fetal adrenal medulla

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Abstract. The catecholamine producing cells of the adrenal medulla of fetal as well as adult mammals contain enkephalins. We have quantified met-enkephalin and the catecholamines, norepinephrine and epinephrine, in human fetal adrenal glands during the late first trimester and throughout the second trimester of intrauterine life. Met-enkephalin (ME) was detectable in human fetal adrenals of 11 to 25 weeks’ gestation by RIA. ME concentrations were low through 14 weeks (mean 279 ± 199 pg/gland), higher but extremely variable from 15 to 20 weeks (mean 1100 ± 1000 pg/gland), and then lower with somewhat less variation through 25 weeks (mean 865 ± 625 pg/gland). In contrast, catecholamine concentrations were below 1100 ng/gland through 16 weeks, then increased markedly by 21 weeks. Approximately equal concentrations of norepinephrine and epinephrine were measured throughout the gestational age period studied. Our data demonstrate that enkephalin is present in the human fetal adrenal at least by 11 weeks’ gestation and suggest that the fetal adrenal may be capable of secreting enkephalins as well as catecholamines. The functional significance of adrenal enkephalin secretion remains to be elucidated.

In the last 10 years, the presence of many of the known neuropeptides has been demonstrated in the adult mammalian adrenal medulla (Bryant et al. 1976; Lundberg et al. 1979, 1980; Saria et al. 1980; Day et al. 1982; Evans et al. 1983; Hishimoto et al. 1984). Among these neuropeptides are several products derived from the processing of proenkephalin including leu- and met-enkephalin (ME) (Lundberg et al. 1979; Alumets et al. 1979; Stern et al. 1979; Viveros et al. 1979). All of these peptides are contained in the chromaffin granules (Stern et al. 1979; Viveros et al. 1979), and are, therefore, co-released with the catecholamines (CA) in response to stressful stimuli (Viveros et al. 1979; Kilpatrick et al. 1980; Ryder & Eng 1981; Farrell et al. 1983).

Other investigators have demonstrated enkephalin in the fetal adrenal of rats (Palmer et al. 1982) and sheep (Dunlap et al. 1985). Our interest is in the ontogeny of the enkephalin and CA secretory systems in the primate fetal adrenal medulla. In a previous study (Wilburn et al. 1986), we reported the co-localization of leu-enkephalin and the CA-synthesizing enzymes dopamine-β-hydroxylase and phenylethanolamine N-methyltransferase in the mature adrenal medullary cells of human and rhesus fetuses. The purpose of the present study was to derive developmental profiles of enkephalin, norepinephrine (NE) and epinephrine (E) concentrations in second trimester human fetal adrenal glands.

Materials and Methods

Extraction

Human fetal adrenal glands were obtained from late first trimester and second trimester abortuses within 2 h of dilatation and evacuation, and frozen in liquid nitro-
gen. Approval for the use of these tissues was given by the Human and Environmental Protection Committee, UCSF. Fetal age was determined on the basis of foot length. Each adrenal was thawed on ice, weighed and then homogenized in 10 ml (volume:weight > 10:1) ice-cold 90% MeOH with a Brinkmann Polytron (Westbury, NY) for 1 min.

For assessment of recovery, 2 glands were homogenized together and divided equally. Two hundred µl (500 pg) of cold ME standard and 30 µl of a solution containing 1 ml/l CA (NE:E = 1:1) were added to one half of this homogenate. ME and CA concentrations in the 2 halves were compared to determine percent recovery.

A 500-µl aliquot from each adrenal homogenate was mixed with 2 ml 0.1 N HClO₄. After 30 min centrifugation at 80000 x g, the resultant supernatant was diluted 1:10 in 0.1 N HClO₄. NE and E concentrations were determined using a modification (Joyce et al. 1983) of the radioenzymatic assay of Peuler & Johnson (1977).

After removal of aliquots for CA assay, adrenal homogenates were shaken in a water bath at 85°C for 10 min to inactivate any enkephalin-degrading enzymes, then shaken an additional 1 h at room temperature to facilitate extraction. Homogenates were centrifuged at 65000 x g for 30 min at 4°C. The supernatant was decanted and the pellet re-extracted overnight with 2 ml 90% MeOH at 4°C and centrifuged again. The supernatants from the 2 spins were combined, dried in a water bath at 60°C under nitrogen, reconstituted with 1 ml assay buffer and frozen at −70°C until assay.

Radioimmunoassay

Human fetal adrenal extracts were assayed for ME with an RIA kit from Immunonuclear Corp (Stillwater, MN). The reported cross-reactivity is 2.8% with leu-enkephalin, 0.1% with α-endorphin and <0.002% with β-endorphin, substance P, dynorphin and α-neo-endorphin. The ME antibody in this kit does not cross-react significantly with ME-arg, ME-arg-phe or ME-arg-gly-leu. Before assay, reconstituted samples were shaken in a water bath at 37°C for 1 h and centrifuged at 85000 x g for 1 h to remove particulates. Samples were then assayed undiluted and also at a dilution of 1:3.

Intra- and inter-assay variations were 6 and 18%, respectively. Average recovery was 60%. The average minimum detectable concentration was 35 ng/l. Only duplicates which had a CV of ≤15% and occurred between 20 and 80% of maximal binding were included in the data.

Results

Concentrations of ME in adrenals from fetuses of 11–25 weeks' gestation varied considerably, particularly in the 15–20 week age range (Fig. 1). Note that ME concentration is expressed per gland rather than per amount of tissue. The latter expression is affected by the growth of the cortex, which is much greater during fetal life than that of the medulla (a plot of ME per tissue vs gestational age actually had a negative slope). To determine whether any particular pattern in ME concentration vs age could be discerned, we grouped ME values by 1 week age intervals (Fig. 2). Although there were no statistically significant dif-

![Graph](https://via.placeholder.com/150)

**Fig. 1.**

Met-enkephalin concentrations in adrenals from human fetuses of 11–25 weeks' gestation.
Met-enkephalin concentrations in adrenals from human fetuses of 11–25 weeks' gestation. Values represent mean ± sd. Number of adrenals in each group indicated above each bar.

Differences between these groups due to the wide variation in ME values at each age, a pattern did emerge: ME concentrations were lowest (152 pg/gland) in the 11 week gland, appeared to reach a peak at 19 weeks (mean 1988 pg/gland), declined somewhat and peaked again at 25 weeks (mean 1824 pg/gland).

The initial regression analysis of ME concentration vs age (Fig. 1) suggested that the 15–20 week interval included more high values, and particularly more variation in values, than the intervals before or after this period. Therefore, we divided the data into three groups (Fig. 3). In the first group, ME was low in all glands. In the second

Fig. 3.
Met-enkephalin concentrations in human fetal adrenal glands grouped by 5-week gestational age intervals. Box plots were chosen to represent these groups because they show clearly the degree of variation within each group. The upper and lower boundaries represent the 25th and 75th percentiles, respectively. The horizontal line within the box represents the median. Points below 10% or above 90% are indicated by open circles. Groups which differ significantly from the 10–15 week group marked by * (P < 0.005).
group, concentrations varied markedly, ranging from 129 to 3808 pg/gland. In the third group, ME levels were again more uniform. Using non-parametric Kruskal-Wallis analysis, ME concentrations in the 10–15 week group were significantly lower than in the other two groups, which did not differ from each other (Fig. 3, Table 1).

The developmental pattern for the CAs (Fig. 4) differed from that observed for enkephalins. Concentrations of both NE and E remained below 1100 ng/gland through 16 weeks. By 21 weeks, NE concentrations had reached 3815 and E, 2660 ng/gland. When the CA data were grouped by 10–15, 15–20 and 20–25 week intervals, the 20–25 week NE and E values were significantly higher than those in the 10–15 week group (Fig. 5, Table 1). Thus, in contrast to ME values, the high CA values occurred during the last third of the age range studied. We could discern no difference in the developmental patterns of the two CAs, except that E levels were slightly lower than NE levels late in gestation. Regression analysis indicated that concentrations of the two CAs were well correlated ($r = 0.94$) over the gestational age range studied.

**Discussion**

In this study, we were able to detect ME in adrenal glands from human fetuses of 11–25 weeks' gestation by RIA. In an earlier study, we localized

<table>
<thead>
<tr>
<th>Gestational age (weeks)</th>
<th>ME (pg/gland)</th>
<th>NE (ng/gland)</th>
<th>E (ng/gland)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10–14.9</td>
<td>279 ± 199*</td>
<td>512 ± 249</td>
<td>476 ± 273</td>
</tr>
<tr>
<td>15–19.9</td>
<td>1100 ± 1000</td>
<td>783 ± 300</td>
<td>714 ± 257</td>
</tr>
<tr>
<td>20–24.9</td>
<td>865 ± 629</td>
<td>4502 ± 1198**</td>
<td>2774 ± 1770**</td>
</tr>
</tbody>
</table>

* Significantly different from the 15–19.9 and 20–24.9 week groups ($P < 0.005$).
** Significantly different from the 10–14.9 week group ($P < 0.01$).
enkephalin in the medullary cells of human and rhesus fetal adrenals (Wilburn et al. 1986). However, during fetal development, the medullary cells are in the process of migrating through the cortex into the center of the gland where they form an irregular mass (Crowder 1957; Keene & Hewer 1927). Therefore, we used the whole gland for ME and CA determinations, rather than trying to separate medulla from cortex.

The mean ME concentrations reported here did not increase linearly with age. Instead, ME levels were low at first, rose to an initial peak, declined somewhat and then rose again over the gestational age period studied. Our observation that ME concentrations varied much more during the period of the initial rise (15–20 weeks) than in the following 5 weeks, even though mean ME levels in the two periods were similar, suggests that adrenal enkephalin production is less tightly regulated in the 15–20 week period. Alternatively, the differences in ME content between individual glands may reflect differences in secretion.

While ME levels started to rises at 15 weeks, CA levels were still low at 16 weeks. Unfortunately, we have no CA data from adrenals of 17- to 20-week fetuses. By 21 weeks, both CAs had risen dramatically. The radioenzymatic assay utilized gave approximately equal concentrations of NE and E throughout most of the second trimester. This finding agrees well with results from previous bio- and fluorimetric assays (Coupland 1953; Greenberg & Lind 1961& von Studnitz 1968). However, in the present study, the two 11-week adrenals contained more NE than E (73 and 78% NE). This observation is consonant with the hypothesis that the NE to E ratio decreases with advancing adrenal medullary development (Comline & Silver 1966).

The physiological role of the CAs in the adult is well documented. Evidence from many laboratories suggests that in the mammalian fetus CAs play an important role in maintaining homeostasis; e.g. both hypoxia and hypoglycemia stimulate CA secretion in the fetal lamb and calf (Comline & Silver 1966). Phillippe (1983) reviewed these data thoroughly and concluded that 1) fetal CAs are important regulators of the cardiovascular system, particularly in the event of asphyxia (such as occurs during parturition); 2) the CAs help prepare the fetal lung for extrauterine life by increasing lecithin synthesis, surfactant secretion and pulmonary blood flow, and decreasing fluid production in the lung; and 3) fetal CAs secreted into the amniotic fluid facilitate parturition by stimulating myometrial contractions. Most studies on fetal CA secretion do not distinguish between adrenal and extra-adrenal CAs.

The physiological role of the adrenal enkephalins in the fetus had not been established. However, studies of the effects of exogenous enkephalins suggest several possible roles for blood-borne enkephalins in the fetus. Both central and peripheral administration of enkephalins and their analogues to conscious adult humans,
dogs and rats increase heart rate and blood pressure (Simon et al. 1978; Stacher et al. 1981; Sander & Giles 1985). Enkephalins have regulatory effects on the pituitary gland, stimulating PRL and GH secretion, but inhibiting secretion of the gonadotropins (Hall et al. 1976; Lien et al. 1976; Bruni et al. 1977; Stubbs et al. 1978). Enkephalin effects on the respiratory, gastrointestinal and immune systems have also been reported, but these are more controversial. Enkephalins and other opiates inhibit nicotine-stimulated adrenal CA secretion (Dean et al. 1982; Saiani & Guidotti 1982). This finding suggests that adrenal enkephalins may exert paracrine effects within the adrenal medulla. The evidence for enkephalin effects in adult animals, combined with our observation that, at least by 11 weeks' gestation, human fetal adrenal glands contain enkephalin, leads us to speculate that the fetal adrenal secretes enkephalins which, in concert with the CAs, act to maintain fetal homeostasis and aid in the adaptation of the newborn to extrauterine life.

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References


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