Human Plasma ACTH, β -Lipotropin and β -Endorphin during Delivery

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FURUHASHI, N., TAKAHASHI, T., FUKAYA, T., KONO, H., SHINKAWA, O. and SUZUKI, M. *Human Plasma ACTH, β -Lipotropin and β -Endorphin during Delivery.* Tohoku J. exp. Med., 1983, 141 (1), 71-76 — Adrenocorticotrophic hormone (ACTH), β -lipotropin (β -LPH) and β -endorphin (β -EP) immunoreactivities were measured by high sensitive radioimmunoassay in maternal and umbilical cord plasma samples which were obtained simultaneously in 12 cases. Mean ACTH levels in cord and maternal plasma were significantly higher than those of 8 normal adults. Mean β -EP to β -LPH molar ratio of 0.19 in maternal plasma was different from that of cord plasma (0.13). There was no significant correlation between β -EP levels, or between β -LPH levels, in the paired fetal and maternal samples. These data suggest that β -LPH and β -EP elevate during labor and delivery in response to the stress. Beta-LPH and β -EP in cord plasma are of fetal and/or placental origin. ——— ACTH; β -lipotropin; β -endorphin; delivery

Beta-endorphin (β -EP) and β -lipotropin (β -LPH) were known to elevate in parallel in maternal blood and umbilical cord plasma during pregnancy, labor and delivery (Csontos et al. 1979; Wardlow et al. 1979; Granat et al. 1980; Wilkes et al. 1980; Goland et al. 1981).

Recently, it was suggested that β -EP is synthesized and released from human placenta (Nakai et al. 1978; Odagiri et al. 1979; Fraioli and Genazzani 1980; Houck et al. 1980; Julliard et al. 1980; Liotta and Krieger 1980). There were few reports measuring maternal and umbilical cord plasma ACTH, β -LPH and β -EP simultaneously to study the correlations among those hormones. It is expected that these three hormones are elevated in parallel during labor and delivery. To confirm this hypothesis, in this study, umbilical cord and maternal plasma ACTH, β -LPH and β -EP were measured simultaneously at delivery. Correlations among these three hormone levels in the paired samples were also investigated.

MATERIALS AND METHODS

Subjects

Twelve term infants and their healthy mothers were studied; all infants were delivered vaginally after spontaneous labor. The mean gestational age was 39.5 weeks (range, 38-41); mean birth weight was 3,355 g. All infants were judged to be healthy. Eight

normal adult women (aged 22–32 years) who were confirmed to be at the follicular phase according to their basal body temperature were also studied for comparison.

Collection of samples

Simultaneous maternal and umbilical cord blood samples were obtained at vaginal delivery in 12 cases. Samples (5–30 ml) were collected in tubes containing 1 mg/ml EDTA (ethylenediamine-tetraacetic acid) on ice and centrifuged at 4°C. Plasma was either extracted for assay immediately or frozen at –20°C. Birth weights were measured immediately after delivery. Blood was collected from the normal adults between 08:00–10:00 h before breakfast.

Plasma extraction and chromatography

Plasma was extracted, chromatographed and assayed for β -EP and β -LPH as reported by Wardlow et al. (1979). Briefly, β -EP and β -LPH were extracted from 2 ml plasma with silicic acid (Mallinckrodt, St. Louis, 100 mesh) 50 mg and then eluted from the silicic acid with 40% acetone–0.1 N HCl (1:1). The acetone–HCl extracts were evaporated and taken up with elution buffer (0.1 M acetone acid, 0.1% BSA).

In 12 pregnant women and 8 normal adults, plasma extracts were chromatographed on 1×30 cm Sephadex G–50 (fine) column with the elution buffer in order to assay β -EP and β -LPH separately. Recovery of added β -EP and β -LPH was 74% and 66% on an average after extraction and 88 to 92% after extraction and chromatography for both peptides. All plasma concentrations of β -EP and β -LPH were corrected for the recovery rate.

Radioimmunoassay

Total β -EP immunoactivity was measured by RIA using synthetic human β -EP (Peptide Laboratories, Osaka) and antiserum to human β -EP (supplied by Dr. A. Sasaki) which has less than 1% cross-reactivity with β -LPH on a weight basis. The current assay sensitivity was 2 pg/tube for β -EP (Fig. 1). The method of the β -LPH assay was identical to that for β -EP, with the same antibody and [125 I] β -EP tracer at the same dilutions, except that β -LPH standards were substituted for β -EP. The cross-reactivity of β -LPH with β -EP under these conditions was constant in all concentrations, i.e. the standard curves were parallel over the whole range (Fig 1). Current sensitivity was 20 pg/tube for β -LPH. Intra- and interassay coefficients of variation were 8.0% and 14.9%, respectively.

Human β -LPH was kindly supplied by Dr. C.H.Li. After chromatography of plasma extracts, fractions representing β -LPH and β -EP peaks were assayed in a similar manner against a β -EP standard, and results were expressed as β -EP immunoactivity. When

Fig. 1. Standard curve for plasma immunoreactive β -LPH (○—○) and β -EP (●—●).

actual β -LPH and β -EP concentrations were determined, fractions representing β -LPH and β -EP peaks had been assayed against their respective standards. Plasma immunoreactive ACTH was measured with a CIS (CEA-IRE-SORIN, Paris) RIA kit. The antisera were raised in rabbits against ACTH₁₋₃₉ (Wintour et al. 1980).

Statistical analysis

Group data were subjected to the Student's *t*-test for significance of difference. Linear regression analysis was done by microcomputer TRS-80 (Radis Shack, Fortworth, Texas, USA).

RESULTS

It appears evident that term infants were normally born with high blood levels of β -LPH and β -EP and that both fetal and maternal concentrations of these peptides in blood were significantly ($p < 0.001$) higher than those in normal adults (Table 1). There were significant differences ($p < 0.05$) between the plasma levels of ACTH in cord or maternal blood and those in normal adults.

TABLE 1. ACTH, β -LPH and β -EP levels measured in the maternal venous plasma and cord venous plasma

Sample	Number of samples	ACTH (pg/ml)	β -LPH (pg/ml)	β -EP (pg/ml)
Maternal plasma				
Total	12	135.9 (35.8) *	690.1 (138.6) †	125.8 (24.0) †
Primipara	4	128.8 (80.2)	700.5 (418.6)	135.5 (75.2) *
Multipara	8	139.5 (40.5)	684.9 (87.9) †	121.0 (11.8) †
Cord plasma				
Total	12	97.1 (17.9) *	1114.8 (94.2) †	130.6 (20.6) †
Primipara	4	100.8 (36.1)	948.3 (263.9) †	128.5 (41.6) †
Multipara	8	95.3 (21.8)	956.0 (100.6) †	131.6 (25.1) †
Normal adult‡	8	48.5 (6.9)	93.0 (8.2)	6.0 (0.9)

Values are mean ($\pm$ S.D.).

Compared with normal adult; * $p < 0.05$, † $p < 0.01$.

‡ Follicular phase in normal woman.

No significant difference in ACTH, β -LPH or β -EP concentration of either maternal or cord plasma was observed between primipara and multipara.

Highly significant positive correlations were present between β -LPH and β -EP levels in maternal plasma ($n=12$, $r=0.9$, $p < 0.005$; Fig. 2) and cord plasma ($n=12$, $r=0.901$, $p < 0.001$; Fig. 3).

There was no significant correlation between maternal and umbilical cord plasma in ACTH, β -LPH or β -EP concentration.

The mean β -EP to β -LPH molar ratio of 0.13 (range, 0.09–0.18) in newborns was different from the ratio of 0.19 (range, 0.06–0.42) in their mothers at delivery (Table 2). The mean β -LPH to ACTH molar ratios and β -EP to ACTH molar ratios measured in maternal and cord plasma were not significantly different.

Fig. 2. Correlation between maternal plasma immunoreactive β -LPH and β -EP levels. Primipara ($\circ$) and multipara ($\bullet$). Coefficient of correlation was $r=0.9$ ($n=12$, $p<0.005$), and the regression line was $Y=0.156X+18.28$.

Fig. 3. Correlation between cord plasma immunoreactive β -LPH and β -EP levels. Primipara ($\circ$) and multipara ($\bullet$). Coefficient of correlation was $r=0.901$ ($n=12$, $p<0.001$), and the regression line was $Y=0.18X-41.34$.

TABLE 2. Mean molar ratio of ACTH, β -LPH and β -EP in the maternal venous plasma and cord venous plasma

Sample	Number of samples	β -LPH/ACTH	β -EP/ β -LPH	β -EP/ACTH
Maternal venous plasma	12	14.41 (6.68)	0.19 (0.03)	2.59 (1.26)
Cord venous plasma	12	14.07 (3.22)	0.13 (0.01)	1.75 (0.35)

Mean ($\pm$ S.E.).

DISCUSSION

There were few reports measuring simultaneously maternal and umbilical cord plasma ACTH, β -LPH and β -EP. The umbilical cord plasma ACTH has been reported to be elevated at delivery (Allen et al. 1973; Winter et al. 1974; Arai et al. 1976). This was confirmed by the present study.

With Sephadex G-50 chromatography, it was possible to measure β -EP and β -LPH levels separately and simultaneously. Both peptides in maternal and umbilical cord plasma were significantly elevated as compared to normal adults, as previously reported (Csontos et al. 1979; Wardlow et al. 1979; Granat et al. 1980; Wilkes et al. 1980; Goland et al. 1981). The ACTH, β -LPH and β -EP levels in maternal and fetal plasma at delivery have been speculated to elevate in response to the stress of labor and delivery, though the exact mechanism of the elevation of these peptides is still unclear.

When both β -EP and β -LPH were measured in 12 paired maternal and fetal plasma samples, there was no significant correlation between the mother and baby as reported by Goland et al. (1981). In addition, the molar ratio of β -EP and β -LPH was investigated and a significant difference in the ratio was found between maternal plasma and fetal plasma.

Wardlow et al. (1979) reported that the umbilical arterial β -EP level was higher than the umbilical venous β -EP level, suggesting that the umbilical cord β -EP was of fetal origin. However, a placental contribution cannot be excluded. This is an important consideration, since it has recently been demonstrated by several investigations (Nakai et al. 1978; Odagiri et al. 1979; Fraioli and Genazzani 1980; Houck et al. 1980; Julliard et al. 1980; Liotta and Kricger 1980) that the human placenta can synthesize β -EP.

The physiological role of the increased β -LPH and β -EP in maternal and fetal plasma at delivery is unknown. Beta-EP injected exogenously has been shown to affect hormone secretion from both the anterior and posterior pituitaries (Gluckman et al. 1980). It is tempting to speculate that the elevated plasma levels of β -EP during delivery may have important peripheral or neuroendocrinological actions. It should be investigated further.

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References

- 1) Allen, J.P., Cook, D.M., Kendakk, J.W. & McGilvra, R. (1973) Maternal-fetal ACTH relationship in man. *J. clin. Endocr. Metab.*, **37**, 230-234.
- 2) Arai, K., Yanaihara, T. & Okinaga, S. (1976) Adrenocorticotrophic hormone in human fetal blood at delivery. *Amer. J. Obstet. Gynecol.*, **125**, 1136-1140
- 3) Csontos, K., Rust, M.M., Holtt, V., Mahr, M., Krumoer, W. & Teshemacher, H.J.

- (1979) Elevated plasma β -endorphin levels in pregnant women and their neonates. *Life Sci.*, **25**, 835-844.
- 4) Fraioli, F. & Genazzani, A.R. (1980) Human placental β -endorphin. *Gynecol. Obstet. Invest.*, **11**, 37-44.
 - 5) Gluckman, P.D., Marti-henneberg, C., Kaplan, S.L., Li, C.R. & Grumbach, M.M. (1980) Hormonal ontogeny in the ovine fetus. X. The effect of β -endorphin and naloxone on circulating GH, PRL and cSM. *Endocrinology*, **107**, 76-80.
 - 6) Goland, R.S., Wardlow, S.L., Stark, R.I. & Frantz, A.G. (1981) Human plasma β -endorphin during pregnancy, labor and delivery. *J. clin. Endocr. Metab.*, **52**, 74-78.
 - 7) Granat, M., Sharf, M. & Weissman, B.A. (1980) Human endorphin in human body fluids during pregnancy. *Gynecol. Obstet. Invest.*, **11**, 214-218.
 - 8) Houck, J.C., Kimball, C., Chang, C., Pedigo, N.W. & Yamamura, H.I. (1980) Placental β -endorphin-like peptides. *Science*, **207**, 78-80.
 - 9) Julliard, J.H., Shibasaki, T., Ling, N. & Guillemin, R. (1980) High-molecular-weight immunoreactive β -endorphin in extracts of human placenta is a fragment of immunoglobulin G. *Science*, **208**, 183-185.
 - 10) Liotta, A.S. & Krieger, D.T. (1980) In vitro biosynthesis and comparative posttranslational processing of immunoreactive precursor corticotropin/ β -endorphin by human placental and pituitary cells. *Endocrinology*, **106**, 1504-1511.
 - 11) Nakai, Y., Nakao, O., Oki, S. & Imura, H. (1978) Presence of immunoreactive β -lipotropin and β -endorphin in human placenta. *Life Science*, **23**, 2013-2018.
 - 12) Odagiri, E., Sherrell, B.J., Mount, C.D., Nicholson, W.E. & Orth, D.N. (1979) Human placental immunoreactive corticotropin, lipotropin and β -endorphin. Evidence for a common precursor. *Proc. nat. Acad. Sci. USA*, **76**, 2027-2031.
 - 13) Wardlow, S.L., Stark, R.I., Baxi, & Frantz, A.G. (1979) Plasma β -endorphin and β -LPH in the human fetus at delivery: Correlation with arterial pH and PO_2 . *J. clin. Endocr. Metab.*, **49**, 888-891.
 - 14) Wilkes, M.M., Stewart, R.D., Bruni, J.F., Quigley, M.E., Yen, S.S.C., Ling, N. & Chretien, M. (1980) A specific homologous RIA for human β -endorphin: Direct measurement in biological fluids. *J. clin. Endocr. Metab.*, **50**, 309-315.
 - 15) Winter, A.J., Oliver, C., Colston, C., MacDonald, P.C. & Porter, J.C. (1974) Plasma ACTH levels in the human fetus and neonate as related to age and parturition. *J. clin. Endocr. Metab.*, **39**, 259-273.
 - 16) Wintour, E.M., Coghlan, J.P., Hardy, K.H., Hennessy, D.P., Lingwood, B.E. & Scoggins, B.A. (1980) Adrenal corticosteroids and immunoreactive ACTH in chronically cannulated ovine fetuses with bilateral adrenalectomy. *Acta endocr. (Kbh.)*, **95**, 546-552.
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