Pediatr. Res. *16:* 95–98 (1982)

Plasma Opioids in the First Hours of Life

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Summary

High concentrations of ACTH are present in the neonatal circulation in response to birth stress. Since ACTH and β-lipotropin (βLPH) have a common precursor, and ACTH and β-endorphin (βEP) are released in parallel in stressful situations, we measured plasma levels of βLPH and βEP in the first 24 hours of life.

Blood samples were taken from the umbilical cord at birth in 27 neonates, subdivided into four groups.

A further blood sample was obtained from the jugular vein after 30 min in nine cases (group A), after 6 hours in six (group B), and after 12 hours in six (group C), and after 24 hours in six (group D).

βLPH and βEP were measured by specific radioimmunoassays after silicic acid extraction of the plasma and Sephadex G-75 column chromatography.

In cord plasma, the mean (±S.E.) βLPH concentrations (pg/ml) varied between 219.5 ± 84.5 (group D) and 241 ± 43.3 (group B), while those of βEP ranged between 70.2 ± 8.2 (group C) and 54.6 ± 14.4 (group D). The βLPH/βEP ratio on a molar basis ranged from 1.12 ± 0.73 (group A) to 1.37 ± 0.60 (group C).

Plasma βLPH concentrations determined at 30 min were 199.1 ± 50.4, and these declined slightly in group C (after 12 hours: 168.2 ± 30.2), but only showed a significant fall after 24 hours (88.5 ± 27.0; $P < 0.01$ in comparison with the previous groups), reaching normal adult levels (71.7 ± 25.1).

The pattern of βEP plasma levels (pg/ml) was very similar to that reported for βLPH. βEP plasma levels only showed a significant decrease after 24 hours (22.7 ± 8.9; $P < 0.01$) when compared to the 12-hour age group C. βEP concentrations at 30 min (76.1 ± 47.7), 6 hours (68.6 ± 29.3), and 12 hours (51.8 ± 18.1) of life did not differ significantly from those found in mixed cord blood and were constantly higher than normal adult levels [24.5 ± 12.3].

Since the half-life of βLPH and βEP is respectively 45 and 37 min, the present data demonstrate that neonates are able to release βLPH and βEP during the first hours of life. Both these molecules may contribute to help the newborn in the transition from a close dependence on the mother, to an autonomous existence.

Speculation

Although the physiologic role of circulating βLPH and βEP has not been fully clarified, the presence of these peptides in neonatal plasma could represent an important analgesic factor in overcoming birth stress. Thus, an abnormal presence of these substances could be related to pathological situations. However, the source, the regulatory mechanisms and the biologic effects of circulating opioids in neonatal life are still unclear and require further investigation.

The transition from intra- to extra-uterine life is probably the most stressful situation that is met during our existence, and many adaptive phenomena are required to overcome this critical period. In particular, an early activation of the hypothalamus-pituitary-adrenal axis is necessary.

The significantly lower plasma cortisol levels found in newborn babies delivered by elective caesarian section, when compared with vaginally delivered neonates, demonstrates the relation between stimulation of the adrenal gland and the stressful events of labour and delivery (5, 35).

Furthermore, it has recently been demonstrated that ACTH plasma levels are elevated during the first 6 hours of life, declining to normal adult values only 18 hours later (4, 20). Accordingly, many authors have observed high plasma cortisol levels during the first 3 hours of life (1, 15, 16, 19, 33), which decrease to normal adult levels after 24–48 hours.

The ability of neonates to secrete cortisol in response to ACTH stimulation (15, 18) or during insulin-induced hypoglycemia (1) and plasma ACTH suppression after dexamethasone, suggest that a Corticotropin Releasing Factor (CRF)-mediated cortisol-ACTH feedback mechanism is present in the neonatal period.

On the other hand, the ACTH molecule in the anterior pituitary is contained in a large molecular weight precursor, the pro-opiocortin (25) which also includes the aminoacid sequences of γ-MSH and β-lipotropin (βLPH). In adults, ACTH, βLPH, and β-endorphin (βEP) (C-terminal residue of βLPH; βLPH 61–91) are secreted concomitantly in stressful situations (14, 21). On this basis, and considering the very high amounts of ACTH (7, 11, 39), βLPH and βEP (7, 9, 12, 13, 27) which circulate in the maternal and cord plasma at delivery, we investigated the changes occurring in βLPH and βEP plasma levels during the first few hours of life.

MATERIALS AND METHODS

SUBJECTS

After informed consent of the parents, 27 neonates, whose mothers were suspected of having contracted an infectious disease during pregnancy or who presented Rh incompatibility and in whom a blood sample was required for routine analysis, were considered in this study.

Gestational age ranged between 38 and 40 wk, the 1-min Agpar score between 8 and 10, and neonatal weight ranged from 2750 to 4000 g.

Mixed cord blood was collected in all cases at delivery and a second sample was taken from the jugular vein after 30 min in nine cases, after 6 hours in six, after 12 hours in six, and after 24 in the remaining six subjects. A further sample was also taken after 24 hours in three of the neonates from whom second samples had been taken at 6 hours, because of suspected (although not confirmed) hypocalcemia.

Heparinized blood samples of 3–4 ml, with the addition of 500 KIU Trasylol, were centrifuged immediately after collection at 2000 g at 4°C for 15 min; the plasma was stored at −70°C and assays were performed within 2 months.

PLASMA PURIFICATION

Plasma, 1.5 to 3 ml, was extracted with 100–200 mg silicic acid (100 mesh) on a rotary mixer for 1 hour. After washing with water

and 1 N hydrochloric acid, the silicic acid was extracted twice with 1 ml of acetone-1 N HCl (9:1).

The acetone mixture was dried under nitrogen, redissolved in 0.5 ml of 0.1 M acetic acid, 0.01% bovine serum albumin and applied to a Sephadex G-75 column (1.5 × 45 cm) eluted with the same solution. In these conditions, standard βLPH and βEP, added to dexamethasone-treated plasma, gave partition coefficients (Kav) of 0.54 for βLPH and 0.82 for βEP. In these conditions, two distinct peaks containing respectively βLPH and βEP were collected from each chromatographed plasma extract; 10–15 ml of eluates were pooled, freeze-dried, and redissolved in 0.4 ml of 0.05 M phosphate buffer pH 7.4, to be tested by radioimmunoassay (RIA) (8).

Since the molecular weight of γ-lipotropin (1–58 βLPH) is near 6300 and its tertiary structure is similar to βLPH, this peptide can be expected to elute in an intermediate position between βLPH and βEP peaks, and that an aliquot of endogenous γLPH will be present in the βLPH fraction to be measured by RIA.

Cold βLPH and βEP, 0.5 ng, added to 24 plasma samples, were recovered in the order of 51.2 ± 7.4 and 57.5 ± 8.1% (mean± S.D.), respectively. Twelve Sephadex columns were eluted simultaneously through a multi-channel peristaltic pump and I^{125}-βLPH and β-EP were added to one plasma sample in order to monitor the recovery in each series of extraction and chromatography.

RIA

Synthetic βEP (Bachem Inc., Torrance, CA) and Human βLPH (donated by Prof. C. H. Li, San Francisco, CA) were used as standard and for labelling with I^{125} using the Chloramine-T method.

Anti-human βEP rabbit serum (C-terminal) and anti-human LPH rabbit serum (N-terminal), both supplied by Prof. C. H. Li, were used at dilutions of 1:3400 and 1:5400, respectively.

Cross-reactivities of the antisera have been reported elsewhere (37, 38).

Since γLPH probably cross-reacts completely with N-terminal anti-βLPH serum, γLPH present in the pooled βLPH fraction obtained by column chromatography may contribute to the amount of IR βLPH apparently measured. Nevertheless, the IR materials present in this fraction and tested with the above described βLPH RIA, will conventionally be referred to as βLPH.

Two double-antibody RIAs were performed as described elsewhere (8).

Sensitivity of the assays was 10 pg for βLPH and 4.5 pg for βEP. Intra- and inter-assay coefficients of variation were respectively 8.4 and 10.6 for βLPH and 7.6 and 11.2 for βEP.

RESULTS

Figure 1 reports the plasma levels of βLPH, βEP and their molar ratio, found in the 27 neonates subdivided into four groups according to neonatal age, and respective cord blood concentrations. The mean (± S.D.) βLPH concentrations in cord plasma in the four groups ranged from 219.5 ± 84.5 to 241 ± 43.3 pg/ml, while single values ranged from 102 to 342 pg/ml. Plasma concentrations in the neonates diminished from 199.1 ± 50.4 pg/ml (range: 102–283 pg/ml) after 30 min, to 168.2 ± 30.3 pg/ml (range: 105–310 pg/ml) after 12 hours, and 88.5 ± 27.0 pg/ml (range: 140–294 pg/ml) after 24 hours. Only the levels found after this interval of time showed a significant difference ($P < 0.01$) in comparison to both the respective cord plasma concentration and the plasma levels found in the group tested after 12 hours ($P < 0.05$).

The mean (± S.D.) βEP concentration in cord plasma varied in the four groups from 70.2 ± 8.2 to 54.6 ± 14.4 pg/ml, while single values ranged between 14 and 101 pg/ml. Plasma levels of βEP in the neonates decreased from 76.1 ± 47.7 pg/ml (range: 18–176 pg/ml) at 30 min, to 66.6 ± 29.3 pg/ml (range: 35–109 pg/ml) at 6 hours, 51.8 ± 18.1 pg/ml (range: 33–79 pg/ml) at 12 hours, and 27.7 ± 8.9 pg/ml (range: 17–38 pg/ml) after 24 hours. These last values were significantly lower ($P < 0.01$) than the respective βEP cord plasma concentrations (54.5 ± 14.4 pg/ml) and those found in neonates after 12 hours ($P < 0.05$).

The βLPH/βEP ratio on a molar basis ranged from 1.12 ± 0.37 to 1.37 ± 0.60 in the four groups of cord plasma, and in the neonatal plasma from 1.22 ± 0.63 to 1.13 ± 0.36. A significant correlation between βLPH and βEP plasma levels was found both in cord plasma ($r = 0.492$; $P < 0.01$) and in neonatal plasma ($r = 0.780$; $P < 0.001$).

Individual plasma levels of the two opioids obtained in the three cases in which two successive samples were taken, are reported in Table 1. These results support the above reported βLPH and βEP patterns in the early hours of neonatal life.

DISCUSSION

With the exception of Goland *et al.* (13) who found constantly normal levels of βEP-like immunoreactive materials, plasma concentrations of βEP have been shown to increase progressively during pregnancy, the highest levels being found at term (7, 12). A further increase has been observed in the first and second stages of labour (7, 9, 13), when βLPH plasma levels also rise in parallel.

High concentrations of both opioids have been demonstrated in mixed cord plasma (7, 9, 12, 13) with a positive gradient from the umbilical artery to vein (36).

The origin of these opioids in the maternal circulation could be attributed not only to the pituitary, but also to the placenta, since the presence (11, 28) and the *in vitro* synthesis and release (23) of both βLPH and βEP have been demonstrated by this endocrine organ. Moreover, in addition to the ability of the fetal pituitary to secrete ACTH (2), immunocytochemical studies have recently demonstrated that fetal anterior pituitary granules contain βLPH, βEP and probably the proopiocortin (22).

The presence in the neonatal circulation, up to the twelfth hour of life, of elevated βLPH and βEP values which are similar to those found in cord plasma, seems to indicate that the neonate is able to release these peptides. In fact, the half-life of βLPH and βEP, calculated as time of disappearance from the plasma, is 45 and 37 minutes, respectively (10). Therefore, if the opioid peptides found in neonates originate from the placental tissue or maternal plasma, it may be expected that they will have disappeared from the circulation after 6 hours.

On the other hand, these data are in perfect agreement with previous demonstrations of raised ACTH levels during the first 6 hours of life (4, 20), and offer further support to the concept that ACTH, βLPH, and βEP are released in parallel in stressful situations (14, 25).

The hypothesis that not only delivery, but also the first hours of extrauterine life may represent a stressful situation, is further supported by the marked metabolic changes occurring at this time, such as hypoglycemia (6), hyponatremia (32), hypocalcemia (30), loss of liquids, etc. These observations also support the supposition that the adrenal hyperactivity found in this critical period is sustained (1, 18, 19, 23) by the high levels of ACTH. On the other hand, the demonstration that βEP stimulates corticosterone release by rat adrenal cells *in vitro* (3), and that βLPH induces aldosterone production by human adrenal cells in culture (26), suggest that βLPH and βEP may also be involved in the adrenal stimulation occurring in the postnatal period.

However, the physiologic significance of the high βLPH and βEP levels at birth and during the first few hours of life still remains unclear.

In consideration of the fact that a significant amount of pituitary hormonal secretion may reach the central nervous system by backflow (29), we can assume [bearing in mind the possible extrapituitary sources of these peptides (3)], that circulating levels could also partially reflect their central effects. Therefore, the three times higher than normal βEP levels found in early postnatal life could play a role in mediating neonatal analgesia which is probably fundamental in overcoming the painful situation of delivery and birth. In fact, it has been shown that βEP in pharmacologic doses possesses a marked analgesic activity when administered both intraventricularly (24) and peripherally (34) in animals, and more

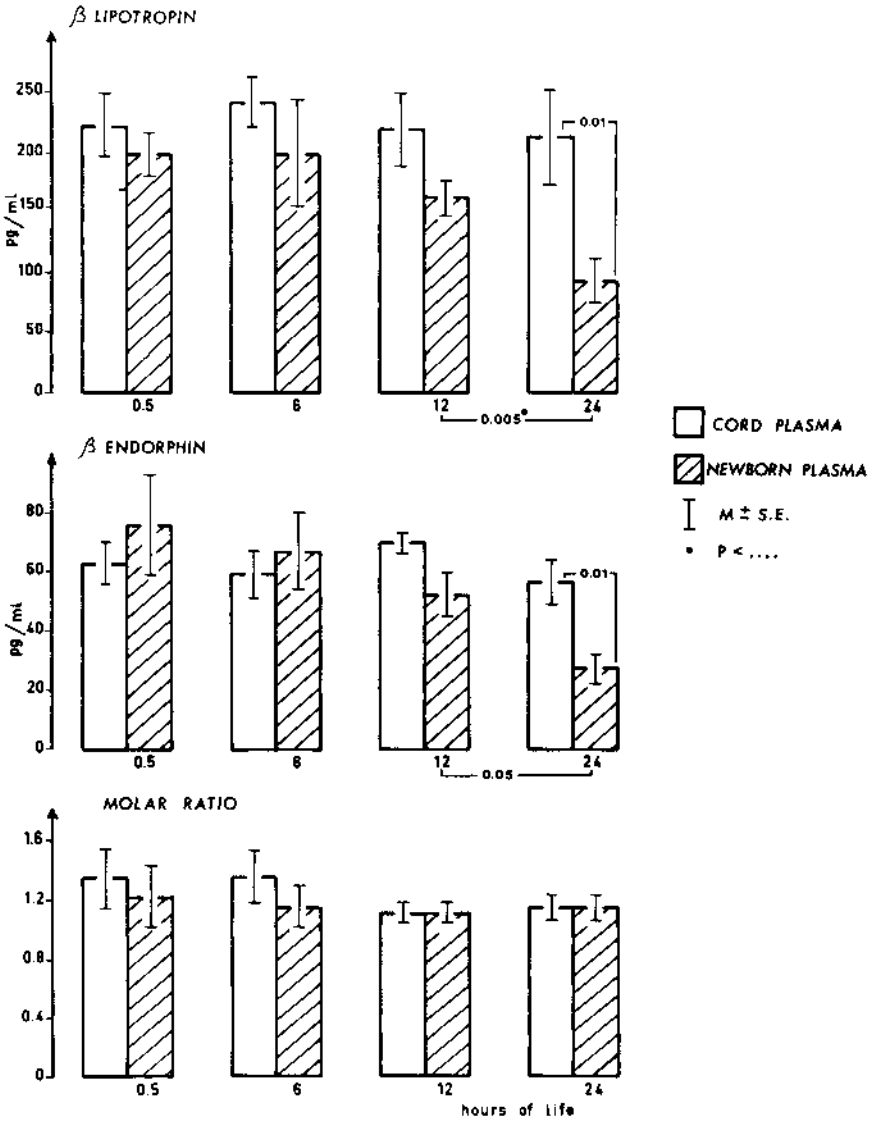

Fig. 1. Plasma levels of β-lipotropin and β-endorphin and their ratio on a molar basis, determined in the four groups of neonates at different hours of life. Opioid concentrations (*shaded bars*) are reported for each group, together with the respective mixed cord plasma levels at birth. The statistical analysis is reported.

Table 1. *β-Lipotropin and β-endorphin concentrations (pg/ml) in mixed cord plasma (MCP) and after 6 and 24 hours of life, in three cases studied longitudinally*

	β-lipotropin			β-endorphin		
Case	MCP	6	24	MCP	6	24
BG	271	222	97	85	66	31
MF	294	211	65	54	45	31
RC	211	147	78	66	51	25

recently pain relief has been demonstrated in humans by intravenous injection of βEP (17).

In conclusion, the present data demonstrate that neonates are able to release βLPH and βEP during the first hours of life. Both these molecules may contribute to help the neonate in the transition from a close dependence on the mother to an autonomous existence.

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41. This research was supported in part by the C.N.R. Project "Biology of Reproduction".
42. Received for publication February 11, 1981.
43. Accepted for publication May 1, 1981

0031-3998/82/1602-0095$02.00/0

Printed in U.S.A.