Opioid peptides of the pituitary and hypothalamus: changes in pregnant and lactating rats

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ABSTRACT

Immunoreactive (Ir) β-endorphin concentrations were determined in plasma, anterior pituitary (AP), neurointermediate pituitary lobe (NIL) and mediobasal hypothalamus (MBH) of pregnant (12–14 and 18–20 days) and fertile control rats, during labour and lactation. Immunoreactive Met-enkephalin concentrations were also evaluated in the MBH.

Concentrations of Ir β-endorphin in plasma, AP and NIL of rats during early and late pregnancy were significantly higher than in controls, the plasma and AP contents showing an increasing pattern in the second half of gestation. During labour, Ir β-endorphin concentrations in plasma and AP reached the highest values, whereas those in NIL remained unchanged. Lactating rats showed Ir β-endorphin concentrations in NIL and plasma in a range similar to that found in pregnant rats, resulting in concentrations in the AP not significantly different from those of non-pregnant controls.

Immunoreactive β-endorphin and Ir Met-enkephalin concentrations in MBH of pregnant rats were almost twice as high as in controls, rising markedly during labour; during lactation levels were in the same range as in non-pregnant controls.

These results indicate that pregnancy and labour are characterized by high plasma, pituitary and hypothalamic concentrations of Ir β-endorphin as well as by high hypothalamic Ir Met-enkephalin levels, and that Ir β-endorphin concentrations vary differently during pregnancy, lactation and labour in the two pituitary lobes, supporting the existence of different control mechanisms in the AP and NIL.

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INTRODUCTION

Endogenous opioids seem to be involved in maternal adaptation to pregnancy and parturition. Several studies have shown that plasma concentrations of immunoreactive (Ir) β-endorphin increase in pregnant women, with a progressive trend toward the third trimester (Csontos, Rust, Höllt et al. 1979; Genazzani, Facchinetti & Parrini, 1981; Goland, Wardlaw, Stark & Frantz, 1981; Browning, Butt, Lynch & Shakespear, 1983; Newnham, Tomlin, Ratter et al. 1983) and a further increase occurs during labour, leading to values approximately tenfold higher (Fletcher, Thomas & Hill, 1980; Facchinetti, Centini, Parrini et al. 1982). It remains to be demonstrated whether this increase is only related to secretion by the placenta, which is able to synthesize β-endorphin (Nakai, Nakao, Oki & Imura, 1978; Fraioli & Genazzani, 1980) or is dependent on hypersecretion in the pituitary gland, the main source of circulating Ir β-endorphin. It has been reported recently that in lactating rats plasma β-endorphin levels are higher than in control females (Riskind, Millard & Martin, 1983).

β-Endorphin-secreting cells are present both in the anterior pituitary (AP) and in the neurointermediate pituitary lobe (NIL) and different neuroendocrine controls regulate their secretion (Vale, Rivier, Yang et al. 1978; Przewlocki, Höllt, Voigt & Herz, 1979; Vermes, Mulder, Smelick & Tilders, 1980; Petraglia, Peñalva, Genazzani & Müller, 1982a; Petraglia, Locatelli, Peñalva et al. 1984).

We have evaluated Ir β-endorphin concentrations in the plasma and in the pituitary lobes (AP and NIL) during pregnancy, at delivery and during lactation, in an attempt to clarify the role of the two pituitary

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lobes in the regulation of peripheral β-endorphin concentrations in such conditions. Furthermore, since opioid peptides of the central nervous system may participate in maternal adaptation to pregnancy, delivery and lactation, in the same experimental models, we have evaluated Ir β-endorphin and Ir Met-enkephalin concentrations in the mediobasal hypothalamus (MBH), the brain area which contains the most β-endorphin- and Met-enkephalin-secreting neurones.

MATERIALS AND METHODS

Pregnant and control fertile Wistar rats were used (180–220 g; Morini, S. Polo D’Enza, Italy). The date of insemination was taken as the day of detection of sperm in the vaginal smears; control rats were used at random stages of the oestrous cycle, excluding those at pro-oestrus. The rats were housed in a climatized room (20–22 °C), with a lighting cycle of 10 h light: 14 h darkness and food and water available ad libitum.

In the first group of experiments we compared Ir β-endorphin concentrations in the plasma, AP, NIL and MBH and Met-enkephalin-like immunoreactivity concentrations in the MBH between groups of rats in mid-(12–14 days, n = 7) or advanced (18–20 days, n = 7) pregnancy and in non-pregnant rats (n = 7). In the second study we measured Ir β-endorphin and Ir Met-enkephalin concentrations in the same fluid or tissues from rats at advanced pregnancy (16–20 days, n = 6), during delivery (n = 6), in lactating rats (4 days post partum, n = 6) and in non-pregnant rats (n = 7).

Rats were killed by decapitation between 09.00 and 11.00 h; blood was collected from the trunk in heparinized cooled plastic tubes, containing aprotonin (1000 kallikrein inactivator units/ml), and immediately centrifuged (1397 g \times 10 \text{min at } 4°C); plasma was separated and stored at −20 °C until assayed.

The brain was removed, placed on dry ice and the MBH carefully separated as described by Glowinsky & Iversen (1966). The AP and NIL were dissected from the isolated pituitary. For extraction of β-endorphin and Met-enkephalin, all tissues were placed in tubes containing 2 ml 0-01 M-acetic acid incubated in a water bath for 15 min at 100 °C. They were then homogenized and centrifuged at 12000 g for 10 min; the supernatant was stored at −20 °C until assay. Protein content was measured according to Lowry, Rosebrough, Farr & Randall (1951) in aliquots of the homogenates.

Plasma samples (2 ml) placed in plastic tubes containing silicic acid (150 mg, 100 mesh; Mallinkrodt, F.R.G.) were shaken with a rotary mixer (60 min) and then centrifuged (1676 g \times 10 \text{min}), the supernatant was discarded, the silicic acid washed twice with distilled water (2 ml) and finally β-endorphin was extracted with acetone: 1 M-HCl (8:2, v/v). The extracts were vacuum-dried, redissolved with 0.4 ml 0.1 M-acetic acid and 0.01% bovine serum albumin, applied on Sephadex G-75 columns (45 × 1.5 cm) and eluted with the same solvent, utilizing a 12-channel peristaltic pump (Desaga, Heidelberg, F.R.G.). On the basis of the elution profile of synthetic β-endorphin (300 fmol) and of 125I-labelled β-endorphin, a 15 ml fraction was collected; the elution pattern of β-endorphin was also confirmed by a detailed evaluation of Ir β-endorphin in 2 ml chromatography fractions of a large pool of plasma extracts (10 ml) from control rats. The recovery of 125I-labelled β-endorphin added to eight plasma samples before extraction was 83.7 ± 11.1% (S.D.) at the end of the entire procedure. The eluted fractions were freeze-dried and redissolved in 0.5 ml distilled water at the time of the assay.

Immunoreactive β-endorphin and Met-enkephalin concentrations were measured by homologous radioimmunoassays. Tissue fragments and plasma of the two experiments were assayed in the same radioimmunoassay. All determinations were carried out in triplicate with three different dilutions.

Immunoreactive β-endorphin

Camel β-endorphin (Peninsula Labs, San Carlo, CA, U.S.A.) was used for iodination with 125I, according to the chloramine T method (Greenwood, Hunter & Glover, 1962) and as standard. Anti-camel β-endorphin (kindly given by Professor A. E. Panerai, Milan, Italy) was used at a final dilution of 1:100,000 for tissue fragments and 1:120,000 for plasma.

The antiserum cross-reacted 100% with ovine β-endorphin, 30% with human β-endorphin and 50% with β-lipotrophin. No cross-reactivity was evident with α- and γ-endorphin, Leu- and Met-enkephalin, α-melanocyte-stimulating hormone (α-MSH), adrenocorticotrophin (ACTH), vasopressin, insulin, glucagon, thyrotrophin-releasing hormone, luteinizing hormone-releasing hormone, bombesin, growth hormone, prolactin, morphine and naloxone. The estimation of Ir β-endorphin in tissues (Ogawa, Panerai, Lee et al. 1979) and plasma (Petraglia, Peñalva, Locatelli et al. 1982b) has been reported previously in detail.

Assay sensitivities were 7 and 2 fmol per tube for tissue and plasma radioimmunoassays respectively; inter- and intra-assay coefficients of variation were 10 ± 1.5 and 5.0 ± 0.5% respectively at 50% binding (seven samples).
Met-enkephalin-like immunoreactivity

[Met⁵]-enkephalin (UCB, Brussels) was used as standard. Anti-Met-enkephalin serum (UCB) was used at a final dilution of 1:25,000; no cross-reaction was observed with [Arg⁶-Phe⁷]Met-enkephalin, [Arg⁶-(Gly⁷)-Leu⁸]Met-enkephalin, Leu-enkephalin, human β-lipotrophin, synthetic human, ovine or camel β-endorphin, β-MSH, luteinizing hormone, prolactin or ACTH. [³H-Met⁵]-enkephalin (New England Nuclear, Baltimore, MD, U.S.A.), antiserum and standard or unknown samples were incubated for 24 h at 4°C in 0.2 ml 0.14 M-phosphate buffer (pH 7.4) containing 0.5% bovine serum albumin. Bound and free [³H-Met⁵]-enkephalin were separated by 0.5 ml charcoal slurry (2.5 g/l), containing dextran T70 (0.25 g/l).

Assay sensitivity was 5 fmol; inter- and intra-assay coefficients of variation were 9.5 ± 0.5 and 6.8 ± 0.7% respectively at 50% binding (seven samples).

Data are expressed as pmol/mg protein for AP and MBH, as pmol/lobe for NIL and as pmol/l for plasma. Statistical analysis of the results was performed utilizing Dunnett’s (1964) t-test for multiple comparisons.

RESULTS

Anterior pituitary

Immunoreactive β-endorphin concentrations (pmol/mg protein) in the AP at 18-20 days of gestation (284.0 ± 26.1) were significantly higher than those seen either at mid-pregnancy (213.1 ± 28.1, P < 0.05) or in non-pregnant control rats (120.6 ± 15.6, P < 0.01); levels during mid-gestation were significantly (P < 0.05) higher than in non-pregnant animals (Fig. 1a). The highest concentrations of Ir β-endorphin in the AP were observed in rats during delivery (379 ± 51.5 pmol/mg protein) and were significantly greater than those found in advanced pregnancy (235.5 ± 40.0), in lactating (94.5 ± 38.1) or in control (108.8 ± 27.7) rats (Fig. 1b).

Concentrations in lactating rats were of the same magnitude as in non-pregnant controls and
significantly \((P<0.01)\) lower than in pregnant rats (Fig. 1b).

**Neurointermediate pituitary lobe**

Immunoreactive \(\beta\)-endorphin concentrations (pmol/mg protein) in the NIL at 12–14 days (275 ± 37) and 18–20 days (238 ± 41) of gestation were significantly higher than those seen in non-pregnant control rats (105 ± 19, \(P<0.05\)) (Fig. 1c). Immunoreactive \(\beta\)-endorphin concentrations in the NIL observed in rats during delivery (186 ± 22 pmol/mg protein) were of the same magnitude as those during advanced pregnancy (229 ± 30) and in lactating rats (207 ± 33), values in these three groups being significantly higher than those in non-pregnant control rats (101 ± 24 pmol/mg protein, \(P<0.05\)) (Fig. 1d).

**Mediobasal hypothalamus**

Concentrations of Ir \(\beta\)-endorphin and Ir Met-enkephalin (pmol/mg protein) in the MBH both in mid- (13.0 ± 1.1 and 17.5 ± 1.15 respectively) and late (12.8 ± 0.51 and 15.8 ± 1.13) gestation were significantly \((P<0.05)\) higher than in non-pregnant rats (8.1 ± 0.80 and 8.9 ± 2.01) (Fig. 2a, c). The highest concentrations of Ir \(\beta\)-endorphin and Ir Met-enkephalin in the MBH were found during delivery (35.5 ± 5.88 and 95.9 ± 15.5), which were significantly \((P<0.01)\) higher than in pregnant (13.9 ± 1.6 and 20.5 ± 2.0) or lactating (8.5 ± 1.1 and 10.0 ± 1.8) rats or in controls (9.1 ± 0.4 and 12.2 ± 1.5) (Fig. 2b, d). Concentrations of Ir \(\beta\)-endorphin and Ir Met-enkephalin in the MBH of lactating rats were significantly \((P<0.05)\) lower than in pregnant rats.

**Plasma**

Plasma Ir \(\beta\)-endorphin concentrations in pregnant rats (12–14 days, 41.2 ± 1.8 pmol/l; 16–20 days, 48.5 ± 4 pmol/l) were significantly \((P<0.05)\) higher than in control cyclic rats (25.1 ± 1.8 pmol/l) (Fig. 3).

Delivering rats showed the highest concentrations (68.1 ± 4.4 pmol/l), which were significantly \((P<0.01)\) higher than in pregnant or lactating (46.3 ± 6.1 pmol/l) rats; the mean plasma Ir \(\beta\)-endorphin concentrations of the latter group were higher than those of control rats (Fig. 3).

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**FIGURE 2.** (a, b) Immunoreactive (Ir) \(\beta\)-endorphin and (c, d) Ir Met-enkephalin concentrations (means ± s.d.) in the mediobasal hypothalamus of non-pregnant control rats (C; \(n=7\)), pregnant (P) rats during mid- (12–14 days, \(n=7\)) and late pregnancy (18–20 days, \(n=7\); 16–20 days, \(n=6\)) and during delivery (D; \(n=6\)) and lactation (L; \(n=6\)). \(*P<0.05, **P<0.01\) compared with C group (Dunnett's t-test).

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DISCUSSION

Our results indicated that Ir β-endorphin concentrations increased in plasma, AP and NIL during pregnancy in rats, suggesting that the secretory events of pituitary Ir β-endorphin-producing cells undergo significant changes during gestation.

The increase in plasma β-endorphin levels during pregnancy may thus be related to the increased activity of β-endorphin-producing cells in both pituitary lobes; however, we cannot exclude a possible placental contribution (Nakai et al. 1978; Fraioli & Genazzani, 1980). The increase of Ir β-endorphin concentrations in the plasma and pituitary lobes of pregnant rats agrees with the progressive rise in circulating β-endorphin during pregnancy (Csontos et al. 1979; Genazzani et al. 1981; Goland et al. 1981; Browning et al. 1983; Newnham et al. 1983). Since ACTH and β-lipotropin, secreted concomitantly with β-endorphin by corticotroph cells in AP (pro-opiomelanocortin-related peptides) (McLoughlin, Lowry, Ratter et al. 1980), also increase in the plasma during pregnancy (Genazzani, Fraioli, Hurlimann et al. 1975; Rees, Burke, Chard et al. 1975; Newnham et al. 1983) and considering that our assay for Ir β-endorphin in AP also detected β-lipotrophin, these data support the fact that during pregnancy there is an increase of the secretory activity of pituitary corticotrophs. The increased concentrations of β-endorphin in plasma and pituitary during pregnancy may be related to high circulating levels of oestrogens and progesterone, which exert a positive effect on the β-endorphin contents of both pituitary lobes (Petraglia et al. 1982b).

The different degree of activation of β-endorphin synthesis in the AP and NIL during gestation confirms the existence of separate control systems for secretion of β-endorphin by AP (corticotrophin-releasing factor) (Vale et al. 1978) and NIL (dopamine, noradrenaline) (Przewlocki et al. 1979; Vermes et al. 1980; Berkenbosch, Tilders & Vermes, 1983) which may undergo different changes in their activity during pregnancy.

The increase of Ir β-endorphin concentrations in MBH during pregnancy agrees with the results obtained in MBH and mid-brain by Wardlaw & Frantz (1983) and in MBH and medial preoptic area by Bridges & Ronsheim (1983), while the concomitant increase in Ir Met-enkephalin concentrations in MBH is described here for the first time. The rise in central endogenous peptides may derive either from the increase in sex steroids and/or from the changes in the turnover of central amines, considering that the hypothalamic concentrations of both opioids show significant changes after the chronic administration of gonadal steroids (Hong, Yoshikawa, Hudson & Uphouse, 1982; Petraglia et al. 1982b) or of serotoninergic (Harsing, Yang, Govoni & Costa, 1982) or dopaminergic (Locatelli, Petraglia, Peñaalva & Panerai, 1983) drugs. Placental or fetal factors do not seem to involve an increase in MBH and mid-brain Ir β-endorphin concentrations, also occurring in pseudopregnant rats (Cholst, Wardlaw & Frantz, 1983).

Delivering rats showed further increases in Ir β-endorphin concentration in plasma and AP, without changes in the NIL, thus indicating an important activation of the release of pro-opiomelanocortin-related peptides from the AP as was indicated also by the significant increase of plasma ACTH in rats during parturition (Voogt, Sar & Meites, 1969).

It has been shown that the selective ablation of AP abolishes the stress-related mobilization of β-endorphin into the systemic circulation and both acute and chronic dexamethasone administration alters the stress-induced changes in β-endorphin levels found in AP and plasma (Przewlocki, Millan, Gramsch et al. 1982; Lim, Oei & Funder, 1983). These results agree with the progressive rise in plasma β-endorphin concentrations during human labour, which reach peak values at delivery (Fletcher et al. 1980; Facchinetti et al. 1982). The observation that Ir β-endorphin and Ir Met-enkephalin showed the highest hypothalamic concentrations in delivering rats supports the concept that opioid peptides play an important role in stress-related responses (Adler, 1980).

The results obtained in lactating rats, that NIL and plasma β-endorphin concentrations were higher than in control rats, are consistent with those obtained by Baizman, Cox, Osman & Goldstein (1979), who showed raised bioactive opioid concentrations in the
NIL of lactating rats, and support the recent findings of high plasma Ir β-endorphin levels in rats after lactation (Riskind et al. 1983), reinforcing the concept that Ir β-endorphin in AP and NIL undergoes different control mechanisms. On the other hand, the decreasing pattern of Ir β-endorphin and Ir Met-enkephalin concentrations in the MBH of lactating rats is also in agreement with the previously reported observations (Panerai, Sawynok, La Bella & Friesen, 1980).

All these data indicate the existence of selective changes in the endogenous opioid system during pregnancy, labour and lactation in rats.

REFERENCES


