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# PLASMA BETA-ENDORPHIN-LIKE IMMUNOREACTIVITY, SELF REPORTED PAIN PERCEPTION AND ANXIETY LEVELS IN WOMEN DURING PREGNANCY AND LABOR

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## Summary

Plasma Beta-endorphin (B-END) immunoreactivity was measured in 19 men, 16 women at midcycle, and ten pregnant women at various points in their pregnancies. There is a significant difference between the levels measured in males and females ($t = 3.74$, $df = 31$, $p = .0007$). Pregnant women demonstrated a steady increase in plasma Beta-End-like immunoreactivity between second and third trimester and through labor. The levels dropped rapidly postpartum. The material being measured is predominantly B-end-sized. Psychological studies indicate that these changes are not strongly correlated with pain perception or self-reported anxiety levels.

Beta-endorphin (B-END) is thought to be involved in the mediation of pain perception in animals and man. Several groups have shown that plasma B-END-like immunoreactivity is elevated during pregnancy and labor (1,2,3,4). Rats demonstrated higher pain thresholds as pregnancy progressed and parturition neared (5). Therefore the increases in endogenous opiates noted during pregnancy may be reflective of the body's response to pain. However, B-END/B-LPH and ACTH are co-produced and co-released by the anterior pituitary into the peripheral blood in response to stress among other things. Therefore, it may be suggested that elevated B-END-like immunoreactivity is reflective of the stress of pregnancy and labor or may be caused by endocrine changes not closely coupled to pain. We have therefore begun a series of experiments to: a) study changes in B-END immunoreactivity in normal males, females, and in pregnancy, labor and postpartum; b) characterize the immunoreactivity chromatographically and by specific antisera to determine the size, acetylation and opiate activity; and c) examine any possible correlations between B-END-like levels and perceived pain or anxiety during pregnancy and labor.

## METHODS

We have developed a very sensitive radioimmunoassay to measure B-END-like immunoreactivity in extracted human plasma (6). The $IC_{50}$ of the assay using antisera Brenda at 1:40,000 final

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concentration is 10.25 fm per assay tube. The extraction procedure uses the Sep-Pak $C_{18}$ cartridge (Waters Associates, Inc). The recovery of [$^{125}$I] $B_h$-END is 90%. We have determined that the addition of plasma extract stripped of B-END-like immunoreactivity by affinity purification caused the $IC_{50}$ of the standard curve to shift to 9.25 fm per assay tube, a 10% shift to the left. To determine the molecular size of the material being measured, we used a 1.5 cm X 90cm Sephadex G-50 superfine column with a 1% formic acid buffer (7).

Anxiety was measured by the Spielberger State Anxiety Inventory (8). Pain perception was measured by a visual analog scale. All subjects were asked to complete both tools just prior to the collection of the blood samples. Blood was collected by venopuncture in non-laboring subjects. Laboring subjects had a heparin lock inserted upon admission to the labor room and all samples were collected from it. Blood was collected in chilled EDTA Vacutainers (Brand, BD). It was immediately placed on ice and centrifuged for 10 minutes at 4°C. serum was pipetted into plastic vials containing 100 microliters of 1 N HCl per ml of serum. The sample was quickly frozen on dry ice and stored at -80°C until it was assayed.

## RESULTS

Plasma samples were collected from 19 male volunteers and 14 females at midcycle. These two groups represented control groups. We found that there is a significant difference between the B-END-like immunoreactivity of these two groups ($t = 3.74$, $df = 31$, $p = .0007$). Five women were studied longitudinally throughout their pregnancies. We found that B-END-like immunoreactivity increased steadily throughout pregnancy (Tau B = .40, N = 23, p = .008). Six other women were studied during labor and at term. In early labor, B-END-like immunoreactivity dipped slightly, but by the last stage of labor B-END-like immunoreactivity rose to the highest level. Then 24 to 48 hours postpartum there was a dramatic and significant ($t = 3.23$, d.f. = 11, $p = .008$) drop in B-END-like immunoreactivity to levels comparable to women at midcycle (see Table I). Although chromatographic studies of the plasma from pregnant women indicated that the majority of the material measured was B-END size, there was only a weak (non-significant) correlation between pain perception and B-END-like immunoreactivity. The same is true of self-reported anxiety levels.

TABLE I

Summary of Plasma B-End-like Immunoreactivity by Group

| | N | Plasma B-End(fm/ml) + S.E.M. | |
|---|---|---|---|
| Male | 19 | 6.8 | .95 |
| Female (Midcycle) | 14 | 2.4 | .45 |
| Pregnant Term | 7 | 7.1 | 1.1 |
| Stage I Labor (0-4cm) | 3 | 5.6 | 2.8 |
| Stage II (5-8cm) | 4 | 6.7 | 1.2 |
| Stage III (9-10cm) | 5 | 8.8 | 1.6 |
| Postpartum | 6 | 2.3 | .8 |

## CONCLUSIONS

We have shown that B-END-like immunoreactivity can be measured in human serum and that changes in experimental conditions can be detected. We have not demonstrated any strong correlations between B-END-like immunoreactivity and self-reported anxiety levels or pain levels. However, the number of subjects studied thus far is small and there is a wide range of variance among them. As additional individuals are studied, we will be able to use more powerful statistical approaches which may help to solve the problem of non-homogeneity of variance.

The finding that the greatest proportion of B-END-like immunoreactivity in the serum of pregnant women is B-END size is important. The amount of B-END produced by the anterior pituitary is usually small compared to the amount of B-LPH produced. The molar ratio of B-LPH to B-END is usually 6:1. During pregnancy the molar ratio changes to 1:6. So, not only is the absolute amount of B-END-like immunoreactivity elevated, there is also a change in the final product. It is not clear if this change is reflective of alterations in the usual biosynthetic pathways in the anterior pituitary or if a novel source such as intermediate pituitary or fetus develops. Since the intermediate lobe of the pituitary processes POMC differently than the anterior lobe producing primarily N-acetylated forms of B-END studies on the state of acetylation of B-END in pregnancy should be revealing. Such studies are currently ongoing.

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