Detection of Immunoreactive Human Placental Oxytocin and its Contractile Effect on the Uterine Muscle

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Abstract

Freshly obtained human placentas from various periods of gestation were quantitatively analysed for their immunoreactive oxytocin (OT) content and its biological activity was examined in a Magnus apparatus by utilizing rat uterus.

The mean values for placental immunoreactive OT per gram tissue increased from the first to the second trimester, maintaining its high level to term. The total content of placental OT also increased continually from the beginning of pregnancy to term. Blood levels of estrogen stimulated neurophysin (ESN) and OT were concomitantly enhanced through gestation. Placental extract and synthetic OT showed similar peaks in the elution pattern of ion-exchange chromatography through a carboxymethyl cellulose column. Synthetic OT and placental extract induced marked uterine contraction in diestrous rats. However, placental extract previously incubated with OT antiserum failed to induce this effect.

Though detection of immunoreactive OT by immunoassay alone does not provide definite identification of pituitary and placental OT, the present study suggests that placental immunoreactive OT could have a contracting effect on the uterine muscle.
incised from each placenta within 10 minutes after delivery or intrauterine curettage. The tissue were thoroughly rinsed 5 times in normal saline and 400-500 mg of the tissues were homogenized for 4 minutes in the 4 volumes of oxytocin buffer (2.0×10⁻³M EDTA, 3.0×10⁻³M NaN₃, 5.0×10⁻⁵M phenanthroline, 0.3% gelatin in 0.01 M phosphate saline buffer, pH 7.2) with an Ultra Turrax homogenizer, then the homogenate was centrifuged at 900×g, at 4°C, for 15 minutes and assayed for OT. For the study on bioactivity of placental tissue, freshly obtained term placenta (approximately 10 gm) immediately after delivery were homogenized in 5 volumes of 0.04 N acetic acid in an Ultra Turrax homogenizer for 4 minutes and centrifuged at 900×g, at 4°C, for 30 minutes, then the pH of the supernatant was adjusted to pH 5.5 by 0.1 N NaOH and lyophylized. The lyophylized placental extract was resuspended in 5 ml modified Ringer’s solution. Mature female rats of SD strain (weighing 200-300 gm, Sankyo Lab. Company Ltd., Tokyo) were kept in an artificially illuminated room (12 hrs light and 12 hrs darkness) at 23°C and fed rat chow and water ad libitum. Daily vaginal smears were observed for at least 2 weeks, then the rats were sacrificed on diestrous and 1 cm lengths of the uterine horns were dissected and fixed in Magnus apparatus filled with modified Ringer’s solution at 25°C in an air supply atmosphere. Either synthetic OT (14 ng/ml solution), placental extract (2 gm/ml solution) or the same amounts of placental extract previously incubated with excess amount of OT antiserum for 2 days at 4°C, were applied to this apparatus with frequent changing of the solution and the uterine contractions were recorded.

Blood samples for OT assay were collected from 29 pregnant women. A small amount of chelating agent was added to the blood samples, then these samples were centrifuged at 4°C and the plasma samples were stored at −20°C until the assay. Samples for neurophysin assay were obtained from 80 pregnant women and sera separated by centrifugation were also stored at −20°C until the assay.

Radioimmunoassay for OT

Radioimmunoassay for OT was carried out according to the method previously reported (Nakayama et al., 1980). Five to ten microgram of synthetic OT was labelled with 1 mCi of ¹²⁵I-Na by a modified chrolamine T method and purified through a carboxymethyl cellulose column (10×150 mm) by ion-exchange chromatography eluted by 0.002 to 0.4 M ammonium acetate buffer (pH 4.0) in a linear gradient. The specific activity of the ¹²⁵I-OT obtained was about 500 ƒÊCi/ƒÊg. Antiserum to OT was generated in rabbits and its immuno-specificity was evaluated against the other peptides shown in Fig. 1. As indicated the immuno-crossreaction of the antiserum with these peptides was less than 0.01%. The limit of detection of the OT assay was 2 pg/assay tube.

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Radioimmunoassay for Neurophysins

Two neurophysins and their antibodies were generous gifts from the National Institute of Arthritis, Diabetis and Digestive and Kidney Diseases, N.I.H., U.S.A. Neurophysins were also labelled with $^{125}$I-Na by chrolamine-T and purified by gel-filtration on a Sepadex G-25 column (10×100 mm). The RIA procedure was identical to that described previously (Nakayama et al., 1979).

Results

Plasma OT levels from 6 to 40 weeks of normal pregnancy are shown in Table 1. As shown in this table, the mean values for plasma OT gradually increased from the first to the third trimester. The concentration of placental immunoreactive OT significantly increased from the $1.6 \pm 0.29$ ng/gm tissue (mean±S.E.) of the first trimester to the $20.8 \pm 1.90$ ng/gm of the second trimester, maintaining this level until the third trimester (Fig. 2). The total placental immunoreactive OT content also increased from $12.7 \pm 5.61$ ng/placenta in the first trimester to $4.1 \pm 0.41$ µg/placenta in the second trimester, and continually increased to $12.5 \pm 1.22$ µg/placenta in the third trimester (Fig. 3).

Serum ESN concentrations in normal pregnancy markedly increased from the beginning of gestation to term (Table 2). In contrast, serum NSN was not enhanced significantly through pregnancy (Table 3). Similar peaks were detected in both synthetic OT and placental tissue extract through CMC column chromatography (Fig. 4).

Synthetic OT and placental extract induced marked uterine muscle contraction, however the placental extract previously incubated

<table>
<thead>
<tr>
<th>Weeks of pregnancy</th>
<th>No. of samples</th>
<th>Plasma OT levels Mean±S.E. (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7—14</td>
<td>8</td>
<td>$84.1 \pm 13.57$</td>
</tr>
<tr>
<td>15—27</td>
<td>9</td>
<td>$101.9 \pm 15.01$</td>
</tr>
<tr>
<td>28—40</td>
<td>12</td>
<td>$119.2 \pm 21.29$</td>
</tr>
</tbody>
</table>

Fig. 2. Concentration of immunoreactive oxytocin in human placenta at each trimester
with OT antiserum failed to induce the same contraction.

Discussion

The exact role of the placenta is still an enigma in human physiology, although it has been well recognized that the human placenta plays an important role in exchanging many substances between the mother and fetus. Recently it has become more evident that hypothalamic or pituitary peptides exist in diverse organs with structural homology and similar immunoreactivity to a generated antiserum. Considerable evidence has also been accumulated showing that additional peptides previously demonstrated in the brain or pituitary exist in the placenta, such as human placental...
Fig. 4. Ion-exchange chromatography on carboxymethyl cellulose column of placental tissue extract and synthetic oxytocin lactogen (hPL, Kaplan and Grunbach, 1964), placental ACTH (Genazzani et al., 1975), TSH (Hershman and Starnes, 1969), as well as hCG (Halban, 1905) and several other peptides. However, few studies on human placental oxytocin have been reported. Cantone and Martini reported oxytocic activity in placental tissue (1954), but no further detailed study has been reported on the OT-like effect of the placental peptide on the uterine muscle. The present study clearly demonstrated that immunoreactive OT was detectable in the human placenta and the total content of immunoreactive OT in this tissue increased from the beginning of pregnancy to term. It is not possible in the present study to tell whether the source of plasma OT is the posterior pituitary or the placenta, through plasma levels of immunoreactive OT and ESN increased concomitantly to term.

Krieger (1982) reported that placental concentrations of pituitary-like peptides are markedly less than those of their pituitary counterpart, usually several orders of magnitude lower than reported for their original sites of production. However it is of interest that the content of immuno-reactive OT in the placenta was significantly higher than its total content in the posterior pituitary.

It should be realized that the detection

Fig. 5. Effect of synthetic oxytocin and placental tissue extract on rat uterine contraction.
of peptides only by immunoassay or immunohistochemistry does not provide absolute identification. Quite recently, Wathes et al. (1982) reported that a compound resembling oxytocin and vasopressin was extracted in a detectable quantity from ovarian tissue, especially from the corpus luteum, suggesting some role in luteolysis. Thus OT can exist in diverse organs apart from its original sites, the hypothalamus and the posterior pituitary.

In this study, similar peaks were detectable in the elution pattern of the placental extract on a CMC column if it is overlapped with the same ion-exchanged chromatography of synthetic OT, indicating the existence of some structural homologies in these two experiments.

At present, it is still an enigma why an oxytocin-like substance exists in the placenta. Pearse (1977) has postulated that all tissues including the placenta which contain similar peptides are of neural crest origin, because the yolk sac endoderm arises as a layer of ectoblast continuous with the ectoblast of neural plate. Therefore the placenta can be developed with this neurally programmed region and some of its cell functions can be similarly programmed.

There is little information with regard to any mechanism of synthesis, release and degradation of placental oxytocin, however an investigation on the chemical composition of an OT degrading enzyme, oxytocinase in the placenta (Yman, 1970), encourages our present study, because it is more physiological that a hormone and its linked enzyme co-exist in same tissue or organ.

The exact role of placental immuno-reactive OT is not clear and further studies are necessary to elucidate the physiology of placental OT, but the data obtained in the present study suggest that placental immuno-reactive OT could have a similar effect on the uterine contraction.

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References

