NOTE
Immunoreactive Oxytocin Synthesis in Human Placental Tissue

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Abstract

The change in the amount of immunoreactive oxytocin in human term placental tissue due to cycloheximide, an inhibitory agent of protein biosynthesis, was observed by a tissue culture method.

The immunoreactive oxytocin content per gram tissue was 1.81 ng/g and significantly decreased to 1.17–1.11 ng/g when the concentrations of cycloheximide were at 1–10 µg/ml in culture medium.

The total quantity of immunoreactive oxytocin including medium content was 1.82 ng/g and significantly increased to 3.06 ng/g in the control group. However, those of the groups in which added cycloheximide was at 1–10 µg/ml, were 1.91–1.85 ng/g and showed no remarkable changes after incubation.

The data suggest that immunoreactive placental oxytocin can be synthesized in the human placenta itself rather than in other tissues.

During the last decade considerable attention has been paid to the investigation of the placenta as an endocrine organ, especially to synthesis and release of the peptides previously demonstrated in other tissues, such as the brain and pituitary.

In previous communications we have reported the existence of a large quantity of oxytocin-like substance which was proved to have immunoreactivity by radioimmunoassay and bioactivity in Magnus apparatus using rat uterus (Makino et al., 1983).

In this study we investigated, by a tissue culture method, the origin of this oxytocin-like substance: whether it is synthesized in other tissues and only stored in the placenta or originally synthesized in the placental tissue.

Materials and Methods

Incubation

Human term placenta freshly obtained by cesarian section before the onset of labor was separated from the cord, amnion and decidua, thoroughly rinsed in 37°C Hanks’ balanced salt solution (pH 7.3) and divided into pieces of about 200 mg wet weight for one sample and again rinsed in the same buffer (Fig. 1). Each sample was transferred to a 20 ml glass flask with 2 ml of control medium of medium 199 with Hanks’ salts and and L-glutamine containing 15% fetal bovine serum, 50 U/ml of penicillin and 50 µg/ml of

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Fig. 1. Procedure for incubation and extraction of immunoreactive oxytocin from placental tissue.

streptomycin, and incubated at 37°C in a water bath for 90 minutes. Then each flask medium was exchanged for 2 ml of new medium containing different doses (0, 1, 10 µg/ml) of cycloheximide (SIGMA Chem. Com. St. Louis USA) and incubated for 24 hours in the same conditions.

**Assay**

After incubation, each piece of tissue was homogenized in 2 ml of 0.04 M acetic acid with an Ultra Turrax homogenizer for 3 minutes. The homogenate was spun at 900×g in a refrigerated centrifuge for 30 minutes and the pH of the supernatant was adjusted to 5.5 with 0.1 N sodium hydroxide. The aliquot was kept at 4°C overnight and recentrifuged.

The immunoreactive oxytocin content of the second supernatant and culture medium was assayed by our own specific radioimmunoassay (Nakayama et al., 1980).

**Results**

As shown in Fig. 2, mean immunoreactive oxytocin concentration per gram tissue after 24 hours incubation without cycloheximide was 1.81±0.09 ng/g, almost equal to 1.82±0.18 ng/g of the preincubation value. It was decreased to 1.17±0.19 ng/g with 1 µg/ml of cycloheximide and significantly to 1.11±0.25 ng/g with 10 µg/ml of cycloheximide.
suggesting that the oxytocin content was decreased dose-dependently by cycloheximide.

Fig. 3 shows the alteration of the total amount of immunoreactive oxytocin per gram tissue including the medium content. The total quantity of immunoreactive oxytocin in the control group significantly increased from 1.82±0.18 ng/g to 3.06±0.38 ng/g (P<0.05). In contrast, no remarkable changes were observed in the cycloheximide treated groups and the values were 1.91±0.16 ng/g-1.85±0.20 ng/g.

Discussion

It is well known that the human placenta synthesizes large quantities of human chorionic gonadotropin and human placental lactogen (Chatterjee et al., 1976; Boime and Buguslawski, 1974) as well as estrogen and progesterone. Oxytocic activity of placental tissue has also been reported (Cantone, 1954), but so far the biosynthesis of oxytocin by the placenta has not been demonstrated. Previously we reported the existence of a large amount of immunoreactive placental oxytocin found by radioimmunoassay (Makino et al., 1983, Nakazawa et al., 1984a), bioactivity like synthetic oxytocin on rat uterus of human placental extract (Nakazawa et al., 1984b) and localization of oxytocin in the syncitiotrophoblast of the placenta by immunohistochemical study (Nakazawa et al., 1984c).

As demonstrated in this study, the immunoreactive oxytocin concentration of placental tissue was unchanged in at least 24 hours of incubation in control medium, but significantly decreased in accordance with the dose of cycloheximide as an inhibitory agent of protein synthesis in cytoplasm. The total quantity of immunoreactive oxytocin including both tissue and medium contents in the control group showed a significant increase, but those in the cycloheximide treated groups remained unchanged. Therefore concerning these paradoxical results it can be speculated that while synthesis of oxytocin and oxytocinase activity were suppressed by cycloheximide, some stored oxytocin in cultured placental tissue discharged into the medium resulting in decreased tissue content of oxytocin and the total amount unchanged.

These data suggest that immunoreactive placental oxytocin is not merely stored but synthesized in the human placenta itself.

Though the exact mechanism of the initiation of labor is still not clear, the possibility of biosynthesis of an oxytocin-like substance in the placental tissue detected in this study can be one of the factors influencing the onset of labor.

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References

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