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OXYTOCIN RECEPTOR IN HUMAN FETAL MEMBRANES AT TERM AND DURING LABOR

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Summary—Human fetal membranes, taken from 30 patients submitted to caesarean section during the final stages of gestation and labor, were examined in order to evaluate the presence and characteristics of the oxytocin receptor.

The presence of oxytocin receptors in human fetal membranes, both in the amnion and in the chorion-decidua, was demonstrated in this study. The receptor binding to oxytocin showed a significant increase during early and advanced labor compared with before the onset of labor. When the pre-labor level was taken as the normalized form (control = 100) the increase with respect to the control (10 cases) for the amnion in early labor (2.27 times ± 0.11, mean ± SEM, $P < 0.001$, 10 cases) and in advanced labor (2.53 times ± 0.15, 10 cases, $P < 0.001$) was highly significant. In the chorion-decidua the increase was 1.61 times ± 0.09, $P < 0.001$ in early labor and 1.66 times ± 0.19, $P < 0.001$ in advanced labor.

Scatchard analysis showed a single receptor site for oxytocin in amnion and chorion-decidua. The dissociation constant (K_d) did not change during the various stages of labor: the mean values found were 0.228 ± 0.02 (mean ± SEM) nM in the amnion and 0.193 ± 0.03 nM in the chorion-decidua respectively.

These findings suggest that human fetal membranes are target organs for oxytocin and that they might play a role in the onset of labor through an increase of receptor binding.

INTRODUCTION

Oxytocin and prostaglandins play an important role in the onset of parturition in women [1], but their interaction is not yet well known. In humans, oxytocin acts on the myometrial contraction mechanism and it also stimulates the production of prostaglandins [2]. The induction of labor with oxytocin is successful only when it is combined with an increase in production of prostaglandins F2α (PgF2α) [3]. The production of prostaglandins in the sheep endometrium is stimulated by oxytocin through a receptor-mediated process [4].

These receptors have been found in the uterus of the rat and sow [5], guinea-pig [6], sheep [4] and humans [2]. In the rat uterus the receptor sites show a rapid increase during labor [7]. In humans the concentration of oxytocin receptors increases in the myometrium of pregnant women and reaches the maximum levels in early labor [8].

In vitro oxytocin increases prostaglandin production in the decidua but not in the myometrium [2]. There is an active production of prostaglandins at the membrane level [9, 10] and oxytocin is probably an important stimulus of their production.

The presence and distribution of oxytocin receptors has been demonstrated in human myometrium during the different stages of gestation and during labor [8], while the presence of oxytocin receptor in human fetal membranes has not yet been well documented.

The aim of our study was to determine whether fetal membranes represent a target for oxytocin.

MATERIAL AND METHODS

Chemicals

Tyrosil-[^{3}H]oxytocin (16.5 Ci/mmol) was obtained from the Radiochemical Center, Amersham, England. Non-radioactive oxytocin was a gift from Sandox, Basel, Switzerland.

Tissue collection and storage

Fetal membranes were obtained from 30 patients who underwent caesarean section at term, between the 39th and 41st weeks of gestation (a) before the onset of labor (10 patients), (b) after 1 h of spontaneous labor (10 patients), (c) in advanced labor (10 patients). None of these patients had medical complications in pregnancy, no amniotomy was performed and no oxytocin was administered in any of the cases.

The fetal membranes were manually separated into amnion and chorion-decidua, blood was removed

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from the tissue samples and the decidua was carefully scraped from the chorion. Nevertheless, contamination of the chorion by the decidua could not be completely excluded and, for this reason, we refer to the chorion as chorion-decidua. The samples were frozen in liquid nitrogen and stored at −80°C until use. Assays were carried out within 4 weeks.

The frozen tissue was pulverized with a dismembrator at 4°C and resuspended in 4 vol of TED buffer (10 mM Tris–HCl, pH 7.4, 1.5 mM EDTA and 0.5 mM di-thiothreitol) and centrifuged at 800 *g* for 10 min. The resulting supernatant was centrifuged at 165,000 *g* for 1 h, and the pellet was resuspended in assay buffer (50 mM Tris–maleate, pH 7.6, containing 5 mM MnCl and 0.1% w/v of gelatin) to obtain a concentration of 2 mg/ml of proteins. An aliquot was taken for protein determination according to Lowry[11] and the remainder used for receptor assay according to Alexandrova and Soloff[12, 13]. The receptor binding was estimated by a single point-assay (0.6 nM of [^{3}H]oxytocin) or by Scatchard analysis [14] (0.6 nM of [^{3}H]oxytocin and increasing concentrations of non-radioactive oxytocin, over a range of 0.6–10 nM total oxytocin). The value of the receptor binding is expressed in fmol/mg protein.

The significance of the difference between groups was determined by Student's *t*-test (2-tailed, unpaired).

RESULTS

The presence of oxytocin receptors was demonstrated in both the amnion and chorion-decidua of human fetal membranes. The different oxytocin receptor binding in the three stages of labor and between the amnion and the chorion-decidua is shown in Table 1.

The level of oxytocin receptor binding in the amnion was 6.2 ± 0.94 (mean ± SEM) fmol/mg protein before the onset of labor and 14.1 ± 1.52 and 15.7 ± 2.37 fmol/mg protein during early and advanced labor respectively. A representative Scatchard analysis of 5 specimens obtained from the amnion of each group showed a straight line, indicating a single class of binding sites with a K_d of 0.271 ± 0.02 nM (mean ± SEM) before the onset of labor, 0.203 ± 0.02 nM during early labor and 0.211 ± 0.03 nM in advanced labor (Fig. 1).

The mean binding of oxytocin to receptors of the chorion-decidua in the three stages of labor were 12.4 ± 1.78 (mean ± SEM), 20.0 ± 1.78 and 20.6 ± 3.97 fmol/mg protein respectively. Scatchard analysis of 5 specimens obtained from the chorion-decidua in different stages of labor showed a K_d of 0.185 ± 0.03 (mean ± SEM) nM before the onset of labor, 0.209 ± 0.03 nM in early labor and 0.187 ± 0.04 nM in advanced labor. A representative Scatchard plot also showed a straight line (Fig. 1).

In amnion and chorion-decidua a significant increase of oxytocin binding to receptors in early and advanced labor, compared with before labor was found (Fig. 2), while no significant differences were detected in K_ds.

The mean binding of oxytocin to receptors of the chorion-decidua before the onset of labor was significantly higher with respect to the amnion, whereas there was no significant difference between cases of early and advanced labor.

To facilitate the comparison among the variation in receptor binding in different stages of labor, the data are shown in a normalized form (control = 100) (Fig. 3).

In the amnion the relative increase was 2.27 times ± 0.11 (mean ± SEM), $P < 0.001$ and 2.53 times ± 0.15, $P < 0.001$ in early and advanced labor respectively.

DISCUSSION

The fetal membranes and uterine decidua play an important role in the metabolic events leading to the onset of parturition. During labor, the human fetal membranes produce large quantities of prostaglandins which, together with oxytocin, are responsible for the onset of myometrial contractions, cervical softening and dilatation [15].

The action of oxytocin is mediated by specific receptors on the plasma membranes of the target cells [16]. In the uterus, the oxytocin receptors seem to be induced by estrogens and inhibited by progesterone [17]. It has been shown that in the rat myometrium the cytoplasmic and nuclear estradiol receptors increase before the oxytocin receptors: this suggests that through their receptors, estrogens induce the synthesis of specific post-receptor sub-

Table 1. Means ± SE of oxytocin receptor binding (OT-R) and dissociation constant (K_d) in human fetal membranes before, in early and advanced labor

	Amnion		Chorion-decidua	
	OT-R	K_d(nM)	OT-R	K_d(nM)
Before labor	6.2 ± 0.94[a] (10)[b]	0.271 ± 0.02 (5)	12.4 ± 1.78 (10)	0.185 ± 0.03 (5)
Early labor	14.1 ± 1.52 (10)	0.203 ± 0.02 (5)	20.0 ± 1.78 (10)	0.209 ± 0.03 (5)
Advanced labor	15.7 ± 2.37 (10)	0.211 ± 0.03 (5)	20.6 ± 3.97 (10)	0.187 ± 0.04 (5)

[a]fmol/mg protein; [b]No. of cases.

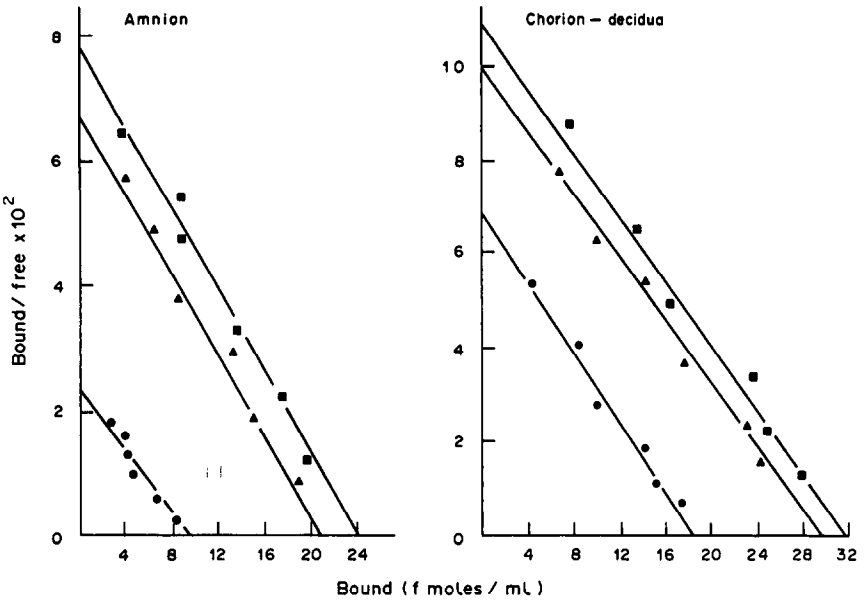

Fig. 1. Scatchard plots of specific [^{3}H]oxytocin binding to human fetal membranes (amnion, chorion-decidua) before (●), in early (▲), and advanced labor (■). The points represent the mean of 5 cases.

stances, which may stimulate the increase of oxytocin receptor binding [18].

Our study showed the presence of oxytocin receptors at different concentations in the amnion and in the chorion-decidua. A higher concentration found in the chorion-decidua may be due to the decidual component that remains attached to the chorion after separation.

The receptor binding to oxytocin rises significantly during early labor with respect to the onset of labor; while the dissociation constant remains unchanged during the different stages of labor. This also takes place in human myometrium during labor. In fact, the contractile activity is correlated with the increase of oxytocin and its receptor rather than with a variation in the affinity of the oxytocin to receptor [19].

The percentage increase of oxytocin receptor bind-

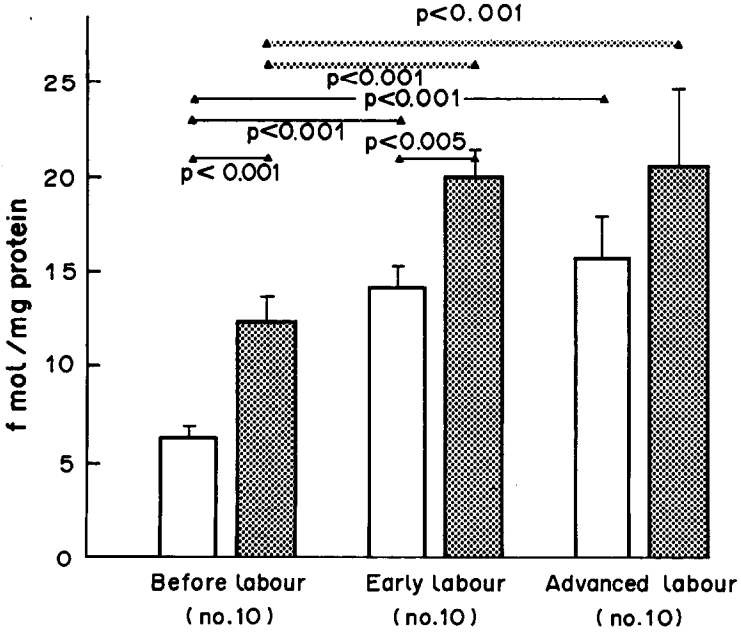

Fig. 2. Oxytocin receptor binding in human fetal membranes, amnion (□) and chorion-decidua (⊡) in each stage of labor. Values of each column represent means ± SE of 10 cases. Significant difference between amnion and chorion-decidua during labor are shown.

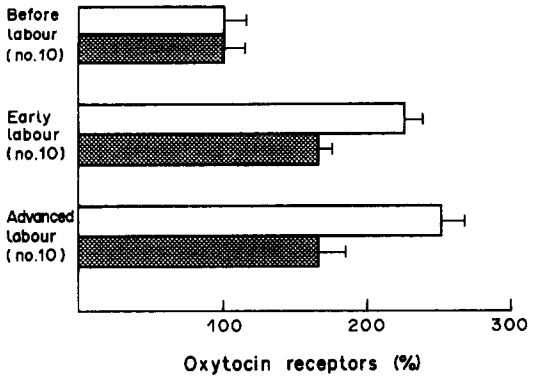

Fig. 3. Oxytocin receptor binding in human fetal membranes, amnion (□) and chorion-decidua (▨) in each stage of labor. The values in normalized form (control = 100) represent the mean ± SE (10 cases for each column) of the percent changes relative to the control values.

ing in early labor is 66% higher in the amnion than in the chorion-decidua, while in advanced labor it is 87% higher.

The higher percentage increase in the amnion may be correlated with a direct action by oxytocin which is produced by the human fetus in higher concentrations during early and advanced labor than before its onset [20].

It has been demonstrated that the induction of labor with oxytocin is only successful when oxytocin-induced contractile activity results in increases of PgF2α production [1]. Oxytocin binding was associated with stimulation of prostaglandin synthetase activity in the decidua of pregnant women at term [2, 8] and in the endometrium of ewes [4].

The increase of oxytocin receptor binding, that we found in the amnion and in the chorion-decidua during labor, could thus represent an important factor in the induction of prostaglandin synthesis.

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