Effects of oxytocin on cervical and uterine connective tissue

Ingrid Wiqvist, Anders Norström and Nils Wiqvist

Department of Obstetrics and Gynecology, University of Göteborg, Göteborg, Sweden

Abstract. The effect of oxytocin on collagen metabolism in the cervix and lower uterine segment of pregnant women was studied by measuring the incorporation of [3H]proline in vitro. Oxytocin had a concentration related inhibitory effect on the labelling with [3H]proline. Addition of indomethacin did not influence the response to oxytocin indicating that the effect was not directly mediated by prostaglandins. Oestradiol-17β potentiated the effect of oxytocin. Vasopressin decreased the incorporation of [3H]proline slightly but the action of this hormone was significantly less than that of oxytocin. The results suggest that oxytocin under in vitro experimental conditions influences cervical connective tissue metabolism which is in contrast to current clinical experiences.

Labour is preceded by functional changes in both the corpus uteri and the cervix (Keirse et al. 1979; Ulmsten & Ueland 1983). Though studied for decades, the exact mechanisms responsible for the initiation of normal labour at term have not been clarified. Among the various hormones which may play a crucial role in this context special interest has been paid to steroids, prostaglandins (PGs) and oxytocin. PGs have been shown to influence the myometrial activity as well as the biochemical composition of the cervix (Bygdeman et al. 1967; Karim et al. 1968; Calder et al. 1974; Hillier & Wallis 1981). IV infusion of oxytocin near term stimulates uterine contractility but seems to have little if any effect on cervical ripening (Ulmsten et al. 1979; Forman et al. 1982).

Cervical maturation includes altered synthesis and degradation of collagenous elements as well as changes in the composition of the ground substance (Danforth et al. 1974; Kleissl et al. 1978; Kitamura et al. 1979; von Maillot et al. 1979; Golichowski 1980). Steroids, PGs and relaxin seem to be the primary regulators of these events (Kroc et al. 1959; MacLennan et al. 1980; Gordon & Calder 1977). The present investigation was carried out to explore, by the use of in vitro methods, whether oxytocin – in spite of available clinical evidence – may have any direct effects on certain biochemical parameters associated to metabolic events within the cervical connective tissue.

Material and Methods

Tissue was obtained from patients admitted for legal abortion in the 7th–12th week of pregnancy and from women undergoing elective caesarean section in the 38th–40th week of gestation. Cervical biopsies (approximately 1.5 × 15–20 mm) were taken from the anterior lip of the cervix by the use of a biopsy needle (Tru Cut, Travenol, Deersfield, Ill., USA) and specimens from the lower uterine segment were excised at the site of uterine incision. The study was carried out following informed consent from each patient and with permission from the Ethical Committee of the Medical Faculty, University of Göteborg.

The specimens were dissected into smaller pieces (5–10 mg wet weight) in chilled oxygenated (5% CO₂ in O₂) Krebs-Ringer bicarbonate (KRB) buffer and preincubated for 1 h at 37°C in KRB buffer fortified with glucose (10 mM). Oxytocin alone (50–200 mU/ml) or in combination with oestradiol-17β (1–10 µg/ml) or indomethacin (5 µg/ml) was added to the incubation medium. The effect of vasopressin (50–200 mU/ml) was studied in a separate series of experiments.
In the presence of the substances under investigation the tissue was incubated for 2 h in freshly gassed KRB buffer containing [3H]proline. Control specimens from the same patients were treated in a similar way but without adding any of the compounds to be tested. Incubations were terminated by rinsing the tissue twice in chilled KRB buffer. Tissue proteins were precipitated and dry weight of the delipidized tissue was determined. Radioactivity (CPM/mg dry weight) was expressed as percent of the control due to variation of the net radioactivity among the patients within the separate experimental groups.

**Chemicals**

Oxytocin (Syntocinon®, 5 IU/ml) was purchased from Sandoz AG, Basel, Switzerland, and oxytocin as dry substance (450 IU/mg) from Calbiochem, Basel, Switzerland; lysine-vasopressin (Postacton®, 20 IU/ml) and arginine-vasopressin as dry substance (453 IU/mg) were supplied by Ferring AB, Malmö, Sweden. Oestradiol-17β (Sigma Chemicals Co., St. Louis, Mi., USA) was dissolved in absolute ethanol and further diluted to appropriate concentration before each experiment. Indomethacin was kindly supplied by Merck, Sharp & Dohme, Rahway, NJ, USA). [3H]proline (139 Ci/mmol, 0.01 μm) was obtained from New England Nuclear Co., Boston, Mass. USA.

**Statistical procedure**

The mean values ± SEM were calculated and the significance of difference from control values was determined using analysis of variance followed by the Student-Neuman-Keuls multiple range test (Wolf 1968).

**Results**

Oxytocin at a concentration of 100 mU per ml incubation medium reduced the incorporation of [3H]proline into cervical specimens from early pregnant women to approximately 40% of the control level (Fig. 1a). In the lower uterine segment of term pregnant women the decrease in radio labelling was dose-dependent and significant at all concentrations between 50 and 200 mU/ml.
Comparison of the influence of oxytocin (same data as in Fig. 1) and vasopressin on radiolabelling with $[^{3}H]$ proline (mean ± SEM). Cervical specimens (cx) were incubated with arginine vasopressin and lower uterine segment specimens (l.u.s.) with lysine vasopressin. There was a significant ($*** P < 0.001$) difference between the effect of oxytocin and vasopressin at concentrations of 100 (cx, n = 4 & l.u.s. n = 6) and 200 (n = 7) mU/ml.

(Fig. 1a). The incorporation of $[^{3}H]$ proline was significantly lower in tissue from the lower uterine segment as compared to that in cervical tissue of the same term pregnant women (Fig. 1b). However, oxytocin reduced the radiolabelling to a similar extent in both tissues.

Since the main two neurohypophyseal hormones, oxytocin and vasopressin, have a similar molecular
configuration and several functional effects in common, the corresponding action of vasopressin was tested in a separate series of experiments. Incubation of cervical tissue with arginine-vasopressin showed that vasopressin was significantly ($P < 0.001$) less effective than oxytocin in reducing the incorporation of $[^3]$H]proline (Fig. 2). Radiolabelling of tissue from the lower uterine segment of term pregnant women with lysine-vasopressin was also considerably less than that in corresponding experiments with oxytocin ($P < 0.001$). The difference in effect of the two hormones was significant at concentrations of 100 and 200 mU/ml (Fig. 2).

When tissue was incubated in media containing combinations of oxytocin and oestradiol-17β the reduction of the net radiolabelling with $[^3]$H]proline was even more pronounced than that with oxytocin alone. The oestradiol-induced reduction was significant and concentration related (Fig. 3).

To investigate whether the effect of oxytocin could be mediated by prostaglandins, tissue was incubated with oxytocin combined with indomethacin (5 µg/ml). At the concentration applied this blocker of PG-synthesis did not influence the effect of oxytocin neither on cervical specimens nor on tissue from the lower uterine segment (Fig. 4).

**Discussion**

The amino acid composition of collagen is characterized by a predominance of certain amino acids of which proline and hydroxyproline represent around 20% of the residues (Miller 1976). Hydroxyproline can be considered specific for collagen but cannot be incorporated into collagen as such. Proline is, on the other hand, the natural precursor of hydroxyproline. Metabolic events in terms of synthesis and degradation of collagen may therefore be reflected by the net incorporation of radiolabelled proline (Minor 1980). The net tissue-bound radioactivity encountered in the present experiments comprises several events involved in the process of protein metabolism: uptake of the radioactive amino acid, its incorporation as $[^3]$H]proline and further hydroxylation in tropocollagen molecules in addition to degradation of the collagen formed and escape out of cells of break-down products. Since it cannot be stated at the present stage to what extent oxytocin affects the amino acid uptake or protein turnover rates, the hormone induced changes of $[^3]$H]proline labelling can just be looked upon as indicative of an altered collagen metabolism.

The functional properties of human cervical connective tissue are influenced by a variety of
hormonal substances, which are responsible for softening and increased dilatability before and during labour as well as restitution of the cervix following parturition. Among the different hormones that theoretically might influence the properties of the cervix oxytocin has been found to have little if any direct effect. This statement is, however, based only on clinical observations in connection with iv oxytocin administration for induction of labour and provides little information on the possible significance of long-term effects of endogenous levels of oxytocin (Valentine 1977; Wilson 1978).

Prostaglandins, and possibly also oestrogen and relaxin have been shown to promote cervical ripening in the human (Calder et al. 1974; Gordon & Calder 1977; MacLennan et al. 1980). The mechanisms underlying this clinically documented effect have earlier been investigated in our laboratory in in vitro experiments by measuring the action of these hormones on the incorporation of [3H]proline (Norström 1982; Wiqvist et al. 1984). At term pregnancy prostaglandins as well as relaxin were found to reduce the net radiolabelling with [3H]proline, indicating inhibition of collagen synthesis as one of several possible metabolic effects. The results obtained in the present study demonstrate that oxytocin has a similar inhibitory effect.

Although the effective concentrations of oxytocin under our experimental conditions are by far higher than those resulting from clinically applied doses the suppression of radiolabelling with [3H]proline by oxytocin was more evident than that induced by any other substance so far tested. The specificity of the oxytocin effect gains support by the finding that the inhibitory action of corresponding concentrations of vasopressin was much less. However, the fact that vasopressin did reduce the net tissue radiolabelling is in line with previous observations that these neurohypophyseal hormones have several biological effects in common, though with varying relative potency (Bisset 1976).

The suppressive action of oxytocin on the incorporation of [3H]proline was significantly enhanced by oestradiol-17β. Oestradiol has been shown to increase the myometrial sensitivity to oxytocin (Pinto et al. 1966). The mechanism for this effect of oestradiol is unknown but some evidence points to the possibility of formation of oxytocin receptors in the myometrium (Alexandrova & Soloff 1980) and increased production of prostaglandins (Kogo et al. 1977). Similar mechanisms might underly the action of oestradiol on cervical and uterine connective tissue.

Since proline is a constituent not only of connective tissue proteins but also of myometrial cell proteins, it cannot be excluded that the effect of oxytocin on [3H]proline labelling refers to metabolic events within the smooth muscle cells. However, the fact that the net proline uptake was greater in the cervix than in the lower uterine segment which contains less connective tissue than the cervix (Danforth et al. 1974) is in line with the concept that this amino acid particularly reflects connective tissue metabolism. Further, the relative reduction in [3H]proline radiolabelling induced by oxytocin, was similar in the cervix and the lower uterine segment which supports the view that the incorporation of [3H]proline mainly refers to the connective tissue cells rather than to the myometrial cells.

Oxytocin has recently been shown to stimulate the formation of prostaglandins in the decidua but not in the myometrium (Husslein et al. 1982). It seems likely that decidual prostaglandins under physiological conditions may influence the connective tissue within the myometrium as well as the smooth muscle cells themselves. Such an indirect action of oxytocin cannot occur under our experimental conditions. However, it might still be possible that the oxytocin effect could be mediated by myometrial prostaglandins. This possibility seems less probable in light of the fact that indomethacin did not influence the action of oxytocin in our system.

The question as to whether the demonstrated effect of oxytocin on connective tissue metabolism has any implication in the in vivo situation still remains unanswered. However, the present results demonstrate that oxytocin has a hitherto unknown metabolic effect on human cervical and uterine connective tissue.

Acknowledgments

The present work was supported by grants from The Swedish Medical Research Council (B83-17X-02019-17A), The Medical Faculty, University of Göteborg, The Medical Society of Göteborg, Tore Nilsson's Foundation, Harald Jeansson's and Greta Jeansson's Foundation and The 'Expressen' Prenatal Research Foundation.

We are indebted to Ms Ingegerd Karlsson for technical assistance and to Mrs Ann-Louise Dahl for secretarial work.
References


Received on October 17th, 1983.