

Loss of myometrial oxytocin receptors during oxytocin-induced and oxytocin-augmented labour

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Oxytocin is used widely for the induction and augmentation of labour, but there is little information about the dynamics of oxytocin receptors in human myometrium during parturition, and the possible effect of oxytocin infusion. This information is important because G protein-coupled receptors, such as the oxytocin receptor, undergo desensitization after prolonged or repeated stimulation. The concentration of myometrial oxytocin receptors and the steady state of its mRNA were measured in patients undergoing Caesarean sections before or during spontaneous or induced labour. The concentration of receptors before labour was 477 (175–641) fmol mg⁻¹ protein (median, quartile range), and decreased to 140 (72–206; $P < 0.05$) and 118 (69–75; $P < 0.01$) fmol mg⁻¹ protein during prolonged oxytocin-augmented and oxytocin-induced labour, respectively. The corresponding oxytocin receptor mRNA concentrations decreased by 60- and 300-fold, respectively. The decrease in receptor binding and mRNA in women receiving oxytocin infusion indicates that homologous receptor desensitization occurs *in vivo*.

Introduction

Oxytocin has been used for many years in obstetric practice for the induction and augmentation of labour, and for the prevention of postpartum haemorrhage (Zeeman *et al.*, 1997). Oxytocin has been effective in improving the chances of vaginal delivery and reducing maternal mortality and morbidity from Caesarean section. The risks associated with oxytocin infusion include hyperstimulation and uterine rupture. Moreover, long episodes of labour induction or augmentation with oxytocin may result in postpartum atony and bleeding. Over the past few years our knowledge of oxytocin receptor biochemistry has increased considerably (Kimura *et al.*, 1996; Zingg, 1996; Mitchell *et al.*, 1998) and oxytocin receptor antagonists have been introduced in clinical practice (Goodwin and Zograbyan, 1998). It is expected that these developments will lead to a better understanding of the role of oxytocin in the physiology of human parturition and of its pharmacological use.

Myometrial oxytocin receptor concentrations increase during human pregnancy and this is reflected in increased sensitivity to oxytocin stimulation (Fuchs *et al.*, 1984; Bossmar *et al.*, 1994). The oxytocin receptor belongs to the G protein-coupled receptor family and is a member of the vasopressin receptor group, which consists of V1a, V1b and V2 vasopressin receptors, and oxytocin receptor (Zingg, 1996). Binding of oxytocin to the myometrial receptor stimulates

uterine contractility by activating phospholipase C, which hydrolyses phosphatidylinositol 4,5-bisphosphate resulting in the production of 1,2-diacylglycerol and inositol 1,4,5-trisphosphate, which mobilizes calcium from intracellular stores (Schrey *et al.*, 1988; Phaneuf *et al.*, 1993). Additional effects on contractility may be mediated through decidual oxytocin receptors modulating intrauterine prostanoid production (Fuchs *et al.*, 1984; Mitchell *et al.*, 1998).

Studies on a variety of G protein-coupled receptors indicate that their repeated or prolonged stimulation results in the loss of hormonal responsiveness, which is termed desensitization. There is evidence for oxytocin receptor desensitization in the myometrium, but little is known about the mechanisms involved in the loss of myometrial response to a variety of agonists. Treatment of cultured human myometrial cells with oxytocin leads to homologous desensitization (Adachi and Oku, 1995; Phaneuf *et al.*, 1997), characterized by a decrease in oxytocin-stimulated activation of the phospholipase C–calcium pathway, a substantial loss of oxytocin binding sites, and by a severe reduction of the mRNA encoding oxytocin receptor (Phaneuf *et al.*, 1998). There is controversy as to whether an increase in the density of myometrial oxytocin receptor precedes normal labour in women (Fuchs *et al.*, 1984), and it is not known whether oxytocin receptor desensitization occurs *in vivo* in women exposed to oxytocin infusion for many hours during labour (Bossmar *et al.*, 1994). The present study was designed to determine the extent to which myometrial oxytocin receptor binding and mRNA concentrations change during labour and the possible influence of oxytocin administration.

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Materials and Methods

This investigation had the approval of the Central Oxfordshire Research Ethics Committee, and all patients gave informed consent. Myometrium free of decidual or peritoneal tissue was taken from the upper border of the uterine incision during Caesarean sections at weeks 37–42 of gestation from the following groups of women:

(i) Before the onset of labour. The indications included previous Caesarean section, cephalopelvic disproportion, malpresentation, and placenta praevia.

(ii) After labour of spontaneous onset. The sections were performed because of failure to progress in labour or fetal distress.

(iii) After labour induced because of post-term pregnancy, pre-eclampsia, fetal growth retardation or for social reasons. The sections were performed because of failed progress of labour, failed labour induction or fetal distress.

The patients were of mixed parity and further clinical details are presented (Table 1). The samples were frozen in liquid nitrogen and stored at -70°C until required (< 6 months).

Labour was managed according to the Delivery Suite Guidelines of the John Radcliffe Maternity Hospital. Patients for induction of labour had their relevant medical and obstetric histories checked and a full abdominal and pelvic examination including cervical scoring (MacKenzie and Burns, 1997). If the cervical score was 0–8, 2 mg prostaglandin E_2 (PGE_2) was administered vaginally as triacetin gel (Prostin E_2 , Pharmacia & Upjohn, Milton Keynes) at 17:00–19:00 h, followed by artificial rupture of the membranes and oxytocin infusion at 09:00 h the next morning if labour was not established. If the cervical score was ≥ 9 , artificial rupture of the membranes was performed at 09:00 h, followed by oxytocin infusion at 10:00 h if labour was not establishing. Oxytocin (Syntocinon, Novartis Pharmaceuticals UK Ltd, Camberley) was prepared at a concentration of 30 mIU ml^{-1} and infused at an initial rate of 1 mIU min^{-1} . The infusion rate was increased at 15–20 min intervals by doubling steps and

the minimum infusion rate that maintained contractions was used. Syntocinon was also administered to women who had been in spontaneous labour for more than 10 h.

Oxytocin binding

Myometrial membranes were prepared and incubated with increasing concentrations of [^3H]oxytocin with and without an excess of non-labelled oxytocin to measure non-specific binding essentially as described elsewhere (Rivera *et al.*, 1990; López Bernal *et al.*, 1995). Binding capacity and affinity (K_d) were calculated from saturation analysis by Scatchard plots. Myometrial membranes from each patient were assayed in duplicate using at least five concentrations of labelled oxytocin. At the mid-point (5 nmol^{-1}), non-specific binding averaged 31% of the total binding. Saturation was achieved both in samples with high and low density of receptors and the Scatchard plots were rectilinear, demonstrating a single class of binding sites (Fig. 1).

RNA isolation and cDNA synthesis

Owing to the limited amount of myometrium available from each patient, it was not possible to measure oxytocin binding and oxytocin receptor mRNA concentrations in the same myometrial samples, but the clinical characteristics of the samples used for either assay were very similar (Table 1). Total RNA was prepared from tissue specimens by the guanidium–isothiocyanate–caesium chloride method (Chirgwin *et al.*, 1979). Oligo dT primers (500 ng) were annealed to 2 μg total RNA at 70°C for 10 min, and cDNA synthesis was carried out with 100 U Moloney murine leukaemia virus (MMLV) reverse transcriptase. The oxytocin receptor cDNA was amplified with oxytocin receptor forward (1230-5'GGACGCCCTTCTTCTCG3'-1247) and reverse primers (1962-5'CTGGAGTCTACCCTCCCC3'-1945), generating a 734 base pair (bp) fragment. The PCR

Table 1. Clinical details of patients

Group	Gestational age (weeks)	Birth weight (kg)	Duration of labour (h)	Cervical dilatation (cm)
All Samples				
Not in labour ($n = 29$)	37.9 ± 1.4	3.08 ± 0.66	–	–
Spontaneous labour ($n = 33$)	38.9 ± 1.7	3.38 ± 0.60	10.2 ± 6.3	5.5 ± 2.9
Induced labour ($n = 30$)	39.5 ± 1.8	3.30 ± 0.70	10.3 ± 5.8	5.2 ± 3.1
Oxytocin binding measurements				
Not in labour ($n = 24$)	38.3 ± 0.8	3.30 ± 0.46	–	–
Spontaneous labour ($n = 16$)	38.7 ± 1.8	3.27 ± 0.44	11.2 ± 6.0	5.9 ± 2.8
Induced labour ($n = 15$)	39.7 ± 1.4	3.28 ± 0.63	9.2 ± 4.5	5.1 ± 3.0
Oxytocin receptor mRNA measurements				
Not in labour ($n = 5$)	38.8 ± 0.4	3.15 ± 0.35	–	–
Spontaneous labour ($n = 17$)	39.3 ± 1.3	3.53 ± 0.65	9.5 ± 6.4	5.5 ± 2.8
Induced labour ($n = 15$)	39.6 ± 1.6	3.44 ± 0.59	11.8 ± 5.9	5.3 ± 3.1

Data are means \pm SD.

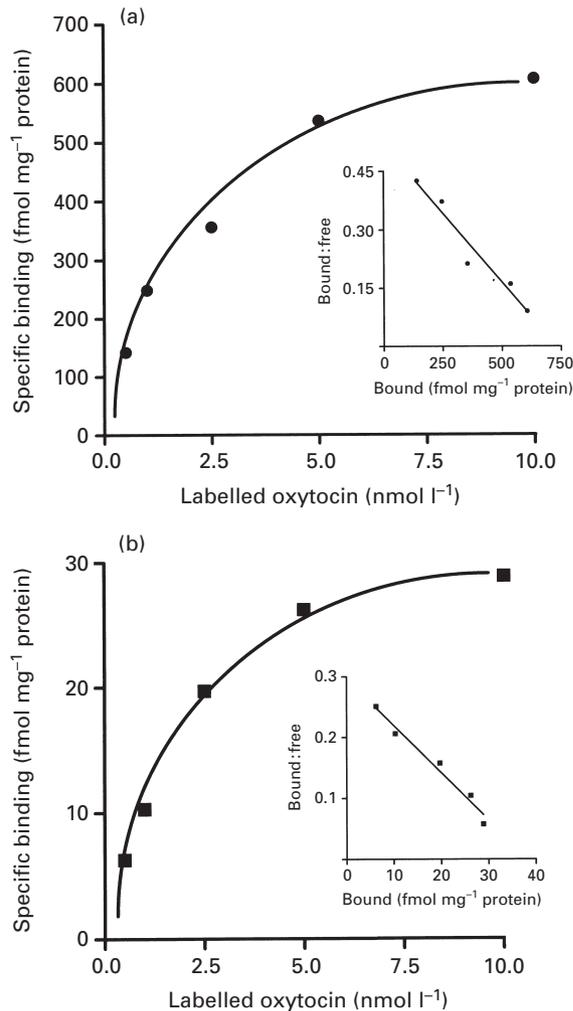


Fig. 1. Binding of [^3H]oxytocin to membrane preparations of pregnant human myometrium. The insets show Scatchard transformations of the data. Two examples from tissues with high and low receptor density are given. Under the conditions used, saturation of radioligand binding occurred at both high ($685 \pm 57 \text{ fmol mg}^{-1} \text{ protein}$; a) and low ($38 \pm 2 \text{ fmol mg}^{-1} \text{ protein}$; b) receptor density.

reaction was 94°C for 5 min, 30 cycles of 94°C for 1 min, 64°C for 1 min, 72°C for 3 min, and a final elongation step at 72°C for 5 min. The primers were shown not to amplify a product from genomic DNA (data not shown).

Homologous competitive RT-PCR

The competitive RT-PCR assay was based on the method of Celi *et al.* (1993) and fully validated by Phaneuf *et al.* (1998). A 679 bp internal cDNA standard or competitor was generated from oxytocin receptor cDNA by PCR, using the following primers: (i) the 18-mer oxytocin receptor forward primer described above, and (ii) a composite 39-mer reverse primer made up of the 18-mer oxytocin receptor reverse primer described above with an additional 21 bases (1871–1891 of

oxytocin receptor) at its 3' end. The cDNA standard (679 bp) was purified with the QIAquick PCR purification kit (Qiagen Ltd, Crawley), quantitated by spectrophotometry, and diluted to 100 amol ml^{-1} in $10 \mu\text{g glycogen ml}^{-1}$. Initially, cDNA obtained from myometrial mRNA was titrated in a first series of competitive PCR assays. A constant amount of a 1:8 dilution of RT products ($0.1 \mu\text{g total RNA ml}^{-1}$) was mixed with consecutive 1:10 dilutions of the DNA standard, down to $0.001 \text{ amol ml}^{-1}$. A second series of competitive PCR assays was carried out with consecutive 1:2 dilutions of the cDNA standard mixed with the constant amount of RT products as described above. The PCR reactions were carried out under the same conditions as described in the previous section. Samples were loaded on 2% (w/v) agarose gels containing $1 \mu\text{g ethidium bromide ml}^{-1}$, and photographed under UV illumination. The result of one experiment using 1:2 dilutions of the cDNA standard for oxytocin receptor on a myometrial sample is presented (Fig. 2). When the intensity of both PCR products is the same after PCR amplification, it is assumed that the amount of starting material was the same for the test cDNA and the cDNA standard. From this method it is concluded that the sample presented has 1 amol oxytocin receptor mRNA (Fig. 2). Myometrial RNA from each patient was assayed three times.

Data analysis

Statistical comparisons were performed with parametric (ANOVA, *t* tests) or non-parametric (Kruskal-Wallis, Mann-Whitney) tests as appropriate. Oxytocin binding and mRNA data were plotted and best-fit lines calculated by regression analysis. Data were plotted and analysed using the Prism-InStat package for PC (GraphPad Software Inc., San Diego, CA). Differences were considered significant if $P < 0.05$.

Results

The group of patients studied was very homogeneous in terms of gestational age and birth weight (Table 1).

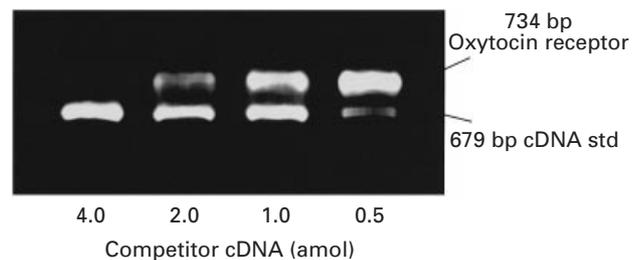


Fig. 2. Analysis of oxytocin receptor mRNA by RT-PCR, using homologous competitive cDNA. Human myometrial mRNA was reverse transcribed, and the resulting cDNA was used as a template for PCR amplification, in the presence of decreasing amounts of a homologous cDNA competitor fragment (679 bp). The oxytocin receptor fragment is 734 bp. The samples were run on a 2% (w/v) agarose gel and stained with ethidium bromide.

Moreover, the spontaneous and induced labour groups had very similar average duration of labour (10 h) and cervical dilatation (5 cm) at the time of Caesarean section. The number of patients receiving oxytocin infusion and the average duration of infusion were significantly higher and longer, respectively, in the induced compared with the spontaneous labour group (Table 2).

Oxytocin receptor binding changes in spontaneous and induced labour

The oxytocin binding affinity of myometrial membranes was very similar in all groups: $K_d = 1.2$ (0.9–1.6) nmol l⁻¹ (medians and quartiles) for women not in labour ($n = 24$); and 1.1 (0.8–2.3) nmol l⁻¹ ($n = 16$) and 1.2 (0.9–5.6) nmol l⁻¹ ($n = 15$) for women in the spontaneous and induced labour groups, respectively. The oxytocin-binding capacity of myometrial samples in all groups was plotted in relation to the duration of labour and cervical dilatation (Fig. 3). The binding capacity in samples from patients not in labour was 477 (175–641) fmol mg⁻¹ protein (median and quartiles). The establishment of labour was not associated with any apparent changes in oxytocin binding sites in either the spontaneous or the induced labour groups. However, those patients who had been in labour for more than 10 h, and had received intravenous oxytocin, had a significant loss of myometrial receptors: 140 (72–206) and 118 (69–175) fmol mg⁻¹ protein (medians and quartiles) in the spontaneous ($n = 9$; $P < 0.05$) and induced groups ($n = 6$; $P < 0.01$), respectively, compared with samples from women not in labour. Overall, the progress of labour was associated with an apparent loss of oxytocin binding sites (Fig. 3a) and this decrease was significant in the induced labour group ($P < 0.02$). In the induced labour group, there was a significant ($P < 0.02$) decrease in oxytocin binding with increasing cervical dilatation (Fig. 3b). However, in the spontaneous labour group cervical dilatation *per se* had no effect on oxytocin binding.

When the binding data were plotted in relation to the duration of oxytocin infusion, irrespective of whether labour was of spontaneous or induced onset, there was a significant ($P < 0.01$) decrease in oxytocin binding with increasing

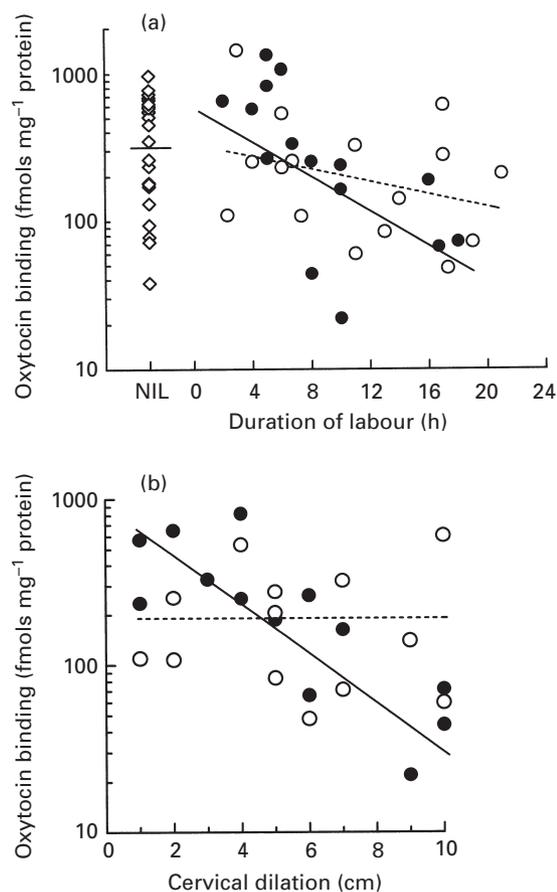


Fig. 3. (a) Oxytocin binding in human myometrium as a function of the duration of labour. NIL, \diamond , women not in labour; \circ , spontaneous labour group; \bullet , induced labour group. The slope of the regression line in the induced labour group (—) was significantly different from zero ($F = 8.1$; $P < 0.02$). The slope of the regression line in the spontaneous labour group (---) was not significantly different from zero ($F = 0.23$; $P > 0.05$). (b) Oxytocin binding in human myometrium as a function of cervical dilatation. \circ , spontaneous labour group; \bullet , induced labour group. The slope of the regression line in the induced labour group (—) was significantly different from zero ($F = 8.14$; $P < 0.02$). The slope of the regression line in the spontaneous labour group (---) was not significantly different from zero ($F = 0.01$; $P > 0.05$).

Table 2. Prostaglandin administration and Syntocinon infusion in the spontaneous and induced labour groups of patients

Group	Number of patients receiving prostaglandin gel	Number of patients receiving oxytocin infusion	Duration of oxytocin infusion (h)	Maintenance dose (miu min ⁻¹)
			Mean \pm SD	Median (range)
Spontaneous labour ($n = 33$)	–	18	4.8 \pm 3.5	12 (1–32)
Induced labour ($n = 30$)	25	26 ^a	7.7 \pm 3.8 ^b	16 (1–48)

^aSignificantly more patients in the induced labour group received oxytocin ($P < 0.05$).

^bSignificantly longer than in the spontaneous labour group ($P < 0.02$).

duration of infusion (Fig. 4a). A decreasing trend was also observed when oxytocin binding was plotted in relation to increasing maintenance dose of oxytocin; however, this trend was not statistically significant (Fig. 4b).

Oxytocin receptor mRNA changes in spontaneous and induced labour

Oxytocin receptor mRNA concentrations in myometrial samples from women who had a Caesarean section early in labour were similar to those from the group of women not in labour (Fig. 5). However, there was a significant decrease ($P < 0.01$) in oxytocin mRNA concentrations with increasing duration of labour in both the spontaneous and induced labour groups, although the decrease was steeper in the

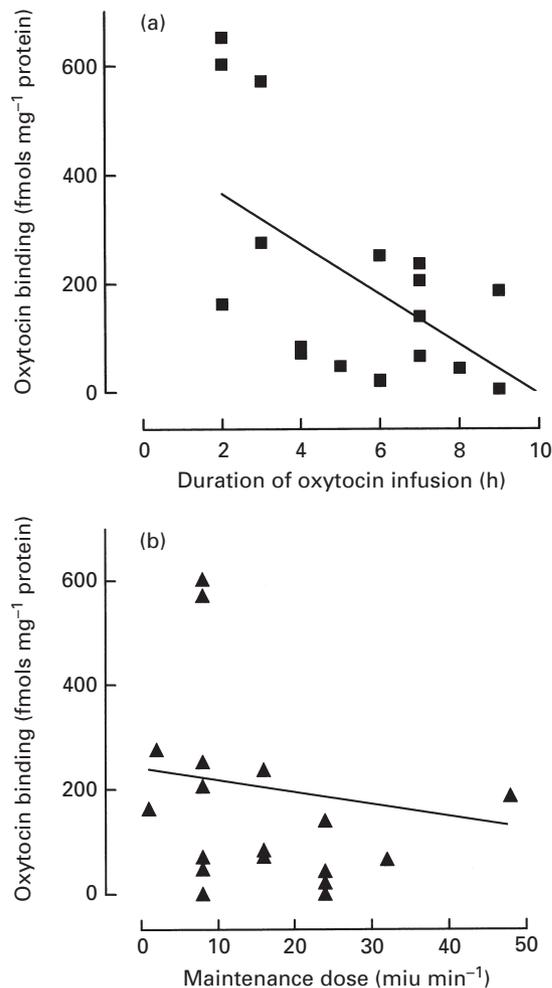


Fig. 4. (a) Oxytocin binding in human myometrium as a function of the duration of oxytocin infusion. Myometrial samples from women who were given intravenous oxytocin either to augment or to induce labour. The slope of the regression line was significantly different from zero ($F = 9.6$; $P < 0.01$). (b) Oxytocin binding in human myometrium as a function of the oxytocin infusion maintenance dose. The slope of the regression line was not significantly different from zero ($F = 0.24$; $P > 0.05$).

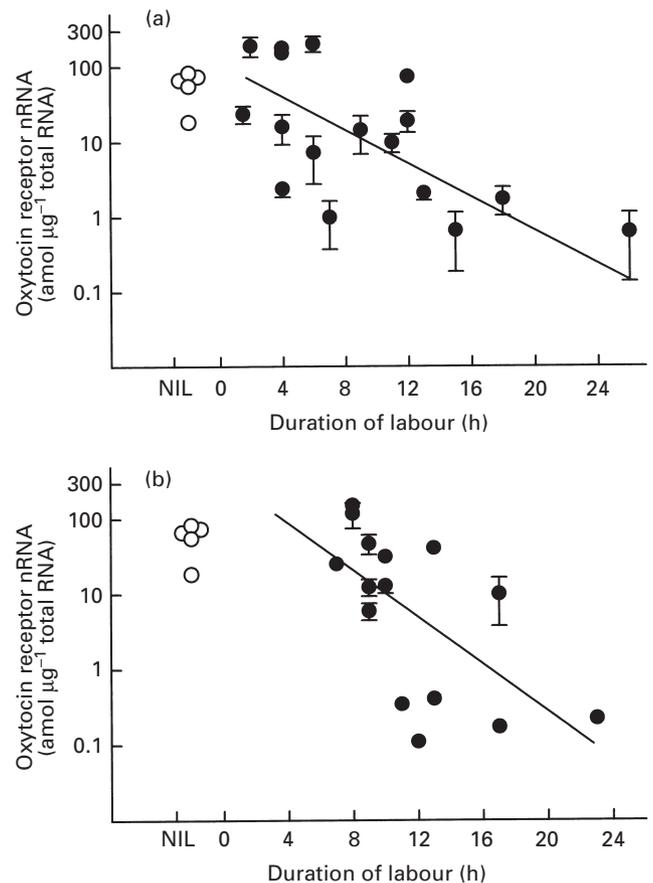


Fig. 5. Oxytocin receptor mRNA concentrations in human myometrium as a function of the duration of labour. (a) Spontaneous labour at term; (b) induced labour. NIL, O, women not in labour. Each point represents the mean (\pm SEM) of three independent determinations on myometrial RNA from each specimen. When not shown, the error bars are hidden by the symbols. The slope of the regression line in both panels was significantly different from zero ($F = 9.46$, $P < 0.01$; $F = 8.15$, $P < 0.02$, respectively).

induced labour group (Fig. 5). In the spontaneous labour group, myometrial oxytocin mRNA concentrations in women who had a Caesarean section after 10 h in labour were approximately 60 times lower than in women not in labour. In the induced group, concentrations were 300 times lower.

Discussion

The present study demonstrates that during the progress of oxytocin-induced and, to a lesser extent, oxytocin-augmented labour, there is a significant reduction in both the concentration of myometrial oxytocin binding sites and oxytocin receptor mRNA. These two parameters are integral parts of myometrial oxytocin receptor desensitization in cultured myometrial cells *in vitro* (Phaneuf *et al.*, 1998). The

progressive decrease in myometrial receptor density and the steep decrease in mRNA in these women indicate that administration of oxytocin leads to homologous receptor desensitization *in vivo*. The data also confirm that the establishment of labour at term is not accompanied by changes in the concentration of myometrial oxytocin receptor or oxytocin receptor mRNA expression (Bossmar *et al.*, 1994; Wathes *et al.*, 1999).

Desensitization of hormonal receptors is a complex physiological process that prevents hyperstimulation by impairing signal transmission during prolonged receptor activation, but also facilitates responsiveness of the tissue to successive multiple extracellular stimuli over time. The loss of β -adrenoceptor responses in human myometrium after β -mimetic tocolytic treatment is well established (Berg *et al.*, 1985; Engelhardt *et al.*, 1997). Oxytocin is released in a pulsatile manner and, during labour, the pulse frequency increases to reach a maximum during the second stage of labour (Fuchs *et al.*, 1991). However, prolonged infusion of oxytocin provokes decreased uterine responsiveness (Crall and Mattison, 1991) probably due to long-term loss of myometrial receptors. The loss of oxytocin receptors, reflected by the decrease in binding sites and the very low mRNA concentrations, may eventually result in uterine atony. A loss of myometrial oxytocin receptor has been described in 'oxytocin-resistant' dystocia (Rezapor *et al.*, 1996). These observations agree with preliminary clinical trials, which have shown that pulsatile administration of oxytocin is more efficient than constant infusion to induce labour (Zeeman *et al.*, 1997). The reported increase in efficiency may be attributed to a reduction in the desensitization of oxytocin receptor, but this assumption needs to be tested by comparing the concentration of myometrial oxytocin receptors in women given continuous and pulsatile oxytocin infusions. The unavoidable cross-sectional design of the present clinical study makes it difficult to rule out the possibility that those patients with low receptor density and low mRNA concentrations possessed these attributes before, rather than as a consequence of prolonged labour and oxytocin infusion. Moreover, this population may represent only those women who ended up having a Caesarean section, and the observed changes may not apply to women who had successful vaginal deliveries after spontaneous or induced labour. Despite these reservations, the data are compatible with the concept that administration of oxytocin leads to homologous receptor desensitization *in vivo*, as has been demonstrated *in vitro* (Phaneuf *et al.*, 1997). Desensitization of oxytocin receptors may contribute to decreased myometrial contractility during long episodes of labour induction or augmentation with oxytocin. The decrease in oxytocin binding sites and oxytocin receptor mRNA is specific for the oxytocin receptor and does not affect the structurally related vasopressin V1 receptor (Helmer *et al.*, 1998).

The mechanisms involved in oxytocin desensitization most probably include phosphorylation of oxytocin receptors by either G protein-coupled receptor kinases (Premont *et al.*, 1995) or by other kinases, for example protein kinase C, followed by the uncoupling of the receptor from the second messenger generation system by arrestins (Lefkowitz, 1998). Prolonged exposure of myometrial cells to oxytocin *in vitro* causes loss of binding without decreasing

the total amount of receptor protein, demonstrating functional desensitization of the receptor (Phaneuf *et al.*, 1994, 1997). Several G protein-coupled receptor kinases are present in human myometrium, including G protein-coupled receptor kinases 2, which is upregulated in pregnant tissue at term (Brennkmeijer *et al.*, 1999). Furthermore, β -arrestin 1 expression in term human myometrium and its association with immunoprecipitated oxytocin receptor has been demonstrated in cultured myometrial cells after a few hours of exposure to oxytocin (C. P. Plested, F. Mayor and A. López Bernal, unpublished). However, the decrease in oxytocin receptor mRNA is likely to be due to destabilization of mRNA by RNA binding proteins and degradation of the mRNA. The loss of functional receptors during labour may be the result of prolonged receptor occupancy by exogenous oxytocin, plus endogenous circulating oxytocin, or by locally synthesized hormone at the decidual-myometrial interface (Mitchell *et al.*, 1998). Moreover, parturition is stressful for the mother and fetus and the possibility of heterologous receptor downregulation due to endocrine or paracrine agonists other than oxytocin, released during labour (for example prostanoids, adrenocorticotrophic hormone, corticotrophin-releasing hormone, cortisol, endorphins, catecholamines) must also be considered (López Bernal *et al.*, 1993). Parturition is associated with increased decidual release of PGF_{2 α} that acts on receptors for prostaglandin F (FP) in human myometrial cells, leading to phosphoinositide hydrolysis and protein kinase C activation (Carrasco *et al.*, 1996). Furthermore, vaginal administration of PGE₂ in the induced labour group in the present study may have led to local changes in myometrial oxytocin receptor dynamics either directly through myometrial receptors for prostaglandin E (EP) or by altering decidual oxytocin release (Mitchell *et al.*, 1998).

In conclusion, the data show for the first time that continuous oxytocin infusion may provoke homologous receptor downregulation *in vivo*. Despite decades of clinical use to facilitate or induce labour, the uterine effects of oxytocin are not fully understood. Further investigations are needed to understand the possible mechanisms involved in myometrial oxytocin receptor downregulation during parturition, in particular the role of several G protein-coupled receptor kinases and β -arrestins. This knowledge will help establish optimal protocols for the effective use of oxytocin in protracted labour.

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