Quantitative immunoconfocal analysis of human myometrial gap junction connexin43 in relation to steroid hormone concentrations at term labour

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The aim of this study was to quantify gap junction expression in the human myometrium in relation to progesterone and oestriadiol concentrations, and to establish whether oxytocin-resistant dystocia is due to an abnormality in gap junction expression. Three groups of patients were investigated: (i) before labour (at term), (ii) normal labour and (iii) oxytocin-resistant dystocia (eight patients per group). For each patient, the concentrations of oestriadiol and progesterone in maternal blood and in myometrial tissue were measured, and the number and area of immunostained connexin43 gap junctions per unit volume of tissue determined by quantitative analysis of digital images obtained by confocal microscopy. No significant difference in connexin43 gap junction content was observed between the three patient groups. When all groups were pooled, there was a significant positive correlation (P < 0.05) between the quantity of immunolabelled gap junctions and the oestriadiol:progesterone ratio, but there was no significant difference in this correlation between the groups. Gap junction immunolabelling was not correlated with the progesterone or oestriadiol concentration in the maternal blood or the myometrium. These data suggest that in human myometrium: (i) dystocia is not due to a reduced level of immunodetectable connexin43 gap junctions, (ii) onset of labour is not associated with a sudden increase in immunodetectable gap junction protein and (iii) gap junctions can be expressed in the presence of high progesterone concentrations.

Key words: gap junctions/human myometrium/laser scanning confocal microscopy/oestriadiol/ progesterone

Introduction

Elaborate signalling mechanisms enable uterine myocytes to communicate with each other and to coordinate their behaviour. During labour, coordinated uterine contractions result from the rapid spread of action potentials through the myometrium (Cunningham et al., 1989). Gap junctions between myometrial cells are the sites of low resistance for the intercellular flow of current (Sims et al., 1982) and are widely believed to provide the basis for improved propagation during labour.

Progesterone and oestriadiol are considered to have a regulatory role in human and animal pregnancy. However, the role of steroid hormones in initiation of labour and coordination of uterine contractility in term pregnant women is still obscure. Measurements of steroid hormone concentrations in human myometrial tissue indicate that term pregnant myometrium has a higher progesterone/oestriadiol ratio than that of the peripheral plasma, thus suggesting a progesterone-dominated myometrium at term (Dawood and Dawood, 1984).

Myometrial gap junctions directly connect uterine smooth muscle cells, and in common with smooth muscle of other tissue, the major component of these junctions has been identified as connexin43 (Cole et al., 1985; Lang et al., 1991; Tabb et al., 1992). This connexin is expressed by the smooth muscle cells responsible for uterine contraction and is detectable using anti-peptide antibodies against connexin43 (Dupont et al., 1994). However, the application of anti-connexin43 antibodies for the immunofluorescence localization of gap junctions, in combination with scanning laser confocal microscopy and image analysis, permits these limitations to be overcome (Green et al., 1993). By means of confocal microscopy, serial optical sections of images that are free from out-of-focus blur may be obtained through a defined volume of a tissue slice, and quantitative analysis of the digital images allows accurate estimation of gap junction content in relatively large samples of myometrial tissue (Kilarski et al., 1993).

The present investigation was designed to establish the relationship between connexin43 gap junction content and the concentration of oestriadiol and progesterone in the myometrium and plasma, and to investigate whether a deficiency in gap junction content underlies dysfunctional labour. Functional dystocia was defined as failure of the cervix to dilate despite normal presentation, adequate oxytocin stimulation and the absence of obvious signs of fetopelvic disproportion.
Materials and methods

**Patients**

In all, 43 term pregnant patients participated in the study. Myometrial tissue was obtained at Caesarean section. The study was approved by the ethics committee of Uppsala University, Sweden, and all women gave informed consent. The gestational age was determined from the first day of the last menstrual period and was confirmed by an ultrasound scan at 16–17 weeks of gestation. Cervical score was assessed 10–60 min before Caesarean section. Uterine activity was registered by external tocography in the non-labour group and, when possible, by internal tocography in the other groups.

The patient material (Table I) was grouped as follows: group 1, patients not in labour (BL), Bishop score (Bishop, 1964) <5 and no uterine activity; group 2, patients in active phase of spontaneous labour (Cunningham et al., 1989) (3–4 uterine contractions/10 min), and cervix dilated >4 cm. Two subgroups could be identified: 2a, normal labour (NL), i.e. patients who had a cervical dilatation progress of 1.2 cm/h, but had to undergo Caesarean section because of signs of fetal distress; and 2b, oxytocin-resistant labour (ORL), i.e. patients with a normal spontaneous start of labour, which gradually developed into a hypotonic uterine contraction pattern and slow progress of cervical dilatation (Table II). These patients received augmentation but did not respond to oxytocin and had to be operated upon because of persistent cervical dilatation arrest.

The adequacy of contractility was judged based on the progress of cervical dilatation, the descent of the fetal presenting part and the uterine contraction pressure. All patients who developed a normal progress of cervical dilatation, according to the descriptions of Friedman (1971) and Phipcott and Castle (1972), and in whom a progressive descent of the presenting fetal part was recognized, were classified as normal labour. Arrest of cervical dilatation in the active phase of labour was considered as inefficient labour or functional dystocia and then treated by oxytocin. Finally, all patients in whom cervical dilatation did not progress for 2 h despite oxytocin infusion were classified as persistent dystocia (oxytocin-resistant labour). All other obstetrical complications were excluded. In this way, from 43 consecutive cases 24 patients entered the study randomly, eight patients in each group.

Oxytocin (10 U/l) was administered i.v.. The initial dose was 1 ml/min and the dose was increased every 15–30 min until an adequate contractile activity was achieved (three contractions/10 min with an amplitude of ~60 cm H2O and a duration of 45–50 s). The progress of cervical dilatation was assessed every hour. Uterine contraction pressures were quantified in Montevideo units. Intrauterine pressure recordings were instituted as soon as possible after rupture of membranes. For further details concerning the clinical course in different groups, see Table II.

Spontaneous rupture of the membrane or therapeutic amniotomy occurred at least 1 h prior to the initiation of oxytocin infusion. Fetopelvic disproportion, malpresentations and other obstetric complications were excluded.

Tissue fixation

Directly after the tissue specimens were obtained, the decidua and serosa were removed. The sample was immersed immediately in ice-cold fixative containing 2.0% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer (pH 7.4) for 2 h (Kilarski et al., 1993). The tissue was then trimmed into small pieces, dehydrated in a graded series of ethanol (from 50 to 100%), cleared in lemon oil and embedded in paraffin wax following standard histological procedures.

Radioimmunoassay of progesterone and oestradiol

The progesterone antiserum was made against progesterone-17-oxime-bovine serum albumin antigen, and was generously provided by Dr K. Martinsson (School of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala), and the oestradiol antiserum was against a 17β-6-oxime derivate purchased from Miles-Yeda (Rehovoth, Israel). One gram of the myometrial biopsy was submitted for measurement of oestradiol and progesterone. The tissue was rinsed in saline solution, blotted, immediately frozen at ~120°C, and stored at ~70°C until assayed. The tissue was weighed and incubated in 95% ethanol for 7 days at 4°C for hormone extraction as described by Bixo et al. (1984). The extraction procedure gives a recovery of 100%. Aliquots of the extract were used for the progesterone and oestradiol radioimmunoassay procedure: 100 ml of serum was incubated with petroleum ether for progesterone analysis, and 400 ml of serum was incubated with diethyl ether for oestradiol analysis.

**Solutions**

(i) Phosphate-buffered saline (PBS): 0.14 M NaCl in 0.01 M phosphate buffer, pH 7.0 (Methiolate 1:10 000). (ii) 0.1 and 5% gel PBS: 0.1% or 0.5% gelatin dissolved in PBS. (iii) Progesterone standards: stock solution 1 mg/ml in absolute ethanol, diluted with ethanol to obtain the desired concentrations. (iv) Progesterone [1,2,6,7-3H], specific activity 109.0 Ci/mmol (New England Nuclear, Boston, MA, USA). (v) Oestradiol [2,4,6,7-3H], specific activity 111.0 Ci/mmol (New England Nuclear). Stock solution: 1 mCi diluted in 100 ml of absolute ethanol. Final solution: ~10 000 cpm/100 ml. (vi) Antiserum was diluted with 0.1% gel PBS. (vii) Dextran-coated charcoal suspension: 1.25 g of Norit A and 125 mg of dextran T-70 in 250 ml of PBS.

Radioimmunoassay procedure

Steroid concentrations were assayed in the tissue extracts as described by Bosu et al. (1976) and Lindberg et al. (1974). Briefly, standard curves were prepared in duplicate, in the range of 10–1000 pg for progesterone and 5–500 pg for oestradiol. The extract was transferred to disposable glass tubes and dried. Then 100 µl of antiserum diluted 1/5000 for the progesterone assay or 1/200 000 for the oestradiol assay was added to all tubes, mixed briefly and left at room temperature for 30 min. Next, 100 µl of tracer was added and incubated overnight at 4°C. Subsequently, 100 µl of 5% gel PBS and 1 ml of dextran-coated charcoal were added. The tubes were left at 4°C for 15 min and then centrifuged at 5000 g at 4°C for 5 min. The antibody-bound fractions were decanted into counting vials, the scintillation fluid (3 ml Ready Safe; Beckman, Stockholm, Sweden) was added, and the samples were assayed in a scintillation counter. All samples were assayed in duplicate. The coefficients of variation for progesterone analysis by radioimmunoassay were 10% intra-assay and 12% interassay. Intra- and interassay coefficients of variation for oestradiol analysis were 9% and 11% respectively.

Immunolabelling of gap junctions

Sections of 10 µm were cut, deparaffinized in xylene, rehydrated in an ethanol series and incubated for 10 min in 0.1% trypsin in 0.2 M Tris buffer containing 0.1% calcium chloride. The trypsin treatment re-exposes antigenic sites that are sensitive to alteration during processing (Harlow and Lane, 1988). After a brief rinse in PBS, the sections were blocked using 0.1 M l-lysine in PBS containing 0.5% Triton X-100. Incubation with primary anti-connexin43 monoclonal antibody, purchased from Zymed Laboratories, Inc. (San Francisco, CA., USA), diluted 1:500 in PBS, followed for 16 h at 28°C. The sections were then given two washes in PBS, treated with biotinylated anti-mouse secondary antibody (raised in sheep; Amersham International, Amersham, UK), given a further two washes in PBS, and incubated with streptavidin–Texas Red (Amersham). In each case,
the reagents were diluted 1:250 in PBS and treatment was for 1 h. After two final washes in PBS, the sections were mounted with Fluoromount–G (Southern Biotechnology Associates, Birmingham, AL, USA) mounting medium. Standard controls were routinely run in parallel, including a positive control using human heart.

**Image recording and analysis**

Images were recorded using a Leica TCS 4D laser scanning confocal microscope (Leica, Milton Keynes, UK). In a preliminary series of experiments, it was found that myometrial smooth muscle cell gap junction distribution was very heterogeneous, and therefore extensive sampling over large areas was required to obtain reliable data. We therefore adopted the following comprehensive approach to analysis. Sixteen non-overlapping but adjacent sets of serial optical sections were recorded from each tissue section selected for analysis. Each set consisted of 10 optical sections (each 107 µm x 107 µm) at intervals of 0.5 µm in the z axis. This sampling exercise (Figure 1) was conducted on one section from each of eight patients, for each of the three experimental groups, giving an overall total of 3840 individual images. Only smooth muscle-rich regions were analysed; areas containing blood vessels, or those high in autofluorescence (due to basement membranes and fibrous tissue) were excluded from the analysis. Projections of each set of optical sections were then prepared, and the image data transferred to IBM PC format for analysis and measurement using PC Image (Foster Findlay Associates, Newcastle, UK). The areas and number of immunolabelled gap junctions were determined within each projection, and the data were analysed using Microsoft Excel or the MINITAB statistical package. Means and SE were calculated on both log e and untransformed data. Log e transformation was performed because the raw data showed distributions slightly skewed from normal. Linear regression analysis was carried out on untransformed data. Critical values corresponded to \( P < 0.05 \).

**Results**

Connexin43 gap junctions were detectable as clearly defined fluorescent spots in all the groups of patients examined (Figure 2). The specificity of the labelling for the detection of gap junctions was confirmed using cardiac muscle as a positive control (Figure 2, lower right panel). The punctate connexin43 labelling observed in the myometrial specimens was typically located at the level of the smooth muscle cell outlines and was readily visualized at microscopy, the series of spots often organized at the tapering tips of adjoining cells (Figure 3). The distribution of the label through the sections was markedly uneven: in single optical sections, some sample areas showed no connexin43 label, while others revealed abundant label.

Even when the sample volume was increased by taking sets of serial optical sections, prepared as projections, this irregular pattern of distribution remained apparent (Figure 3). Thus, to obtain a reliable sample of the quantity of connexin43 signal in each tissue section, 16 separate projections per section were used for analysis. Only by adopting this extensive sampling approach was it possible to make reliable comparisons of the quantity of connexin43 labelling between the patient groups.

Quantitative analysis of the three groups, ORL, BL, and NL, using the sampling procedure outlined in Figure 1, showed that the area of immunolabelled gap junctions per mm² of tissue was 2.1 ± 0.1 (ORL), 2.2 ± 0.1 (BL) and 1.8 ± 0.3 (NL) x 10⁵ (µm²). The number of immunolabelled gap junctions calculated per mm² of myometrium was 1.0 ± 0.9 (ORL), 0.8 ± 0.5 (BL) and 0.9 ± 0.1 (NL) x 10⁶. Neither the number of spots nor the area occupied by the spots was significantly different between the groups.

Correlation matrices were prepared to examine the relationship between immunolabelled gap junctions and hormone concentrations. The plots in Figure 4 illustrate the relationship between gap junction number versus the ratio of oestradiol to progesterone in maternal blood (Em/Pm). When all groups were pooled, a significant positive correlation \( (r = 0.786, \text{ critical value for } r \text{ at } P < 0.05 = 0.423) \) was found between the number of gap junctions and the Em/Pm ratio (Figure 4).
Figure 1. Diagram illustrating the sampling procedure used for the confocal analysis of gap junction protein. From each tissue section, 16 adjacent sets of serial optical sections were taken (top diagram). Each of these sets contained 10 optical sections (each $107 \mu m \times 107 \mu m$ in area), at intervals of 0.5 $\mu m$ (bottom left). Projected images of each of the 16 sets of optical sections were prepared (bottom right), giving a total sample volume of $9.16 \times 10^5 \mu m^3$ from each tissue section for analysis of gap junction immunofluorescence.

Figure 2. Examples of projected immunofocal images from each of the three patient groups, presented at optimal magnification for viewing labelled gap junction proteins. Gap junction proteins, visualized with anti-connexin43 monoclonal antibody followed by biotinylated secondary antibody and streptavidin–Texas Red, are seen as sharply defined spots, similar to those seen in human cardiac muscle, which was used as a positive control (lower right). In cardiac muscle cells, gap junction proteins have a characteristic, easily recognized pattern, at intercalated disks situated at the end-on abutments between cells. BL = before labour; NL = normal labour; ORL = oxytocin-resistant labour (dystocia). Scale bar = 25 $\mu m$. 

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Immunoconfocal analysis of connexin43 in myometrium

Figure 3. Example of a set of 16 projections, each formed from 10 serial optical sections, prepared for analysis using PC Image software, following the procedure described in the text. The sharp small bright spots represent immunostained connexin43 gap junctions, visible against the background of distinctive smooth muscle cell profiles. Each of the 16 images represents a tissue volume of 57 000 µm³. The quantity of gap-junctional signal varies considerably from one image sample to the next. The extensive sampling procedure was designed to take account of this variation. The example shown here comes from the before labour (BL) group. Scale bar = 25 µm.

The individual groups showed a similar positive correlation between the number of gap junctions and the Em/Pm ratio, with the following correlation coefficients: ORL group, $r = 0.941$ (critical value 0.707); BL group, $r = 0.660$ (critical value 0.754); NL group, $r = 0.457$ (critical value 0.707). There was no significant difference between these correlations for the different patient groups. There was a highly significant correlation ($r = 0.877$, critical value 0.412) between the number of gap junctions and gap junction area, and an analysis of the correlation between gap junction area and Em/Pm ratio resulted in the same conclusions as given above for the correlations between gap junction number and Em/Pm ratio.

There was no significant correlation between gap junction number (or area) and the progesterone (Pm) or oestradiol (Em) concentrations in maternal blood.

There was also a significant, positive correlation between the number (or area) of gap junctions and the ratio of oestradiol and progesterone in the myometrium (Emy/Pmy; for the pooled groups, $r = 0.480$ for gap junction number and $r = 0.518$ for gap junction area, critical value 0.412), but there was no significant correlation between gap junction number (or area) and the progesterone (Pmy) or oestradiol (Emy) concentrations in myometrium.

Table III summarizes the concentrations of oestradiol and progesterone in myometrium from the different groups of patients. The analysis of variance test showed no significant heterogeneity for oestradiol and progesterone in myometrium of the women from the different groups studied, nor did the progesterone/oestradiol ratio show any difference between the groups studied ($F = 3.23$, df $= 1,11$; $P = 0.3$).

Discussion

Direct cell-to-cell interactions mediated by intercellular junctions are increasingly being recognized to play important roles in the cellular events underlying the human reproductive process. While recent studies have implicated changes in the molecular constituents of adhesive junctions in, for example, endometrial shedding during menstruation (Tabibzadeh et al., 1995a,b), the present study focuses on a different category of cell-to-cell contact, the gap junction, responsible for cell-to-cell communication. The constituent proteins of gap junctions, connexins, form a multigene family of conserved proteins, 12 members of which have so far been reported in mammalian cells. One of these connexins, connxin32, has recently been reported in human endometrium (Jahn et al., 1995); another
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progesterone concentration in both maternal blood and in the

myometrium suggests that, in contrast to the situation in rodents (Garfield

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and cumbersome, and has a number of technical disadvantages, e.g. if the section is too thick, it may become impossible to
discriminate small gap junctions. Consequently, there may be
errors in the quantitative determination of the gap junctions
because of their size and the plane of sectioning, and standard
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of distribution apparent from the present study, accurate
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member, connexin43, has previously been identified as the
principal protein of human myometrial gap junctions (Tabb

et al

1992; Chow and Lye, 1994). In the present study, a
comprehensive immunoconfocal quantitative three-dimen-
sional analysis was conducted to determine the connexion43
gap junction content of myometrial tissue from patients before
labour (at term), in normal labour and in oxytocin-resistant
dystocia. The relationships between these data and the oestra-
diol/progesterone ratio, and the concentration of progesterone
and oestradiol individually, were analysed in the maternal
blood and in myometrial tissue.

Our finding that the gap junction content did not differ
significantly between the three groups investigated indicates
that dystocia is not due to decreased expression of immuno-
detectable gap junctions. This suggests that dystocia arises
either from factor(s) entirely unrelated to gap junctions, or to
factors involved in the regulation of gap junction channel
function that are undetectable by immunolabelling. Our results
are consistent with evidence that, in humans, the increased
expression of connexion43 required for labour occurs relatively
early compared with other species (Chow and Lye, 1994). The finding that gap junctions were expressed despite a high
progesterone concentration in both maternal blood and in the
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processed in parallel with the myometrial sections confirmed
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Table III. Progesterone and oestradiol concentrations in human term pregnant myometrium, and in maternal and fetal serum in three patient groups (n = 8 in each group). Values are means ± SE. Factorial analysis of variance showed no significant differences between the groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Myometrium (ng/g wet weight of tissue)</th>
<th>Maternal serum (nmol/l)</th>
<th>Fetal serum (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Progesterone</td>
<td>Oestradiol</td>
<td>Progesterone</td>
</tr>
<tr>
<td>Before labour (BL)</td>
<td>36 ± 12</td>
<td>3.8 ± 0.0</td>
<td>409 ± 82</td>
</tr>
<tr>
<td>Active phase of labour</td>
<td>35 ± 6</td>
<td>4.2 ± 0.0</td>
<td>305 ± 46</td>
</tr>
<tr>
<td>Normal labour (NL)*</td>
<td>36 ± 4</td>
<td>3.3 ± 0.3</td>
<td>410 ± 110</td>
</tr>
</tbody>
</table>

*Definitions based on Friedman (1971) and Philpott and Castle (1972).
defined assemblies of these proteins. In particular, it should be noted that immunofluorescence may potentially detect gap junction protein in transit to but not yet inserted in the plasma membrane, or not fully assembled in the form of gap-junctional plaques.

In a preliminary study using manual quantification of single optical sections of limited samples, we observed that numerous gap junctions were present in the myometrium of women at term and in labour despite a relatively high concentration of progesterone in maternal blood (Kilarski et al., 1993). Both that study and the results reported here provide no clear evidence that progesterone inhibits gap junction expression in the human myometrium. However, in the present study we present novel evidence that there is a significant positive correlation between immunolabelled gap junctions and the ratio of oestradiol to progesterone both in maternal blood and in the myometrium. From the pooled data in Figure 4 it might be thought that the correlation between gap junctions and Em/Pm is influenced mainly by one point, a patient with a very high Em/Pm ratio. This patient’s clinical history did not differ significantly from those of the other patients in this group, and although her Em value was high and her Pm value was low, these values as such were not outside the range found for other patients in this study. There is thus no obvious reason to exclude this patient from the data, but even when this is done, a positive correlation (P < 0.05) between gap junction area or number and Em/Pm persists (r = 0.447 and r = 0.568 respectively, with a critical value of 0.422).

The formation of functional myometrial gap junctions is widely reported to coincide with the onset of labour, and effective labour requires the synthesis of connexin43 during the days preceding labour (Garfield et al., 1978; Sims et al., 1982; Cole et al., 1985; Tabb et al., 1992). The process by which gap junctions come to play a role in the electrical coupling necessary for labour may be divided into two stages. The first stage would begin with the expression of the connexin43 gene and continue with synthesis of connexin protein in the endoplasmic reticulum and transport to and processing through the Golgi apparatus, a process that may take place towards the end of pregnancy. The second stage involves the trafficking from the Golgi, with insertion of the gap junction protein in the plasma membrane and the formation of functional gap junction plaques (Hendrix et al., 1995). Either or both parts of the process are thought to be hormone dependent, and our results appear to show that it is the oestradiol-to-progesterone ratio rather than the individual hormone concentrations that is relevant for the hormonal control of gap junction formation. If, during pregnancy, sufficient gap junction proteins have been synthesized and there is no inhibitory effect of high progesterone concentrations on the insertion of gap junction proteins in the membrane and on labour itself, this would explain why spontaneous labour may occur in women even in the presence of high concentrations of progesterone.

Our finding of similar concentrations of connexin43 immunolabelling in the three patient groups may at first sight seem inconsistent with earlier results from transmission electron microscopy (Kilarski et al., 1993). One possible explanation for this apparent discrepancy is that, in ORL and BL patients, gap junction proteins may have been synthesized in normal amounts but not yet inserted or fully assembled in the membrane as a functional cell coupling system. This would imply halting of the process at some step distal to production of gap junction protein. At this stage, it cannot be excluded that connexins other than connexin43 may contribute to gap-junctional intercellular communication in the experimental groups examined, though additional connexin isoforms have yet to be reported in human myometrium. It should further be noted that our immunocytochemical data, while reflecting the relative capacity for cell-to-cell communication via connexin43 gap junctions, do not directly indicate the functional state of these gap junctions; opening and closure of gap-junctional channels may well be influenced by steroid hormones (Ciray et al., 1996).

There are many examples in which steroids have been shown to exert their effects through regulation of gene transcription (Evans, 1988). The data of Saito et al. (1985) in rats, showing that the relationship between the changes in oestrogen and progesterone concentrations and the appearance of gap junctions at term is paralleled by changes in nuclear receptors for these steroids, are also consistent with an effect at the level of transcription. It is possible that progesterone may exert direct effects on the rate of connexin43 transcription. This steroid could act directly, either by reducing the number of oestrogen receptors (Clark and Peck, 1979) or by decreasing the number of nuclear acceptor sites for the oestrogen receptor and increasing the rate of turnover of the oestrogen receptor (Leavitt et al., 1987). MacKenzie and Garfield (1986) reported that administration of oestrogen postpartum was unable to maintain high numbers of gap junctions in the myometrium. In the rat myometrium the number of steady-state transcripts encoding connexin43 in the rat myometrium is reported to be regulated positively by oestradiol and negatively by progesterone (Petrocelli and Lye, 1993; Lye et al., 1993). However, from the present study, it would appear that data in rats may not be extrapolated to the situation in women; our finding that immunolabelled gap junction numbers were not significantly different in women despite differences in clinical and functional status emphasizes that further investigation into gap junction formation and regulation in human labour is warranted. It should also be mentioned that gap junction formation is just one component of the multifaceted process of labour. Events taking place at the start of labour include the synthesis of other contraction-associated proteins, ion channels and stimulant receptors as well as appropriate changes in cervical structure.

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