

CONTROL OF MYOMETRIAL CONTRACTILITY

AN EXPERIMENTAL STUDY IN THE CHRONICALLY
INSTRUMENTED EWE

REGULATIE VAN MYOMETRIUM CONTRACTILITEIT

EEN EXPERIMENTEEL ONDERZOEK BIJ HET CHRONISCH
GEINSTRUMENTEERDE SCHAAP

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I

General introduction

Myometrial contractility and its control constitute areas of intense scientific interest since several decades. The regulation and modulation of myometrial activity during pregnancy and parturition present the foremost example of hormonal control of smooth muscle activity. From a relatively quiescent state during pregnancy the myometrium is transformed into an organ that displays the vigorous and coordinated activity characterizing labor. There is growing evidence that prostanoids play a pivotal part in the initiation and maintenance of parturition. The name prostaglandins was coined by Von Euler in 1935 (55), 5 years after the first report (27) of their biological action on uterine contractility, and 30 more years passed before the structure and biosynthesis of this extensive group of "äther- und wasserlösliche Substanzen" were gradually discovered (4,48). It became apparent that all these active substances are derived from 20-carbon polyunsaturated fatty acids, but that major differences exist between the chemical structures of these compounds, which are now collectively termed eicosanoids (12). The term prostanoids is usually applied to all products derived from prostaglandin endoperoxides and includes the prostaglandins A through F, prostacyclin (PGI₂) and the thromboxanes.

Although the role of prostanoids in the initiation and progression of labor seems well established (7), the factors regulating prostanoid synthesis and release have not yet been elucidated in all species (29). In sheep the fetal pituitary-adrenal axis controls the beginning of labor (28). The increase in fetal cortisol activates placental 17 α -hydroxylase which promotes the conversion of progesterone to estrogen, resulting in an elevation of the estrogen:progesterone ratio (14). The shift in placental steroid synthesis with the development of estrogen dominance serves as a trigger for the production of prostaglandin F_{2 α} and initiation of labor. In humans the contribution of prostaglandins to the initiation and progression of parturition is well recognized, but the mechanisms controlling prostanoid synthesis are still obscure (56).

In concert with the increase in estrogen and prostaglandin synthesis that marks the beginning of labor in the pregnant ewe, there is an increase in coordination and synchronization of myometrial electrical and mechanical activity. Half a century ago Bozler (5) was the first to demonstrate that estrogens improve the excitability and propagation of impulses in the myometrium. He postulated that excitation was directly conducted from one muscle cell to another. In later years, ultrastructural studies showed the presence of specialized structures for junctional contact between cells

(20). These structures, so-called gap junctions, connect the cytoplasm of two adjacent cells, thereby providing the possibility of electrical coupling and metabolic cooperation. Physiologic, biochemical and morphologic studies have established that gap junctions are sites of electrical and metabolic communication (20,22,40).

Gap junctions are almost absent during pregnancy, when myometrial activity is minimal. Prior to the onset of labor an increase occurs in the number of gap junctions between myometrial smooth muscle cells; gap junctions were shown to be present in large numbers during term or preterm labor in all species studied, including humans (20,50). Gap junction formation in the myometrium appears to be controlled by estrogens, progesterone and prostanoids (17,20). In several species, including ewes, the estrogen dominance that develops prior to labor induces the formation of large numbers of gap junctions (20,50). It has also been demonstrated that gap junction permeability can be regulated by physiologic systems (10,20).

This thesis presents the results of experimental studies to evaluate the functional relationship between estrogen and prostanoid action, formation of gap junctions, and myometrial activity. The following questions were specifically addressed:

- are estrogen-induced changes in myometrial activity related to the formation of gap junctions ?
- is formation of gap junctions associated with improved coordination of electrical and mechanical myometrial activity?
- what is the role of prostanoid synthesis in the modulation of myometrial activity and formation of gap junctions as related to the effects of estrogen ?

The chronically instrumented oophorectomized ewe was used as the experimental model to assess the effects of pharmacologic manipulation of electrical and mechanical myometrial activity in the absence of endogenous ovarian hormones. A separate study was designed to analyse the possible effects of instrumentation with an intrauterine pressure catheter on myometrial activity in the chronically instrumented oophorectomized ewe. The assessment of electrical and mechanical signals of myometrial activity obtained in vivo required the development of a program for computer-aided analysis which is also presented in this thesis.

II

Electrical and mechanical myometrial activity and gap junctions in the estrogen-treated oophorectomized ewe

Introduction

Gap junction formation during labor has been suggested to be a necessary step in facilitating coordination of contractile forces in the myometrium (16). In a previous study in sheep it was shown that the rise in the number of gap junctions during parturition is related to increased coordination of myometrial activity (52). The formation of gap junctions is thought to be regulated by steroid hormones and prostaglandins (18). In sheep the number of gap junctions is low during pregnancy. During parturition it shows a rise, which appears to be related to a fall in progesterone and an increase in estradiol concentrations in plasma (52). In non-pregnant oophorectomized ewes administration of estradiol-17 β induces an initial decrease in myometrial activity, followed by an increase (33,51). Together with the increase in frequency of intrauterine pressure cycles, the shape of the cycles is also changed after administration of estradiol, as shown by an increased rate of rise of the intrauterine pressure cycles (61).

The aim of the present study was to investigate whether the changes in myometrial activity following administration of estradiol are related to gap junction formation. The chronically instrumented non-pregnant ewe is a useful model to investigate the regulation of myometrial gap junction formation and the influence of gap junctions on myometrial contractility, as it allows a much easier manipulation of the factors that seem to control gap junction formation than does the pregnant animal. In this study electrical and mechanical myometrial activity in oophorectomized ewes was investigated before and after administration of a single dose of 0.1 mg of estradiol-17 β , and myometrial gap junction area was quantitated.

* The main substance of this chapter was published in: Verhoeff A, Garfield RE, Ramondt J, Wallenburg HCS. *Am J Obstet Gynecol* 1986;155:1192-1196.

Material and Methods

Animals and instrumentation

Studies were performed in six chronically instrumented non-pregnant Texel ewes. The ewes were oophorectomized and instrumented under general anesthesia with 500 mg of ketamine hydrochloride, 0,5 mg of atropine and 300-500 mg of sodium thiopental administered intravenously. The animals were intubated and ventilated with 40% oxygen and 60% nitrous oxide, and 0.5-4 volume % enflurane. A polyvinyl catheter was inserted into the abdominal aorta through a femoral artery. A lower midline laparotomy was performed, uterus and adnexa were exposed, and both ovaries were removed. Three pairs of bipolar, silver-chloride coated silver electrodes were fixed to the anterior part of the myometrium in the fundal, medial and cervical regions of both uterine horns for recording of the electrical myometrial activity. The needles of electrodes were 3 mm long with a diameter of 0.2 mm; the distance between the needles was 2 mm. Sponge-tipped pressure catheters to record intrauterine pressure were inserted into the cavity of both uterine horns through a small incision in the cervical region. The wires and catheters were passed subcutaneously to a pouch attached to the ewe's flank. The ewes were allowed to recover from the operation for at least one week before experiments were started. The arterial catheter was intermittently flushed with saline containing heparine.

Recording procedures

During the recording periods the ewes were housed in wooden boxes of 2.2 square meters in a quiet environment, with the recording equipment situated in an adjoining room. The electrical signals were filtered by a band-pass filter; for the lower and higher cutoff frequencies (-3dB) 1 and 30 Hz were selected. Intrauterine pressure was measured by a Gould Statham P 23 ID pressure transducer. The myometrial electromyogram (EMG) from three different regions as well as one intrauterine pressure (IUP) signal were recorded on a Gould Brush 2800 eight-channel polygraph with a paper speed of 25 mm/min, and stored on magnetic tape for off-line computer analysis.

Experimental protocol

Electrical and mechanical myometrial activity were recorded continuously from 16-24 hours before to 72 hours after the intraarterial administration of 0.1 mg of estradiol-17 β in 10% ethanol. Myometrial activity was again recorded from 140 to 150 hours after the administration of estradiol. Fifty IUP cycles obtained before and 6, 24, 48, 72 and 144 hours after the administration of estradiol were used for computer analysis. Three to six myometrial biopsies (5 by 2 by 1 mm) were taken before and 6, 24, 48, 72 and 144 hours after the administration of 0.1 mg of estradiol. The procedure was performed under spinal anesthesia with 6-10 ml of 0.5% bupivacain after sedation with ketamine hydrochloride. Biopsies were obtained from two animals at each sampling time; for obvious reasons not all tissue samples could be taken from each animal.

Analytic procedures

The IUP signals and the EMG were analyzed by computer. The algorithms for the analysis of the intrauterine pressure and electrical myometrial activity signals are described in detail in chapter 6. After detection of intrauterine pressure cycles (a temporal rise of 10 mmHg above the tonus), the maximum rate of rise (calculated in a moving window of 2 seconds), the peak pressure (maximum pressure minus the tonus) and the active pressure area (area under the IUP curve corrected for the tonus) were determined. The EMG was analyzed for periods of distinct electrical activity, called single bursts.

The processing of the tissues and electron microscopic quantitation of gap junctions has been outlined extensively before (17,19). Briefly, the length of the plasma membrane was determined in 20 electron micrographs (at x 33,600 magnification) from each tissue. Each possible gap junction was further enlarged to x 100,000 magnification for identification and measurement. From these measurements the percentage of gap junction area relative to the area of plasma membrane was determined according to a procedure which has been reported in detail (19).

Student's t-test was used for statistical analysis of differences between values of maximum rate of rise, peak pressure, active pressure area and percentage gap junction area before and after administration of estradiol. Values of $p < 0.05$ were considered significant.

Results

Recordings of two ewes were incomplete because of catheter malfunctioning; these recordings were not used for analysis. In all animals marked spontaneous myometrial activity was present before administration of estradiol. The electrical activity was characterized by a rhythmic pattern of trains of bursts of electric activity corresponding with intrauterine pressure cycles with a frequency of 0.75 to 1 per min. Following the administration of estradiol, myometrial activity disappeared within one hour. Electrical activity returned after a silent period of approximately two hours, mechanical activity reappeared after five to seven hours. Figure 2.1 shows a composite of recordings of electrical and mechanical activity before and at several time intervals after administration of estradiol. Before and 30 to 40 hours following the injection of estradiol, the IUP cycles usually showed several small peaks; so-called "small waves". These small waves were related to bursts of electrical activity. The frequency of the bursts of electrical myometrial activity and the frequency of the IUP cycles in one ewe, from 12 hours to 72 hours after the administration of estradiol, are shown in figure 2.2. In all animals the frequency of bursts in the EMG and IUP cycles following the administration of estradiol, show periods of high activity alternating with periods of low activity. This pattern gradually returned to the pattern observed before the administration of estradiol.

Figure 2.3 presents the mean values of the maximum rate of rise, peak pressure and active pressure area calculated from 50 cycles before, and at various times after the administration of estradiol.

The maximum rate of rise showed a sharp increase at 24 hours after estrogen administration followed by a gradual decline. The peak pressure was increased at 24, 48 and 72 hours after injection of estradiol. At 144 hours after estrogen administration peak pressure had returned to the level measured before. The active pressure area was low at 24 hours, and showed an increase at 48 and 72 hours. At six hours after estrogen administration three ewes showed no myometrial activity at all.

Figure 2.4 presents the means of the percentage increase of the maximum rate of rise, peak pressure and active pressure area calculated in four sheep, together with the changes in gap junction area. The maximum rate of rise reached its highest level at 24 hours, and peak pressure and active pressure area at 48 and 72 hours, respectively, following the administration of estradiol.

The results of the quantitative evaluation of the tissues for gap junction area are presented in table 2.1. Gap junctions were present in low numbers in tissues taken prior to the administration of a single intraarterial injection of 0.1 mg of estradiol-17 β . Following injection of

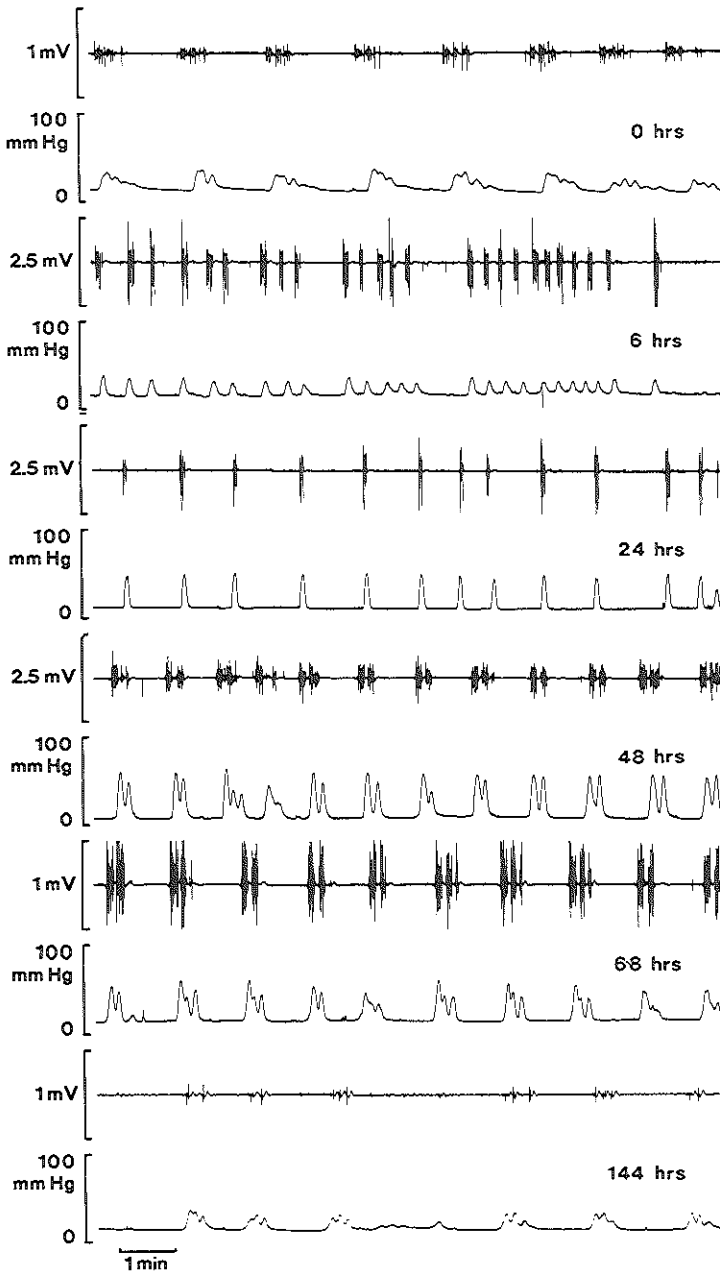


Figure 2.1 Myometrial activity before (0 hrs), and 6, 24, 48, 72 and 144 hours after administration of 0.1 mg estradiol-17 β recorded in one ewe. Top tracing of each set shows the EMG, bottom tracing the IUP.

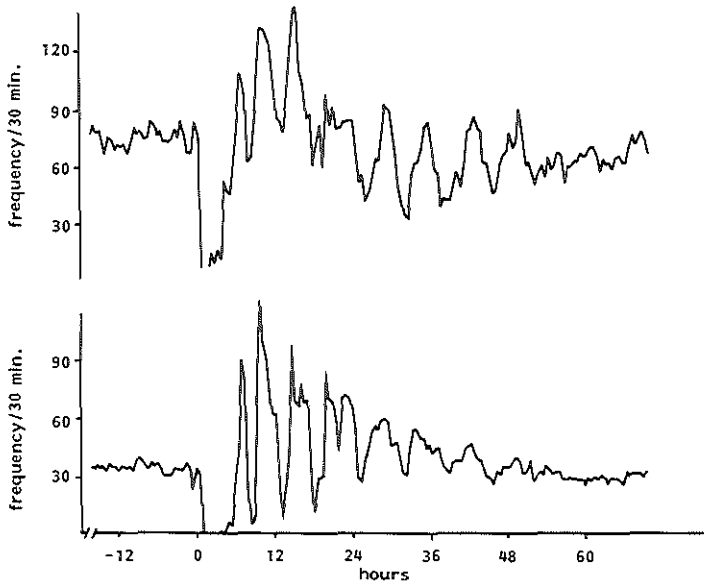


Figure 2.2 Frequency of bursts (top) and of intrauterine pressure cycles (bottom) in one ewe from 12 hours before to 72 hours after administration (at 0 hours) of 0.1 mg estradiol-17 β .

estradiol there was a rise in gap junction area, which reached maximum levels at 24 hours. Thereafter, a gradual fall was observed, and pretreatment levels were reached at about 72 hours.

Discussion

This study in the chronically instrumented oophorectomized ewe confirms earlier observations that marked electrical and mechanical myometrial activity occurs in the absence of endogenous ovarian hormones (51). It cannot be excluded that the instrumentation applied in these experiments, in particular the presence of intrauterine pressure catheters, could influence the occurrence and pattern of spontaneous myometrial activity. Estradiol-induced changes in myometrial activity in non-pregnant oophorectomized sheep appear to be associated with formation of gap junctions. Intraarterial administration of a single dose of 0.1 mg of estradiol-17 β is followed, after one hour, by a quiescent period of approximately two hours, after which electrical activity returns

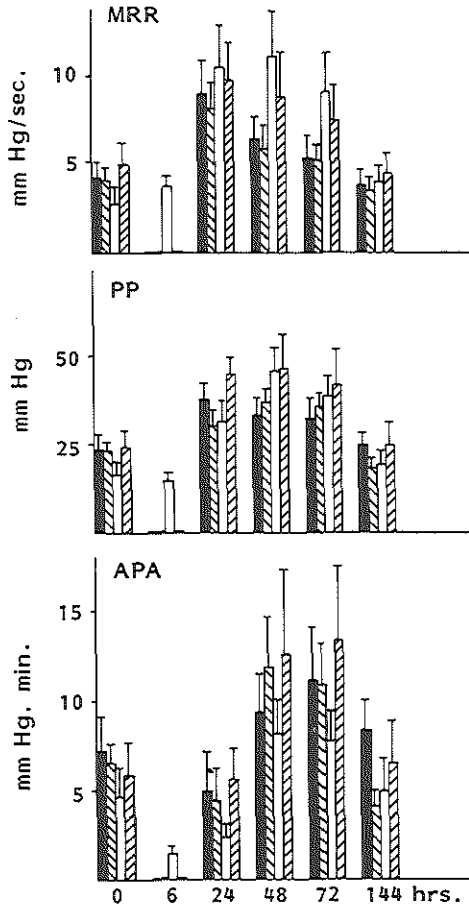


Figure 2.3 Mean values (+ S.D.) of maximum rate of rise (MRR), peakpressure (PP) and active pressure area (APA) in 50 intrauterine pressure cycles in four ewes before (0) and 6, 24, 48, 72 and 144 hours after administration of 0.1 mg estradiol-17 β .

first, followed by the reappearance of mechanical activity three to five hours later. These observations confirm previous studies from this laboratory (51). The observed occurrence of a high frequency of intrauterine pressure cycles alternating with periods with a low frequency has also been reported by others (32,33,61). The discrepancy between the frequency of the intrauterine pressure cycles and the frequency of the bursts in the present study (figure 2.2), can in part be explained by the

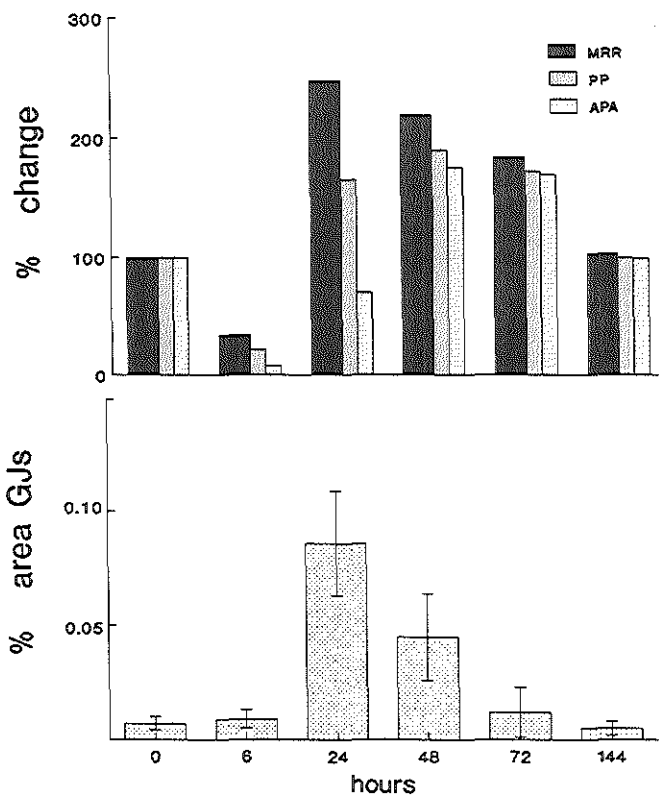


Figure 2.4 Upper graph: relative change of maximum rate of rise, peak pressure, and active pressure area; means from four ewes. Lower graph: means (+ S.E.M.) of the percentage gap junction area. 0.1 mg estradiol-17 β administered at 0 hours.

Table 2.1. NUMBER AND AREA OF MYOMETRIAL GAP JUNCTIONS (GJs) IN OOPHORECTOMIZED EWES BEFORE AND AFTER ADMINISTRATION OF ESTRADIOL-17 β

hr after estradiol	No. GJs	total length GJ membrane (μm)	total length non-GJ membrane (μm)	relative area GJ membrane (% \pm S.E.M.)	P**
0	4	0.420	12,330	0.007 \pm 0.003	-
6	8	0.840	18,766	0.009 \pm 0.004	NS
24	34	3.720	13,370	0.086 \pm 0.023	<.01
48	14	2.100	9,510	0.045 \pm 0.019	NS
72	3	0.300	4,752	0.012 \pm 0.011	NS
144	3	0.195	8,184	0.005 \pm 0.003	NS

** As compared with control (0) values

method of burst detection that was applied. Bursts as detected by the computer occur concomitantly with "small waves" in the intrauterine pressure signal (see figure 2.1). In addition, bursts also occur after the quiescent period without any elevation in IUP or in association with a rise in IUP of less than 10 mg Hg, which is below our criterium for detection.

An increase in the rate of rise of IUP cycles after estradiol administration has also been observed by other investigators in sheep (61) and rats (57). The rate of rise can be regarded as a function of the recruitment of myometrial smooth muscle cells (13). The maximum increase in rate of rise occurs 24 hours after administration of estradiol and corresponds with the maximum increase in myometrial gap junction area. This suggests that the observed increase in myometrial gap junction area enhances the coordination of the myometrial muscle cells. Similar observations were made in a study in oophorectomized postpartum rats equipped with a balloon catheter, in which the number of gap junctions showed an increase concomitant with an increase in the rate of rise of IUP cycles, 15 hours after estrogen treatment (57). Windmoller et al. (61) found a significantly increased response to a bolus challenge with oxytocin or prostaglandin $F_{2\alpha}$, 30 hours after estradiol infusion in oophorectomized sheep. This corresponds with the period of time in which gap junction area was found to be significantly elevated in the study reported here.

It is remarkable that maximal peak pressure and maximal active pressure area occurred at 48 and 72 hours after the administration of estradiol, respectively (figure 2.4), when gap junction area appeared to be not significantly greater than before administration of estradiol. This may be explained by the fact estrogens also stimulate uterine synthesis of prostaglandins (32), receptors (23), contractile filaments (13), uterine blood flow (26), and the number and size of myometrial cells (9). Each of these factors is known to affect uterine contractility.

The decline in the number of gap junctions from a maximum level at 24 hours to a low level at 72 hours after a single bolus of estradiol suggests a life span of gap junctions in vivo of approximately 48 hours. This is in agreement with studies in various pregnant animals which show a marked decline in the number of gap junctions 24 to 48 hours after parturition (53).

The close relationship between gap junction area and changes in the pattern of myometrial activity, in particular the maximum rate of rise, in non-pregnant ewes, provides further evidence to support the hypothesis that gap junctions improve the coordination of contractile forces in the myometrium.

In the pregnant uterus, the physiologic role of gap junctions seems to be to facilitate the spread of electrical activity during parturition. As yet the physiologic role of gap junctions in the non-pregnant uterus remains to be established.

III

Effects of chronic instrumentation on electrical and mechanical myometrial activity in the oophorectomized ewe

Introduction

In the study in oophorectomized ewes, chronically instrumented with myometrial electrodes as well as with intrauterine pressure catheters, reported in chapter 2, marked spontaneous electrical and mechanical uterine activity, showing a continuous rhythmic pattern, was observed, in the absence of endogenous ovarian steroid hormones. In oophorectomized ewes instrumented with an intrauterine pressure catheter a pattern of continuous low amplitude and low frequency intrauterine pressure cycles was present (33). A quite different pattern of electrical activity, showing low-amplitude bursts of electrical activity, lasting five to ten minutes at a frequency of three to four per hour, was reported by Rousseau and Prud'homme (43) in oophorectomized ewes instrumented with electrodes only. Since on the basis of the previous experiments it could not be excluded that instrumentation, in particular the presence of the intrauterine pressure catheter, could have had an influence on the occurrence and pattern of spontaneous myometrial activity, the present study was designed to investigate the effect of instrumentation on the pattern of spontaneous uterine activity in oophorectomized ewes.

Materials and methods

Animals and instrumentation

Eight chronically instrumented, oophorectomized Texel ewes were used for this study. The surgical procedures and methods of instrumentation are described in detail in chapter 2. Polyvinyl catheters were inserted into the abdominal aorta through a femoral artery and into a femoral vein. In four ewes three bipolar, silver chloride-coated silver electrodes were fixed to the anterior part of the myometrium in the tubal, middle and cervical regions of both uterine horns, and a sponge-tipped pressure

** The main substance of this chapter was published in: Ramondt J, Verhoef A, Wallenburg HCS. Eur J Obstet Gynecol reprod Biol 1987;25:77-85.

catheter was introduced into the cavity of the left horn (catheterized, C ewes). The other four ewes were only equipped with three electrodes on the left uterine horn, without an intrauterine catheter (non-catheterized, NC ewes). The ewes were allowed to recover from surgery for two weeks before experiments were started. The arterial and venous catheters were regularly flushed with saline containing heparin.

Recording procedures

The electrical signals were filtered by a band-pass filter, with a lower and higher cutoff frequency of 1 and 30 Hz, respectively. Intrauterine pressure was measured by a Gould Statham P 23 ID pressure transducer. All signals obtained were recorded on a Gould Brush 2800 eight-channel polygraph.

Experimental protocol

In the four NC ewes the myometrial electromyogram (EMG) was recorded continuously for 24 hours to establish the presence or absence of a pattern of diurnal variation. After 24 hours 0.1 mg of estradiol-17 β was administered in 10 ml of 10% ethanol as an intraarterial bolus and electrical activity was again recorded for 24 hours.

Two NC ewes underwent a second laparotomy 6 weeks after the previous operation, and three electrodes were fixed to the right horn and a sponge-tipped catheter was inserted into the left horn (NC-C ewes). Following a recovery period of two weeks electrical and mechanical myometrial activity were recorded for 10 hours.

In the four C ewes the EMG and intrauterine pressure (IUP) were recorded for 10 hours before intraarterial administration of 0.1 mg of estradiol-17 β and for 24 hours thereafter. One week later the same protocol was repeated with, in addition, intravenous infusion of the prostaglandin synthetase inhibitor naproxen sodium (4 g in 100 ml of 0.1 M phosphate buffer, pH 8) starting after the first four hours of recording. The infusion pump was set to deliver 160 mg/hr.

Analytic procedures

Each EMG was analyzed visually for epochs of distinct electrical activity, referred to as bursts. Bursts were defined as episodes in which the amplitude of EMG signals showed an increase of at least 25% of the maximum amplitude in that recording period for more than 15 seconds.

Episodes occurring less than 15 seconds apart were taken to represent one burst. The bursts were analyzed for duration in minutes and frequency per hour. The IUP signals were qualitatively analyzed for the presence or absence of periodic elevations of intrauterine pressure. In contrast to the studies described in chapters 2 and 4, the recordings were not analyzed by computer, since this study was designed to compare patterns of electrical activity rather than to obtain a detailed quantitative analysis. The Mann-Whitney U test was used for statistical comparison of the duration of bursts before and at various time intervals after administration of estradiol-17 β .

Results

All ewes remained in good condition and no malfunction of electrodes or catheters occurred throughout the experiments. In general, two distinct patterns of myometrial electrical activity were recognized: a pattern with short bursts (duration of less than 1 min) and a pattern with long bursts (duration of more than 3 min).

NC ewes

Spontaneous electrical myometrial activity in the NC ewes was characterized by long bursts of electrical activity with an average duration of 5 to 7 min and with irregular spikes of electrical activity between the bursts. The bursts occurred with a frequency of 1.7 to 3.3 per hours. The maximum amplitudes of spikes in these bursts ranged from 0.4 to 0.6 mV. There were no obvious differences between amplitude, frequency and duration of the bursts in the recordings from different regions of the uterus. Figure 3.1 shows long bursts, recorded from the tubal middle part of the left horn. No pattern of diurnal variation with regard to frequency or duration of the bursts was apparent in any of the 24 hour tracings.

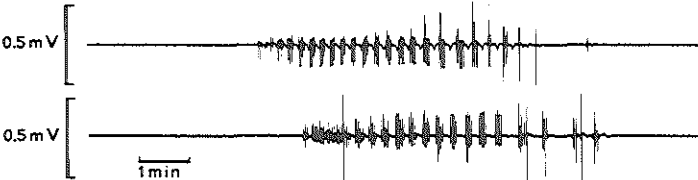


Figure 3.1 EMG recorded from the tubal (top) and middle (bottom) part of the left uterine horn in an non-catheterized ewe before administration of estradiol-17 β .

Intraarterial administration of 0.1 mg of estradiol-17 β resulted in disappearance of electrical myometrial activity within one hour in all ewes. The silent period lasted approximately one hour in two animals and two to three hours in the other ewes. The electrical activity returned in increasingly frequent, irregularly occurring short bursts which gradually grouped into long bursts. Twelve hours after administration of estradiol-17 β the short bursts between the long bursts had almost disappeared. Table 3.1 shows the duration and frequency of the long bursts before and during various time intervals after administration of estradiol-17 β . The duration of the long bursts was significantly elevated in all animals for up to 24 hours after administration of estradiol ($p < 0.05$). The frequency of the long bursts also showed an increase, which could not be statistically evaluated due to the small total number of bursts. The amplitude of the spikes in the bursts increased to 0.7 to 1.2 mV after estradiol. Figure 3.2 shows a composite of two recordings of electrical myometrial activity before and after estradiol treatment.

Table 3.1. DURATION (D, in minutes \pm S.D.) AND FREQUENCY (F, per hour) OF LONG BURSTS OF ELECTRICAL ACTIVITY, (data obtained from four oophorectomized, non-catheterized ewes, from 24 hr before to 24 hr after intraarterial administration of 0.1 mg of estradiol-17 β)

time before estradiol		sheep 1	sheep 2	sheep 3	sheep 4
8.00-14.00	D	6.2 \pm 1.7	5.5 \pm 1.6	7.2 \pm 3.1	6.4 \pm 2.3
	F	1.7	2.5	2.5	3.2
14.00-20.00	D	6.7 \pm 1.2	6.4 \pm 1.9	6.9 \pm 1.9	5.6 \pm 2.1
	F	1.7	2.2	2.5	3.3
20.00- 2.00	D	5.3 \pm 1.0	6.9 \pm 1.7	6.4 \pm 1.6	5.3 \pm 2.0
	F	1.8	2.0	2.3	3.3
2.00- 8.00	D	5.0 \pm 1.5	5.5 \pm 1.1	6.4 \pm 2.6	5.5 \pm 1.6
	F	2.2	2.5	2.7	3.0
hours after estradiol					
1 - 6		cessation of activity within one hour and return after two to four hours, with increasing spiking activity, gradually grouping into long bursts			
7 - 12	D	7.8 \pm 1.6	5.4 \pm 2.0	10.4 \pm 2.0	7.5 \pm 2.4
	F	3.7	3.0	3.0	2.8
13 - 18	D	7.3 \pm 1.4	8.1 \pm 1.5	9.9 \pm 2.1	7.7 \pm 1.9
	F	3.3	2.7	3.3	4.3
19 - 24	D	7.3 \pm 0.7	6.9 \pm 1.4	8.5 \pm 1.6	7.8 \pm 2.2
	F	2.0	2.0	3.4	2.8

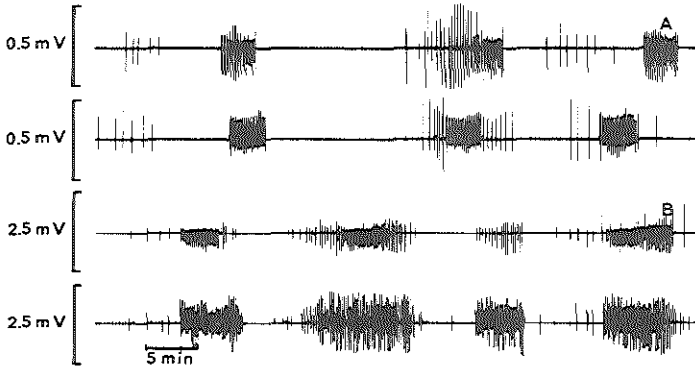


Figure 3.2 EMG in a non-catheterized ewe before (A) and 16 hours after administration of estradiol-17 β (B), recorded from the middle (top) and the tubal (bottom) part of the left horn.

NC-C ewes

The EMG recorded from the left horn of the two ewes after secondary introduction of a pressure catheter into the left horn was markedly different from the EMG recorded before insertion of the IUP catheter, and comparable with the recordings obtained from the catheterized horn of the four ewes that were equipped with an IUP catheter during the first operation. The tracings showed bursts of electrical activity, corresponding with IUP cycles, with a duration of 0.3 to 0.5 min and a frequency of 30 to 60 per hour. No long bursts of electrical activity were observed. The EMG of the right horn in NC-C ewes was comparable to the EMG obtained in the NC ewes. Figure 3.3. shows a recording of the

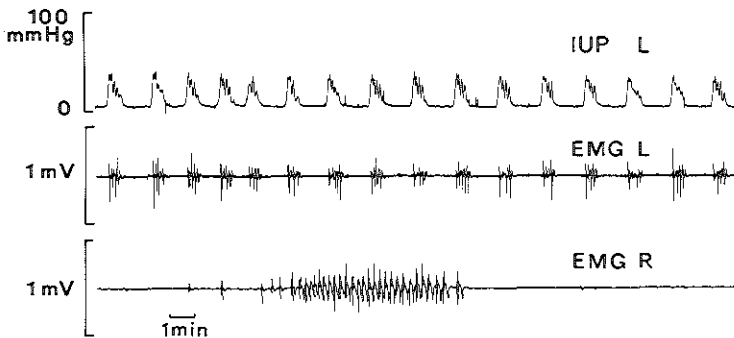


Figure 3.3 EMG recorded from the middle part of the left (catheterized) and the right (non-catheterized) uterine horn with the IUP recording from the left horn in a ewe equipped with a catheter during the second surgery, before administration of estradiol-17 β .

IUP and EMG of the left horn and the EMG of the right horn, obtained from a ewe instrumented with an IUP catheter in the left horn.

C ewes

The EMG of the right horn of the C ewes showed bursts of long duration similar to those in the EMG of the NC ewes. The long bursts occasionally disappeared for one or two hours, to be replaced by irregular occurring short bursts. The EMG of the left horn showed a contrasting pattern with regularly occurring short bursts of electrical activity (duration less than 1 min) corresponding with IUP cycles with an amplitude of 15 to 40 mm Hg. The tracing shown in figure 3.4 was obtained from a C ewe before administration of estradiol-17 β .

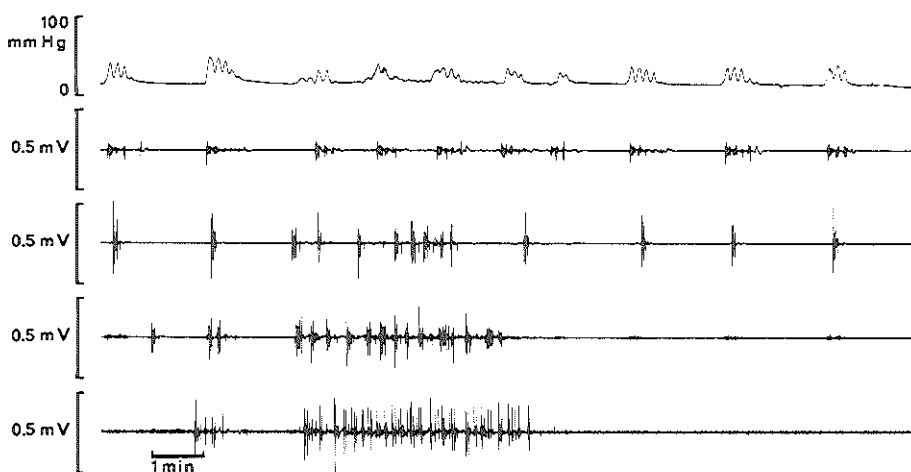


Figure 3.4 IUP and EMG recorded from a catheterized ewe before administration of estradiol-17 β . From top to bottom: IUP left (catherized) horn, EMG middle part left horn, EMG tubal part left horn, EMG middle part right (non-catheterized) horn, EMG tubal part right horn.

Myometrial activity in the C ewes disappeared within approximately one hour following administration of estradiol. Electrical activity reappeared in both horns in a similar pattern, after a silent period of two to three hours, and mechanical activity returned after six to seven hours. The frequency of the IUP cycles in the left and of the EMG bursts in both horns increased to a higher level than observed before administration of estradiol. An EMG and an IUP recording obtained 24 hours after estradiol treatment is presented in figure 3.5. Within the pattern of short

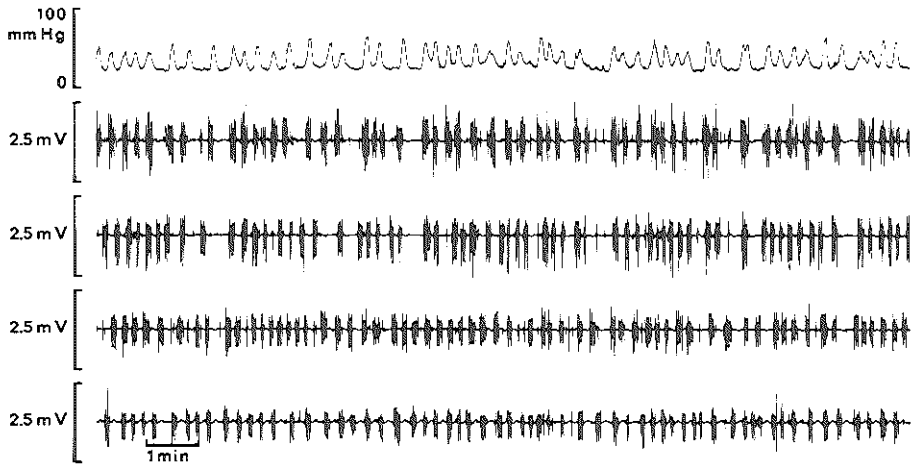


Figure 3.5 IUP and EMG recorded from a catheterized ewe 24 hours after administration of estradiol- 17β . Tracings arranged as in figure 3.4.

EMG bursts and corresponding IUP cycles a few long bursts occurred, concurrently with periods of sustained elevated basal pressure. The long bursts appeared with a low frequency (less than 0.5 per hour) in the period between 12 and 24 hours after administration of estradiol- 17β . Infusion of naproxen sodium in C ewes was followed by a fall in electrical myometrial activity recorded from the catheterized horn. After an infusion period of three to four hours, bursts of long duration, with corresponding elevations in IUP, began to appear in the catheterized horn. The pattern of long bursts in the non-catheterized horn was not interrupted. A recording, made 16 hours after the start of naproxen infusion, is presented in figure 3.6. The electrical activity of the myometrium following administration of estradiol, during naproxen infusion, was similar to that in NC ewes following estradiol although the short bursts did not disappear completely. The electrical activity increased two to three hours after administration of estradiol. Figure 3.7 shows the EMG and IUP recording obtained 24 hours after administration of estradiol- 17β in a C ewe treated with naproxen sodium. The figure shows long bursts of electrical activity in both horns. The IUP recording shows a sustained elevation of intrauterine pressure during the long burst.

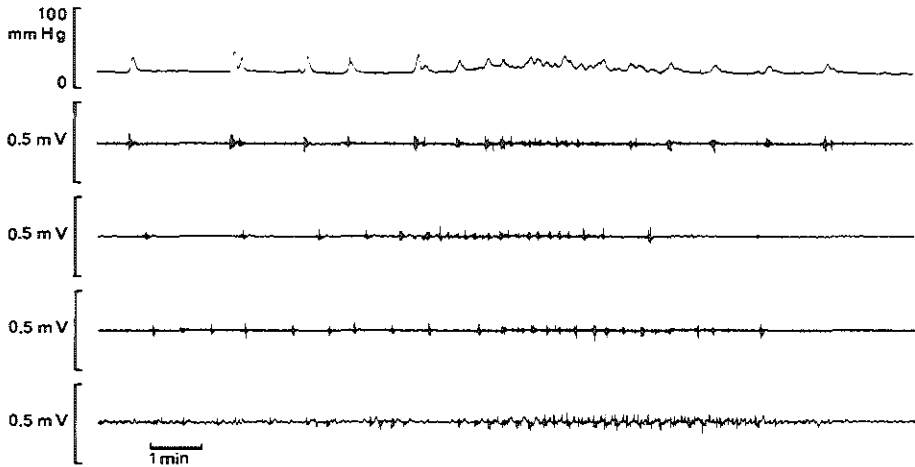


Figure 3.6 IUP and EMG recorded from a catheterized ewe 16 hours after the start of continuous naproxen infusion. Tracings arranged as in figure 3.4.

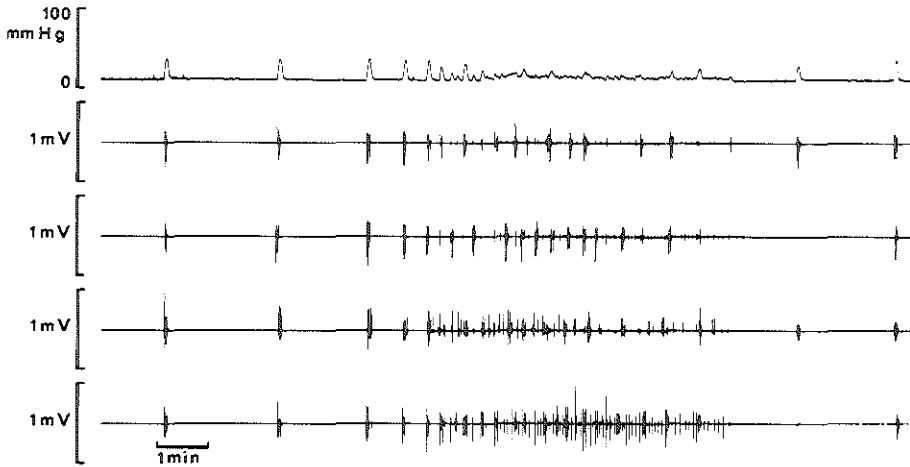


Figure 3.7 IUP and EMG recorded from a catheterized ewe 24 hours after administration of estradiol-17 β during continuous naproxen infusion. Tracings arranged as in figure 3.4.

Discussion

The results of this study indicate that myometrial activity in chronically instrumented ewes is markedly influenced by the presence of an intrauterine pressure recording device.

The presence of long bursts of electrical activity in oophorectomized NC ewes without steroid treatment confirms observations reported by Rousseau and Prud'homme (43), who described low-amplitude bursts of electrical activity, lasting five to ten minutes at a frequency of three to four per hour. However, Van der Weyden (49) observed no activity in oophorectomized NC ewes without steroid substitution, and Garcia-Villar et al. (15) found long bursts of electrical activity in the cervix but not in the uterine horns. The reason of these disparate results is not clear, but they may be due to differences between electrodes and recording equipment used in the various studies.

The gradual increase in myometrial activity following an initial period of quiescence after intraarterial administration of estradiol-17 β is in agreement with previous observations in ewes equipped with an intrauterine catheter (32,33,41,51,54) and in non-catheterized ewes (15). In the present study a striking difference was observed between patterns of elevated myometrial activity in catheterized and non-catheterized ewes following estradiol treatment. The EMG in C ewes showed mainly short bursts with a frequency (up to 2 per minute) occurring in conjunction with IUP cycles, with an occasional long burst. In NC ewes the EMG showed long bursts with a low frequency as compared with that of the short bursts in C ewes.

Infusion of naproxen sodium caused the characteristic pattern of myometrial activity in the C ewes to change to a pattern with long bursts, similar to that obtained from a horn without a catheter, both before and after administration of estradiol. This indicates involvement of prostanoid synthesis through the cyclo-oxygenase pathway, most likely induced by the presence of the IUP catheter. This observation is in agreement with the results of studies in non-oophorectomized ewes that showed elevated endometrial and uterine venous plasma concentrations of prostaglandin F_{2 α} in the presence of an intrauterine device (47). Involvement of the lipoxygenase pathway has also been suggested by several authors (32,34,58). There is a striking resemblance between the long bursts or episodes of activity recorded in oophorectomized NC ewes (21), in intact ewes during estrous (49), and the long bursts associated with contractures in the pregnant ewe (21,39). Infusion of meclofenamic acid in parturient ewes (38) changed the myometrial activity from a labor-like pattern to a pre-labor like pattern. This resembles the change induced by naproxen in non-pregnant catheterized oophorectomized ewes, in which the labor-

like pattern with frequent short bursts concomitant with IUP cycles with high amplitudes, induced by the presence of an intrauterine catheter, is converted to a pattern with infrequent long bursts and low amplitude IUP cycles.

As reported in chapter 2 administration of estradiol induces gap junction formation. The similarity between the electrical activity in both horns of the C ewes after administration of estradiol-17 β may be accounted for by the presence of gap junctions (46), which allow better propagation of electrical activity. Before administration of estradiol, in the absence of gap junctions, both horns show a different pattern of electrical activity, whereas after estradiol the electrical activity in one horn appears to be influenced by that in the other.

The results of this study show that myometrial activity in the chronically instrumented oophorectomized ewe is markedly influenced by the presence of an intrauterine pressure catheter, which may affect myometrial activity by stimulating prostaglandin synthesis. This effect is to be taken into account when conclusions are drawn from results of studies on myometrial activity recorded with the use of intrauterine catheters.

IV

Effects of estrogen treatment and inhibition of prostanoid synthesis on electrical and mechanical myometrial activity and gap junction formation in the oophorectomized ewe

Introduction

Oophorectomized ewes have been used to study the influence of various factors that are thought to modulate myometrial activity in vivo (32,33,42,43,51). These studies have shown that estradiol increases myometrial activity, after an initial quiescent period (32,33,51). The estradiol-induced increase in myometrial activity is associated with the formation of myometrial gap junctions (chapter 2), which facilitate the spread of electrical activity and coordinate the contractile forces of the myometrium (37,52). In pregnant animals gap junctions are required for the conversion of the myometrium to the active state of labor (16), which is also associated with an increase in prostanoid synthesis by fetal and uterine tissues (29). Inhibitors of prostanoid synthesis reduce myometrial activity in non-pregnant animals (32,42, chapter 3) but, on the other hand, were shown to stimulate estrogen-induced formation of gap junctions (35).

The experimental study in the oophorectomized ewe, described in this chapter, was designed to elucidate the functional relationship between estradiol-induced changes in electrical and mechanical myometrial activity, uterine prostanoid synthesis, and gap junction formation in vivo.

Material and Methods

Animals and instrumentation

The study was performed in six chronically instrumented oophorectomized Texel ewes. The surgical procedures and methods of instrumentation are reported in detail in chapter 2. Three pairs of bipolar, silver-chloride coated silver electrodes were fixed to the anterior part of the myometrium in the tubal, medial and cervical regions of both uterine horns for recording of the electromyogram (EMG). A sponge-tipped catheter to record intrauterine pressure (IUP) was inserted into the cavity of the left uterine horn through a small incision in the cervical

region. Silicon catheters were inserted into the abdominal aorta through a femoral artery, into the vena cava through a femoral vein, and into a large parametrial vein on both sides of the uterus. The wires and catheters were passed subcutaneously to a pouch attached to the ewe's back. The ewes were allowed to recover from the operation for at least one week before experiments were started. The venous catheters were kept open by a continuous saline infusion. The arterial catheter was flushed intermittently with saline without heparin.

Recording procedures

The technical details of the recording procedures are described in chapters 2 and 6. Electrical myometrial signals recorded by two electrodes on the left horn and one electrode on the right horn of the uterus, and the intrauterine pressure of the left uterine horn were recorded on a Gould eight channel polygraph with a paperspeed of 25 mm/min, and stored on magnetic tape for off-line computer analysis.

Experimental protocol

Experiments consisted of three parts. During the first part, EMG and IUP signals were recorded and stored on magnetic tape during one hour, at 1 hour before, and at 24, 48, and 72 hours after intraarterial administration of 0.1 mg estradiol-17 β in 10% ethanol. Blood samples were drawn from the aorta and from both uterine veins before (3 samples) and at 1, 2, 4, 8, 12, 24, 48 and 72 hours after administration of estradiol for determination of concentrations of prostaglandin (PG) F_{2 α} , PGE₂, tromboxane (Tx) B₂, and 6-ketoPGF_{1 α} . In the second part of the study the same protocol was followed one week later with, in addition, continuous intravenous infusion of the prostaglandin synthetase inhibitor naproxen sodium (4 g in 100 ml of 0.1 M phosphate buffer, pH 8) at a rate of 160 mg/hr, starting 24 hours before the first recording. During both parts of the experiment myometrial activity was continuously recorded on the polygraph. In the third part of the study, performed two weeks later, 3 to 6 myometrial biopsies (5 by 2 by 1 mm) were taken before and 24, 48, 72 and 96 hours after administration estradiol with, in addition, intravenous infusion of naproxen sodium at a rate of 160 mg/hr, starting 24 hours before the first sampling time. Biopsies were obtained under general anesthesia from at least two animals at each sampling time.

Analytic procedures

The methods used for computerized analysis of the EMG and IUP signals will be described in detail in chapter 6. In the IUP signal the tonus was calculated in a moving window of one minute. After detection of intrauterine pressure cycles (a temporal rise of at least 10 mm Hg above the tonus), the maximum rate of rise (calculated in a moving window of 2 seconds), the peak pressure (maximum pressure minus the tonus) and the active pressure area (area under the intrauterine pressure cycle curve corrected for the tonus) were determined. The EMG was analyzed for periods of distinct electrical activity called single bursts. These were analyzed for maximum Root Mean Square (RMS) level, mean RMS level, period time and RMS content (mean RMS x period time). After detecting the single bursts, trains of single bursts were defined by clusters of single bursts with their end and starting points less than 20 seconds apart. When the period time of a train of single bursts was less than 2 minutes it was defined as a short burst; when the period time was more than two minutes it was termed a long burst. In the short and long bursts, maximum RMS, mean RMS and RMS content, i.e. the sum of RMS content of the single bursts within the short or long burst, were determined. Burst frequency was calculated from the burst-to-burst time intervals. In order to obtain an estimate of conduction of electrical activity, the starting points of the single and short bursts from one recording site were compared with the starting points of single and short bursts from the other two recording sites. "Conduction times" in the left horn (CTLL) and from the left to the right horn (CTLR) were computed from the time lag between the starting points of the bursts recorded in two regions.

Blood samples were drawn in plastic tubes containing 4.5 mM EDTA and indomethacin, and immediately centrifuged at -20°C . A Sep-Pack C18 cartridge (Waters Assoc., Milford MA, U.S.A.) was prewashed with 10 ml abs. ethanol, 10 ml distilled water and 2 ml air. Two ml plasma were applied to the column, followed by 2 ml distilled water and 2 ml air. Prostanoids were then extracted with 2 ml abs. ethanol, followed by 2 ml air, and the extract was stored at -80°C until assay. The specific radioimmunoassays of PGE_2 , $\text{PGF}_{2\alpha}$, TxB_2 and 6-Keto $\text{PGF}_{1\alpha}$ used in this study were described in detail elsewhere (62).

The preparation of the tissue samples and the electron microscopic quantitation of gap junctions was performed as described in chapter 2. The length of the plasma membrane was determined in 20 electron micrographs (at x 33,600 magnification) from each tissue sample. Each possible gap junction was further enlarged to x 100,000 magnification for identification and measurement.

For statistical analysis the SPSS/PC+ V2.0 program (SPSS Inc. Chicago, Illinois, U.S.A.) was used. The Mann-Whitney U test was used for statistical analysis of differences between values of variables obtained at various recording or sampling times. Values of $p < 0.005$ were considered to represent statistical significance comparing variables of myometrial activity, and values of $p < 0.05$ in the statistical assessment of concentrations of prostanoids, gap junction areas, and correlations between measured variables. Interaction between burst frequency and CTLL was tested with the ANOVA (analysis of variance) procedure in which the effects of the covariate, burst frequency, were assessed first.

Results

All ewes survived 8 to 10 weeks after the initial operation and did well. Recordings from one ewe were incomplete due to intrauterine catheter and electrode malfunctioning and were not used for analysis. In one ewe (C) data of uterine activity at 72 hours after administration of estradiol are missing because of temporary failure of the recording equipment. Blood sampling from both uterine veins during the first two parts of the experiment was completely successful in one ewe only. In two ewes one uterine vein could be sampled throughout. The selection of electrodes for recording of the EMG from different regions of the uterus had to be changed during the experiment in two ewes.

Myometrial activity

Electrical and mechanical uterine activity before and after administration of estradiol showed patterns as described in chapter 2 and 3 in all ewes. Spontaneous myometrial activity in the catheterized horn before administration of estradiol-17 β was characterized by a rhythmic pattern of short bursts corresponding with intrauterine pressure cycles with a frequency of 0.75 to 1 per min. The EMG of the non-catheterized horn showed a different pattern of long bursts with a frequency of 0.5 to 3 per hour and irregular short bursts in between. Intraarterial administration of 0.1 mg estradiol-17 β caused complete inhibition of electrical and mechanical myometrial activity in both horns after 45 minutes. Electrical activity reappeared after one to three hours, and intrauterine pressure cycles returned after 5 to 7 hours. When myometrial activity returned, short bursts and IUP cycles, recorded from the catheterized horn, initially showed a pattern of periods of high activity alternating with periods of low activity. After the silent period, the EMG of the right

horn showed increasingly frequent short bursts, with a gradual transformation over a period of 24 hours to the pattern with long bursts observed before estradiol administration.

During naproxen infusion the patterns of electrical and mechanical myometrial activity recorded from the catheterized horn became similar to those obtained from the non-catheterized horn. A composite of EMG and IUP recordings before and 24 hours after estradiol administration is presented in figure 4.1. Figure 4.2 shows recordings before and 24 hours after estradiol during naproxen infusion.

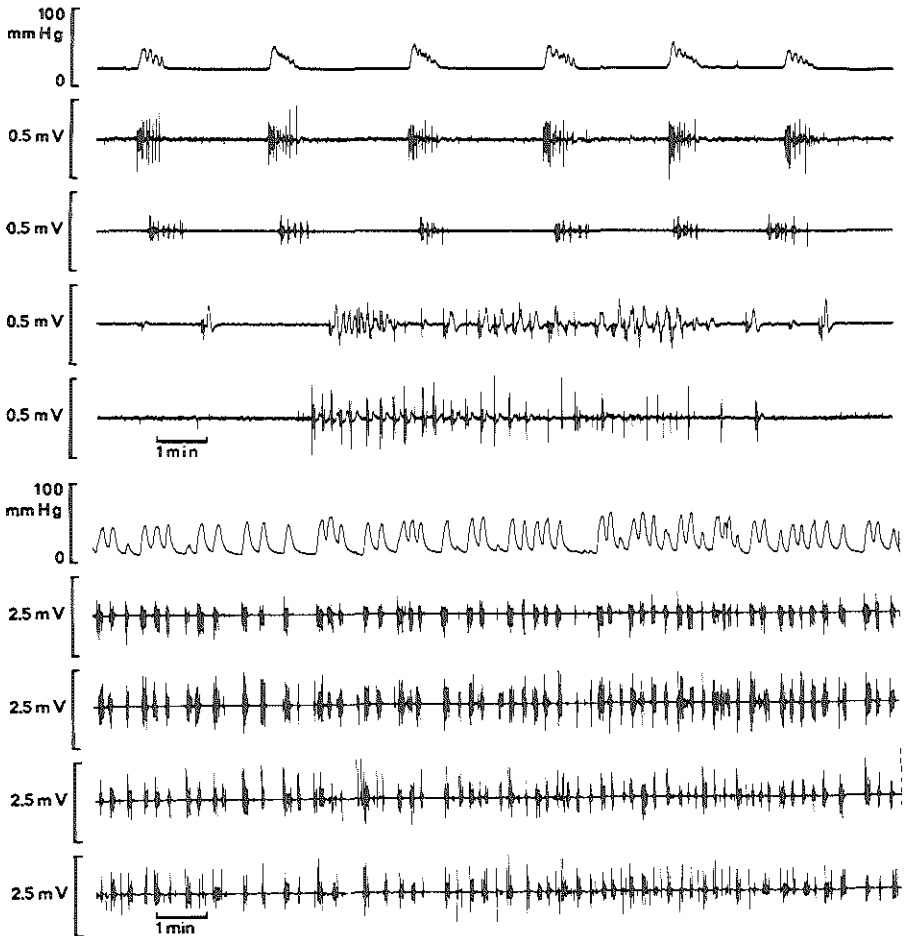


Figure 4.1 Composite of recordings of myometrial activity before (top 5 tracings), and 24 hours after (bottom 5 tracings) administration of estradiol-17 β . Tracings from top to bottom in each recording: IUP left (catheterized) horn, EMG tubal part of left horn, EMG middle part of left horn, EMG middle part of right (non-catheterized) horn and EMG tubal part of right horn.

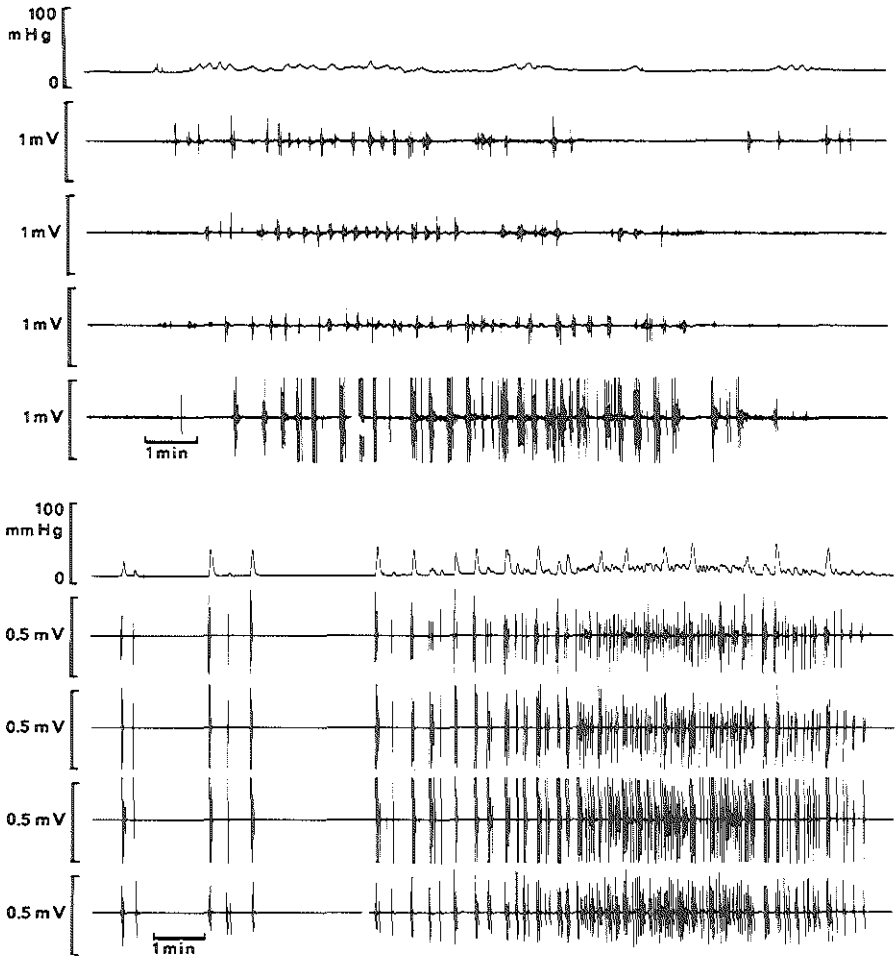


Figure 4.2 Composite of recordings of myometrial activity 24 hours after the start of naproxen infusion (top 5 tracings) and 24 hours after administration of estradiol-17 β during naproxen infusion (bottom 5 tracings). Tracings are arranged as in figure 4.1.

The frequency of short bursts is presented in figure 4.3. Within recording periods of one hour rather large fluctuations in frequency occurred as shown by the standard deviation.

Table 4.1 presents conduction times between two regions in the left horn (CTLL), calculated for short bursts recorded during one hour recording periods, in 5 ewes. In all animals CTLL showed a significant fall at 24 hours after administration of estradiol, before as well as during naproxen

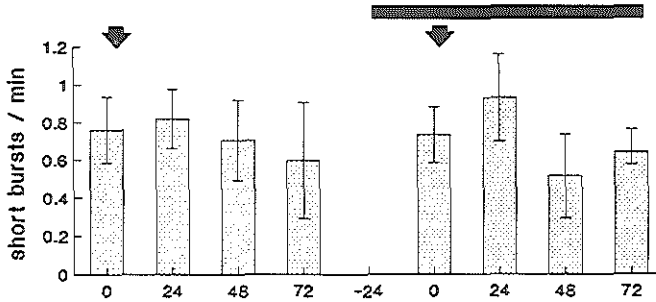


Figure 4.3 Frequency of short bursts in 6 ewes, (mean \pm s.d. during a one hour recording period) before, and 24, 48 and 72 hours after administration of estradiol-17 β ; and also during naproxen infusion before, and 24, 48 and 72 hours after administration of estradiol-17 β . Arrows indicate administration of 0.1 mg of estradiol-17 β , horizontal bar indicates continuous infusion of naproxen sodium, 160 mg/hr.

Table 4.1 CONDUCTION TIME OF SHORT BURSTS IN THE LEFT UTERINE HORN (mean \pm s.d.)

recording period *	sheep A	sheep B	sheep C	sheep D	sheep E
spontaneous activity	9.3 \pm 9.3	24.2 \pm 12.6	23.4 \pm 15.8	18.6 \pm 26.3	20.4 \pm 17.2
EA + 24 hr	2.6 \pm 4.8	3.8 \pm 8.5	3.8 \pm 5.6	6.2 \pm 12.8	2.8 \pm 3.6
EA + 48 hr	9.5 \pm 7.1	14.7 \pm 9.8	13.8 \pm 9.4	8.7 \pm 16.0	8.9 \pm 8.6
EA + 72 hr	14.1 \pm 17.1	22.9 \pm 12.5	**	35.1 \pm 35.9	19.0 \pm 18.0
N + 24 hr	39.3 \pm 34.3	47.0 \pm 50.6	24.9 \pm 13.5	17.8 \pm 14.9	29.6 \pm 23.5
NEA + 24 hr	8.1 \pm 21.5	2.1 \pm 2.3	7.9 \pm 8.8	4.6 \pm 13.5	6.9 \pm 11.7
NEA + 48 hr	28.8 \pm 26.4	33.1 \pm 64.5	10.9 \pm 8.6	31.6 \pm 57.2	26.8 \pm 39.3
NEA + 72 hr	24.8 \pm 18.1	60.9 \pm 96.0	28.8 \pm 22.5	25.5 \pm 24.1	34.8 \pm 42.9

* EA + 24, 48, and 72 hr is 24, 48, and 72 hours, respectively, after intraarterial administration of estradiol-17 β (EA); N + 24 hr is 24 hours after start of intravenous infusion of naproxen sodium (N); NEA + 24, 48, and 72 hr denotes 24, 48, and 72 hours, respectively, after EA during N.

** Data missing because of malfunctioning recorder.

infusion, followed by a gradual but variable return to pretreatment values. The frequency of the short bursts was significantly correlated with CTLL in two ewes. When corrected for burst frequency as a covariate in the analysis of variance, the fall in CTLL 24 hours after estradiol remained significant in all animals. CTLL calculated for single

bursts showed a similar pattern of a significant fall 24 hours after estradiol, before and during infusion of naproxen (table 4.2).

Table 4.2. CONDUCTION TIME OF SINGLE BURSTS IN THE LEFT UTERINE HORN (mean \pm s.d.)

recording period *	sheep A	sheep B	sheep C	sheep D	sheep E
spontaneous activity	4.2 \pm 3.5	7.0 \pm 7.1	6.1 \pm 7.0	6.6 \pm 5.6	5.0 \pm 6.6
EA + 24 hr	3.6 \pm 3.4	1.8 \pm 1.8	3.7 \pm 9.7	3.6 \pm 4.2	3.1 \pm 2.5
EA + 48 hr	10.1 \pm 8.1	6.2 \pm 6.4	7.1 \pm 5.9	3.8 \pm 3.3	4.2 \pm 4.8
EA + 72 hr	7.5 \pm 8.4	6.8 \pm 7.6	*	6.8 \pm 7.6	12.9 \pm 15.8
N + 24 hr	25.5 \pm 26.3	18.6 \pm 88.8	26.0 \pm 25.3	11.6 \pm 11.6	10.8 \pm 0.1
NEA + 24 hr	3.7 \pm 5.4	3.3 \pm 3.8	3.6 \pm 3.9	2.0 \pm 2.8	2.5 \pm 2.6
NEA + 48 hr	20.3 \pm 20.1	5.0 \pm 6.7	19.2 \pm 19.5	8.0 \pm 25.7	6.0 \pm 12.2
NEA + 72 hr	15.1 \pm 14.6	9.8 \pm 30.0	12.6 \pm 12.4	12.6 \pm 12.5	9.2 \pm 21.8

* and ** see footnote table 4.1.

The frequency of the single bursts was correlated with CTLL for single bursts in all ewes. When adjusted for burst frequency, CTLL for single bursts still showed a significant decrease at 24 hours after estradiol, both with and without naproxen infusion. Conduction times from left to right (CTLR), calculated for single bursts recorded by electrodes on the left and right horn, displayed a similar pattern, (table 4.3).

Table 4.3. CONDUCTION TIME OF SINGLE BURSTS IN THE LEFT AND RIGHT UTERINE HORN (mean \pm s.d.)

recording period *	sheep A	sheep B	sheep C	sheep D	sheep E
spontaneous activity	15.0 \pm 0.5	4.7 \pm 6.9	12.3 \pm 11.8	6.9 \pm 7.3	11.1 \pm 16.8
EA + 24 hr	4.0 \pm 8.5	3.8 \pm 4.9	5.7 \pm 11.7	3.4 \pm 3.5	4.3 \pm 4.0
EA + 48 hr	11.4 \pm 12.9	6.0 \pm 14.0	20.2 \pm 21.3	12.8 \pm 19.5	20.0 \pm 32.8
EA + 72 hr	15.2 \pm 18.4	4.7 \pm 7.0	**	4.7 \pm 7.0	10.6 \pm 16.7
N + 24 hr	18.8 \pm 25.5	31.5 \pm 99.4	23.7 \pm 41.1	0.6 \pm 12.7	8.2 \pm 0.8
NEA + 24 hr	1.7 \pm 3.3	8.4 \pm 27.5	1.8 \pm 1.8	4.0 \pm 11.7	6.5 \pm 19.8
NEA + 48 hr	23.9 \pm 40.7	4.5 \pm 4.6	26.8 \pm 41.7	9.3 \pm 16.7	10.3 \pm 27.7
NEA + 72 hr	16.8 \pm 24.3	11.9 \pm 35.9	15.0 \pm 25.7	18.0 \pm 27.1	0.1 \pm 19.8

* and ** see footnote table 4.1.

The CTRLR of short bursts was difficult to evaluate as the number of short bursts recorded from the right horn was low during some of the recording periods. The mean RMS level of the short bursts is presented in table 4.4. At 24 and 48 hours after estradiol administration mean RMS levels were significantly increased. During naproxen infusion mean RMS levels were significantly elevated in all ewes at 24 hours after estradiol, and returned to pretreatment values at 48 to 72 hours.

Table 4.4. MEAN RMS LEVEL OF THE SHORT BURSTS IN THE LEFT UTERINE HORN (mean \pm s.d.)

recording period *	sheep A	sheep B	sheep C	sheep D	sheep E
spontaneous activity	6.1 \pm 1.7	7.4 \pm 2.3	8.8 \pm 2.0	10.1 \pm 3.4	7.6 \pm 2.4
EA + 24 hr	24.2 \pm 5.8	19.4 \pm 3.3	60.2 \pm 9.5	34.5 \pm 52.8	30.8 \pm 5.5
EA + 48 hr	14.8 \pm 4.2	14.4 \pm 4.3	26.8 \pm 4.2	40.5 \pm 8.0	12.3 \pm 2.7
EA + 72 hr	9.2 \pm 2.6	7.8 \pm 2.4	**	7.8 \pm 2.4	8.1 \pm 1.7
N + 24 hr	4.3 \pm 2.5	2.1 \pm 0.8	5.0 \pm 2.0	3.9 \pm 1.0	4.6 \pm 1.1
NEA + 24 hr	17.1 \pm 3.9	18.5 \pm 5.1	16.4 \pm 3.2	39.3 \pm 11.8	14.2 \pm 3.6
NEA + 48 hr	5.8 \pm 2.4	12.9 \pm 4.3	4.7 \pm 1.1	19.0 \pm 5.0	11.2 \pm 2.8
NEA + 72 hr	3.9 \pm 1.0	5.6 \pm 2.1	3.5 \pm 0.8	8.1 \pm 0.9	7.8 \pm 1.9

* and ** see footnote table 4.1.

The patterns of Maximum RMS levels and RMS contents of short bursts and single bursts recorded from both horns were similar to those of the mean RMS levels.

The active pressure area (APA) of the IUP cycles was significantly reduced at 24 hours after estradiol in all animals, due to a marked decrease in period time (table 4.5). Naproxen infusion caused a significant reduction in APA in all recording periods.

The maximum rate of rise (MRR) was significantly increased at 24 and 48 hours after estradiol in all ewes (table 4.6), without as well as with naproxen infusion. The mean values of MRR were significantly correlated with the mean values of CTLL in all animals ($p < 0.001$).

Prostanoids

Plasma concentrations of PGE₂, PGF_{2 α} , TxB₂, and 6-KetoPGF_{1 α} in the aorta, left uterine vein and right uterine vein before administration of estradiol-17 β and after 24 hours of infusion of naproxen are presented

Table 4.5. ACTIVE PRESSURE AREA OF INTRAUTERINE PRESSURE CYCLES (mean \pm s.d.)

recording period *	sheep A	sheep B	sheep C	sheep D	sheep E
spontaneous activity	4.3 \pm 1.8	4.9 \pm 1.8	6.7 \pm 2.2	3.5 \pm 1.4	7.7 \pm 2.1
EA + 24 hr	1.8 \pm 1.0	0.9 \pm 0.5	1.4 \pm 0.8	1.6 \pm 0.8	3.6 \pm 2.0
EA + 48 hr	5.6 \pm 1.4	5.1 \pm 1.4	5.6 \pm 1.8	5.0 \pm 2.0	10.2 \pm 1.3
EA + 72 hr	5.3 \pm 2.1	5.1 \pm 1.8	**	4.3 \pm 1.4	9.7 \pm 1.0
N + 24 hr	2.4 \pm 0.8	3.4 \pm 2.5	0.9 \pm 0.4	1.9 \pm 1.0	0.8 \pm 0.5
NEA + 24 hr	1.3 \pm 0.7	2.8 \pm 1.3	0.8 \pm 0.3	1.0 \pm 0.3	1.9 \pm 0.9
NEA + 48 hr	2.4 \pm 0.8	4.1 \pm 3.1	1.0 \pm 0.7	1.5 \pm 0.5	4.4 \pm 1.5
NEA + 72 hr	2.5 \pm 1.0	3.9 \pm 2.4	2.1 \pm 1.0	1.7 \pm 1.1	3.0 \pm 2.3

* and ** see footnote table 4.1.

Table 4.6. MAXIMUM RATE OF RISE OF INTRAUTERINE PRESSURE CYCLES (mean \pm s.d.)

recording period *	sheep A	sheep B	sheep C	sheep D	sheep E
spontaneous activity	3.8 \pm 0.8	2.7 \pm 0.8	3.6 \pm 1.3	4.1 \pm 1.4	3.9 \pm 1.2
EA + 24 hr	5.4 \pm 1.7	5.1 \pm 1.2	4.3 \pm 1.2	5.4 \pm 1.7	8.0 \pm 3.1
EA + 48 hr	5.4 \pm 1.5	4.2 \pm 0.9	5.4 \pm 1.0	4.4 \pm 1.1	5.5 \pm 1.3
EA + 72 hr	3.0 \pm 1.1	2.9 \pm 0.8	**	4.3 \pm 1.4	3.7 \pm 1.3
N + 24 hr	4.0 \pm 2.3	1.8 \pm 1.7	2.5 \pm 0.5	3.2 \pm 1.7	2.3 \pm 2.0
NEA + 24 hr	5.7 \pm 1.5	4.9 \pm 1.8	4.3 \pm 0.9	5.4 \pm 1.6	4.3 \pm 1.9
NEA + 48 hr	4.3 \pm 1.3	4.7 \pm 1.8	3.4 \pm 1.3	4.6 \pm 1.5	4.3 \pm 2.7
NEA + 72 hr	3.7 \pm 2.1	4.2 \pm 1.6	4.2 \pm 1.3	2.5 \pm 0.9	3.4 \pm 1.9

* and ** see footnote table 4.1.

in table 4.7. In four ewes plasma concentrations in samples obtained from the left and right uterine vein could be compared in the first part of the experiment. Concentrations of PGE₂ and TxB₂ in the left uterine vein were significantly higher in 4 animals; concentrations of PGF₂ α were higher in 3 animals and concentrations of 6-KetoPGF₁ α in 2 animals. Figure 4.4 shows the changes in the plasma levels of PGE₂, PGF₂ α , TxB₂ and 6-KetoPGF₁ α in the left uterine vein and concentrations of PGE₂ and PGF₂ α in the right vein measured in the first part of the experiment.

Table 4.7. PLASMA CONCENTRATIONS OF PGE₂, PGF_{2α}, TxB₂ AND 6-ketoPGF_{1α} (pg/ml, mean ± s.d.)

	PGE ₂	PGF _{2α}	TxB ₂	6-ketoPGF _{1α}
before administration of estradiol-17β				
aorta	7 ± 1	21 ± 3	58 ± 8	90 ± 8
left uterine vein	219 ± 54	410 ± 87	122 ± 15	159 ± 9
right uterine vein	61 ± 25	301 ± 54	73 ± 14	128 ± 11
after 24 hours of naproxen infusion				
aorta	3 ± 3	22 ± 25	106 ± 59	109 ± 55
left uterine vein	6 ± 4	76 ± 63	124 ± 18	117 ± 29

Concentrations of all four prostanoids were significantly reduced 1 to 4 hours after estradiol administration, and showed a temporary increase above baseline between 8 to 24 hours after estradiol administration.

During naproxen infusion arterial and venous blood could be sampled in 3 ewes; plasma concentrations of PGE₂, PGF_{2α} and 6-KetoPGF_{1α} in the uterine venous samples were significantly reduced in all 3 ewes, TxB₂ was decreased in 2 ewes. Administration of estradiol during naproxen infusion did not increase the low uterine venous levels of PGE₂ and PGF_{2α}. Arterial concentrations of TxB₂ and 6-KetoPGF_{1α} were not significantly decreased during infusion of naproxen.

Gap junctions

The results of the quantitative analysis of the myometrial biopsies for gap junction area are presented in table 4.8. Low numbers of gap junctions were found 24 hours after the start of the naproxen infusion, before administration of estradiol. Administration of estradiol was followed by an increase in gap junction area, which was maximal after 24 hours and gradually declined thereafter. Figure 4.5 shows the changes in APA, MRR, mean RMS, CTLL⁻¹ (the reciprocal of CTLL) and gap junction area after administration of estradiol during naproxen infusion. These data are combined with those obtained after administration of the same dose of estradiol-17β without naproxen infusion as reported in chapter 2. MRR, mean RMS and gap junction area are maximal 24 hours after administration of estradiol with as well as without naproxen infusion; at the same time CTLL is at its lowest.

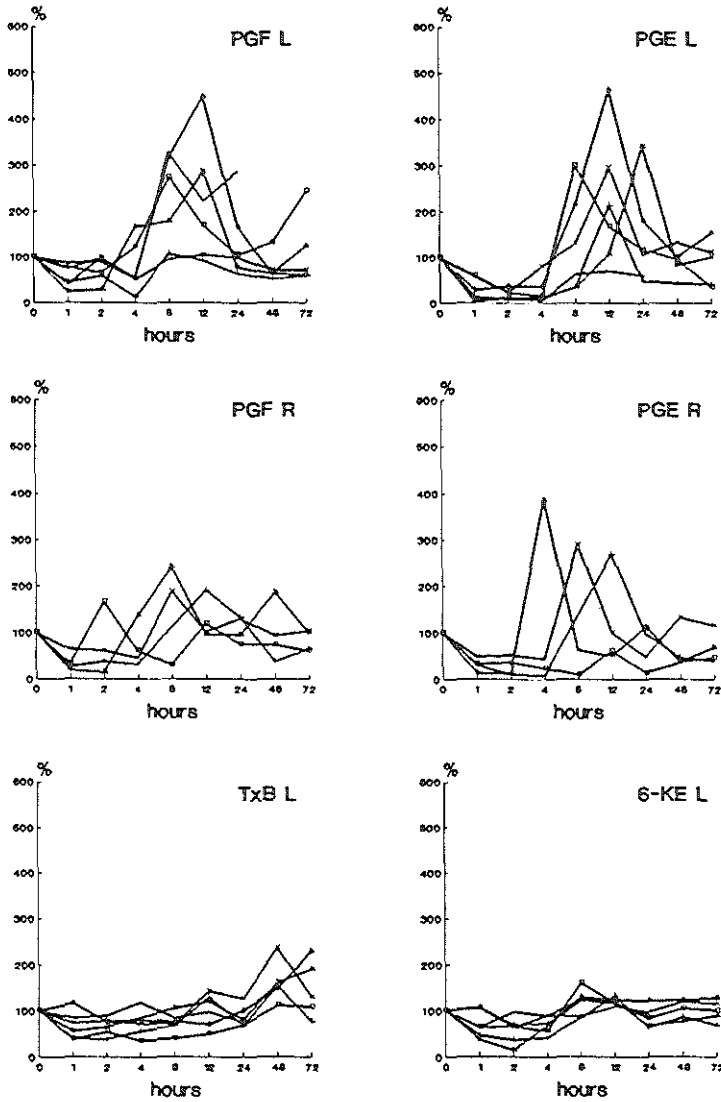


Figure 4.4 Changes in concentrations of PGE_2 , $PGF_{2\alpha}$, TXB_2 and 6-Keto $PGF_{1\alpha}$, in the left uterine vein (L) in 6 ewes, and PGE_2 and $PGF_{2\alpha}$ in the right uterine vein (R) in 4 ewes, expressed as percentages of the concentration at 0 hours, following the administration of 0.1 mg estradiol-17 β .

Table 4.8. AREA OF MYOMETRIAL GAP JUNCTIONS (GJs) IN OOPHORECTOMIZED EWES, DURING NAPROXEN INFUSION, BEFORE AND AFTER TREATMENT WITH ESTRADIOL-17 β .

sampling period *	number of sheep	area of GJs / area of total membrane % \pm S.E.M.
N + 24 hr	3	.006 \pm .002
EA + 24 hr	3	.4 \pm .030
NEA + 48 hr	5	.053 \pm .007
NEA + 72 hr	3	.022 \pm .005
NEA + 96 hr	2	.015 \pm .004

* see footnote table 4.1.

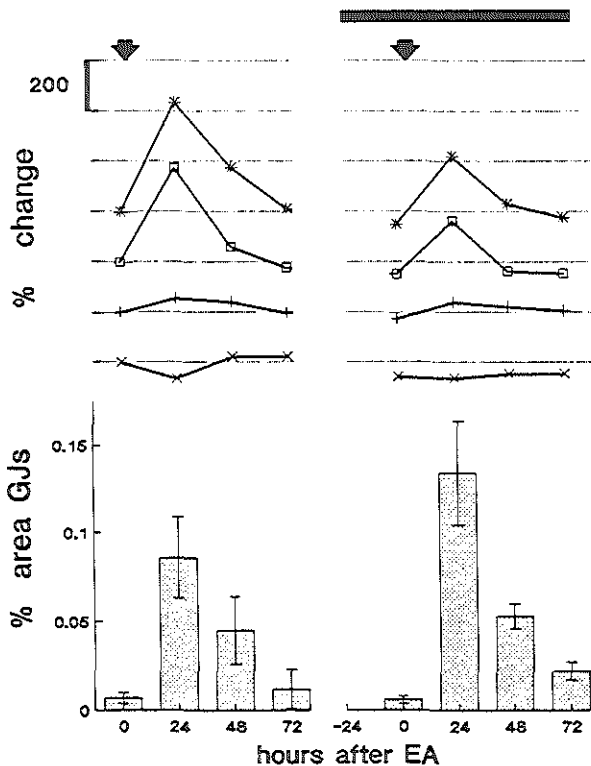


Figure 4.5 Upper graph: changes in (from top down): mean RMS (*), the reciprocal of conduction time (CTLL) (□), maximum rate of rise (+) and active pressure area (x), expressed as percentages of the values obtained before the first administration of estradiol-17 β (EA); means from 5 ewes. Lower graph: means (\pm S.E.M.) of the percentage gap junction area. Arrows indicate administration of 0.1 mg of estradiol-17 β , horizontal bar indicates continuous infusion of naproxen sodium, 160 mg/hr.

Discussion

The patterns of electrical and mechanical uterine activity reported in this study before and after administration of estradiol are in accordance with those observed previously in oophorectomized ewes (32,51) and those described in chapter 2 and 3. Conduction time, i.e. the period of time between the onset of bursts recorded from different sites in the uterus was used, to assess the spread of electrical activity across the myometrium. Because the distances between the electrodes at the time of measurement were not known and since it could not be ascertained that conduction of electrical activity occurs in a straight line, conduction velocity was not used as a variable. The MRR of the intrauterine pressure cycles is considered to represent the degree of coordination of myometrial activity (11). The amplitude of the spikes recorded within bursts is reflected in the mean RMS level and is thought to depend on the synchronous action of the smooth muscle cells adjacent to the electrode (24). Therefore, the increases in MRR, and mean RMS, together with the decrease in conduction time between single and short bursts recorded from the same as well as from different uterine horns, observed 24 hours after intraarterial administration of estradiol-17 β , indicate improved coordination of myometrial activity. This effect of estradiol was not suppressed by inhibition of prostanoid synthesis by means of naproxen, although the active pressure area of the intrauterine pressure cycles, a measure of the "work" performed, was significantly reduced. The observation that uterine venous concentrations of PGE₂ and PGF₂ α were low during infusion of naproxen and remained so after administration of estradiol, suggests that the increase in myometrial activity observed 24 hours after estradiol during naproxen infusion is independent of prostanoid synthesis.

Data presented in chapter 2 indicate that improvement of the coordination of myometrial activity is closely associated with an increase in gap junction area. The results of this study show that continuous infusion of naproxen stimulates the estradiol-induced formation of myometrial gap junctions. A similar effect of a prostanoid synthetase inhibitor was observed in rats (35). The formation of gap junctions in pregnant rats at term was not affected by treatment with naproxen sodium whereas labor was delayed (8). Permeability of gap junctions is modulated by various physiologic systems (20). The observation that inhibition of prostanoid synthesis suppressed myometrial activity, whereas coordination of myometrial activity was not altered, indicates that gap junction permeability in vivo is not controlled by prostanoid action.

The higher concentrations of prostanoids measured in the left uterine

vein as compared with those in the right vein may reflect increased prostanoid synthesis induced by the presence of the pressure catheter in the left uterine horn. Catheter-induced prostanoid synthesis may explain the observed differences between the patterns of myometrial activity in the catheterized and those in the non-catheterized horn. This concept is supported by the observation that pharmacologic inhibition of prostanoid synthesis abates those differences, (chapter 3). The finding that concentrations of TxB_2 and 6-Keto $\text{PGF}_{1\alpha}$ in the aorta remained unchanged after infusion of naproxen whereas uterine prostanoid synthesis was reduced suggests preferential inhibition of uterine prostanoid synthesis with the dose of naproxen used in our experiments. No reference to such an effect of naproxen could be found in the literature, and the number of animals in the study reported here is too small to draw firm conclusions. The variability of the rise in prostanoid concentrations in both uterine veins 8 to 12 hours after administration of estradiol may be explained by previous observations in ewes, indicating that estradiol does not stimulate PGF synthesis in the absence of progesterone priming (2,31).

In conclusion, the results of this study indicate that prostanoids, generally accepted as important endogenous myometrial stimulants, are not primarily involved in the estradiol-induced increase in coordination and synchronization of electrical and mechanical activity of the nonpregnant myometrium.

V

Sensitivity of ovine myometrial tissue to various eicosanoids in vitro

Introduction

Previous studies have shown that estradiol causes an increase in myometrial activity after an initial quiescent period in non-pregnant oophorectomized ewes, chronically instrumented with an intrauterine pressure catheter combined with myometrial electrodes (32,51, chapter 2 and 4) as well as in ewes equipped with myometrial electrodes only (chapter 3). It was shown in chapter 4 that these changes in myometrial activity are accompanied by variable changes in prostanoid synthesis as determined by the measurement of prostanoid concentrations in the uterine veins. Pharmacologic inhibition of the cyclooxygenase pathway of prostanoid synthesis by means of intravenous administration of naproxen sodium appeared to alter the pattern of myometrial activity in ewes instrumented with a catheter (chapter 3) but did not abolish the estradiol-induced increase in myometrial activity, although prostanoid concentrations in the uterine veins were shown to be significantly decreased. The lack of a demonstrable influence of inhibition of the cyclooxygenase pathway of prostanoid synthesis on the estradiol-induced changes in myometrial activity in non-catheterized ewes raises the question whether the lipoyxygenase pathway, with formation of leukotrienes, could be involved in the modulation of myometrial activity in the non-pregnant ewe. In an attempt to answer that question the response was investigated of myometrial smooth muscle to administration of prostaglandin (PG) $F_{2\alpha}$, PGE_2 , a thromboxane analog (U-44069), leukotriene (LT) C_4 and LTD_4 in vitro.

Material and methods

Myometrial tissue was obtained from 8 nonpregnant Texel ewes who were oophorectomized and instrumented with an intrauterine pressure catheter in the left uterine horn 2-3 weeks earlier (chapter 2). Four of the ewes had received an intraarterial dose of 0.1 mg of estradiol-17 β in 10% ethanol 24 hours before the tissue samples were obtained. A longitudinal strip of myometrium, 3 - 4 mm in diameter and 15-20 mm in length, was obtained from the cervical, medial and tubal regions of the right uterine horn, and from the tubal and medial regions of the left horn. The five tissue strips were suspended in superfusion chambers and superfused at

37 °C with Krebs solution (NaCl 118 mM, KCl 4.7 mM, CaCl₂ 2.5 mM, MgSO₄ 1.18 mM, KH₂PO₄ 1.18 mM, NaHCO₃ 25 mM and glucose 5.6 mM) at a rate of 2 ml/min. The superfusion fluid was oxygenated with 95% O₂ and 5% CO₂. Contractions were recorded isotonicly using a Harvard smooth muscle transducer and an isotonic load of 1.5 g. The muscle strips were equilibrated for 60 min. All strips developed spontaneous contractions, of which a median peak level was determined. Spontaneous contractile activity was then abolished by adding indomethacin to the superfusion fluid to a concentration of 252 µM/L. Next PGE₂, PGF_{2α}, U-44069, LTC₄ and LTD₄ were added to the tissues in incremental doses from 5 to 2000 ng until the tissues responded with a contraction reaching a peak of 50% of the previously determined median peak level of the spontaneous contractions.

All myometrial strips from all ewes were exposed to PGE₂, PGF_{2α} and U-44069. Myometrial tissue from four ewes who had not received estradiol was exposed to LTC₄, whereas tissues from three untreated and three estradiol-treated ewes were exposed to LTD₄.

PGE₂ and PGF_{2α} were obtained from Sigma (U.S.A.); the thromboxane mimetic U-44069 from Upjohn, U.S.A.; LTC₄ and LTD₄ were gifts from Dr J. Rokach (Merck Frosst, Canada).

The Mann-Whitney U test was used for statistical analysis of differences in response to administration of PGE₂, PGF_{2α}, U-44069, LTC₄ and LTD₄. Values of $p < 0.01$ were considered to represent statistical significance.

Results

Experiments lasted four to five hours, after which the viability of the tissues, as tested by administering PGE₂ and PGF_{2α}, appeared to be good in all strips. The number of myometrial strips from ewes not treated with estradiol responding to the various doses of PGE₂, PGF_{2α}, U-44069, LTC₄ and LTD₄ with a contraction reaching at least 50% of the median peak level of spontaneous contractions (R₅₀), is shown in figure 5.1. The R₅₀ was reached in all strips with all agents except with LTC₄ (9 of 20 strips) and LTD₄ (3 of 15 strips). The dose required to obtain the R₅₀ increased from PGE₂ to PGF_{2α}, U-44069, LTD₄ and LTC₄. The differences between the doses needed for the R₅₀ were significant for all agents ($p < 0.003$), except for LTD₄ and LTC₄. Figure 5.2 presents data obtained with myometrial tissues from estradiol-treated ewes. Also in this group the R₅₀ was reached in all strips with all agents, except with LTD₄. Comparison of the doses required to elicit the R₅₀ showed significant ($p < 0.008$) differences between all substances, with the lowest dose needed

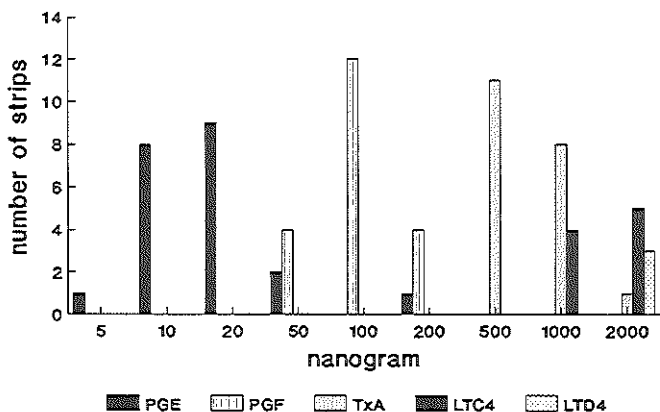


Figure 5.1 Number of myometrial muscle strips from untreated oophorectomized ewes, responding with a contraction reaching at least 50% of the median peak level of spontaneous contractions to administration of PGE₂, PGF_{2α}, U-44069, LTC₄ and LTD₄ in increasing dosages.

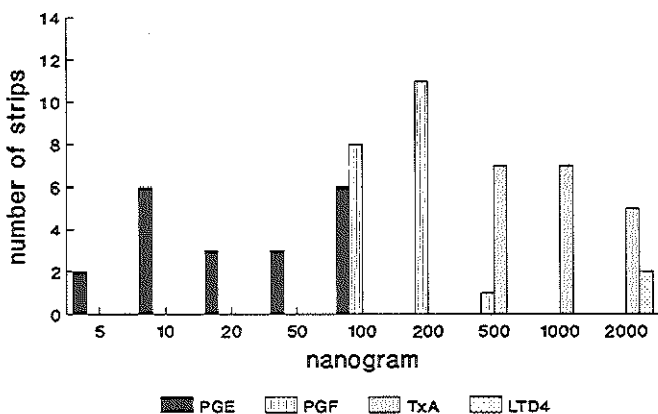


Figure 5.2 Number of myometrial muscle strips obtained from oophorectomized ewes 24 hours after administration of estradiol-17β, responding with a contraction reaching at least 50% of the median peak level of spontaneous contractions to administration of PGE₂, PGF_{2α}, U-44069, and LTD₄ in increasing dosages.

for PGE₂, and increasing doses for PGF_{2α}, U-44069 and LTD₄. The responsiveness to PGE₂, U-44069 and LTD₄ was not different between tissues from ewes with and without estradiol treatments, but the dose of PGF_{2α} required to reach the R₅₀ was elevated in estradiol-treated ewes (p = .003). No statistical differences were observed between responses of myometrial tissues obtained from the various regions, nor between tissues from the left (catheterized) or right (non-catheterized) horn.

Discussion

The responses of ovine myometrial smooth muscle to PGF_{2α} and PGE₂ obtained in this study are similar to those observed in guinea pigs (58), rats, and pregnant human myometrium (30), with PGE₂ being 5 to 10 times more potent than PGF_{2α}. Thromboxane A₂ has been shown to stimulate human myometrial contractility in vitro (60), but in these experiments the thromboxane analog U-44069 was shown to be significantly less potent than PGF_{2α}. The marked increase in myometrial activity and the change in the pattern of the electromyogram observed in vivo after administration of estradiol-17β in oophorectomized ewes during infusion of a prostanoid synthesis inhibitor (chapter 4), raised the question whether leukotrienes could be involved in modulation of myometrial activity in this animal. The results of this study do not support a physiologic involvement of leukotrienes in the regulation of myometrial activity in the ewe. In the presence of inhibition of the cyclooxygenase pathway of prostanoid synthesis relatively high doses of LTD₄ and LTC₄ appear to have a rather small stimulating effect on myometrial contractility. Studies on the effect of leukotrienes on myometrial activity in other species have produced variable results. In guinea pigs LTC₄ and LTD₄ appeared to stimulate myometrial activity ex vivo with a relative potency that was 10 times less than that of PGE₂ (58). LTB₄ did not have an effect on lower segment myometrium ex vivo, but 5-hydroxyeicosatetraenoic acid had a small stimulating effect, 10 times less than that of PGF_{2α}. Studies ex vivo in the pregnant as well as the nonpregnant human myometrium and in the rat, have shown that LTC₄ and LTD₄ have no influence ex vivo on myometrial contractility (30). The results of the present study indicate that leukotrienes are less important than the primary prostaglandins in the modulation of myometrial contractility.

VI

Computer analysis of electrical and mechanical myometrial activity

Introduction

The study of the electrical and mechanical activity of the uterine myometrium requires qualitative and quantitative analysis. Electrical signals from the smooth muscle cells of the uterus in various animal species *in vivo* are usually obtained by means of surface or needle electrodes fixed to the myometrium. These electrodes record action potentials that originate in a large number of cells, depending on the dimensions of the electrode and on the physiologic state of the myometrium (25). Action potentials which depolarize the cell membranes are followed by contraction of muscle cells. The simultaneous contraction of an adequate number of cells results in an increase in intrauterine pressure which can be measured by means of an intrauterine pressure catheter (13).

Assessment of mechanical myometrial function in animal experiments or in humans in clinical conditions is performed by most investigators by manual quantitative analysis. This is a subjective and time-consuming procedure, with inherent risk of observer bias. The results of visual assessment may differ considerably from those obtained by computer analysis (6).

Recordings of electrical activity, generally obtained in animal experiments to study the physiology of myometrial function, are usually subjected to qualitative analysis only. A semi-automatic quantitative analysis of electrical activity was described by Harding et al. (21).

This chapter describes the algorithms, developed in an experimental study, for continuous computer-aided analysis of intrauterine pressure and electric signals. These algorithms were shown to be satisfactory in several experiments (51; 54; chapter 2). At a later stage the program was extended at a later date and applied to data obtained in a more recent study (chapter 4); these modifications are separately referred to in the text.

*** The main substance of this chapter was published in: Ramondt J, Van Kooten C, Verhoeff A, Wallenburg HCS. *Med & Biol Eng & Comput* 1986;24:351-355.

Data acquisition

Intrauterine pressure (IUP) and electrical myometrial signals (EMG) were obtained from chronically instrumented ewes, as described in detail in chapter 2 (54). Briefly, intrauterine pressure was measured with an open-ended sponge-tipped catheter connected to a Gould Statham P23 pressure transducer and a Gould 13-4615-50 transducer amplifier. The EMG was recorded by means of silver-chloride coated bipolar silver needle electrodes, fixed to the myometrium from the outside. The electrical signals were amplified by a Gould 13-4615-56 universal amplifier. The IUP and EMG were simultaneously recorded on a 14-channel Racal analogue instrumentation recorder and written on a Gould Brush 2800 eight-channel polygraph for qualitative review. Examples of original tracings of IUP and EMG in various experimental conditions are shown in chapters 2, 3 and 4.

A transitory rise above the basal pressure level or tonus of the intrauterine pressure is called an IUP cycle. Usually several pressure peaks can be distinguished within one IUP cycle. In the analysis of the IUP signal these peaks as well as pressure peaks occurring separately are called "small waves". The EMG shows periods of quiescence and "bursts" of activity consisting of a rapid succession of electrical spikes. These spikes are supposed to be caused by action potentials arising in the cells adjacent to the electrodes.

Digitalization of the analog signals was performed using a PDP 11/34 minicomputer under the RT-11 operating system. The minicomputer was equipped with an LPS11 (Laboratory Peripheral System) extension, including an LPS11-S basic unit, a 12 bits A/D converter (LPSAD), a real time clock (LPSKW), a digital I/O unit (LPSDR) and a display unit (LPSVC). A general purpose computer program written in Macro-11 was used for digitalization. The electrical signals were pre-processed using a analog band-pass filter. For the lower and higher cutoff frequencies (-3dB), 1 and 10 Hz, respectively, were used. The sampling frequency of IUP and EMG was set at 5 HZ and 25 Hz, respectively. Data files containing a one hour period of recording were stored for analysis on a PDP 11/70 minicomputer under the RSX-11M PLUS operating system. The computer program for analysis of the data was written in Fortran PLUS. Figure 6.1 shows a diagrammatic overview of the system.

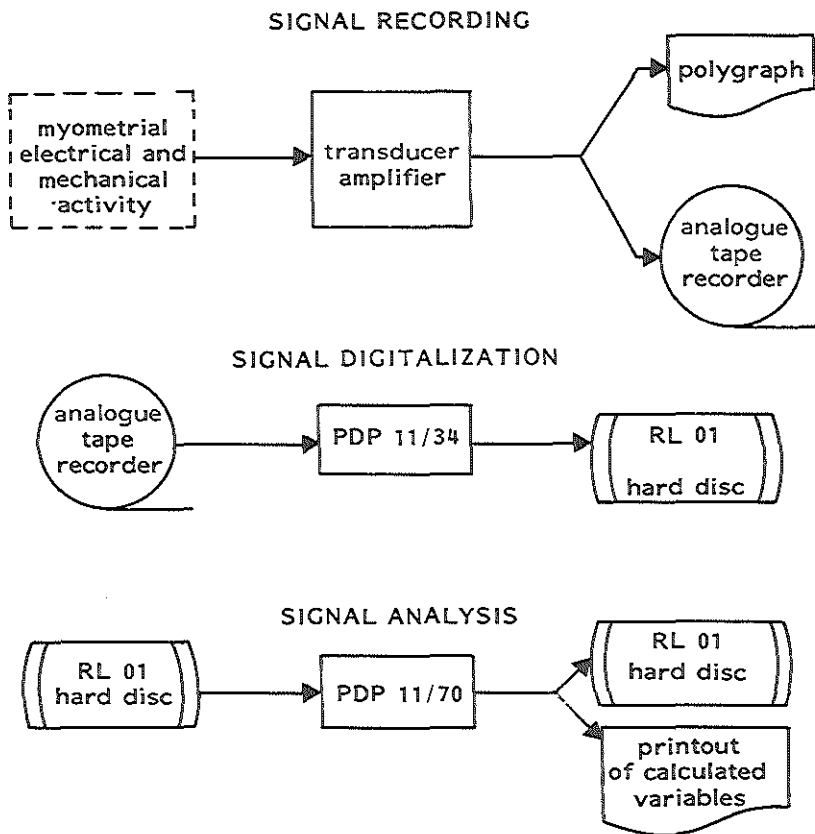


Figure 6.1 Diagrammatic overview of the system.

Data analysis

The algorithms for analysis of IUP and EMG were developed with the use of recordings obtained from various experiments, as described in previous chapters. The criteria used were empirically chosen.

Intrauterine pressure

In a recording period of one hour the basal pressure level (tonus) was calculated first. Due to the fact that the tonus was not stable in some

segments, the tonus was calculated in a one minute window with an overlap of one minute on each side of the segment. In each window containing 300 data points, the mean value of the 75 data points (25%) with the lowest pressure level was calculated to obtain minute levels. By linear interpolation of the midpoints of consecutive minute levels the tonus in each second was determined.

The fluctuating components of the IUP signal were detected by filtering the original signal by a differentiating band-pass filter. Digital differentiation algorithms are widely used for biological signal processing in cardiovascular (36) and neurophysiological (1,59) research. Biological signals have low frequency components, which may be contaminated by intrinsic biological noise as well as by wide-band noise introduced by the use of the measuring equipment. Analog-to-digital conversion for data analysis on a digital computer also introduces wide-band noise. Full-band differentiation amplifies such noises, in particular at higher frequencies (36).

In view of these considerations, a band-limited differentiation algorithm was used, which, by its smoothing effect, avoids distortion of the derivative by higher frequencies. The filter is described by:

$$y_i = \frac{f_s}{m} \cdot \frac{\sum_{k=1}^m (x_{i+k} - x_{i-k})}{2 \sum_{k=1}^m k}$$

in which x is the value of the original signal and f_s is the sampling frequency. Empirically m was chosen as 10, in accordance with the range of the frequencies of the fluctuating components. In the derived curve a search was made for positive-to-negative zero crossings, each of them indicating a curve maximum in the original signal. A curve in the original signal that produces such a maximum is defined as a "small wave" if it reaches a level of 5 mm Hg above the corresponding tonus for at least two seconds. A small wave or a complex of small waves is then designated an intrauterine pressure cycle according to the following criteria:

a. If the minimum between two small waves is higher than the tonus, the small waves are considered to constitute one complex, unless the duration of the curve under the tonus + 5 mm Hg level is longer than five seconds.

b. The maximum of the small wave or of the small wave complex must reach a level of at least 10 mm Hg above the tonus.

Intrauterine pressure cycles and small waves which have their end points within a selected segment were included in the calculations. In each segment the following variables were calculated:

- a. Small wave frequency per minute.
- b. Intrauterine pressure cycle frequency per minute.
- c. Intrauterine pressure cycle period time, i.e. the time from starting point to end point of an intrauterine pressure cycle at the level of tonus + 5 mm Hg, in minutes.
- d. Peak pressure, i.e. the maximum pressure level of the IUP cycles corrected for the corresponding tonus, in mm Hg.
- e. Active pressure area, i.e. the area under the curve of the IUP cycles, above the tonus, in mm Hg.min.
- f. Overall rate of rise of the IUP cycles, i.e. the maximal pressure corrected for tonus + 5 mm Hg, divided by the time between start point and maximum, in mm Hg/sec.
- g. Maximum rate of rise of the IUP cycles, i.e. calculated in a sliding window of two seconds along the rising slope of an intrauterine pressure cycle, in mm Hg/sec.

The calculated variables are shown in a diagram of an IUP cycle (figure 6.2). The detection level of 10 mm Hg for the IUP cycles was chosen in accordance with other investigators (44). In some experiments the original signal showed elevations in intrauterine pressure between 5 and 10 mm Hg. In these examples small wave frequency reflects the presence of mechanical activity at a low level. The 5 mm Hg level for the starting and end points of the IUP cycles was chosen to be able to determine the area and period time of the intrauterine pressure cycles more accurately than by using a 10 mm Hg level. The 5 mm Hg level also allows calculation of the rate over a longer section of the curve, which is of importance when the IUP cycles hardly reach the 10 mm Hg level.

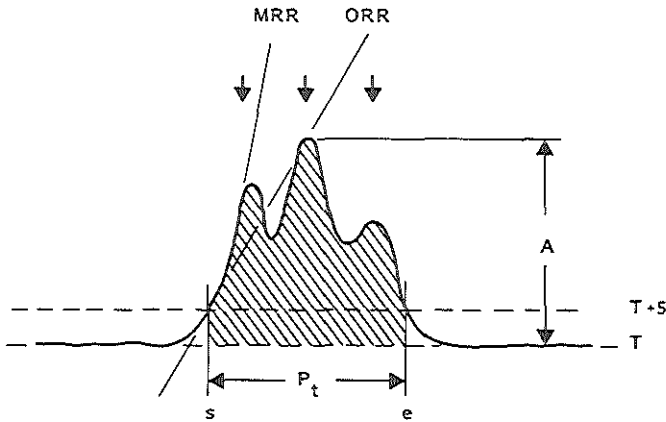


Figure 6.2 Diagrammatic representation of an intrauterine pressure cycle and the calculated variables. T, tonus; T + 5, tonus + 5 mm HG; s, starting point; e, end point; MRR, maximum rate of rise; ORR, overall rate of rise; A, peak pressure; Pt, period time. The arrows indicate small waves.

Electromyogram

As in the analysis of intrauterine pressure, recording periods of one hour were chosen. The root mean square (RMS) value, which is a measure of the average signal intensity (3), was calculated from the data points within each five minutes' segments, according to Bendat and Piersol (3) by using the following formula:

$$RMS_i = \sqrt{\frac{\sum_{j=i-k}^{i+k} x_j^2}{2k+1}}$$

in which x represents the signal magnitude at the sample points in the original recording. A moving time interval of one second was empirically chosen ($k=12$). The range between the highest and lowest RMS values was calculated within each five minutes' segment. A single burst was detected when an RMS curve reached a level calculated by: lowest RMS value + $0.25 \times$ RMS range and remained above this level for at least two seconds. When the single bursts were defined the following variables were calculated:

- a. Single burst frequency per minute.
- b. Single burst period time, i.e. the time from starting to end point of an RMS curve (burst) at the detection level, in minutes.
- c. Mean single burst RMS level, i.e. the mean of the RMS values within a single burst corrected for the detection level, in microvolt.
- d. Maximum single burst RMS level, i.e. the maximum RMS level within a single burst corrected for the detection level, in microvolt.

An example of an EMG and the corresponding RMS curve is shown in figure 6.3. The variable detection level was used to discriminate between bursts and noise.

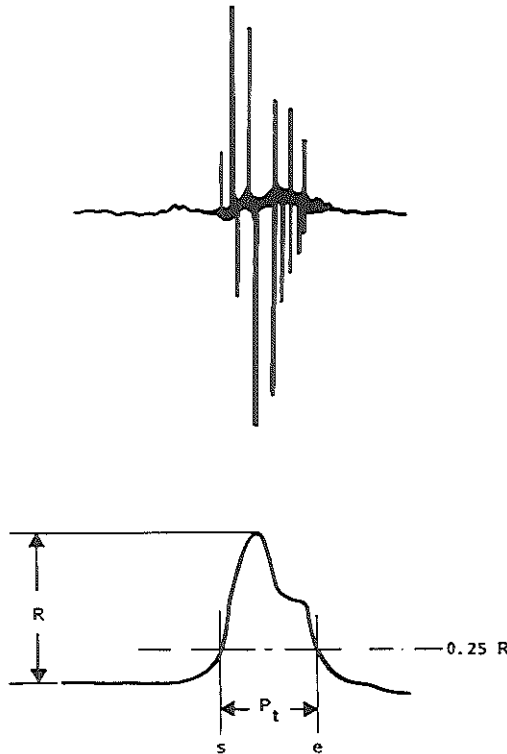


Figure 6.3 Diagrammatic representation of a burst of electrical activity and calculated RMS wave. R , range between minimum and maximum RMS level; $0.25 R$, minimum RMS level + $0.25 \times$ RMS range; s , starting point; e , end point; P_t period time.

Application

The computer prints of two recordings of IUP and EMG obtained in an oophorectomized ewe before and after administration of estradiol, as described in chapter 2, are presented in figure 6.4. The vertical bars above

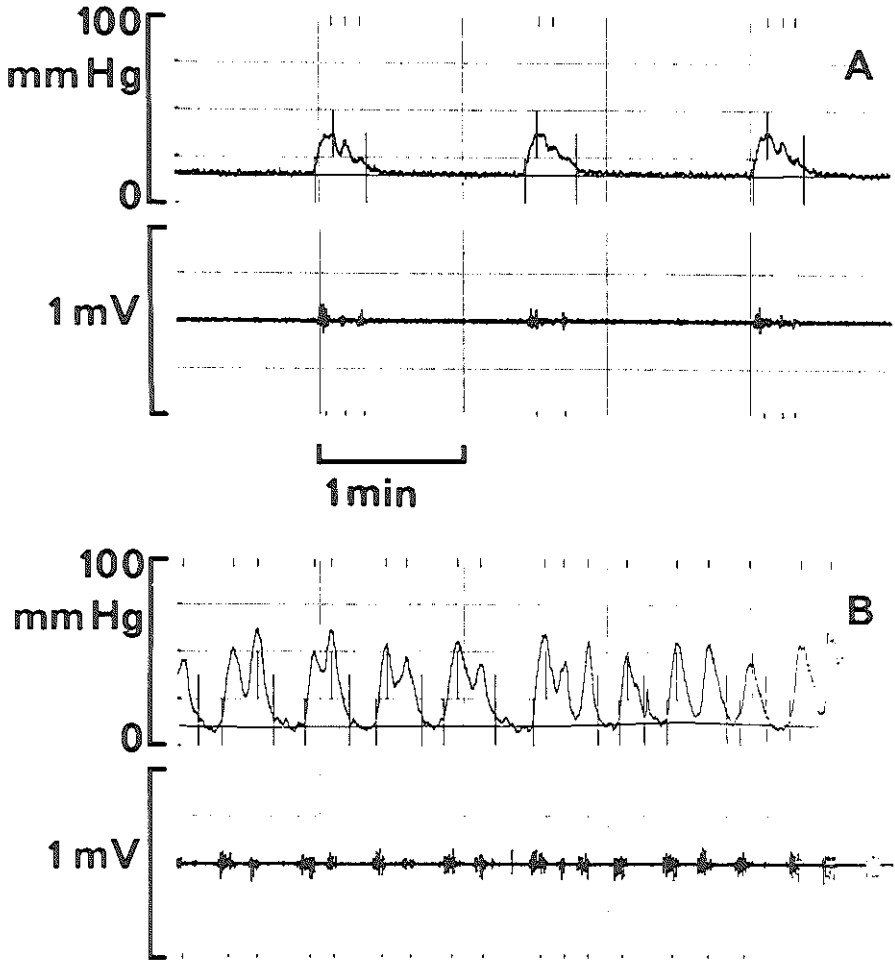


Figure 6.4 Computer analysis of two sets of recordings of intrauterine pressure (IUP, upper tracings) and electromyogram (EMG, lower tracings) obtained in an oophorectomized ewe before (A) and 36 hours after (B) administration of 0.1 mg of estradiol-17 β . The vertical bars in the IUP signal indicate peak pressure and the starting and end points of an IUP cycle, the bars above the IUP signal indicate detection of a small wave. The vertical bars below the EMG indicate detection of a single burst.

the IUP signal indicate the detection of a small wave. The bars in the intrauterine pressure signal represent the starting and end points and the maxima of the IUP cycles. The vertical bars below the EMG indicate the detection of a single burst. Table 6.1 presents the variables that were calculated from the recordings. The applications of the method described here have been published (51, 54) and are described in chapters 2 and 4.

Table 6.1. RESULTS OF THE ANALYSIS OF A 5 MINUTES SEGMENT OF THE INTRAUTERINE (IUP) RECORDING AND THE ELECTROMYOGRAM SHOWN IN FIGURE 6.4A AND 6.4B.

variable	unit	figure 6.4A	figure 6.4B
small wave frequency	min ⁻¹	1.6	4.0
IUP cycle frequency	min ⁻¹	0.6	2.0
mean IUP cycle periodtime	min	0.35	0.34
mean peakpressure	mm Hg	23.2	44.5
mean active pressure area	mm Hg.min	4.9	8.4
mean overall rate of rise	mm Hg.sec ⁻¹	3.1	6.7
mean maximum rate of rise	mm Hg.sec ⁻¹	5.4	12.1
single burst frequency	min ⁻¹	1.6	3.8
mean single burst periodtime	min	0.09	0.1
mean single burst RMS level	μV	3.1	20.5

Modifications

After detecting the single bursts, trains of single bursts were defined as one or more single bursts with end and starting points less than 20 seconds apart. When the period time of a train of single bursts was less than two minutes it was defined as a short burst; when the period time was more than two minutes it was termed a long burst. For the short and long bursts maximum RMS, mean RMS and RMS content i.e. the sum of RMS content (i.e. single burst period time x mean single burst RMS level) of the single bursts within the short or long burst were calculated. Single and short burst frequency (in min⁻¹) was calculated from the burst-to-burst time intervals. The modifications described here were applied in the study reported in chapter 4.

Discussion

In this chapter the development is described of a program for computer-aided analysis of electrical and mechanical myometrial activity. The algorithms were designed for use in experiments in which the characteristics of the recorded signals may show considerable variation. The calculated variables are a reflection of myometrial activity, although the physiologic significance of each of the intrauterine pressure variables is still debated (11,13,45).

We employed the RMS method to analyze the electromyogram. The variable detection level was used to discriminate between bursts and noise. The burst period time, the average RMS level and, to a lesser degree, burst frequency are influenced by the detection level. This implies that care should be taken to compare recordings with different noise levels.

The computer analysis described in this chapter allows the investigation of the temporal relationship between electrical and mechanical myometrial activity and comparison of the variables calculated from both signals, which is of physiologic interest. The accurate automated analysis of recordings of electrical and mechanical uterine activity provides a large data base of several physiologically important variables, which can be rapidly statistically evaluated. Therefore, it may facilitate physiologic and pharmacologic research on myometrial activity.

The program as such is not suitable for clinical monitoring of uterine activity during human labor. First, although intrauterine pressure recordings are widely used to monitor human labor, the electrical myometrial activity cannot be easily obtained in humans. Second, the obstetrician needs on-line analysis of intrauterine pressure signals. However, conversion of the off-line analysis described here to on-line analysis with the use of a microcomputer system seems feasible.

VII General discussion

Progesterone and prostaglandins are the main determinants in the control of myometrial contractility (7,13). Progesterone seems to be the most important factor in maintaining the quiescent myometrial state of pregnancy, whereas prostaglandins appear to have a central role in the initiation and progression of labor (13,29,56). The formation of gap junctions between myometrial smooth muscle cells was shown to be indispensable for the development of the coordinated activity that characterizes effective labor (20).

This thesis presents a combined approach using electrophysiologic, endocrinologic and ultrastructural methods to assess the control of myometrial contractility. The chronically instrumented oophorectomized ewe was used as an experimental model because it allows manipulation and investigation of many of the factors involved. As reported in chapter 3, the introduction of an intrauterine pressure catheter induces a pattern of myometrial activity resembling that of labor. The myometrial activity induced by the intrauterine pressure catheter can be suppressed by progesterone (51) and by inhibition of prostanoid synthesis, factors in control of pregnancy maintenance and termination.

The results reported in chapter 2 indicate that in the chronically instrumented oophorectomized ewe estradiol induces the formation of gap junctions in the myometrium. The increase in the number and area of gap junctions is associated with an increase in the coordination of myometrial activity as described in chapter 4. Inhibition of prostanoid synthesis does neither interfere with the estradiol-induced increase in the number of gap junctions, nor with the improvement in the coordination of myometrial activity, although myometrial contractility is reduced. Prostanoid concentrations measured in the uterine veins indicate that prostanoid synthesis was effectively inhibited with the dose of naproxen sodium used. The unaltered coordination of myometrial activity in the presence of inhibited prostanoid synthesis suggests that gap junction permeability is not affected by prostaglandins. Pharmacologic inhibition of prostaglandin synthesis inhibition is increasingly employed in the treatment of preterm labor. Extrapolation of the observations in chronically instrumented oophorectomized ewes to humans, would suggest that the mechanism of inhibition of labor by means of inhibition of prostaglandin synthesis should not be sought in the suppression of gap junction formation or reduction in gap junction permeability.

The increase in myometrial activity observed after estradiol treatment in spite of inhibition of prostanoid synthesis raised the question whether

other eicosanoids could be involved in the modulation of myometrial activity. In an attempt to answer that question, the sensitivity of ovine myometrial smooth muscle to various eicosanoids, including leukotrienes, was investigated *in vitro*. The results of the investigations reported in chapter 5 suggest that the primary prostaglandins are the main modulators of myometrial contractility. No evidence was obtained to support the concept of involvement of leukotrienes in the regulation of myometrial activity.

The computer program, described in chapter 6, was developed for analysis of signals of electrical and mechanical myometrial activity. It allowed more accurate analysis of myometrial activity in the animal model that was used in the studies presented in the thesis. This model provides an opportunity to study myometrial gap junction formation and permeability *in vivo*. The presence of gap junctions is mandatory for the occurrence of physiologic labor. Defining the factors that control gap junction formation and permeability may lead to a further understanding of the physiologic control of labor.

At present there is no causal treatment of preterm labor, which still constitutes one of the main causes of perinatal morbidity and mortality. Pharmacologic inhibition of prostaglandin synthesis, although frequently applied in clinical practice, is often not effective. Despite the progress in our understanding of the mechanisms involved in the control of term and preterm labor, there is still a challenge ahead.

VIII Summary

The studies presented in this thesis deal with control of myometrial contractility. The *in vivo* experiments were carried out in chronically instrumented oophorectomized ewes.

Chapter 1 is an introduction to the thesis. The main factors thought to be involved in the control of myometrial activity with respect to the initiation of parturition are briefly reviewed. Against this background the studies described in the thesis were performed to assess the functional relationship between estrogen and prostanoid action, formation of gap junctions, and myometrial activity.

Chapter 2 describes an experimental study on the influence of estrogen treatment on electrical and mechanical myometrial activity and gap junction formation *in vivo*. Intraarterial administration of estradiol-17 β induced a period of myometrial quiescence followed by a significant increase in maximum rate of rise, peak pressure, and active pressure area of the intrauterine pressure cycles and frequency of bursts of electrical activity. Gap junction area was also increased after administration of estradiol-17 β . The maximum increase in gap junction area occurred 24 hours after estradiol and was associated with the greatest increase in maximum rate of rise. These results provide further evidence that gap junctions improve the coordination of myometrial activity.

Chapter 3 presents a study on the influence of the intrauterine pressure catheter on electrical and mechanical myometrial activity in the chronically instrumented oophorectomized ewe. The electromyogram of non-catheterized ewes was characterized by long bursts of electrical activity, similar to myometrial activity in pregnant ewes, whereas catheterized ewes showed short bursts of electrical activity with a much higher frequency, similar to myometrial activity during labor. Administration of estradiol-17 β was followed by an increase in myometrial activity, after a period of quiescence. The pattern of electrical activity in catheterized and non-catheterized ewes was not markedly changed by estradiol. Pharmacologic inhibition of prostanoid synthesis by means of intravenous infusion of naproxen sodium in catheterized ewes resulted in a change in electrical activity to a pattern resembling the electromyogram of non-catheterized ewes. It is concluded that the presence of an intrauterine pressure catheter affects myometrial activity by stimulating prostanoid synthesis.

Chapter 4 describes a study on the effect of inhibition of prostanoid synthesis on estradiol-induced coordination of electrical and mechanical myometrial activity and gap junction formation *in vivo*. Administration of estradiol-17 β was followed by a significant increase in maximum rate of rise of the intrauterine pressure cycles and a significant decrease in

conduction time of single and short bursts of electrical activity. This response was not altered by pharmacologic inhibition of prostanoid synthesis. Concentrations of prostaglandins E_2 and $F_{2\alpha}$ were reduced by naproxen infusion and remained low after administration of estradiol. Administration of estradiol during pharmacologic inhibition of prostanoid synthesis by means of continuous infusion of naproxen increased gap junction area. Prostanoids appear not to be primarily involved in the estradiol-induced increase in coordination and synchronization of electrical and mechanical myometrial activity.

Chapter 5 presents an *in vitro* study of the sensitivity of sheep myometrial tissue to prostaglandins $F_{2\alpha}$, E_2 , the thromboxane analog U-44069, and leukotrienes C_4 and D_4 in a superfusion system. Tissues were obtained from oophorectomized ewes, with or without pretreatment with estradiol- 17β . The dose needed to induce a contraction with a peak level of 50 % of the median peak level of spontaneous contractions, increased from PGE_2 to $PGF_{2\alpha}$, U-44069, LTC_4 and LTD_4 . The difference in the dose required was significantly different for all compounds except between LTC_4 and LTD_4 . Estradiol pretreatment caused an increase in the required dose for $PGF_{2\alpha}$. The results of this study do not support the hypothesis that leukotrienes are involved in the regulation of myometrial activity.

Chapter 6 describes a method for computer analysis of electrical and mechanical myometrial signals. Two algorithms were developed for analysis of both electrical and mechanical myometrial activity. A combination of maxima- and level detection was used to define pressure cycles in the intrauterine pressure signal. The electrical signal was assessed by calculating the root mean square (RMS) value in a moving time interval. A threshold value was applied to the obtained RMS curve to detect single bursts of electrical activity present in the original signal. Short and long bursts were then defined. By comparing recordings from various sites of the uterus conduction time of single and short bursts can be calculated. The algorithms were applied to recordings obtained from chronically instrumented ewes in various experiments and were shown to contribute to the accurate automated analysis of recordings of electrical and mechanical myometrial activity.

Chapter 7 presents a general discussion of the results of the studies described in the thesis. The chronically instrumented oophorectomized ewe proved to be a useful model to investigate control of myometrial contractility. Instrumentation with an intrauterine pressure catheter induces a prostanoid-mediated change in electrical and mechanical myometrial activity. Prostanoids are not involved in the estradiol-induced formation of gap junctions. Pharmacologic inhibition of prostanoid synthesis does not seem to affect gap junction permeability and estradiol-induced improvement in coordination of myometrial activity.

Samenvatting

In dit proefschrift wordt onderzoek beschreven naar de regulatie en controle van de contractiliteit van het myometrium. De in vivo experimenten werden uitgevoerd bij chronisch geïnstrumenteerde, niet-zwangere oöien.

Hoofdstuk 1 geeft een kort overzicht over de belangrijkste factoren die betrokken zijn bij de controle van de contractiliteit van het myometrium en het op gang komen van de baring. Tegen deze achtergrond werden de in het proefschrift beschreven onderzoeken naar de functionele verbanden tussen de werking van oestrogenen en prostaglandines, de vorming van gap junctions en de activiteit van het myometrium uitgevoerd.

Hoofdstuk 2 beschrijft een onderzoek naar de invloed van oestradiol op elektrische en mechanische myometriumaactiviteit en op de vorming van gap junctions in het myometrium. Door toediening van 17β -oestradiol werd de activiteit van het myometrium tijdelijk onderdrukt. Deze periode van relaxatie werd gevolgd door een significante toename van de stijgsnelheid van de intrauteriene drukcurves en van de hoogte en het oppervlak van de drukcurves. Toediening van oestradiol induceerde de vorming van gap junctions in het myometrium. Het maximum van de toeneming van het aantal gap junctions viel samen met het maximum van de toeneming van de stijgsnelheid van de intrauteriene drukcurves. Dit ondersteunt de stelling, dat gap junctions essentieel zijn voor een goede coordinatie van myometriumaactiviteit.

Hoofdstuk 3 omvat een onderzoek naar de invloed van de catheter voor de meting van de intrauteriene druk op de elektrische en mechanische myometriumaactiviteit bij chronisch geïnstrumenteerde geovariëctomeerde oöien. Het electromyogram van oöien zonder drukcatheter werd gekarakteriseerd door lange bursts van elektrische activiteit, vergelijkbaar met de myometriumaactiviteit die tijdens de zwangerschap wordt gezien. Het electromyogram van oöien met een drukcatheter was gekenmerkt door korte bursts van elektrische activiteit met een veel hogere frequentie, vergelijkbaar met de myometriumaactiviteit tijdens de baring. Door het toedienen van oestradiol nam de myometriumaactiviteit toe, na een periode van relaxatie. Na het toedienen van oestradiol veranderde het patroon van het electromyogram bij oöien met of zonder drukcatheter niet in belangrijke mate. Het patroon van de elektrische activiteit bij oöien met een drukcatheter werd door remming van de prostaglandinesynthese door middel van intraveneuze infusie van naproxen-natrium zodanig veranderd, dat het vergelijkbaar werd met dat

van oöien zonder drukcatheter. Hieruit kan worden geconcludeerd dat een intrauteriene drukcatheter de myometriümaciviteit beinvloedt door stimulatie van de prostaglandinesynthese.

Hoofdstuk 4 beschrijft een onderzoek naar de invloed van remming van de prostaglandinesynthese op de door oestradiol geïnduceerde vorming van gap junctions in het myotriüium en de daarmee samenhangende toeneming van de coordinatie van myometriümaciviteit. Toediening van 17β -oestradiol veroorzaakte een significante toename van de stijgsnelheid van de intrauteriene drukcurves en een significante afname van de geleidingstijd van bursts van elektrische activiteit. Dit effect werd niet beïnvloed door farmacologische remming van de prostaglandinesynthese. Infusie van naproxen-natrium verlaagde de concentraties van prostaglandine E_2 en $F_{2\alpha}$, zowel voor als na toediening van oestradiol. Na toediening van oestradiol tijdens farmacologische remming van de prostaglandinesynthese met naproxen nam het aantal gap junctions toe. Prostaglandines lijken dus niet primair te zijn betrokken bij de door oestradiol geïnduceerde verbetering van de coordinatie en synchronisatie van elektrische en mechanische myometriümaciviteit.

Hoofdstuk 5 betreft een onderzoek in vitro naar de gevoeligheid van het myotriüium voor prostaglandine E_2 en $F_{2\alpha}$, de tromboxaan analoog U-44069 en de leukotrienes C_4 en D_4 in een superfusiesysteem. Myotriüium werd verkregen van geovariectomeerde oöien, waarvan de helft was voorbehandeld met 17β -oestradiol. De dosis nodig om een contractie te bereiken met een hoogte van 50 % van de mediane waarde van de hoogte van spontane contracties nam toe van PGE_2 tot $PGF_{2\alpha}$ en vervolgens U-44069, LTC_4 en LTD_4 . De verschillen tussen de benodigde doses waren significant voor alle agentia, met uitzondering van LTC_4 en LTD_4 . Door voorbehandeling met oestradiol werd de benodigde dosis van $PGF_{2\alpha}$ hoger. De resultaten van dit onderzoek de bieden geen steun aan de hypothese dat leukotrienen een rol spelen bij de regulatie van myometriümaciviteit.

Hoofdstuk 6 beschrijft het computerprogramma dat werd gebruikt voor de analyse van myometriümaciviteit. Er werden twee algoritmen ontwikkeld voor analyse van de elektrische en de mechanische signalen van myometriümaciviteit. Voor het definiëren van intrauteriene drukcurves werd een combinatie van maxima- en niveaudetectie gebruikt. Van het elektrische signaal werd de "root mean square" (RMS) waarde berekend. Om de bursts van elektrische activiteit te definiëren werd een drempelwaarde berekend in de RMS curve, waarna single bursts werden gedetecteerd. Voor het onderzoek beschreven in hoofdstuk 4 werden na detectie van single bursts korte en lange bursts gedefinieerd. Door de elektrische signalen van verschillende delen van de uterus te vergelijken werd daarna de geleidingstijd van bursts bepaald. Het

computerprogramma werd gebruikt voor de analyse van in verschillende experimenten verkregen registraties van elektrische en mechanische myometriëactiviteit en bleek een nauwkeurige geautomatiseerde bewerking mogelijk te maken.

Hoofdstuk 7 geeft een algemene bespreking van de resultaten van de in dit proefschrift beschreven experimenten. De chronisch geinstrumenteerde geovariëctomeerde ooi blijkt een goed bruikbaar model te vormen voor onderzoek naar de regulatie en controle van myometriëactiviteit. Een intrauteriene catheter veroorzaakt door prostaglandines geïnduceerde veranderingen van de activiteit van het myometrium. Prostaglandines zijn niet betrokken bij de door oestradiol geïnduceerde vorming van gap junctions. Farmacologische remming van de prostaglandinesynthese lijkt geen invloed te hebben op de permeabiliteit van gap junctions, noch op de door oestradiol geïnduceerde toeneming van de coordinatie van de activiteit van het myometrium.

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- 1991 Chef de Clinique, "St Claraziekenhuis" Rotterdam.
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