MYOMETRIAL CONTRACTILITY AND GAP JUNCTIONS

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AN EXPERIMENTAL STUDY IN CHRONICALLY INSTRUMENTED EWES

MYOMETRIUM CONTRACTILITEIT EN GAP JUNCTIONS
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GEINSTRUMENTEERDE SCHAPEN

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To the memory of my mother
To my father
To Marijke, Thomas and Bruno

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

The conversion of the uterus from the relatively inactive state during pregnancy to the active state of labor is one of the major topics in obstetric research. Scientific attention has focused mainly on the endocrinological control of myometrial activity and initiation of parturition.

In 1956 Csapo (1) postulated the progesterone-block hypothesis. He suggested that progesterone blocks myometrial activity during pregnancy by inhibiting the excitation of the smooth muscle cells and the conduction of action potentials. Labor is to occur only after the withdrawal of progesterone. This hypothesis, intended to be a unifying concept for all species, was mainly based on observations in rabbits. As knowledge expanded with investigations in other species, Csapo revised his much debated hypothesis into the seesaw theory (2) in which he proposed that myometrial activity was controlled by a balance between stimulation (prostaglandins and other factors) and inhibition (progesterone). The initiation of parturition was suggested to be caused by an increase in stimulating factors and a decrease in progesterone.

In contrast to Csapo who concentrated mainly on myometrial events to define the initiating steps leading to labor, Liggins (3,4) directed his investigations to the role of the fetus. His pioneering experimental work showed the involvement of the fetal pituitary-adrenal axis in sheep (3). In this species labor was shown to be initiated by the activation of the fetal pituitary-adrenal axis leading to an increase in fetal cortisol secretion (4). The increase in cortisol is thought to induce a change in placental steroid production with a decrease in progesterone and an increase in estradiol secretion. The changes in maternal plasma and tissue levels of progesterone and estradiol starting 3-5 days before labor are associated with an increase in prostaglandin production by the uterine tissues and with a gradual change in myometrial activity, from a pattern of long bursts of electrical activity occurring one to three times per hour to the short frequent bursts characterizing labor (5).

An increase in prostaglandin production by uterine and fetal tissues has also been demonstrated in humans (6), but its regulation is not yet clear. The changes in steroid hormones observed in sheep and other animal species such as the cow, goat, rabbit and rat at the end of gestation have not been demonstrated to occur in human

pregnancy. Also the initiating events that control the onset of labor in humans are still obscure (7).

In addition to endocrinological and electrophysiological investigations of pregnancy and labor, recent electron microscopic studies revealed that significant ultrastructural changes take place to facilitate myometrial activity. Garfield (8-10) demonstrated that gap junctions, which connect the cytoplasm of two adjacent smooth muscle cells and thus provide low-resistance pathways for electrical and metabolic coupling, are almost absent during pregnancy but can be demonstrated in high numbers during labor (8). Such an increase in myometrial gap junctions was shown to occur in all species studied, including humans (9). Garfield proposed that the formation of gap junctions is a necessary step in the initiation of parturition (10). In vitro and in vivo studies suggested an endocrine control of gap junction formation by estradiol, progesterone and prostaglandins. In vitro studies also showed that gap junctions improved electrical coupling as postulated on the basis of theoretical considerations (11).

Gap junctions provide low-resistance pathways for the propagation of action potentials from one cell to another. Their presence should facilitate the spread of electrical activity across the uterus, thereby improving the coordination of the myometrial muscle cells. For obvious reasons, the relationship between gap junction formation, electrical and mechanical myometrial activity and steroid hormone levels cannot be assessed quantitatively in pregnant women. Therefore, studies were performed in chronically instrumented ewes. The influence of various factors that are suggested to control gap junction formation is difficult to study separately in vivo in the pregnant animal, as these factors show interrelated changes at the end of gestation. The nonpregnant ewe may serve as a useful model to investigate gap junction formation as it allows a much easier manipulation of the factors that seem to control gap junction formation in the pregnant animal.

Following a review of the literature on structure and organization of the myometrial smooth muscle cells, with special emphasis on the structure, function and development of myometrial gap junctions, this thesis presents the separately published results of experimental studies in pregnant and nonpregnant sheep. This compilation of articles represents an effort to investigate in vivo the relationship between gap junction formation and coordination of myometrial activity in pregnant ewes, and to develop a nonpregnant animal model in which the control of gap junction formation can be investigated in relation to myometrial activity. The sixth chapter is a description of the computer analysis of electrical and mechanical activity as used in our studies in the nonpregnant sheep model.

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CHAPTER 2

ULTRASTRUCTURE OF THE MYOMETRIUM AND THE ROLE OF GAP JUNCTIONS IN MYOMETRIAL FUNCTION

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I. Introduction

The uterus is a heterogeneous organ composed of cells of many types, with a predominance of smooth muscle cells in the myometrial layer. Knowledge of various types of cells, their functions, relationships, and interactions is necessary for a complete appreciation of uterine physiology. The changes which occur in uterine muscle during pregnancy to activate it at term are important physiological considerations. The events which bring about the initiation of parturition and the conversion of the myometrium from inactive to active have been debated for many years (1-6). It is evident from many studies that changes in the levels and binding of several hormones, including steroid hormones, oxytocin, and prostaglandins are important determinants of parturition in many species (1-9). Recent ultrastructural studies of the myometrium have shown that gap junctions develop in large numbers between smooth muscle cells during labor in response to steroid hormones and prostaglandins and it has been proposed that these junctions may initiate parturition (10-21).

This chapter will describe the structure of the uterus with emphasis on the ultrastructure of the myometrium. Components of the uterine wall will be discussed, including the arrangement of the muscle layers. Ultrastructural features of the smooth muscle cell will be presented. Particular attention will be devoted to recent studies of the presence and function of gap junctions in the myometrium.

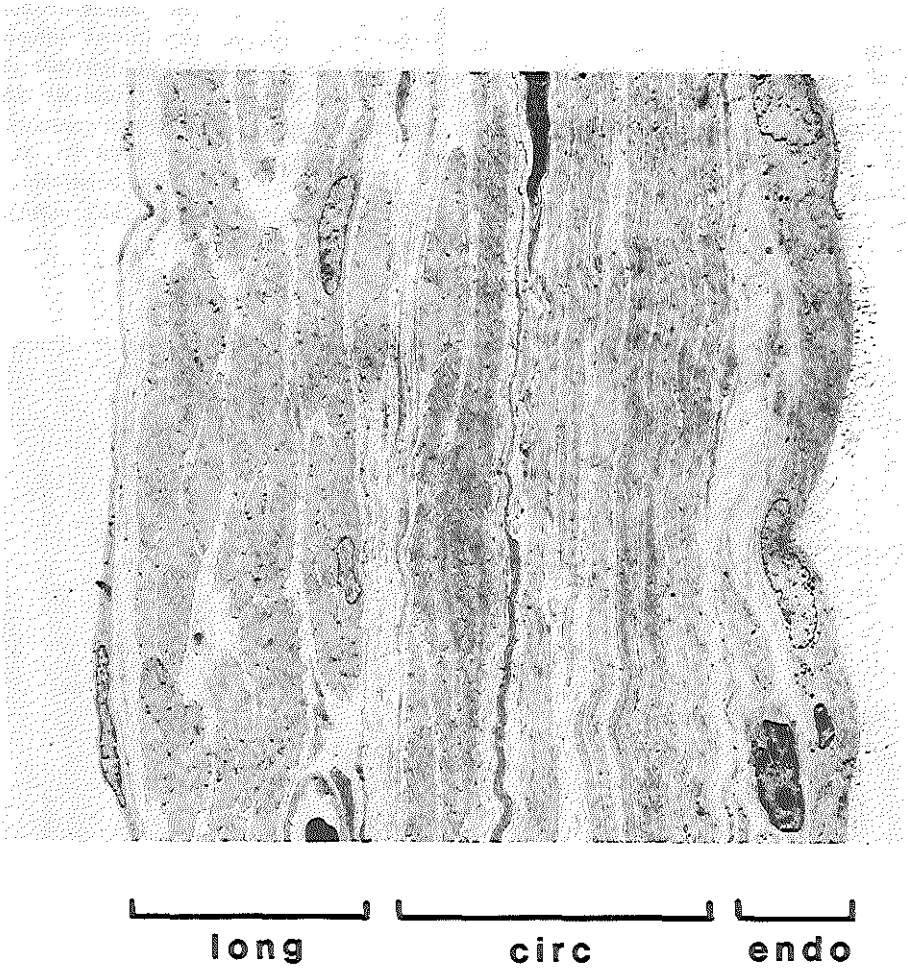

Figure 2.1 Cross-section of the uterine wall from a 20-day pregnant rat. The uterine lumen is to the right of the field and is bordered by the endometrium (endo) composed of epithelium, connective tissue, and blood vessels. The circular muscle layer (circ) is shown in longitudinal section and the longitudinal muscle (long) in transverse orientation. The total wall thickness in this distended, term uterus is approximately 65 μm (magnification x 1700).

II. Structure and organization of the myometrium

The uterine wall is composed of three distinct layers in most species. The inner layer, the endometrium, lines the lumen of the organ. The myometrium makes up the other two layers: the outer longitudinal muscle layer and the inner circular layer. Varying degrees of organization into circular and longitudinal layers of cell bundles are apparent in different species. The longitudinal muscle layer consists of bundles of smooth muscle cells that are generally oriented in the long axis of the uterus. The bundles interconnect to form a network on the surface of the uterus (22). Contraction of the longitudinal muscle would tend to shorten the uterus and constrict the lumen.

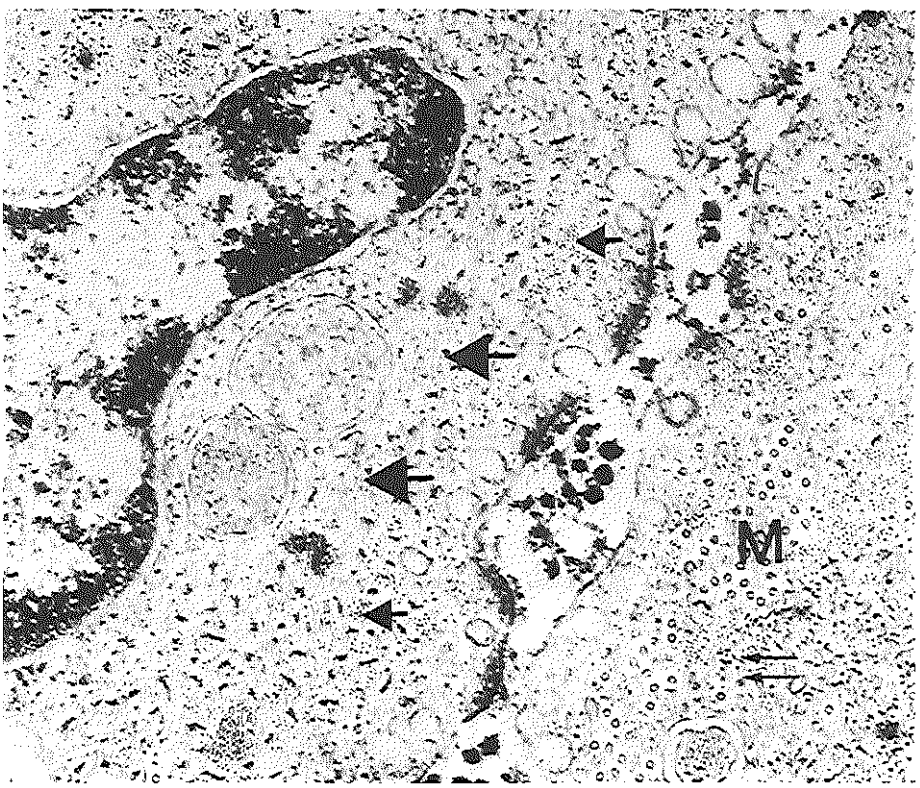

Figure 2.2 High magnification micrograph of a portion of a smooth muscle cell from rat myometrium showing the myofilaments. Myosin filaments, large arrows; actin, small arrows; intermediate, double arrows. Microtubules (M) are also evident within the cell (magnification x 55,000).

Muscle cells of the circular muscle layer are arranged concentrically around the longitudinal axis of the uterus. The muscle cells are arranged more diffusely and the bundle arrangement, if present, is not as apparent as that of the longitudinal layers. Contraction of the circular muscle layer constricts the uterine lumen. Functional and structural studies in the pregnant rat indicate that the longitudinal layer is continuous with the circular layer (23,24).

A low magnification electron micrograph showing the entire wall of the uterus from a pregnant rat is shown in figure 2.1. Muscle cells of both muscle layers occupy a major part of the uterine wall. The extracellular space between the muscle cells is occupied by collagen

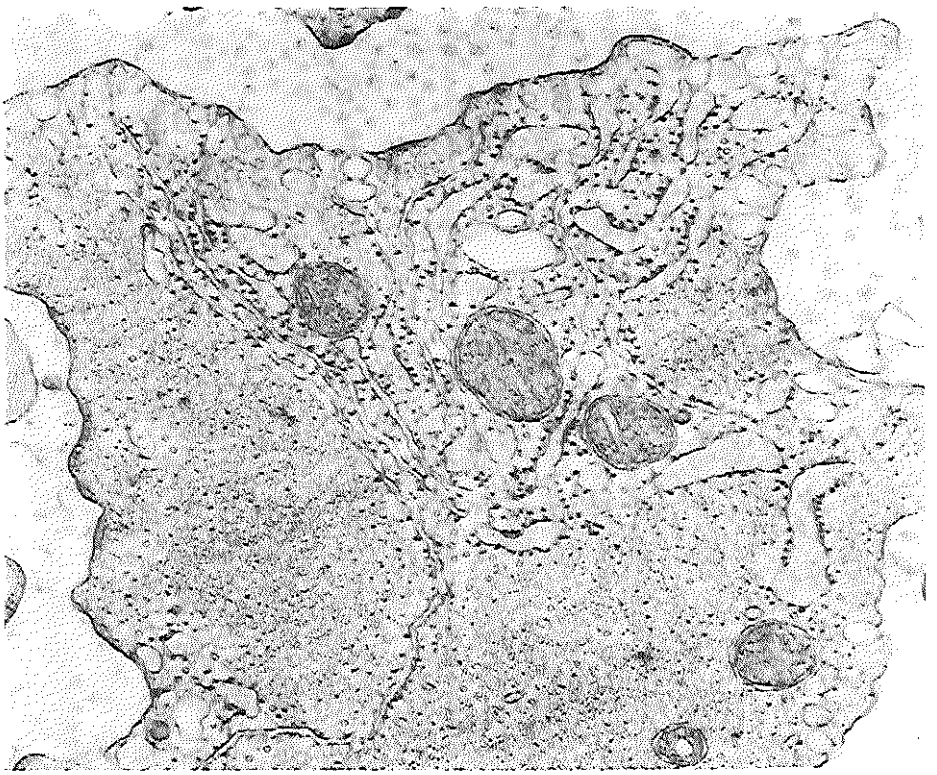

Figure 2.3 Transverse section through a myometrial cell from a pregnant guinea pig uterus. Note the continuity between granular and agranular sarcoplasmic reticulum and the close association between vesicles, reticulum, plasma membrane, and mitochrondia (magnification x 40,500).

and other cells including fibroblasts, blood and lymphatic vessels, and nerves. The smooth muscle cell, together with its matrix of connective tissue elements, is the functional unit for uterine contractility.

III. Features of smooth muscle cells

A. Contractile apparatus

Myometrial smooth muscle cells are long, spindle-shaped cells. The cells are largest during the later stages of gestation and their size

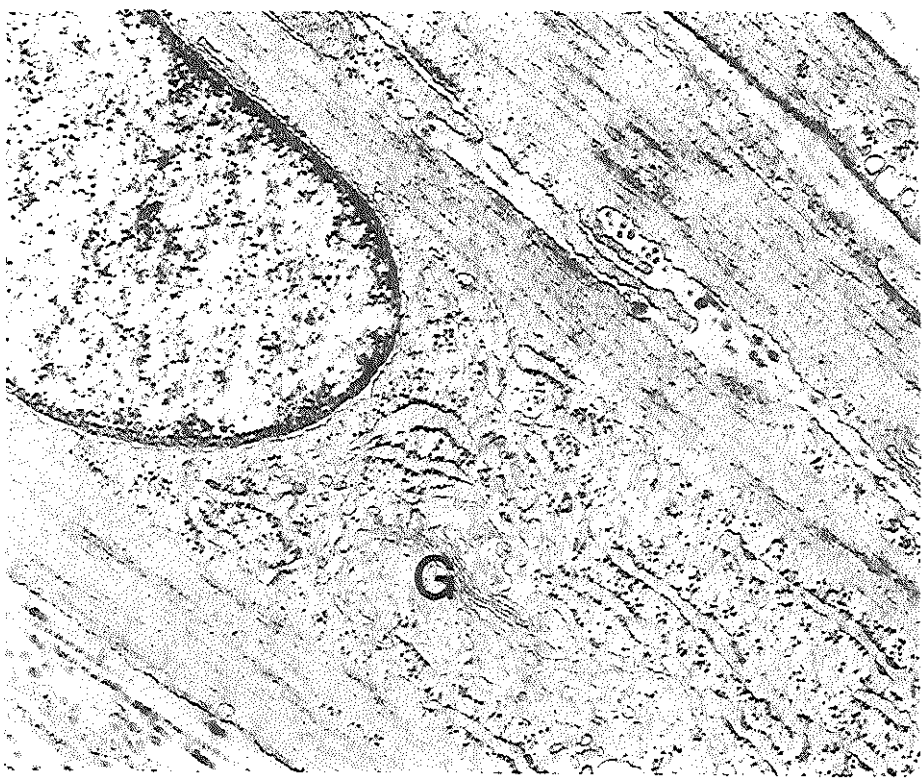

Figure 2.4 Longitudinal section from pregnant rat myometrium showing extensive granular sarcoplasmic reticulum of muscle cells and Golgi apparatus (G) located perinuclearly (magnification x 26,000).

and number is thought to be regulated by steroid hormones and distension (25,26). It should be mentioned that the size of the myometrial cells probably varies considerably between species.

The protein components of the cell that respond to the Ca^{2+} fluctuations and utilize the chemical energy of ATP to result in either shortening or tension development are termed collectively the contractile apparatus. In smooth muscle, the major contractile proteins are myosin, actin, and tropomyosin, and minor components of the contractile apparatus include the proteins that are involved in the Ca^{2+}-dependent regulatory mechanism (27).

At least three different types of myofilaments have been identified in uterine smooth muscle cells by electron microscopy. Figure 2.2 is

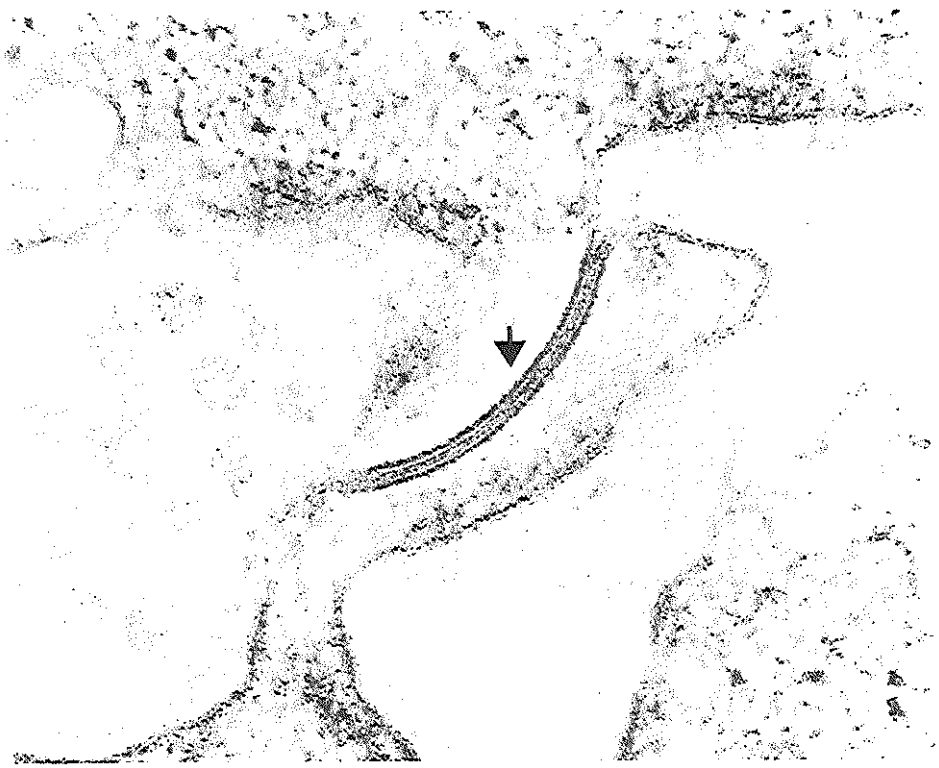

Figure 2.5 Gap junction (arrow) between two muscle cells from the myometrium of a ewe fixed during parturition as seen in thin section at high magnification (magnification x 180,000).

an electron micrograph of a uterine smooth muscle cell showing thick (myosin, 15 nm), thin (actin, 5 to 6 nm), and intermediate (10 nm) filaments as well as microtubules.

B. Plasma membrane

The plasma membrane of uterine smooth muscle cells, the sarcolemma, is a trilaminar structure of approximately 8 nm in thickness, as in other cells (28), and is thought to be composed of phospholipids and proteins. Intramembranous protein particles, about 9 nm in diameter, are seen in freeze-fracture replicates of the plasma membrane. These particles are more numerous on the protoplasmic- than on the external-face of the membrane (11), as in other types of smooth muscle (29,30).

Surface vesicles or caveolae (50 to 80 nm in diameter) populate the

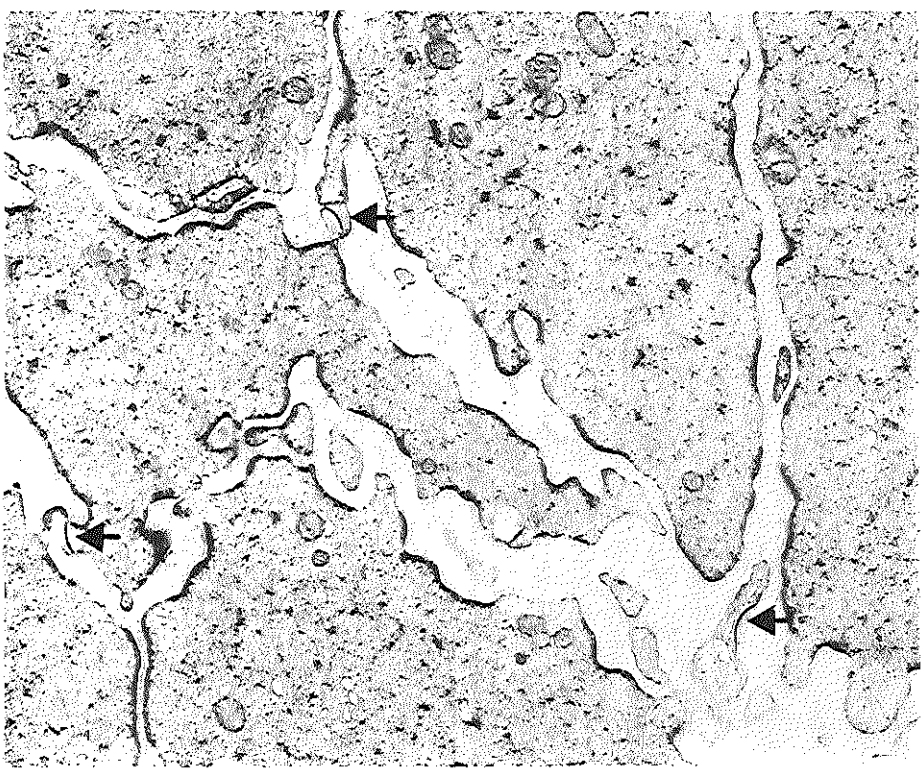

Figure 2.6 Low magnification electron micrograph of sheep myometrium during labor illustrating gap junctions (arrows) between muscle cells (magnificaton x 11,000).

surface of the smooth muscle cells (figure 2.3). These flask-shaped invaginations of the plasma membrane contribute substantially to the cell surface area. In uterine smooth muscle cells the vesicles have been estimated to occupy 31% of the surface area (31). The vesicles are not randomly scattered over the surface of the cells but are arranged in longitudinal rows along the cell surface as seen by freeze-fracture microscopy (29-33).

Although these vesicles have been suggested to be sites for ion

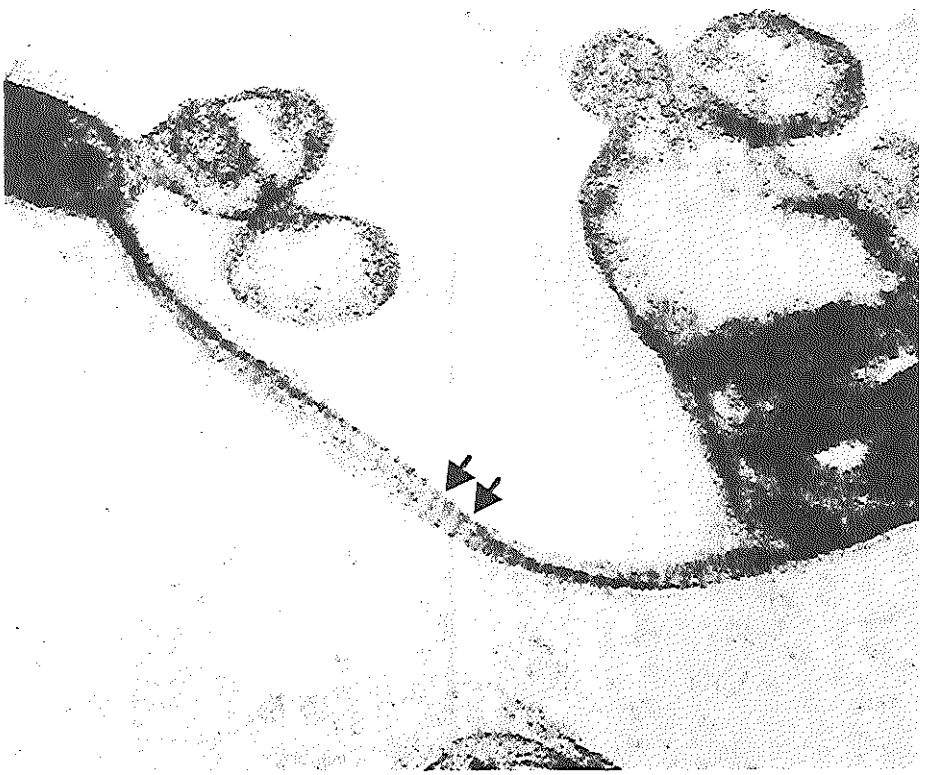

Figure 2.7 Gap junction sectioned tangentially after fixation in glutaraldehyde + 8% tannic acid. Note that the electron-dense tannic acid penetrates into the exterior portion of the junction and delineates a periodicity (arrows) which corresponds to the external part of the membrane particles in alignment between the two cell membranes (magnification x 295,000).

transport and binding (34,35), their function is not known. It is suggested that they perform a similar function as the T-tubules in striated muscle. The vesicles are often seen in close association to mitochondria and sarcoplasmic reticulum (figure 2.3) (31,32,36). The vesicles are not likely to participate in any pinocytotic or endocytotic function as they have never been shown to be interiorized. Electron-dense tracers readily penetrate into the vesicles when added either before or after fixation (30), suggesting that they are all open to the exterior.

C. Sarcoplasmic reticulum

Uterine smooth muscle cells have an extensive system of sarcoplasmic reticulum consisting of a network of tubules and sacs within the cytoplasm (figures 2.3 and 2.4). The volume of the sarcoplasmic reticulum has been estimated to be 2 to 7.5% of the cell

Figure 2.8 The protoplasmic-face of a freeze-fracture replicate showing portions of two gap junctions from rat myometrium. The gap junctions appear as an aggregation of membrane particles (large arrows) (magnification x 89,000).

volume in other types of smooth muscle (31). The granular reticulum and agranular reticulum are continuous and the agranular reticulum makes close contact with surface vesicles, plasma membrane, and gap junctions (figure 2.3) (15).

The function of the sarcoplasmic reticulum is poorly understood. The granular reticulum most probably is involved in synthetic processes within the cell. Estrogen treatment and pregnancy increase the content of granular reticulum in uterine smooth muscle suggesting a stimulation of protein synthesis (25,37). The agranular reticulum may be involved in calcium storage and release and, therefore, control of muscle contractility. If the sarcoplasmic reticulum is a storage reservoir for Ca^{2+}, then ionic changes that occur in the smooth muscle cell during excitation may result in voltage-dependent changes in permeability of the sarcoplasmic reticulum and the release of Ca^{2+} to produce contraction. If this hypothesis is correct, it is clear that it would be advantageous to have the sarcoplasmic reticulum close to the plasma membrane or gap junctions to be more effective. The close proximity of the sarcoplasmic reticulum to gap junctions may sequester Ca^{2+} locally to avoid closing of the pores (see below).

D. Gap junctions

1. Structure and function of gap junctions

Extensive reviews on the structure and function of gap junctions in various cells, including those of the myometrium (12), have been published in the past few years (38-47).

A gap junction is a structure composed of two symmetrical portions of the plasma membrane from two opposing cells (38,40,46). When examined by thin section electron microscopy, gap junctions consist of regions where the plasma membranes of two cells appear to be approximated at a distance of about 2 nm after en bloc staining (figures 2.5 and 2.6). It is now clear that intramembraneous particles (proteins) which protrude through each membrane span the gap between the membranes (38,40,44,46). After fixation in tannic acid, which fills the extracellular portion of the gap junction, a periodicity is observed which probably corresponds to the exterior portion of particles making up the junction (figure 2.7).

Gap junctions appear as an aggregation of membrane particles or an array of pits in freeze-fracture replicates (40,45,46). The particles represent partially exposed membrane proteins and the pits are the impression left by the particles when the membrane is cleaved (40,46). In a fracture face through a region of a gap junction (figure 2.8), the P-face (protoplasmic portion of membrane) of the fracture usually contains a circular array of protruding particles about 7 nm in diameter with a 7 to 14 center-to-center spacing of particles (38,45,46,48). This array of particles may vary upon functional states (see below). The E fracture face, extracellular face of membrane, contains the pits. Generally, the structure of myometrial gap junctions as seen in thin sections and freeze-fracture replicates (figures 2.5, 2.6, and 2.7) is similar to that described in other cells.

Gap junction proteins within the opposed cell membranes are thought to align themselves and create channels (about 1.5 nm) from the cytoplasm of one cell to the cytoplasm of the other cell (38,40,42,46,48). The channels in the gap junctions are supposed to be the sites of electrical and metabolic coupling between cells (42,44,46). There is evidence that gap junctions form a pathway between coupled cells to provide for the passage of current (electrical coupling or ionic coupling); i.e., gap junctions are a low-resistance or low-impedance contact between cells (38,48). Gap junctions also provide a pathway for the direct exchange of metabolites (metabolic coupling) between cells (42,44,49). Whether gap junctions are the only type of cell contact which can accomplish electrical coupling is a matter considered by all investigators, but other cell contacts have not been suggested to be involved in metabolic coupling.

Recently, it has become apparent that cells connected by gap junctions may not necessarily show a free exchange of dyes, isotopes, and current (41,42,44, 46,50). Electrophysiologic studies show that gap junctions can rapidly switch from a low- to a high-resistance (44,46). It is now recognized that the channels or pores created by the gap junction may not always be in the open state (44,46,51,52). There may be times when the channels are open and times when they are closed. This uncoupling mechanism may be a safety device to uncouple injured cells (44) or to uncouple cells when metabolically or electrically desirable. Closing the channels or uncoupling has been achieved by changes in internal and external Ca^{2+} and pH (41, 44,46,50-52).

Gap junctions are present between most types of cells at least during some stage of the cell cycle, except between cells which are not part of an organized tissue (i.e. blood cells). Though present throughout the animal kingdom, the number of gap junctions varies considerably depending on the tissue type (40,46). Changes in the number, size, and distribution of gap junctions have been described as part of many developmental cell processes including growth and maturation (38-42,44,46,53-55). Gap junctions appear to be dynamic structures in most cell systems. The formation of gap junctions has been studied primarily in vitro where cells are separated and allowed to reaggregate and form communicating junctions (43,49,54). In cell systems where gap junctions are believed to be dynamic structures, gap junction degradation is supposed to occur by either dispersal of gap junction particles within the plasma membrane or internalization of the entire gap junction within one of the cells by endocytosis and degradation by lysosomes (40,43). The latter mechanism has been suggested because internalized gap junctions (annular) have been observed in many cell types (43).

2. Development of gap junctions in the myometrium during labor

Gap junctions have only recently been demonstrated satisfactorily in uterine smooth muscle (10-21,56). It has been shown that gap junctions are present between myometrial cells in pregnant animals

only during parturition (10-21). We have recently demonstrated that gap junctions develop in the myometrium of nonpregnant, ovariectomized sheep treated with estrogen (56). Similar results were obtained in studies on rats in a number of laboratories (55,57,58).

Quantitative morphometric analysis of thin sections were used to determine the number and area of gap junctions in the myometrium. The area of gap junctions as percentage of myometrial plasma membrane area during pregnancy, parturition, and postpartum in various animals is shown in figure 2.9. Studies in rats (10,11,19), guinea pigs (18), sheep (12,13,20), baboons (21), and studies in the human (16) demonstrate that (a) myometrial gap junctions are absent or present in low frequency throughout pregnancy; (b) at the end of term, gap junction area increases; (c) number and size of gap junctions increase during delivery of the fetus; (d) the gap junctions begin to disappear within 24 hour after delivery. The junctions are also present in increased numbers in tissues from animals induced to deliver prematurely (11,17,18) and in myometrium from women in premature labor (16).

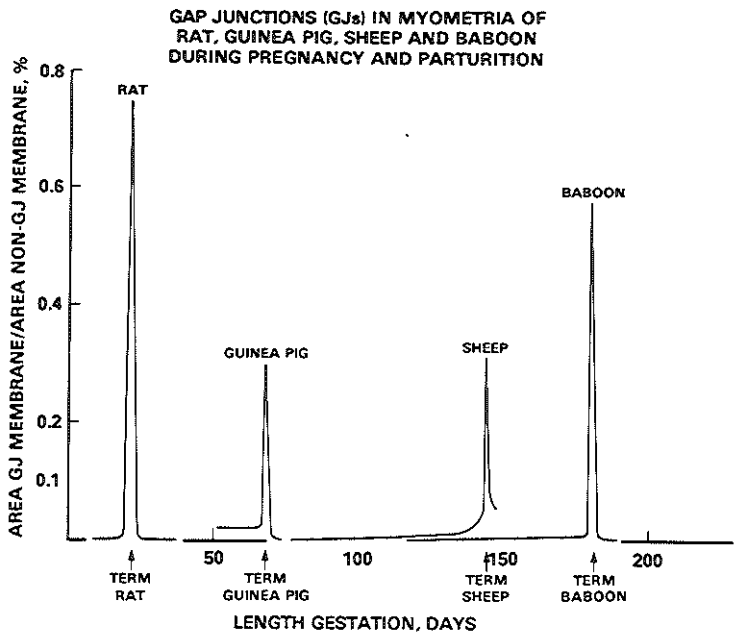

Figure 2.9 Relative area of myometrial gap junction (GJ) membrane (percentage area of GJ membrane per area of plasma membrane) vs. time during pregnancy, delivery and postpartum for rats, guinea pigs, sheep and baboons. For purposes of illustration, tissues from animals either delivering or postpartum were referenced to the term point regardless of their actual day of gestation.

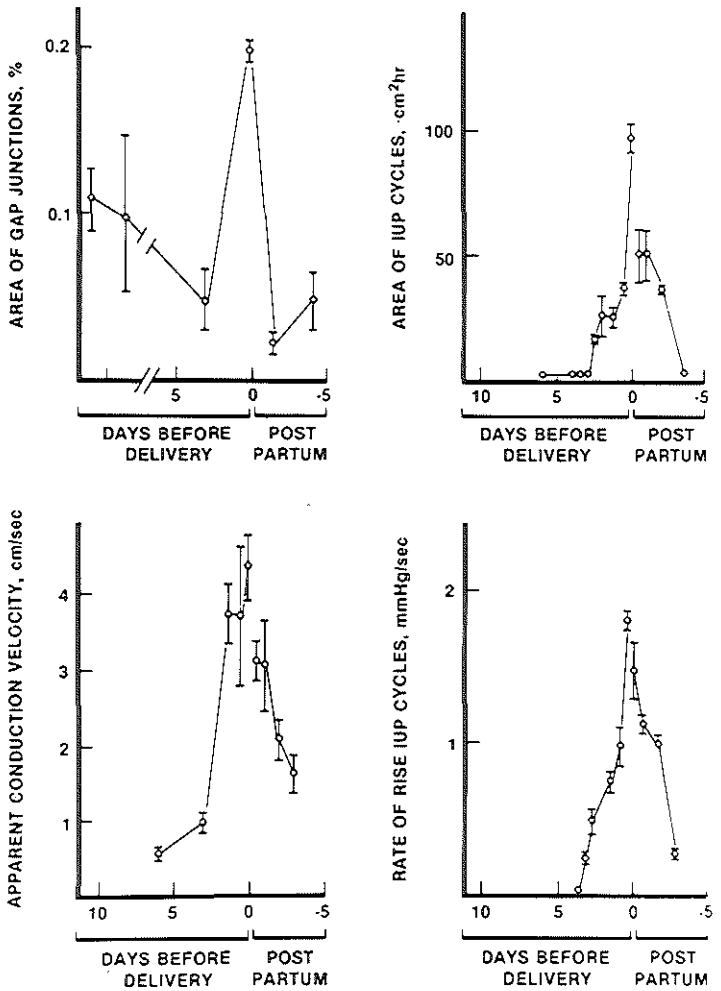

Figure 2.10 Relationship between percentage gap junction area, area of intra-uterine pressure (IUP) cycles, apparent conduction velocity of bursts of electrical activity and rate of rise of IUP cycles in sheep before, during, and following parturition. All values are means $\pm$ S.E.M. Note the correspondence between changes in area of gap junctions, changes in electrical conductance (conduction velocity) and mechanical activity (area and rate of rise of IUP cycles).

3. Role of myometrial gap junctions

The presence of gap junctions between uterine smooth muscle cells, limited to the period just prior to, during, and immediately following normal and premature parturition has significant implications for the maintenance and termination of pregnancy. The absence of gap junctions between smooth muscle cells throughout gestation may maintain pregnancy by limiting electrical communication between cells, thereby preventing coordinated contractions of the uterus. The formation of gap junctions in the muscular portion of the uterus may initiate or allow initiation of parturition by providing low-resistance pathways between muscle cells, thus allowing rapid, synchronized spread of action potentials, leading to well-coordinated contractions. In all species parturition is accompanied by rapidly propagating trains of electrical discharges which synchronously activate billions of individual muscle cells that compose a major portion of the uterine wall (1,7). The presence of gap junctions between myometrial cells may be the basis for conversion of uterine activity from inactive to active at the end of pregnancy.

To evaluate the relationship between electrical coupling in the myometrium and the development of increased gap junctional area, two independent methods have recently been used (59). Cable properties of the myometrium were determined in myometrial tissues before and during labor. These studies revealed that the space constant (λ) was significantly increased in tissues from delivering animals (3.7 ± 1.0 mm) as compared to similar animals that were not delivering (2.6 ± 0.8 mm) and which had low numbers of gap junctions.

Measurements of electrical impedance in the myometrium also showed lower junctional resistance in tissues from animals during delivery (139 Ω cm) compared to junctional resistance immediately prior to delivery (375 Ω cm) and 1.5 to 2 days postpartum (1450 Ω cm). We recently found (20) a good association between the increase in gap junction area in the sheep uterus and the increase in apparent conduction velocity of electrical signals, the rate of rise of intrauterine pressure cycles, and the increase in area of intrauterine pressure cycles. These parameters showed a significant increase during labor, together with the increase in gap junctional area, with a decline in the postpartum period (figure 2.10). These studies provide electrophysiological and mechanical evidence for better coupling of myometrial cells during delivery when gap junctions are present.

Possibly, another important role for gap junctions in the myometrium during labor is to connect the cells for metabolic cooperation (42). Studies of the relationship between gap junctions and the diffusion of metabolites between myometrial cells or other types of smooth muscle have not been reported. However, the presence of gap junctions between muscle cells probably allows the passage of small molecules between muscle cells which may synchronize metabolic and contractile activity.

The gap junctions also probably increase the response of the myometrium to drugs and influence the response to stimulation of the

receptor mechanism (60). Burnstock (61) has proposed that gap junctions are necessary for coupling of smooth muscle cells in sparsely innervated tissues. Nerves are thought to terminate only on a few smooth muscle cells (key cells) in a muscle bundle. The electrical response that is generated in these cells by the release of transmitter and interaction with receptors is thought to be transmitted from cell to cell by gap junctions. Nerves in uterine tissue are abundant, except in late pregnancy when they are thought to degenerate in response to hormones and stretch (3,62-65).

Similarly, myometrial tissue with gap junctions would be expected to be more sensitive to exposure to a given stimulant than tissue without gap junctions irrespective of the presence of nerves. Perhaps this is an important mechanism responsible for increased reactivity of the myometrium to oxytocin or other stimulants at term (60,66,67).

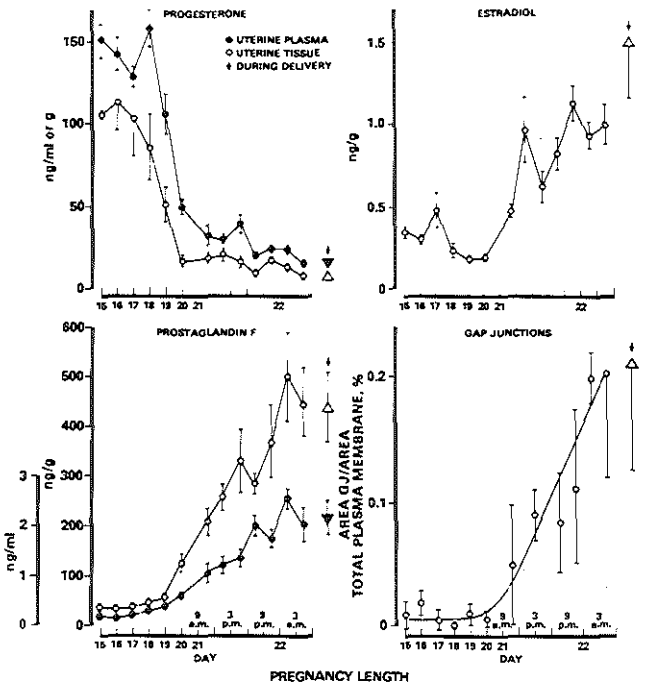

Figure 2.11 Changes (mean ± S.E.M.) in the levels of progesterone, estradiol and prostaglandin $F_{2\alpha}$ in uterine vein plasma (●) and uterine tissue (o) and the myometerial gap junctional area during the latter days of pregnancy and during delivery (△) in rats. Note the axis of the time scale is expanded from day 21 onwards to delivery. (From Puri CP, Garfield RE, Biol Reprod 1982;27:967. Reproduced with permission.)

4. Regulation of myometrial gap junctions

a. Steroid hormones

Prior to normal or premature labor in most animal species, there are changes in the synthesis of several hormones which are reflected in fluctuations of their plasma or tissue levels (1,2,4-6,8,9,20,68). The development of gap junctions in the myometrium is probably regulated by these hormonal changes. Understanding of the mechanisms which regulate gap junction formation and function may lead to effective procedures to initiate or inhibit labor.

Garfield et al. (10-21) have suggested previously that changes in the steroid hormones which precede labor initiate gap junction formation in the myometrium. Evidence from studies of rat tissues in vivo and in vitro indicate that progesterone inhibits, whereas estradiol stimulates gap junction formation (10-19). In sheep and rats a good correlation exists between gap junction formation and an increase in estradiol, a decrease in progesterone, and an increase in the estradiol/progesterone ratio measured in maternal and fetal blood and in uterine tissues (13,17,19,20).

In the rat, progesterone levels decline on day 19 of pregnancy (figure 2.11). This is followed by an increase in estradiol and prostaglandin $F_{2\alpha}$ levels (19) after day 20 (figure 2.11). An increase in gap junction area was apparent on day 21, increasing further until delivery at day 22 (figure 2.11). In sheep, progesterone declines four days before labor and estradiol increases 12 hours before labor, and gap junction area increases 48 to 60 hours before labor (20). Thus, gap junctions increase before the rise in estradiol but after progesterone levels fall.

Treatment of rats with progesterone, starting on day 19 of gestation, prevents the increase in gap junctions and delivery of the fetuses (11). Other studies have shown that ovariectomy of pregnant rats on day 16 results in decline in progesterone, development of gap junctions, and premature labor and delivery (17). Treatment of similar ovariectomized animals with progesterone prevented the fall in progesterone, the formation of gap junctions, and delivery (17). Furthermore, studies of myometrial tissues in vitro indicate that progesterone inhibits and estrogens stimulate the formation of gap junctions (11,14,15).

We have demonstrated the formation of gap junctions in ovariectomized nonpregnant sheep after estradiol treatment (56). We also found a relative high number of gap junctions prior to labor in the pregnant sheep uterus (20). This might be due to the fact that in some parts of the sheep uterus there are no placental cotelydons which produce local progesterone. In the regions covered by placental tissue there is local progesterone production from the placenta, which is probably responsible for the prevention of gap junction formation in sufficient levels to initiate or support labor. These results are consistent with the hypothesis (10-21) that changes in the levels of hormones which precede (progesterone withdrawal) or accompany (increase in estradiol and prostaglandins) the development of gap

junctions regulate their appearance.

The changes in the levels of steroid hormones may initiate synthesis of proteins associated with gap junctions. It is well known that estradiol and progesterone bind to specific cytoplasmic receptors, move to the nucleus, and regulate receptor and protein synthesis (60,69). In the myometrium, estradiol stimulation is required to achieve full development of gap junctions as well as to permit progesterone to inhibit their formation (15,17). This may be the result of estradiol stimulating the formation of receptors for progesterone which then allows it to enter the cell and express an inhibitory effect on protein synthesis. Cycloheximide treatment prevents the development of gap junctions in myometrial tissues in vitro, confirming that protein synthesis is essential for gap junction formation (15).

b. Prostaglandins

The studies of Garfield and co-workers also suggest that prostaglandins directly influence gap junction formation in the myometrium. Prostaglandin synthesis increases prior to and during labor (figure 2.11) in various animals (1,2,4-6,9). Some prostaglandins directly stimulate the myometrium to contract (1,2,4-6) and indirectly affect the myometrium by altering steroid synthesis (70). Administration of these prostaglandins to humans and animals usually initiates termination of pregnancy after a period of time (4-6).

Indomethacin, a prostaglandin synthesis inhibitor, prevents gap junction formation in myometrial tissues in vitro (14,15). Also, both an antagonist and an inhibitor of thromboxane A_2 synthesis, as well as prostacyclin analog (carbacyclin), prevent gap junction development (15). These studies demonstrate that prostaglandins play an important role in gap junction formation. It has been proposed that the prostaglandins may control the aggregation of the gap junction proteins by effecting cross-linking reactions or by changing the fluidity of the membrane (15). Another possibility is that prostaglandins regulate steroid receptors within the myometrial cell.

c. Control of gap junction permeability

Recently, several studies have shown that cells structurally coupled by gap junctions are not necessarily functionally coupled (44,46). Loewenstein (44) has shown that increased intracellular calcium concentrations lead to decreased gap junction permeability and uncoupling of cells with no apparent gross structural alterations in the cell junctions. Peracchia (46) has proposed that high calcium concentrations on the intracellular surface of the gap junction lead to an altered packing of the junctional proteins associated with uncoupling. Other studies also indicate that there are conditions in which gap junction channels are either open or closed (42,44,46).

It has been implied in studies of the myometrium that the presence of gap junctions in myometrial tissues during periods of intense

uterine contractility indicated that the muscle cells were functionally coupled and the pores in the junctions were open (10-21). Garfield et al. have recently demonstrated electron-dense deposits associated with gap junctions following treatment of tissue in vitro with β-adrenergic agents which affect adenylate cyclase and result in relaxation of the muscle (15). These deposits may represent a reaction product of an enzyme associated with gap junctions. In other tissues similar deposits have been observed. They are thought to represent calcium phosphate crystals formed during hydrolysis of ATP by an enzyme linked with gap junction proteins (43). Gap junctions may be aggregates of hormone receptors or aggregates of adenylate cyclase, as there is a good correlation between binding of some hormones, the activation of adenylate cyclase, and the presence of gap junctions in a variety of tissues (43).

It is most likely that pores in the myometrial gap junction can be functionally opened and closed, as they can in other cell systems (42,44,46). It is tempting to speculate that gap junctions may be receptor sites for oxytocin binding, as oxytocin receptor sites increase at the same time as gap junctions during parturition and both are thought to be regulated by the same mechanisms (8,71). If oxytocin does bind to gap junction proteins which are partially exteriorized, the binding may result in a conformational change in the proteins and alter the permeability of the gap junction channels.

d. Degradation of gap junctions

Gap junctions, once formed in the myometrium during labor, disappear following parturition (10,11,18,72). Ovarian presence or function is not necessary for disappearance of gap junctions from rat myometrium after parturition (72). Ovariectomy on day 21 of gestation delayed parturition slightly but did not prevent the disappearance of gap junctions in less than 24 hours after delivery. Also, gap junctions in nonpregnant, ovariectomized sheep myometrium disappear without hormonal treatment (56). These studies suggest that estradiol or progesterone are not required for the destruction of gap junctions.

The exact mechanisms for gap junction degradation is not understood (72). One possibility is that the junctional protein components detach any connection they have between adjacent cells and become dispersed within the cell membrane (40). Another possibility is that gap junctions become interiorized within one of the cells connected by the junction and are digested by an endocytotic-lysosomal mechanism (43). Studies by Garfield et al. (15) support the latter mechanism.

e. Model for control of gap junctions

Evidence from studies of the myometrium demonstrates that gap junctions are dynamic structures, regulated by a synthetic and a degradative process. These processes could be modulated by steroid hormones, prostaglandins, and calcium. A diagrammatic model for regulation of gap junctions is presented in figure 2.12. It is proposed that the steroid hormones regulate gap junctions in the

myometrium by controlling protein synthesis as described above. Once the proteins (connexins) are synthesized, they are inserted into the plasma membrane to interact with each other via disulfide bridges or by other means of cross-linking to form aggregates. The aggregates may be functional proteins associated with adenylate cyclase, hormone binding, or a calcium transport or binding mechanism. The

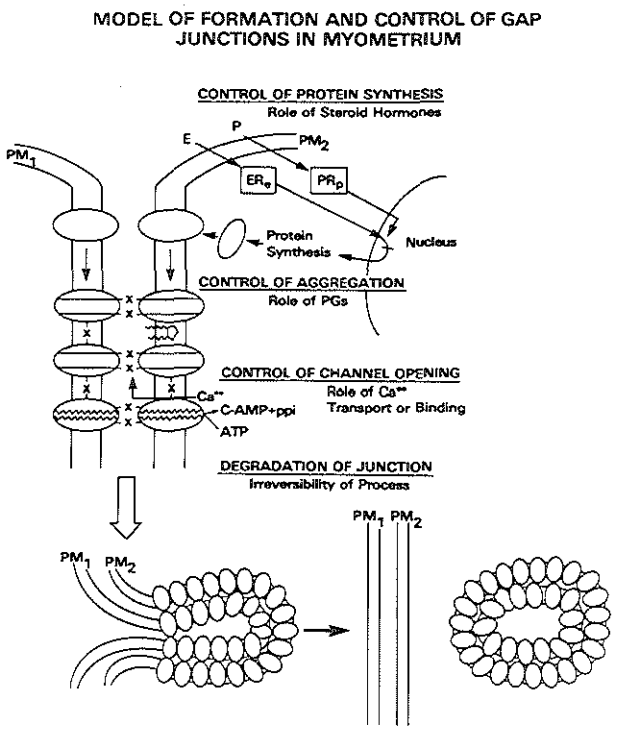

Figure 2.12 Schematic representation of possible events controlling formation, functional coupling, and destruction of gap junctions between plasma membranes (PM_1 and PM_2) of smooth muscle cells of the uterus. Shown are possible roles for (1) estrogen (E) and progesterone (P) interacting with their receptors (Re and Rp) to control protein (connexin) synthesis; (2) prostaglandins in controlling cross-linking or connexin-connexin aggregation (X); (3) Ca^{2+} in regulating channel opening and possible sites for transport or binding of Ca^{2+} and (4) an irreversible endocytotic pathway for degradation of the junction.

intercellular channel created by the functional proteins may be in either an open or closed state. When the aggregates have grown to sufficient size to become superfluous, they are incorporated into one of the connected cells by an endocytotic mechanism where they are digested by a lysosomal process. Thus, this dynamic system has at least four separate sites for the control of the junctions and possible control of labor. The sites include: (a) the steroid hormones and their receptors for control of protein synthesis, (b) the prostaglandins and effects on membranes or steroid receptors, (c) the opening and closing of gap junction channels, and (d) a degradative pathway.

IV. Conclusions

In this review we have attempted to briefly describe the structural features of the uterus that are important in control of myometrial function. The organization of the muscle layers was presented. The ultrastructural appearance of the smooth muscle cells was discussed. A substantial section of this survey deals with quantitative studies of the development, role, and regulation of gap junctions in the myometrium during labor. Gap junctions are regions of intercellular channels through which ions and molecules can pass from one cell to another. Cells having gap junctions are electrically coupled; i.e., there is a pathway for flow of electrical current carried by ions. Gap junction formation may be the basis of the synchronous contractility observed during labor by providing low-resistance pathways for rapid spread of action potentials in the myometrium. Thus, gap junctions may convert the uterus into an active organ and induce excitability and increased contractile force. It is suggested that the hormonal changes which precede labor promote the synthesis of gap junctions and possibly of other subcellular structures. Further studies towards clarifying the regulation of gap junctions and labor, especially the role of prostaglandins, may lead to clinically useful means to control term and preterm labor.

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CHAPTER 3

ELECTRICAL AND MECHANICAL UTERINE ACTIVITY AND GAP JUNCTIONS IN PERIPARTAL SHEEP

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Introduction

Labor in various species is preceded by or associated with hormonal changes, which proceed sequentially to achieve normal parturition (1-3). In sheep a decrease in progesterone levels in plasma and uterine tissue, followed by an increase in estrogen and prostaglandins is thought to lead to increased and coordinated contractility of the myometrium which eventually results in delivery (1,2). It has been proposed that the spread of electrical activity between muscle cells of the myometrium, to synchronize their contractility during labor, may be facilitated by the development of gap junctions (4). Gap junctions are specialized cell-to-cell contacts which may provide sites of low-resistance to the propagation of electrical signals between myometrial cells (4-6). The formation of gap junctions is accompanied by an augmentation of the electrical coupling between rat myometrial cells as measured in vitro (6). It has been shown that the area of gap junction contact between myometrial cells is small prior to labor, increases during labor and declines shortly after delivery in rats (4,5,7), rabbits (8), sheep (9), and humans (10). The results of various studies in rats (5,7,11,12) and sheep (9) indicate that the development of gap junctions between myometrial cells may be modulated by changes in tissue levels of steroid hormones. However, the relationship between myometrial electrical and mechanical activity, changes in steroid hormone levels, and the development of myometrial gap junctions before and during labor has not been investigated in vivo. The increase in myometrial effort which leads to delivery, in relation to gap junction formation, can only be investigated in vivo. In vitro studies do not provide information on the activity of the

entire myometrium and, in addition, the factors which control contractility in vivo may be altered in vitro.

Therefore, the present study was designed to compare myometrial activity, plasma levels of estradiol-17β and progesterone, and the amount of myometrial gap junctions before, during, and after parturition in chronically instrumented ewes.

Material and methods

Studies were carried out in ten chronically instrumented unanesthetized pregnant ewes. Seven ewes had a single fetus and three ewes had a twin pregnancy. At 110–124 days gestation operation was performed with the animal under general anesthesia with 500 mg of ketamine hydrochloride, 0.5 mg of atropine and 300–500 mg of sodium thiopental intravenously. The animals were intubated and ventilated with 40% oxygen, 60% nitrous oxide and 0.5 - 4 volume % enflurane. A polyvinyl catheter was inserted into a femoral artery and advanced into the descending aorta. A lower midline laparotomy was performed and a pregnant horn was exposed. For recording of electrical myometrial activity, three bipolar, silver-chloride coated, silver needle electrodes were fixed to the anterior part of the myometrium in the fundal, medial and cervical regions of the pregnant horn. The needles of the electrodes were 3 mm long, and had a diameter of 0.2 mm. The distance between electrodes (20–30 cm) was measured. A sponge-tipped catheter was inserted into the amniotic cavity for recording of intrauterine pressure. The wires and catheters were passed subcutaneously to a pouch attached to the ewe's flank.

During the operation longitudinal strips of myometrium (5 by 2 by 1 mm) were taken from the fundal and medial regions of the pregnant horn for electron microscopy.

After a recovery period lasting at least four days, electrical and mechanical uterine activity were recorded daily for at least two hours, with the ewe in a quiet environment. 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 P23 ID pressure transducer. Electrical signals from three different regions as well as the intrauterine pressure signal were recorded on a Gould Brush eight-channel polygraph with a paper speed of 25 mm/min and stored on magnetic tape.

Aortic blood samples were drawn for assay of the concentrations of progesterone and estradiol at four day intervals from the fourth day after operation. When labor appeared to be imminent as judged from the electrical myometrial activity and intrauterine pressure signals, samples were drawn at 12 hour intervals. The samples were centrifuged immediately at 1380 x g for 10 min at 4°C; the plasma was stored at -20°C until analysis.

Additional myometrial biopsies for electron microscopy were obtained from the pregnant horn in three ewes before parturition, in

eight animals during the second stage of labor, and in eight animals after parturition. The procedure was performed under epidural anesthesia with 6-10 ml of 5% bupivacain after sedation with ketamine hydrochloride. On these occasions the distance between the electrodes was again measured. The lambs were removed from the ewes after birth.

Analytic procedures

The electrical myometrial activity was analyzed visually for intermittent epochs of distinct electrical activity, referred to as bursts. Bursts were defined as episodes in which the amplitude of the electromyographic signals showed an increase to three times the baseline signal or more for longer than 15 seconds. Episodes occuring less than 15 seconds apart were taken as one burst. The bursts were analyzed for duration in minutes and frequency per hour. The apparent conduction velocity in cm/sec was calculated by dividing the measured distance between the electrodes by the phase lag between the onset of bursts. The intrauterine pressure signals were analyzed for periodic elevations of intrauterine pressure of at least 3.5 mm Hg, with return to baseline level, which are called intrauterine pressure cycles. The intrauterine pressure cycles were analyzed for duration in minutes and frequency per hour. The active pressure area, i.e. the area of intrauterine pressure cycles minus the basal tone, was measured by means of a digitizing tablet (Laboratory Computer System, Inc., Cambridge, MA). Readily recognizable artifacts caused by bearing down efforts of the ewe during labor were omitted. The areas were added during one hour periods and expressed in cm^2/hr (1 cm^2 = 500 mm Hg.sec). The rate of rise of the intrauterine pressure cycles (mm Hg/sec) was calculated by dividing the maximum amplitude of the intrauterine pressure cycle by the time needed to reach it.

The methods for processing the tissue samples for electron microscopy and for quantitative determination of myometrial gap junctions have been published elsewhere (10,11). 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. The area of gap junction membrane relative to the area of plasma membrane was calculated from measurements of the respective lengths and expressed as a percentage.

Radioimmunoassay of estradiol and progesterone was carried out as previously described (13).

All data were time-related to parturition. The paired Student's t-test was used for statistical analysis of differences between apparent conduction velocity, rate of rise, and active pressure area of intrauterine pressure cycles before, during and after parturition. Percentages of gap junction area in myometrial biopsies obtained at different days before and after parturition were compared with those obtained during labor using Student's t-test. Values of $p < 0.05$ were considered significant.

Results

The preparation lasted two to three weeks but because of malfunction of the electrodes only five ewes provided complete recordings before, during and after parturition, which were used for subsequent analysis. Figure 3.1 shows tracings of the intrauterine pressure signals and the electromyograms from fundal, medial and cervical regions recorded at various time intervals before, during and after labor in a single ewe. The sequence of onset of bursts from the three regions appeared to be variable. The bursts of electrical activity corresponded to changes in intrauterine pressure. More than 30 hours before delivery the bursts and the intrauterine pressure cycles were infrequent (1-2 cycles/hour) and of long duration (5-12 min).

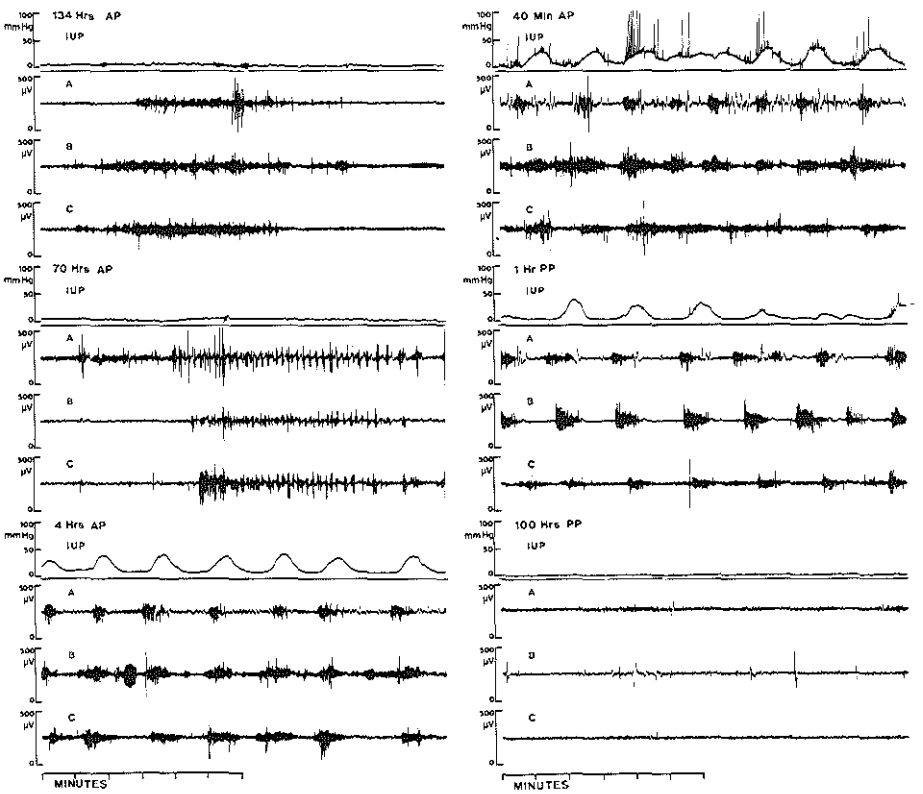
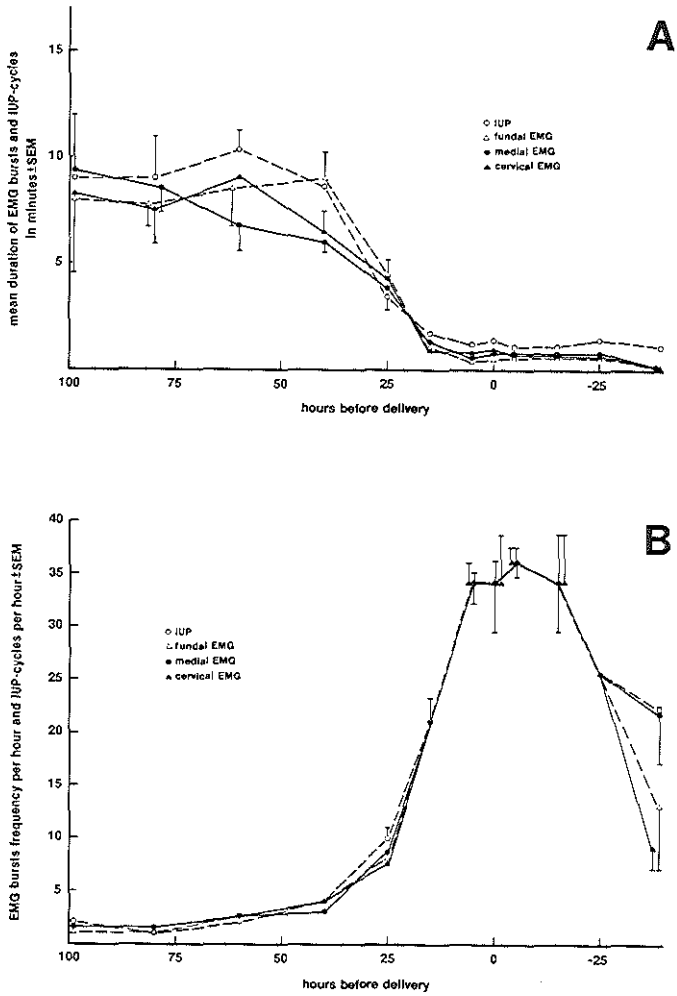


Figure 3.1 Composite of six recordings from a single ewe at different times before (AP) and after (PP) delivery. Each tracing shows, from top to bottom, the intrauterine pressure signal and the electromyograms from the fundal (A), medial (B), and cervical region (C).

Figure 3.2A shows the duration and figure 3.2B the frequency of the bursts at the various regions and intrauterine pressure cycles before, during and following labor. Although some variation was observed between different ewes, there was a consistent decrease in duration and an increase in frequency of bursts of electrical activity and intrauterine pressure cycles in all animals, starting approximately one day before delivery to reach minimum (duration) and maximum



Figures 3.2A and B. Means (± S.E.M.) of duration (A) and frequency (B) of intrauterine pressure (IUP) cycles and bursts of electrical activity (EMG) in fundal, medial and cervical regions, recorded before, during and after delivery in five ewes.

(frequency) values during labor. After delivery the frequency of bursts and intrauterine pressure cycles gradually decreased: four days following parturition no activity could be demonstrated. There were small and inconsistent differences between the durations of the bursts of electrical activity from the three regions obtained

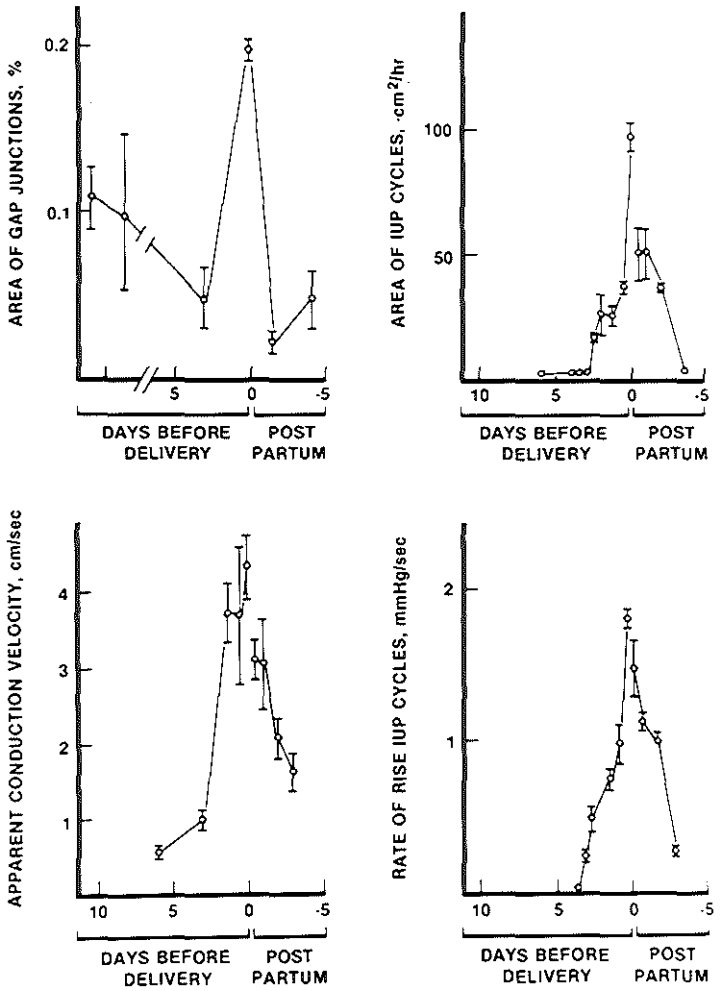


Figure 3.3 Means (± S.E.M.) of relative gap junction area from all regions, area of intrauterine pressure (IUP) cycles, apparent conduction velocity, and rate of rise of intrauterine pressure cycles before, during and after delivery. Note that peak values occurred during delivery.

until two days before delivery. From that time, through delivery and during the first two days after delivery the electrical myometrial activity variables obtained from the three regions were equal, and closely related to intrauterine pressure.

The apparent conduction velocity, the active pressure area and the rate of rise of the intrauterine pressure cycles are shown in figure 3.3. All three variables increased significantly one to two days before delivery to reach maximum values at delivery, and to decline within three days thereafter. The three variables show a close temporal relationship to the changes in relative gap junction area.

Plasma levels of progesterone and estradiol measured before, during and after parturition are summarized in figure 3.4. Progesterone levels began to fall at approximately four days before delivery, but the steepest fall occurred at approximately 12 hours before delivery, coinciding with a sharp rise of the estrogen levels.

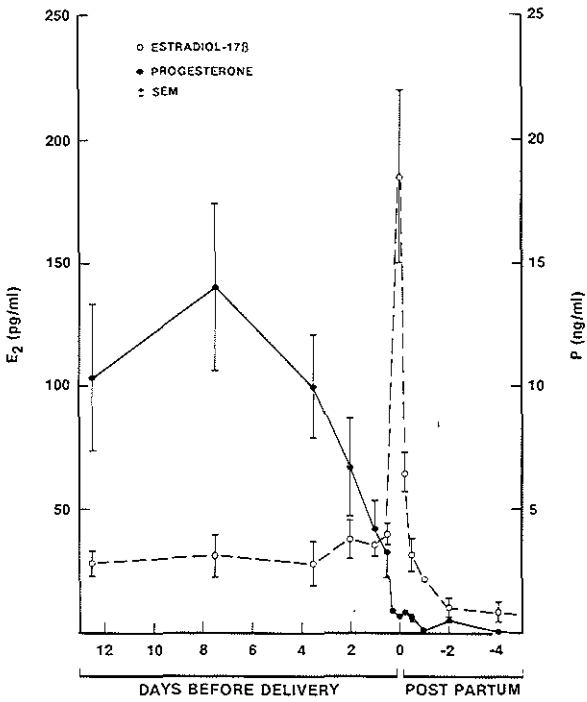


Figure 3.4 Means (± S.E.M.) of maternal plasma levels of progesterone (P) and estradiol-17β (E_2) before, during and after delivery. Compare to gap junction area in figure 3.3.

TABLE 3.1. NUMBER AND AREA OF GAP JUNCTIONS IN PREGNANT AND POSTPARTUM SHEEP MYOMETRIUM

	DAY OF SAMPLING						
	DAY OF GESTATION (FIRST OPERATION)			DAYS BEFORE DELIVERY	DURING LABOR	DAYS AFTER DELIVERY	
	110–113	117	122–124	3–2		1–2	4–5
No animals	3	3	4	3	8	5	3
No tissues	8	8	10	7	42	11	5
No gap junctions	18	24	23	11	147	7	8
Total length plasma membrane surveyed (μm)	7,613	7,596	9,455	6,251	33,638	8,470	4,864
Total length gap junction membrane (μm)	3.105	4.110	4.680	1.815	32.365	0.815	1.200
Relative area gap junction membrane (%),	0.078	0.108	0.099	0.048	0.198	0.021	0.049
S.E.M.	0.027	0.019	0.048	0.019	0.006	0.011	0.017
p*	<0.004	<0.02	<0.05	<0.001	-	<0.001	<0.001

*p Values indicate levels of significance between gap junction areas during delivery compared to those at other times

Table 3.1 shows the total length of the plasma membrane surveyed, the total length of gap junction membrane, and the relative gap junction area. Gap junctions were found at all times but the relative gap junction area was significantly elevated during parturition as compared to other times before and following delivery. The increase in relative gap junction area appeared to start three to two days before delivery. Maximal levels were reached during labor. Within one to two days after parturition, the number of gap junctions had declined to a low level. Figures 3.3 and 3.4 show the close temporal relationship between gap junction area, the course of variables of uterine activity, and steroid hormone levels in the peripartum period.

Figure 3.5 shows a high magnification photograph of a gap junction between myometrial cells of fundal tissue, taken immediately postpartum. No differences between the volume occupied by muscle cells nor between their basic structures were apparent in the various regions examined.

Discussion

The present study shows that labor in the ewe is associated with changes in plasma levels of progesterone and estrogen, an increase in myometrial gap junction area and by an increase in electrical and mechanical activity of the uterus. From the results of studies in rats and rabbits, Csapo and Takeda (14) concluded that electrical and mechanical events of the myometrium were local, non-propagating and asynchronic prior to labor and that the onset of labor was characterized by the gradual evolution of synchronized myometrial activity. In the present study in chronically instrumented pregnant ewes we observed a good correspondence between long lasting bursts of electrical activity recorded from various sites of the myometrium before the animals were in labor. Similar observations in ewes have been reported by others (15). In rabbits a similar pattern of long bursts occurring during pregnancy has been reported (8). These bursts are not necessarily propagated events. Harding et al. (15) suggest

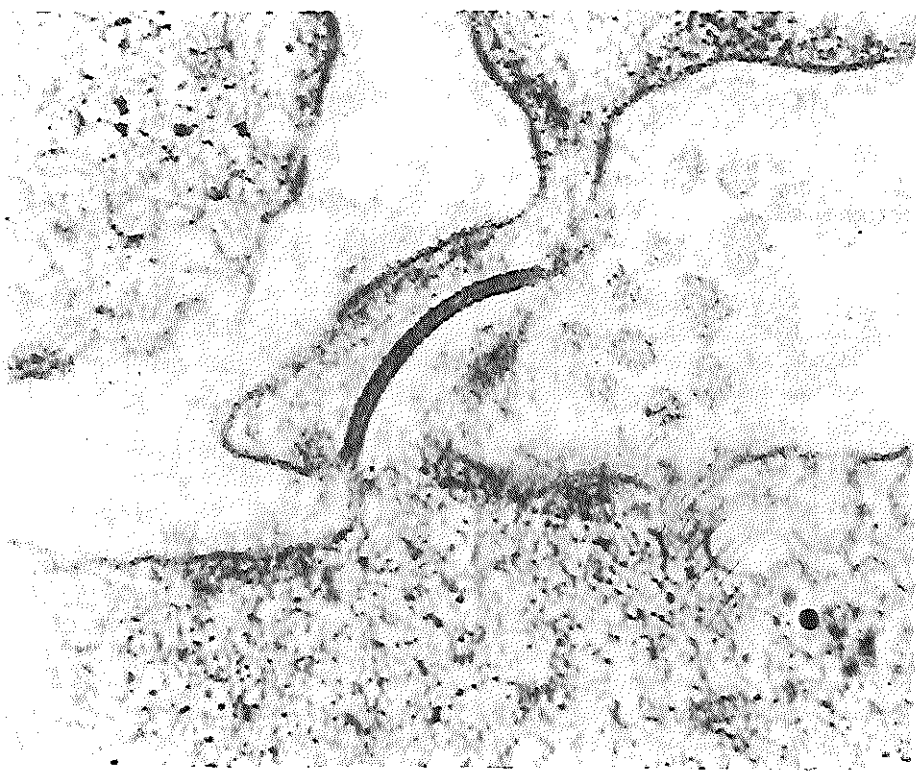


Figure 3.5 Electron micrograph of myometrial tissue sample taken during labor showing a gap junction with a typical 7-lined appearance (magnification x 130,000).

humoral control or stimulation by a nerve net, because an of a isolated part nonpregnant horn showed bursts occurring synchronously with those in the body of the uterus. The relatively low amplitude of corresponding intrauterine pressure cycles observed in pregnancy until a few days before labor may be explained by uncoordinated contraction of the myometrial muscle cells.

The apparent conduction velocity measured in our experiments is similar to that reported previously for the rabbit (14). The observation of a close relationship between apparent conduction velocity and percentage gap junction area (figure 3.3) suggests that the increase in gap junction area improves spread of electrical activity across the myometrium. However, the values obtained should be interpreted with care, since the primary electrical event could be initiated between two electrodes and the pathway of conduction need not be straight. For this reason the term "apparent" conduction velocity is used. We could not demonstrate a dominant direction of conduction, which may be due to the relative large distance between the electrodes (20–30 cm). Other investigators (16) reported a greater propagation in the tubo-cervical direction during labor.

The total active pressure area of the intrauterine pressure cycles (figure 3.3) during one hour is a reflection of the myometrial effort during that time. The active pressure area and the rate of rise of intrauterine pressure cycles measured after delivery are not comparable with the same variables obtained before delivery. The wall tension needed to build up a certain intrauterine pressure will be less as the uterine cavity becomes smaller, and the myometrial muscle cells are shortened. This must also be taken into account in the interpretation of the values of apparent conduction velocity after delivery.

The rate of rise of the intrauterine pressure cycles is considered to reflect recruitment of muscle cells (3). There appears to be a close relationship between the rate of rise and relative gap junction area (figure 3.3), which suggests that the formation of gap junctions improves the coordination of the smooth muscle cells.

The hormonal changes in plasma observed in this study are similar to those reported by others (1,2,9). The present study does not prove that the changes in hormone levels are responsible for the increase in myometrial gap junction area. The temporal relationship between the changes in hormone levels and the increase in gap junction area shortly before labor must be interpreted with care, as only one sample was taken for measurement of gap junctions in the days immediately prior to labor. However, as in previous studies (9) there is a good relationship between changes in hormone levels and the increase in gap junctions. Previous studies show that progesterone inhibits and estrogens stimulates the development of gap junctions (5,11,12). Thus, our results confirm previous investigations supporting the hypothesis that steroid hormones regulate the formation of myometrial gap junctions.

Our study shows that the area of gap junctions in delivering

animals is approximately twice that present before and after labor (table 3.1). In other studies very low numbers of gap junctions were found in the antepartum and postpartum periods in sheep (9) and other animals (4,5). The higher number of gap junctions prior to labor in this study cannot be attributed to either operation or the introduction of recording devices because the values were already relatively high at the time of first operation. However, these levels were not sufficient to initiate or support labor. The area of gap junctions found during labor of approximately 0.2%, (table 3.1), is similar to that reported for various animals (6,7,8,9) and humans (10) indicating that there is a critical and similar number of gap junctions needed to sustain labor in different species.

The present study shows that labor in the ewe is associated with changes in plasma levels of progesterone and estrogen, with a concomitant increase in myometrial gap junction area as well as in electrical and mechanical activity of the uterus. These results support the hypothesis that changes in plasma and tissue levels of steroid hormones lead to an increase in muscle cell gap junction area, which improves conditions favoring coordination of contractile activity of the uterus during parturition (4). It is likely that the increase in gap junction area is one of several events necessary to increase uterine contractility.

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CHAPTER 4

MODULATION OF SPONTANEOUS MYOMETRIAL ACTIVITY IN CHRONICALLY INSTRUMENTED OVARIECTOMIZED SHEEP

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Introduction

The modulation of electrical and mechanical activity of the uterus by various hormones has been the subject of experimental studies in a variety of animals. Chronically instrumented sheep have been extensively used to study electrical and/or mechanical myometrial activity during the estrous cycle (1-4).

Such an experimental design does not allow one to distinguish between the intricate effects of endogenous ovarian hormones on myometrial behavior and the intrinsic myometrial activity which may occur due to spontaneous contractile events in uterine smooth muscle cells. For this reason ovariectomized ewes have been used to assess the effects of steroid and peptide hormones on myometrial activity (2,5,6). However, these reports provide little quantitative and qualitative information on the occurrence of spontaneous, intrinsic myometrical activity in the absence of ovarian hormones. Results of a preliminary study in our laboratory suggested that marked and protracted myometrial activity does occur in chronically instrumented ewes following ovariectomy (7).

The aim of the present experimental study was to assess further the intrinsic spontaneous electrical and mechanical myometrial activity in ovariectomized ewes, to investigate the underlying mechanisms, and to assess the effects of estradiol and progesterone.

Material and Methods

Animals and instrumentation

Fourteen ovariectomized Texel ewes were used. Operation was performed with the animal under general anesthesia induced with 500 mg of ketamine hydrochloride, 0.5 mg of atropine and 300-500 mg of sodium thiopental intravenously. The animals were intubated and ventilated with 40% oxygen and 60% nitrous oxide, and 0.5-4 volume % of 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. The needles of the electrodes were 3 mm long with a diameter of 0.2 mm. The distance between the needles was 2 mm. Sponge-tipped pressure catheters were inserted into the cavity of both uterine horns via 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 operation for at least one week before experiments were started.

Arterial blood samples (10 ml) were drawn daily during seven days following ovariectomy. The samples were centrifuged immediately at 1380 x g for 10 min at 4°C, and the plasma was stored at -20°C until analysis. Radioimmunoassay of estradiol-17β and progesterone was carried out as previously described (8,9).

Recording and computer analysis

Electrical and mechanical activity of the uterine horns were recorded with the ewe standing quietly in its cage. The recording equipment was placed in an adjoining room, and care was taken not to disturb the animal. Electrical and mechanical activity were measured continuously during experiments. The electrical signals were filtered by a band-pass filter. For the lower and higher cutoff frequencies (-3 dB) 1 and 10 Hz were selected. Intrauterine pressure was measured by a Gould Statham P23 1D pressure transducer. The electrical myometrial activity and intrauterine pressure signals were recorded on a Gould Brush 2800 eight-channel polygraph and stored on magnetic tape by means of a Racal instrumentation recorder.

The analogue signal on the magnetic tapes was digitalized using a PDP 11/34 minicomputer with LPS extension. Sampling rates were 5 Hz for the intrauterine pressure signal and 25 Hz for the electrical signal. Analysis of both signals was performed on a PDP 11/70 minicomputer. Each recording was divided into 5 min periods. The following intrauterine pressure variables were calculated: mean peak pressure, mean tonus and intrauterine pressure cycle frequency. An intrauterine pressure cycle was defined as an increase in intrauterine pressure of at least 10 mm Hg above mean tonus. The burst frequency was calculated from the electromyogram. The procedures are described in detail elsewhere (10).

Experimental protocols

Electrical myometrial activity and intrauterine pressure were recorded continuously during 24 hours in four ewes prior to any other experiments, to assess the presence or absence of a circadian pattern. Before starting the protocols mentioned below, spontaneous uterine activity was recorded during 2-4 hours to obtain baseline values.

The role of adrenergic and cholinergic receptors in the maintenance of spontaneous myometrial activity was studied in three animals using the following blockers: propranolol, phentolamine and atropine. The effects of alpha-adrenergic stimulation were investigated by infusing norepinephrine. Beta-adrenergic stimulation was obtained by infusing ritodrine. The following protocol was applied to the intraarterial administration of the adrenergic blockers and agonists: first, ritodrine was infused at a dose of 500 µg/min during 35 min; after a 1-hour interval propranolol was infused (200 µg/min) during 30 min, immediately followed by ritodrine infusion (500 µg/min) for 30 min. After an interval of another hour a bolus of noradrenaline (0.5 mg) was given, followed by phentolamine infusion (2 mg/min) for 30 min. At the end of the phentolamine infusion a second bolus of noradrenaline (0.5 mg) was injected. Atropine (0.5 mg) was injected as a bolus 30 min after the last dose of noradrenaline.

Oxytocin in an incremental dose of 5 to 320 mU/min and 1 mg prostaglandin $F_{2\alpha}$ were administered intraarterially in four animals to assess the influence of these uterotonic agents on spontaneous uterine activity. Indomethacin (100 and 200 mg, rectal suppositories) was used in four animals to study the effect of inhibition of endogenous prostaglandin production on spontaneous myometrial activity.

After administration of adrenergic blockers or agonists, oxytocin, prostaglandin and indomethacin, an interval of at least one day was allowed before the following experiments were performed. The effect of estradiol in 10% ethanol in doses of 0.001 mg (n=4), 0.01 mg (n=4), 0.1 mg (n=7) injected as a bolus into the aorta was studied in 12 ewes. To examine the effects of ethanol (10%) alone on myometrial activity up to twice the amounts used to dissolve estradiol were injected. The mechanism of the observed suppression of myometrial activity by estradiol was investigated using the above schemes of administration of adrenergic and cholinergic blockers, agonists, oxytocin and prostaglandin, starting 90 min after administration of 0.1 mg of estradiol. Following administration of estradiol as part of the protocol, a time interval of at least 10 days was taken before the next experiment was performed.

In addition, we compared the effects of estradiol to those of progesterone. Progesterone (100 mg in oil) was administered intramuscularly in four sheep. To compare the responsiveness of the uterus to uterotonics during progesterone- and estradiol-suppression, oxytocin and prostaglandin were administered intraarterially in the doses mentioned previously (n=4). Progesterone was administered to sheep at least three weeks after estradiol treatment, or three weeks after ovariectomy. Plasma levels of estradiol or progesterone

following injection were not measured.

In the text all variables are expressed as mean values with standard deviations throughout.

Results

Most ewes remained in good condition during approximately 4 months following surgery and could be used for different protocols. Failures occurred because of malfunctioning of the electrodes. From the first day following ovariectomy plasma concentrations of estradiol and progesterone were below the detection levels of 10 pg/ml and 0.1 ng/ml, respectively.

Spontaneous myometrial acitivity

Marked spontaneous myometrial activity was present in all ovariectomized animals. The electrical activity was characterized by a

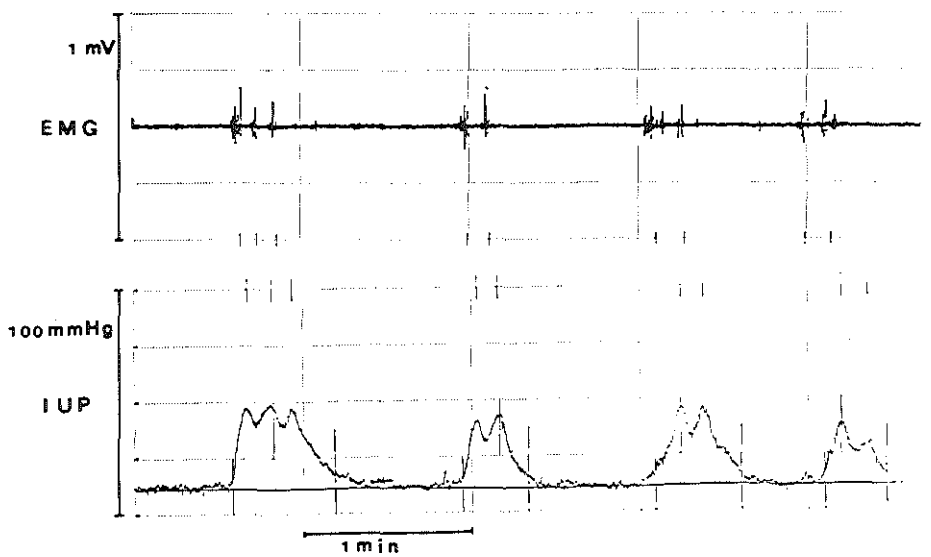

Figure 4.1 Computer analysis of 5 min of spontaneous myometrial activity. The vertical bars in the intrauterine pressure (IUP) signal indicate the starting and end points of an IUP cycle; the bars above the IUP signal indicate detection of a small wave superimposed on an IUP cycle. The vertical bars below the electromyogram (EMG) indicate detection of a burst.

rhythmic pattern of trains of bursts of action potentials corresponding with changes in intrauterine pressure, with a frequency of 0.75 to 1 per min. Each train of bursts was composed of two to four separate small bursts. Computer analysis showed that each of the small bursts was associated with a separate small pressure peak in one intrauterine pressure cycle. (figure 4.1). We observed this pattern of activity from seven days up to two months after ovariectomy without estradiol treatment. We could not demonstrate a circadian rhythm in the frequency of bursts of electrical activity nor in the frequency of intrauterine pressure cycles. Figure 4.2 shows the intrauterine pressure cycle frequency over a 24 hour period in four sheep.

Effects of sympathetic and parasympathetic agonists and inhibitors

An example of the effects of beta- and alpha-adrenergic and cholinergic blockade on mean peak pressure, burst frequency and intrauterine pressure cycle frequency of uterine activity is shown in figure 4.3. Administration of the beta and alpha-agonists ritodrine and noradrenaline depressed electrical myometrial activity and intrauterine pressure activity. With infusion of ritodrine the separate small peaks on the intrauterine pressure cycles disappeared and the corresponding train of bursts changed to single bursts. This

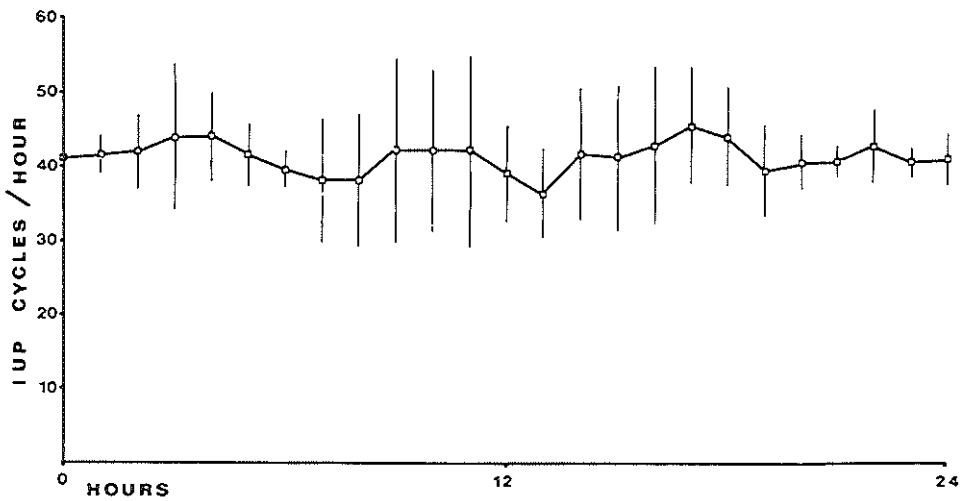

Figure 4.2 Mean intrauterine pressure (IUP) cycle frequency (± S.D.) in four ovariectomized ewes over a 24 hour period. No circadian pattern is apparent.

effect is apparent from figure 4.3, which shows a fall in burst frequency following ritodrine, whereas the intrauterine pressure cycle frequency shows hardly any change. The effects of both agonists were abolished by propranolol and phentolamine, indicating that proper pharmacological blockade was obtained. We could not demonstrate any effect of alpha- and beta-blockade on spontaneous electrical and mechanical activity. Also atropine did not change electrical and mechanical activity.

Oxytocin and prostaglandins

Administration of oxytocin (figure 4.4) and prostaglandin resulted in an increase in the frequency of bursts of electrical activity and intrauterine pressure cycles and a rise in mean uterine tonus.

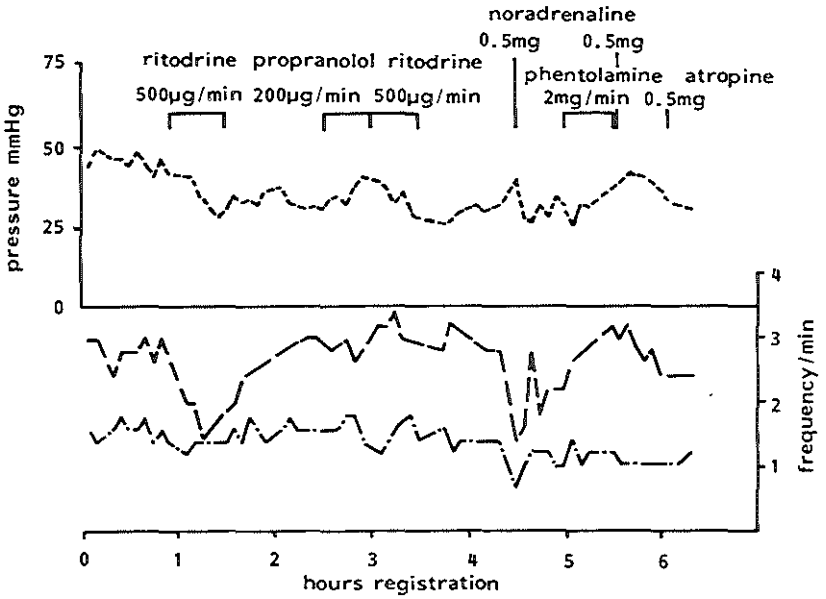

Figure 4.3 Representative analysis of mean peak pressure (------, top), burst frequency (— — —, middle) and intrauterine pressure cycle frequency (·—·—·—·, bottom) in one sheep, during pharmacological sympathetic or parasympathetic stimulation or inhibition.

The response of uterine activity to 100 and 200 mg of indomethacin was not consistent. In some sheep electrical and mechanical myometrial activity showed a marked decline, whereas no effect could be demonstrated in other animals.

Steroid hormones

Following intraarterial administration of estradiol a fall in uterine activity was observed. Infusion of ethanol (10%) alone in the amounts used to dissolve estradiol had no effect on uterine activity. Uterine activity did not disappear completely with 0.001 mg estradiol. With doses of 0.1 and 0.01 mg of estradiol the period of time needed for complete inhibition of uterine activity and the time needed for reappearance of activity appeared to be related to the dose of estradiol given. Administration of 0.01 or 0.1 mg estradiol resulted in complete inhibition after 156 ± 19 min and 64 ± 7 min,

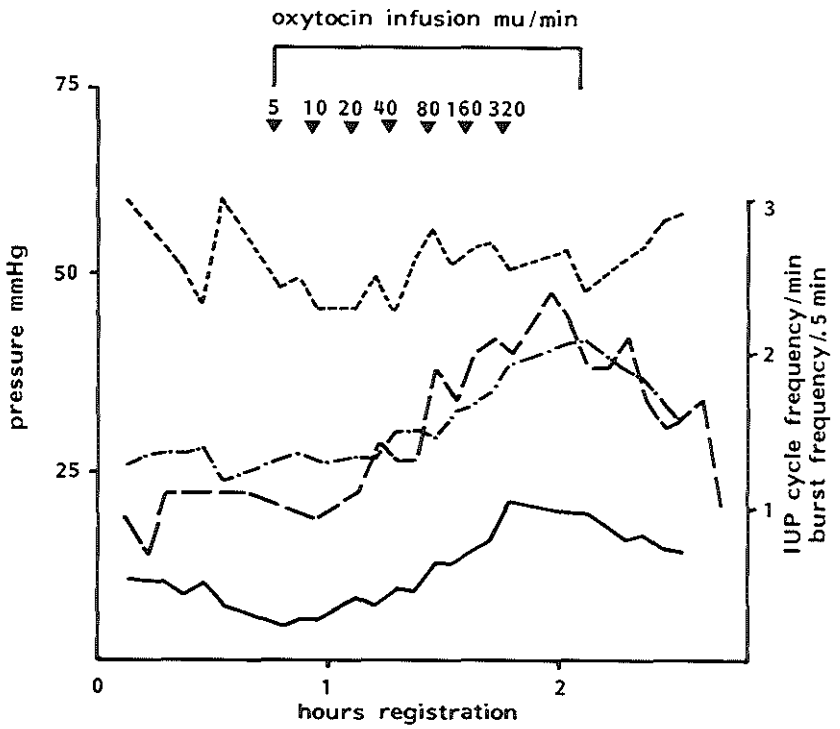

Figure 4.4 Response of myometrial activity to oxytocin in one ovariectomized ewe. Computer analysis of mean peak pressure (------), intrauterine pressure (IUP) cycle frequency (—·—·—) per min, burst frequency (— — —) per 0.5 min, and mean tonus (————).

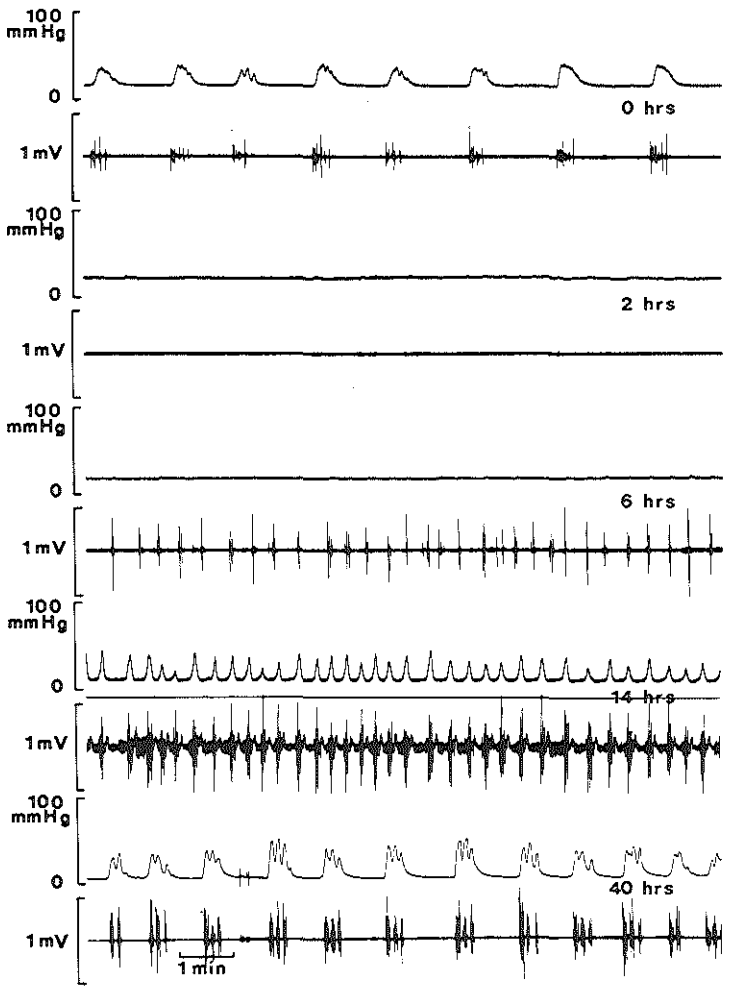

Figure 4.5 Response of myometrial activity of one sheep before, and 2, 6, 14 and 40 hours after administration of 0.1 mg of estradiol-17β.

respectively. Electrical activity always reappeared before mechanical activity at the end of the quiescent period.

Intrauterine pressure cycles returned after 205 ± 23 min in sheep treated with 0.01 mg of estradiol, electrical activity returned after 83.5 ± 7.9 min. In sheep receiving 0.1 mg, mechanical activity returned after 351 ± 64 min, whereas electrical activity returned after 210 ± 50 min in this group. After the quiescent period the burst frequency and the intrauterine pressure cycle frequency increased to a level higher than that present before administration of estradiol. Also the shape of the intrauterine pressure cycles changed after estradiol administration (figure 4.5). The two uterine horns of each

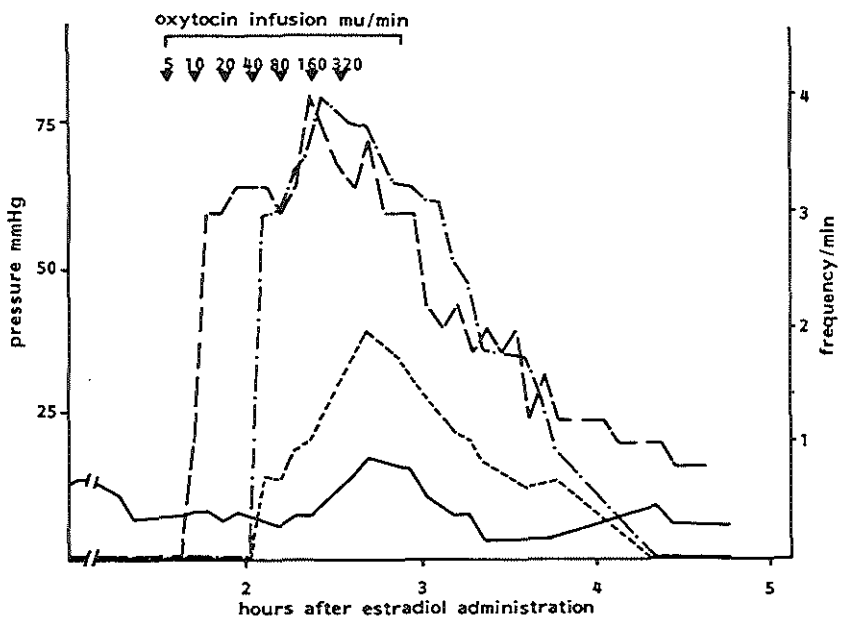

Figure 4.6 Myometrial activity following oxytocin administration during the estradiol-induced silent period; computer analysis of mean peak pressure (– – – –), intrauterine pressure cycle frequency (—·—·—), burst frequency (— — —) and mean tonus (————).

ewe behaved similarly, i.e., both were quiescent or active at the same time. Following administration of progesterone (100 mg) electrical and mechanical myometrial activity started to decrease after approximately four hours and all activity had disappeared after five to six hours. The uterus remained inactive for at least three to four days. We did not determine the exact time of reappearance of uterine activity after progesterone treatment.

Alpha- and beta-receptor blockade during the quiescent period induced by estradiol did not change the silent pattern. Administration of oxytocin (figure 4.6) and prostaglandin (figure 4.7) during the quiescent period induced by estradiol resulted in the recurrence of bursts of electrical activity and intrauterine pressure cycles. Oxytocin and prostaglandin did not activate the progesterone-suppressed myometrium in the amounts administered in this study. No electrical or mechanical myometrial activity could be demonstrated either before or after administration of these compounds.

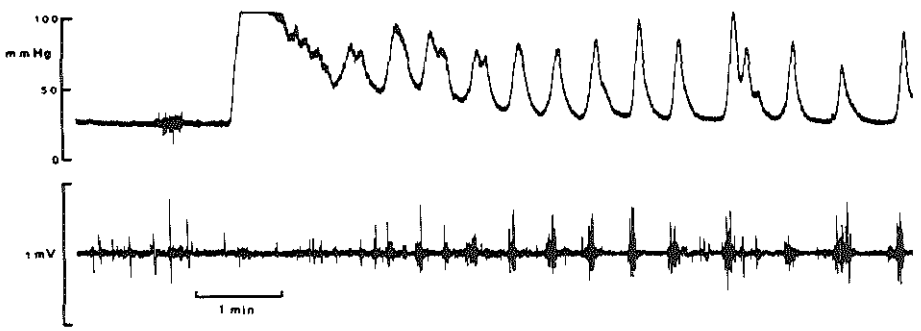

Figure 4.7 Response of uterine activity to 1 mg prostaglandin $F_{2\alpha}$ during the estradiol-induced silent period.

Discussion

The present study, performed in chronically instrumented, ovariectomized ewes, shows that electrical and mechanical myometrial activity occurs in the absence of ovarian hormones. Even two months after ovariectomy, without any steroid hormone treatment, this activity could still be observed. These results are in agreement with observations in rats and rabbits, which also show spontaneous mechanical uterine activity after ovariectomy (11-13). On the basis of

our experiments we cannot exclude the possibility that the instrumentation may have had an influence on the occurrence and the pattern of spontaneous uterine activity.

We were unable to demonstrate a myometrial response to alpha- and beta-receptor blockade during spontaneous uterine activity and during estradiol-induced myometrial suppression. This suggests that the observed spontaneous myometrial activity and the estradiol-induced suppression are not mediated by the autonomic nervous system, although estrogens are known to increase the content of neurotransmitters in the uterus (14). These findings are in agreement with results of studies in rats (15). The results of our study make it also unlikely that spontaneous activity and estradiol-induced suppression depend on the cholinergic system. The lack of a consistent effect of indomethacin could be due to varying degrees of absorption of rectal suppositories in sheep. This finding awaits further investigations on the effect of indomethacin administered by other routes.

In our experiments there was a marked difference in the shape of the intrauterine pressure cycles before estradiol treatment compared to those after the return of uterine activity following the silent period (see figure 4.5). This finding might be explained by estrogen-induced formation of gap junctions (16,17). The increase in activity after the quiescent period may be related to the influence of estradiol on synthetic processes in the myometrial cells. Estradiol is known to increase the synthesis of contractile proteins (18), receptors (19), gap junctions (16,20), and prostaglandins (21), and is also known to increase the number and size of myometrial cells (22).

The inhibition of uterine activity by estradiol has been observed by several other investigators in sheep (6,21,23,24), in rabbits (11), and in rats (12,13). We found that the latency period to reach complete quiescence was shorter and the quiescent period was of longer duration with a higher dose of estradiol. The suppression of uterine activity after administration of estradiol has been attributed to an increase in inhibitory prostaglandins (21), and to changes in relaxin levels (6).

Suppression of myometrial activity by estradiol in rats can be prevented by administration of actinomycin D, which inhibits DNA-dependent RNA synthesis, suggesting that protein synthesis is involved in the inhibitory mechanism, possibly by increasing relaxin concentrations (25). On the other hand, one might expect that contractile activity may be suppressed in the myometrial cells to conserve energy for synthetic processes. Also the known effect of estradiol on uterine blood flow (26) may have a direct or indirect influence on uterine activity.

The inhibition of the myometrium and the abolishment of its response to oxytocin and prostaglandin by progesterone in estradiol-treated sheep is well established (5). Our study shows that this effect also occurs in the absence of estradiol pretreatment. Progesterone may directly, independent of its receptors, increase binding of Ca^{2+} within the smooth muscle cells (27), thereby

inhibiting contractile activity. The inhibitory mechanisms of estradiol and progesterone appear to be different, as is apparent from the different responses to oxytocin and prostaglandin during the period of inhibition. However, it should be emphasized that our experiments are of a pharmacological nature. Plasma levels of estradiol and progesterone following administration of these steroids were not measured and may well have been outside the physiologic range.

The return of electrical activity before the reappearance of mechanical activity after administration of estradiol is of interest, as in this period electrical activity is not related to mechanical activity. This phenomenon may be explained by assuming that excitation is not coupled to contraction, or that the electrical events remain local and non-propagating.

Our studies indicate that the chronically instrumented ewe is a valuable model to investigate electrical and mechanical activity of the uterus. Further studies of the mechanisms responsible for the effects of estradiol, progesterone and prostaglandins are necessary to gain an understanding of myometrial behavior in physiological and pathological conditions.

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CHAPTER 5

ELECTRICAL AND MECHANICAL UTERINE ACTIVITY AND GAP JUNCTIONS IN ESTROGEN TREATED OVARIECTOMIZED SHEEP

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Introduction

Gap junction formation during labor has been suggested to be a necessary step in facilitating coordination of contractile forces in the myometrium (1). In a previous study in sheep we showed that the rise in the number of gap junctions during parturition is related to increased coordination of myometrial activity (2). The formation of gap junctions is thought to be regulated by steroid hormones and prostaglandins (3). 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 (2).

In nonpregnant ovariectomized ewes administration of estradiol induces an initial decrease in myometrial activity, followed by an increase (4,5). 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 (6).

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 nonpregnant 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 we investigated electrical and mechanical myometrial activity in ovariectomized ewes before and after administration of a single dose of 0.1 mg of estradiol-17β, and we quantitated myometrial gap junction area.

Material and Methods

Animals and instrumentation

Studies were performed in six chronically instrumented nonpregnant Texel ewes. The ewes were ovariectomized 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 the 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 operation for at least one week before experiments were started.

Recording procedures.

During the recording periods the ewes were housed 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 P23 ID pressure transducer. Electrical myometrial activity signals from three different regions as well as one intrauterine pressure signal were recorded on a Gould Brush 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-17β in 10% ethanol. Myometrial activity was again recorded from 140 to 150 hours after the administration of estradiol. Fifty consecutive intrauterine pressure 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 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 we could not take all tissue samples from each animal.

Analytic procedures.

In the intrauterine pressure signals 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 two 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 electromyogram was analyzed for periods of distinct electrical activity, called bursts. The algorithms for the computerized analysis of the intrauterine pressure and electrical myometrial activity signals are described in detail elsewhere (7, chapter 6).

The processing of the tissues and the electron microscopic quantitation of gap junctions has been outlined extensively before (8,9). 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 (9).

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 myometrial activity before administration of estradiol was characterized by a rhythmic pattern of bursts of electrical 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 5.1 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 intrauterine pressure cycles usually showed several small peaks ("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 intrauterine pressure cycles in one ewe, from 12 hours before to 72 hours after the administration of estradiol, are shown in figure 5.2. In all animals, the frequency of bursts and intrauterine pressure cycles following the

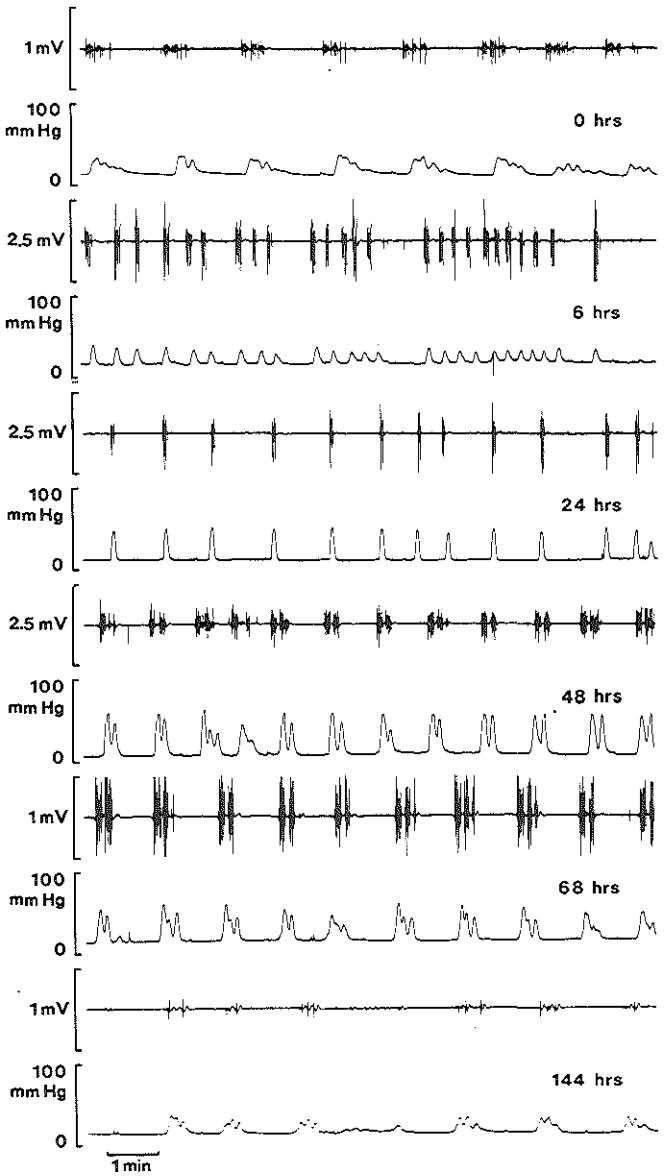

Figure 5.1 Myometrial activity before (0 hrs), and 6, 24, 48, 72 and 144 hours after administration of 0.1 mg of estradiol-17β recorded in one ewe.

TABLE 5.1. NUMBER AND AREA OF MYOMETRIAL GAP JUNCTIONS (GJs) IN OVARIECTOMIZED SHEEP BEFORE AND AFTER ESTROGEN TREATMENT

Hours after estradiol*	No. GJs	Total length GJ membrane (μm)	Total length non-GJ membrane (μm)	Relative area GJ membrane (% ± S.E.M.)	P**
0	4	0.420	12,330	0.007 ± 0.003	-
6	8	0.840	18,766	0.009 ± 0.004	NS
24	34	3.720	13,370	0.086 ± 0.023	<0.01
48	14	2.100	9,510	0.045 ± 0.019	NS
72	3	0.300	4,752	0.012 ± 0.011	NS
144	3	0.195	8,814	0.005 ± 0.003	NS

* For each experiment, two sheep were used
** As compared with control values

administration of estradiol shows periods of high activity alternating with periods of low activity. This pattern gradually returned to the pattern observed before the administration of estradiol.

Figure 5.3 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 5.4 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 administration of estradiol.

The results of the quantitative evaluation of the tissues for gap junction area are presented in table 5.1. 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-17β . Following injection of 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.

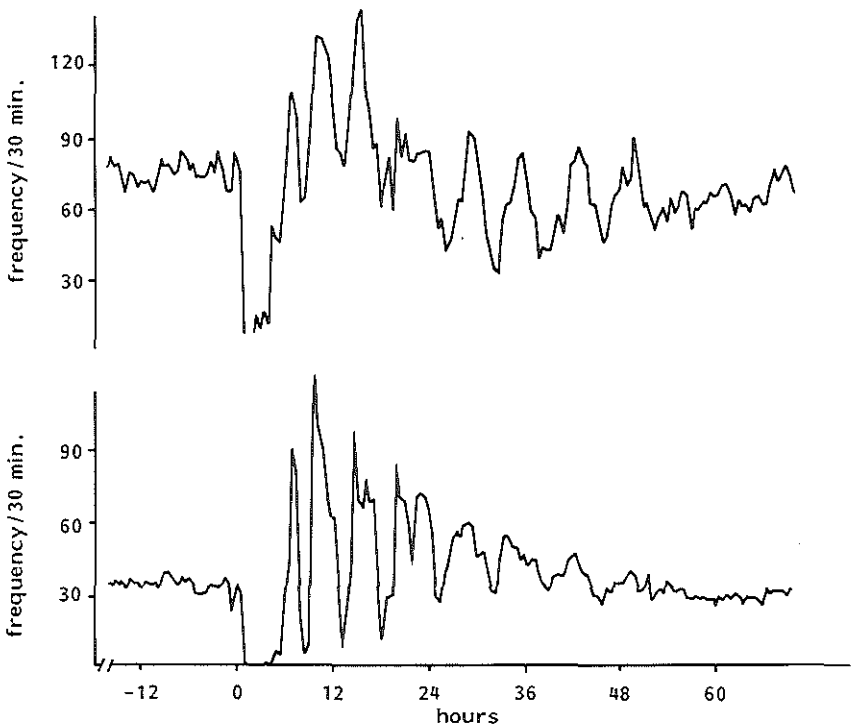

Figure 5.2 Frequency of bursts (top) and of intrauterine pressure cycles (bottom) in one ewe from 12 hours before to 72 hours after administration (0 hours) of 0.1 mg estradiol-17β .

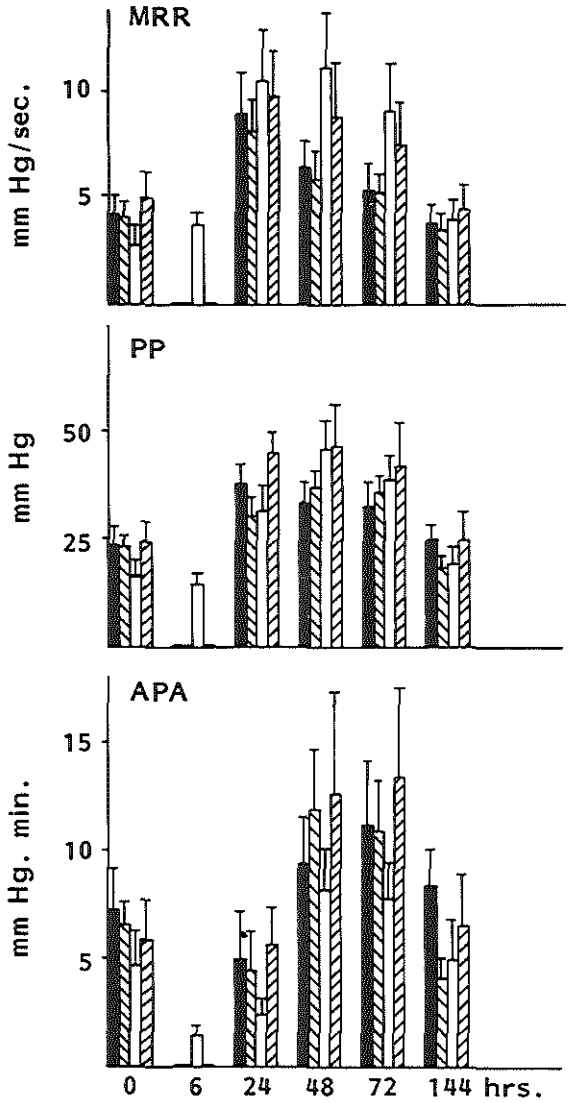

Figure 5.3 Mean values (+ S.D.) of maximum rate of rise (MRR), peak pressure (PP) and active pressure area (APA) in 50 intrauterine pressure cycles in four sheep before (0) and 6, 24, 48, 72, and 144 hours after administration of 0.1 mg of estradiol-17β.

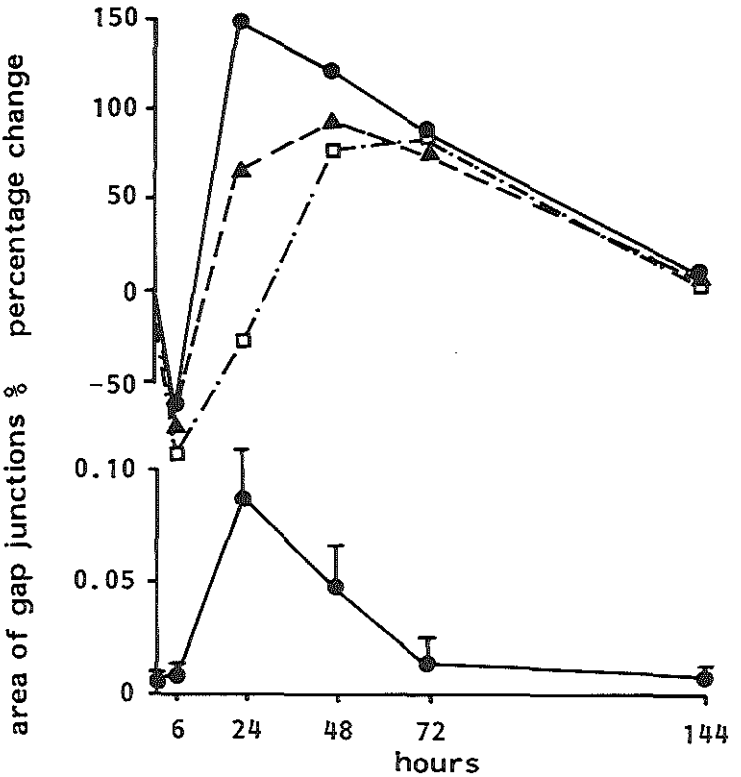

Figure 5.4 Top: means of the percentage increase of maximum rate of rise (●), peak pressure (▲), and active pressure area (□). Bottom: means (+ S.E.M.) of the percentage gap junction area. 0.1 mg of estradiol-17β administered at 0 hours.

Discussion

The results of this study indicate that estradiol-induced changes in myometrial activity in nonpregnant ovariectomized sheep are associated with formation of gap junctions. Intraarterial administration of a single dose of 0.1 mg of estradiol is followed, after one hour, by a quiescent period of approximately two hours, after which electrical activity returns first, followed by the reappearance of mechanical activity three to five hours later. These observations

confirm previous studies from this laboratory (5). The observed occurrence of a high frequency of intrauterine pressure cycles alternating with periods with a low frequency has also been reported by others (4,6,10). The discrepancy between the frequency of the intrauterine pressure cycles and the frequency of the bursts in our study (figure 5.2), can in part be explained by the method of burst detection that we applied. Bursts as detected by the computer occur concomitant with "small waves" in the intrauterine pressure signal (see figure 5.1). In addition, bursts also occur after the quiescent period without any elevation in intrauterine pressure or in association with a rise in intrauterine pressure of less than 10 mm Hg, which is below our criterium for detection.

An increase in the rate of rise of intrauterine pressure cycles following estradiol administration has also been observed by other investigators in sheep (6) and rats (11). The rate of rise can be regarded as a function of the recruitment of myometrial smooth muscle cells (12). 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 ovariectomized 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 intrauterine pressure cycles, 15 hours after estrogen treatment (11). Windmoller et al. (6) found a significantly increased response to a bolus challenge with oxytocin or prostaglandin $F_{2\alpha}$ 30 hours after estradiol infusion in ovariectomized sheep, which corresponds with the period of time in which gap junction area was found to be significantly elevated in our study.

It is remarkable that maximal peak pressures and maximal active pressure areas occurred at 48 and 72 hours after the administration of estradiol, respectively (figure 5.4), when gap junction area appeared to be not significantly greater than before administration of estradiol. This may be explained by the fact that estrogens also influence uterine synthesis of prostaglandins (10), receptors (13), contractile filaments (12), uterine blood flow (14), and number and size of myometrial cells (15). 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 (16).

The close relationship between gap junction area and changes in the pattern of myometrial activity, in particular the maximum rate of rise, in nonpregnant 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 nonpregnant uterus remains to be established.

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CHAPTER 6

COMPUTER ANALYSIS OF MECHANICAL AND ELECTRICAL UTERINE ACTIVITY

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Med Biol Eng Comput (Accepted for publication)

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 physiological state of the myometrium (1). 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 (2).

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 an inherent risk of observer bias. The results of visual assessment may differ considerably from those obtained by computer analysis (3).

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-automatical quantitative analysis of electrical activity was described by Harding et al. (4).

In this paper we present the algorithms, developed in an experimental study, for continuous computer-aided analysis of intrauterine pressure and electrical signals. These algorithms were shown to be satisfactory in several experiments.

Data acquisition

The intrauterine pressure and electrical signals were obtained from chronically instrumented sheep. The animal model has been described in detail elsewhere (5, chapter 4). Intrauterine pressure was measured with an open-ended sponge-tipped catheter connected to a Gould Statham

P23 ID pressure transducer and a Gould 13-4615-50 transducer amplifier. The electrical myometrial activity was recorded by means of silver-chloride coated bipolar silver needle electrodes fixed to the myometrium from the outside. The needles of the electrodes were 3 mm long with a diameter of 0.2 mm, and the distance between the needles was 2 mm. The electrical signals were amplified by a Gould 13-4615-56 universal amplifier. The intrauterine pressure and electrical signals were simultaneously recorded on a 14-channel Racal analogue

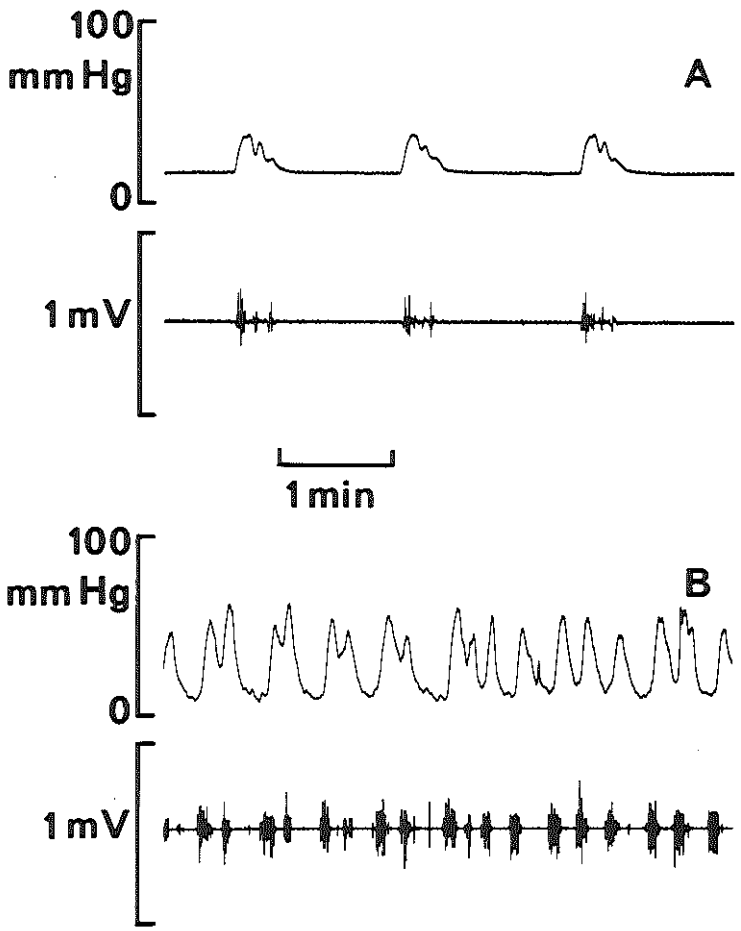

Figure 6.1 Original recording of mechanical (upper tracings) and electrical (lower tracings) myometrial activity in an ovariectomized ewe before (A) and 36 hours after (B) administration of 0.1 mg of estradiol-17β.

instrumentation recorder and written on a Gould Brush 2800 eight-channel polygraph for qualitative review. Original tracings of intrauterine pressure and electrical myometrial activity in sheep in various experimental conditions are shown in figure 6.1.

A transitory rise above the basal pressure level or tonus of the intrauterine pressure is called an intrauterine pressure cycle. Usually several pressure peaks can be distinguished within one intrauterine pressure cycle. In the analysis of the intrauterine pressure signal these peaks as well as pressure peaks occurring separately are called "small waves". The electromyogram 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.

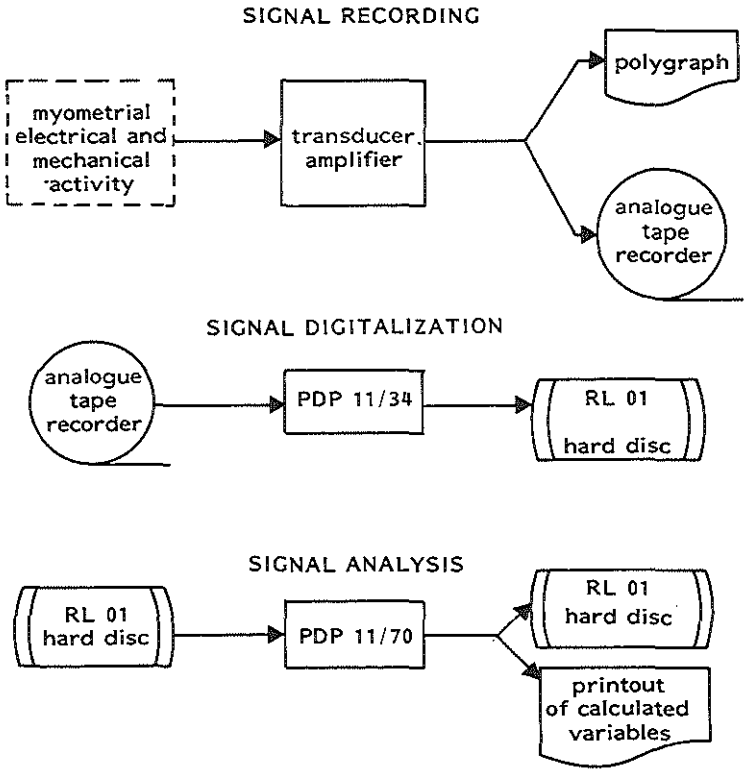

Figure 6.2. Diagrammatic system overview.

Digitalization of the analogue 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 preprocessed using an analogue band-pass filter. For the lower and higher cutoff frequencies (-3dB), 1 and 10 Hz, respectively, were used. The sampling frequency of the intrauterine pressure and electrical signals was set at 5 Hz and 25 Hz, respectively. Data files containing a one hour period of recording were stored for analysis on RL01 hard discs. Analysis was performed 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-IV PLUS. Figure 6.2 shows a diagrammatic overview of the system.

Data analysis

The algorithms for analysis of the intrauterine pressure and electrical myometrial activity were developed with the use of recordings obtained from various experiments. The criteria used were empirically chosen.

Intrauterine pressure

A recording period of one hour was divided into segments of five minutes in which 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 the consecutive minute levels the tonus in each second was determined.

The fluctuating components of the intrauterine pressure 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 (6) and neurophysiological (7,8) 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. Analogue-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 (6).

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

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

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 five minutes' 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. Mean 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. Total intrauterine pressure cycle period time, i.e. the sum of the period times of the intrauterine pressure cycles, in minutes.

e. Mean amplitude, i.e. the maximum pressure level of the intrauterine pressure cycles corrected for the corresponding tonus, in mm Hg.

f. Mean intrauterine pressure cycle area, i.e. the area under the curve of the intrauterine pressure cycles above the tonus, in mm Hg.min.

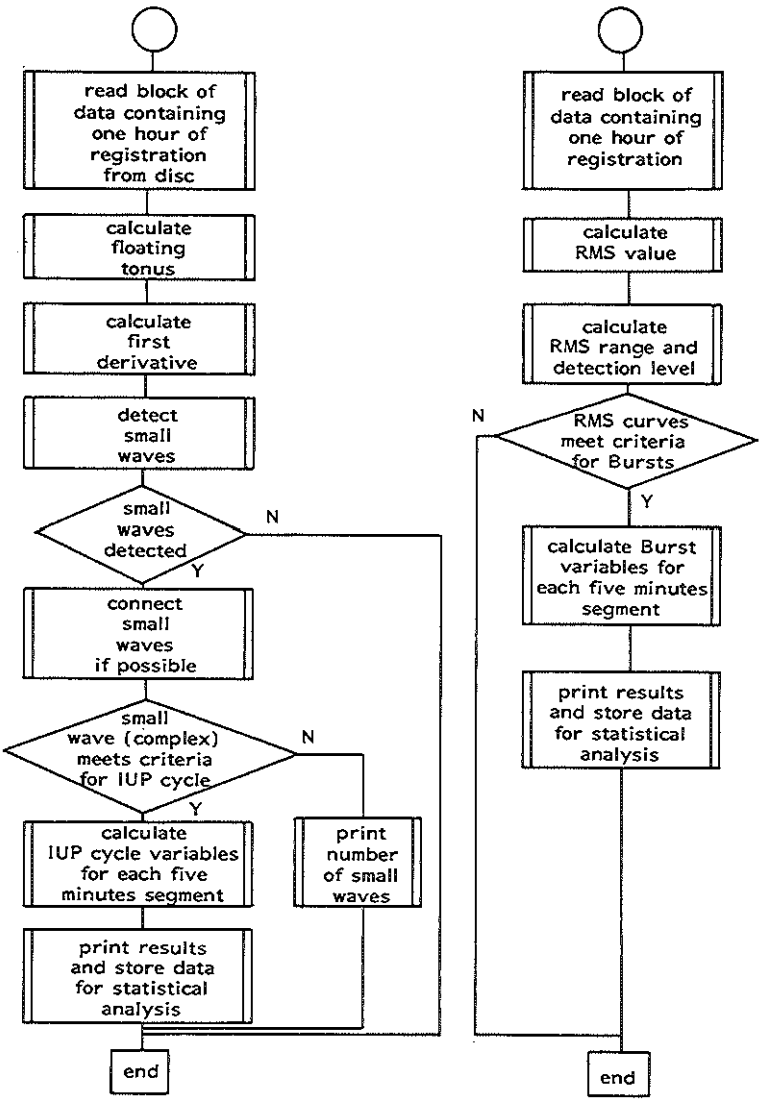

Figure 6.3 Flow diagram of the intrauterine pressure (IUP) and electromyogram (EMG) analysis program.

g. Total intrauterine pressure cycle area, i.e. the sum of the areas of the intrauterine pressure cycles, in mm Hg.min.

h. Mean overall rate of rise of the intrauterine pressure cycles, i.e. the maximal pressure corrected for tonus + 5 mm Hg, divided by the time between starting point and maximum, in mm Hg/sec.

i. Mean maximum rate of rise of the intrauterine pressure 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 flow diagram in figure 6.3 shows the elementary steps in the computer program. The calculated variables are shown in a diagram of an intrauterine pressure cycle (figure 6.4). The detection level of 10

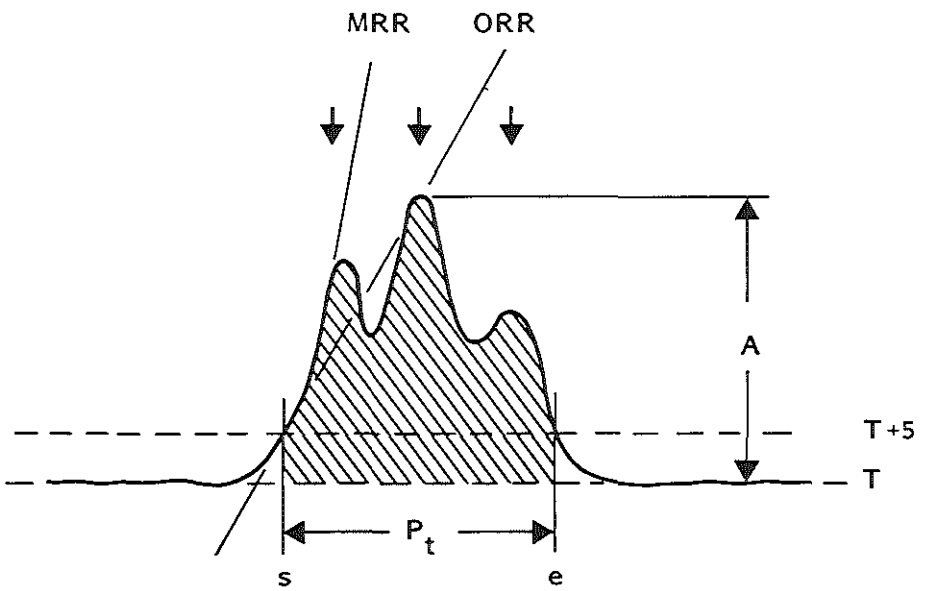

Figure 6.4 Diagrammatic representation of an intrauterine pressure cycle and the calculated variables. T, tonus; T + 5, tonus + 5 mm Hg; s, starting point; e, end point; MRR, maximum rate of rise; ORR, overall rate of rise; A, amplitude; Pt, period time. The arrows indicate small waves.

mm Hg for the intrauterine pressure cycles was chosen in accordance with other investigators (9). 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 intrauterine pressure 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 intrauterine pressure cycles hardly reach the 10 mm Hg level.

Electromyogram

As in the analysis of the intrauterine pressure, recording periods of one hour of an electromyogram were divided into segments of five minutes in which the variables were calculated. The root mean square (RMS) value, which is a measure of the average signal intensity (10), was calculated from the data points in each five minutes' segment, according to Bendat and Piersol (10) by using the following formula:

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

in which x_j 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 minute' segment. A burst was detected when a RMS curve reached a level calculated by: lowest RMS value + 0.25 x RMS range and remained above this level for at least two seconds. When the bursts were defined the following variables were calculated:

a. Burst frequency per minute.

b. Mean burst period time, i.e. the time from starting to end point of a RMS curve (burst) at the detection level, in minutes.

c. Total burst period time, i.e. the sum of the period times of the bursts.

d. Mean of the average burst RMS levels, i.e. average RMS values in a burst corrected for the detection level, in microvolts.

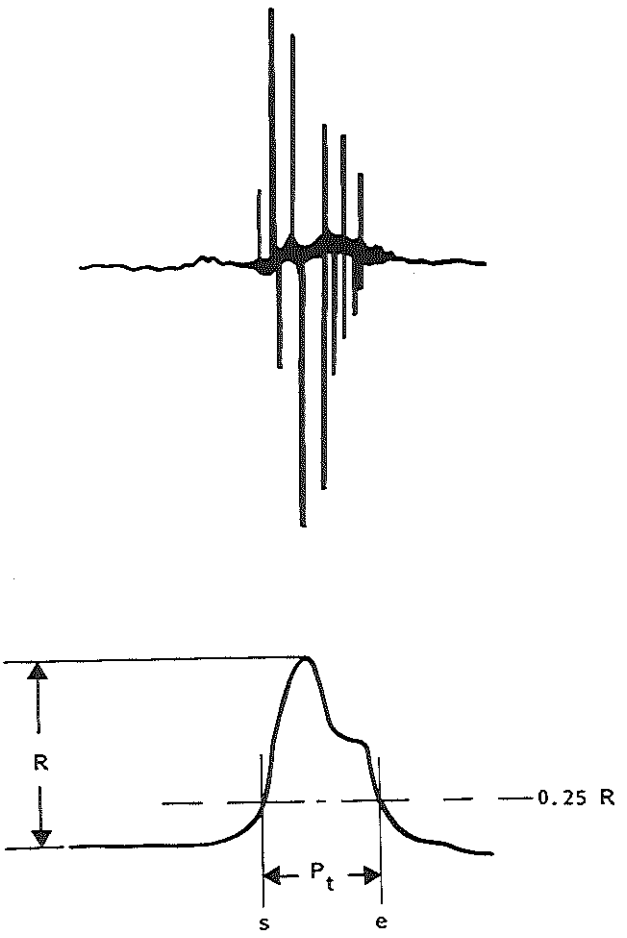

Figure 6.5 Diagrammatic representation of a burst of electrical activity and the calculated RMS wave. R, range between minimum and maximum RMS level; 0.25 R, minimum RMS level + 0.25 x RMS range; s, starting point; e, endpoint; Pt, period time.

An example of an electromyogram, of the RMS curve, and of the calculated variables is shown in figure 6.5. The variable detection level was used to discriminate between bursts and noise.

Application

The original tracing of electrical and mechanical myometrial activity shown in figure 6.1A was obtained from a chronically instrumented ovariectomized ewe. Figure 6.1B shows myometrial activity in an ovariectomized ewe 36 hours after intraarterial administration of 0.1 mg of estradiol-17β. The computer prints of the recordings shown in figures 6.1A and B are presented in figures 6.6A and B, respectively. The vertical bars above the intrauterine pressure signal indicate the detection of a small wave, the bars below the intrauterine pressure signal indicate the detection of an intrauterine pressure cycle. The bars in the intrauterine pressure signal represent the starting and end points of intrauterine pressure cycles, and the maxima of the

TABLE 6.1. RESULTS OF ANALYSIS OF THE 5 MINUTES SEGMENTS OF THE IUP AND EMG SIGNALS SHOWN IN FIGURE 6.1A AND B*

Parameter	Unit	Figure 6.1A	Figure 6.1B
Small wave frequency	min^{-1}	1.6	4.0
IUP cycle frequency	min^{-1}	0.6	2.0
Mean IUP cycle period time	min	0.35	0.34
Total IUP cycle period time	min	1.1	3.4
Mean amplitude	mm Hg	23.2	44.5
Mean IUP cycle area	mm Hg.min	4.9	8.4
Total IUP cycle area	mm Hg.min	14.6	84.4
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
Burst frequency	min^{-1}	1.6	3.8
Mean burst period time	min	0.09	0.10
Total burst period time	min	0.15	0.4
Mean average burst RMS level	μV	3.1	20.5

* IUP = intrauterine pressure, RMS = root mean square, EMG = electromyogram

intrauterine pressure cycles. The vertical bars below the electromyogram indicate the detection of a burst. Table 6.1 represents the variables that were calculated from the recordings shown in figure 6.1A and B, respectively. The first application of the method described here, in a study on the effects of pharmacological inhibition and stimulation of myometrial activity in chronically instrumented ovariectomized sheep, has been published recently (5, chapter 4).

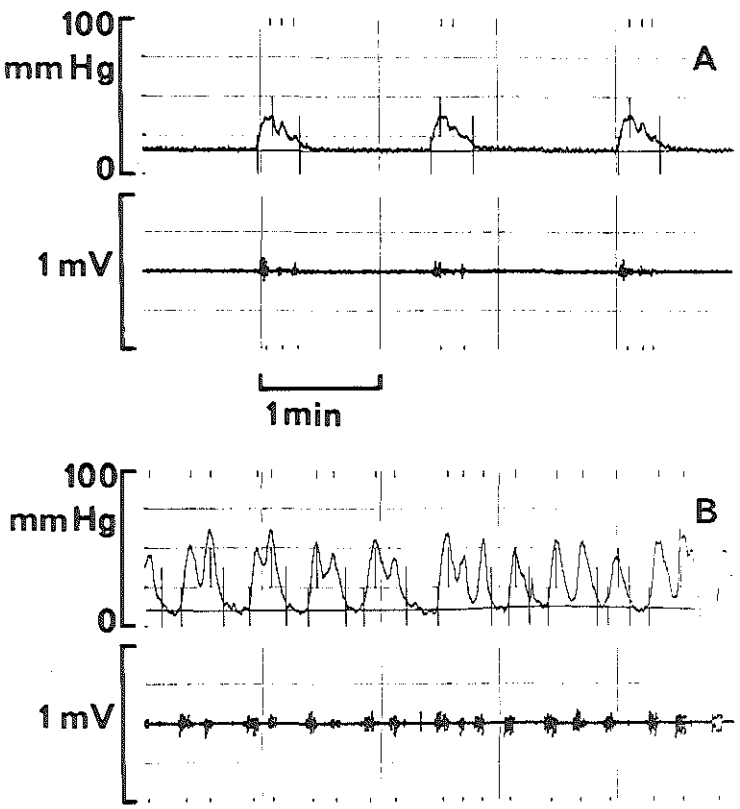

Figure 6.6 Computer analysis of the recording shown in figure 6.1A (A) and 6.1B (B). The vertical bars in the intrauterine pressure (IUP) signal indicate peak pressure and the starting and end points of an IUP cycle, the bars above the IUP signal indicate detection of a small wave. The vertical bars below the electromyogram indicate detection of a burst.

Conclusion

The aim of the present study was to develop 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 physiological significance of each of the intrauterine pressure variables is still debated (2,11,12).

We employed the RMS method to analyse 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 paper 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 physiological 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 physiological and pharmacological research on myometrial activity.

The program as such is not suitable for clinical monitoring of uterine activity during human labor. Firstly, although intrauterine pressure recordings are widely used to monitor human labor, the electrical myometrial activity cannot be easily obtained in humans. Secondly, 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.

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CHAPTER 7

GENERAL DISCUSSION

This thesis presents a combined approach using ultrastructural, electrophysiological and endocrinological methods to investigate factors which control myometrial contractility in vivo in the period of time around parturition, in an attempt to integrate various recent research findings. These findings indicate the importance of steroid hormones, prostaglandins and myometrial gap junctions in the control of myometrial contractility during pregnancy and labor.

Our study in pregnant ewes shows the relationship between the presence of gap junctions during labor and the observed increase in myometrial contractility. Gap function formation, which facilitates the spread of electrical activity across the myometrium, appears to be associated with enhanced coordination of contractile activity of the myometrial smooth muscle cells. The increase in the number of gap junctions which occurs in all species investigated, also in humans, seems to be a necessary step in the initiation of parturition.

The results of our study, as well as those of studies by others in sheep, rats and rabbits, indicate that gap junction formation is regulated and modulated by steroid hormones. Progesterone inhibits and estradiol stimulates the formation of gap junctions. Since investigation in vivo of the regulation of gap junction formation by steroid hormones, and by other factors such as prostaglandin synthesis, causes considerable difficulties in pregnant animals, we developed a nonpregnant animal model in which several factors could be manipulated more easily.

In this model, the chronically instrumented ovariectomized ewe, the myometrium shows spontaneous activity which appears to be independent of the autonomic nervous system. Progesterone suppresses myometrial activity in this model for a prolonged period of time. Estradiol-17β initially inhibits myometrial activity, but the quiescent period is followed by a pronounced increase in electrical and mechanical activity. We demonstrated that the increase in myometrial activity is associated with an increase in the number and area of gap junctions. Also in the nonpregnant uterus gap junctions seem to improve the coordination of contractile activity of the myometrial smooth muscle cells. The chronically instrumented ovariectomized ewe may serve as a useful model for further studies towards clarifying the regulation of gap junctions as an important step in the initiation of labor.

Gap junctions have also been shown to be present in the myometrium in humans during term as well as preterm labor. If the presence of gap junctions is, as we suggest, necessary for labor to occur, the control

of their formation or functioning may be an important clinical tool to induce or inhibit labor. The progression of threatened premature labor in humans may well depend on the formation of a large number of gap junctions. In our study the presence of gap junctions lasted approximately 48 hours after they reached maximum numbers following the initiating stimulus. For that reason, it may be postulated that the success of treatment of premature labor will depend on the inhibition of the contraction of individual myometrial smooth muscle cells as well as on preventing the spread of electrical signals across the myometrium during 48 hours. During this period of time, the initiating stimulus for the formation of gap junctions, if still present, should be counteracted.

The experimental studies reported in this thesis were performed with the hope that the results could add to our understanding of the mechanisms involved in term and preterm labor. In the future, animal experimental studies may result in the development of better methods of treatment of premature labor, which still constitutes one of the main causes of perinatal morbidity and mortality.

SUMMARY

This thesis consists of seven chapters dealing with combined ultrastructural, electrophysiological and endocrinological investigations into the mechanisms involved in the control of myometrial contractility in vivo. All experiments were carried out in chronically instrumented pregnant and nonpregnant ewes.

Chapter 1 is an introduction to this thesis. It gives a brief review of the major recent advances in the study of myometrial contractility in pregnancy and during labor. The objectives of this thesis are outlined.

Chapter 2 presents a review of the structural features of the uterus which are considered to be important in the control of myometrial function. A substantial section of this survey deals with quantitative studies of the development, role and regulation of gap junctions in the myometrium during labor. Gap junctions are intercellular channels through which ions and molecules can pass from one cell to another. Cells having gap junctions are electrically coupled; i.e. there is a pathway for flow of electrical current carried by ions. Myometrial gap junctions are present in low frequency or absent throughout pregnancy. The number and size of the gap junctions increase during delivery. Gap junction formation may be the basis of the synchronous contractility observed during labor, by providing low-resistance pathways for rapid spread of action potentials in the myometrium. Thus, gap junctions may convert the uterus into an active organ, inducing excitability and increasing contractile force. It is suggested that the hormonal changes which precede labor promote the synthesis of gap junctions and possibly of other subcellular structures.

Chapter 3 deals with an experimental study in chronically instrumented pregnant sheep. Myometrial electrical activity and intrauterine pressure were measured and plasma levels of estradiol-17β and progesterone were determined in late pregnancy, and during and after labor. Myometrial biopsies were obtained to determine gap junction area. Plasma progesterone levels fell four days before delivery, the concentration of estradiol rose sharply 12 hours before delivery. Towards delivery the pattern of the electrical myometrial activity changed from infrequent bursts of long duration to frequent bursts of short duration. The active pressure area of the intrauterine pressure cycles, the apparent conduction velocity, and the rate of rise of intrauterine pressure cycles increased in association with the changes in hormone levels. These changes were related to an increase in gap junction area. The results of this study support the hypothesis that changes in concentrations of steroid hormones lead to an increase in myometrial gap junction area, which improves the coordination of contractile activity of the uterus during labor.

Chapter 4 describes an experimental study in chronically instrumented ovariectomized sheep. Electrical activity and intrauterine pressure were investigated as indicators of myometrial activity. In all animals marked spontaneous myometrial activity was observed, characterized by rhythmic patterns of trains of bursts of electrical activity accompanied by intrauterine pressure waves. Administration of adrenergic (propranolol or phentolamine) or cholinergic (atropine) blocking agents had no effect on spontaneous uterine activity. Both oxytocin and prostaglandin $F_{2\alpha}$ appeared to stimulate spontaneous myometrial activity. Estradiol-17β temporarily depressed uterine activity in a dose-dependent fashion. The period of relaxation was followed by a pronounced increase in activity. Administration of progesterone resulted in long-term suppression of myometrial activity. Oxytocin and prostaglandin $F_{2\alpha}$ increased electrical and mechanical activity during estradiol suppression, but not after progesterone treatment. These results indicate that (1) the myometrium is spontaneously active in chronically instrumented, ovariectomized ewes; (2) the autonomic nervous system or its receptors do not play a role in the maintenance of spontaneous myometrial activity; (3) estradiol and progesterone suppress myometrical activity, but by different mechanisms.

Chapter 5 presents an experimental study on the effects of a single intravascular dose of 0.1 mg of estradiol-17β on myometrial electrical activity, intrauterine pressure, and myometrial gap junctions in chronically instrumented ovariectomized ewes. Maximum rate of rise, peak pressure, and active pressure area were determined, together with the frequency of intrauterine pressure cycles and the burst frequency, and related to gap junction area. Estradiol temporarily depressed uterine activity. The period of quiescence was followed by a pronounced increase in the variables of electrical and mechanical activity. Estradiol also increased gap junction area. The greatest increase in maximum rate of rise occurred 24 hours after administration of estradiol and was associated with the maximum increase in gap junction area. The results of this study indicate that a single dose of estradiol-17β induces formation of gap junctions, which may facilitate the spread of electrical activity across the myometrium and improve coordination of uterine contractility.

Chapter 6 gives a description of the computer analysis of electrical and mechanical activity as used in our studies in the nonpregnant sheep model. To facilitate the study of myometrial activity two algorithms were developed for continuous analysis of both electrical and mechanical (intrauterine pressure) activity. A combination of maxima- and level detection was used to define cycles in the intrauterine pressure signal, in which frequency, period time, area of the cycle, amplitude, overall- and maximum rate of rise were calculated. 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 bursts of electrical activity present in the original signal. Burst frequency, burst period

time, and mean burst RMS value were calculated from the RMS curve. The algorithms were applied to recordings obtained from chronically instrumented ewes in various experiments. 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 physiological and pharmacological studies of myometrial activity.

Chapter 7 is a brief general discussion of the results of the studies presented in this thesis. The hope is expressed that the results obtained in chronically instrumented ewes may add to our understanding of the mechanisms involved in labor in women. These and further animal experimental studies may result in the development of better methods for treatment of premature labor, which still constitutes one of the main causes of perinatal morbidity and mortality.

SAMENVATTING

Dit proefschrift bestaat uit zeven hoofdstukken, waarin een gecombineerd ultrastructureel, electrofysiologisch en endocrinologisch onderzoek naar de regulatie van de contractiliteit van het myometrium in vivo wordt beschreven. De experimenten werden uitgevoerd met chronisch geinstrumenteerde zwangere en niet-zwangere schapen.

Hoofdstuk 1 is de inleiding van het proefschrift. Het geeft een kort overzicht van de belangrijkste recente ontwikkelingen in het onderzoek naar de contractiliteit van het myometrium gedurende zwangerschap en baring. De doelstellingen van het proefschrift worden uiteengezet.

Hoofdstuk 2 geeft een overzicht van structurele eigenschappen van de uterus die waarschijnlijk van belang zijn voor de controle van de functie van het myometrium. Een belangrijk deel van dit overzicht betreft kwantitatieve onderzoeken van de ontwikkeling, de functie en de regulatie van "gap junctions" in het myometrium gedurende de baring. Gap junctions zijn intercellulaire kanalen, waardoor ionen en moleculen zich van de ene cel naar de andere cel kunnen bewegen. Cellen met gap junctions zijn daardoor electrisch gekoppeld, dat wil zeggen dat er een verbinding is voor electrisch geladen ionen. Tijdens de zwangerschap zijn gap junctions niet of in gering aantal aanwezig in het myometrium. Tijdens de baring neemt hun aantal en grootte toe. De vorming van gap junctions zou de basis kunnen zijn voor het ontstaan van gesynchroniseerde uteruscontracties tijdens de baring. Zij vormen immers intercellulaire verbindingen met een lage weerstand, waardoor een snelle verspreiding van actiepotentialen door het myometrium wordt mogelijk gemaakt. Op deze wijze kunnen gap junctions de uterus omvormen tot een actief orgaan met een verhoogde prikkelbaarheid en contractiekracht. Verondersteld wordt dat hormonale veranderingen die voorafgaan aan de baring de vorming van gap junctions induceren.

Hoofdstuk 3 beschrijft een experimenteel onderzoek in chronisch geinstrumenteerde zwangere schapen. Aan het einde van de zwangerschap en gedurende en na de baring werden de electrische activiteit van het myometrium en de intra-uteriene druk gemeten en werden de concentraties van 17β-oestradiol en progesteron in plasma bepaald. Om het aantal gap junctions te bepalen, werden biopten van het myometrium genomen. De progesteronconcentratie in plasma daalde vier dagen voor de bevalling, terwijl de concentratie van oestradiol een scherpe stijging liet zien 12 uur voor de partus. Bij het naderen van de partus veranderde de electrische activiteit van het myometrium van weinig frequente, langdurige electrische ontladingen (bursts) in frequente, kortdurende bursts. De oppervlakte onder de intra-uteriene

drukcurve, de relatieve voortplantingssnelheid van de electrische potentialen en de stijgsnelheid van de intra-uteriene druk namen toe, gelijktijdig met de veranderingen in de hormoonspiegels in het plasma. Deze veranderingen waren gerelateerd aan een toename van het aantal gap junctions. De resultaten van dit onderzoek steunen de hypothese dat veranderingen in concentraties van steroidhormonen voor het begin van de baring aanleiding geven tot een toename van het aantal gap junctions in het myometrium, hetgeen een verbetering van de coordinatie van de contractiliteit van de uterus tot gevolg heeft.

Hoofdstuk 4 handelt over een experimenteel onderzoek bij chronisch geïnstrumenteerde geövariëctomeerde schapen. De electrische activiteit van de uterus en de intra-uteriene druk werden geanalyseerd als indicatoren van myometriumactiviteit. De spontane electrische activiteit die werd gezien was gekarakteriseerd door een ritmisch patroon van opeenvolgende bursts vergezeld van intra-uteriene drukgolven. Toediening van adrenergische (propranolol of phentolamine) of cholinergische (atropine) blokkerende middelen had geen effect op deze spontane uterusactiviteit. Zowel oxytocine als prostaglandine $F_{2\alpha}$ veroorzaakten een toename van de spontane activiteit van het myometrium. Toediening van 17β-oestradiol onderdrukte, dosis afhankelijk en tijdelijk, de uterusactiviteit. Deze periode van relaxatie werd gevolgd door een duidelijke toename van de activiteit van de uterus. Toediening van progesteron had een langdurige onderdrukking van de activiteit van het myometrium tot gevolg. Oxytocine en prostaglandine $F_{2\alpha}$ verhoogden de myometriumactiviteit tijdens de door oestradiol geïnduceerde relaxatieperiode. Dit was echter niet het geval na relaxatie ten gevolge van behandeling met progesteron. Deze resultaten geven aan dat (1) het myometrium spontaan actief is in chronisch geïnstrumenteerde, geövariëctomeerde ooien; (2) het autonome zenuwstelsel of de receptoren daarvan geen rol van betekenis spelen bij het onderhouden van deze spontane myometriumactiviteit; (3) oestradiol en progesteron beide de spontane myometriumactiviteit onderdrukken, maar via verschillende mechanismen.

Hoofdstuk 5 presenteert een experimenteel onderzoek naar de effecten van een enkelvoudige intravasculaire toediening van 0,1 mg 17β-oestradiol op de electrische activiteit van het myometrium, de intra-uteriene druk en het aantal gap junctions in het myometrium in chronisch geïnstrumenteerde geövariëctomeerde ooien. De maximale stijgsnelheid van de intra-uteriene druk, de top van de intra-uteriene drukcurve en de oppervlakte onder deze curve werden bepaald, samen met de frequentie van de intra-uteriene drukcycli en de frequentie van de bursts. Deze variabelen werden gerelateerd aan het aantal gap junctions. Oestradiol onderdrukte tijdelijk de uterusactiviteit. Deze periode van relaxatie van de uterusactiviteit werd gevolgd door een duidelijke toename van de variabelen van zowel de electrische als de mechanische activiteit. Oestradioltoediening veroorzaakte ook een stijging van het aantal gap junctions. De grootste toename van de maximale stijgsnelheid van de intra-uteriene druk vond plaats 24 uur na toediening van oestradiol, gelijktijdig met de maximale toename van

het aantal gap junctions. De resultaten van dit onderzoek geven aan dat een enkelvoudige dosis van oestradiol de vorming van gap junctions induceert, hetgeen waarschijnlijk een betere voortgeleiding van electrische activiteit in het myometrium mogelijk maakt en daarmee een betere coördinatie van de contractiliteit van de uterus.

Hoofdstuk 6 geeft een beschrijving van het computerprogramma voor de bewerking van electrische en mechanische myometriumactiviteit zoals wij dat gebruikten bij ons onderzoek bij niet-zwangere schapen. Om de bestudering van de myometriumactiviteit te vereenvoudigen werden twee algorithmen ontwikkeld voor continue analyse van zowel de electrische activiteit van het myometrium als van de mechanische (intra-uteriene druk) signalen. Een combinatie van maxima- en niveaudetectie werd gebruikt om de intra-uteriene druksignalen te definieren. Hierin werden frequentie, tijdsduur, oppervlakte, amplitude en gemiddelde en maximale stijgsnelheid van de intra-uteriene drukgolf berekend. Van het electrische signaal werd de "root mean square" (RMS) waarde in een verschuivend tijdsinterval berekend. Om de bursts van electrische activiteit die aanwezig waren in het originele signaal op te sporen, werd een drempelwaarde aangebracht in de verkregen RMS-curve. De frequentie, de tijdsduur en de gemiddelde RMS-waarde van de bursts werden berekend in de RMS-curve. De algorithmen werden toegepast op meetgegevens, verkregen uit experimenten bij chronisch geinstrumenteerde schapen. De nauwkeurige geautomatiseerde analyse van meetgegevens van electrische en mechanische uterusactiviteit verschaffen een uitgebreide verzameling gegevens over verschillende fysiologisch belangrijke variabelen, die snel statistisch kunnen worden geëvalueerd. Het beschreven computerprogramma kan daardoor van nut zijn voor fysiologische en farmacologische studies van de myometriumactiviteit.

Hoofdstuk 7 is een korte discussie van de resultaten van de studies die in dit proefschrift worden beschreven. De hoop wordt uitgesproken dat de bij chronisch geinstrumenteerd schapen verkregen resultaten een bijdrage zullen leveren aan het begrip van de mechanismen die ten grondslag liggen aan de baring bij de vrouw. Deze en andere dierexperimentele onderzoeken kunnen leiden tot de ontwikkeling van betere behandelingsmethoden van de voortijdige baring, een van de belangrijkste oorzaken van perinatale morbiditeit en mortaliteit.

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The ultrastructural studies were carried out at the Department of Neurosciences, McMaster University Health Sciences Centre, Hamilton, Ontario, Canada. Bob Garfield, Professor of Neurosciences, Obstetrics and Gynecology authoritatively guided me into the ultrastructure of the myometrium, first during my stay at his Laboratory, and later during his visits and telephone conversations. His always expert advice was worth the telephone bill. I thank him for his friendship and example as a scientist. The gap between Rotterdam and Hamilton could not disrupt the junction between us, which lasted significantly ($p<0.001$) longer than the gap junctions in our sheep.

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