

MECHANISMS OF CONTRACTION /
RELAXATION OF THE HUMAN UTERUS –
NEW DEVELOPMENTS

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Mechanisms of contraction / relaxation of the human uterus – new developments



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I declare that the attached work is entirely my own, other than in the sections where all sources are clearly and properly indicated.

Abstract

This is a literature research based on the review of 115 papers written over the last ten years. Our interest on the mechanisms of uterine contractility and relaxation was based on the importance of such mechanisms from the clinical but also scientific point of view. We know that the mechanisms of contractility and relaxation of the human myometrium are very important for understand the pathophysiology of term and preterm labour and also helps as to identify common pathways and differences in between them. In this dissertation it was given a sort description of the uterine embryology and anatomy and also there is a description of our background information on the role of different receptors. Also there is a description of the mechanochemical, electrochemical and pharmacomechanical theories of activation of the contractility mechanisms in myometrium. Some biochemical aspects like G-protein and different other molecules involved in these mechanisms were discussed. Our aim was to review the new developments on the research of the mechanisms of contractility and relaxation in human myometrial cells. We focused on the oxytocin action and the up -regulation and down regulation mechanisms of the correlated receptor. A very important part of the literature research was correlated with the mechanisms of calcium homeostasis and the new developments over the last years Also we focused on relaxation mechanisms which is a field of the research not much developed.

We also had an extensive review of the new developments on the mechanisms of term and preterm labour and correlated theories. Is very positive the improvement over the last few years on the correlation of genetic factors and preterm labour and new methods of research like genomics and proteomics helps a lot. The clarification of these mechanisms will be give as the knowledge to create new safer tocolytic drugs in the future not only to improve the neonatal outcome in cases of premature labour but also in correlation with other mechanisms that uterine relaxation could be a benefit (embryo transfer after IVF).

Introduction

As suggested by the title, the aim of this review is to present the data from various journals over the last ten years. This thesis concentrates on the mechanisms of relaxation and contractility of the human myometrium, a very important and complicated part of the human female physiology. These mechanisms are important because of their correlation with premature labours.

Prematurity, is the major cause of mortality and morbidity in the neonates all over the world. In U.K, 10% of the pregnancies result in preterm labour. It is therefore very important to understand the mechanisms of contractility and relaxation in the human myometrium as this understanding can help us create new drugs to combat preterm labour and its complications which emotionally and financially distress our modern society.

An understanding of the exact mechanisms of human contraction and relaxation can provide us with a better understanding of term and probably preterm labour, allowing us to assess whether preterm labour is a deviation of normal labour or if a similar mechanism is triggered early in gestation by pathological circumstances.

To date, many groups around the world are researching the mechanisms of human myometrial contractility and relaxation, using animal models and isolated human myometrial cells in culture from hysterectomies and caesarean sections.

An understanding of these complicated mechanisms is vital to clarify differences through animal and human myometrial contractility and relaxation. However, since it is ethically more difficult to obtain human tissue, most studies are carried out using animal models.

This dissertation will focus on human myometrial tissue and will exclude any animal model work.

1. Background Information ---

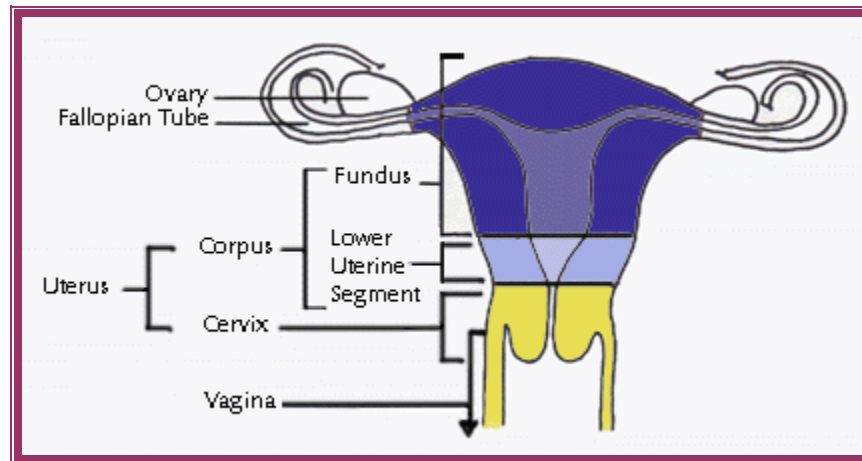
1.1 Embryology

In the female, the paramesonephric ducts are in contact at the level of the posterior wall of the pelvic urethra. This structure is termed sinusal trabecle, and its fusion with the paramesonephric ducts forms the genital canal, which forms the uterus and the superior part of the vagina. All these structures are of mesonephric origin (Human Embryology, Larsen, third edition)

1.2 Anatomy

The human uterus is a pear-shaped organ, composed of two distinct anatomic regions: the cervix and the corpus. The corpus is further divided into the lower uterine segment and the fundus. The cervix is a narrow cylindrical passage which connects at its lower end with the vagina. At its upper end, the cervix widens to form the lower uterine segment (isthmus); the lower uterine segment in turn widens into the uterine fundus. The corpus is the body of the uterus which grows during pregnancy to carry a fetus. Extending from the top of the uterus on either side are the fallopian tubes (oviducts); these tubes are continuous with the uterine cavity and allow the passage of an ova (egg) from the ovaries to the uterus where the egg may implant if fertilized.

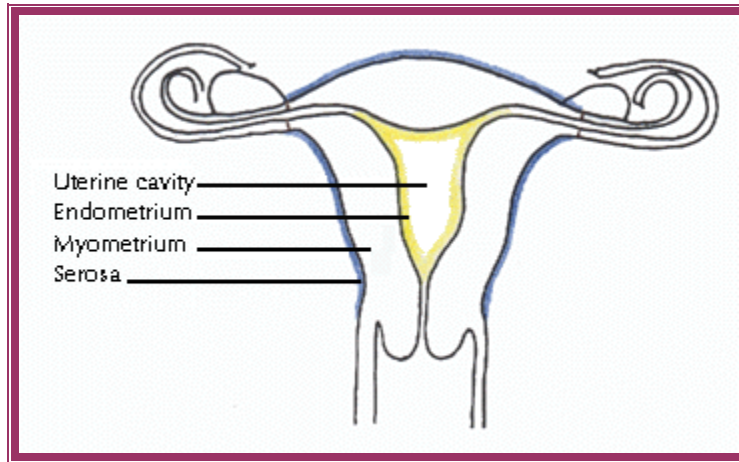
Figure 1. Basic anatomy and physiology of the uterus



Source: www.fibroids.net

The thick wall of the uterus is formed of three layers: endometrium, myometrium and serosa. The endometrium (uterine mucosa) is the inner most layer that lines the cavity of the uterus. Throughout the menstrual cycle, the endometrium grows progressively thicker with a rich blood supply to prepare the uterus for potential implantation of an embryo. In the absence of implantation, a portion of this layer is shed during menstruation. The myometrium is the middle and thickest layer of the uterus and is composed of smooth (involuntary) muscle. The myometrium contracts during menstruation to help expel the sloughed endometrial lining and during child birth to propel the foetus out of the uterus. The outermost layer, or serosa, is a thin fibrous layer contiguous with extrauterine connective tissue structures such as ligaments that give mechanical support to the uterus within the pelvic cavity.

Figure 2. Basic anatomy and physiology of the uterus



Source: www.fibroids.net

The posterior surface of the uterus is more concave than the anterior one. The fallopian tubes are attached to the upper corners of the uterus; at the point of junction between the lateral and superior margins of the uterus. The fallopian tubes open in the uterine cavity in their proximal end and in the pelvis on their distal end. Anterior to the uterus is the bladder and posterior, the rectum. The Pouch of Douglas is found between the posterior part of the uterus and the rectum.

Uterine size varies with age and number of pregnancies, but is approximately 8cm long and weighs about 70g. During puberty, the uterus is 3-4cm long; accounting for the smaller body mass and during term pregnancy can increase to 1100g, accounting for increased body mass as a result of hypertrophy.

The uterus is composed of bundles of smooth muscle, which is responsible for its contractility, and connective tissue. The anterior and posterior walls of the uterine cavity are more muscular, as is the inner uterine wall. The cervix is mainly composed of collagen and elastic tissue, and contains 10% muscle tissue.

Three types of uterine ligaments, the broad ligaments, the cardinal ligaments, and the round ligaments form the lateral and superior part of the organ running laterally and downwards to the inguinal ligament and stop at the labium majus. On the posterior and lower part of the uterus we can see the uterosacral ligaments.

The blood vessels are derived from the uterine arteries main branch of the internal iliac artery and from the ovarian artery. The main vein is the uterine vein branch of the internal iliac vein.

The lymphatic from the cervix arrive to the hypogastric nodes, from the body into the internal iliac nodes and some in the periaortic nodes.

The innervation of the uterus is from the autonomic nervous system.

The parasympathetic system derives from the pelvic nerves (second, third and fourth sacral nerves).

The sympathetic system arises from the internal iliac plexus.

The uterus has the characteristic that it can permit, in case of pregnancy, a huge distension gradually over forty weeks of pregnancy. During this time an extraordinary increase of its size and weight occurs. The myometrium is formed from smooth muscle tissue arranged in bundles. These cells have a close relation with connective tissue and more specific with collagen.

The ultrastructure of the myometrium shows presence of actin and myosin filaments. These are not organised like in case of the striate muscle and they are forming bundles. Smooth muscle has pacemaker regions. These regions can generate contractions. There are also gap junctions in between the cells which help in the in the spread of the electrical depolarization from cell to cell.

The smooth muscle does not have cross striations and the fibers are smaller than in striate muscle. Another characteristic of the smooth muscle is the lack of interconnecting bridges in their multiunit form.

Desmin and vimentin are specific proteins in the intermediate filaments characteristic of smooth muscle tissue.

1.3 Receptors –Their role in uterine contractility

The regulation of endocrine physiology is dependent on the presence of different receptors, which regulate cellular activity of various hormones, neurotransmitters and cellular factors. Receptors are activated by binding to a specific hormone. This binding can alter its structure, resulting in the activation of a cascade of other molecules (second messengers) and changes in activities of various enzymes resulting in altered gene expression. The result of the activation of a receptor is the gene transcription alteration, the changes of specific mRNAs and the formation of new proteins.

The myometrium consists of many different receptors such as, receptors on the surface of the smooth muscle cells, oxytocin; prostaglandins receptors and specific receptors for hormones, such as neurotransmitters etc, correlated with the G-proteins. Heptameric receptors also exist and have different loops passing in and out of the cell membrane.

There are many receptors involved in the mechanisms of human myometrium contractility. These include: prostanoid receptors, peptide hormone receptors (angiotensin, oxytocin, endothelin, etc), adrenoceptors, histaminic and muscarinic receptors. In most of these receptors there are agonists and antagonists and also partial agonists. The identification of the mechanisms of their action is very important in the design of new drugs for use in cases of preterm labour.

In the next few paragraphs we will examine some of the most important groups of receptors involved in the mechanisms of uterine contractility and relaxation.

1.4 Peptide Hormone Receptors

The mechanism of uterine contractility and relaxation is largely influenced by peptide hormones. These include oxytocin, angiotensin and vasopressin. Vasopressin is similar to oxytocin and is localised on the surface of both pregnant and not pregnant human myometrium. Endothelin is made up of two kinds of receptors: a dominant receptor ETA and a non dominant receptor ETB. ETA can increase the production of cAMP and result in uterine relaxation in some animals. A type of endothelin called endothelin -1 in human myometrium can increase the action of oxytocin near term (Valenzuela GJ, *et al*, 1995).

1.5 Adrenoreceptors

There are two types of adrenoreceptors in humans, inhibitory and stimulatory ($\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$). The $\alpha 1$ receptor can increase uterine contractility, activating the PLC and the $\alpha 2$ receptors can both increase and decrease myometrium contractility.

The $\beta 2$ receptors can relax the myometrium by increasing the production of cAMP and also by activating the K^+ channels, lowering that way the intracellular concentration of Ca^{2+} (Brenninkmeijer PABC *et al*, 1999).

The use of agonists to these receptors is very common for the treatment of preterm labour but because of the lack of selectivity for the myometrium we have many side effects like tachycardia increase of blood sugar levels etc.

1.6 Prostanoid Receptors

The human myometrium consists of many forms of prostanoid receptors. These receptors are found in higher concentrations in non-pregnant than in pregnant (term) myometrium. Arachidonic acid is the most important precursor for the synthesis of prostaglandins. Prostaglandins are vital in uterine contractility mechanisms, and are produced and released near the myometrium to escape catabolic degradation.

In obstetrics, synthetic prostaglandins are used for inducing labour. Indomethacin is commonly used as an inhibitor of their synthesis in the treatment of preterm labour. Thromboxane receptors produce stimulation. In addition, histamine receptors type H1 and H2, exist. Type H1 increase contractility by activating the PLC and type H2 relax the myometrium by reacting with the AC.

Many different prostaglandins are found in the myometrium. PGE₂ is a prostaglandin can have two different actions on the myometrium. It can cause both contraction and relaxation by using both the MLCK activation pathway and Ca²⁺ increase and cAMP increase (Asboth G et al, 1996). PGF₂ α has a stimulatory effect in the myometrium. It acts by stimulating the FP receptor and coupling with the PLC molecule. It was found that the FP receptor in humans is a classic heptameric receptor (Lake. S *et al*, 1994), (Abramovitz M, *et al*, 1994).

The stimulation of PLC when we use a selective FP agonist, such as fluprostenol, shows that it is PT resistant. This shows the role of Gq-PLC- β and also confirms the role of an isoform of PLC- γ (Carrasco MP, *et al*, 1995).

There is a possibility that there are two receptors acting on the same PLC- β , which can explain the probability of a mechanism of interaction between oxytocin and PGF₂ α in labour and parturition. There are different types of EP receptors, EP1, EP2, EP3 and EP4. EP1 and EP3 cause contraction, and EP2 and EP4 cause relaxation. EP3 consists of different isoforms.

The clarification of the mechanisms of action of the different receptors involved in human myometrial contractility and relaxation will be of vital importance when designing new, receptor specific drugs.

1.7 Mechanisms of Contractility-Relaxation of the Myometrium

There are three main mechanisms known to be involved in uterine contractility: electrochemical, pharmacomechanical, and mechanochemical.

1.7.1 Electrochemical

In this case, the autonomic nervous system which innervates the uterus can generate a depolarization of the cell membrane which results in an influx of Ca^{2+} in the cytoplasm from the extracellular space. This can create an increase of intracellular Ca^{2+} concentration and the contraction of the myofilaments due to activation of the MLCK from the complex Ca^{2+} / calmoduline and the phosphorylation of LC. The human myometrium has two different types of calcium channels: T (transient) and L (long lasting). L is responsible for Ca^{2+} influx and T is responsible for action potential waves. During resting periods, the concentration of Ca^{2+} is 0.1 μM and after stimulation can increase 100 fold.

Gap junctions in human smooth cells improve the transmission of second messengers and electric waves from cell to cell. In cultured human myometrium, oxytocin and estradiol increase the concentrations of the protein connexin-43, one of the main proteins involved in the formation of gap junctions. hCG and FSH are known to decrease the concentration of connexin-43 (Ambus G *et al*, 1994).

1.7.2 Pharmacomechanical

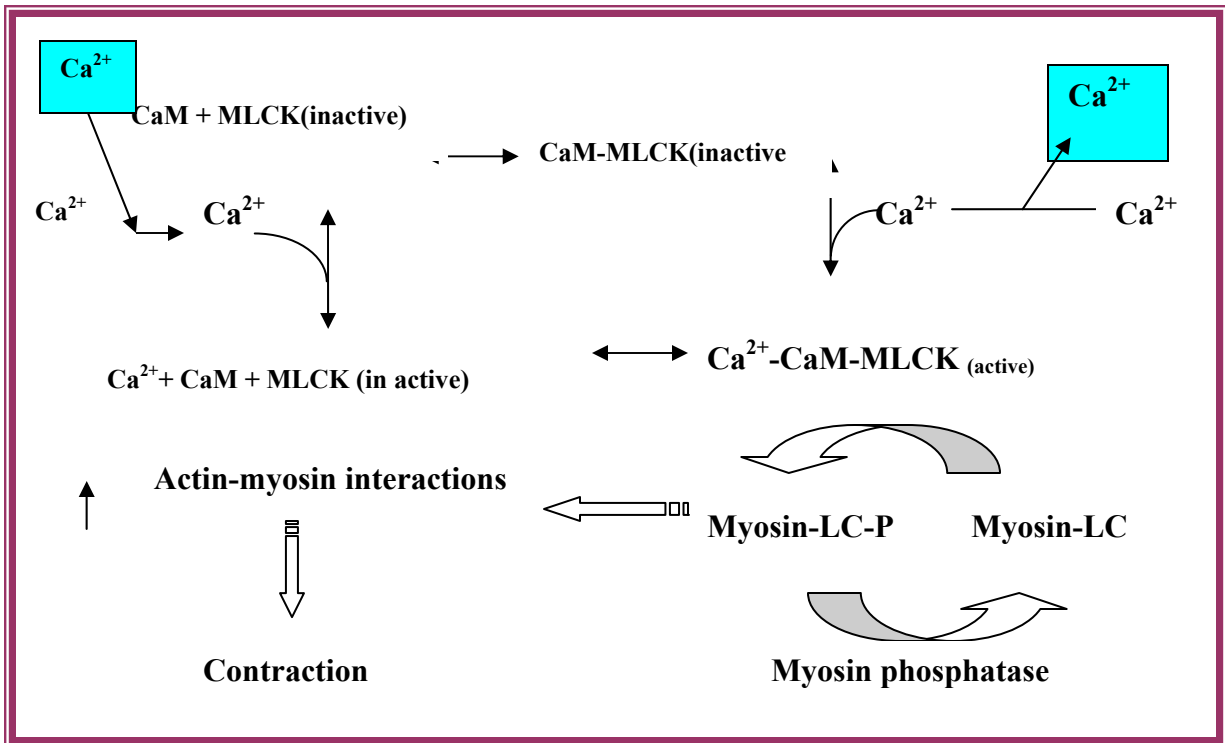
This mechanism is independent of the change in membrane potential. It is based on the activation of receptors by different drugs and hormones resulting in muscle contraction as a result of Ca^{2+} being released from an internal source, the sarcoplasmic reticulum.

The concentration of Ca^{2+} in the cytoplasm is extremely important for the interaction between actin and myosin. The phosphorylated myosin interacts with actin resulting in the contraction of the smooth muscle.

In human myometrium, there is a correlation between concentration of Ca^{2+} and phosphorylation of the MLC. The complex Ca^{2+} - calmodulin has a double role in smooth muscle tissue (Tansey MG *et al*, 1994). Firstly, it improves the MLCK action and secondly, the negative control on MLCK by activation of an enzyme the (CaM Kinase II) acts on a specific serine residue of MLCK and reduces the sensitivity of this enzyme for Ca^{2+} (Amano M *et al*. 1996).

MLCP is an important enzyme in the mechanism of relaxation- contraction (Shirazi *et al* 1994). The dephosphorylation of MLCP is the key point in the whole mechanism of relaxation of the smooth muscle. Hence, another mechanism of increasing smooth muscle contractility is to decrease the activity of MLCP.

Figure 3. Mechanism of contraction of smooth muscle



In pharmacomechanical mechanisms, the superficial receptor of the cell is activated by different drugs, hormones or neurotransmitters. This superficial receptor has a heptahelical structure consisting of seven transmembrane domains. There are three loops on the outside of cell and three on the inside of the cell's membrane.

Binding to the receptor causes conformational changes and there is an interaction through the guanine nucleotide binding protein (G-protein) and the third intracellular loop (Milligan G. *et al*, 1995). The G-protein is a protein complex comprised of three different subunits, α , β and γ . The α - subunit is connected with a GDP molecule and is inactive.

During stimulation, GTP instead of GDP binds with the α - subunit, separating it from the subunits β and γ (Barany and Barany 1996b). As a result, the receptors reacting much better with the heteromeric form than the whole trimeric complex. Alternatively, the α -subunit can convert the GTP to GDP, inactive form. This can happen because of the hydrolytic activity of the above subunit.

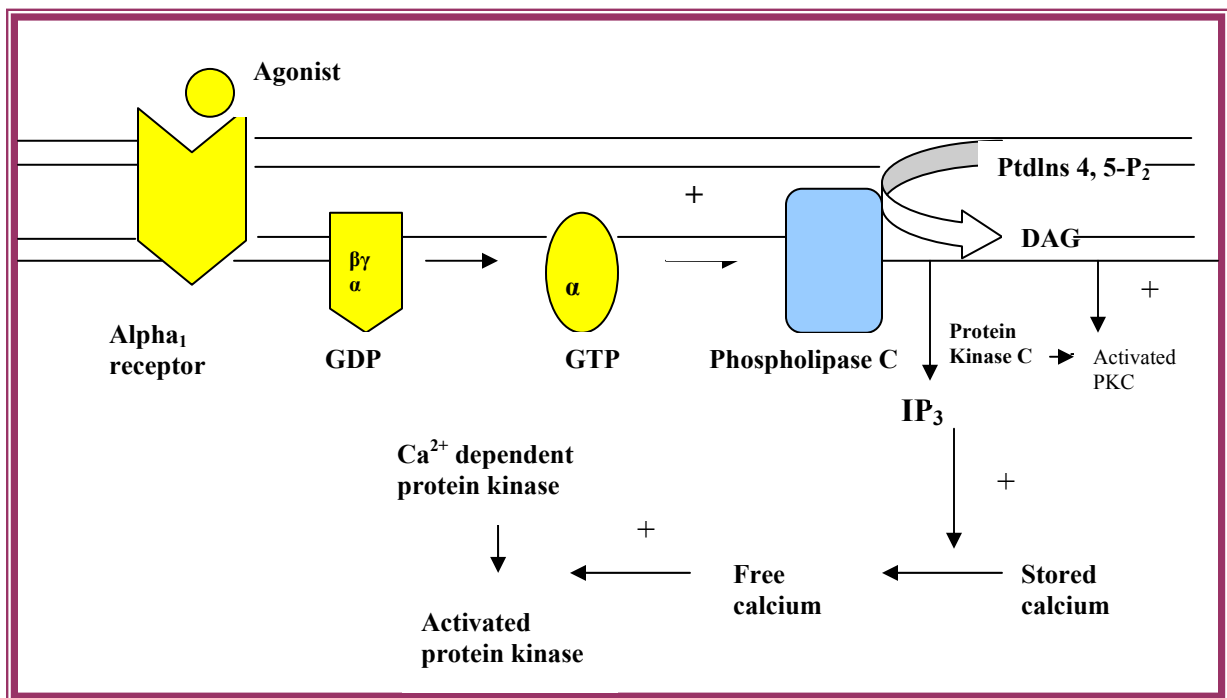
Phosphoinositide- specific phospholypase C PI (PLC) are enzymes that differ from each other in the number of the phoshoryl groups they have in their inositol ring. Some of them have the action of hydrolyse the PIP₂ (triphosphorylated phosholipid) to form two

messengers IP3 and DAG. IP3 is involved in the mechanism of Ca^{2+} released from the SR and increases the cytoplasmic Ca^{2+} concentration. DAG activates the protein kinase C (PKC) and increase the contractility of the smooth muscle. DAG can also hydrolyse some lipases (diacyl and monoacylglycerol), resulting the production of arachidonic acid, an important component of the prostaglandins (Hertelendy F, *et al*, 1995). DAG can be transformed into phoshatidic acid and have a role in the reformation of PIP2.

Several enzymes called effectors system enzymes are involved in generating a second messenger response.

In smooth muscle, myosin has to be phosphorylated for actin in order for activation to occur.

Figure 4. α_1 receptors stimulation



There are different receptors involved with the G-protein. Several, can couple with AC (adenyl cyclase) and increase the relaxation of the smooth muscle by increasing the cAMP. There are many different receptors in the myometrial cells that can decrease or

increase the activity of AC.receptors. An increase in activity of AC receptors increases contractility, hence, a decrease in its concentration is going to improve relaxation.

A very important role is that of cAMP, which if activated in human can decrease the frequency and amplitude of the contractions by unknown mechanism (Buhimschi I et al, 1995).

In case of an increase of cAMP and through the activation of the PKA, MLCK is phosphorylated and its affinity for Ca^{2+} / calmoduline is decreased, resulting muscle relaxation. In addition, PKA improves relaxation of the smooth muscle by helping the SR to collect Ca^{2+} again.

Other receptors associated with PLC produce IP3 from PIP2 and increase the Ca^{2+} concentration in the cytosol by removing it from the SR. Hence DAG is produced causing protein phosphorylation and contraction.

1.7.3 Mechanochemical

This mechanism involves phosphorylation of LC and uterine muscle contraction after stretching of the uterine muscle fibres. Probably indicating that there are receptors activated by mechanical stretching which can have some kind of interaction with the SR. Ca^{2+} channels activate receptors to pump Ca^{2+} on the cytosol resulting in endogenous Ca^{2+} increase in the contraction of the smooth muscle.

In order to observe a contraction we have to phosphorylate the LC (Barany and Barany 1996) and for relaxation to occur we have to dephosphorylate the LC.

The uterine muscle consists of myosin light chain kinase (MLCK), an enzyme activated by the Ca^{2+} / calmoduline complex. MLCK transfers a phosphate group (terminal) of ATP to phosphorylate the LC (light chain) (Stull *et al.*, 1996). Another enzyme, myosin light chain phosphatase (MLCP) can also dephosphorylate the LC (Hartshorne *et al.* 1998). MLCP has three subunits: one subunit of 110-130-KDa that influences the association with myosin, a subunit of 20 KDa with an unknown role and catalytic subunit of 37-38 KDa at the end of the molecule. If this end subunit is phosphorylated, MLCP loses its properties. An antagonist action exists between MLCP and MLCK, thus, increased MLCP action causes increased contractility, whilst increased MLCK action

causes increased relaxation. There is evidence that a system which regulates MLCP exists in smooth muscle. This system is called the Rho system and the most important component of this system is the enzyme Rho – kinase. The action of this enzyme is the phosphorylation of the 130 KDa subunit of the MLCK and the inhibition of its activity. The result of this inhibition of MLCP activity causes an increase in the contractility force, a phenomenon called sensitivity increase (Somlyo and Somlyo 1994).

Protein kinase C (PKC) can also phosphorylate LC.

During relaxation, the main goal is to decrease the Ca^{2+} intracellular concentration. There are two mechanisms involved in this transition. The first mechanism includes an SR pump transporting ATPase, resulting in the transport of Ca^{2+} from the cytoplasm to the SR. The second mechanism involves the plasma membrane pumps to transfer Ca^{2+} from the cytosol to the extracellular compartment. Relaxation is increased by the increase of c-

AMP dependent protein kinase. This enzyme can phosphorylate MLCK and as a result we decrease the affinity of the known calmodulin / Ca^{2+} complex.

A negative feedback mechanism which increases the MLCK phosphorylation causes relaxation.

It is of vital importance to note the role of the sarcoplasmic reticulum (SR) in all three mechanisms mentioned above. There are two kinds of receptors in the SR: the ryanodine receptor and the InsP3 receptor, both of which are involved in the Ca^{2+} entrance and exit from the SR. Many subtypes of InsP3 receptor exist, even in human myometrium (Newton CL ed al, 1994), (Wojcikiewicz RJ, et al, 1995).

There are several mechanisms involved in decreasing Ca^{2+} . One is the uptake from the SR and another is the exchange of Na^+ and Ca^{2+} . Also the activation of K^+ channels causes uterine relaxation.

1.8 Biochemical Aspects of the Mechanisms of Labour

The myometrium has a few characteristic differences when compared to skeletal muscle. These are probably because of the particular role of this organ in the human parturition. The myometrium has a higher degree of shortening than the skeletal muscle. Also the forces in the myometrium are multidirectional and not unidirectional as in case of straight muscles. In the myometrium, thin and thick filaments are organised in bundles.

The exact mechanisms of human parturition still remain unknown. Extensive research has been carried out on animal models such as goats and sheep, but for ethical reasons the research in human parturition is limited.

There are two main mechanisms that significantly influence parturition: the dilatation and cervical ripening and the increase in intensity and frequency of the uterine contractions.

There is a mechanism that increases the sensitisation of Ca^{2+} , which in turn activates Rho kinase, the enzyme involved in the inhibition of MLCP and improved uterine contractility. Other mechanisms such as G-protein pathways of different receptors and effectors can also increase the uterine contractility. Conversely the increase of cAMP concentration can improve uterine relaxation.

In sheep, there is an activation of the adrenal pituitary axis, increase of cortisol and induction of P450 placental enzymes. There is then a conversion of progesterone to oestrogens and a decline in progesterone concentration, causing a production of prostaglandins and initiation of labour.

In rabbits, there is an active corpus luteum, and in this case a late luteolysis reduces the progesterone concentration, initiating labour.

In humans, the corpus luteum disappears early in pregnancy thus its role in the initiation of labour is unlikely.

Another factor of importance is the foetal pituitary-adrenal axis. This probably has a supportive role in the timing of parturition. Research, has to date concentrated on the electrophysiological mechanisms of uterine contractility to explain the exact mechanism of parturition in humans. There are complex mechanisms of regulation of calcium activated potassium channels and voltage regulated calcium channels. The regulation of these channels could possibly be a key mechanism for increasing human myometrium contractility (Sanbourne BM *et al*, 2000).

The most common receptors in the human myometrial membrane are the oxytocin receptors. These are G-protein, heptameric couple receptors.

It is still unclear if the parturition is due to loss of uterine quiescence or due to activation of stimulatory pathways. A possible mechanism of initiation of labour is the 'switch off' of the main pathway of relaxation, to decrease cAMP and cGMP. The signalling for cAMP is very complex because there are many isoforms expressed in the human myometrium. Hence, research in this field has proved to be complicated since most of these receptors are found in smooth muscle tissue.

During labour, there is an increase in the production of prostaglandins from the residua. The main type of prostaglandins produced is PGF₂ α , produced by local macrophages.

This mechanism could be correlated with preterm labour because of the increase of macrophages and the related production of prostaglandins.

Human parturition must involve a number of complex mechanisms correlating the mother and the foetus. It is possible that some foetal organs and the placenta are involved in these complex mechanisms

Perhaps, the maturation of some of the foetal organs during the advances of the gestation age contributes to the signalling pathways of the initiation of labour (foetal adrenals, lungs etc).

The aim of the research in this field is to identify the mechanisms of initiation of human parturition and to understand the relation of these mechanisms to preterm labour. We have to identify similarities and possible differences.

Therefore it is very important to understand if the pathophysiology of preterm labour is different to that of full term labour and if there is an early onset of the same mechanisms during full term as in preterm labour.

2. Methods

In order to carry out this literature review, I used the library facilities of Imperial College, London and the library at Chelsea and Westminster Hospital, London. Pubmed, Medline and Google search engines were used to search for papers in this field of research using key words such as parturition, preterm labour, contraction, relaxation, myometrium, G-proteins, ion channels, and calcium homeostasis. All research papers published within ten years of today (1994-2005) were included in this thesis.

My objectives were to find primary papers as well as reviews. The reason being that study reviews expand on a particular topic and I could identify new key words include in my search.

My research concentrated on human myometrium; hence, studies on animal models were omitted. The search keywords also included names of authors, expert in this field, namely, Prof. A. López Bernal, R. Tribe, S. Wray, A. Gilman, H. Bourne and M. Simon. I focused on creating couples or triplets of key words and tried to identify papers in correlation with the mechanisms of relaxation and contraction in human myometrium.

After the initial selection of papers, the abstract gave me a clear indication as to whether the paper should be included in the study or not. Several other authors were identified within the references of core papers, and used in the search criteria.

When using particular websites, I carefully assessed the quality of the website and noted when it was last updated.

In total, I have reviewed 115 papers, which are included in this study (82 primary papers and 33 reviews).

At this point I would like to thank my supervisor, Professor Andrés López Bernal for his suggestions on how to organise and plan this thesis. His advice and time were valuable in writing this review.

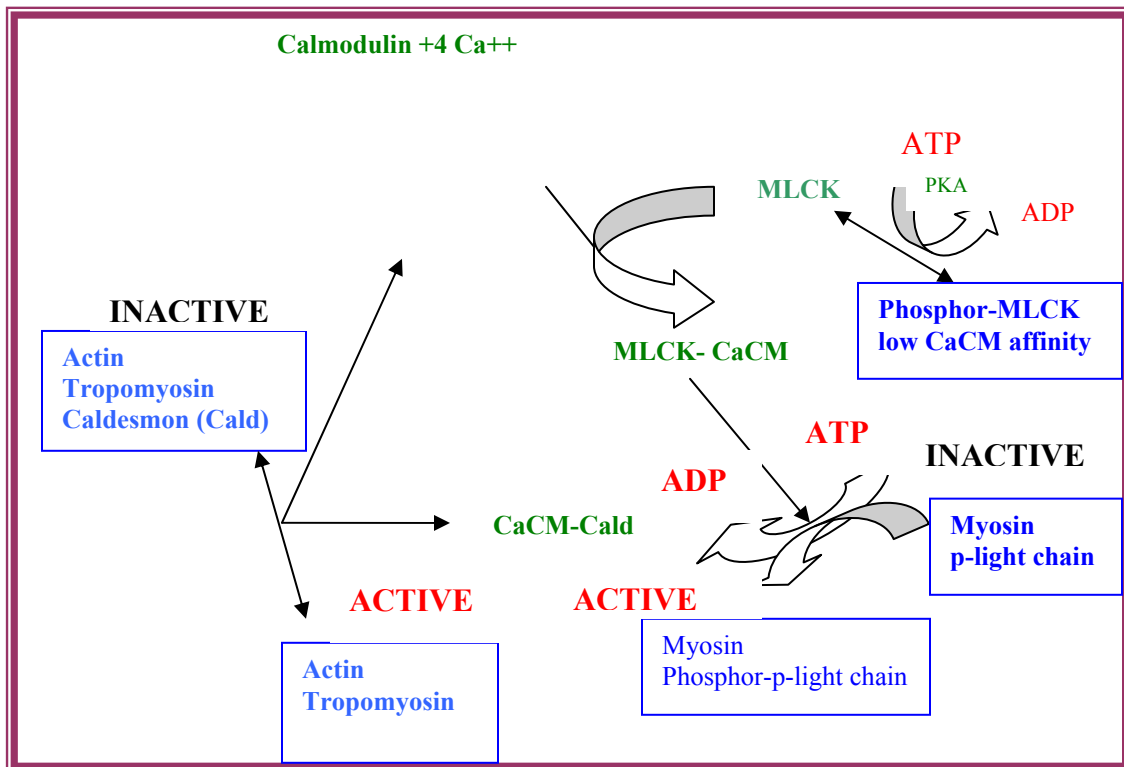
3. Results - New Developments

The understanding of the different mechanisms of contraction – relaxation in human myometrium is essential to improve the clinical management involving uterine contractility in humans.

Stimulating the myometrium with agonists, results in the elevation of the Ca^{2+} concentration in the cytoplasm. This increase also involves the IP3 pathway and the SR release of Ca^{2+} . An increase in Ca^{2+} concentration is connected with calmoduline in a complex which has an increased affinity for another important enzyme, MLCK and its activation. The activated MLCK phosphorylates the MLC (myosin light chain) and as a result we have the reaction through myosin and actin and contraction.

Another mechanism to increase contractility due to agonists is the sensitising of myometrium to activate Ca^{2+} . The most important molecule in this pathway is a small g-protein called RhoA and its effector molecule called ROK (Lee.H .Y et al, 2001).

Figure 5. Smooth muscle contraction mechanism



In resting myofilaments, RhoA is present diffusely in the cytoplasm (Yu and A López Bernal, 1998). In uterine muscle the major sites of RhoA /ROK mediated Ca^{2+} sensitization is closely related to the plasma membrane.

Specific proteins called caveolins, which are involved in the co-ordination of signalling, form regions called caveoli on the plasmalemma and are involved in interactions through external signals and the internal cascade.

The exact mechanism of their action is still not clear.

The use of isoform-specific antibodies for caveolins was found in human myometrium but had no correlation with the gestational status (Taggart *et al*, 2000).

3.1 Oxytocin and its Action on Myometrium

Oxytocin is a nonapeptide produced in the hypothalamus and released through the posterior hypophysis, known also as neurohypophysis. The pulses of this hormone are increased during labour. It is often clinically used for augmentation and induction of labour in humans but also for increasing uterine contractility in cases of uterine atony and post partum haemorrhage. Oxytocin has a contractile effect *in vitro*. The sensitivity for oxytocin is also increased in pregnancy.

The receptors in uterus are increased in pregnancy, mainly at term. The receptor is on the cell surface and is associated with the G-proteins. Oxytocin acts by coupling G - proteins, activating PLC and as a result producing $InsP_3$ and exit of Ca^{2+} from the SR to the cytoplasm.

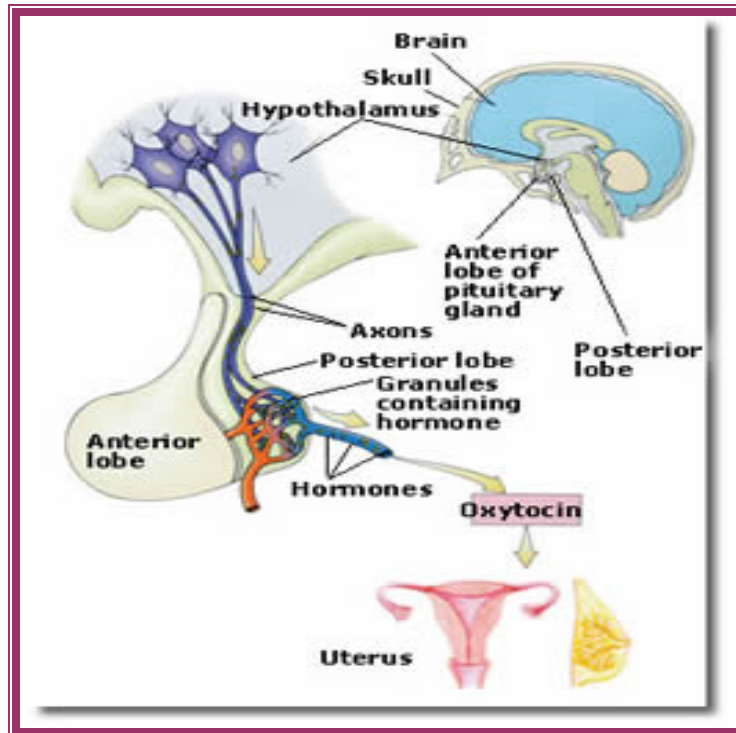
Experiments have shown that oxytocin reacts with a minimum of two different G-proteins, one which is resistant for the Pertussis Toxin called $G_{\alpha q}/G_{\alpha 11}$ and one which is part of the G_i family and is shown to be sensitive for the Pertussis Toxin.

There are also many different types of PLC- β and G-proteins involved in oxytocin action on myometrium (Phaneuf S *et al*, 1996).

The activation of the oxytocin receptor can also produce DAG. DAG can react with PKC which can phosphorylate different proteins and control uterine contractility. A phenomenon called desensitization of the oxytocin receptors has also been described.

This phenomenon suggests a loss in sensitivity of receptors exposed to oxytocin for prolonged periods of time, possible by receptors internalization. (Phaneuf S, *et al* 1994).

Figure 6. Production and role of Oxytocin



Source: <http://www.harunyahya.com>

3.2 Oxytocin Action and Receptor

We know that oxytocin is a nonapeptide (nine aminoacids) which can stimulate myometrial cells and also induce the prostaglandins production from amnion and endometrial cells. We know that the concentration of oxytocin on the maternal plasma is constant during gestation and increases during the late second stage of labour. It is also known that the presence of OTR in human myometrium commences by the 13th week of pregnancy and is influenced positively by estradiol and negatively by progesterone.

At term, the concentration of OTRs is higher in the upper uterus the body but decreased in the lower uterus and the cervix.

At term there is not only an increase of OTRs in each cell in the myometrium, but there is also an increase of the myometrial cell that are expressed on there surface of OTRs (Iveell R *et al*, 2001). There is also evidence of gene activation and differentiation of the myometrial cells during the final stages of gestation and parturition.

Oxytocin is used in labour induction and augmentation IV (intra venous). Oxytocin needs 40 minutes to gain maximum levels in the blood and its half life is 12 minutes. The main characteristic of oxytocin is that it does not bind to proteins in the plasma, hence, increase it's concentration in the extracellular space independently of plasma proteins.

As previously mentioned, Oxytocin is a very important drug in clinical practice for the induction and augmentation of labour. It is also used in cases of atonic uterus with the aim to increase contractility and decrease bleeding in cases of post partum haemorrhage. It is very important to know what happens in the myometrium from the point of view of desensitization of the OTRs in humans. A study using human myometrium measured the levels of OTRs and mRNA in patients having spontaneous and elective caesarean sections. The results of this study showed a decrease of mRNA and OTRs in cases of prolonged use of oxytocin infusion. This is evidence of *in vivo* desensitization (lost of hormonal responsiveness) of OTRs (Phaneuf S *et al*, 2000). Another study by Phaneuf S *et al*, 1998, showed a 50 fold decrease in OTR's and m RNA in samples obtained from patients that had been in labour for 12 hours, compared to samples obtained from

patients in labour for less time. The same study showed that OT treatment for up to six hours has a small effect in OT binding capacity. Prolonged use of OT dramatically decreased the binding capacity. It is evident that we still don't know many key points of the mechanisms of down regulation and desensitization of the OTRs in human myometrium. The role of G-protein coupled receptor kinases and the role of β -arrestins is still unclear, but obviously the clarification of these mechanisms will dramatically improve the clinical effectiveness of oxytocin use in labour.

It has been suggested that the interaction of oxytocin and PLC is mediated by G α_q and G α_{11} (Chun K *et al*, 1995). The same study suggested that the phospholypase, PLC β has a central role in this process.

It is known that oxytocin activates the myometrium in humans by interacting with three PLC β isoforms and two or more different types of G-proteins (Phaneus S *et al*, 1996).

A study by Monga *et al*, 1999 used immortalised myometrial cells and concluded that oxytocin increases the entry of Ca^{2+} capacitance, however not in pregnant human myometrium.

Experiments carried out by Helmer *et al*, 1998 in human myometrium, showed that the desensitization of the OTRs has no evident effect in the vasopressin's receptor regulation. In human myometrium, the desensitization of OTRs has no effect on the PLC activation cascade and no effect on the prostaglandins agonist effect on G-protein stimulation (Phauneuf *et al*, 1994).

Atosipan is an antagonist of the oxytocin receptor and is found to prevent oxytocin induced desensitization (Phanaeur S *et al*, 1995). It is currently used clinically in cases of preterm labour and uterine hypertony. This drug is much safer than most of the drugs commonly used, such as ritrodine, because of the reduced side effects to the patients.

OTR coupling with G_i results in the inhibition of cell proliferation and has the opposite effect in cases of G_q coupling. Atosipan has an agonistic effect on the coupling of OTR with G_i and an antagonistic effect on the coupling of OTR and G_q in human prostate carcinoma (Reversi A *et al*, 2005).

It was found that term human myometrium obtained during caesarean sections, expressed higher levels of OTR mRNA than in preterm myometrium (Watches D C, *et al*, 1999).

In the same study authors concluded that there was no increase of OTR mRNA in cases of onset of labour. Moreover, the decidual OTR was significantly lower than the myometrial and did not increase during gestation. OTR was also found to be absent in the placenta but present in corion. Furthermore, authors concluded that the increase of the OTRs during late stages of pregnancy is not correlated with the mechanism of timing of the initiation of labour in humans.

Another study on isolated human pregnant myometrial, found that 10 Nm of oxytocin can increase the sensitivity of Ca^{2+} by the proteins involved in the contractile process (McKillen K *et al*, 1999). This increase in sensitivity starts after the beginning of a

contraction (falling phase of phasic contractions). The possible mechanism is by inhibition of the activity of the MLCP (myosin light chain phosphatase).

The oxytocin receptor is a heptameric superficial receptor with a -COOH terminal in the intracellular space and a -N extracellular terminal. The OTR is a protein formed by 388 amino acids. The presence of this receptor is also evident in different tissues and organs, namely the pancreas, breast, liver and CNS.

As we already know, oxytocin alters the concentration of Ca^{2+} by binding to the heptameric (seven parts) G-protein coupled receptor which is part of the family of vasopressin receptors.

The activation of this receptor stimulates $G\alpha q/I$ proteins resulting in an increase of PI and Ca^{2+} concentrations (Sanborn et al, 1998). The increase of Ca^{2+} concentration is fast as is the decrease of its concentration due in the pumping mechanism in the inner part of SR and to the extracellular space.

Some data suggest that the N-terminal region of the fourth domain (intracellular), is responsible for the coupling (Hoare *et al*, 1999).

Other authors suggest that the third domain is involved in coupling with $G\alpha q$ (Quian et al, 1998). We know that pertussis toxin can be involved in decreasing the activity of oxytocin and can also be indirectly involved in the cAMP pathway.

Oxytocin can also activate MAPK interacting with the third intracellular domain (Hoare *et al*, 1999).

Experiments on human myometrial cells show that oxytocin can interact with Mitogen-activated protein kinase (MAPK) and COX-2 gene expression. As a result of this interaction, the production of prostaglandins in the myometrium is increased (Miklos M *et al*, 1999).

In cases of pregnant myometrial cells, oxytocin can increase the concentration of Ca^{2+} , dependent on the mechanism of extracellular Ca^{2+} partially present (Monga *et al*. 1999).

This is an evidence that both extracellular and SR Ca^{2+} have an important role in the increase in concentration of the plasmalemma Ca^{2+} and the increase of contractility.

The mechanism of regulation of the contribution of the two different sources of Ca^{2+} to the mechanism of contraction remains unknown.

Oxytocin can increase the Ca^{2+} entry to the cell by different mechanisms. In non-pregnant myometrium, the role of L-type calcium channels is very important, but in pregnant human myometrium the concentration of these channels is decreased resulting in a lack of effect in specific blockers (Sanborn M B, 2001).

A very important mechanism is the capacitate Ca^{2+} entry.

There is partial dependence on the extracellular Ca^{2+} from the oxytocin stimulated increase of Ca^{2+} . Also inhibitors of PLC such as NCDC affect the abolition of increased oxytocin induced Ca^{2+} concentrations (Sanborn MB, 2001).

Trp proteins have a role to promote the increase of Ca^{2+} by improving its entry. (Putney *et al*, 1999). In humans, there are more than four channels for capacitative Ca^{2+} entry.

The Trp proteins are most likely regulated by different molecules such as PKC, DG, arachidonic acid, etc (Hoffman *et al*, 1999).

IP3 receptor was found to stimulate Ca^{2+} entry (Yao *et al*, 1999).

Oscillations of Ca^{2+} concentration in the cell is a natural phenomenon. It was found that using blockers for L-type channels, such as caffeine, in intracellular human myometrial cells and human myometrial cells in culture, stores depletion and the frequency of the oscillations start decreasing (Burghardt *et al*, 1999).

In the absence of extracellular Ca^{2+} or in the blockage of PLC, a decrease of the frequency of the oscillations of the Ca^{2+} concentration is observed. The oscillations increase in frequency if ryanodyne is used (Burghardt *et al*, 1999).

We also know that PKC can reduce or even block PLC action (Ali *et al*, 1997).

SERCA inhibitors can also block the oscillation process (Fu *et al*, 2000). Is it a fact that during pregnancy the myometrium is much more inactive than active in terms of contractility?

During labour and parturition everything changes and the mechanisms of contractility are switched on.

There is an up-regulation of contractile mechanisms and a down regulation of the mechanisms which increase relaxation (López Bernal *et al*, 1995). There is an observation that specific G-proteins which lead to the formation of AC and relaxation induction are decreased during labour and parturition (Europe. F *et al*, 1996). It is known

that some GPCRs are able to pass through a process of desensitisation. There are two known mechanisms of desensitisation, homologous and heterogeneous desensitisation.

In homologous desensitisation the effect is on the same GPCRs, whereas in heterogeneous desensitisation the effect is also amplified to responses from other heterogenous GPCRs. The exposure to beta- mimetic drugs for long periods of time can result in the internalisation of the receptors (Engelhardt *et al*, 1997).

It is possible that the phosphorylation of GPCRs is involved in the initiation of desensitisation (Pitcher *et al*, 1998). Some molecules called β -arrestins are also involved in the internalisation of different GPCRs, but we are unsure of their role in the process of internalisation of the OTR. Endocytosis is also a possible mechanism of desensitisation (Cerea *et al*, 2000).

Another group of proteins involved in the desensitisation mechanisms are the GRK or G-protein-coupled receptor kinase.

It was found that in human myometrium there are different subunits of these molecules namely, type 2, 5, 6 and 4 γ . There is evidence that GRK2 is present in full term pregnant myometrium and absent in pregnancy. There is also evidence to suggest that two subtypes of GRKs, specifically type 2 and 6 have a possible key role in the regulation of myometrial contractility at term. Their role in preterm labour and in other conditions which involve contractility is unknown (C B A P Brenninkmeijer *et al*, 1999).

This mechanism is unclear and extensive research needs to be carried out to determine the role of these mechanisms in relation to a better understanding of the exact action of tocolytic drugs clinically used on the myometrium. This knowledge can change the way these drugs are currently used clinically.

Schmit *et al*, 2001, demonstrated the implication of IL-1 β and IL-6 on the human oxytocin receptor transcription. This is a role of inhibition of the receptor transcription (ORT).

The role of oxytocin on human physiology is extremely complicated. The reason being that this hormone is involved in many mechanisms related to reproductive physiology. The involvement of oxytocin in lactation, sexual and maternal behaviour, anorexia, male orgasm and memory are probably a few of the involvements of this nonapeptide (nine

aminoacids) in the human physiology. In addition oxytocin is possibly involved in the interaction of different mechanisms

There is evidence that OTRs are present in human vascular endothelial cells (Thibonnier *M et al*, 1999). It was found that these receptors are identical to the uterine receptors and their existence is correlated with vasodilatation of Ca^{2+} dependent mechanisms involving nitric oxide, once again illustrating oxytocin's multi-directionality mechanisms of action. Oxytocin probably has a cross-talk relationship with many different molecules. A study by Grammatopoulos *et al*, 1999, proposed that in term human myometrium there is a remodulation of the CRH receptor resulting in the inhibition of its activity. As a result of this remodelling there was a reduction of cAMP and an increase in contractility.

A study conducted by Phaneur, *et al*, 1994 suggested that oestradiol has a positive effect on the response of the myometrium to oxytocin. In this study human myometrial cells were used and the possible mechanism of oestradiol action and its improvement on the post receptor level activation of the PLC (phospholypase C) was investigated. Antagonistic action on this mechanism is support by tamoxifen, a known antiestrogen. Tamoxifen can inhibit both pertusis sensitive and non sensitive PLC activation, possibly by double action in G_i and G_q levels. These mechanisms are still not clearly understood and need further research for clarification.

Many of these experiments were carried out on myometrial cell cultures. Some authors tried to produce myometrial cells lines *in vitro* as they would be more ideal for the research on the responses of the myometrium to oxytocin stimulation (Monga *M et al*, 1996). These experiments were carried out in both the presence and absence of extracellular Ca^{2+} .

It has been established that oxytocin uses different mechanisms to increase the Ca^{2+} concentration. One of the main mechanisms is due to the G-protein receptor activation of the PLC and the production of IP₃ which can increase the release of Ca^{2+} from the sarcoplasmatic reticulum.

Research has shown that the mechanism of PLC activation could be inhibited by the action of cAMP. This mechanism is based on the reaction of protein kinase A (Sanbourn BM, *et al*, 1998).

A study conducted by Burghardt *et al*, 1999 used immortalised myometrial cells to measure the Ca^{2+} oscillations after stimulation with oxytocin. Using Fast Fourier Transform (FTT), they found that the IP3 pathway is responsible for about 60% of the frequencies and that the rest of the frequencies were due to the action of the Ca^{2+} entering from the extracellular space. The former was probably due to the plasma membrane channel activation. In frequencies higher than 6 MHz, the role of the ryanodine-sensitive Ca^{2+} was evident. The frequencies were also noted to increase with the use of prostaglandins type E1 and E2. These experiments are extremely important to clarify the mechanisms that regulate Ca^{2+} oscillations.

A study conducted on non-pregnant myometrium by Domali *et al*, 2001, performed on human myometrial cells of pre and post menopausal women showed that KCL and endothelin -1 can influence the uterine spontaneous contractility in different ways by using different mechanisms. Ovarian steroids have an important role in regulating these mechanisms. However, these studies were conducted *in vitro* and the correlation of these findings with the myometrial physiology *in vivo* still remains unknown.

Oxytocin and lysophosphatidic acid was found to induce stress fiber formation in human myometrium. This pathway involves the action of Rho-kinase (Goatee W *et al*, 2001). The use of oxytocin antagonist on the formation of stress fibres was eliminated. This mechanism most likely involved Rho-kinase, a mechanism of maintenance for uterine contractility for longer periods of time during labour and parturition and may also have a key role on the increase of the sensitivity for oxytocin during in humans at the end of the gestation.

3.3 Calcium and its Homeostasis

A trial to explain and connect the single organ function with the cellular physiology was carried out by Young R *et al*, 2000. It has been established that there is a propagation of intracellular action potentials that result in Ca^{2+} waves. One of the reasons of excitability is the electromechanical stimulation of the myometrial cell. This electrophysiologic effect on the other hand can not clearly explain the physiology of the contractility observed in human myometrium. Hence, there must be another mechanism that acts on the complicated intercellular communication physiology. The theory of the Ca^{2+} waves was used as a model to explain the gap in the electrophysiology of human uterine contractility.

The reason for this is their ability of long signal propagation, increasing Ca^{2+} in the cytoplasm and decreasing the speed of propagation.

We know that the increase of intracellular Ca^{2+} is directly correlated with uterine contractility and that the return of the Ca^{2+} concentration in the previous levels is correlated with the relaxation of the myometrium. The hypothesis of calcium waves is designed to support the mechanisms of action on myometrial tissue already undergoing contractility and that can not be connected with the initiation of the contractile phenomenon.

Myometrial cells communicate with gap junctions which can support the electrical activity and communication between adjacent myometrial cells.

The concentration of gap junctions increases during pregnancy in humans, suggesting that this increase is very important for labour and parturition.

The ion channels seem to be actively involved in the contractility of the uterine smooth muscle (Garfield RE *et al*, 1994). Moreover, the single unit hypothesis stated in main text books of physiology is not possible to explain the experimental observations of different authors. The way to explain the single unit theory is to make the hypothesis that the action potential is moving slowly in the human myometrial cells.

Intracellular waves described by different authors in various tissues, such as cardiac myocytes and vascular smooth muscle, etc. The mechanism of propagation seems to need a 'physical' contact of adjacent cells to use certain messenger molecules. Groups of

myocytes are organised into two different bundles: cylindrical and sheet like bundles. They are organised in fascicule and the bundles run in parallel to form the axis of a fascicule (Young RC, *et al*, 1999).

We still don't know the expansion of this model in the 'micro-geography' of the human uterus but there are probably some differences in this model in different areas of the pregnant human uterus. Further research is required to clarify the microanatomy of the human uterine smooth muscle.

Some key points on the Ca^{2+} wave theory include:

- 1) The myocyte can remain in the contraction status as long as there is elevated Ca^{2+} concentration. The concentration of Ca^{2+} is the result of the single cell metabolism status.
- 2) All the myometrial cells are synchronised to start contracting by the propagation of action potentials and the subsequent initiation of Ca^{2+} waves.
- 3) Gap junctions are extremely important for the process of propagation of the action potentials.
- 4) There are no cross boundaries between Ca^{2+} waves in bundles (Young RC *et al*, 1999).

In cases where the Ca^{2+} waves- action potentials theory is in control of the myometrial activation during labour, we have to clarify that there are probably two mechanisms.

The first mechanism is the action potential propagations which control the frequency of the contraction and the second mechanism is the Ca^{2+} waves that control the strength of the contraction.

Some experiments showed that when Ca^{2+} was removed from the solution of the cultured myometrial cells the Ca^{2+} wave speed was unaffected (Young ,2002). This is probably because of the use of intracellular Ca^{2+} sources released from the sarcoplasmic reticulum (SR).

These observations are clinically very important. The reason being that we can start thinking about the frequency and the contractile strength of the myometrium, as factors

correlated with different mechanisms of control and the possibility of using different tocolytic drugs in correlation with the clinical picture must be considered.

We know that the contractility of the uterine muscle is strictly correlated with the increase of the intracellular Ca^{2+} concentration. On the other hand the return of Ca^{2+} concentration back to the rest concentration guides the whole mechanism to relaxation. A very important role of the increase of the Ca^{2+} concentration in human myometrium is the ability to capacitate Ca^{2+} entry. It was found that certain proteins from the family of Trp isoforms have an important role in the entry of capacitated Ca^{2+} (Ming Y *et al*, 2002). It was suggested that these proteins form Ca^{2+} channels in human myometrium. There is also a negative feedback between some Trp proteins which is blocked by calmoduline and/or Ca^{2+} .

In human myometrial cultured cells, there is evidence of TrpC proteins (transient receptor potential channel) which may have an important role in the pregnant uterus at term regulating SOCE (store- operated calcium entry).

Data exists to support the activity of endogenous hTrpC in human myometrium (Shlykov S *et al*, 2003).

There is evidence that diacylglycerol *in vitro* can activate (myometrial cell lines) cation channels. This activity is found to be independent of Ca^{2+} - ATPase and PKC, and results in the increase of the Ca^{2+} concentration in the cytoplasm (Sergiy G *et al*, 2004).

It is evident that Ca^{2+} is one of the most important factors in the mechanisms regulating human parturition.

Extensive research is being carried out by various scientific groups around the world to try find the correlation of this cation with the possible mechanisms of contractility and relaxation in humans.

Most of these studies have been carried out on human myometrial cells *in vitro*, obtained by caesarean sections or hysterectomies. A study using myometrial cell cultures found that IL-1 β can have a key role on the modulation of the mobilization of Ca^{2+} (Tribe R *et al*, 2003). Cytokines probably have a very important role in calcium homeostasis and the

increase in Ca^{2+} concentration before and during labour. This results in increased uterine contractility. A possible mechanism that connects the IL-1 β and Ca^{2+} homeostasis is the effect on the SERCA (sarcoplasmic reticulum ATPases).

Studies conducted by Tribe *et al*, 2000 have shown that SERCA isoforms are extremely important in labour in regulating contractility.

SERCAs are very important in the sequestration of the Ca^{2+} in the cytoplasm. Thus, it is a very important modulator in the mechanisms of contractility. This study also demonstrated the existence of SERCA 2 in human myometrium and its role in the physiology of tension.

The importance of this study was that it was the first study to demonstrate there are some changes in Ca^{2+} homeostasis during labour in humans. Inhibition of SERCA in myometrium not in labour had no effect on the spontaneous contraction frequency. This was evidence that in cases of non- labour human myometrium at term, the Ca^{2+} liberated from the SR can not influence the phasic activity. The explanation on the apparent different response to SERCA in cases of labouring and non- labouring human myometrium is that both these mechanisms or only one of them are possibly up - regulated during labour.

Progesterone can also directly modify the receptor (OX, ET) mediated rise in Ca^{2+} concentration during pregnancy resulting in relaxation (Fomin V *et al*, 1999).

3.4 Mechanical Stretch

During labour there is a production of inflammatory molecules and an infiltration of the myometrium and the membranes. We know that there is an increase in the production of interleukin-8 (IL-8) at the level of membranes and myometrium. Research has been carried out on the stretching of the human myometrial smooth cells *in vitro* (Remier *et al*, 1998).

A study by Loudon *et al*, 2004 found that stretching of the human myometrium increases the IL-8 production in the human myometrium.

In case of non- pregnant myometrium there was an increase in IL-8 synthesis. This increase was higher in pregnant myometrium during labour and lower in case of pregnant myometrium not in labour.

These results are probably due to the mechanism of the suppression of IL-8 expression by progesterone (Loudon *et al*, 2003). We know that progesterone is reduced in humans during labour, which explains the previous findings.

It is evident that there is a suppression of the mechanism of production of IL-8 during pregnancy as well as it's up regulation during labour.

It is also evident that stretching is caused as a result of increased production of cytokines and probably has a very important role in the initiation of labour. This data is of vital importance in understanding the possible mechanisms of regulation of the onset of labour and the implications on this from a very important mechanical factor, the myometrial stretch.

Further research in this field tried to evaluate the correlation of human myometrial stretching to COX 2 and OTRs. A recent study by (Sooranna *et al*, 2004), tried to measure the expression of COX -2 in human myometrium obtained from non- pregnant women, pregnant women and women in labour. The results showed that the concentration of COX-2 mRNA were comparable in non-pregnant women and women in labour. The concentration of the COX-2 mRNA in pregnant women not in labour was much lower than the previous two groups. In the same study, they studied myometrial cells not in labour after a 6 hour stretching period. They measured the levels of COX-2, PGE2 and PGF2 α after stretching. There was an increase in the concentration of COX-2 and PGE2 but a decrease in the concentration of PGF2 α . Studies carried out using EMSA

(electrophoretic mobility shift assays) have shown an increase of the activator protein-1(AP-1). These results can be explained by the increasing concentration of COX-2 as a result of the stretching of the myometrial cells with the possible use of AP-1 pathway.

The same study group tried to measure OTR expression in the previous groups. They found that the concentration of OTR was higher in the group of pregnant women in labour, lower in the non pregnant women and much lower in the group of pregnant women not in labour.

After stretching, the myometrial cells of the pregnant women not in labour were up-regulated and no effect was observed in the last two groups. These results concluded that stretching prior to labour in pregnant myometrium can induce OCR up- regulation maybe by a mechanism involved in over expression of C/EBP and not AP-1(Terzidou B, *et al* 2004).

It is also been established that in cases of cyclic mechanical stretching in human myometrial cells of pregnant women in culture, there is an increase of the prostacyclin production (PGI₂) and concentration in the maternal plasma (Korita *et al*, 2005). The mechanism of the prostacyclin increase is due the increase of the COX-1 expression and the parallel expression of PGIS protein. This increase mainly occurs just before the initiation of labour in humans. The stretching up- regulates the PGIS production which results in an increased concentration of PGI₂ (Challis J *et al*, 2002).

3.5 Relaxation Mechanisms

It is known that the calcitonin generated peptide, PGE₂ and beta-adrenoceptor agonists such as ritodrine have a relaxant effect on the human uterine myometrium. The mechanism of this effect is by increasing the intracellular c-AMP. We know that AC has many different isoforms. AC isoforms were identified in pregnant and non-pregnant (Price S *et al*, 2001). The findings of this study suggested the presence of eight different types of AC in pregnant and non- pregnant myometrium. These findings demonstrate how complex the mechanisms of cAMP regulation are and how many different pathways exist. In this study, a list of myorelaxants was proposed, such as CRH (corticotrophin releasing hormone).

This hormone is possibly involved in the process of regulating labour in humans (Grammatopoulos D *et al*, 1999). Another molecule, urinary trypsin inhibitor (UTI), probably involved in the relaxation of the human myometrium was also mentioned. UTI is found in the amniotic fluid during gestation. The binding site of UTI is hyaluronidase sensitive and is found on the myometrial cells surface. However, the physiology of its action and the regulation during different gestational ages is unknown. The correlation of UTI with preterm labour is also unknown (Kobayashi H *et al*, 2000).

The regulation of cAMP requires PKA. This protein has two catalytic and two regulatory subunits. A recent study on human myometrial cells identified an increase of the regulatory protein, R α , at the start of the second trimester. During labour these proteins are decreased. This is a mechanism of regulation of the uterine activity until the moment of initiation of labour (McDougall M *et al*, 2003).

We know that the pathway of interaction of β -adrenoreceptor and channels type BK α is very important in the process of uterine quiescence. Some studies suggest both components described above decrease in pregnant myometrium. This is probably one of the mechanisms of quiescence regulation during pregnancy (Chanrachakul B *et al*, 2004). Another very important group of molecules which have a role in the mechanisms of human myometrial relaxation are the polyamines. These are components found in all cells and assist in the differentiation and growth processes. It was suggested that polyamine spermine is a strong Ca²⁺ antagonist and increases the relaxation effect during pregnancy (Houlihan *et al*, 2002). This relaxant effect was also found in non- pregnant myometrium. Further studies need to be carried out to identify the role of its expression at different gestational ages.

The mechanism of oxidative phosphorylation is very important in cellular physiology. This mechanism is blocked partially in cases of hypoxia during labour and delivery. The effect of this is the reduction of activity of oxytocin which results in uterine relaxation (Bishty M *et al*, 2003).

Research has been carried out on the identification of the role of different genes involved in the modulation of different pathways in the human myometrial relaxation. CREB,

(cAMP-response element binding) and modulators like CREM are some proteins involved in this process (Bailey J *et al*, 2005).

We know that potassium channels are extremely important in the regulatory processes of the cellular membrane potentials.

In normal conditions Na^+ , Ca^{2+} and Cl^- ions are increased in concentration in the extracellular space and K^+ is at a higher concentration inside the cytoplasm. The action of different ion channels has a direct or indirect effect on the Ca^{2+} concentration. The regulation of these channels is a way to partially control the mechanisms of contractility and relaxation (Sanborn BM *et al*, 2000).

A study was designed to examine the presence of specific proteins on the potassium channels (ATPase sensitive) in three different situations: non-pregnant myometrium, pregnant at term and term myometrium in labour. The results of this study show that the main potassium channels subtypes of human myometrium are Kir6.1 and SUR2B. The concentration of these subtypes was higher in non pregnant than in pregnant ladies. There is evidence that there is a possible decrease of the concentration of these channels during pregnancy which could be beneficial (Curley M *et al*, 2002).

It was suggested that potassium channel openers, such as cromakalim, could be used in case of dysmenorrhoea due to the effect on both myometrium and uterine vessels (Kostrzewska *et al*, 1996).

Another very important kind of potassium channel is the calcium-sensitive channel. This channels increases during pregnancy. These channels are very important targets for the development of new drugs acting on the mechanism of closing or opening channels (Khan N. R *et al*, 1998).

Evidence from studies on myometrial cells *in vitro* shows that 17β -estradiol in pregnancy has an inhibitory effect on potassium channels and that progesterone has the completely opposite effect on myometrial cells (Knock *et al*, 2001).

We know that nitric oxide has a relaxation effect on the myometrium. This effect has also been proven in other tissues such as endothelium tissue in the respiratory system. The role of NO (nitric oxide) in parturition has still not been established but NO donors have an effect on myometrial relaxation.

In humans NO is produced in the female genital tract during pregnancy (Buxton *et al*, 2000). The main action of NO is the stimulation of cGMP (guanosine 3-5- cyclic monophosphate) by guanylate cyclase resulting in the decrease of Ca²⁺ concentration. This is probably an important mechanism of relaxation during pregnancy and a possible alteration of this mechanism could be considered as a possible mechanism of initiation of labour (Norman J, 1996; Buhimschi I *et al*, 1995)). This mechanism in correlation with cGMP has not been proven in cases of non- pregnant women (Bradley K *et al*, 1998; Norman J *et al* 1997; Modzelewska B *et al*, 1998; Hoffman P *et al*, 2003).

It has also been proven that apamin-sensitive K⁺ channels exist in human myometrium during pregnancy. The possible role of these channels is the myometrial modulation in cases where NO donors are used (Modzelewska B *et al*, 2003).

Nitric oxide synthetase (iNOS) has been identified at increased concentrations in human myometrial cells in pregnant preterm women not in labour. In case of term women the concentration decreased by 75%. There was no evidence of iNOS in non-pregnant myometrium. Evidence exists for the role of NO on the regulation of relaxation/contractility mechanism in humans (Bansal R *et al*, 1997).

Conversely, authors tried to define the result of endogenous NO in the contractility of preterm and preterm myometrium. They used human myometrial cells treated with L-arginine, an inhibitor of NO. This had no effect on the contractility of the myometrium (Jones, GD *et al*, 1997; Ekerhovd E *et al*, 1999).

3.6 Preterm Labour

Preterm labour is defined when delivery takes place at a gestational age less than 37 weeks. In the United Kingdom, preterm deliveries account for (6-7) % of all deliveries. In developed countries preterm labour accounts for 70% of neonatal deaths and 50% of neurological disabilities in children. There is an increased possibility of children born handicapped, if delivered at less than 28 weeks of gestation. The aetiology is multifactorial and should also consider uterine factors, infections, socio-economical factors and reproductive history:

Maternal history

- Age < 15, >35
- Smoking
- Race (black > White)
- Socioeconomic level
- Weight <50Kgr
- Drug abuse
- Pyrexia - infection
- Trauma-pelvic/abdominal
- Uterine abnormalities

Foetal and placental history

- Multiple pregnancy
- Foetal abnormalities
- PV bleeding
- Chorioamnionitis
- Polyhydramnios

Previous history

- Preterm labour (35% recurrence)
- PV bleeding
- Cone biopsy

As in full term labour, preterm labour also experiences cervical effacement and dilatation, increasing uterine contractility. Pro-inflammatory cytokines and prostaglandins are also involved. Preterm labour can be divided into two categories: ‘late’ after 32 weeks and ‘early’ before 32 and after 24 weeks of gestation.

Late preterm labour is often caused by polyhydramnios and multiple pregnancies, as a result of uterine stretching and increase of CRH in earlier gestational age due to increase in placental size in multiple pregnancies. Early preterm labours may be caused by cervical insufficiency or infection. In cases of infection, a cascade of cytotoxins may have an important role in prostaglandin production. In case of cervical insufficiency, it is probable that the stretching of the cervical smooth muscle can activate some genes specific to the initiation of labour (Aarthi R *et al*, 2004). Approximately 30% of preterm births are caused by an infection, 50% are idiopathic. Some studies suggest that prostaglandin production as a result of infection is the result of preterm labour and not the cause. The fact is that in cases of preterm labour, there is an increase in the concentration of cytokines in amniotic fluid. It is possible that bacterial infections start from the vagina and ascend into the uterine cavity resulting in chorioamnionitis.

Phospholipases produced from bacteria aid the local production of arachidonic acid and prostaglandins as a final product.

The local production from the bacteria of endotoxins, such as PS can increase prostaglandin production and the production of TKFs, IL, and other cytokines. This vicious cycle can continue with the increase of macrophages and direct production of more prostaglandins (John R.G *et al*, 2000). The most common infections are ureoplasma, bacterial vaginosis and trichomonas vaginalis.

Some clinicians suggest measuring the fibronectin in the cervical and vaginal secretions. This is a protein which is responsible for the adherence of the membranes.

Some authors like McDonald believe that the cytokine concentration is the result of the preterm labour and not the cause of it. They believe that there is an invasion of bacteria after the cervical canal dilates occurring as a result of the exposure of the amniotic sac to the vaginal bacterial flora. Contradictory evidence exists regarding this hypothesis.

In 50% of the suspected cases of preterm labour the contractions will cease after some time without the use of drugs. Neonatal outcome is improved with gestational age. Alveolar capillaries form between 24-26 weeks of gestation; hence, neonates born after 26 weeks have a better chance of survival.

Drugs are mainly used during preterm labour to allow the neonate a prolonged intrauterine life, with the hope that corticosteroids can assist in the maturation of the lungs. Intramuscular glucocorticoids are administered in two doses at a twelve hour interval. The maximum of action is after 48 hours of the administration of the second dose.

The use of antibiotics has not shown to improve the outcome of preterm labour and is only recommended after prolonged rupture of the membranes. There are different drugs we can use in preterm labour but many of them have serious side effects and the clinicians must be very careful in monitoring the patients during their treatment.

There is no evidence that suggests these drugs can reduce the rates of premature delivery, but as mentioned previously, the main reason is to allow some more time for the glucocorticoids to develop foetal lung maturation.

The different categories of drugs include:

Beta-Adrenergic Agonists

This category consists of salbutamol, ritodrine and terbutaline. These drugs have a direct effect on beta - adrenoreceptors and are characterised by many serious side effects such as, hypotension, cardiac arrhythmia, tachycardia, pulmonary oedema, hypocalcaemia, hypoglycaemia etc. The receptors for these drugs are not specific to the myometrium, but may be found in smooth muscle in other parts of the human body.

Calcium Channel Blockers

This category consists of nifedipine. This drug is safer than the beta- adrenergic antagonists and its mechanism of action is the inhibition of the influx of Ca^{2+} from the Ca^{2+} channels. Nifedipine can have some side effects such as hypotension and face flushes and it can be use by oral administration.

Glycerol Nitrate

Glycerol Nitrate is not commonly used in the U.K. It is a NO donor and is used in the form of patches.

Prostaglandin Synthetase Inhibitors

Prostaglandin synthetase inhibitors include indomethacin. This drug exhibits an inhibitory effect in prostaglandin production by blocking the action of specific enzymes, COX-1 and COX-2, which have an important role in prostaglandin biosynthesis.

As we know prostaglandins are very important for the initiation of labour and the uterine contractility. The side effects of indomethacin can affect the newborn by producing necrotising enderocolitis, early closure of the ductus arteriosus and intracranial haemorrhage (Macones E.T *et al*, 2001).

Oxytocin Antagonists

Oxytocin antagonists are the latest category of drugs involved in preterm labour. The most common oxytocin antagonist is Atosiban, which has the same efficacy as ritodrine but is safer and has fewer side effects. This drug is administered intravenously and is still very expensive.

There are some contraindications in using tocolytic drugs. These include:

- Maternal heart disease
- Antepartum haemorrhage
- Abnormalities on CTG
- Chorioamnionitis
- Foetal death
- Lethal malformation.

Currently, the main problems experienced with the use of these drugs include lack of myometrium selectivity, side effects and the fact that the effects of the drugs are not long lasting. The main aim for the research is to understand the mechanisms of initiation of preterm labour and try to design new drugs that are more specific, longer lasting, have no side effects and are more affordable.

On the other hand the cost of treatment of preterm neonates is extremely high in the western world, despite the high rate of mortality and morbidity. The neonatal intensive care units are very expensive and not available to every body even in Western Europe and in the U.S. Perinatal mortality and morbidity is significantly increased in the developing world where these facilities are not available.

The problem is not only the very sophisticated machinery but also the lack of specialised personnel (nurse, doctors) to run these units. The follow-up of these children is still debateable as the long term result needs to be assessed. The design of new drugs which are safer and more specific to the myometrium, based on the understanding of the

mechanisms of preterm labour, is the solution in resolving all these ethical and financial problems.

There is increased evidence that correlates preterm labour with the pre-inflammatory cytokines and prostaglandins. We also know that there is a complicated mechanism to correlate the equilibrium between relaxation and contractility. We are still unsure if the onset of labour at term or at pre-term is the loss of this equilibrium or a result of another reason involving other events. We also don't know if parturition is the result of increased myometrium sensitivity on the existing stimulation (TabyaRaja R. L *et al*, 2000).

A hypothesis exists, suggesting that a possible reason for idiopathic preterm labour is the decreased concentration of the endogenous inhibitor of Ca^{2+} channels in fetal corion and amnion (Caroll E *et al*, 2001).

Another study studied the permeability of the fetal membranes to Ca^{2+} and Mg^{2+} ions. This study was carried out on preterm labour human membranes. This study concluded that there was reduced permeability for both cations, suggesting a possible initiation of myometrial activation (Lemancewicz A *et al*, 2000).

In humans, progesterone is not decreased in term as in other animal models. There is the possibility that this rise in progesterone levels affects the topographic differences in uterine activity, resulting in the relaxation of the lower segment of the human uterus and the increased activity of the fundal segment (Challis J *et al*, 2000). Progesterone was found to have a double effect on the myometrium at term. This effect could be inhibitory or even stimulatory on the uterine smooth muscle (Fu X *et al*, 1996).

There is also a theory supporting the fact that in humans, cortisol is produced by the foetus, which upregulates prostaglandin synthesis. In addition, cortisol can increase the placental synthesis of CRH (corticotrophin releasing hormone). This hormone also affects uterine contractility (Challis J *et al*, 1999; Sehringer B *et al*, 2004).

CRH also has an effect on the production of PGD from placental and decidual tissues and is also involved in placental vessel dilatation. Another important role of CRH is its involvement in the regulation of organ maturation (Hillhouse E *et al*, 2002).

The regulation of uterine contractility is a complex mechanism. There is some evidence that TGF β (transforming growth factor) has an important role in this regulation (Hatthachote P *et al*, 1999).

It is suggested that cGMP dependent protein kinase does not improve uterine relaxation and that there is a redistribution of calponin and caldesmon proteins in the thin filaments during pregnancy. This is probably due to the changes in the cytoskeleton and the myofilaments tone to adapt to pregnancy (Cornwell T *et al*, 2001).

It was also proposed that a down regulation of Gas decreases the cAMP effect on the myometrium and is possibly involved with the initiation of labour mechanisms (Europe N *et al*, 1994).

4. Discussion

The mechanisms of uterine contractility and relaxation are influenced by many factors. It is important to understand the complexity of these mechanisms. To date, only a small part of these complex interactions has been revealed. Further research is still required to completely clarify the different pathways and the possible interactions of these mechanisms, with the aid to combat the severe consequences of preterm labour and delivery. Preterm labour is associated with different types and grades of neonatal handicap and involves immense emotional, long term circumstances for the families involved as well as financial constraints.

Preterm neonates require a high level of care, involving very sophisticate machinery and highly specialised health professionals to support the needs of the newborns.

It is evident that the need of clarification of the pathways of human myometrial contractility and relaxation is also clinically indicated and has a huge impact on the clinical management of preterm labour.

We have to understand the mechanisms because this is the only way to realise which level of these complex pathways we can try and block or even promote.

This understanding is the key to developing new drugs, which are more specific and efficient. The main concept of intervention is to improve selective myometrial relaxation and/or block the myometrial contractility.

There are many different pathways in humans to promote contractility and relaxation. There are possible mechanisms of interaction between these pathways.

Once the role of these pathways is understood, the next step is to try design new drugs that can interact with different enzymes and receptors at the myofilament level.

Research has been extensively carried out throughout the world to study the mechanisms of uterine contractility and relaxation. Experiments have been designed *in vitro*, that can possibly explain what is happening at the molecular level *in vivo*.

For ethical reasons, most of the research carried out is based on animal models, such as sheep, rodents, goats, etc, who have different mechanisms of initiation of labour compared to humans. The only way to use human myometrial cells is by biopsy during

hysterectomies and caesarean sections because of the different aetiology. Patients don't consent easily to collect these samples, hence, research on human relaxation and contractility mechanisms is limited.

To date different drugs are used in clinical practice. The most recent drug used is atosiban, an oxytocin antagonist with much less side effects for the patient. The disadvantage of using this drug is that it is not more effective than previous drugs used and it is very expensive.

On the other hand 'classic' drugs used, such as ritodrine, have not been shown to have a selective effect on the myometrium and can create many and very dangerous side effects such as tachycardia, hyperglycaemia and pulmonary oedema.

Hence, it is of vital importance to design new more selective drugs for the human myometrium. For example, we know that RhoA, ROCK1 and ROCK2 are present in pregnancy in the human myometrium. We also know that the inhibition of Rho kinase/Rho kinase-mediated calcium sensitization can cause relaxation of the uterine activity in humans. This is probably a new field of research to create selective drugs for blocking this pathway and improve uterine relaxation (Moran CJ *et al*, 2002).

There are many other pathways waiting to be clarified and many other hormones and proteins probably to be analysed.

A very important field of research is the regulatory mechanisms of the different ion channels like Ca^{2+} , K^{+} etc. This is an attractive possibility to the future for the use of new drugs. The regulatory mechanisms of the main channels just a few years back have started to be clarified but have still left a lot of information to be investigated.

In conclusion, despite extensive research carried out, further research is required to completely understand the complex mechanisms of human relaxation and contractility. Probably the relaxation pathways need to be investigated much more because most of the research to date was concentrated on the mechanisms of uterine contractility.

The aims of the research should be targeted at developing longer lasting drug effects as well as improving the specificity of these drugs to decrease possible side effects. To date we have focused on clinical practice, to prolong time '*in utero*' before delivery to improve the action of corticosteroids on foetal lung maturation. In the future, we need to focus on developing longer standing drugs, aimed to add a few weeks of intrauterine life

as opposed to a few days or hours in order to allow for the relevant maturational , and certain better quality of extra uterine life.

It is suspected that different mechanisms are responsible for preterm labour initiation. These include uterine distension, inflammation, decidual haemorrhage and premature activation of the hypothalamus-pituitary- adrenal axis. The initiation of preterm labour could be the result of the activation of one or more than one of these mechanisms .A very interesting field of the research on preterm labour is the trial to identify the genes which are possibly involved in the initiation of the preterm labour. It is suspected that a combination of genes and proteins are involved in this process (Esplin Sean M *et al*, 2005).

Two new disciplines, genomics and proteomics try to help us identify genes and proteins that may be involved in the mechanisms of preterm labour. These two disciplines have evolved over the past couple of years and are a new tool in the understanding of the pathophysiology of preterm labour initiation.

With these methods we can measure multiple markers in different samples and most importantly we can measure these markers at different chronological points, before and after the clinical manifestation of preterm labour. This can help us understand the possible regulation of the mechanisms of initiation of preterm and term labour and we can try to correlate the findings in order to find common areas and differences on the pathophysiology of preterm and term labour initiation.

Genomics is the method of evaluation of a large number of genes and the interaction of these genes with the different biological pathways which have a role in preterm labour initiation. Micro array and suppression subtraction hybridization (SSH) techniques are used. Conversely, proteomics uses mass spectrometry (MS) for the identification of different proteins present in different samples taken at different chronological stages of preterm labour. We can measure many markers simultaneously and correlate the findings with the possible pathophysiological mechanisms. We can identify new proteins which are present or absent in different chronological stages in relation to the clinical evidence of preterm labour.

A paper published by Buhimschi *et al*, 2002 was performed on amniotic fluid proteomic investigations. Samples were taken from 58 women that had a preterm birth along with

19 controls that had term deliveries. The authors used four different markers and identified the ladies in preterm labour with a specificity and sensitivity of 100%.

The most important challenge for the future using these two methods on the research of pathophysiology of preterm labour is the identification of the genes and associated proteins which are directly correlated with the mechanisms of initiation of preterm labour, and to identify which of these proteins and genes are indirectly correlated or are the result of the initiation of these pathways.

With the use of these methods, it is very important to identify different aetiologies of preterm labour and organise a sub classification based on different aetiologies. This will help us in the future to find different directions of new treatments based on the individual reasons of initiation of preterm labour.

Research in pharmacogenomics will be extremely important in the future development of new patient specific drugs.

It important to say that there is a possibility of a genetic predisposition in some women to have a preterm labour and this is more possible in cases of spontaneous preterm labour. In the future it may be possible to identify different genotypes at risk of preterm labour and to possibly use of genetic treatment to prevent, instead of treat preterm labour.

Another field of research developed in this area is pharmacogenetics which is the study of the correlation of different individual genetic factors and their response to different drugs in use. These new developments will help us in the future in tailoring the prevention or active treatment for each individual woman based on her genetic profile.

The clarification of the different mechanisms of human myometrial contractility / relaxation and their interactions is extremely important in understanding the mechanisms of the preterm and term labour as well as dysmenorrhoea. There are suggestions that this phenomenon of painful periods is based on pathological increased uterine contractility. If this explanation is true for this clinical phenomenon, the design of new specific drugs in the future based not only on analgesia but also on the decrease of contractility will be beneficial.

Other fields in obstetrics and gynaecology can benefit from the understanding of these different mechanisms of uterine contractility. Assisted Reproduction Techniques (ART) could be one of them. We know that the final stage of IVF/ICSI treatment is the embryo

transfer. This is the last step in the treatment and is based on the transfer of two or three embryos in the uterine cavity. This procedure is carried on with out the use of any form of analgesia. A small soft catheter is passed through the cervix and under trans-abdominal ultrasound scan the embryos are placed one centimetre from the fundus of the uterus. The technique of this procedure is very important and the catheter should be introduced gently into the cervix. We must also be very careful to not touch the uterine fundus because this can cause increased uterine contractility. It has been demonstrated that 1 hour embryo transfer only, 45% of the embryos were present in the uterine cavity (Mansour.R.T *et al*, 2002).

What is the reason for this? What happens to the other 55% of embryos? All these embryos probably expelled from the uterine cavity because of uterine contractions. What are the reasons for these contractions? It has been proven that the cervical stimulation during the procedure affects uterine contractility by increasing the production of oxytocin (Dorn et al, 1999). At this point we can suggest the possibility of using 'safe' tocolytic drugs such as Atosiban during the embryo transfer procedure, which can decrease the probability of uterine contractions and possibly increase the number of embryos to remain in the uterine cavity and indirectly increase the implantation and pregnancy rates (Sykoutris A, author's hypothesis July 2005). As we can see there are other fields to benefit from the deeper understanding of the mechanisms of human uterine contractility and relaxation. We not only have to identify the role of uterine contractility in labour or preterm labour, but also identify the involvement of lower grade contractility in other aspects of the female physiology and pathophysiology. We also need to identify the mechanisms of stimulation and production of oxytocin. It is very important to identify the different internal and external stimuli that can increase oxytocin production and its release.

Over the last few years, genetics has had a crucial role in the research of this complicated field of human female pathophysiology. New more specific drugs will probably be designed to be used in the future. Furthermore, a very important aspect for the future is the prevention of preterm labour and the possibility of correcting the distort pathway due to early genetic diagnosis even before the initiation of a pregnancy. A serial of markers

will probably be used in the future to monitor pregnancies and identify any abnormalities at early stages.

Thus, prevention and early correction of the distorted pathways resulting in preterm labour will be the aim of future clinicians and scientists with the assistance of genetics and improvement of research on human physiology.

The research on preterm labour is not only concentrated on the prevention and treatment but also on the protection of the neonate's central nervous system. There have been new developments over the last years on the pharmacological management of preterm neonates. One of these developments is the use of surfactant to improve the neonatal respiratory system effectiveness. In addition, there is still a huge ongoing research in different areas to improve the pharmacological and technological support of preterm infants. One very important area of research is the design of new drugs for neuroprotection of the neonatal central nervous system. Also the debate on ethics and the limits on the resuscitation of extremely premature babies is ongoing.

Over the last twenty five years, Assisted Reproduction Techniques (ARTs) have led to an increase in multiple pregnancy rates. In the United Kingdom, the HFEA (Human Fertilization and Embryology Authority) is the governing body for all IVF units, which controls all ART procedures. This authority only authorises a maximum of two embryos to be transferred in women that are below 40 years of age, and a maximum of 3 embryos in women that are above 40 years of age, to combat the occurrence of multiple pregnancies and their associated complications.

It has been well established that multiple pregnancies are one of the factors of preterm labours caused by over distention of the uterine cavity mechanism.

Unfortunately this limitation on the number of the embryos to transfer is not followed world wide and many countries still transfer more than three embryos, resulting in increased multiple pregnancies and their associated complications. In many cases of multiple pregnancies the babies need to be delivered prematurely. Future developments of improved tocolytic treatment will allow us to deliver one of the babies first and delivery the other one later on, improving the lung maturity and the possibility of reducing complications in extrauterine life.

Our aim for the future is to try to understand the mechanisms of human myometrial contractility and relaxation in order to create new methods of early diagnosis and treatment of preterm labour. It is important for our modern societies to decrease the preterm labour rates and to discover new drugs to decrease the neonatal complications. We know that the psychological effects on the families are huge and also huge are the financial implications.

It is vital for us to create new tocolytic drugs based on the model of the ideal tocolytic: Effective at 23 to 29 weeks of gestation, long term lasting, possibility of oral administration and no side effects to mother and babies.

But probably the most important aim for this research is the prevention of the preterm labour based on the deep understanding of its pathophysiology.

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