CHAPTER 2

LITERATURE REVIEW

Anatomy of Uterus

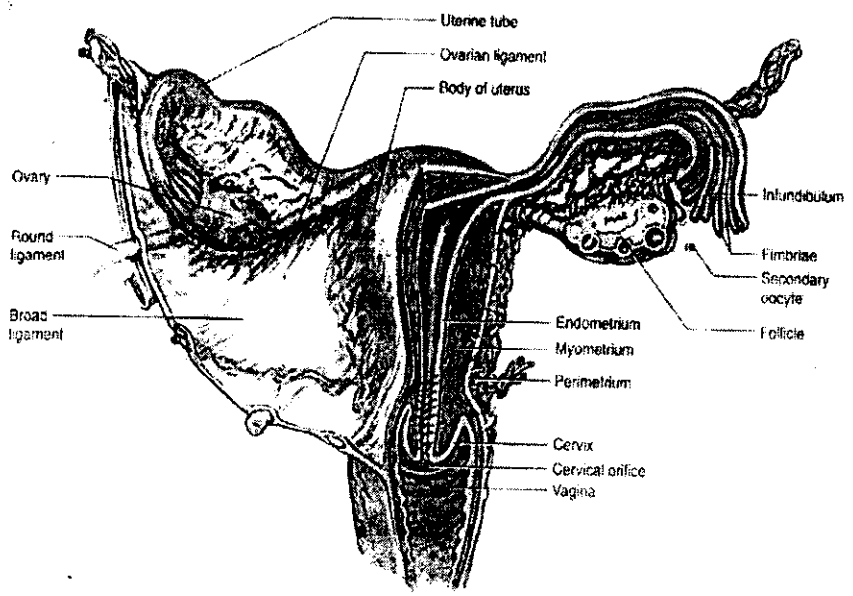


Figure 1. The funnel-shape infundibulum of the human uterine tube partially encircles the ovary (Source: Shier et al., 1999, pp. 857)

Uterus is in front of ileum and behide urinary bladder. It is the organ that composes of smooth muscle and involves the labor and menstruation. The uterine cavity is the place for embryo and fetus to develop during pregnancy. It can be expanded about 3 – 6 times of normal size (Shier et al., 1999). The uterus can be divided into three main parts; fundus, body, and cervix (as shown in Figure 1).

The body of the uterus consists of three layers: perimetrium, myometrium and endometrium. The perimetrium is the outer serous coat, consisting of peritonium that supported by thin layer of connective tissue. The myometrium is the middle muscular coats, becomes greatly distended (more extensive but thinners) during pregnancy. The main branches of the blood vessels and nerves of the uterus are located in this layer. It contains three parts, circular muscle, diagonal muscle and longitudinal muscle. The endometrium layer is the inner mucous coat which is firmly adherent to the underlying myometrium. It is changed follow the menstruation cycles and regulated by the ovarian hormone (Shier et al., 1999).

Innervation of the Uterus

The nerves of the uterus are derived from the uterovaginal plexus, which travels with the uterine artery at the junction of the base of the peritoneal broad ligament and the superior part of the fascial transverse cervical ligament. The uterovaginal plexus is one of the pelvic plexuses that extend to the pelvic viscera from the inferior hypogastric plexus, sympathetic, parasympathetic, and visceral afferent fibers pass this plexus.

Sympathetic innervation originates in the lower thoracic spinal cord segments and passes through lumbar splanchnic nerves and the intermesenteric/hypogastric series of plexuses. The function of sympathetic nerve that is supplying the uterus may involve vasodilatation and uterine relaxation.

Parasympathetic innervation originates in the S_2 through S_4 spinal segments and passes through the pelvic splanchnic nerves to the inferior hypogastric/uterovaginal plexus. The major function of the parasympathetic nerve is the pain perception. The other function of uterine parasympathetic nerve is to constrict blood vessel and stimulate uterine contraction (Shier et al., 1999; reviewed by Wray, 1993).

Physiology of Uterine Contraction

Electrical activity

The uterus is an organ that composed of special smooth muscle. During pregnancy, it can greatly expanded which is reversible (reviewed by Riemer and Heyman, 1998). It is very excitable and can generate spontaneous rhythmic contraction that varies in frequency and amplitude throughout the menstrual cycle (Lammer et al., 1994). The mechanism of spontaneous activity is still not know, but specialized pacemaker cells have been hypothesized as the initiator of activity (Lammer et al., 1994; Lammer et al., 1996). Uterine contraction occurs after action potentials which was generated by the pacemaker cells. The action potentials then spread throughout the uterus via the low resistance pathway called gap junction (Coleet al., 1985).

The contractility of the uterus is dependent on the resting membrane potential, which is determined primarily by permeability of Na^+, Ca^{2+}, K^+, and Cl^-. The concentrations of Na^+, Ca^{2+} and Cl^- are higher outside the cell while K^+ concentration is higher inside the cell (Monga and Sanborn, 1992). In general, the resting membrane potential of myometrium is approximately -40 to -50 mV (Parkington and Colemman, 1990). It becomes more negative

(~-60mV) during pregnancy and increase to approximately -45 mV near term (Monga and Sanborn, 1992).

The rhythmic contraction of myometrium involves the alteration of the membrane potential in the terms of slow wave. Whenever the membrane potential increased to the threshold, a fast depolarization occurs to generate an action potential on the top of slow wave (Parkington and Colemman, 1990). This depolarization is due predominantly to Na^+ channel and Ca^{2+} entry. The repolarization stage of the action potential is due to inactivation of Ca^{2+} channel and activation of K^+ channel, lead to K^+ efflux and then the potential drops to the resting potential (reviewed by Riemer and Heyman, 1998).

Ca^{2+} and Na^+ channel. It is well known that a rise in intracellular Ca^{2+} is central to control of contractile activity of the uterus. Ca^{2+} channels which are expressed in the myometrium are both L-type voltage sensitive channels and T-type voltage-inactivated Ca^{2+} channels (Sanborn, 2000). However, The L-type voltage sensitive channel is considered the major pathway of Ca^{2+} influx in myometrium (reviewed by Karaki et al., 1997). The effects of Ca^{2+} channels in the myometrium will be described in more details in the following section.

Although Ca^{2+} is a major charge carrier in myometrium, at the end of pregnancy, Na^+ also plays a role. A fast Na^+ currents have been measured in the pregnant human, rat tissues and cells, and it is found that the voltage-gated Na^+ channel mRNAs are expressed in the human and rat uterus. The numbers of this channel are increased before parturition in rat (Sanborn, 2000).

K^+ channel. K^+ channels contribute to the repolarization stage of action potential which results in a reduction of potential to resting stage. Little is known about K^+ channels expression in the myometrium, however the specific K^+ channel shown in myometrium is the Ca^{2+}-activated K^+ channels or maxi-K channel (K_{ca}) (as shown in Figure 2) (Sanborn, 2000). The K^+ channel are activated by several agents such as cAMP-generating agents (e.g. beta-adrenergic agonist), cGMP-generating agents (e.g. NO) or NO itself which result in uterine relaxation. The mechanism which regulates the K^+ channel involves receptor-channel coupling, channel phosphorylation or modification of critical sulfhydryl groups by oxygen radicals such as NO (reviewed by Wray, 1993; Riemer and Heyman, 1998).

Gap junction

The uterine contraction happens when the action potential triggered in pacemaker regions spread throughout the uterus. Gap junction is a low resistance pathway which allows the spreading of action potential between individual myometrial cells to happen (Cole et al., 1985). Gap junctions consist of pores which is formed by connexin protein. The major components of myometrial gap junction is the 42-kD protein, connexin 43 (Cx 43) (Chen et al., 1994). Each gap junction may consist of a few to thousands of channels and each channel is constructed from a group of six connexin proteins symmetrically aligned in an adjacent cell. These channels allow current and molecules up to 1000 daltons to pass between cells (Riemer and Heyman, 1998; Monga and Sanborn, 1992). The function of gap junction is regulated by the number of gap junction (structural coupling), their permeability (function

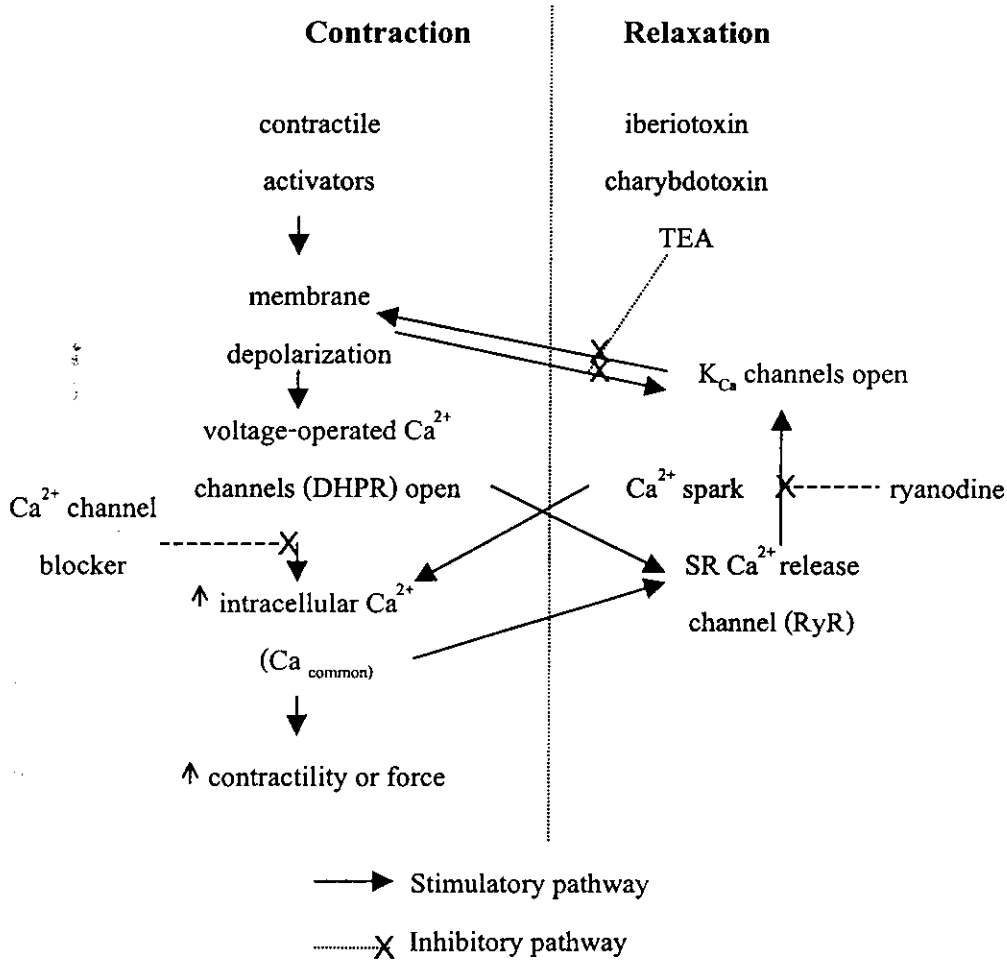


Figure 2. Roles of Ca^{2+} and K^{+} channels in controlling smooth muscle activity/contractility and relaxation. The two types of channel are thought to act in reciprocal manner to control contractility. Calcium entry via the dihydropyridine receptor/VOC activates the contractile apparatus and also activates Ca^{2+} release from SR stores, providing a locally elevated Ca^{2+} concentration in the region of sarcolemmal K_{Ca} channels. The locally elevated Ca^{2+} (sparks) are sufficient to activate K_{Ca} channels, which repolarize the sarcolemma through potassiun efflux, and thereby eliminate the voltage potential driving the VOCs. DHPR, dihydripyridine receptor; TEA, tetraethylammonium chloride (Source: Riemer and Heyman, 1998, pp. 5)

coupling) and their degradation (Cole and Garfield, 1986). In human myometrium the number of gap junction increase in woman in spontaneous labor when compare to the number in nonpregnant woman or pregnant woman not in labor (Monga and Sanborn, 1992).

In many species, progesterone appears to suppress the number and permeability of gap junction whereas estrogen act to increase both of them (Chen, et al., 1994; Cole and Garfield, 1986). Gap junctions rapidly disappear after delivery probably by internalization, endocytocis and digestion resulting in a decrease in excitability and contractile function of myometrium smooth muscle (Monga and Sanborn, 1992).

Mechanism of Contraction

For the contractility of myometrium, Ca^{2+} is considered to be the major charge carrier, except at the end of pregnancy which Na^{+} may also play a role (reviewed by Sanborn, 2000; Parkington and Colemman, 1990). It is well known that the rise in intracellular free Ca^{2+} level ($[Ca^{2+}]_i$) is a major determinant of myometrium contractility (reviewed by Sanborn, 2000; Horowitz et al., 1996). The structural basis of contraction is the relative movement of thick and thin filament in the contractile apparatus (reviewed by Riemer and Heyman, 1998).

Thick filament of contractile apparatus is composed of myosin molecule. Smooth muscle myosin is a hexamer consisting of two heavy chain subunit (~200 kD) and two pairs each of 20- and 17-kD light chains (as shown in Figure 3). Each heavy chain has a globular head that contains actin-binding site and adenosine triphosphate hydrolysis (ATPase) activity. A neck region

connects globular head to the remainder of each myosin molecule, which consists of a long α-helical tail that interacts with the tail of the other heavy chain subunit. Multiple myosin molecules interact via the α-helical tail in a coiled rod, forming the thick filament from which the globular heads protrude (Monga and Sanborn, 1992; Horowitz et al., 1996).

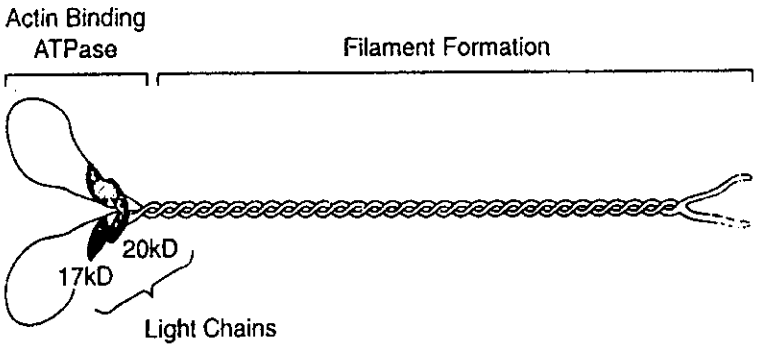


Figure 3. Schematic representation of smooth muscle myosin showing the globular head region of each 200-kD heavy-chain, with associated 17- and 20-kD light chain subunits, and the filamentous tail region that interacts with similar regions of other myosin molecules to from the thick filament. (Source: Monga and Sanborn, 1992, pp. 3)

Thin filaments are composed of actin, polymerized into a double helical strand and associated proteins. When myosin head interacts with actin, ATPase in myosin head is then activated. The active ATPase then hydrolyzed ATP to generate energy that allows the myosin head to move in the neck region, changing the relative position of the thick and thin filaments. The myosin head then detaches and can reattach at another site on the actin filament when reactivated (Monga and Sanborn, 1992).

The actin-myosin interaction is regulated by $[Ca^{2+}]_i$ when Ca^{2+} interacts with Ca^{2+}-binding protein calmodulin (CaM) to form the Ca^{2+}-CaM complex (Horowitz et al., 1996). The complex binds to and increase the activity of MLCK by a mechanism that decreases the influence of an autoinhibitory region of the kinase (Monga and Sanborn, 1992). Active MLCK phosphorylate the myosin 20-kD light chain in a specific serine residue near N-terminals. The phosphorylation of myosin correlates with increase in actomyosin ATPase activity, the enzyme that facilitate the actin-myosin interaction by increasing the flexibility of the head/neck junction (as shown in Figure 4) (reviewed by Monga and Sanborn, 1992; Riemer and Heyman, 1998).

Modulation of the Sensitivity of Contractile Apparatus

Ca^{2+}-dependent activation of MLCK and phosphorylation of MLC are the mechanism that explains for smooth muscle contraction (Karaki et al., 1997). However, the variation in the relation between $[Ca^{2+}]_i$ and contraction from many reports have explained the contraction in terms of "Ca^{2+} sensitivity of phosphorylation" (reviewed by Wray, 1993; Karaki et al, 1997; Riemer and Heyman, 1998). There are four proposed mechanisms for changes in the Ca^{2+} sensitivity of phosphorylation (as shown in Figure 5).

1. Activation of Ca^{2+}-calmodulin dependent protein kinaseII

Ca^{2+} which rises in intracellular binds with CaM to form Ca^{2+}-CaM complex. However, an increase in intracellular Ca^{2+} able to activate Ca^{2+}-calmodulin dependent protein kinaseII, the enzyme that phosphorylates MLC kinase, resulting in a reduction of MLCK activity (Monga and Sanborn, 1992; Karaki et al., 1997). Furthermore, an active Ca^{2+}-calmodulin dependent protein

kinaseII is also able to decreases affinity of Ca^{2+}-CaM complex leading to a utilization of more Ca^{2+} to activate contractile system. This phenomenon is called in term of negative feed-back (Monga and Sanborn, 1992).

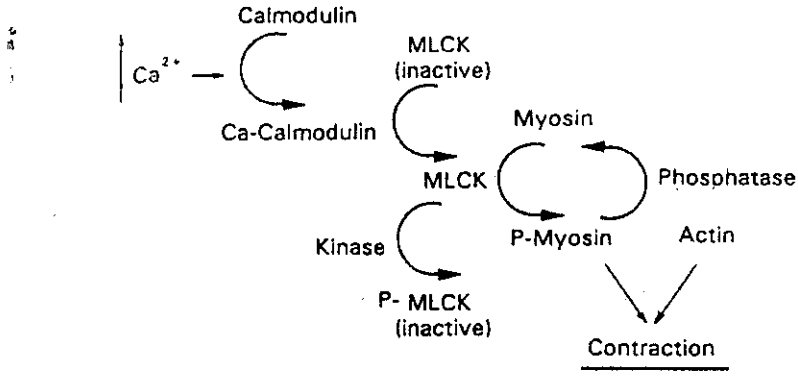


Figure 4. Scheme of contraction in uterine smooth muscle. Rise in $[Ca^{2+}]_i$, produced either spontaneously or by agonist, cause Ca^{2+} to bind to calmodulin (Ca-calmodulin). This then allows activation of enzyme myosin light-chain kinase (MLCK). This kinase phosphorylate light chains on myosin (P-myosin). This allows actin binding and activates myosin Mg^{2+}-ATPase, and thus contraction can occur with hydrolysis of ATP. P-myosin is dephosphorylated by phosphatases, leading to relaxation. If MLCK is phosphorylated, e.g., by Ca^{2+}-calmodulin-dependent protein kinase II, then it is much less efficient at phosphorylating myosin, and force falls. Reduction of $[Ca^{2+}]_i$ will also promote relaxation. (Source: Wray, 1993, pp. C5).

2. Inhibition of MLC-phosphatase (PPase)

Phosphatase is an enzyme that removes phosphate group out of MLC, MLCK, calponin and caldesmon (Haeberle et al., 1985). Thus it plays an importance role in determining the sensitivity of the contractile apparatus and

change in $[Ca^{2+}]_i$ (Wu et al., 1996). The phosphatase enzyme is inhibited by two mechanisms. 1) The agonists that activate phospholipase A_2 to cleave arachidonic acid from membrane phospholipids which turn to inhibits MLC phosphatase (Karaki et al., 1997). 2) The receptor agonists activate rho, a small GTP binding protein, which directly or indirectly inhibit MLC phosphatase, and/or activate protein kinase C and tyrosine kinase (Hirata et al., 1992; Fujita et al., 1995).

3. Activation of phosphorylation-independent mechanism

The other proteins, tropomyosin, caldesmon and calponin are the actin-binding protein which may regulate the actin (thin filament). Both tropomyosin and caldesmon increase the binding of actin to myosin, while both also bind to Ca^{2+}-CaM complex. When they are binding with Ca^{2+}-CaM, the effect of caldesmon on the actin-myosin was then decreased. Furthermore calponin can inhibit actomyosin ATPase activity, and the Ca^{2+}-CaM complex reverses this inhibition. Although, the pathways of this actin-binding protein (tropomyosin, caldesmon and calponin) is still not clear but believed that these proteins are involved in the regulation of the actin-myosin interaction, associated ATPase activity and are implicated in the regulation of cross-bridge cycling (Marston et al., 1998; Monga and Sanborn, 1992; Horowitz et al., 1996).

4. Increase free calmodulin concentration

The regulation of Ca^{2+}-calmodulin complex on the MLC kinase activity is well established. However, many studies suggested that the regulation of contractility is possible through the alteration of not only Ca^{2+} concentration

but also calmodulin concentration. Thus the changes in calmodulin concentration may determine the Ca^{2+}-sensitivity of contractile apparatus (reviewed by Karaki et al, 1997).

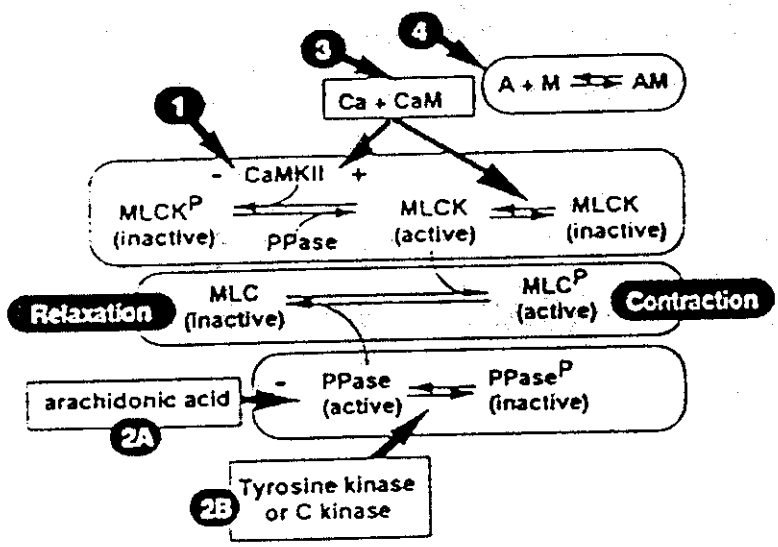


Figure 5. Mechanisms of agonist-induced Ca^{2+} sensitization in smooth muscle. Stimulation of a receptor increase $[Ca^{2+}]_i$, activates MLC kinase (MLCK), phosphorylation of MLC, and induces contraction. This process is modulated by four different mechanisms. The first mechanism is the inhibition of Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII), which phosphorylates MLC kinase and inhibits its activity (1). The second mechanism is the inhibition of MLC phosphatase (PPase) (2). Arachidonic acid, produced by receptor-mediated activation of phospholipase A_2, may directly inhibit phosphatase (2A). C kinase and tyrosine kinase may also inhibit phosphatase by inhibiting the endogenous inhibitor of phosphatase (2B). The third mechanism is to increase free calmodulin concentration (3). The fourth mechanism is to activate actin independently of MLC phosphorylation (A = actin, M = myosin) (4). (Source:Karaki et al., 1997, pp. 179)

Ca^{2+} Mobilization

In myometrium, the increase in Ca^{2+} influx contribute to depolarization stage of action potential, while the increase in K^+ efflux contribute to repolarization or hyperpolarization stage of action potential (as shown in Figure 2). It is well known that the rise in intracellular Ca^{2+} is a central control activity in myometrium (Parkington and Colemman, 1990). Generally, the resting level of intracellular Ca^{2+} free ionized is approximately 130 nM. Whenever the activation leading to the rise of this resting level of Ca^{2+} toward 200-400 nM, it can activate MLCK to increase myosin ATPase activity, cross-bridge cycling and then muscle cell shortening.

The mechanism of the rise in $[Ca^{2+}]_i$

The pathway which increase in $[Ca^{2+}]_i$ may involve voltage-dependent calcium channels, receptor operated calcium channel, sodium-calcium exchange and the release of Ca^{2+} from the internal storage (reviewed by Wray, 1993; Riemer and Heyman, 1998). Details of each pathway are as follows.

1. Voltage-dependent calcium channel

Two subtypes of Ca^{2+} channels are expressed in the myometrium including L-type voltage sensitive channel and T-type inactivated Ca^{2+} channel (Riemer et al., 2000; Sanborn, 2000). However only the L-type Ca^{2+} channel is considered to be a major Ca^{2+} influx pathway (reviewed by Karaki et al., 1997). This channel is activated by membrane depolarization. Under resting condition (membrane potential is -40 to -50 mV and Ca^{2+} free ionized is 130 nM) (Godfriand et al., 1986; Monga and Sanborn, 1992; Riemer and Heyman, 1998), the voltage-dependent Ca^{2+} channel are closed. Upon excitation, a rapid

depolarization of the cell membrane follows, voltage-dependent Ca^{2+} channel is then opened, and Ca^{2+} enter the cell. This is followed by inactivation (closing) of the channels. The next depolarization will occurs when the Ca^{2+} channel reverse from inactivation and be ready to open from a resting (closed) conformation (as shown in Figure 6) (Vaghy, 1998). The L-type channel exhibits voltage-dependent inactivation with half inhibition at approximately 45 mV, but can be returned to the resting stage at more negative potential (Parkington and Colemman, 1990). Therefore, both membrane potential changes and increase intracellular Ca^{2+} inactivate the channel in terms of negative feedback regulate channel activity (Sanborn, 2000). This channel is inhibited by Ca^{2+}channel blocker such as nifedipine and verapamil (Sanborn, 2000; Hess et al., 1984).

Agonists such as oxytocin and acetylcholine also open this channel by depolarizing the cell membrane through activation of nonselective cation channel (Bolton, 1979). Furthermore, agonists may open the L-type channel directly or indirectly through GTP-binding proteins in the absence of membrane depolarization (Karaki et al., 1997).

Ca^{2+}channel agonist and antagonist. Both of the Ca^{2+}channel antagonist such as verapamil or nifedipine and Ca^{2+}channel agonist such as Bay K 8644 or CGP28392 are the dihydropyridine. Furthermore, their structures are also very similar (Schramm et al., 1983; Brown et al., 1984; Vaghy, 1998). However, their effects on Ca^{2+}channel are closely diametrically opposite. The Ca^{2+}channel agonist opens Ca^{2+}channel, the following Ca^{2+}influx result in muscle contraction, whereas Ca^{2+}channel antagonists close this channel and

produce muscle relaxation. This phenomenon can be explained with the change in conformation of Ca^{2+} channel. Generally, behaviors of transmembrane Ca^{2+} channel in the absence of drugs have three modes, activated (open) mode, inactivated (close) mode and resting (close) mode. The dihydropyridine Ca^{2+} channel agonist (Bay K 8544) enhance Ca channel current by promoting activated mode, while Ca^{2+} channel antagonist (nifedipine or verapamil) promotes in the inactivated (close) mode of Ca^{2+} channel (as shown in Figure 6) (Hess et al., 1984; Vaghy, 1998).

2. Receptor-operated calcium channel

Much less is known about receptor operated calcium channel than voltage-operated calcium channel. This ion channel is controlled or operated by a receptor for a stimulant substance (as show in Figure 7) (Bolton, 1979). Generally, different stimulants may have their own receptor, each receptor type operates its own ion channels with their particular ionic selectivity (Bolton, 1979). However, when it is operated, they allow the ions including Na^+, K^+ and Ca^{2+} to enter, but the permeability of this channel for Ca^{2+} is less than that for Na^+ and other ions. It is partially inhibited by dihydropyridine (reviewed by Karaki et al., 1997; Wray, 1993). The ionic conductance which changed by receptor operated channel will have effects on the membrane potential and turn to activate or inhibit voltage-operated channel (reviewed by Wray, 1993)

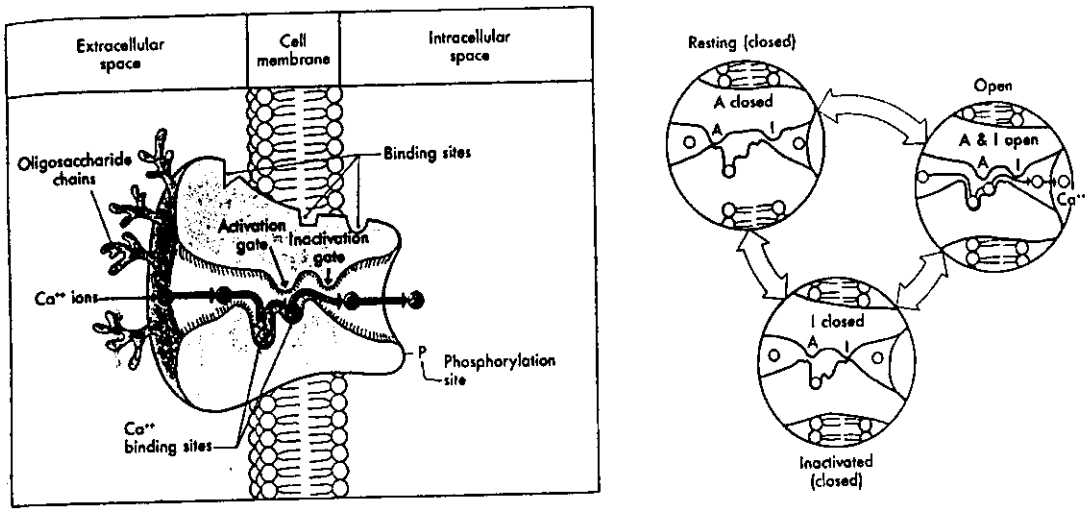


Figure 6. **(A)** Ca^{2+}-channel glycoprotein positioned in cell membrane. The ion channel is assumed to contain activation and inactivation gates that are moved or altered by the potential difference across the membrane and drugs so as to open or close the channel to transmembrane flux of Ca^{2+}. A site of phosphorylation is shown. The detail configuration and mechanism of the channel are still speculative. **(B)** They are voltage-dependent conformations (states) of Ca^{2+}-channels. The ion channel (pore) is "open" to the transmembrane flux of Ca^{2+} only when both the propose activation (A) and inactivation (I) sites are open. In the "closes" states, either the A or I sites are not open (Source: Vaghy, 1998, pp. 229)

3. Ca^{2+}/Na^{+} exchange reaction

The Ca^{2+}_{i}/Na^{+}_{o} exchange reaction exchanges one internal Ca^{2+} ion for 3 external Na^{+} ions via a membrane carrier molecule. This reaction is facilitated by ATP, while ATP is not hydrolyzed in this reaction. The energy for the moving of Ca^{2+} against its large electrochemical gradient comes from the Na^{+}

electrochemical gradient. The exchange reaction depends on relative concentrations of Ca^{2+} and Na^{+} on each side of membrane and relative affinities of the binding site to Ca^{2+} and Na^{+}. When the membrane is depolarized during the action potential plateau, the exchange carrier will exchange the ions in reverse, namely internal Na^{+} for external Ca^{2+}, resulting in increase Ca^{2+} influx. The net effect of this mechanism is to elevate $[Ca^{2+}]_i$ (Sperelakis, 1995). However, this mode is not operated under normal condition (reviewed by Wray, 1993).

4. Ca^{2+} release from the sarcoplasmic reticulum

Sarcoplasmic reticulum (SR) is the major internal Ca^{2+} storage site of smooth muscle including myometrium (Shmigol et al., 1999; reviewed by Riemer and Heyman, 1998). The release of Ca^{2+} from intracellular stores is controlled by two families of channel; ryanodine receptor (RyRs) and inositol 1,4,5-triphosphate receptor ($InsP_3R$) (Boothman and Berridge, 1995; Teng et al., 1996). Both $InsP_3R$ and RyRs have a bell-shaped sensitivity to the cytosolic Ca^{2+} concentrations; low Ca^{2+} concentration prompt more Ca^{2+} release (positive feed-back), at high Ca^{2+} concentrations, Ca^{2+} release is inhibited (negative feed-back). The positive feed-back is also called Ca^{2+}-induced Ca^{2+} release (CICR). (Boothman and Berridge, 1995; Riemer and Heyman, 1998; Karaki et al., 1997).

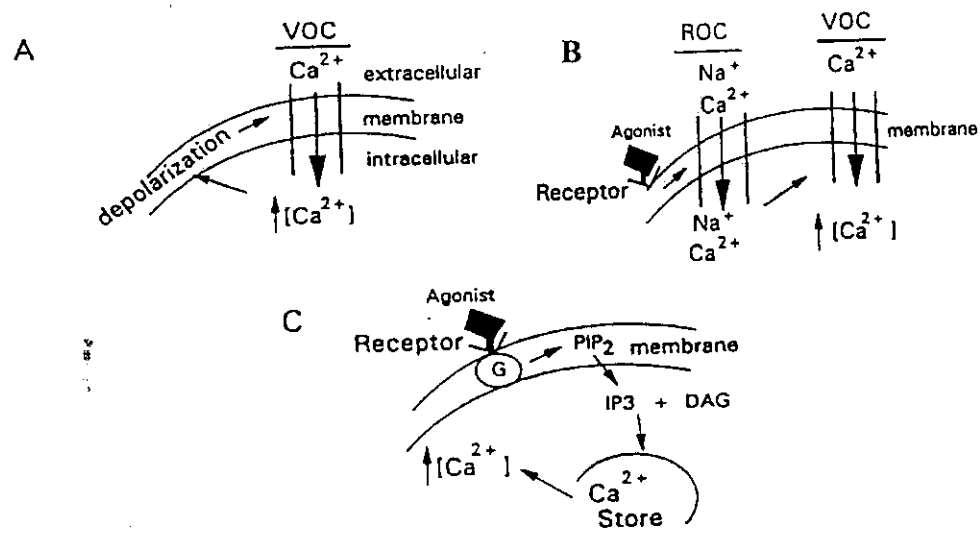


Figure 7. Diagrammatic representation of mechanisms for exciting uterus. (A) Ca^{2+} entry via voltage-operated channels (VOC) after depolarization, which can arise spontaneously within myometrial membrane. Entry of Ca will lead to further depolarization. (B) Increase in intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ produced by agonists binding to a receptor, as occurs in pharmacological coupling. Binding to a receptor stimulates receptor-operated channels (ROC) that allow cations to enter. This will cause depolarization, which will activate VOC, and leading to Ca^{2+} entry. (C) Increase in $[Ca^{2+}]_i$ produced by agonist-induced Ca^{2+} release from internal stores. Binding of agonist stimulates a G protein that activates phospholipase C, which produces inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) from phosphatidylinositol 1,4,5-biphosphate (PIP_2) in membrane. IP_3 then cause Ca^{2+} to be released from internal store, and thus $[Ca^{2+}]_i$ is increased. Effects of DAG are not shown (Source: reviewed by Wray, 1993, pp. C3)

IP_3 sensitive store. A Ca^{2+} channel that composed of four identical subunits, each containing an IP_3-binding sites in the large N-terminal cytosolic domain. When IP_3 bind to its binding site, it induces opening of this channel allow Ca^{2+} ions to exit from SR into the cytosol (as shown in Figure 8). However, this channel is inhibited by higher intracellular Ca^{2+} concentration, in term of negative feedback (reviewed by Riemer and Heyman, 1998).

Ryanodine receptor mediated release of Ca^{2+} (RyRs). This channel is sensitive to ryanodine, the plant alkaloid, so this channel is called ryanodine receptor. It is a homotetramer of four 565-kD subunits that mediates Ca^{2+} release from SR (Teng et al., 1996). It is unclear how these Ca^{2+} channels open in response to a voltage change or neurotransmitter (as shown in Figure 9). However, Chen et al., (1997) has shown that this channel was activated by Ca^{2+} at about 100 nM and inactivated at about 10 mM. Furthermore, they also showed that RyRs was activated by ATP, caffeine and inhibited by Mg^{2+}. Recent studies indicates that cyclic ADP-ribose, a newly identified intracellular signaling molecule may be an endogeneous ligand which promote the release of Ca^{2+} via RyRs (Riemer and Heyman, 1998; Boothman and Berridge, 1995).

Store-operated channel (SOCs). Elegant patch-clampimg studies have revealed a certain plasma membrane Ca^{2+} channel called store-operated channels (SOCs). It is opened in response to a depletion of intracellular Ca^{2+} stores (as shown in Figure 8 and 10). Although specific signal that promotes the opening

of SOCs has not yet been identified, the opening of this channel is critical to cellular response induced by elevated cytosolic Ca^{2+} (Lodish et al., 2000).

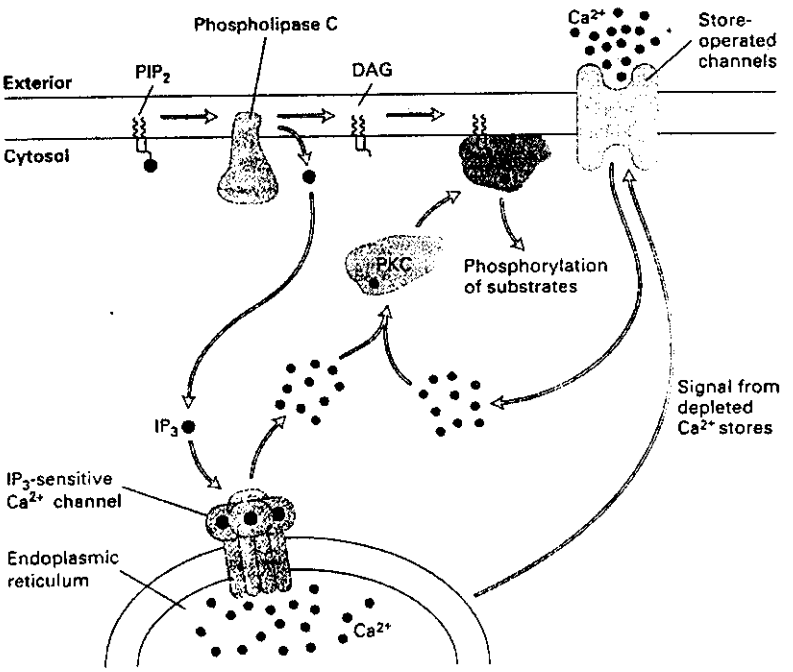


Figure 8. Elevation of cytosolic Ca^{2+} via inositol-lipid signaling pathway. Binding of a hormone to its receptor leads to activation of the G protein (Gq), which in turn activates phospholipase C by a mechanism analogous to activation of adenylyl cyclase. Phosholipase C then cleaves PIP_2 to IP_3 and DAG. The IP_3 diffuses through the cytosol and interacts with IP_3-sensitive Ca^{2+} channels in the membrane of endoplasmic reticulum, causing release of stored Ca^{2+} ions, which mediate various cellular responses. Release of intracellular Ca^{2+} stores promote influx of extracellular Ca^{2+} via store-operated channels. (Source: Lodish et al., 2000, pp. 739).

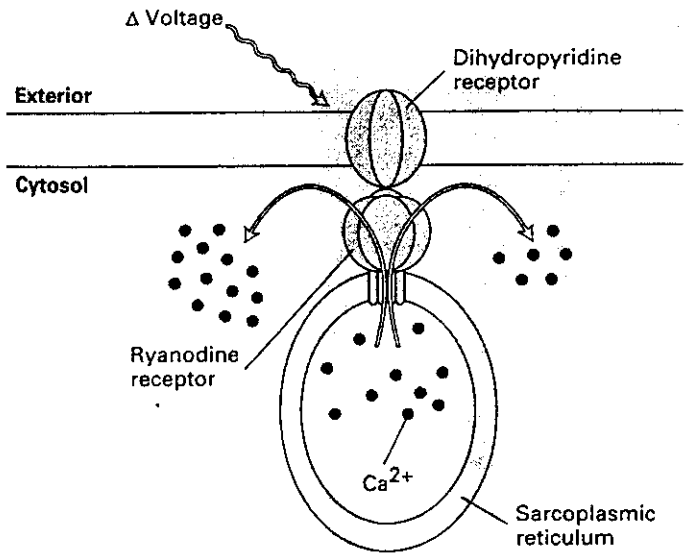


Figure 9. Release of Ca^{2+} stores mediated by ryanodine receptors (RyRs) in skeletal muscle. Votage-sensing dihydropyridine receptors in the plasma membrane contact ryanodine receptors located in the membrane of the sarcoplasmic reticulum. In response to a change in voltage, the dihydropyridine receptors undergo a conformational change; this produces a conformational change in the associated RyRs, opening them so that Ca^{2+} ions can exit into the cytosol. (Source: Lodish et al., 2000, pp. 891).

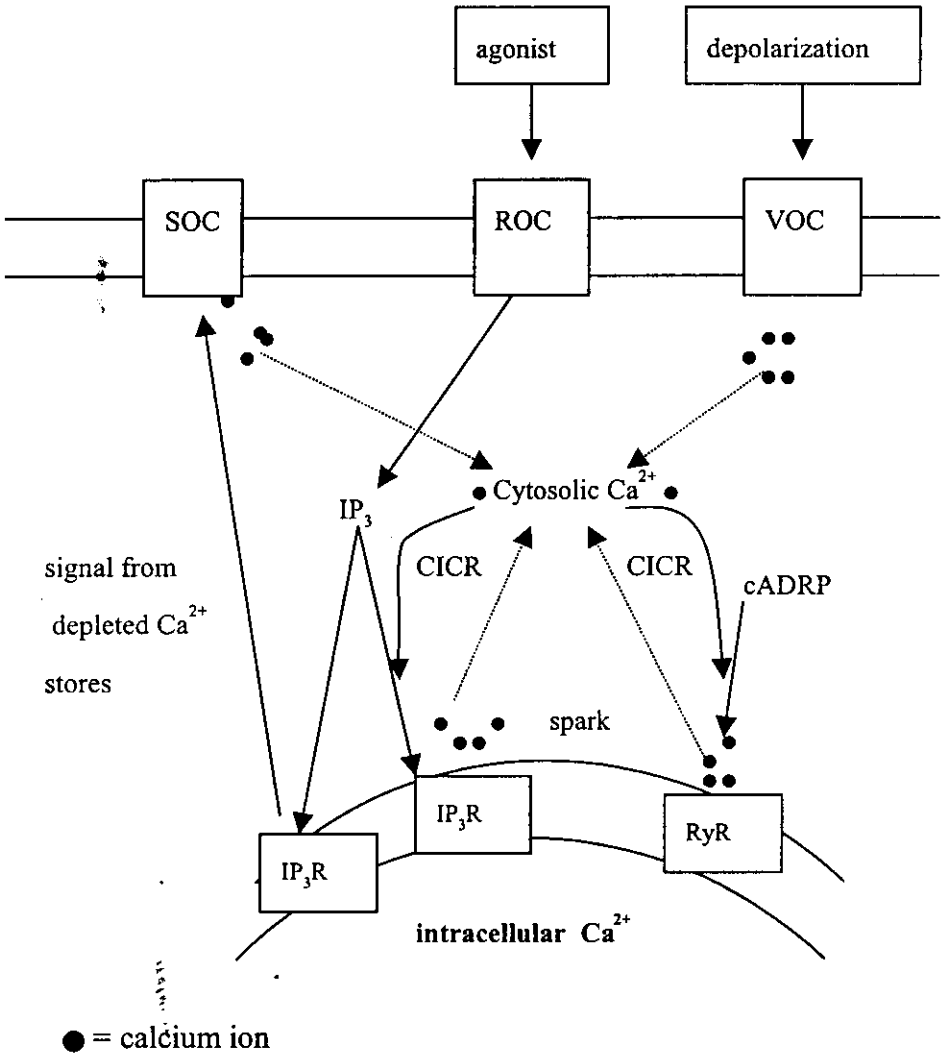


Figure 10. Mechanisms of fine control of intracellular Ca^{2+} mobilization in smooth muscle; role of receptors/channels. When SR Ca^{2+} stores are depleted, IP_3R activation is coupled activation of store-operated Ca^{2+} channel, leading to replenishment of SR Ca^{2+} stores. cADPR, cyclic ADP ribose; CICR, Ca^{2+}-induced Ca^{2+} release; ROC, receptor-operated Ca^{2+} channel; SOC, store-operated Ca^{2+} channel (Source: Riemer and Heyman, 1998, pp. 6).

Other source of Ca^{2+}. Another potential internal source of Ca^{2+} is a sarcolemmal-bound Ca^{2+}. Grover et al., (1983) has shown that there is a high-affinity pH-dependent intracellular Ca^{2+} binding site on the plasma membrane. The membrane bound Ca^{2+} may contribute the rise in $[Ca^{2+}]_i$ required for contraction, however, there is no evidence indicating the role of this Ca source. It is likely that this Ca^{2+} source may be responsible for the effect of intracellular pH alteration on the contraction, perhaps, due to the competition between Ca^{2+} and H^+ on their binding sites on the plasma membrane.

The mechanism of the reduction in $[Ca^{2+}]_i$

Immediately after elevation of $[Ca^{2+}]_i$ level, it can restore to the resting level by homeostatic mechanism (reviewed by Riemer and Heyman, 1998). The homeostatic mechanism can lower cytosolic Ca^{2+} from the cell by the sarcolemmal Ca-ATPase, Na^+-Ca^{2+} exchange, taken up into the intracellular Ca^{2+} store by the Ca^{2+}-ATPase pump on the SR membrane and therefore bind to plasma membrane (shown in Figure 11 and 12) (reviewed by Horowitz, et al., 1996).

Since Ca^{2+} extrusion through Na^+-Ca^{2+} exchange mechanism would ultimately be limited by (Na^+-K^+)-ATPase activity and it must lower affinity for Ca^{2+}, it is suggested that plasmalemmal Ca^{2+} ATPase plays a more important role in Ca^{2+} extrusion than Na^+-Ca^{2+} exchanger (reviewed by Karaki et al., 1997; Monga and Sanborn, 1992). In smooth muscle, there are two type of Ca^{2+}-ATPase

pump, sarcolemmal Ca^{2+}-ATPase and SR Ca^{2+}ATPase (as shown in Figure 11 and 12) (Katraki et al., 1997).

1. Plasma membrane Ca^{2+}-ATPase. It is a sarcolemmal pump with a single-polypeptide enzyme of molecular weight ~130,000-150,000. In myometrium under physiological condition, Ca^{2+}-ATPase has been shown to be able to extrude large amount of Ca^{2+}, thus it may play the most important role of removing Ca^{2+} from the myometrium cell. This pump transports Ca^{2+} to the extracellular space and uses ATP as its energy sources and it can be activated by protein kinase C or cGMP (reviewed by Wray, 1993; Sperelakis, 1995; Karaki et al., 1997) and inhibited by vanadate (Ca^{2+}-ATPase inhibitor) (Nechay, 1984).

2.The SR Ca^{2+}-ATPase. It has a molecular weight ~100,000 (Wray, 1993), and play an important role for actively translocates Ca^{2+} at cytosol into SR system and preventing Ca^{2+} overload. (Monga and Sanborn, 1992). This enzyme transports Ca^{2+} using ATP as its energy source similar to the sarcolemmal Ca^{2+}-ATPase. The Ca^{2+}-ATPase inhibitor, thapsigargin (Bremen, 2000) and cyclopiazonic acid (Maggi et al., 1995; Sperelakis, 1995; Uyama et al., 1993) or vanadate (Nechay, 1984) can inhibit this pump.

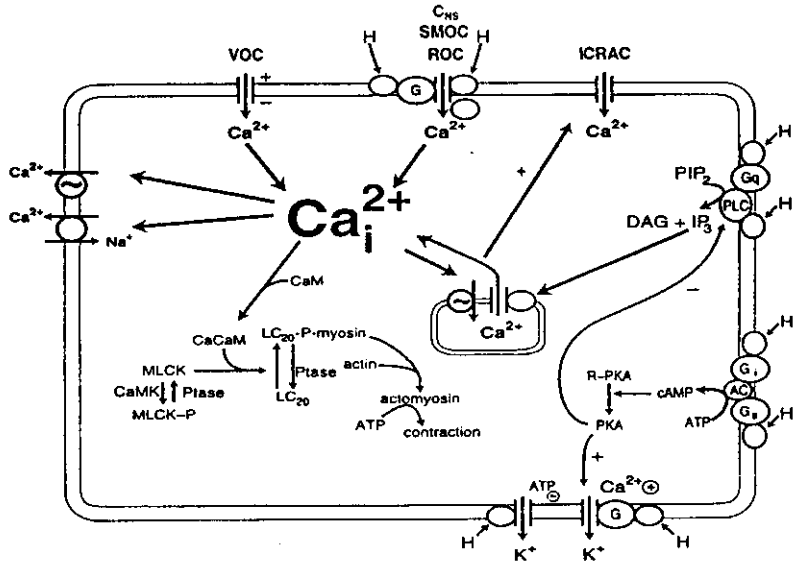


Figure 11. Summary of Ca^{2+} mobilization in smooth muscle. AC = adenylyl cyclase; ATP = adenosine triphosphate; C_{NS} = nonselective cations channels; SMOC = second messenger operated channels; H = hormone; Ptase = phosphatase; CaM = calmodulin; CaMK = Ca^{2+}-calmodulin light chain kinase; cAMP = cyclic adenosine monophosphate; PLC = phospholipase C; DAG = diacylglycerol; MLCK = myosin light chain kinase; ICRAC = intracellular calcium release-activated channel; G = GTP-binding protein; IP_3 = inositol 1,4,5-triphosphate; LC_{20} = myosin light chain; PKA = protein kinase A; ROC = receptor-operated channel; VOC = voltaged-operated Ca^{2+} channel, Ca^{2+} transport ATPases; Na^+/Ca^{2+} exchanger, Ca^{2+}-activated K^+ channel, ATP-sensitive K^+ channel. (Source: Monga and Sanborn, 1992, pp. 97).

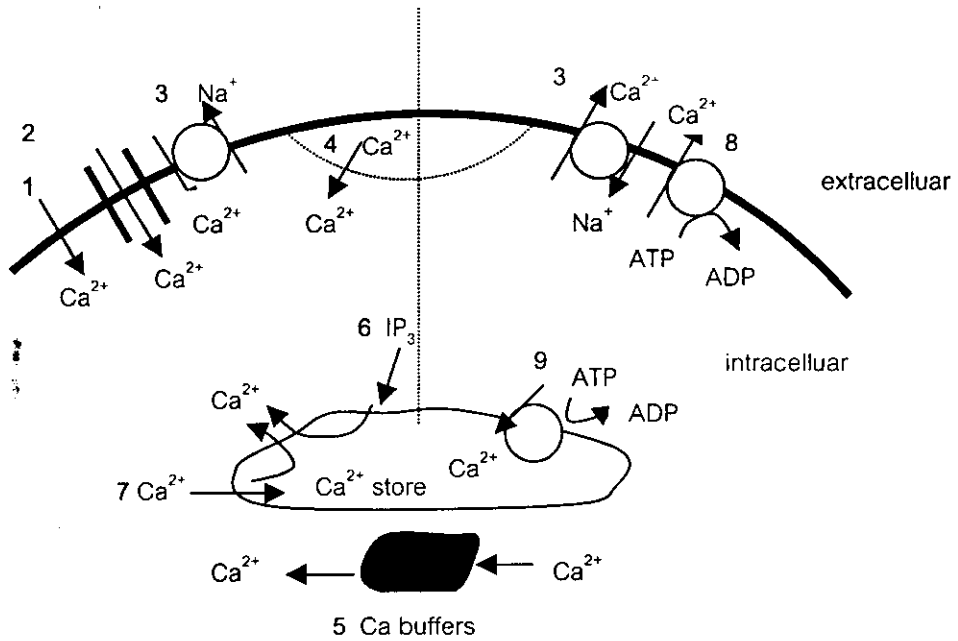


Figure 12. Summary of the route for the increasing (left) and decreasing (right) $[Ca^{2+}]_i$. 1) Passive entry is simply leakage across myometrial sarcolemma. 2) Entry via ROC and VOC. 3) Na^+-Ca^{2+} exchange; in its normal mode of operation it will remove Ca^{2+} but can operate in reverse to bring Ca^{2+} into cell. This exchanger is not thought to be quantitatively very important in uterus. 4) Sarcolemmal-bound Ca^{2+} stores. This may be large in uterus and is pH dependent. 5) Ca^{2+} buffers exist within smooth muscle cell, and their capacity will change under physiological and pathological conditions (e.g., decreased pH_i or increased Mg^{2+}). 6) IP_3 produced as result of agonists binding to sarcolemmal membrane causes Ca^{2+} to be released from internal stores. 7) Increased $[Ca^{2+}]_i$, e.g., as result of VOC activation, may cause Ca^{2+} to be released from internal stores (Ca^{2+}-induced Ca^{2+} release). It is not known whether this occurs in uterus. 8) Sarcolemmal Ca^{2+}-ATPase, this will remove Ca^{2+} from cell at expense of ATP. 9) Internal Ca^{2+} store Ca^{2+}-ATPase, this will remove $[Ca^{2+}]_i$ by sequestering into store at expense of ATP (Source: reviewed by Wray, 1993, pp. C6).

Modulator of Uterine Contraction

The uterine contraction is modulated by several ways such as the neuronal modulation, hormonal modulation and metabolic modulation. The final effect of these modulations are result in changed of the frequency, duration and amplitude of uterine contraction, however, the mechanism of action of the modulators in each modulation may be not similar (reviewed by Wray, 1993). The details of each modulation are discussed as follows.

1. **Neuronal modulation**

The neuronal role which contribute to the uterus is still not clear. It has been shown that postglangionic fibers innervate the uterus both in endometrium and myometrium layer, however, it does not appear to be close in apposition of nerve ending at the myometrium cells. It is suggested that the neuronal effect which modulate the contractility of uterus is less important than hormonal effect (reviewed by Wray, 1993; Riemer and Heyman, 1998).

1.1 Adrenergic receptor. All four types of adrenergic receptor (α_1,α_2, β_1, β_2) appear to be present on the uterus. In general, α-activation causes contraction, while β-activation causes relaxation (Segal, et al., 1998). α_1-activation has been linked to phosphoinositide breakdown and IP_3 formation produces uterine contraction (Breuiller-Fouche et al., 1991). The activation of α_1 was largely dependent on Ca^{2+} influx from extracellular which is mediated via G_i transduction pathway (Kitazawa et al., 2000). Whereas β_2-activation causes the generation of cAMP which produces uterine relaxation (Kitazawa et al., 2001).

The proportion of the receptors will differ with hormonal state, species and muscle layer. Generally, high progesterone levels will increase the number and also produces the formation of high-affinity β_2 receptor (Robert et al., 1977; Williams et al., 1977). However, at term, there is rapid desensitization leading to a loss of myometrium response to β agonist (Bylund et al., 1994). The action of β-adrenergic stimulation on uterine contraction will be described more details in the uterine relaxation section.

1.2 **Cholinergic receptor.** The role of parasympathetic innervation has received more attention to pain perception than motor activity. Although uterine contraction is stimulated by acetylcholine, but it is well established that contraction and expulsion the fetus can be occurred in the absence of nerve activation. Thus the role of these nerves in the contractility of uterus may involve only a small function such as a coordination of activity (reviewed by Wray, et al., 1993).

In myometrium, two subtypes of muscarinic receptor has been identified which are M_2 and M_3 (Choppin et al., 1999; Kitazawa et al., 1999). These muscarinic receptor are G protein-coupled receptors linked to phosphoinositide break down, increasing IP_3 and hence elevating $[Ca^{2+}]_i$ with produces contraction (Bolton, 1979). However external Ca^{2+} is also required for the contraction and many mechanisms for elevating $[Ca^{2+}]_i$ is considerably similar to the action of oxytocin (reviewed by Wray, 1993).

2. Hormonal Modulation

Cyclical changes in steroid hormonal levels in females have profound effects on uterine force production as well as other uterine physiology such as implantation. It has been long known that the ovarian hormone, estrogen and progesterone influence the contractility of the uterus throughout menstrual periods and pregnancy.

2.1 Estrogen and progesterone

Progesterone plays a role for maintaining the uterus in a quiescent stage, while estrogen plays a role for increasing the sensitivity of the contractile response (reviewed by Wray, 1993). In general, during late pregnancy, estrogen promote uterine contractility via the effects on contractile protein, gap junction formation, and increase responsiveness of uterus to agonist such as oxytocin and $PGF_{2\alpha}$ (Challis et al., 1994). Batra (1986) have shown that Ca^{2+} uptake in the isolated rabbit uterus which had been treated by estrogen was more than double amount of cellular Ca^{2+} uptake. Estrogen also increases the sensitivity of contractile response of uterus to oxytocin by increase the number of oxytocin receptor (Monga and Sanborn, 1992), and increase gap junction expressions in the human myometrium (Di et al., 2001).

Progesterone maintains the uterus in a quiescent stage which is thought to be mediated through suppression of the spontaneous generation and propagation of action potentials (Challis et al., 1994). Its effects may involve the inhibitory of

gap junction (Shinohara, et al., 2001) and the suppression on the number of oxytocin receptor (Monga and Sanborn, 1992).

2.2 Oxytocin

Oxytocin is a powerful stimulator of contraction in the uterus which increase in force, frequency and duration of contraction (Rall, 1991; Michale, 1998). The response of uterus to oxytocin is dependent on the number of its receptor rather than its concentration. Oxytocin receptor concentration in myometrial cells is increased by an elevation of estrogen and suppressed by progesterone (Monga and Sanborn, 1992). Thus, the effect of this hormone is higher in the last trimester, because the ratio of estrogen to progesterone is high resulting in an increase in the number of oxytocin receptor (Monga and Sanborn, 1992). Oxytocin also has an important role in third stage labor (expulsion of placenta) which prevent hemorrhage. The hormone is also an importance hormone for lactation (Challis, et al., 1994).

Myometrial oxytocin receptor is a G protein-coupled receptor. The binding of oxytocin to this receptor results in interaction with the GTP-binding protein (G-$\alpha_{q/11}$ subfamilies) which stimulates phospholipase C. This enzyme increase production of IP_3 and diacylglycerol (DAG) (see in Figure 13) (Monga and Sanborn, 1992; Rall,. 1991). DAG, in turn, stimulates protein kinase C that phosphorylates and activates specific effector protein with contribute to muscle contraction, while IP_3 promotes the release of Ca^{2+} from internal storage (Rall, 1991).

Although oxytocin induces hydrolysis of phosphoinositides, there is an evidence to show that Ca^{2+} channel blocker markedly inhibit the effect of oxytocin on contractile response. This evidence suggested that the increase of intracellular Ca^{2+} by IP_3 may play a relatively minor role. Direct depolarization induced activation of voltage-dependent Ca^{2+} channel by oxytocin may be of greater importance (Vaghy, 1998). Furthermore, oxytocin has been shown to inhibit Ca^{2+} extrusion at sarcolemma by inhibiting the Ca^{2+}ATPase, whereas it can inhibit SR Ca^{2+}ATPase which pump Ca^{2+} in cytosol to the internal storage site (Wray, 1993). The inhibition of Ca^{2+} extrusion and internal uptake will prolong the rise in Ca^{2+} and promote contraction of the uterus.

Oxytocin can also induce uterine contraction in terms of "Ca^{2+} independent-induced contraction". It has been known for several years that oxytocin and some agonist (acetylcholine, angiotensin II) can cause continued contraction of uterus in Ca^{2+} free, ethylene glycol-bis (β-aminoethyl-ester)-N,N,N',N'-tetra acetic acid (EDTA)-containing solution. The amplitude of contraction is only 5-15% of that obtained with Ca, but this contraction is persistence for many hours (reviewed by Wray, 1993). The contractions occur without measurable increase in $[Ca^{2+}]_i$ or myosin light chain phosphorylation (Oishi, et al., 1991). It is suggested that the contraction is probably due to a phosphorylation either a contractile or cytosol protein.

2.3 Prostaglandins

Two actions of PGs which involve smooth muscle cells are contractile action and relaxant action (Bolton, 1979). In uterine smooth muscle, $PGF_{2\alpha}$ and PGE_2 receptor are capable of profoundly modulating uterine contraction (MacDonald and Casey, 1993).

An increase in contractile activity by PGs is associated with a rise in free $[Ca^{2+}]_i$ as determined by fura-2 measurement in a single cultured human myometrial cells (Mackenzie et al., 1990). Philippe et al. (1977) and Fu et al. (2000) has shown that $PGF_{2\alpha}$ stimulates uterine contraction by generating phosphatidylinositol-signal pathway which results in an increase of Ca^{2+} release from IP_3 sensitive site. PGs may also open nonselective cation channel which could cause the depolarization of plasma membrane leading to Ca^{2+} influx (Bolton, 1979).

Furthermore, PG may have an interaction with other agonists. Oxytocin is the agonist which could stimulate the synthesis and release of contractile PG. DAG which was released by binding of oxytocin to its receptor will stimulate arachidonic acid release and, hence, PG synthesis (Wray, 1993).

2.4 Endothelin

Endothelin receptor has been present in amniotic, chorion, endometrium and myometrium (Monga and Sanborn, 1992). All three endothelin (ET) peptides (ET-1, ET-2 and ET-3) have been shown to cause uterine contraction in both pregnant and nonpregnant uterus (Bolton, 1979). Myometrial endothelin receptor

is increased during delivery and may contribute to an enhancement of the contractile response to ET at term (Monga and Sanborn, 1992).

The effect of endothelin to increase the force of contraction is due to a rise in $[Ca^{2+}]_i$ and produce myosin phosphorylation. Endothelin increases $[Ca^{2+}]_i$ by an activation of receptor-PLC coupling and also by increased Ca^{2+} influx via nonselective cation channels leading to an activation of voltage-dependent L-type channels as a result of in depolarization (Sakata and Karaki, 1992)

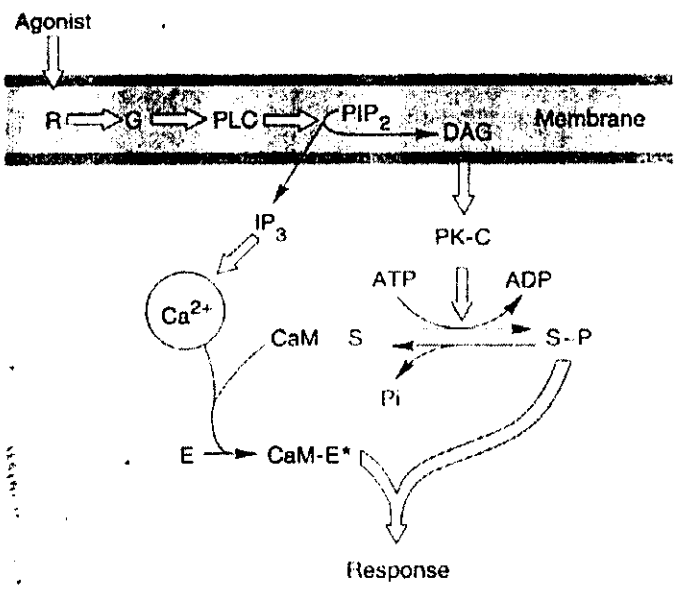


Figure 13. The Ca^{2+}-phosphoinositide signaling pathway. Key proteins include hormone receptor (R), a G protein (G), a phosphoinsitide-specific phospholipase C (PLC), protein kinase C (PK-C), substrates of the kinase (S), calmoulin (CaM), and calmodulin-binding enzymes (E), including kinase, phosphodiesterase, etc. (PIP_2, phosphatidylinositol-4,5-biphosphate: DAG, diacylglycerol. Asterisk denotes activated state. Open arrows denote regulatory effects.) (Source: Bourne and Zastrow, 1998, pp. 26)

2.5 High-K^+ solution

High-K^+ solution induced uterine contraction with biphasic contraction. The initial peak or short peak of contraction is called phasic contraction. When the initial peak reach a maximum, it is slightly declined to a relative steady at low level. This steady stage is called tonic contraction (Bolton, 1979). High-K^+ solution induces sustained contraction which was totally abolished by removing external Ca^{2+} and also by agents that block Ca^{2+} channel. It is suggested that high-K^+ increase transmembrane Ca^{2+} influx, then increase $[Ca^{2+}]_i$ and results in contraction (Kalsner, 1997). The uterine contraction activated by high-K^+ can prolonged its effect on tonic contraction because it reduces the potassium gradient across the membrane (Bolton, 1979).

Although the KCl-induced contraction is mainly mediated by Ca^{2+} influx via voltage-sensitive channel, some recent reports suggested an effect of KCl on the release of Ca^{2+} from intracellular storage and an increase in IP_3. It may contribute to an increase in a Ca^{2+} sensitivity of the contractile apparatus (Kalsner, 1997). Moreover it may be involved to a variety of enzymes such as protein kinase C or phospholipase A_2 (PLA_2) (Somlyo and Somlyo, 1994). However, Trujillo et al. (2000) has shown that high K^+ solution may produce contraction through the effect on Ca^{2+} influx, and activate other processes leading to an increase in the Ca^{2+} sensitivity of the contractile machinery by a mechanism independent of extracellular Ca^{2+}. The authors suggested that arachidonic acid and

its metabolites derived from cyclooxygenase pathway might be possible candidates to be involved in KCl-induced Ca^{2+} sensitization.

Uterine Relaxant

1. β_2-adrenergic receptor and cAMP

β_2-adrenergic receptors are present on the myometrium smooth muscle. β_2-mimetics such as ritodrine, salbutamol terbutaline or isoproterenol display for membrane-bound β_2-adrenergic receptors in the myometrium, results in activation of the adenylate cyclase enzyme via stimulation of G-α_s protein which increase intracellular cAMP concentration (shown in Figure 14 and 15) (Fortier et al., 1983). The increases in cAMP activate cAMP dependent kinase that phosphorylate protein and cause relaxation. In such a case, the phosphorylayted protein by cAMP dependent kinase may be MLCK. When MLCK is phophorylated, it markedly decreases in its affinity to Ca-calmodulin leading to a reduction of phosphorylation of myosin light chain (Scheid et al., 1979).

The other mechanism of cAMP involving to relaxation may also contribute to $[Ca^{2+}]_i$ (Verma and McNeill, 1976). It may occur by; 1) increased extrusion of Ca^{2+} by stimulation of Ca^{2+} transport and plasma membrane Ca^{2+}ATPase. 2) activating the Na^+-K^+ pump results in lowering $[Na^+]_i$ which turn to enhance Ca^{2+} efflux in Na^+-Ca^{2+} exchange. 3) causing internal sequestering of Ca^{2+}, 4) inhibiting Ca^{2+} influx and increase K^+ conductance (Schied et al., 1979; Monga and Sanborn, 1992).

The cAMP is degraded to 5'AMP by phosphodiesterase enzyme, thus the phosphodiesterase inhibitor such as rolipram or caffeine could elevate $[cAMP]_i$ which lead to muscle relaxation (Bourne and Zastrow, 1998).

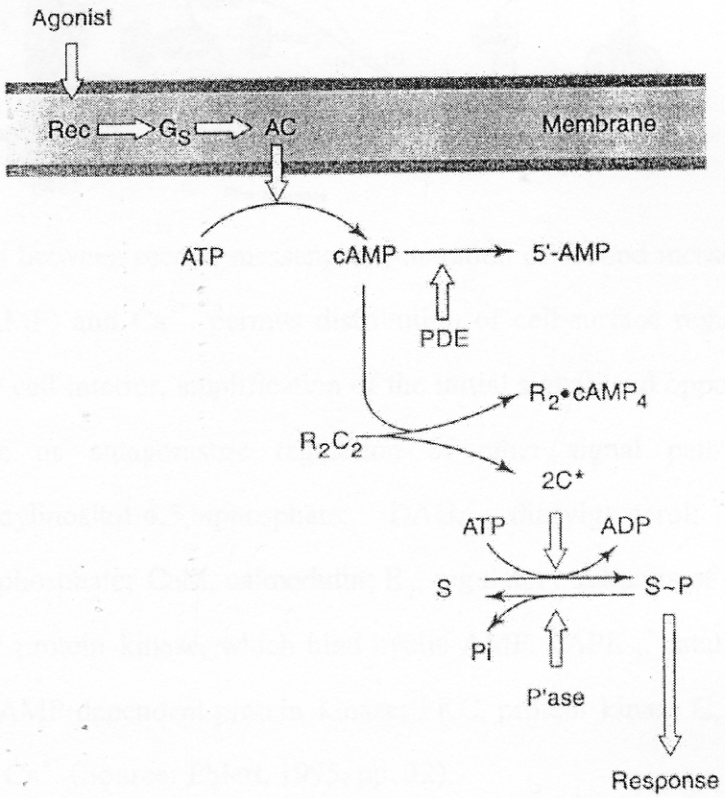


Figure 14. The cAMP second messenger pathway. Key protein include hormone receptors (Rec), a stimulatory G protein (G_i), catalytic adenyl cyclase (AC), phosphodiesterase (PDE) that hydrolyze cAMP, cAMP-dependent kinases, with regulatory (R) and catalytic (C) subunits, protein substrate (S) of the kinase, and phosphatase (P'ase), which remove phosphates from substrate proteins. Open arrows denote regulatory effects (Source: Bourne and Zastrow, 1998, pp. 25).

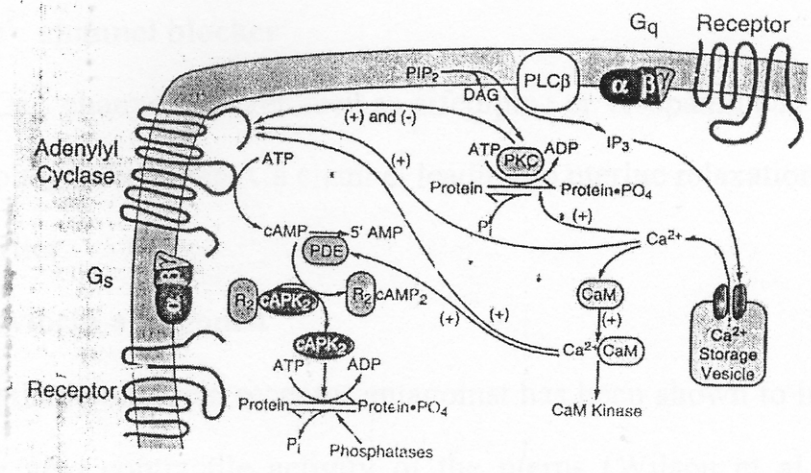


Figure 15. Interaction between second messenger, generation of second messenger cyclic AMP (cAMP) and Ca^{2+}, permits distribution of cell-surface regulatory input within the cell interior, amplification of the initial signal, and opportunities for synergistic or antagonistic regulation of other signal pathways. PIP_2, phosphatidylinositol-4,5,biphosphate: DAG, diacylglycerol; IP_3, 1,4,5 inositoltriphosphate; CaM, calmodulin; R_2, regulatory subunits of cyclic AMP-dependent protein kinase, which bind cyclic AMP; $cAPK_2$, catalytic subunits of cyclic AMP-dependent protein kinase; PKC, protein kinase C, activated by DAG and Ca^{2+} (Source: Ehlert, 1995, pp. 32).

2. Mg^{2+} ions

High extracellular Mg^{2+} concentration (10 nM) gradually increase in magnesium levels inhibit Ca^{2+} influx into myometrial cells via L-type and T-type voltage operated calcium channel, and increase sensitivity of K^+ channel to Ca^{2+}. Thus it can rapidly produced repolarization or hyperpolarization resulting in uterine relaxation (Monga ang Sanborn, 1992; Bolton, 1979).

3. Ca^{2+} channel blocker

The Ca^{2+} channel blocker such as nifedipine or verapamil can inhibit Ca^{2+} influx via voltage-dependent Ca channel leading to uterine relaxation as already reviewed earlier.

4. Oxytocin antagonist

A synthetic oxytocin receptor antagonist has been shown to inhibit both *in vitro* and *in vivo* contractile activity of the uterus (Wilson et al., 1997). The mechanisms may involve a competitive inhibition of oxytocin binding to its receptor leading to an inhibition of oxytocin signal pathway and Ca^{2+} entry. This would result in uterine relaxation (Monga and Sanborn, 1992).

PAPAYA TREE

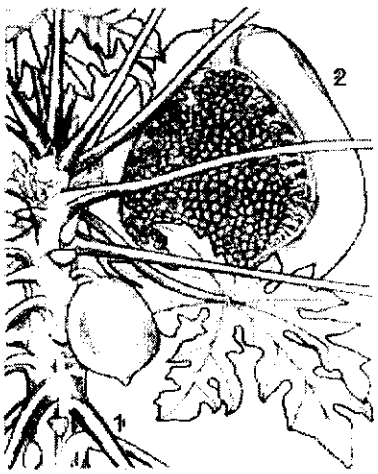


Figure 16. *Carica papaya* L. – 1) part of flowering and leaves, 2) part of fruit

Carica papaya (family Caricaceae, genus *Carica* L.) is believed to be originating in Southern Mexico and Central America. It was then brought to the Caribbean and South East Asia during the Spanish exploration in the 16th Century. It spreaded rapidly to India, Oceania, Africa, and, today, it is widely distributed throughout the tropical and warmer subtropical areas of the world (Morton et al., 1977; Hartman et al., 1981). Common name for papaya trees etc., papaya, pawpaw, melon tree (English) papayier, arbre de melon (French) papaya, gedang (Indonesia) papaya, betex katalah (Malaysia), malakor, loko, makuai thet (Thailand) (Brouk, 1975; Morton et al., 1977).

Description. A perennial, herbaceous plant, with copious milky latex reaching to 20 or 30 ft. (6 to 30 m), the stem to 10 in. (25 cm) thick, simple or branched above the middle and roughened with leaf-scars. Leaves, clustered around the apex of the stem and branches, have nearly cylindrical stalks, green, purple-streaked or deep-purple, 10 to 40 in. (25 to 100 cm) long; the left blade has 7 to 11 main and some secondary, irregular, pointed lobes and

prominent veins; leaf surface is yellow-green to dark-green above, paler beneath. Usually male and female flowers are born on separate plants, but hermaphrodite (bisexual) flowers often occur; and a male plant convert to a female after beheading. Flowers emerge singly or in clusters from the main stem among the lower leaves, the female short-stalked, the male with drooping peduncles 10 to 40 in (25 to 100 cm) long. Corolla is ½ to 1 in. (1.25 to 2.5 cm) long, with 5 oblong, recurred white petals. Fruit is extremely variable in form and size; it may be nearly round, pear-shaped, oval or oblong; that of wild plants may be as small as a hen's egg, white, in cultivation the fruit ranges from 5 in. (12.5 cm) to 2 ft. (60 cm) in length and up to 8 in. (20 cm) thick. The fruit's skin is smooth, relatively thin, deep yellow to orange or salmon-red, sweet and more or less mushy. The central cavity of the fruit is lined with a dryish, pulpy membrane to which adhere with numerous black, rough, peppery seeds, each with a glistening, transparent, gelatinous coating (Williams, 1975; Brouk, 1975; Morton et al., 1977).

Uses. The ripe papaya is eaten raw, cooked or preserved; the green fruit is cooked as a vegetable. Young papaya leaves are cooked and eaten as spinach. In the tropics, tough meat is tenderized by washing it with the diluted latex or wrapping it for a few hours in the bruised leafed, or by cooking the meat with papaya leaves or with the green fruit (Brouk, 1975). Papaya latex is an ingredient in chewing gum and confection. In some countries papaya is grown in sizeable plantations for the extraction of papain, a proteolytic enzyme present in the latex, collected mainly from the green fruit. Papain has uses in the beverage, food and pharmaceutical industries, in chill-proofing

beer, tenderizing meat, drug preparations for digestive ailments and treatment of gangrenous wounds. It is also used in bathing hides, degumming silk and softening wool (Morton et al., 1977).

Medicinal uses. Various parts of the plant have been used medicinally in different parts of the world. It is believed that the efficacy of treatment with *Carica papaya* is dependent on the quantity of the different compounds in the preparation. The quantity of the compound is also different in the fruit, latex leaves and roots and varies with the extraction method, age of plant part, and the cultivation and sex of tree (Morton et al., 1977). In the Northern Nigeria, a cold water decoction of the ripe fruit is used to control and calm mentally agitated individuals. Crushed leaves and seeds have been used for anthelmintic purpose and fever. In Iboland and Ghaha, the yellow red of the dried leaves is used to treat gastric problems (see reviewed by Gupta et al., 1990). Many reports have shown the pharmacological properties of papaya latex with anthelmintic activity and antifungal action (Giordani et al., 1993; Satrija et al., 1994). The unripe papaya has been shown to have antimicrobial and antioxidant activities (Osato et al., 1993). Studies with the alcoholic and benzene extract of papaya seeds have shown their antifertility effects in male and female rats (Lohiya et al., 1992; Hrasha and Chinoy, 1996).

Toxicity. The fresh latex is acrid and can cause severe eye inflammation. It can provoke irritation and blisters if it is allowed to remain in contact with skin. Papaya harvesters have to wear gloves and aprons or overalls to avoid dermatitis, the latex will digest the tissue and cause sores. Internally, it is a severe gastric irritant and has been employed in malicious

poisoning. Some people are acutely allergic to any parts of the papaya plant such as, its pollen, fruit and latex. Particularly sensitive persons react to meat tenderized by papain and to papain administered in any form or manner as medication. Pharmacists may experience rhinitis, asthma and other allergic reactions from handling papain preparations (Morton et al., 1977).

Chemistry. As discussed earlier, *Carica papaya* contains many biologically active compounds, the level of compounds vary in fruit, latex and roots. In addition, plant parts from male and female trees, or the cultivation can also cause a variation in the quantity of compounds (Morton et al., 1977). Biochemical information of *Carica papaya* is shown in Table 1.

Apart from nutritional substances, many biologically active compounds in *Carica papaya* have been reported. Papaya leaves contain glycoside, carposide, and alkaloids (will be discussed later). Fresh leaves latex contains 75% water, 4.5% caoutchouc-like sbstance, 7% pectinous matter and salts, 0.44% malic acid, 5.3% papain, 2.4% fat, and 2.9% resin. The fatty oil of seeds contains 16.97% saturated acids (11.3% palmitic, 5.25% steraric, and 0.31% arachidic), and 78.63% unsaturated acids (76.5% oleic, and 2.13% linoleic). The seeds, air-dried, yield 660-760 mg/100 g of bactericidal aglycone of glucotropaeolin benzyl isothiocyanate (BITC), a glycoside, sinigrin, the enzyme myrosin, and carpasemine (Morton et al., 1977; Sharma and Ogbeide, 1982)

Table 1 The biochemistry of various parts of *Carica papaya* L.

Biochemistry	Per 100 gm			
	Green fruit	Ripe fruit	seeds	leaves
Calories	26	32-45	-	74
H_2O (gm)	92.1	87.1-90.8	-	77.5
Protein (gm)	1.0	0.4-0.6	24.3	7.0
Fat (gm)	0.1	0.1	25.3	2.0
total carbohydrate (gm)	6.2	8.3-11.8	32.5	11.3
Fiber (gm)	0.9	0.5-0.9	17.0	1.8
Ash (gm)	0.6	0.4-0.6	8.8	2.2
Mineral (mg)				
Ca	38	20-24	-	344
Fe	0.3	0.3-0.7	-	0.8
P	20	15-22	-	142
Na	7	3-4	-	16
K	215	221-234	-	652
Other chemistry				
β-carotene equivalent (μg)	15	710-1,050	-	11,565
thiamine (mg)	0.02	0.03-0.04	-	0.09
riboflavin (mg)	0.03	0.03-0.05	-	0.48
niacin (mg)	0.3	0.3-0.4	-	2.1
ascorbic acid (mg)	40	52-73	-	140
Vitamin E (mg)	-	-	-	136

(Source: Sharma and Ogbeide, 1982, pp. 872)

The alkaloid of *Carica papaya*. Three alkaloids have been isolated from alcoholic extracts of *Carica papaya*. They are carpaine, pseudocarpaine, and nicotine. There is also an unidentified alkaloid (see reviewed by Gupta, 1990). Carpaine is the major alkaloid found in *Carica papaya* and occurs in all green parts of the tree (see reviewed by Burdick, 1971). The yield of carpaine from Nigerian, *Carica papaya* is of 0.0115% of the dried weight of the leaves as opposed to 0.2% in Malayan plants and the 0.4% in Hawaii plants (reviewed by Burdick, 1971; Gupta, 1990). Several chemical derivatives of carpaine are known, such as methyl carpaine, ethyl carpaine, carpaine chloroauate, carpaine hydrochloride, nitro-carpaine and dehydrocarpaine I and II (see reviewed by Burdick, 1971; Gupta, 1990).

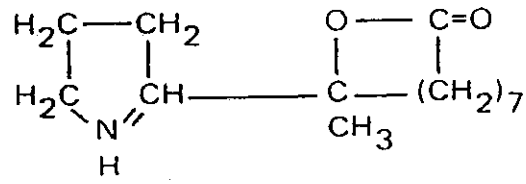
Its chemistry and pharmacology have pose many interesting and perplexing problems since its first isolation by Greshoff in 1890 (see reviewed by Burdick, 1971). The following are a review of some studies of structural of carpaine alkaloid.

1890 Carpaine was first isolated by Greshoff, after then Merck & Company reported the structure of carpaine as $C_{14}H_{27}NO_2$ (see reviewed by Pellletier, 1970).

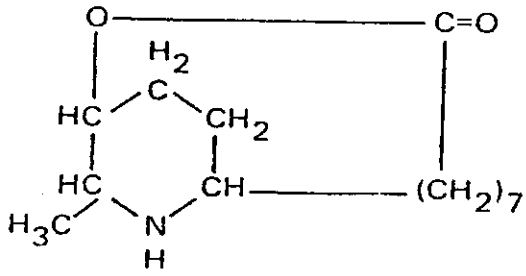
1953 Rapoport and co-worker reinvestigated the structure of carpaine and found that it was $C_{14}H_{50}N_2O_4$ (see reviewed by Pellletier, 1970).

1964 Spiteller-Friedmann and Spiteller proposed the structure of carpaine as $C_{14}H_{25}NO_2$.

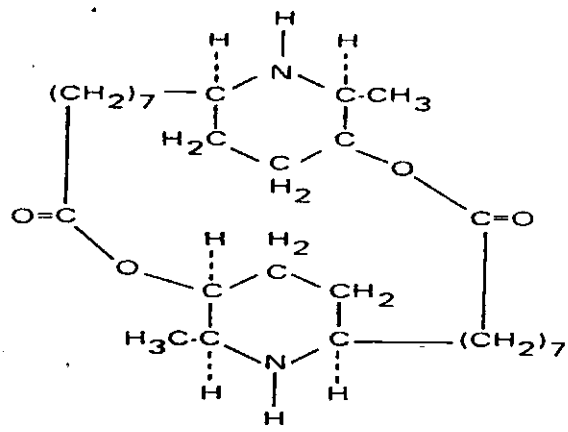
1969 Coke and Rice reported that the structural formula of carpaine was $C_{28}H_{50}N_2O_4$. This structural formula has been established to the "absolute configuration" with accept to the right formula structure until now (see reviewed by Burdick, 1971).



Carpaine, according to Barger et al.



Carpaine, according to Rapoport et al.



CARPAINE (1969)

Figure 17. The chemical formula of carpaine alkaloid (Source: see reviewed by Burdick, 1971, pp. 364)

Pharmacological Properties of Carpaine. Action of carpaine are quite similar to those of both digitalis and emetine. However carpaine possesses no most of their bad side effects (reviewed by Burdick, 1971). Van Ryn, (1987) (Hurnick et al., 1978; reviewed by Gupta et al., 1990) reported that carpaine and pseudocarpaine have a dose-dependent action on heart depression. Plain muscle is generally depressed. The uterus shows marked relaxation by this alkaloid (reviewed by Burdick, 1971). Studies with alcoholic papaya leaves extract, it induced a dose-dependent sedative effect and also induced central muscle relaxation (Gupta et al., 1990). Carpaine is shown to be a peripheral vasodilator and can decrease blood pressure in intact animal (Mulkijanyan et al., 1991). To and Kyu (1934) reported that carpaine causes respiratory depression, and may be effective in the treatment of amoebic dysentery (reviewed by Gupta et al., 1990). Ramaswamy and Srisi, (1960) demonstrated its antituberculosis activity by demonstrating its activity on the inhibition of *Mycobacterium tuberculosis*. Furthermore, carpaine also have been reported to have anti-tumor activity and antihelmintic activity (reviewed by Burdick, 1971).