CHAPTER 2

REVIEW LITERATURE

***Carica papaya* L.**

Family: Caricaceae

Genus: *Carica*

Common name: papaya, pawpaw, melon tree (English) papayier, arbre demelon (French) papaya, gedang (Indonesia) papaya, betex katalah (Malaysia) malakor, loko, makuai thet (Thailand)

Papaya have always held an attraction for people; there is a great economic importance to tropical regions where it is widely grown for its edible fruit and latex. *C. papaya*, a small group of trees with their leaves in the terminal clusters and latex vessels throughout their tissue. There are about 40 species of the genus *Carica* in the American tropics and subtropics and papaya originated in Southern Mexico and Central America. The early Spanish and Portuguese explorers carried it to the Caribbean and South East Asia during the Spanish exploration in the 16th Century. It distributed rapidly to India, Oceania, Africa, and today it is grown throughout the tropical and warmer subtropical areas of the world (Morton, 1977; Hartman et al., 1981).

Botanical Descriptions

C. papaya is a perennial, herbaceous plant, with copious milky latex reaching to 20 or 30 ft. (6-10 m) tall, the stem cylindrical to 10 in. (25 cm.) thick, hollow, usually unbranched above the middle and roughened with leaf-scars. Leaves, clustered around the apex of trunk and branches, have nearly cylindrical stalks, hollow, green, purple-streaked or deep-purple,10 to 40 in. (25 to 100 cm) long; the leaf 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) flower 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 is1/2 to 1 in. (1.25 to 2.5 cm) long, with 5 oblong, recurred white petals. Fruit is extremely variable in shape and size according to variety; 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 musky. 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; Cobley, 1976; Morton, 1977).

Uses

Papaya is cultivated for both fruit and latex. The ripe fruits are consumed as a fruit and as ingredient in jams, preserves, or cooked in various ways; the unripe fruit is cooked as a vegetable (Brouk, 1975). 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 a few hours in the bruised leafed, or by cooking the meat with papaya leaves or with the green fruit (Morton, 1977). Small latex vessel ramify throughout all the tissues of the plant and exude latex contain two protease, papain and chymopapain when they are cut. These proteolytic enzymes are harvested with the latex by scoring or injuring the unripe fruit. It is collected in earthenware and then dried and sold in pulverized form. Papain has been used in the beverage, food and pharmaceutical industries: in chill-proofing beer, tenderizing meat, drug preparations for digestive ailments and treatment of gangrenous wound. It is also used in bathing hides, degumming silk and softening wool. Papain is also used in the manufacture of easily digestible children's food and in chewing gum (Brouk, 1975; Cobley, 1976; Morton, 1977).

Medicinal uses

Various parts of the plant have been used in traditional medicine in different parts of the world. The efficacy of treatment with *C. 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, 1977). In West and Central Africa, the fruit extracts are used by traditional healers for the treatment of hypertension and for the prevention of miscarriages in women (personal communication), and is known to produce uterine relaxation in the rat (reviewed by Eno et al., 2000). In the Northern Nigeria, a cold water decoction of the ripe fruit is used to control and calm mentally agitated individuals (Gupta et al., 1990). The unripe papaya has been shown to have antimicrobial and antioxidant activities (Osato et al., 1993). Moreover, the methanol extract of unripened fruit markedly depressed the blood pressure and heart rate in rats (Eno et al., 2000). Many reports have shown the pharmacological properties of papaya latex with anthelmintic activity, antifungal action and

bacteriostatic effects on a number of infectious organism (Giordani et al., 1991; Satrija et al. 1994; Cherian, 2000). The latex has many applications in folk medicine. It is used as a styptic and vermifuge, an anti-chigger application and remedy for freckles, warts, corns, ringworm, infected wounds, malignant tumors and the pain of burns. Small dose of latex and sugar are taken as a digestive, emmenagogue and for whooping cough. In India and Malaya, the latex is smeared on the mouth of the uterus to induce abortion (reviewed by Morton, 1977). Cherian (2000) have been shown that the crude papaya latex contain an uterotonic principle which can evoke sustained contraction of the uterus. Crushed leaves and seeds have been used for anthelmintic purpose and fever. In Ibolnd and Ghaha, the yellow red of the dried leaves is used to treat gastric problems (see reviewed by Gupta et al., 1990). Intraperitoneal injection of alcoholic extract of papaya leaf was shown to be effective as sedative, central muscle relaxation and anti-convulsant property in rat (Gupta et al., 1990). Papaya seeds are used against intestinal parasite in humans and farm animals in India, Central and South America and elsewhere. The extracts of papaya seeds have been shown to be effective against helminthes *in vitro* and in infected animals (reviewed by Wilson et al, 2002). Studies with the chloroform, alcoholic and benzene extract of papaya seeds have shown their antifertility effects in male rats, mice and rabbits. The reversal of fertility occurred within 15 days to a month, and the compound was free of side effect (reviewed by Lohiya et al., 2000). Furthermore, the pentane extracts of papaya seed caused relaxation of mammalian vascular smooth muscle (Wilson et al, 2002). Sripanidkulchai et al. (2001) reported that the decoction of papaya root is employed by practitioners of Northeast of Thailand for treatment of dysuria. A similar extract also showed diuretic activity in rats. The diuretic activity of *C. papaya* may be due to the high salt content of its extract (Sripanidkulchai et al., 2001).

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 should 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 parts of the papaya plant such as, its pollen,

fruit and latex. Particularly, sensitive persons react to meat tenderized by papain administration in any form or manner as medication. Pharmacists may experience rhinitis, asthma and other allergic reactions from handling papain preparations (Morton, 1977).

Chemistry

As discussed earlier, *C. papaya* contains many biologically active compounds, the level of compounds varies in fruit, latex, seed, leave and root. In addition, plant parts from male and female tree, or the cultivation can also cause a variation in quantity of compounds (Morton, 1977). Biochemical information of *C. papaya* is shown in Table 1.

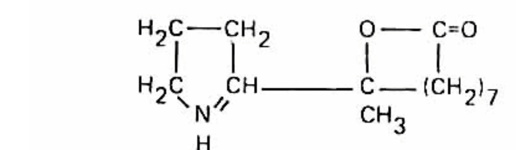
Apart from nutritional substances, many biologically active compounds in *C. papaya* have been reported. Papaya leaves contain glycoside, carposide, and alkaloids (will be discussed later). Fresh leaves latex contains 75% water, 4.5% caouchouc-like substance, 7% pectinous matter and salts, 0.44% malic 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% arachicic), 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 isotiocyanate (BITC), a glycoside, sinigrin, the enzyme myrosin and carpasemine (Morton, 1977; Sharma and Ogbide, 1982).

The alkaloids of *C. papaya*

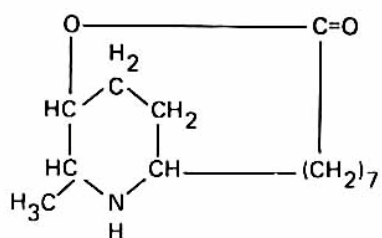
Four alkaloids have been isolated from alcoholic extracts of *C. papaya*. They are carpaine, psuedocarpaine and nicotine and an unidentified alkaloid (see reviewed by Gupta et al., 1990). Choline, a quaternary alkaloid also found in this plant. The yield of choline was 0.02% of the dried leaf weight (Ogan, 1971). Carpaine is the major alkaloid found in *C. papaya* and occurs in all green parts of the tree and in the seeds (Burdick, 1971). The yield of carpaine from Nigerian *C. papaya* was 0.0115% of the dried weight of leaves as opposed to 0.2 % in Malayan plants (Ogan, 1971) and 0.4% reported by Burdick (1971). However, Tang (1979) isolated dehydrocarpaine I and II in the higher concentrations than carpaine from the leaves of Hawaii plants. In this regard, it is suggested that the yield of carpaine in the different papaya varieties (Tang, 1979), age of the used leaves (Ogan, 1971), the region where this plant grows (Morton et al., 1977) are taken into consideration, together with other possible

factors. Apart from carpaine, pseudocarpaine, nicotine and unidentified alkaloids have been found in papaya leaves (Gupta et al., 1990); however, the yield of these alkaloid extracts has not been published. Furthermore, several chemical derivatives of carpaine are known, such as pseudocarpaine, a stereoisomer (Govindachari et al., 1965), dehydrocarpaine I and II (Tang, 1979) and carpaine hydrochloride, (Table 2) (reviewed by Burdick, 1971).

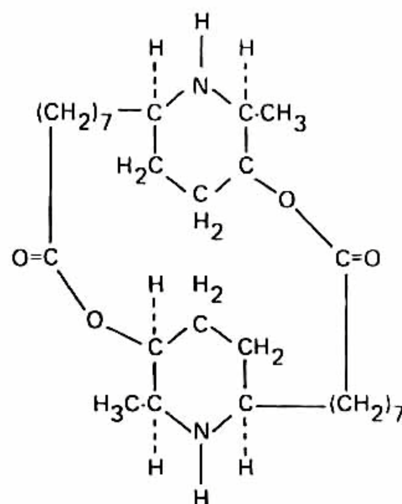
The alkaloid carpaine ($C_{28}H_{50}N_2O_4$) consists of two identically substituted piperidine rings linked together by two ester groups. First isolated in 1890 from papaya leaves by Greshoff, the determination of the structure of carpaine was not completed until 1965 by Coke and Rice (reviewed by Burdick, 1971; Tang, 1979). The following is a review of some studies of structural of carpaine alkaloid.



Carpaine, according to Barger et al. (1937)



Carpaine, according to Rapoport et al. (1953)



Carpaine, according to Coke and Rice (1965)

Figure 2. The chemical formula of carpaie alkaloid (Source: reviewed by Burdick 1971, pp. 364).

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

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

Biochemistry	Per 100 g			
	Green fruit	Ripe fruit	seeds	leaves
Calories	26	32-45	-	74
H_2O (g)	92.1	87.1-90.8	-	77.5
Protein (g)	1.0	0.4-0.6	24.3	7.0
Fat (g)	0.1	0.1	25.3	2.0
total carbohydrate (g)	6.2	8.3-11.8	32.5	11.3
Fiber (g)	0.9	0.5-0.9	17.0	1.8
Ash (g)	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 chemicals				
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)

1893 van Rijn corrected this to $C_{14}H_{25}NO_2$ and the chemical identity was well established some 40 years ago.

1937 Barger and co-worker investigated various degradation products of carpaine and isolated carpamic acid ($C_{14}H_{27}NO_3$) and the structure formula carpaine ($C_{14}H_{25}NO_2$) was proposed as a lactone containing 7 methylene groups attached to a pyrolidine nucleus.

1953 Rapoport and co-worker reinvestigated the structure of carpaine and found that it has a piperidine structure instead of the pyrolidine.

1964 Spiteller - Friedmann and Spiteller using mass spectrometry showed that molecular weight of carpaine ($C_{28}H_{50}N_2O_4$) was 478, which the structure formed a 26-membered cyclic diester.

1965 Coke and Rice accomplished the "absolute configuration of carpaine" (reviewed by Burdick, 1971).

Pharmacological properties of carpaine.

Action of carpaine are quite similar to those of both digitalis and emitine. However carpaine possesses no most of their bad side effects (reviewed by Burdick, 1971). Tuffley and Williams (1951) found that carpaine reduces blood pressure, heart rate movement of the intestinal strips. It also causes the uterus marked relaxation and the bronchioles dilatation. Van Ryn, (1987) (reviewed by Gupta et al., 1990) reported that carpaine and pseudocarpaine have a dose-dependent action on heart depression. Plain muscle is generally depresses. Studies with alcoholic papaya leaves extract has shown a dose-dependent sedative effect and central muscle relaxation (Gupta et al., 1990). Carpaine is shown to be 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 on the inhibition of *Mycobacterium tuberculosis*. Furthermore, carpaine also has been reported to have anti-tumor activity and antihelmintic activity (see reviewed by Burdick, 1971).

Table 2. Chemistry properties of carpaine and its derivative alkaloid from the leaves of *C. papaya* (Source: Chapman & Hall. Dictionary of Natural Products on CD-ROM, 1998).

Name	Derivative synonym	Molecular Formula	Molecular Weight	Physical Description	Melting point (^oC)
Carpaine	-	$C_{28}H_{50}N_2O_4$	478.714	Cubes	119-120
	1,2-dehydro Dehydrocarpaine I	$C_{28}H_{48}N_2O_4$	476.698	Viscous oil	-
	1,1',2,2'-Tetradehydro Dehydrocarpaine II	$C_{28}H_{46}N_2O_4$	474.682	Viscous oil	-
	2-Epimer Psuedocarpaine	$C_{28}H_{50}N_2O_4$	478.714	Crystal	65-68
	2-Epimer; hydrochloride	-	-	Crystal	295

The Uterus

Anatomy of Uterus

Uterus is in front of ileum and behind urinary bladder. It is the organ that is composed 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 3).

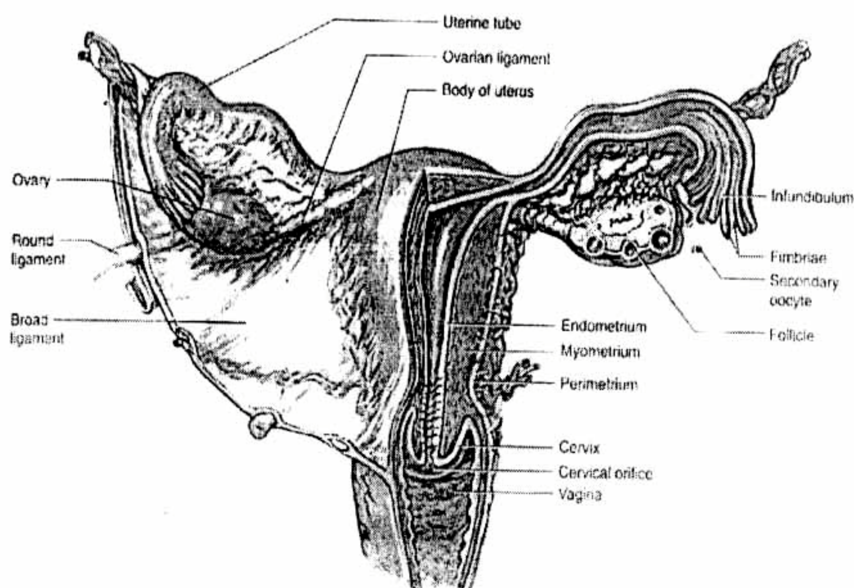


Figure 3. The funnel-shape infundibulum of human uterine tube partially encircles the ovary (Source: Shier et al., 1999, pp. 857)

The body of the uterus consists of three layers: perimetrium, myometrium and endometrium. The perimetrium is outer serous coat, consisting of peritonium that supported by thin layer of connective tissue. The myometrium is the middle muscular coat, become 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 menstrual 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 passing 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 supplied the uterus may involve vasodilation and uterine relaxation.

Parasympathetic innervation originates in the S_2 through S_4 spinal segments and passes through the splanchnic nerves to the inferior hypogastric/uterovaginal plexus. The major function of 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 is composed of special smooth muscle. During pregnancy it can greatly expanded which is reversible (review 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 known, but specializd 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 pacemaker cells.

The action potentials then spread throughout the uterus via the low resistance pathway called gap junction (Cole et al., 1985).

The contractility of the uterus is dependent on the membrane potential (E_m) and the permeability of the channel for the particular ion. At the resting membrane potential (RMP), the concentration of Na^+, Ca^{2+} and Cl^- are higher outside the cell while K^+ concentration is higher inside the cell (Monga and Sanborn, 1992; Saborn, 2000). Na^+, K^+, Cl^- and Ca^{2+} are the most important ions, which can affect the E_m. For each ionic species distributed unequally across the cell membrane by the concentration gradient for that particular ion and the membrane potential difference. Therefore, equilibrium potential (E_i) can be calculated by the Nernst equation,

$$E_i = -RT/zF(\ln C_i/C_o)$$

where C_i is the internal concentration of the ion, C_o is the extracellular concentration, R is the gas constant (8.3 J/mol.^{o}K), T is the absolute temperature in degree Kelvin (^{o}K = 273 + ^{o}C), F is the Faraday constant (96,500 coul/eq) and z is the valence (Sperelakis, 1995; ; Saborn, 2000). However, a better understanding of the E_m is obtained from the Goldman-Hodgkin-Katz constant -field equation, where concentration of the most important ions and their permeability are taken into account:

$$E_m = -RT/F[\ln((P_k[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_i) / (P_k[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_o))]$$

where P_k, P_{Na} and P_{Cl} are the membrane permeabilities for K^+, Na^+ and Cl^- respectively. However, when Cl^- is passively distributed, it is not considered because resting potential cannot be determined by Cl^-. As a result, equation can be reduced to

$$E_m = -2.303RT/F[\log(([K^+]_i + P_{Na}/ P_k [Na^+]_i / ([K^+]_o + P_{Na}/ P_k [Na^+]_o))]$$

Constant field equation that common use for calculating the E_m or P_{Na}/P_K ratio (Sperelakis, 1995). These calculated RMP is very close to the equilibrium potential for K^+ (Sanborn, 2000). In general, the resting membrane potential of myometrium is approximately -40 to -50 mV (Parkington and Colemman, 1990). It becomes more negative (-60 mV) during pregnancy and increases to approximately -45 mV near term (Monga and Sanborn, 1992).

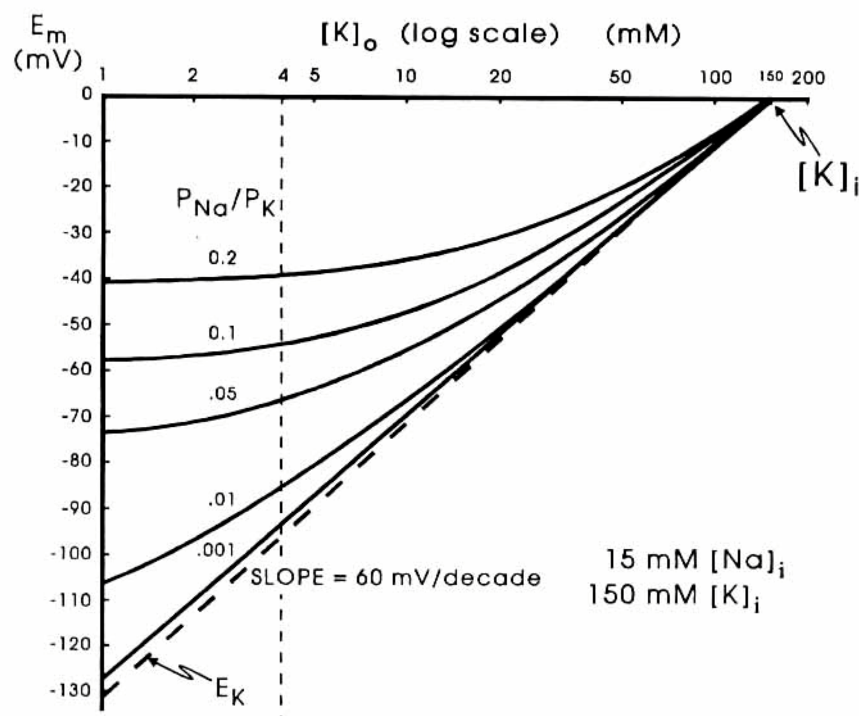


Figure 4. Theoretical curves calculated form the Goldman constant-field equation for resting potential (E_m) as a function of $[K]_o$. Family of curves is given for various P_{Na}/P_K ratios (0.001, 0.01, 0.05, 0.1, and 0.2). K^+ equilibrium potential (E_K) calculated from the Nernst equation (broken straight line). Curves calculated for a $[K]_i$ of 150 mM and a $[Na]_i$ of 15 mM. Calculations made holding $[K]_o$ + $[Na]_o$ constant at 154 mM; that is as $[K]_o$ was elevated, $[Na]_o$ was lowered by an equimolar amount. Change in P_k as a function of $[K]_o$ was not taken into account for these calculations. Point at which E_m is zero gives $[K]_i$. The potential reverse in sign when $[K]_o$ exceeds $[K]_i$. (Source: Sperelakis, 1995, pp. 73)

Contraction of smooth muscle is regulated by the cytosolic Ca^{2+} level and the sensitivity to calcium of contractile machinery in response to change in the environment surrounding the cell (Karaki et al., 1997; Riemer and Heyman, 1998). The rhythmic contraction of myometrium involves the alteration of the E_m 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 Coleman, 1990). This depolarization 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)

Contractile proteins

It is well known that 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 (150 A° in diameter) and thin filament is composed of actin molecule (60 A° in diameter). Smooth muscle myosin is a hexamer consisting of two heavy chain subunits (~200 kD) and two pairs each of 20-kD and 17-kD light chains. The heavy chains from α-helices and two globular heads that contain actin–binding site and adenosine triphosphate hydrolysis (ATPase) activity are present at the amino terminal end of the myosin molecule. 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). Myometrial actin molecules are globular (45-kD) and polymerized into a double helical strand and associated proteins such as tropomyosin, caldesmon and calponin (Monga and Sanborn, 1992). The effects of contractile proteins in the myometrium contraction will be described in more details in the biochemical pathways of myometrial contraction section.

Ion channels

Electrically excitable cells use gradients of inorganic cations like Na^+ and K^+ to generate electrical signals including the firing of action potentials. Voltage-gated ion channels mediate these ions flowing at rates close to diffusion limits (Varshey and Methew,

2003). The myometrium expresses a variety of ion-specific channels within the cell membrane that act to regulate the excitability of this smooth muscle.

Voltage-gated sodium channel. Na^+ channels are known to mediate the early increase in Na^+ flux underlying initial depolarization of action potential in many excitable cells. It is well known that Na^+ is a main component contributing to maintain of the RMP (Challis and Lye, 1994). Na^+ channels have been identified in the human and rat myometrium. Inoue and Sperakis (1995) reported that this channel increased the average current densities for Na^+ in longitudinal rat myometrial cell: the expression of these channels was found to progressively increase from midgestation until term. It is also found that the voltage-gated Na^+ channel mRNAs are expressed in the pregnant human, rat tissues and cells (Sanborn, 2000). Insertion of Na^+ channel into the membrane would increase the speed of propagation over the uterus and might also lead of an elevation of intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ (Inoue and Speralakis, 1995).

Calcium channels. The Ca^{2+} channel has a pore-forming α_1-subunit and several auxiliary subunits, including the β subunit on the cytoplasmic side and an β_2/δ subunit located extracellularly (reviewed by Wray et al., 2003). Two subtype 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; Wray et al., 2003). However only the L-type Ca^{2+} channel is considered to be a major Ca^{2+} influx pathway (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.

Potassium channels. K^+ channels are a diverse and ubiquitous group of ion channels that play an important role in the modulation of cell excitability. The efflux of K^+ is a mechanism for recovering (repolarization), maintaining, and/or enhancing (hyperpolarization) the RPM of the cells. The functions of these channels depend on the great variety of different K^+ channels that are cloned by numerous genes (reviewed by Kuriyama et al., 1998; Lawson, 1996; Wickenden, 2002).

Figure 5. Role 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 potassium efflux, and thereby eliminate the voltage potential driving the VOCs. DHPR, dyhydropyridine receptor; TEA, tetraethylammonium chloride (Source: Riemer and Heyman, 1998, pp. 5).

K^+ channel classifications and their pharmacological properties have been reviewed extensively (reviewed by Kuriyama et al., 1998; Wickenden, 2002). However, a few K^+ channel types have been identified in myometrial smooth muscle which exhibit differential hormone regulation and pregnancy stage (Sanborn, 2000; Kafali et al., 2002; Wray et al., 2003). The most common characterized are the Ca^{2+}-activated K^+ channels (K_{Ca}), delayed rectifier K^+-channel (K_v), adenosine triphosphate-sensitive K^+ channel (K_{ATP}) and inward rectifier K^+-channel (K_{ir}) (Piper and Hollingworth, 1995; Lowson, 1996; Sanborn, 2000; Kafali et al., 2002). K_v channels have been shown to be tetrameric assemblies, each subunit consisting of six transmembrane segments and contributing a re-entrant Pore-lining loop (P-loop) to the ion conduction pathway. This type of K^+ channel has a transmembrane segment (S4) in its structure that gives it voltage dependency (reviewed by Varshey and Methew, 2003). Other classes of K^+ channels, such as K_{ir} and K_{ATP} channels have a different architecture, with only two transmembrane segments and included P-loop per channel subunit (reviewed by Kuriyama et al., 1998; Varshey and Methew, 2003). Thus, the subtypes of K^+ channel that have been associated with synthetic and endogenous opener of these channels will be described as follows.

Ca^{2+}-activated K^+ channels (K_{Ca}). K_{Ca} channel are activated by membrane depolarization and by increase in $[Ca^{2+}]_i$. Three subtypes of K_{Ca} channels have been described on the basis of their single-channel conductance and sensitivity to specific pharmacological blocker (Cook, 1990; Kuriyama et al., 1998; Wickendel, 2002). Maxi-K^+ (big, high; BK_{Ca}) is sensitive to charybdotoxin and iberotoxin; intermediate conductance (IK_{Ca}) is blocked by high concentration of charybdotoxin and small conductance (SK_{Ca}) K^+ channels are potently blocked by apamin. However, Ca^{2+}-dependent K^+ channels (3 subtypes) are also voltage-dependent K^+ chennel (Lawson, 1996; Okabe et al., 1999; Kafali et al., 2002; Kuriyama et al., 1998). BK_{Ca} channels have been described in myometrial cells from a number of species, including humans (Lawson, 1996; Sanborn, 2000). Iberotoxin increased contractile activity in rat and human myometrium and depolarized human myometrial cells in association with an increase in $[Ca^{2+}]_i$ as a result of activation of voltage-sensitive L-type Ca^{2+} channel (Anwer et al., 1993). Tetraethylammonium (TEA) a nonselective K^+ blocker also

stimulates the mechanical activity of rat myometrial, most likely because of its ability to block K_{Ca} channel (Kafali et al., 2002). These data suggest that K_{Ca} channels are involve in setting both RMP and the shape and duration of the action potential in myometrium. Although, during labor, a BK_{Ca} which is channel insensitive to voltage or Ca^{2+}, and may permit an increase $[Ca^{2+}]_i$ without hyperpolarization, has been described in human myometrium (Sanborn, 2000).

Adenosine triphosphate-sensitive K^+ channels. K_{ATP} has been implicated in myometrial function, but has not been measured directly (Sanborn, 2000; kafali et al., 2002). The primary structure of typical K_{ATP} channels containing two transmembrane domains with intracellular ATP binding site. Activation of K_{ATP} channel increases K^+ efflux, resulting in hyperpolarization and decreasing the likelihood of action potential generation in the cell (Monga and Sanborn, 1992). These channels inhibited by steady-state ATP concentration, and their activity is also modulated by nucleotide diphosphates and by phosphorylation (Quast, 1993; Sanborn, 2000; Wickendel, 2002). K_{ATP} channel openers, cromakalim and levcromakalim depressed contractant-stimulated uterine contraction and were selective antagonized by glibenclamide. Although, they were more potent in nonpregnant than in pregnant rat and human myometrium (Piper and Hollingworth, 1995; Sanborn, 2000; Kafali et al., 2002).

Delayed rectifier K^+-channels. The predominant K_v current in myometrium is a voltage-dependent, slowly activated delayed rectifier ($K_{v1.5}$). This K^+-channel gene was exhibited estrogen-dependent regulation (Sanborn, 2000). In addition, the K_v current was maintained the driving force for Ca^{2+} entry during activation of resting cells. These current was inhibited by 4-aminopyridine (4-AP), that exhibited slow recovery from inactivation (Okabe et al., 1999; Wickendel, 2002). According to measurement of E_m using patch-clamp method, TEA completely blocked this K^+ current in pregnant rat myometrium (Okabe et al., 1999).

Inward rectifier K^+-channels. K_{ir} has a different gating mechanism that involves the voltage-dependent plugging of the channel pore by intracellular Mg^{2+} and polyamines (Varshey and Mathew, 2003). In addition to K_v channel, data exist to support the

presence of inward rectifier (ROMK1 or $K_{ir1.1}$) in myometrium. The expression of the mRNA for K_{ir} is highest in midpregnancy in rat myometrium (Sanborn, 2000). Elevation of $[Ca^{2+}]_i$ was shown to activate an inwardly rectifying K^+ current, which was blocked by charybdotoxin (Wickendel, 2002).

Nevertheless, K^+ channels are activated by endogenous substances. Several endogenous openers such as β-adrenergic agonist, relaxin and other uterine relaxants that increase intracellular cyclic-adenosine mono-phosphate (cAMP), activate K_{Ca} channel. Cyclic-guanosine mono-phosphate (cGMP)-generating agents (e.g. NO) or NO itself which result in uterine relaxation. The mechanism which regulates the K^+ channel involved in receptor-channel coupling, channel phosphorylation or modification of critical sulfhydryl groups by oxygen radicals such as NO (reviewed by Wray, 1993; Lawson, 1996; Riemer and Heyman, 1998).

Chloride channel. Cl^--channels are membrane proteins that mediate passive transport of chloride ions across the lipid bilayer by forming an aqueous diffusion pore (Pusch and Jentsch, 1994). The chloride equilibrium potential in many smooth muscle is usually more positive than the resting membrane potential. Thus, activation of Cl^--channels lead to depolarization (Cl^- leaves the cell), enhanced excitability and contraction (Carl et al., 1996). A large number of different chloride channels belong to voltage-activated Cl^- (Cl_v) channels that were dependent on membrane potential. Cl_v channels were identified in myometrium from a number of species, including human (Carl et al., 1996; Pusch and Jentsch, 1994). Another important class of Cl^--channels is activated by elevated intracellular calcium, calcium-activated chloride (Cl_{Ca}) channel (Pusch and Jentsch, 1994; Wray et al., 2003). Cl_{Ca} channels have been described in rat myometrium stimulated with oxytocin. Therefore, these channels may be activated by Ca^{2+} release from SR, nevertheless, extracellular Ca^{2+} entry can also activate these channels. Increasing in $[Ca^{2+}]_i$ in cells gives to chloride current proportional to the Ca^{2+} current and this current is abolished by niflumic acid, an inhibitor of Cl^--channels (Wray et al., 2003).

Cell-cell communication

Excitation produced in a pacemaker cell must be communicated to neighboring cells to be effective, which requires highly developed cell-to-cell coupling (Challis and Lye, 1994; Wray et al., 2003). These intercellular (or gap) junctions are formed connexin proteins and organized in hexagonal channel connecting the cytoplasm of the adjacent cell. These channels provide sites for low-resistance electrical or ionic coupling between cells and provide a pathway for transport of metabolite (less than 1-1.7 kDa) directly between cells. Action potential is also rapidly propagated from cell to cell through tissues (Cole et al., 1985; Monga and Sanborn, 1992; Wray, 1993; John et al., 1994; Riemer and Heyman, 1998). In the uterus, connexin 43 (C_x43) appears to be the most abundant connexin, but others, including C_x45 and C_x26, have been identified (reviewed by Wray, et al., 2003). The function of gap junction is regulated by the number of gap junction (structural coupling), their permeability (function coupling) and their degradation (Cole and Garfield, 1986). The number of gap junction might be gestationally regulated. Stretch on the myometrial cell membrane also increases the number of gap junctions and connexins (reviewed by Wray et al., 2003).

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; Cloe and Garfield, 1986). Gap junction rapidly disappears after delivery by internallization, endocytocis and digestion resulting in a decrease in excitability and contractile function of myometrium smooth muscle (Monga and Sanborn, 1992).

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

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 pathway which increases 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

Under resting condition (–40 to –50 mV), the voltage-dependent Ca^{2+} channel is closed. Upon excitation, a rapid depolarization of the cell membrane follows, voltage-dependent Ca^{2+} channel is then opened, and Ca^{2+} enters the cell. This is followed by inactivation (closing) of the channel. The next depolarization will occurs when the Ca^{2+} channel reverse form inactivation and be ready to open form a resting (closed) conformation (as shown in Figure 6) (Vaghy, 1998). The L-type Ca^{2+} channel (now also referred to as Ca_v 1.2 channels) exhibits voltage-dependent inactivation with half inhibition at approximately 45 mV, but can be returned to the resting stages at more negative potential (Parkington and Colemman, 1990; Wray et al., 2003). Therefore, both membrane potential changes and increase intracellular Ca^{2+} inactivate the channel in term of negative feedback regulate channel activity (Sanborn, 2000). T-type Ca^{2+} channels are also voltage sensitive but open at more negative potentials than L-type Ca^{2+} channels (-60 mV compared with -40 mV, respectively), have a smaller conductance, and have peak currents at around -30 mV compared with +10 mV for L-type channels. They have been shown to be present in human uterine cells but not those of the rat. The presence of T-type Ca^{2+} channels is potentially very interesting as they have been associated with action potential transmission and pacemaker activity (Wray et al., 2003).

Agonist such as oxytocin and acetylcholine also open this channel by depolarizing the membrane through activation of nonselective cation channels and GTP-binding protein 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 CG28392 are the dihydropydridine. 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 results 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 drug have three modes, activated (open) mode, inactivated (close) mode and resting (close) mode. The dihydropydridine Ca^{2+} channel agonist (Bay K 8644) enhances Ca^{2+} 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 be their own receptors, each receptor type operates its own ion channels with their particular ions selectivity (Bolton, 1979). However, when it is operated, they allow the ionic 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 (reviewd 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).

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

The Ca^{2+}_i / Na^+_o exchange reaction exchanges one internal Ca^{2+} ion for 3 external Na^+ ion 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 form 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). Calcium release from the SR store can occur through inositol triphosphate gated channels or ryanodine gated channels. Inositol 1,4,5-triphosphate (IP_3) is generated when agonists such as oxytocin, bind to their receptors on the surface membrane. Ryanodine channels are physiologically activated by calcium itself giving rise to calcium induced calcium -release (CICR) (Boothman and Berridge, 1995; Kupittayanant et al., 2002; Teng et al., 1996; Wray et al., 2003). Both RyRs and $InsP_3R$ have a bell-shaped sensitivity to the cytosolic Ca^{2+} concentration; low Ca^{2+} concentration prompted more Ca^{2+} release (positive feed-back), at high Ca^{2+} concentration, Ca^{2+} release is inhibited (negative feed-back). (Boothman and Berridge, 1995; Riemer and Heyman, 1998; Karaki et al., 1997).

IP_3 sensitive store.

IP_3 levels increase when agonists bind to their G-coupled receptors and stimulate the hydrolysis of phosphatidylinositol. The IP_3-sensitive Ca^{2+} channel in the membrane of ER that composed of four identical subunits, each containing IP_3-binding sites in the large N-terminal cytosolic domain. When IP_3 binds to its binding site, it induces opening of this channel, allowing, Ca^{2+} ions to exit from SR into the cytosol (As shown in Figure 7) (reviewed by Riemer and Heyman, 1998; Wray et al., 2003). Nevertheless, IP_3-induced Ca^{2+} release can be modulated through its receptor, by Ca^{2+} (providing a negative feedback mechanism), by calmodulin (decreasing Ca^{2+} release), by cyclic guanosine monophosphate via its kinase, and the IP_3 receptor-associated cyclic guanosine monophosphate kinase substrate, which is found in uterus and other tissues and causes decreased release (Wray et al., 2003).

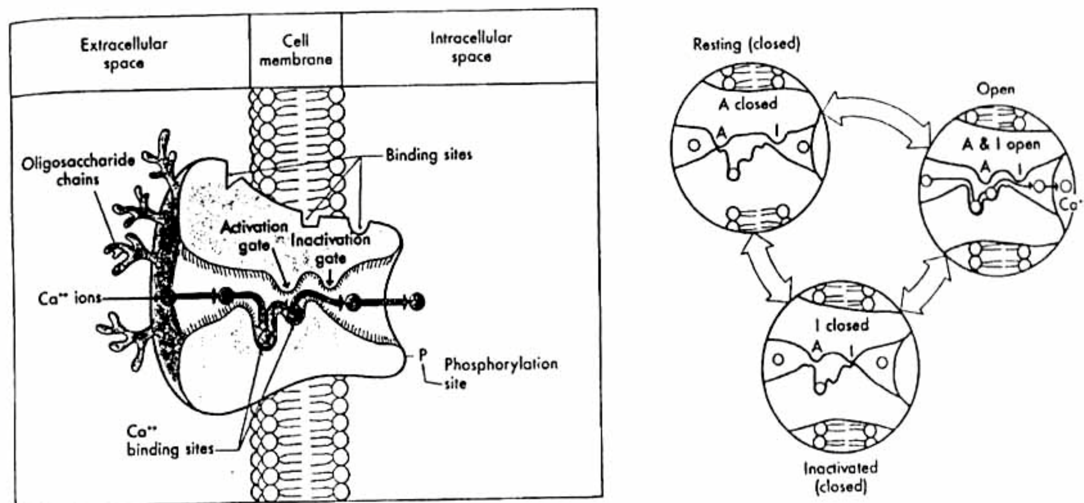


Figure 6. (A) Ca^{2+}-channel glycoprotein positioned in cell membrane. The ion channel is assumed to contain activation and in activation gates that are moved or altered by the potential diffrence across the membrane and drugs so as to open or close the channel to transmembrane flux Ca^{2+}. A site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

Ryanodine receptor mediated release of Ca^{2+} (RyRs)

This channel is sensitive to ryanodine, a 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). In many excitable tissues, Ca^{2+} can also be released from the SR by Ca^{2+} itself. In intact myometrium this process does not appear to operate, at least in a functionally discernable way. In addition, ryanodine has no appreciable effect on the increase in $[Ca^{2+}]_i$ seen in zero-Ca^{2+} solution, when agonists are applied to the uterus (Kupittayanant et al., 2002; Wray et al., 2003). Furthermore, caffeine, an agonist for CICR, failed to elicit either an increase in $[Ca^{2+}]_i$ or contraction in the myometrium (Kupittayanant et

al., 2002). This finding, however, may be due in part to the fact that, of the three types of ryanodine receptor, the one that is most highly expressed in the uterus, type 3, is most insensitive to caffeine (Kupittayanant et al., 2002; Wray et al., 2003). It appears that although the myometrium expresses RyRs, a translation to functional effects via CICR is not presented for any species studied so far (rat, human, and mouse), excluding studies on cultured cells (reviewed by Wray et al. 2003).

Store-operated channel (SOCs)

Elegant patch-clamping studied have revealed a certain plasma membrane Ca^{2+} channel a called store-operated channels (SOCs). The emptying of SR elicits Ca^{2+} entry via SOCs, a process known as capacitative or stroe-operated Ca^{2+} entry. 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 elevated cytosolic Ca^{2+} and that it may regulate contraction as well as refill the SR (reviewed by Lodish et al., 2000; Wray et al., 2003).

5. 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^{2+} sources. 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 site on the plasmamembrane.

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 (reviewed by Riemer and Heyman, 1998). The homeostatic mechanism can lower cytosolic Ca^{2+} from the cell by the sarcolemmal Ca^{2+}-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 8) (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+} exchange (reviewed by Karaki et al., 1997; Monga and Sanborn, 1992). In smooth muscle, there are two type of Ca^{2+} -ATPase pump, Ca^{2+}-ATPase sarcolemmal and SR Ca^{2+}-ATPase (as shown in Figure 9) (Karaki 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 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; Sperlakis, 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 activity 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.

Biochemical pathways of myometrial contraction

Contraction in smooth muscle is a result of the formation of covalent cross-linking bonds between actin and myosin filament. The consequences of increase in $[Ca^{2+}]_i$ levels in the myometrial smooth muscle cell are the binding of Ca^{2+} to protein calmodulin (CaM) and the resulting Ca^{2+}-CaM complex. The complex binds to myosin light-chain kinase (MLCK), conformation changes lead to the formation of activated MLCK (as shown in Figure 10)(reviewed by Wray, 1993; Horowitz et al., 1996; Wray, et al., 2003).

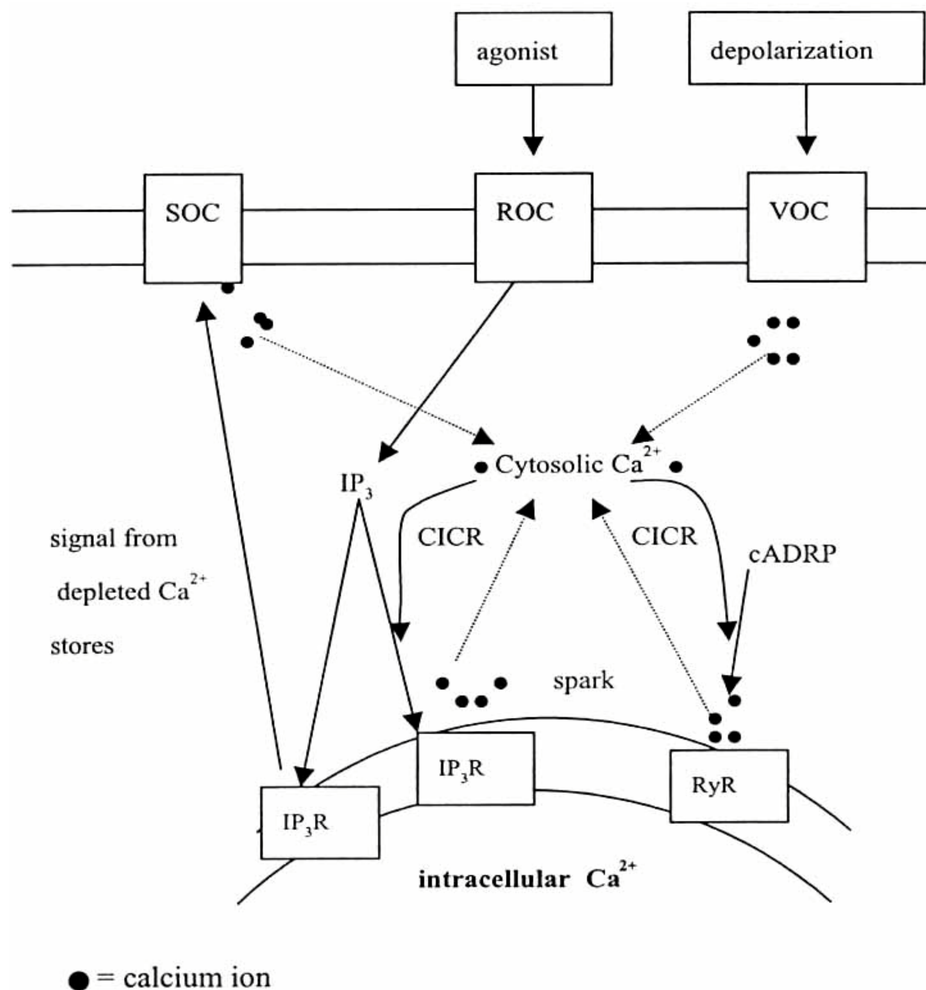


Figure 7. Mechanism of fine control of intracellular Ca^{2+} mobilization in smooth muscle; role of receptors/channels. When SR Ca^{2+} stores are depleted, IP_3R activation is coupled activation of store-operated Ca^{2+} channel, Ca^{2+}-induced Ca^{2+} release; ROC, receptor-operated Ca^{2+} channel; SOC, store-operated Ca^{2+} channel (Source: Rimer and Heyman, 1998, pp. 6).

Active MLCK phosphorylates the myosin 20-kD light chain in a specific serine at position 19. 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 (reviewed by Monga and Sanborn, 1992; Riemer and Heyman, 1998; Wray, et al., 2003). 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).

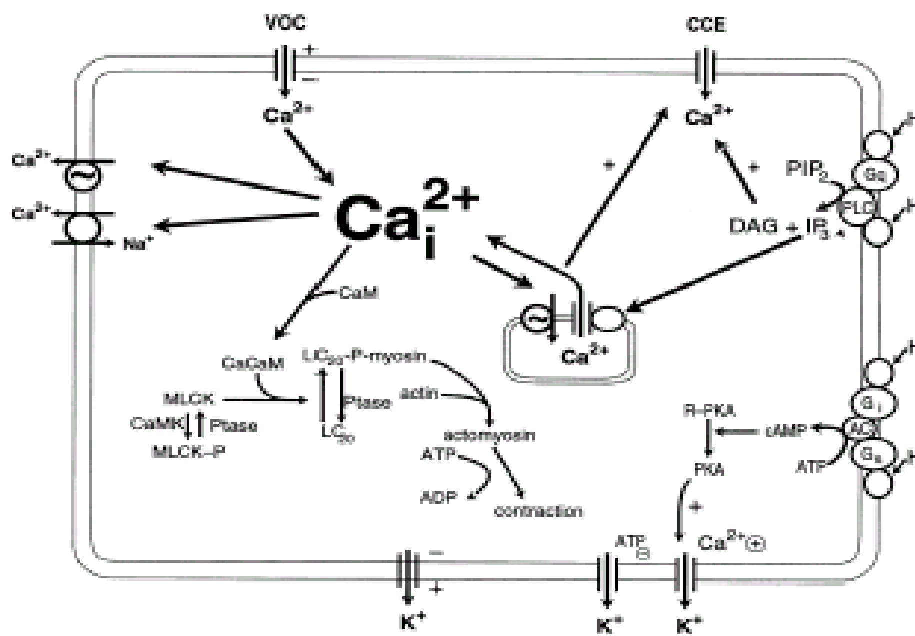


Figure 8. Schematic representation of pathways leading to control of Ca^{2+} in smooth muscle such as myometrium. Components include calmodulin (CaM), myosin light chain kinase (MLCK), myosin light chain (LC20), protein phosphatase (Ptase), L-type voltage-operated Ca^{2+} channel (VOC), capacitative channel entry (CCE), heterotrimeric G proteins (G), phospholipase C (PLC), inositol 1,4,5-triphosphate (IP_3), diacylglycerol (DAG), adenylyl cylase (AC), protein kinase A (PKA), Ca^{2+} transport adenosine triphosphate (ATP)ases, Na/Ca exchanger, Ca^{2+}-activated K^+ channel, ATP-sensitive K^+ channel, and voltage-sensitive K^+ channel. (Source: Sanborn, 2000, pp. 7)

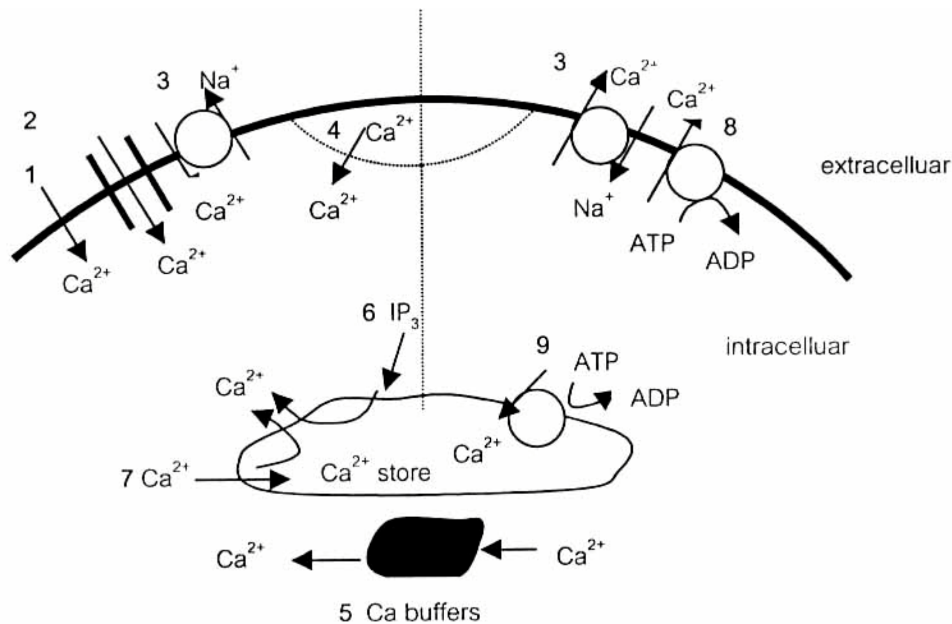


Figure 9. Summary of the route for the increasing (left) and decreasing (right) $[Ca^{2+}]_i$. 1) passive entry is simply leakage across myometrial sarcolemma. 2) Entry via ROC and VOC. 3) Na^+-Ca^{2+} exchange; in its normal mode of operation it will remove Ca^{2+} but can operate in reverse to bring 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 uuterus and is pH dependent. 5) Ca^{2+} buffers exist within smooth muscle cell, and their capacity will change under physiological and pathological condition (eg., 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 stroes. 7) Increased $[Ca^{2+}]_i$, eg., as result of VOC activation, may cause Ca^{2+} to be released from internal (Ca^{2+}-induced Ca^{2+} release). It is 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).

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 concentration has explained the contraction in term 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 11).

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

Ca^{2+} which rises in intracellular binding with CaM to form Ca^{2+}-CaM complex. However, an increase in intracellular Ca^{2+} able to activate Ca^{2+}-calmodulin dependent protein kinase II (CAM kinase II), PKA, or PKC greatly reduced MLCK activity by increasing the amount of Ca^{2+}-calmodulin required to activate it to 10-fold (Monga and Sanborn, 1992; Karaki et al., 1997; Wray, et al., 2003). The reduction in activity via CAM kinase II may be part of a temporal feedback mechanism limiting contraction, as Ca^{2+} first activats MLCK via calmodulin and then inactivate it via CAM kinase II (Monga and Sanborn, 1992; Wray, et al., 2003).

2. Inhibition of MLC–phosphatase (MLCP)

In addition to Ca^{2+} activation of MLC kinase, the state of MLC phosphorylation is further regulated by MLC phosphatase, which removes the high-energy from the light chain of myosin to promote smooth muscle relaxation (reviewed by Webb, 2003; Wray, et al., 2003). Thus it plays an importance role in determining the sensitivity of the contractile apparatus and changing in $[Ca^{2+}]_i$ (Wu et al., 1996; Wray, et al., 2003). The small G protein rho-A and its downstream target rho kinase play an important role in the regulation of MLCP. The rho kinase, a serine/threonine kinase, phosphorylates the myosin-binding subunit of MLCP, inhibiting its activity and thus promoting the phosphorelated state of the myosin light chain (reviewed by Webb, 2003; Wray, et al., 2003). The myometrium contains rho-1A, and it has been reported to translocate from the interior to the cell membrane with stimulation. Furthermore, it has been shown that rho kinase is expressed in human myometrium and that this expression increases with pregnancy (Wray et al., 2003). The other

mechanism for influencing MLCP, including agonist that activates phospholipase A_2 to cleave arachidonic acid, and mediator of DAG/ PKC, CPI-17 (Kiraki et al.,1997; Webb, 2003; Wray, et al., 2003).

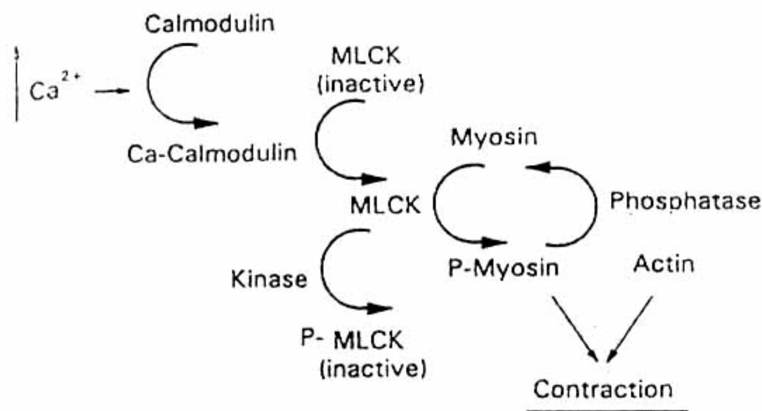


Figure 10. Scheme of contraction in uterine smooth muscle. Rise in $[Ca^{2+}]_i$, produced either spontaneously or by agonist, cause Ca^{2+} to bind to calmodulin (Ca-calmodulin). This then allows activation of enzyme myosin light-chain kinase (MLCK). This kinase phosphorylate light chains on myosin (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, eg., 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)

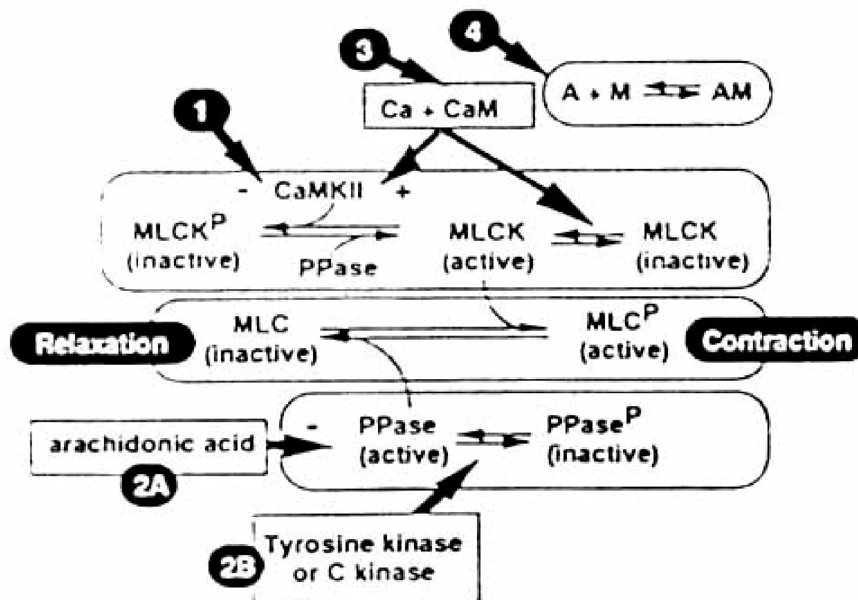


Figure 11. Mechanism of agonist-induced Ca^{2+} sensitization in smooth muscle. Stimulation of a receptor increase $[Ca^{2+}]_I$, activates MLC kinase (MLCK), phosphorylation of MLC, and induces contraction. This process is modulated by four different mechanisms. The first mechanism is the inhibition of Ca^{2+}- calmodulin-dependent protein kinase II (CaMKII), which phosphorylates MLC kinase and inhibits its activity (1). The secound mechanism is the inhibition of MLC phosphatase (PPase) (2). Arachidonic acid, produced by receptor-mediated activation of phospholipase A_2, may directly inhibit phosphstase (2A). C kinase and tyrosine kinase may also inhibt 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)

3. Activation of phosphorylation-independent mechanism

Actin-binding proteins, such as caldesmon and calponin, also appear to be able to regulate contraction. Both proteins have an inhibitory effect on the myosin ATPase. The inhibitory actions of caldesmon and calponin can be reversed by phosphorylation. Caldesmon can be phosphorylated by PKC, p21-activated kinase (PAK), and extracellular-regulated kinase-1 (ERK1). Calponin has been shown to associate with ERK and PKC during agonist stimulation. Thus, it is proposed that a part of the mechanism whereby agonists may modulate smooth muscle force reducing the inhibitory action of thin filament–associated proteins, and that this can occur by their phosphorylation and subcellular redistribution (Wray, et al., 2003).

4. Increase free calmodulin concentration

The regulation of Ca^{2+} -calmodulin complex on the kinase activity is well established. However, many studies suggested that 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 change the Ca^{2+}-sensitvity of contractile apparatus (Reviewed by Karaki et al., 1997).

Pathways to myometrial relaxation

For relaxation to occur, the signal to Ca^{2+} entry should be terminated, and myosin light chains should become dephosphorylated. As discussed earlier, changes in membrane potential lead to the opening of L-type Ca^{2+} channels and Ca^{2+} entry. This entry is curtailed by at least two mechanisms; inactivation of the Ca^{2+} channels and repolarization of the membrane potential. These processes are interlinked; ie, repolarization of the membrane will reduce Ca^{2+} entry because of deactivation of the channels even if no inactivation had occurred. Inactivation will reduce depolarization of the membrane because of a reduction in inward (depolarizing) current. There are two chief mechanisms of Ca^{2+} channel inactivation: Ca^{2+} dependent and voltage/time dependent. Both mechanisms are present in uterine cells (Wray, et al., 2003). Repolarization will occur as Ca^{2+} entry decreases and K^+ channel activity is stimulated, as discussed earlier. Activation of Ca^{2+} -dependent K^+ channels by propagating

Ca^{2+} waves has been recorded in human myometrial cells by using a combination of patch-clamping and digital imaging. There was a time delay between the peak of the Ca^{2+} wave and the actual increase in the K^+ channel activity (Young et al., 2001).

When $[Ca^{2+}]_i$ starts to decrease, the Ca^{2+}-calmodulin activation of MLCK is stopped as Ca^{2+} dissociates from calmodulin. The phosphorylated light chains are dephosphorylated by MLCP. Haeberle et al.(1985) were the first to demonstrate in skinned uterus that dephosphorylation of myosin light chains by MLCP produces relaxation. MLCP does not depend on Ca^{2+} for its activation, but its activity is regulated by phosphorylation of its subunits. There are three subunits of MLCP: the 110–130-kD myosin phosphatase targeting subunit (MYPT), the 37-kD catalytic subunit and a 20-kD variable subunit of unknown function (Reviewed by Webb, 2003; Wray, et al., 2003). Dephosphorylation of Ser-19 results in a much reduced (100 times) myosin ATPase rate, and hence cross-bridge cycling returns to basal levels. There is significant interest in the regulation of MLCP via phosphorylation of MYPT, as this is now considered a physiologically important route for calcium sensitization (Wray, et al., 2003). Furthermore, several mechanisms are implicated in the removal of $[Ca^{2+}]_i$ and involving the SR and the plasma membrane ,as discussed earlier.

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 change 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 contributed to the uterus is still not clear. It has been shown that postganglionic fibers innervate the uterus both in endometrium and myometrium layer, however, it does not appear to be close apposition of nerve ending at the myometrium

cells. It is suggested that the neuronal effect which modulated the contractility of uterus is less important than hormonal effect (reviewed by Wray, 1993; Rimer 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 (Robert et al., 1997; Williams et al., 1997). 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 stimulant on uterine contraction will be described more details in the uterine relaxant section.

1.2 Cholinergic receptor.

The role of parasympathetic innervation has received more attention to pain perception than motor activation and 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 have been identified which are M2 and M3 (Choppin et al., 1999; Kitazawa et al., 1999). These muscarinic receptor are G protein-coupled receptors linked to phosphoinositide break down, increasing IP3 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 hormones, estrogen and progesterone influence the contractility of the uterus through menstrual periods and pregnancy.

2.1 Estrogen and progesterone

Progesterone plays a role in maintaining the uterus in a quiescent stage, while estrogen plays a role in increasing the sensitivity of the contractile response (reviewed by Wray, 1993). In general, during late pregnancy, estrogen promotes 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 and Lye, 1994). Batra (1986) has 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+}. 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 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 while 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, the time at which 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 prevents hemorrhage. The hormone is also an important hormone for lactation (Challis and Lye, 1994). The action oxytocin on uterine contraction will be described more details in the uterine stimulant section.

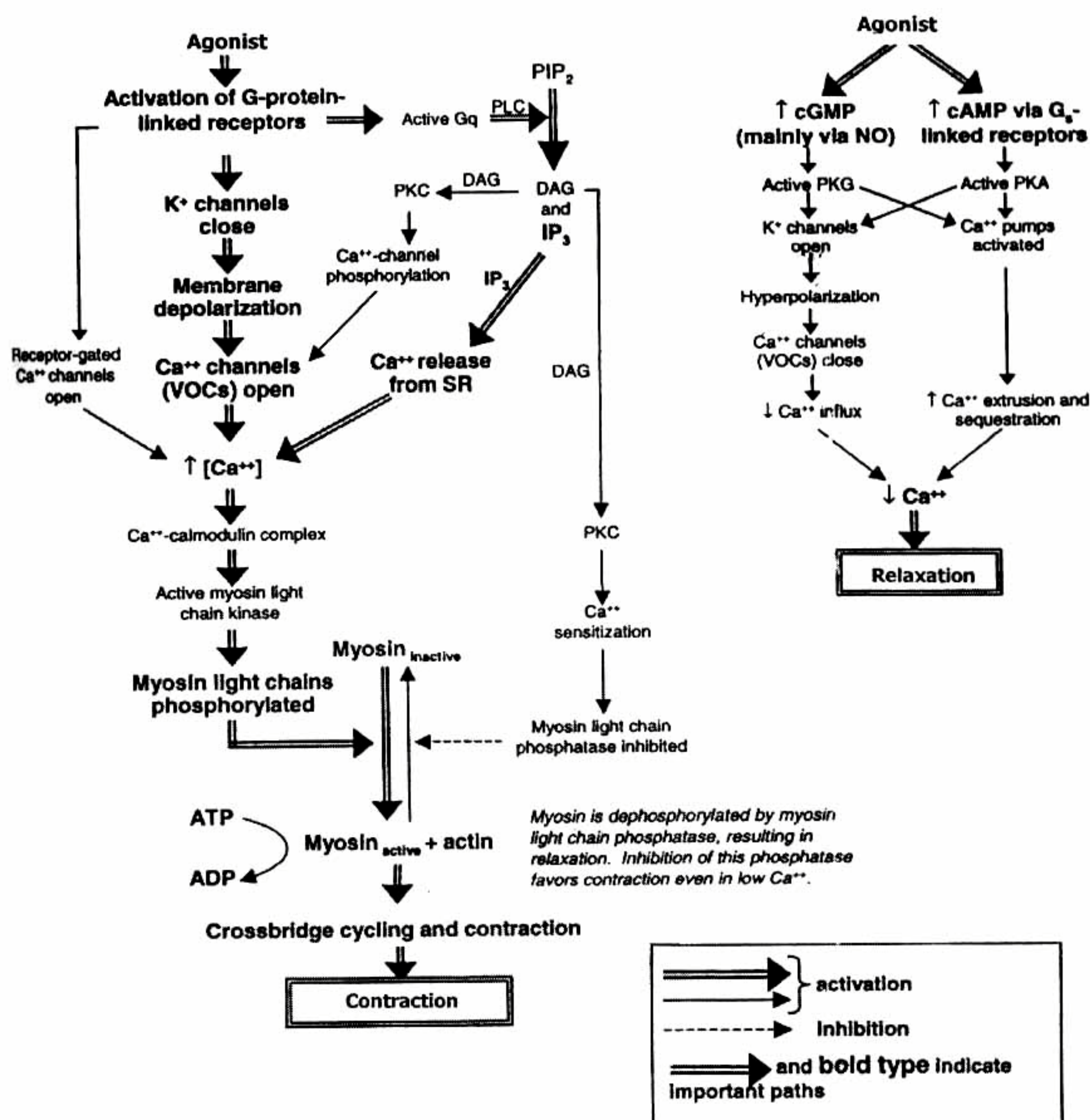


Figure 12. Mechanism of agonists-induced contraction and relaxation (Source: Poonpanang, 2004, pp 51).

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. $PGF_{2\alpha}$ stimulates uterine contraction by generating phosphatidylinositol-signal pathway which results in an increase of Ca^{2+} release from IP3 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 contracrile 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 enchancement 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 increase Ca^{2+} influx via nonselective cation channels leading to an activation of voltage-dependent L-type channels as a result of depolarization (Sakata and Karaki, 1992).

Agents inducing smooth muscle contraction

1. Role of $CaCl_2$ in the contraction of uterine smooth muscle

The factors that control intracellular calcium in uterine myocytes is crucial to elucidating the mechanism of labor, as uterine contraction are triggered by increases in $[Ca^{2+}]_i$. This is derived from Ca^{2+} entry through the surface membrane calcium channels, as well as Ca^{2+} releases from the SR, as already reviewed earlier.

2. Oxytocin-induced contraction

Myometrial oxytocin receptors are functionally coupled to $Gq/11_{\alpha}$ class GTP binding proteins that stimulate together with $G_{\beta\gamma}$ the activity of phospholipase C-β isoforms. This leads to the generation of inositol trisphosphate (IP_3) and 1,2-diacylglycerol (DAG). IP_3 triggers Ca^{2+}release from intracellular stores, whereas DAG stimulates protein kinase C, which phosphorylates unidentified target proteins. Finally, in response to an increase of $[Ca^{2+}]_i$, a variety of cellular events are initiated (Monga and Sanborn, 1992; Rall 1991; Gimpl and Fahrenholz, 2001).

Oxytocin-induced $[Ca^{2+}]_i$ increase is greater in the presence of extracellular Ca^{2+} than that in its absence. This suggests that oxytocin has also effects on calcium influx through voltage gated or receptor-coupled channels. 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 (Vaghy, 1998; Gimpl and Fahrenholz, 2001). 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). This could sustain transient increases in intracellular Ca^{2+} concentrations and thereby prolong the effects of oxytocin (Gimpl and Fahrenholz, 2001).

Oxytocin can also induce uterine contraction in term of "Ca^{2+} independent-induced contraction". It has been known for several years that oxytocin and some agonists (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^{2+}, but this contraction is persistence for many hours (reviewed by Wray, 1993). The contraction 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.

3. KCl-induced contraction

Depolarization of the cell membrane is achieved by increasing K_e over the threshold level which is approximately 40 mM concentration of K^+. An increase in the K_e will change the E_m and this will increase probability of other important channels such as the Na^+, Cl^-, and Ca^{2+} channels to open. Opening the K^+ channels can no longer close VOC. Since the extracellular concentration of Ca^{2+} is high, Ca^{2+} will enter the cell and induce contraction (reviewed by Riemer and Heyman, 1998). However, in the permanent presence of high K_e, most of the K^+ channels cannot affect the E_m before the K_e concentration is decreased and the VOC become closed. When K_e is decreased gradually, these K^+ channels will be effective again as the E_m changes closer and closer to the resting potential and the cell membrane becomes hyperpolarized (Quast, 1993; Riemer and Heyman, 1998).

High-K^+ solution induced uterine contraction with biphasic contraction. The initial peak of contraction is called phasic contraction. When the initial peak reaches a maximum, it is slightly declined to a relative steady at low level. This steady state is called tonic contraction (Bolton, 1979). The relative sizes of the "phasic" and "tonic" components vary, depending among other factors on the concentration of potassium applied. However, low-K^+ concentrations (20-40 mM KCl), are known to produce partial depolarization and cause a rise in tension that was maintained in rat uterus (Bolton, 1979; Piper and Hollingsworth, 1995). It is plausible, although it has not been shown in detail, that the tension produced by High-K^+ declines in uterus after initial peak, because depolarization is sufficient to abolish action potential discharge and maintained depolarization is less able to generate tension. Whereas, with low-K^+ concentration, action potential discharge is continuous and tension is better maintained (Bolton, 1979). The uterine contraction activation by high-K^+ can prolonged its effect on tonic contraction because it reduces the potassium gradient across the

membrane (Bolton, 1979). Nevertheless, this 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^+ increases transmembrane Ca^{2+} influx, then increases $[Ca^{2+}]_i$ and results in contraction (Kalsner, 1997).

Although the KCl-induced contraction is mainly mediated by Ca^{2+} influx via voltage-sensitive channel, some 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 process leading to an increase in the Ca^{2+} sensitivity of the contractile machinery by a mechanism independent of extracellular Ca^{2+}.

Agents inducing uterine smooth muscle relaxation

1. K^+ -channel openers

As Ca^{2+} increases in myometrial cells and promotes contraction, it will activate the K_{Ca} -channel, resulting in K^+ efflux and promoting repolarization (Sanborn, 2000). The efflux of K^+ is a mechanism for recovering (repolarization, maintaining (clamping) and/or enhancing (hyperpolarization) the resting potential of the cell. Thus, the opening of K^+-channels is a physiological means for counteracting, restricting, or preventing depolarizing activity caused by inward currents, due to entry of Ca^{2+} and Na^+ and the efflux of Cl^- ions (Karaki et al., 1997; Lawson, 1996; Quast, 1993). Thus, the relaxation of uterine smooth muscle via K^+ channel activation may play an important role in the treatment of premature labour and dysmenorrhoea.

The standard criteria that initially identified a compound as a K^+ channel opener has been its ability to relax an *in vitro* smooth muscle preparation contracted with low, but not high, concentrations of extracellular K^+ ions (Lowson, 1996; Quast, 1993). The opening of K^+ channel by these compounds and subsequent efflux of K^+ ions from the cytosol leads to membrane repolarization and/or hyperpolarization (Lowson, 1996; Quast, 1993;

Karaki et al., 1997). This change in membrane potential is followed by a reduction in cytosolic free Ca^{2+} and/or an inhibition of mechanism producing increase in cytosolic free Ca^{2+}. The outcome of these effects is a reduction in membrane and cell excitability, resulting in a greater cellular resistance to activation by excitatory stimuli. Although, it was assumed that hyperpolarization caused by K^+ efflux produced closure of VOCs, preventing depolarization-induced Ca^{2+} entry into the cell, but other mechanisms may also contribute to the effects produced by K^+ channel openers (Lowson, 1996). The mechanism of action of K^+ channel openers at the cellular level is complex and has been reviewed extensively (Lawson, 1996; Quast, 1993, Karaki et al., 1997; Kuriyama et al., 1998). Thus, the mechanism of hyperpolarization mediated by K^+ channel openers may be summarized as follows: 1) preventing depolarization -induced Ca^{2+} entry into the cell; 2) reduced the uptake into SR and inhibited the release of Ca^{2+} from the SR; 3) inhibition of the production of IP_3 and, hence, Ca^{2+} release from intracellular stores; 4) linked with a reduction in the sensitivity of contractile elements to Ca^{2+}; and 5) interact with an enzyme system involved in intracellular phosphorylation (Lowson, 1996; Quast, 1993).

K^+ channel comprises the most diverse group of ion channels and a large number of agents have been shown to modulate their activities. Therefore, classification of K^+ channels opener has largely defined as a consequence of their biological effects being sensitive to block (Quast, 1993; Lawson, 1996; Wickenden 2002; Karaki et al., 1997; Kuriyama et al., 1998). However, the determination of a common site of action for these compounds is complicated by at least; the existence of subtypes of K^+ channel, evidence of these compounds opening several K^+ channel and the sensitivity to K^+ channel blocker (Lawson, 1996). It may be divided into three basic groups: K_{ATP} channel openers, K_{Ca} channel openers and endogenous channel openers (Lawson, 1996)

K_{ATP} channel openers. Cromakalim, levcromakalim, pinacidil, aprialim, nicrorandil, diazoide and minoxidil are structurally diverse agents which have been reported to open membrane K_{ATP} channels (see reviewed by Lawson, 1996). K_{ATP} channel openers have largely defined as a consequence of their biological effect being sensitive to blockade by sulphonylurease (glibenclamide, tolbutamide). Glibenclamide has shown specificity to block

K_{ATP} channels in variety of preparations and has therefore proved to be a useful tool in attempting to characterize the K^+ channel subtypes opened in various preparations by a range of agent (Lawson, 1996; Karaki et al., 1997; Quast, 1993). In addition, the pharmacology of the K^+ channel openers, cromakalim, levcromakalim and other K^+ channel modulating agents, have been studies on wide variety of muscle preparations (reviewed by lawson, 1996).

K_{Ca} channel openers. These opening properties of K_{Ca} channel openers have been described on the sensitivity to pharmacological blockers, quaternary ammonium compounds which their specificities are in the order; charybdotoxin > iberotoxin > apamin > TEA. Two major chemical groups benzimidazoles (NS-004, NS-1619) and imidazol (1, 2-a) pyrazines (SCA40) have been reported to exhibit BK_{Ca} channel opening properties. These agents directly activated the charybdotoxin-sensitive BK_{Ca} channel in many preparations (reviewed by Lowson, 1996) NS-1619 was reported to inhibit the K_V channel over the same concentration range that activated the BK_{Ca} channel. NS-004 induced relaxations was sensitive to charybdotoxin and iberotoxin but not glibenclamide (K_{ATP} channel), dofetilide (inward rectifier) or apamin (SK_{Ca}), suggesting activation of only BK_{Ca} being responsible for this functional response. The K_{Ca} channel opener profile of SCA 40 may be due to an indirect action on the cAMP-dependent protein kinase pathway (reviewed by Lowson, 1996; Karaki et al., 1997). Although several BK_{Ca} openers have been identified, including NS-4, NS-8, NS-1608, CGS-7148, and BMS-204352 (reviewed by Wickenden, 2002), there is a little published information regarding selective BK_{Ca} opener on uterine function.

Endogenous channel openers. Endogenous openers of K^+ channels can be divided into tree basic groups: 1) ligands that modulate the gating of K^+ channels following interaction with plasmalemma receptors, 2) intracellular second messengers (cAMP, cGMP, IP_3) and 3) extracellular substances believed to act directly on the channel. Endogenous modulators, such as adenosine, NO, EDHF (endothelium derived hyperpolarizing factor) and VIP (vasoactive intestinal peptides) have been shown to relax vascular smooth muscle by opening K^+ channel (Lowson, 1996; Karaki et al., 1997).

2. Cyclic nucleotide monophosphate (cNMP)

Contraction and relaxation of smooth muscle is a tightly regulated process involving numerous endogenous substances and their intracellular second messengers. We briefly review the current article regarding cNMP generation and degradation, while focusing on the recent identification of the molecular mechanisms underlying cNMP-mediated smooth muscle relaxation.

The intracellular second messengers involved in SM relaxation are the cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), generated by the activity of adenylate cyclases (AC) and guanylate cyclases (GC), respectively. They exert their intracellular effects by activating a specific protein kinase: cAMP-dependent protein kinase (PKA) or cGMP-dependent protein kinase (PKG) and degradation by cyclic nucleotide phosphodiesterases (PDEs) (Karaki et al., 1997; Carvajal et al. 2000; Schwede et al. 2000). Cyclic AMP is the dominant mediator of smooth muscle relaxation stimulated by β-adrenergic drugs, whereas cGMP-mediated relaxation is triggered by nitric oxide (NO) and natriuretic peptides. Classically, cAMP was seen as the principal second messenger involved in smooth muscle relaxation (Ross, 1999; Carvajal et al. 2000; Schwede et al. 2000).

Proteins involved in formation and degradation of cAMP and cGMP

The AC family consists of at least nine membrane-associated isoforms (AC1–AC9) activated by G-proteins, and represents the major pathway to generate increased cAMP levels in intact cells or tissues, since extracellular cAMP is considered impermeable to the cell membrane. In contrast to the AC isozymes, GCs are divided into soluble forms (GC-S$\alpha_{1-3}\beta_{1-3}$), predominantly activated by nitric oxide (NO), and membrane-receptor forms (GC-A to GC-G, retGC) for natriuretic peptides family (Carvajal et al. 2000; Schwede et al. 2000; Montorsi et al., 2004). PDEs are the only known enzymes responsible for the intracellular degradation of cAMP and cGMP to their corresponding inactive 5′-nucleotide monophosphates 5′-AMP and 5′-GMP. The family of PDEs is large, consisting of complex proteins that differ in substrate affinity, kinetic properties, subcellular localization, and Ca^{2+} sensitivity. In mammalian cells, at least 20 genes and 50 different PDE proteins are described. They are

grouped into seven gene families named PDE I to PDE VII (Table 3). Each family has several distinct genes, alternative splice variants, and characteristic tissue expression. PDE action can be modified by changes in protein concentration or by the modification of enzymatic activity typically via phosphorylation/dephosphorylation mechanisms (Carvajal et al. 2000; Schwede et al. 2000).

PDE IV and PDE VII are highly specific for degradation of cAMP, while PDE V and PDE VI are cGMP-specific. The cGMP-stimulated PDE II and the cGMP-inhibited PDE III preferentially hydrolyze cAMP. In addition, they are regulated by cGMP through a competitive (PDE III) or allosteric (PDE II) mechanism. Therefore, these two isozyme families represent excellent targets for crosstalk between cAMP and cGMP signaling (Carvajal et al. 2000; Schwede et al. 2000).

Target proteins of cyclic AMP and cyclic GMP

PKAs constitute an ubiquitous enzyme family presented in all eukaryotic cells, responsible for the mediation of most biological effects of cAMP. PKA exists as an inactive holoenzyme complex of a dimeric regulatory (2R) subunit and two monomeric catalytic (2C) subunits in the absence of cAMP. Two major forms of PKA, Type I and Type II (PKA-I and PKA-II), have been identified in the uterine smooth muscle. Activation of PKA holoenzyme depends on binding of two molecules of cAMP to each R-subunit and subsequent dissociation of the C-subunits. After liberation, the active free C-subunit can phosphorylate target proteins. This results in specific cellular responses initiated by an extracellular first messenger (hormone, neurotransmitter, etc.) and mediated by cAMP as second messenger. (Ross, 1999; Schwede et al. 2000)

cGMP is able to regulate numerous receptor proteins; cGMP regulateed ion channels, cGMP-binding PDEs and cGMP-dependent PKGs . Because cGMP directly regulated ion channels have not yet been reported in smooth muscle, they are not discussed here as a possible target for cGMP-mediated smooth muscle relaxation. Two classes of PKG with similar substrate specificity are described elsewhere (Carvajal et al. 2000). In general, type I PKG is located in the soluble fraction of the cell, whereas type II PKG is membrane bound and expressed only in intestinal epithelial cells, kidney, and brain. Type I PKG is a

homodimer. Each subunit contains a dimerization domain; two cGMP-binding domains; and an autophosphorylation-autoinhibitory domain, the relevance of which is poorly understood. It is thought that the autophosphorylation of PKG after activation by cGMP prolongs the duration of the activated state. Type II PKG is a monomeric kinase with a catalytic domain at its COOH-terminal and two cGMP-binding domains at its NH_2-terminal. PKG is selectively activated by cGMP binding. Once activated, it phosphorylates several intracellular target proteins. Phosphorylation modulates the activity of these proteins and accounts for many of the cellular events induced by cGMP; for instance, smooth muscle relaxation, inhibition of platelet aggregation, inhibition of endothelial cell permeability, and reduction of cardiac myocyte contractility (Carvajal et al. 2000; Schwede et al. 2000).

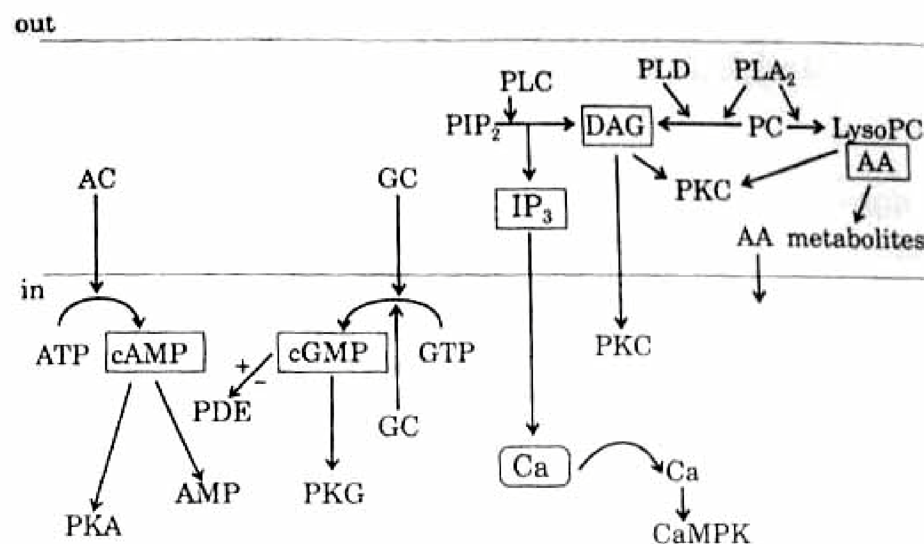


Figure 13. Second messenger pathways in muscle cells, Horizotal lines, cell membrane; AC adenyl cyclase; GC, guanyl cyclase; PDE, cGMP-activated (+) or inhibited (-) phosphodiesterase; PLA_2, phospholipase A_2; PLC, phospholipase C; PLD, phospholipase D; AA, arachidonic acid; PC, phosphaytidylcholine; lysoPC, lysophosphaytidylcholine; PIP_2, phosphatidylinositol diphosphate; DAG, diacylglycerol; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; AMP, adenosine monophosphate; GTP, guanosine triphosphate; cGMP, cyclic guanosine monophosphate; PKG, PKC, protein kinase C; IP_3, inositol 1,4,5- triphosphate; Ca, calcium; CaMPK, Ca/ calmodulin protein kinase II (source: McDonald et al., 1994, pp 407)

Table 3. Phosphodiesterase families, characterization, and inhibitors

PDE type	Name	Inhibitors
Type I	Ca^{2+}/CaM dependent	Papaverine, Vipocentine, Zaprinast
Type II	cGMP stimulated	EHNA*
Type III	cGMP inhibited	Nicorandil, Milrinone*
Type IV	cAMP specific	Papaverine, Rolipram*, Butein*
Type V	cGMP specific	Zaprinast, WIN65579, DMMPO*, E4021*
Type VI	Rod outer segment	Zaprinast, Dypiridamole
Type VII	High affinity cAMP specific	Not reported

Cyclic nucleotides phosphodiesterases (PDE) transform cyclic nucleotides (cAMP and cGMP) into inactive monophosphate nucleotides (AMP or GMP) and thus are involved in the modulation of cGMP cellular action. There are a large number of PDE proteins grouped in seven gene families, which differ in characteristics and substrate affinity. Several pharmacological inhibitors of PDE activity have been developed and tested. Some (*) are selective (Source: Carvajal et al., 2000, pp.412).

Mechanism of cNMP-mediated smooth muscle relaxation

Based on our current understanding of smooth muscle contraction, we propose the existence of two mechanisms for smooth muscle relaxation: the first is the reduction of intracellular Ca^{2+}. cNMP triggers a reduction in $[Ca^{2+}]_i$ through the activation of PKs. Activated PKs phosphorylate several key target proteins, including ion channels, ion pumps, receptors, and enzymes, all involved in the control of $[Ca^{2+}]_i$. The second is the reduction of the sensitivity of the contractile system to the Ca^{2+}. cNMP reduces the sensitivity of the contractile system to Ca^{2+}, such that a contraction is not triggered even by suprathreshold levels of $[Ca^{2+}]_i$. The evidence supporting the conclusion that cNMP induces SM relaxation is described below.

cAMP

PKA is activated by cAMP produced by activation of AC. The β-agonist and forskolin are widely used to activate this enzyme. Inhibitors of PDE also increase cAMP. The effects of these agents on Ca^{2+} movement in smooth muscle are variable (Karaki et al., 1997). Activated PKs phosphorylates several key target proteins, including ion channels, ion pumps, receptors, and enzymes, all involved in the control of intracellular Ca^{2+} concentration, for instance; activation of Ca^{2+} -activated K^+ channels, direct inhibition of membrane Ca^{2+} channel activity, activation of Ca^{2+}/ATPase pump in the plasma membrane and the sarcoplamic reticulum and inhibition of the IP_3 receptor reduce $[Ca^{2+}]_i$ and result in relaxation of smooth muscle (Kuriyama et al., 1998; Carvajal et al. 2000). However, inhibition of MLCP could produce contraction in the absence of increased $[Ca^{2+}]_i$ (increased sensitivity to Ca^{2+}). Alternatively, an increase in MLCP activity could produce relaxation by decreasing MLC phosphorylation without changing $[Ca^{2+}]_i$ (reduced Ca^{2+} sensitivity) (Carvajal et al. 2000). and inhibition of phosphodiesterase, respectively, resulting in increasing cAMP accumulation (Revuelta et al., 1997).

Many compounds affect the cNMP pathways through specific or nonspecific inhibition of PDEs (Table 4). Forskolin a direct stimulation of the catalytic subunit of adenylyl cyclase inhibited high K^+ or agonists induced contraction accompanied by the decrease in both $[Ca^{2+}]_i$ and Ca^{2+} sensitivity (Revuelta et al., 1997). A cAMP- specific PDE inhibitor, loprinone hydrochloride, increased the cAMP and decreased $[Ca^{2+}]_i$. These results suggest that cAMP induced relaxation is caused by the cAMP mediated decreased in $[Ca^{2+}]_i$ due to indirect inhibition of the L-type Ca^{2+} channel, possibly mediated by activation of K^+ channels and resulting membrane hyperpolarization, and also inhibition of the receptor-coupled signal transduction. The decreases in both $[Ca^{2+}]_i$ and Ca^{2+} sensitivity were elicited by the cAMP-specific PDE inhibitor (reviewed by Karaki et al., 1997). In rat aorta, papaverine a non-specific PDE inhibitor relaxed high K^+ induced contraction accompanied by a decreased $[Ca^{2+}]_i$ and a decrease in Ca^{2+} sensitivity (D'Ocon, 1997).

Table 4. Compounds affecting the cAMP and cGMP pathways (Source: Montorsi et al., 2004, pp. 324).

Activity	Compounds
Adenylate cyclase activator	Forskolin
Activator of PKA and PKG, cyclic nucleotide gated channels and GEFs	cAMP and cGMP analogs
Inhibitors of PKA and PKG	ATP analogs
Nitric oxide donating agent	Sodium nitroprusside
PDE1 inhibitor	Vinpocetine
PDE3 inhibitor	Cilostamide, enoximone, milrinone, quazinone, trequinsin
PDE4 inhibitor	Rolipram, RP73401 (Aventis Pharmaceutical Inc.)
PDE5 inhibitor	Sildenafil, vardenafil, tadalafil, zaprinast
Nonselective PDE inhibitor	Dipyridamole, papaverine, theophylline

ATP = adenosine triphosphate; cAMP = cyclic adenosine monophosphate; cGMP = cyclic guanosine monophosphate; GEFs = guanine nucleotide exchange factors; PKA = protein kinase A; PKG = protein kinase G.

There are some reports indicating that cAMP may increase $[Ca^{2+}]_i$ in noncontractile compartment and either decrease or do not change $[Ca^{2+}]_i$ in the contractile compartment. In addition, cAMP may decrease the Ca^{2+} sensitivity of the contractile elements. In some types of smooth muscle, either a decrease in the $[Ca^{2+}]_i$ or decrease in Ca^{2+} sensitivity play an important role whereas both of these mechanisms are important for relaxation in other types of smooth muscle. Also there may be a concentration-dependent differences in the mechanisms of action of cAMP (reviewed by Karaki et al., 1997).

Mechanism of relaxant effects mediated by cAMP may be summarized as follows: 1) inhibition of the receptor-mediated signal transduction resulting in the inhibition of

all effects of agonists including Ca^{2+} release, Ca^{2+} influx and Ca^{2+} sensitization; 2) dissociation of contraction from MLC phosphorylation; 3) increase in SR Ca^{2+} uptake; 4) decrease in Ca^{2+} sensitivity of MLC phosphorylation possibly by activating MLCP; and 5) increase in non-contractile $[Ca^{2+}]_i$ which may result in activation of K^+ channels and membrane hyperpolarization. A part of these may be mediated by PKG but not by PKA (reviewed by Karaki et al., 1997).

cGMP

PKG is activated by cGMP produced by stimulation of guanyl cyclase (GC) by NO, atrial natriuretic peptide and nitro-vasodilators (reviewed by Carvajal et al., 2000). Similar to PKA, effects of PKG on Ca^{2+} movement are diverse (Karaki et al., 1997). There are some reports indicating that phosphorylation of membrane Ca^{2+} channels decreases $[Ca^{2+}]_i$, leading to relaxation of SM (reviewed by Carvajal et al., 2000). NO donors decrease, in a dose-dependent fashion, Ca^{2+} channel availability in several isolated smooth muscles and cultured smooth muscle cells via a cGMP-PKG-mediated mechanism. These observations suggest that PKG phosphorylates either the Ca^{2+} channel or a closely associated regulatory protein, reducing the probability that the channel remains in its open state (reviewed by Karaki et al., 1997; Carvajal et al., 2000). Several investigators have shown in several smooth muscle that cGMP-PKG pathway activation increases the activity of K_{Ca} channels by phosphorylation of either the channel protein or a related regulatory protein (reviewed by Carvajal et al., 2000; Rosenfeld et al., 2000). Similar observations were made in uterine smooth muscle, demonstrating that the NO-cGMP-PKG pathway induces smooth muscle relaxation by a K_{Ca} channel–dependent mechanism (Kuenzli et al., 1998; Modzelewska et al., 2003; Bradley et al., 1998).

Ca^{2+} extrusion across the plasma membrane is thought to be a mechanism mediating smooth muscle relaxation via $[Ca^{2+}]_i$ reduction. An indirect mechanism of cGMP increases the Na^+-independent Ca^{2+} efflux via activation of the Ca^{2+}/ATPase pump has also been proposed. Furthermore, it has been postulated that cGMP activated of Ca^{2+}/ATPase pump in the SR. The reduction in $[Ca^{2+}]_i$ induced by cGMP is, at least in part, mediated by the activation of this Ca^{2+}/ATPase pump. cGMP-activated PKG phosphorylates the protein

phospholamban that increases Ca^{2+}/ATPase pump activity and sequestration of Ca^{2+} into the SR, thereby inducing smooth muscle relaxation. Moreover, cGMP has been demonstrated that induces an increase in MLCP activity without affecting MLCK activity via the cGMP-PKG pathway. This shift in the MLCK/MLCP equilibrium results in a net decrease in MLC phosphorylation and, thus, a reduction in the sensitivity of the contractile system to Ca^{2+}, which relaxes smooth muscle (reviewed by Carvajal et al., 2000).

However, mechanism of relaxant effects mediated by cGMP are similar to those of cAMP: 1) inhibition of the receptor-mediated signal transduction resulting in the inhibition of all effects of agonists including Ca^{2+} release, Ca^{2+} influx and Ca^{2+} sensitization; 2) increase in SR Ca^{2+} uptake; 3) decrease in Ca^{2+} sensitivity of MLC phosphorylation possibly by activating MLCP; and 4) dissociation of contraction from MLC phosphorylation. Difference between the effect of cAMP and cGMP are that 1) cGMP augments Ca^{2+} extrusions by activating membrane Ca^{2+} pump and 2) cGMP does not increase but decrease the noncontractile Ca^{2+} (reviewed by Karaki et al., 1997).

Second -messenger cross talk

It was thought that cAMP and cGMP activate PKA and PKG, respectively, with a high degree of specificity. However, recent informations indicate that the cAMP and cGMP second messenger pathways function not only as separated mechanisms, but can interfere vice versa at several target proteins. The cyclic nucleotide pathways can interact at several levels: 1) influencing synthesis or degradation of its counterpart; 2) cross-activating protein kinases; and 3) phosphorylating common-target proteins through kinase activation. Although these three potential levels of interaction have been subject to some study, it is unclear whether they have a physiological role. Cross talk at the level of kinase activation has been the most extensively studied in smooth muscle. For instance, cGMP is reported to inhibit platelet-derived growth factor-evoked proliferation of cultured smooth muscle cells and to stimulate intestinal Cl^- transport by activation of PKA. cAMP can activate PKG and mediate smooth muscle relaxation in pig coronary arteries or phosphorylate the IP_3 receptor in intact rat aorta. Both cAMP and cGMP can modulate Ca^{2+} channels in rabbit portal vein by activation of the opposing protein kinase. In some tissues or cells, such as heart and adrenal,

gland activation of cGMP-stimulated PDE II is an accepted or proposed mechanism of cGMP-controlled regulation of cAMP hydrolysis (reviewed by Schwede et al., 2000; Carvajal et al., 2000).

3. Endogenous NO production and NO-induced relaxation

Nitric oxide (NO) is involved in the regulation of multiple physiological and pathophysiological processes in various cells and tissues. NO is synthetized from L-arginine via the catalytic action of the enzyme, the NO synthase (NOS) (Norman, 1996; Batra et al., 2003). Three types of NOS have been identified and characterized at the protein and cDNA level. The constitutive isoforms, neuronal NO synthase (nNOS or NOS I) and endothelial NO synthase (eNOS or NOS III), are both calcium-calmodulin dependent and both produced small amounts of NO (picomoles) for short periods. On the other hand, inducible NO synthase (iNOS or NOS II) is calcium-calmodulin independent and produced large quantities of NO (nanomoles) for long periods when stimulated by cytokines and other agents (reviewed by Norman, 1996; Norman and Carmeron, 1996; Batra and Al-Hijji, 1998; Ekerhovd et al, 1999; Hare, 2003).

The production of NO by the action of NOS can be inhibited competitively by analogues of L-arginine, including N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine methyl ester (L-NAME). These agents have provided powerful and specific tools to determine the role of NOS in physiology and pathology. The effects of L-NMMA and L-NAME on the synthesis of NO can be attenuated by large doses of L-arginine when endogenous supplies of this amino acid are limited (Norman, 1996).

An endogenous NO system is present in the rat, rabbits and human uterus and is up-regulated during pregnancy, followed by down-regulated during labor (Ekerhovd et al., 1999; Batra et al., 2003). Uterine NOS originates from several cell types including nerve endings, vascular smooth muscle, endometrial cells, endothelial cells, fibroblasts and macrophages(Batra and Al-Hijji, 1998). Studies on the human and animal species uterus have been demonstrated myometrial NOS activity. NO plays an important role in the regulation of myometrial activity, uterine and fetoplacental blood flow, quiescence of myometrium and ripening of cervix (Ekerhovd et al., 1999; Batra et al., 2003). Evidence that

NO has an important role in myometrial function comes largely from studies showing that NO has a relaxing effect on the myometrium and this relaxation is specifically blocked by inhibitors of NOS (Norman, 1996; Batra et al., 2003). The action of NO is mediated by activation soluble guanyl cyclase (sGC), thereby increasing cGMP. This substance in turn activates PK, which ultimately leads to relaxation of smooth muscle (Ekerhovd et al., 1999; Batra et al., 2003).

In uterus, NO is produced by inflammatory cells, and nerves fibers. NO synthesis nerve fibers have been demonstrated in the non-pregnant uterus of the rat, mouse and human. Although some nerve fibers were present in the myometrium of the body of the uterus, the density of nerve fibers was greatest in the cervix (Norman, 1996). Further studies have suggested that these nerves are autonomic and sensory. Rounded cells staining with an antibody raised against an iNOS specific peptide have also been demonstrated in the myometrium. The number of positively stained cell was greatest during diestrus-I, and further staining identified the majority of the iNOS cells within the myometrium as mast cells (Norman, 1996). Batra et al. (2003) reported that the expression of eNOS and nNOS in the myometrium tissue and eNOS and nNOS isoforms was concentrated in the particulate and cytosolic fraction, respectively. However myometrial blood vessels showed positive staining for eNOS, but no eNOS staining was seen in the myometrial smooth muscle cells (Norman, 1996). These studies suggest that within both animal and human uterus, neural and vascular tissue and cell of immune system all may generate NO and impact on physiology. Myometrial NOS varies during the estrus cycle, presumably under the control of steroid hormones (Norman, 1996; Ekerhovd et al., 1999; Batra et al., 2003).

NO has a short half-life. It is rapidly oxidized to nitrite (NO_2^-) both *in vivo* and *in vitro.* Oxidised NO may also form nitrosate molacules with sulphydry-containing compound, giving biological active molecules that are more stable than NO itself. Nitric oxide also reacts with superoxide anions (O_2^-) to form peroxynitrite ($ONOO^-$), which in turn is oxidized to nitrate (NO_3^-). Thus the effects of NO are reduced in the presence of compounds that generate superoxide anions (such as xanthine and xanthine oxidase), and they are potentiated in the presence of superoxide dismutase (SOD), a widely located enzyme that inactivates

superoxide anions. NO exerts its smooth muscle relaxant effect by stimulating sGC, thereby increasing cGMP (Norman et al. 1996; Batra et al., 2003). Furthermore, several recent studies have postulated the involvement of K^+ channels in the relaxation induced by NO donors (Modzelewska et al., 2003; Hare, 2003).

Table 5. NO within the uterus in non-pregnant subjects, during late pregnancy and labor (Source: Norman, 1996, pp. 97)

	Potential source of NO within the uterus	Relative amount of NO production within the uterus	Sensitivity of myometrium to tocolytic effects of NO
Nonpregnant	Blood vessels Nerves Cells of immune system Endometrium	Low	Low
Late pregnancy	Blood vessels Nerves Cells of immune system Placenta Decidua	High	High
In labour	Blood vessels Nerves Cells of immune system Placenta Decidua	Low	Low

Mechanism of action of NO with the myometrium

Most information on mechanism of action of NO comes from studied in the vascular tissue. There are three potential mechanisms by which NO may cause smooth muscle relaxation: the activation of GC, the stimulation of K_{Ca} channels and ADP ribosylation.

The most important mechanism is probably the activation of soluble GC, which catalyses the formation of cGMP (as reviewed earlier). This is supported by data showing that in myometrium, as in other smooth muscle, the relaxant actions of NO or L-arginine are mimicked by 8-bromo-cGMP (a plasma permeable form of cGMP) and inhibited by methylene blue (an inhibitor of GC). However, a number of investigators have previously attempted to uncover a role for cGMP in the regulation of uterine smooth muscle contractility, and their findings suggest that cGMP plays a minor role in the regulation of rodent and human uterine contractility (Kuenzli et al., 1998). The cGMP-independent mechanism of action of NO in the myometrium is not clear, although there have been a number of studies demonstrating cGMP-independent effects of NO in variety of tissues (Kuenzli et al., 1998; Modzelewska et al., 2003). One possible mechanism by which NO could cause smooth muscle relaxation via activation of K_{Ca} channel. Blockage of K_{Ca} channel causes a reduction in the cell membrane potential, an increase in intracellular Ca^{2+} and stimulation of contraction in isolated myometrium strips (Norman, 1996; Kuenzli et al., 1998; Modzelewska et al., 2003).

4. β_2-adrenergic agonist

β_2-adrenergic receptors are present on the myometrium smooth muscle. β_2-mimetics such as ritodrine, salbutamol, terbuline or isoproterenol display for membrane-bound β_2-adrenergic receptors in the myometrium, results inactivation of the adenylate cyclase enzyme via stimulation of G-α_s protein which increase intracellular cAMP concentration (Fortier et al., 1983). The increase in cAMP activate cAMP dependent kinase that phodphorylated, it markedly decrease in its affinity to MLCK. When MLCK is phosphorylated, it markedly decreases in its affinity to Ca^{2+}-calmodulin leading to a reduction of phosphorylation of myosin light chain (Scheid et al., 1979).

The other mechanism of cAMP involving to relaxation may contribute to $[Ca^{2+}]_i$ (Verma and Mcneill, 1976). It may occur; 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 turns to enhance Ca^{2+} efflux in Na^+-Ca^{2+} exchange, 3) causing internal sequestering of Ca^{2+}, 4) inhibiting Ca^{2+} influx and increase Na^+ conductance (Schied et al., 1979; Monga and Sanborn, 1992).

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

5. Mg^{2+} ions

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

6. Ca^{2+} channel blocker

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

7. Oxytocin antagonist

A synthetic oxytocin receptor antagonist has been shown to inhibit both in *vitro* and in *vivo* contractile 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).