CHAPTER 5

DISCUSSION

Alkaloids have been considered to be an important group of chemicals for their potential in drug discovery and drug development in the future (Cordell et al., 2001). Indeed, there are several alkaloids and their derivatives which are used as pharmaceutical agent. Several studies have shown the effects of alkaloid on many systems most of which involved the action of alkaloid on numerous receptors and ion channels (Cordell et al., 2001). In our laboratory, previous studies have shown inhibitory effects of papaya leaves alkaloid on the contraction of isolated rat uterus (Keereevong, 2002). This finding has led us to study further on the purified component of the alkaloid which could cause the effects. It is evident from the previous studies that crude alkaloid of *C. papaya* possesses spasmolytic activity that occurs predominantly via interference with calcium channels. This interpretation came from the observation that the alkaloid caused an inhibition of KCl (56.3 mM) and $CaCl_2$-induced contractions on isolated rat uterus. Crude alkaloid inhibited not only the contraction induced by either KCl or $CaCl_2$ but also the contraction produced by oxytocin, acetylcholine and $PGF_{2\alpha}$ in a concentration-dependent manner (Keereevong, 2002). In addition, crude extract of *C. papaya* leaves could markedly reduced blood pressure and heart rate, depress movement of the intestinal strips, relax uterine muscle, dilate the bronchioles (Tuffley and Williams, 1951), and relax muscle (Gupta et al. 1990). It is suggested that the crude alkaloid extract from papaya leaves may act as a general relaxant and have common mechanisms to relax these smooth muscle

Recently calcium antagonistic activity has been reported in various groups of natural products such as alkaloids, terpenes, coumarins, lignans and flavonoids (reviewed by Vuorela et al. 1997; Revuelta et. al., 2000; D'Ocon et al., 1991). The extract used in these investigation was crude extract which contained many compounds such as alkaloids, cardiac glycoside (Gupta et al., 1990) and flavonoids (Runnie et al., 2004). At this stage it is not

possible to state which of the alkaloid from *C. papaya* leaves is responsible for spasmolytic activity. However, the effects of papaya extract on bronchiole dilatation, vasorelaxation and uterine relaxation (Keereevong, 2002; Gupta et al., 1990; Tuffley and Williams, 1951) could be attributed to one or more of the above mentioned compounds. The experiments in this regard are underway to find out the actual compound responsible for these activities. Thus, the aim of this study is to investigate the role of carpaine, a major alkaloid of *C. papaya* leaves, on uterine smooth muscle relaxation.

The absolute configuration of carpaine seems to be concluded by Coke and Rice, that its formula is $C_{28}H_{50}N_2O_4$ and consist of a dimeric, macrocyclic lactone with two piperidine units incorporated into the ring (reviewed by Burdick, 1971; Tang, 1978). The crystals of carpaine in the present study had the melting point (122-123 ˚C) and NMR spectra were in agreement with the reported data (Tang, 1978; Godavindachari et al., 1965). Carpaine have been reported to be a major alkaloid of *C. papaya* and found in all green parts of the plants and in the seeds (Burdick, 1971). The yield of carpaine from papaya leaves varied widely ranging from 0.0115-0.4% (Burdick, 1971; Ogan, 1971; Tang, 1978). In this regard, it is suggested that the yield of carpaine in the different papaya varieties (Tang, 1978), age of the used leaves (Ogan, 1971), the region where this plant grows (Morton et al., 1977) can be taken into consideration, together with other possible factors. The total yield of crude alkaloid in the present study was approximately 0.284% of the dried leaves and 0.1194% carpaine of the crude alkaloid weight. The result indicated that the inbred line of *C. papaya* L. common in southern of Thailand contained relatively high content of carpaine and more abundant basic constituent of this source than other alkaloids. This purified carpaine was used to investigate its effects on uterine relaxation throughout this study.

The aim of this study was to examine the mechanism of carpaine-induced relaxation in uterine smooth muscle. In order to accomplish this, we tested the effect of the alkaloid, carpaine, on KCl or oxytocin-induced contraction as well as its modification by different drugs in order to clarify the mechanism of action. Our preliminary study has shown the inhibitory effect of carpaine on the uterine contraction precontracted by depolarizing solution. Crude alkaloid and purified carpaine were able to inhibit uterine contraction

completely in a dose-dependent manner. It is indicated that carpaine may act directly on the smooth muscle via interference with Ca^{2+} influx through voltage-dependent Ca^{2+} channels. The interference of carpaine with the voltage operated Ca^{2+} channels were then study further in $CaCl_2$-induced contraction preparation. The uterine smooth muscles were treated with different concentrations of $CaCl_2$ in calcium-free-high K^+ (60 mM) solution. Under such conditions, the contractions of smooth muscles which are induced by the presence of high K^+ are dependent upon movement of Ca^{2+} into the cell through voltage operated Ca^{2+} channels (Bolton, 1979). Both mechanisms of depolarizing solution are contributed to voltage-dependent Ca^{2+} channel. In depolarizing solution, the high KCl concentration (56.3 mM) depolarized the cell membrane, change the ions conductance of the cell which turned to activate voltage-dependent Ca^{2+} channel leading to Ca^{2+} influx inside the cell and the stimulated uterine contraction. This contraction is inhibited by the Ca^{2+} channel antagonist such as verapamil (Revuelta etal., 1997) which is consistent to the effect of verapamil in this study. Similarly, in Ca^{2+}-free-high K^+ solution, KCl also changed ions conductance which turned to activate voltage -dependent channel to open stage (Vaghy, 1998). Although Ca^{2+} channel was opened, the lack of Ca^{2+} in Ca^{2+}-free solution could not stimulate the uterus to contract. The addition of $CaCl_2$ to the organ bath caused the Ca^{2+} to enter the cell via opened Ca^{2+} channel and stimulated the uterine contraction. The result of this study showed that carpaine was able to completely inhibit uterine contraction induced by depolarizing solution and also inhibited $CaCl_2$ -induced contraction in Ca^{2+}-free-high K^+ solution. It is suggested that the effect of carpaine may be related to a reduction in Ca^{2+} influx. In addition, the inhibitory effect of carpaine on $CaCl_2$-induced uterine contraction in Ca^{2+}-free-high K^+ solution is similar to the effect of verapamil, a calcium channel antagonist (Vaghy, 1998). This result suggested that the inhibitory effect of carpaine on Ca^{2+} influx may occur through voltage-dependent Ca^{2+} channel in the same manner as verapamil.

Oxytocin induced the isolated rat uterus to contract rhythmically in Jalon-ringer solution. The force of contraction produced by this stimulant was stable throughout the experiment. Although the frequency of contraction declined slowly with time, it has never been less than 80 % of the initial frequency at the end of the experiment. In such a case, statistical

analysis was performed using ANOVA to assure if the reduction of frequency of contraction was due to the drug treatment or the time effect.

In contrast to KCl-induced depolarization, the contraction elicited by oxytocin is due to an increase in intracellular Ca^{2+} resulting from both Ca^{2+} influx via plasma membrane and Ca^{2+}-release from internal stores (Monga and Sanborn, 1992). The agonists bind to its G-protein coupling receptors at plasma membrane, activates receptor-linked channel to open and allows cations such as Na^{+} or Ca^{2+} to enter the cell, and /or generate signal pathway which induces Ca^{2+} release from internal storage. The latter action of oxytocin that induced Ca^{2+}-release from internal storage is meditated by IP_3 signaling pathway. The interaction between oxytocin to its receptor on plasma membrane leading to an activation of G-protein coupled to PLC, hydrolysis of PIP_2 to IP_3 and then IP_3 will activate its receptor at SR resulting in Ca^{2+}-release form internal storage. However, the contractile effect of oxytocin is markedly inhibited by substance within a group of Ca^{2+} channel blocker. It is suggested that the major action of oxytocin to induce uterine contraction is mediated by an increase in Ca^{2+}-influx from extracellular space via voltage-dependent channel and receptor-operated channel whereas the action that mediated Ca^{2+}-release from internal stores plays a minor role (Bolton, 1979; Marc et al., 1988; McDonal et al., 1994: Rall, 1991). It is therefore concluded that the action of oxytocin to induce an increase in intracellualr Ca^{2+} is mediated by both Ca^{2+} influx via plasma membrane and Ca^{2+} release from internal stores but the major source of Ca^{2+} is the influx via plasma membrane. The result of this study showed that carpaine was able to inhibit contractile effect of oxytocin, similar to the action of verapamil, a Ca^{2+} channel blocker, because it inhibit the effect on Ca^{2+} influx via voltage-dependent Ca^{2+} channel (Bolton, 1979; Karaki et al, 1997). It is suggested that the inhibition pathway of carpaine may occur through the inhibition on Ca^{2+}-influx via plasma membrane. However, its effect on the Ca^{2+} release from internal storage can not be excluded.

Carpaine and verapamil have shown to inhibit rhythmic contraction induced by oxytocin in the isolated rat uterus on both frequency and force of contraction. The effect of carpaine or verapamil on the frequency of contraction indicated that carpaine or verapamil might somehow affects the action potential generating system on the uterine muscle cell. It is

known that the basal membrane potential undergoes rhythmic electrical changes called slow wave. At the threshold potential, there is a fast depolarization that generates an action potential on top of the slow wave. The action potential is attributed primarily to entry of Ca^{2+} (and Na^{+} in late pregnancy), through voltage sensitive Ca^{2+} channels (and also through fast Na channel in late pregnancy) (Moga amd Sanborn, 2000). At the end of the action potential the membrane repolarized, which is thought to involve the opening of K^{+} channels. Shortly after the repolarization, the depolarized action processes begin again and a new action potential occurs. This cycle continues again and again causing rhythmic excitation of tissue (Guyton, 1986; Karaki et al., 1997). Oxytocin is known to stimulate Ca^{2+} influx across the plasma membrane. However, the action of this stimulant on action potential is not fully understood. It is known that action potential occurs in uterine muscle cells by an increase in permeability of Na^{+} or Ca^{2+}. If any substrate acts to decrease the permeability of Na^{+} or Ca^{2+}, the depolarized phase of action potential could be largely delayed resulting in a decrease in frequency of contraction. In addition, an increase in the permeability of plasma membrane to K^{+} efflux contributes to repolarization phase of action potential. The next depolarization phase of action potential can not happen if K^{+} channels is still activated (Metha et al., 1995; Anwer et al., 1993). If substrate acts to promote opened stage of K^{+} channel, it may delay the action potential leading to a reduction of frequency of contraction. From this point of view, it is therefore, suggested that carpaine may act to decrease plasma membrane permeability to Ca^{2+} or Na^{+}, or to increase the permeability of membrane to K^{+}. This would cause a delay of the action potential and then, slower the frequency of contraction.

A number of specific K^{+} channels types have been described in myometrium. More than one type has been detected in individual myometrial cells from a number of species. The most common K^{+}-channel are the Ca^{2+}- sensitive K^{+} channel and ATP-sensitive K^{+} channel. These channels are reported to play important roles in modulation of uterine contractility (Anwer et al., 1993; Kafali et al., 2002). The actions of K^{+} channel opener in rat myometrium are dependent on the extracellular K^{+} concentration and/or membrane potential. K^{+} channel opener such as cromakalim selectively inhibited spasm to low (20 mM) but not high (60 mM) concentration of KCl and this is a unique feature of drug acting as K^{+} channel

openers. The uterine relaxation effect of the K^+ channel openers is abolished in media containing high K^+ concentration (> 50 mM) because high K^+ concentration results in a shift in membrane potential towards the equilibrium potential for K^+ and this is the mechanism by which K^+ channel openers normally elicits smooth muscle relaxation (Lowson, 1996).

The mechanism by which K^+-channel openers relax KCl or oxytocin-induced smooth muscle contraction is believed to involve repolarization and/or hyperpolarization of the cell membrane resulting in the prevent Ca^{2+} entry through voltage operated Ca^{2+} channel. Although, it was assumed that hyperpolarization caused by K^+ efflux produced closure of VOCs, preventing depolarization-induced Ca^{2+} entry into the cell. The other mechanisms may also contribute to the effects produced by K^+ channel openers such as reduced the uptake into and inhibited the release of Ca^{2+} from the SR, inhibited the production of IP_3 and, hence, Ca^{2+} release from intracellular store and linked with a reduction in the sensitivity of contractile elements to Ca^{2+} (Lowson, 1996).

In isolated uterine smooth muscle, the prototype K^+ channel opener, cromakalim preferentially relaxed contractions induced by low K^+ (25 mM) compared with high K^+ (60 mM). The same effect profile was found for carpaine, although it relaxed the contractions induced by low K^+ (25 mM) and high K^+ (60 mM) equally. In addition, glibenclamide, the K_{ATP} blocker suppressed the relaxant response to cromakalim by the shift of the concentration response curves to the right but did not influence the relaxation produced by carpaine. TEA, a K_{Ca} blocker shifted the concentration-relaxation curves for cromakalim and carpaine to the right. In addition, cromakalim was more potent than carpaine in the inhibition of rhythmic contractions induced by oxytocin in a dose dependent manner, however, in contrast to carpaine, cromakalim did not completely inhibit this contraction at the highest dose used. Furthermore, in oxytocin-induced uterine contraction, glibenclamide suppressed the relaxant response to cromakalim but did not influence the relaxation produced by carpaine. Nevertheless, the uterine relaxation produced by carpaine and cromakalim was significantly attenuated in the presence of TEA. These slight differences may suggest differences in the mechanism of action of carpaine and cromakalim in their effect on

contraction evoked by KCl compared with contractions elicited by oxytocin. Glibenclamide is well documented to be a potent blocker of the ATP-sensitive K^+ channel and a large number of pharmacological studies have involved the use of glibenclmind to antagonize the relaxant effects of the K^+ channel openers (Davies et al, 1996). In the present study, glibenclamide antagonized the relaxant response of cromakalim on 25 mM KCl and oxytocin induced contraction. Although glibenclamide was relatively potent antagonist of these K^+ channel openers, its failed to influence carpaine induced uterine relaxation. TEA, a blocker mainly of Ca^{2+}-activated K^+ channels, produced a parallel shift of the carpaine concentration relaxation curve to the right. However, TEA is not a specific K^+-channel blocker subtype and show a blocking action on many types of K^+ channels (Davies et al, 1996). TEA also antagonized the relaxant responses to cromakalim and this antagonism was more pronounced than that against carpaine. These results suggest that the lack of effect of glibenclamide on the relaxation produced by carpaine demostrated that carpaine does not act by activation of ATP-sensitive K^+ channels and that the uterine relaxation mechanism therefore differs from that utilized by K^+ channel opener. The inhibition of uterine smooth muscle induced by carpaine seems to be mediated, at least in part, via activation of Ca^{2+}-activated K^+ channels, because the inhibition was reduced by TEA. In addition, phosphorylation and dephosphorylation of ion channel protein are known mechanism for modulation of ion channels including Ca^{2+}-activated K^+ channels (Bang et al., 1998) and might be involved in carpaine mediated activation of Ca^{2+}-activated K^+ channels.

The inhibitory activity of carpaine in the uterine tissue may inhibit Ca^{2+} entry through the L-type Ca^{2+} channels, but also from other effects such as the opening of Ca^{2+}-activated K^+ channels and decrease of intracellular free Ca^{2+} levels. It is possible that carpaine may act by activation of AC or inhibition of PDE and thereby cause an accumulation of intracellular cAMP which further leads to uterine relaxation.

cAMP is an important intracellular second messenger in many tissues and mediates the effect of multiple drugs and hormones. It is known that cAMP produces relaxation of smooth muscle by activation of cAMP-dependent protein kinase (PKA) which interferes with several processes involved in smooth muscle contraction (Perez-Vallina et al.,

1997). cAMP may also cause relaxation by: 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 noncontractile $[Ca^{2+}]_i$ which may result in the activation of K^+ channels and membrane hyperpolarization (Karaki et al., 1997).

Rp-cAMPS is a soluble membrane-permeable analogue of cAMP with enhanced metabolic stability and is not a substrate for cAMP phosphodiesterase. Rp-cAMPS behaves as an antagonist of cAMP and does not cause the release of the catalytic subunit of the enzyme, although it binds to the regulatory subunit of PKA (Perez-Vallina et al., 1997). The KCl (60 mM)-induced tonic contraction is maintained by calcium influx through voltage-dependent calcium channel and is relaxed by drugs that inhibit calcium influx or increase of intracellular cAMP (Perez-Vallina et al., 1997). The activator of adenylyl cyclase forskolin and the phosphodiesterase inhibitors papaverine relaxed KCl-induced contractions in rat uterus. The alkaloid carpaine also produced a concentration-dependent relaxation on KCl-induced tonic contraction. The inhibitors of PKA, Rp-cAMPS, antagonized carpaine-induced relaxation and its effect is significantly antagonized with highest concentrations of Rp-cAMPS. Rp-cAMPS also inhibited the smooth muscle relaxation elicited by forskolin and papaverine . This results suggest that the relaxing effect on uterine contraction of carpaine may be related to the activation of adenylyl cyclase or inhibition of phosphodiesterase. This effects induced the cascade of events that modulated by cAMP or PKA. Accordingly, it seems likely that carpaine could increase cAMP accumulation and produces the relaxing effect. This possibility was confirmed by further experiment which Rp-cAMPS significantly shifted the concentration-response curves of carpaine-induced relaxation to the right. Similar results were obtained for forskolin and papaverine. Our results of the two drugs are consistent with the study by Revuelta et al. (2000) and the authors also suggested that Rp-cAMPS also inhibited the smooth muscle relaxations elicited by drug that increase cAMP or cAMP-dependent protein kinase. In addition, Pérez-Vallina et al. (1997) demonstrated the inhibition of KCl- and vanadate-induced uterine contraction by papaverine, the non-specific phosphodiesterase

inhibitors and Rp-cAMPS antagonized its effect. Rp-cAMPS antagonized the relaxation produced by the activator of adenylyl cyclase forskolin on KCl contraction, this suggests a cAMP-dependent effect. However, the relaxation on vanadate contraction in Ca^{2+}-free solution is not antagonized by Rp-cAMPS, which suggests a cAMP-independent effect. Moreover, D'Ocon et al. (1991) reported that papaverine was capable of relaxing the contraction induced by oxytocin and vanadate in Ca^{2+}-free solution. However, the mechanisms involved relaxation in KCl-depolarized uterus are different for the different drugs. As it is known, verapamil inhibit the contraction by reduction of extracellular calcium influx through L-type Ca^{2+} channels, whereas forskolin produces direct stimulation of the catalytic subunit of adenylyl cyclase resulting in cAMP accumulation (Revuelta et al., 1997; Revuelta et al., 2000). It is well known that papaverine relaxed smooth muscle through non-specific mechanisms related to a modification of the sensitivity of contractile machinery to Ca^{2+}, a decrease in the cytosolic free Ca^{2+} levels and the inhibition of cAMP phosphodiesterase (D'Ocon, 1997). In addition, guanylyl cyclase and cGMP have been shown to play a role in the regulation of myometrial contractility (Batra et al 2003), the inhibitory effect of carpaine on uterine contraction is anticipated to involve this mediator. This led me to test the effect of carpaine on this pathway.

It is well established that NO-cGMP system plays an important role in myometrial function. This information comes largely from studies showing that NO has a relaxing effect on the myometrium. NO exerts its smooth muscle relaxing effect by stimulating soluble guanylyl cyclase, thereby increase cGMP (Batra et al 2003). We evaluated the ability of NO in the forms of NO-donor, SNP and NO-substrate, L-arginine, to modulate uterine contraction induced by high K^+ solution.

The results of these studies showed that a NO-donor, SNP, caused lesser (20-30%), concentration-dependent relaxation in KCl-depolarized uterus, and the inhibitor of guanylyl cyclase, methylene blue, significantly modified the relaxing effect of SNP. Inhibition of guanylyl cyclase caused a reduction in cGMP content of uterine smooth muscle stimulated with NO. In the same experiment condition, a relaxation is also observed when L-arginine is applied to the uterine smooth muscle. This relaxation is slightly blocked by the inhibitors of

NOS, NOARG, resulting in a reduction of NO production. On the other hand, carpaine more strongly inhibited the high K^+-induced contraction than SNP and L-arginine in rat uterus. Furthermore, both methylene blue and NOARG did not antagonize this relaxation. Consequently, it seems reasonable to suggest that the relaxing effect of carpaine is not mediated by NO-cGMP pathway. Under the present experimental condition, the activator of GC, SNP and the precursor of NO, L-arginine slightly relaxed the uterine contraction. NO may produce its effects through an activation of GC (Izumi and Garfield, 1995; Pérez-Vallina et al. 1998). Complete inhibition of spontaneous and agonist-induced, but not KCl-induced, myometrium contractility was also affected by the application of SNP (Izumi and Garfield, 1995; Pérez-Vallina et al. 1998). Similarly, L-arginine inhibited spontaneous and agonist induced, but not KCl induced, myometrial contraction (Izumi and Garfield, 1995; Pérez-Vallina et al. 1998). This inhibition of contractility was abolished by inhibitor of NOS, such as NOARG, or by inhibitor of GC such as methylene blue (Izumi and Garfield, 1995; Pérez-Vallina et al. 1998). The most important mechanism of action of NO is probably the activation of soluble GC, which catalyses the formation of cGMP. This is supported by an evidence showing that, in myometrium, as in other smooth muscle, the relaxant actions of NO or L-arginine are mimicked by 8-bromo-cGMP and inhibited by methylene blue. The rise in intracellular cGMP then activates PKG which, in turn, phosphorylates protein and causes uterine relaxation. Mechanism of relaxant effects mediated by cGMP 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) 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 5) augments Ca^{2+} extrusions by activating membrane Ca^{2+} pump (Karaki et al., 1997). Apart from NO-cGMP pathway, NO may cause smooth muscle relaxation via an alternative mechanism by an activation of K_{Ca} channel leading to a series of action on mention above.

It is well established that uterine responses to contractile agonist may be due to the opening of two types of Ca^{2+} channels: potential -and/or receptor-operated channels (Bolton, 1979). These Ca^{2+} channels have unique characteristics in that the voltage-

dependent channels are selectively inhibited by the organic Ca^{2+} channel blocker such as verapamil or dihydropyridines whereas the receptor-linked channels are not sensitive to Ca^{2+} antagonists or more weakly inhibited than voltage-dependent channels (Godfriend, 1985). We therefore selected two agonists acting via different channel: KCl-induced contraction involves Ca^{2+} entry via VOC (Bolton, 1979) and oxytocin-induced rhythmic contractions involve the opening of ROC (D'Ocon et al., 1989) for used in this study.

The present finding suggests that the substances tested act as smooth muscle relaxants but their mechanisms of action are not similar. It is well known that verapamil inhibit the contraction of smooth muscle of several organs, including rat uterus, by reduction of extracellular calcium influx through direct action in structural proteins of L-type channels (Godfraind et al., 1986). This relaxant effect on KCl-induced tonic contraction in rat uterus is counteracted by increasing the $CaCl_2$ concentration in the incubation medium (Vaghy, 1998). As is known that papaverine relaxes smooth muscle contractions induced by the opening of VOC or ROC through non specific mechanisms related to a modification of the sensitivity of contractile machinery to Ca^{2+}, a decrease in cytosolic free Ca^{2+} levels and the inhibition of cAMP phosphodiesterase, thus increasing cAMP accumulation (Montorsi et al., 2004), whereas forskolin produces direct stimulation of the catalytic subunit of adenylyl cyclase resulting in increase in cAMP (Revuelta et al., 1997). The increase in cAMP activates PKA that interferes with several processes involved in smooth muscle contraction (Karaki et al., 1997). The relaxation elicited by the K^+ channel opener, cromakalim, could be produced by repolarization and/or hyperpolarization of the cell membrane resulting in prevention of Ca^{2+} entry through voltage-operated Ca^{2+} channel (Lawson, 1996; Quast, 1993). This relaxant effect on agonist induced contraction in rat uterus is counteracted by glibenclamide, TEA or increasing the KCl concentration in the incubation medium (Lawson, 1996; Okabe et al., 1999).

The results of the present study show that carpaine inhibited myometrium contractions induced by oxytocin and KCl, implying that the effect of carpaine is independent from the stimulants. Verapamil, a Ca^{2+} antagonist with non-selectivity for Ca^{2+} entry through VOC, carpaine, also gave similar relaxant effects by increasing the $CaCl_2$ concentration in the

incubation medium. Consequently, it seems reasonable that the K^+ opener involved in the relaxant effect of carpaine. The fact that K^+ opener, cromakalim, relaxed contractions elicited by low but not high K^+ induced contractions and was antagonized by various K^+ channel blockers such as glibenclamide and TEA (Piper and Hollingsworth, 1995). Carpaine relaxed contractions induced by 25 mM and 60 mM K^+ and glibenclamide did not influence the relaxation produced by carpaine. The only evidence relating the mechanism underlying the inhibitory effect of carpaine was that TEA prevented the inhibitory effect of carpaine on oxytocin or 25 mM KCl induced contraction suggesting involvement of Ca^{2+} activated K^+ channels. However, K_{Ca} channel was activated by phosphorylation via PKA, suggesting modulation of the channel opening by cAMP or adenylate cyclase (Kuriyama et al., 1998). Our results provide evidence of the implication of cAMP in the effect of carpaine. Rp-cAMPs significantly modified the relaxing effect of carpaine as did the inhibitor of cAMP-dependent protein kinase. It is possible that carpaine may act somehow to increase cAMP accumulation leading to uterine relaxation. The mechanism to cause cAMP accumulation could occur by the inhibition of PDE enzyme or the activation of adenylate cyclase. Moreover, carpaine may directly activate PKA and cause a cascade of action mimicked to cAMP. On the other hand, we cannot rule out the possibility that carpaine might interfere with intracellular calcium homeostasis through PKA activator or other mechanisms (PDE inhibitor and AC activator).

The present report clearly shows the uterine relaxing effects of carpaine isolated from the leaves of *C. papaya* L. in estrogen-primed rat myometrium and are summarized as follows.

1. Carpaine may blockage of calcium movements across the plasma membrane. This effect may be mediated mainly through a reduction on Ca^{2+} influx probably through voltage dependent Ca^{2+} channel.

2. The effect of carpaine may involve the opening of K_{Ca} channel. Which is likely to be due to the direct action of carpaine on the channel or a consequence of an increase in $[Ca^{2+}]_i$.

3. Other possible mechanism of carpaine in uterine tissue may involve an accumulation of cAMP through an activation of AC, inhibition of PDE or activation of PKA.

4. It is unlikely that the relaxing effect of carpaine occurs through an activation of NO-cGMP pathway.