CHAPTER IV

DISCUSSION

In the first part of the present study, most myometrial preparations exhibited spontaneous contractile activity. The pattern of the contraction of specimens obtained from women during proliferative phase of the menstrual cycle mostly appeared to be high frequency and low amplitude, which is consistent with that observed by Lohsiriwat and Anumanrajadhon (1986). Tyrode's solution was similarly used as physiological solution whereas Krebs-Ringer's solution was used by some authors (Odum and Broughton Pipkin, 1988; Morizaki et al., 1989).

The results in the first part of the present study demonstrated both excitatory and inhibitory effects of allicin on the contraction of nonpregnant human myometrial preparations from women during proliferative phase of the menstrual cycle. Allicin exhibited significant increase ($p<0.05$) in the force and stress of contraction at a dose of 0.4 ml of 4 mg/ml while at high doses of 0.6 and 0.8 ml of 4 mg/ml, allicin significantly decreased the force and stress of contraction ($p<0.05$) (Figs. 4 b-d, 5). At doses of 0.2, 0.3, and 0.5 ml, allicin was without significant effect on the response (Fig. 5). Thus, a dose of 0.4 ml of 4 mg/ml seemed to be optimal for contractile response to allicin. However, rate and waveforms were not affected.

The dose responses to allicin in this study seemed to be a selective manner, resembling that described by Borvonsin, Rerksngarm, and Chumpolbunchorn (1989), even though the doses, phase and species model are different. Such study was conducted on rat uterine motility during diestrus which is approximately the same period as of the menstrual phase in human. Three doses, 2, 4, and 8 mg of garlic solution were fed to the animal. It was shown that garlic solution at doses of 2 and 4 mg (equivalent to 0.1 and 0.2 mg of allicin, respectively) increased amplitude and regulated rhythmicity and form of uterine contraction while a dose of 8 mg (equivalent to 0.4 mg of allicin) did not affect the uterine contraction. The effect was more potent at a dose of 4 mg of garlic solution.

Contrary to the present study, the experiment in isolated rat uterus obtained from estrus phase of the estrous cycle of rat, 8 to 10 weeks of age, have shown that 3.5 mg/ml allicin significantly ($p<0.05$) increased the amplitude of contraction at doses of 0.4, 0.8, and 1.6 ml in a dose-dependent manner while rate and waveforms of contraction were not affected (Permpintong, 1991). The dose responses of allicin were different despite the same procedure of allicin extraction. This may be due to difference in species, since the innervations of human and rat uterus are not similar in the density of nerves (Morizaki et al., 1984).

In vitro study on human myometrial preparations revealed different types of contraction waveforms either singly or in combination, which seemed to relate to dates of the menstrual cycle (Lohsiriwat and Anumanrajadhon, 1986). The myometrial contraction is certainly affected by levels of estrogen, progesterone, prostaglandins, oxytocin and many other factors (Pritchard and MacDonald, 1980). Likewise, contractile response to drugs or chemical agents may be also affected during different phases of the menstrual cycle. Thus, it has been further proposed that contractile responses to allicin of human myometrium from women during ovulatory, secretory, and menstrual phases of the menstrual cycle are probably different because of changes in hormonal levels during the cycle. Further studies on the effects of allicin in the presence or absence of some hormones on the myometrial contraction of humans and animals during different phases of the menstrual and estrous cycle, respectively, are suggested.

In the second part of the present study, the mechanism of action of allicin on the contraction of isolated human myometrium was investigated. Atropine and phentolamine were used as a muscarinic and alpha-adrenergic antagonist, respectively, at doses of 0.2 ml of 10^{-4} M both. Such doses of atropine and phentolamine were followed those previously described by Morizaki et al. (1989). It was shown that atropine and phentolamine significantly ($p<0.05$) decreased the force and stress of contraction (Figs. 6,7). These results are in accord with the finding that human

myometrium is innervated by cholinergic and alpha-adrenergic excitatory motor nerves by electrical field stimulation study (Morizaki et al., 1989). 0.2 ml of 10^{-4} M atropine and phentolamine were without significant effects on the response to 0.4 ml of 4 mg/ml allicin. As a consequence, it is suggested that allicin acts on neither muscarinic nor alpha-adrenergic receptors.

Using propranolol as a beta-adrenergic antagonist at a dose of 0.2 ml of 10^{-4} M (as previously described by Morizaki et al., 1989), it was found that propranolol only slightly affected the contraction of human myometrium. The contractile response to allicin was not enhanced by propranolol, suggesting that allicin does not exert its action via beta-adrenergic receptor. The findings are in agreement with those described by Permpintong (1991).

Myometrial contractility is generally known to be initiated by an increase in the concentration of intracellular free calcium and is heavily dependent on the availability of extracellular calcium (Bolton, 1979; Forman et al., 1986) so that agents which inhibit the flow of calcium across the cell membrane would be expected to inhibit uterine contraction. Calcium channel blockers, such as verapamil, nifedipine, and nitrendipine inhibit myometrial contractility *in vitro* and *in vivo* (Ulmsten et al., 1978; Odum and Broughton Pipkin, 1988; Morizaki et al., 1989) by preventing the increase in intracellular free calcium. The

increase in intracellular free calcium is the result of the influx of extracellular calcium and/or the release of intracellular storage sites. Myometrial smooth muscle appears primarily to be activated by the influx of calcium from the extracellular fluid, rather than by the release of intracellular stores (Maigaard et al., 1986).

The influx of extracellular calcium across the cell membrane is through the channels called potential-dependent channel (PDC), which is activated by membrane depolarization, and receptor-operated channel (ROC), which is activated by specific receptor (Triggle, 1981). Four types of potential-dependent channels have been described (Zelis and Moore, 1989). These are the T (transient of "fast"), the N (neuronal), the L (long-lasting or "slow") and the omega-conotoxin (CTX) sensitive channels (Zelis and Moore, 1989; Cohen, Jones, and Angelides, 1991). The activity of the L channel, neither T nor N channels, causes an increase in membrane potential to the threshold potential.

The data presented here show that 0.2 ml of 10^{-5} M verapamil and nifedipine significantly inhibited the force and stress of myometrial contraction. The findings are in agreement with Odum and Broughton Pipkin (1988), and Permpintong (1991). 0.2 ml of 10^{-5} M verapamil and nifedipine also had significant inhibitory effects on myometrial response to 0.4 ml of 4 mg/ml allicin. In this study, verapamil completely blocked the contractile responses to

allicin while partial blockade was observed by Permpintong (1991). Since the effect of allicin at a dose of 0.4 ml of 4 mg/ml, which induced a maximal contractile response, was completely blocked by nifedipine, the dihydropyridine calcium antagonist, it is therefore indicated that allicin may exert its action through dihydropyridine-sensitive calcium channel, that is, the L-channel. Furthermore, the results in the first part of this study revealed that at doses of 0.2, 0.3, and 0.5 ml of 4 mg/ml allicin, the responses were slightly increased without significance. Allicin at such doses may exert its action through T- and/or N- type channels by which the membrane potential does not rise to the threshold potential. At higher doses of 0.6 and 0.8 ml of 4 mg/ml allicin, contractile responses were inhibited. Allicin at high doses might cause membrane depolarizing blockade and negative feedback loop of calcium, which in turn cause a decrease in extracellular calcium influx and hence inhibition of smooth muscle contraction. It is proposed that omega-CTX sensitive channel probably plays a role to some extent in the contractile responses to allicin because in pathological conditions, the production of toxins may occur even though normal myometria were selected to use in the study. However, age, parity, phases of the menstrual cycle, and individual factors might be involved in contractile responses of the human myometrium to allicin.