Effect of allicin on rat uterine contraction

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The purpose of this study was to investigate the effects of allicin and its mechanisms of action on the contraction of rat uterine muscle. Four different doses (0.004, 0.008, 0.017 and 0.034 mM) of allicin were added to 20 ml. of De Jalon’s solution in which the segment of uterine horn was suspended. The uterine horn used obtained from the estrus phase of the estrous cycle of rats of 8–12 weeks of age. One end of 1 centimeter length of each horn was ligated to a glass rod in the bath solution which was aerated with a 95% O₂ and 5% CO₂ mixture, the other end was connected to the transducer. The mechanism studies of the effect of allicin on muscarinic, alpha and beta receptors were conducted by the application of atropine, phentolamine and propranolol, respectively. Verapamil was used to study the effect of allicin on Ca-channel. The contractions were recorded with a Dynograph in terms of force, rate, rhythmicity and form.

From the results of our study, it appears that allicin significantly increases the force of contraction (p<0.01). The higher dose of allicin, the more force of contraction. Furthermore, atropine did not inhibit the action of the allicin. (p<0.01). Thus it appears that the allicin may or may not exert its function via muscarinic receptors. The regimen employed for phentolamine caused no inhibitory effect of the allicin action, by which indicating no exertion on alpha receptors (p>0.01). Testing for beta adrenergic receptors by the application of propranolol indicated that the propranolol neither enhanced the effect of allicin nor possessed permissive actions (p<0.01). It may be suggested that allicin does not act via the beta receptor. In the application of verapamil, a calcium blocker, the allicin overcame the effect of the verapamil in a dose-dependent manner (p<0.025). The conclusion has been drawn that allicin may induce an opening of calcium channel and/or activate intracellular calcium mobilization.

Keywords: Allicin, Rat uterine muscle, Muscarinic, Alpha and beta receptors, Ca-channel.

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การวิจัยครั้งนี้ มีวัตถุประสงค์ที่จะศึกษาฤทธิ์และกลไกการทำงานของอัลลิชินต่อการขาดด้านของเด็กเห่า 哒ค์ ปัญญา 18-12 ดัคค์ 1 นาที 体นาทีestrus ศึกษา อัลลิชิน ขนาด 0.004, 0.008, 0.017 และ 0.034 มิลิมิเตอร์ 12 มิลิลิตรในวิก De Jalon's solution ใน water jacket organ bath ที่มีอุณหภูมิ 1 ชม. ลูกกังค์ติดอยู่กับ transducer ของเครื่อง Dynograph ใช้ atropine, phentolamine และ propanololเพื่อศึกษาการออกฤทธิ์ของอัลลิชินผ่านทาง muscarinic, alpha และ beta receptors ตามลำดับ และใช้ verapamil เพื่อศึกษาการทาง Ca channel บันทึกผลในรูปของความแรง อัตรา จังหวะและรูปลักษณะของการขาดด้าน

จากการศึกษาพบว่า อัลลิชินเพิ่มความแรงการขาดด้านของเด็กเห่า 18-12 ผ่านทาง ถ้าที่ (p<0.01) โดยการขาดด้านจะสูงขึ้นตามปริมาณอัลลิชินที่เพิ่มขึ้น พบว่า atropineไม่สามารถยับยั้งฤทธิ์ของอัลลิชินได้ (p<0.01) ซึ่งเชื่อว่าอัลลิชินอาจไม่ได้ออกฤทธิ์ผ่าน muscarinic receptor แต่จะ alpha adrenergic receptor ของเด็กเห่า โดยใช้ phentolamine พบว่า phentolamineไม่สามารถยับยั้งฤทธิ์ของอัลลิชินได้ (p<0.01) เชนเดียวกัน จึงเชื่อว่าสารสกัดไม่มีผลกระทบ alpha adrenergic receptor สำหรับ propanolol ไม่สามารถเสริมฤทธิ์หรือรับรองการทำงานอัลลิชิน ซึ่งแสดงว่าอัลลิชินไม่มีผลกระทบbeta adrenergic receptor (p<0.01) แต่พบว่า อัลลิชินสามารถเพิ่มการขาดด้านของเด็กเห่า 18-12 จากการใช้ verapamil แบบ dose-dependent (p<0.025) ซึ่งเชื่อว่าอัลลิชินอาจทำหน้าที่เป็นการเปิด calcium channel และ/หรือ ทำให้เกิด การเคลื่อนย้าย calcium รายในเซลล์
Garlic (*Allium sativum*, Linn.) has been prescribed as a dermatologic agent, anthelmintic and diuretic in Thai traditional medicine. It was also used as an emmenagogue and abortifacient. A number of reports have claimed the estrogenic activity of garlic on uterine motility, i.e., in ovariectomized rats, immature rats and mice. On isolated guineapig uterus, 31–50 mg/ml of garlic extract demonstrated an equivalence of 0.003 IU oxytocin on contractile action. A study in rats, 40 days of age, fed garlic solution at doses of 1, 5, 10, 50, 100 and 150 mg/day for 7 and 10 days did not significantly change the weight of the ovaries and uteri. It was concluded that garlic did not stimulate the growth of ovary and uterus tissue and/or induce the secretion of pituitary gonadotropins. By feeding 0.5 ml (3 mg) of commercial garlic to rats, it was demonstrated that garlic solution dramatically regulated the rhythmicity, form and amplitude of contraction. In vitro study demonstrated increasing the rate of contraction during proestrus and diestrus.

The effect of garlic on uterine contraction has been studied by various methods. Water extract appeared to decrease uterine motility in pregnant and non-pregnant albino rats and guineapig. 95% alcoholic extracts and petroleum ether extracts of garlic at doses of 150–200 mg/kg showed no antifertility effect in female albino rats. In humans, alcohol extracts of garlic increased the contractions of non-pregnant uterus. The contrary effects may be due to different solvents used for the preparation of the garlic, and thus different derivative products and purities of the active substances obtained. Distillation of vapourized substance by high pressure yields an active substance called diallyl disulfide oxide or allicin. Extraction by chloroform obtained pure allicin whereas by ethyl or methyl alcohol other substances contaminated the allicin. The effects of allicin, the most active substance of garlic on the contraction of uterine muscle have rarely been reported. Furthermore, only a little analysis for the mechanism of action of allicin has been postulated.

The objectives of our study were:

1. To investigate the effects of allicin extracted from garlic on the contraction of isolated rat uterine muscle during the estrus stage.
2. To investigate the mechanism of the action of allicin on the contraction of isolated rat uterine muscle via muscarinic receptor, alpha-adrenergic receptor, beta-adrenergic receptor and/or calcium channel.

**Materials and Methods**

1. **preparation of allicin and allicin solution**

The procedures of allicin extraction followed those described by Poolsanong. By such preparation 5% of allicin was obtained and the total mixture was 7 gm/100 gm of crude garlic.

One gram of the prepared mixture was dissolves in 10 ml of distilled water. The concentration of allicin was thus 70 mg/ml. Then the supernatant was diluted 1:20 to make a concentration of 3.5 mg/ml and stored in a refrigerator for subsequent use.

2. **Tissue preparation and experimental procedure**

Female Wistar rats, 8–12 weeks of age, weights of 150–230 gm, during their estrus stage used in the experiment. Ten rats, each with both uterine horns, were used in each treatment. A
one centimeter length of each uterine horn was excised and immersed in De Jalon's solution (NaCl, 0.9%; KCl, 0.042%; CaCl₂, 0.006%; glucose, 0.05%; NaHCO₃, 0.05%) and aerated with a mixture of 95% O₂ and 5% CO₂. The clear and clean segments were suspended in a 20 ml organ bath perfused with the De Jalon's solution and aerated with the same mixture of gases. The solution temperature was thermostatically controlled at 37 °C. One end of the uterine segment was ligated to a glass retaining rod at the bottom of the organ bath whereas the other end was connected to the transducer of a Dynograph. The spontaneous contraction of the tissue was measured by an isotonic force transducer applying a resting tension of 1 gm equilibrated.

In each treatment the segments were allowed to equilibrate in the bath for 30 minutes. The contractile activity was recorded following the equilibration. The segments were washed three times by draining and the addition of another 20 ml of De Jalon's solution after each treatment. Doses of 3.5 mg/ml of prepared allicin were added to 20 ml of De jalon's solution for each treatment. The contractile responses were expressed in terms of force, rate, rhythmicity, and form.

3. Chemicals

The following drugs were used: propranolol HCl, phentolamine, acetylcholine chloride, isoproterenol, atropine sulphate, norepinephrine, and verapamil. All of the drugs used were products of well-known pharmaceutical manufacturers.

4. Experimental protocol

The experiments were conducted as follows:

4.1 Effect of allicin on uterine muscle contraction

Four doses of allicin, 0.2, 0.4, 0.8 and 1.6 ml of 3.5 mg/ml (i.e. 0.004, 0.008, 0.017 and 0.034 mM, respectively) were added to an organ bath containing 20 ml of De Jalon's solution where the uterine segments were suspended. Each dose was used for 5 minutes. The segments were washed after each treatment.

4.2 Study on the mechanisms of the action of the allicin

4.2.1 Effect of allicin on muscarinic receptor

Atropine was used in order to determine the action of allicin on muscarinic receptor. 0.2 ml of 10⁻⁴ M atropine was added to the organ bath where the uterine horn segment was suspended and allowed to act for 5 min. Then 0.004, 0.008, 0.017 and 0.034 mM doses of prepared allicin solution were added sequentially. Prior to each of the aforementioned doses being applied, the medium was drained off and the muscle was allowed to return to a steady state of normal contraction.

4.2.2 Effect of allicin on alpha-receptor

The dose of allicin that caused 90-95% of maximal uterine contraction was 0.017 mM. This was used as the control dose. The control dose of allicin was added to the bath fluid and allowed to act for 5 min. The segment was then washed 3 times and the muscle allowed to regain its control of spontaneous motility.

Phentolamine, an alpha-antagonist, was used for studying the effect of allicin on alpha-receptor. 0.2 ml of 10⁻⁷ M of
phenolamine was added to the muscle bath and allowed to act for 5 min and then followed by the control dose of allicin

4.2.3 Effect of allicin on beta-receptor

Propranolol was used in order to determine the action of allicin on beta-receptor. Propranolol, 0.2 ml, 10^{-5} M was added to the muscle bath and allowed to act for 5 min and then followed with the control dose of allicin. All steps of the treatment were as performed in 4.2.2

4.2.4 Effect of allicin on Ca-channel

The responses to doses of verapamil, a calcium channel antagonist, (10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} M.) were conducted. Each concentration was allowed to act for 9 min. The dose which relaxed 80% of control, 10^{-8} M, was employed as the standard dose in the treatment. The standard dose of verapamil was added into the muscle bath and allowed to act for 9 min. Following this, 0.004, 0.008, 0.017 and 0.034 mM of allicin were added to the bath for each treatment.

5. Statistical analysis

The results are presented as mean and standard error of the mean (SEM). The student's paired t-test was used to evaluate the levels of significant difference of the mean values. Probability values of less than 0.05 were accepted to be significant.

Results

Effect of allicin on uterine muscle contraction

The tests with the 0.004, 0.008, 0.017, and 0.034 mM doses of allicin were found to increase the force of contraction of the uterine muscle. The higher the dose of allicin, the more force of contraction. The 0.008-0.034 mM doses significantly increased the contractions (p<0.025-0.01). Rate, rhythmicity and form of contraction did not respond to the allicin applied (Fig 1 and Table 1).

<table>
<thead>
<tr>
<th>Table 1. Effects of various doses, 0.004, 0.008, 0.017, and 0.034 mM of allicin on uterine muscle contraction. (mean ± SEM)</th>
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<tr>
<td><strong>Treatment</strong></td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>control (n=10)</td>
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<tr>
<td>Allicin 0.004</td>
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<td>Allicin 0.008</td>
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<td>Allicin 0.017</td>
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<td>Allicin 0.034</td>
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NS, non-significant; *p<0.025; **p<0.01
Figure 1. Effect of allicin at 4,8,17 and 34 μM on contraction of the rat uterine muscle.

1. Effect of allicin on muscarinic receptor

Figure 2 shows the effects of allicin at doses of 0.004–0.034 mM and 0.2 ml of 10⁻⁴ M atropine. It appears that with the presence of atropine the muscular contraction remained responsive in a dose-dependent manner to the allicin (p<0.01). That is, atropine did not affect the contraction resulting form the allicin. This indicates that allicin may not act on uterine muscle via muscarinic receptor.

Figure 2. Effect of allicine, at different doses on uterine contraction followed the application of 0.2 ml, 100 μM atropine

- allicin 4–34 μM

** p<0.01
2. Effect of allicin on alpha-receptor

In the presence of phentolamine in the bath solution, allicin still elicited the uterine muscle contraction (p<0.01). The contractile activity was similar to that of without phentolamine (Fig. 3). It was shown that phentolamine did not inhibit the action of the allicin. This led to the postulation that allicin did not act on rat uterine smooth muscle through alpha-receptor.

![Graph showing force (g) vs. treatment](image)

**Figure 3.** Effect of allicin, 17 μM on uterine contraction followed the application of 0.2 ml, 100 μM phentolamine  
**p<0.01; NS, non-significant**

3. Effect of allicin of beta-receptor

Figure 4 depicts the uterine muscle contractions in response to the standard dose of allicin, with and without propranolol in the bath solution. Propranolol, per se significantly decreased the amplitude of contraction (p<0.025). Adding allicin after the application of propranolol did not significantly change the contractile response activated by the allicin.

4. Effect of allicin to Ca blocker

Verapamil at $10^{-9}$ to $10^{-6}$ M doses was used to study calcium channel. It was found that all doses significantly decreased uterine contraction (p<0.01). Verapamil at a $10^{-4}$ M dose that decreased 80% of contractions was applied as the control. It was shown that 0.017 and 0.034 mM of allicin significantly reversed the effect of the verapamil, p<0.025 and p<0.01 respectively (Fig.5). It may be postulated that allicin acts on uterine smooth muscle via the calcium channel.
**Figure 4.** Effect of allicin, 17 μM on uterine contraction followed the application of 0.2 ml, 10 μM propranolol
**p<0.01; *p<0.025; NS, non-significant**

**Figure 5.** Effect of allicin, 4–34 μM followed the application of 0.2 ml, 0.1 μM verapamil
**p<0.01; *p<0.025; NS, non-significant**
Discussion

The present study demonstrates that 0.004–0.034 mM allicin increased the contraction of uterine muscle in a dose dependent manner, as shown in figure 1 and Table 1. The force of contraction significantly increased as the concentration of allicin increased from 0.008 mM (p<0.025) to 0.017 and 0.034 mM (p<0.01). A series of investigations\(^4\)\(^\text{14,15,20}\) indicates that alcohol-extract garlic acts as a uterine stimulant in non-pregnant female uterus and in vitro. Sharaf\(^{13}\) found that the water extract garlic stimulated the uterus of non-pregnant and pregnant guineapigs. Mateo Tinao\(^4\)\(^\text{14,15}\) reported that 2 mg/animal of alcohol extract garlic action is similar to that of 0.001 i.u. pituitrin in vitro in guineapig uterine muscle. In addition, Saha and Kasinathan\(^5\) showed that 50 mg/ml. of water-extract garlic had a similar effect as that of 0.003 i.u. oxytocin in guineapigs. Groups of workers\(^6\)\(^\text{9,15}\) have fed 2 mg of garlic or used subcutaneous injection in ovariectomized rats and found an estrogenic effect. The result found in our study indicates that allicin extracted by chloroform acts as a uterine stimulant as shown in the previous studies. The more allicin, the higher the contraction. Form, rhythmicity and rate of contraction were not affected by allicin in this study. The unchanged events of the above may be due to the chemical property of allicin responsiveness and the estrous phase of the rat uterine muscle. However, allicin increases the degree of uterine contractility as that elicited by oxytocin, estrogens and acetylcholine. It was suggested the garlic might induce the secretion of acetylcholine\(^7\)\(^\text{15}\) or inhibit cholinesterase activity.\(^21\) This has led to the interesting question of what is the exact mechanism of allicin on the contraction of uterine muscle.

Atropine is a competitive antagonist of the action of acetylcholine and other muscarinic agonists.\(^22\) In this study the action of acetylcholine on rat uterus was performed as pretreatment. It was found that 0.2 ml. of acetylcholine (10\(^{-3}\) \text{-}10\(^{-5}\) M) significantly increased the amplitude of contraction. The 10\(^{-4}\) M dose induced 90–95% of maximal contraction and was used to test with 0.2 ml atropine, 10\(^{-4}\) \text{-}10\(^{-5}\) M. The results showed that atropine at 10\(^{-3}\) and 10\(^{-2}\) M significantly relaxed the uterine muscle (p<0.025 and 0.01 respectively). It indicated that rat uterus possesses muscarinic receptors. As shown in figure 2, atropine (0.2 ml., 10\(^{-4}\) M) that inhibited the action of acetylcholine did not inhibit the action produced by allicin at doses of 0.008, 0.017 and 0.034 mM (p<0.001). Previous studies demonstrated muscarinic receptor subtypes, i.e. M\(_1\) on cerebral cortex, M\(_2\) on heart, M\(_3\) on salivary glands\(^{23}\) and M\(_4\) on ileum.\(^{24}\) Eglen\(^{25}\) suggested M\(_2\) receptor on guineapig uterus. The excitatory response is mediated through the M\(_2\) receptor which is evidently different from other smooth muscles. They suggested that based on different subtypes on muscarinic receptors, the contractile response depends upon different affinities of selective responses to that of guineapig uterus. Therefore, atropine did not inhibit the contraction stimulated by allicin. In addition, the dose of atropine used in this study was probably insufficient to reverse the effect of allicin. The conclusion may be drawn that allicin may or may not exert its action via muscarinic receptor.

Ahluquist\(^{26}\) reported that uteri of dogs, cats, rabbits and rats have two adrenotropic receptors, one excitatory in function and the other inhibitory. The response varies by receptor domi-
nance, physiological condition (pregnant or nonpregnant), procedure of experiment (isolated, or intact) and species difference. The estrogenized rat uterus possesses excitatory alpha-1 adrenergic receptors to which the contraction may be induced by methoxamine. Norepinephrine is a potent agonist at alpha receptors and phenotolamine is a non-selective alpha-1 +alpha-2 adrenoceptors antagonist. Phentolamine was found to inhibit the activity caused by norepinephrine. The pilot study of this experiment exhibited that 0.2 ml. of phenotolamine, $10^{-4}$ M presented no decreasing effect on uterine contraction (Fig.3). The similar dose was found to have an inhibitory effect on rat uterus. Furthermore, its identical dose insignificantly inhibited the excitatory effect of 0.2 ml. norepinephrine, $10^{-4}$ M which caused 90% of maximal contractions. The result of this pre-treatment was not exactly in agreement with those reported by former elaborators. Presumably this is due to the insufficient dose of phenolamine applied in this experiment or due to physiological conditions. However, it appears that phenotolamine decreased the contraction excited by norepinephrine. The application of allicin after phenotolamine did not decrease the force of contractions elicited by the allicin (Fig.3). This indicated that allicin activity on rat uterus did not act on alpha receptors.

Isoproterenol, the most potent of the sympathomimetic amines that act almost exclusively on beta adrenergic blocking agent were used to confirm the activity of beta receptors on rat uterus in this experiment. Isoproterenol, 0.2 ml at $10^{-4} - 10^{-5}$ M was applied. It was found that the isoproterenol used in this study significantly relaxed the force of the contractions. The dose of $10^{-4}$ M, which relaxed the contraction at 50%, was chosen to test with propranolol. Propranolol at the concentration of $10^{-5}$ M is the best beta antagonist on rat uterus. Propranolol, 0.2 ml., $10^{-5}$ M was used. The application of propranolol following isoproterenol exhibited that propranolol significantly converted the relaxing action of isoproterenol. In contrast, propranolol significantly decreased the force of contraction induced by allicin. On the other hand, application of allicin following propranolol modestly increased the force of contraction (Fig.4). Propranolol is a beta antagonist. Most beta antagonists are partial agonists. Beta adrenergic blocking agents antagonize relaxation of the uterus of catecholamines, but they have no effect under conditions in which the response is excitatory. In this experiment, allicin had an excitatory effect and thus the activity of propranolol appeared to decrease the contraction. In addition, propranolol did not modify the contractile effect of allicin. This result may suggest that propranolol partially acts as an agonist on rat uterus. It is also a partial antagonist under the condition of allicin effect. Thus it is postulated that allicin activity does not exert the contraction via beta receptors.

The activity of uterine smooth muscle is mediated by actin-myosin interaction, the dominant role of intracellular calcium and second messengers. Among the established intracellular second messengers are cyclic AMP, $Ca^{2+}$, diacylglycerol, cyclic GMP and inositol-1, 4, 5-triphosphate. The common denominator for this activity is cytoplasmic free calcium. The cytoplasmic free calcium for contractile activation is the result
of an influx of the ion through the cell membrane and/or by releasing of calcium from the sarcoplasmic reticulum. Experimental and clinical experience have shown potent inhibitors of uterine contractile activity which are inorganic polyvalent cations, calcium antagonists, nicardipine, nifedipine and other related compounds like diltiazam, verapamil and methoxyverapamil (D 600). Verapamil and D 600 effectively counteract myometrial activation in vitro but their clinical use for uterine relaxation is limited by cardiac side effects.

The results of the pretreatment showed that the greater the concentration of verapamil, the more significant the decrease in both the rate and force of contraction of uterine muscle. The dose that abolished the contraction at 80% of maximum was 10^-5 M and it was used to study the activity of allicin. It was show that 0.017 and 0.034 mM of allicin significantly reversed the force of contraction (Fig 5).

Previous studies showed contraction of canine trachea induced by acetylcholine and rabbit aorta and mesenteric artery by norepinephrine after application of verapamil. The led to the postulation of intracellular calcium mobilization caused by those neurotransmitters through potential dependent channels. Studies using atropine and phentolamine demonstrated that allicin presumably did not exert its action through muscarinic and alpha adrenergic receptor, respectively. Also, experiments with propranolol suggested that allicin did not exert its action on beta receptor. Bond et al. showed that in vascular smooth muscle in a calcium-free solution, through the cell membrane, intracellular stores of calcium are sufficient to elicit maximal contraction through recycling of intracellular stores. The present study indicated that allicin may elicit activity similar to that of acetylcholine and norepinephrine. And it may act on rat uterine muscle by calcium channel and activate releasing of intracellular calcium. However, in order to elucidate what is (are) the exact the mechanism(s) of allicin on rat uterine contraction, other substances, e.g. prostaglandins (E2 and F2-alpha) and oxytocin with which inhibit calcium uptake and enhance calcium release, remain for further study. Furthermore, the use of fluorescent chelate probes such as Quin-2 would have made it possible to elaborate on the mechanism of action of allicin on uterine contraction.

Conclusion

Our experiments suggested that:

1. Allicin induced the contraction of rat uterine muscle.

2. The mechanism of action of allicin may not act via muscarinic receptors since atropine failed to inhibit the action of allicin.

3. Phentolamine did not inhibit the rat uterine contraction when allicin was applied. It is believed that allicin exerts no effect on alpha adrenergic receptor of the rat uterus.

4. Allicin did not affect the beta receptor since propranolol neither augmented the contraction nor indicated permissive effects.

5. Allicin reversed the effect of a calcium antagonist on uterine contraction. It is concluded that allicin possibly opens the calcium channel or mobilized intracellular calcium.

6. Allicin had no effect on rhythmicity, rate and form of uterine contraction in this study.

References

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