กลไกการเพิ่มฤทธิ์ของกลูทาไธโอนต่อแบรดดิไคนินในการกระตุ้นการหดตัวกล้ามเนื้อเรียบลำไส้เล็กที่แยกจากกายหนูตะเภา

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POTENTIATING MECHANISM OF GLUTATHIONE ON BRADYKININ MEDIATED CONTRACTION IN ISOLATED GUINEA PIG ILEUM

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Bradykinin (BK) เป็นสาร nonapeptide ในกลุ่ม kinin มีผลต่อการทำงานของอวัยวะต่างๆในร่างกาย เช่น ระบบหลอดเลือดและหัวใจ ทำให้เกิดการคลายตัวของหลอดเลือด นอกจากนี้ยังมีผล ทำให้กล้ามเนื้อเรียบการหดตัว BK เป็นสารที่มีครึ่งชีวิตน้อยกว่า 1 นาที และถูกทำลายได้ง่ายโดยเอนไซม์ kininases หรือ angiotensin converting enzyme (ACE) มีรายงานการศึกษาแสดงให้เห็นว่าฤทธิ์ของ BK สามารถเพิ่มขึ้นได้จากสารหลายชนิดรวมทั้งสารที่มีกลุ่ม-SH เป็น functional group ในการศึกษานี้จึงมุ่งที่จะตรวจสอบความสามารถและกลไกการออกฤทธิ์ของ glutathione (GSH) ในการเพิ่มฤทธิ์ของ BK ในการกระตุ้นการหดตัวกล้ามเนื้อเรียบลำไส้เล็กที่ได้มาจากหนูตะเภาสายพันธุ์ Dunkin Hartley Guinea pig เพศผู้ น้ำหนัก 250-350 กรัม ผลการศึกษาพบว่า ความเข้มข้นของ GSH ทำให้ผลสูงสุดในการเพิ่มการหดตัวของกล้ามเนื้อเมื่อกระตุ้นด้วย BK ได้แก่ 20.21 μM โดยที่การเพิ่มความเข้มข้นของ GSH ไม่มีผลเพิ่มการการหดตัวเมื่อกระตุ้นด้วย BK นอกจากนี้ GSH 20.21 μM ไม่มีผลทันทีในการทำให้กล้ามเนื้อเพิ่มการหดตัวเมื่อกระตุ้นด้วย BK (3 nM) แต่เมื่อเพิ่มเวลาในการ incubate เนื้อเยื่อกับ GSH เป็นเวลา 5 นาที พบว่า GSH สามารถเพิ่มการหดตัวของกล้ามเนื้อต่อ BK ได้ 39.5 ± 7.41 % ($P < 0.05$) อย่างไรก็ตาม เมื่อเพิ่มเวลาเป็น 30 นาที GSH ไม่มีผลเพิ่มการหดตัวของกล้ามเนื้อเมื่อกระตุ้นด้วย BK เมื่อเทียบกับเวลา 5 นาทีได้อย่างมีนัยสำคัญทางสถิติ นอกจาก BK แล้ว GSH ไม่มีผลเพิ่มการตอบสนองของกล้ามเนื้อลำไส้ที่มีต่อสารกระตุ้นอื่นๆ ได้แก่ acetycholine, histamine, $BaCl_2$ และ serotonin โดยที่ GSH สามารถเพิ่มการหดตัวของกล้ามเนื้อเมื่อกระตุ้นด้วย BK ได้ 52.5 ± 4.83 % ($P < 0.05$) แม้จะมีสาร QSA (10^{-6}M) ร่วมอยู่ด้วย แต่อย่างไรก็ตามฤทธิ์ของ GSH ไม่เปลี่ยนแปลงเมื่อมี L-NAME (10^{-4}M) อยู่ด้วย และ GSH ออกฤทธิ์ยับยั้งการหดตัวของกล้ามเนื้อเมื่อกระตุ้นด้วย BK ในสภาวะที่ปราศจาก Ca^{2+} นอกจากนี้ สารที่มีกลุ่ม -SH เป็น functional group เช่น N-acetycysteine, homocysteine, dithiothreitol และ captopril ที่ความเข้มข้น 20.21 μM มีผลเพิ่มการหดตัวของกล้ามเนื้อต่อ BK อย่างมีนัยสำคัญทางสถิติเช่นกัน จากการศึกษาจึงสรุปได้ว่า GSH สามารถเพิ่มฤทธิ์ของ BK ได้อย่างจำกัดโดยขึ้นกับเวลาและความเข้มข้นของ GSH ซึ่งการออกฤทธิ์ดังกล่าวมีความจำเพาะต่อการกระตุ้นการทำงานของกล้ามเนื้อด้วย BK โดยที่กลไกการออกฤทธิ์ของ GSH นอกจากเกี่ยวข้องกับการยับยั้งการสลายตัวของ BK แล้วยังอาจมีกลไกอื่นๆร่วมด้วย เช่น การเพิ่มความไวของตัวรับ อย่างไรก็ตามการออกฤทธิ์ของ GSH ในการศึกษานี้ไม่พบว่ามีความสัมพันธ์กับการเปลี่ยนของ NO-pathway และการเคลื่อนที่ของ Ca^{2+} ภายในเซลล์

สาขาวิชา เภสัชวิทยา (สหสาขาวิชา) ลายมือชื่อนิสิต.....สุพจนา เจริญสิน.....

ปีการศึกษา 2548 ลายมือชื่ออาจารย์ที่ปรึกษา.....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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KEYWORD: BRADYKININ / GLUTATHIONE / POTENTIATING EFFECT

SUPOCHANA CHAROENSIN: POTENTIATING MECHANISM OF GLUTATHIONE ON BRADYKININ MEDIATED CONTRACTION IN ISOLATED GUINEA PIG ILEUM

THESIS ADVISOR: ASST. PROF. SUREE JIANMONGKOL, Ph.D., THESIS CO-ADVISOR: ASST. PROF. NOPAMART TRAKRANRUNGSIE, D.V.M, Ph.D., 84 PP.

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Bradykinin (BK) is an endogenous nonapeptide of kinin system with multi-biological effects including vasodilation in cardiovascular system and contraction of smooth muscle. BK has a relative short half life of less than 1 minute because it can be destroyed by plasma kininases or angiotensin converting enzyme (ACE). Several compounds including thiol-containing compounds have been reported their ability in potentiating BK actions. In this study, the investigated potentiating mechanisms of glutathione (GSH) on BK mediated contraction were in isolated guinea pig ileum. The ileum was isolated from male Dunkin Hartley Guinea pigs (250 – 350 g). The results showed that GSH at the concentration of 20.21 µM elicited the maximum potentiation on BK-induced contraction. In addition, an increase in contractile responses did not correlate with an increase in concentration of GSH. GSH (20.21 µM) did not cause an immediate potentiating response to BK (3 nM). However, exposure to GSH for 5 minutes incubation significantly potentiated contractile responses to BK by 39.5 ± 7.41 % ($P < 0.05$). An increase in incubation time from 5 minutes to 30 minutes did not significantly enhance the ileal contraction in response to BK. Furthermore, the potentiation effects of GSH was not observed when cumulative addition of various contractants including acetycholine, histamine, $BaCl_2$ and serotonin were used instead of BK. In the presence of QSA (10^{-6}M), the potentiation effect of GSH increase significantly by 52.5 ± 4.83 % ($P < 0.05$). The potentiation effects of GSH , however, did not increase in the presence of L-NAME or in the absence of Ca^{2+} in Tyrode' s solution. Moreover, thiol-containing compounds including N-acetycysteine, homocysteine, dithiothreitol and captopril (20.21 µM) significantly potentiated contractile responses to BK. In conclusion, the potentiating effects of GSH on BK-mediated ileal contraction appeared to be restrictive to time and concentration. In addition, the potentiating effects of GSH on ileal contraction was specific to BK. It is possible that the potentiation effects of GSH was attributed to receptor sensitization and other mechanism in addition to ACE inhibiton. The potentiating mechanisms of GSH was not found to be correlated with an alteration NO pathway and intracellular Ca^{2+} under the present conditions.

Field of study Pharmacology (Inter-Department) Student's signature Supochana Charoensin

Academic year 2005 Advisor's signature S. Jianmongkol

Co-advisor's signature N. Trakranrungsie

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LIST OF ABBREVIATIONS

Ca^{2+}	calcium ion
AC	adenylate cyclase
GTP	guanosine 5' - triphosphate
PLC	phospholipase C
IP3	inositol 1,4,5 - trisphosphate
DAG	diacylglycerol
ATP	adenosine 5' - triphosphate
cAMP	cyclic adenosine 3',5'-monophosphate
cGMP	cyclic guanosine 3',5'-monophosphate
EDRF	endothelium-derived relaxing factor
NO	nitric oxide
SR	sarcoplasmic reticulum
MLC	myosin light chain
MLCK	myosin light chain kinase
BK	bradykinin
GSH	glutathione
CICR	Ca^{2+}- induced Ca^{2+} release
IICR	IP_3- induced Ca^{2+} release
DTT	dithiothreitol
QSA	8-hydroxyquinoline-5-sulfonic acid
PMA	phorbol-12-myristate-13-acetate
NAC	N-acetycysteine
ACh	acetylcholine
M	molar
mM	millimolar
μM	micromolar

CHAPTER I

INTRODUCTION

Bradykinin (BK) is an endogenous nonapeptide (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) of the kinin system (Figure 1). It participates in wide number of physiological and pathological states through the binding of its specific B_1 and B_2 receptors. BK has a relative short half-life of less than 1 minute because it can be destroyed by plasma kininase I and kininase II (angiotensin converting enzyme, ACE) (Regoli and Barabe, 1980).

Certain compounds and peptides can increase the pharmacological effects of BK. Various peptides have been demonstrated for their BK potentiating activity, for example an oligopeptide isolated from the snake venom (*Bothrops jararaca* and *Agkistrodon halysblomhofii)* (Cushman, *et al*.,1973 ; Kato and Suzuki, 1969) as well as those from the hydrolysis of serum proteins (Yamafuji, *et al*.,1996), hemoglobin (Ivanov, *et al*.,1997), milk (Henriques, *et al*.,1987) and wheat germ (Matsui, *et al*.,1999). In addition, thiol-containing compounds such as cysteine and penicillamine also elicited BK potentiating property (Erdos and Wohler, 1963).

Glutathione (GSH) (Figure 2) is the most prevalent cellular thiol and the most abundant low molecular-weight peptide present in the cell (Boyland and Chasseaud, 1969 ; Dickinson and Forman, 2002). GSH has been known as an endogenous antioxidant which participates in detoxification reactions for xenobiotics (Burk, *et al*., 1983) and free radicals (Arrick, *et al*., 1982 ; Deneke, *et al*.,1983). In addition, it has been reported that GSH regulates the intensity and duration of action of kinin which may due to its inhibitory effect on kininase

enzymes (Erdos and Wohler, 1963 ; Werle, *et al*., 1964 ; Edery and Grundfeld, 1969 ; Mita, *et al*., 1978).

The purpose of this study was to characterize the BK-potentiating effect of GSH and to investigate its underlying mechanisms of potentiation, using the *in-vitro* model of isolated guinea pig ileum.

Hypothesis

GSH, a thiol-containing compound, can increase the response of ileal smooth muscle to BK-mediated contraction. It is possible that GSH exerts its potentiating actions via the mechanisms in addition to kininase inhibition.

Objectives

1. To characterize the potentiating effect of GSH on BK-induced contraction such as specificity as well as the dependency on GSH concentration and exposure time.

2. To compare the potentiating effects of GSH and other thiol-containing compounds on BK-induced contraction.

3. To investigate the potentiating modes of action of GSH beside the kininase inhibition. These mechanisms of action may involve the intra-cellular signalling pathways such as protein kinase C, the nitric oxide pathway as well as intracellular Ca^{2+}.

Significance

This study will further provide knowledge of the characteristics and potentiating mechanisms of action of GSH on BK-induced contraction in isolated guinea pig ileum. The information may be useful for the treatment of gastrointestinal diseases example muscle spasm or proposed as an antihypertensive agent.

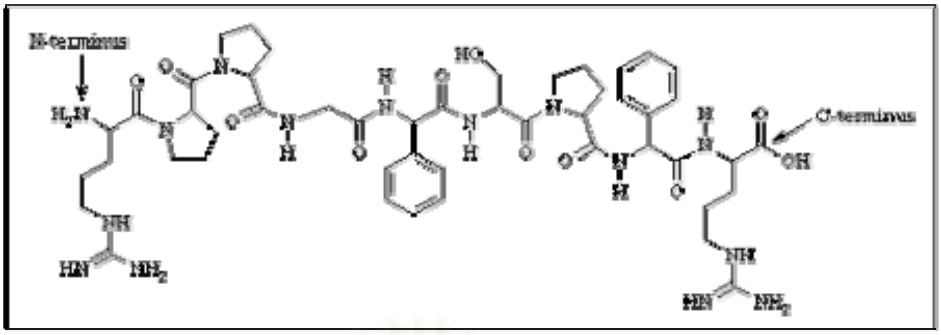

Figure 1 The structure of BK.

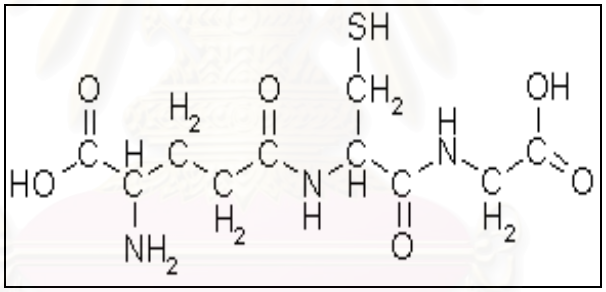

Figure 2 The structure of GSH.

CHAPTER II

LITERATURE REVIEWS

The kinin system

Kinins are a group of potent vasodilator peptides. They are formed enzymatically by kallikreins or kininogenases with as its substrate. There are two forms of kininogens. One is the high molecular weight (HMW) kininogen which is mostly found in plasma. The other is low molecular weight (LMW) kininogen which is mostly found in tissue. Hydrolysis of HMW kininogen generates BK whereas hydrolysis of LMW kininogen produces kallidin. Kinins are destroyed by the kininase enzymes. Plasma kininases are comprised of kininase I and kininase II isoforms. Kininase I is a carboxypeptidase that releases the carboxyl terminal at the arginine residue. Kininase II or angiotensin converting enzyme (ACE) inactivates kinins by cleaving the carboxyl terminal dipeptide between phenylalanyl-arginine (Figure 3) (Ward, 1991).

Kinins exert their biological actions through specific receptors located on the membranes of cells. Two types of kinin receptors, B_1 and B_2 receptors, have been characterized. The B_1 receptors appear to have a very limited distribution in mammalian tissues. Although, they are found mostly during inflammatory insults, the functional roles have not been well established. By contrast, the B_2 receptors have a widespread distribution which is consistent with the multitude of its biologic effects such as vasodilation, activation of inflammation, control of blood pressure and contraction of smooth muscle. So far, the expression of B_2 receptors have been found in smooth muscle and

some neurons. The B_2 receptors are members of the G protein-coupled family which regulates the cellular function via activation of phosphatidylinositol-specific phospholipase C signalling pathway (Hall, 1992 ; Farmer and Burch, 1992).

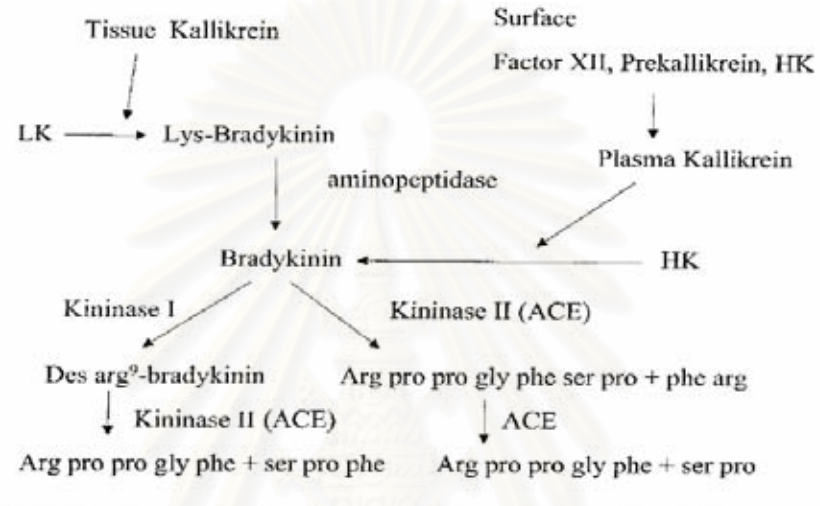

Figure 3 The kinin system. (Kaplan, *et al.*, 2002)

Control of smooth muscle contraction

Contraction of smooth muscle can be one of the pharmacological tools to screen the actions of drugs or xenobiotics as well as to study the mechanism of its actions. Generally, the contractile response is regulated by the concentraction of intracellular Ca^{2+} and the sensitivity of the contractile elements to an increase of Ca^{2+} (Karaki, *et al*., 1997) (Figure 4). The overall control of smooth muscle is shown in figure 4. Contractile agonists elicit contraction by either increasing intracellular Ca^{2+} concentration, or increasing the sensitivity of myofilaments to Ca^{2+}. Intracellular Ca^{2+} concentration is increased by: (1) Receptors coupled to phospholipase C (PLC), which leads to inositol 1,4,5-trisphosphate (IP_3) production and release stores of Ca^{2+}. (2) Voltage-gated Ca^{2+} channels, which open in response to depolarization. (3) Receptor-operated channels, which allow Ca^{2+} entry and also depolarization. The other way to increase intracellular Ca^{2+} concentration is by decreasing myosin phosphatase activity, which then causes contraction via Ca^{2+} sensitization. Agents that cause relaxation may produce this effect by reducing intracellular Ca^{2+} concentration, or directly affecting the contractile mechinery. (4) Through K^+ channel (sensitive to intracellular ATP) openers such as diazoxide that cause hyperpolarization, and thus prevent opening of voltage-gated Ca^{2+} channels. (5) ANP occupying a receptor that is directly coupled to membrane-bound guanylated cyclase. (6) Receptors (e.g. for PGI_2, adenosine) coupled to adenylated cyclase, activation of which cause increased cAMP production. This acts via protein kinase A (PKA) and myosin light chain kinase (MLCK) to inhibit contraction. Inhibitors of phosphodiesterase (PDE) protect cAMP or cGMP from degradation. (7) Stimulation of soluble guanylate cyclase by NO increases cGMP formation.

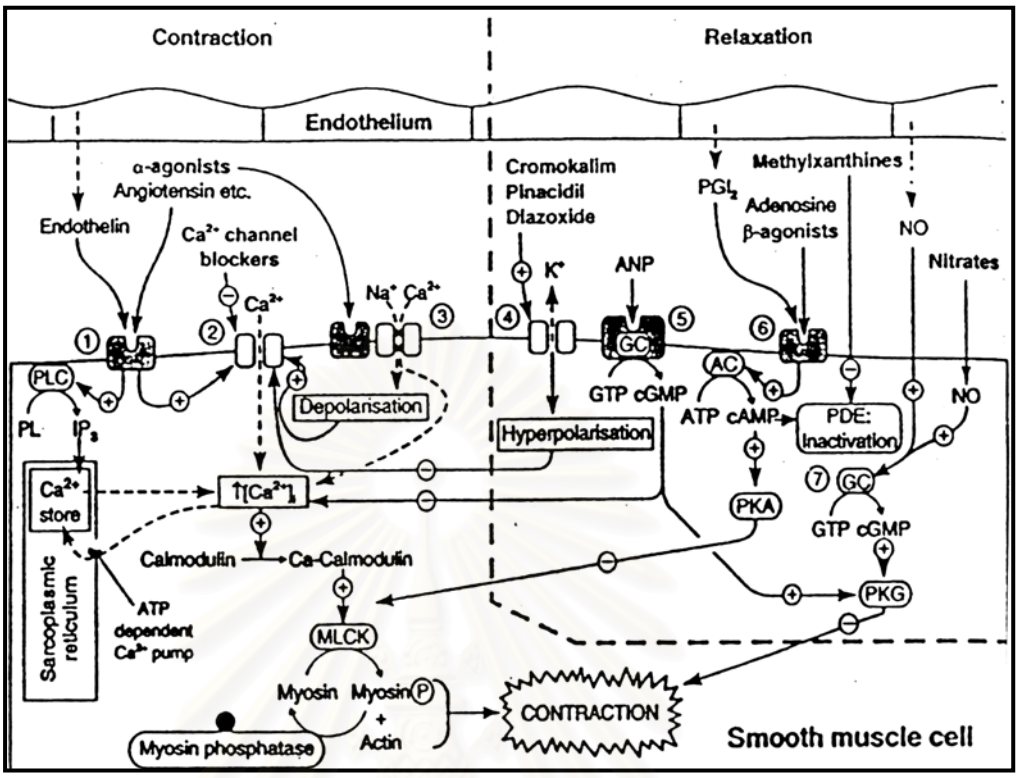

Figure 4 Control of smooth muscle. (Rang, *et al*., 1999)

The activation of B_2 receptors causes an increase of intracellular Ca^{2+} through the phospholipase C (PLC) pathway. It is well known that PLC hydrolyzes phosphatidylinositol-4,5-biphosphate (PIP_2) generating diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). IP_3 acts on its receptors on the sarcoplasmic reticulum to release Ca^{2+} into the cytoplasm. DAG activates protein kinase C, leading to the phosphorylation of several enzymes involved in muscle contraction (Farmer and Burch,1992). Intracellular Ca^{2+} exerts its action through Ca^{2+}-calmudulin complex, which activates the myosin light chain kinase (MLCK) (Westfall, *et al*., 1998). Subsequently, activated MLCK phosphorylates myosin light chains, which in turn interacts with actin to induce contraction (Figure 5) (Silverthorn, 1998).

In addition to receptor-mediated contraction, the NO-cGMP system plays an important role in regulating smooth muscle tone (Figure 5). In smooth muscle, NO exerts its effect by activation of guanylyl cyclase causing an increased formation of cGMP. The cGMP activates a kinase that subsequently leads to the inhibition of Ca^{2+} influx into the smooth muscle cell, a decreased Ca^{2+}-calmudulin stimulation of myosin light chain kinase (MLCK), and relaxation (Westfall, *et al*., 1998).

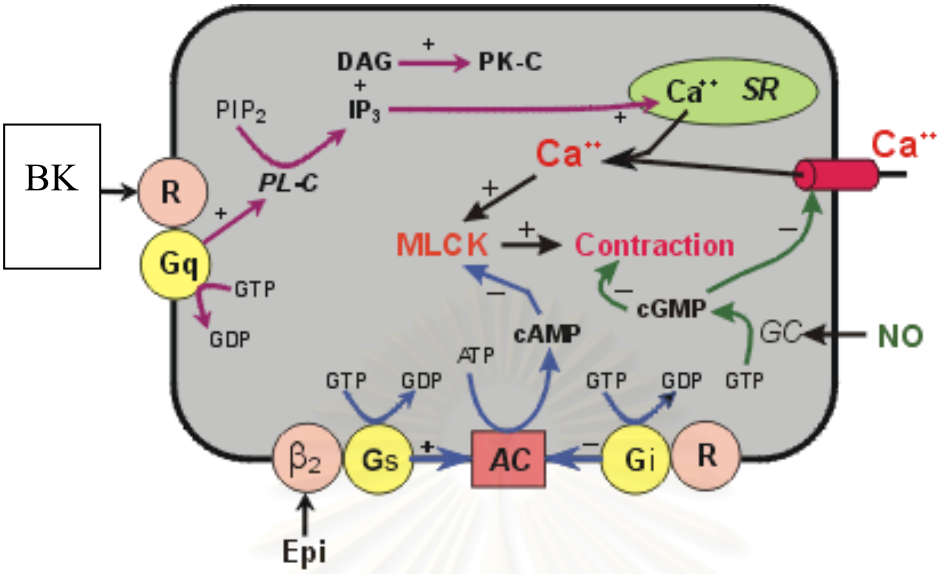

Figure 5 The signal transduction mechanisms that modulate intracellular Ca^{2+} concentration. (Richard, 2002)

The potentiating-activity of xenobiotics on BK-induced contraction

It has been hypothesized that the effect of BK on isolated smooth muscle may be potentiated by different mechanisms as follows: (1) inhibition of kininase, an enzyme in degradation process of BK (Ferreira and Rocha e Silva, 1962; Hamberg, *et al*., 1969), (2) sensitization of the BK receptors (Camargo and Ferreira, 1971) and (3) interference of signalling pathways (Muller, *et al*., 2005).

Various classes of substances, mainly peptides, can potentiate the actions of BK via different mechanisms. For example, peptides isolated from snake venom *Bothrops jararaca* increased the effects of BK on guinea pig ileum and arterial blood pressure by blocking the action of ACE/kininase II

(Ferreira, *et al*., 1965). Proteases such as chymotrypsin and cathepsin G elicited the potentiating effects on BK-mediated contraction by selectively uncovering specific BK receptors by splitting peptide bonds in the smooth muscle membranes (Edery, 1965). ACE inhibitors such as enalaprilat, quinaprilat and ramiprilat potentiated the BK effects by inhibition of ACE and activation of the B_2 receptors on guinea pig ileum (Minshall, *et al*., 2000).

Effects of thiol-containing compounds on BK actions

In addition to peptides, several thiol-containing compounds have been reported to increase the effects of BK both in the *in-vivo* and *in-vitro* model (Fontaine, *et al*., 1984). For example, cysteine and penicillamine increased the hypotensive effect of BK through the mechanism of kininase inhibition (Erdos and Sloane, 1962). In addition, L-cysteine, dimercaprol and thioglycolic acid increased the effects of BK, which had been linked to inhibition of enzymatic BK-destruction in plasma (Ferreira and Rocha e Silva, 1962 ; Erdos and Woher, 1963 ; Aureswald and Doleschel, 1967). Although there are several models to study the potentiation effects on BK actions, the *in-vitro* model of an isolated preparation of smooth muscle has been frequently employed. This may due to its effectiveness as a quick screening bioassay. Among the various models of isolated smooth muscle, it has been found that the potentiation effects on BK-mediated contraction can be most effectively determined in isolated preparation of guinea pig ileum (Vogel, *et al*., 1970). YS-980 (thiol-containing ACE inhibitor) has been reported its ability to potentiate the ileal response to BK (Iso, *et al*., 1979).

Effect of GSH on BK actions

GSH is tripeptide which consists the three amino acids including glutamic acid, cysteine and glycine. It is the most prevalent cellular thiol and the most abundant low molecular-weight peptide present in the cell. *In-vivo* study demonstrated that GSH increased hypotensive responses to doses of BK in rabbits (Takeya and Hotta, 1979). Furthermore, GSH significantly enhanced the acute edema of rat hind paws in response to BK treatment (Takeya and Hotta, 1979). In an *In-vitro* study, GSH specifically potentiated the effects of BK-mediated contraction in guinea pig ileum. In addition, GSH had no effect on the ileal contraction induced by other contractants including acetylcholine and histamine (Takeya and Hotta, 1979). Like the effects of other thiol-containing compounds, the potentiating mechanisms of GSH has been linked to inhibition of kininase II (Takeya and Hotta, 1979).

In addition to the inhibition of kininase, it has been hypothesized that thiol-containing compounds such as GSH, dithiothreitol, mecaptoethanol, monothioglycerol and cysteine increase the BK-induced contractile responses by directly reducing disulphide bonds located on the membrane surface of the smooth muscle. In addition, it has been reported that the potentiating effect of thiol-containing compounds is not limited to BK-induced contraction. (Fontaine, *et al*., 1984).

Taken together, the evidence suggests that it is possible that GSH may increase the effect of BK through mechanisms other than inhibiting kininase. Hence, the purpose of this study was to further characterize the BK-potentiating effect of GSH as well as to investigate the underlying mechanism of potentiation, using the *in- vitro* model of isolated guinea pig ileum.

CHAPTER III

MATERIALS AND METHODS

Experimental animals

Male Dunkin Hartley Guinea pigs of body weight between 250-350 g were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom. The animals were housed in the animal care facility at the Faculty of Pharmaceutical Sciences, Chulalongkorn University and acclimatized for 4 days before the experimentation.

This study was approved by the Ethical Committee of Faculty of Pharmaceutical Science, Chulalongkorn University.

Experimental instruments

1. Double walled Harvard type organ bath (Figure 6) were used. The organ baths were made of glass and comprised of inner and outer chambers. An inner chamber with the capacity of 20 ml is for suspending the isolated tissue in physiological solution aerated with carbogen gas (95%O_2 and 5%CO_2). An outer chamber is connected to and perfused by the water bath for temperature control at 37 ± 0.5 ^{0} C.

2. Water bath and thermoregulation water pump (Heto®, Model HWT 100, Jouan Nordic, Gydevang, Denmark).

3. Isometric transducers (Harvard Appararus Ltd., England) and force transducer (Model MLT 050/A, ADInstruments, Castle Hill, Australia).

4. Powerlab/ 4sp connected to a computer with program SCOPE CHART 5 V.0.2. (ADInstruments, Castle Hill, Australia).

5. Carbogen gas (95%O_2 ± 5% CO_2) (T.I.G., Bangkok, Thailand).

Chemicals

Contractants in this study included BK (MW = 1060.2), acetylcholine (Ach), histamine, barium chloride ($BaCl_2$) and serotonin. Thiol-contaning compounds included GSH (MW = 307.33), N-acetycysteine (NAC), homocysteine, dithiothreitol (DTT) and captopril. Other principal chemicals included dimethyl sulfoxide (DMSO), L-valine, enalapril, 8-hydroxyquinoline-5-sulfonic acid (QSA), phorbol-12-myristate-13-acetate (PMA) and N^G-nitro-L-arginine methyl ester (L-MAME). All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.)

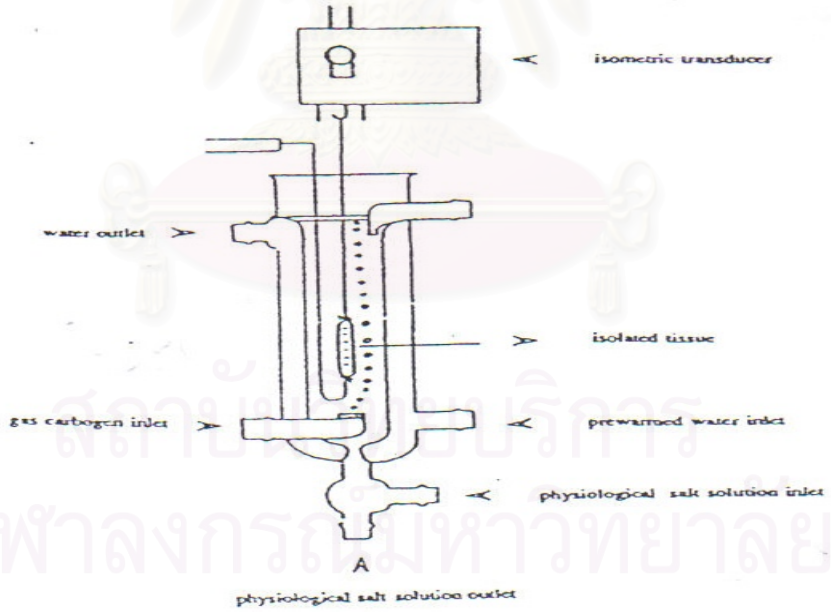

Figure 6 Illustration of instrument and organ bath for isolated guinea pig ileum

Figure 7 Ileocecal junction and preparing of isolated guinea pig ileum

Preparation of isolated guinea pig ileum

The animals were fasted 24 hours prior to use. On the day of the experiment, guinea pigs were euthanized in CO_2 chamber and killed by cervical dislocation. The ileal segments of 4-7, 7-10, 10-13, 13-16 cm. were cut from ileocecal junction and cleaned of fat and connective tissue (Figure 7). The ileal segment was suspended in a 20 ml-organ bath containing Tyrode's solution at 37°C, gassed with 95% of O_2 and 5% of CO_2. An initial load of 1 g was applied to tissue and maintained throughout the experiment. The ileum was incubated in Tyrode's solution for 30 minutes until the tension was stable before starting the experiment. Tension was recorded isometrically via force-displacement transducers, which was connected to Powerlab/ 4sp equipped with an analysis program.

Experimental procedures

1. The effects of GSH on BK-induced contraction

After the equilibration period, BK at the concentrations of 0.1 to 3000nM was added cumulatively to induce contraction. The contraction response upon addition of 3000 nM of BK was referred as maximum contraction in this study. The effects of GSH on BK-induced contraction were studied by addition of GSH at various concentrations (6.64 to 20.21 μM) 5 minutes prior to the cumulative addition of BK. The contractile responses were calculated as a percentage of contraction induced by 3000 nM of BK.

The effects of GSH-incubation time were also investigated by varying the GSH-incubation from 5 minutes to 0 and 30 minutes prior to addition of BK. The "zero" incubation was defined as the immediate response of tissue toward GSH in the presence of BK. In order to determine the "zero" incubation time, the experimental procedures were modified from the above mentioned protocol by an immediate addition of GSH (20.21 μM) at the peak response of BK (3 nM)-induced contraction. Contractile responses were calculated as a percentage of contraction induced by 3 nM of BK.

2. The potentiating effects of GSH on contraction induced by other contractants

Another series of experiments with the similar procedures were carried out using various contractants including acetycholine, histamine, barium chloride, and serotonin instead of BK. Tissues were pretreated with 20.21 μM GSH for 5 minutes prior to cumulative addition of various contractants at the concentration range of $1x10^{-9}$-$1x10^{-5}$M. Contractile responses were calculated

as percentages of maximum contraction induced by the highest concentration of each contractant.

3. Comparative effects between GSH and other thiol-containing compounds on BK-induced contraction

In order to examine the influence of the SH-group on BK-induced contraction, the effects of various thiol-containing compounds were carried out under the same experimental procedures as mentioned above. In this study, the thiol-containing compounds included N-acetycysteine (20.21 μM), homocysteine (20.21 μM), dithiothreitol (20.21 μM) and captopril (20.21 μM) were used instead of GSH for 5 minutes incubation time, followed by addition of BK (3 nM). In addition, L-valine (20.21 μM) and enalapril (20.21 μM), which were compounds without SH-groups, were also used as controls. Contractile responses were calculated as a percentage of BK (3 nM)-induced contraction.

4. Potentiating mechanisms of GSH on BK-induced contraction in isolated guinea pig ileum

4.1 Inhibition of ACE

In order to investigate the influence of ACE inhibition on the BK-potentiating action of GSH, the contractile responses were determined in the presence of QSA which is an ACE inhibitor. The effects of GSH in the presence of QSA were studied by incubating QSA (10^{-6} M) for 5 minutes prior to addition of GSH (20.21 μM). After 5 minutes incubation with GSH, BK (3nM)

was added and the tension was recorded. Contractile responses were calculated as a percentage of BK (3nM)-induced contraction in the absence of QSA.

4.2 Involvement of protein kinase C

The involvement of protein kinase C in GSH-potentiating effects on smooth muscle contraction was also studied using phorbol-12-myristate-13-acetate (10^{-5} M, PMA) as contractant. As have been shown, PMA caused smooth muscle contraction by stimulating PKC activity. Hence GSH may potentiate the effect of PMA, suggesting the involvement of an increase in PKC activity. The effect of GSH on PMA-mediated contraction was examined by pretreating the ileal segment with GSH (20.21 μM) for 5 minutes, followed by PMA to induce contraction. Contractile responses were calculated as a percentage of PMA (10^{-5} M)-induced contraction.

4.3 Inhibition of nitric oxide pathway

Another study focused on the influence of nitric oxide on the BK-potentiating action of GSH was studied using a NOS inhibitor, N^{G}-nitro-L-arginine methyl ester (L-NAME). The effect of GSH in the presence of L-NAME was determined by incubating L-NAME for 30 minutes prior to addition of GSH (20.21 μM) for another 5 minutes. Then BK (3 nM) was added and the tension was recorded. Contractile responses were calculated as a percentage of BK (3 nM)-induced contraction in the absence of L-NAME.

4.4 Influence of intracellular Ca^{2+}

The influence of intracellular Ca^{2+}on GSH action was investigated using the model of a Ca^{2+}-free condition. First, the ileal segment was incubated in Ca^{2+}-containing solution for 30 minutes and recorded its response to BK (3 nM). The contractile response to 3 nM BK in Ca^{2+}-containing solution was referred to as the maximum contraction in this study. Next, the medium was changed to Ca^{2+}-free, EDTA-containing solution for 15 minutes, followed by addition of BK (3 nM) for the record of the response. The effects of GSH was studied by pretreatment the tissue with GSH (20.21 μM) for 5 minutes in Ca^{2+}-free condition prior to addition of BK (3 nM). Contractile responses were calculated as a percentage of BK (3 nM)-induced contraction in Ca^{2+}-containing solution.

Statistical analysis

Data are presented mean ± S.E.M. for *n* separated experiments. Statistical significances were tested either by Student's *t*-test for paired data or by one way ANOVA followed by post-hoc Dunnett tests, where appropriate. The *p* values less than 0.05 were considered significant.

CHAPTER IV

RESULTS

1. Effect of GSH on BK-induced contraction

BK-induced contraction was characterized by slow-developing isometric contraction (Figure 8). As shown in Figure 9-12, cumulative addition of BK at the concentration range of 0.1 to 3000 nM generated a concentration-dependent contractile responses in all ileal segments. The maximum contraction of each ileal segment which was induced by BK at the concentration of 3000 nM was shown in Table 1. GSH at the concentration of 6.67 to 26.68 μM increased the ileal responsiveness to BK as seen by the parallel shift of concentration-response curve of BK to the left, whereas the maximum response remained unchanged (Figure 9-12). Each anatomically different ileal segment responsed to the GSH-potentiating effect at a different magnitude. In this study, an ileal segment of 4-7 cm. from ileocecal junction was the least responsive toward GSH-potentiation on BK-induced contraction. The most responsive ileal segment was the segment of 13-16 cm. from ileocecal junction. In addition, the effects of GSH on an increase in BK-mediated contraction was restrictive and independent of the concentration of GSH. As shown in Figure 11, an increase in contractile responses did not correlate with an increase in concentration of GSH (6.67 to 26.68 μM). The best GSH effect on BK-induced contractile responses were observed in ileal segment of 13-16 cm with GSH at the concentration of 20.21 μM and BK at the concentration of 3 nM (Figure13). In this experiment, GSH at the concentration of 20.21 μM with 5 minutes incubation significantly increased

the contractile effect of 3 nM BK by 39.5 ± 7.41 % ($P < 0.05$, n=4) (Figure 13,14).

Figure 15 (a, b), demonstrated the contractile profiles of the effects of GSH at various incubation times. GSH did not cause an immediate response to BK (Figure 15a). The 30 minutes of incubation time with GSH led to an increase the contractile response to BK by 53 ± 10.46 % (n=4) (Figure 16). However, an increase in incubation time from 5 minutes to 30 minutes did not significantly enhance the ileal contraction in response to BK (Figure 16).

2. Potentiating effects of GSH on contraction induced by other contractants

As seen in Figure 17 (a-d), the effects of GSH were not observed when cumulative acetylcholine, histamine, barium chloride and serotonin were contractants instead of BK. These findings suggested that the potentiating effects of GSH on ileal contraction was specific to BK.

3. Comparative effects between GSH and other thiol-containing compounds on BK-induced contraction

The contractile profiles of the effects of other thiol-containing compounds on BK-induced contraction are shown in Figure 18 (a-d). As seen in Figure 19, pretreatment of ileal segment with thiol-containing compounds such as N-acetycysteine, DTT, captopril and homocysteine at the concentraction of 20.21 μM after 5 minutes incubation caused an increase in BK-induced contraction. Among the thiol-containing compounds in this study, captopril was the most potent in potentiating the effect of BK. The magnitude

of potentiation effect of captopril was 45.5 ± 14 % (n=4). L-valine and enalapril, which were the compounds without thiol groups and were also employed in this study under the same testing conditions. L-valine did not affect the BK-mediated contraction. The contractile profiles of the effects of other thiol-containing compounds at various incubation times are shown in Figure 20 (a-d) and Figure 21 (a-d). As seen in Figure 22, none of thiol-containing compounds, except captopril, was able to elevate the contractile response upon its addition at the peak of BK-induced contraction. Like GSH, an increase in preincubation time from 5 minutes to 30 minutes had no influence on the potentiating effects of thiol-containing compounds in this study (Figure 22). These findings suggested that the potentiating effects of GSH and other thiol-containing compounds, except captopril, were comparable to BK-induced contraction.

4. Potentiating mechanisms of GSH on BK-induced contraction in isolated guinea pig ileum

4.1 Inhibition of ACE

The contractile profiles of the effects of ACE inhibition on the BK-potentiating action of GSH are shown in Figure 23. The influence of ACE which is an enzyme to degrade BK was determined in this study. The presence of QSA, an ACE inhibitor, caused a significant increase in BK-induced contraction by 26.58 ± 2.09 % (n=12). This might be due to an increase in BK availability in the system. The effect of GSH on BK-induced contraction was enhanced by 52.5 ± 4.83 % (n=4) in the presence of QSA.

Moreover, the difference in degree of GSH-potentiation in the presence and absence of QSA was significant (Figure 24). These findings suggested that GSH might exert its potentiating effect via multimechanisms in addition to ACE inhibition.

4.2 Involvement of protein kinase C

The contractile profiles of the effects of protein kinase C in GSH-potentiating effects on smooth muscle contraction were shown in Figure 25. The influence of GSH on protein kinase C-mediated smooth muscle contraction was determined in this study. The presence of PMA, a protein kinase C activator, caused contraction at 1.82 ± 0.34 g (n=6) with a dose of 10^{-5} M. GSH significantly inhibited the PMA-mediated contraction by 38.83 ± 4.87 % (n=6) (Figure 26).

4.3 Inhibition of nitric oxide pathway

The contractile profiles of the effects of nitric oxide on the BK-potentiating action of GSH were shown in Figure 27. The influence of nitric oxide on the BK-potentiating action of GSH pathway was determined this study. The presence of L-NAME, a specific inhibitor of NO synthesis, caused an increase in BK-induced contraction by 79.25 ± 16.24 % (n=8). The effect of GSH on BK-induced contraction was enhanced by 69.75 ± 26.82 % (n=8) in the presence of L-NAME, an insignificant decriment. Hence, the results showed that GSH did not affect the BK-mediated contraction in the presence of L-NAME (Figure 28).

4.4 Influence of intracellular Ca^{2+}

The contractile profiles of the effects of intracellular Ca^{2+} on GSH action were shown in Figure 29. The magnitude of contraction induced by BK in Ca^{2+}-free condition was 40.5 ± 15.75 % (n=4) of BK response observed in Ca^{2+}-containing solution. GSH significantly inhibited the BK-induced contraction in Ca^{2+}-free condition by 21.7 ± 13.8 % (n=4) is shown in Figure 30. These findings suggested that GSH might exert its potentiating effect did not correlated with intracellular Ca^{2+}.

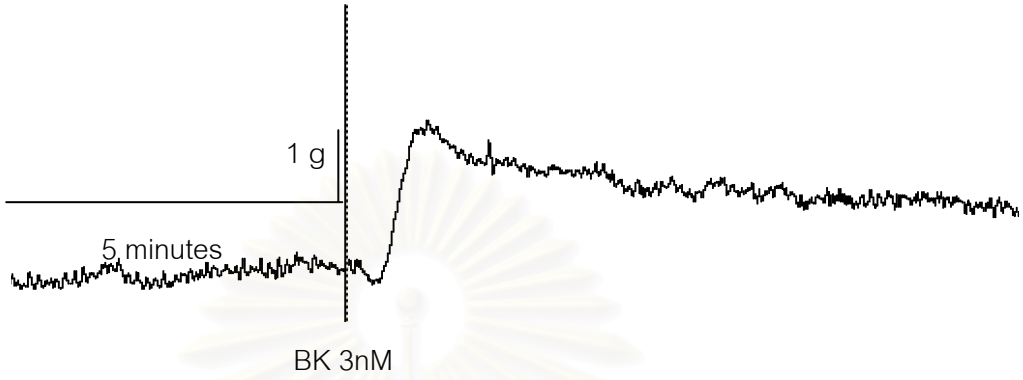

Figure 8 The profiles of the effect of 3nM BK-induced contraction.

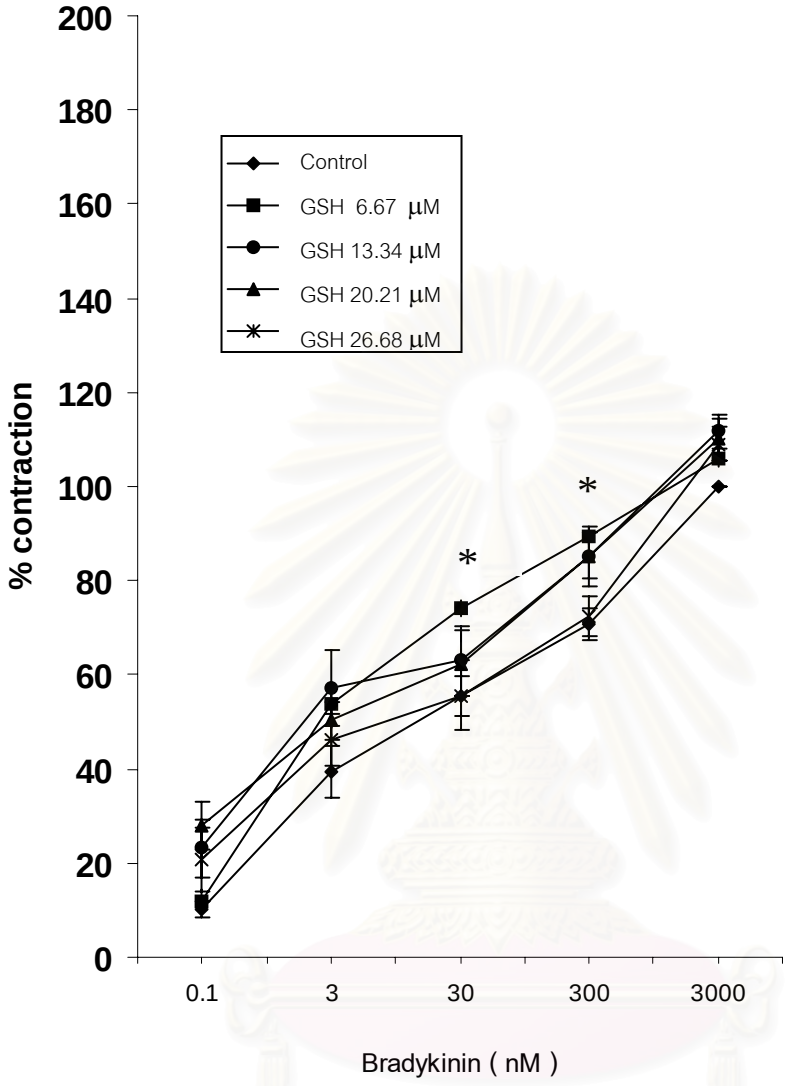

Figure 9 Effect of GSH at various concentration adding 5 minutes prior to BK (0.1 nM to 3000nM) in ileal of segment 4-7 cm.

Data were presented as mean ± S.E.M., n=4.

**P < 0.05*, significant difference from control (the group without GSH).

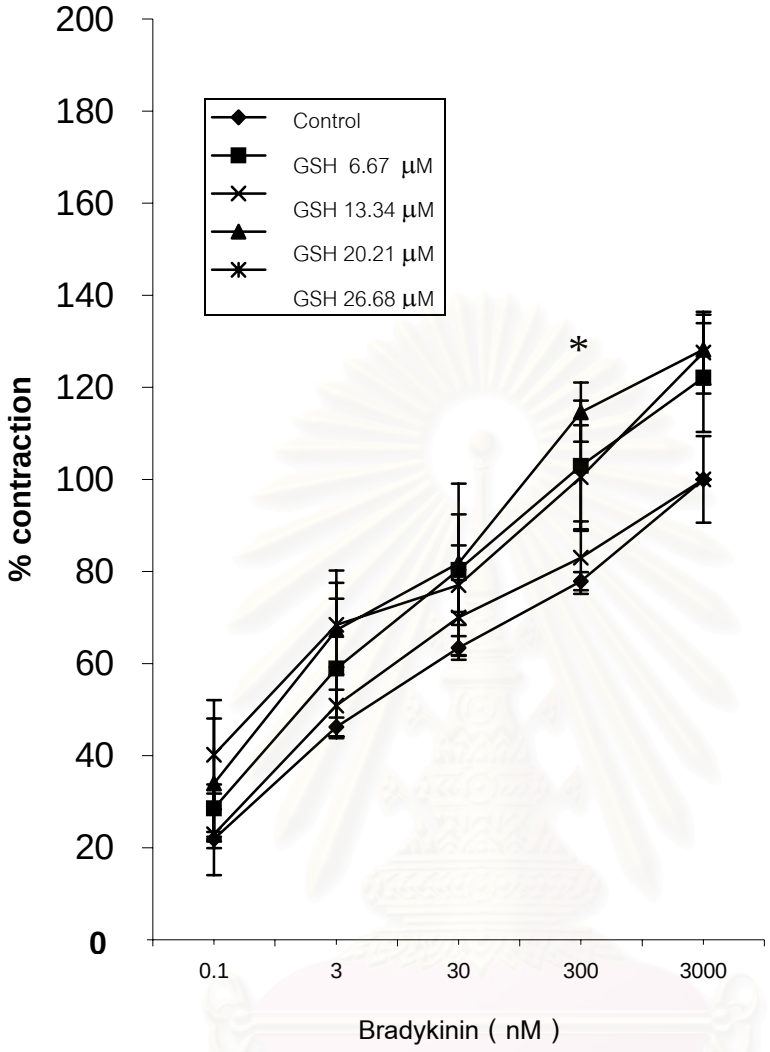

Figure 10 Effect of GSH at various concentration adding 5 minutes prior to BK (0.1 nM to 3000nM) in ileal of segment 7-10 cm.

Data were presented as mean ± S.E.M., n=4.

**P < 0.05*, significant difference from (the group without GSH).

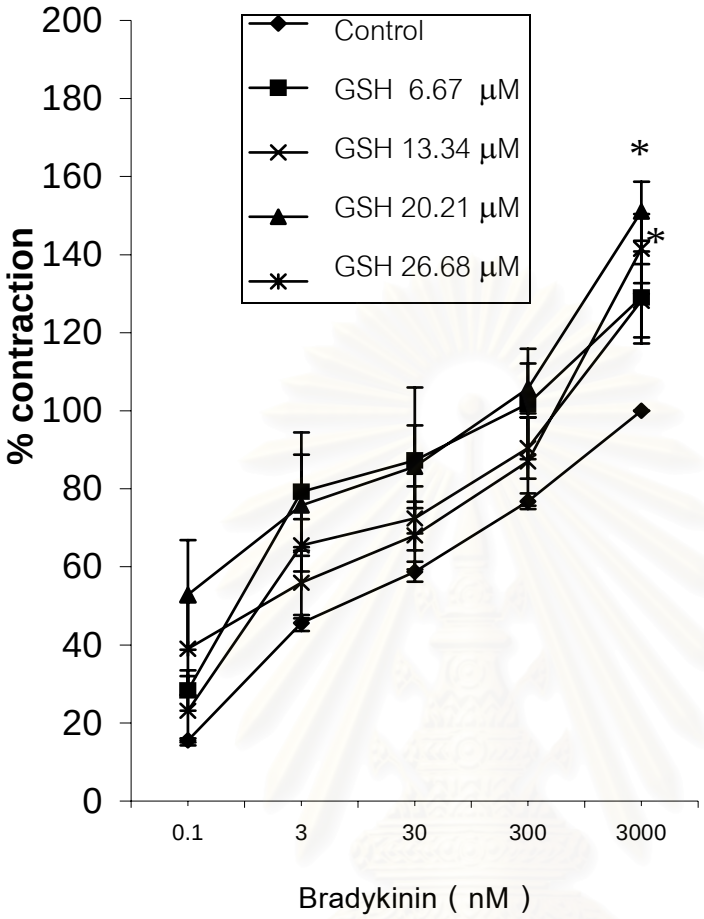

Figure 11 Effect of GSH at various concentration adding 5 minutes prior to BK (0.1 nM to 3000nM) in ileal of segment 10-13 cm.

Data were presented as mean ± S.E.M., n=4.

**P < 0.05*, significant difference from control (the group without GSH).

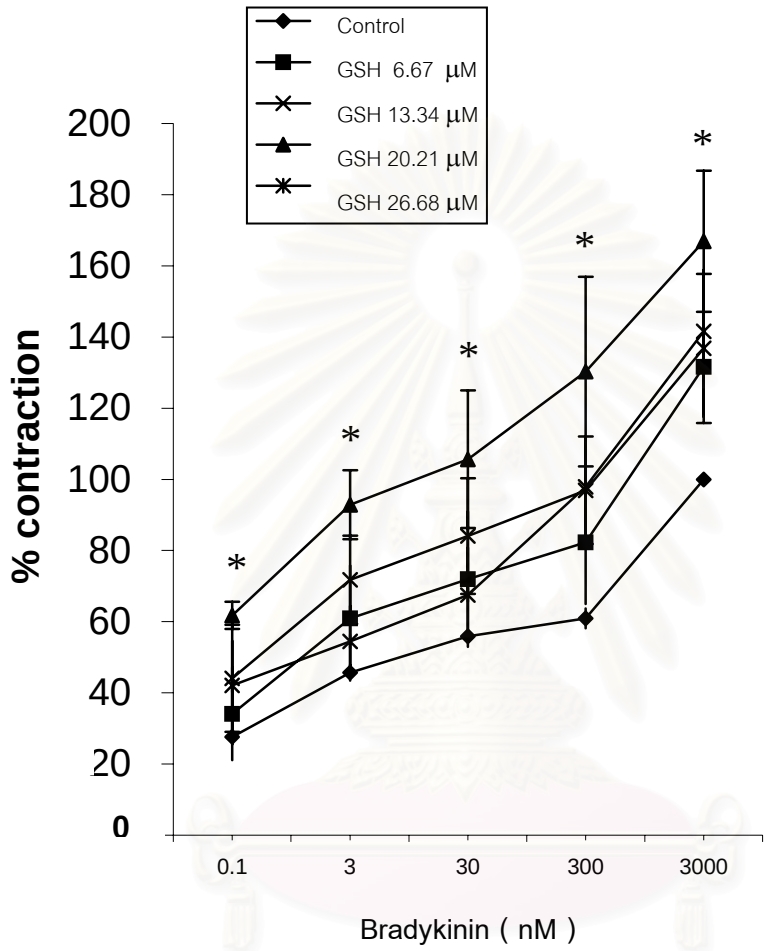

Figure 12 Effect of GSH at various concentration adding 5 minutes prior to BK (0.1 nM to 3000nM) in ileal of segment 13-16 cm.

Data were presented as mean ± S.E.M., n=4.

**P < 0.05*, significant difference from control (the group without GSH).

Table 1 The maximum contraction (g) of guinea pig ileum induced by 3000 nM BK in various segments of ileum

Segments of ileum	Force of contraction
4-7 cm.	3.89 ± 0.39 g (n=10)
7-10 cm.	3.90 ± 0.48 g (n=13)
10-13 cm.	3.90 ± 0.35 g (n=13)
13-16 cm.	3.35 ± 0.37 g (n=11)

All values represent mean ± S.E.M, n=number of experiment.

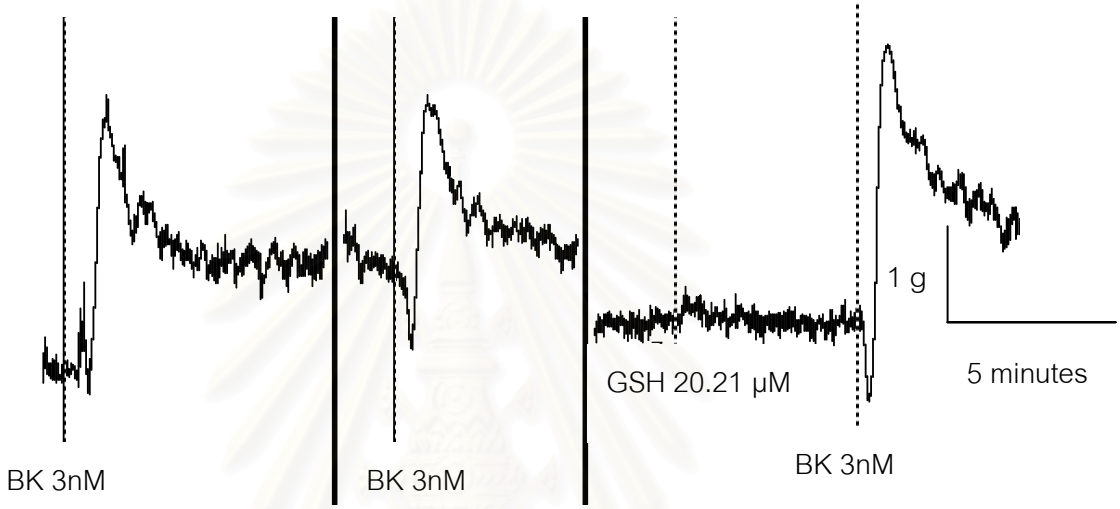

Figure 13 The contractile profiles of the effects of 20.21 µM GSH at 5 minutes incubation time on 3nM BK-induced contraction.

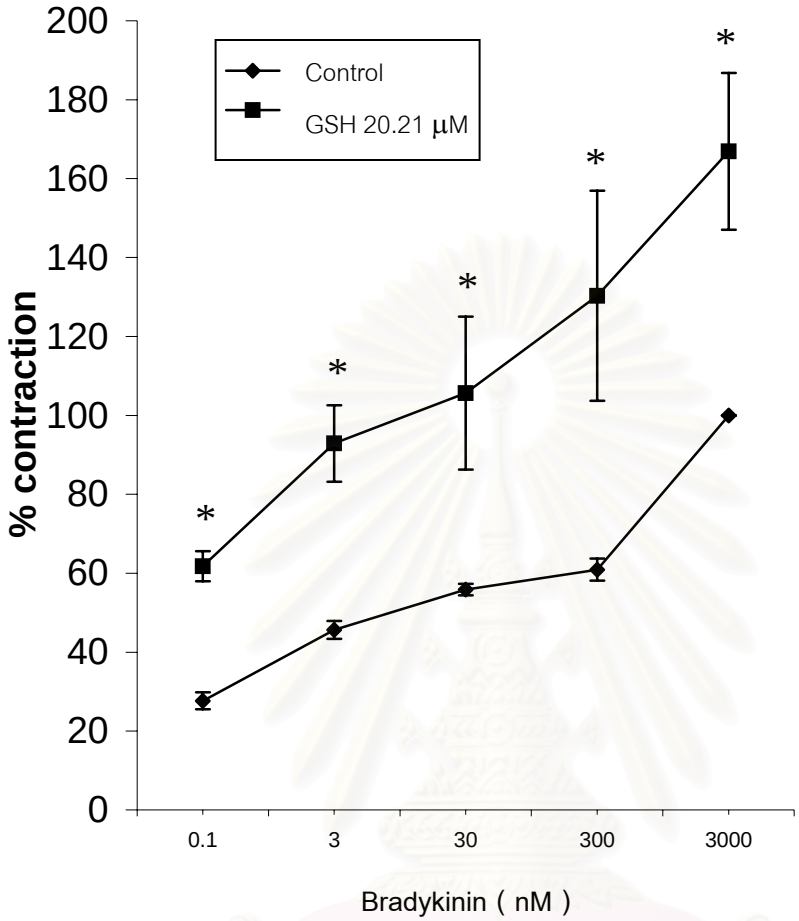

Figure 14 Effect of GSH (20.21 µM) on the responsiveness of guinea pig ileum toward cumulative addition of BK.

Data were presented as mean ± S.E.M., n=4.

**P < 0.05*, significant difference from control (the group without GSH).

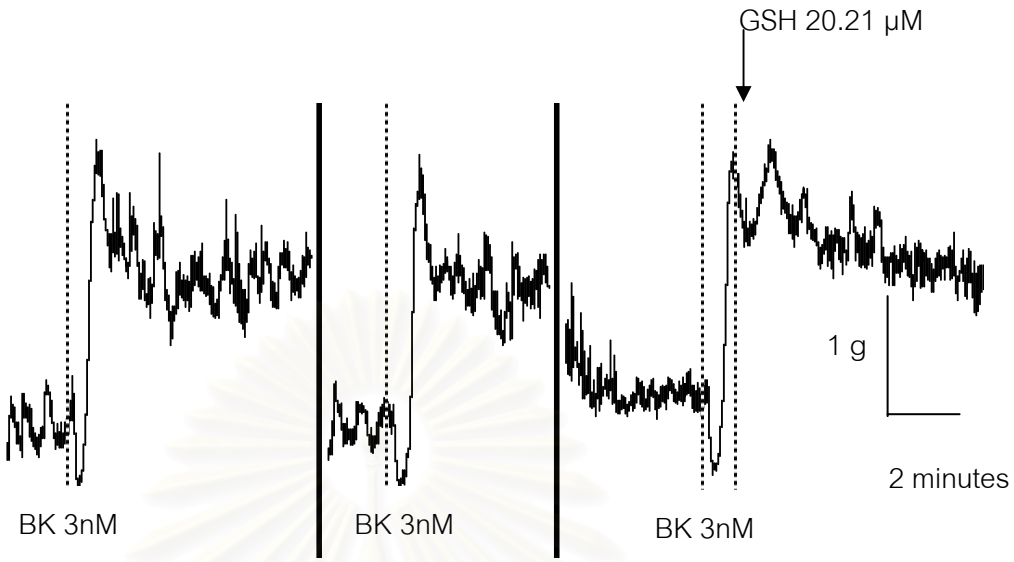

Figure 15a The contractile profiles of the effect of 20.21 µM GSH at 0 minute incubation time on ileal contraction induced by 3nM BK.

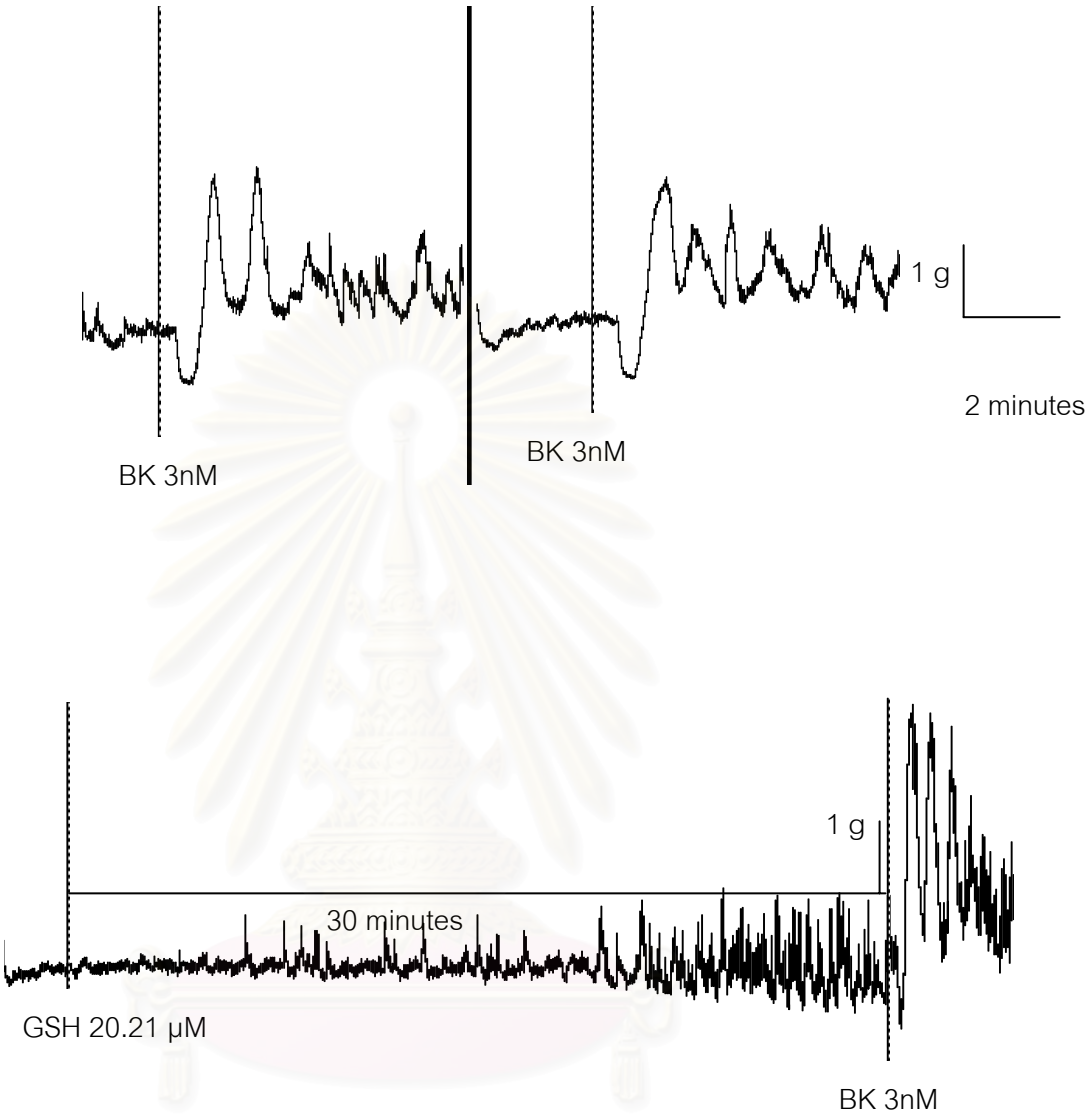

Figure 15b The contractile profiles of the effects of 20.21 μM GSH at 30 minutes incubation time on ileal contraction induced by 3nM BK.

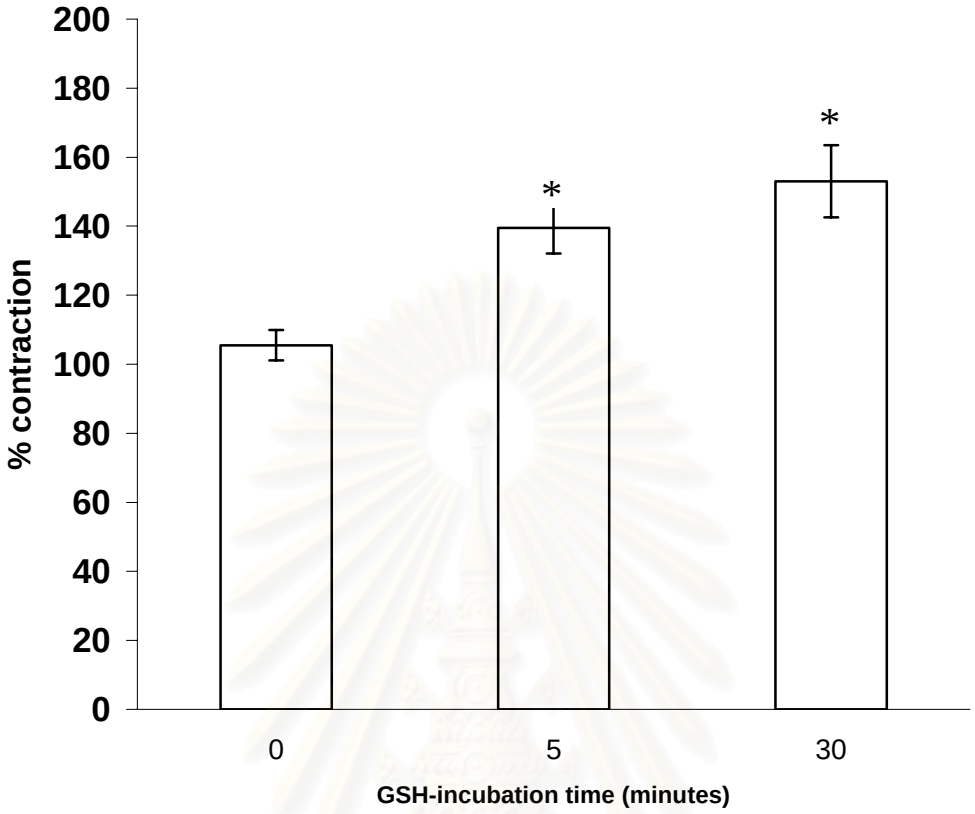

Figure 16 Effect of GSH (20.21μM) at various incubation time prior to addition of BK (3 nM).

Data were presented as mean ± S.E.M., n=4.

*$P < 0.05$, significant difference from control.

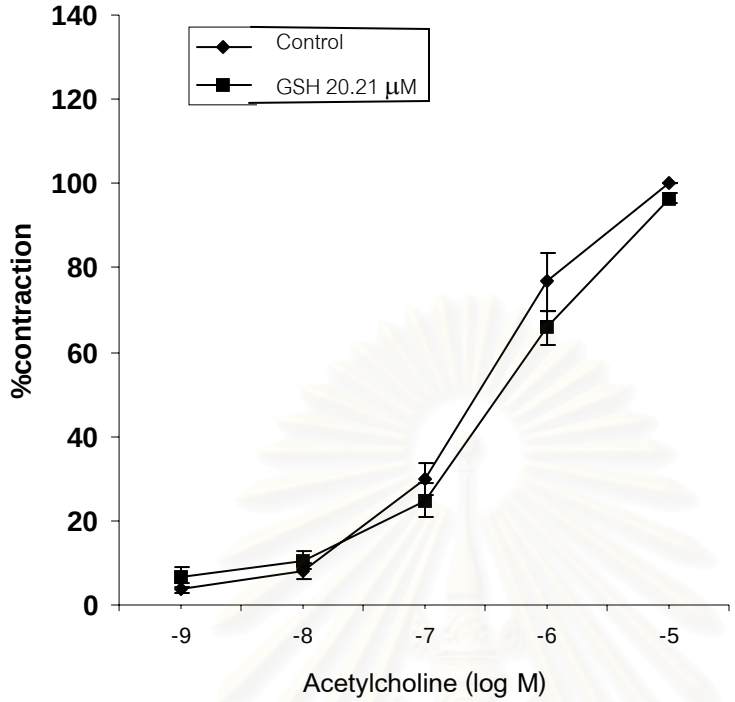

Figure 17a Effects of GSH (20.21µM) on contraction induced by cumulative acetylcholine.

Data were presented as mean ± S.E.M., n=4.

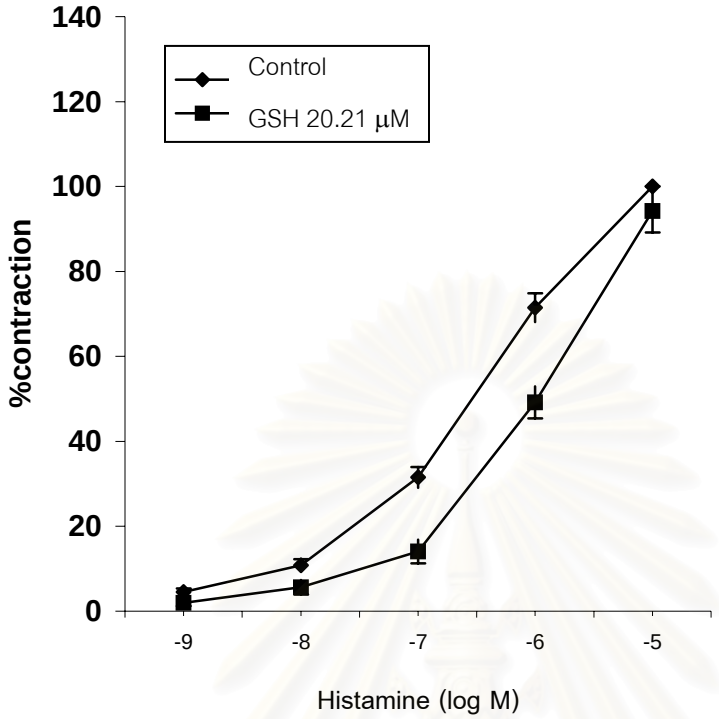

Figure 17b Effect of GSH (20.21μM) on contraction induced by cumulative histamine.

Data were presented as mean ± S.E.M., n=4.

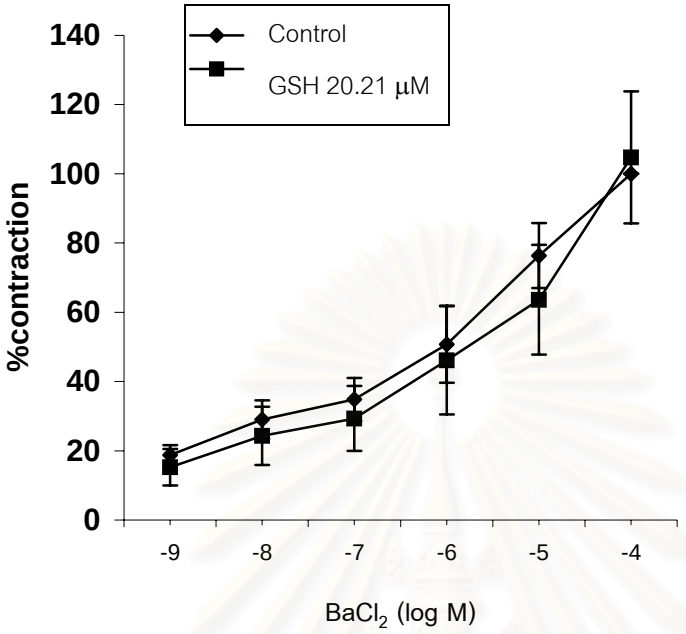

Figure 17c Effect of GSH (20.21μM) on contraction induced by cumulative barium chloride.

Data were presented as mean ± S.E.M., n=4.

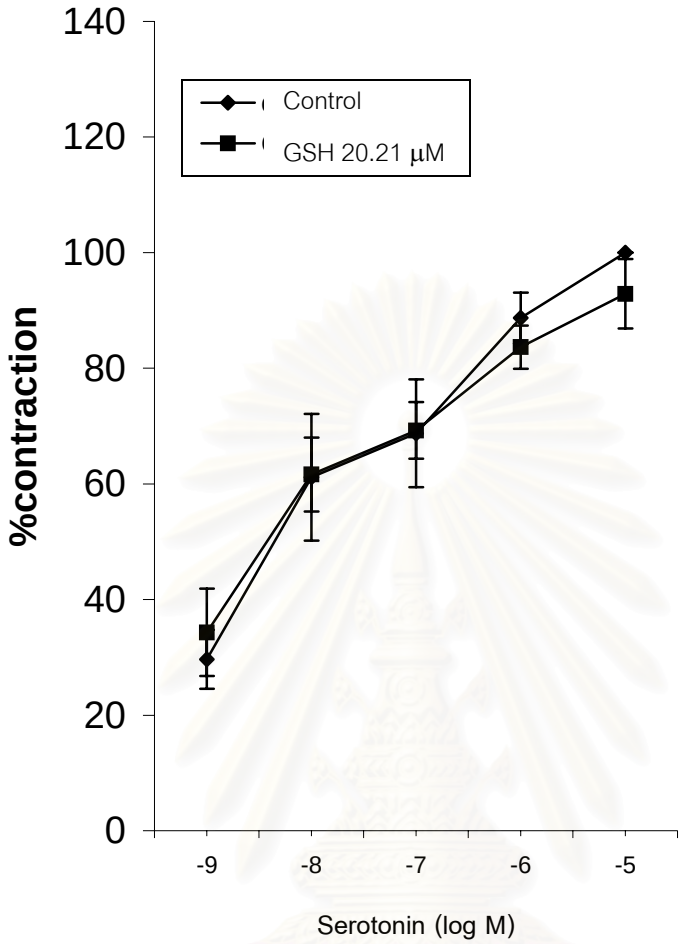

Figure 17d Effect of GSH (20.21µM) on contraction induced by cumulative serotonin.

Data were presented as mean ± S.E.M., n=4.

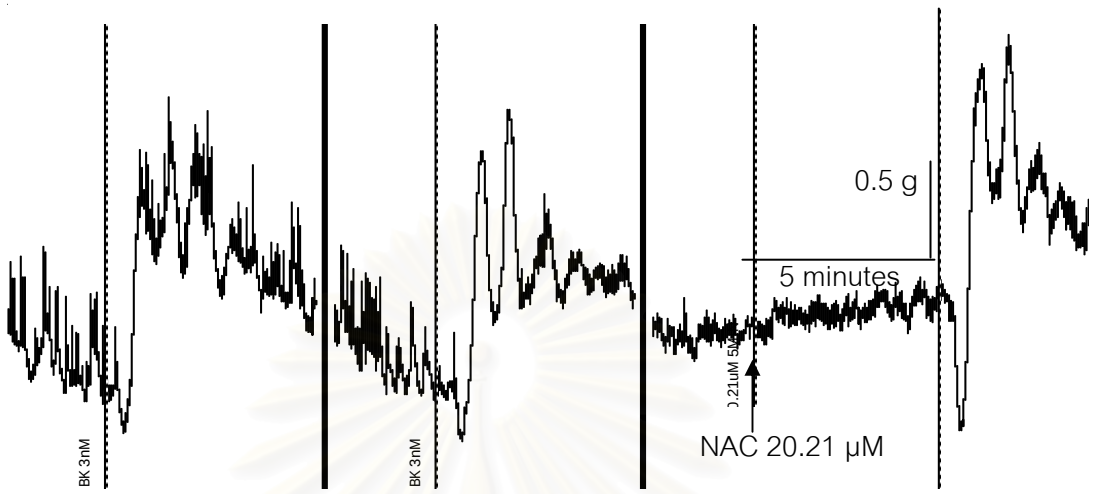

Figure 18a The contractile profiles of the effects of 20.21 µM NAC at 5 minutes incubation time on 3nM BK-induced contraction.

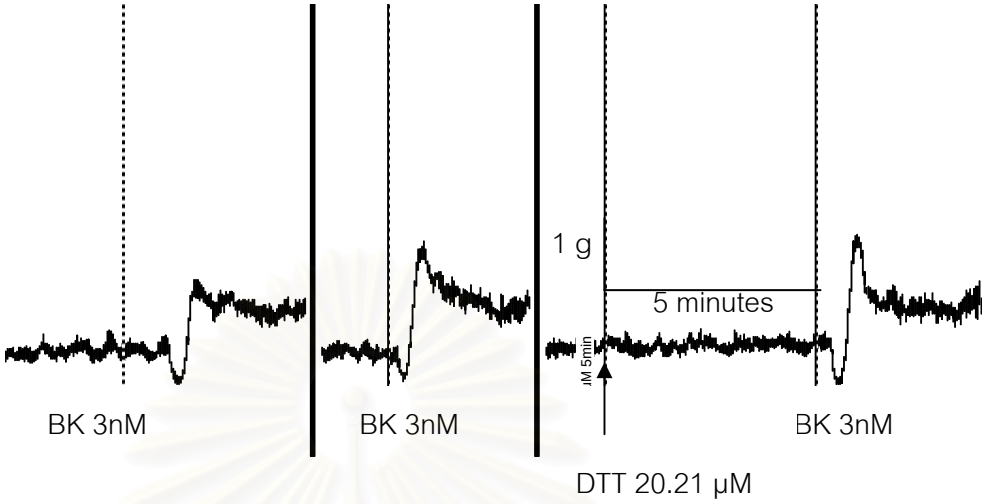

Figure 18b The contractile profiles of the effects of 20.21 μM DTT at 5 minutes incubation time on 3nM BK-induced contraction.

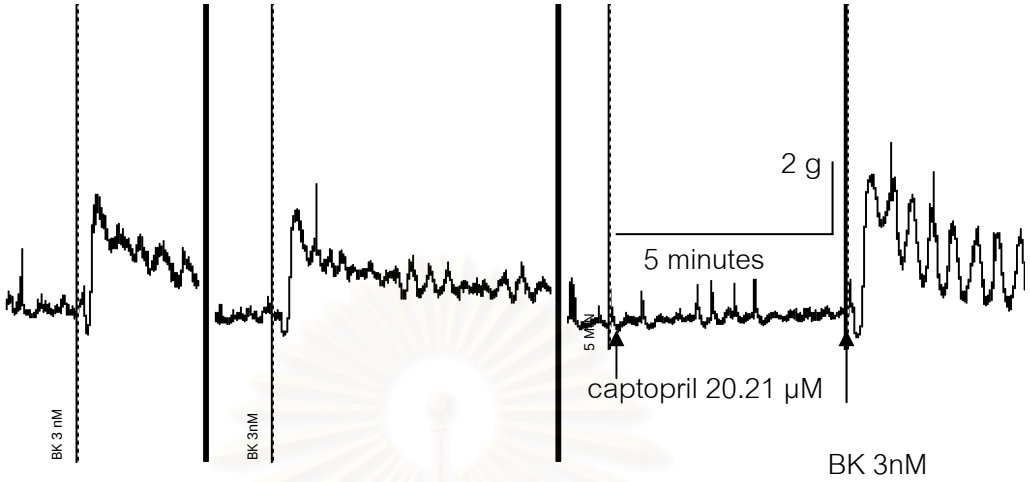

Figure 18c The contractile profiles of the effects of 20.21 µM captopril at 5 minutes on 3nM BK-induced contraction.

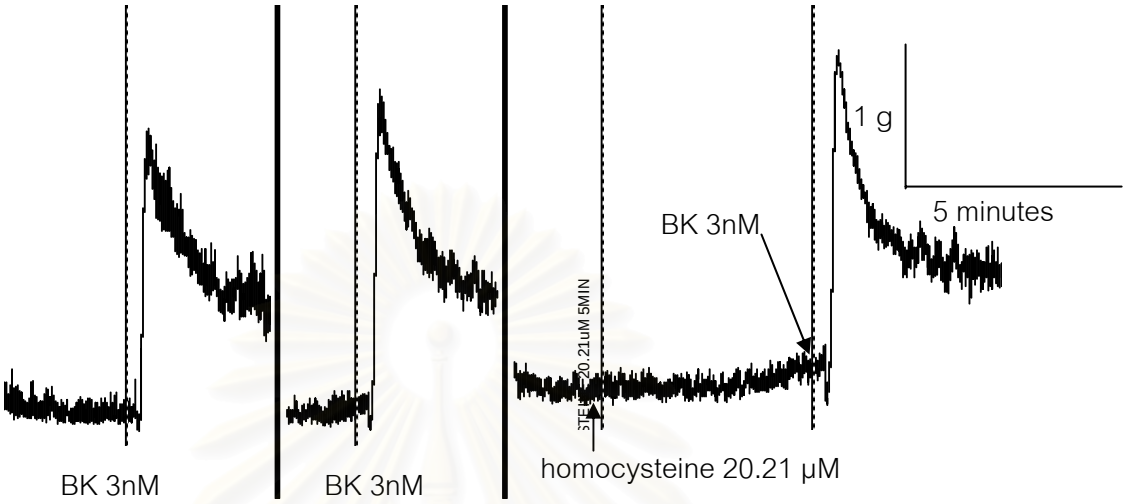

Figure 18d The contractile profiles of the effects of 20.21 μM homocysteine at 5 minutes on3nM BK-induced contraction.

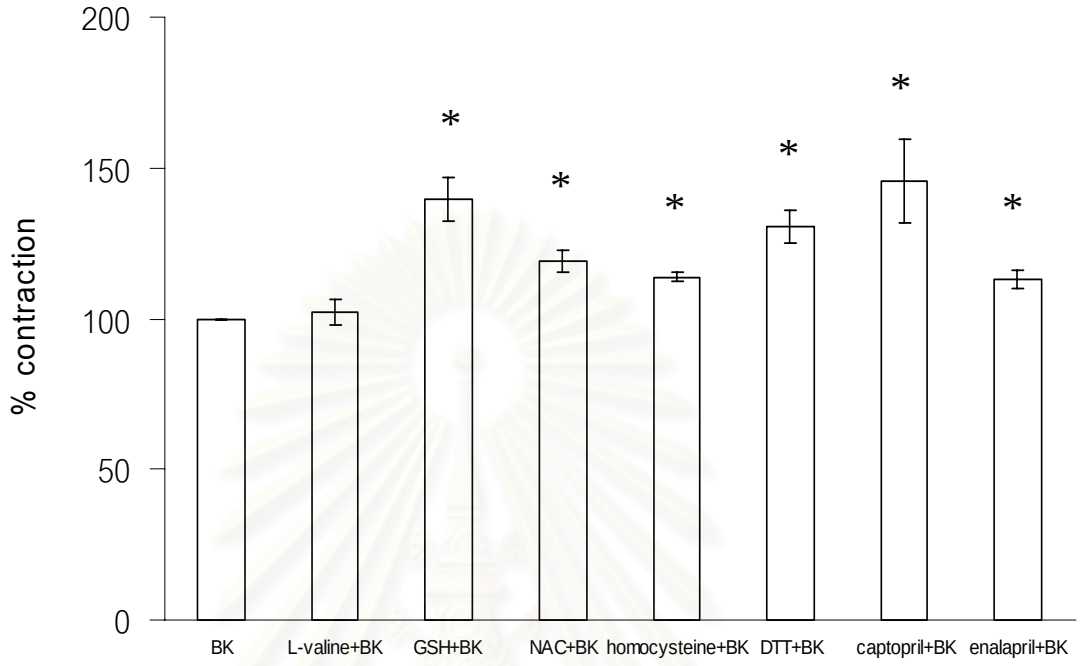

Figure 19 Effect of thiol-containing compounds on BK-induced contraction. Ileal segment were pretreatmented with several thiol-containing compounds at the concentration of 20.21 μM at 5 minutes incubation time prior to addition of BK (3 nM)-induced contraction.

Data were presented as mean ± S.E.M., n=4.

** $P < 0.05$*, significant difference from BK (3 nM)-induced contraction.

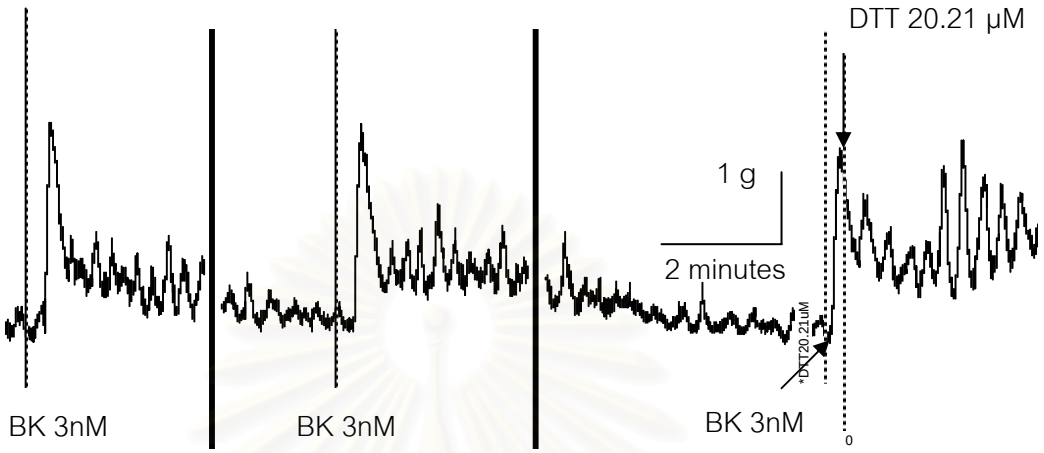

Figure 20a The contractile profiles of the effect of 20.21 µM DTT at 0 minute incubation time on ileal contraction induced by 3nM BK.

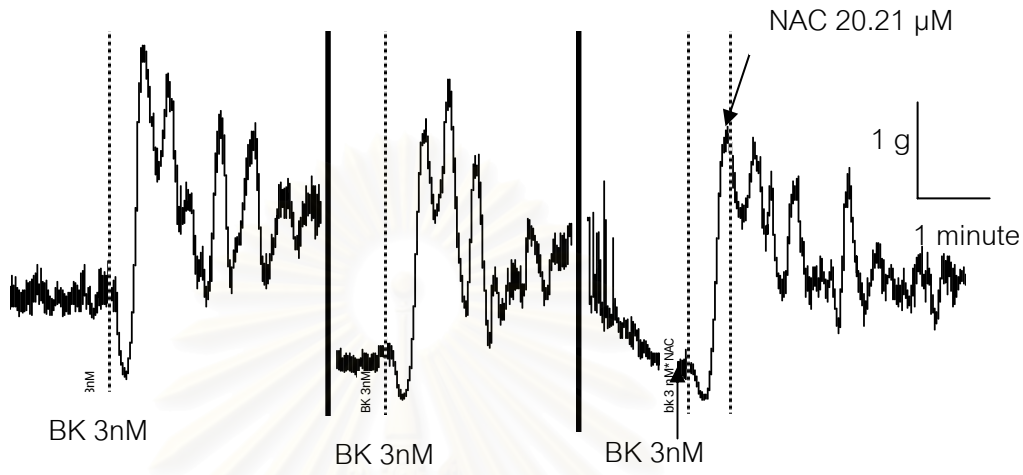

Figure 20b The contractile profiles of the effect of 20.21 µM NAC at 0 minute incubation time on ileal contraction induced by 3nM BK.

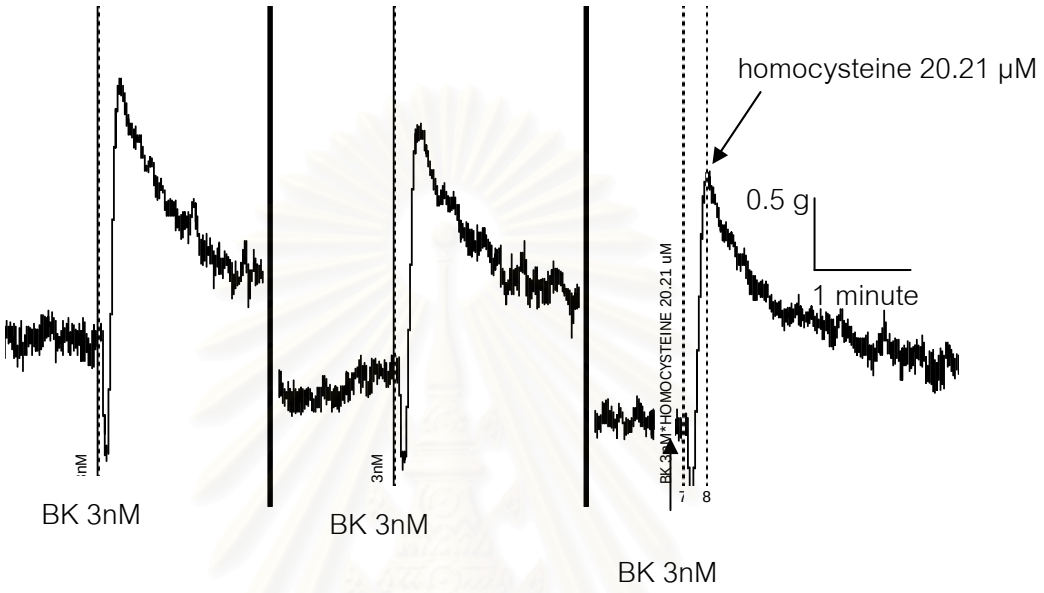

Figure 20c The contractile profiles of the effect of 20.21 µM homocysteine at 0 minute incubation time on ileal contraction induced by 3nM BK.

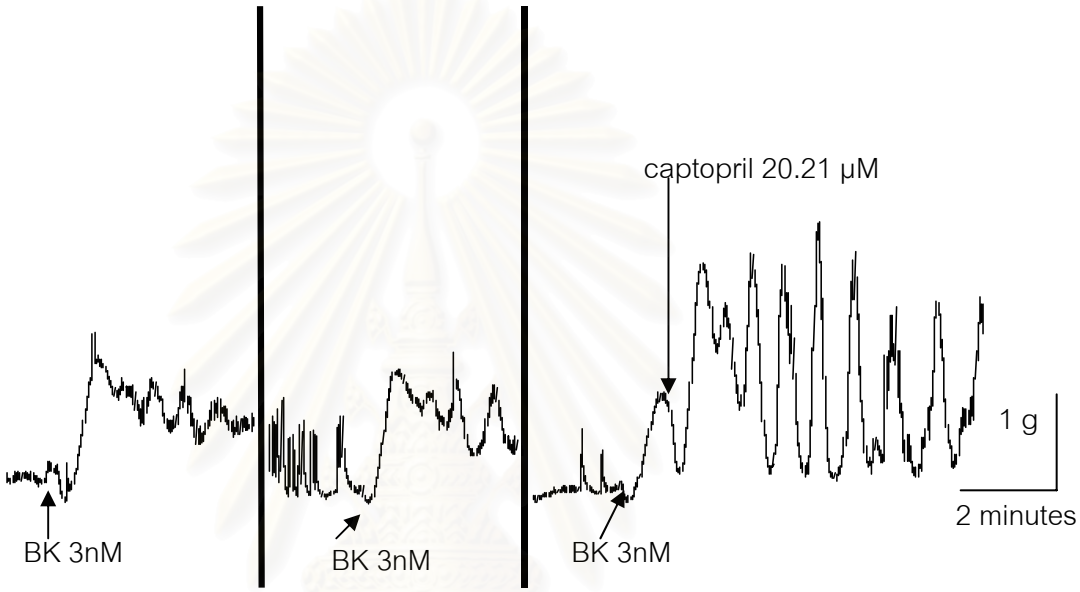

Figure 20d The contractile profiles of the effect of 20.21 μM captopril at 0 minute incubation time on ileal contraction induced by 3nM BK.

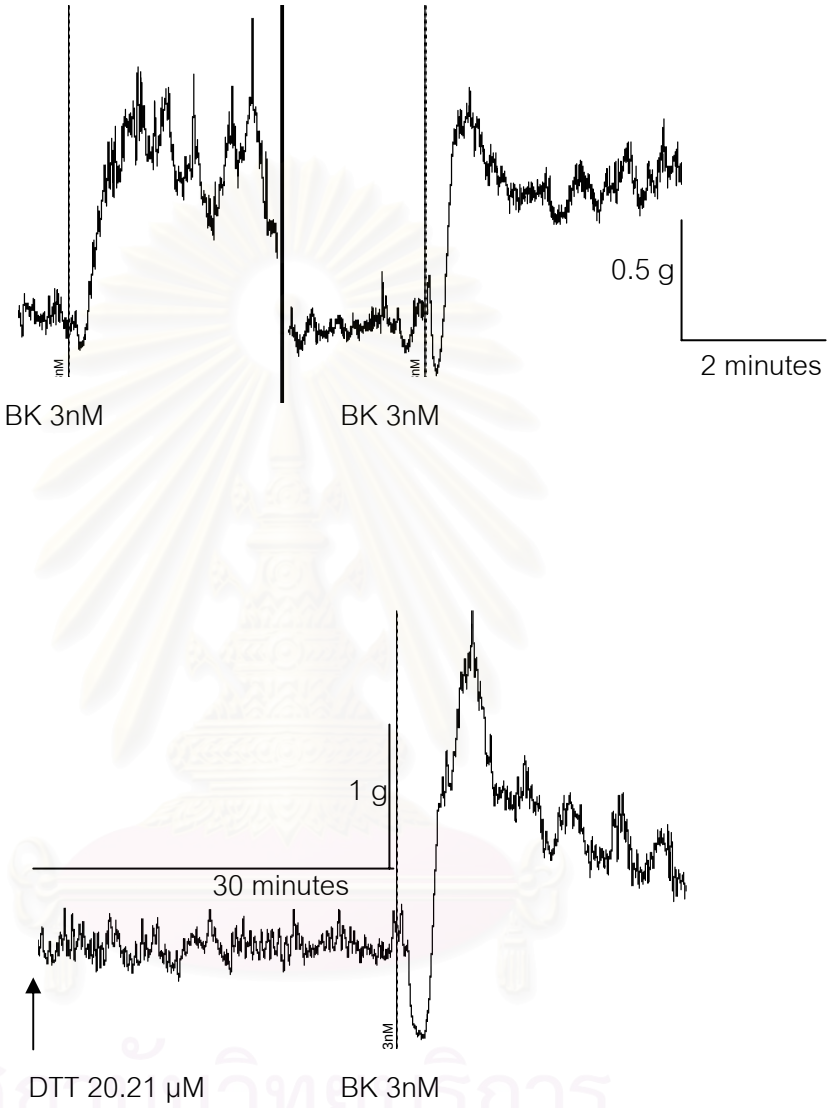

Figure 21a The contractile profiles of the effects of 20.21 µM DTT at 30 minutes incubation time on ileal contraction induced by 3nM BK.

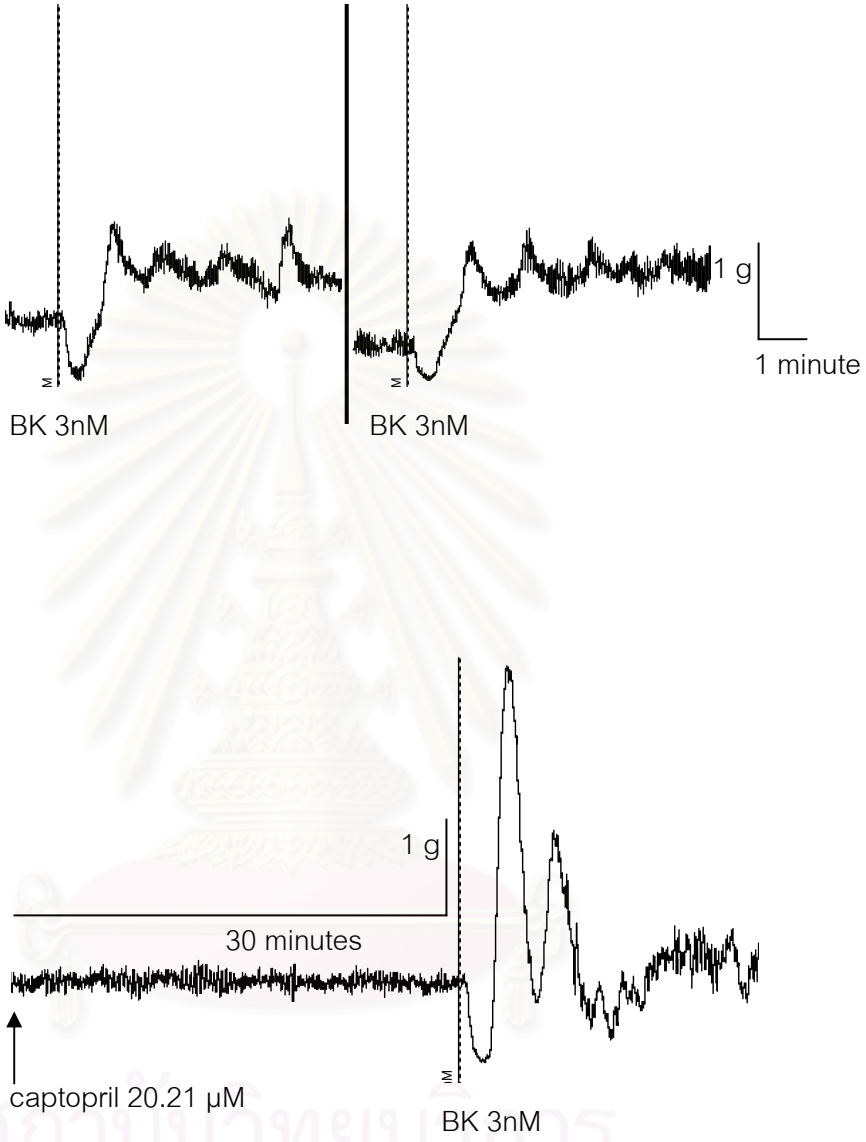

Figure 21b The contractile profiles of the effects of 20.21 μM captopril at 30 minutes incubation time on ileal contraction induced by 3nM BK.

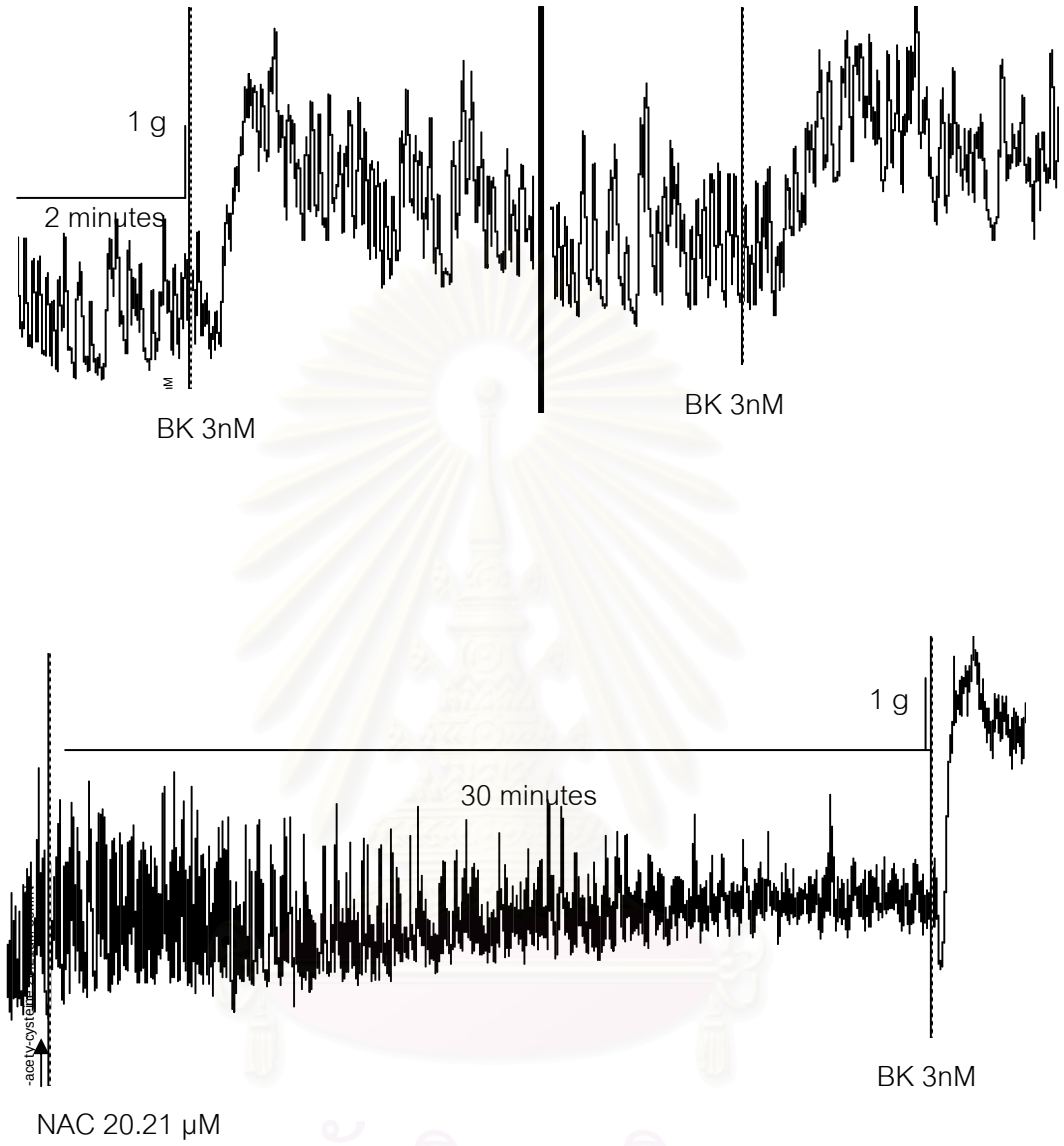

Figure 21c The contractile profiles of the effects of 20.21 µM NAC at 30 minutes incubation time on ileal contraction induced by 3nM BK.

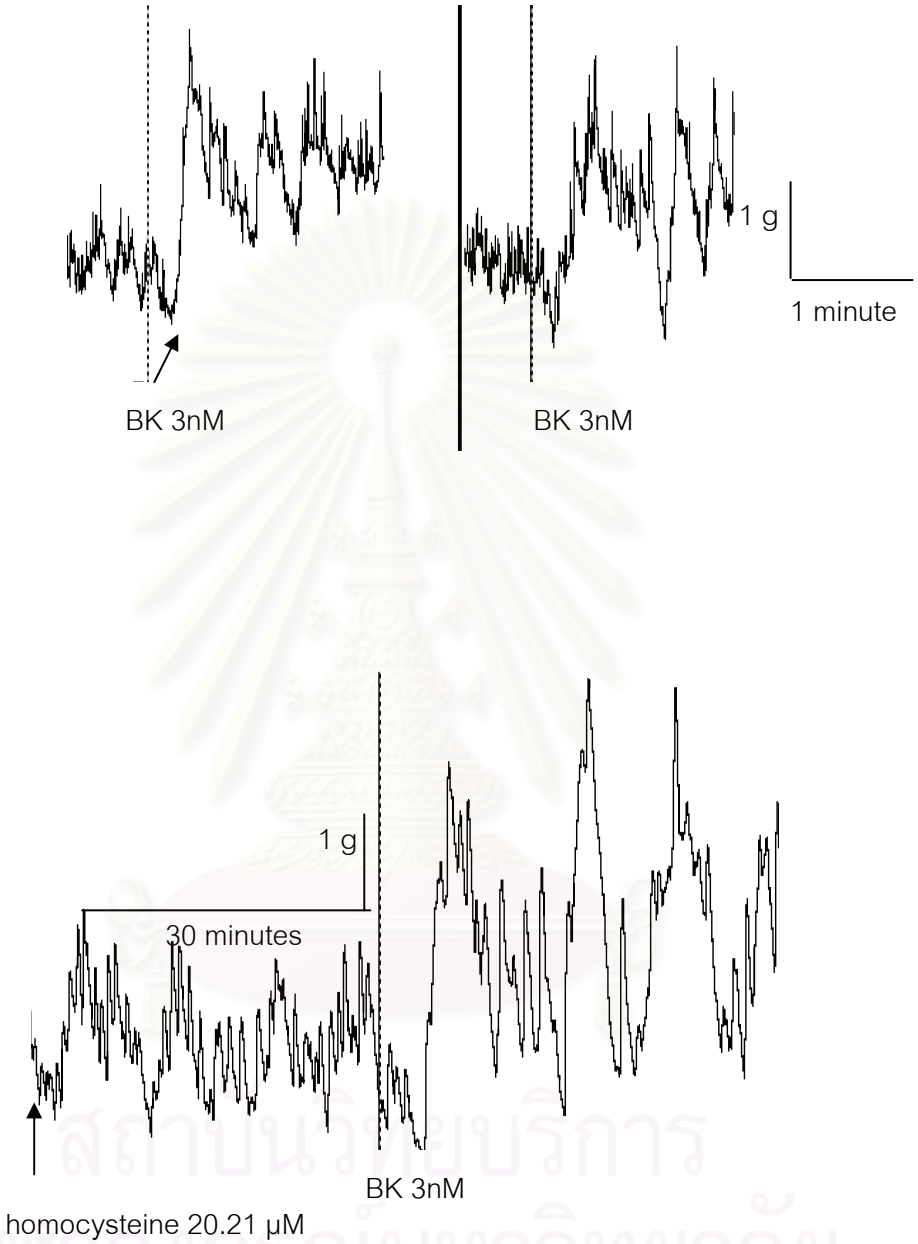

Figure 21d The contractile profiles of the effects of 20.21 μM homocysteine at 30 minutes incubation time on ileal contraction induced by 3nM BK.

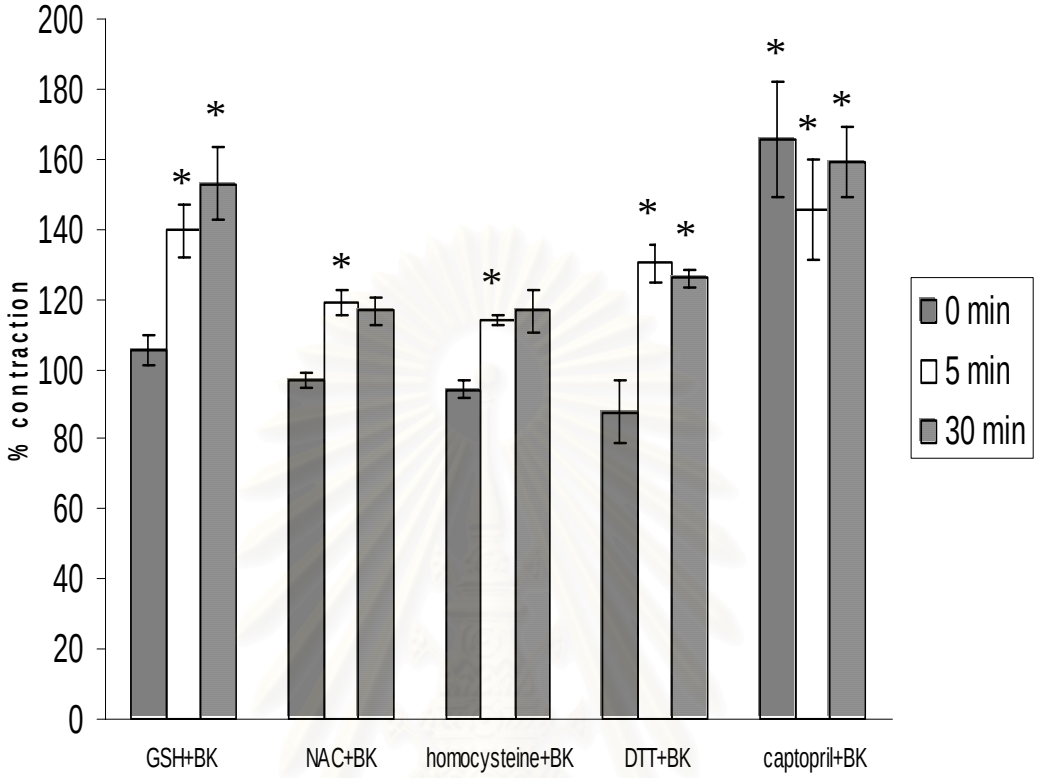

Figure 22 Effect of thiol-containing compounds on BK-induced contraction. Ileal segment were pretreatmented with several thiol-containing compounds at the concentration of 20.21 μM at various incubation time prior to addition of BK (3 nM)-induced contraction.

Data were presented as mean ± S.E.M., n=4.

*$P < 0.05$, significant difference from BK (3 nM)-induced contraction.

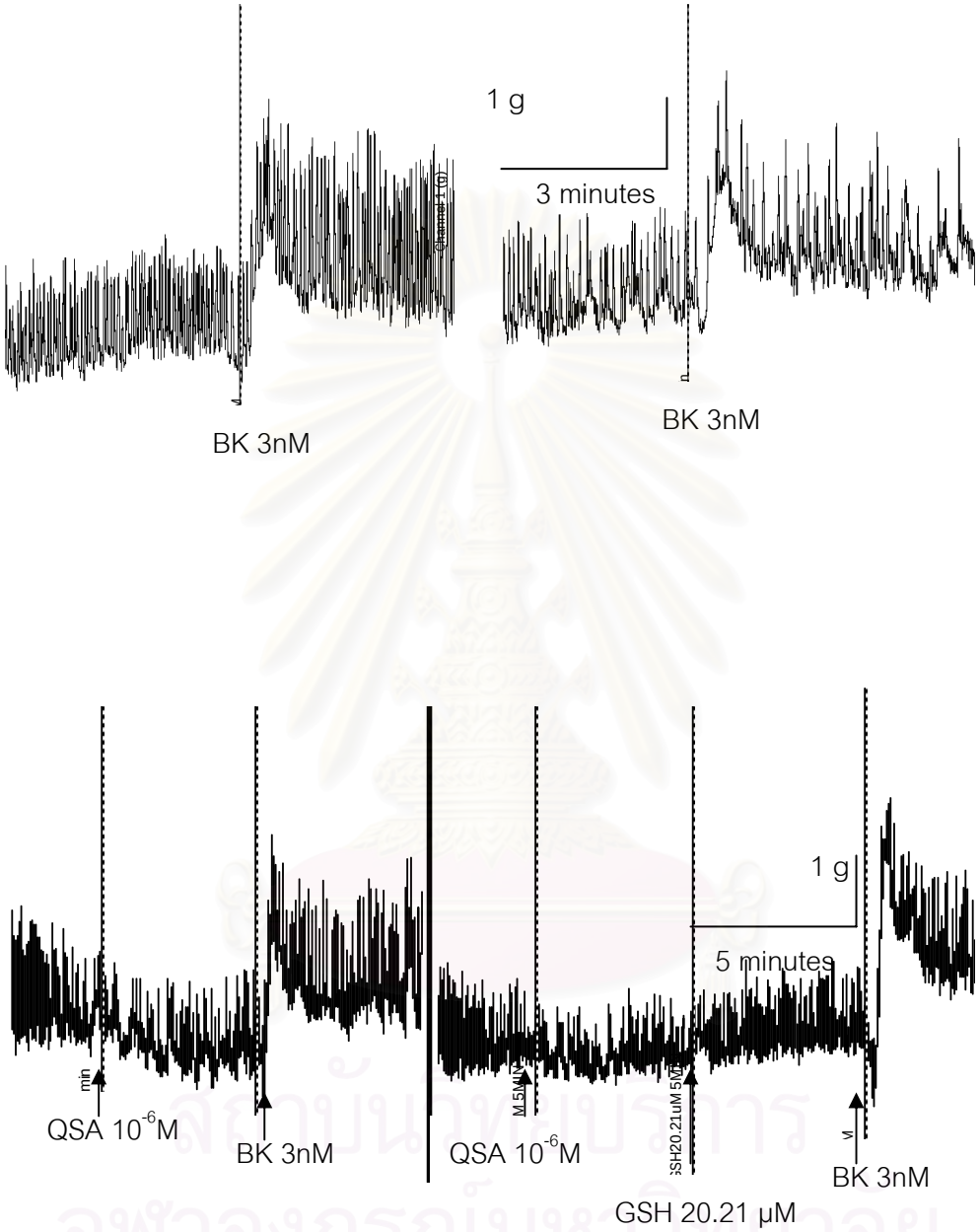

Figure 23 The contractile profiles of the effects of ACE inhibition (10^{-6}M QSA) on the 3nM BK-potentiating action of 20.21 µM GSH.

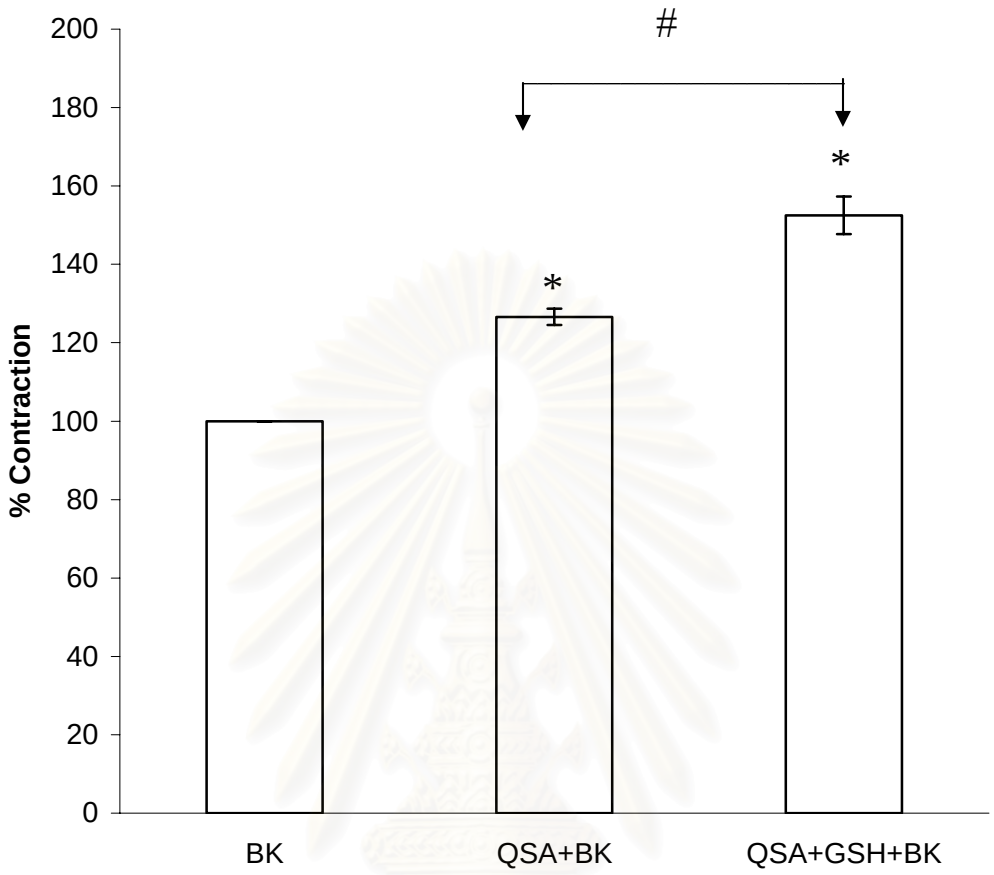

Figure 24 Effect of GSH (20.21μM) on BK (3nM)-induced contraction in the presence of 10^{-6}M QSA.

Data were presented as mean ± S.E.M., n=4.

*$P < 0.05$, significant difference when compared to BK-induced contraction.

# $P < 0.05$, significant difference between QSA+BK and QSA+GSH+BK.

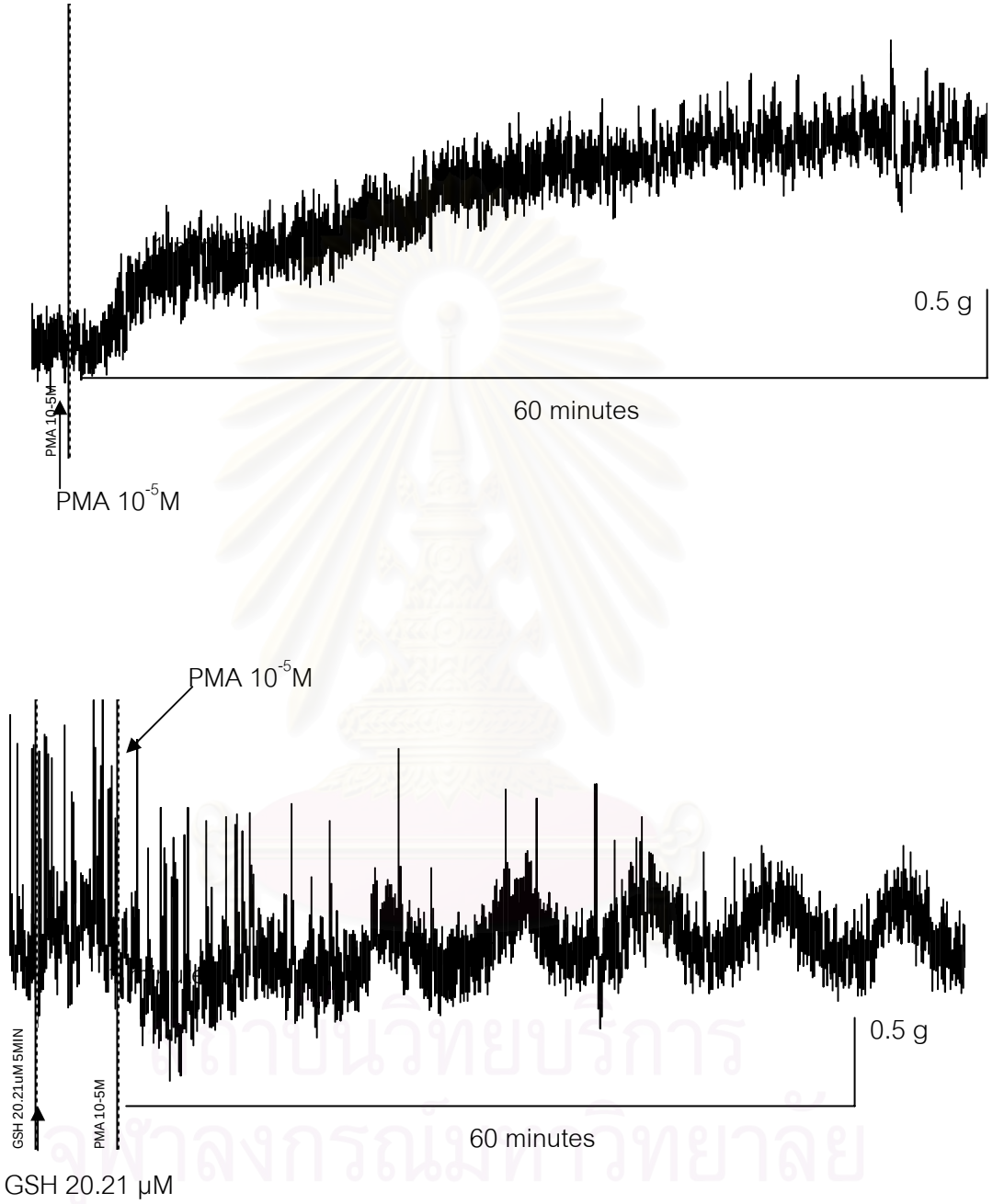

Figure 25 The contractile profiles of the effects of protein kinase C (10^{-5}M PMA) in 20.21 µM GSH-potentiating effects on the smooth muscle contraction.

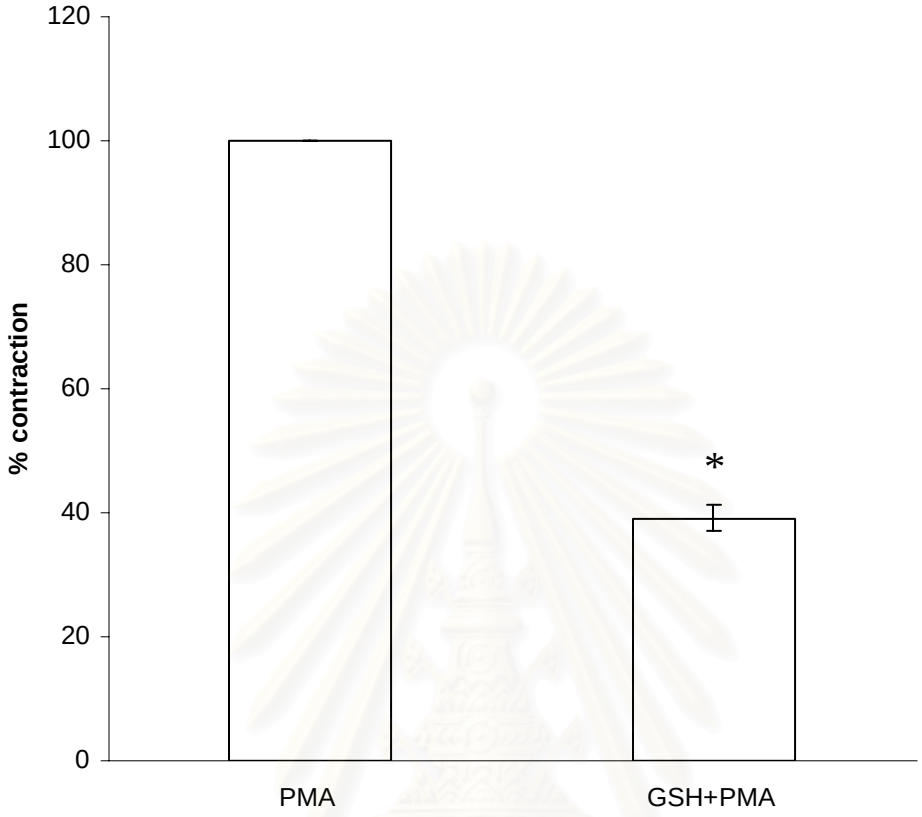

Figure 26 Effect of GSH on PMA (10^{-5} M)-induced contraction.

Data were presented as mean ± S.E.M., n=6.

**P < 0.05*, significant difference from PMA (10^{-5} M)-induced contraction.

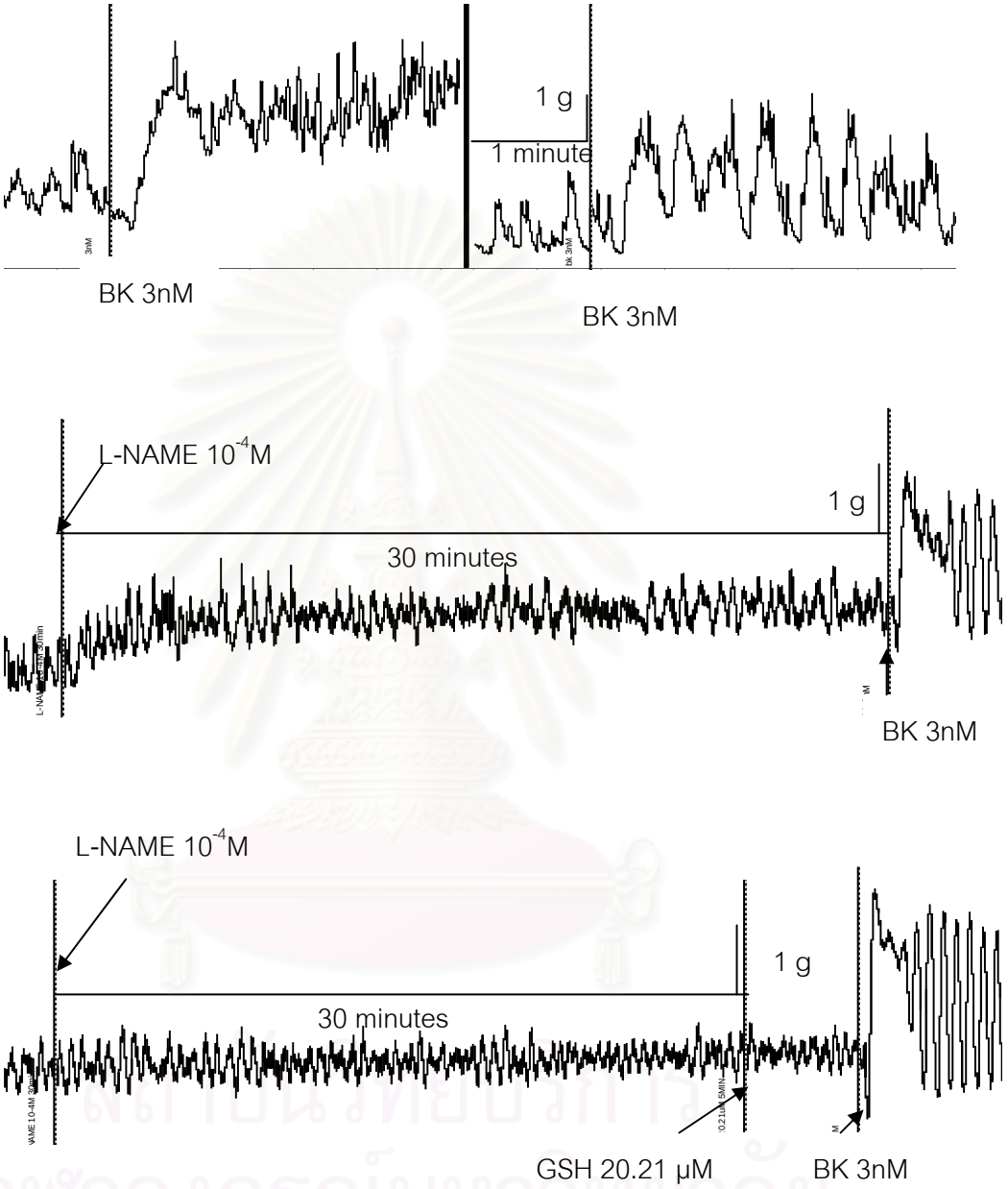

Figure 27 The contractile profiles of the effects of nitric oxide (10^{-4}M L-NAME) with 30 minutes on the 3nM BK-potentiating action of 20.21 µM GSH.

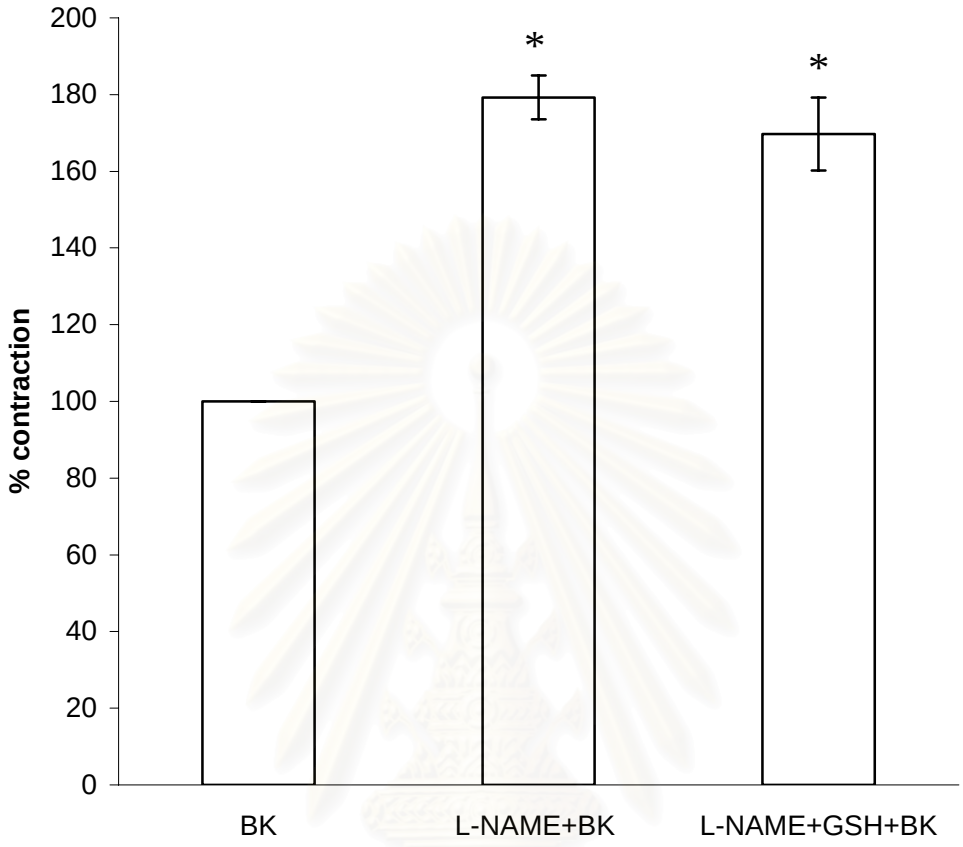

Figure 28 The effect of GSH (20.21μM) on BK (3nM)-induced contraction in the presence of 10^{-4}M L-NAME.

Data were presented as mean ± S.E.M., n=6.

**P < 0.05*, significant difference from BK (3nM)-induced contraction.

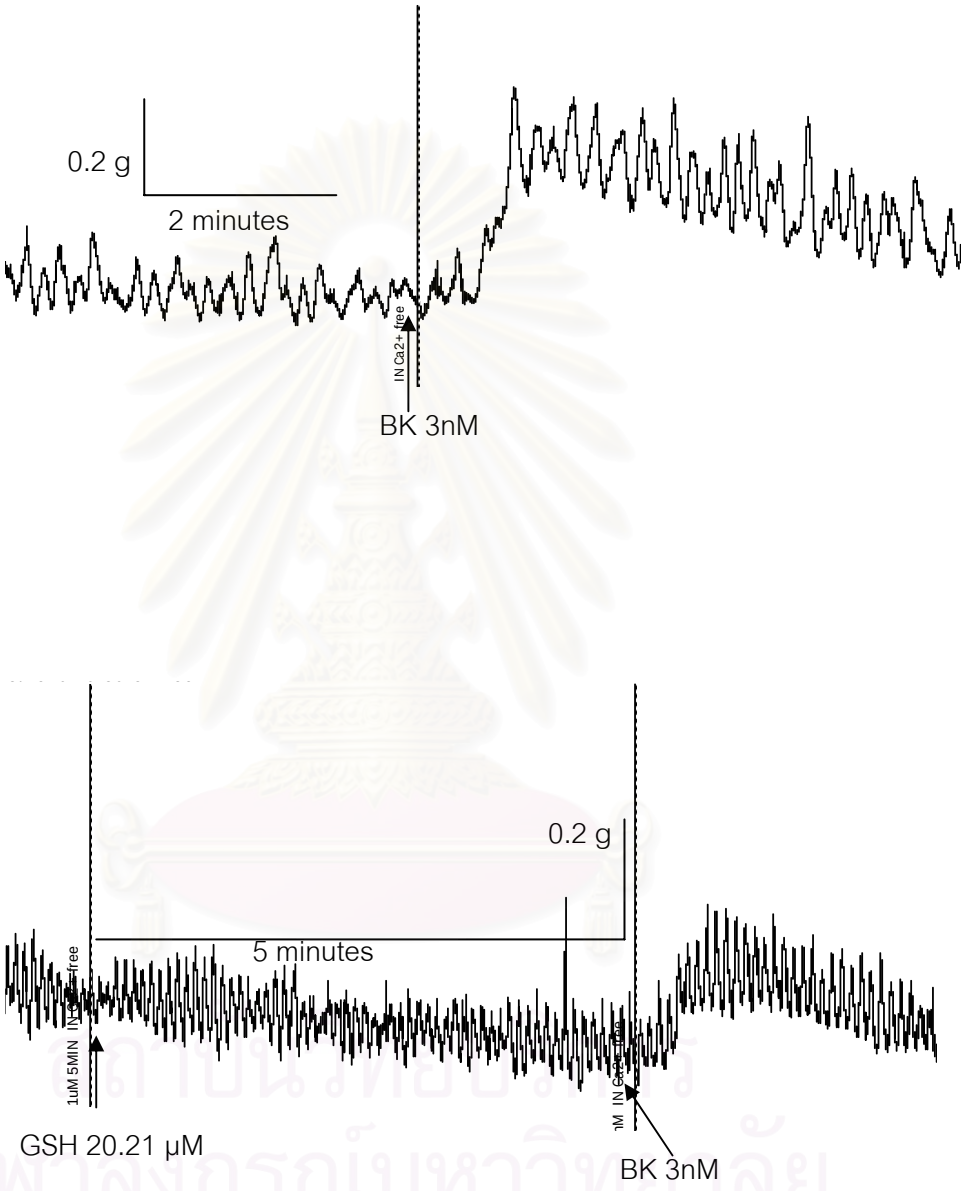

Figure 29 The contractile profiles of the effects of intracellular Ca^{2+} on 20.21 µM GSH action.

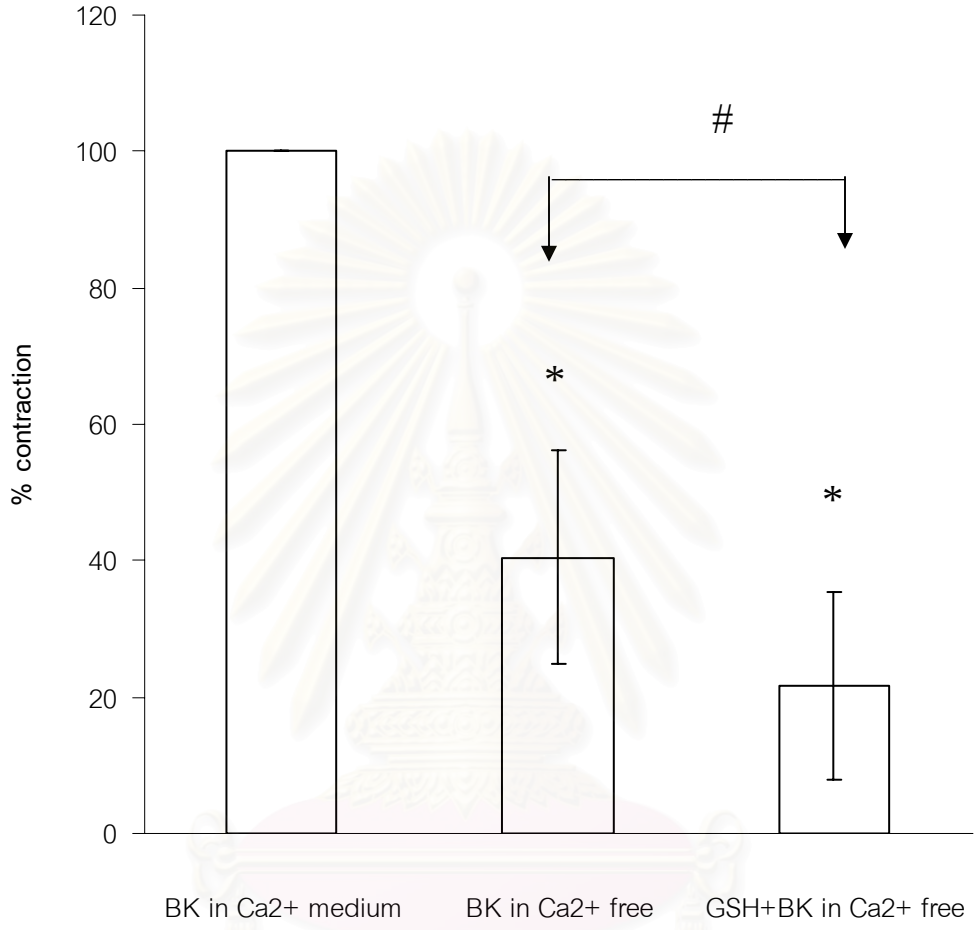

Figure 30 Effect of GSH (20.21μM) prior to BK (3nM)-induced contraction in Ca^{2+}- free medium.

Data were presented as mean ± S.E.M., n=4.

**P < 0.05*, significant difference from BK-induce contraction in Ca^{2+}-medium.

\# *P < 0.05*, significant difference between BK in Ca^{2+} free and GSH+BK in Ca^{2+} free medium.

CHAPTER V

DISCUSSION AND CONCLUSION

The study aimed to investigate the effect of GSH on BK-induced contraction in isolated guinea pig ileum and to characterize the mechanism of GSH. In order to establish the *in-vitro* model of guinea pig ileal-contraction for further studies, the responsiveness of different segments of ileum toward BK were tested. The responsiveness of each of the ileal segments toward BK-induced contraction in the presence of GSH was different. It is likely that there are some discrepancies in receptor distribution in each segment of ileum. In addition, GSH shifted the concentration-response curve of BK parallel to the left, whereas the maximum response remained unchanged. This result was similar to those of peptides A-VI-5 and BPP_{5a} which were reported as example of BK-potentiator. These potentiators exerted its action by sensitization of BK receptor (Ufkes, *et al*., 1984). Hence, it is possible that GSH mediated the potentiating effect on BK-induced contraction by causing the sensitization of BK receptor. In addition, these potentiating of GSH effects were restrictive and uncorrelated with the concentration of GSH.

The potentiating effect of GSH on BK-induced contraction did not take place immediatedly after GSH was added to the tissue bath, but developed after minutes. However, the effects of GSH were not significantly enhanced after longer contact with the tissue. Chymotrypsin and cathepsin G were example of BK-potentiators which exerted its action by selectively uncovering specific BK receptors by splitting peptide bonds in the smooth muscle membranes (Edery, 1965). The increase of incubation time of tissue with

chymotrypsin from 5 to 30 minutes significantly enhance the BK-induced contraction by 2.5 folds (Minshall, *et al*., 2000). Hence, the result in this study suggested that the effect of GSH was not linked to selectively uncovering specific BK receptors by splitting peptide bonds in the smooth muscle membranes at the receptor level.

The potentiating effect of GSH was specific to BK-induced contraction. This was evidenced by that GSH did not enhance the contraction induced by other contractile agonists such as acetycholine, histamine, barium chloride and serotonin. This result was similar to other previous studies demonstrating that GSH did not shift the concentration-response curves of histamine and acetycholine (Takeya and Hotta, 1979). Other thiol-containing compounds including N-acetycysteine, homocysteine, DTT and captopril also significantly increased BK-induced contraction. It is likely that the mechanism of potentiating effect of GSH involved the –SH group in the compounds and acted directly on the smooth muscle. These results supported the finding in another study which demonstrated that thiol-containing compounds increase BK-induced contraction in guinea pig ileum. However, the potentiating effect of thiol-containing compounds on agonists such as acetylcholine, 5-hydroxytryptamine, nicotine and prostaglandin E_2 were reported (Fontaine, *et al*., 1984).

The effect of BK on isolated smooth muscle may be potentiated by different mechanisms: (1) inhibition of kininase enzyme to increase BK availability (Ferreira and Rocha e Silva, 1965; Hamberg, *et al*., 1969), (2) sensitization of the BK receptor (Camargo and Ferreira, 1971) and (3) through signal pathway (Muller, *et al*., 2005). As in this study, GSH significantly increased BK-induced contraction in the presence of QSA, suggested that

GSH exerted its potentiating effects via other mechanisms in addition to inhibition of BK degradation. Although an attempt to elucidate the mechanism of potentiation of GSH was not successful, the results suggested that GSH did not exert its action via NO-pathway. In this study, GSH did not significantly increase the BK-induced contraction in the presence of L-NAME, a specific inhibitor of NO synthesis. In addition, GSH did not significantly increase the BK-induced contraction in Ca^{2+}-free medium. This suggested that the potentiation of GSH was not correlated with intracellular Ca^{2+}.

The activation of B_2 receptors caused an increase intracellular Ca^{2+} through the phospholipase C (PLC) pathway. As known, PLC hydrolyzes phosphatidylinositol-4,5-biphosphate (PIP_2) generating diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). IP_3 acts on its receptors on the sarcoplasmic reticulum to release Ca^{2+} into cytoplasm. DAG activates protein kinase C, leading to the phosphorylation of several enzymes involved in muscle contraction (Farmer and Burch,1992). Interestingly, GSH did not attenuated increase PMA-mediated contraction, suggesting that GSH may block or neutralize PMA before penetrating into a cell because GSH act as an antioxidant and participates in detoxification reactions for xenobiotics (Arrick, *et al*., 1982 ; Burk *et al*., 1983).

In conclusion, GSH increased the ileal segment contraction in response to BK and this appeared to be restrictive to time and concentration. In addition, the potentiating effects of GSH on the ileal segment contraction was specific to BK. Moreover, the potentiating effects of GSH and other thiol-containing compounds, except captopril, were comparable on BK-induced contraction. The potentiating effects of GSH on BK-induced contraction was

also observed in the presence of kininase inhibitor, suggesting that GSH may exert its potentiating effect via multimechanisms in addition to ACE inhibition.

It is possible that the potentiation effects of GSH was attributed to receptor sensitization and other mechanism in addition to ACE inhibiton. The potentiating mechanisms of GSH was not found to be correlated with an alteration NO pathway and intracellular Ca^{2+} under the present conditions.

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APPENDIX

Table 2 The structure of thiol-containing compounds

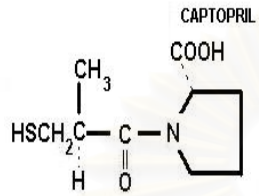
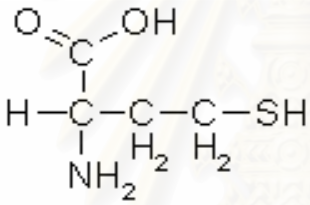
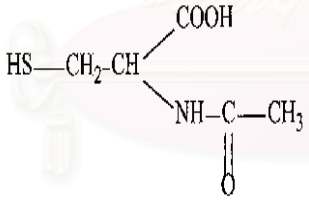
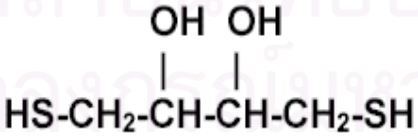
Structure	Name
	Captopril
	Homocysteine
	N-acetycysteine
	Dithiothreitol

Table 3 Compound of Physiological solution (mM/L)

Chemicals	Physiology solution	
	Tyrode's solution	Ca^{2+}-free Tyrode's solution
NaCl	137	137
KCl	2.68	2.68
$MgSO_4$ ($7H_2O$)	0.1	0.1
$NaHCO_3$	1.16	1.16
KH_2PO_4	0.36	0.36
$CaCl_2$	1.8	-
Glucose	5.56	5.56
EDTA	-	0.1

Table 4 The contraction (g) of guinea pig ileum induced by GSH at concentration of 6.67μM to 26.68 μM

GSH concentration (μM)	Force of contraction
6.67 (n=4)	0.08 ± 0.03
13.34 (n=4)	0.18 ± 0.05
20.21 (n=4)	0.3 ± 0.07
26.68 (n=4)	0.43 ± 0.13

Table 4 The effect of GSH on the percentage of contraction induced by adding cumulatively BK in the segment of 4-7 cm. from ileocecal junction

GSH concentration (μM)	BK concentration (nM)				
	0.1	3	30	300	3000
0 (n=4)	10.03 ± 1.67	39.33 ± 5.63	55.42 ± 4.33	70.75 ± 3.31	100
6.67 (n=4)	11.96 ± 2.42	53.64 ± 4.99	74.18 ± 1.69*	89.38 ± 1.75*	105.93 ± 1.58
13.34 (n=4)	23.13 ± 6.14	57.25 ± 8.14	62.94 ± 7.57	85.36 ± 4.85	111.82 ± 3.56
20.21 (n=4)	27.83 ± 5.13	50.30 ± 3.91	62.37 ± 6.97	85.28 ± 6.39	110.01 ± 4.33
26.68 (n=4)	20.75 ± 6.86	46.33 ± 5.53	55.59 ± 7.48	72.47 ± 4.34	109.08 ± 3.61

* $P < 0.05$, significant difference from control (the group without GSH).

Table 5 The effect of GSH on the percentage of contraction induced by adding cumulatively BK in the segment of 7-10 cm. from ileocecal junction

GSH concentration (μM)	BK concentration (nM)				
	0.1	3	30	300	3000
0 (n=4)	22 ± 0.55	46 ± 2.06	63 ± 2.57	78 ± 1.99	100
6.67 (n=5)	29 ± 5.17	59 ± 15.17	80 ± 18.69	103 ± 14.15	122 ± 11.8
13.34 (n=4)	23 ± 8.86	51 ± 6.71	70 ± 8.2	83 ± 7.84	100 ± 9.39
20.21 (n=4)	34 ± 14.07	67 ± 12.95	82 ± 10.6	115 ± 6.43*	128 ± 7.56
26.68 (n=4)	40± 11.86	68 ± 9.14	77 ± 8.65	100 ± 11.3	128 ± 8.87

* $P < 0.05$, significant difference from control (the group without GSH).

Table 6 The effect of GSH on the percentage of contraction induced by adding cumulatively BK in the segment of 10-13 cm. from ileocecal junction

GSH concentration (μM)	BK concentration (nM)				
	0.1	3	30	300	3000
0 (n=4)	16 ± 2.51	46 ± 4.52	59 ± 2.18	77 ± 1.05	100
6.67 (n=4)	28 ± 7.66	79 ± 10.94	87 ± 8.18	102 ± 6.70	129 ± 12.99
13.34 (n=4)	23 ± 4.45	66 ± 9.06	72 ± 9.61	90 ± 9.28	128 ± 12.48
20.21 (n=5)	53 ± 10.9	76 ± 12.12	86 ± 11.36	106 ± 11.85	151 ± 15.74*
26.68 (n=4)	39 ± 11.68	56 ± 5.69	68 ± 5.56	87 ± 10.53	142 ± 9.52*

* $P < 0.05$, significant difference from control (the group without GSH).

Table 7 The effect of GSH on the percentage of contraction induced by adding cumulatively BK in the segment of 13-16 cm. from ileocecal junction

GSH concentration (μM)	BK concentration (nM)				
	0.1	3	30	300	3000
0 (n=4)	28 ± 2.17	46 ± 2.26	56 ± 1.44	61 ± 2.79	100
6.67 (n=4)	34 ± 13.03	61 ± 14.88	72 ± 19.02	82 ± 17.32	132 ± 14.15
13.34 (n=4)	44 ±15.04	42 ± 12.39	84 ± 16.24	97 ± 15.09	137 ± 20.92
20.21 (n=4)	62 ± 3.78*	92 ± 9.69*	106 ± 19.36*	130 ± 26.66*	167 ± 19.88*
26.68 (n=4)	42 ± 12.51	54 ± 9.96	68 ± 13.32	98 ± 12.98	142 ± 17.34

* $P < 0.05$, significant difference from control (the group without GSH).

Table 8 The effect of GSH-incubation time on ileal contraction induced by BK

Incubation time (minutes)	The percentage of contraction induced by 3 nM BK in the presence of 20.21 μM GSH
0 (n=4)	105.5 ± 4.42
5 (n=4)	139.5 ± 7.41*
30 (n=4)	153 ± 10.46*

*$P < 0.05$, significant difference from BK-induced contraction.

Table 9 The effect of thiol-containing compounds-incubation time on the percentage of contraction induced by adding 3nM BK

Incubation time (minutes)	Thiol-containing compounds at concentration of 20.21 μM			
	NAC (n=4)	homocysteine (n=4)	DTT (n=4)	captopril (n=4)
0	97 ± 2.27	94.25 ± 2.17	87.5 ± 9.09	165.75 ± 16.31*
5	119 ± 4.56*	113.75 ± 1.43*	130.25 ± 5.43*	145.5 ± 14*
30	116 ± 3.92*	116.5 ± 6.11*	112 ± 2.51*	159 ± 10*

*$P < 0.05$, significant difference from BK-induced contraction.

Table 10 The effect of GSH on the percentage of contraction induced by adding cumulatively acetycysteine

GSH concentration (μM)	acetycysteine concentration (M)				
	$1x10^{-9}$ (n=4)	$1x10^{-8}$ (n=4)	$1x10^{-7}$ (n=4)	$1x10^{-6}$ (n=4)	$1x10^{-5}$ (n=4)
0	3.93 ± 1.12	7.83 ± 1.89	29.86 ± 3.92	76.80 ± 6.94	100
20.21	6.53 ± 2.45	10.58 ± 2.27	24.82 ± 4.12	65.81 ± 4.07	96.41 ± 1.2

Table 11 The effect of GSH on the percentage of contraction induced by adding cumulatively histamine

GSH concentration (μM)	histamine concentration (M)				
	$1x10^{-9}$ (n=4)	$1x10^{-8}$ (n=4)	$1x10^{-7}$ (n=4)	$1x10^{-6}$ (n=4)	$1x10^{-5}$ (n=4)
0	4.49 ± 0.86	10.82 ± 1.43	31.52 ± 2.47	71.48 ± 3.36	100
20.21	1.93 ± 0.74	5.67 ± 1.72	14.08 ± 2.81	49.17 ± 3.79	94.23 ± 5.05

Table 12 The effect of GSH on the percentage of contraction induced by adding cumulatively barium chloride

GSH concentration (μM)	barium chloride concentration (M)					
	$1x10^{-9}$ (n=4)	$1x10^{-8}$ (n=4)	$1x10^{-7}$ (n=4)	$1x10^{-6}$ (n=4)	$1x10^{-5}$ (n=4)	$1x10^{-4}$ (n=4)
0	18.79 ± 2.80	29.03 ± 5.52	34.86 ± 6.17	56.77 ± 11.15	76.37 ±9.37	100
20.21	15.21 ± 5.27	24.31 ± 8.43	29.33 ± 9.38	46.15 ± 15.6	63.62 ± 15.85	104.74 ± 19.10

Table 13 The effect of GSH on the percentage of contraction induced by adding cumulatively serotonin

GSH concentration (μM)	serotonin concentration (M)				
	$1x10^{-9}$ (n=4)	$1x10^{-8}$ (n=4)	$1x10^{-7}$ (n=4)	$1x10^{-6}$ (n=4)	$1x10^{-5}$ (n=4)
0	29.64 ± 5.10	61.16 ± 10.95	68.75 ± 9.34	88.68 ± 4.39	100
20.21	34.32 ± 7.55	61.63 ± 6.39	69.24 ± 4.91	83.64 ± 3.74	92.86 ± 5.99

CURRICULUM VITAE

Miss Supochana Charoensin was born in July 16, 1982 in Bangkok, Thailand. She graduated with Bachelor of Science in 2004 from Medical Technique in Radiological Technology, Mahidol University, Thailand. After graduation, she studies a master degree in interdepartment of pharmacology, Chulalongkorn University, Thailand.