ผลของเคอร์เซตินและนารินเจนินต่ออัตราการเต้นและแรงบีบตัวของหัวใจห้องบนขวาและซ้าย
ที่แยกจากกายหนูขาว

นางสาว นุชนาท ประมาคะเต

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EFFECTS OF QUERCETIN AND NARINGENIN ON RATE AND FORCE OF CONTRACTION OF ISOLATED RAT RIGHT AND LEFT ATRIA

Miss Nuchanat Pramakatay

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จากรายงานการศึกษาพบว่าสารในกลุ่ม flavonoids มีผลต่อการทำงานของหัวใจ จึงต้องการศึกษาผลของสาร flavonoids 2 ตัว คือ quercetin และ naringenin ต่ออัตราการเต้นและแรงบีบตัวของหัวใจห้องบนขวาและซ้ายที่แยกจากกายหนูขาว ทำการศึกษาในหนูขาวสายพันธุ์ Wistar เพศผู้ น้ำหนัก 250-300g โดยการแยกหัวใจห้องบนขวาและซ้ายออกจากกัน แล้วทำการวัดอัตราการเต้นและแรงบีบตัวของหัวใจห้องบนขวาและซ้าย ผลการศึกษาพบว่าสาร flavonoids 2 ตัวนี้ให้ผลต่อการทำงานของหัวใจที่แตกต่างกันโดยพบว่า quercetin (100 μM) มีผลเพิ่มแรงบีบตัวของหัวใจห้องบนขวาและซ้าย 33.32 ± 4.13 % และ 45.75 ± 4.90 % ตามลำดับ และมีผลเพิ่มอัตราการเต้นของหัวใจคิดเป็น 17.62 ± 6.51%. ในขณะที่ naringenin (100 μM) มีผลลดแรงบีบตัวของหัวใจห้องบนขวาและซ้าย 6.16 ± 3.02 % และ 10 ± 3.51% ตามลำดับ และมีผลลดอัตราการเต้นของหัวใจห้องบนขวา 30% ± 5.32 % ซึ่งผลของ quercetin ในการเพิ่มแรงบีบตัวของหัวใจนั้นเป็นผลจากการออกฤทธิ์ของ quercetin โดยตรงต่อกล้ามเนื้อหัวใจห้องบน และ สามารถยับยั้งได้ด้วย propranolol, verapamil และ pinacidil ผลรวม naringenin ในการลดแรงบีบตัวของหัวใจดังกล่าวไม่สามารถยับยั้งได้ด้วย atropine ตลอดจนไม่มีผลต่อการออกฤทธิ์ของ pinacidil ทั้งนี้การออกฤทธิ์ของสาร flavonoids ทั้ง 2 ตัวไม่เกี่ยวข้องกับการหลั่งแคลเซียมจากแหล่งสะสมภายในเซลล์ จากการศึกษาครั้งนี้สรุปได้ว่า quercetin มีผลต่อ pacemaker และการทำงานของกล้ามเนื้อหัวใจทำให้เกิดการเพิ่มแรงบีบตัวและอัตราการเต้น โดยการออกฤทธิ์ผ่านการกระตุ้น ß- adrenoceptor เพิ่ม Ca^{2+} influx และมีกลไกบางส่วน เกี่ยวข้องกับการปิดของ K^+ channel ในขณะที่ naringenin นั้นมีผลลดแรงบีบตัวและอัตราการเต้นของหัวใจ โดยที่กลไกการออกฤทธิ์ไม่เกี่ยวข้องกับการกระตุ้น cholinergic receptor

สาขาวิชาเภสัชวิทยา (สหสาขาวิชา) ลายมือชื่อนิสิต นุชนาท ประมาคะเต

ปีการศึกษา 2548 ลายมือชื่ออาจารย์ที่ปรึกษา สุรีย์ เจียรณ์มงคล

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม ประสาน ธรรมอุปกรณ์

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KEYWORD: INOTROPIC EFFECT / CHRONOTROPIC EFFECT / QUERCETIN / NARINGENIN

NUCHANAT PRAMAKATAY: EFFECTS OF QUERCETIN AND NARINGENIN ON RATE AND FORCE OF CONTRACTION OF ISOLATED RAT RIGHT AND LEFT ATRIA. THESIS ADVISOR: ASSIS. PROF. SUREE JIANMONGKOL, Ph.D. THESIS CO-ADVISOR: ASSOC. PROF. PRASAN DHUMMA-UPAKORN, Ph.D. 92 pp. ISBN 974-14-3282-8.

Flavonoids have been known for their cardiotonic actions. This study investigated the effects of quercetin and naringenin, one of the most abundant dietary flavonoids, on rate and contraction of isolated rat atria. The right and left atria were isolated from male Wistar rats (250 – 300g), attached to force transducer and suspended in a 20 ml - organ bath containing Krebs-Henseleit solution at 37^0C, gassed with carbogen. The results showed that these two flavonoids elicited different cardiotonic activities. Quercetin (100 μM) increased the contraction of the right and left atria by 33.32 ± 4.13 % and 45.75 ± 4.90%, respectively. In addition, it caused an increased in the rate of the right atria by 17.62 ± 6.51%. In contrast, naringenin (100 μM) slightly decreased the contraction of the right and left atria by 6.16 ± 3.02 % and 10 ± 3.51%, respectively. It also markedly reduced the rate of right atria by 30% ± 5.32 %. Quercetin possessed intrinsic inotropic activity which did not relate to a release of synaptic catecholamine. Moreover, the positive inotropic effect of quercetin was blocked by propranolol (10 μM), verapamil (1 μM) and pinacidil (100 μM). The negative inotropic property of naringenin was not change in the presence of atropine (10 μM) and pinacidil (100 μM). Furthermore, the actions of both flavonoids were not related to Ca^{2+}release from SR. The positive inotropic effect of quercetin may be due to the activation of beta-adrenoceptor, an increase Ca^{2+} influx. In addition, quercetin may interfere function of K^+ channel. Furthermore, naringenin caused the negative inotropic and chronotropic effects of which were not related cholinergic receptor. In conclusions, quercetin and naringenin exerted their cardiotonic actions on pacemaker and cardiac muscle.

Field of Study Pharmacology (Inter - Department) Student's Signature Nuchanat Pramakatay

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Co-Advisor's Signature Prasan Dhumma-upakorn

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List of Abbreviations

Ach	=	acetylcholine
BMP	=	beat per minute
Ca^{2+}	=	calcium ion
CICR	=	calcium- induced calcium release
DMSO	=	dimethyl sulfoxide
K^{+}	=	potassium channel
KCL	=	potassium chrolide
Kg	=	kilogram
KHS	=	Krebs- Henseleit solution
L	=	litre
M	=	molar
Min	=	minute
MW	=	molecular weight
mg	=	milligram
ml	=	milliliter
mM	=	millimolar
Na^{+}	=	sodium ion
NE	=	norepinephine
n	=	number of experiments
PRC	=	post-rest contraction
SA	=	sinoatrial
SEM	=	standard error of mean
SR	=	sarcoplasmic reticulum
ß	=	beta
µ	=	micro
^{0} C	=	degree of Celsius
v/v	=	volume by volume
%	=	percent
p	=	probability

CHAPTER I

INTRODUCTION

Recently, cardiovascular disease has been recognized as one of the major causes of mortality and morbidity in many countries. Its prevalence is markedly rising in the last decade. Nowadays, the goals of therapy in cardiovascular diseases are relieving symptom, preventing progression and improving survival rates.

Flavonoids are a major class of natural polyphenolic compounds which are ubiquitous in plants, vegetables, fruits and plants origin. Flavonoids are known to exhibit various pharmacological and biological effects such as anti-inflammatory, antiallergic reaction, inhibition of cell proliferation, antihepatotoxicity, antifungal and bactericidal and antioxidant (Halliwell, 1994; Sangku *et al*., 2003). In addition, pharmacological effects of flavonoids in the cardiovascular system have been reported including antihypertensive action, antiarrhythmia, antiaggregation and reduction of low-density lipoproteins levels in plasma (Formica and Regelson, 1999). Several epidemiological studies revealed an inverse association between flavonoids intake and reducing in occurrence of cardiovascular disease such as myocardial infarction (Knekt *et al*., 1996).

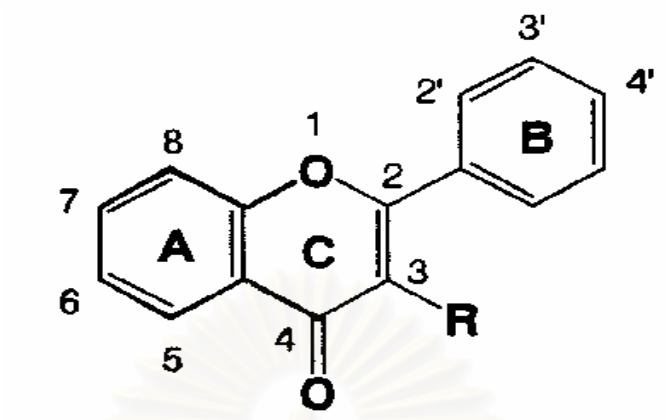
Quercetin and naringenin (Figure1) are in a class of flavonol and flavonone group of flavonoids. These compounds are found in several plants and natural products such as red wine, grape juice, onions, kale, broccoli, apple, peanut, cherries, berries, tea, parsley, thyme, citrus, tomato and coffee (Pierpoint, 1996). It has been reported that quercetin and naringenin caused endothelium-dependent and endothelium-independent vasorelaxation in the model of isolated rat aorta (Fitzpatrick *et al.,* 1993). The mechanisms underlying the effects on vasorelaxation have been linked to an inhibition of Ca^{2+} influx, a release of Ca^{2+} from intracellular store and an activation of ß-adrenoceptor (Ajay *et al.,* 2003).

Flavonoids also elicited its cardiotonic effects. The study of structure-activity relationship (SAR) of cardiotonic flavonoids as determined by contraction of guinea-pig

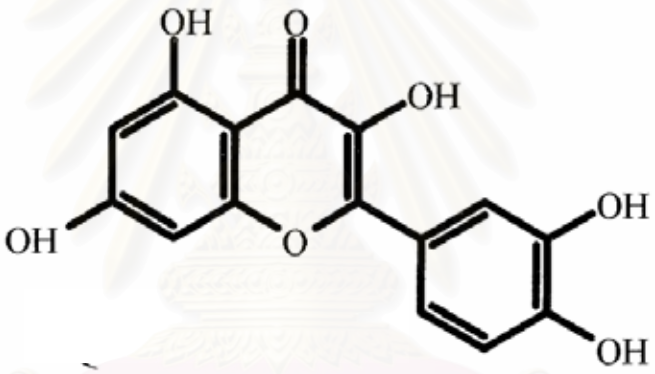
papillary muscle revealed that quercetin was one of the most potent inotropic flavonoids. The relative order of potency was quercetin > morin = kaemferol = HEPTA > luteolin = apigenin > natsudaidain = fisetin = galangin > catechin > naringenin. The inotropic potency of these flavonoids has been related to the completeness of flavonoid nucleus (Itoitawa *et al*., 1999). Naringenin also demonstrated its positive inotropic activity although at a lessor degree than quercetin (Itoitawa *et al*., 1999). Interestingly, naringenin has been associated with QT prolongation in clinical studies (Zitron *et al*., 2005).

Cardiotonic activity of flavonoids compounds especially quercetin and naringenin has been of interest in several research groups. However, knowledge of its underlying mechanisms on inotropic and chronotropic actions is still limited. Most of the current studies have been done in whole heart or ventricular papillary muscle, which may be difficult to distinguish the direct effects of xenobiotics on pacemaker activity and force of contraction. Hence, this study aimed to investigate the effects of quercetin and naringenin on pacemaker activity and heart muscle, using the model of isolated rat right and left atria. In addition, the study was to examine the possible underlying mechanisms of these two flavonoids actions.

A. Flavonoid



B. Quercetin



C. Naringenin

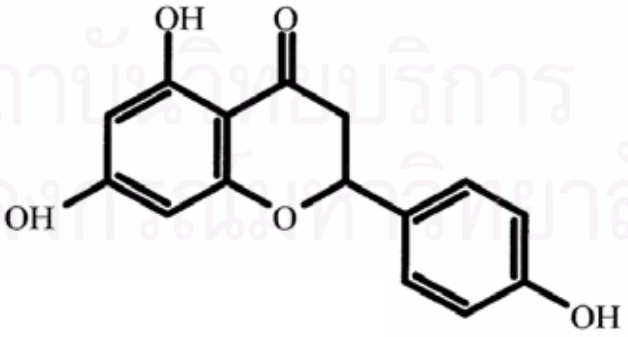


Figure1 The structure of general flavonoid (A), quercetin (B) and naringenin (C).

Hypothesis

Based on its polyphenolic flavonoids structure, quercetin and naringenin are alike in its positive inotropic and chronotropic activities. These two compounds may exert its direct action on the heart via activation of sympathetic pathway.

Objective

1. To investigate and compare the pharmacological effects of quercetin and naringenin on rate and force of contraction.
2. To investigate the mechanisms of action on of quercetin and naringenin on rate and force of contraction. For example, these two compounds may affect the activation of ß -adrenoceptor, the increase in intracellular Ca^{2+} or the activation of K^{+} channel of the heart.

Expected benefit and applications

This study further provided pharmacological knowledge and mechanisms of action of quercetin and naringenin on rate and force of contraction. The information from this study will be useful for the new drug development especially for the cardiovascular agent. In addition, this knowledge may help to encourage an use flavonoids as dietary supplement and may lead to new dietary recommendation for patients at risk of cardiovascular events.

CHAPTER II

LITERATURE REVIEW

The flavonoids are a group of naturally polyphenolic compounds that are ubiquitous in plants, vegetables, fruits, and beverage of plants origin. Flavonoids can be classified based on upon its molecular structure into 4 groups as follow (Table 1):

1. Flavonols such as quercetin, kaemferol, morin, rutin.
2. Flavones such as luteolin, apigenin, flavone.
3. Flavonones such as naringenin, naringin.
4. Flavanes such as epigallocatechin gallate, catechin.

Numerous epidemiological studies have found beneficial effects of flavonoids consumption on overall cardiovascular mortality (Hertog *et al.,* 1993). Flavonoids apparently reduced the incidence of fatal complications associated with acute myocardial infarction and the post infarction period (Zitron *et al*., 2005).

Flavonoids are known to exhibit various pharmacological effects including anti-inflammatory, antiallergic reaction, inhibition of cell proliferation, antihepatotoxicity, antifungal and bactericidal, antioxidant (Sangku *et al*., 2003), antihypertensive, antiarrhythmia, antiaggregation and reduction of low-density lipoproteins levels in plasma (Formica and Regelson, 1999). Because this study aimed to investigate the cardiotonic effects of quercetin and naringenin, the literature review emphasized on the recent knowledge of both compounds on the heart.

Quercetin

The flavonoids consists of the three rings structure (A, B and C-ring) (Figure1) with a diphenyl propane skeleton (C-6-C3-C-6) (Formica *et al.,* 1995). The study of structure-activity relationship (SAR) of cardiotonic flavonoids on the guinea- pig papillary muscle revealed that the complete flavonoid nucleus with cardiotonic effects consists of a hydroxyl group at C-4' in B ring and the double bond at C 2-3 position in the C-ring (Itoitawa *et al*., 1999). Quercetin and naringenin are in a different subclass with a few

difference in its structure (Figure1). Quercetin has the double bond at C 2-3 position in the C-ring whereas naringenin lack double bond at this position. These differences reflected on the discrepancy in inotropic activity of these compounds (Itoitawa *et al*., 1999). The positive inotropic effects of quercetin has been partially mediated by the ß-adrenoceptor (Itoitawa *et al.,* 1999). Another study by Kubota *et al*., 2002, which investigated the effects of dietary supplements on isolated rat atria, showed that quercetin exerted a positive inotropic effect without any effect on the rate of rat atria (Kubota *et al*., 2002). In addition, quercetin elicited a positive chronotropic effects in the isolated guinea-pig right atria, but the inotropic activity was not described (Laekeman *et al.,* 1986). The positive inotropic action of quercetin was also demonstrated on spontaneous contraction in a dose- dependent manner. Furthermore, quercetin prevented the negative inotropic evoked by acetylcholine in cardiac and skeletal muscle isolated preparation (Apisariyakul *et al.,* 1999).

Naringenin

Like other flavonoids, naringenin has been reported its inotropic activity. This compound was found mostly in citrus fruit and grapefruit (Hertog *et al.,* 1996). In the 5-year study by Wilcox *et al*., 1999, the correlation between flavonoids consumption and cardioprotective activities was established in 805 men from the Netherlands, aged 65-84 years. The results suggested that flavonoids consumption caused a reduction in cardiovascular mortality. Naringenin was one of the most important flavonoids in grapefruit which was the main components in the flavonoid compounds. However, these was an clinical report of QT prolongation in healthy volunteer having flavonoids containing naringenin (Zitron *et al*., 2005). In the study, ten healthy volunteers drank 1 L of freshly grapefruit juice with half and hour in the morning. The 12 lead ECG was recorded continuously starting 30 minute before a drink and 12 hours afterward. The results suggested that naringenin was associated with QT prolongation in those healthy volunteer (Zitron *et al*., 2005). In the wake of an interest in cardioprotective effects of dietary flavonoids, this phenomenon and its underlying mechanism are needed to be elucidated.

The regulation of cardiac function

The cardiovascular system consists of three anatomical components: the autonomic nervous system, the heart, and the vasculature. These three components interact in a complex manner to control blood flow to organ throughout the body. In the cardiac function, the autonomic system were regulation the heart rate and contraction in the heart.

The autonomic nervous system

The autonomic nervous system is widely distributed throughout the body and controls a variety of bodily functions, including blood pressure and heart rate. The efferent peripheral autonomic nervous system is composed of two opposing subsystems, the sympathetic nervous system and the parasympathetic nervous system.

1. The sympathetic nervous system

The sympathetic nervous system is diffuse and innervates many components of the cardiovascular system. The primary neurotransmitter of postganglionic sympathetic nerve fibers is norepinephrine. The target organs of the sympathetic nervous system contain receptors for norepinepherine and epinephrine; these receptors are known as adrenergic receptors. The response of the heart to sympathetic stimulation, was increased contractility via $beta_1$-adrenergic and $beta_2$-adrenergic; increased heart rate via $beta_1$-adreneregic (Figure 2).

2. The parasympathetic nervous system

The parasympathetic nervous system innervation of the cardiovascular system is essentially just the innervation of the heart by the vagus nerve. This innervation is relatively discrete, being limited to the sino-atrial (SA) node (pacemaker) and the atrioventricular (AV) junction. There is little or no innervation of the cardiac ventricles.The postganglionic neurotransmitter of the parasympathetic nervous system is acetylcholine. Acetylcholine released by the parasympathetic nervous system binds to muscarinic cholinergic receptors on target tissues. The effects of parasympathetic stimulation on the heart were major cholinergic muscarinic (M) receptor subtypes. These results effects on SA node and AV junction were decreased heart rate (Katzung, 2001).

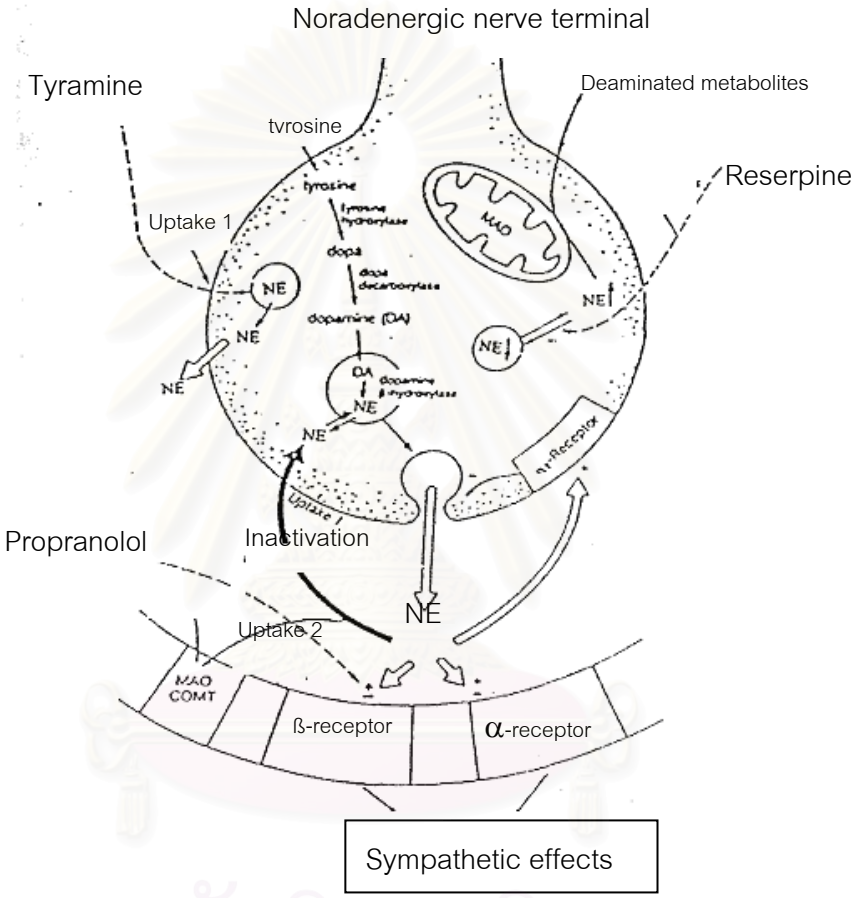


Figure 2 Synaptic events of sympathetic nervous system (Neal, 1997)

Cardiac excitation-contraction coupling

Excitation-contraction coupling (ECC) is the process by which an action potential triggers a myocyte to contract. When a myocyte is depolarized by an action potential, calcium ions enter the cell during phase 2 of the action potential through L-type calcium channels located on the sarcolemma. This calcium triggers a subsequent release of calcium that is stored in the sarcoplasmic reticulum (SR) through calcium-release channels (ryanodine receptors) (Figure 3). Calcium released by the SR increases the intracellular calcium concentration from about 10^{-7} to 10^{-5} M. The free calcium binds to troponin-C (TN-C) that is part of the regulatory complex attached to the thin filaments. When calcium binds to the TN-C, this induces a conformational change in the regulatory complex such that troponin-I (TN-I) exposes a site on the actin molecule that is able to bind to the myosin ATPase located on the myosin head. This binding results in ATP hydrolysis that supplies energy for a conformational change to occur in the actin-myosin complex. The result of these changes is a movement between the myosin heads and the actin, such that the actin and myosin filaments slide past each other thereby shortening the sarcomere length. Ratcheting cycles occur as long as the cytosolic calcium remains elevated. At the end of phase 2, calcium entry into the cell slows and calcium is sequestered by the SR by an ATP-dependent calcium pump (SERCA, sarco-endoplasmic reticulum calcium-ATPase), thus lowering the cytosolic calcium concentration and removing calcium from the TN-C. To a quantitatively smaller extent, cytosolic calcium is transported out of the cell by the sodium-calcium-exchange pump. The reduced intracellular calcium induces a conformational change in the troponin complex leading, once again, to TN-I inhibition of the actin binding site. At the end of the cycle, a new ATP binds to the myosin head, displacing the ADP, and the initial sarcomere length is restored.

Mechanisms that enhance the concentration of cytosolic calcium increase the amount of ATP hydrolyzed and the force generated by the actin and myosin interactions, as well as the velocity of shortening. Physiologically, cytosolic calcium concentration are influenced primarily by beta-adrenoceptor-coupled mechanisms. Beta-adrenergic

stimulation, as occurs when sympathetic nerves are activated, increases cAMP which in turn activates protein kinase to increase in calcium entry into the cell through L-type calcium channels. Activation of the IP_3 signal transduction pathway also can stimulate the release of calcium by the SR through IP_3 receptors located on the SR. Furthermore, activation of the cAMP-dependent protein kinase phosphorylates a protein (phospholamban) on the SR that normally inhibits calcium uptake. This inhibition of phospholamban leads to an increased rate of calcium uptake by the SR. Therefore, beta-adrenergic stimulation increases the force and shortening velocity of contraction (i.e., positive inotropy), and increases the rate of relaxation (i.e., positive lusitropy) (Figure 4).

In the heart, potassium channels are important for cardiac functions. Numerous potassium channels have been reported. On the basis of the amino acid sequence of the pore-forming α- subunit, potassium channels can be classified into two main superfamilies: the inward rectifier (K_{IR}) superfamily (including receptor-coupled, ATP-sensitive and voltage-dependent channels) and the *Shaker*-related superfamily (which includes Ca^{2+}-dependent channels). Potassium channels, particularly those regulated by intracellular ATP are ubiquitous in the heart and are important modulators of cardiovascular function (Purcell, 1999). Opening potassium channels cause increased efflux of potassium ion from the cell, subsequently the resting membrane potential is shift towards more negative valves (hyperpolarization). This effect leads to an inhibition of calcium influx or indirect calcium antagonism, causing a fall in intracellular calcium concentration (Purcell, 1999).

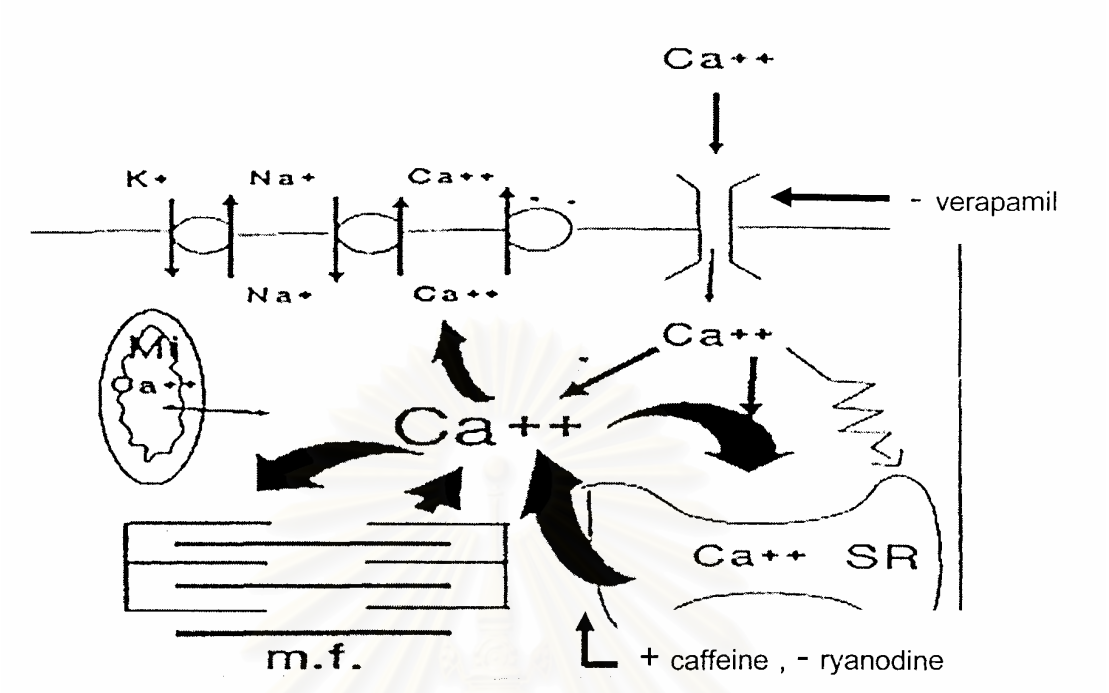


Figure 3 Processes of excitation - contraction coupling in cardiac- myocytes (Neal, 1997)

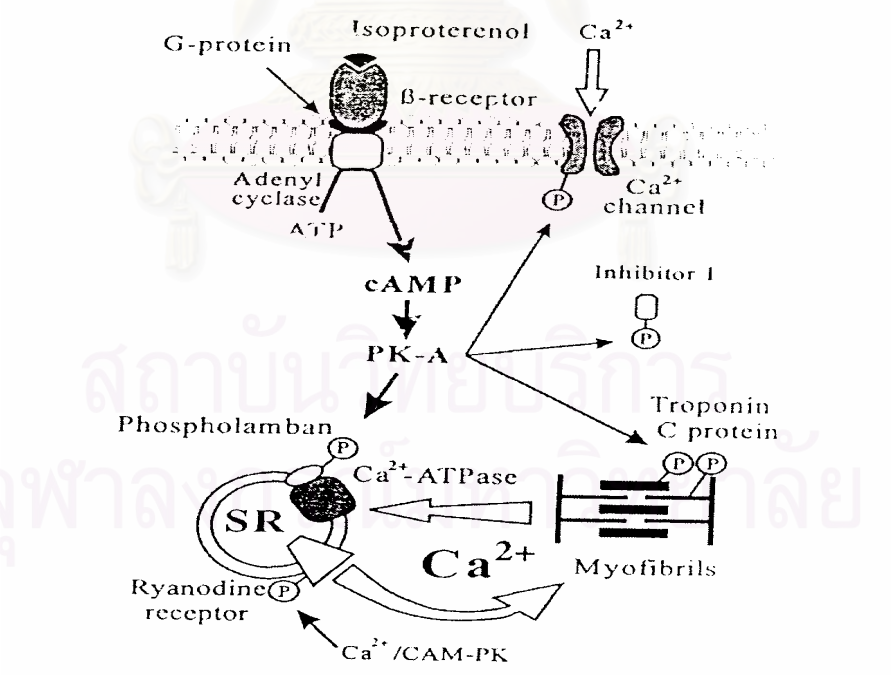


Figure 4 Mechanism of cardiac contraction mediated through beta –adrenoceptor. (Katzung, 2001)

CHAPTER III

MATERIALS AND METHODS

Experimental animals

Adult male Wistar rats of body weight 250-300 g were obtained from National Labaratory Animal Center, Mahidol University, Salaya, Nakornpathom. The animals were housed in animal care facility at the Faculty of Pharmacuetical Sciences, Chulalongkorn University. The rats were housed in a temperature at 25 ^{0}C and allowed free access to water and food. In addition, the rats were acclimatized for at least 1 week before the experiment.

Chemicals

Flavonoids used in this research were quercetin (2-(3, 4-dihydroxyphenyl) - 3, 5, 7-trihydroxy-4H-1-benzopyran-4-one), (MW 338.26) and naringenin (2, 3- dihydro-5, 7-dihydroxy- 2(4-hydroxyphenyl)-4 H- 1-benzopyran-4-one) (MW 272.27). The principal chemicals used in this research included dimethyl sulfoxide (DMSO) 99.5% V/V, norepinephine, propranolol, atropine, verapamil, caffeine, tyramine, reserpine and pinacidil. Flavonoids and principal chemicals were purchased from Sigma Aldrich (St.Louis,MO, U.S.A). Other chemicals for Krebs-Henseleit solution such as NaCl, $CaCl_2$, KCl, $NaHCO_3$, $MgSO_4$, KH_2PO_4 and glucose were purchased from Merck KGaA (Darmstadt, Germany).

Experimental instruments

1. Double- wall organ bath. The organ bath made of glass comprises an inner and outer chamber. An inner chamber with the capacity of 25 ml is for suspended the isolated tissue in physiological solution. The reservoir shoud also be constantly aerated with carbogen gas. An outer chamber is for temperature control from water bath at 37 ^{0} C.

2. Water bath and thermoregulation water pump (Heto®, Model HWT100, Jouan Nordic, Gydeuang, Denmark).
3. Isometric force transducer model MLT 050/A (ADInstrument, Castle Hill, Australia).
4. Powerlab/ 4 sp equipped connected to a computer with program SCOPE CHART 5 V. 2.0 (ADInstrument, Castle Hill, Australia).
5. Tank of carbogen gas (95%O_2 ± 5% CO_2) (T.I.G, Bangkok, Thailand).

Experimental methods

Preparation of isolated rat atria

Male Wistar rats (250-300g) were sacrified by cervical dislocation, rapidly removed heart, and immediately placed in a Krebs-Henseleit solution. The atria was cut to separate right and left sides, and then attached to force transducer with a-one-gram initial load of resting tension. The left atria was also connected with electrical stimulation to induce contraction by electrical pacing (4.2 Hz, 5 ms, 5 voltage). All experiment, the tissue were equilibrated for at least 30-45 min or until the tension was stable before starting the experiment.

1. Determination of inotropic and chronotropic effects of quercetin and naringenin

The right and left atria were prepared and incubated 30-45 min in Krebs-Henseleit solution until the tension stable. Quercetin and naringenin were freshly dissolved in DMSO before the experiment. The final concentration of DMSO was 0.03% v/v, which had no significant effect on rate and force of contraction. The experiment started with addition of quercetin and naringenin cumulatively at the concentration range of 0.1μM-1000 μM.

The rate and force of contraction of the right atria were measured through isometric transducer. The force of contraction of the left atria was determined through isometric transducer under the electrical stimulation at 4.2 Hz, impulse of 5 ms duration with threshold at 5 voltage. The transducer was coupled to powerlab4/sp, ADInstrument, Castle Hill, Australia, which was connected to the computer equipped

with program SCOPE CHART 5 V. 2.0 (ADInstrument, Castle Hill, Australia) to analyze the rate and force of contraction. The contraction of left and right atria in Krebs-Henseleit solution were expressed in mg of developed tension. The basal contractile activities were referred as 100 %. The responses were calculated as a change in percentage of contraction or heart rate from the basal activities.

2. Determination the mechanisms of action of quercetin and naringenin.

2.1 Effects of quercetin and naringenin on activation of ß–adrenoceptor, muscarinic receptor, the increase in intracelular Ca^{2+} and the activation of K^{+}channel on the heart

The inotropic and chronotropic effects of quercetin and naringenin were further investigated for its underlying mechanism. After equilibration period of 30-45 min, the flavonoids was immediately added to the tissue, and recorded the responses. In order to investigate the involvement of ß–adrenoceptor, muscarinic receptor, the increase in intracellular Ca^{2+} and the activation K^{+}channel, propranolol 10 µM, atropine 10 µM, verapamil 1 µM or pinacidil 100 µM were added 5 min prior to addition of the flavonoids. The responses were calculated as a change of percentage of contraction or heart rate from the basal activities.

2.2 The inotropic effect of quercetin and naringenin on Ca^{2+} release from internal store.

This experiment procedures were designed to determine the inotropic effect of quercetin and naringenin on the rest state contraction (RSC) of isolated left atria. The rest state contraction was induced by continuous "on-off'' electrical stimulation. In brief, electrical stimulation started at pacing 4.2 Hz, 5 ms, 5 voltage, then stopped for a short periods ranging from 10 seconds to 5 minute. The effects of flavonoids were tested by incubation the flavonoids with the tissue 5 minute prior to a stimulation. In this study, caffeine (10mM) was also used as positive control because of its known activity to release Ca^{2+} from SR. The initial contractile response of flavonoids on resumption of stimulation after 10 seconds to 5 minute of rest period (Ti) was compared with the steady- state response (Tss) as shown in Figure 5.

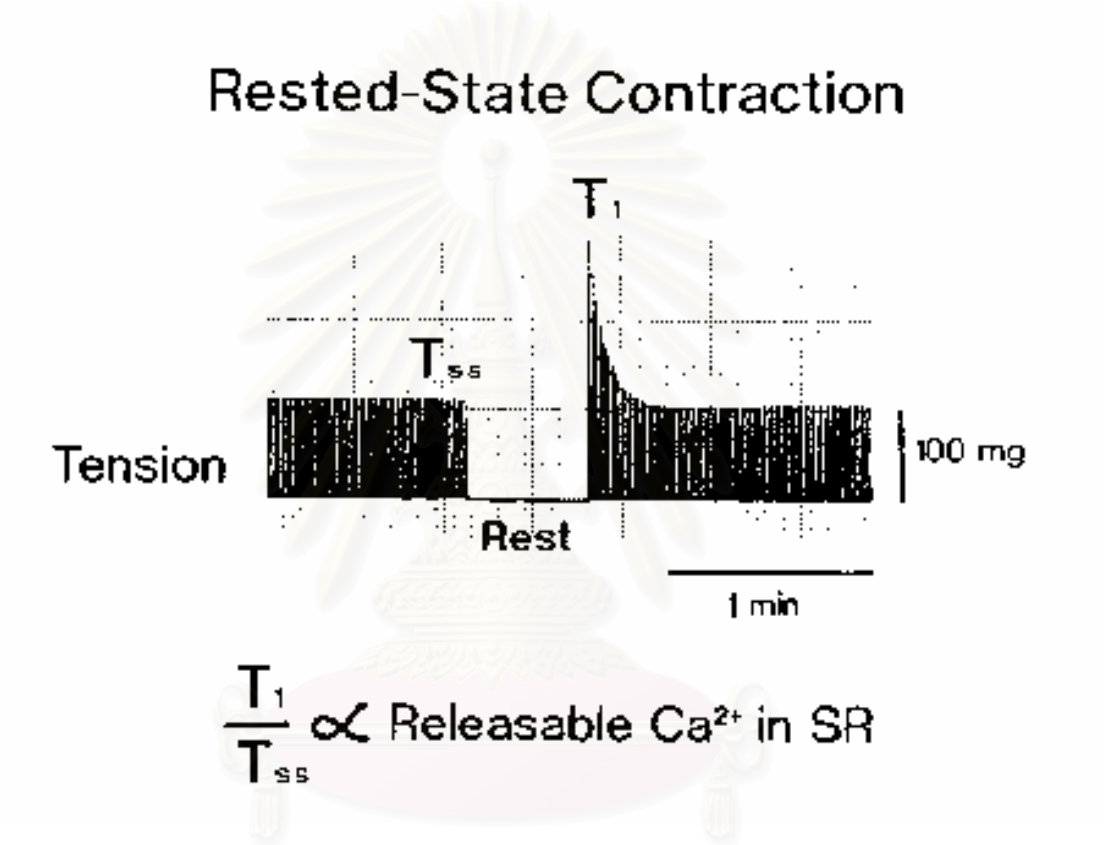


Figure 5 An example of rest- state contraction in papillary muscle. The muscle preparation was stimulated at a fixed stimulation for a certain period and the steady-state tension (Tss) was record. After stopped stimulation, an initial tension (Ti) was measured as RSC. (Ti) / (Tss) was used as an index of releasable Ca^{2+} in sarcoplasmic reticulum (SR) (Yamato *et al*., 1996).

2.3 Role of the storage catecholamine

These experiments were performed in atria isolated from reserpine- treated rats (5 mg/kg, i.p. for 2 days) in order to investigate the effects of flavonoids on storage catecholamine. Tyramine exerted sympathomimetic action indirectly. This compound caused positive inotropic and chronotropic effects by releasing storage catecholamine from adrenergic nerve ending. The depletion of catecholamine in its storage, which may be induced by reserpine treatment could result in the lack of tyramine cardiotonic actions. Hence, in this experiment the atria was isolated from reserpine-treated rats to exclude the effect of the storage catecholamine. Following the procedures of atria preparation, tyramine (10 μM) was added to confirm a lack of noradrenaline from adrenergic nerve ending. The success of reserpine treatment was elicited by the absence of inotropic or chronotropic effects upon addition of tyramine. Then, the direct effect of quercetin on rate and force of contraction was examined by addition of quercetin to the reserpine-pretreated tissue which had no response toward tyramine. The responses were calculated as a change of percentage of contraction or heart rate of the atria preparation in the absence of flavonoid.

Data analysis

Contraction of right and left atria in Krebs- Henseleit solution was expressed in mg of developed tension. The basal tension which was measured in the absence of any compound was referred to a 100 percent. The developed tension upon addition of flavonoids was calculated as a percentage of basal activities as percentage difference. The results were reported as the mean ± S.E.M. (n=6). Statistical significance was evaluated by Student's *t* - test for paired or unpaired data. Data were considered significant difference when *p* value was less than 0.05.

CHAPTER IV

RESULTS

1. The intrinsic inotropic and chronotropic effects of quercetin and naringenin

The basal contraction of right and left atria were 0.5 ± 0.05 g and the basal rate of right atria was 250 ± 10 beats / min. Quercetin and naringenin were freshly dissolved in DMSO before each experiment. The final concentration of DMSO was 0.03 % V/V, which had no significant effects on rate and force of contraction (Figure 6 and 7). The concentration-response curves of quercetin and naringenin were plot as a change in percentage of response against logarithmic concentration of flavonoids. Quercetin caused positive inotropic and chronotropic effects in a concentration-dependent manner (Figure 8A). In contrast, naringenin caused negative inotropic and chronotropic effects in a concentration-dependent manner (Figure 8B). Quercetin at the concentration of 100 μM significantly increased the contraction of the right and left atria by 33.32 ± 4.13 % and 45.75 ± 4.90%, respectively. In addition, it caused an increase in the rate of the right atria by 17.62 ± 6.51%. (n=6) (Figure 9 and 10). In contrast, naringenin at the concentration of 100 μM slightly decreased the contraction on the right and left atria by 6.16 ± 3.02 % and 10 ± 3.51%, respectively. It also markedly suppressed the rate of right atria by 30% ± 5.32(n=6) (Figure 11 and 12).

2. The positive inotropic and chronotropic of quercetin

2.1 An involvement of ß- adrenoceptor

At the concentration of NE 1 μM significantly increased the basal heart rate and contraction on right and left atria as shown in Figure 14 and 16A. At this concentration, NE increased the basal of contraction of right and left atria by 33.74 ± 4.33 % (0.35 ± 0.04 g) and 69.42 ± 4.33 % (0.41 ± 0.05 g), respectively. It also increased the rate of the right atria from the basal rate 216 beat / min to 383 beat / min (77.32 ± 9.32 %). In this study, propranolol at the concentration of 10 μM suppressed both basal rate and force of atria (Figure 13 and 16B). At the concentration of 10 μM, propranolol blocked

effects of NE on the rate and force of contraction (Figure 15 and 17). In addition, the positive inotropic and chronotropic of quercetin were completely blocked by propranolol (Figure 18 and 19). This finding suggested that quercetin may exerted its cardiotonic action via activation of ß- adrenoceptor.

2.2 Role of Ca^{2+}entry

Figure 24-28 illustrated the inotropic and chronotropic effects of quercetin in the presence and absence of verapamil (1 µM). Although, over 15 min quercetin (100 µM) increased the rate and force of the right atria by 17.31 ± 6.94 % and 33.30 ± 4.13 %, respectively, the sign of arrhythmia was not observed (Figure 24). In this study, verapamil at the concentration of 1 µM suppressed the basal force of contraction of right and left atria by 18.88 ± 11.85 % and 30.94 ± 8.08 %, respectively. It also suppressed the basal rate of right atria by 53.17 ± 10.9 % (Figure 25). It appeared that the presence of verapamil (1 µM) for 5 min produced a sign of arrhythmia as seen in Figure 27. This phenomenon was observed in 3 out of 6 of the right atria in this experiment. As seen in Figure 26 and 28, the positive inotropic and chronotropic effect of quercetin was significantly blocked by verapamil. Interesting, verapamil triggered arrhythmia at 5 min after addition of quercetin (100 µM) to the right atria. This occurrence was observed in 3 out of 6 of the right atria in the experiment. This finding suggested that the inotropic and chronotropic effects of quercetin was due to Ca^{2+} influx. In addition, the concurrent use of quercetin and calcium channel blocker potentially trigger an arrhythmia.

2.3 Role of Ca^{2+}release from SR

This experiment employed caffeine (10 mM) to induce Ca^{2+} release from sarcoplasmic reticulum (SR), resulting the positive inotropic effect. As seen in Figure 29 B and 30 shown the relationship between Ti / Tss and the resting interval in the presence of caffeine. The ratio of Ti / Tss in the presence of caffeine was markedly inhibit the post rest contraction in all the time of range 10 seconds to 5 minute. Figure 29C and 31 shown the relationship between Ti / Tss and the resting interval in the presence of quercetin. The ratio of Ti / Tss in the presence of quercetin did not

significantly inhibit the post rest contraction in all the time of range 10 seconds to 5 minute. When compared the inotropic effects of quercetin and caffeine on the post rest contraction. Quercetin significantly different on the post rest contraction. This finding suggested that the positive inotropic of quercetin may not involved the calcium release from sarcoplasmic reticulum (SR).

2.4 An involvement of K^+ channel

Pinacidil at the concentration of 100 μM suppressed the basal rate and contraction of the right atria by 11.29 ± 1.09 % and 13.00 ± 4.07 %, respectively. It also suppressed the basal force of contraction of left atria by 20.41 ± 4.10 % (Figure 33 and 34). At the concentration of 100 μM, pinacidil significantly blocked the positive inotropic and chronotropic effects of quercetin (Figure 35 and 36), but did not significantly block the effects of NE on the rate and force of contraction (Figure 37 and 38). This findings suggested that quercetin exerted its cardiotonic actions through the K^+ channel.

2.5 Effects of the storage catecholamine

Tyramine has indirect sympathomimetic action. Its caused positive inotropic and chronotropic effects by releasing of storage catecholamine from adrenergic nerve ending. In isolated preparation of left and right atria, treatment of tyramine produced an increased in the rate and force of contraction due to the increase in availability of synaptic norepinephine (Figure 41 and 43). However, the positive inotropic and chronotropic effects of tyramine markedly decreased in the atria isolated from rats pretreated with reserpine (Figure 42). This was due to the effects of reserpine in rat pretreated with reserpine leading to a deprivation of presynaptic storage norepinephine. With the use of atria isolated from reserpine-treated rats, quercetin was able to cause the inotropic and chronotropic effects (Figure 44 and 45). In addition, the cardiotonic activity of quercetin was not statistically different between the atria isolated from normal rats and from reserpine treated rats (Figure 46). This data suggested that quercetin exerted the cardiotonic actions directly the activation of ß- adrenoceptor.

3 The cardiotonic effects of naringenin

3.1 An involvement of parasympathetic system

Naringenin at the concentration of 100 μM decreased the contraction on the right and left atria by 6.16 ± 3.02 % and 10 ± 3.51%, respectively. It also markedly suppressed the rate of right atria by 30% ± 5.32 % as shown in Figure 10 and11. It was possible that this compound activated muscarinic receptor on the heart. However, pretreatment of atropine (10 μM) did not significantly affect the action of naringenin (Figure 20 and 22). This result suggested that naringenin exerted its cardiotonic actions via a mechanism not involving the parasympathetic system.

3.2 Role of Ca^{2+} release from SR

The relationship between the Ti / Tss and the resting interval in the presence of caffeine was shown in Figure 29B and 30. The ratio of Ti / Tss in the presence of caffeine was markedly inhibit the post rest contraction in all the time of range 10 seconds to 5 minute. As seen in Figure 29D and 32 shown the relationship between Ti / Tss and the resting interval in the presence of naringenin. The ratio of Ti / Tss in the presence of naringenin did not significantly inhibit the post rest contraction in all the time of range 10 seconds to 5 minute. When compared the inotropic effects of naringenin and caffeine on the post rest contraction. There were significantly different. This finding suggested that the negative inotropic of naringenin may not involve decreased the calcium release from sarcoplasmic reticulum (SR).

3.3 An involvement of K^+ channel

Pinacidil at the concentration of 100 μM decreased the contraction on the right and left atria by 13.00 ± 4.07 % and 20.41 ± 4.10 %, respectively. It also suppressed the basal rate and contraction of the right atria by 11.29 ± 1.09 % as shown in Figure 33 and 34). In addition, naringenin at the concentration of 100 μM decreased the contraction on the right and left atria by 6.16 ± 3.02 % and 10 ± 3.51%, respectively. It also markedly suppressed the rate of right atria by 30% ± 5.32 % as shown in Figure 10 and 11. Although, these two compounds significantly suppressed the rate and force of

contraction, but pretreatment of pinacidil (100 μM) did not significantly affect the actions of naringenin (Figure 39 and 40). This results suggested that the used of naringenin with potassium channel opener did not additive effects on pacemaker and heart conduction.

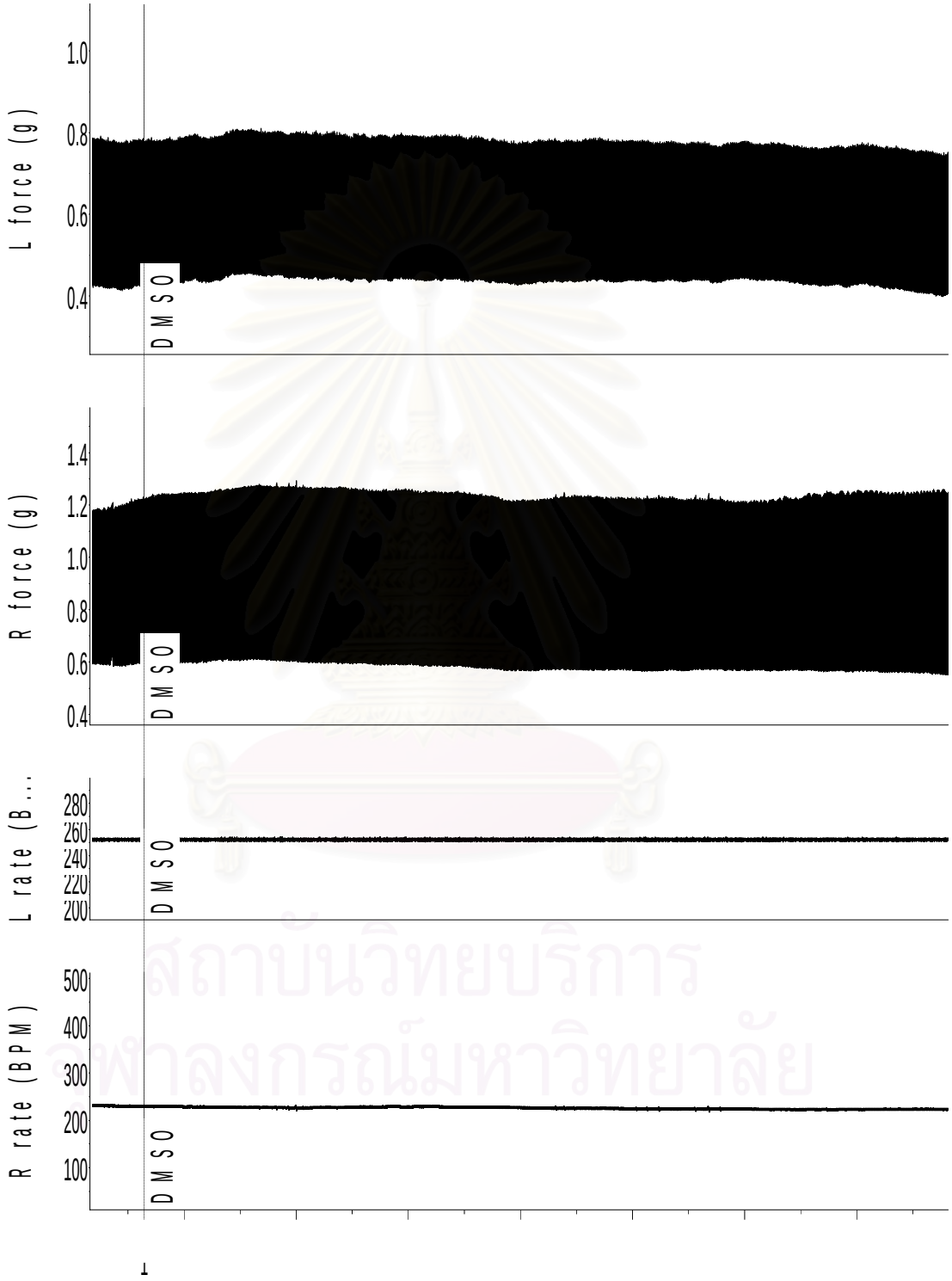


Figure 6 The inotropic and chronotropic response on the right and left atria in the presence of DMSO (0.03% V/V).

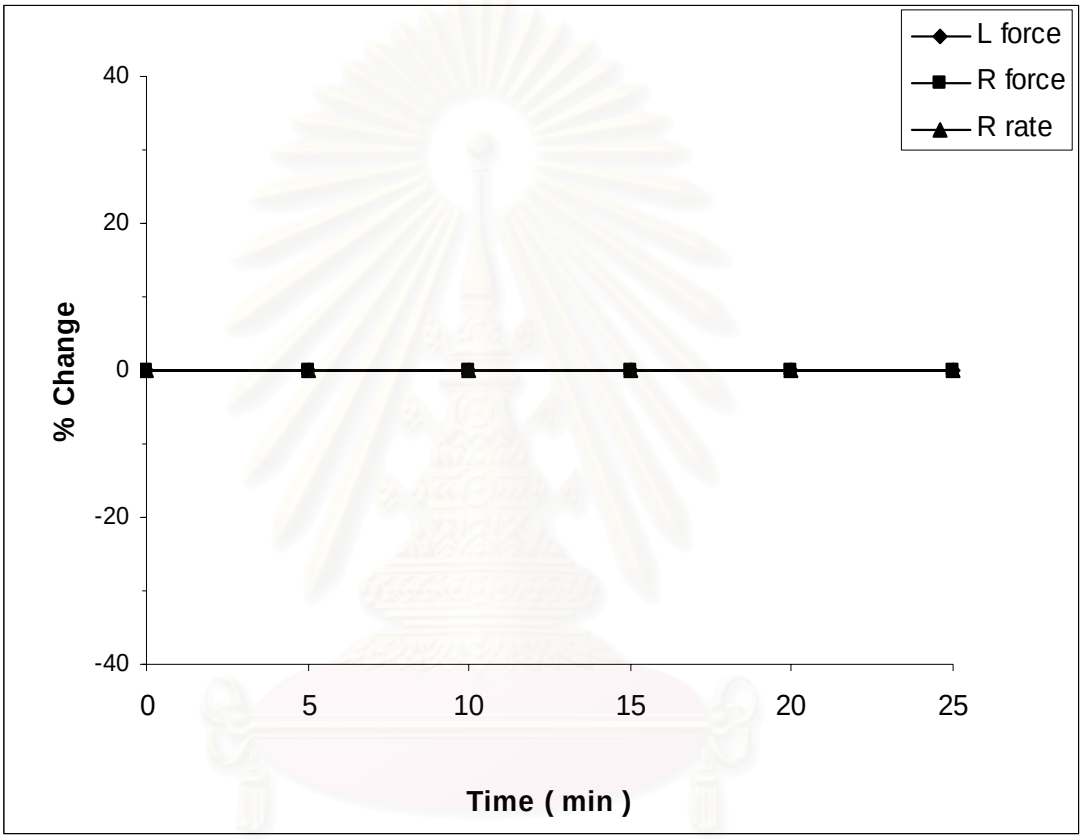
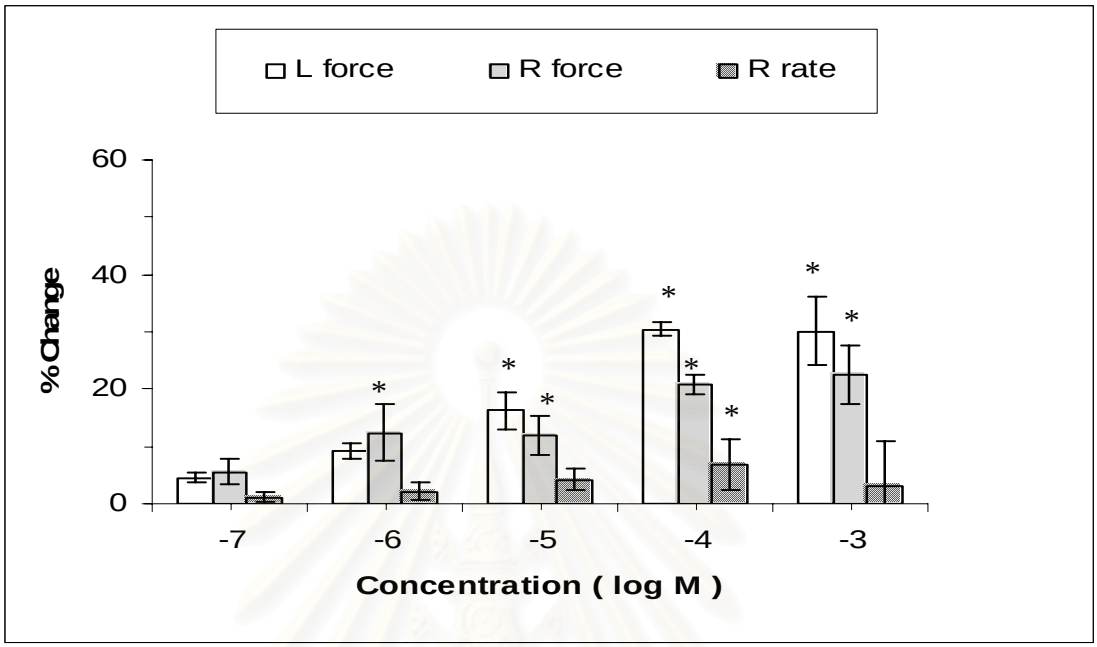


Figure 7 The inotropic and chronotropic response on the right and atria in the presence of DMSO (0.03% V/V) (n=6).

A. Quercetin



B. Naringenin

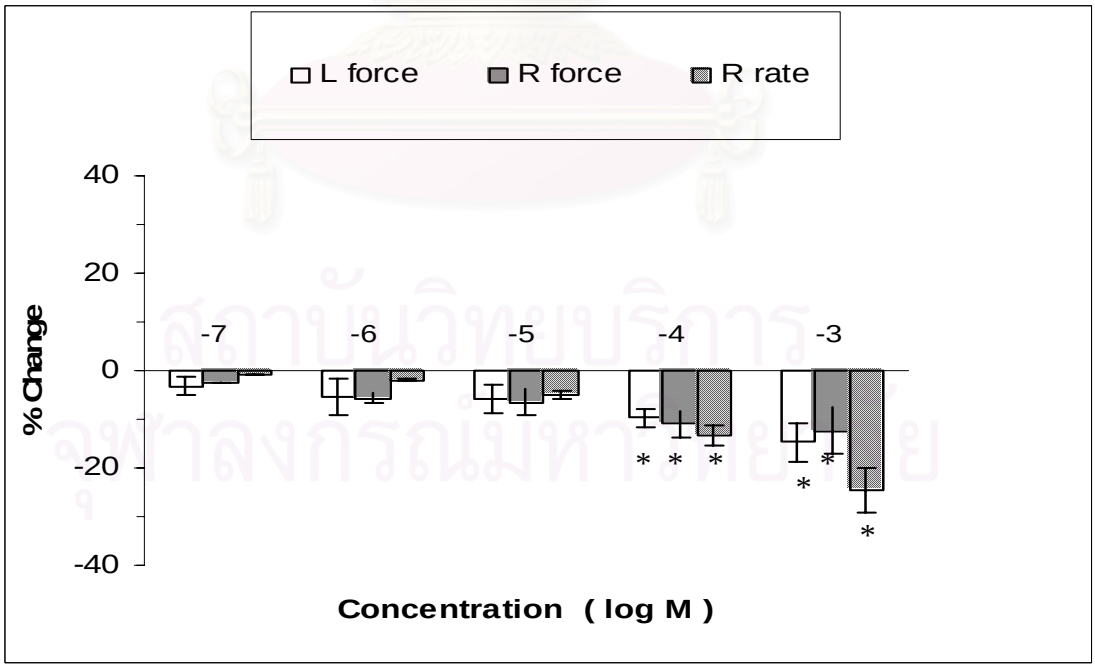


Figure 8 Concentration- response curve of quercetin (A) and naringenin (B) on heart rate (R rate) and contraction of left (L force) and right (R force) atria. n=6, mean ±S.E.M, *$p < 0.05$, significantly different from DMSO group (unpaired *t* - test).

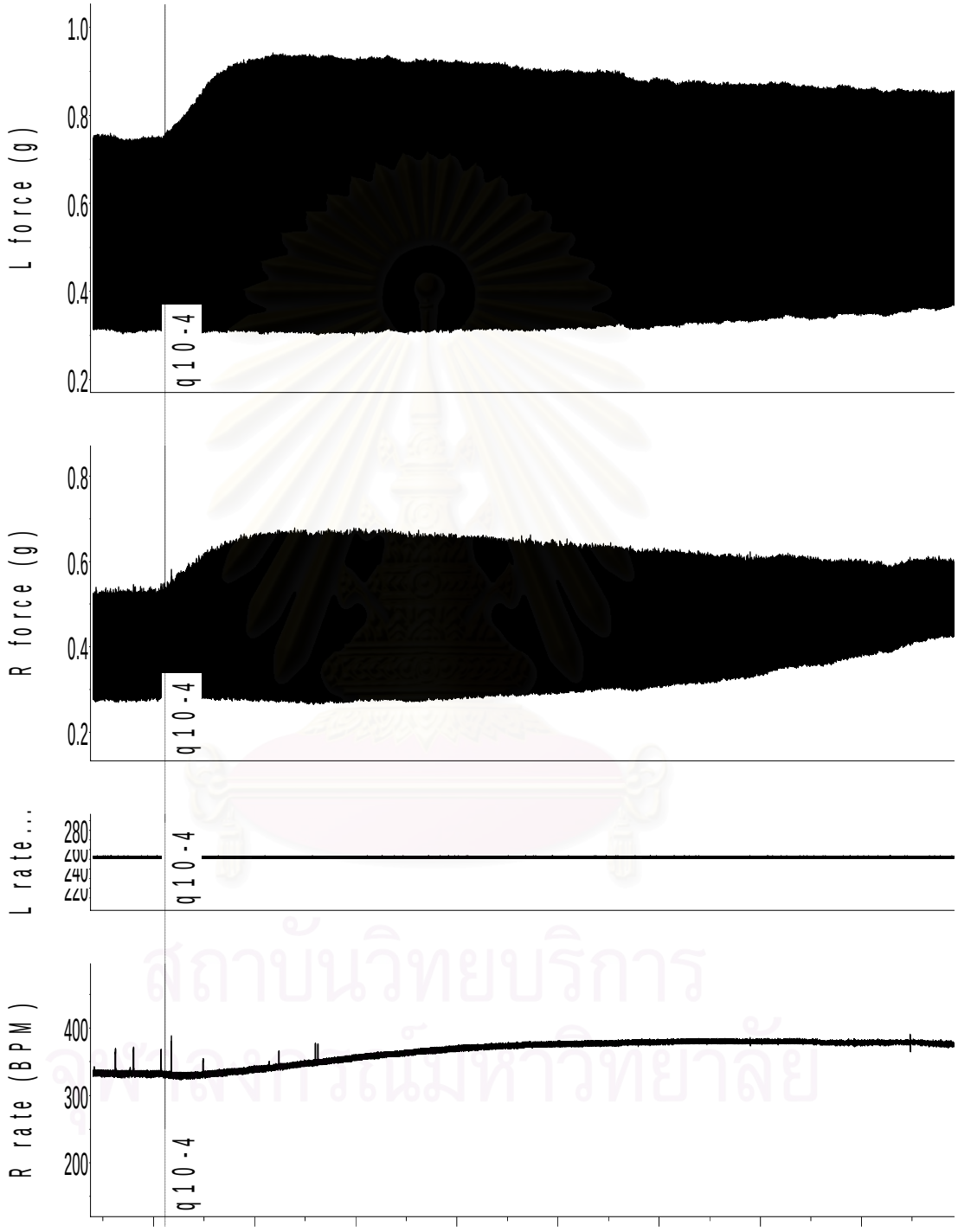


Figure 9 The inotropic and chronotropic response on the right and left atria in the presence of quercetin (100 μM).

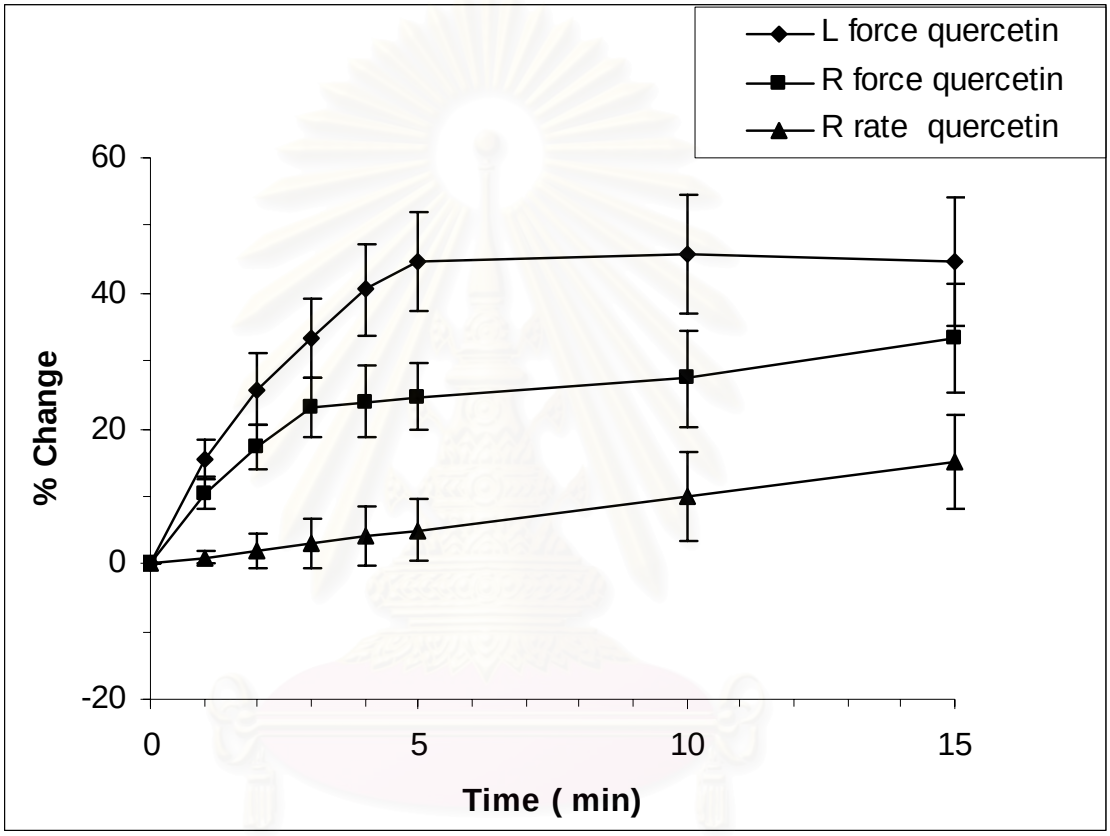


Figure 10 The inotropic and chronotropic response on the right and left atria in the presence of quercetin (100 μM), n=6, mean ± SEM.

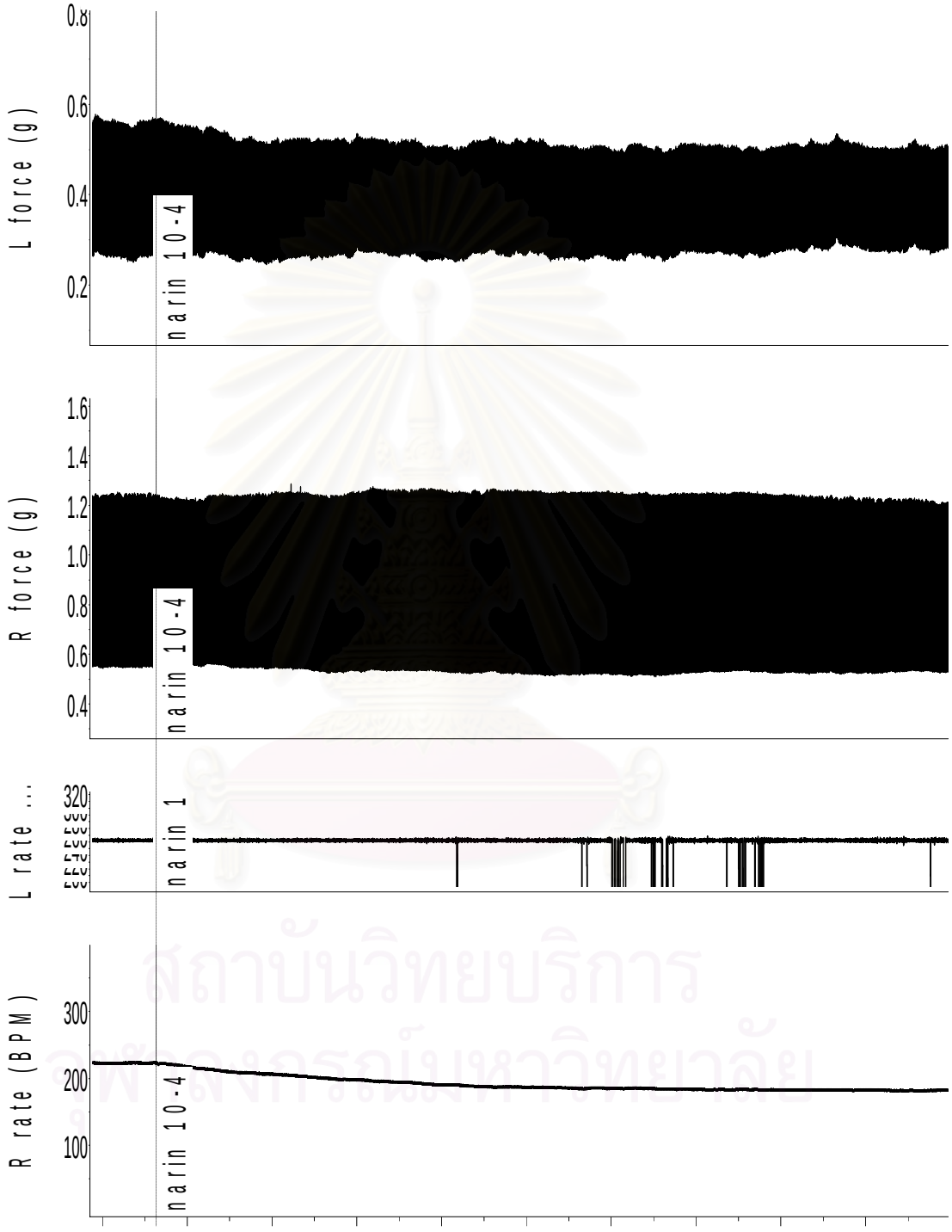


Figure 11 The inotropic and chronotropic response on the right and left atria in the presence of naringenin (100 μM)

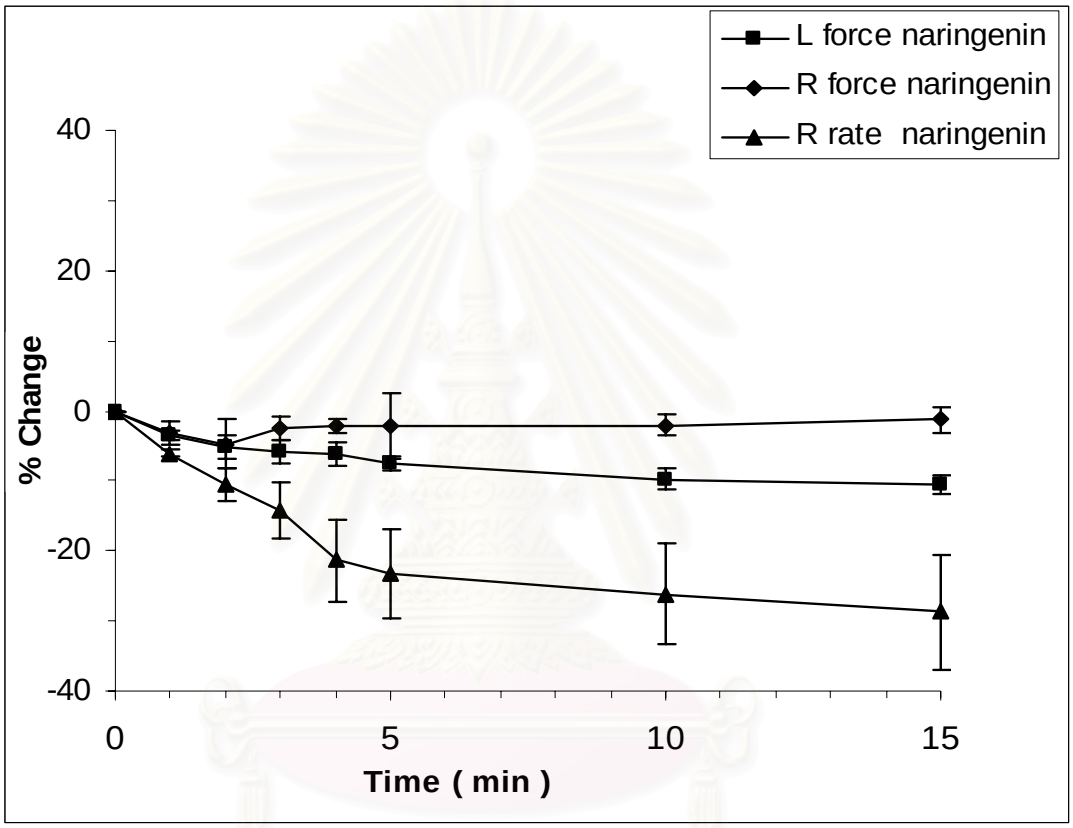


Figure12 The inotropic and chronotropic response on the right and left atria in the presence of naringenin (100 μM), n=6, mean ± SEM.

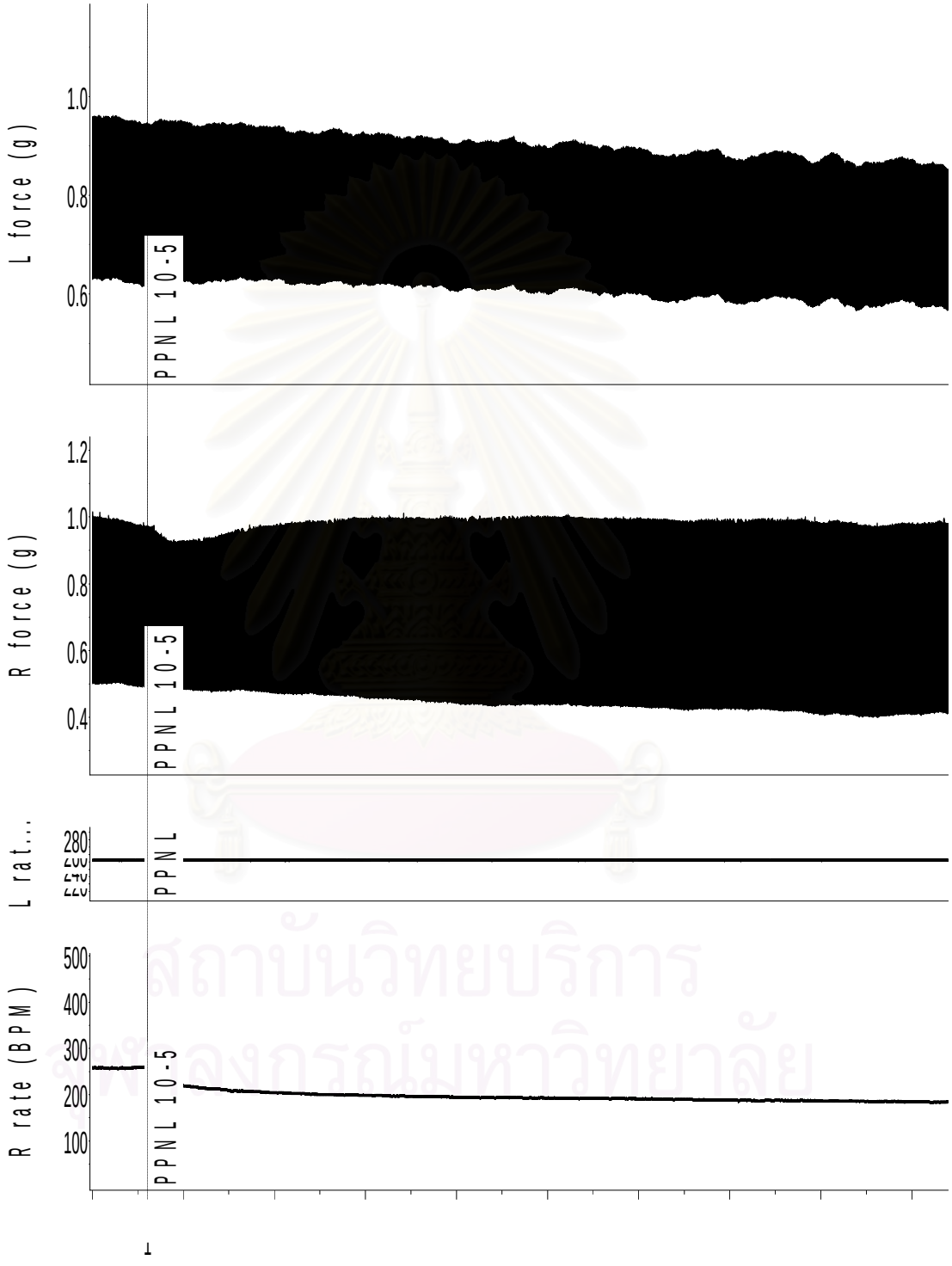


Figure 13 The inotropic and chronotropic response on the right and left atria in the presence of propranolol (10 μM).

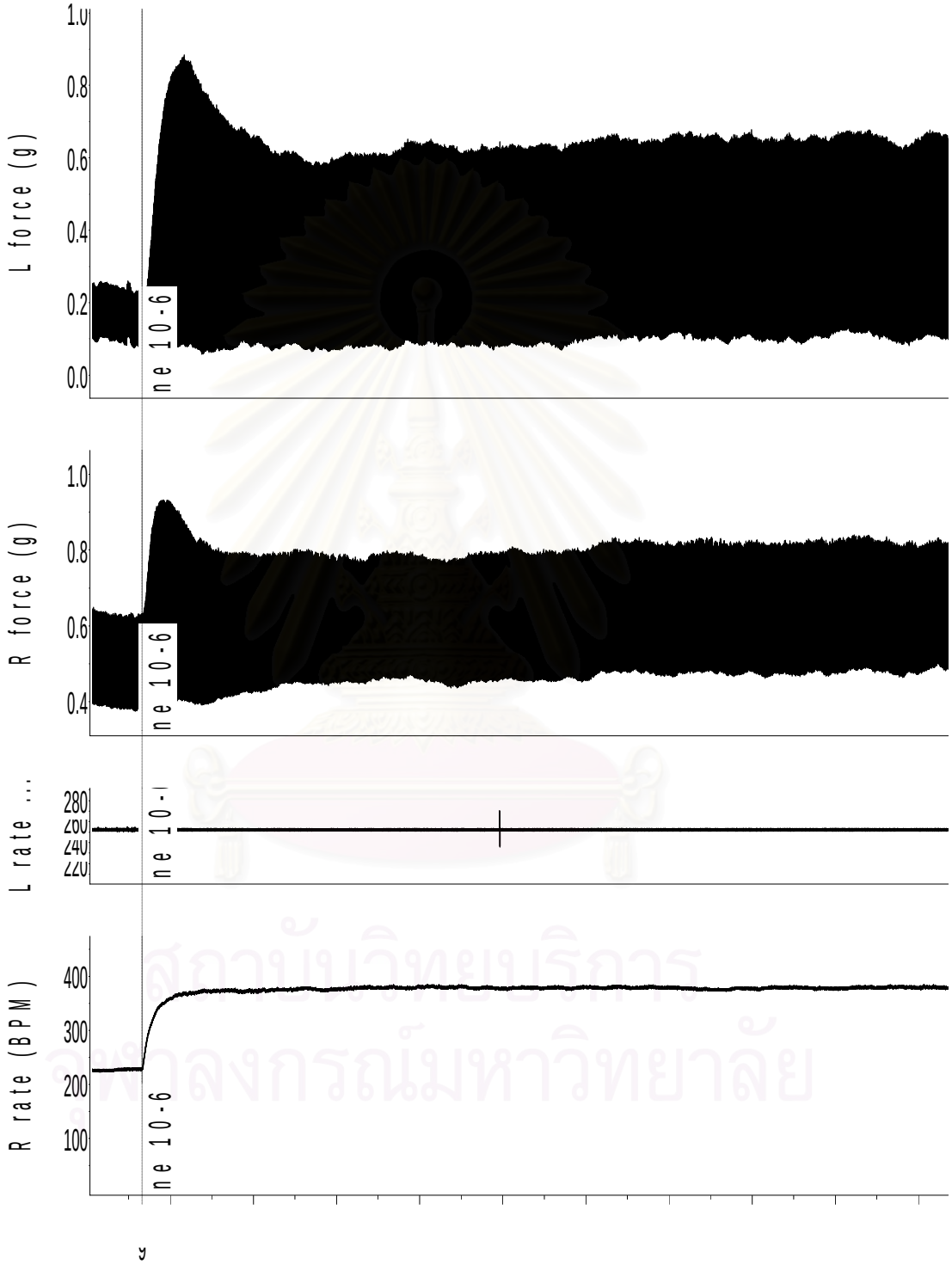


Figure 14 The inotropic and chronotropic response on the right and left atria in the presence of NE (1 μM).

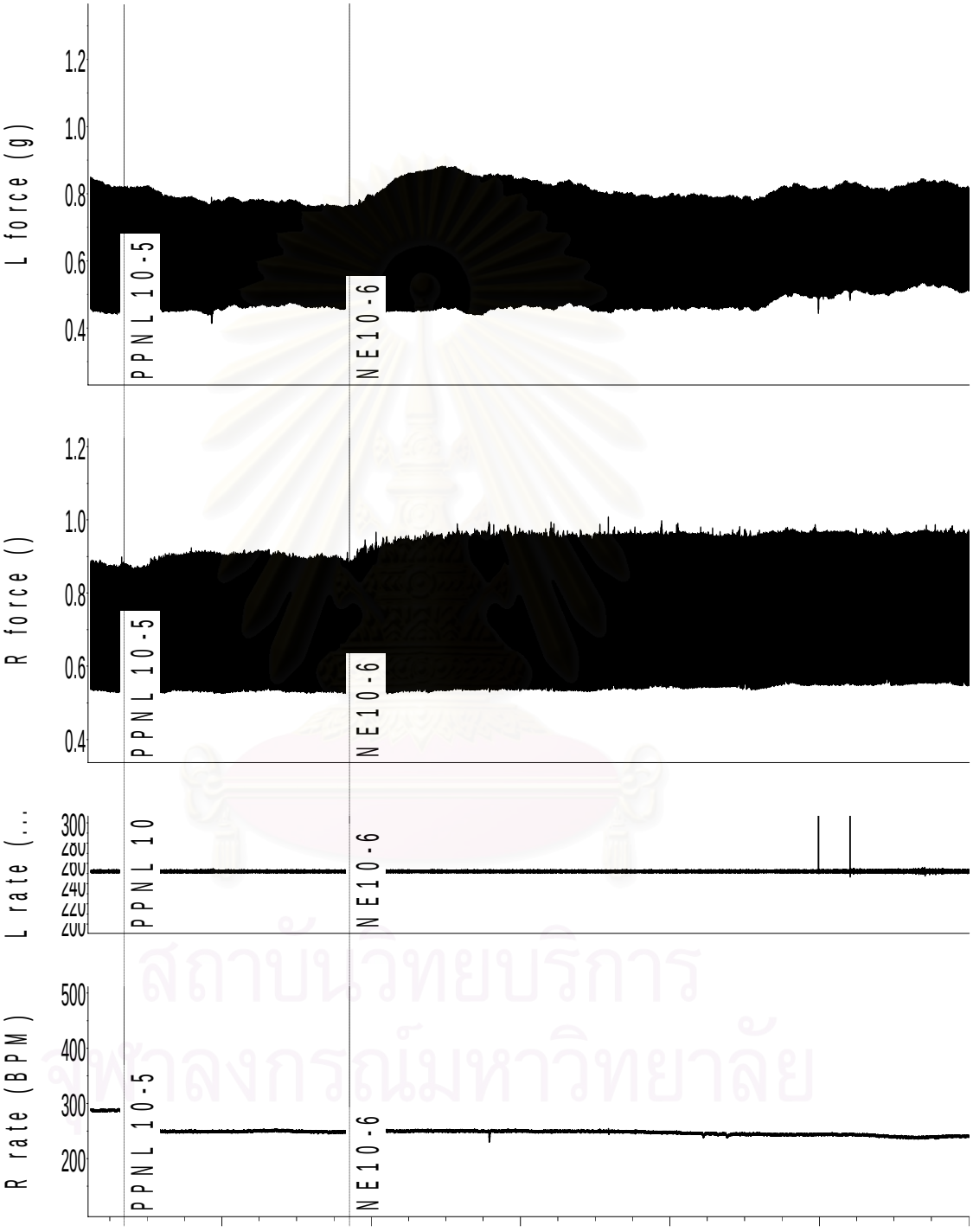
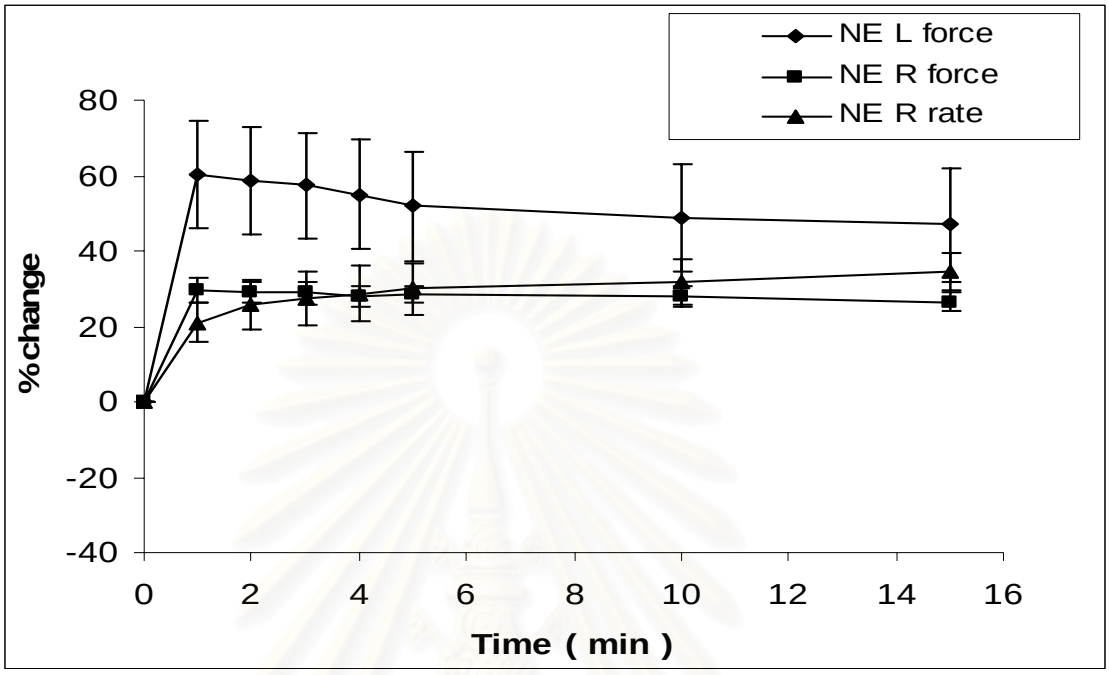


Figure 15 The inotropic and chronotropic response of NE (1 μM) on the right and left atria in the presence of propranolol (10 μM).

A. Norepinephine.



B. Propranolol.

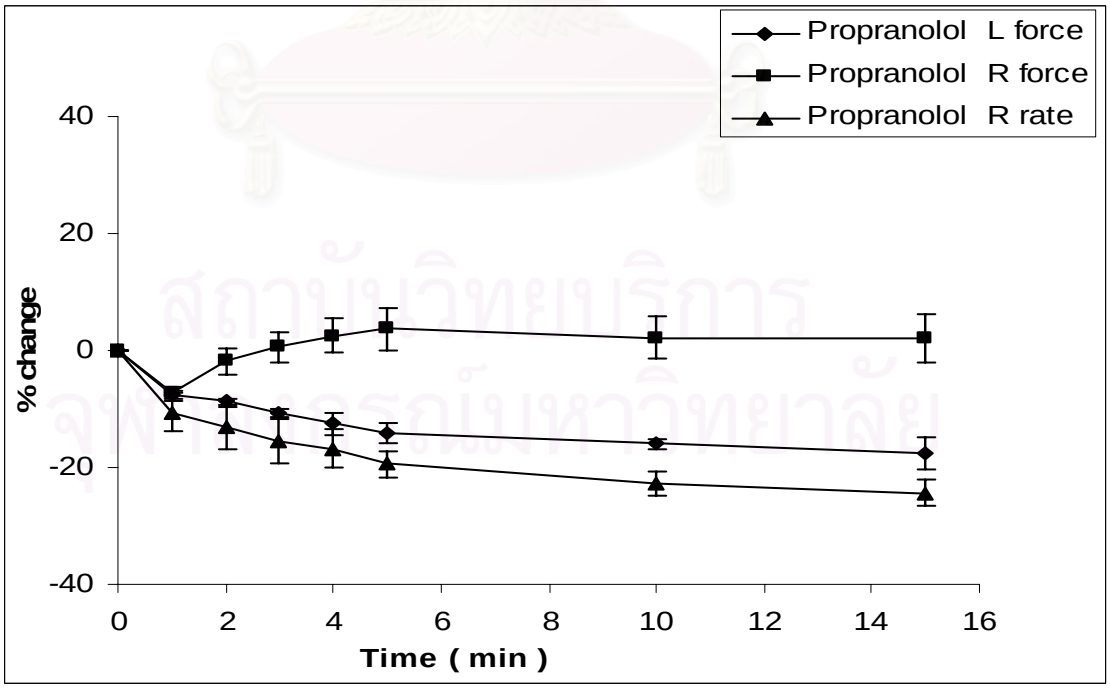
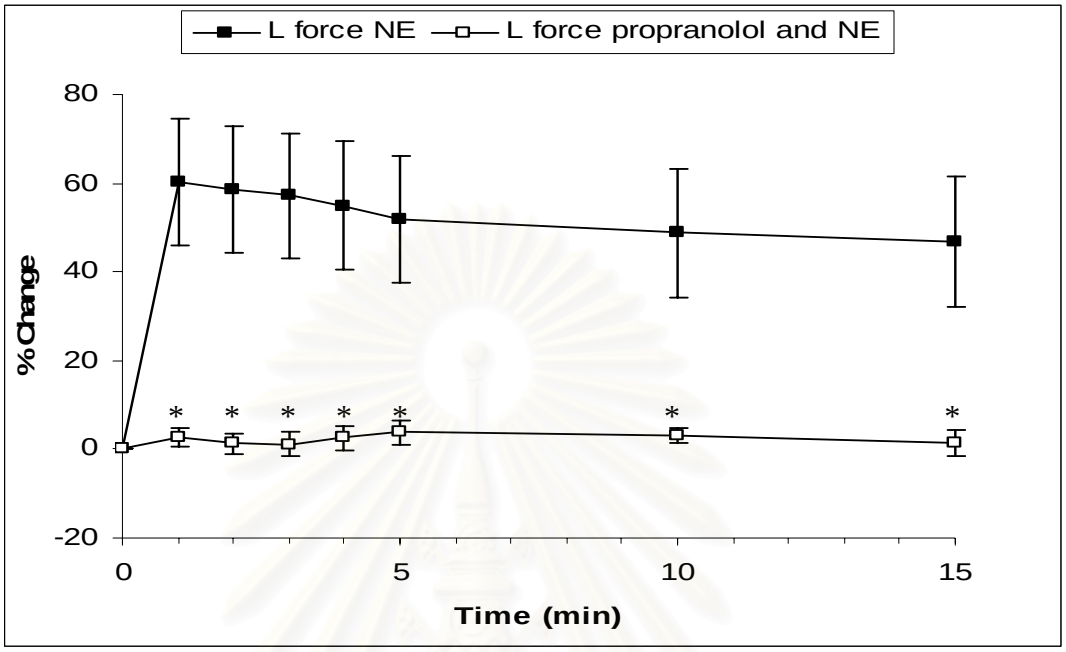


Figure 16 The inotropic and chronotropic response of NE (1 μM) (A) and propranolol (10 μM) (B)on the right and left atria, n=6, mean ± SEM.

A. Left atria



B. Right atria.

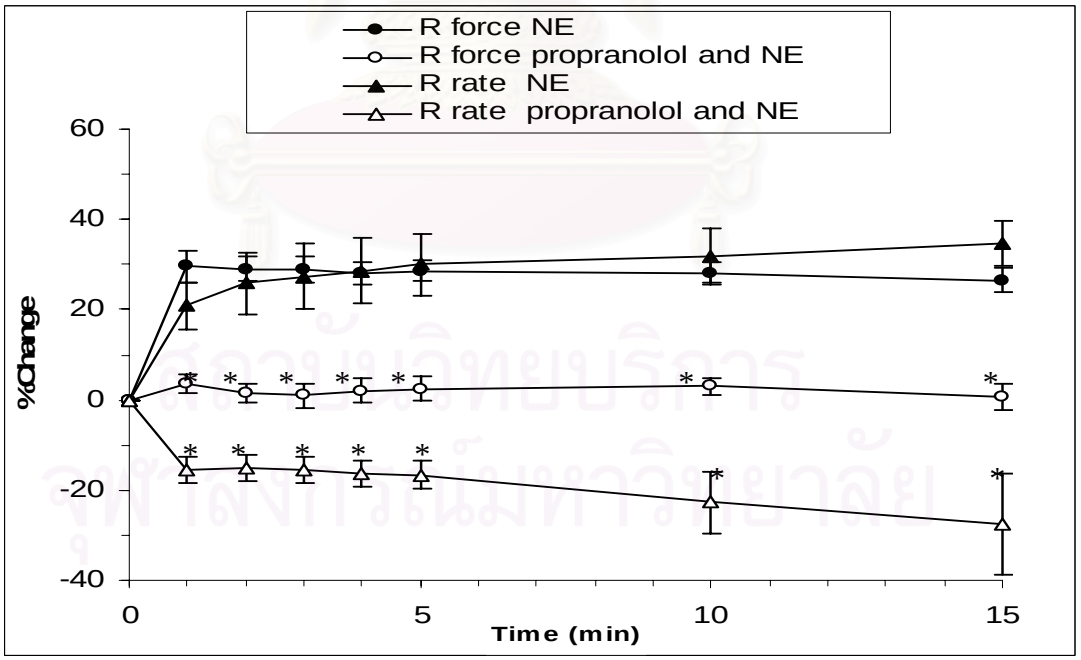


Figure 17 The inotropic and chronotropic response of NE (1 μM) on left (A) and right (B) atria in the absence and presence of propranolol (10 μM), n=6, mean ± S.E.M * $p<0.05$, significantly different from NE group in the absence of propranolol (paired *t* - test).

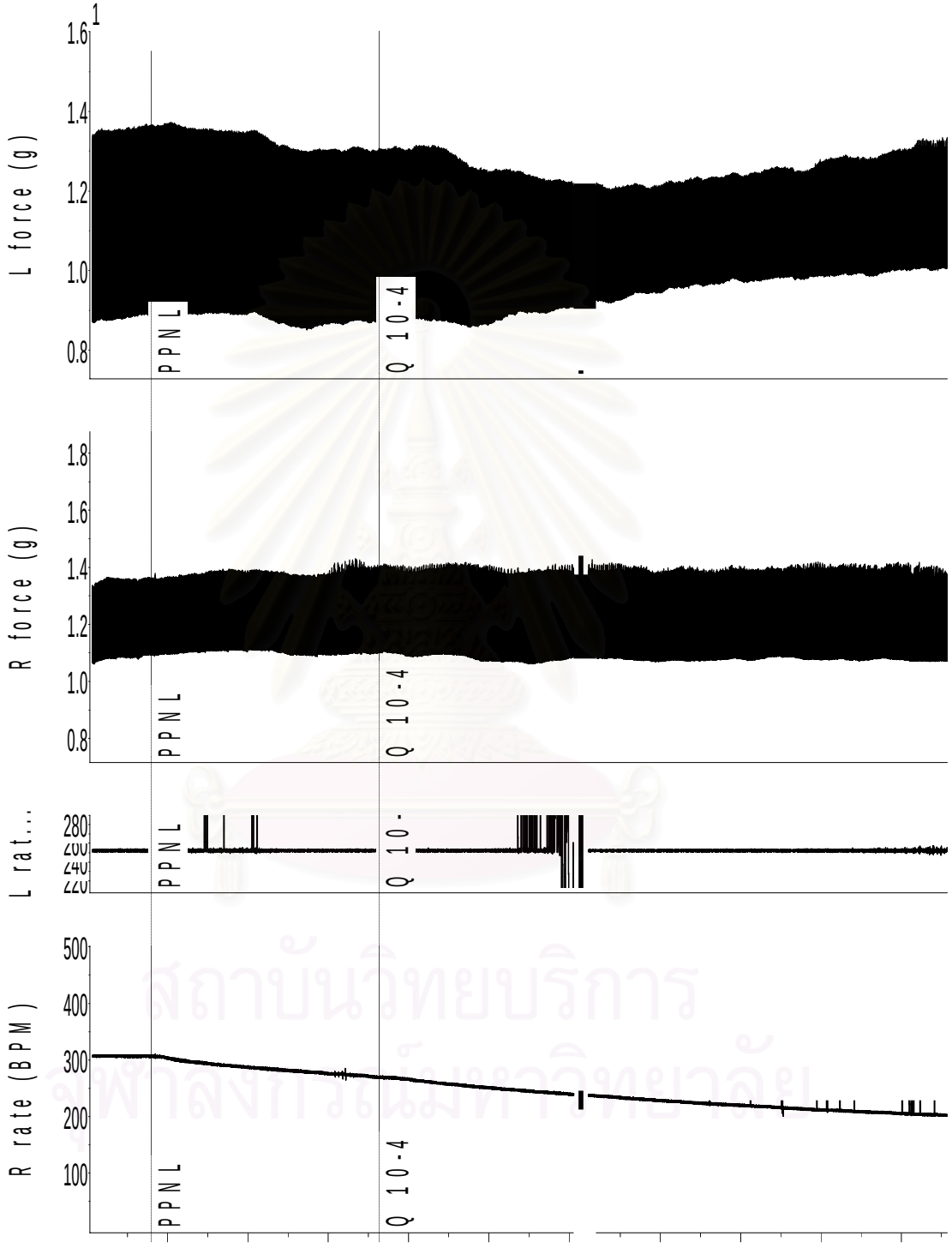
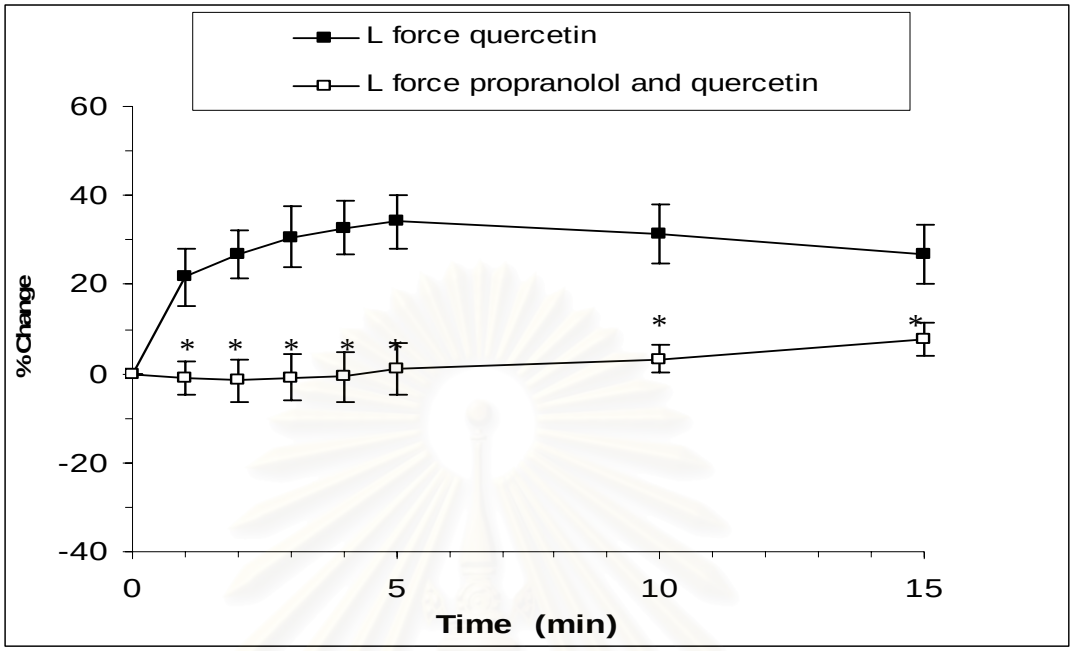


Figure 18 The inotropic and chronotropic response of quercetin (100 μM) on left and right atria in the presence of propranolol (10 μM).

A. Left atria.



A. Right atria.

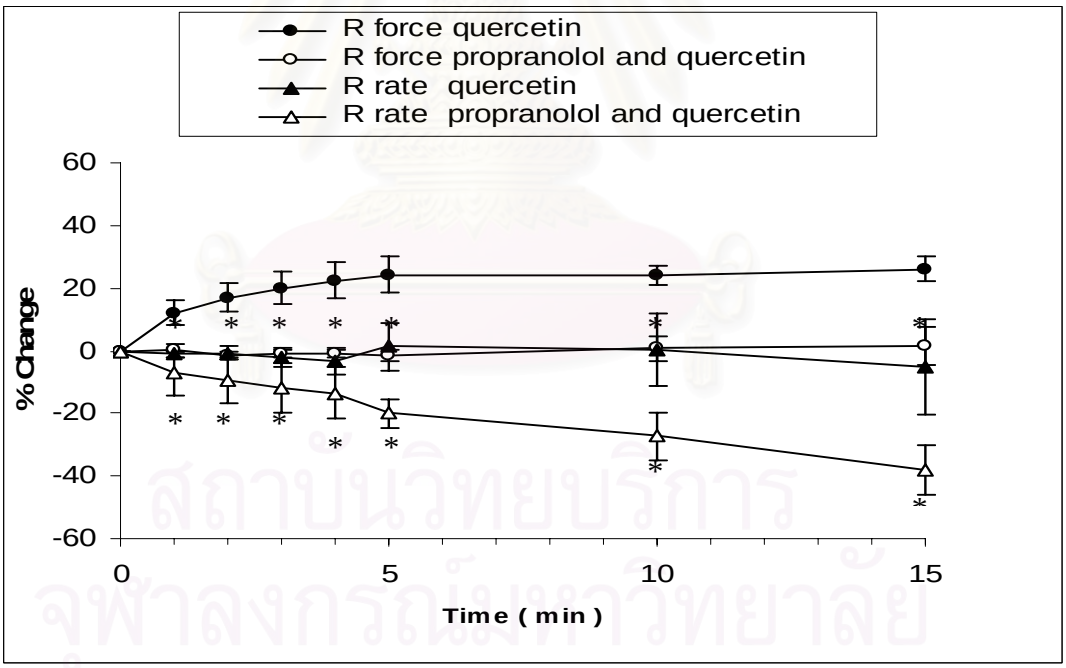


Figure 19 The inotropic and chronotropic response of quercetin (100 μM) on left (A) and right (B) atria in the absence and presence of propranolol (10 μM), n=6, mean ± S.E.M *$p < 0.05$, significantly different from quercetin group in the absence of propranolol (paired *t* - test).

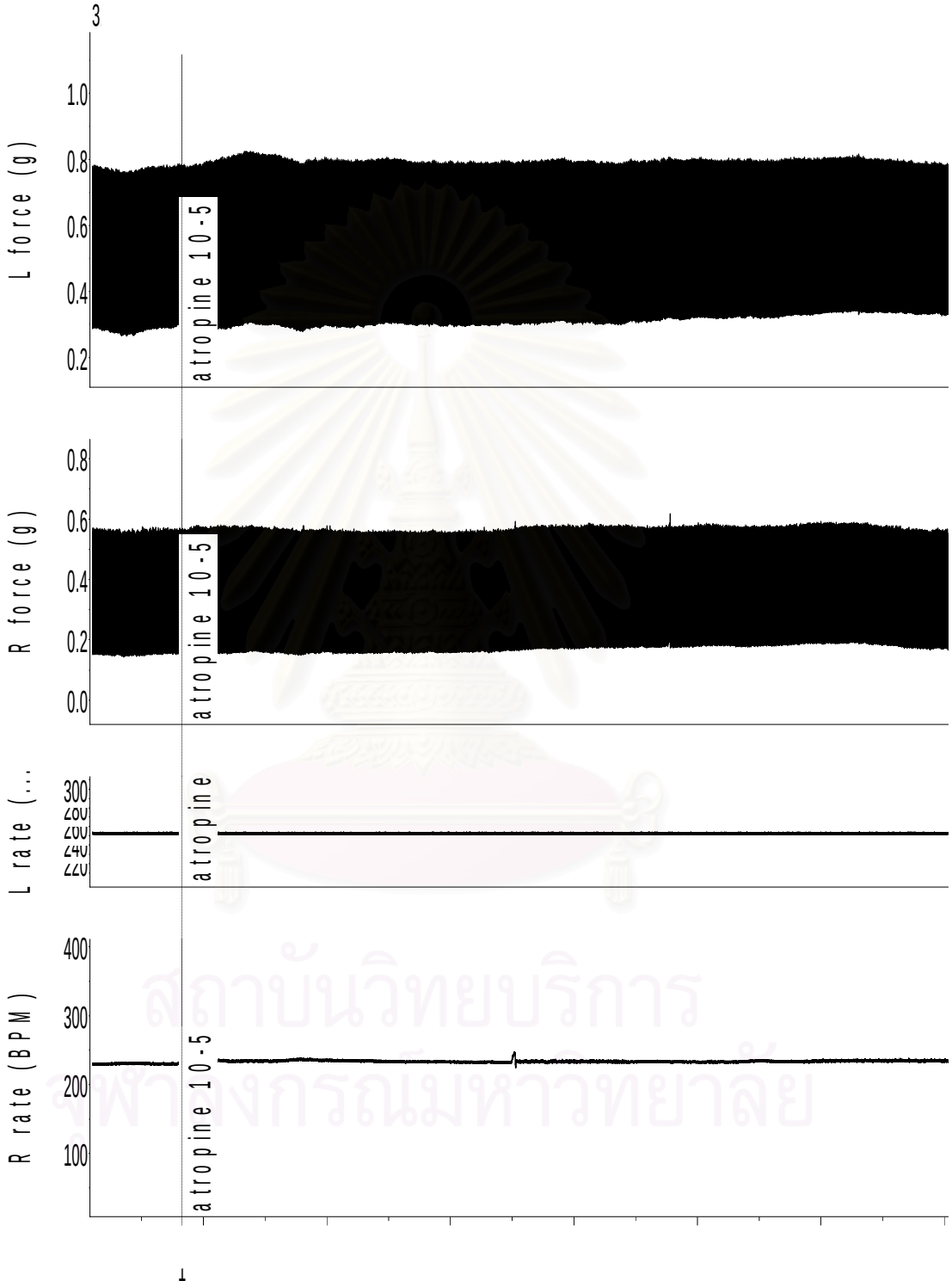


Figure 20 The inotropic and chronotropic response on the right and left atria in the presence of atropine (10 μM).

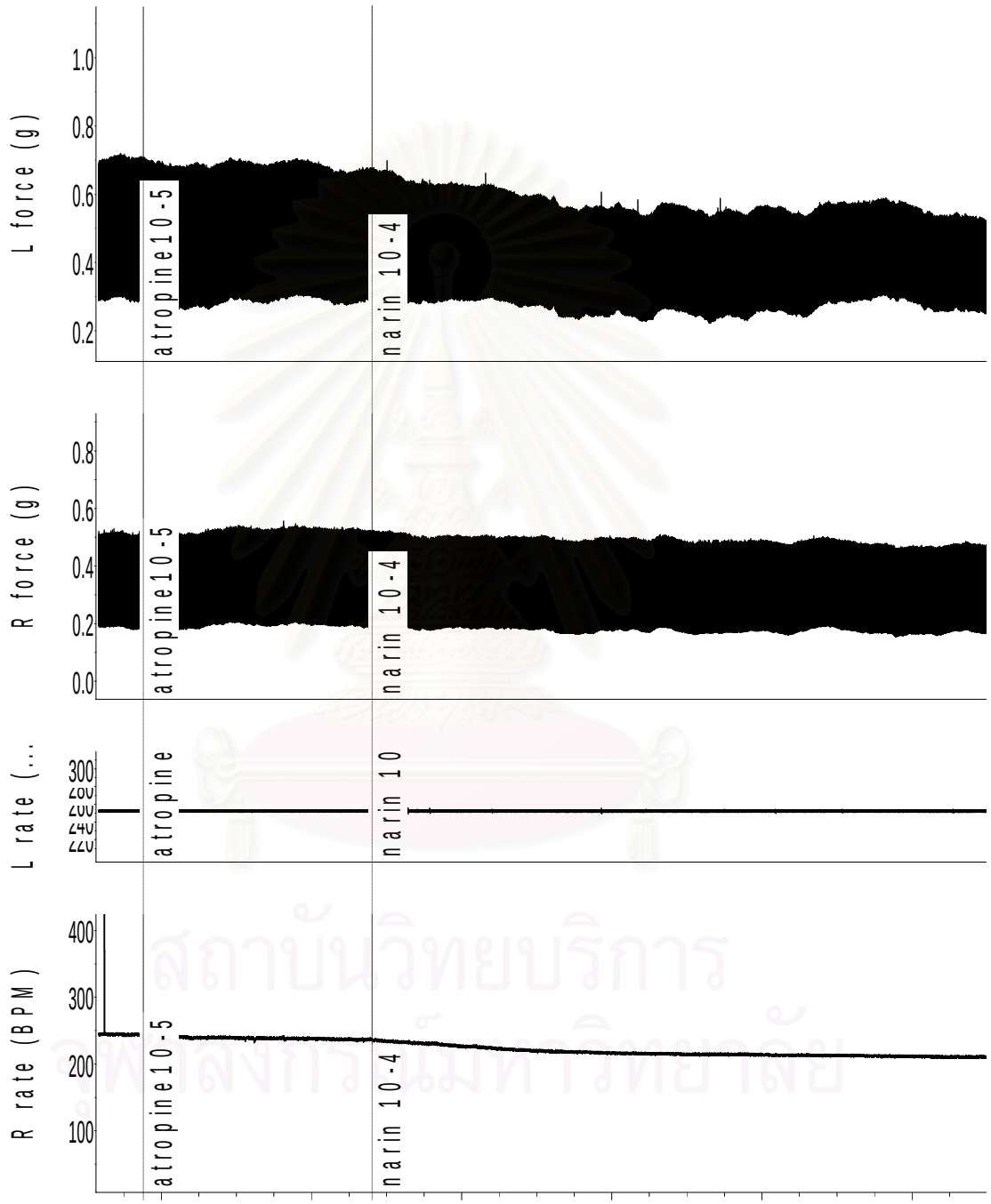


Figure 21 The inotropic and chronotropic response of naringenin on the right and left atria in the presence of atropine (10 μM).

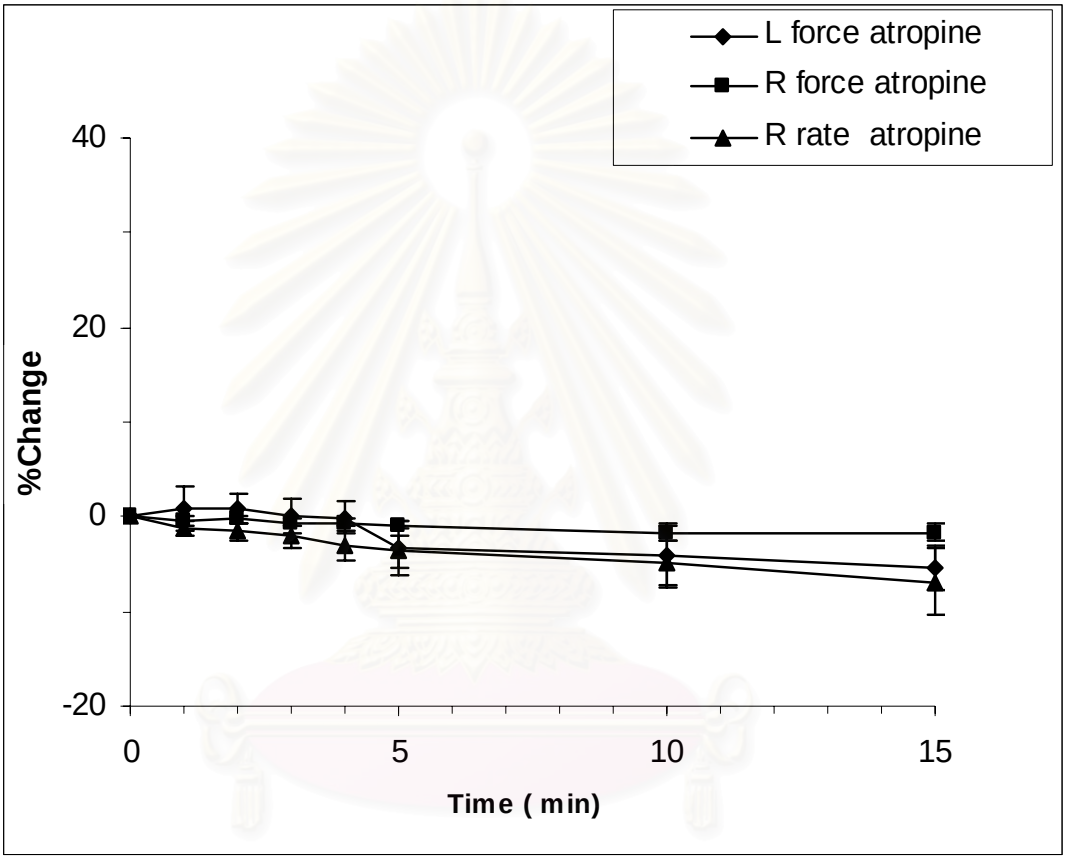
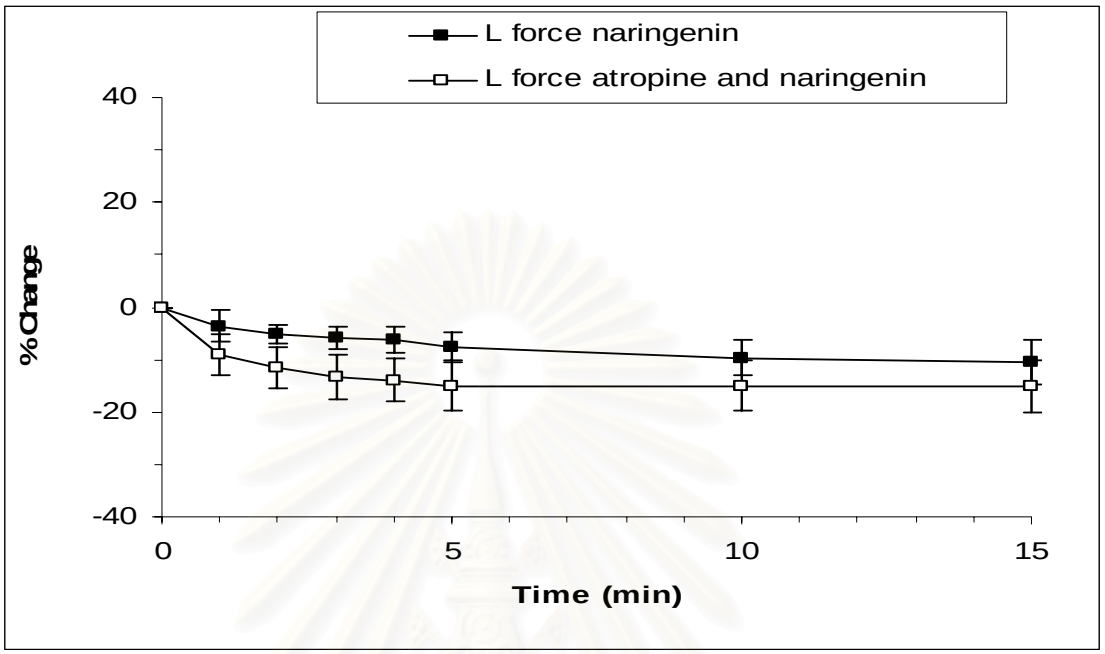


Figure 22 The inotropic and chronotropic response of atropine (10 μM) on the right and left atria, n=6.mean ± SEM.

A. Left atria.



B. Right atria.

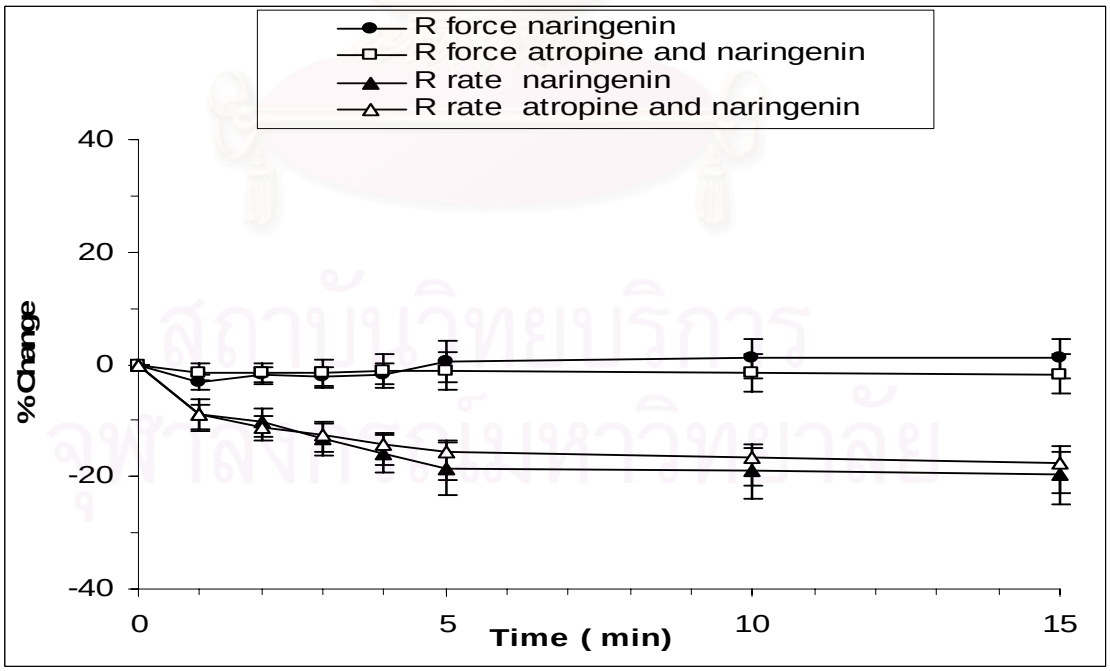


Figure 23 The inotropic and chronotropic response of naringenin (100 μM) on left (A) and right (B) atria in the absence and presence of atropine (10 μM), n=6, mean ± S.E.M.

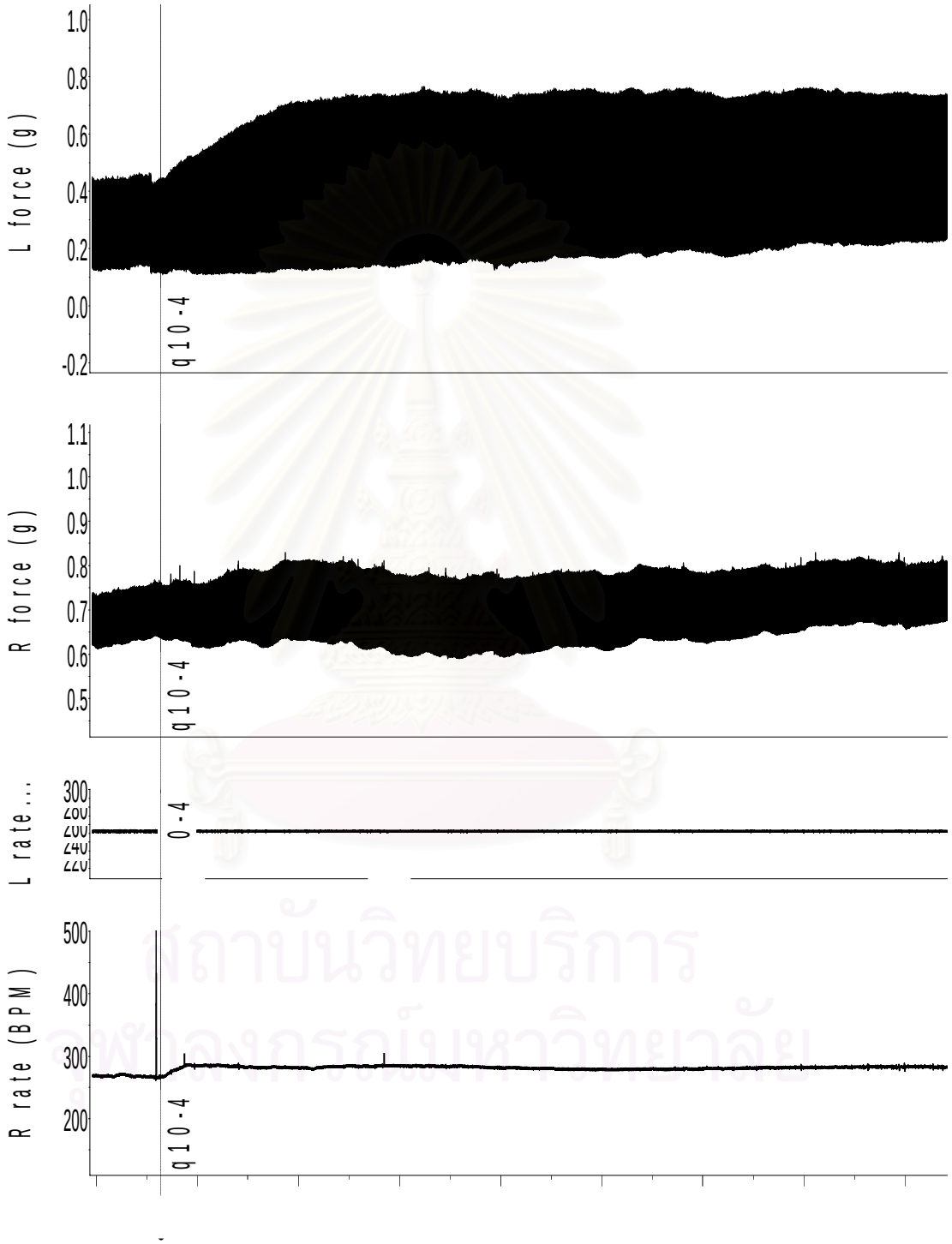


Figure 24 The inotropic and chronotropic response on the right and left atria in the presence of quercetin (100 μM).

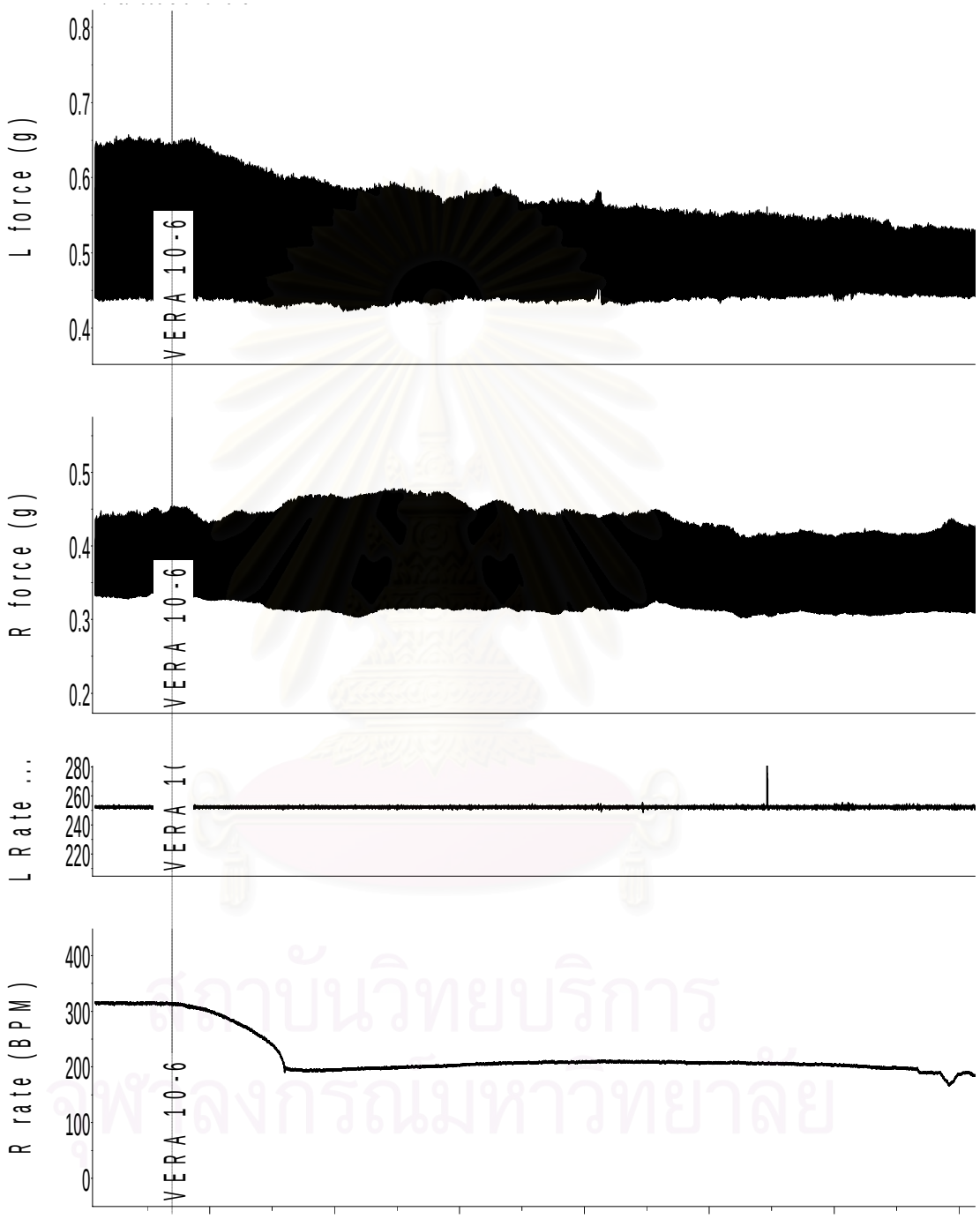


Figure 25 The inotropic and chronotropic response on the right and left atria in the presence of verapamil (1 μM).

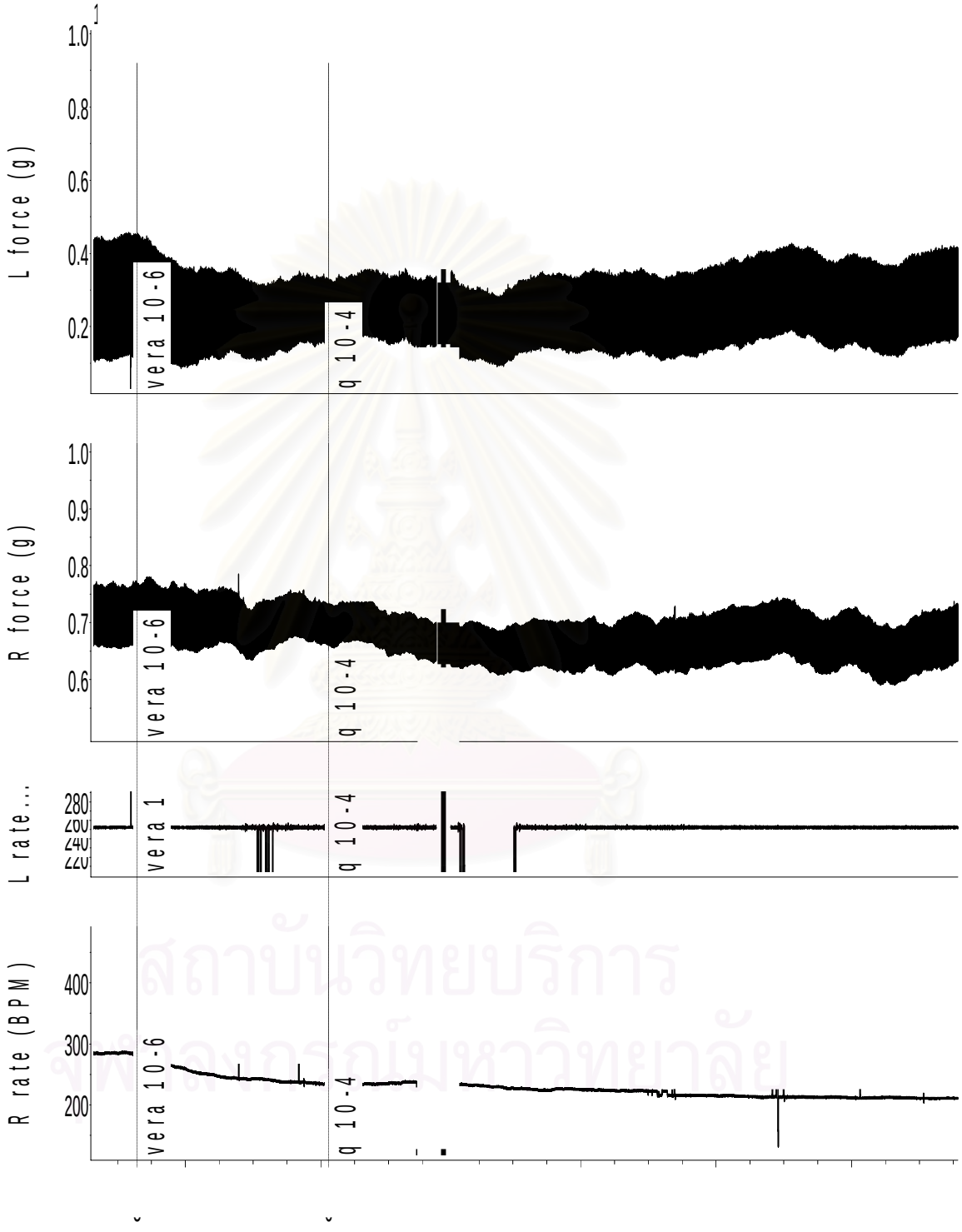


Figure 26 The inotropic and chronotropic response of quercetin (100 μM) on right and left atria in the presence of verapamil (1 μM).

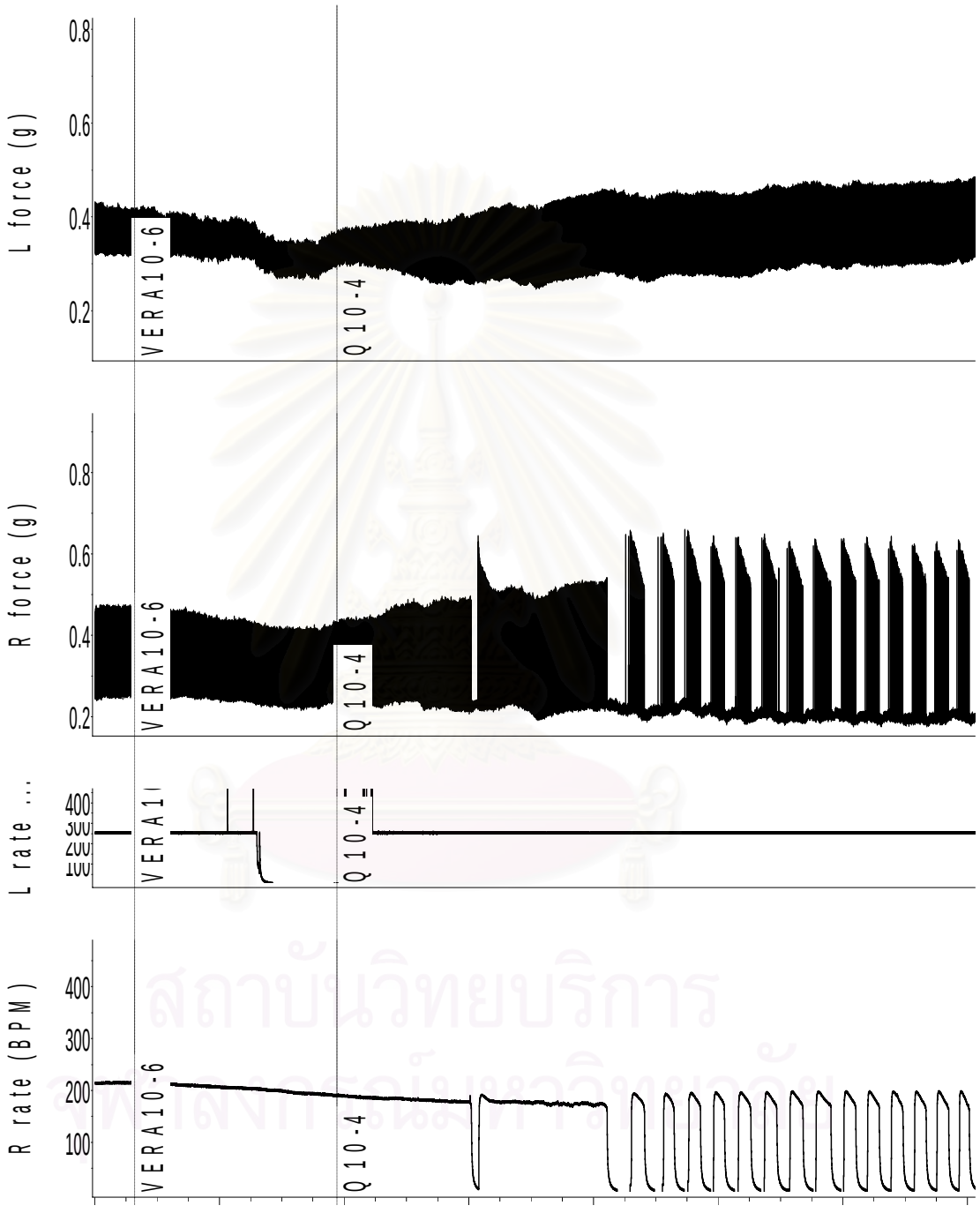
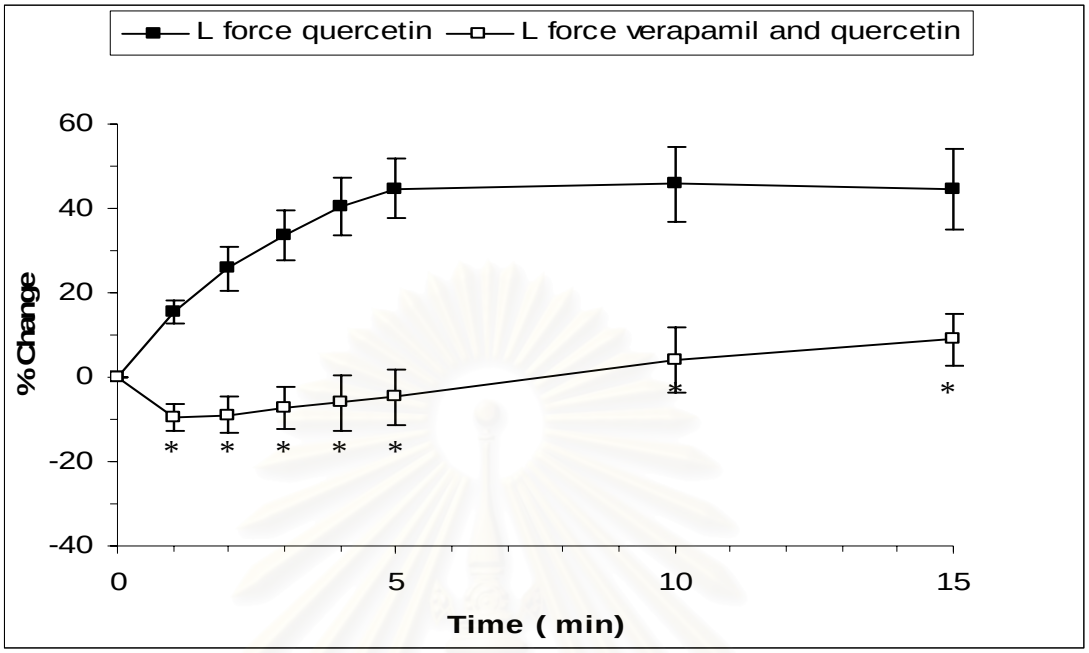


Figure 27 The inotropic and chronotropic response of quercetin (100 μM) on left and right atria in the presence of verapamil (1 μM).

A. Left atria.



B. Right atria.

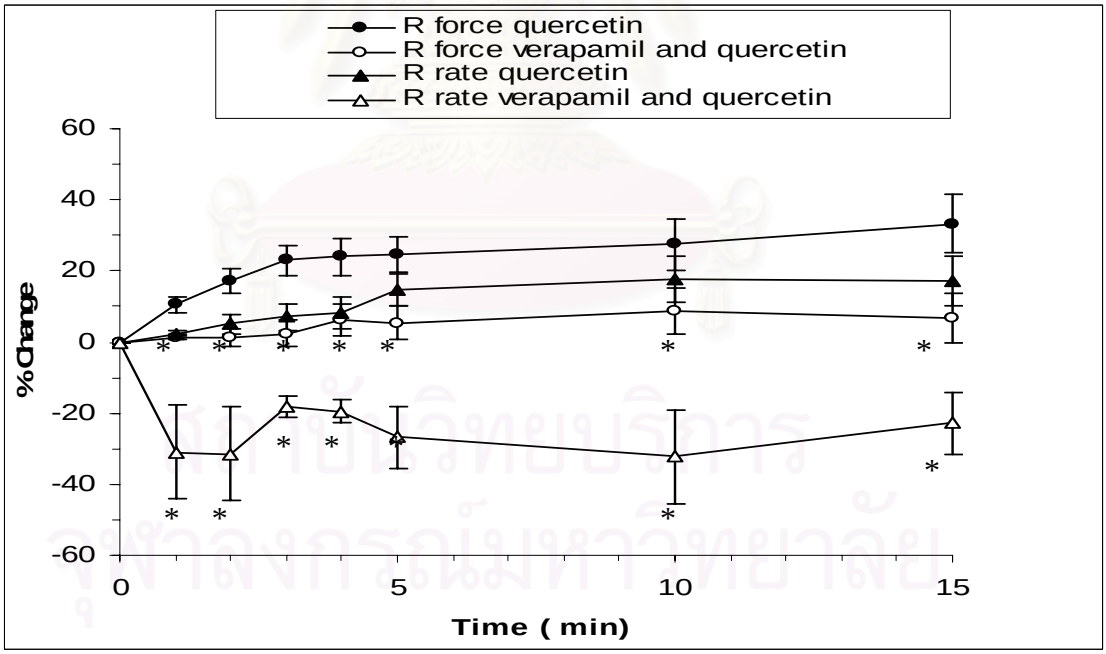


Figure 28 The inotropic and chronotropic response of quercetin (100 μM) on left (A) and right (B) atria in the absence and presence of verapamil (1 μM), n=6, mean ± S.E.M, * $p<0.05$, significantly different from quercetin group in the absence of verapamil (paired *t* - test).

A. Control

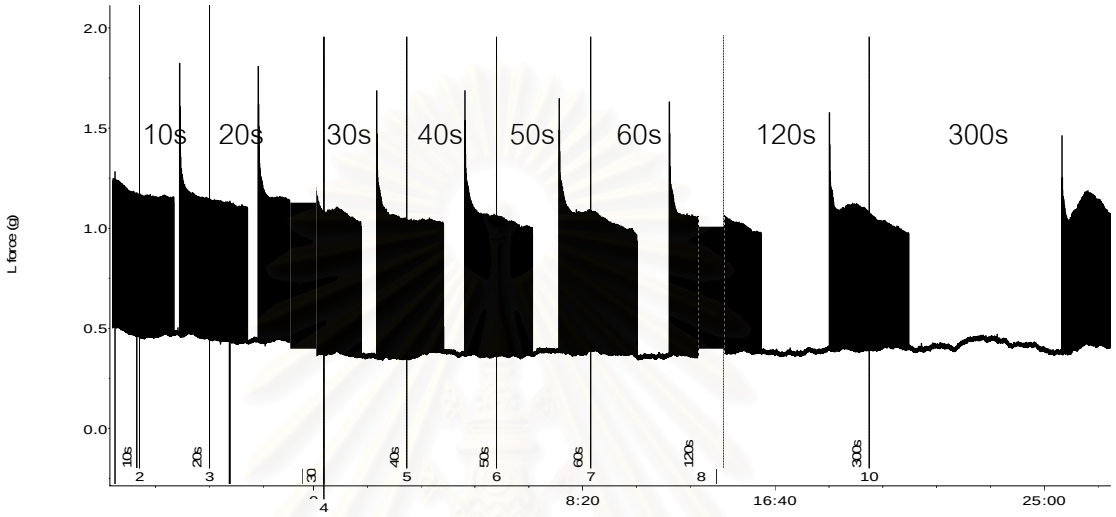


Figure 29A A representative example on inotropic response of control on the rest interval of the range10 to 300 seconds.

B. Caffeine

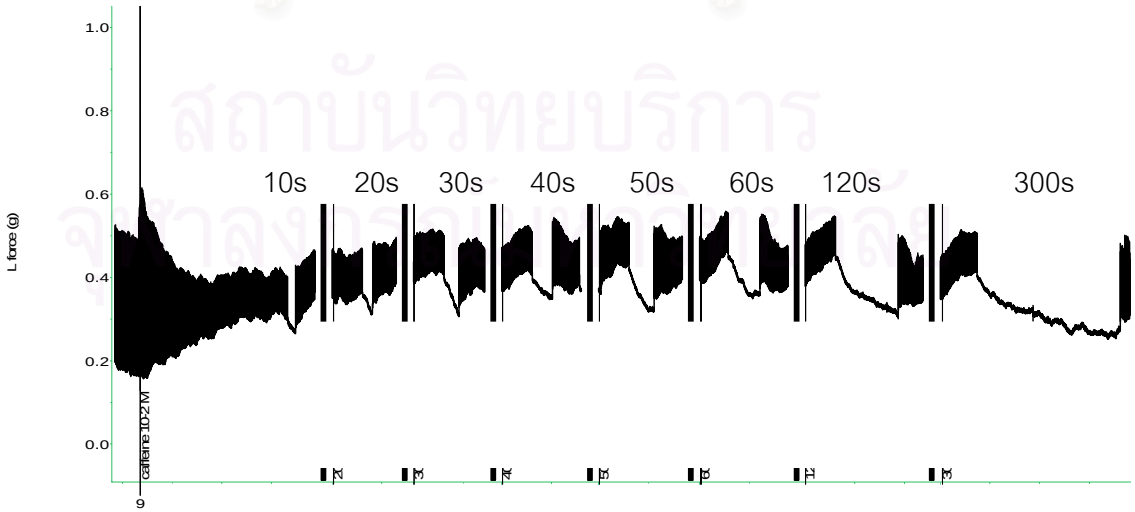


Figure 29B A representative example on inotropic response of caffeine on the rest interval of the range10 to 300 seconds.

C. Quercetin.

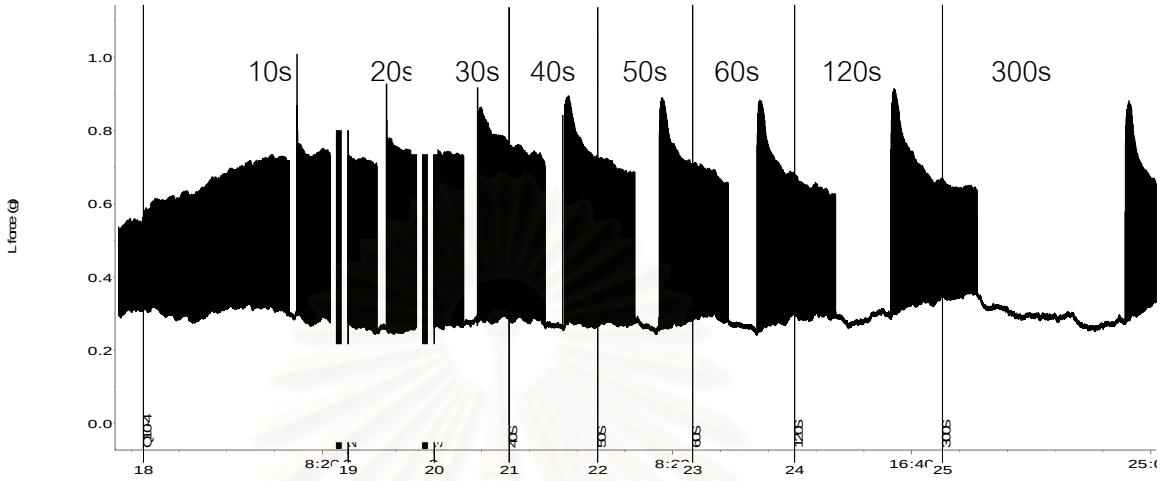


Figure 29C A representative example of inotropic response of quercetin on the rest interval of the range10 to 300 seconds.

D. Naringenin

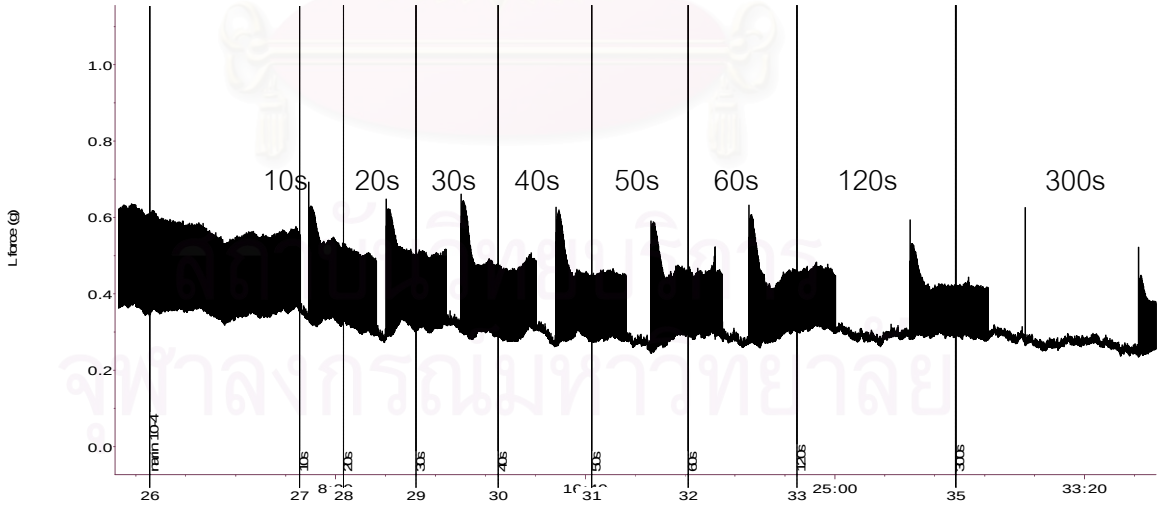


Figure 29D A representative example of inotropic response of quercetin (C) and naringenin (D) on the rest interval of the range10 to 300 seconds.

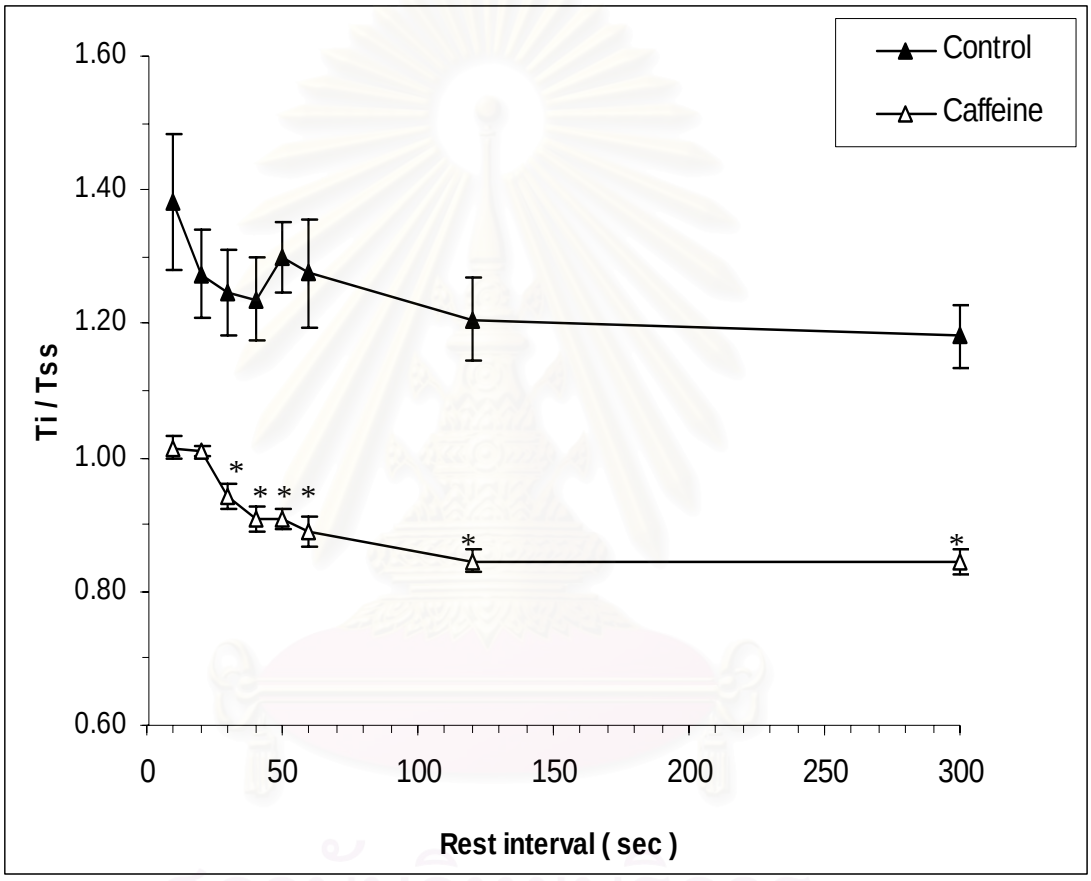


Figure 30 Effect of caffeine on the relationships between the Ti / Tss and the rest interval of range 10 to 300 seconds. n=6, mean ± S.E.M.

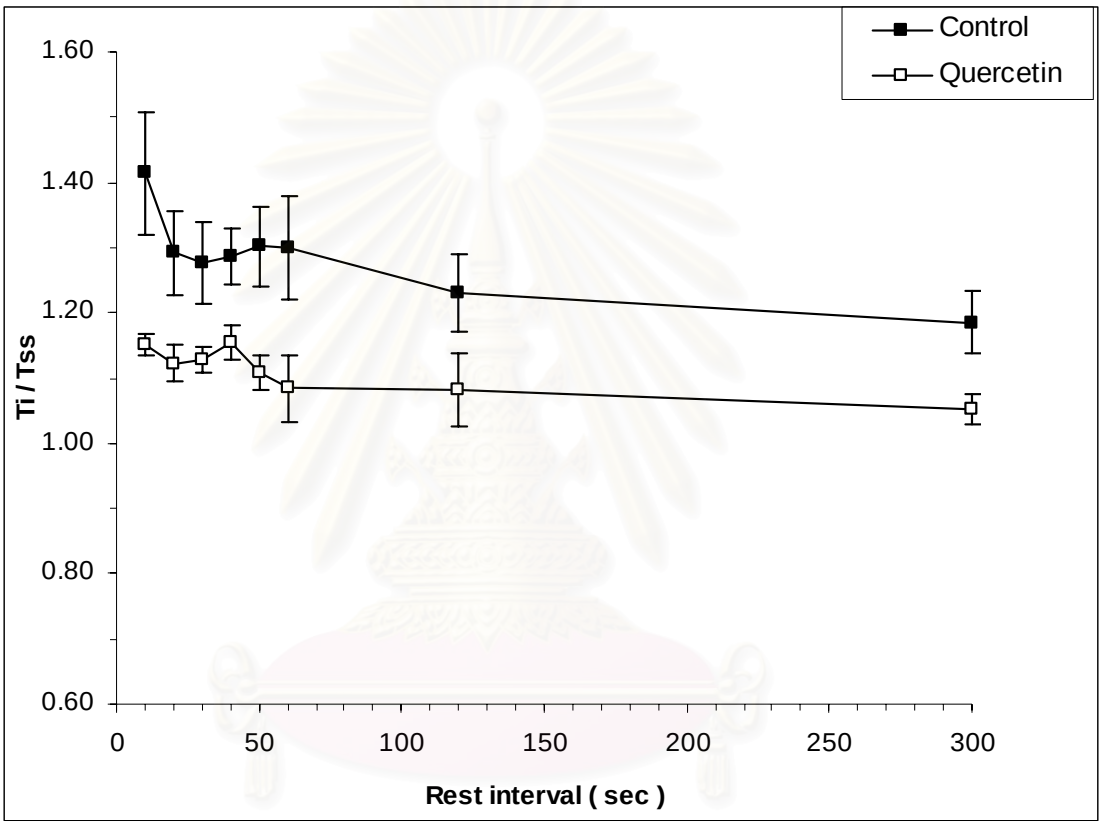


Figure 31 Effect of quercetin on the relationships between the Ti / Tss and the rest interval of range 10 to 300 seconds. n=6, mean ± S.E.M.

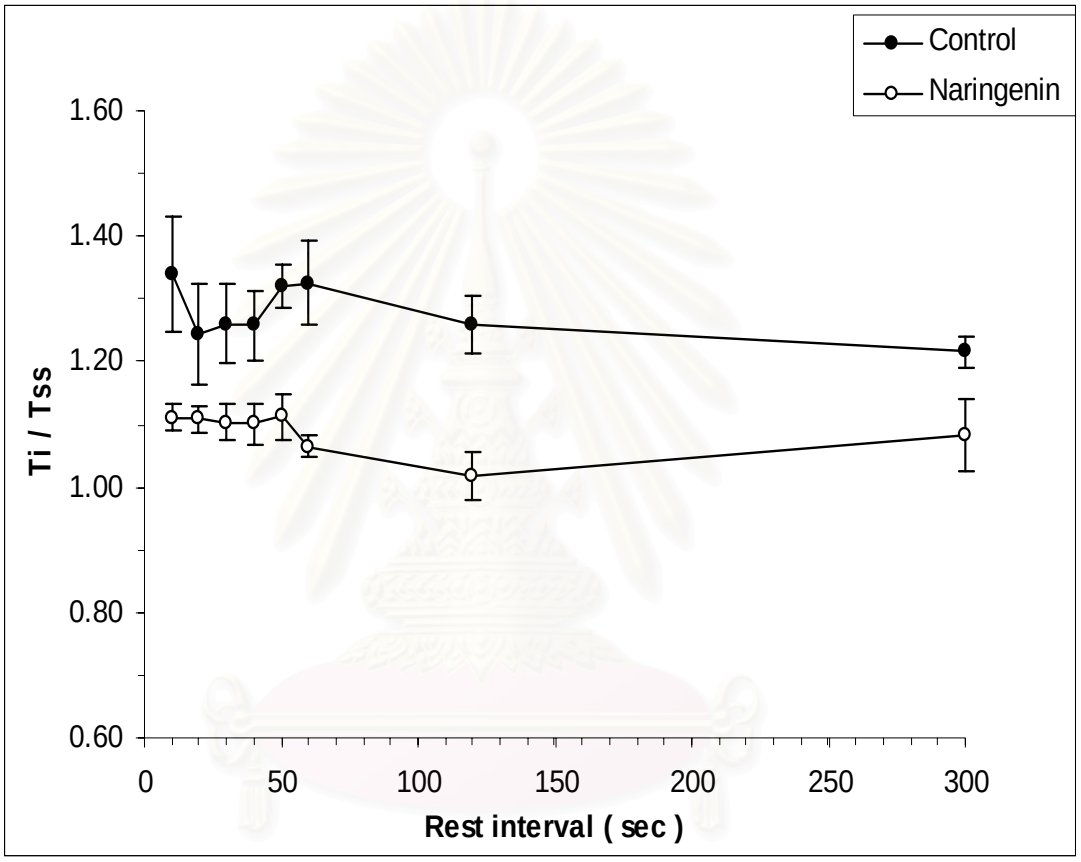


Figure 32 Effect of naringenin on the relationships between the Ti / Tss and the rest interval of range 10 to 300 seconds. n=6, mean ± S.E.M.

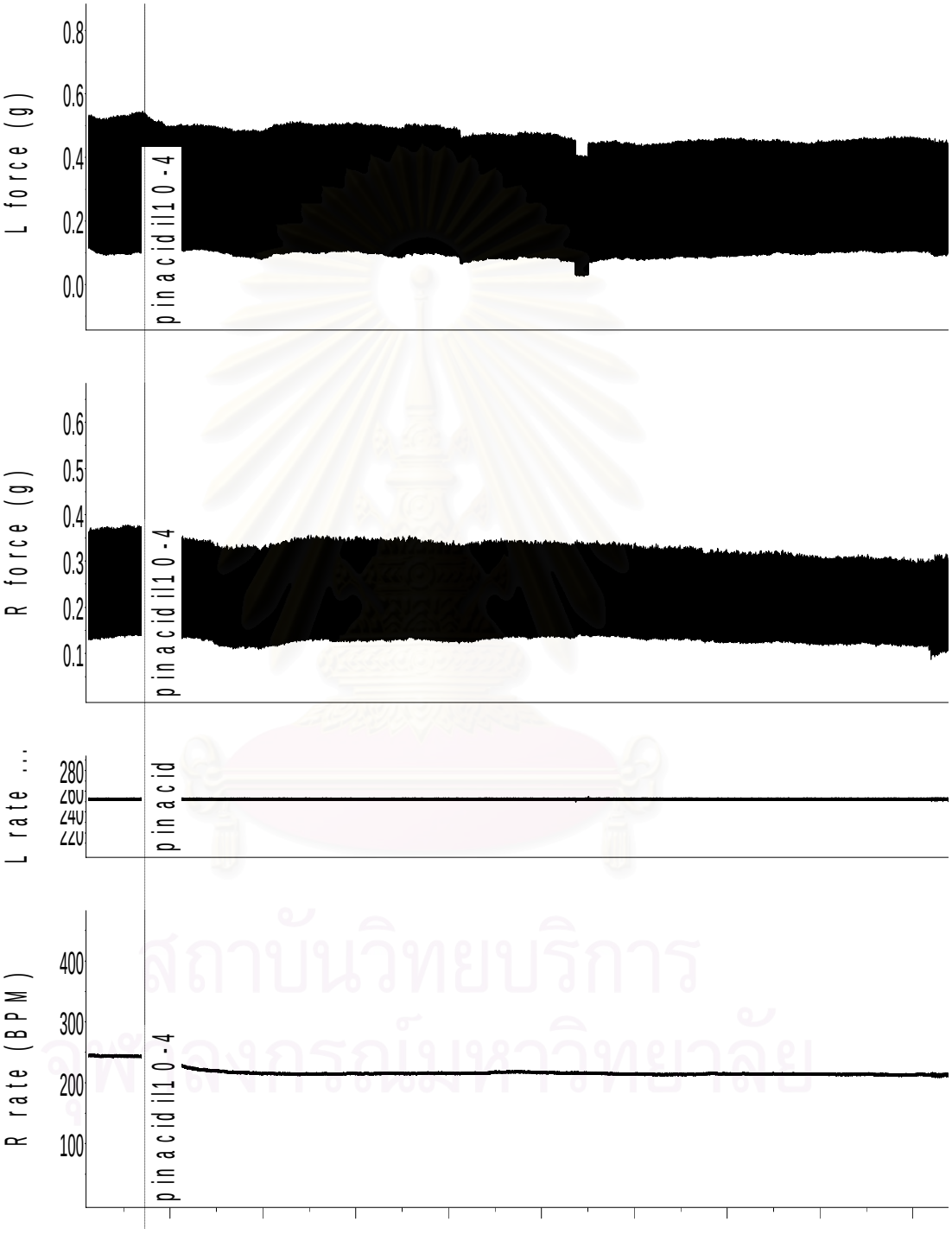


Figure 33 The inotropic and chronotropic response on the right and left atria in the presence of pinacidil (100 μM).

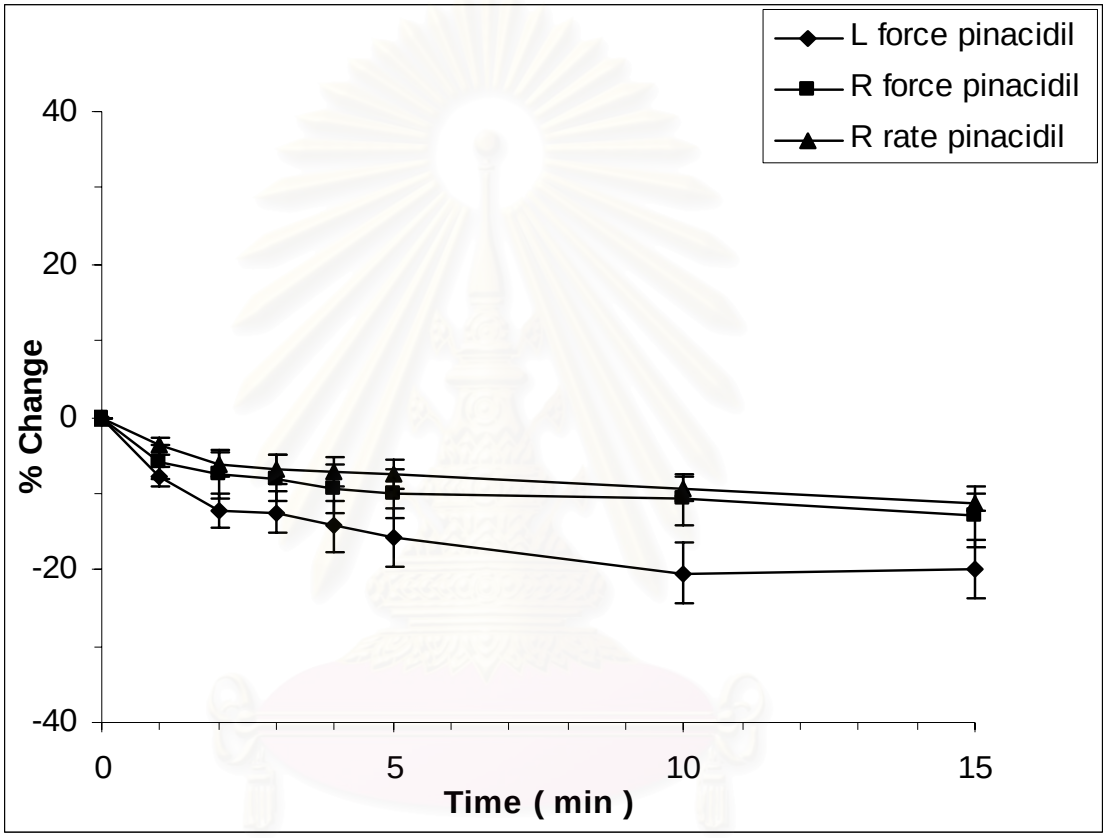


Figure 34 The inotropic and chronotropic response on the right and left atria in the presence of pinacidil 100 μM. n=6, mean ± S.E.M.

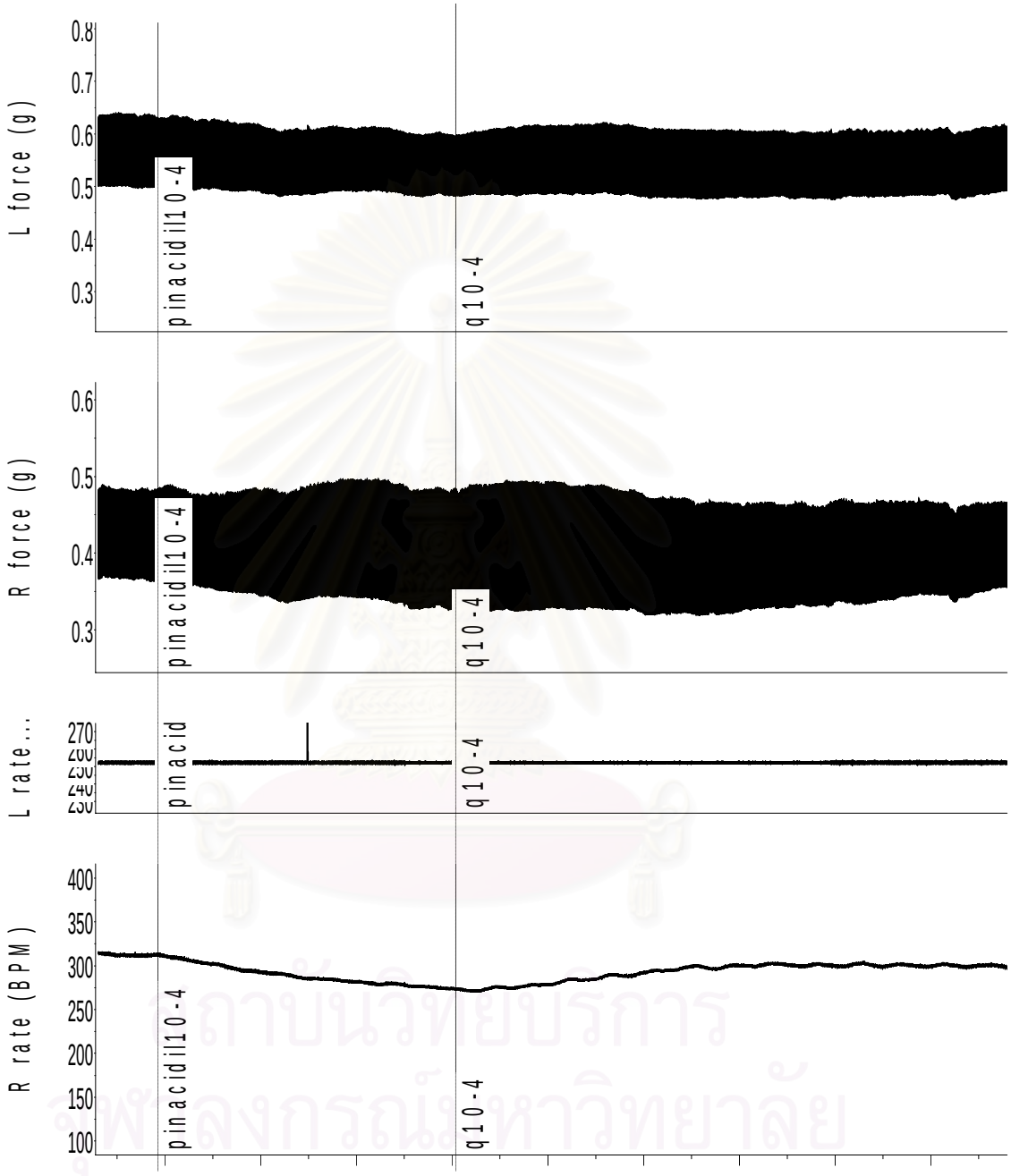
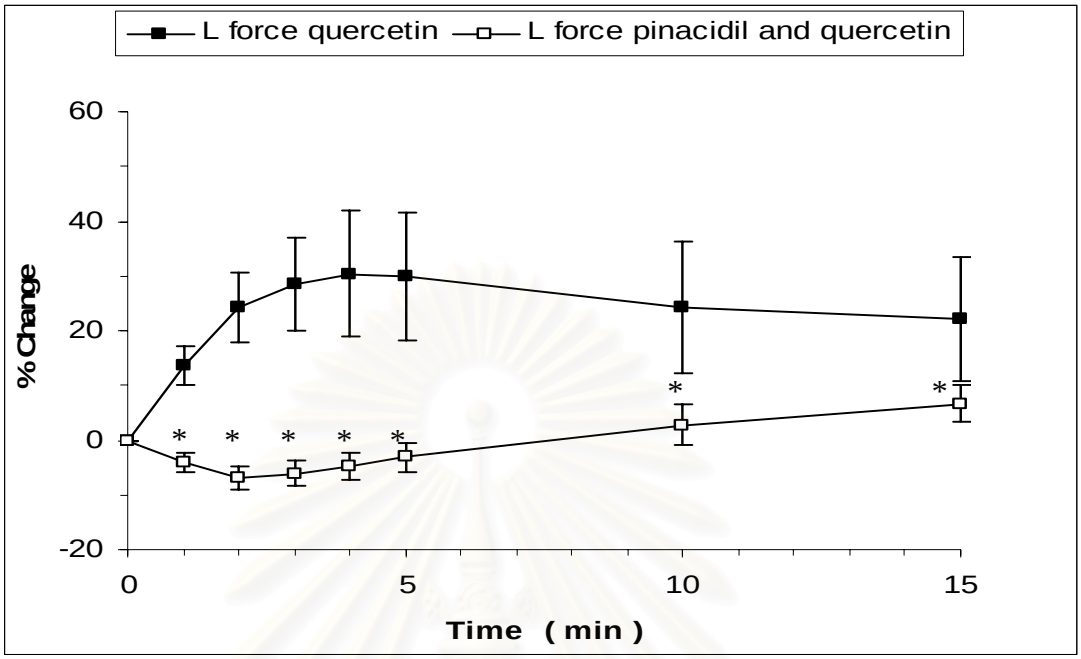


Figure 35 The inotropic and chronotropic response of quercetin (100 μM) on the right and left atria in the presence of pinacidil (100 μM).

A. left atria.



B. Right atria

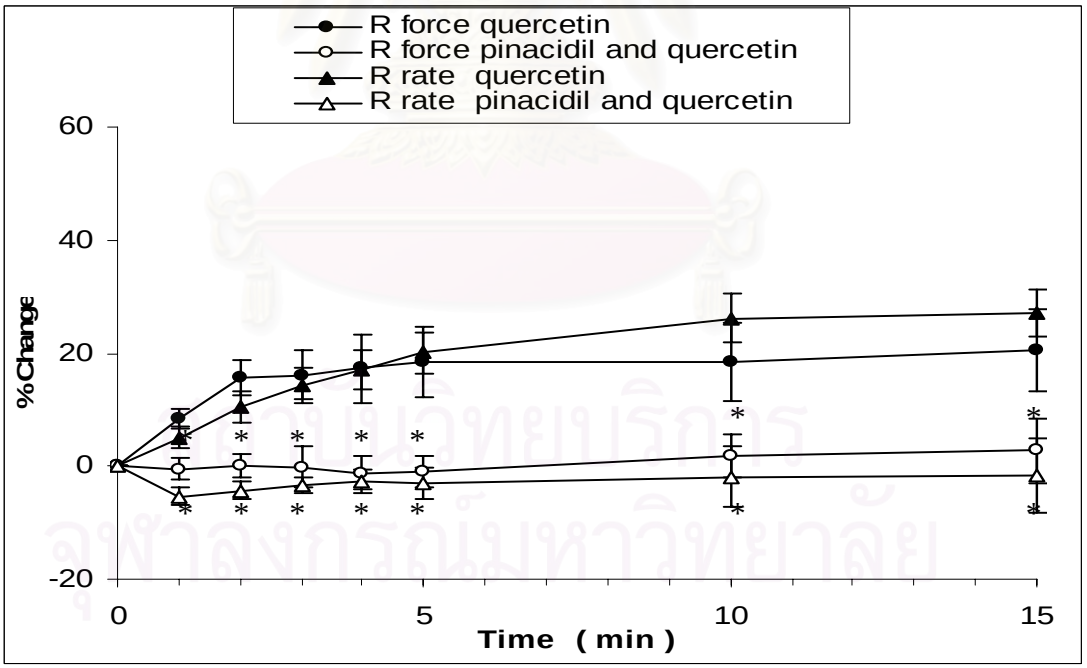


Figure 36 The inotropic and chronotropic response of quercetin (100 μM) on the left (A) and right (B) atria in the presence and absence of pinacidil (100 μM). n=6, mean ±S.E.M. * $p<0.05$, significantly different from quercetin group in the absence of pinacidil (paired *t* – test).

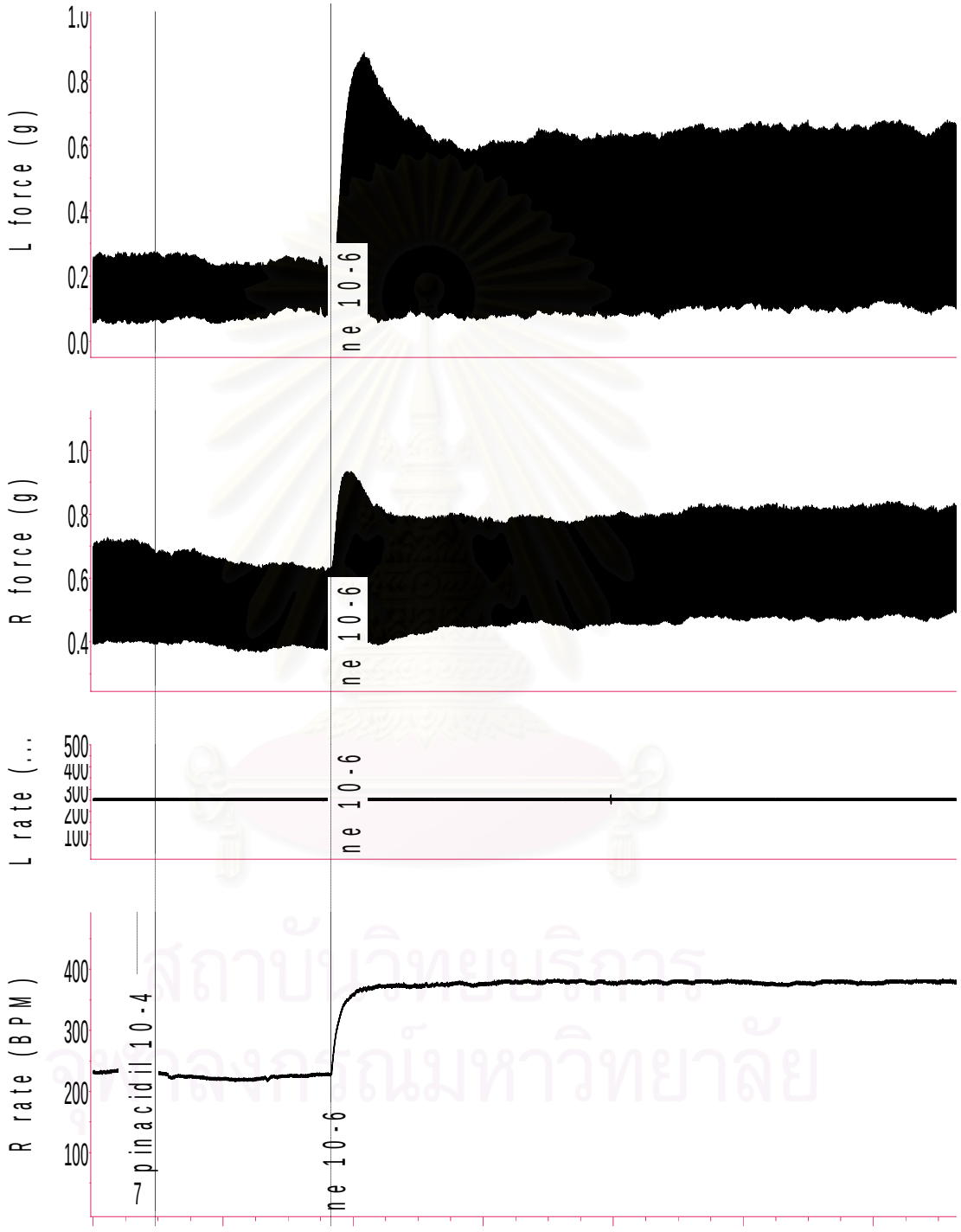
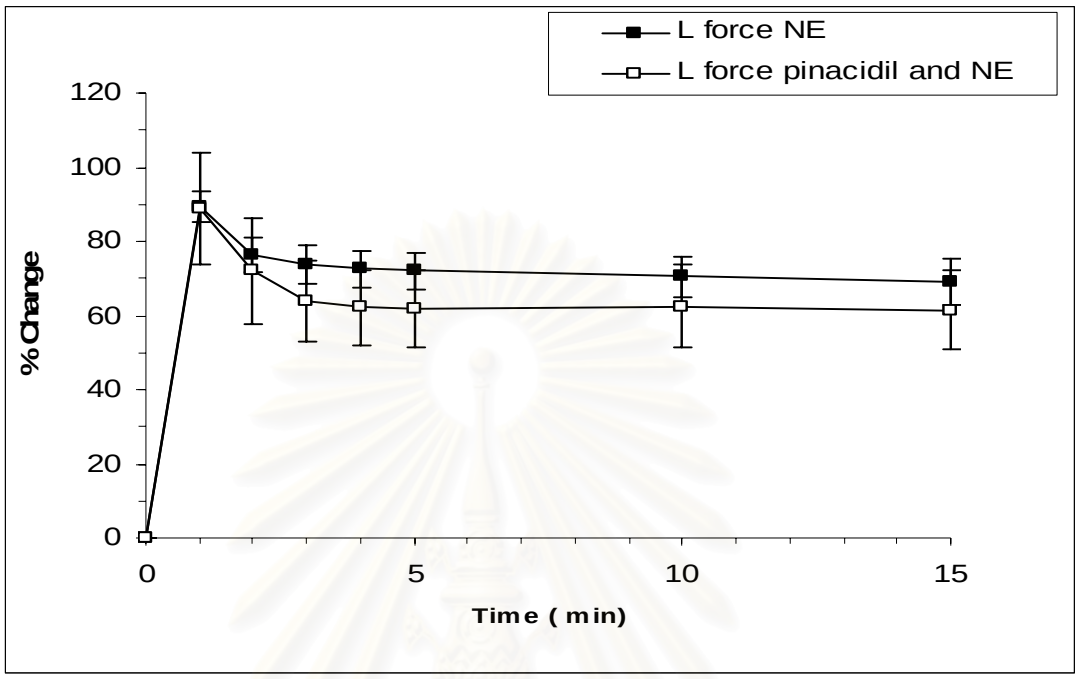


Figure 37 The inotropic and chronotropic response on the right and left atria in the presence of NE (1 μM).

A. Left atria



B. Right atria.

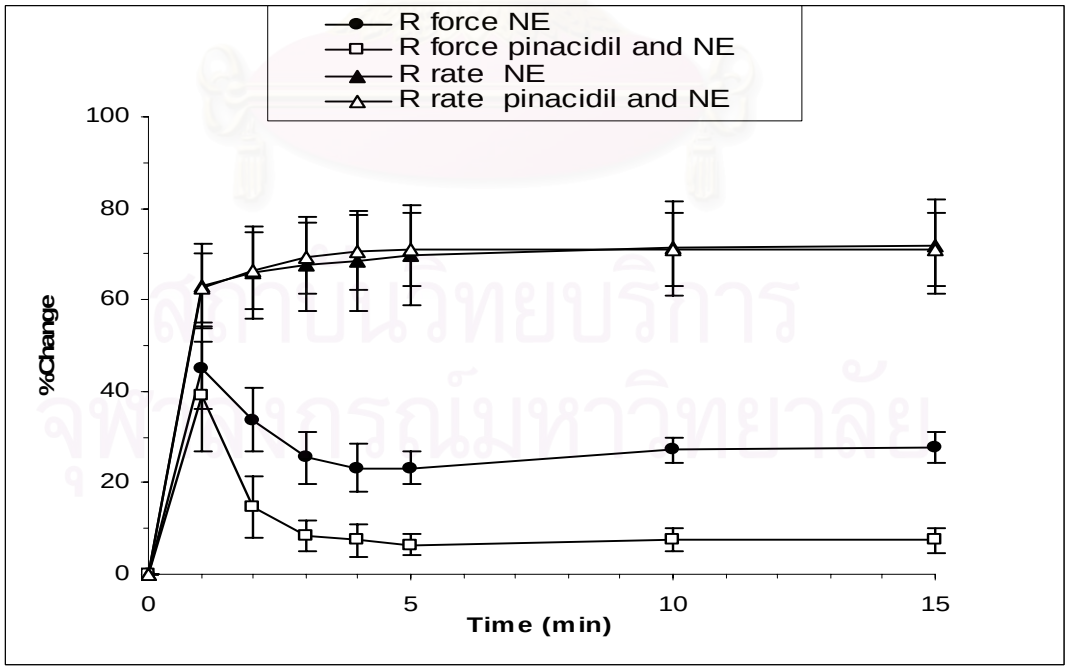


Figure 38 The inotropic and chronotropic response of NE (1 μM) on the left (A) and right (B) atria in the presence of pinacidil (100 μM). n=6, mean ± S.E.M.

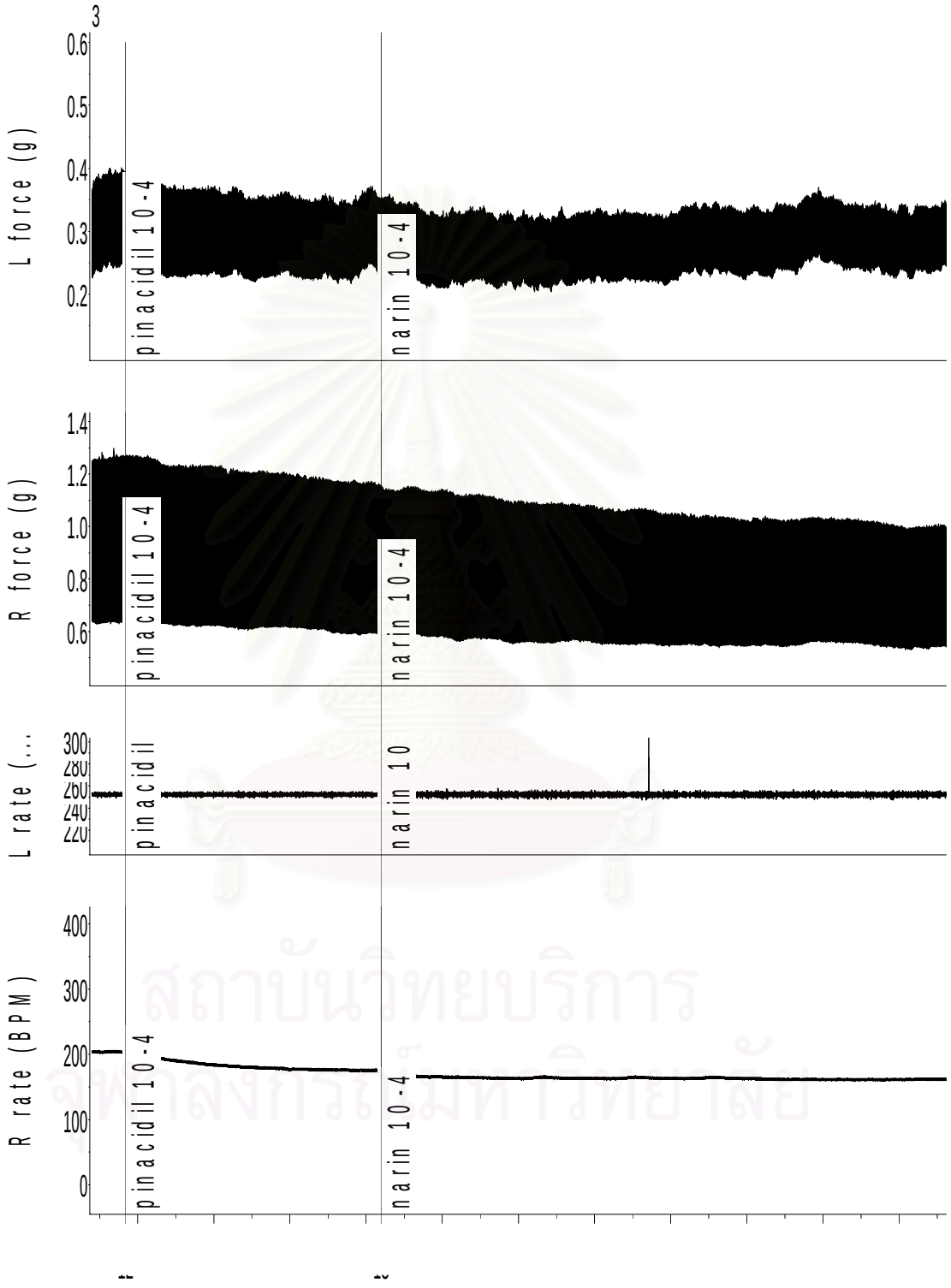
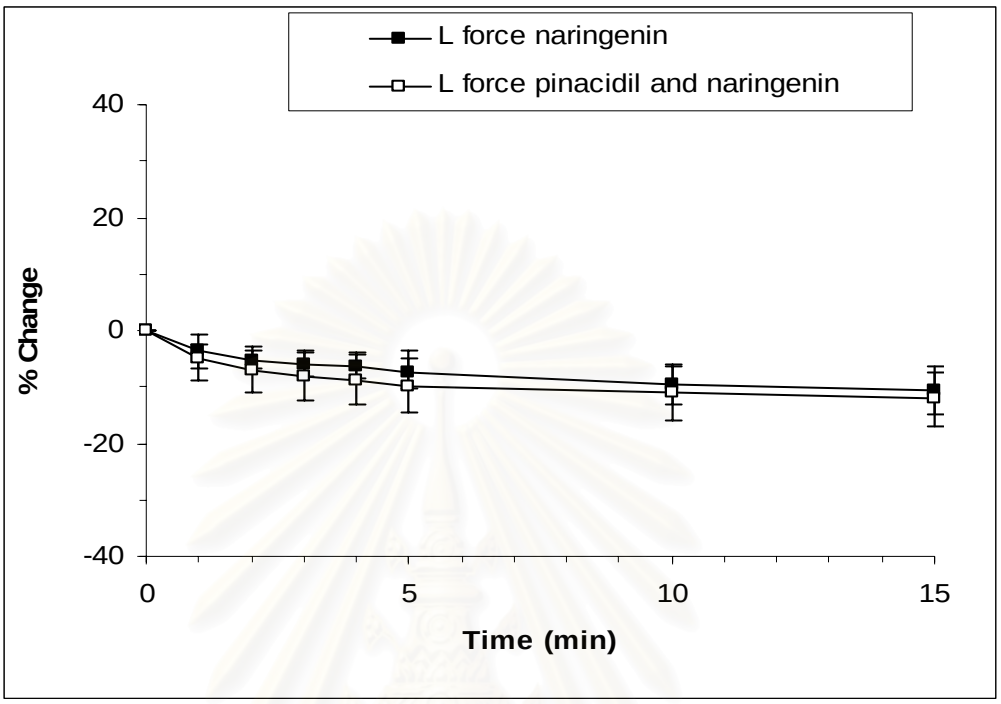


Figure 39 The inotropic and chronotropic response of naringenin (100 μM.) on the right and left atria in the presence of pinacidil (100 μM).

A. Left atria.



B. Right atria.

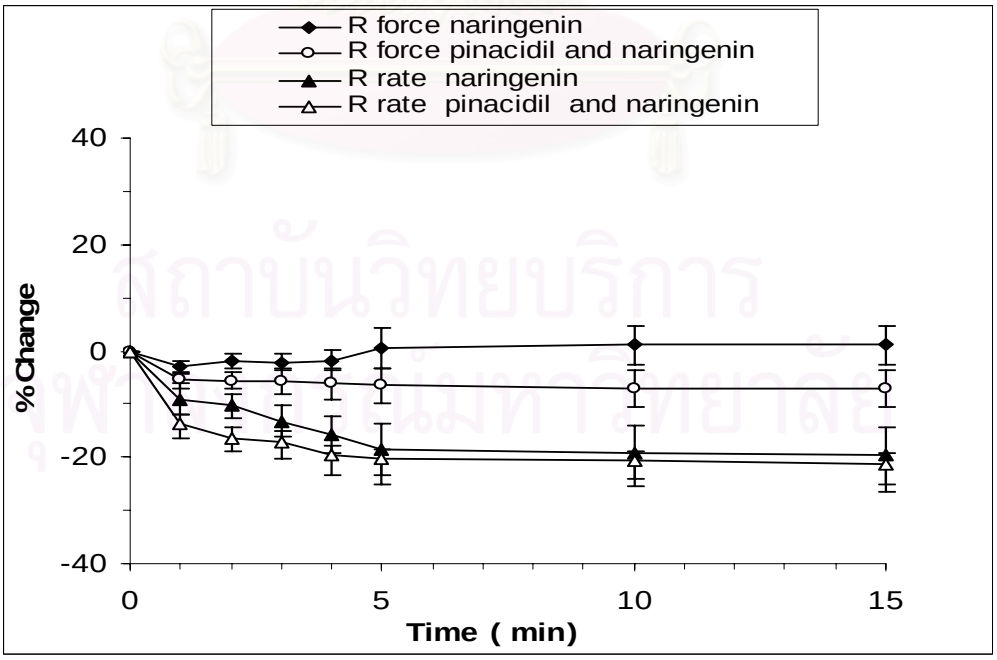


Figure 40 The inotropic and chronotropic response of naringenin (100 μM) on the left (A) and right (B) atria the presence of pinacidil (100 μM), n=6, mean ± S.E.M.

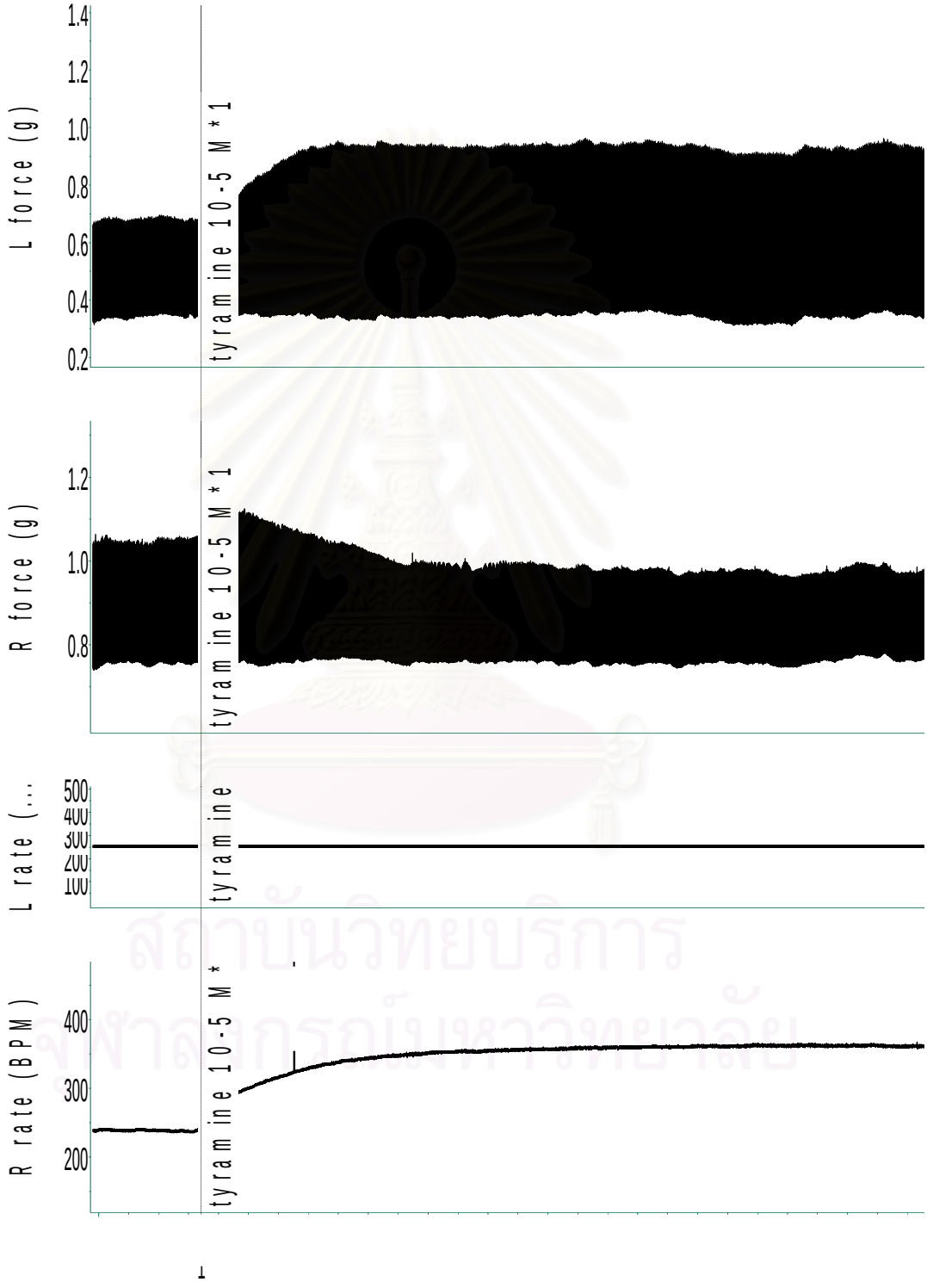


Figure 41 The inotropic and chronotropic response of tyramine (10 μM) in normal rats.

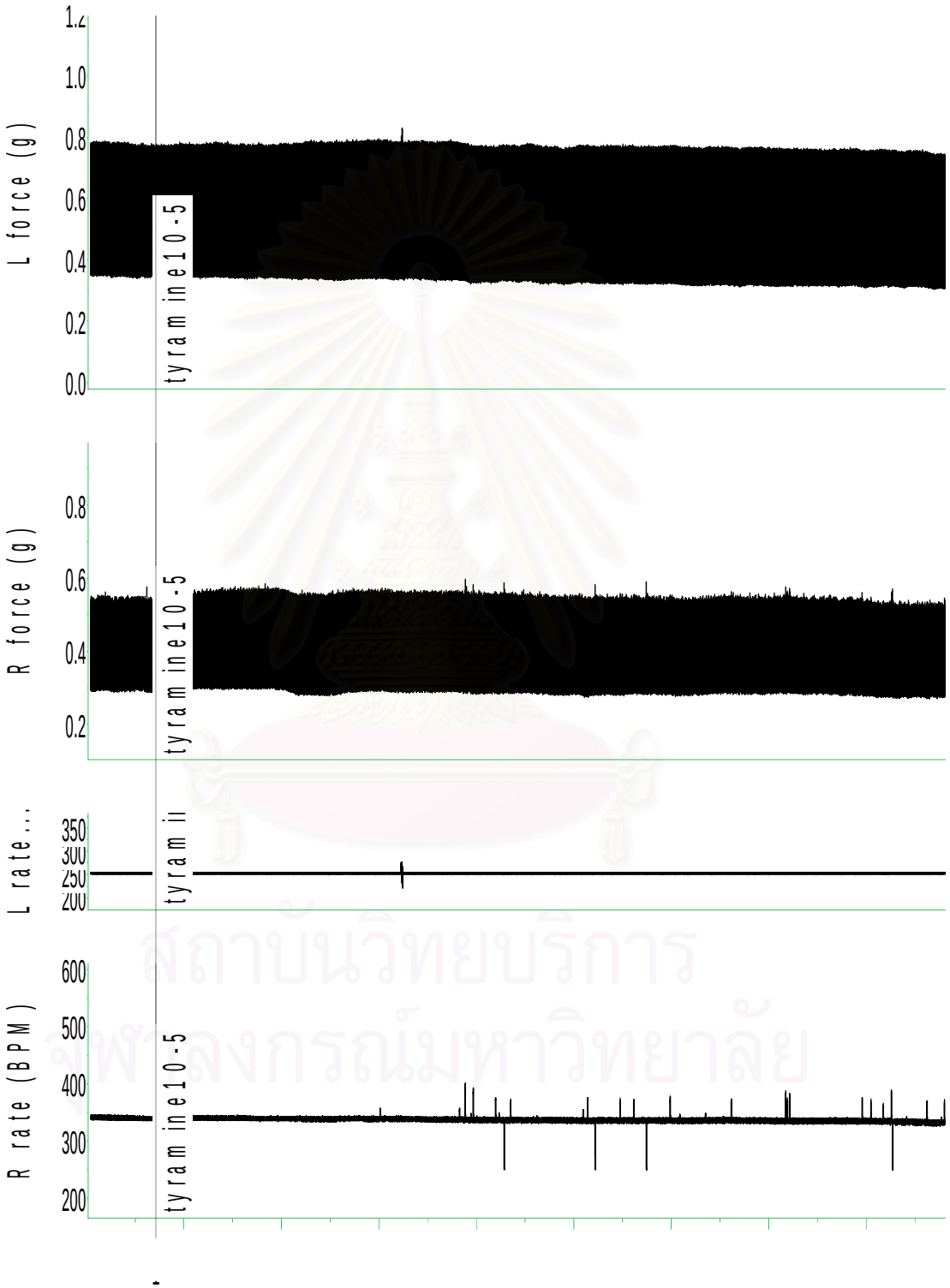
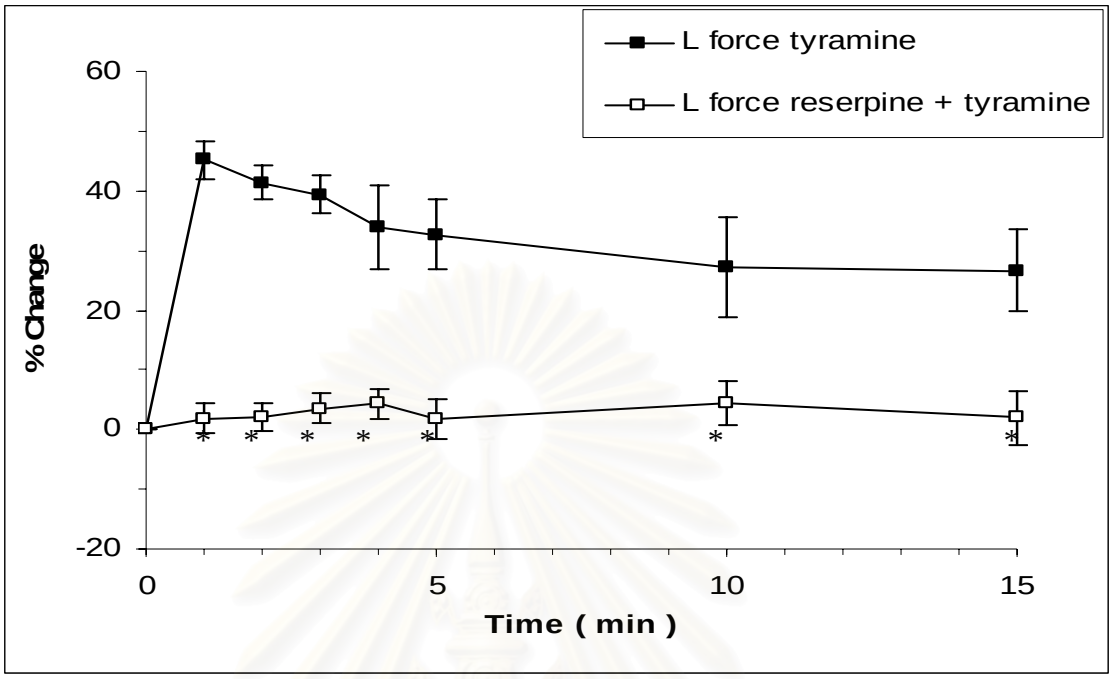


Figure 42 The inotropic and chronotropic response of tyramine (10 μM) in rats pretreated with reserpine (5 mg / kg, i.p. for 2 days).

A. Left atria.



B. Right atria.

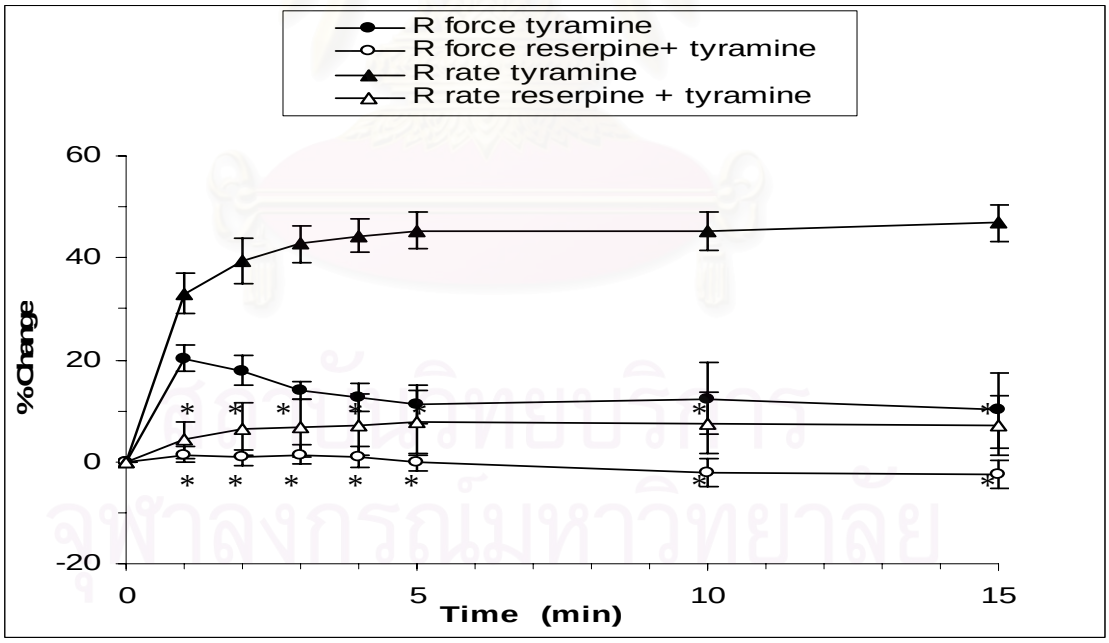


Figure 43 The inotropic and chronotropic response of tyramine (10 μM) on left (A) and right (B) atria in normal rats and rats pretreated with reserpine (5 mg / kg, i.p.for 2 days). N=5, mean ±S.E.M. * $p < 0.05$, significantly different from tyramine group in normal rats (paired *t* - test).

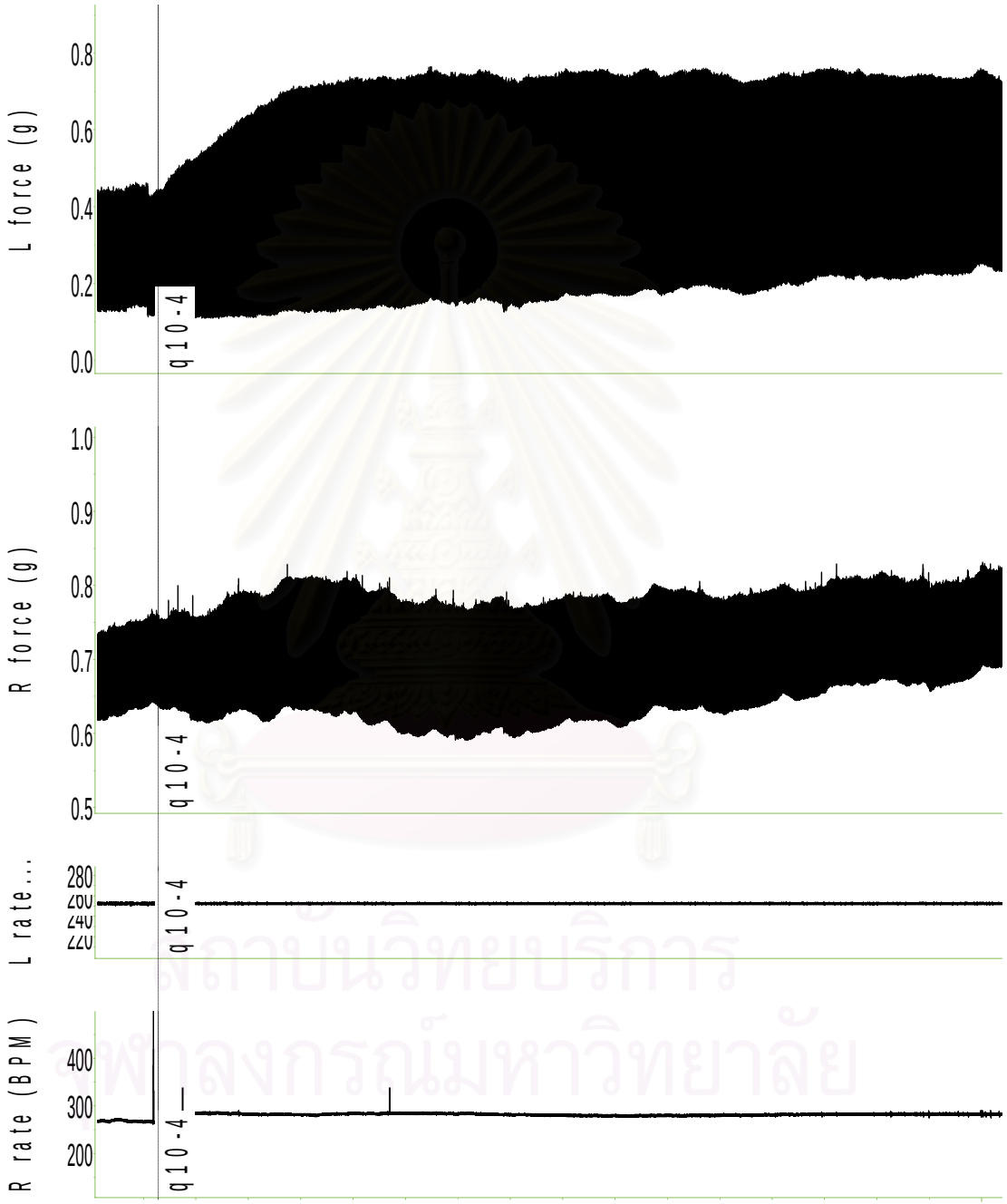


Figure 44 The inotropic and chronotropic response of quercetin (100 μM) in the normal rats.

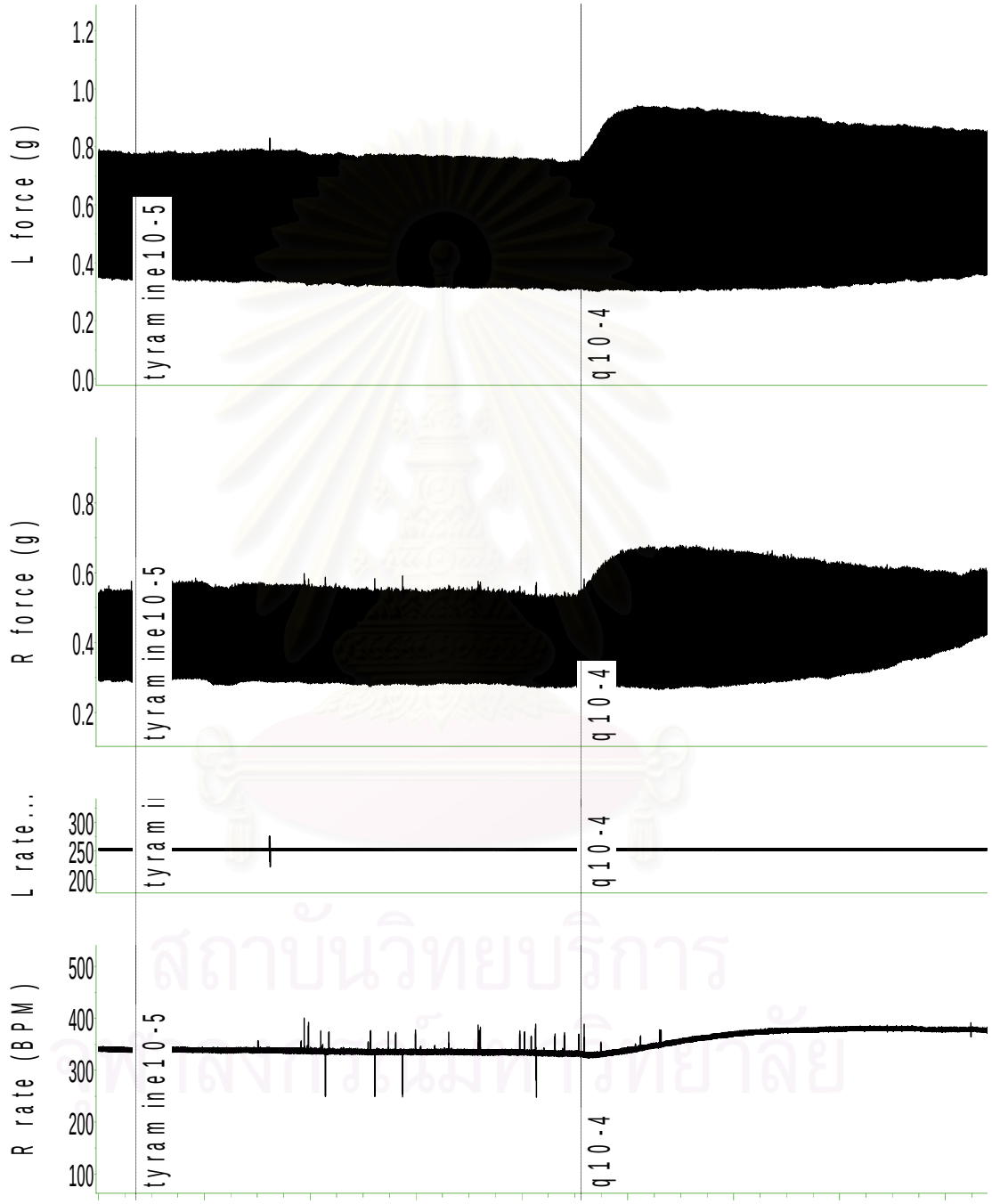
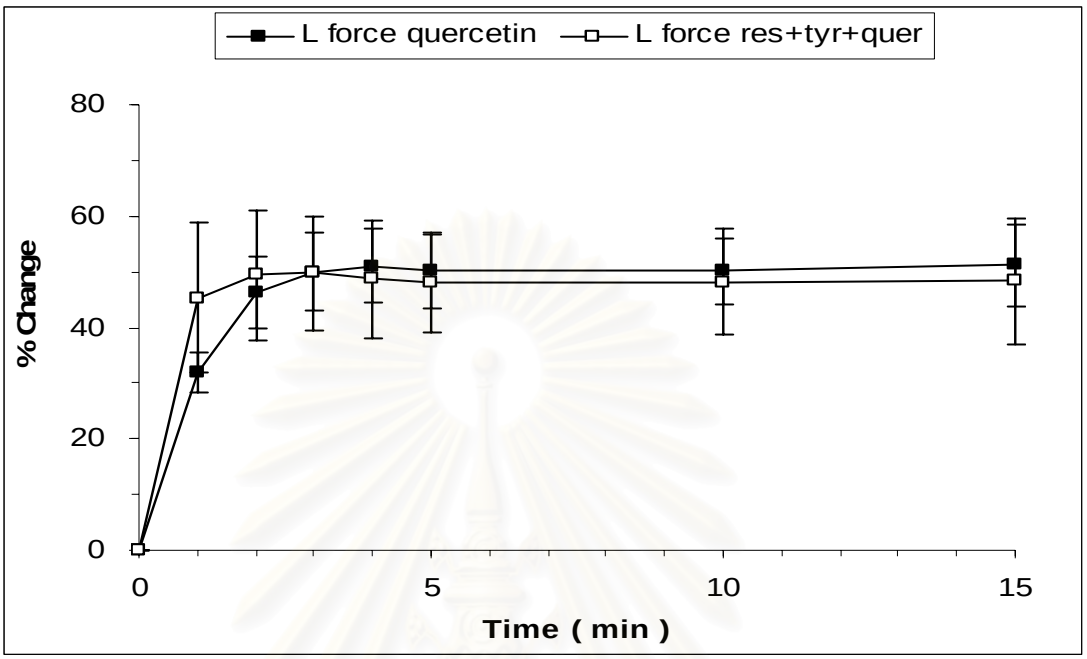


Figure 45 The inotropic and chronotropic response of quercetin (100 μM) in the rats were pretreated with reserpine (5 mg / kg, i. p.for 2 days).

A. Left atria.



B. Right atria

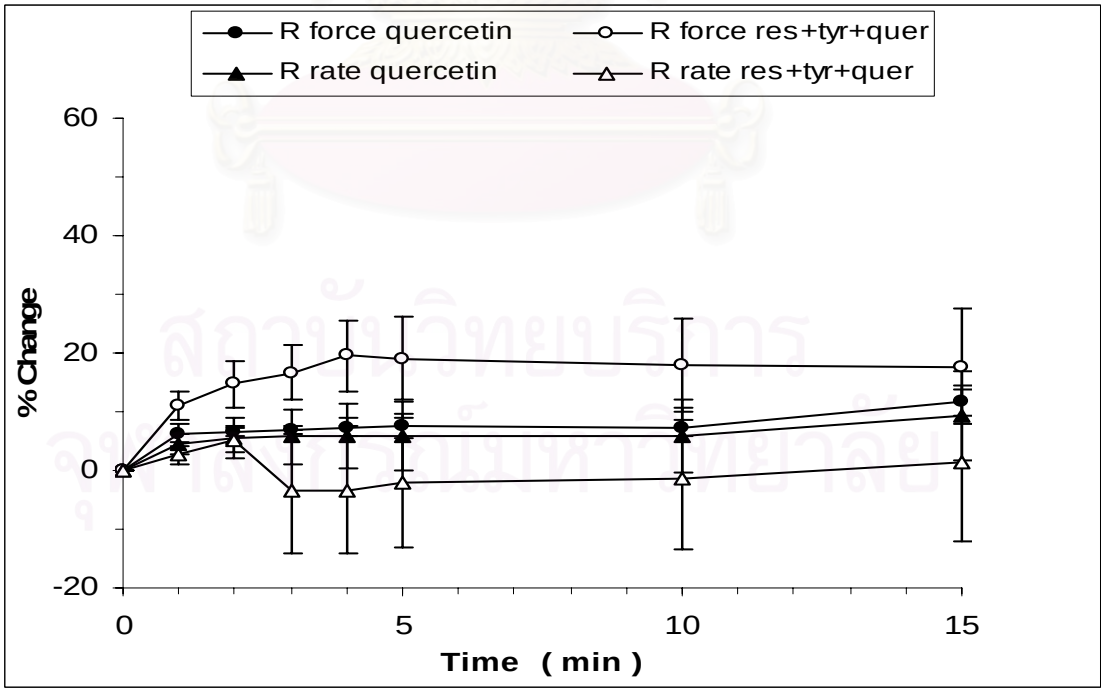


Figure 46 The inotropic and chronotropic response of quercetin (100 μM) on left (A) and right (B) atria on rats pretreated with reserpine (5 mg/kg, i.p.for 2 days). n=5, Mean ± S.E.M.

CHAPTER V

DISCUSSIONS AND CONCLUSIONS

The results of this study showed that two flavonoids exerted a different cardiotonic action. Quercetin caused positive inotropic and chronotropic effects whereas naringenin caused negative inotropic and chronotropic effects. This results supported the structure-activity relationship of cardiotonic action of flavonoids. The positive inotropic effect required flavonoid nucleus, which was presence in quercetin (Itoigawa *et al*., 1999). The positive inotropic and chronotropic effects of quercetin was linked to the activation of ß- adrenoceptor. In this study, these positive effects seen in the right and left atria suggested that quercetin and naringenin directly affect the pacemaker activity as well as the cardiac muscle. Moreover, these effects were blocked by propranolol (ß- adrenoceptor blocker) and verapamil (Ca^{2+}channel blocker). Interesting, these was an interaction between quercetin and Ca^{2+} channel blocker to producing potential arrhythmia in the right atria. This arrhythmia occurrence was not found in the left atria, which may be due to the contraction of the left atria was exogenously controlled by electrical pacing with continue rate of 252 beats per minute. This observation implied the direct effects of compounds on SA node and heart conductivity, and suggested the potential interaction of the concurrent used of quercetin or dietary supplements with calcium channel blockers such as verapamil.

The inotropic effects of quercetin on the Ca^{2+} release from SR was investigated in isolated left atria preparation. The present study modified the method from the study of the Ca^{2+} handling function in SR of rats ventricular papillary muscle by Yamato *et al* 1996. When compared the effects of quercetin on the rest- state contraction which those of caffeine, quercetin was sinificantly different from that of caffeine, suggested that the inotropic effects of quercetin may not involved Ca^{2+} release from SR.

In the heart, potassium channels are important for cardiac functions. Potassium channel opener such as pinacidil increased efflux of potassium ion from the cardiac muscle cell and an inhibition of calcium influx or indirect calcium antagonism, caused a fall in intracellular calcium concentration (Purcell, 1999). The present study,

the positive inotropic and chronotropic effects of quercetin was blocked by pinacidil. However, pretreatment of pinacidil (100 μM) did not significantly affect the action of NE. This results suggested that quercetin exerted its cardiotonic actions through the K^+ channel.

The sympathetic drugs such as norepinephine or epinephrine act by the direct effects on the activation of ß- adrenocepor. Others sympathomimetic drugs such as tyramine act indirectly dependent on the release of endogenous catecholamine. Some drug such as phenylthylamine had both direct and indirect actions on the heart function. The positive inotropic and chronotropic of quercetin on the storage of the catecholamine were investigate, quercetin was able to cause the inotropic and chronotropic effects. This finding suggested that quercetin act by the direct effects on the activation of ß-adrenocepor.

Naringenin has been reported its positive inotropic in guinea- pig papillary muscle (Itoitawa *et al*., 1999). However, in this study naringenin elicited negative inotropic and chronotropic effects. This results may be due to the different in the method and isolated preparation were used in the experiments. The mechanisms of cardiotonic actions of naringenin was investigate. The present study suggested that the negative inotropic and chronotropic of naringenin may not involved of the muscarinic receptor, and did not involved decrease calcium release from sarcoplasmic reticulum (SR). In addition, naringenin did not additive effects on potassium channel opener.

In conclusions, this results showed that these two flavonoids were different cardiotonic action. Quercetin caused positive inotropic and chronotropic effects whereas naringenin elicited negative inotropic and chronotropic effects on the isolated rat atria. The positive inotropic effect of quercetin on the isolated rat atria may be related to the activation of beta-adrenoceptor, increase in Ca^{2+} influx and may interfere function of K^+ channel. Furthermore, naringenin caused the negative inotropic and chronotropic effects of which were not related cholinergic receptor. In summary, quercetin and naringenin exerted their cardiotonic actions on pacemaker and cardiac muscle.

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APPENDICES

Table1 Structure and hydroxylation pattern of various flavonoids.

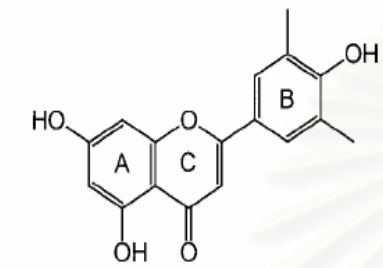
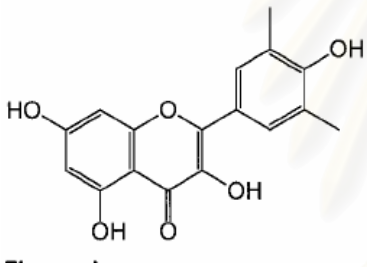
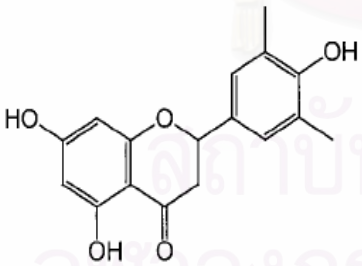
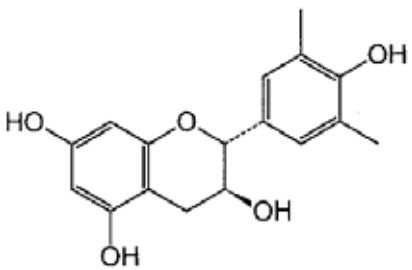
Generic structure	Flavonoid	Hydroxylation pattern					
		3	5	7	3'	4'	5'
Flavonols 	Quercetin	OH	OH	OH	OH	OH	H
	Rutin	OR	OH	OH	OH	OH	H
	Kaemferol	OH	OH	OH	OH	OH	OH
Flavones 	Apigenin	H	OH	OH	H	OH	H
	Luteolin	H	OH	OH	OH	OH	H
Flavonone 	Naringin	H	OH	OR	OH	OH	H
	Naringenin	H	OH	OH	H	OH	H
	Hesperetin	H	OH	OR	OH	OCH_3	H
Flavanes 	catechin	OH	OH	OH	OH	OH	H

Table 2 Chemical compound for Krebs- Henseleit Solution (mM/L)

Chemicals	mM/L
Sodium chloride	119.0
Potassium Chloride	4.7
Magnesium Sulfate	1.0
Calcium Chloride	2.5
Sodium Bicarbonate	25
Potassium Dibasic Phosphate	1.2
Glucose	11.1

Table 3 Effects of quercetin at the concentration range of 0.1 μM-1000 μM on rate and force of contraction of right and left atria (n=6) (mean ± SEM).

Concentration (M)	Left force (g)	Right force (g)	Rate right (BPM)
control	0.38 ± 0.03	0.63 ± 0.05	258 ± 0.79
10^{-7}	0.40 ± 0.03	0.66 ± 0.05	260 ± 1.61
10^{-6}	0.43 ± 0.03	0.66 ± 0.05	262 ± 1.85
10^{-5}	0.46 ± 0.03 *	0.67 ± 0.04	264 ± 1.85
10^{-4}	0.57 ± 0.04 *	0.73 ± 0.04 *	270 ± 4.40 *
10^{-3}	0.55 ± 0.04 *	0.74 ± 0.04 *	270 ± 7.71 *

*$p<0.05$, significantly different from DMSO group (student unpaired *t* – test)

Table 4 Effects of naringenin at the concentration range of 0.1μM-1000 μM on rate and force of contraction of right and left atria (n=6) (mean ± SEM).

Concentration (M)	Left force (g)	Right force (g)	Rate right (BPM)
control	0.43 ± 0.02	0.81 ± 0.04	299 ± 0.20
10^{-7}	0.42 ± 0.02	0.79 ± 0.03	290 ± 0.87
10-^{6}	0.40 ± 0.02	0.77 ± 0.03	274 ± 2.21
10^{-5}	0.39 ± 0.03	0.76 ± 0.05	255 ± 4.44
10^{-4}	0.35 ± 0.02 *	0.67 ± 0.05 *	236 ± 4.44 *
10^{-3}	0.32 ± 0.03 *	0.64 ± 0.05 *	234 ± 7.44 *

*$p<0.05$, significantly different from DMSO group (student unpaired *t* – test)

Table 5 Effects of quercetin (100 μM) on rate and force of contraction of right and left atria (n=6) (mean ± SEM).

Time	Left force (g)	Right force (g)	Rate right (BPM)
control	0.50 ± 0.05	0.62 ± 0. 04	265 ± 2.33
1 min.	0.62 ± 0.05 *	0.68 ± 0.04	272 ± 2.34
2 min.	0.65 ± 0.05 *	0.72 ± 0.03	279 ± 5.07
3 min.	0.69 ± 0.05 *	0.76 ± 0.03 *	285 ± 7.06 *
4 min.	0.72 ± 0.05 *	0.76 ± 0.05 *	286 ± 8.15 *
5 min.	0.75 ± 0.05 *	0.77 ± 0.03 *	303 ± 7.66 *
10 min.	0.75 ± 0.04 *	0.78 ± 0.04 *	311 ± 8.62 *
15 min.	0.75 ± 0.04 *	0.82 ± 0.05*	309 ± 8.31 *

*$p<0.05$, significantly different from DMSO group (student unpaired *t* – test)

Table 6 Effects of naringenin (100 μM) on rate and force of contraction of right and left atria (n=6) (mean ± SEM).

Time	Left force (g)	Right force (g)	Rate right (BPM)
control	0.56 ± 0.05	0.85 ± 0.06	240 ± 3.56
1 min.	0.53 ± 0.05	0.82 ± 0.06	208 ± 3.70
2 min.	0.52 ± 0.05	0.81 ± 0.06	206 ± 3.78
3 min.	0.51 ± 0.05	0.81 ± 0.05	198 ± 3.49 *
4 min.	0.50 ± 0.05	0.81 ± 0.06	192 ± 3.07 *
5 min.	0.48 ± 0.05 *	0.80 ± 0.06	188 ± 3.15 *
10 min.	0.49 ± 0.05 *	0.80 ± 0.06	182 ± 2.67 *
15 min.	0.49 ± 0.04 *	0.80 ± 0.06	174 ± 2.78 *

*$p<0.05$, significantly different from DMSO group (student unpaired *t* – test)

Table 7 Effects of NE (1 μM) on rate and force of contraction of right and left atria (n=6) (mean ± SEM).

Time	Left force (g)	Right force (g)	Rate right (BPM)
control	0.52 ± 0.04	0.57 ± 0.05	224 ± 5.21
1 min.	0.87 ± 0.04 *	0.74 ± 0.05 *	272 ± 6.77 *
2 min.	0.86 ± 0.04 *	0.74 ± 0.05 *	283 ± 7.25 *
3 min.	0.86 ± 0.04 *	0.74 ± 0.06 *	286 ± 7.25 *
4 min.	0.84 ± 0.04 *	0.73 ± 0.06 *	289 ± 7.03 *
5 min.	0.82 ± 0.04 *	0.73 ± 0.06 *	292 ± 6.05 *
10 min.	0.80 ± 0.04 *	0.73 ± 0.06 *	296 ± 5.01 *
15 min.	0.79 ± 0.04 *	0.72 ± 0.06 *	302 ± 6.25 *

*$p<0.05$, significantly different from control group (student unpaired t – test)

Table 8 Effects of propranolol (10 μM) on rate and force of contraction of right and left atria (n=6) (mean ± SEM).

Time	Left force (g)	Right force (g)	Rate right (BPM)
control	0.57 ±0.06	0.55 ± 0.06	229 ± 3.36
1 min.	0.53 ± 0.06	0.52 ± 0.05	206 ± 3.28 *
2 min.	0.52 ± 0.06	0.54 ± 0.05	200± 3.63 *
3 min.	0.50 ± 0.06 *	0.56 ± 0.05	195 ± 3.79 *
4 min.	0.49 ± 0.06 *	0.57 ± 0.05	192 ± 3.33 *
5 min.	0.48 ± 0.06 *	0.57 ± 0.06	185 ± 2.20 *
10 min.	0.47 ± 0.05 *	0.56 ± 0.05	177 ± 2.09 *
15 min.	0.47 ± 0.05 *	0.56 ± 0.05	174 ± 2.22 *

*$p<0.05$, significantly different from control group (student unpaired *t* – test)

Table 9 Effects of NE (1 μM) in the presence of propranolol (10 μM) on rate and force of contraction of right and left atria (n=6) (mean ± SEM).

compound \ time	Left force (g)	Right force (g)	Right rate (BPM)
control	0.86 ± 0.05	0.73 ± 0.05	256 ± 3.56
Propranolol 1 min.	0.82 ± 0.05	0.69 ± 0.05	225 ± 3.58
2 min.	0.81 ± 0.05	0.71 ± 0.05	215 ± 3.96
3 min.	0.80 ± 0.05	0.72 ± 0.05	209 ± 5.32
4 min.	0.79 ± 0.05	0.73 ± 0.05	207 ± 5.96
5 min.	0.78 ± 0.06	0.75 ± 0.05	206 ± 6.10
NE 1 min.	0.85 ± 0.06 *	0.75 ± 0.05 *	212 ± 5.71 *
2 min.	0.85 ± 0.06 *	0.75 ± 0.05 *	214 ± 5.77 *
3 min.	0.84 ± 0.06 *	0.75 ± 0.05 *	212 ± 5.89 *
4 min.	0.84 ± 0.06 *	0.75 ± 0.06 *	211 ± 6.15 *
5 min.	0.85 ± 0.06 *	0.75 ± 0.06 *	210 ± 6.36 *
10 min.	0.84 ± 0.06 *	0.76 ± 0.06 *	195 ± 6.89 *
15 min	0.83 ± 0.05 *	0.76 ± 0.06 *	184 ± 11.23 *

*$p < 0.05$, significantly different from NE group in the absence of propranolol (student paired *t* - test)

Table 10 Effects of quercetin (100 μM) in the presence of propranolol (10 μM) on rate and force of contraction of right and left atria (n=6) (mean ± SEM).

time / compound	Left force (g)	Right force (g)	Right rate (BPM)
control	0.84 ± 0.04	0.89 ± 0.06	235 ± 0.89
Propranolol 1 min.	0.81 ± 0.04	0.87 ± 0.06	229 ± 0.56
2 min.	0.81 ± 0.04	0.87 ± 0.06	220 ± 2.05
3 min.	0.80 ± 0.05	0.88 ± 0.06	216 ±2.36
4 min.	0.79 ± 0.04	0.87 ± 0.06	214 ± 2.50
5 min.	0.78 ± 0.05	0.88 ± 0.05	212 ± 2.64
quercetin 1 min.	0.82 ± 0.05 *	0.87 ± 0.05 *	215 ± 7.01 *
2 min.	0.82 ± 0.05 *	0.88 ± 0.05 *	209 ± 7.37 *
3 min.	0.82 ± 0.05 *	0.88 ± 0.05 *	203 ± 7.86 *
4 min.	0.83 ± 0.05 *	0.88 ± 0.05 *	199 ± 8.10 *
5 min.	0.84 ± 0.05 *	0.88 ± 0.05 *	187 ± 4.72 *
10 min.	0.84 ± 0.05 *	0.90 ± 0.05 *	169 ± 7.36 *
15 min	0.87 ± 0.05	0.91 ± 0.05 *	147 ± 7.71 *

*$p < 0.05$, significantly different from quercetin group in the absence of propranolol (student paired *t* - test).

Table 11 Effects of atropine (10 μM) on rate and force of contraction of right and left atria (n=6) (mean ± SEM).

Time	Left force (g)	Right force (g)	Right rate (BPM)
control	0.60 ± 0.04	0.51 ± 0.03	231 ± 0.79
1 min.	0.61 ± 0.04	0.51 ± 0.03	228 ± 0.79
2 min.	0.61 ± 0.04	0.51 ± 0.03	227 ± 0.90
3 min.	0.61 ± 0.04	0.51 ± 0.03	226 ± 1.15
4 min.	0.60 ± 0.04	0.50 ± 0.03	223 ± 1.54
5 min.	0.58 ± 0.04	0.50 ± 0.03	222 ± 1.68
10 min.	0.58 ± 0.04	0.50 ± 0.03	219 ± 2.40
15 min.	0.57 ± 0.03	0.50 ± 0.04	214 ± 3.56

Table 12 Effects of naringenin (100 μM) in the presence of atropine (10 μM) on rate and force of contraction of right and left atria (n=6) (mean ± SEM).

compound \ time		Left force (g)	Right force (g)	Right rate (BPM)
control		0.53 ± 0.03	0.80 ± 0.04	230 ± 1.45
Atropine	1 min.	0.53 ± 0.03	0.81 ± 0.06	228 ± 1.70
	2 min.	0.53 ± 0.04	0.81 ± 0.06	227 ± 2.39
	3 min.	0.53 ± 0.04	0.82 ± 0.05	226 ± 1.71
	4 min.	0.52 ± 0.04	0.81 ± 0.04	226 ± 3.56
	5 min.	0.51 ± 0.04	0.81 ± 0.04	223 ± 3.45
naringenin	1 min.	0.49 ± 0.05	0.81 ± 0.04	210 ± 1.60
	2 min.	0.48 ± 0.05	0.80 ± 0.04	204 ± 1.86
	3 min.	0.47 ± 0.06	0.80 ± 0.04	201 ± 2.11
	4 min.	0.46 ± 0.06	0.80 ± 0.04	197 ± 1.83
	5 min.	0.46 ± 0.06	0.81 ± 0.04	194 ± 1.75
	10 min.	0.45 ± 0.05	0.81 ± 0.04	192 ± 1.80
	15 min	0.44 ± 0.05	0.81 ± 0.04	190 ± 2.14

Table 13 Effects of verapamil (1 μM) on rate and force of contraction of right and left atria (n=6) (mean ± SEM).

Time	Left force (g)	Right force (g)	Right rate (BPM)
control	0.62 ± 0.03	0.66 ± 0.02	313 ± 0.86
1 min.	0.59 ±0.03 *	0.64 ±0.03	284 ± 0.85 *
2 min.	0.55 ± 0.03 *	0.64 ± 0.02	252 ± 2.59 *
3 min.	0.54 ± 0.03 *	0.64 ± 0.03	241 ± 3.82 *
4 min.	0.52 ± 0.03 *	0.64 ± 0.03	235 ± 4.35 *
5 min.	0.50 ± 0.03 *	0.64 ± 0.02	233 ± 4.66 *
10 min.	0.47 ± 0.03 *	0.61 ± 0.03	157 ± 6.51 *
15 min.	0.45 ± 0.03 *	0.59 ± 0.03 *	141 ± 6.94 *

*$p < 0.05$, significantly different from control group (student unpaired *t* - test)

Table 14 Effects of quercetin (100 μM) in the presence of verapamil (1 μM) on rate and force of contraction of right and left atria (n=6) (mean ± SEM).

compound \ time		Left force (g)	Right force (g)	Right rate (BPM)
control		0.45 ± 0.03	0.66 ± 0.02	272 ± 1.90
Verapamil	1 min.	0.41 ± 0.03	0.64 ± 0.02	254 ± 1.92
	2 min.	0.39 ± 0.03	0.65 ± 0.02	207 ± 6.15
	3 min.	38 ± 0.03	0.63 ± 0.02	201 ± 5.70
	4 min.	0.37 ± 0.03	0.63 ± 0.02	190 ± 6.03
	5 min.	0.36 ± 0.05	0.63 ± 0.02	189 ± 6.21
quercetin	1 min.	0.40 ± 0.05 *	0.67 ± 0.04 *	186 ±10.17 *
	2 min.	0.40 ± 0.05 *	0.67 ± 0.04 *	184 ±10.31 *
	3 min.	0.40 ± 0.05 *	0.67 ± 0.04 *	0.220 ± 3.05 *
	4 min.	0.40 ± 0.05 *	0.68 ± 0.04 *	217 ± 3.31 *
	5 min.	0.41 ± 0.05 *	0.70 ± 0.04 *	196 ± 8.73 *
	10 min.	0.42 ± 0.05 *	0.76 ± 0.04 *	178 ±13.02 *
	15 min	0.44 ± 0.05 *	0.73 ± 0.04 *	203 ± 8.53 *

*$p < 0.05$, significantly different from quercetin group in the absence of verapamil (student paired t – test)

Table 15 Effect of caffeine (10 mM), quercetin (100 μM) and naringenin (100 μM) on the relationships between the Ti / Tss and the rest interval of range 10 to 300 seconds. (n=6) (mean ± SEM).

Rest interval second	Post- rest contraction (PRC)					
	control	caffeine	control	quercetin	control	naringenin
10	1.38±0.10	0.98±1.02 *	1.41±0.09	1.15±0.01	1.34±0.02	1.11±0.02
20	1.27±0.06	1.00±1.01 *	1.29±0.06	1.12±0.02	1.24±0.02	1.11±0.02
30	1.25±0.06	0.98±0.94 *	1.28±0.06	1.13±0.02	1.26±0.03	1.10±0.01
40	1.24±0.05	0.85±0.91 *	1.29±0.04	1.15±0.02	1.26±0.03	1.10±0.03
50	1.30±0.08	0.95±0.91 *	1.30±0.06	1.11±0.02	1.32±0.01	1.11±0.03
60	1.28±0.06	0.88±0.89 *	1.30±0.07	1.08±0.05	1.33±0.04	1.07±0.05
120	1.21±0.06	0.88±0.85 *	1.23±0.06	1.08±0.05	1.26±0.05	1.02±0.02
300	1.18±0.06	0.90±0.84 *	1.19±0.04	1.05±0.05	1.22±0.05	1.08±0.02

*$p < 0.05$, significantly different from control group (student unpaired *t* - test)

Table 16 Effects of pinacidil (100 μM) on rate and force of contraction of right and left atria (n=6) (mean ± SEM).

Time	Left force (g)	Right force (g)	Right rate (BPM)
control	0.49 ± 0.03	0.47 ± 0.04	262 ± 1.12
1 min.	0.46 ± 0.03	0.44 ± 0.04	252 ± 1.35
2 min.	0.43 ± 0.03 *	0.43 ± 0.04	246 ± 1.72 *
3 min.	0.43 ± 0.02 *	0.43 ± 0.04	244 ± 2.05 *
4 min.	0.43 ± 0.02 *	0.42 ± 0.04 *	243 ± 1.94 *
5 min.	0.43 ± 0.02 *	0.42 ± 0.04 *	242 ± 1.96 *
10 min.	0.40 ± 0.02 *	0.42 ± 0.04 *	237 ± 1.65 *
15 min.	0.40 ± 0.02 *	0.41 ± 0.04 *	233 ± 1.09 *

*$p < 0.05$, significantly different from control group (student unpaired *t* - test).

Table 17 Effects of NE (1 μM) in the presence of pinacidil (100 μM) on rate and force of contraction of right and left atria (n=6) (mean ± SEM).

compound \ time		Left force (g)	Right force (g)	Right rate (BPM)
control		0.43 ± 0.05	0.85 ± 0.04	225 ± 1.50
pinacidil	1 min.	0.42 ± 0.05	0.84 ± 0.04	221 ± 1.52
	2 min.	0.41 ± 0.05	0.83 ± 0.04	217 ± 2.10
	3 min.	0.40 ± 0.05	0.83 ± 0.04	214 ± 2.96
	4 min.	0.40 ± 0.05	0.82 ± 0.04	210 ± 1.60
	5 min.	0.39 ± 0.06	0.81 ± 0.04	208 ± 1.64
NE	1 min.	0.80 ± 0.06	1.17 ± 0.04	361 ± 1.46
	2 min.	0.72 ± 0.06	0.98 ± 0.04	369 ± 1.44
	3 min.	0.69 ± 0.06	0.94 ± 0.04	376 ± 2.02
	4 min.	0.69 ± 0.05	0.93 ± 0.04	378 ± 2.64
	5 min.	0.69 ± 0.05	0.92 ± 0.05	379 ± 5.33
	10 min.	0.68 ± 0.05	0.93 ± 0.05	380 ± 6.47
	15 min	0.69 ± 0.05	0.93 ± 0.05	379 ± 6.47

Table18 Effects of quercetin (100 μM) in the presence of pinacidil (100 μM) on rate and force of contraction of right and left atria (n=6) (mean ± SEM).

compound \ time		Left force (g)	Right force (g)	Right rate (BPM)
control		0.55 ± 0.03	0.67 ± 0.06	305 ± 1.57
Pinacidil	1 min.	0.52 ± 0.03	0.65 ± 0.06	294 ± 2.10
	2 min.	0.51 ± 0.03	0.66 ± 0.05	288 ± 2.22
	3 min.	0.51± 0.03	0.65 ± 0.05	285 ± 3.41
	4 min.	0.50 ± 0.03	0.65 ± 0.04	285 ± 3.75
	5 min.	0.50 ± 0.03	0.65 ± 0.05	285 ± 7.73
quercetin	1 min.	0.53 ± 0.04 *	0.67 ± 0.06 *	289 ± 8.36 *
	2 min.	0.51 ± 0.05 *	0.66 ± 0.04 *	291 ± 7.80 *
	3 min.	0.51 ± 0.05 *	0.65 ± 0.05 *	294 ± 7.78 *
	4 min.	0.52 ± 0.05 *	0.65 ± 0.05 *	295 ± 7.76 *
	5 min.	0.53 ± 0.05 *	0.65 ± 0.05 *	296 ± 8.32 *
	10 min.	0.56 ± 0.05 *	0.67 ± 0.05 *	296 ± 8.65 *
	15 min	0.58 ± 0.05 *	0.67 ± 0.04 *	296 ± 7.56 *

*$p < 0.05$, significantly different from quercetin group in the absence of pinacidil (student paired *t* - test)

Table 19 Effects of naringenin (100 μM) in the presence of pinacidil (100 μM) on rate and force of contraction of right and left atria (n=6) (mean ± SEM).

compound \ time		Left force (g)	Right force (g)	Right rate (BPM)
control		0.55 ± 0.05	0.67 ± 0.03	305 ± 3.20
Pinacidil	1 min.	0.52 ± 0.05	0.65 ± 0.03	294 ± 3.56
	2 min.	0.51 ± 0.05	0.66 ± 0.03	288 ± 3.85
	3 min.	0.51 ± 0.05	0.65 ± 0.03	285 ± 3.65
	4 min.	0.50 ± 0.05	0.65 ± 0.03	285 ± 5.12
	5 min.	0.50 ± 0.05	0.65 ± 0.04	285 ± 6.54
naringenin	1 min.	0.53 ± 0.05	0.67 ± 0.04	289 ± 3.70
	2 min.	0.51 ± 0.04	0.66 ± 0.04	291 ± 3.78
	3 min.	0.51 ± 0.04	0.65 ± 0.04	294 ± 3.49
	4 min.	0.52 ± 0.04	0.65 ± 0.04	295 ± 3.07
	5 min.	0.53 ± 0.04	0.65 ±0.05	296 ± 3.15
	10 min.	0.56 ± 0.04	0.67 ± 0.05	296 ± 2.67
	15 min	0.58 ± 0.04	0.67 ± 0.05	296 ± 2.78

Table 20 Effects of tyramine (10 μM) on rate and force of contraction of right and left atria in normal rats. (n=6) (mean ± SEM).

Time	Left force (g)	Right force (g)	Right rate (BPM)
control	0.42 ± 0.02	0.69 ± 0.02	268 ± 3.95
1 min.	0.60 ± 0.02 *	0.81 ± 0.02 *	356 ± 3.95 *
2 min.	0.59 ± 0.02 *	0.80 ± 0.02 *	372 ± 4.49 *
3 min.	0.58 ± 0.02 *	0.77 ± 0.02 *	382 ± 3.54 *
4 min.	0.55 ± 0.03 *	0.77 ± 0.02 *	386 ± 3.23 *
5 min.	0.55 ± 0.03 *	0.77 ± 0.02 *	389 ± 3.61 *
10 min.	0.53 ± 0.02 *	0.76 ± 0.02 *	389 ± 3.81 *
15 min.	0.52 ± 0.02 *	0.75 ± 0.02 *	392 ± 3.75 *

*$p < 0.05$, significantly different from control group (student unpaired *t* - test)

Table 21 Effects of tyramine (10 μM) on rate and force of contraction of right and left atria in rats were pretreated with reserpine. (n=6)(mean ± SEM).

Time	Left force (g)	Right force (g)	Right rate (BPM)
control	0.51 ±0.04	0.75 ± 0.03	310 ± 3.50
1 min.	0.52 ± 0.04	0.75 ± 0.03	323 ± 3.56
2 min.	0.52 ±0.04	0.75 ± 0.03	333 ± 5.19
3 min.	0.53 ± 0.05	0.75 ± 0.03	332 ± 5.64
4 min.	0.53 ± 0.05	0.75 ± 0.03	330 ± 5.89
5 min.	0.52 ± 0.05	0.75 ± 0.03	332 ± 6.10
10 min.	0.52 ± 0.05	0.74 ± 0.03	334 ± 6.05
15 min.	0.52 ± 0.05	0.72 ± 0.03	333 ± 5.82

Table 22 Effects of quercetin (100 μM) on rate and force of contraction of right and left atria in rats were pretreated with reserpine (n=6) (mean ± SEM).

Time	Left force (g)	Right force (g)	Right rate (BPM)
control	0.50 ± 0.02	0.70 ±0.03	306 ± 3.50
1 min.	0.70 ± 0.02	0.77 ±0.03	313 ± 3.50
2 min.	0.72 ± 0.02	0.80 ± 0.03	320 ± 5.19
3 min.	0.73 ± 0.02	0.82 ± 0.03	294 ±5.64
4 min.	0.72 ± 0.03	0.84 ± 0.03	295 ± 5.89
5 min.	0.72 ± 0.03	0.84 ± 0.03	298 ± 6.10
10 min.	0.72 ± 0.03	0.84 ±0.03	300 ± 6.05
15 min.	0.72 ± 0.03	0.84 ±0.03	306 ± 5.82

CURRICULUM VITAE

Miss Nuchanat Pramakatay was born in August 26, 1979 in Mahasarakham, Thailand. She graduated with a Bachelor of Nursing in 2001 from the Faculty of Nursing, Mahidol University, Thailand. After graduation, she worked as a nurse in Sirisaj Hospital, Thailand, for 2 years.