CHAPTER I

INTRODUCTION

Glutathione (GSH) is the major representative of nonprotein thiols in human body that acts as a reducing agent and an antioxidant (Dickinson and Forman, 2002; Voskoboinik *et al*, 1998). GSH serves as a reservoir for cysteine and participates in detoxification reaction for xenobiotics and metabolism of numerous cellular compounds (Deneke and Fanburg, 1989; Kojda and Harrison, 1999). Depletion of GSH results in perturbation of the nitric oxide (NO) system and severe hypertension which correlate to induction with oxidative stress (OS) (Vaziri *et al.*, 2000). Furthermore, it has been reported that depletion of GSH enhances contraction and attenuates endothelium-dependent relaxation of isolated rat aorta (Ford *et al.*, 2006).

Vascular endothelium has a major regulatory role in maintain homeostasis of the vascular circulation. Endothelium regulates the vascular tone by synthesizing and releasing several vasoactive substances such as nitric oxide (NO), prostacyclin (PGI_2), endothelium-derived hyperpolarizing factor (EDHF), endothelin-1 (ET_1), prostaglandin H_2 (PGH_2) and thromboxane A_2 (TXA_2) (Guerci *et al.*, 2001). Oxidative stress or chemical assaults at the vascular wall targeting either at endothelium or smooth muscle can impair vascular functions (Kojda and Harrison, 1999). It has been well established that endothelial dysfunction is a hallmark of the cardiovascular diseases such as atherosclerosis, essential hypertension, diabetes and heart failure (Mombouli and Vanhoutte, 1999).

One feature of endothelial dysfunction is an impairment of endothelium-dependent vasodilation which can be attributed to a decrease of NO bioavailability (Kalinowski and Malinski, 2004; Forgione *et al.*, 2002). Infusion of exogenous GSH has been reported to improve endothelial dysfunction and NO- mediated vasodilation

(Prasad *et al.*, 1999). It is possible that GSH may directly exert the vasodilatation via activation NO-cGMP mechanism. In addition, it has been hypothesized that GSH may also interact with NO to form S-nitroso-glutathione (GSNO) which is responsible for the vasodilatation effect (Cheung and Schulz, 1997). However, this hypothesis is inconclusive. It has been reported that the mechanism of GSH-induced relaxation in guinea pig trachea was not involved with the formation of GSNO (Kloek *et al.*, 2002). The vasorelaxation may be linked to the antioxidant activity of sulhydryl group of GSH, mediating the redox status of the K^+ channel (Kloek *et al.*, 2002; Lacampagne *et al.*, 1995). Hence, there could be several mechanisms involved with GSH-induced vasorelaxation.

Vasodilatation can be mediated through endothelium-dependent or/and -independent mechanims. Endothelium-dependent vasorelaxation can be caused by three major mechanisms including (1) NO-cGMP pathway, (2) K^+ channel-activated hyperpolarization pathway and (3) metabolites of arachidonic acid pathway (Bachschmid *et al.*, 2005). Endothelium-independent vasorelaxation involves with an alteration of contractility of smooth muscle via either inhibition of Ca^{2+} entry or Ca^{2+} release from the sacroplasmic retriculum (SR) in vascular smooth muscle cells (Kajioka *et al.*, 1991; Carl *et al*, 1996). In addition, the activation of K^+ channel on smooth muscle cell can be one of mechanisms of endothelium-independent vasorelaxation (Kwan *et al.*, 2003).

The change in intracellular Ca^{2+} concentration is very crucial for the regulation of vascular contractility. The vasomotion effects of an increase in intracellular Ca^{2+} in smooth muscle cells are opposite to those in endothelial cells (Wang *et al.*, 1999; Buluc and Yilmaz, 2006). In endothelium-intact vessels, a rising of intracellular Ca^{2+} in endothelium triggers NO synthesis and release, leading to activation of guanylate

cyclase (GC) and vasorelaxation. In contrast, a rising of intracellular Ca^{2+} in smooth muscle cells causes an activation of contractile elements, leading to vasoconstriction. Hence, the direct effects of GSH on modulating vascular tone may be dependent on the presence of endothelial cells.

This research aimed to investigate the mechanism of GSH-induced vasorelaxation in endothelial intact and endothelial denude blood vessels by using the model of isolated rat thoracic aorta. Furthermore, this study elucidated the influence of GSH on alteration of intracellular Ca^{2+} as well as the influence of endothelium on the effect of GSH in modulating vascular tension.

Hypothesis:

Glutathione modulates the vascular tone via endothelium-dependent and endothelium-independent pathways. In endothelium-dependent pathway, GSH may target at endothelium and exerts its vasorelaxation effects via NO-cGMP pathway. In addition, GSH may also affect the activation of K^+ channel or Ca^{2+} entry and mobilization in vascular smooth muscle, resulting in a change in vascular tension.

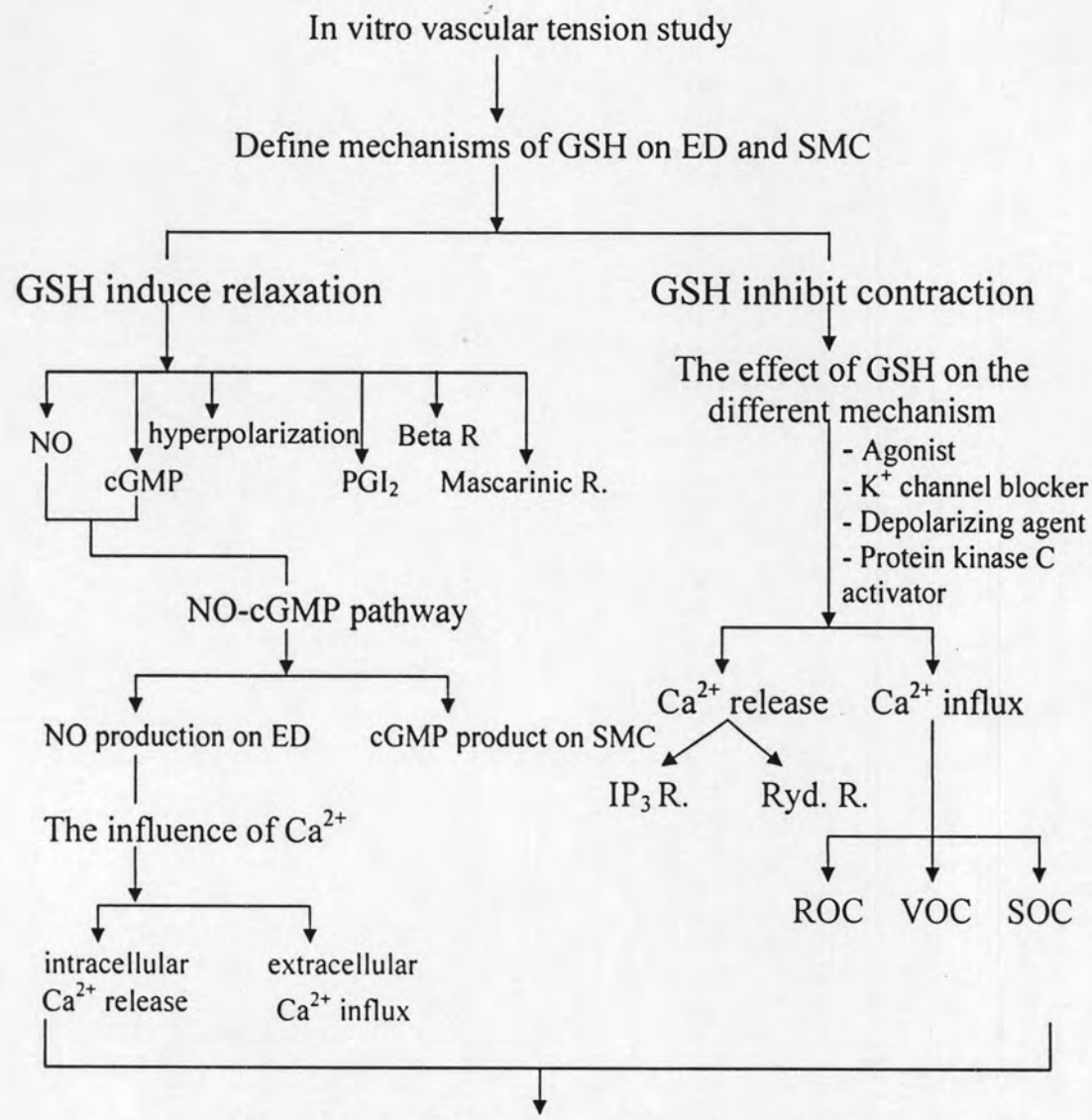
Objectives:

1. To investigate the potential effects of GSH in modulating vascular tension.
2. To investigate the mechanisms of GSH-induced endothelium-dependent and -independent relaxation pathway.
3. To investigate the effects of GSH on the Ca^{2+} storage within the cell and the Ca^{2+} entry, which may involve with the mechanisms of receptor operated Ca^{2+} channels (ROC), voltage-gated Ca^{2+} channels (VOC) and store-operated Ca^{2+} channels (SOC) on smooth muscle cell.

Significances:

This research will elucidate the direct effect of GSH on modulation of vascular tone via mechanisms other than antioxidant. These data provide better understanding about effect of GSH and its mechanisms of action which may be applied for its use to improve endothelium dysfunction in cardiovascular disease such as hypertension and atherosclerosis.

Conceptual Framework



- Study the effect of thiol compounds including GSH, N-acetylcysteine, homocysteine and captropril, using L-valine as a control group
- Comparing the relationship of Ca^{2+} and vascular tone on endothelium and smooth muscle cell

IP_3 R.: inositol 1, 4, 5-triphosphate receptor

Ryd R.: ryanodine receptor

ED: endothelium

SMC: smooth muscle cell

ROC: receptor-operated Ca^{2+} channel

VOC: voltage-operated Ca^{2+} channel

SOC: store- operated Ca^{2+} channel