CHAPTER II

LITERATURE REVIEWS

Glutathione (GSH) (Fig. 1) is the endogenous antioxidant which was found in the millimolar range in most cells (Dickinson *et al.*, 2002). GSH can be recycled from its oxidized state (GSSG, glutathione disulfide) to its reduced state (GSH, glutathione) (Deneke and Fanburg, 1989; Ford *et al.*, 2006). Intracellular GSH has an important role in protection endothelial cells from oxygen free radicals, leading to prevention against endothelial dysfunction (Kugiyama *et al.*, 1998). Dysfunction of the vascular endothelium in a number of cardiovascular disorders has been associated with a descrease in bioavailability of vasodilator nitric oxide (NO) (Kojda and Harrison, 1999; Ford *et al*, 2006). Furthermore, GSH has been reported to improve endothelial dysfunction and increase NO bioavailability. It is possible that GSH interact with NO to generate S-nitrosoglutathione (GSNO) adduct. In addition, formation of GSNO has been hypothesized to be a mean of NO-storage because its half-life is considerably longer than that of NO (Prasad *et al.*, 1999; Kugiyama *et al.*, 1998). However, there were several studies demonstrated that GSNO caused vascular relaxation through mechanisms unrelated to NO production. For example, GSNO was able to induce relaxation in denude rat aorta by activation of K_{ATP} and K_{Ca} channel (Ceron *et al.*, 2001). Moreover, GSNO is a potent airway smooth muscle relaxant with at least its partial effect occurrs through stimulation of cGMP (Jansen *et al.*, 1992). GSH has been shown its ability to relax several smooth muscles including guinea pig trachea and thoracic aorta (Kloek *et al.*, 2002; Jansen *et al.*, 1992; Ceron *et al.*, 2001). The proposed mechanisms of GSH on vasorelaxation through formation of GSNO were inconclusive. Moreover, it was possible

that the effect of GSH were mediated via its antioxidant action rather than activation of NO production or formation of GSNO (Kloek *et al.*, 2002).

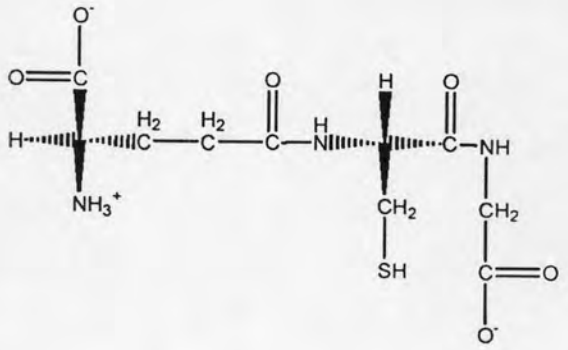

Fig.1 Structure of Glutathione (Dickinson *et al.*, 2003)

GSH has one cysteine in its molecular structure to play important role in antioxidant defense, protein folding and signal transduction (Dickson and Forman 2002). The sulfhydryl group on cysteine portion of thiol compounds may be important to direct its several actions on smooth muscle including the reduction of disulfide bonds of receptors, ion channels, or enzymes (Fujioka *et al.*, 1993; Cheung and Schulz, 1997). Furthermore, the thiol compounds could interact with cysteinyl residue within basic regions of the channel protein on K_{Ca} channel (Cai and Sauve, 1997; Lang and Harvey, 2002). This modulation may be the effect of GSH on the activation of membrane K^+ channel in airway smooth muscle cell (Kloek *et al*, 2002). Alteration of ion channel via sulfhydryl modification could affect the activity of these channels directly (Lacampagne *et al.*, 1995). In addition, a change in cellular redox status might also affect channel function (Fearon *et al.*, 1999). An alteration of the K^+ current leads to a change in the membrane potential of the vascular smooth muscle cell. Consequently, this could influence Ca^{2+} entry to the cells. Moreover, it was reported that K^+ current was regulated

by an oxidation and reduction reaction on cysteine residue on the cytoplasmic side of the membrane (Ha *et al.*, 2000).

Supplementation of exogenous GSH by intraperitoneal or intravenous injection has been demonstrated a limited and controversial effect on increasing GSH level in the tissue (Ramires and Ji, 2001). Although extracellular GSH is not effectively transported into the cells, addition of exogenous GSH is shown to cause substantial increase of intracellular GSH and cysteine concentration in cultured endothelial cells and in humans (Kugiyama *et al*, 1998). There were several studies on the effects of exogenous GSH and GSH monoethyl ester (GSH-Mee) on the enhancement of endothelial GSH concentration. For example, both GSH and GSH-Mee markedly increased the intracellular concentration of GSH in endothelial cells, and GSH-Mee was more potent than GSH (Tsan *et al.*, 1989). It has been reported that exogenous GSH and GSSG could induce coronary vasodilation (Chueng and Schulz, 1997). This effect involved with a NO and soluble guanylate cyclase dependent mechanism. Moreover, it has been suggested that the mechanism of GSH may also involve with the reaction between GSH and peroxynitrite to form GSNO which is an NO donor and possibly increases bioavailability of NO (Chueng and Schulz, 1997; Prasad *et al.*, 1999; Kloek *et al*, 2002). Furthermore, exogenous GSH could attenuate coronary constriction of acetylcholine (Ach) in patients with coronary spastic angina, whereas it had no significant effect in control subjects (Kugiyama *et al.*, 2001). In spontaneous hypertensive rat (SHR), administration of GSH improved vasorelaxation of isolated rat aorta possibly via endothelium-dependent mechanisms. By contrast, GSH effects were not seen in aortic ring isolated from Wistar- Kyota rat (WKY) (Akpaffiong and Taylor, 1998). Hence, GSH has been shown to improve vascular

endothelium function in certain condition. In addition, the influence of GSH on the endothelium-dependent relaxation may vary depending on the degree of endothelium impairment.

Control of vascular smooth muscle tone

Vascular smooth muscle tone is controlled by balance between the cellular signaling pathways that mediate the generation of tension and the release of tension. (Woodrum and Brophy, 2001).

The contraction of smooth muscle starts with an increase of intracellular Ca^{2+} from Ca^{2+} influx through membrane Ca^{2+} channels and from Ca^{2+} release from SR. The free cytosolic Ca^{2+} binds to a special calcium binding protein called calmodulin. The Ca^{2+}-calmodulin complex activates myosin light chain kinase (MLCK), an enzyme that is capable of phosphorylation myosin light chain (MLC) in the presence of ATP (Hill *et al.*, 2001). The phosphorylation of MLC leads to cross-bridge formation between the myosin heads and the actin filaments to induce smooth muscle contraction. Relaxation is usually initiated by a decrease in intracellular Ca^{2+}, leading to dephosphorylation of the myosin light chain by myosin phosphatase. In addition to the MLCK system contraction of smooth muscle can be regulated not only by the Ca^{2+} / calmodulin /myosin light chain kinase system but also by modulation of Ca^{2+} sensitivity such as changing concentrations of freely diffusible calmodulin (CaM), inhibition of phosphatase (PPase) activity from receptor agonist or phorbal esters, thin filament (Caldesmon and Calponin) regulation and myosin light chain phosphorylation elicited by rho kinase (Karaki and Hori , 1998).

Ion channel and vascular tone

The contractile activity of vascular smooth muscle cells is the major determinant of the resistance to blood flow through the circulation. Regulation of the contractile activity of vascular smooth muscle cells in the systemic circulation depends on a complex interplay of vasodilator and vasoconstrictor stimuli from circulating hormones, neurotransmitters, endothelium-derived factors, and blood pressure. Vascular smooth muscle uses Ca^{2+} as the trigger for contraction. Influx of Ca^{2+} through membrane Ca^{2+} channels and release of Ca^{2+} from intracellular stores are the major sources of intracellular Ca^{2+}. An alteration of membrane potential, along with increase of intracellular Ca^{2+} concentration, lead to Ca^{2+} entry through membrane Ca^{2+} channels, and consequently to provoke the tissue contraction (Jackson, 2000).

Regulation of vascular tone by K^+ channels and voltage-gated Ca^{2+} channels

Potassium channels are the ion conductive pathways in vascular muscle cells. The opening of K^+ channels results in diffusion of this ion out of the cells, causing membrane hyperpolarization. Voltage-gated Ca^{2+}channels play a central role in controlling Ca^{2+} entry into the cells. Its activity depends on an alteration of membrane potential. Hyperpolarization closes these Ca^{2+} channels, leading to vasodilatation. By contrast, depolarization results in an opening of the Ca^{2+} channels, leading to vasoconstriction (Fig. 2). A change in membrane potential dose not only regulate voltage-gated Ca^{2+} channels, but also influence the Ca^{2+} sensitivity of the contractile apparatus (Jackson, 2000).

The K^+ channels in the microcirculation, as in other vascular muscles are classified 4 different types of K^+ channels (Fig. 3) expressed in vascular smooth muscle.

They are ATP-sensitive $K^+(K_{ATP})$ channels, large-conductance Ca^{2+}- activated K^+(BKca) channels, voltage-activated K^+(Kv) channels, and inward rectifier $K^+(K_{IR})$ channels (Jackson, 2000). The activation of these K^+ channels lead to membrane hyperpolarization followed by closure of voltage-gated Ca^{2+} channels. Consequently, Ca^{2+} entry is quenched.

Voltage-gated Ca^{2+}channels are modulated by protein kinase C pathway. The vasodilators that stimulated production of cAMP and activated protein kinase A have been reported to both activate and inhibit these Ca^{2+} channels. Voltage-gated Ca^{2+} channels are inhibited by increases in intracellular Ca^{2+} and activation of cGMP-dependent protein kinase (Jackson, 2000; Garland *et al.*, 1995).

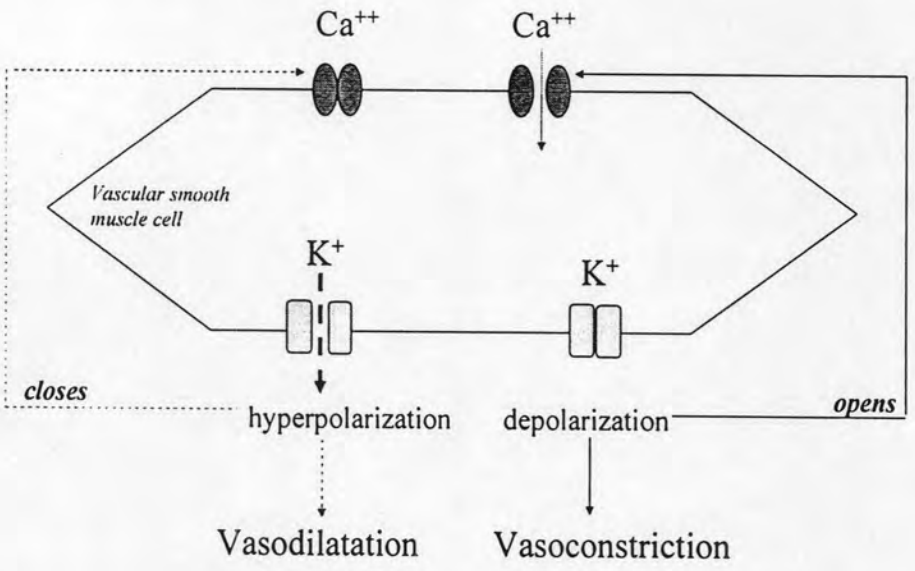

Fig. 2 Regulation of vascular tone by K^+ channels and voltage-gated Ca^{2+} channels. The opening K^+ channels leads to diffusion of K^+ ions out of the cell, membrane hyperpolarization, closure of voltage-gated Ca^{2+} channels, decreased intracellular Ca^{2+}, which leads to vasodilatation. Closure of K^+ channels has the opposite effect, which lead to vasoconstriction (Adapted from Jackson, 2000).

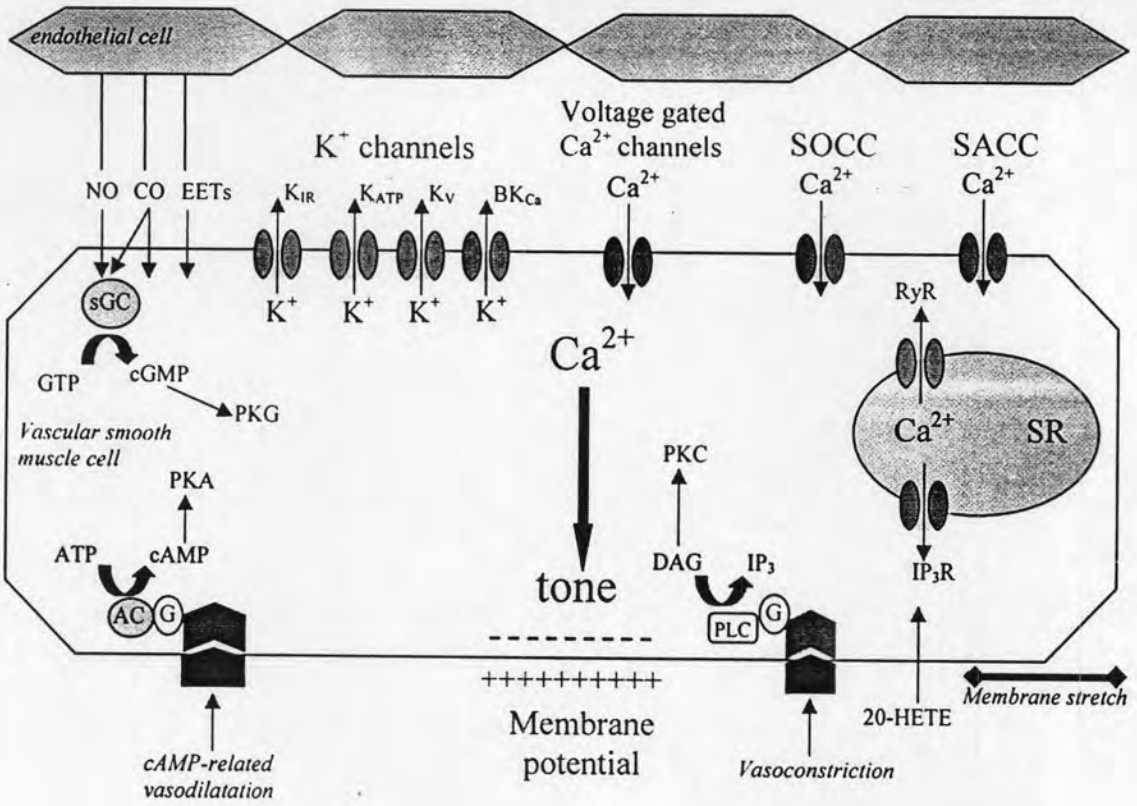

Fig. 3 Ion channels and vascular tone. Schematic of a cross section through part of a vascular muscle cell. The outer membrane are shown K_{IR}, K_{ATP}, K_V, and BK_{Ca} channels, voltage-gated Ca^{2+} channels, SOC channels (SOCC) and SAC channels (SACC). The membranes of the sarcoplasmic reticulum (SR) are ryanodine receptors (RyR) and inositol 1,4,5-trisphosphate receptors (IP_3R). The signals that are known to modulate the function of the ion channels depicted. AC indicates adenylate cyclase; PKA, cAMPdependent protein kinase; sGC, soluble guanylate cyclase; PKG, cGMP-dependent protein kinase; EETs, epoxyeicostetraenoic acid; PLC,phospholipase C; DAG, diacylglycerol; PKC=protein kinase C; and 20-HETE, 20-OH-arachidonic acid (Adapted from Jackson, 2000).

Receptor-operated calcium channels (ROCCs)

In addition to an alteration of membrane potential, activation of certain receptors could lead to calcium entry into smooth muscle cells. These receptors are G-protein-coupled receptors with an essential role to link receptor and cation channels. Several

agonists such as serotonin, epinephrine and histamine activate phospholipase C to generate IP_3 and DAG which initiate signal transduction cascade involving protein kinase. IP_3 can trigger the Ca^{2+} release from SR and provoke transient contraction (McFadzean and Gibson, 2002). Increase in intracellular Ca^{2+}, in turn, caused an open of membrane Ca^{2+} channel, resulting in Ca^{2+} entry. Consequently, the contraction can be sustained for longer period (Fig. 3).

Store-operated and stretch-activated Ca^{2+} channels

In addition to Ca^{2+} entry through permeable Ca^{2+} channel, intracellular Ca^{2+} increased through store-operated Ca^{2+} channels (SOC) (Beech, 2002) and stretch-activated Ca^{2+} channels (SAC) (Jackson,2000) (Fig.3). SOC channels can be activated when intracellular calcium stores are empty. Another type of Ca^{2+} channel which can be activated by mechanic stretch which is called stretch-activated Ca^{2+} channel. Studies in isolated porcine coronary cells have provided evidence for SAC channels permeable to Ca^{2+} in response to stretch-induced depolarization of these vascular muscle cells. Consequently an influx of Ca^{2+} is sufficient to increase intracellular Ca^{2+} (Jackson, 2000).

Role of nitric oxide and other endothelium-derived factors

The endothelium regulate the underlying smooth muscle layer and vascular tone by releasing endothelium-derived relaxing factors (EDRF) such as nitric oxide (NO), prostaglandins, and endothelium-derived hyperpolarizing factor (EDHF) as well as vasoconstricting factors such as endothelin, superoxide (O_2^-), and thromboxane. The main receptor for NO is guanylyl cyclase in smooth muscle cell, leading to formation of smooth muscle cyclic guanosinmonophosphate (cGMP) and vascular relaxation. EDHF is an endothelium-derived factor causing vasorelaxation of the underlying smooth muscle

layer by membrane hyperpolarization. The nature of EDHF is still unknown, but several candidates for EDHF have been proposed such as potassium ions, hydrogen peroxide, and epoxyeicosatrienoic acids (Stankevicius *et al.*, 2003).

Prostacyclin and hyperpolarization of vascular smooth muscle

Prostaglandins such as prostacyclin and prostaglandin E_2 bind to specific receptors, followed by increases in cyclic adenosinmonophosphate (cAMP) and vasorelaxation. Certain prostaglandins constrict the vessels by activation of thromboxane and endoperoxidase receptors (Stankevicius *et al.*, 2003) (Fig.4).

The hyperpolarization signals of vascular smooth muscle included exogenous prostacyclin and the components of endothelium-derived hyperpolarization sensitive to blockers of cyclooxygenase (COX). Hyperpolarization is associated with an increase in membrane conductance of which current reverse is closed to the equilibrium potential for potassium. Prostacyclin is also capable of relaxing vascular smooth via the mechanisms independent of changes in membrane potential such as reducing cytoplasmic calcium and suppressing the sensitivity of the contractile apparatus to calcium. In addition, hyperpolarization may play an additional role in relaxing muscle. Interaction between prostacyclin and its receptor in the plasma membrane of vascular smooth muscle leads to activation of adenylyl cyclase and an increase in the production of cAMP which further activates protein kinase A, resulting in phosphorylation of selected target proteins and vascular relaxation (Bachschmid *et al.*, 2005).

Release of arachidonic acid from membrane-bound phospholipids by phospholipases is the basis of endothelial prostanoid production (Fig. 4). Arachidonic acid is metabolized by the major enzymes systems. They are Lipoxygenase gives rise to

lipoxides, which are mainly vasoconstrictor. Epoxygenases, various isoforms of cytochrome P450, give rise to a variety of products with intrinsic ability to modulate vascular tone. In particular, the epoxyeicosatrienoic acid deriatives (EETs) and the hydroxyeicosatrienoic acid derivatives (HETEs) are vasoconstrictors or, importantly, form the basis of the vasodilation attributed to EDHF in some vessels (Parkington *et al.*, 2004)

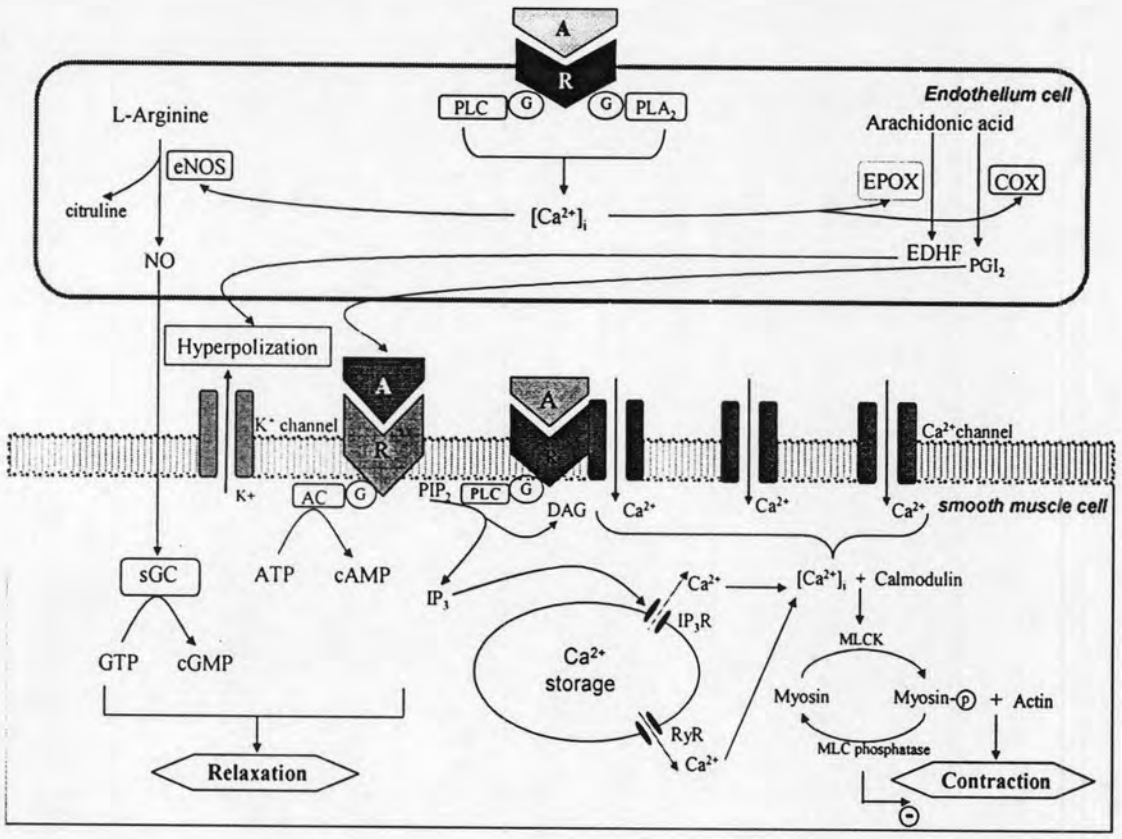

Fig. 4 The signaling pathways in regulation of vascular tone. Abbreviations: A, agonist; AC, adenylyl cyclase; EPOX, epoxygenase; COX, cycloxygenase; eNOS, NO synthase; G, G-protein; R, receptor; P, protein phosphorylation; PGI_2 , prostacyclin; PIP_2, phosphatidylinositol-4,5 bisphosphate; PLA_2, phospholipase A_2; PLC, phospholipase C; DAG, diacylglyceral; IP_3, inositol 1,4,5-trophosphate; Ry, ryanodine; sGC, soluble guanylyl cyclase.