CHAPTER I

INTRODUCTION

Review Literature

Adrenomedullin (AM) is a novel hypotensive peptide that was recently isolated and identified from human phaeochromocytoma arising from the adrenal medulla (Kitamura et al., 1993a; Kitamura et al., 1993b). The peptide consists of 52 amino acids, and has one intramolecular disulfide bond.

Tyr-Arg-Gln-Ser-Met-Asn-Phe-Gln-Gly-Leu-Arg-Ser-Phe-Gly-Cys-Arg-Phe-Gly-Thr-Cys-Thr-Val-Gln-Lys-Leu-Ala-His-Gln-Ile-Tyr-Gln-Phe-Thr-Asp-Lys-Asp-Lys-Asp-Asn-Val-Ala-Pro-Arg-Ser-Lys-Ile-Ser-Pro-Gln-Gly-Tyr-NH_2

(Disulphide bond between Cys^{16} and Cys^{21})

At present, structures of AM in several species have been characterized. The ring structure and carboxy terminal amide structure, which are essential for biological activity of AM, are well conserved between species. Sequence analysis of cloned human AM cDNA showed that human AM precursor is 185 amino acids in length, including a putative signal peptide (Kitamura et al., 1993b). AM shows homology in chemical structure with the sensory nerve-derived vasodilator, calcitonin gene-related peptide (CGRP), one of the most potent vasodilator known. They share a six residue ring structure formed by an intramolecular disulphide linkage and a slight

homology in the C-terminal amide sequence (Kitamura et al., 1993a; Kitamura et al., 1993b).

Although many studies confirm the vasodilator action of AM, its physiological mechanism is still not clear. This effect has been reported to be either a direct action on the smooth muscle cells (Eguchi et al., 1994; Ishizaka et al., 1994) or to be mediated by endothelium-derived nitric oxide (Feng et al., 1994; Fiscus et al., 1994). The potent hemodynamic actions of this peptide together with its high circulating plasma levels, suggest that it may be an important hormone in regulating the cardiovascular system (Kitamura et al., 1993a; Kitamura et al., 1994). Several biological effects including natriuretic activity (Jougasaki et al., 1995) and inhibition of aldosterone secretion (Yamaguchi et al., 1994; Maggocchi et al., 1996) have also been elucidated. Accumulating evidences have tended to indicate that, besides its role in cardiovascular system, AM is also important in regulation of other systems. Northern blot analysis revealed that AM mRNA is expressed not only in the adrenal medulla, cultured endothelial cells, aorta and cultured vascular smooth muscle cells (VSMCs), but also in various organs including heart, lung, kidney, stomach, intestine, thyroid gland and cerebral cortex (Sakata et al., 1993; Ichiki et al., 1994). Other physiological actions of AM are attracting a number of researchers. These actions include bronchodilation, renal regulation, hormone regulation, and cerebrovascular dilatation. The pathophysiological actions of AM are also of interested.

Effect of adrenomedullin on cardiovascular system

It has been found that AM is pressent in a considerable concentration in human plasma (Kitamura et al., 1993a; Kitamura et al., 1994). AM circulates in the plasma of healthy humans at a concentration sufficiently high (**19±5.4 fmol/ml**) to suggest a role for this peptide in blood pressure regulation. The source of AM in plasma is unclear. Nishikimi et al (1994) observed no significant increase in the concentration of the peptide in venous drainage from various organs, including the adrenals, as compared with arterial levels. Sugo et al (1994) demonstrated secretion of AM from both endothelial cells and VSMCs. Endothelial cells secreted AM at a rate 5.8 times higher than VSMCs. The synthesized AM from endothelial cells was not stored but constitutively secreted (Isumi et al., 1998). VSMCs expressing AM mRNA at levels 40-fold higher than that in the adrenal medulla (Sugo et al., 1994). The level of gene transcription of AM in the cultured endothelial cells was 20 times higher than that of adrenal gland in the rat (Isumi et al., 1998). Together with the discovery of specific receptors on endothelial cells and VSMCs (Eguchi et al., 1994; Kato et al., 1995), these findings raise the possibility that the peptide may function as a paracrine and/or autocrine factor as well as a circulating hormone. In addition, AM was shown to exert an antiproliferative action on endothelial and mesangial cells and was suggested its role as a local modulator of endothelial and mesangial functions (Michibata et al., 1998). Interestingly, Kato and his colleagues (1997) demonstrated a role of AM as an apoptosis survival factor for rat endothelial cells.

The predicted sequence of human AM is 52 amino acids. Porcine AM is identical to the human isoform except a single base replacement of Gly for Asn at position 40 (Kitamura et al., 1994). Rat AM is shorter than human AM in which consisting of 50 rather than 52 amino acids (Sakata et al., 1993; Kitamura et al., 1993b). However, intravenous injections of both AM isoforms have been shown to dramatically decrease systemic arterial pressure in the adult rat (Kitamura et al., 1993; Perret et al., 1993) and in the newborn piglet (DeVito et al., 1995), and to decrease vascular resistance in the preconstricted pulmonary vascular bed of the intact cat (Lippton et al, 1994). Other data affirm that these effects are produced with little or no concomitant alterations in heart rate or in cardiac output (Perret et al., 1993; DeVito et al., 1995), and that these hemodynamic properties of AM are highly conserved among species (Hao et al., 1994).

AM caused a rapid-onset and long-lasting depressing response in the anaesthetized rat (Kitamura et al., 1993a; Sakata et al., 1993) due to peripheral vasodilation (Ishiyama et al., 1993). The studies in conscious animals (He et al., 1995; Parkes DG, 1995) showed that AM had a potent and sustained vasodilating action. This peptide can reduce blood pressure and promote large increases in both cardiac output and heart rate. The data suggested that AM might have both direct and indirect actions on the heart to increase cardiac function, and the overall result of elevated plasma AM is to reduce cardiac afterload. Tsuruda et al. (1998) demonstrated that cultured neonatal rat cardiomyocytes produced and secreted AM. The secreated AM inhibited the protein synthesis of these cells. Thus AM may act on cardiomyocytes as a factor modulating the cardiac growth. In addition, AM was also shown to exert its mitogenic activity via protein tyrosine-kinase mediated mitogen-activated protein kinase activation in quiescent rat

vascular smooth muscle cells (Iwasaki et al., 1998). Both AM and binding sites for this peptide have been found in cardiac tissue, indicating the possible existence of an autocrine or paracrine system of AM in the heart (Ichiki et al., 1994). Furthermore, Nishikimi and his coworkers (1998) demonstrated that both cardiac myocytes and nonmyocytes of the ventricle of neonatal Wistar rats secrete almost equal amounts of AM and that AM increases cAMP levels possibly via different receptors in myocytes and nonmyocytes.

The role of AM as a vasoactive agent has been extensively studied. The vasodilating action of AM is believed to be mediated by increasing levels of adenosine 3',5'-cyclic monophosphate (cAMP) in VSMCs (Eguchi et al., 1994a, b; Shimekake et al., 1995; Yoshimoto et al., 1998) in a manner similar to CGRP (Kubota et al., 1985). There are evidences suggesting that AM and CGRP may activate the same receptors in the vasculature (Eguchi et al., 1994; Ishizaka et al., 1994). Two subtypes of CGRP receptors have been identified, $CGRP_1$ receptor and $CGRP_2$ receptor. The CGRP receptor antagonist, CGRP[8-37], which possesses a high affinity for $CGRP_1$ receptor has been shown to block the AM-induced elevation of cAMP level in rat cultured VSMCs (Eguchi et al., 1994; Ishizaka et al., 1994). CGRP [8-37] also attenuated the vasodilator response of AM in the perfused mesenteric artery of the rat (Nuki et al., 1993). However, the study of Champion et al. (1997) suggested that vasodilating responses to CGRP and AM were mediated by different receptors. Furthermore, the study by Nandha et al. (1996) suggested that the hypotensive effect of AM might be mediated via specific AM binding sites. In addition, the study of Okamura et al. (1997) concluded that AM had an ability to inhibit adrenergic neuronal transmission by $CGRP_1$ receptors independent mechanism in the peripheral vasculature.

This inhibition partly participated in potent hypotensive action of AM. Taken together, these studies suggested different mechanisms between species and organs as well as the regional variations in adrenomedullin receptor specificity.

The direct effect of AM on isolated arteries has been demonstrated by Nakamura et al. (1995). They demonstrated that AM can produced concentration dependent relaxation of basilar, mesenteric, coronary, renal and femoral arteries. The relaxing effects were slightly greater in endothelium-intact arteries than those in denuded ones. The study in isolated canine central retinal arteries showed that AM-induced relaxation was endothelium-independent (Okamura et al., 1997). This endothelium-independent relaxation to AM may be mediated primarily by intracellular cAMP by stimulation of $CGRP_1$ receptors and partially by cGMP since cGMP was unlikely to be produced by methylene blue-sensitive soluble guanylate cyclase. Prostanoids, NO, and ATP-sensitive potassium channels opening did not appear to be involved in the AM-induced relaxation in isolated canine central retinal arteries. It has also been observed that physiological effects of AM are somehow connected with those produced by nitric oxide (Feng et al., 1994; Nossaman et al., 1996; Hayakawa et al., 1999). This relationship may be due to the cross-talk in the target cell between the signal transduction pathways for AM, which increases cAMP, and those for nitric oxide, which increase cGMP.

In addition to arterial vasodilating effect, AM was also shown to cause the relaxation in veins (Barber et al., 1997). In the experiment, rings of canine femoral vein with and without endothelium were suspended in organ chambers for measurement of isometric force. The result showed that AM

produced concentration-dependent relaxation only in veins with endothelium. The effect was not associated with activation of CGRP receptors or AM receptors. Further, relaxation are not mediated by NO, indomethacin-sensitive prostanoids, tetraethylammonium-sensitive hyperpolarizing factors, oxygen free radicals, or accumulation of cyclic nucleotides.

Effect of adrenomedullin on pulmonary system

Immunohistochemical studies demonstrated the presence of AM in human lung. In addition, a Northern blot analysis indicated that rat AM mRNA was expressed in many organs including lung (Kitamura et al., 1993b). The lung also expresses AM receptors on endothelial and vascular smooth muscle cells (Lin et al., 1994; Kapas et al., 1995; Kato et al., 1995). The study by Heaton et al. (1995) showed that AM dilated the pulmonary vascular bed in isolated rat lung by a mechanism that did not depend on cyclooxygenase products, endothelium-derived relaxing factor, serotoninergic receptors, angiotensin receptor, ATP-sensitive potassium channel, and CGRP receptor. Nossaman et al. (1996) showed that AM was threefold more potent in decreasing pulmonary vascular resistance in the cat than in the rat. They also found that the vasodilating effect was dependent on the release of nitric oxide in the rat but not in the cat. They suggested that responses to the peptide were mediated by different mechanisms in the pulmonary vascular bed of these two animals.

Yang and his coworkers (1996) investigated the effect of AM on rat pulmonary arterial rings under normoxic and hypoxic conditions. During normoxia, AM caused a concentration-dependent relaxation of preconstricted pulmonary arterial rings which was abolished by the NO synthesis inhibitor

and by deendothelialization. During hypoxia, AM produced the modest relaxation which was abolished by pretreatment with indomethacin, an inhibitor of cyclooxygenase pathway. In isolated main bronchi and pulmonary artery of guinea-pig, AM was demonstrated to be a potent vasodilator of the pulmonary artery without any bronchomotor effect (Pinto et al., 1996). This vasorelaxant actions of AM were not mediated via the activation of $CGRP_1$ receptors.

Recently, the study in fetal sheep showed that AM decreased the main pulmonary arterial pressure and increased the left pulmonary arterial blood flow (de Vroomen et al., 1997; Takahashi et al., 1999). AM-induced increase in pulmonary arterial blood flow of fetal sheep was suggested to depend largely on NO release and partly on ATP-sensitive potassium channels activation and does not involve CGRP receptors or cyclooxygenase-mediated mechanisms (Takahashi et al., 1999).

Effcet of adrenomedullin on renal function

The study by Jougasaki et al. (1995) demonstrated the presence of AM in the glomeruli, cortical distal tubules and medullary collecting duct cells of the canine kidney. The study also demonstrated that intrarenal administration of AM resulted in a significant natriuretic and diuretic responses in normal dogs. This natriuresis is associated with increases in glomerular filtration rate (GFR) and fractional sodium excretion as well as a decrease in distal tubular sodium reabsorption. These data suggest that AM is an important natriuretic peptide, which may play a physiological role in the regulation of sodium excretion as well as in the regulation of renal vascular tone. The current study extended previous report and demonstrated that the

natriuresis mediated by AM was completely abolished by the inhibition of prostaglandin synthesis since AM-mediated increased in glomerular filtration rate and decreased in distal tubular sodium reabsorption were blocked by meclofenamate, an inhibitor of prostaglandin synthesis (Tougasaki et al., 1997). This study demonstrated an important mechanistic role for the renal prostaglandin system as a mediator of AM-mediated natriuresis at the level of the glomerulus and terminal nephron.

In addition, intrarenal infusion of AM resulted in an increase in urinary cAMP excretion. Thus, the inhibition of increase in glomerular filtration rate and decrease in distal tubular sodium reabsorption by meclofenamate was associated with an inhibition of second messenger cAMP (Eguchi et al., 1994; Ishigaka et al., 1994). This observation may suggest that the increase in urinary cAMP excretion with AM could be the result of AM-mediated release of prostaglandin E_2 in the medullary collecting duct (Teitebaum I, 1992). Renal arterial infusion of non-hypotensive doses of AM into anaesthetized rats produced renal vasodilation, increased GFR, diuresis and increased absolute but not fractional sodium excretion. The peptide did not affect potassium excretion or urine osmolality (Elhawary et al., 1995). These efects are shown not to be mediated via the action of $CGRP_1$ receptors (Elhawary et al., 1995; Heynes and Cooper, 1995). The study by Osajima et al. (1995) suggested that the receptors for AM were preferentially expressed in renal tubular basolateral membranes and AM increased cAMP in renal tubular cells.

The videomicroscope analysis revealed that AM increased the diameters of both afferent and efferent arterioles. In the isolated perfused rat kidney, AM decreased renal vascular resistance (Hirata et al., 1995). This

vasodilatation was associated with a dose-dependent increase in NO release, which was measured with a chemiluminescence method. The study by Miural et al. (1995) suggested that AM has an arginine-derived NO-mediated renal vasodilator action in anaesthetized dogs. The effect does not involve the glibenclamide-sensitive potassium channel. Very recently, Hayakawa and his colleagues (1999) examined the effects of a cGMP-specific phosphodiesterase inhibitor on vasorelaxation induced by AM in renal vessels of rats. The result suggeted that the NO-cGMP pathway is involved in the mechanism of AM-induced vasorelaxation in rat kidney. The expression of AM mRNA in porcine renal arterial smooth muscle cells suggests an important role of AM in the regulation of the renal vascular tone, not only as a circulating hormone, but also as an autocrine transmitter (Segusshi et al., 1995).

Effect of adrenomedullin on cerebral circulation

Plasma and tissue concentrations of AM appear to increase in several disease states including brain ischemia (Wang et al., 1995). Studies of vascular rings *in vitro* suggested heterogeneous sensitivity to AM, with greater responses in canine basilar and mesenteric arteries than in renal, coronary, and femoral arteries (Nakamura et al., 1995). In contrast, another study reported only modest relaxation to AM in isolated dog basilar and middle cerebral arteries (Baskaya et al., 1995). AM exhibited profound vasodilating effects on dog vertebral and basilar arteries *in vivo* which was inhibited by the CGRP receptor antagonist (Baskaya et al., 1995). The study also ruled out the possibility that AM produced cerebral vasodilation by a mechanism that involved production of nitric oxide or vascular prostanoids. Lang et al. (1997) demonstrated that AM produced substantial dilatation of

cerebral arterioles and such effect was mediated by activation of $CGRP_1$ receptors. Activation of both ATP-sensitive and calcium-dependent potassium channels appeared to be important in mediating microvascular responses to AM. However, the precise physiological role of AM in the cerebral circulation remains uncertain because of the lack of specific pharmacological probes that separate the effects of AM from CGRP.

Effect of adrenomedullin on hormone secretion

Adrenal medulla is one of the major production sites of AM. The investigation in the interaction of AM wth adrenal cortical steroidogenesis showed the possibility that AM is a novel inhibitory peptide of aldosterone secretion (Yamaguchi et al., 1995). These investigators reported that AM specifically inhibits angiotensin II-stimulated aldosterone production but does not affect basal or ACTH-stimulated one. Mazzocchi et al. (1996) demonstrated that the inhibitory effect was mediated by CGRP receptor. However, autoradiography showed the presence of abundant [125I] AM binding sites in the zona glomerulosa cells of human adrenals (Belloni et al., 1998), which was concluded to be AM (22-52)-sensitive receptors. The aldosterone antisecretagogue acitons of AM (1-52) were shown to be counterated by AM (22-52). The six-membrane ring structure and C-terminal, but not N-terminal, amino-acid sequence are both seem to be essential for AM (1-52) to exert its antimineralocorticoid action. The C-terminal sequence is probably needed for AM (1-52) to bind its zona glomerulosa receptors, while the ring structure is required for the receptor activation (Belloni et al., 1998).

In addition, AM has been reported to influence the secretion rate of several hormones including catecholamine (Kato et al., 1995) and ACTH (Samson et al., 1995). The study by in situ hybridization analysis, showed that AM and its receptor is homogeneously distributed throughout the islets of Langerhans (Martine et al., 1996). The experimental data on isolated rat islets clearly demonstrated the inhibitory role of AM on insulin secretion in a dose dependent manner. However, there is another report where stimulation of insulin was observed (Mulder et al., 1996). These studies may implicate AM as a newly defined factor of the insulin regulatory system that could be involved in disorders such as diabetes and obesity. There is still a controversy in the studies dealing with the action of AM on secretion of many hormones. However, many studies showed that AM is a potent regulator of hormone secretion. Further studies on the receptor systems affected by AM and signal transduction pathways involved are necessary in order to fully understand its precise role in regulating normal and pathological processes.

Pathophysiological action of adrenomedullin

Plasma and tissue concentrations of AM appear to be altered in several disease states, including hypertension, renal failure, congestive heart failure (CHF), cirrhosis, endotoxin shock, diabetes, and brain ischemia (Ishimitsu et al., 1994; Jougasaki et al., 1995; Shiji et al., 1995; Sugo et al., 1995; Wang et al., 1995; Kohno et al., 1996; Guevara et al., 1998). Plasma concentration of AM in patients with essential hypertension, renal failure and cirrhosis is found to be significantly increased in relation to severity of the disease when compared to those in control patients (Kitamura et al., 1994). AM has also been suggested to participate in the pathophysiology of salt dependent

hypertension in rat and played a role in cardiac hypertrophy (Shimokubo et al., 1996). The plasma levels of AM are increased in patients with CHF (Ikeda et al., 1996; Jougasaki et al., 1996; Kato et al., 1996). Tissue levels of AM peptide and mRNA have been shown to be increased in the heart, kidney and lungs of heart failure rat (Nishikimi et al., 1997). Jougasaki and his coworkers (1996) demonstrated that the failing human heart secretes AM and results in the increase in plasma AM. The finding indicates an additional endocrine system of cardiac origin which is activated in human CHF. The increased plasma AM in CHF is associated with fluid retention in these patients (Kato et al., 1996). Considering its vasodilating and/or natriuretic actions, plasma AM may play a defensive role against further deterioration of CHF. In addition, AM reduced ventricular preload and afterload and improved cardiac output with CHF.

Despite the decrease in arterial pressure, AM increased creatinine clearance and sodium excretion and maintained urine output (Rademaker et al., 1997). The study by Nakaya and his colleagues (1999) revealed that intravenous infusion of AM exerted diuresis and natriuresis without inducing hypotension, and in the higher dose, produced beneficial hemodynamic and renal vasodilator effects in rats with compensated heart failure. The investigation of the pathophysiological significance of AM in patients with acute myocardial infarction indicated that plasma AM increased in the early phase of acute myocardial infarction (Yoshitomi et al., 1998). The increase in plasma AM was in proportion with clinical severity (Miyao et al., 1998). The volume expansion may be one of the additional stimuli for the release of AM in patients with acute myocardial infarction complicated by CHF (Yoshitomi et al., 1998). These results imply an important pathophysiological role of AM in the regulation of pressure and volume in

heart failure and raise the possibility of a new therapeutic approach to the treatment of this disease.

Various hemodynamic changes are observed in thyrotoxicosis, including an increase in cardiac output and heart rate with a concomitant decrease in peripheral vascular resistance (Taniyama et al., 1996). The plasma concentration of AM was elevated in hyperthyroid patients. The correlation between the plasma AM and serum free thyroid hormone levels was marginally significant. The mean blood pressure was relatively low while the plsma AM level was elevated. AM may therefore be responsible for the vasodilatation observed in thyrotoxicosis (Taniyama et al., 1996).

Increased in plasma AM and the expresion of AM mRNA has been observed in response to focal cerebral ischemia (Wang et al., 1995). The potency of this novel peptide, as well as recent report describing increased expression after cerebral ischemia, implies the potenital for an important role of AM in cerebrovascular physiology or pathophysiology.

Plasma AM concentration in hyperglycemic patients was found to be significantly increased. The rise in plasma AM is believed to be due to a hyperglycemia-induced increase of AM expression in the vasculature, which may provide a link between hyperglycemia and alteraction of vascular function (Hayashi et al., 1997). In patients with non-insulin dependent diabetes mellitus, the plasma adrenomedullin increased dependent on the severity of diabetic nephropathy and retinopathy (Nakamura et al., 1998). Up to now, the physiological and pathophysiological actions of AM have been studied by groups of researcher. However, the actions of AM in some topics are still unclear and remain to be determined. There is a tendency that many

researches on AM will be done and the unclear actions are probably be elucidated. A better understanding of the interactions of AM in normal physiology and in different pathological states, such as congestive heart failure, hypertension, renal failure, and diabetes, may help define new areas of therapeutic intervention to obliterate these disorders.

Adrenomedullin and adenosine 3′, 5′ -cyclic monophosphate

Adenosine 3′, 5′ -cyclic monophosphate (cAMP) is a second messenger for many hormones and many tissue factors. One of the means by which hormones and the tissue factors exert intracellular actions is to cause the second messenger, cAMP, to be formed inside the cell membrane. Then the cAMP in turn causes all or most of the intracellular effect. Figure 1.1 shows the function of the cAMP mechanism in more detail. The stimulating factor first binds with a specific receptor for that factors on the membrane surface of the target cell. The specificity of the receptor determines which factor will affect the target cell. After binding with the membrane receptor, the portion of the receptor that protrudes to the interior of the cell membrane is activated to become the protein enzyme adenyl cyclase. This enzyme in turn causes immediate conversion of a small amount of the cytoplasmic adenosine triphosphate into cAMP.

Once cAMP is formed inside the cell, it activates other enzymes. In fact, it usually activates a cascade of enzymes. That is, a first enzyme is activated, which then activates another enzymes, which activates still a third, and so forth. The importance of this mechanism is that only a few molecules of activated adenyl cyclase immediately inside the cell membrane can cause many more molecules of the next enzyme to be activated, which can cause

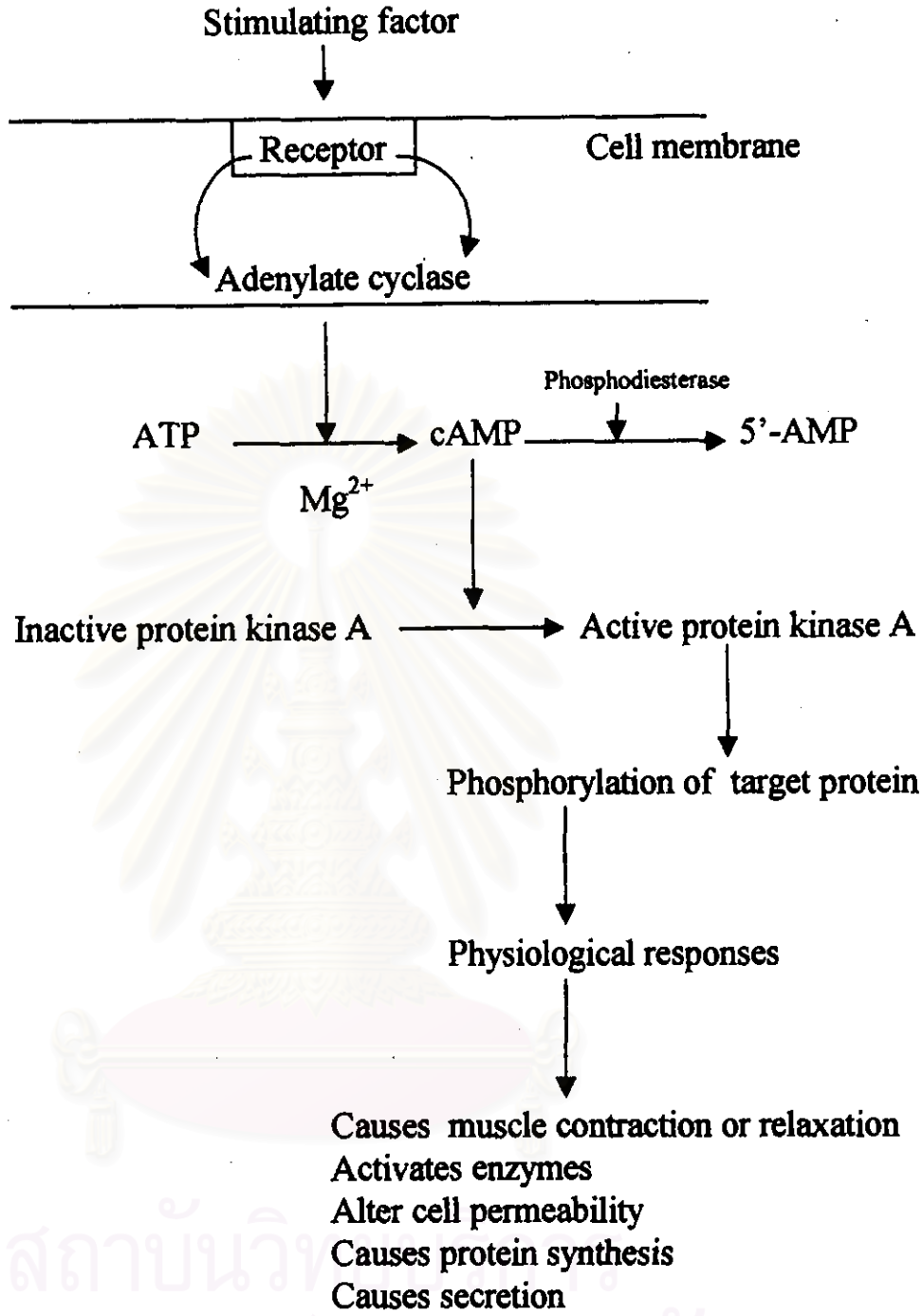

Figure 1.1 Cyclic 3',5'-adenosine monophosphate (cAMP) mechanism

still many times that many molecules of the third enzymes to be activated, and so forth. In this way, even the slightest amount of factors acting on the cell surface can initiate a powerful cascading activating force for the entire cell. The specific action that occurs in response to cAMP in each type of target cell depends on the nature of the intracellular machinery, some cells having one set of enzymes and other cells having other enzymes. Therefore, different functions are elicited in different target cells-such functions as initiating synthesis of specific intracellular chemicals, causing muscle contraction or relaxation, initiating secretion by the cells, and altering the cell permeability.

AM was initially discovered by monitoring the ability of human pheochromocytoma extracts to increase platelet cAMP levels (Kitamura et al., 1993a). The structure of AM shows some homology with calcitonin gene related peptide (CGRP) (Kitamura et al., 1993a), which is known to be a potent vasorelaxant (Brain et al., 1985; Shoji et al., 1987). The hypotensive effects of AM and CGRP were demonstrated to be due to the vasodilation mediated via the increase in the intracellular cAMP (Kubota et al., 1985; Eguchi et al., 1994). Both AM and CGRP have been shown to increase cAMP in various tissues, including smooth muscle cells (Kitamura et al., 1993a, b; Ishizaka et al., 1994; Eguchi et al., 1994a, b; Shimekake et al., 1995), which is thought to lead to a subsequent vasorelaxation. Previous studies have shown that the elevation of cAMP induced by AM was antagonized by CGRP(8-37) in rat cultured smooth muscle cells (Eguchi et al., 1994a; Ishizaka et al., 1994), suggesting that AM and CGRP share the $CGRP_1$ receptor. However, in other tissues, like human umbilical vein endothelial cells and rat aorta cells, CGRP(8-37) failed to antagonize the elevation of cAMP induced by AM (Kato et al., 1995; Yoshimoto et al.,

1998). These results are consistent with previous *in vivo* and *in vitro* studies (Kato et al., 1995; Heaton et al., 1995; Edward et al., 1996; Nandha et al., 1996; Pinto et al., 1996; Champion et al., 1997). These investigations imply the presence of subtype of the AM receptor. Accumulation of cAMP in smooth muscle leads to an inhibition of contraction by deceasing $[Ca^{2+}]_i$ and Ca^{2+} sensitivity of contraction elements of smooth muscle (Karaki H 1989; Karaki et al., 1997). The study by Yoshimoto and his colleagues (1998) showed that AM act directly on smooth muscle of porcine coronary artery, increased cAMP and decreased both $[Ca^{2+}]_i$ and muscle tension at the resting or contracted muscle. It is likely that AM relaxes the porcine coronary artery by increasing cAMP, resulting in decreases in $[Ca^{2+}]_i$ and Ca^{2+} sensitivity.

In addition to the direct effect on vascular smooth muscle to promote the cAMP formation, it has also been demonstrated that AM increases cAMP more potently than CGRP in human vascular endothelial cells (Kato et al., 1995). Although the relaxant potencies of AM and CGRP were comparable, CGRP increased endothelial $[Ca^{2+}]_i$ almost twice as much as AM in rat aorta (Yoshimoto et al., 1998). In general, agonist-induced activation of NO synthase requires an elevation of endothelial $[Ca^{2+}]_i$ (Moncada et al., 1991). In addition, Gray and Marshall (1992 b,c) have suggesed that the elevation of endothelial cAMP induced by CGRP and other cAMP elevating agents, such as β-adrenoceptor agonists and forskolin, results in activation of NO synthase. It is possible, therefore, that AM release NO from the endothelium by increasing both endothelial cAMP and $[Ca^{2+}]_i$ resulting in the relaxation of rat aorta (Yoshimoto et al., 1998).

Jougasaki et al. (1995) localized AM-like immunoreactivity to glomeruli, cortical distal tubules, and medullary collecting tubules of dog

kidney. They also found that intrarenal administration of AM produced increases in glomerular filtration rate and fractional sodium excretion. AM was shown to modulate the renal tubular functions presumably by increasing cAMP (Osajima et al., 1995). The study by Edwards and his coworkers (1996) demonstrated that AM produced concentration-dependent increase in cAMP levels in both the glomerulus and distal convoluted tubule. Although additional studies are needed to identify the cell type within the glomerulus that is the target for AM, other agonists that increase glomerular cAMP have generally been shown to induce mesangial cell relaxation, which may alter the filtration characteristics of the glomerulus (Kreisburg and Hassid, 1986). In dogs, intrarenal administration of a low dose of AM that had no effect on renal blood flow or glomerular filtration rate decreased distal fractional reabsorption of sodium without affecting proximal reabsorption (Jougasaki et al., 1995). This latter observation is particularly significant, in view of a distal but not a proximal effect of AM on cAMP levels.

AM immunoreactivity can be detected in the plasma (Nishikimi et al., 1994) and is widely distributed in various normal tissues, including the heart (Sakata et al., 1994). Expression of mRNA for AM has also been demonstrated in these tissues (Nuki et al., 1993; Kitamura et al., 1993b). Furthermore, abundant binding sites also exist in the ventricular tissue (Owji et al., 1995). These findings suggest that AM could be a locally acting hormone rather than a systemic agent. Thus, AM may exert its effects on the heart via autocrine or paracrine mechanism. Although the cardiac actions of AM have not been extensively examined, Perret and his coworkers (1993) showed that it had a negative inotropic effect in the isolated perfused heart. The signal transduction system for AM in myocytes remains unclear. Cyclic

AMP has been suggested as a second messenger for AM in a variety of cultured cells, including smooth muscle cells (Eguchi et al., 1994; Ishizaka et al., 1994), endothelial cells (Shimikake et al., 1995) and glomerular mesangial cells (Chimi et al., 1995). Similarly, AM has been reported to stimulate cAMP formation in isolated cardiac myocytes (Ikeda et al., 1996; Sato et al., 1997). These observations suggest that activation of the adenylate cyclase-cAMP system, which is one of the major pathways for the regulation of cardiac contractility in the mammatian heart (Morgan JP, 1991) may also mediate the cardiac effects of AM.

However, recent findings that AM inhibits adrenocorticotropin secretion in anterior pituitary cells (Samson et al., 1995), and increases intracellular Ca^{2+} levels in aortic endothelial cells (Shimekake et al., 1995) independently of its effect on cAMP suggest the involvement of other signal transduction pathways in mediating the effects of AM, at least in some tissues. Furthermore, the study in adult rabbit ventricular myocytes revealed a negative inotropic effect of AM (Ikenouchi et al, 1997). The result showed the significant increase in intracellular content of cGMP, a second messenger of NO, but not that of cAMP. This study indicated that such an AM-NO-cGMP system may be responsible for the effects of this peptide on cardiac myocytes.

Adrenomedullin and the vascular endothelium

The endothelium is a confluent monolayer of thin, flattened, rhomboid-shaped cells lining the intimal surface of all blood vessels and, thus, is situated at the vital interface between the circulating blood and the body's tissues. Endothelial cells are multifunctional cells playing a key role

in the local control of vascular tone (Furchgott and Vanhoutte, 1989). They can sense changes in their mechanical, chemical, and humoral environments, process these signals, and respond by the synthesis and release of a vast number of factors (Davies and Hagen, 1993). Perhaps the greatest advances in endothelial physiology has been the discovery and characterization of endothelium vasoactive factors. In general, seven families of compounds have been associated with endothelium mediated vasomotor responses : Prostanoids, NO and NO containing compounds, oxygen free radicals, endothelins, angiotensin, endothelium-derived hyperpolarizing factor (EDHF), and other uncharacterized endothelium-derived constriction factors (Vanhoutte PM, 1988) (Figure 1.2 and Figure 1.3). In practice, endothelial cells receive and transform signals coming from the blood, and then transmit them to vascular smooth muscle cells to regulate the degree of contraction. Several factors produced by endothelial cells have been reported to regulate vascular smooth muscle cells functions.

It has been demonstrated that endothelial cells actively synthesize and secrete AM, a novel vasorelaxant peptide (Kitamura et al., 1993). Gene transcription levels of AM in cultured rat endothelial cells are about 20 times higher than that of adrenal gland and rat endothelial cells secretes AM at a rate comparable with that of endothelin-1 (Sugo et al., 1994a; Sugo et al., 1994b). The secretion rate of AM from endothelial cells is about 5 times higher than that of vascular smooth muscle cells. Furthermore, AM specific receptors coupled with an adenylate cyclase have been shown to be expressed on endothelial cells (Kato et al., 1995; Shimekake et al., 1995). These findings suggested that AM secreted from endothelial cells plays an important role in the regulation of vascular tone. A number of studies have established the important role of the vascular endothelium in mediation of

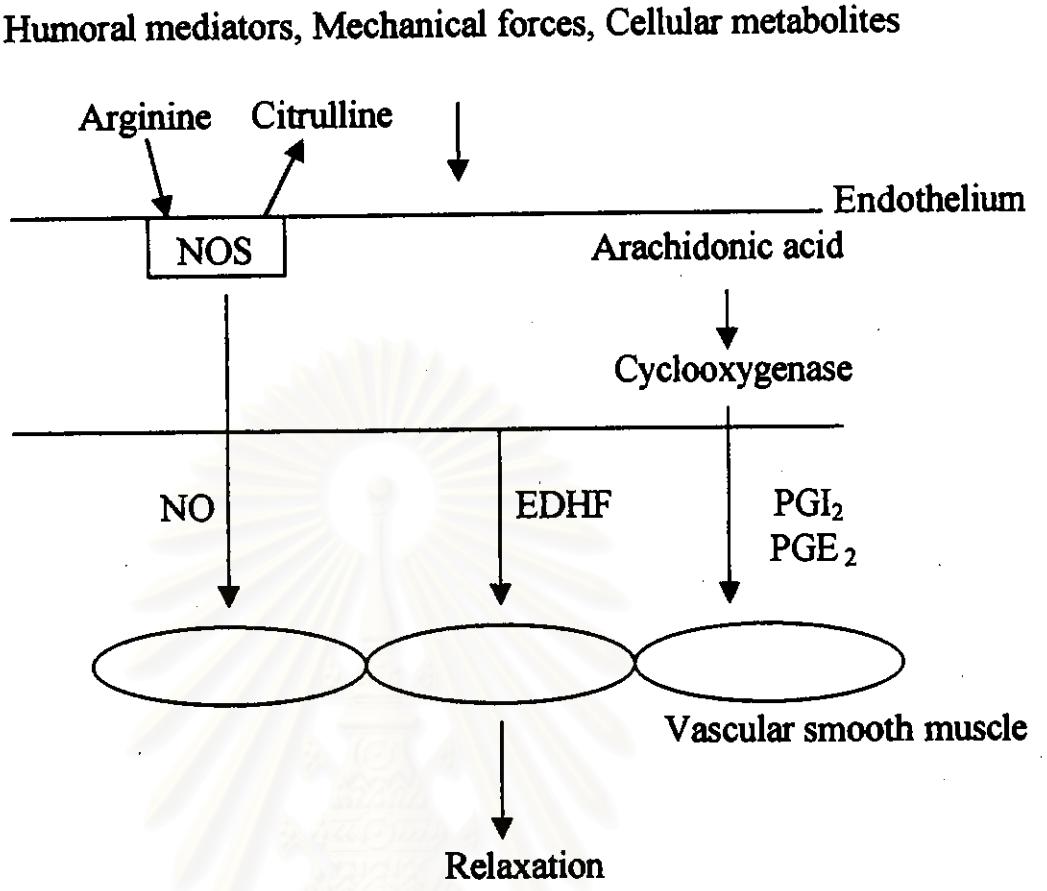

Figure 1.2 Endothelium-derived relaxation. Relaxation is mediated by nitric oxide (NO), prostacyclin (PGI_2) and prostaglandin E (PGE_2), and endothelium-derived hyperpolarizing factor (EDHF). Endothelium-derived relaxation can be influenced by alterations in local, physical, chemical,humoral, and cellular factors.

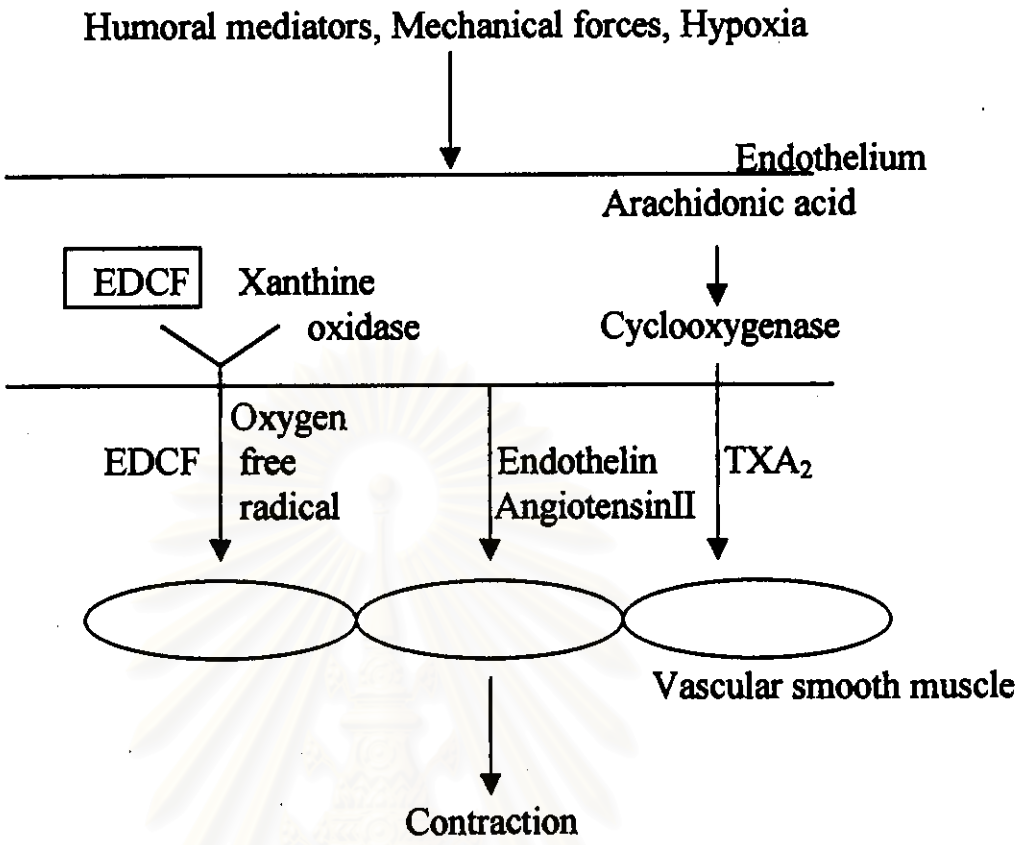

Figure 1.3 Endothelium-derived contraction. Vasocontraction can be induced by a reduction in the release of vasorelaxant factors and by thromboxane A_2 , oxygen free radicals, angiotensin II, endothelins, and endothelium-derived contracting factors (EDCFs). Endothelium-derived contraction can be influenced by alterations in local, physical, chemical,humoral, and cellular factors.

relaxation to various vasoactive substance including AM. However, the controversy as to whether AM-induced relaxation is endothelium-dependent or not still persists.

Nakamura and his colleagues (1995) studied the vasodilating effects of AM in basilar, mesenteric, coronary, renal and femoral arteries isolated from the dog. The result showed that AM induced concentration-dependent relaxation of arteries with and without endothelium, and the relaxing effects were slightly greater in endothelium-intact arteries than in denuded one. The study suggested that a minor component of AM-induced relaxation may be mediated by endothelium-derived relaxing factors. The study in pulmonary vascular bed of rats also demonstrated that the vasorelaxant response to AM on rat pulmonary arterial rings was inhibited by endothelium removal (Gumuse et al., 1998). The data revealed that AM dilated the pulmonary vascular bed of the rat by activating AM receptors on the endothelium and promoting the release of NO and cGMP-dependent K^+ channel activation. Furthermore, the relaxant effects of AM in rat thoracic aortic rings contracted with norepinephrine were abolished by removal of endothelium (Yoshimoto et al., 1998). The results also suggested that the rat aortic endothelium seems to express the AM-specific receptor, and that AM increases the endothelial $[Ca^{2+}]_i$, activates NO synthase and releases NO, without a direct action of smooth muscle. More recently, the study in the same model of rat aortic rings indicated that AM relaxed the aorta preconstricted with phenylephrine in a dose-dependent manner, and denudation of endothelium attenuated the vasodilatory action of AM (Hayakawa et al., 1999). This finding supported that AM-induced vasorelaxation is, at least in part, endothelium-dependent.

However, conflicting data exist on the role of endothelium in mediation of vasorelaxation induced by AM. In isolated canine retinal arteries, changes in isometric tension to AM were recorded in helical strips of the arteries with and without the endothelium (Okamura et al., 1997). The AM-induced relaxation was shown to be endothelium-independent and unaffeced by indomethacin, a cyclooxygenase pathway inhibitor; N^G-nitro-L-arginine, a NO synthase inhibitor; and glibenclamide, K_{ATP} channels blocker. In addition, the study in porcine coronary artery demonstrated that the peptide induced endothelium-independent relaxation (Yoshimoto et al., 1998). Their investigations demonstrated that AM seem to act directly on smooth muscle, increases cAMP content, decreases the smooth muscle $[Ca^{2+}]_i$ resulting in vasodilatation.

These findings raise the possibility that the endothelium-dependency or-independency of the action of AM depends on differences in vascular beds.

Adrenomedullin and prostanoids

Vane and associates (1976) reported that vascular endothelial cells could synthesize and release an I series prostaglandin, prostacyclin (PGI_2), which was subsequently shown to be a potent platelet antiaggregator, a vasodilator, and a profibrinolytic agent (Armstrong et al., 1978). Thus, it conveys both a thromboresistant and vasodilatory property to the endothelium.

PGI_2 acts on smooth muscle cells through receptor-mediated activation of adenylate cyclase. In smooth muscle cells, increased cAMP concentration

activates intracellular kinases and brings about relaxation. PGI_2 is the major product of arachidonic acid in large vessels; however, the generation of PGE_2 and PGD_2 takes precedence over PGI_2 production in tissue culture (Furchgott and Vanhoutte, 1989). With PGI_2 production, endothelial cells also generate a small amout of thromboxane A_2 (TXA_2), a proaggregating vasoconstrictor (Figure 1.4). A variety of other eicosanoids, such as monohydroxy-, dihydroxy-, and epoxy- derivatives of arachidonic acid that are formed by the cyclooxygenase, lipooxygenase, and cytochrome P450 dependent mono-oxygenation pathways, also influence vascular tone (Vanhoutte PM, 1988; Furchgott and Vanhoutte, 1989). A number of studies have established that the vascular endothelium has an important role in the mediation of relaxation to various vasoactive substance via prostaglandins and other endothelium-derived factors. However, the involvement of prostaglandins in the action of AM is still controversial. It has been shown that AM dose-dependently increased intracellular cAMP concentration in cultured rat vascular smooth muscle (Eguchi et al., 1994; Ishizaka et al., 1994). Whereas, cyclooxygenase inhibitor, indomethacin, failed to inhibit the stimulatory effect by AM. Therefore, it is suggested that AM-induced cAMP formation in vascular smooth muscle cells is not due to a prostanoid-dependent mechanism but directly mediated by specific AM receptors functionally coupled to adenylate cyclase (Eguchi et al., 1994). The study in isolated canine retinal arteries also showed that AM-induced relaxation was unaffected by indomethacin (Okamura et al., 1997). Furthermore, the endothelium-dependent relaxations induced by AM in rat aorta were attenuated by NO synthase inhibitor but not by indomethacin (Yoshimoto et al., 1998). In addition, AM dilating effect on the rat pulmonary vascular bed was also shown to be independent of cyclooxygenase product (Heaton et al., 1995; Gumusel et al., 1998).

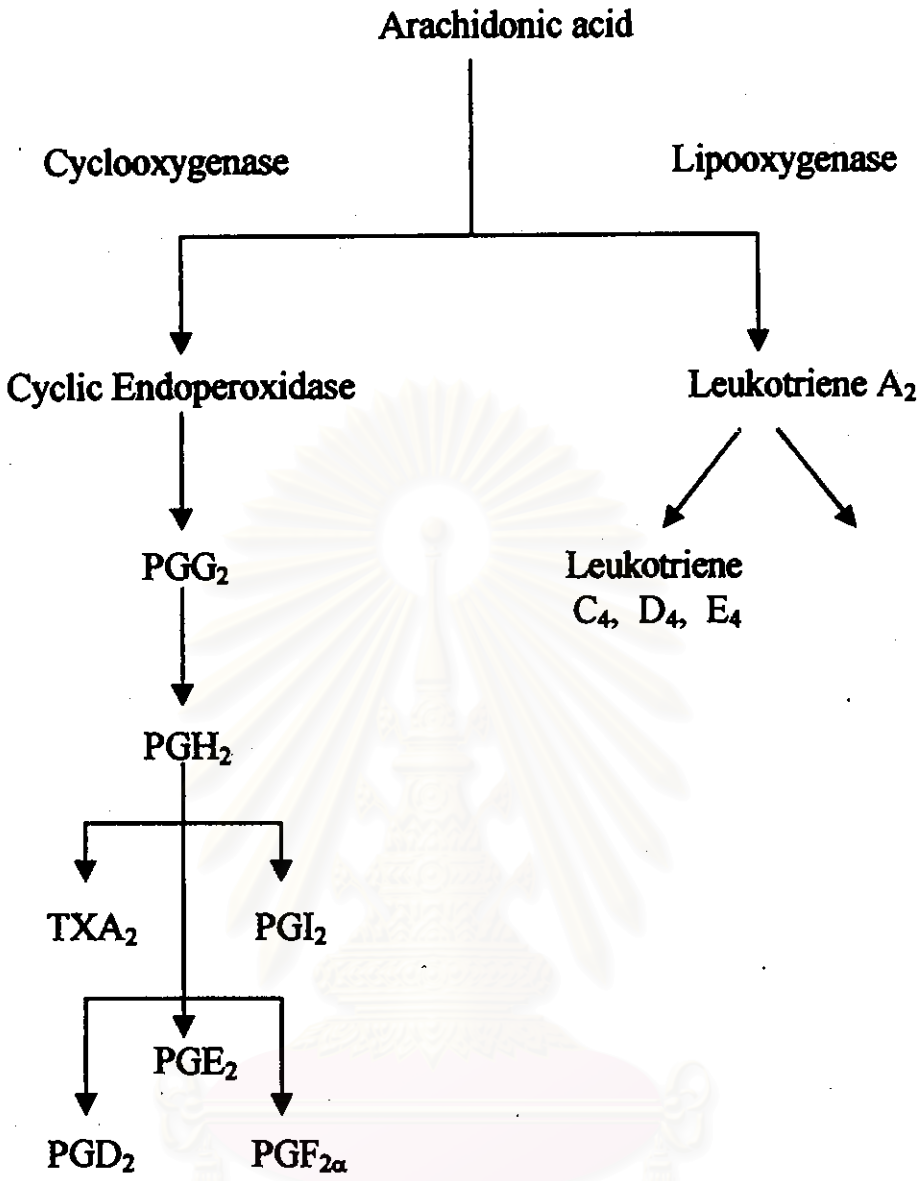

Figure 1.4 Prostaglandin synthesis in the vascular wall. Endothelial cells produce eicosanoids from arachidonic acid through the cyclooxygenase , peroxidase, and lipooxygenase enzyme pathways. In general, vasodilation is mediated by prostacyclin (PGI_2) and by prostaglandin E (PGE_2). Vasoconstriction can be produced by thromboxane A_2 (TXA_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and several leukotrienes. The balance of prostaglandin production in the vessel wall is towards vasodilatory products.

However, the vasodilator effect of AM on hypoxic pulmonary artery was revealed to mediate through an indomethacin-sensitive pathway (Yang et al., 1996). Jougasaki and his colleagues (1995) have reported that AM is present in glomeruli, cortical distal tubules and medullary collecting duct of the kidney and when infused intrarenally, results in renal vasodilatation and increases urinary sodium excretion. This natriuretic action is associated with increases in glomerular filtration rate and decreases in distal tubular sodium reabsorption. Recently, the renal prostaglandin was shown to play an important role as a mediator of AM-mediated natriuresis at the level of the glomerulus and terminal nephron (Jougasaki et al., 1997).

Adrenomedullin and nitric oxide pathway

Nitric oxide (NO) is a major mediator of endothelium-dependent relaxations in various vascular bed (Furchgott RF, 1983; Moncada et al., 1991). In biological systems, NO is generated from L-arginine. The enzymes that responsible for NO synthesis are NO synthase (NOS). NOS activity has been reported in many tissues, including endothelium, vascular smooth muscle, myocardium, brain, peripheral nerves, macrophages, neutrophils and microglia of several species (Knowles and Moncada, 1994; Forstermann et al., 1994). NOS is demonstrated to exist for at least three isoforms (Knowles and Moncada, 1994). Endothelial NOS (eNOS) and neuronal NOS (nNOS) are constitutive, Ca^{2+}/calmodulin-dependent and release NO from endothelium and neurons, respectively. The third isoform is inducible NOS (iNOS) and generally Ca^{2+} independent. Inducible NOS releases NO from macrophages, astrocytes, microglia and vascular smooth muscle cells for long period and in large amounts.

NO is a small lipophilic gas molecule with an ultrashort half-life, in the range of 5-30 second in biological tissues. Because of a very small lipophilic molecule, NO can rapidly diffuse through biological membrane barriers and thereby reach the intracellular compartments of nearby cells. It is generally accepted that NO and exogenous nitrovasodilators relax smooth muscle cells by activation of the soluble form of guanylate cyclase resulting in accumulation of guanosine 3′, 5′- cyclic monophosphate (cGMP) (Figure 1.5) (Ignarro and Kadowitz, 1985; Moncada et al., 1991), and activation of a cGMP-dependent protein kinase. This mechanism can stimulate vasorelaxation through several mechanisms that decrease intracellular calcium levels. However, the precise mechanisms underlying vascular smooth muscle relaxation via NO and cGMP still remain to be defined. In recent years, electrophysiological and pharmacological studies demonstrated an important role of K^+ channels in the hyperpolarization and relaxations of smooth muscle cells (Brayden JE, 1990; Cohen and Vanhoutte, 1995; Nelson and Quayle, 1995). Several studies have suggested that native endothelium-derived relaxing factor/ NO and NO liberated from nitrovasodilators can activate K^+ channels in blood vessels. Although NO reportedly activates large-conductance K_{Ca} through cGMP-dependent protein kinase (Williams et al., 1988; Robertson et al., 1993; Archer et al., 1994), it has been also demonstrated that NO itself can directly (independently of cGMP) activate K_{Ca} in rabbit aortic smooth muscle cells (Bolotina et al., 1994). This investigation suggested that in addition to the direct effect of NO on vascular smooth muscle cells, it is possible that endothelium-derived NO potentiates or stimulates other receptors or channels to promote further relaxation in response to NO.

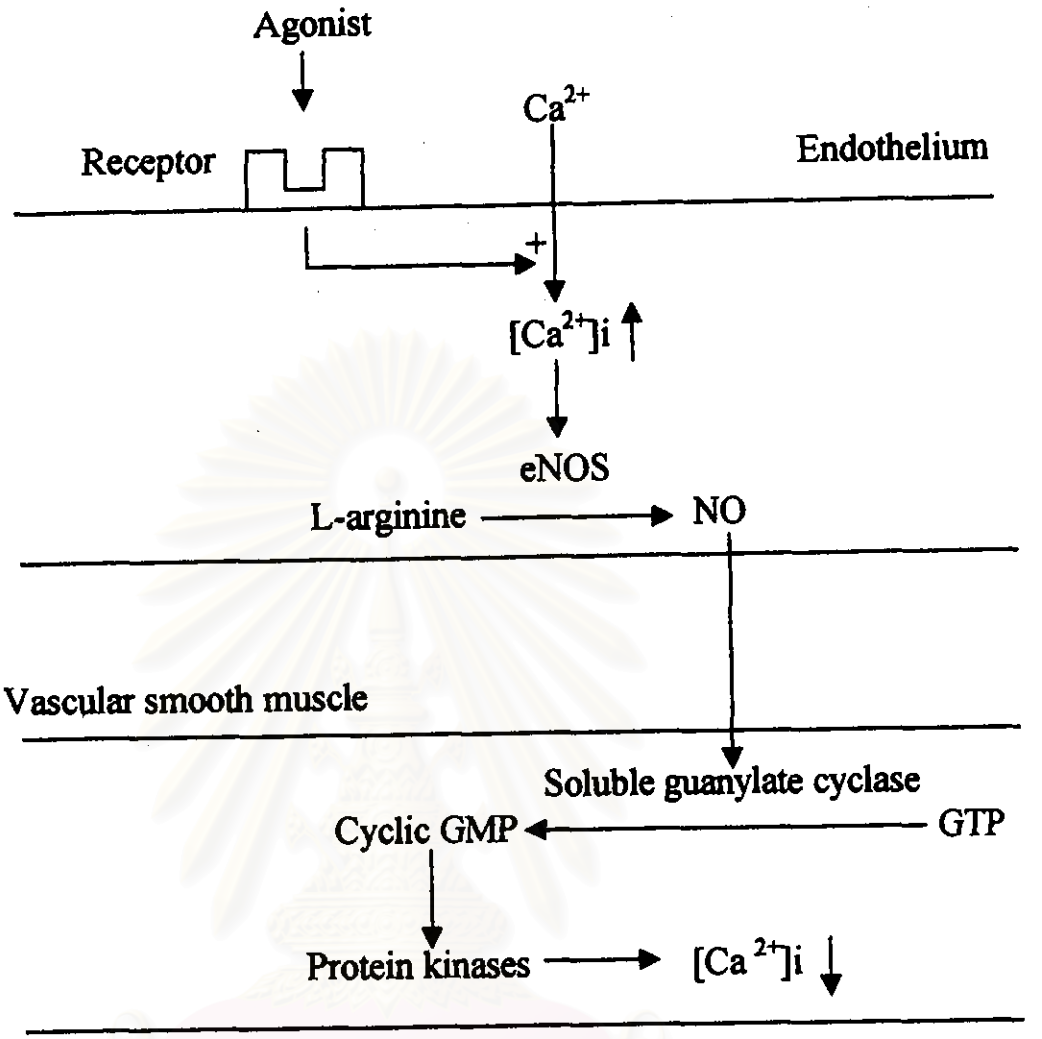

Figure 1.5 Endothelium-derived synthesis of NO. Endothelium nitric oxide synthase (eNOS) is stimulated by an increase in intracellular calcium. This increase may be caused by shear stress and receptor stimulation (e.g. histamine, bradykinin, substance P, and acetylcholine). NO diffuses from endothelial cells to smooth muscle cells and activates the soluble guanylate cyclase. This in turn leads to an increase in cyclic guanosine monophosphate (cGMP) and via activation of protein kinases and subsequent poorly understood intermediately processes stimulates the membrane bound Ca^{2+} ATPase . Ca^{2+} then diffuses out of the cell, eventually leading to smooth muscle relaxation and vasodilation.

NO-cGMP pathway is demonstrated to involve in the mechanism of many vasoactive peptides. Adrenomedullin, a potent vasodilator peptide, is reported to increase intracellular cAMP in cultured vascular smooth muscle cells (Eguchi et al., 1994; Ishizaka et al., 1994) and mesangial cells (Kohno et al., 1995). It is well established that the increase in intracellular cAMP of vascular smooth muscle cells is associated with endothelium-independent vasorelaxation. On the other hand, Shimekake and his colleagues (1995) observed that AM increased cGMP in rat aortic strips and that this effect was suppressed by pretreatment with N^G-monomethyl-L-arginine, a NOS inhibitor. This finding suggested that the NO-cGMP pathway may be involved at least in part in the mechanism of AM-induced vasodilatation.

AM has also been reported to decrease renal vascular resistance in the rat isolated perfused kidney (Hirata et al., 1995). This vasodilatation was associated with a dose-dependent increase in NO release, which was measured with a chemiluminescence method. In addition, Miura and his coworkers (1995) reported that renal vasodilation caused by intra-arterial administration of AM in dogs was suppressed by N^G-nitro-L-arginine, a NOS inhibitor, and that an excessive amount of L-arginine restored this suppression.

The experiment in the isolated perfused rat lung showed that the vasodilating response to AM are reduced after treatment with the NOS inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME) (Nossaman et al., 1996). This data provide support for the hypothesis that vasodilating response to AM is mediated in part by the release of NO from the endothelium in the pulmonary vascular bed of the rat. Furthermore, the pulmonary vasodilating response to AM in intact rat and the vasorelaxant

response to AM in rat pulmonary arterial rings were also reported to be inhibited by AM (26-52), an AM receptor antagonist; L-NAME; methylene blue, an inhibitor of soluble guanylate cyclase activation and endothelium removal (Gumusel el al., 1998). The data suggested that AM acts on receptors in the pulmonary vascular bed that are coupled to endothelium-derived NO release and cGMP formation.

During the transition from fetal to neonatal circulation, pulmonary vascular resistance falls rapidly and pulmonary arterial blood flow increases approximately 8-to 10-fold (Heymann and Soifer, 1989; Teilel et al., 1990). It has been demonstrated that endogenous vasoactive substances play an important role in the transition (Abman et al., 1990). Recently, AM was shown to decrease pulmonary arterial blood flow in fetal sheep, which suggested that this peptide might be another vasoactive substance involved in the transition in the pulmonary circulation (Takahashi et al., 1999). The effect was implied to be depend largely on NO release and partly on K_{ATP} channel activation.

In addition, AM was shown to induce endothelium-dependent relaxation in rat aorta. The relaxation was likely to be mediated by the release of NO (Yoshimoto et al., 1998). Most studies evaluated the endothelium-dependency of AM-induced vasodilatation with NOS inhibitors. However, because the NOS inhibitors sometimes increase baseline vascular tone, it is difficult to get a clear-cut conclusion. Therefore, very recently, Hayakawa and his coworkers (1999) explored whether the NO-cGMP pathway is involved in AM-induced vasorelaxation by examining the effects of cGMP-specific phosphodiesterase inhibitor on AM-induced vasodilatation in rat thoracic aortic rings and isolated perfused kidneys. Their observations

also supported the possibility that cGMP-mediated mechanism is involved in vasodilatory action of AM. Furthermore, vascular relaxation by AM was attenuated by denudation of the endothelium, a guanylase cyclase inhibitor, or a NOS inhibitor (Hayakawa et al., 1999). It is well established that when guanylate cyclase is stimulated by NO, cGMP is increased in vascular smooth muscle cells. These findings implied that AM-induced vasorelaxation is, at least in part, mediated by NO-cGMP pathway in the thoracic aortas and renal arteries of rats.

Numerous studies have revealed that NO exerts its vasodilatory effect via activation of guanylate cyclase and the accumulation of cGMP. However, the effects of NO on cadiac performane are still controversial. It has been suggested that NO and cGMP could regulate the calcium current (I_{Ca}) in cardiac myocytes (Mery et al., 1991; Mery et al., 1993). There is a study reported that NO decreases the I_{Ca} in myocytes, possibly via activation of the guanylate cyclase/protein kinase C system (Mery et al., 1993). Such reports suggest a potential influence of NO on cardiac performance. NO is produced by NOS, which is widely distributed in endothelial cells. A recent study revealed that cNOS is also present in cardiac myocytes (Balligand et al., 1995). Previous investigations have detected mRNA for AM in heart tissue (Nuki et al., 1993). Furthermore, abundant binding sites of AM also exist in the ventricular tissue (Owji et al., 1995). These findings provide a molecular biological basis for a possible AM-NO-cGMP system in cardiac tissue. This hypothesis was supported by the study in isolated adult rabbit cardiac ventricular myocytes (Ikenouchi et al., 1997). The result showed that AM has a negative inotropic effect and decrease both $[Ca^{2+}]_i$ and I_{Ca}, with these effects being at least partly mediated via the L-arginine-NO pathway.

Adrenomedullin and ATP – sensitive potassium channels

Membrane potential of vascular muscle is a major determinant of vascular tone, and activity of potassium channels is a major regulator of membrane potential (Nelson and Quayle, 1995). Activation or opening of these channels increases potassium efflux, thereby producing hyperpolarization of vascular muscle. Membrane hyperpolarization closes voltage-dependent calcium channels and thereby causes relaxation of vascular muscle (de Weille IR, 1992; Nelson MT, 1993; Nelson and Quayle, 1995) (Figure 1.6). Several types of potassium channels, including ATP-sensitive potassium channels (K_{ATP} channels), calcium-activated potassium channels, delayed rectifier potassium-channels, and inward rectifier potassium channels, have been identified in blood vessels.

K_{ATP} channels, which were defined by their sensitivity to intracellular ATP, were first described in cardiac muscle (Noma A, 1983) and have also been found in skeletal muscle (Spruce et al., 1985), pancreatic β cells (Ashcroft et al., 1984; Cook and Hales, 1984) and neurons (Ashford et al., 1988). Recently, K_{ATP} channels have also been identified in vascular muscle (Standen et al., 1989; Nelson et al., 1990) and in endothelial cells (Janigro et al., 1993; Katnik and Adams, 1997). These channels are closed by ATP binding to an intracellular site and are opened by the dissociation of ATP from this site (Nelson et al., 1990; Edwards et al., 1993). Thus, reduction of intracellular ATP opens the channels and produces vasodilation. Activity of K_{ATP} channels is also affected by other factors, including adenosine diphosphate (Pfrunder et al., 1993) and reductions in Po_2 or pH (Davies NW, 1990). Thus, activity of K_{ATP} channels may reflect the metabolic state of cells.

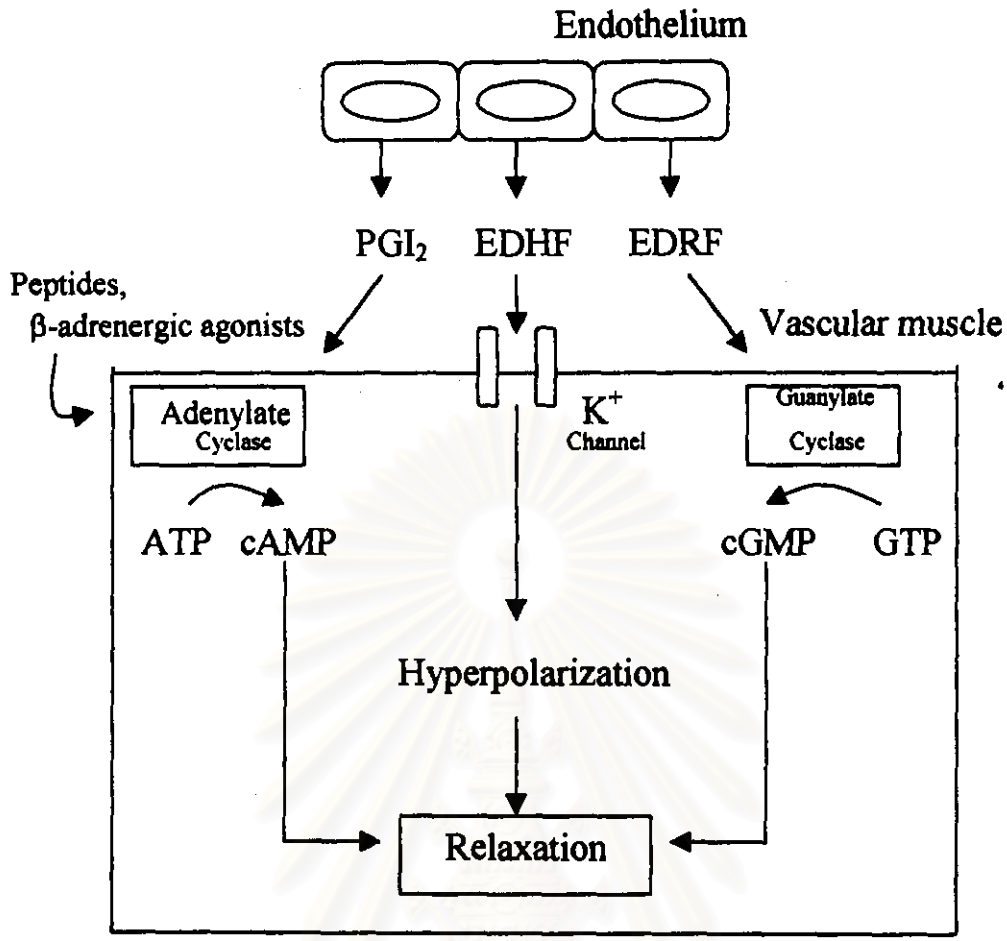

Figure 1.6 Schematic diagram shows three major mechanisms of vasodilatation involving activation of adenylate cyclase, potassium channels, and guanylate cyclase. Both endothelium-dependent and endothelium-independent mechanisms can produce vasodilatation. Several peptides, β -adrenergic agonists, and nitrovasodilators such as nitroglycerin act directly on vascular muscle to produce relaxation. Prostacyclin (PGI_2), which is released from endothelium, β -adrenergic agonists, and several peptides activate adenylate cyclase and thereby produce vasodilatation. Vasodilatation in response to EDRF (nitric oxide) is mediated by activation of guanylate cyclase. EDHF and potassium channel openers increase the open probability of potassium channels, hyperpolarize vascular muscle, and thereby relax blood vessels.

An important feature of K_{ATP} channels is that the channel can be activated by synthetic openers of potassium channel openers (Richer et al., 1990; McPherson et al., 1993) and inhibited by sulfonylureas (Nelson et al., 1990; Standen NB, 1992; Edwards et al., 1993). Potassium channel openers are a chemically diverse group of compounds that produce hyperpolarization and thereby cause relaxation of vascular muscle (Quast U, 1992; Quast U, 1993). Sulfonylureas such as glibenclamide, which are hypoglycemic agents that are widely used in the treatment of diabetes mellitus, inhibit K_{ATP} channels in blood vessels (de Weille et al., 1989; Bean BP, 1992). The exact mechanism by which sulfonylureas inhibit K_{ATP} channels is still not clear, but it has been suggested that the binding site for glibenclamide is coupled in a negative allosteric manner to the binding site for the potassium channel openers (Bray and Quast, 1992; Quast et al., 1993).

Many stimuli that produce hyperpolarization bind to receptors on vascular muscle and activate potassium channels through an endothelium-independent mechanism. Some agonists, however, produce relaxation of vascular muscle by endothelium-dependent hyperpolarization (Taylor and Weston, 1988; Garland et al., 1995). Several lines of evidence have suggested that a substance, which is distinct from nitric oxide (endothelium-derived relaxing factor, EDRF) or prostanoids, is released from endothelium and produces hyperpolarization of vascular muscle (Chen and Suguki, 1991; Nagao and Vanhoutte et al., 1991). The chemical nature of this substance, which is called endothelium-derived hyperpolarizing factor (EDHF) is still not clear (Garland et al., 1995).

Endothelium plays an important role in regulation of vascular tone by releasing several substances, including EDRF, EDHF, and prostacyclin

(Gryglewski et al., 1991; Moncada et al., 1991; Faraci FM, 1992). Production of some endothelium-derived vasoactive substances, is dependent on the concentration of cytoplasmic Ca^{2+} (gryglewski et al., 1991; Moncada et al., 1991). Because endothelium does not express voltage-dependent calcium channels, increases in cytoplasmic Ca^{2+} concentrations in endothelium are achieved by mobilization of Ca^{2+} from intracellular stores and/or Ca^{2+} entry through receptor-operated cation channels. Ca^{2+} influx through receptor-operated Ca^{2+} entry is activated by membrane hyperpolarization, which may be achieved by activation of potassium channels (Chen and Cheung, 1992; Ohno et al., 1993). ATP-sensitive potassium channels have been described in aortic endothelium and brain microvascular endothelial cells (Janigro et al., 1993). Thus, activation of K_{ATP} channels in endothelium may be an important mechanism that contributes to vasodilatation in response to several endothelium-dependent stimuli (Figure 1.7)

The involvement of K_{ATP} channels in AM induced vasodilatation has been reported in several studies. Lang and his colleagues (1997) have provided evidence that vasodilating responses of cerebral blood vessels to AM are mediated by activation of K_{ATP} channels. They measured the diameter of cerebral arterioles in response to the topical application of AM (10^{-7} and 10^{-6} mol/L) by using a closed cranial window in anesthetized rats. The result showed that glibenclamide, an inhibitor of K_{ATP} channels, reduced the responses of the arterioles to AM. In addition, in anesthetized open-chest dogs, bolus injections of AM as well as those of adenosine into the coronary artery caused significant increases in coronary blood flow in a dose-related fashion, without altering systemic hemodynamic measurements, which were blocked by intracoronary injection of U 37883A, an antagonist of K_{ATP}

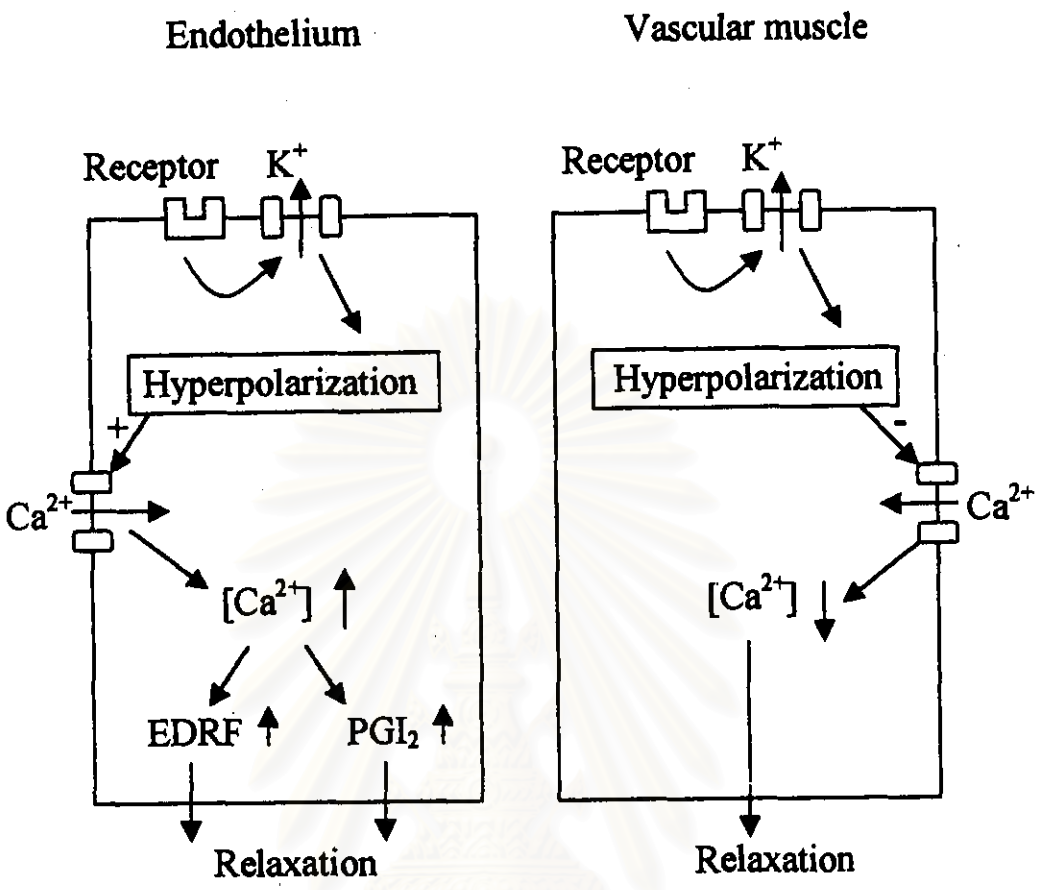

Figure1.7 Schematic diagrams show possible functions of potassium channels in endothelium and smooth muscle. Vasodilator may activate receptors that open potassium channels and produce hyperpolarization of endothelium. Because endothelium does not express voltage-sensitive calcium channels, hyperpolarization of endothelium enhances calcium influx through receptor-operated calcium channels. Increase in cytoplasmic calcium concentration leads to production of EDHF and/or prostacyclin (PGI_2) from endothelium. Receptor-mediated hyperpolarization of smooth muscle (eg. by norepinephrine and CGRP) reduce open probability of voltage-sensitive calcium channels and decreases cytoplasmic calcium concentration of vascular muscle. Decreases in cytoplasmic calcium concentration reduce calcium-dependent contraction of vascular muscle and produce vasodilatation.

channels (Sabates et al.,1997). Thus, it appears that AM acts in part through activation of K_{ATP} channels, similar to adenosine (Daut et al.,1990).

Recently, in the whole-cell voltage clamp experiments using single cells of the rat mesenteric artery, AM produced increases of inward current in a concentration-dependent manner. The AM-induced current was suppressed markedly by glibenclamide (Sakai et al., 1998). Additionally, the reversal potentials of the glibenclamide-sensitive currents in the presence of AM was approximately -19.6 mV, near the theoretical potassium equilibrium potential. Taken together, the findings may indicate that AM-induced currents were through K^+ selective channels.

More recently, the study by Takahashi and his coworkers (1999) also indicated the existence of K_{ATP} channels mediated mechanism of AM induced vasodilatation in the fetal pulmonary circulation. Thus, the experiment supports the suggestions by Lang and colleagues (1997), Sabates and Colleagues (1997), and Sakai and colleagues (1998) that the vasodilatory effect of AM is in part linked with K_{ATP} channels. Interestingly, to examine synergistic interactions among naturally occuring vasodilators, Sakai and his coworkers (1998) also investigated the effects of iv. infusion of AM on adenosine-induced vasodepression in anesthetized male Sprague-Dawley rats. The result showed that AM significantly potentiates the adenosine-induced vasodepression. The potentiation was not observed after treatment with glibenclamide, suggesting that it is at least in part coupled to K_{ATP} channels. As AM possesses a potent vasodilating property (Baskaya et al., 1995; Lang et al., 1997), it is possible that this agent may contribute to the physiological regulation of blood flow and vascular tone in the cardiovascular system.

Rationale

The Isolated Perfused Heart Model

The isolated perfused heart preparation developed by Langendorff is a model which eliminates systemic reflex effects. This model is free of central neural and hormonal effects. It has been employed to study basic questions in cardiac biochemistry, pharmacology, pathology, and physiology. This model has also been used in a number of applied studies of cardiac protection and preservation. Because of the general acceptance and use of this model, the isolated perfused heart was chosen as an experimental model in this study.

The Dorsal Skinfold Chamber Model

The dorsal skinfold chamber model was designed for direct, quantitative studies of the acute change in hemodynamic in the microcirculation such as red blood cell velocity and vasodilation or vasoconstriction. It consists of implanting a modified chamber in the dorsal skin flap of the animals. Due to the poor contrast between blood cells, blood capillaries and surrounding tissue, microvascular beds were visualized using fluorescent microscopy after intravenous injection (i.v.) of 5% fluorescein-isothiocyanate (FITC)-Dextran 150. The combination of optical elements and low amounts of FITC-Dextran improved the contrast of the televised image without changing macro- and micro- hemodynamic parameters, and blood plasma was delineated as bright structure against the substantially darker background of red blood cells and surrounding tissue. This permitted

the quantitative study of practically all blood vessels within a given field. Since i.v. injection of drugs and systemic pressure measurements are possible in this model, it provides a unique mean for studying the reactivity of the microcirculation over a prolonged period.

Adrenomedullin (AM) is a vasodilator peptide, originally discovered in human phaeochromocytoma. The peptide consisting of 52 amino acids, has one intramolecular disulfide bond and shows slight homology with calcitonin gene related peptide (CGRP) (Kitamura et al., 1993a; Kitamura et al., 1993b). By the present, structures of AM in several species have been clarified. The ring structure and carboxy terminal amide structure, both of which are essential for biological activity of AM, are well conserved between species. AM and its binding sites have been found to exist in vascular endothelium and smooth muscle cells indicating that the peptide may function as a paracrine and/or autocrine factor as well as a circulating hormone.

The study of cardiovascular actions of AM in conscious sheep and rats showed the hypotensive response and a marked increase in heart rate (HR) and strong cardiostimulating effect to increase cardiac output (CO) (Parkes DG, 1995; He et al., 1995). The large increase in CO may be partly mediated by AM producing an effect to increase cardiac contractility since the increase in left ventricular contractility and aortic flow were observed. However, other factors such as a reflex enhancement of myocardial to supplement CO by increasing HR, as well as the observed fall in cardiac afterload, may also contribute to the overall increase in CO. AM elicits a potent and long lasting hypotensive effect in anesthetized rats (Kitamura et al., 1993a; Sakata et al., 1993) and produces a much smaller increase in CO

when compared with conscious animals and there is no change in HR suggesting that the tachycardia may play a large part in increasing cardiac function in conscious animals. Furthermore, the lack in any change in HR in anesthetized rats may be mediated by anesthesia-induced inhibition of sympathetic baroreflex leading to a much larger and sustained reduction in blood pressure (BP) following AM injection.

To date, the cardiovascular effects of AM have been examined only in anesthetized and conscious animals, in which both are the *in vivo* study that systemic reflex and neurohormonal effects may be involved. AM and its binding sites have been found in the heart indicating that AM may function as an endogenous regulator of cardiac function. However, the direct effects of AM on caridac functions are unknown. Therefore, the present study will access the effects of AM on cardiac functions and coronary circulation directly by using the isolated perfused rat heart model.

Moreover, it has been reported that AM causes a vasodilatory response in variety of vascular beds, however, the vasodilator action of AM is not clear. A number of studies have been reported that the AM action is a direct action by stimulate cAMP formation in smooth muscle cells (Eguchi et al., 1994; Ishizaka et al., 1994b) in some papers and to be mediated by endothelium-derived nitric oxide (Nakamura et al., 1995; Feng et al., 1994) or endothelium-derived prostaglandins (Jougasaki et al., 1997) in the others. In addition, AM has also been reported to induce hyperpolarization in the isolated rat mesenteric resistance arteries (Goto et al.,1997). The hyperpolarization is abolished by glibenclamide, a specific blocker of ATP-sensitive K^+ channel. The findings suggest that AM hyperpolarizes the

membrane through the opening of ATP-sensitive K^+ channel, which might contribute to the vasodilatory action of AM.

Therefore, the experiments will be carried out to assess the biological responses of both cardiac performance and coronary circulation to AM in isolated perfused rat heart model. In addition, the possible mechanism(s) of the AM on coronary vasodilation was also employed. By using different inhibitors, the mode of actions of AM on coronary vessel were able to identified. The study is also designed to study the hypotensive effect of AM in anesthetized rat and to investigate the vasodilatory response in rat skin microcirculation by using the dorsal skinflod chamber model.

Objective of this study

The aim of the present study are :-

1. To study the effect of AM on systemic arterial pressure.
2. To study the effect of AM on rat skin microcirculation.
3. To study the effect of AM on cardiac performance and coronary circulation in isolated perfused rat heart model.
4. To study the mechanism of action of AM on coronary circulation.