CHAPTER IV
DISCUSSION

AM has been proposed as a vasodilator peptide in a variety of vascular bed. However the nature of the mechanism (s) through which AM exerts its vasoactive properties is still under investigation. In this study, a series of experiments was conducted to investigate the possibility of the mechanism that may involve to the effect of AM. In a preliminary series of experiments, the study were performed to confirm the hypotensive and the vasodilator effects of AM. The hypotensive property of AM was investigated by the intravenous injection of AM (1 nmol/kg BW) in anesthetized rats. The dorsal skinfold chamber model was used to demonstrate the vasodilator response by topical application of AM (10^{-7} M) on the rat skin microcirculation. To clarify the cardiac effect of AM and mechanism (s) involved in the coronary vasodilator response to AM, the experiments were carried out in the isolated perfused rat heart model. Various bolus doses of AM were injected to the coronary circulation in order to select the appropriate dose in this study. The minimal dose that cause the coronary vasodilation was 20 μg and was chosen for this experiment. In isolated perfused rat heart model, we studied the AM – induced coronary vasodilator response after pharmacological inhibition of the pathways believed to be implicate in the effect of AM and also after damage to the coronary endothelium. In addition, the involvement of cAMP and the effect of AM on electrocardiogram were also investigated.

1. The effects of AM on systemic arterial pressure

The present study revealed that intravenous injection of AM caused a rapid and marked hypotension (MAP decreased by 29.9±3.3 mmHg in 30 second). In previous study (Kitamura et al., 1993 a; Ishiyama et al., 1993) AM in a dose of 1 nmol/kg as used in the present study was also demonstrated to reduce blood pressure in anesthetized rat. In their study a significant correlation was found between changes in blood pressure and total peripheral resistance, peripheral vasodilation may play a primary role in the hypotensive action of this peptide. Several studies have suggested possible mechanisms of AM-induced vasodilation, e.g. through contributing actions of cAMP, NO, K_{ATP} channels, CGRP receptors, or prostaglandin (Eguchi et al., 1994; Feng et al., 1994; Ishizaka et al., 1994; Entzeroth et al., 1995; Hirata et al., 1995; Jougasaki et al., 1997; Sabate et al., 1997). These mechanisms are still controversial, and differences in effect are reported between organs and between species (Eguchi et al., 1994; Nossaman et al., 1996) as we will discuss later. However, previous studies have demonstrated that AM dose - dependently inhibited angiotensin II - induced secretion of aldosterone from dispersed rat zona glomerulosa cells and reduced plasma aldosterone levels (Yamaguchi et al., 1995). The possible inhibitory effects of AM on the angiotensin II – induced secretion of aldosterone may have contributed to its natriuretic and diuretic actions as shown by Jougasaki et al. (1995) and Elhawary et al. (1995). Anyway, it is unlikely that the reduction in plasma volume , caused by natriuretic and diuretic actions of AM , contributed to the reduction in MAP observed in the present study, because the reduction in MAP was rapid in onset and reached its peak effect in thirty seconds.

Interestingly, AM did not cause reflex tachycardia despite a marked reduction in mean blood pressure. This effect also agree with the work of Kitamura et al (1993a; 1993b). The lack of any change in HR in the study on anesthetized rats may be mediated by anesthesia-induced inhibition of sympathetic baroreflex (Fluckier et al., 1985; Kannan et al., 1989). However, the direct chronotropic effect of AM have to be clarify. The direct effect of AM on cardiac performance can be examined in the isolated heart model as we have done and will discuss then.

2. The effects of AM on rat skin microcirculation

By using the dorsal skinfold chamber model and intravital fluorescent microscopy, the topical application of AM (10^{-7} M) produced the relaxation of skin microcirculation, % increase is 4.8±1.3% for the second order arteriole and 14.2±3.6% for the third order arteriole. The results appear to agree with the study in hamster cheek pouch, % increase in diameter of arteriole preconstricted by endothelin-1 was 140±25% with AM at the dose of 0.4 nmol. (Hall et al., 1995) and in rat cerebral arterioles, % increase in diameter was about 20% with AM at the dose of 10^{-7} M (Lang et al., 1997).

The potency of AM in the present study may appear to be somewhat low, this may be due to the application technique. In our experiment, AM was applied extraluminally, which may limit access of AM to the vascular smooth muscle. Under physiological conditions, AM is mainly released by the endothelium and vascular smooth muscle itself (Sugo et al., 1994; Isumi et al., 1998), which does not require passing of the adventitia. However, this

study demonstrated the vasodilatory action of AM in the microvasculature of rat skin.

3. The effects of AM on cardiac performance and coronary circulation in isolated perfused rat heart

3.1 The effect of AM on cardiac contractility

Despite the increase in coronary blood flow, we found a small decrease in cardiac contractility. Because the rate of pressure increment ($+dP/dT_{max}$) and the rate of pressure decrement ($-dP/dT_{max}$) were reduced to the same extent, the ratio $(-dP/dT_{max})/(+dP/dT_{max})$ remained basically constant over the course of the observation. This pattern of changes indicated that myocardial rate of contraction and relaxation were affected by AM in the same degree. The mild negative inotropic effect observed in this study is in agreement with the study by Perret and coworkers (1993). In a preliminary series of rat experiments, these investigators showed that intravenous injections of AM produced the expected decline in mean systemic arterial pressure. Then, using an *ex vivo* isolated perfused rat heart model in another set of experiments, the addition of AM (50μg) to the perfusate first produced a mild increase, followed by a decline, in left ventricular peak systolic pressure, indicating a mild negative inotropic effect as seen in our study.

The negative inotropic effect of AM found in the present study was in the same line with result of ventricular cAMP accumulation. Although there is an intimate relation between AM-induced biological effects and the increased cAMP level in several cell types (Eguchi et al., 1994; Ishizaka et

al., 1994; Yoshimoto et al., 1998), AM failed to increased cAMP content in the ventricles of the isolated perfused rat heart in our study . Recently, a negative inotropic effect of AM has been reported in isolated adult rabbit cardiac ventricular myocytes (Ikenouchi et al., 1997). In their investigation, superfused the myocytes with AM had a concentration-dependent negative inotropic effect. AM was also demonstrated to produce the decrease in both intracellular cardiac myocytes calcium concentration ($[Ca^{2+}]_i$) and calcium current (I_{Ca}). This reduction in $[Ca^{2+}]_i$ and I_{Ca} may explain the negative inotropic effect of AM in cardiac cells, although there is still a possibility that myofilament sensitivity to Ca^{2+} may change simultaneously (Ikenouchi et al., 1994). However, our experiments do not directly address this speculative explanation.

An alternative explanation for the inotropic effect of AM is the role of NO-cGMP system. Activation of this system by AM was recently reported in kidney (Hirata et al., 1995) and bovine aortic endothelial cells (Shimekake et al., 1995). In addition, previous investigations have detected mRNA for AM in heart tissue (Nuki et al., 1993), and there is also evidence that constitutive nitric oxide synthase (cNOS) activity is present in the heart (Balligand 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 a recent study by Ikenouchi and his coworkers (1997). They found the negative inotropic effect of AM in rabbit cardiac myocytes which was blocked by NO synthase inhibitor, L-NMMA, and addition of L-arginine restored the effect. Furthermore, after exposure to AM, the intracellular content of cGMP increased in concentration-dependent manner, suggesting the possible contribution of NO and subsequent cGMP production to the negative inotropic effect of AM. In general, agonist-induced activation

of NO synthase requires an elevation of $[Ca^{2+}]_i$ (Moncada et al., 1991). In endothelial cells, a transient increase in $[Ca^{2+}]_i$ may activate cNOS (Hirata et al., 1995). In cardiac myocytes, $[Ca^{2+}]_i$ is decreased after superfusion with AM (Ikenouchi et al., 1997). It seem unlikely, therefore, that a transient increase in $[Ca^{2+}]_i$ triggers the activation of NO synthase, so an alternative pathway for NOS activation may exist in cardiac myocytes. The precise mechanism underlying the cGMP-evoked negative inotropic effect remains unclear. However, recent studies have provided evidence that NO or cGMP regulates the calcium current in cardiac myocytes (Mery et al., 1993; Ikenouchi et al., 1997). The reduction of I_{Ca} could also contribute to the negative inotropic effect of AM.

AM level is reported to be increased in patients with hypertension, especially hypertension associated with organ failure, or renal dysfunction and that the increase is in proportional to the plasma norepinephrine level (Ishimitsu et al., 1994), suggesting that AM may act to counterbalance sympathetic activity. A recent report indicated that AM immunoreactivity is markedly increased in ventricles of patients with congestive heart failure and that plasma AM levels are also increased (Jougasaki et al., 1995), suggesting a potential role for this peptide in the neurohumoral activation that characterizes human congestive heart failure. It is also uncertain whether or not the negative inotropic effect of AM in such a setting is beneficial. A recent study using an ischemic heart model showed that intravenous infusion of SPM-5185, an NO donor, significantly reduced the area of myocardial necrosis after coronary occlusion, suggesting the possibility that supply of NO could be beneficial (Lefer et al., 1993). AM may act as an in situ NO donor in the heart, but further investigations are necessary to determine the clinical importance of this peptide.

3.2 The effect of AM on heart rate

In the present study, a bolus injection of AM can produce the increase in heart rate of the isolated perfused rat heart. This result is consistent with recent data in conscious sheep (Parkes et al., 1995; Parkes and May, 1997). However, AM-induced tachycardia was not observed in anesthetized rats (Kitamura et al., 1993b) and any of the *in vitro* study (Perret et al., 1993; Szokodi et al., 1996). The previous study in conscious sheep demonstrated that this tachycardia is mediated via autonomic baroreflex since it was abolished during treatment with an autonomic glanglion blockade (Matsunaga et al., 1996; Parkes and May, 1997). Because anesthesia is known to profoundly affect the cardiovascular and autonomic nervous systems (Fluckier et al., 1985; Kannan et al., 1989), this may explain the conflicting results between conscious sheep and anesthetized rats. It is difficult to explain the differences between the present and previous *in vitro* studies regarding the effects of AM on heart rate, but differences in the experimental preparation and the method used may be involved. In the previous study by Perret and his colleagues (1993), since AM at the bolus dose of 50 μg was infused in the left atrium of the isolated rat heart, it is uncertain as to how much of the agent reached the coronary circulation. They did not find any significant alteration in heart rate. Whereas, Szokodi and his coworkers (1996) dilated the vasculature by decreasing the perfusion rate before the start of the experiment in order to exclude any secondary effects caused by the vasorelaxation of the coronary arteries induced by AM. Their result showed that heart rate was not affected by the continuous infusion of AM (0.03-1mM) for 30 minute. However, despite that the coronary arteries were almost maximally dilated under their experimental

conditions, AM decreased the coronary perfusion pressure slightly, but significantly.

Based on the result which show the increase in the rate of heartbeat together with the normal electrocardiogram observed after AM injection, this tachycardia appear to be mediated by the increase in sinoatrial node firing (sinus tachycardia). The pesistence of sinus rhythm during all responses was confirmed by the continued presence of P waves and 1:1 correspondence of P and R waves in the electrocardiogram. In our pilot experiment, AM at the dose that did not cause the significant increase in coronary blood flow produced the increase in heart rate. This finding demonstrated that heart rate is likely to be more sensitive than coronary vasodilatation to exogenously administered AM. Therefore, for further study of the direct positive chronotropic and the mechanism that may involve, the lower dose of AM which produce the only increase in HR without the effect on CBF should be selected.

3.3 The effect of AM on coronary circulation

As we have reported that intravenous injection of AM caused a rapid and marked hypotension and the peripheral vasodilation was suggested to play a primary role in this effect (Ishiyama et al., 1993). In addition, the local vasodilation in response to AM was also observed in skin microcirculation. Now, we investigated the vasodilatory effect of AM on coronary circulation. Data from the present study also indicate the marked increases in coronary blood flow in response to direct injections of AM (20 μg), with the % increase of 18.93±1.42%. Because coronary perfusion pressure was held constant, changes in coronary blood flow directly reflect changes in coronary

vascular resistance. The vasodilatory effect of AM observed in this study is consistent with several previously published studies (Nuki et at., 1993; Hirata et al., 1995; Heaton et al., 1995; Nakamura et al., 1995; Lang et al., 1997).

The coronary vasodilatory action of AM in isolated rat heart model has not been defined. However, a number of studies have been reported that the vasodilator action of AM is a direct action by stimulate cAMP formation in smooth muscle cells in some papers (Eguchi et al., 1994; Ishizaka et al., 1994; Osajima et al., 1995; Yoshimoto et al., 1998) and to be mediated by endothelium-derived NO (Feng et al., 1994; Miura et al., 1995; Nakamura et al., 1995; Nossaman et al., 1996; Gumused et al., 1998; Hayakawa et al., 1999; Takahashi et al., 1999.) or endothelium-derived prostaglandins (Jougasaki et al., 1997) in the others. In addition the opening of K_{ATP} channels was also shown to contribute to the vasodilatory action of AM (Goto et al., 1997; Lang et al., 1997; Sabates et al., 1997; Sakai et al., 1998; Takahashi et al., 1999). In the next series of our experiment, the mechanisms involved in the coronary vasodilatory action of AM in isolated perfused rat heart model were investigated and the results will be discuss.

3.4 The mechanism of action of AM on coronary vasodilation

To define the mechanisms involved in the response of AM on coronary vasodilation, we studied AM-induced coronary vasodilatation after physical damage to the coronary endothelium, and also after pharmacological inhibition of the pathways believed to be implicated. In other words, the experimental procedure in this session has been set up in order to verify whether AM is a coronary vasodilator via endothelium-dependent or endothelium-independent fashion.

3.4.1 Is AM an endothelium-independent coronary vasodilator ?

To test the possibility that endothelial-independent mechanisms are involved in AM-induce coronary vasodilation, the experiments in which the endothelium was physically damaged by Triton X-100 were carried out. This procedure was previously found to increase the basal coronary tone and abolish the vasodilating effect of acetylcholine in an isolated heart model (Li et al., 1993). In the present study, treatment with Triton X-100 caused the marked decrease in CBF, reflecting impairment of the basal release of endothelium-derived vasodilating substances. The efficacy of the Triton X-100 treatment was also demonstrated by its complete inhibition of response to bradykinin, an endothelium-dependent vasodilator. However, as shown by the vasodilatation in response to SNP, the endothelium-independent response was not impaired. Consequently, after endothelial degradation, the slightly but significantly relaxing response to AM was observed (CBF increase by 4.64±1.50% in endothelium-damage; by 15.70±0.91% in endothelium-intact). A number of studies have been reported that the AM action is a direct action by stimulating cAMP formation in smooth muscle cells (Eguchi et al., 1994a, b; Ishizaka et al., 1994; Shimekake et al., 1995). Therefore, the present study confirmed that AM-induced vascular relaxation could be assessed through the direct effect on smooth muscle cells (Figure 4.1).

3.4.2 Is AM an endothelium-dependent coronary vasodilator?

From the experimental result in 3.4.1, it was observed that coronary vasodilation was attenuated after endothelial damaging using Triton X- 100 (CBF increase by 4.64±1.50% in endothelium-damage; by 15.70±0.91% in endothelium-intact). Therefore, the possible mechanisms of AM on

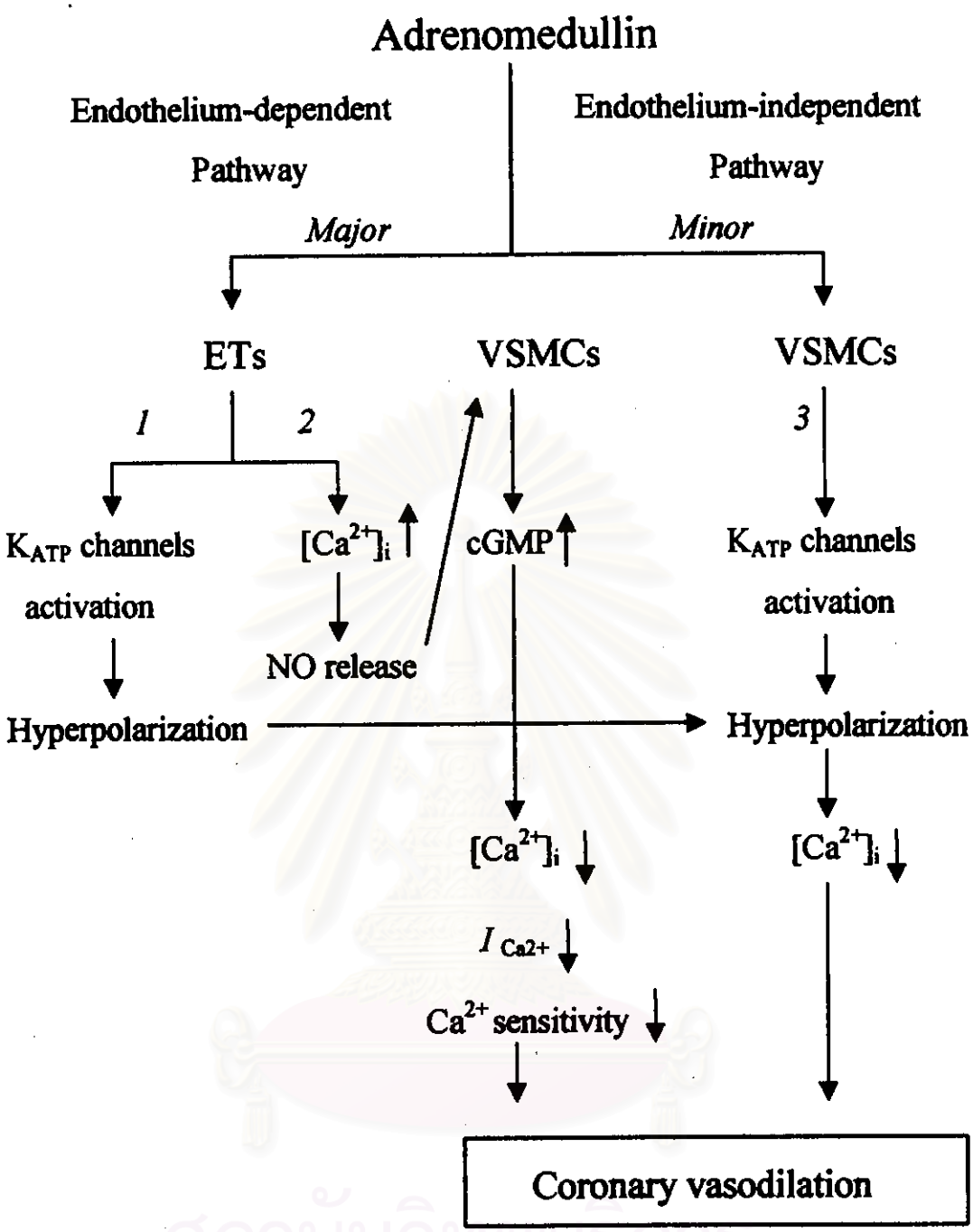

Figure 4.1 The diagram shows the proposed mechanisms of adrenomedullin on coronary vasodilation in isolated perfused rat heart model. 1) The direct effect of AM on endothelial cell caused the hyperpolarization of endothelial cell membrane and then smooth muscle cell membrane. 2) The direct effect of AM on endothelial cell that caused the release of NO. 3) The direct effect of AM on smooth muscle that caused the hyperpolarization of smooth muscle without the involvement of endothelium.

endothelial vasodilating function were further investigated. Up to now, the three major pathways for endothelial controlling vasodilation are as follows : 1) through the PGI_2 2) through the EDRF (NO) and 3) through the activation of K_{ATP} channels.

Three key blockers were chosen in the followed experimental protocol in order to assess those three possible mechanisms (Figure 4.2)

3.4.2.1 Role of the cyclooxygenase pathway in the coronary response to AM

The involvement of prostanoid production in the coronary vasodilatory response to AM was tested using the cyclooxygenase inhibitor, indomethacin, which was previously shown to inhibit the release of the products of the cyclooxygenase pathway in an isolated heart preparation (Minkes et al., 1973). In the present study, indomethacin did not alter the coronary response to AM. Thus demonstrating that prostaglandins release or other cyclooxygenase products is not involved the coronary vasodilatation induced by AM in the isolated pesfused rat heart model.

3.4.2.2. Role of the NO pathway in the coronary response to AM

From Table 3.15, we can see that AM caused the smaller increase in CBF in the presence of L-NNA, which has been shown to inhibit NO synthesis in isolated heart model (Lamontagne et al., 1991), when compared to control (AM alone) which then makes the involvement of an NO-mediated mechanism likely (CBF increase by 14.90±1.22% in AM+L-NNA group; by 18.93±1.42% in AM alone group). Our result is in agreement with previous findings (Feng et al., 1994; Miura et al., 1995; Nakamura et al., 1995;

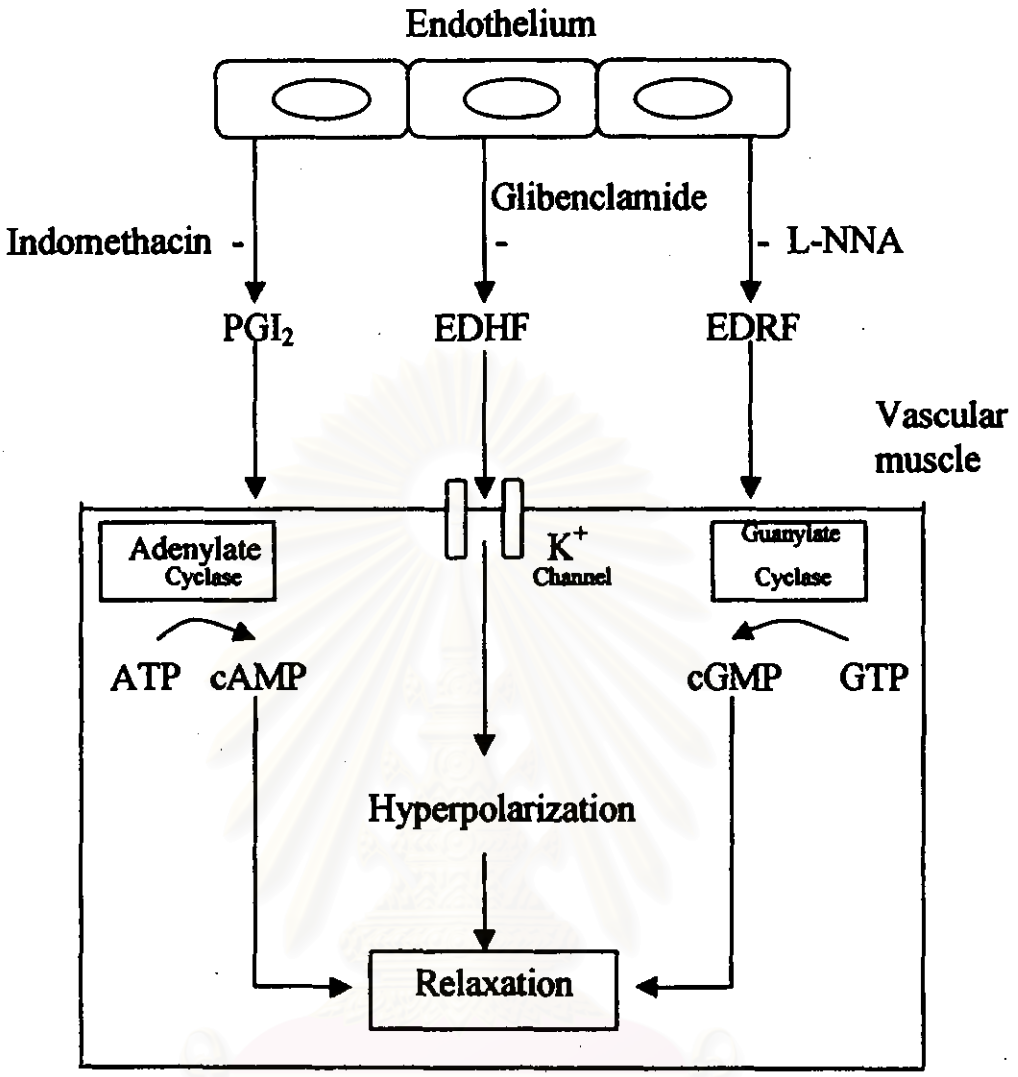

Figure 4.2 Schematic diagram shows three major mechanisms of vasodilation involving activation of adenylate cyclase, potassium channels, and guanylate cyclase. Prostacyclin (PGI_2), which is released from endothelium activate adenylate cyclase and thereby produce vasodilation. This pathway can be blocked by indomethacin. Vasodilation in response to EDRF (NO) is mediated by activation of guanylate cyclase. The formation of NO can be inhibited by NOS inhibitor, L-NNA. EDHF and potassium channel openers increase the open probability of potassium channels, hyperpolarize vascular muscle, and thereby relax blood vessels. Glibenclamide is the blocker for K_{ATP} channels.

Nossaman et al., 1996; Yoshimoto et al., 1998; Hayakawa et al., 1999; Takahashi et al., 1999). However, the precise biochemical process responsible for NO-mediated coronary vasodilation caused by AM is not clear in this study . It has been shown that an inhibitor of NO synthase reduced the AM-induced hypotension in systemic arterial pressure and hindquarters perfusion pressure of the rat (Feng et al., 1994; Nossaman et al., 1996). Furthermore, the study by Yoshimoto and his colleagues (1998) demonstrated that AM caused a concentration-dependent relaxation in the rat aorta. The relaxant effects of the peptide was significantly attenuated by an inhibitor of NO synthase N^G-monomethyl-L-arginine (L-NMMA). Their investigation also showed that AM increase the endothelial $[Ca^{2+}]_i$. This result is consistent with a previous study demonstrating that AM increased $[Ca^{2+}]_i$ in bovine cultured endothelial cells (Nakamura et al., 1995). Because agonist-induced activation of NO synthase requires an elevation of endothelial $[Ca2+]_i$ (Moncada et al., 1991), these findings may support the idea that AM induced the release of NO from endothelium. In addition, Gray and Marshall (1992b, c) have suggested that the elevation of endothelial cAMP induced by α-CGRP and other cAMP-elevating agents results in activation of NO synthase. It has also been demonstrated that AM increases cAMP more potently than α-CGRP in human vascular endothelial cells (Kato et al., 1995). It is possible, therefore, that AM may release NO from the endothelium by increasing both endothelial cAMP and $[Ca^{2+}]_i$. However, we cannot discount the possibility that the production of NO by AM is dependent in part to the activation of K_{ATP} channels, since our investigation also showed that glibenclamide, an inhibitor of K_{ATP} channels, reduce the coronary vasodilatory effect of AM. This result implied the involvement of K_{ATP} channels to the AM-induced coronary vasodilation. In addition, based on a recent study, hyperpolarization of endothelial cells in response to

activation of K^+ channels leads to increased cytoplasmic calcium concentration via calcium entry through receptor-operated cation channels (Kitazono et al., 1995; Kuriyama et al., 1995) and thus may potentially enhance release of NO (Figure 4.2). In vascular muscle, NO activates soluble guanylate cyclase resulting in accumulation of guanosine 3′, 5′ cyclic monophosphate (cGMP) and activation of cGMP-dependent protein kinase. cGMP is degraded and inactivated by phosphodiesterase in vascular smooth muscle cells. Hayakawa and his coworkers (1999) demonstrated that cGMP – specific phosphodiesterase inhibition augmented AM–induced vasorelaxation in aortic rings and isolated perfused kidneys. Their results confirm the suggestion that the NO-cGMP pathway is involved in the mechanism of AM-induced vasorelaxation. This mechanism can stimulate vasorelaxation through several mechanisms that decrease intracellular calcium levels and/or decreased calcium sensitivity of the contractile apparatus (Twort and Breeman, 1988; McDaniel et al., 1992). The possible contribution of AM to cardiac performance via the NO-cGMP system was also demonstrated as discussed previously (Ikenouchi et al., 1997).

3.4.2.3 Role of the K_{ATP} channels on coronary response to AM

The involvement of K_{ATP} channel in AM-induced vasodilation has also been reported in several studies (Goto et al., 1997; Lang et al., 1997; Sabates et al., 1997; Sakai et al., 1998). In the whole-cell voltage clamp experiments using single cells of the rat mesenteric artery, AM produced increase in inward current in a concentration-dependent manner. The AM-induced current was suppressed markedly by glibenclamide, an antagonist of K_{ATP} channels (Sakai et al., 1998). Additionally, the reversal potential of the glibenclamide-sensitive currents in the presence of AM was approximately

-19.6 mv, being closed to the theoretical potassium equilibrium potential. In the present study, coronary vasodilation by AM was attenuated in the presence of the K_{ATP} channel blocker, glibenclamide as reported in rat cerebral blood vessels (Lang et al., 1997) and in dog coronary artery (Sabates et al., 1997). These results, together with ours, suggest that the AM-induced coronary vasoditation was at least in part linked to K_{ATP} channels. The mechanism whereby glibenclamide inhibits AM's coronary vasodilative actions is not clear. The susceptibility of AM-induced coronary dilatation to antagonism by glibenclamide suggests the involvement of a hyperpolarizing K^+ current. Glibenclamide has been shown to inhibit the acetylcholine-induced hyperpolarization of smooth muscle in arterial rings (Standen et al., 1989). Since this hyperpolarization is though to be mediated by a factor released from the vascular endothelium (endothelium-derived hyperpolarizin factor, or EDHF), it is possible that glibenclamide might have opposed the action of Ach at the endothelial cell, rather than directly at the smooth muscle cell. A similar action may underlie glibenclamide's antagonism of AM-induced coronary vasodilatation in the present study. Although AM is known to have direct actions on vascular smooth muscle, it has been suggested that AM also induced vasodilatation indirectly through an endothelium-mediated process (Hirata et al., 1995; Majid et al., 1995; Miura et al., 1995; Nakamura et al., 1995). In our study, AM produce a small but significantly increase in coronary blood flow after physical damage of endothelium by Triton X-100. This result may imply that the coronary dilatation effect of AM is mediated by an endothelium dependent mechanism as well as the direct effect on vascular smooth muscle cells. Activation or opening of K^+ channels increase K^+ efflux, thereby producing membrane hyperpolarization. There are evidences suggested that the effect of membrane hyperpolarization differs in endothelium and vascular smooth

muscle. In vascular smooth muscle, membrane hyperpolarization closes voltage-dependent calcium channels, thus decreasing the concentration of cytoplasmic calcium and resulting in vasodilatation (Nelson and Quayle, 1995). Whereas endothelial cells express both K^+_{ATP} channels and calcium-activated K^+ channels (Kitazono et al., 1995; Kuriyama et al., 1995). Hyperpolarization of endothelial cells in response to activation of K^+ channels leads to increased cytoplasmic calcium concentration via calcium entry through receptor-operated cation channels (Kitazono et al., 1995; Kuriyama et al., 1995). The increase in cytoplasmic calcium concentration may potentially enhance release of NO, which is thought to lead to a subsequent vasorelaxation.

The present study demonstrated that both L-NNA and glibenclamide attenuated the coronary vasodilation induced by AM. This result is similar to the study by Takahashi and his colleagues (1999) which indicated the existence of both a NO-mediated and a K_{ATP} channel-mediated mechanism of AM-induced vasodilatation in the fetal pulmonary circulation. In our study, L-NNA was far less efffective than glibenelamide in decreasing the vasodilation (CBF increase by 14.90±1.22% in AM+L-NNA group; by 5.46±0.67% in AM+glibenclamide group). In addition, after endothelium degradation by Triton X-100, AM still cause the coronary vasodilation but in the less extent. Together with the discovery of specific receptor for AM on vascular smooth muscle (Eguchi et al., 1994) and that K_{ATP} channels have also been identified in vascular muscle (Standen et al., 1989; Nelson et al., 1990). These results, providing support for the hypothesis that AM may produce the opening of K_{ATP} channels both in endothelium and vascular smooth muscle.

However, mechanisms other than the opening of K_{ATP} channels might be involved in the direct effect of AM on vascular smooth muscle. Many studies have considered cAMP as a primary second messenger for AM. In vascular smooth muscle cells, increases in cAMP results in activation of protein kinase A, increasing in turn Ca^{2+} efflux through the Ca^{2+} pump. Previous studies have shown that AM elevated cAMP in cultured smooth muscle cells from rat aorta (Eguchi et al., 1994a, b; Ishizaka et al., 1994; Shimekake et al., 1995). There are also evidences that accumulation of cAMP in smooth muscle leads to an inhibition of contraction by decreasing $[Ca^{2+}]_i$ and Ca^{2+} sensitivity of contractile elements of smooth muscle (Karaki H, 1989; Karaki et al., 1997). Recently, AM has been shown to increase cAMP and decrease both $[Ca^{2+}]_i$ and muscle tension at the resting or contracted muscle of porcine coronary artery (Yoshimoto et al., 1998). In addition, AM decrease $[Ca^{2+}]_i$ and Ca^{2+} sensitivity of contractile elements in pig coronary arterial smooth muscle (Kureishi et al., 1995). Taken together, it is likely, therefore, that AM may also partly relax the coronary artery by increasing cAMP in vascular smooth muscle cells, resulting in a decrease in $[Ca^{2+}]_i$ and Ca^{2+} sensitivity. However, ventricular cAMP accumulation was not affected by AM in our study. This may implied that in isolated rat heart model, coronary vasodilatory effect of AM is not mediated via the cAMP pathway.