Review

Heart and Vessels

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It is time to ask what adenosine can do for cardioprotection

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Summary. Prevention and attenuation of ischemia and reperfusion injury in patients with acute coronary syndrome are critically important for cardiologists. To save these patients from deleterious ischemic insults, there are three different strategies. The first strategy is to increase ischemic tolerance before the onset of myocardial ischemia; the second is to attenuate the ischemia and reperfusion injury when an irreversible process of myocardial cellular injury occurs; the third is to treat the ischemic chronic heart failure that is caused by acute myocardial infarction. Adenosine, which is known to be cardioprotective against ischemia and reperfusion injury, may merit being used for these three cardioprotection strategies. First of all, adenosine induces collateral circulation via induction of growth factors, and triggers ischemic preconditioning, both of which induce ischemic tolerance in advance. Secondly, endogenous adenosine may mediate the infarct size-limiting effect of ischemic preconditioning, and exogenous adenosine is known to attenuate ischemia and reperfusion injury. Thirdly, we also revealed that adenosine metabolism is changed in patients with chronic heart failure, and increases in adenosine levels may attenuate the severity of ischemic heart failure. Therefore, adenosine therapy may improve the pathophysiology of ischemic chronic heart failure. Taking these factors together, we hereby propose potential tools for cardioprotection attributable to adenosine in ischemic hearts, and we postulate the use of adenosine therapy before, during, and after the onset of acute myocardial infarction.

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Introduction

It is critically important to consider how cardioprotection is achieved in ischemic heart diseases, because mortality and morbidity of heart disease has increased in Japan and Western countries, and the burden in terms of not only individual patients but also social and economical aspects is increasing as we move towards the 21st century. To decrease this burden, it is essential to protect the heart against ischemia and reperfusion injury. There are three different aspects to achieve cardioprotection in the diseased hearts. First, the acquisition of tolerance against ischemia and reperfusion injury is effective for patients with coronary artery disease or coronary risk factors. For example, the development of collateral circulation in advance will reduce the severity of ischemia even when coronary occlusion occurs. To prevent rupture of the atheroma at the coronary vessels is another paradigm for attenuating the incidence of acute coronary syndrome. Furthermore, if the trigger mechanisms of cardioprotection due to ischemic preconditioning could be elucidated, they could be applied in patients at a high risk for acute myocardial infarction. In other words, we need to develop a prevention method against acute coronary syndrome. Second, it is also important to develop tools for the treatment of ischemic and reperfusion injury. To our knowledge, we do not have any drugs or tools to decrease either infarct size or cardiac remodeling in patients with acute myocardial infarction, except for recanalization therapy. If the mediators of cardioprotection due to ischemic preconditioning were eluci-

dated, we could administer drugs to reduce the size of the myocardial infarction. Even if patients do not die following acute myocardial infarction, chronic ischemic heart failure may occur and increase mortality or morbidity. Therefore, as the third paradigm, we need to consider drugs to treat chronic ischemic heart failure. Angiotensin converting enzyme (ACE) inhibitors and β-blockers are proven to attenuate the mortality and morbidity of chronic heart failure. However, we do not believe that these drugs are powerful enough to treat chronic heart failure satisfactionly.

Adenosine, an autacoid produced and released mainly during myocardial ischemia and reperfusion, has multifactorial action in the heart. Adenosine is produced not only in cardiomyocytes but also in endothelial cells, and is known to be cardioprotective via activation of adenosine receptors [1–3]: (1) attenuation of release of catecholamine, β-adrenoceptor-mediated myocardial hypercontraction, and myocardial Ca^{2+} overload via adenosine A_1 receptors; (2) increases in coronary blood flow, and inhibition of platelet and leukocyte activation via adenosine A_2 receptors. Furthermore, adenosine inhibited both renin and tumor necrosis factor-α (TNF-α) production in experimental models [4, 5]. It increases the transcriptional levels of vascular endothelial growth factor (VEGF) in endothelial cells [6], and is believed to contribute to angiogenesis, suggesting an important role for the development of collateral vessels to the ischemic area [7–9]. These effects of adenosine may synergistically inhibit the deleterious sequelae of ischemic heart diseases. Thus, adenosine is believed to (1) develop the collateral circulation, (2) trigger and mediate cardioprotection in ischemic preconditioning, and (3) attenuate the severity of chronic heart failure. Therefore, we will discuss here the cardioprotective role of adenosine in ischemic heart disease.

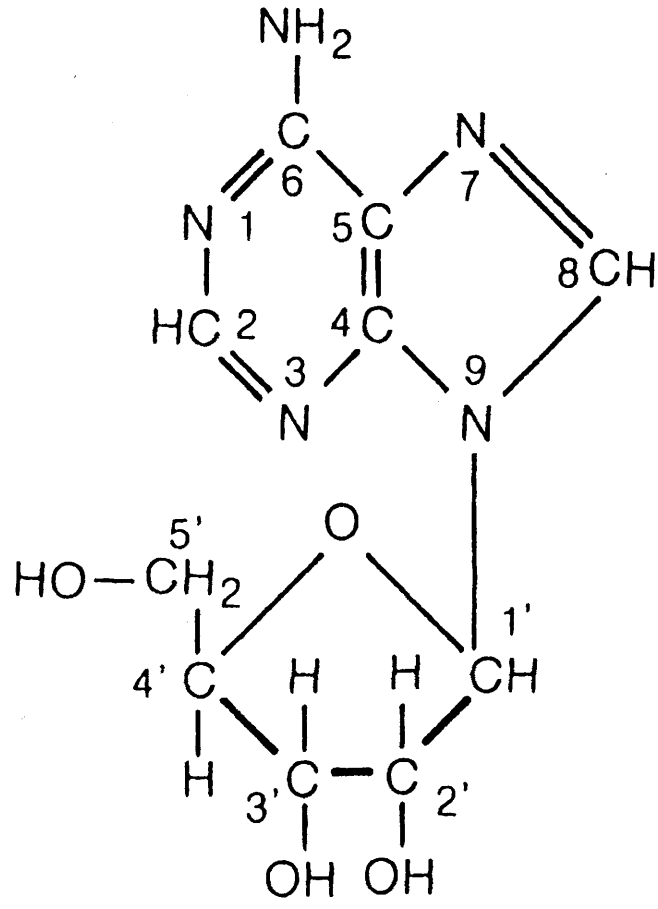

Fig. 1. The molecular structure of adenosine [10]

What is adenosine?

Adenosine, a metabolite of adenine nucleotides, is a ubiquitous biological compound found in every cell of the human body. Figure 1 shows its molecular structure [10], the molecular weight being 267.

Receptors

Although adenosine can enter the cardiomyocytes directly and modulate cellular function as the substrate for ATP resynthesis, its physiological actions are mainly attributable to the activation of adenosine receptors, which are classified into three subtypes [11]. Four adenosine receptor subtypes have been cloned, namely A_1, A_{2a}, A_{2b}, and A_3, and all subtypes are coupled to guanine nucleotide-binding (G) protein. Adenosine A_1 receptors are responsible for inhibition of adenylate cyclase activity via activation of G_i proteins, and A_2 receptors are responsible for stimulation of this enzyme activity via activation of G_s proteins [12]. A_3 receptor activation is thought to activate G_o or G_q proteins, which may increase phospholipase C activity. Since A_1 and A_3 receptors are distributed mainly among myocardial cells, and A_2 receptors among coronary vascular smooth muscles in the heart [13], adenosine may substantially modulate cardiac function as a whole.

The sensitivity of adenosine receptors may be altered by pathophysiological conditions. Exposure to exogenous adenosine desensitizes the adenosine A_1 receptors, and decreases the cellular responsiveness to adenosine, and adenosine receptor antagonist increases the number of A_1 receptors, and thus the cardiac response to adenosine. As for adenosine A_2 receptors, chronic intake of caffeine in humans sensitizes platelets to the antiaggregator actions of adenosine via adenosine A_2 receptors. H^+ also increases the sensitivity of adenosine A_2 receptors in the coronary vessels [14].

We have also found that α_2-adrenergic activity modifies the vasodilatory action of adenosine. A low dose of the α_2-adrenoceptor agonist, clonidine, enhances adenosine-induced coronary vasodilation [15–17] and low doses of yohimbine and rauwolscine, α_2-adrenoceptor antagonists, attenuate the coronary flow response to either endogenous or exogenous adenosine [15, 16]. This is consistent with earlier studies of Nayler et al. [18]. They observed that phenoxybenzamine, a nonspecific α-adrenoceptor antagonist, blocks the vasodilatory action of adenosine in isolated rat and guinea pig hearts. Furthermore, attenuation of ischemia-induced myocardial damage by administration of clonidine in coronary hypoperfusion and in coronary microembolization strongly suggests that adenosine plays an important role by dilation of the coronary arterial bed; clonidine significantly increased coronary blood flow in both ischemic models without augmentation of adenosine release [17].

Adenosine production in the heart

Major pathways of adenosine formation are the dephosphorylation of 5′-AMP by 5′-nucleotidase (EC 3.1.3.5) and the hydrolysis of *S*-adenosylhomocysteine (SAH) by SAH-hydrolase (EC 3.3.1.1) [19]. During normoxia, a major source of adenosine is SAH formed from *S*-adenosylmethionine (SAM) through the transfer of the methyl group to a variety of methyl acceptors [20]. SAH is hydrolyzed by SAH-hydrolase to adenosine and homocysteine. Adenosine is phosphorylated by adenosine kinase or deaminated by adenosine deaminase. The rate of adenosine production is reported to be approximately 800 pmol/min per g in the isolated perfused guinea pig heart, which is very close to the hydrolysis rate of SAH (750 pmol/min per g) [21]. This finding suggests that most of the synthesized adenosine is derived from SAH during the normoxic condition. The basal level of adenosine does not play a major role in the regulation of coronary blood flow because the basal coronary blood flow does not change during infusion of an adenosine receptor antagonist or adenosine deaminase. During ischemia or hypoxia, however, the major pathway of adenosine production is shifted to the 5′-AMP pathway [22], because adenosine production is markedly attenuated by the inhibitor of ecto-5′-nucleotidase. In perfused hearts, there is a close relationship between tissue levels of adenosine, the rate of release of adenosine into the perfusate, and coronary blood flow during hypoxia [23]. The adenosine receptor antagonist, theophylline, decreases coronary blood flow during hypoperfusion [24–26]. Also, a significant attenuation of the increase in coronary flow during systemic hypoxia has been observed after intracoronary administration of adenosine deaminase [27–29]. These findings indicate that adenosine plays a major role in the regulation of coronary blood flow in the ischemic heart.

We have reported that the α_1-adrenoceptor antagonist, prazosin, markedly attenuates the release of adenosine from the ischemic myocardium either during hypoperfusion [30] or after coronary microembolization [31]. Administration of a low dose of prazosin, which did not affect basal coronary blood flow, reduced coronary blood flow and further exaggerated the ischemic damage, i.e., increase in lactate production and decrease in regional myocardial segment shortening. Since the contribution of α-adrenergic activity to the release of adenine nucleotides has also been reported in endothelial cells [32], it is likely that the activation of protein kinase C (PKC) by the α_1-adrenergic stimulation is involved in the production of adenosine in the hypoxic heart [33, 34]. We found that ecto-5′-nucleotidase is activated by PKC in rat cardiomyocytes (Fig. 2) and that it increases the adenosine levels in cardiomyocytes. Hermann and Feigl also observed that adrenergic receptor blockade attenuates adenosine concentration

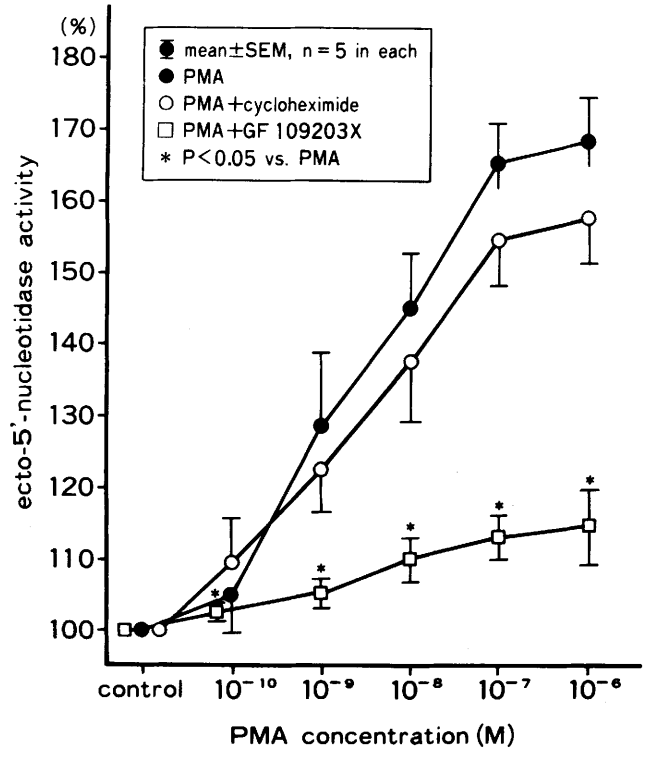

Fig. 2. The dose–response relation between phorbol 12-myristate 13-acetate (*PMA*) and ecto-5′-nucleotidase activity with and without either GF109203X (an inhibitor of protein kinase C) and cycloheximide (an inhibitor of protein synthesis) in rat cardiomyocytes. Ecto-5′-nucleotidase activity in the control conditions was 6.44 ± 0.89, 5.96 ± 0.78, and 5.81 ± 0.44 nmol/mg protein per min in the PMA, PMA with GF109203X, and PMA with cycloheximide groups, respectively [56]

and coronary vasodilation during hypoxia in dogs [35]. Therefore, PKC activation via α_1-adrenoceptor stimulation may mediate adenosine production in the ischemic heart via activation of ecto-5′-nucleotidase.

Treatment with adenosine before the onset of acute myocardial infarction

In subjects with high risk factors for acute myocardial infarction such as hyperlipidemia, smoking, and hypertension, pretreatment to attenuate ischemia and reperfusion injury is an effective method. This is similar to immunization therapy for infectious diseases such as mumps. There are two aspects of adenosine pretreatment for either prevention or attenuation of ischemia and reperfusion injury.

Collateral circulation in ischemic heart diseases

An important strategy to attenuate the severity of myocardial ischemia even when coronary arterial occlusion occurs is to increase the collateral circulation. The FGF (fibroblast growth factor) family, TGF-β (transforming growth factor-β), and VEGF (vascular endothelial growth factor) are known to be important growth factors for angiogenesis [36–38]. Recently, basic FGF has proved to be responsible for the development of the collateral circulation [39]. Yanagisawa et al. [39] clearly showed that basic FGF levels increase after the onset of myocardial infarction. Heparin is also known to cause angiogenesis since heparin activates HB-EGF, which then activates the migration and proliferation of smooth muscle cells [40, 41]. Furthermore, VEGF is also known to cause potent angiogenesis due to proliferation and migration of endothelial cells [42].

Adenosine is known to increase the mRNA and the protein levels of VEGF [6], suggesting its important role in the development of the collateral circulation. Furthermore, adenosine increases the proliferation and migration of endothelial cells in vitro [7–9]. In vivo, adenosine stimulates angiogenesis on the chick chorioallantonic membrane [7], and dipyridamole increases the adenosine-induced angiogenesis. Finally, repeated chronic treatment with dipyridamole increases the regional myocardial flow of the ischemic area compared with the control, and this effect cannot be mimicked by diltiazem [43]. This result suggests that coronary vasodilation per se does not affect the development of collateral circulation, but the enhancement of adenosine during administration of dipyridamole can increase the development of collateral flow. There are no reports linking VEGF and adenosine from the viewpoint of angiogenesis in vivo. However, one of the major methods for inducing ischemic tolerance may be to treat the hearts with an adenosine-related compound. Another method for increasing ischemic tolerance is to induce cardioprotection by ischemic preconditioning.

The trigger mechanisms of ischemic preconditioning

What is ischemic preconditioning?

Recently, ischemic preconditioning, first described by Murry et al. [44], has received much attention from both basic researchers and clinicians. Results to date have shown that ischemic preconditioning limits infarct size to 10%–20% of the risk area in the reperfused ischemic myocardium [44–47]. Liu et al. [45] have implicated endogenous adenosine in ischemic preconditioning by demonstrating that administration of 8-phenyltheophylline abolishes the salutary effect of ischemic preconditioning. These investigators have hypothesized that ischemic preconditioning occurs via adenosine A_1 receptor activation. Adenosine A_1 receptor activates PKC via activation of phospholipase C, and several investigators, including our group, found that activation of PKC is transiently observed after the procedure of ischemic preconditioning [48–52]. Furthermore, the inhibition of PKC blunts the infarct size-limiting effect of ischemic preconditioning [48, 51, 52]. Therefore, at present, activation of PKC is believed to be a common pathway to trigger cardioprotection.

Cardioprotection due to the ischemic preconditioning procedure disappears in several hours, and this disappearance is attributable to dephosphorylation of the cardioprotective proteins or enzymes. Interestingly, cardioprotection will reappear in 24–48 h after the ischemic preconditioning. This fact has been revealed by other groups, independently, and is called the second window of ischemic preconditioning [53, 54]. Marbar et al. [53] reported that HSP-72 induction is important to mediate the infarct size-limiting effect of ischemic preconditioning, and Kuzuya et al. [54] reported that Mn-superoxide dismutase (Mn-SOD) induction may contribute to the cardioprotection in ischemic preconditioning. We also revealed that induction of ecto-5′-nucleotidase also contributes to cardioprotection. Suzuki et al. [55] reported that transfection of HSP-72 induces the activation of ecto-5′-nucleotidase, and that HSP-72-induced cardioprotection is abolished by the inhibitor of ecto-5′-nucleotidase, suggesting that these factors are linked to each other to synergistically mediate cardioprotection. We also reported that SOD increases ecto-5′-nucleotidase [55].

PKC and adenosine

The question arises how PKC is activated during ischemic preconditioning. In the canine heart, we ob-

served that PKC-α is activated due to the ischemic preconditioning procedure. Since ischemic preconditioning increases release of norepinephrine from the presynaptic vesicles, we tested the role of α_1-adrenoceptor activation in cardioprotection with ischemic preconditioning [57]. Using open-chest dogs, we found that prazosin attenuated both the activation of ecto-5′-nucleotidase and the infarct size-limiting effect of ischemic preconditioning, and that methoxamine, an α_1-adrenoceptor stimulant, mimicked the infarct size-limiting effect of ischemic preconditioning. Several other stimulants of PKC, e.g., adenosine, bradykinin, endothelin, and angiotensin II, are also reported to activate PKC in different species, suggesting that various stimuli during the ischemic preconditioning procedure contribute to the activation of PKC.

The next question is how PKC activation triggers the infarct size-limiting effect of ischemic preconditioning. PKC is reported to open ATP-sensitive K^+ (K_{ATP}) channels [58], which may have a cardioprotective effect against ischemia and reperfusion injury. This is because (1) the opening of K_{ATP} channels decreases the action potential duration of the membrane and attenuates the Ca^{2+} inward via voltage-dependent Ca^{2+} channels, (2) nicorandil and cromakalim attenuate the infarct size even without preconditioning, and (3) glibenclamide attenuates the infarct size-limiting effect of ischemic preconditioning. Recently, the opening of mitochondrial K_{ATP} channels was also activated by PKC [59, 60], suggesting that mitochondrial K_{ATP} channels are responsible for cardioprotection. However, if either sarcolemmal or mitochondrial K_{ATP} channels are opened by PKC and these openings may be mediators of cardioprotection, it is unclear how the opening of either sarcolemmal or mitochondrial K_{ATP} channels exert their physiological actions only during ischemia and reperfusion, but not during the preischemic period.

On the other hand, we showed that activation of PKC increases ecto-5′-nucleotidase activity (Fig. 2), and mediates the cardioprotection via enhancement of adenosine production in ischemic preconditioning [47, 49, 61]. Since adenosine can be produced only when the substrate (AMP) is supplied, i.e., during myocardial ischemia, and released adenosine can cause several beneficial actions, the activation of ecto-5′-nucleotidase does not necessarily cause the contractile and metabolic changes of the myocardium before the sustained ischemia after the preconditioning procedure. Activation of ecto-5′-nucleotidase increases adenosine levels only during sustained myocardial ischemia and reperfusion, which may primarily merit cardioprotection. This hypothesis is different from the idea of Downey's group: Downey hypothesized that adenosine activates PKC, but our hypothesis is that ischemic preconditioning activates PKC, which phosphorylates ecto-5′-nucleotidase, and thereby causes cardioprotection. We also do not refute Downey's hypothesis and many investigators suggest the existence of this pathway, but our idea is whether protein kinase C can also activate the adenosine-dependent mechanisms. Some investigators confirm our hypothesis [62–64], and others do not [65, 66]. Further elucidation is necessary concerning the role of ecto-5′-nucleotidase on the infarct size-limiting effect of activation of PKC.

Disappearance of ischemic preconditioning

The cardioprotective effect of ischemic preconditioning disappeared within 1–3 h (Fig. 3), and this process may be attributable to the dephosphorylation of cardioprotective proteins or enzymes. There are no data on the time courses of deactivation or dephosphorylation of K_{ATP} channels, but we have revealed that deactivation of ecto-5′-nucleotidase occurs within several hours following the ischemic preconditioning procedure (Fig. 4) [67, 68]. Since dephosphorylation of ecto-5′-nucleotidase is mediated by phosphatase, inhibition of phosphatase may prolong the cardioprotection of ischemic preconditioning [69]. Indeed, okadaic acid, which inhibits phosphatase, inhibits the deactivation of ecto-5′-nucleotidase and prolongs the infarct size-

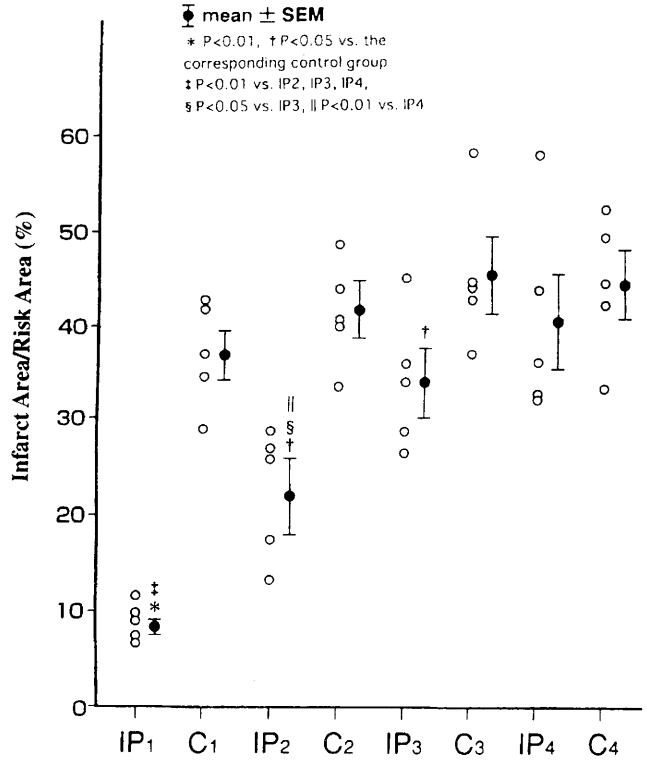

Fig. 3. Infarct size in the ischemic preconditioning (*IP*) and control groups. Data were analyzed by the modified Bonferroni's test [69]

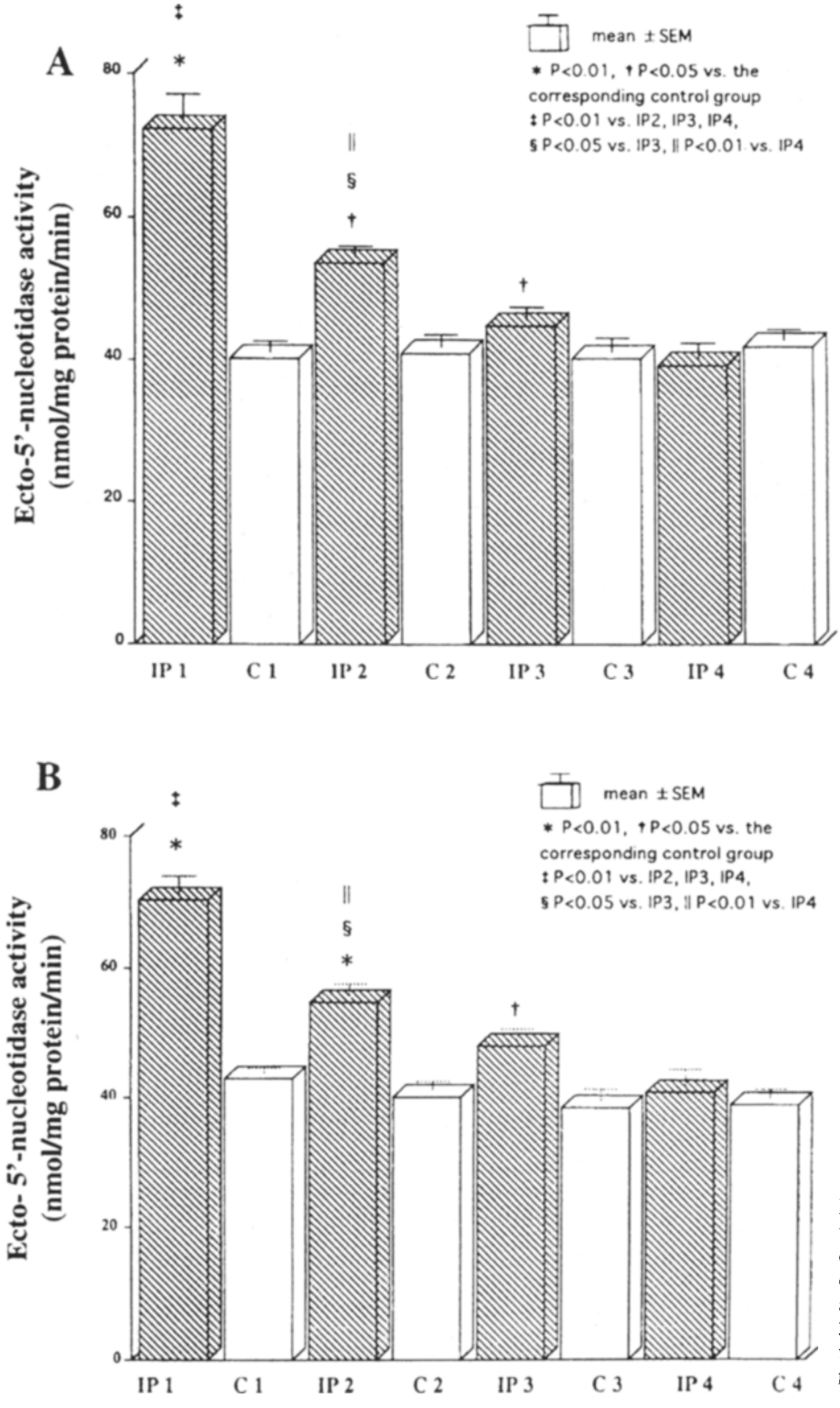

Fig. 4. Ecto-5′-nucleotidase activity in the epicardium (**A**) and the endocardium (**B**) in the preconditioned and control myocardium obtained immediately after preconditioning or after a 30-min, 60-min, or 120-min recovery time. Data were analyzed by the modified Bonferroni's test [69]

limiting effect [70]. Another method is to keep the activation of adenosine receptors or PKC until the onset of sustained myocardial ischemia. We administered dipyridamole or dilazep to increase adenosine levels during this period, and we found that both the activation of ecto-5′-nucleotidase and the infarct size-limiting effect was prolonged compared with the no-treatment group (Fig. 5) [71]. Therefore, since we need to prolong the infarct size-limiting effect to realize the cardioprotection of ischemic or pharmacological preconditioning

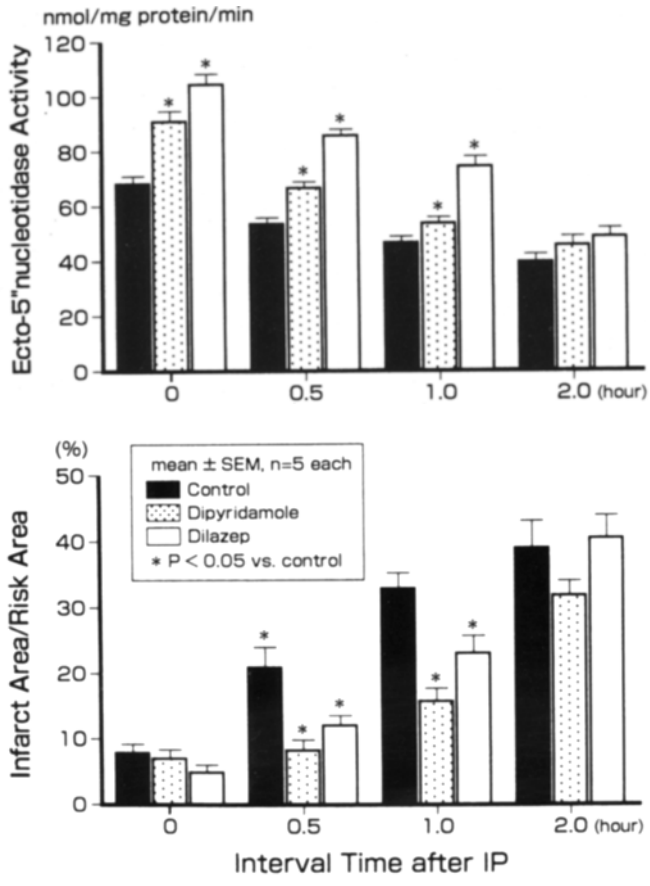

Fig. 5. Effects of dipyridamole and dilazep on the deactivation of ecto-5′-nucleotidase and the disappearance of the infarct size-limiting effect of ischemic preconditioning [69]

in the clinical setting, we may propose the sustained elevation of adenosine levels after the ischemic or pharmacological preconditioning procedure as a method of prolonging cardioprotection.

Treatment of acute myocardial infarction with adenosine

The mediators of the infarct size-limiting effect of ischemic preconditioning

There are two ways of finding a method to directly decrease ischemia and reperfusion injury. One is to find the mediator of the infarct size-limiting effect of ischemic preconditioning, and the other is to invent drugs to attenuate the ischemia and reperfusion injury independent of cardioprotection of ischemic preconditioning.

Preconditioning and ecto-5′-nucleotidase

Since the activation of ecto-5′-nucleotidase may predict the elevation of myocardial adenosine levels during ischemia and reperfusion, we measured adenosine levels during sustained ischemia and reperfusion. In anesthetized open-chest dogs, we observed that adenosine concentration in the coronary venous blood was higher in the group with ischemic preconditioning compared with the untreated control group. We observed that AOPCP (α,β-methyleneadenosine 5′-diphosphate), a potent and selective inhibitor of ecto-5′-nucleotidase, completely abolished both elevation of the adenosine levels in coronary venous blood and the infarct size-limiting effect of ischemic preconditioning [47]. Therefore, we suggest that this enhanced adenosine release during ischemia and reperfusion from the preconditioned myocardium may decrease infarct size, since it has been proven that exogenously administered adenosine decreases infarct size [72].

There are two criticisms of our hypothesis. The first is that adenosine may be produced in large enough amounts to attenuate infarct size even without ischemic preconditioning. However, endogenous adenosine is fully released during ischemia and the reperfusion period, indicating that further elevation of adenosine may not mediate further cardioprotection. Second, there is a report indicating that increases in adenosine concentration in the interstitial space are not augmented during sustained ischemia in the ischemic preconditioning group [65]. We also observed increases in the adenosine levels in the coronary venous blood during sustained ischemia but not in the interstitial space in the ischemic preconditioning group in our experiment. One possible explanation for this difference is that the adenosine level in the coronary venous blood is largely affected by endothelial cells; in turn, the interstitial adenosine levels may be largely affected by myocardial ecto-5′-nucleotidase. Thus, ischemic preconditioning has a different effect on ecto-5′-nucleotidase located at the endothelial cells and that at cardiomyocytes. Secondly, it is possible that even if the adenosine concentration in the microenvironment surrounding ecto-5′-nucleotidase on the myocardial cellular membrane is increased by the activated ecto-5′-nucleotidase, the alteration of the interstitial volume determined by myocardial cellular swelling and the rate of washout due to the lymphatic stream may change the interstitial adenosine concentration. In any of these possible situations, the temporal and topical increases in the adenosine concentration surrounding ecto-5′-nucleotidase may be able to directly activate the adenosine receptors located on the same cellular membrane, which may not contradict Van Wylen's observation [65]. This close juxtaposition may explain how ecto-5′-nucleotidase activates the adenosine receptors. Indeed, the regulatory systems, including the ATP receptors, G proteins, ecto-5′-nucleotidase, and K_{ATP} channels, are closely linked with each other and are present in a single patch of no more than 1 mm² [72].

Ecto-5′-nucleotidase and PKC

Since the activation of ecto-5′-nucleotidase may mediate the infarct size-limiting effect via modulation of adenosine metabolism during sustained ischemia and reperfusion, both the activation of ecto-5′-nucleotidase and the infarct size-limiting effect should be blunted by an inhibitor of PKC. We observed that activation of ecto-5′-nucleotidase due to ischemic preconditioning is blunted by GF109203X, an inhibitor of PKC, in canine hearts, and inhibition of PKC by GF109203X blunted the infarct size-limiting effect of ischemic preconditioning. We have also revealed that threonine and serine residues of ecto-5′-nucleotidase are phosphorylated in the preconditioned myocardium. Therefore, we speculate that phosphorylation of ecto-5′-nucleotidase due to PKC may change the characteristics of the active site of ecto-5′-nucleotidase or induce a conformational change in the structure of 5′-nucleotidase.

Prazosin blunted the increases in ecto- and cytosolic 5′-nucleotidase activities due to ischemic preconditioning, and completely abolished the infarct size-limiting effect of ischemic preconditioning. Methoxamine increased both ecto- and cytosolic 5′-nucleotidase activities to the levels obtained by ischemic preconditioning, and it attenuated infarct size to the level seen with ischemic preconditioning. This observation is consistent with previous studies. It is reported that α_1-adrenoceptor activation is intimately involved in attenuation of the severity of ischemia and reperfusion [2, 30] and ischemic preconditioning [57]. Therefore, these results may support the hypothesis that α_1-adrenoceptor stimulation via activation of PKC mediates the cardioprotection seen in ischemic preconditioning, which is attributable to activation of ecto-5′-nucleotidase. We also found that activation of PKC due to ischemic preconditioning is α-PKC in canine hearts [49, 50].

In summary, we propose our hypothesis that the linkage between PKC–ecto-5′-nucleotidase–adenosine production plays the role of mediating cardioprotection seen in ischemic preconditioning. It is intriguing that the backward (PKC – adenosine) and forward (adenosine – PKC) mechanisms amplify each others' mechanisms, and contribute to the cardioprotection afforded by ischemic preconditioning. A future aim in the field of ischemic preconditioning is to examine intracellular mechanisms, and several investigators home suggested the involvement of p38MAP kinase and p70 S6 kinase, and sarcolemmal and mitochondrial K_{ATP} channels.

Adenosine and ischemic heart diseases

Since enhanced release of adenosine via activation of ecto-5′-nucleotidase may be one of the mediators of cardioprotection in ischemic preconditioning, exogenous adenosine may be effective for the treatment when myocardial ischemia occurs.

It has been thought that stimulation of adenosine A_2 receptors activates adenylate cyclase in the coronary arteries to produce cyclic adenosine monophosphate (cAMP), and relaxes coronary vascular smooth muscles [74]. Several studies suggest that low concentrations of adenosine relax vascular smooth muscles, primarily by decreasing intracellular Ca^{2+} levels, due to either reduction of sarcolemmal permeability to Ca^{2+} [75] or to enhancement of Ca^{2+} sequestration [76]. Increases in cAMP may increase the uptake of Ca^{2+} into the sarcoplasmic reticulum, and cause vasorelaxation. Furthermore, cAMP may open K_{ATP} channels, and decrease Ca^{2+} inward into the smooth muscle cells. Indeed, adenosine-induced coronary vasodilation is attenuated by glibenclamide, an inhibitor of the opening of K_{ATP} channels [77, 78].

The endothelium is also involved in the vasodilatory action of adenosine [79]. The vasodilatory effect of adenosine is attenuated by removal of the endothelium in the isolated canine coronary artery, and this effect is greater when adenosine is exposed to the luminal side than when applied to the adventitial side [80]. Furthermore, NO is released due to the stimulation of adenosine A_1 receptors [81]. Indeed, adenosine activates guanylate cyclase and increases the intracellular cyclic guanosine monophosphate (cGMP) [81]. These observations are compatible with the concept that endogenous adenosine released from the cardiomyocytes may act on the coronary vascular smooth muscles (A_2-receptor mediated) in a different way from the exogenous adenosine acting on the endothelial cell receptors (A_1-receptor mediated). Furthermore, we have shown that adenosine is required to maintain the NO synthase (NOS) activity of the endothelial cells; in cultured endothelial cells, adenosine A_2 receptor blockade decreased NOS activity.

In the ischemic heart, adenosine-induced coronary vasodilation is beneficial for preserving mechanical and metabolic function during myocardial ischemia [1, 2]. However, this is not the only effect of adenosine in the ischemic myocardium. Thromboembolism in small coronary arteries, which is believed to be one of the causes of the "no-reflow phenomenon" of the reperfused myocardium, may worsen the severity of acute myocardial infarction. Small coronary microembolizations are caused by platelet aggregation, and stimulation of the adenosine A_2 receptors has been reported to inhibit the platelet aggregation induced by norepinephrine in vitro [83, 84]. We have investigated whether endogenous adenosine inhibits thromboembolism secondary to platelet aggregation in in vivo ischemic hearts (Fig. 6) [83]. We further examined the cellular mecha-

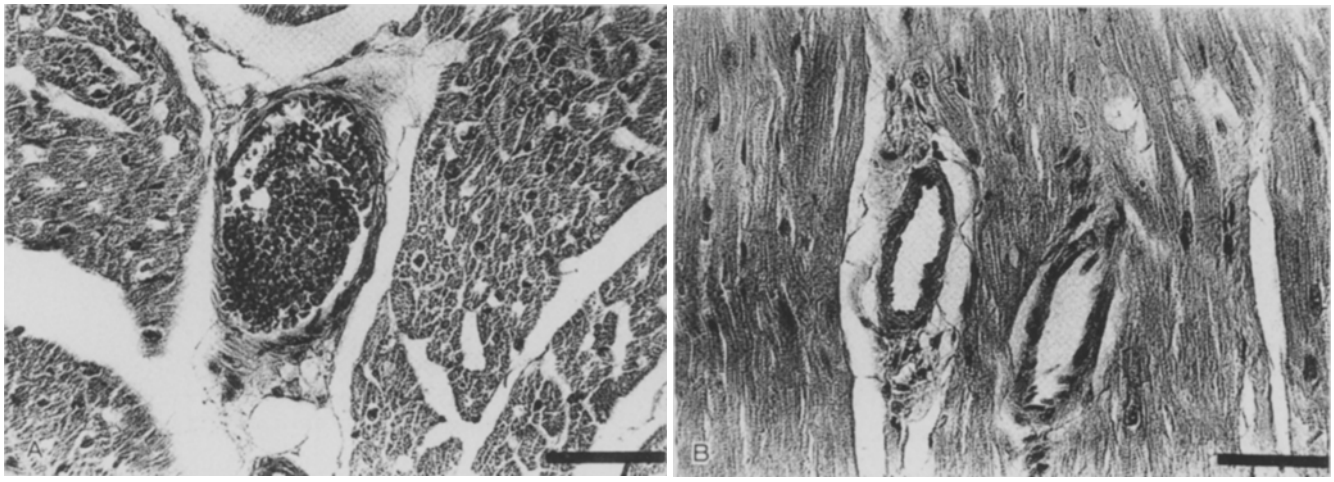

Fig. 6. Photomicrograph of hypoperfused coronary arteries without (**A**) and with (**B**) the intracoronary administration of 8-phenyltheophylline during coronary hypoperfusion (38 ± 2 mmHg). 8-Phenyltheophylline is a potent antagonist of intracoronary adenosine receptors and induced thrombosis in the small coronary arteries. Tissue excised following in situ perfusion fixation for 3 min following the onset of ischemia. *Bars*-50 μm (hematoxylin and eosin) [83]

nisms of platelet aggregation when adenosine receptors were inhibited (Fig. 6). The appearance of P-selectin in the platelets increased due to 8-sulfophenyltheophylline treatment (Fig. 7), and the inhibitor of P-selectin inhibited the platelet aggregation with leukocytes, and thus with endothelial cells [84]. Thus, endogenous adenosine released in the ischemic myocardium inhibited the activation of platelet P-selectin, and inhibited the microembolization in the small coronary vessels.

Adenosine also inhibits leukocyte chemotaxis [85] and the production of oxygen-derived free radicals [86] through the stimulation of adenosine A_2 receptors. This decrease in the inflammatory response may also be cardioprotective [87]. Interestingly, the activation of leukocytes decreases ecto-5′-nucleotidase activity [88], which may decrease adenosine production and further activate leukocytes. These "vicious circles" in leukocytes may enhance the injury in ischemic hearts by release of oxygen-derived free radicals, and adhesion to the endothelial cells to obstruct small coronary arteries.

Adenosine also attenuates the increase in myocardial contractility induced by β-adrenoceptor stimulation [89]. We have shown that this phenomenon actually occurs in ischemic hearts [90]. Attenuation of an increase in myocardial contractility prevents a further increase in the discrepancy between energy supply and demand. This phenomenon appears different from adenosine-induced inhibition of norepinephrine release from sympathetic nerve terminals, as it was not abolished in the hearts denervated with 6-hydroxydopamine [91]. Adenosine-induced inhibition of norepinephrine release may also prevent catecholamine-induced injury caused by excess amounts of norepinephrine. In this sense, norepinephrine release causes two opposite effects. The amount of norepinephrine released during ischemia may enhance adenosine production and adenosine-induced coronary vasodilation through α_1- and α_2-adrenoceptor stimulations [1, 2]. However, high norepinephrine concentrations associated with severe prolonged ischemia may mask the adenosine-related cardioprotection [92].

Adenosine and reperfusion injury

If ischemic heart muscle is reperfused before irreversible injury occurs, contractility remains impaired for a long period, a phenomenon known as myocardial stunning. We have reported that endogenous and exogenous adenosine attenuates myocardial stunning in the canine model via adenosine A_1 and A_2 receptors [93, 94].

It would be of interest to know the mechanisms by which adenosine is decremental to myocardial stunning. Stimulation of adenosine A_1 receptors has been shown to inhibit the β-adrenoceptor-mediated inotropic response and intracellular Ca^{2+} influx [89, 95]. However, since propranolol does not affect the severity of myocardial stunning [94], inhibition of Ca^{2+} influx by adenosine [96] appears to modulate myocardial stunning. Indeed, several experiments have supported Ca^{2+} overload as featuring prominently in the pathogenesis of myocardial stunning [97, 98]. On the other hand, stimulation of adenosine A_2 receptors augments hyperemia and inhibits the activation of neutrophils and platelets [83–87, 99, 100]. However, when we increased hyperemia with papaverine instead of adenosine, myo-

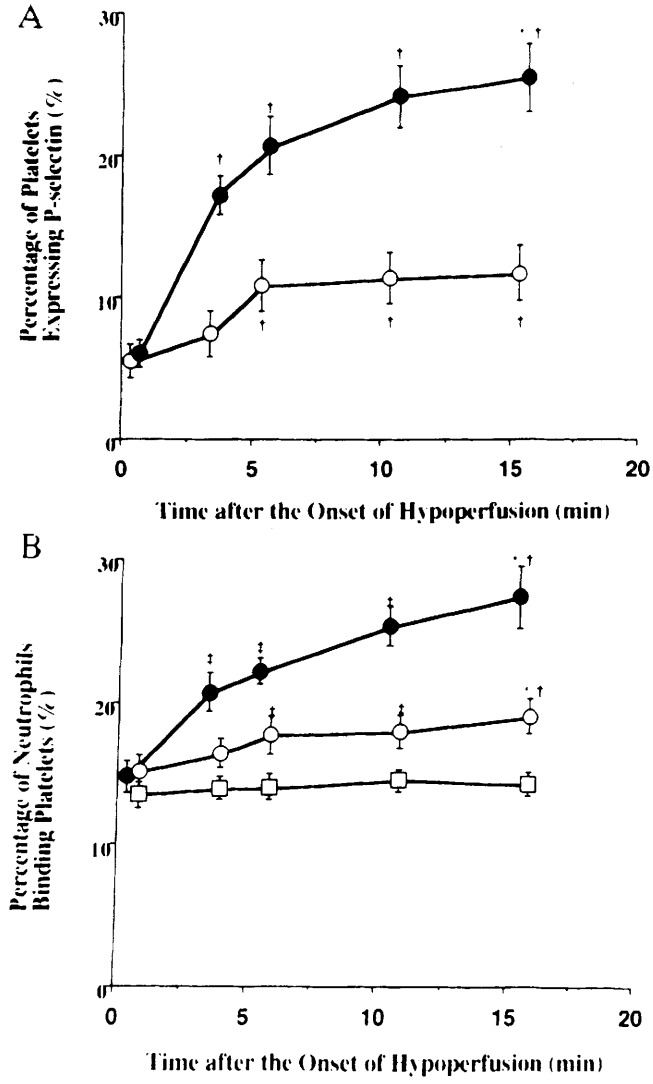

Fig. 7. Changes in the percentage of platelets that expressed P-selectin (**A**) and of neutrophils that bound to platelets (**B**) during coronary hypoperfusion in dogs. 8-Sulfophenyltheophylline (8-SPT) increased the percentage of platelets that expressed P-selectin and of neutrophils that bound to the platelets during coronary hypoperfusion. CY1747 partially inhibited the percentage of neutrophils that bound to the platelets. Each point represents the mean ± SEM of five distinct experiments. *Open circles*, controls; *closed circles*, 8-SPT; *open squares*, 8-SPT + CY1747 [84]

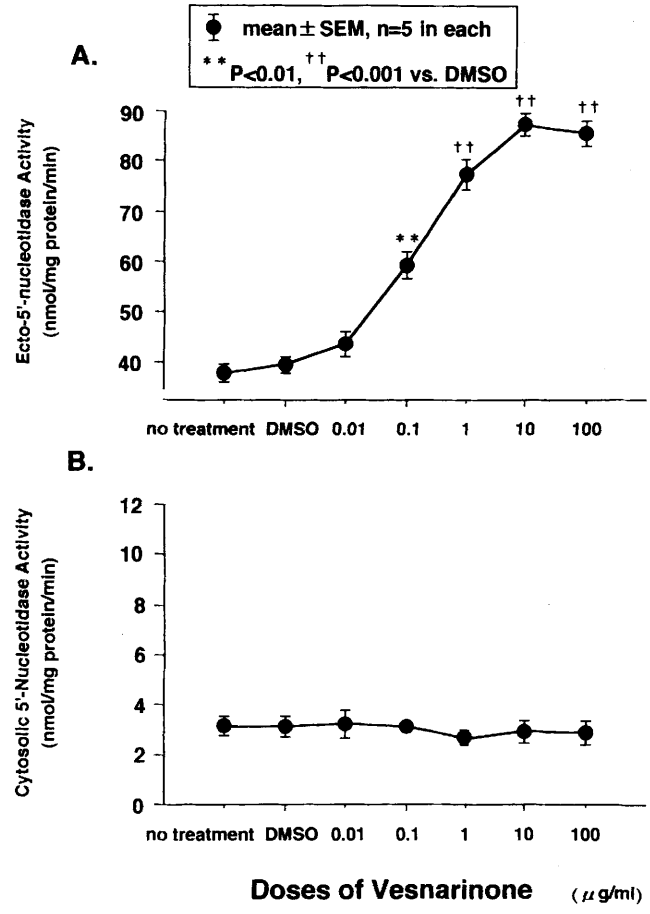

Fig. 8. Ecto- and cytosolic 5′-nucleotidase activity in the presence and absence of vesnarinone. Ecto-5′-nucleotidase is increased, dose-dependently, without changing cytosolic 5′-nucleotidase activity. Statistical significance was tested by ANOVA [119]

cardial stunning did not improve [94], suggesting that the increase in coronary blood flow due to adenosine may not be related to the reduced severity of myocardial stunning.

Microcirculatory disturbances in myocardial stunning improves with the administration of adenosine [101, 102]. Adenosine-induced inhibition of neutrophil and platelet activation may play a role in reducing myocardial stunning, especially since activated neutrophils have been shown to generate oxygen-derived free radicals [87], and the administration of SOD attenuates myocardial stunning. Intriguingly, oxygen-derived free radicals have been reported to reduce ecto-5′-nucleotidase activity and adenosine production during ischemia [56, 102]. Thus, decreased generation of oxygen-derived free radicals due to adenosine may preserve 5′-nucleotidase activity and the capacity for adenosine production.

Adenosine may also attenuate the irreversible myocardial cell injury after reperfusion in various species of animals [72, 104]; intracoronary infusion of adenosine results in a 75% reduction in myocardial infarct size in dogs [104], and attenuates contractile dysfunction in rats [105]. This beneficial effect may be attributed to one or more of the following mechanisms: (1) preservation of ATP, (2) inhibition of neutrophil activation, (3) inhibition of platelet aggregation, and (4) an increase in coronary blood flow. Most of all, the preservation of myocardial ATP levels seems to be the most important

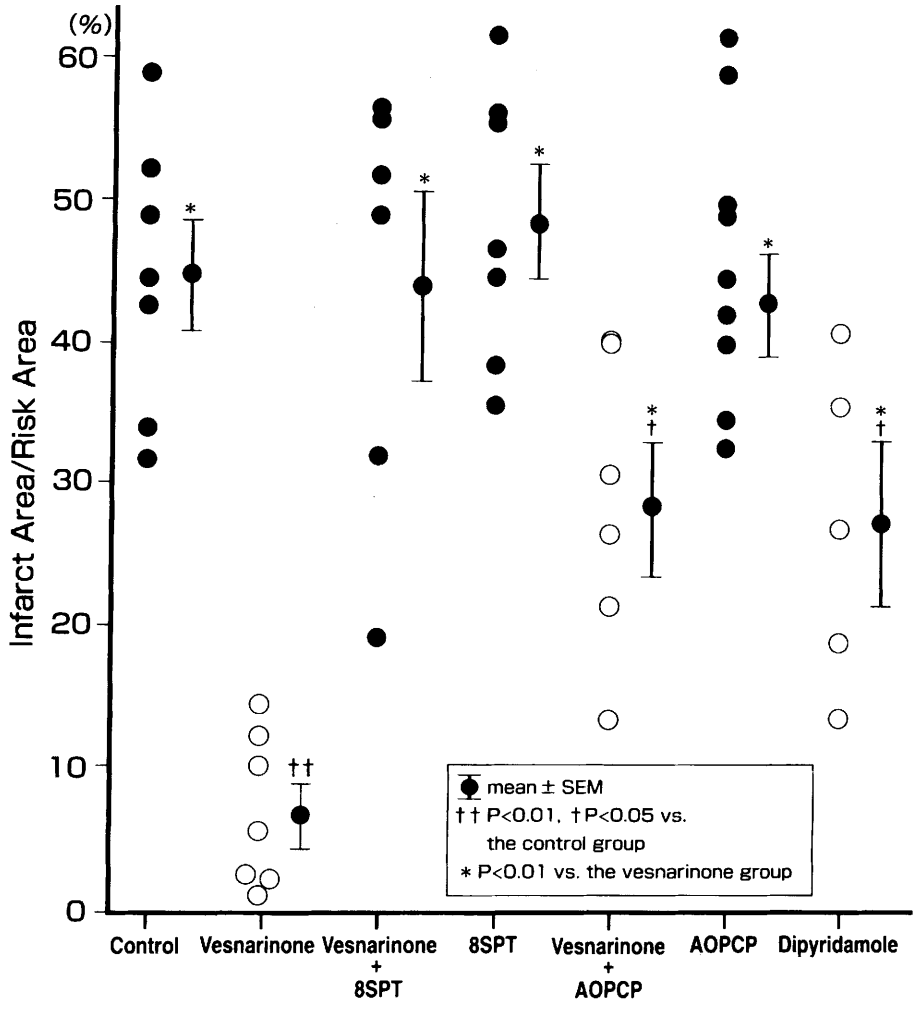

Fig. 9. Infarct size expressed as a percentage of the risk area. Infarct size was markedly decreased in the vesnarinone group compared with the control group, which was completely blunted by 8-SPT and was partially blunted by α,β-methyleneadenosine 5′-diphosphate (AOPCP). The size of the infarct in the dipyridamole group was slightly smaller than in the control group and in larger than in the vesnarinone group, and was similar to that of the vesnarinone with AOPCP group. Statistical significance was tested by ANOVA [119]

factor for cardioprotection against irreversible myocardial cell injury. When adenosine was administered throughout the ischemic and reperfusion periods, a 90-fold increase in ATP synthesis was obtained in the reperfused myocardium [109]. It is known that (1) adenosine stimulates glycolysis in rat hearts [110–112], (2) intracoronary infusion of adenosine increases glucose uptake [113], and (3) dipyridamole enhances glucose uptake accompanied by an increase in myocardial ATP in the newborn lamb [114]. Thus, enhanced glucose metabolism by adenosine may contribute, in part, to a decrease in the rate of ATP depletion during ischemia. A 90% decrease in ATP coincidentally develops the irreversible deterioration of the myocardium [115], leading to the idea that depletion of ATP content in the reperfused myocardium may be a critical factor for the process of irreversible injury.

Since adenosine potently attenuates the ischemia and reperfusion injury, it needs to be tested in the clinical setting. The AMISTAD trial revealed that adenosine administration is effective for the treatment of acute myocardial infarction [115], and we are also planning an ATP administration trial for patients with acute myocardial infarction (COAT trial) [116].

Adenosine-related compounds

Since adenosine is effective in reducing ischemic and reperfusion injury, adenosine-related compounds may effectively provide cardioprotection against ischemia and reperfusion injury. Vesnarinone, a new inotropic agent, has recently been reported to inhibit adenosine uptake in immune cells [117]. Furthermore, we observed that vesnarinone inhibits adenosine uptake in myocytes, endothelial cells, and smooth muscle cells [118]. We also observed that vesnarinone activates ecto-5′-nucleotidase via PKC-independent mechanisms (Fig. 8). These data suggest that vesnarinone may be effective for the treatment of acute myocardial infarction. We tested this hypothesis, and observed that vesnarinone limits infarct size, which is blunted by an antagonist of adenosine receptors (Fig. 9) [119].

Furthermore, both methotrexate and sulfasarazine, drugs for rheumatic arthritis, are reported to attenuate

inflammation via adenosine and ecto-5′-nucleotidase [120–123]. We also tested whether the analog of methotrexate mediates cardioprotection, and found that it does mediate the infarct size-limiting effect, which is completely abolished by either an antagonist of adenosine receptors or AOPCP (unpublished data). AICA riboside also increased the activity of ecto-5′-nucleotidase, which may merit its use for cardioprotection.

It is, therefore, important to discover compounds that increase the activity of ecto-5′-nucleotidase or decrease the activity of adenosine deaminase or kinase.

Treatment with adenosine in chronic heart failure after acute myocardial infarction

What is chronic heart failure?

Chronic heart failure, the end-state of the ischemic heart, is characterized by a reduction in cardiac performance relative to the oxygen demand of the body. However, several neurohormonal factors are reported to ameliorate the severity of chronic heart failure [124]. Catecholamine, renin-angiotensin, and cytokines are thought to be involved in the pathophysiology of chronic heart failure [125–127]. Indeed, chronic heart failure is effectively treated by β-adrenoceptor antagonists and angiotensin converting enzyme (ACE) inhibitors [125, 126], and these drugs have proved to be effective for the treatment of chronic heart failure in mass studies. Interestingly, activation of PKC due to norepinephrine and angiotensin II activates ecto-5′-nucleotidase, and cytokines increase the transcriptional and protein levels of ecto-5′-nucleotidase [127], both of which may increase plasma adenosine levels. Adenosine, produced not only in cardiomyocytes but also in endothelial cells, is known to be cardioprotective via adenosine receptors [1, 2]: (1) by attenuation of release of catecholamine, β-adrenoceptor-mediated myocardial hypercontraction, and Ca^{2+} overload via A_1 receptors; (2) by increases in coronary blood flow, and inhibition of platelet and leukocyte activation via A_2 receptors. Furthermore, adenosine inhibits renin release and TNF-α production in experimental models [4, 5]. However, there are no reports in the literature of the metabolism of endogenous adenosine in chronic heart failure.

Role of endogenous adenosine in pathophysiology of chronic heart failure

Interestingly, we observed that plasma adenosine levels increased according to the NYHA (New York Heart Association) classification in patients with chronic heart failure [128]; there were no significant differences in the plasma adenosine levels in the patients with ischemic and nonischemic (valvular diseases and dilated cardiomyopathy) chronic heart failure. The plasma norepinephrine levels were also increased according to NYHA classification, and there was a correlation ($r = 0.46$, $P < 0.01$) between the plasma adenosine and norepinephrine levels in the patients with chronic heart failure. These results indicate that plasma adenosine levels increase in patients with chronic heart failure, and that this increase in plasma adenosine level correlates well with the functional classes of the severity of chronic heart failure in NYHA classification. Since endogenous norepinephrine levels increase due to the progression of the severity of chronic heart failure, and endogenous norepinephrine increases the activity of ecto-5′-nucleotidase, the enzyme responsible for adenosine production [55], the increased plasma norepinephrine may contribute to the increases in adenosine production. Interestingly, norepinephrine is believed to worsen chronic heart failure, and adenosine attenuates the cardiovascular effect of norepinephrine. Therefore, adenosine may contribute to negative feedback mechanisms against the progressive loop between norepinephrine and heart failure.

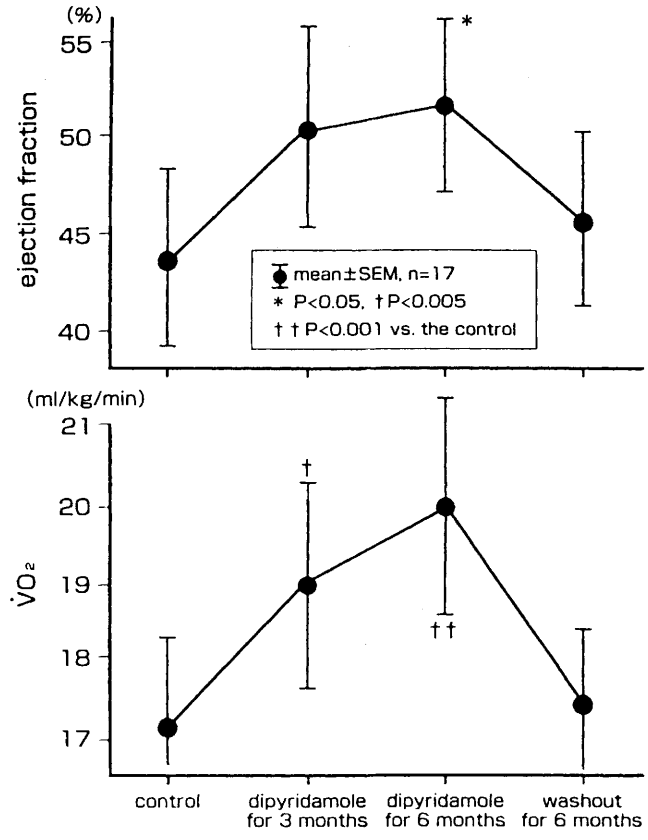

Fig. 10. Changes in ejection fraction and oxygen consumption during the bicycle exercise test in patients with chronic heart failure who were treated with dipyridamole for 6 months [129]

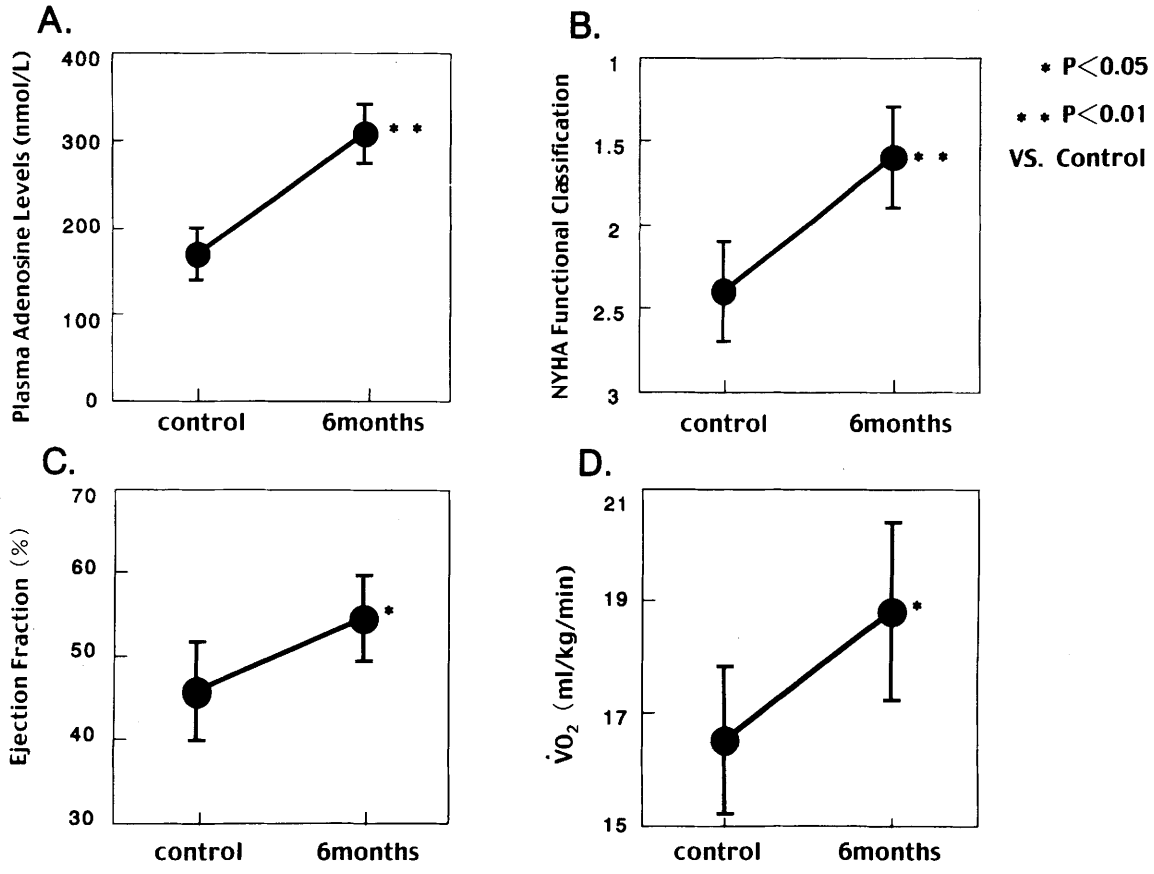

Fig. 11. Changes in plasma adenosine levels (**A**), the New York Heart Association functional classification (**B**), ejection fraction (**C**), and oxygen consumption during the bicycle exercise test (**D**) in patients with chronic heart failure who were treated with dilazep for 6 months [129]. $*P < 0.05$, $**P < 0.01$ vs controls

We further tested whether elevation of the adenosine levels due to dipyridamole or dilazep for 6 months modulates the pathophysiology of chronic heart failure [129]. Twenty-two patients (mean 58 ± 4 years old, range 42–74 years) attending a specialized chronic heart failure clinic over 6 months and judged as being in the NYHA function class II or III were examined. We administered 300 mg/day dipyridamole (n = 17) or 300 mg/day dilazep (n = 7) for 6 months, and then discontinued the drugs for another 6 months. Dipyridamole increased the plasma adenosine levels for 6 months and improved the severity of chronic heart failure; both EF and VO_2 were also increased (Fig. 10). Dilazep also increased the plasma adenosine levels and improved the severity of chronic heart failure after 6 months (Fig. 11). These results indicate that plasma adenosine levels increase in patients with chronic heart failure, and that this increase in plasma adenosine levels attenuates the severity of chronic heart failure.

Since adenosine is reported to attenuate the sympathetic nervous system, the renin-angiotensin system, and the cytokine system [4, 5], the elevation of adenosine levels may largely contribute to the beneficial treatment of chronic heart failure. Indeed, the present study revealed that the elevation of adenosine levels due to the two different drugs equally improves chronic heart failure. Since myocardial ischemia is one of the causes of chronic heart failure, the elevation of adenosine levels may have attenuated the severity of myocardial ischemia, stunning, or hibernation. This is because even nonischemic chronic heart failure may be attributable to the latent myocardial ischemia and hypoxia caused by coronary microvascular spasm, myocardial external compressive forces on the small coronary arteries, increased diffusion distances of oxygen due to myocardial distention, and the reduced perfusion gradients from the epicardium and endocardium with increased myocardial stress in dilated cardiomyopathy. Therefore, elevation of adenosine levels in patients with chronic heart failure may be a novel and effective strategy for the treatment of chronic heart failure. These drugs need to be tested to decrease mortality and mobidity in the mass study.

Future direction of investigation of adenosine in the heart

Since, in ischemic hearts, many factors that cause deterioration of the heart are activated, it is important to inhibit these factors. One strategy is to administer corresponding drugs to attenuate them, for example, SOD for oxygen-derived free radicals. However, this strategy is not realistic in the clinical setting, since we would need to administer many drugs to cope with the numerous deleterious factors. Another possibility is to administer adenosine-related substances, because adenosine is believed to be a potent cardioprotective substance via its multiplicity of the action via adenosine A_1–A_3 receptors. Furthermore, adenosine may be effective for other cardiac disorders such as myocarditis, and it is known to be an anti-inflammatory agent. Adenosine administration may become part of a new strategy for the treatment of diseased hearts. Clinical investigation of adenosine for heart disease will be required in the future. Since we have asked what stress to the heart can do for adenosine, it is now time to ask what adenosine can do for patients with heart disease.

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