The beneficial effects of atrial natriuretic peptide on arrhythmias and myocardial high-energy phosphates after reperfusion

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Abstract

Objectives: The aim of this investigation was to test whether the administration of atrial natriuretic peptide (ANP) has cardioprotective effects against ischaemic and reperfusion injury. Methods: Thoracotomized dogs underwent a 30 min left circumflex coronary artery occlusion and 60 min of reperfusion (control group; n = 16). The ANP group (n = 9) received a 20 μg bolus injection of synthetic α-human ANP (SUN 4936) followed by infusion at a dose of 0.1 μg/kg/min from the beginning of coronary occlusion to the end of the procedure. Results: Administration of exogenous ANP increased plasma ANP immediately and maintained levels at 3000 pg/ml, resulting in an 8-fold increase in plasma cyclic guanosine monophosphate (cGMP) levels. Plasma ANP and plasma cGMP levels did not change at all in controls. There were no significant differences in haemodynamic parameters during ischaemia and reperfusion between the groups. In the ANP group, the prevalence and frequency of ventricular extrasystoles within 10 min after reperfusion decreased markedly [ANP 22% vs. control 100%, P < 0.01, and ANP 1 (1) vs. control 92 (50), P < 0.05, respectively]. No dog in the ANP group had ventricular fibrillation (VF), but the incidence of VF was not statistically significant between the groups [ANP 0% vs. control 25%]. ATP content in the inner layers of the ischaemic myocardium in the ANP group was higher than in controls (P < 0.05) [1.92 (0.28) vs. 1.18 (0.13) μmol/g wet weight]. There was no significant difference in the content of myocardial tissue angiotensin II between the groups. Conclusions: These data show that the infusion of ANP has cardioprotective effects on myocardial ischaemia and reperfusion in this model. These beneficial effects are probably due to direct effects through cGMP rather than haemodynamic changes.

Keywords: Atrial natriuretic factor; cGMP; Myocardial ischemia; Reperfusion; Arrhythmias; High-energy phosphates; Dog, anesthetized

1. Introduction

It is well known that atrial natriuretic peptide (ANP) is a cardiac hormone secreted mainly in response to atrial distension, and has natriuretic and vasoactive properties and suppresses the activity of the renin–angiotensin–aldosterone system [1–3]. Plasma ANP levels in patients with congestive heart failure are elevated and correlate with pulmonary capillary wedge pressure [4]. ANP has been reported to improve cardiac performance by reducing both preload and afterload and therefore is thought to play an important role in the compensatory mechanism of congestive heart failure. There are numerous reports that the administration of this peptide could be potentially valuable in patients with congestive heart failure [5,6]. There are several reports of increases in plasma ANP levels in patients with acute myocardial infarction [7,8]. Tan et al. indicated that the phenomenon of ‘hypo-ANP-ism’ would occur after rapid elevation of plasma ANP levels in a very early stage of myocardial infarction. They hypothesised that excessive release of the peptide resulting in the depleted state of the atrial-stored granules would readily lead to retention of fluid and sodium even in patients with uncomplicated myocardial infarction [7]. Nakamura et al. indicated that low-dose infusion of synthetic ANP may be a beneficial adjunctive therapy for the
management of water–sodium retention in acute myocardial infarction [9].

ANP is thought to cause vasodilatation by activating a guanylate cyclase and increasing the production of cyclic guanosine monophosphate (cGMP) in vascular smooth muscle [10]. Several reports have shown that ANP has a coronary vasodilatory effect in animals and humans [11,12]. Moreover, there are clinical reports that infusion of synthetic α human ANP attenuates exercise-induced myocardial ischaemia in patients with effort angina and hyperventilation-induced attacks in patients with variant angina [13,14]. These studies suggested that administration of ANP could be beneficial not only for congestive heart failure, but also for ischaemic heart disease.

Experimental studies in dogs revealed increases of plasma ANP levels in myocardial infarction without congestive heart failure [15]. Elevated ANP levels in the very early stage after coronary reperfusion paralleled adenosine levels in the coronary sinus in dogs. Uusimaa et al. have shown that myocardial metabolic changes regulate the secretion of ANP in vitro and in vivo [16]. However, the physiologic importance of endogenous ANP in ischaemia and reperfusion is uncertain. The present study aimed to determine whether the intravenous infusion of ANP has cardioprotective effects preventing reperfusion arrhythmias and preserving high-energy phosphates during myocardial ischaemia and reperfusion in dogs.

2. Methods

2.1. Experimental preparations

Mongrel dogs of both sexes, weighing 12–16 kg (n = 46) were used for all experiments. All experiments were performed in accordance with the guidelines of the committee on animals of Tokyo Medical College and with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1985). Dogs were heparinised (1000 U intravenously) and premedicated with intramuscular ketamine hydrochloride (20 mg/kg) and pentobarbitone sodium (25 mg/kg, intravenously). Dogs were intubated and ventilated with a mixture of room air and oxygen (3 l/min). Respiratory rate and tidal volume were controlled, keeping the pH and pCO₂ of arterial blood gases within the physiological range. A cannula was inserted into the femoral vein for drug administration, and another in the femoral artery to measure arterial pressure and for blood sampling. A microtip catheter (Millar Instruments, Houston, TX, USA) was inserted into the left ventricle to measure left ventricular pressure and its first derivative, dP/dt, and a Swan-Ganz catheter (Baxter Healthcare Corp, Edwards Division, Santa Ana, CA, USA) in the pulmonary artery to measure pulmonary capillary wedge pressure, mean pulmonary artery pressure and right atrial pressure, and a catheter was also inserted into the bladder. The heart was exposed through a left thoracotomy incision at the fourth intercostal space. The pericardium was opened, the left auricular appendage was gently elevated, avoiding distension. An occluder with a snare loop was placed proximally in the left circumflex coronary artery. Coronary blood flow was monitored continuously using an ultrasonic transit-time flow probe placed on the distal side of the left circumflex coronary artery (Transonic system 208, Ithaca, NY, USA).

2.2. Experimental protocol

Following a 20-min stabilization period, all dogs underwent a 30-min left circumflex artery occlusion followed by 60 min of reperfusion. In the ANP group (n = 9) 20 µg synthetic human α-ANP (SUN4936, Suntory, Gunma, Japan) was given intravenously as a bolus and then infused at a dose of 0.1 µg/kg/min (infusion volume 1.5 ml/kg/h) from immediately after coronary occlusion until 60 min after reperfusion through a femoral vein using a microsyringe pump. It was dissolved in 5% glucose and adjusted to a concentration of 10 µg/ml. In the control group (n = 16), the dogs received an infusion of the vehicle (5% glucose) at an equivalent volume. No dog received any anti-arrhythmic agents. Dogs that developed ventricular fibrillation or no significant ST elevation on electrocardiogram before reperfusion were excluded from this study. Reperfusion was confirmed by reactive hyperaemia in coronary flow with a rapid return of S-T segment to baseline. A sham operation group (n = 6) was performed to compare the ATP myocardial content in the two groups.

2.3. Measurements

Electrocardiograms were recorded using lead II of the standard limb lead placement system and analysed for the number of ventricular extrasystoles (VEs) and for the incidence of ventricular fibrillation (VF). Ventricular arrhythmias were defined according to the Lambeth Conventions [17]. They were counted during a 10-min period after reperfusion. All haemodynamic data were recorded on a polygraph (Polygraph 360 System, San-ei, Tokyo, Japan). Heart rate, arterial pressure, right atrial pressure, pulmonary arterial pressure and left ventricular pressure were measured before coronary occlusion, immediately before reperfusion, 30 min and 60 min after. Serial blood samples for measurement of plasma ANP and cGMP concentrations were collected from the femoral artery at the same periods. Left ventricular end-diastolic pressure was determined at the peak of the R-wave on lead II and the max positive dP/dt determined with left ventricular pressure curves. Urine volume was measured at 60 min after reperfusion. After completing the experimental procedure, the heart was excised immediately and the content of high-en-
nergy phosphates and angiotensin II in the myocardium was measured.

2.4. Biochemical analyses

Blood samples for measurement of plasma ANP and cGMP concentrations were transferred to chilled disposable tubes containing ethylenediamine tetra-acetic acid (1 mg/ml) and aprotinin (1000 kallikrein inactivator units/ml). The blood samples were immediately placed on ice, centrifuged at 40°C for 15 min at 5,000 rpm and plasma was stored at −80°C until assay. Plasma ANP levels were determined by radioimmunoassay using a specific commercial kit (Eiken Chemical Co., Ltd. Tokyo Japan). Plasma cGMP levels were measured by radioimmunoassay using a specific commercial kit (Yamasa Shoyu Co. Ltd., Choshi, Japan). The left circumflex artery was occluded again after 60 min reperfusion and 5% fluorescein-Na (1 mg/kg) was injected in a bolus into the femoral vein at 30 s before the heart was excised. The heart was immediately placed in a solution of KCl at 0°C. The left ventricle was incised and the non-fluorescent zone which was indicated as the area at risk of infarction was distinguished from the non-ischaemic area under ultraviolet light [18]. Specimens of 100 mg of ventricular transmural tissue were taken from (1) the inner layer of the posterior wall which was at the centre of the ischaemic area, (2) the outer layer at the same site, (3) the edge of the ischaemic area, and (4) the anterior papillary muscle in a non-ischaemic area. The specimens were weighed, homogenized in 3.6% perchloric acid and centrifuged at 0°C for 30 min at 2000 rpm (Tomy Seiko Co, Ltd, Tokyo, Japan). The aliquots were adjusted to pH 5.0 ~ 6.0 by the addition of a solution of KOH/K₂CO₃ and centrifuged at 0°C for 5 min at 10,000 rpm. ATP and its metabolites were determined using high-performance liquid chromatography (LC-6A, Shimadzu Co, Kyoto, Japan) by the method of Anderson and Murphy [19]. The specimens of 200 mg for measurement of angiotensin II were taken from the centre of the ischaemic area and from the non-ischaemic area. The homogenates in 0.1 N hydrochloric acid were centrifuged at 14,000 rpm for 30 min. Angiotensin II measurement was performed by high-performance liquid chromatography coupled with radioimmunoassay [20].

Table 1
Haemodynamic and plasma atrial natriuretic peptide (ANP) and cyclic guanosine monophosphate (cGMP) concentration variables in the control group and the ANP-treated group

<table>
<thead>
<tr>
<th></th>
<th>Pre-occlusion</th>
<th>Occlusion</th>
<th>Reperfusion</th>
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<tbody>
<tr>
<td></td>
<td>30 min</td>
<td>1 h</td>
<td></td>
</tr>
<tr>
<td>HR (beats·min⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>144 (12)</td>
<td>135 (12)</td>
<td>146 (13)</td>
</tr>
<tr>
<td>Treated</td>
<td>128 (14)</td>
<td>122 (12)</td>
<td>124 (11)</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>97 (11)</td>
<td>85 (7)</td>
<td>92 (9)</td>
</tr>
<tr>
<td>Treated</td>
<td>100 (9)</td>
<td>80 (7)</td>
<td>79 (8)</td>
</tr>
<tr>
<td>MRP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Treated</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>MPP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10 (2)</td>
<td>11 (1)</td>
<td>11 (2)</td>
</tr>
<tr>
<td>Treated</td>
<td>9 (1)</td>
<td>10 (1)</td>
<td>10 (1)</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3 (1)</td>
<td>6 (1)</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Treated</td>
<td>3 (0)</td>
<td>4 (1)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>dP/dt MAX (mmHg·s⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2313 (311)</td>
<td>2310 (138)</td>
<td>2172 (144)</td>
</tr>
<tr>
<td>Treated</td>
<td>2698 (614)</td>
<td>2060 (255)</td>
<td>2038 (274)</td>
</tr>
<tr>
<td>RPP/1000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>19.4 (3.5)</td>
<td>16.1 (2.2)</td>
<td>19.4 (3.0)</td>
</tr>
<tr>
<td>Treated</td>
<td>17.5 (3.3)</td>
<td>13.6 (2.2)</td>
<td>13.7 (2.3)</td>
</tr>
<tr>
<td>ANP (pg·ml⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>61 (4)</td>
<td>70 (7)</td>
<td>63 (4)</td>
</tr>
<tr>
<td>Treated</td>
<td>89 (17)</td>
<td>7819 (414)</td>
<td>3001 (422)</td>
</tr>
<tr>
<td>cGMP (pmol·ml⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>13.9 (2.4)</td>
<td>14.9 (1.4)</td>
<td>14.6 (2.8)</td>
</tr>
<tr>
<td>Treated</td>
<td>14.8 (1.8)</td>
<td>114.4 (11.8)</td>
<td>118.2 (9.8)</td>
</tr>
</tbody>
</table>

Values are means (s.e.m.) HR = heart rate; MAP = mean arterial pressure; MRP = mean right atrial pressure; MPP = mean pulmonary arterial pressure; LVEDP = left ventricular end-diastolic pressure; dP/dt MAX = maximum rate of left ventricular pressure fall; RPP = rate-pressure product; ANP = plasma ANP concentration; cGMP = plasma cGMP concentration. Pre-occlusion measurements were taken 3 min before coronary occlusion. Post-occlusion measurements were taken 3 min before coronary reperfusion.

* P < 0.01 control vs. treated; ** P < 0.01 pre-occlusion vs. occlusion, reperfusion.
2.5. Data analysis

Variables are expressed as means (s.e.m.) or percentage of incidence.

Haemodynamic data were analyzed by two-way analysis of variance for repeated measurements and the comparisons between pre-occlusion and each time following occlusion were done by Dunnett’s multiple comparison test. The comparisons of haemodynamics, frequency of VEs, blood and tissue data between the control group and the ANP group, and also ATP content between each group and the sham operation group were made using the Mann-Whitney U-test. Prevalence of VEs and incidence of VF were compared by Fisher’s test. A probability value of < 0.05 was considered significant.

3. Results

Of the 46 dogs initially anaesthetized, 15 were excluded for the following reasons. One dog had extensive pleural adhesion and plasma ANP level at pre-occlusion was very high (530 pg/ml), and 4 did not have ST elevation in lead II during occlusion or defect of fluorescein-Na take-up in the infarct-risk area, probably due to collaterals to the left circumflex artery. Ten dogs died of VF during occlusion (7 in the control group and 3 in the ANP group). Six dogs were used in the sham operation group. A total of 25 dogs were used for comparison of the incidence of reperfusion arrhythmias. Of these, 21 survived and the haemodynamics, content of high-energy phosphates and angiotensin II were compared between the groups.

3.1. Plasma concentration of ANP and cGMP

The difference in plasma ANP or cGMP concentration was not statistically significant between the groups before occlusion. In the ANP group, however, the plasma ANP levels increased significantly \((P < 0.01)\) from 89 (17) pg/ml to 2819 (414) pg/ml at 30 min after administration of synthetic ANP, and was maintained at approximately 3000 pg/ml until the end of the protocol. The plasma cGMP concentration increased 8 times from 14.4 (1.8) pmol/ml to 111.4 (11.8) pmol/ml \((P < 0.01)\) just before reperfusion and 118.2 (9.8) pmol/ml at 60 min after reperfusion. In the control group plasma ANP and cGMP level did not change during occlusion or reperfusion (Table 1, Fig. 1).

3.2. Effect of ANP on haemodynamic variables

The administration of ANP did not show a statistically significant effect on heart rate, mean arterial pressure, mean right atrial pressure, mean pulmonary artery pressure, positive dP/dt, left ventricular end-diastolic pressure or rate–pressure product (Table 1). There were no differences between the groups in any of these variables at any time point during the experiment. There was no difference in urine volume at the end of the experiment between the groups under acute conditions (data not shown).

3.3. Effect of ANP on reperfusion arrhythmias

The 25 dogs studied with regard to reperfusion arrhythmias consisted of 16 control animals and 9 in the ANP group. All 4 dogs (25%) in the control group that developed VF in the early phase after reperfusion died. None in the ANP group developed VF after reperfusion and none of them died until sacrifice. However, there was no statistically significant difference between the groups. Fig. 2 illustrates the prevalence and frequency of VEs in the control group \((n = 12)\) and the ANP group \((n = 9)\). The prevalence indicates the percentage of dogs in which VEs occurred every 2 min. The frequency indicates the number of VEs every 2 min per dog. Since few dogs developed ventricular arrhythmias after 10 min of reperfusion, we evaluated them for the first 10 min after reperfusion. VEs occurred in all dogs (100%) in the control group, while VEs occurred in 4 of 9 dogs (44%) in the ANP group. There was no statistically significant difference between the groups. Particularly between 2 and 10 min, however, VEs occurred in only 2 of 9 dogs in the ANP group (22%) compared with all control dogs (100%) \((P < 0.01)\). Compared with controls, the mean number of VEs per dog between 2 and 10 min in the ANP group was low: 1 (1) vs 92 (50) \((P < 0.05)\) (Table 2).

3.4. Effect of ANP on high-energy phosphate content in myocardium

Fig. 3 shows ATP content at each of the sites of myocardium studied in the control group \((n = 16)\) and the
**Fig. 2.** Effect of atrial natriuretic peptide (ANP) on reperfusion arrhythmias. (A) Effect on prevalence. (B) Effect on frequency. Prevalence indicates the percentage of dogs in which ventricular extrasystoles (VEs) occurred every 2 min. Frequency indicates the number of VEs every 2 min per dog. VEs within 10 min after reperfusion were counted.

ANP group (n = 9) and the sham operation group (n = 6). The ATP content of the ischaemic lesions in the control group and the ANP group was significantly lower than that in the sham operation group (P < 0.01). The ATP content in the inner layer of the ischaemic myocardium in the ANP group was better preserved than in the control hearts, at 1.92 (0.28) vs. 1.18 (0.13) μmol/g wet weight (P < 0.05). This preservation of high-energy phosphate levels in ANP-treated hearts was also reflected in total adenine nucleotide (TAN = ATP + ADP + AMP). The TAN at the same sites of ANP-treated hearts was higher than in the control hearts, at 3.50 (0.43) vs. 2.34 (0.21) μmol/g wet weight (P < 0.05). The difference in ATP levels in the outer layer or the edge of the ischaemic myocardium in ANP-treated hearts did not reach statistical significance compared to controls, 2.52 (0.26) vs. 1.92 (0.20) μmol/g wet weight, 2.12 (0.29) vs. 1.61 (0.22) pmol/g wet weight, respectively.

**3.5. Angiotensin II concentration in tissue**

Fig. 4 shows the angiotensin II concentration in the myocardium at the end of the 60-min reperfusion period. In both groups, the difference of the angiotensin II concentration between the ischaemic area and non-ischaemic area was not significant. In the ischaemic lesions, the difference of the angiotensin II concentration between the control group and the ANP group was not significant [80.20 (16.11) vs. 78.39 (19.59) pg/g].

**4. Discussion**

The present study showed that the infusion of ANP clearly inhibited reperfusion-induced ventricular arrhythmias and preserved the ATP content in the inner layer of canine ischaemic myocardium. Chowdrey et al. [21]...
demonstrated that ANP has no significant influence on heart rate and coronary flow or on ventricular arrhythmias during regional myocardial ischaemia in the isolated rat heart. However, they did not examine reperfusion-induced arrhythmias. In the present study using thoracotomized dogs, there were no significant differences in haemodynamic variables and the rate–pressure product, which is an index of myocardial oxygen consumption. Although VEs during myocardial ischaemia in our study were not inhibited, which is the same result as their study, VEs after reperfusion were markedly inhibited. Therefore, we consider that the beneficial effects of ANP are related to its effects in relation to the phenomenon of coronary reperfusion rather than an anti-ischaemic effect mediated through haemodynamic variables. We recently reported that plasma ANP levels increase in the early stage after coronary reperfusion but do not increase during the ischaemic phase, suggesting that endogenous ANP has a physiologically important effect after reperfusion [15]. The maintenance of high levels of plasma ANP concentration at reperfusion might have contributed to the beneficial effects in the present study.

The mechanisms producing reperfusion arrhythmia are thought to be multiple. Many experiments have suggested that reperfusion injury might be caused mainly due to calcium overload. Lazdunski et al. [22] and Dennis et al. [23] demonstrated that an intracellular Ca\(^2\)+ overload after reperfusion may be caused by an involvement of H\(^+\)/Na\(^+\) and Na\(^+\)/Ca\(^2\)+ exchange. These are potentially important mechanisms of reperfusion-induced arrhythmias. Recently, it was shown that ANP binds to the ANP receptors that are associated with particulate guanylate cyclase activity, mostly ANP-A receptors [24]. ANP decreases cytosolic Ca\(^2\)+ concentration by increasing the synthesis of cGMP in vascular smooth muscle [10]. Although signal transduction of cGMP in cardiac cells remains unclear, it has been reported that ANP decreases cardiac Ca current through activation of cGMP-dependent protein kinase [25]. Furthermore, cGMP is thought to modulate Ca\(^2\)+ efflux by stimulating Na\(^+\)/Ca\(^2\)+ exchange and the ATP-dependent Ca pump [26]. The present study showed that plasma levels of cGMP increased 8-fold after infusion of ANP, suggesting that cGMP plays an important role during ischaemia and reperfusion. Therefore, the reduction of Ca\(^2\)+ overload through the effects of cGMP, in particular, as a stimulator of Na\(^+\)/Ca\(^2\)+ exchange might contribute to the prevention of reperfusion-induced arrhythmias.

In the present study, the reperfusion-induced arrhythmias appearing instantaneously on reperfusion were not completely inhibited by ANP, however, those occurring between 2 and 10 min after reperfusion were markedly inhibited. Kaplinsky et al. [27] characterized reperfusion-induced arrhythmias by the time course of their appearance with specific electrophysiological mechanisms. In their study involving a 30 min period of occlusion and reperfusion model as in our experiment, arrhythmias that occurred instantaneously (onset at 0–1 min) on reperfusion were characteristic of a re-entrant mechanism and were very closely associated with the occurrence of ventricular arrhythmias during the antecedent period of coronary artery occlusion. In contrast, the delayed arrhythmias (2–7 min after reperfusion) were infrequent in relation to ventricular fibrillation and more characteristic of enhanced automaticity, which might be associated with increased Ca\(^2\)+ influx. Bolli et al. [28] measured the efflux of oxygen-free radicals from myocardium after reperfusion, which might be one of the mechanisms of reperfusion arrhythmias, and showed that the efflux increased rapidly at a peak of 2 min after 15 min of ischaemia and then decreased gradually. These studies indirectly support the idea that suppression of delayed arrhythmias by the infusion of ANP was associated with Ca\(^2\)+ overload, rather than re-entrant or oxygen-free radical mechanisms.

The present study showed that the infusion of ANP significantly preserved the ATP content in the ischaemic myocardium after reperfusion. There were no statistical differences between the groups concerning haemodynamic changes at any time points during ischaemia and reperfusion. Moreover, there was no relation between the ATP content and the change in rate–pressure product at any time points in both groups. Thus it is highly unlikely that the infusion of ANP preserves ATP content by reducing myocardial oxygen consumption associated with haemodynamic changes. The effects of ANP on coronary haemodynamics have been reported by several investigators [11,12]. Rosenthal et al. demonstrated that injection of synthetic ANP into the coronary artery in humans without coronary artery disease caused dilation of the proximal coronary artery to the same degree as nitroglycerin [29]. Chu et al. demonstrated that infusion of ANP reversed subendocardial hypoperfusion and significantly improved the subendocardial-to-subepicardial ratio in the ischaemic zone in coronary flow-limited conscious dogs in the absence of intrinsic vascular reactivity or native collateral flow [30].
present study, the ATP content was statistically more preserved in the inner layer of ischaemic myocardium in the ANP group, and the level of total adenine nucleotide was also higher, which indicated recovery of ATP after reperfusion. These results suggest that the preservation of oxidative phosphorylation in mitochondria might be due to an improvement in subendocardial blood flow. It is also thought that the increased cytosolic Ca\(^{2+}\) during the process of myocardial ischaemia and reperfusion causes consumption of the ATP on buffering Ca\(^{2+}\) and decreases ATP synthesis in mitochondria. Thus there is a possibility that ANP causes a decrease of cytosolic Ca\(^{2+}\) through cGMP, resulting in preservation of ATP content.

Angiotensin II may contribute to arrhythmia by decreasing coronary flow and by reducing the high-energy phosphate reserve [31]. Linz et al. [32] demonstrated that exogenous ANP may have cardioprotective effects against exogenous angiotensin II which may promote reperfusion-induced arrhythmia in the isolated working rat heart. However, in the present study, there was no difference in tissue angiotensin II concentration between the groups.

Noma hypothesized that the activation of ATP-sensitive K\(^{+}\) channels may play an important role on myocardial ischaemia or hypoxia [33]. A recent study, using glibenclamide to block or pinacidil to activate, has shown that the activation of ATP-sensitive K\(^{+}\) channels protects against ischaemia/reperfusion damage [34]. Sakuta et al., using the ANP receptor antagonist HS142-1, demonstrated that ANP potentiates K\(^{+}\) currents of cromakalin or Y-26763 which open the ATP-sensitive K\(^{+}\) channels by stimulating intrafollicular accumulation of cGMP in Xenopus oocytes [35]. It has been reported that ANP modulates the activity of ATP-sensitive K\(^{+}\) channels in vascular smooth muscle cells through cGMP [36]. These points may be related to the cardioprotective mechanism of ANP.

Thrombolytic agents, which allow early reperfusion in the acute phase of MI, have the potential to minimize the infarct size and decrease the mortality rate, are now regarded as essential therapy for acute myocardial infarction [37]. However, the incidence of VF is high in patients with acute myocardial infarction who undergo pre-hospital thrombolytic therapy [38]. Moreover, spontaneous reperfusion after silent myocardial ischaemia or coronary artery spasm could initiate potentially fatal arrhythmias [39,40]. Thus the problem of fatal arrhythmias, including ventricular tachycardia or fibrillation and myocardial damage on reperfusion, has not been solved. Therefore, the management of reperfusion injury including reperfusion arrhythmias must be established. The present study showed that ANP has cardioprotective effects on ischaemia and reperfusion. However, further studies are needed to examine whether smaller doses of ANP are also effective in reperfusion, and to elucidate the mechanisms of the cardioprotective effects yielded by ANP infusion.

In summary, the present study showed that administration of synthetic ANP in myocardial ischaemia and reperfusion was cardioprotective, preventing reperfusion arrhythmias and preserving high-energy phosphates in ischaemic myocardium. It is suggested that infusion of ANP could be a beneficial adjunctive component of reperfusion therapy for acute myocardial infarction.

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References


