Intrapericardial Angiotensin II Stimulates Endothelin-1 and Atrial Natriuretic Peptide Formation of the *In Situ* Dog Heart

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Angiotensin (AT) II, endothelin (ET)-1, and atrial natriuretic peptide (ANP) play an important role in cardiovascular regulatory processes under physiologic and pathophysiologic conditions. All of these agents are present in the pericardial fluid, and alteration of their pericardial concentrations mirror changes in the myocardial interstitium. Moreover, the composition the pericardial fluid may also reflect the myocardial interaction of these agents. The local myocardial effects of AT II on cardiac ET-1 and ANP production, as well as on cardiovascular function, was studied by intrapericardial (ip) administration of AT II (0.125–1.0 μg/kg) to the *in situ* dog heart ($n = 8$). Big ET, ET-1, and ANP [1–28] fragment concentrations were measured by enzyme-linked immunosorbent assay in pericardial infusate samples and in peripheral blood before and after an AT II treatment of 15 mins. Systemic blood pressure (BP), heart rate (HR), and left ventricular contractility (dP/dt) were also recorded. In our studies, the pericardial big ET (but not ET-1) concentration was increased to a maximum value of 139 ± 28 versus 74 ± 12 pg/ml (control; $P < 0.02$) with ip AT II administration, with parallel elevations of the pericardial ANP levels (36.8 ± 7.2 vs. 24.4 ± 3.6 ng/ml; $P < 0.05$). The ip administration of AT II did not influence HR, and it elicited moderate changes in BP (BP_{max}, +14 ± 2 mm Hg, $P < 0.001$; dP/dt_{max}, +10 ± 3%, $P < 0.02$). The plasma levels of big ET, ET-1, and ANP did not change significantly. The results suggest that AT II promotes production of big ET and ANP in the heart. However, no detectable conversion of big ET-1 to ET-1 was observed within 15 mins. The myocardial formation of big ET-1 and ANP occurred, at least in part, independently of the changes in cardiovascular function. Exp Biol Med 231:847–851, 2006

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Introduction

Angiotensin (AT) II, endothelin (ET)-1, and atrial natriuretic peptide (ANP) are important regulators of cardiovascular function and act in concert with each other in different cardiovascular diseases, such as hypertension, cardiac hypertrophy, ischemia, and heart failure. In addition to their classic effects exerted on vascular tone, neuroendocrine function and fluid homeostasis, they have local autocrine and paracrine actions in the heart. The main source of the natriuretic peptide is the atrial and ventricular myocytes (1) and, as with ANP, AT II and ET-1 are also produced in the heart (2, 3). Myocardial AT II and ET-1 have synergistic effects on growth promotion of vascular and cardiac cells (4) and on the induction of the production of endothelium- and myocyte-derived regulators and inflammatory cytokines (5). ANP exerts antagonistic effects, such as antigrowth, antiproliferative properties (6), and, thus, may act against cardiac and vascular remodeling. At the same time AT II, ET-1, and ANP show multiple interactions, they modulate cardiovascular actions and the myocardial production of each other. A number of *in vitro* studies demonstrated that AT II induces ET-1 and ANP formation and release from cardiac tissue (7–9), and that ET-1 is also a potent activator of the myocardial release of ANP (10). In turn, ANP can decrease the effects and production of ET-1 and AT II in the heart (6, 11). It has been suggested that ET-1 mediates the effects of myocardial stretch on ANP release (12), as well as the proliferative action of AT II in the cardiac and vascular tissue (13, 14). AT II may also be released from stretched cardiomyocytes and elevate the cardiac ANP production (15).

The pericardial fluid, which is myocardial transudate, contains a large number of endogenous agents, such as natriuretic peptides (16, 17), ET (18), AT II (19), adenine nucleosides (20, 21), catecholamines (22), ferritin (23),

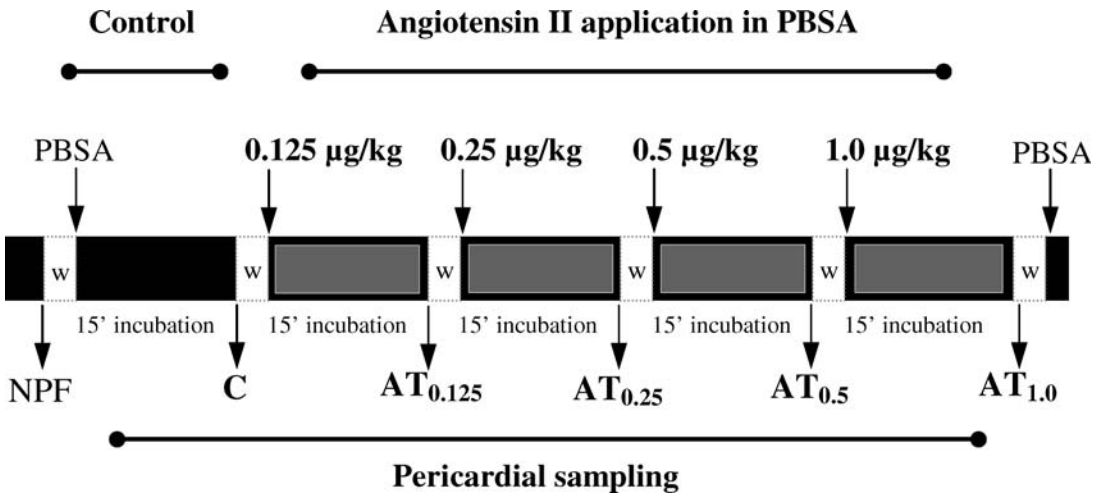

Figure 1. Time table of AT II administration and pericardial sampling procedure in the experiments. NPF, native pericardial fluid; C, control pericardial sample; $AT_{0.125}$, $AT_{0.25}$, $AT_{0.5}$, and $AT_{1.0}$, pericardial samples after administration of 0.125, 0.25, 0.5, and 1.0 µg/kg AT II into the pericardial infusate, respectively; W, pericardial washing five times with 5 ml PBSA.

cytokines and growth factors (24), etc. More importantly, the concentrations of these regulators in the pericardial fluid were found to be several times higher than in the plasma, indicating that they originate from the myocardial tissue. It was demonstrated that pericardial alterations of ANP (17, 25), ET-1 (26), adenosine, and inosine (27, 28) concentrations mirror changes in the myocardial interstitium. Moreover, the composition of pericardial fluid may also reflect the myocardial interaction of these agents.

In this study, we examined the local myocardial effects of intrapericardial (ip) administration of AT II on cardiac ET-1 and ANP production and release *via* measuring big ET-1, ET-1, and ANP concentrations in fluid samples obtained from the pericardial space of the *in situ* dog heart. The cardiac and hemodynamic effects of ip administration of AT II were also studied by monitoring the heart rate (HR), left ventricular contractility (dP/dt), and blood pressure (BP) during the experiments.

Materials and Methods

Animal Preparation. Experiments were performed on anesthetized dogs (intravenous administration of 30 mg/kg Nembutal; CEVA, Libourne Cedex, France; $n = 8$). The chest was opened transsternally in the 5th intercostal space and the animals were intubated and ventilated with room air (Cape CV2424 ventilator; Cape Engineering Co., Warwick, England) and kept at 37°C with a heating pad during the experiments. The right femoral artery was cannulated for monitoring arterial BP (Electromedics XD003 probe; Electromedics Inc., Englewood, CO) and the right carotid artery was prepared for introducing a catheter into the left ventricular cavity to measure the ventricular contractile force (Cordis pig-tail catheter 4F; Cordis Corp., Miami, FL). Needle electrodes were inserted into the limbs for standard electrocardiogram recording (CU12; Madaus Schwarzer, München, Germany). The closed pericardial sac was cannulated in a water tight manner *via* a small incision. The catheter was positioned and fixed in the lowest region of the pericardial sac (oblique sinus). This catheter was used to obtain pericardial fluid samples and for AT II application. Animal procedures were carried out in accordance with the relevant Hungarian National Legislation and with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (29).

Experimental Protocol and Sample Processing. After a 20-min stabilization period, the native pericardial fluid was aspirated completely from the pericardial sac at the beginning of the experiment. A standard quantity of pericardial infusate fluid (0.75 ml/kg), consisting of phosphate-buffered physiologic saline with 0.5% w/v bovine serum albumin (PBSA) was left in the pericardium as a replacement for the native pericardial fluid. Each infusate sample was incubated for 15 mins in the pericardial sac for the standardization of biochemical changes. After control sampling, four consecutive infusate samples were obtained from the pericardial sac after ip applications of AT II in increasing doses of 0.125, 0.25, 0.5, and 1.0 µg/kg. After each sampling, the pericardial sac was rinsed five times with 5 ml PBSA (Fig. 1). Arterial blood samples were taken in parallel with pericardial infusate sampling. All samples were centrifuged (1500 *g* for 15 mins at 4°C) and stored at –20°C until biochemical measurements of big ET-1, ET-1, and ANP concentrations using enzyme-linked immunosorbent assay (Biomedica GmbH, Wien, Austria). Data were corrected by the volume of the native pericardial fluid and were standardized for 100 g of cardiac tissue.

Statistical Analysis. The statistical analyses were performed using Student's *t* test for hemodynamic variables and the Wilcoxon signed rank test or the Mann-Whitney *U* test for big ET-1, ET-1, and ANP data. Differences were considered statistically significant at the level of $P < 0.05$.

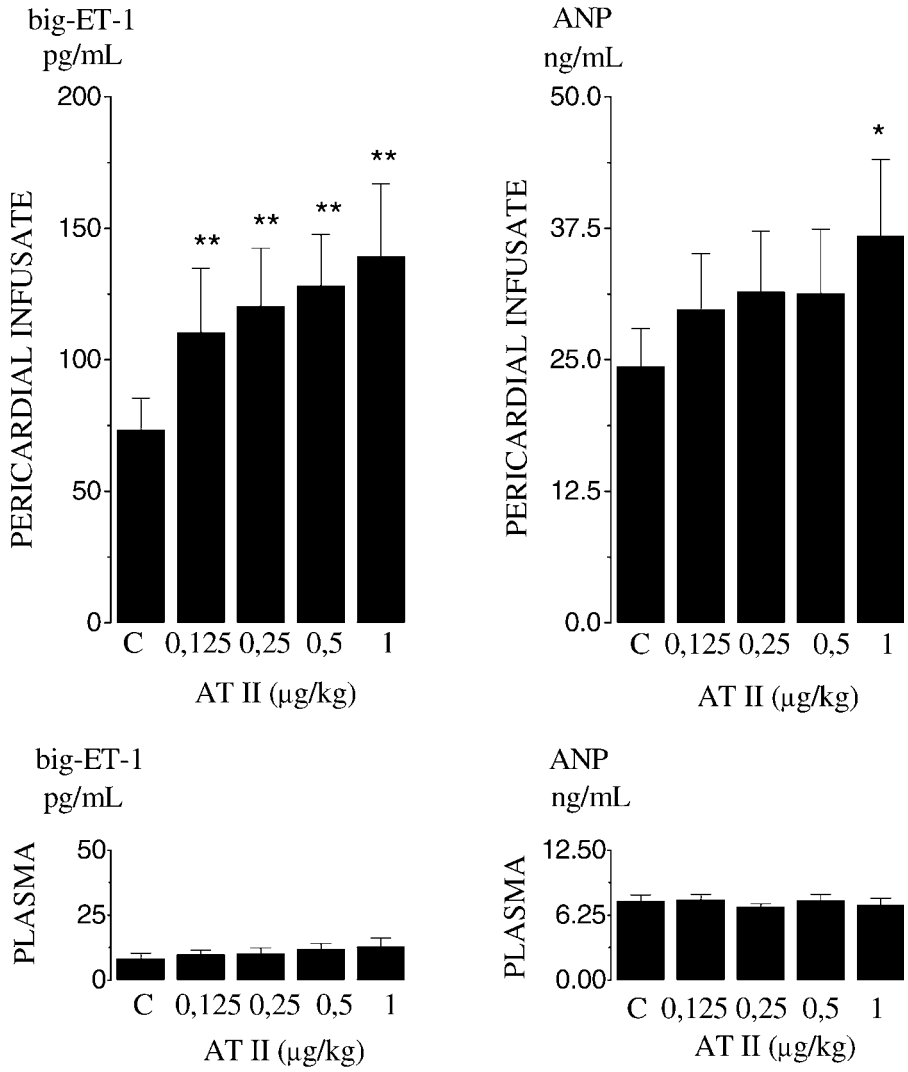

Figure 2. Concentrations of big ET-1 and ANP in the pericardial fluid and in the arterial plasma before (control [C]) and after administration of 0.125–1.0 μg/kg AT II into the pericardial sac. Values are presented as mean ± SEM. *$P < 0.05$ and **$P < 0.02$ vs. control; $n = 8$.

Results

Under basal conditions, the pericardial fluid contained more ET-1 and ANP than the plasma (13.4 ± 0.7 vs. 1.7 ± 0.5 pg/ml, $P < 0.02$; and 24.4 ± 3.6 vs. 7.6 ± 0.6 ng/ml, $P < 0.02$), in accordance with previous results. Similarly, we found higher big ET-1 concentrations in the pericardial fluid compared with the plasma levels (73.5 ± 12 vs. 9.9 ± 2.4 pg/ml; $P < 0.05$). Administration of AT II, ip, even in the smallest dose, caused a significant elevation in the pericardial concentration of big ET-1 (110.6 ± 24.2 vs. 73.5 ± 12; $P < 0.02$). Big ET-1 increased further, in a dose-dependent manner, whereas the plasma big ET-1 levels did not change under the same conditions. However, we did not observe elevations in the ET-1 concentrations in the pericardial fluid (control vs. AT $II_{1.0}$, 13.4 ± 0.7 vs. 13.7 ± 0.7 pg/ml), indicating that no significant conversion of the extra big ET-1 to ET-1 occurred during the AT II effects. At the same time, the pericardial ANP concentrations showed slight increases at lower doses of ip administration of AT II, and the ANP levels were elevated significantly, by approximately 50%, in response to the largest dose of AT II (36.8 ± 7.2 vs. 24.4 ± 3.6 ng/ml; $P < 0.05$), without parallel alterations of ANP in the plasma samples (Fig. 2). The basal values of the hemodynamic variables (HR, 183 ± 8 beats/min; dP/dt_{max}, 2087 ± 228 mm Hg/sec; and mean BP, 121 ± 3 mm Hg) did not change (HR) or increased moderately (dP/dt_{max} and BP) in response to ip administration of AT II, when the highest dose of the agent was applied (Table 1).

Discussion

The major finding of the present study is that ip application of AT II effectively stimulates big ET and ANP production and release in the *in situ* dog heart, demonstrated by the markedly significant elevations of their concentrations in pericardial sac fluid samples. According to

Table 1. Changes of the Cardiovascular Variables in Response to Intrapericardial AT II Administration at the Time of Pericardial Sampling ($n = 8$)[a]

	AT II ip (μg/kg)			
	0.125	0.25	0.5	1.0
Mean BP (Δ mm Hg)	0 ± 4	2 ± 2	7 ± 3	14 ± 2**
HR (Δ bpm)	−3 ± 1	−1 ± 1	1 ± 2	6 ± 4
dP/dtmax (Δ%)	−5 ± 1*	1 ± 3	7 ± 3	10 ± 3*

[a] dP/dt_{max}, left ventricular contractile force.
*$P < 0.02$; **$P < 0.001$.

previous studies (16–24), the pericardial fluid reflects the composition of cardiac interstitial fluid, and alterations in the pericardial concentrations of endogenous agents of cardiac origin may follow the changes in their production and release from the myocardium (17, 25–28). In addition, our previous experiments with the same protocol used in this study proved that, under control conditions, reproducible data can be measured after repeated incubation periods (26, 28). This indicated that 15 mins is sufficiently long to achieve equilibrium.

Because molecules of up to 40-kDa molecular weight can diffuse through the epicardium (30) and because the studied peptides are well within this limited range, we assumed that ip administration of AT II could pass into the heart and that any changes of big ET-1 and ANP concentrations detected in the pericardial infusate samples reflect the result of ongoing interactions in the heart.

Based on our results, we can assert that, 15 mins after ip AT II administration, the pericardial big ET-1 levels increased significantly in a dose-dependent manner. The pericardial concentrations of ANP were also elevated in response to large doses of AT-II. The basic ANP levels were comparatively high in these experiments, probably because of the anesthetic agent (pentobarbital), which stimulates ANP release (31, 32). However, exposure to AT II still induced a further increase. Accordingly, the ip AT II administration stimulated both the ET and natriuretic peptide system in the heart. At the same time, the pericardial ET-1 concentration did not rise significantly in response to any of the AT II dosages. Of interest is the observation that changes in pro-ET-1 levels were not followed by increases of pericardial ET-1 levels. It is conceivable that the stimulation of AT II needs a more prolonged time for the conversion of big ET-1 to ET-1. Another possibility is that AT II induced a dose- and time-dependent inhibition of ET-converting enzyme-1 expression, as demonstrated in human umbilical vein endothelial cells (33). Still another possibility, although speculative, is an accelerated elimination of ET-1 via AT II–stimulated degrading enzymes.

Considering the fact that the myocardial release of ET-1 was not elevated in response to administration of AT II, the stimulation of ANP liberation seems to be a direct effect of AT II rather than an ET-1–mediated response. On the other hand, we cannot exclude the involvement of other endogenous agents activated by AT II in the release of ANP. Because ip AT II administration did not elicit significant cardiovascular effects, except when the highest dose was applied, the AT II–induced increase of big ET-1 and ANP release occurred, at least in part, independently of the changes of cardiovascular functions.

In conclusion, ip AT II application exerts characteristic effects on myocardial big ET-1 and ANP production and release, providing new evidence for the *in vivo* local AT-ET and -ANP interactions in the heart.

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