Pulmonary Elimination of Angiotensin-I after Experimental Damage to the Myocardium

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In a rat model of heart failure induced by microembolization of coronary vessels pulmonary elimination of angiotensin-I directly depends on the degree of myocardial damage, the clearance of angiotensin-I being mediated through angiotensin-converting enzyme-independent factors. Insufficient release of angiotensin-II from the lungs into circulation in embolized animals can be partially responsible for activation of peripheral renin-angiotensin systems and aggravation of heart failure.

Key Words: *angiotensin-converting enzyme; lung clearance; cardiac sclerosis; heart failure*

Activation of the renin-angiotensin system (RAS) depends on the degree of myocardial damage and the severity of heart failure [8]. Increased plasma renin activity at the late [5] and, under physical strain, early stages of heart failure [9] probably results from accelerated elimination of angiotensin-I (AT-I) in the circulation. Plasma concentrations of AT-I can remain unchanged because its production and utilization are compensated. The aim of the present study was to evaluate the relationship between the degree of myocardial damage induced by embolization of the coronary vessels and the rate of AT-I clearance.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 330-370 g. Coronary embolism was induced by injecting plastic 15-μ microspheres (NEN) into the left ventricle with occluded ascendant aorta [6]. Controls were sham-operated rats injected with the same volume of physiological saline via the same route. The intensity of AT-I elimination into pulmonary circulation was assessed 21 days postoperation. To this end, arterial and venous catheters were implanted, and on the next day ^{125}I-AT-I (14×10^5 cpm^2, 50 ng/min) was injected intravenously until attaining the equilibrium concentration. Arterial blood was sampled into the medium for peptide extraction [3]. The vortexed mixture was centrifuged, the supernatant was diluted with distilled water to 5 ml, and peptides were extracted on a Sep-pak C18 microcolumn (Waters Ass.) [2]. The peptide fraction was eluted with 2 ml ethanol and dried under a nitrogen stream. Quantitative analysis of ^{125}I-AT-I and ^{125}I-AT-II was performed using high-performance liquid chromatography followed by radiometry of the corresponding minute eluate fractions. Pulmonary clearance was calculated as the ratio of infusion rate to blood concentration of ^{125}I-AT-I.

For morphological analysis, the hears were fixed in 10% neutral formaldehyde and embedded in paraffin. Microtome longitudinal sections (5-7 μ) through the atria and ventricles were stained with hematoxylin and eosin and picrofuchsin by the van Gieson method. The following pathological phenomena were assessed: the number of scars per section, focal cardiosclerosis, and cardiomyocyte hypertrophy and dystrophy. Each phenomenon was scored using a 5-point scale. The data were processed

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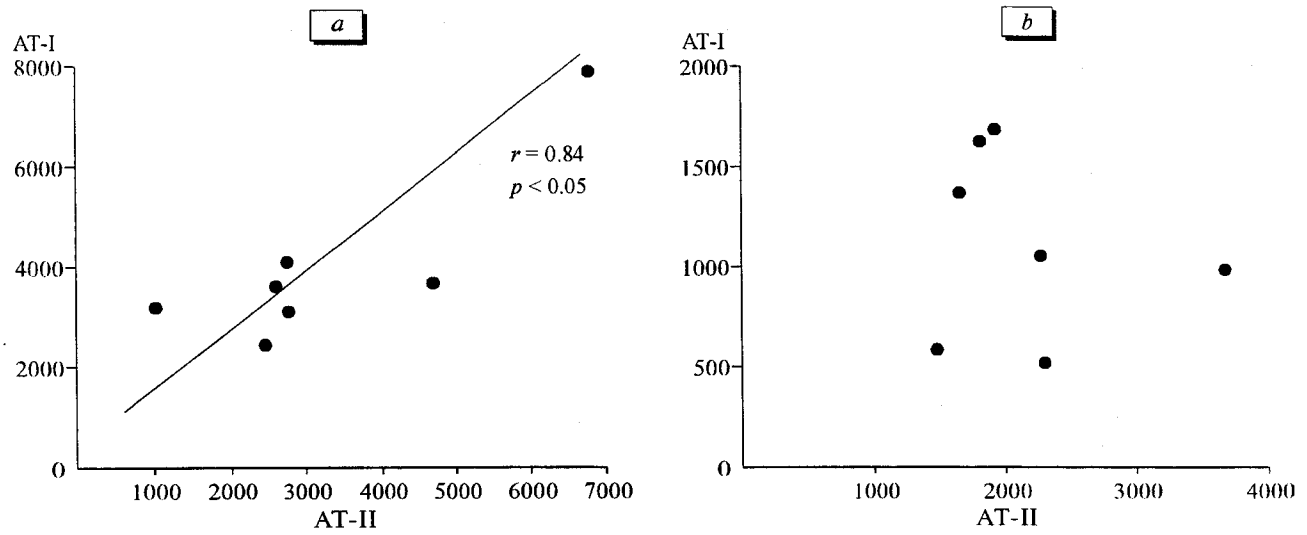

Fig. 1. Concentration (cpm/g blood) of angiotensin-II (AT-II) as a function of arterial concentration AT-I in sham-operated (*a*) and embolized (*b*) rats after infusion of labeled AT-I.

statistically using the Student *t* test and Mann—Whitney test for assessing the intergroup differences.

RESULTS

The equilibrium blood concentration of labeled AT was attained on the 5th min of infusion. Blood pressure remained unchanged during a 20-min infusion. Pulmonary elimination of AT-I was evaluated from the equilibrium concentration of ^{125}I-AT-I and pulmonary clearance of I-AT-I. Angiotensin-converting enzyme (ACE) activity in pulmonary circulation can be indirectly assessed by the concentrations of ^{125}I-AT-I and ^{125}I-AT-II and their ratio, since newly formed venous AT-II totally derived from AT-I is delivered by arterial blood [1]. The concentration of ^{125}I-AT-I in embolized animals was 5-fold lower than in sham-operated controls. This was not due to its enhanced conversion into ^{125}I-AT-II, since the concentration of ^{125}I-AT-II was also decreased. These findings suggest either predominance of alternative metabolic pathways of ^{125}I-AT-I [1] or its more intense uptake in the lungs. The concentration of AT-I influx to the cardiopulmonary region calculated from the cardiac output [7] and ^{125}I-AT-I infusion rate was 10,000 and 11000 cpm/ml for sham-operated and embolized rats, re-spectively. After measuring arterial concentrations of AT-I and AT-II, we found that in 37 and 18% of sham-operated and embolized rats, respectively, ^{125}I-AT-II is converted into ^{125}I-AT-I.

Thus, conversion of ^{125}I-AT-I in pulmonary circulation in embolized animals decreased 2-fold in comparison with the control. This indirectly attests to reduced regional activity of ACE and predominance of alternative (ACE-independent) metabolic pathways of ^{125}I-AT-II. Different metabolic pathways in experimental and control groups are confirmed by a strict correlation between the con-

TABLE 1. Equilibrium Concentrations of Labeled AT, Their Ratio, and Pulmonary Clearance in Sham-Operated and Embolized Rats (*M*±*m*, *n*=7)

Parameter	Sham-operated rats	Embolized rats
^{125}I-AT-I, cpm/g	4135±780	789±160*
^{125}I-AT-II, cpm/g	3670±700	2037±330*
AT-II/AT-I	1.236±0.110	0.507±0.08*
Pulmonary clearance of AT-I, ml/min*	314.4	1479

Note. *Calculated from the mean group values. *$p<0.05$ compared with sham-operated controls.

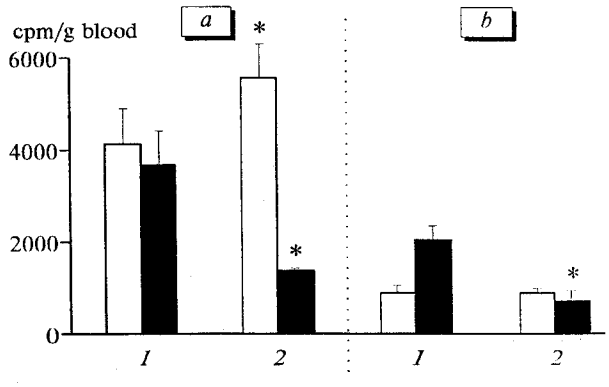

Fig. 2. Blood equilibrium concentrations of angiotensin-I (open bars) and angiotensin-II (shaded bars) in sham-operated (*a*) and embolized (*b*) rats before (*1*) and after (2) bolus injection of captopril. *$p<0.05$ compared with the control.

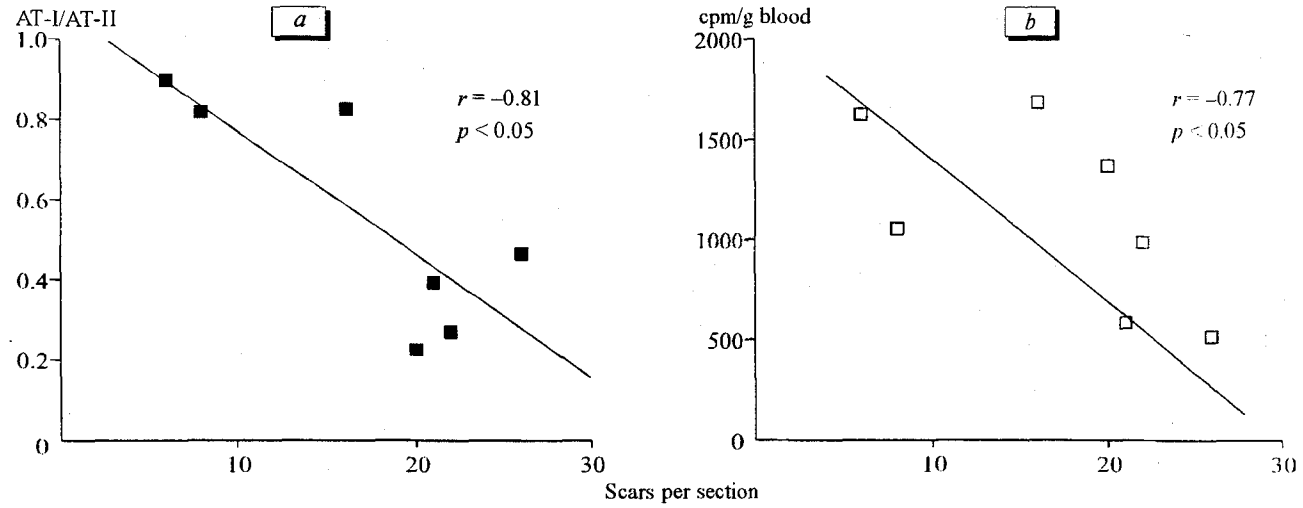

Fig. 3. Arterial equilibrium concentrations ratio of labeled angiotensin (AT) (*a*) and equilibrium AT-I concentration (*b*) as a function of the degree of myocardial damage in embolized rats (regression).

centrations of ^{125}I-AT-I and ^{125}I-AT-II in sham-operated animals and the absence of this correlation in embolized rats (Fig. 1).

Additionally, to evaluate the contribution of ACE to the AT-I elimination, the ACE inhibitor captopril was intravenously injected against the background of ^{125}I-AT-I infusion, which produced a decrease in ^{125}I-AT-II concentration in embolized and control animals by 63 and 64%, respectively. Simultaneously, the concentration of ^{125}I-AT-I increased by 34% in sham-operated animals and remained unchanged in controls. Consequently, ACE-independent pathways of AT-I elimination dominate under pathological conditions.

Histological analysis of the myocardium 21 days after embolization revealed the following typical changes: scars (17.0±2.85 per section), focal cardiosclerosis (2.6±0.3 points), and dystrophy and hypertrophy of cardiomyocytes (1.6±0.2 and 1.7±0.2 points, respectively). By comparing individual morphological and biochemical data we found that the number of scars significantly correlated with arterial concentration of ^{125}I-AT-I (Fig. 3) and more strictly with ^{125}I-AT-I/^{125}I-AT-II ratio. The latter parameter more adequately than the concentrations of different AT reflects the state of RAS, which is consistent with the data of others [4].

Thus, our findings suggest that the intensity of AT-I elimination in the lungs directly depends of the degree of myocardial damage and that the accelerated AT-I clearance is effected though ACE-independent processes. Insufficient release of AT-II from the lung into systemic circulation can be partially responsible for activation of peripheral RAS and aggravation of heart failure.

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