Protein remodeling of extracellular matrix in rat myocardium during four-day hypoxia: the effect of concurrent hypercapnia

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Abstract. The combination of long-term hypercapnia and hypoxia decreases pulmonary vascular remodeling and attenuation of right ventricular (RV) hypertrophy. However, there is limited information in the literature regarding the first stages of acclimatization to hypercapnia/hypoxia. The purpose of this study was to investigate the effect of four-day hypoxia (10% O_2) and hypoxia/hypercapnia (10% O_2 + 4.4% CO_2) on the protein composition of rat myocardium. Expression of the cardiac collagen types and activities of matrix metalloproteinases (MMPs) and of their tissue inhibitor TIMP-1 were followed. The four-day hypoxia changed protein composition of the right ventricle only in the hypercapnic condition; remodeling was observed in the extracellular matrix (ECM) compartments. While the concentrations of pepsin-soluble collagenous proteins in the RV were elevated, the concentrations of pepsin-insoluble proteins were decreased. Furthermore, the four-day hypoxia/hypercapnia increased the synthesis of cardiac collagen due to newly synthesized forms; the amount of cross-linked particles was not affected. This type of hypoxia increased cardiac collagen type III mRNA, while cardiac collagen type I mRNA was decreased. MMP-2 activity was detected on the zymographic gel through appearance of two bands; no differences were observed in either group. mRNA levels for MMP-2 in the RV were significantly lower in both the hypoxic and hypoxic/hypercapnic animals. mRNA levels for TIMP-1 were reduced in the RV of both the hypoxic and hypoxic/hypercapnic animals. Hypoxia with hypercapnia increased the level of mRNA (6.5 times) for the atrial natriuretic peptide (ANP) predominantly in the RV. The role of this peptide in remodeling of cardiac ECM is discussed.

Key words: Heart muscle — Hypoxia — Hypercapnia — Extracellular matrix — Natriuretic peptide

Introduction

Adaptation of rats to chronic hypoxia in a barochamber (intermittent high-altitude hypoxia to 7000 m, 8 h/day, 24 exposures) induces structural changes in their pulmonary vessels, hypertension in pulmonary circulation, and hypertrophy of right ventricles (RV). Furthermore, the profiles of both myofibrillar and extracellular matrix (ECM) proteins are altered in the hearts. All these morphological and biochemical changes are localized predominantly in the hypertrophied RV. The mechanisms involved in protein remodeling of chronic hypoxic myocardium appear to be complex and diverse (Urbanova et al. 1977; Pelouch and Jirmar 1993). Even less is known about protein composition of myocardium in the early phase of acclimatization to chronic hypoxia (Pelouch et al. 1993a).

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Several studies have shown the effect of concurrent long-term hypercapnia and hypoxia. In contrast with chronic hypocapnic hypoxia, decreased pulmonary vascular remodeling and attenuation of RV hypertrophy was observed (Ooi et al. 2000); the higher amount of CO_2 in the hypoxic chamber altered the rebuilding of different cardiac compartments.

However, no data are available on the first stages of acclimatization to hypoxia/hypercapnia. Therefore, the aim of the present study was to analyze the effect of four-day hypoxia/hypercapnia on protein composition of rat myocardium. The expression of cardiac collagen types, the activities of different matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) were followed. In addition, gene expression of atrial natriuretic peptide (ANP) in myocardium was analyzed, as this peptide might play an important role in the remodeling of ECM proteins (Perhonen et al. 1997; Tsutamoto et al. 2001).

Materials and Methods

Experimental model

Two groups of adult male Wistar rats were exposed to hypoxia (10% of oxygen) in an isobaric chamber for 4 days:

a) the first group of animals (n = 12) was in the hypoxic chamber where CO_2 was fully absorbed (CO_2 was not measurable) – hypoxia (H),
b) the second group (n = 12) was in the hypoxic chamber where CO_2 level in inspired air was increased by decreasing its recirculation (CO_2 = 4.4%, range 4.0–5.2) – hypoxia/hypercapnia (HC).

Control rats (n = 12) were kept in atmospheric air (C).

The isobaric chamber wasn't opened during the experimental run. All animals had free access to water and to a standard laboratory diet. All procedures and experimental protocols were approved by the Veterinary Council of the Czech Republic and conform with the European Convention on Animal Protection.

All animals were sacrificed within 12 h after the end of the experiment. The animals were anesthetized with sodium pentobarbital (50 mg/kg) and their hearts were quickly removed. The aorta and pulmonary artery were carefully excised close to the ventricular surface, the RV and the left ventricle (LV) were separated, blotted dry, weighed, immersed in liquid nitrogen, and stored at –70°C until assayed.

Protein profiling

Protein profiling was performed as described previously (Pelouch et al. 1995). The samples of RV and LV (approximately 50 mg/ventricle, n = 6 for each group) were homogenized in 20 volumes of buffer A (0.05 mol·l^{-1} phosphate buffer, 0.01 mol·l^{-3} EDTA, 1% Triton X-100, pH 7.4). The samples were extracted at 4°C (24 h continuous stirring), and then centrifuged at 10,000 × g for 10 min. The supernatant was recovered (a fraction of metabolic proteins contained predominantly different enzymes of glycolytic and aerobic metabolic pathways) and the pellet was dissolved in 10 volumes of buffer B (0.9 mol·l^{-1} NaCl, 0.05 mol·l^{-1}, Tris-HCl, pH 7.5), stirred for 24 h at 4°C and then centrifuged at 10,000 × g for 15 min. The supernatant was recovered, the pellet was washed with 0.5 mol·l^{-1} acetic acid and then subjected to pepsin digestion (two treatments of 24 h duration at 4°C using 10 mg pepsin·g^{-1} wet weight in 0.5 mol·l^{-1} acetic acid). The samples were centrifuged (at 14,000 × g for 15 min); both the pellet and the supernatant (containing pepsin-soluble ECM proteins) were stored. Pepsin digestion and centrifugation were repeated and the supernatant was combined with the previous one. This and the previous pellets were combined and then solubilized in hot 1.1 mol·l^{-1} NaOH for 15 min (the fraction contained insoluble ECM proteins).

Protein determination in the individual fractions was carried out using the Lowry method (Lowry et al. 1951); 750 nm wavelength was used for higher sensitivity. The values were expressed as mg·g^{-1} wet weight of tissue; the concentration of 4-hydroxyproline (as a marker of collagen) was determined in both ECM fractions by the same method as described previously (Pelouch et al. 1993b). The solubility of cardiac collagen was expressed as a ratio of hydroxyproline in soluble and insoluble fractions of ECM proteins.

RNA extraction and Northern blot analysis

Total RNA was isolated from ventricular tissues by the CsCl method (Chirgwin et al. 1979). The frozen samples (6 from each group, approximately 100 mg) were rapidly homogenized and the tissue homogenates were subjected to CsCl extraction. RNA pellets were dissolved in TE buffer and RNA concentration was calculated from absorbance at 260 nm prior to size fractionation. For the Northern blot analysis, 20 μg of total RNA was electrophoresed in a 1% agarose/formaldehyde gel and transferred to a nitrocellulose filter (Hybond – N RPN.203N, Amersham Pharmacia, UK). The filters were air-dried at 80°C for 2 h.

The complementary DNA (cDNA) probes used were mouse procollagen α_1 (I) and rat procollagen α (III) (a generous gift from Dr. Timo Takala from the Dept. of Biology of Physical Activity, University of Jyväskylä, Finland), rat TIMP-1 and MMP-2 (both were the gift of Dr. L. Eklund from the Dept. of Biochemistry, Oulu University, Finland), rat ANP (full-length probes), and rat 18S RNA (482-bp

Table 1. Effect of chronic hypoxia and hypercapnic hypoxia on body and heart weights

Group	Rats	BW	HW	RV	LV	HW/BW	RV/BW	LV/BW	RV/LV
	n	(g)	(mg)	(mg)	(mg)	($mg \cdot g^{-1}$)	($mg \cdot g^{-1}$)	($mg \cdot g^{-1}$)	
C	12	267.50 ± 0.93	749 ± 18	167 ± 14	410 ± 20	2.80 ± 0.06	0.59 ± 0.03	1.50 ± 0.04	0.40 ± 0.03
H	12	242.83 ± 3.26 ^{A}	755 ± 16	178 ± 6	377 ± 10	3.12 ± 0.09 ^{A}	0.74 ± 0.02 ^{A}	1.54 ± 0.05	0.49 ± 0.02 ^{A}
HC	12	228.50 ± 3.68 ^{AB}	784 ± 27	194 ± 7 ^{A}	405 ± 20	3.43 ± 0.10 ^{AB}	0.85 ± 0.04 ^{AB}	1.77 ± 0.06 ^{AB}	0.48 ± 0.02 ^{A}

Values are presented as mean ± SEM; C, controls; H, hypoxia/hypocapnia; HC, hypoxia/hypercapnia; BW, body weight; HW, absolute heart weight; RV, right ventricular weight; LV, left ventricular weight (without septum); HW/BW, relative HW; RV/BW, relative RV; LV/BW, relative LV (without septum); RV/LV, right ventricle/left ventricle ratio. Significance: ^{A} $p < 0.05$ difference from control; ^{B} $p < 0.05$ difference from the H group (analysis of variance).

cDNA probe) (Magga et al. 1997). The cDNAs were labeled with [α-^{32}P]dCTP with a random prime labeling system (Rediprime^{TM} II, Amersham Pharmacia, UK). The pre-hybridization of filters was carried out in an ultrasensitive hybridization solution (ULTRAhyb^{TM}, Ambion, USA) for 30 min at 42°C. The hybridization was performed for 24 h at 42–52°C using the same buffer as for pre-hybridization. After hybridization, the membranes were washed three times in 0.1 × SSC + 0.1% SDS at 60°C for 20 min and exposed to storage phosphor screens. The results of autoradiographs from the Northern blot analysis were quantified by densitometry (Bio-Rad GS 670 imaging densitometer). The signals of individual mRNAs were normalized to those of the 18S ribosomal RNA to compensate for differences in loading and/or transfer of mRNA.

Zymography

MMP activity in cardiac tissue extracts was determined by gelatin zymography performed by standard procedures using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) matrix containing gelatin (1 mg/ml) (Tyagi et al. 1993). The cardiac tissue (20 mg) was minced into small fragments, agitated for 24 h at 4°C in 10 volumes of buffer containing 10 $mmol \cdot l^{-1}$ cacodylic acid, 150 $mmol \cdot l^{-1}$ NaCl, 1 $\mu mol \cdot l^{-1}$ $ZnCl_2$, 20 $mmol \cdot l^{-1}$ $CaCl_2$, 3.0 $mmol \cdot l^{-1}$ NaN_3, and 0.01% Triton X-100. Then the samples were centrifuged (4°C, 15 min, 10,000 × *g*). The final protein concentration of the tissue extract was determined using the Lowry method and the extracts were aliquoted and stored at –70°C. Each sample was mixed with the Laemmli SDS sample buffer in the absence of the reducing agent and electrophoresed on 8% polyacrylamide gel. After electrophoresis, the gels were washed two times for 20 min in 2.5% Triton X-100 to remove SDS and permit enzyme renaturation. The gel was then placed in substrate buffer (50 $mmol \cdot l^{-1}$ Tris-HCl, pH 7.8; 10 $mmol \cdot l^{-1}$ NaCl; 10 $mmol \cdot l^{-1}$ $CaCl_2$) and incubated for 14 h at 37°C. The gels were stained with 0.3% Coomassie Brilliant Blue and distained.

Data analysis

All data are expressed as mean ± SEM. The control, hypoxia, and hypercapnic hypoxia groups were compared using analysis of variance (ANOVA) and Fischer's least significant difference test. The data were accepted as statistically significant at the level of $p < 0.05$.

Results

Body and heart weights

The four-day hypoxia decreased body weight (BW) (Table 1) in both experimental groups (–9.2% for the H and –14.6% for the HC, respectively). The absolute heart weights (HW) were not affected in either experimental group; however, the HW/BW ratios were increased in both experimental groups; in the H it was due to elevation of the relative weight of the RV only, in the HC the relative weights of both the RV and LV were elevated. Furthermore, the RV/LV ratio was elevated in both experimental groups.

ANP gene expression

mRNA expression of ANP in the RV of the HC animals was 6.5 times higher relative to the control level; no change was induced in the H rats. Gene expression of ANP in the LV was not affected (Fig. 1).

Protein composition of the RV and LV: concentration and protein content

The concentration of ECM proteins (Fig. 2) was not affected in the RV of H animals. In contrast, a higher concentration

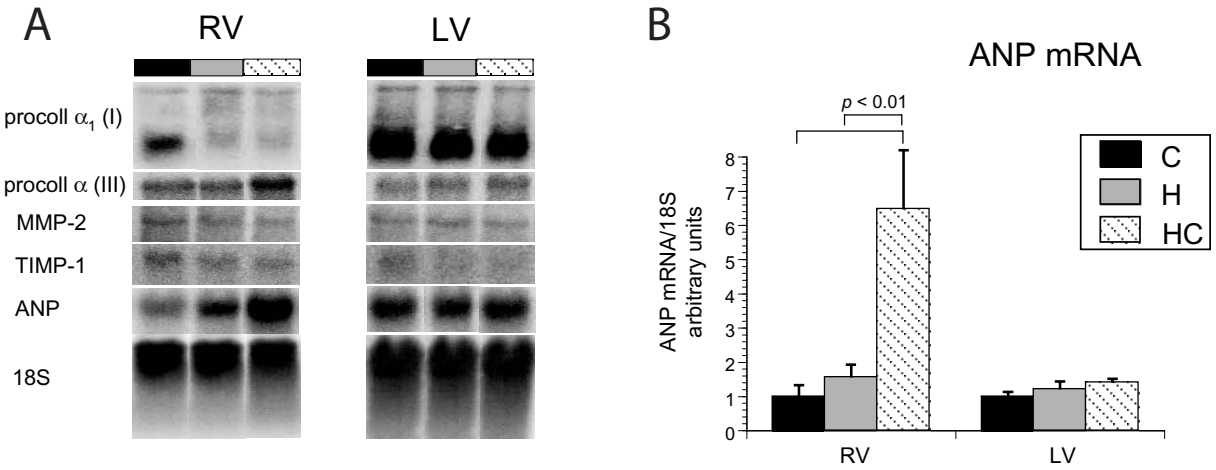

Figure 1. Representative autoradiographs from the Northern blot analysis (A) and the effect of hypoxia and hypoxia/hypercapnia on atrial natriuretic peptide (ANP) mRNA (B). RNA was extracted from the cardiac tissue. Electrophoresis, transfer of RNA, and washing were carried out as described in Materials and Methods. The charts shown are the result of densitometry scanning of autoradiographs. The same blot was sequentially hybridized with procollagen α_1 (I), procollagen α (III), MMP-2, TIMP-1, ANP and 18S probes. Equal RNA loading was demonstrated using the expression of the 18S oligonucleotide. C, controls; H, hypoxic animals; HC, hypoxic/hypercapnic animals; RV, right ventricle; LV, left ventricle.

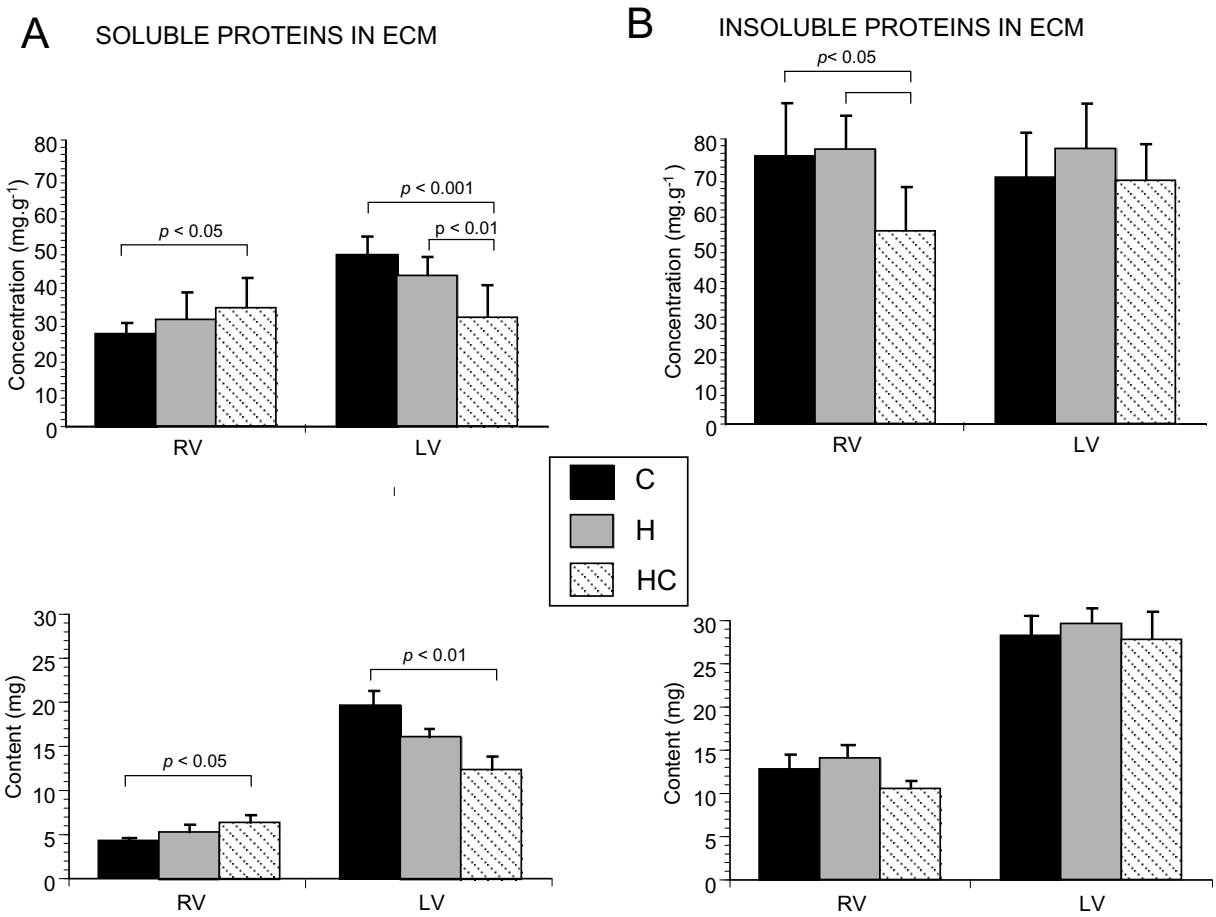

Figure 2. Concentration and content of soluble (A) and insoluble (B) proteins in extracellular matrix (ECM) The concentrations are expressed in mg·g^{-1} of wet weight, the content in mg. Values are means ± SEM.

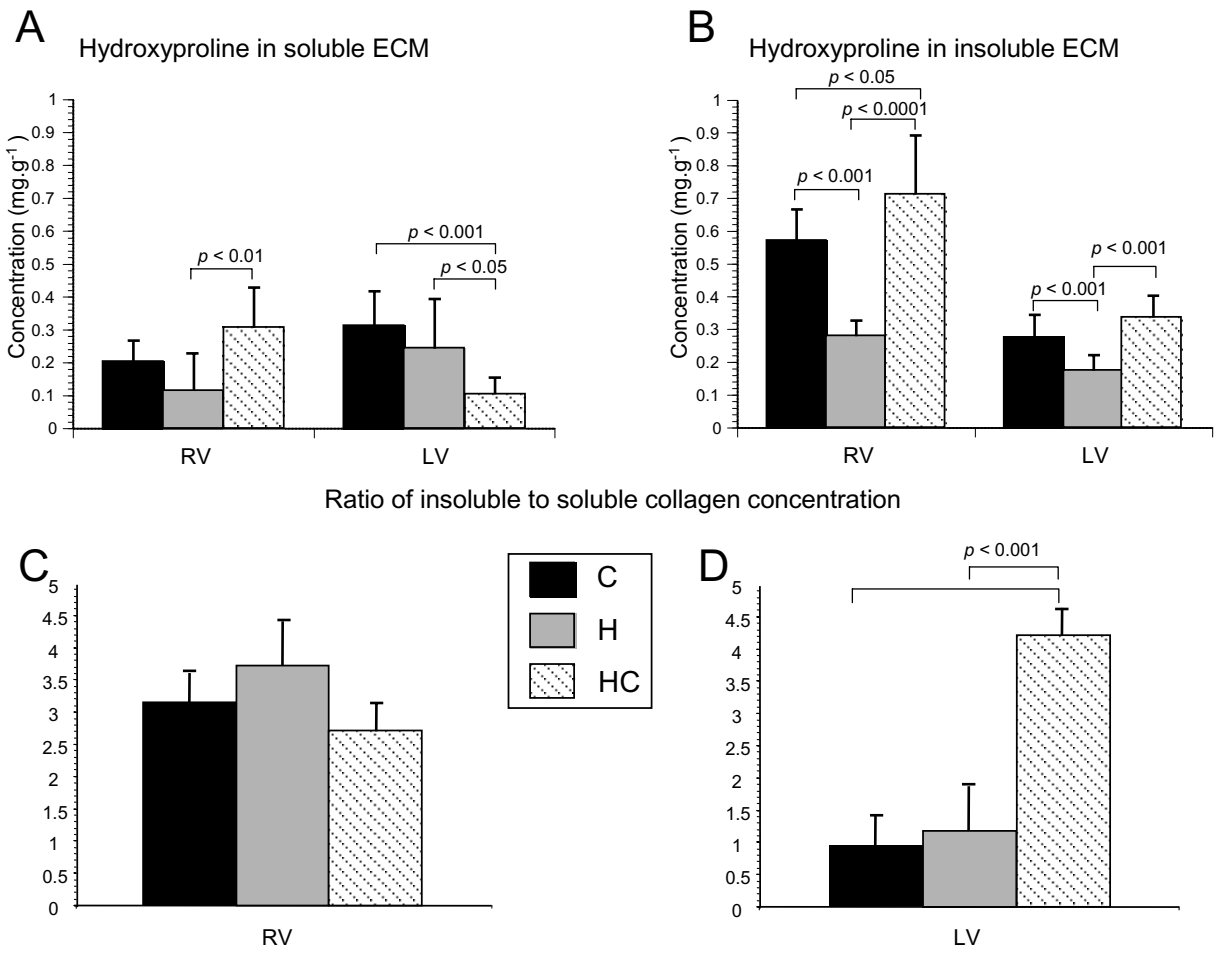

Figure 3. Collagen concentration in the fraction of soluble (A) and insoluble (B) extracellular matrix (ECM) proteins. Collagen concentration was determined by hydroxyproline concentration (mg·g^{-1} of wet weight). The ratios of insoluble to soluble cardiac collagen in the RV (C) and in the LV (D) were derived from the hydroxyproline concentrations. Values are means ± SEM.

of soluble collagenous proteins was found in the RV of HC animals. The concentration of insoluble collagenous proteins was decreased in the RV of HC rats. Lower concentration of soluble collagenous proteins was observed in the LV of these rats (Fig. 2).

Cardiac collagen: concentration, solubility, and gene expression

The concentration of hydroxyproline in the RV and LV differed even in the C rats; it was due to a higher amount of hydroxyproline in the fraction of insoluble ECM proteins derived from the RV. Hypoxia decreased the concentration of hydroxyproline in the fraction of insoluble ECM proteins; this occurred in both the RV and LV. In contrast, hypoxia/hypercapnia elevated the concentration of hydroxyproline in the fractions derived from both the soluble and insoluble ECM proteins of the RV; in the LV it was only in insoluble ECM proteins (Fig. 3A,B). The ratio of insoluble-to-soluble cardiac collagen differed even in the controls. This ratio was elevated in the LV of the hypoxia/hypercapnia animals (Fig. 3C,D).

The level of mRNA for procollagen α_1 (I) was decreased in the RV of hypoxic animals (both the H and HC groups); no change was observed in the LV. The level of mRNA for procollagen α (III) was higher only in the RV of the HC group (Fig. 4A,B and Fig. 1A).

Activities of cardiac MMP, MMP-2 and TIMP-1 gene expression

MMP-2 activity was detected on the zymographic gel by the appearance of two bands: 72 kDa (pro-form) and 66 kDa (active form). No differences were observed in either group. Furthermore, activities of both MMP-9 (92 kDa) and MMP-1

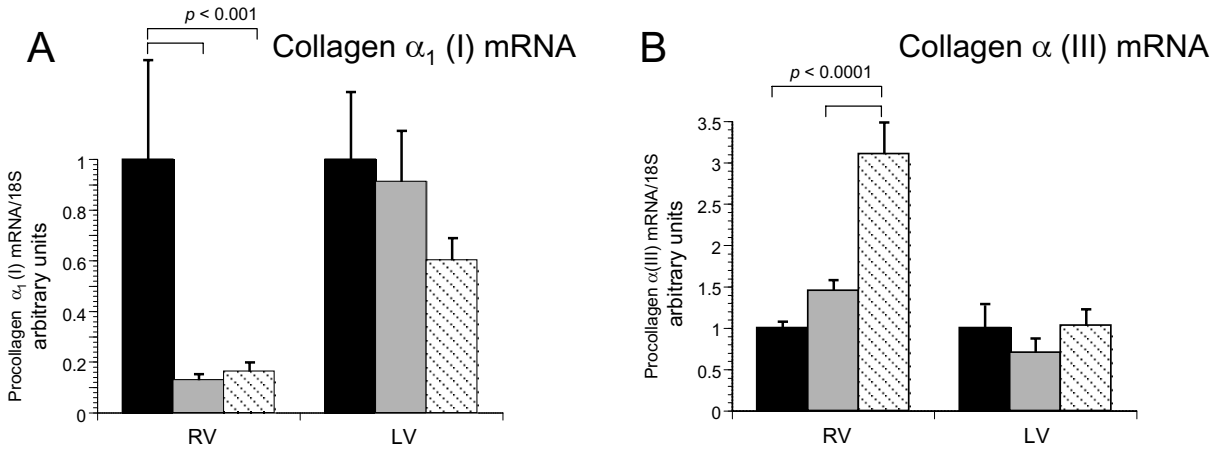

Figure 4. Representative autoradiographs from the Northern blot analysis (A) and the effect of hypoxia and hypoxia/hypercapnia on procollagen α_1 (I) mRNA (A) and procollagen α (III) mRNA (B). RNA was extracted from the cardiac tissue.

(53 kDa) were not quantified due to their very low apparent activities (Fig. 5A,B). The levels of mRNA for MMP-2 in the RV were significantly lower in both H and HC animals; no differences were observed in the LV in either group (Fig. 5C and Fig. 1A). The levels of mRNA for TIMP-1 were reduced in the RV of both H and HC animals; no differences were observed in the LV (Fig. 5D and Fig. 1A).

Discussion

The present study demonstrated that the exposure of rats to four-day hypoxia and hypercapnic hypoxia induced changes in weight parameters, in the protein composition of the cardiac ECM, in collagen concentration and collagen gene expression, and in the expression and activity of matrix metalloproteinase.

Increased pressure load due to the hypoxia-induced pulmonary vasculature resistance is known to lead to RV hypertrophy (Pelouch et al. 1997). Increased RV weights have been shown after 7 days, 10 days, and 3 weeks exposure to isobaric (normobaric) hypoxic conditions (Johnson et al. 1983; Stockmann et al. 1988; Kukacka et al. 2002). Although some authors found RV hypertrophy after 5 days (Sheedy et al. 1996), we now present a novel finding that an even shorter exposure to hypoxia (4 days) leads to RV hypertrophy. Furthermore, the four-day exposure to hypoxic conditions induced a decrease in BW in both experimental groups. We can speculate that it was induced by smaller food and water intakes in animals living in the normobaric chamber. In our experimental set, the growth retardation was much more pronounced in animals living in higher concentrations of CO_2; hypercapnia apparently contributed to the decrease in BW. Whereas it acted as a stress factor in the initial part of acclimatization to chronic hypoxia, hypercapnia in combination with chronic hypoxia had a protective character; the difference in the BW between the controls and the experimental rats living in hypoxia/hypercapnia for a long period is far less pronounced as compared with hypoxic animals in hypocapnia (Neckar et al. 2003).

The absolute weight of myocardium was not affected in our hypoxic rats; but the relative weights of the total myocardium as well as those of its parts were increased. This was the result of the lower BW in both the hypocapnic and hypercapnic experimental rat groups. The elevated relative HW in hypocapnia was due only to the higher relative weight of RV; in hypercapnia, it was due to the relative weights of both the RV and LV. No direct experimental data related to the elevation of pressure in pulmonary circulation in briefly hypoxic animals are available; however, depressed reactivity to acute hypoxic challenges (in the range of 0–10% O_2) and perfusion pressure-flow relationship (resistive properties) were shifted towards higher pressure in hypoxic animals exposed to isobaric hypoxia for 5 days (Lachmannova and Herget 2002). On the other hand, chronic hypoxia in eucapnia induced RV cardiomegaly due to hypertension in pulmonary circulation as a result of hypoxic pulmonary vasoconstriction and morphological and biochemical remodeling of pulmonary vessels (Adnot et al. 1991; Ooi et al. 2000). Similar results were observed in animals even after chronic intermittent hypercapnic hypoxia (McGuire and Bradford 2001), however, RV hypertrophy was less pronounced as compared with eucapnic hypoxic animals (Nattie et al. 1978; Baudouin and Bateman 1989).

Hypoxia is a potent stimulus for ANP secretion from the heart and indeed, some studies demonstrated *in vitro* that

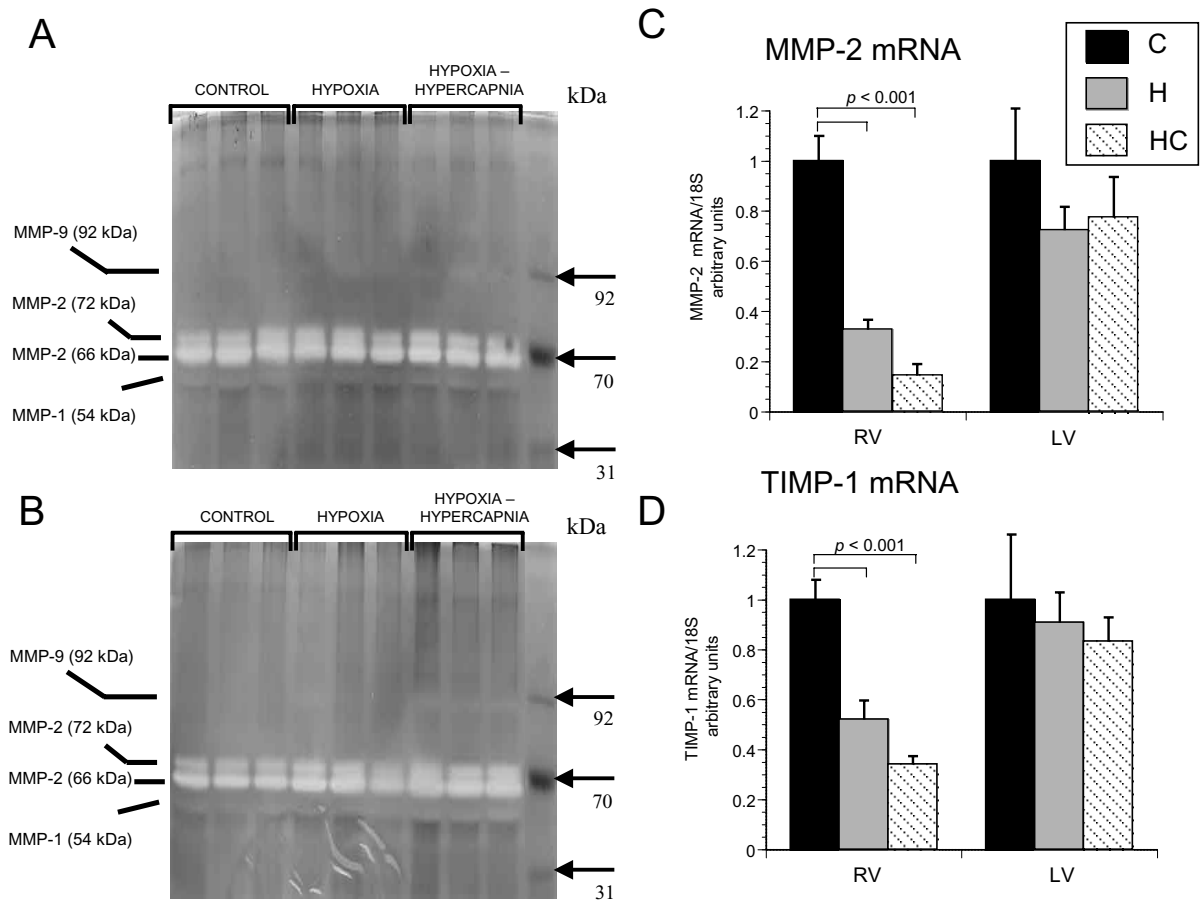

Figure 5. Representative zymographic gel of the cardiac metalloproteinase activity in the control, hypoxic and hypoxic/hypercapnic RV (A) and LV (B), and the effect of hypoxia and hypoxia/hypercapnia on the MMP-2 mRNA (C) and TIMP-1 mRNA (D). The charts shown are the result of densitometry scanning of autoradiographs.

hypoxic exposure can directly stimulate ANP gene expression in the absence of hemodynamic, neural and hormonal influences (Lew and Baertschi 1989; Chen et al. 1997). We analyzed the mRNA for ANP primarily as a validation of the hypoxic stimulus on heart ventricles, but also as a sensitive indicator of the hypertrophic process, because some studies have demonstrated the RV ANP mRNA expression and ANP synthesis during the development of RV hypertrophy (Ruskoaho et al. 1997). The lack of response of the ventricles to ANP mRNA expression in the hypoxic-only animals in the current study may be a result of an insufficient hypoxic stimulus. Indeed, some studies *in vivo* have shown that hypoxic episodes shorter than 4 days have no influence on ANP gene expression (Johnson et al. 1997). Our study possesses a limitation in that we did not examine the influence of isolated hypercapnia on ANP expression or plasma concentration. Yet other animal studies have shown ANP concentration to increase by more than 100% under hypercapnic conditions (Clozel et al. 1989). In this study, we observed highly increased ANP mRNA levels in the right heart of hypoxic/hypercapnic rats, indicating a significant hemodynamic alteration in this part of the heart. This could be due to a rise in the cardiac filling pressures as reported by Chabot et al. (1995).

The changes in the weight of the cardiac muscle were accompanied by protein remodeling of ventricular musculature. Moreover, gene expression of cardiac collagens and matrix metalloproteinases was observed. Chronic hypoxia is an important factor for qualitative and quantitative rebuilding of cellular and subcellular compartments of the myocardium. It was demonstrated here that even a brief hypoxia changed the protein composition, but only in the group of animals living in the hypercapnic conditions. The ECM compartment of the heart muscle is composed of different collagens, proteoglycans, glycoproteins and elastin; all these collagenous proteins have different structural subtypes (e.g. there are two major

collagens in myocardium – types I and III). Furthermore, both the battery of enzymes responsible for degradation of ECM proteins and growth factors responsible for their synthesis are localized in this compartment (Kukacka et al. 2005). The isolation of pepsin-soluble and pepsin-insoluble fractions of collagenous proteins, and subsequent assays of both proteins and hydroxyproline, along with molecular biology techniques (mRNAs for different collagens and MMPs) make it possible to analyze biochemical pattern of ECM and its remodeling under the hypoxic condition. Three different alterations of protein composition could be anticipated in a hypoxic myocardium: a) quantitative changes of ECM proteins; b) quantitative changes of the total collagen and/or its types; c) qualitative changes of ECM proteins. Four days of hypoxia affected the protein profile of the ECM only in the HC condition and predominantly in the RV. Protein remodeling of the ECM compartment was characterized by a higher concentration of proteins in the pepsin-soluble fraction; this fraction is occupied by newly synthesized ECM proteins. It followed from both the hydroxyproline and the mRNA analyses that it is due to a higher amount of the more elastic cardiac collagen type III. Elevation of collagen type III is typical for developing cardiac hypertrophy (Pelouch and Jirmar 1993). However, it was observed in this study in a time period when no change of absolute ventricular weights was detected. The functional significance of different cardiac collagen types is not yet fully understood. Interestingly, the concentration of total hydroxyproline in the RV of the HC rats decreased, which is an indication of a disproportional growth of non-collagenous and collagenous myocardial elements in this time period. The short-term hypoxia-induced synthesis of cardiac collagen had a higher amount of cross-linked material; this can probably be attributed to reduced collagen degradation. The amount of collagenous proteins in myocardium results from the dynamic balance between their synthesis and degradation. Different collagenases (e.g. MMP-1, MMP-8, MMP-13) or gelatinases (MMP-2 and MMP-9) together with their specific inhibitors (TIMPs) remodel the biochemical composition of ECM in hypoxic myocardium (Tyagi et al. 1997; Rouet-Benzineb et al. 1999; Kukacka et al. 2005); this event can be activated in hypoxic myocardium by reactive oxygen species or by the level of natriuretic hormones or cytokines (Rajagopalan et al. 1996; Kinugawa et al. 2000). MMP-9 is inactivated though forming a specific complex with TIMP-1; TIMP-1 is inactivated by different proteinases. However, increased mRNA for TIMP-1 does not necessarily correspond to a decrease in activities of MMPs (Nishikawa et al. 2003). Down-regulation of levels of mRNAs for MMP-2 and TIMP-1 was observed during both hypoxia and hypoxia/hypercapnia in the RV. We can not assume that mRNA levels were accompanied by changes in the protein content because the three genes MMP-2, MMP-9 and TIMP-1 typically do not show positive correlation between the protein and the mRNA (Lichtinghagen et al. 2002; Greenbaum et al. 2003). Additionally, matrix metalloproteinases are affected by many complicated and variable post-transcriptional mechanisms involved in turning the mRNA into a protein. They may differ substantially in their *in vivo* half lives; and these enzymes are activated or inhibited *in vivo* by many independent mechanisms (Kukacka et al. 2005). In conclusion, with regard to the zymography results we could speculate that MMP-2 has a higher specific activity in the RV of the control group. From the present study, it follows that the decreased expression of both the mRNA for MMP-2 and for TIMP-1 is characteristic of the RV in animals exposed to 4-day hypoxia; it elevates the concentration of cardiac collagen in animals after a short time in a hypoxic chamber. Furthermore, it is independent on the CO_2 level in the chamber.

The role of ANP in protein remodeling in a diseased myocardium seems to be a rather speculative subject. It was demonstrated that an increase in ANP suppressed collagen synthesis induced by hypoxia in the cardiac tissue (Ogawa et al. 2000, 2001). Synthesis of type I and type III collagens by rat cardiac fibroblasts was stimulated when the cells were cultured in hypoxic conditions and the stimulative effects were attenuated by the presence of ANP or BNP (brain natriuretic peptide) in the culture medium (Nakagawa et al. 1995). In spite of our completely new findings that the RV hypertrophy in the hypercapnic/hypoxic rats is associated with an increase in ANP mRNA levels and accompanied by an increase in collagen type III mRNA, we cannot conclude whether these changes in ANP are directly responsible for the biochemical remodeling of the ECM compartment. Only a further analysis could clarify this aspect of protein remodeling of myocardium induced by acute hypoxia and different levels of pCO_2.

Acknowledgements. We wish to thank Mrs. Pavlina Santorova for her kind technical assistance. This study was supported by the grants GAUK 45/2001/2.LF and by the Academy of Finland, the Sigrid Juselius Foundation, and the Finnish Cardiovascular Research Foundation.

References

Adnot S., Raffestin B., Eddahibi S., Braquet P., Chabrier P. E. (1991): Loss of endothelium-dependent relaxant activity in the pulmonary circulation of rats exposed to chronic hypoxia. J. Clin. Invest. **87**, 155–162

Baudouin S. V., Bateman N. T. (1989): Contractility of papillary muscle from rats exposed to 28 days of hypoxia, hypercapnia, and hypoxia with hypercapnia. Thorax **44**, 808–811

Clozel J. P., Saunier C., Hartemann D., Allam M., Fischli W. (1989): Effects of hypoxia and hypercapnia on atrial natriuretic factor and plasma renin activity in conscious dogs. Clin. Sci. (London) **76**, 249–254

Greenbaum D., Colangelo Ch., Williams K., Gerstein M. (2003): Comparing protein abundance and mRNA expression levels on a genomic scale. Genome Biol. **4**, 117

Chabot F., Mertes P. M., Delorme N., Schrijen F. V., Saunier C. G., Polu J. M. (1995): Effect of acute hypercapnia on alpha atrial natriuretic peptide, renin, angiotensin II, aldosterone, and vasopressin plasma levels in patients with COPD. Chest **107**, 780–786

Chen Y. F., Durand J., Claycomb W. C. (1997): Hypoxia stimulates atrial natriuretic peptide gene expression in cultured atrial cardiocytes. Hypertension **29**, 75–82

Chirgwin J. M., Przybyla A. E., MacDonald R. J., Rutter W. J. (1979): Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**, 5294–5299

Johnson T. S., Young J. B., Landsberg L. (1983): Sympathoadrenal responses to acute and chronic hypoxia in the rat. J. Clin. Invest. **71**, 1263–1272

Johnson D. D., Singh M. B., Cheung C. Y. (1997): Effect of three hours of hypoxia on atrial natriuretic factor gene expression in the ovine fetal heart. Am. J. Obstet. Gynecol. **176**, 42–48

Kinugawa S., Tsutsui H., Hayashidani S., Ide T., Suematsu N., Satoh S., Utsumi H., Takeshita A. (2000): Treatment with dimethylthiourea prevents left ventricular remodeling and failure after experimental myocardial infarction in mice: role of oxidative stress. Circ. Res. **87**, 392–398

Kukacka J., Bíbová J., Ruskoaho H., Pelouch V. (2002): Chronic hypoxia alters expression of extracellular matrix components in rat. Phys. Res. **51**, 21

Kukacka J., Prusa R., Kotaska K., Pelouch V. (2005): Matrix metalloproteinases and their function in myocardium. Biomed. Pap. Med. Fac. Univ. Palacky, Olomouc, Czech Republic **149**, 225–236

Lachmannova V., Herget J. (2002): N-acetylcysteine (NAC) but not L-NAME inhibits pulmonary vascular effects of 5 days hypoxia in rats. Physiol. Res. **51**, 68

Lew R. A., Baertschi A. J. (1989): Mechanisms of hypoxia-induced atrial natriuretic factor release from rat hearts. Am. J. Physiol., Heart Circ. Physiol. **257**, H147–156

Lichtinghagen R., Musholt P. B., Lein M., Romer A., Rudolph B., Kristiansen G., Hauptmann S., Schnorr D., Loening S. A., Jung K. (2002): Different mRNA and protein expression of matrix metalloproteinases 2 and 9 and tissue inhibitor of metalloproteinases 1 in benign and malignant prostate tissue. Eur. Urol. **42**, 398–406

Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J. (1951): Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**, 265–275

Magga J., Vuolteenaho O., Tokola H., Marttila M., Ruskoaho H. (1997): Involvement of transcriptional and posttranscriptional mechanisms in cardiac overload-induced increase of B-type natriuretic peptide gene expression. Circ. Res. **81**, 694–702

McGuire M., Bradford A. (2001): Chronic intermittent hypercapnic hypoxia increases pulmonary arterial pressure and haematocrit in rats. Eur. Respir. J. **18**, 279–285

Nakagawa O., Ogawa Y., Itoh H., Suga S., Komatsu Y., Kishimoto I., Nishimo K., Yoshimasa T., Nakao K. (1995): Rapid transcriptional activation and early mRNA turnover of brain natriuretic peptide in cardiocyte hypertrophy. Evidence for brain natriuretic peptide as an emergency cardiac hormone against ventricular overload. J. Clin. Invest. **96**, 1280–1287

Nattie E. E., Bartlett D. Jr., Johnson K. (1978): Pulmonary hypertension and right ventricular hypertrophy caused by intermittent hypoxia and hypercapnia in the rat. Am. Rev. Respir. Dis. **118**, 653–658

Neckar J., Szarszoi O., Herget J., Ostadal B., Kolar F. (2003): Cardioprotective effect of chronic hypoxia is blunted by concomitant hypercapnia. Physiol. Res. **52**, 171–175

Nishikawa N., Yamamoto K., Sakata Y., Mano T., Yoshida J., Miwa T., Takeda H., Hori M., Masuyama T. (2003): Differential activation of matrix metalloproteinases in heart failure with and without ventricular dilatation. Cardiovasc. Res. **57**, 766–774

Ogawa E., Saito Y., Harada M., Kamitani S., Kuwahara K., Miyamoto Y., Shikawa M., Hamanaka I., Kajiyama N., Takahashi N., Nakagawa O., Masuda I., Kishimoto I., Nakao K. (2000): Outside-in signalling of fibronectin stimulates cardiomyocyte hypertrophy in cultured neonatal rat ventricular myocytes. J. Mol. Cell. Cardiol. **32**, 765–776

Ogawa Y., Tamura N., Chusho H., Nakao K. (2001): Brain natriuretic peptide appears to act locally as an antifibrotic factor in the heart. Can. J. Physiol. Pharmacol. **79**, 723–729

Ooi H., Cadogan E., Sweeney M., Howell K., O'Regan R. G., McLoughlin P. (2000): Chronic hypercapnia inhibits hypoxic pulmonary vascular remodeling. Am. J. Physiol., Heart Circ. Physiol. **278**, H331–338

Pelouch V., Jirmar R. (1993): Biochemical characteristics of cardiac collagen and its role in ventricular remodelling following infarction. Physiol. Res. **42**, 283–292

Pelouch V., Dixon I. M., Golfman L., Beamish R. E., Dhalla N. S. (1993a): Role of extracellular matrix proteins in heart function. Mol. Cell. Biochem. **129**, 101–120

Pelouch V., Milerova M., Ostadal B., Samanek M., Hucin B. (1993b): Protein profiling of human atrial and ventricular musculature: the effect of normoxaemia and hypoxaemia in congenital heart diseases. Physiol. Res. **42**, 235–242

Pelouch V., Milerova M., Ostadal B., Hucin B., Samanek M. (1995): Differences between atrial and ventricular protein profiling in children with congenital heart disease. Mol. Cell. Biochem. **147**, 43–49

Pelouch V., Kolar F., Ost'adal B., Milerova M., Cihak R., Widimsky J. (1997): Regression of chronic hypoxia-induced pulmonary hypertension, right ventricular hypertrophy, and fibrosis: effect of enalapril. Cardiovasc. Drugs Ther. **11**, 177–185

Perhonen M., Wang W., Han X., Ruskoaho H., Takala T. E. (1997): Right ventricular collagen type III and IV gene expression increases during early phases of endurance training in hypobaric hypoxic condition. Basic. Res. Cardiol. **92**, 299–309

Rajagopalan S., Meng X. P., Ramasamy S., Harrison D. G., Galis Z. S. (1996): Reactive oxygen species produced by

macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases *in vitro*. Implications for atherosclerotic plaque stability. J. Clin. Invest. **98**, 2572–2579

Rouet-Benzineb P., Buhler J. M., Dreyfus P., Delcourt A., Dorent R., Perennec J., Crozatier B., Harf A., Lafuma C. (1999): Altered balance between matrix gelatinases (MMP-2 and MMP-9) and their tissue inhibitors in human dilated cardiomyopathy: potential role of MMP-9 in myosin-heavy chain degradation. Eur. J. Heart Fail. **1**, 337–352

Ruskoaho H., Leskinen H., Magga J., Taskinen P., Mantymaa P., Vuolteenaho O., Leppaluoto J. (1997): Mechanisms of mechanical load-induced atrial natriuretic peptide secretion: role of endothelin, nitric oxide, and angiotensin II. J. Mol. Med. **75**, 876–885

Sheedy W., Thompson J. S., Morice A. H. (1996): A comparison of pathophysiological changes during hypobaric and normobaric hypoxia in rats. Respiration **63**, 217–222

Stockmann P. T., Will D. H., Sides S. D., Brunnert S. R., Wilner G. D., Leahy K. M., Wiegand R. C., Needleman P. (1988): Reversible induction of right ventricular atriopeptin synthesis in hypertrophy due to hypoxia. Circ. Res. **63**, 207–213

Tsutamoto T., Wada A., Maeda K., Mabuchi N., Hayashi M., Tsutsui T., Ohnishi M., Fujii M., Matsumoto T., Yamamoto T., Wang X., Asai S., Tsuji T., Tanaka H., Saito Y., Kuwahara K., Nakao K., Kinoshita M. (2001): Relationship between plasma level of cardiotrophin-1 and left ventricular mass index in patients with dilated cardiomyopathy. J. Am. Coll. Cardiol. **38**, 1485–1490

Tyagi S. C., Kumar S., Katwa L. (1997): Differential regulation of extracellular matrix metalloproteinase and tissue inhibitor by heparin and cholesterol in fibroblast cells. J. Mol. Cell. Cardiol. **29**, 391–404

Tyagi S. C., Matsubara L., Weber K. T. (1993): Direct extraction and estimation of collagenase(s) activity by zymography in microquantities of rat myocardium and uterus. Clin. Biochem. **26**, 191–198

Urbanova D., Pelouch V., Ostadal B., Widimsky J., Ressl J., Prochazka J. (1977): The development of myocardial changes during intermittent high altitude hypoxia in rats. Cor. Vasa **19**, 246–250

Final version accepted: February 23, 2007