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RAPID COMMUNICATION

Protein Profiling of the Myocardium Exposed to Pressure Overload From Birth

J. ČERNOHORSKÝ, V. PELOUCH, M. MILEROVÁ, B. OŠŤÁDAL

Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

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Summary

Aortic banding induced in 2-day-old (A2) and 6-day-old (A6) male rats increased the left ventricular (LV) weight after 60 days; right ventricular (RV) enlargement occurred in the A2 group only. The concentration of collagenous proteins in the LV was elevated in both experimental groups (more in the A2 rats) at the expenses of sarcoplasmic proteins. Aortic banding also affected the proportion of collagen types (lower collagen I, higher collagen III, V) and myosin light chains (higher LC1/LC2) in the LV. Similar changes of proteins in the RV were less pronounced.

Key words

Myocardium – Pressure overload – Aorta banding – Types of collagen – Myosin light chains – Protein profile – Collagenous proteins – Non-collagenous proteins

Whereas morphological, biochemical and functional changes of pressure-overloaded myocardium in adulthood were described by many authors (Korecký and Rakušan 1978, Imamura *et al.* 1990, Carabello *et al.* 1992, Villareal and Dillmann 1992), much less is known about structural and functional parameters of cardiac muscle overloaded in early postnatal development. In the rat this ontogenetic period is characterized by a sustained stage of hyperplasia (up to 4 days of age), followed by a transitional period from hyperplastic to hypertrophic growth (from postnatal day 6 to 14), and, finally, by the stage of hypertrophic growth of ventricular musculature (second and third postnatal weeks) (Bugaiski 1991). The aim of our study was, therefore, to investigate if the pressure overload of cardiac muscle, induced in two different early postnatal periods, could modify protein composition of the left (LV) and right ventricle (RV). It has been shown previously (Borg and Burgess 1992/1993, Pelouch 1995) that various qualitative and quantitative changes of cardiac proteins play an important role in the modulation of heart contractility.

Gradual pressure overload was induced in either 2-day-old (A2) or 6-day-old (A6) male Wistar rats by banding the abdominal aorta. The aorta was constricted by silk suture around a steel wire (diameter 0.25 mm in A2 and 0.45 mm in A6 group) which was removed (Černohorský *et al.* 1994). At 60 days of age, no significant difference were found in the body weight between experimental and control groups (Table 1). On the other hand, aortic banding affected heart weight; the increase was observed not only in the LV, but also in the RV of the A2 group. However, LV enlargement (63 %) was of much greater extent than that of RV (13 %). In the A6 group, the same procedure induced enlargement of the LV (29 %) only; RV weight was not affected (Table 1).

Samples of both the control and the experimental ventricles were treated consecutively with different buffers (Pelouch *et al.* 1993). The procedure yielded 3 basic fractions: a) sarcoplasmic proteins (fraction containing metabolic proteins – various enzymes of aerobic and anaerobic metabolic pathways), b) contractile proteins (complex of proteins transducing the chemical energy of ATP to mechanical work), c) collagenous proteins (fraction containing a mixture of different proteinaceous material of extracellular matrix – e.g. collagens, elastins, proteoglycans and glycoproteins).

Table 1
Body weight (BW), heart weight (HW) as well as the weight of the left (LV) and right (RV) ventricles of experimental (A2, A6) and control (C) 60-day-old rats.

Group	C (n=22)	A6 (n=25)	A2 (n=20)
BW (g)	245.2±5.3	241.2±4.5	236.2±7.7
HW (mg)	664.9±19.7	804.0±29.3*	986.5±51.1*#
LV (mg)	389.4±11.5	503.8±21.2*	634.6±37.6*#
RV (mg)	149.8±5.2	147.5±4.0	169.4±8.0*#
RV/LV	0.38±0.01	0.30±0.01*	0.28±0.01*

*Values are means ± S.E.M. Significant differences ($p<0.05$): * A2 (A6) vs. C; # A2 vs. A6*

There were no significant changes of either non-collagenous or collagenous proteins in 30-day-old animals. The 50 % enlargement of LV (data are not shown) was not associated with quantitative protein remodellation. However, at the age of 60 days, LV enlargement (63 % – see Table 1) was associated with a significantly increased concentration of collagenous proteins in both experimental (banded) groups (Fig. 1). The increased concentration of these extracellular proteins in the A2 group corresponded with a greater cardiomegaly of these animals as compared with the A6 group. On the other hand, lower concentration of non-collagenous proteins was found in the LV of both experimental groups (Fig. 1) due to a decreased level of sarcoplasmic proteins; the concentration of contractile proteins remained unaffected (Černohorský *et al.* 1994).

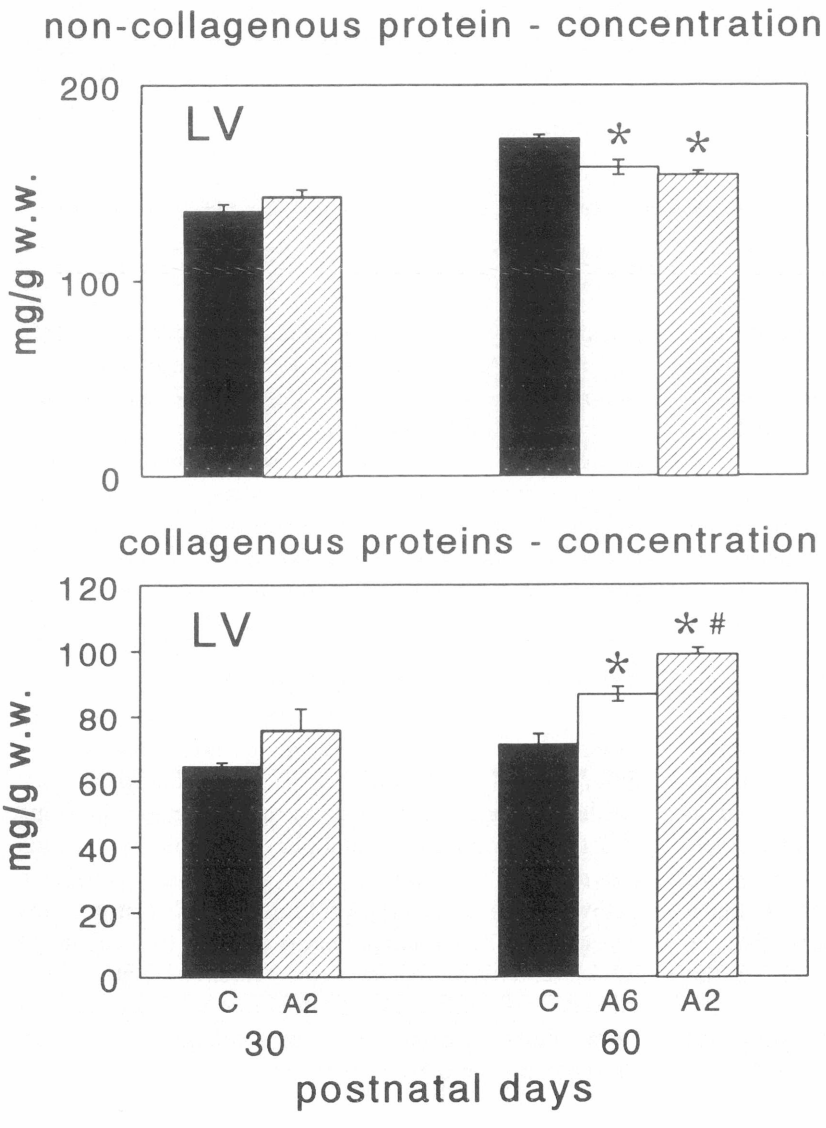

Fig. 1
Protein profiling of the left ventricle (LV). Concentration of non-collagenous (upper part) and collagenous proteins (lower part) in the LV of experimental (A2, A6) and control (C) rats at the age of 30 and 60 days. Values (expressed as mg of proteins/wet weight of the left ventricle) are means ± S.E.M. Student's t-test was used for statistical evaluation. Significant differences ($p<0.05$) from: * A2 (A6) vs. C; # A2 vs. A6

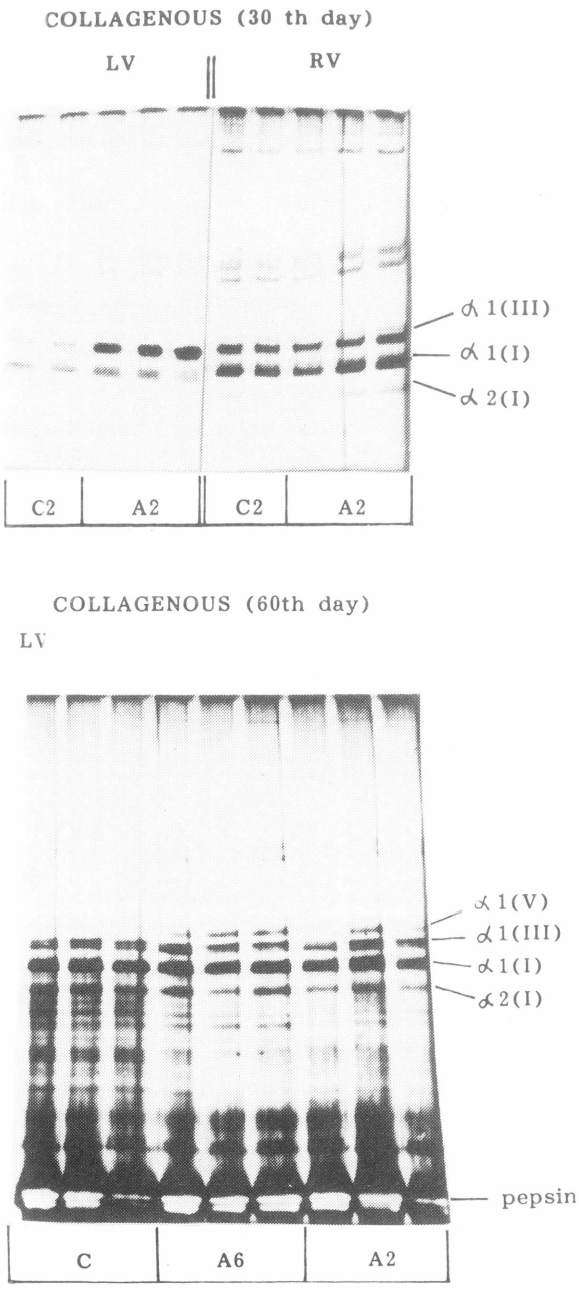

Fig. 2
Qualitative alterations of collagenous proteins in the left (LV) and right (RV) ventricle of 30-day-old (upper part) and 60-day-old (lower part) experimental (A2, A6) and control (C) rats were estimated by using SDS electrophoresis performed on polyacrylamide gel (5–15 % gradient). Different α chains of collagen types were characterized by using collagen standards (Sigma).

The results of qualitative analyses imply that pressure overload affected the proportion of different collagen types in the myocardium (Fig. 2). Whereas at 30 days of age a higher amount of collagen III and a lower amount of collagen I was observed in the LV of the A2 group, higher synthesis of collagen V was found in the LV of both experimental groups at 60 days of age. Our results are in a good agreement with the findings of Pelouch *et al.* (1992) that the ratio of collagen types I and III increases during postnatal development. We have shown that this was especially due to elevated synthesis of the collagenous α2(I) chain the amount of which was significantly higher in the RV than in the LV of both experimental and control rats aged 30 day. Furthermore, remodelling of myosin light chains (an increased synthesis of myosin LC1 as compared with LC2) was observed in the LV of the A2 animals at the age of 30 days (Černohorský *et al.* 1995).

In addition to these biochemical analyses, measurements of various physiological parameters were also carried out (Kolář *et al.* 1994). These results show that, unlike the A6 group, A2 animals exhibit signs of decompensation at the age of 60 days. We conclude that the remodelling of both collagenous and non-collagenous proteins induced by aortic banding in neonatal rats depends on the onset of the pressure overload.

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Reprint Requests

Mgr. J. Černohorský, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic.