Volume 332, number 1,2, 31–34 FEBS 13064 October 1993

Increased production of endothelin-1 in the hypertrophied rat heart due to pressure overload

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Received 30 July 1993

Endothelin-1 (ET-1) has been demonstrated to induce hypertrophy in cultured cardiac myocytes. We investigated the production of ET-1 in the heart of aorta-banded rats in vivo. Seven days after the banding of the abdominal aorta, rats developed a significant left ventricular hypertrophy. The tissue content of mature ET-1 and the level of expression of prepro ET-1 mRNA were higher in the left ventricle of aorta-banded rats than in those of sham-operated rats. The expression of prepro ET-1 mRNA in the right ventricle was not different between the two groups. These findings indicate that the production of ET-1 increased in the hypertrophied left ventricle, thereby suggesting the possible involvement of endogenous ET-1 in the development of cardiac hypertrophy due to pressure overload.

Preproendothelin-1 mRNA; Gene expression; Aortic banding; Sandwich enzyme immunoassay

1. INTRODUCTION

Cardiac hypertrophy, which occurs during a pressure- and/or volume overload to the heart, is generally viewed as a compensatory process that normalizes the increased wall stress [1]. However, cardiac hypertrophy frequently fails to functionally compensate, and results in contractile dysfunction of the myocardium [1]. Therefore, cardiac hypertrophy is considered to be an important process in the progression of heart diseases.

The banding of the rat abdominal aorta is a model of the cardiac hypertrophy caused by a pressure overload. It has been demonstrated that chronic pressure overload to the heart alters the expression of genes for contractile elements, growth factors, and proto-oncogenes in the heart [2–4].

Endothelin-1 (ET-1), originally identified as an endothelial cell-derived 21 amino acid-residue potent vasoconstrictor peptide, shows extensive tissue distribution, including the heart [5–7]. It has been demonstrated that the cardiac myocytes produce ET-1 in culture [8]. ET-1 shows potent positive inotropic and chronotropic effects on heart muscle [9,10]. Furthermore, ET-1 has been recently demonstrated in cultured ventricular myocytes to have a potent hypertrophic activity, i.e. an increase of cell size, and an activation of the expression of contractile protein gene and several proto-oncogenes [11]. However, there is no evidence that the production of ET-1 alters in the hypertrophied heart. In the present study, we investigated both the peptide level of mature ET-1 and the expression of prepro ET-1 mRNA in the hypertrophied heart of rats with aortic banding.

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2. MATERIALS AND METHODS

2.1. *Animals and surgical procedures*

Nine-week-old male Wistar rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the aorta was exposed through a midline abdominal incision. The abdominal aorta was constricted between the right and left renal arteries, using a 22-gauge needle (outside diameter = 0.70 mm) to establish the diameter of the ligature. Sham-operated control rats underwent an identical procedure except for the ligature. Seven days after surgery, rats were anesthetized with sodium pentobarbital. The right carotid artery and the left femoral artery were cannulated for measurement of arterial pressure proximal and distal to the aortic constriction. After the measurement of arterial pressure, a blood sample was collected from the carotid arterial cannula and rats were sacrificed. The heart was excised and the atria were removed. The ventricles were divided into the left ventricle, including intraventricular septum, and the right ventricular free wall, and they were rinsed with cold Kreb's–Ringer's solution, weighed, and quickly frozen in liquid nitrogen. Left ventricular hypertrophy was assessed by the left ventricular wet weight-to-body weight ratio.

2.2. *Sandwich-enzyme immunoassay (EIA) for ET-1 in plasma and the heart*

ET-1 concentration in plasma and myocardial tissue was determined by a sandwich-EIA as previously described [7,12,13]. The blood sample was placed in a chilled tube containing aprotinin (300 kIU/ml) and EDTA (2 mg/ml) and plasma was separated from the blood sample by centrifugation at 3,000 × *g* for 15 min at 4°C. The plasma was stored at −80°C until used. The left ventricle was homogenized with a polytoron homogenizer for 60 s in 10 vols. of 1 M acetic acid containing 10 μg/ml pepstatin (Peptide Institute, Osaka, Japan), and immediately heated to 100°C for 10 min. After chilling, the homogenate was centrifuged at 23,000 × *g* for 30 min at 4°C, and the supernatant was stored until used.

The plasma and the supernatant were subjected to a sandwich-EIA for ET-1 as previously described, using immobilized mouse monoclonal antibody AwETN40, which recognizes the N-terminal region of ET-1, and peroxidase-labeled rabbit anti-ET-1 C-terminal peptide (15–21) Fab' [7,12,13].

2.3. *Northern blot analysis for prepro ET-1 mRNA in the heart*

Total RNA was prepared from the myocardium by selective precipitation in 3 M lithium chloride/6 M urea [6]. Total RNA (15 μg/lane) from the left and right ventricles was separated by formamide/1.1% agarose gel-electrophoresis and transferred onto a nylon membrane. The membrane was prehybridized in a solution containing 5 × SSC, 5 × Denhardt's solution, 1% SDS, 50% formamide and 150 μg/ml fragmented salmon sperm DNA, and then hybridized with ^{32}P-labeled probe in the same solution for 24 h at 42°C. A full-length rat prepro ET-1 cDNA (2 kb, *Eco*RI insert of λrET1–2, see [6]) used as a probe was labeled by random priming with [α-^{32}P]dCTP. After hybridization, the filter was washed in 0.1 × SSC/0.1% SDS at 50°C and autoradiographied at −80°C for 1 week.

2.4. *Data analysis*

Data were expressed as mean ± S.E.M. Statistical comparison of aorta-banded rats with sham-operated rats was made using Student's *t*-test for an unpaired value. When a *P* value was smaller than 0.05, the difference in the mean was considered statistically significant.

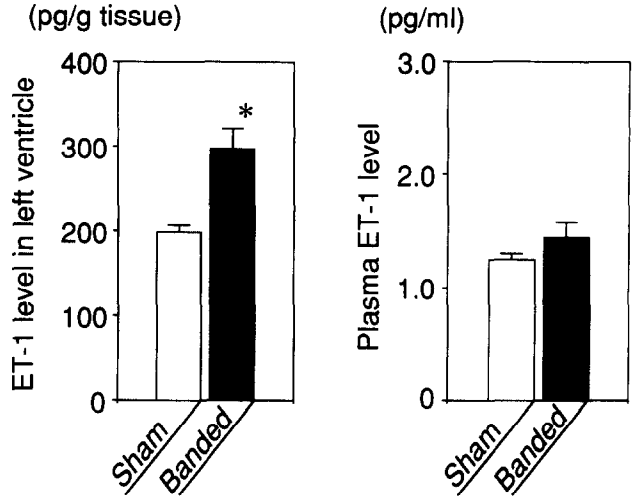

Fig. 1. Left ventricular tissue levels (left panel) and plasma levels (right panel) of ET-1 in aorta-banded rats (hatched column) and sham-operated rats (open column). Each column and bar represents the mean +S.E.M. of 6 rats, respectively. *$P < 0.05$ compared with sham-operated rats.

3. RESULTS

Hemodynamic parameters and heart weight-to-body weight ratio measured in aorta-banded rats and sham-operated rats are summarized in Table I. Mean carotid arterial pressure was significantly higher in aorta-banded rats than in sham-operated rats. In aorta-banded rats, the femoral arterial pressure was normotensive. The left ventricular weight-to-body weight ratio was significantly higher in aorta-banded rats than in sham-operated rats, indicating that aorta-banded rats developed a left ventricular hypertrophy. The right ventricular weight-to-body weight ratio was not significantly different between the two groups.

We measured the peptide level of ET-1 in the left ventricle by a sandwich-EIA specific for ET-1. The ET-1 level in the left ventricle of sham-operated rats was 199 ± 8 pg/g tissue (left panel in Fig. 1), which was in approximate accordance with previous reports [7,14]. The levels of ET-1 in the left ventricle were significantly higher in aorta-banded rats than in sham-operated rats (left panel in Fig. 1). The plasma levels of ET-1 were not significantly different between the two groups (right panel in Fig. 1).

The expression of prepro ET-1 mRNA in the ventricles of both aorta-banded and sham-operated rats was determined by Northern blot analysis (Fig. 2). The level of expression of prepro ET-1 mRNA was markedly higher in the left ventricle of aorta-banded rats than that of sham-operated rats (left panel in Fig. 2). On the other hand, the level of expression of prepro ET-1 mRNA in the right ventricular free wall of aorta-banded rats was similar to those of sham-operated rats (right panel in Fig. 2).

4. DISCUSSION

The present study demonstrates that aorta-banded rats developed a significant left ventricular hypertrophy. A sandwich-EIA specific for ET-1 revealed that the peptide levels of ET-1 increased in the hypertrophied left ventricle. Furthermore, Northern blot analysis showed that the expression of prepro ET-1 mRNA was

Table I

Hemodynamic parameters and heart weight-to-body weight ratios in rats 7 days after aortic banding and sham operation

	n	mCAP (mmHg)	mFAP (mmHg)	HR (beats/min)	LV/BW (mg/g)	RV/BW (mg/g)	BW (g)	Hmt (%)
Sham	6	114 ± 5	–	372 ± 13	1.91 ± 0.03	0.59 ± 0.02	355 ± 5	52 ± 1
Banding	6	163 ± 5**	126 ± 8	434 ± 13**	2.45 ± 0.07**	0.61 ± 0.01	326 ± 9*	55 ± 1

mCAP, mean carotid arterial pressure; mFAP, mean femoral arterial pressure; HR, heart rate; LV/BW, left ventricular wet weight-to-body weight ratio; RV/BW, right ventricular wet weight-to-body weight ratio; BW, body weight; Hmt, hematocrit; *n*, number of rats; –, not measured. Data are expressed as mean ± S.E.M.

*$P < 0.05$, **$P < 0.01$ compared with sham-operated rats.

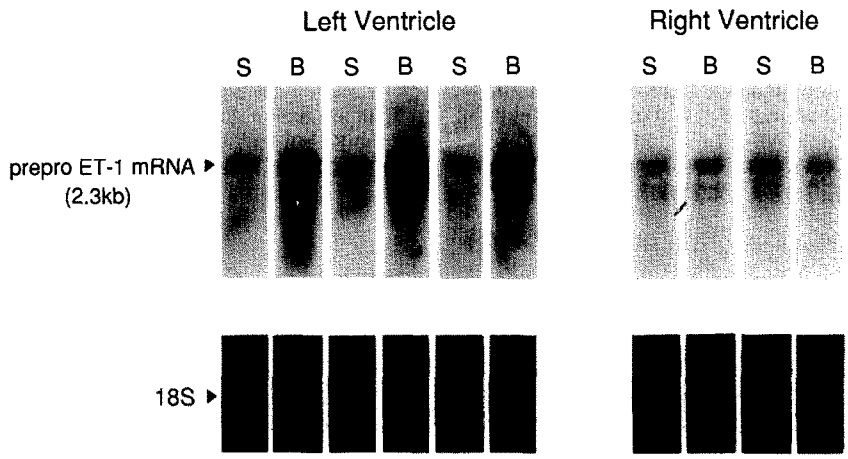

Fig. 2. Northern blot analysis for prepro ET-1 mRNA in the left or right ventricle in aorta-banded rats (B) and sham-operated rats (S). Lower panels show ethidium bromide staining of 18 S ribosomal RNA (rRNA) for a control. The prepro ET-1 mRNA (2.3 kb) and 18 S rRNA are indicated by arrows. Three and 2 independent experiments for the left and right ventricle, respectively, are illustrated. The expression of prepro ET-1 mRNA was markedly enhanced in the left ventricle, but not in the right ventricle, of aorta-banded rats. Total RNA (15 μg) from these tissues, i.e. left and right ventricles, was transferred onto one membrane.

greatly enhanced in the hypertrophied left ventricle but not in the right ventricle. Thus, the increase in the tissue content of mature ET-1 is probably due to the increase in the level of prepro ET-1 mRNA. These findings indicate that the production of ET-1 increases in the hypertrophied myocardium due to pressure overload.

It has been demonstrated that there is an 8-bp DNA sequence that conforms with a consensus of AP-1/Jun-binding sites, which is necessary for the phorbol ester-induced expression of the prepro ET-1 gene in cultured endothelial cells [15,16]. Furthermore, expression of c-*fos* and c-*jun* proto-oncogenes has been shown to be induced by pressure overload to the myocardium [4]. We can, therefore, consider that pressure overload may increase the expression of prepro ET-1 mRNA in the heart at least partly via the activation of *trans*-acting transcription factors Fos and Jun.

Myocardial cells express two distinct subtypes of ET receptors, termed ET_A and ET_B receptor, and these receptors have been reported to be coupled to phospholipase C via G-proteins [17–19]. The activation of phospholipase C results in the activation of protein kinase C through the formation of diacylglycerol, an enzyme thought to play an important role in myocardial cell hypertrophy [20,21]. This signaling pathway is proposed as the mechanism underlying ET-1-induced myocardial cell hypertrophy [11].

The peptide level of ET-1 in the left ventricle was significantly higher in aorta-banded rats. However, there was no significant difference in plasma ET-1 concentrations between aorta-banded and sham-operated rats (right panel in Fig. 1). Thus, the increased ET-1 in the left ventricle may act as a locally acting mediator rather than as a circulating hormone.

The present study demonstrates that the production of ET-1 significantly increased in the hypertrophied rat ventricle due to pressure overload. Since ET-1 has been reported to induce hypertrophy in cultured cardiac myocytes [11], the present findings suggest the possibility that the up-regulation of ET-1 synthesis may be involved in the development of cardiac hypertrophy due to pressure overload.

Acknowledgements: This work was supported by a grant from Special Research Project on Circulation Biosystems of the University of Tsukuba.

REFERENCES

[1] Cooper, G. (1987) Annu. Rev. Physiol. 49, 501–518.
[2] Izumo, S., Lampre, A.M., Matsuoka, R., Koren, G., Schwartz, K., Nadal-Ginard, B. and Mahdavi, V. (1987) J. Clin. Invest. 7, 970–977.
[3] Villarreal, F.J. and Dillmann, W.H. (1992) Am. J. Physiol. 262, H1861–H1866.
[4] Komuro, I., Kurabayashi, M., Takaku, F. and Yazaki, Y. (1988) Circ. Res. 62, 1075–1079.
[5] Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K. and Masaki, T.(1988) Nature 332, 411–415.
[6] Sakurai, T., Yanagisawa, M., Inoue, A., Ryan, U.S., Kimura, S., Mitsui,Y., Goto, K. and Masaki, T. (1991) Biochem. Biophys. Res. Commun. 175, 44–47.
[7] Matsumoto, H., Suzuki, N., Onda, H. and Fujino, M. (1989) Biochem. Biophys. Res. Commun. 164, 74–80.
[8] Suzuki, T., Kumazaki, T. and Mitsui, Y. (1993) Biochem. Biophys. Res. Commun. 191, 823–830.
[9] Ishikawa, T., Yanagisawa, M., Kimura, S., Goto, K., Masaki, T. (1988) Am. J. Physiol. 255, H970–H973.
[10] Ishikawa, T., Yanagisawa, M., Kimura, S., Goto, K. and Masaki, T. (1988) Pflugers Arch. 413, 108–110.
[11] Shubeita, H.E., McDonough, P.M., Harris, A.N., Knowlton, K.N., Glembotski, C.C., Brown, J.H. and Chien, K.R. (1990) J. Biol. Chem. 265, 20555–20562.
[12] Suzuki, N., Matsumoto, H., Kitada, C., Masaki, T. and Fujino, M. (1989) J. Immunol. Methods 118, 245–250.
[13] Miyauchi, T., Yanagisawa, M., Tomizawa, T., Sugishita, Y.,

Suzuki, N., Fujino, M., Ajisaka, R., Goto, K., Masaki, T. (1989) Lancet ii, 53–54.
[14] Kitamura, K., Tanaka, T., Kato, J., Ogawa, T., Eto, T. and Tanaka, K. (1989) Biochem. Biophys. Res. Commun. 162, 38–44.
[15] Inoue, A., Yanagisawa, M., Takuwa, Y., Mitsui, Y., Kobayashi, M. and Masaki, T. (1989) J. Biol. Chem. 264, 14954–14959.
[16] Lee, M.E., Dhadly, M.S., Temizer, D.H., Clifford, J.A., Yoshizumi, M. and Quertermous, T. (1991) J. Biol. Chem. 266, 19034–19039.
[17] Lin, H.Y., Kaji, E.H., Winkel, G.K., Ives, H.E. and Lodish, H.F. (1991) Proc. Natl. Acad. Sci. USA 88, 3185–3189.
[18] Sakurai, T., Yanagisawa, M., Takuwa, Y., Miyazaki, H., Kimura, S., Goto, K. and Masaki, T. (1991) Nature 348, 732–735.
[19] Hori, S., Komatsu, Y., Shigemoto, R., Mizuno, N. and Nakanishi, S. (1992) Endocrinology 130, 1885–1895.
[20] Dunnmon, P., Iwaki, K., Henderson, S.A., Sen, A. and Chien, K.R. (1990) J. Mol. Cell Cardiol. 22, 901–910.
[21] Chien, K.R., Knowlton, K.U., Zhu, H. and Chien, S. (1991) FASEB J. 5, 3037–3046.