FEBS Letters 346 (1994) 185–188

FEBS 14078

Gene expression of atrial natriuretic peptide in rat papillary muscle

Rapid induction by mechanical loading

Costa Jarygin[a], Jörg Hänze[b], Rudolf E. Lang[b,*]

[a]*Russian Academy of Sciences, Moskow, Russian Federation*
[b]*Institut für Normale und Pathologische Physiologie, Philipps-Universität Marburg, Deutschhausstraße 2, 35037 Marburg, Germany*

Received 14 March 1994; revised version received 25 April 1994

Abstract

The effect of mechanical stretch on protein synthesis and the expression of the gene for atrial natriuretic peptide (ANP) was examined in electrically paced, isolated papillary muscles from rat heart. Incorporation of [^{3}H]phenylalanine into protein increased only in stretched but not in unloaded muscles. Five hours of stretching increased ANP mRNA levels more than threefold as compared to freshly excised papillary muscles. A drastic fall in ANP mRNA levels was observed in unloaded muscles over this time. These data indicate that papillary muscles similar to other ventricular tissue are capable of activating ANP gene expression in response to increased load. The effect occurs in vitro and does not depend on circulating or nervous factors. The unexpected rapid induction of ANP gene expression in such a particular structure of the heart raises the possibility of local actions of ventricular ANP.

Key words: Atrial natriuretic peptide; Gene expression; Rat papillary muscle; Mechanical stretch

1. Introduction

Chronic hemodynamic overload induces a variety of adaptational events in the heart with the purpose to compensate for the increase in cardiac work. Characteristic changes are an increase in the individual myocyte volume, enhanced rates of protein synthesis and total RNA transcription and a shift in the expression of a series of proteins to their embryonic isoforms [1]. The gene expression of such fetal proteins in response to hemodynamic stress is relatively slow in onset, taking 1–2 days for contractile proteins such as skeletal α-actin or β-isomyosin heavy chain [2,3]. A similar time course of expression has been reported for the biosynthetic precursor of atrial natriuretic peptide (ANP), another cardiac protein synthesized in response to increased ventricular wall stress [4]. In the adult heart ANP production is confined to the atria and the ventricular impulse conduction system [5]. Ventricular myocytes synthetize ANP during the fetal life, but lose this property within a few days after birth [6]. However, when hypertrophy develops ventricular ANP synthesis starts again, predominantly in cells of the subendocardial layer where wall stress is particularly high [7–9].

The aim of this study was to examine whether the ability to activate ANP gene expression in response to a hemodynamic load is a property shared by any muscle from the ventricle, whether it is intrinsic to the muscle cells themselves or whether it involves additional, e.g. humoral or nervous factors. Another goal was to determine the time course of this activation. For this purpose, papillary muscles isolated from the rat heart, were subjected to mechanical stretch and the accumulation of ANP mRNA in the tissue was measured.

2. Materials and methods

Male Wistar rats weighing 240–280 g were sacrificed by decapitation, a midline incision was made through the sternum and the hearts were removed and placed in Dulbecco's modified Eagles medium (DMEM) at room temperature. Left ventricles were opened and papillary muscles excised. Each muscle was fixed at both ends to the bottom of a circular bath and perfused with DMEM. A glass hook connected to an isometric force transducer was inserted under the central portion of the muscle. Lifting of the hook by means of a micrometer allowed to stretch the muscle under the control of the isometric force transducer. Papillary muscles were constantly stimulated by means of two semicircular electrodes positioned at the periphery of the bath. 'Control' muscles were stimulated without stretching. 'Loaded' muscles were gradually stretched by lifting the glass hook. The force of con-traction increased to maximally 400–600 mg. The muscles were then maintained at 80–90% of the length where maximal isometric tension had been observed (L_{max}). During an equilibrium period of 45 min muscles were stimulated at a frequency of 0.33 Hz with a voltage 10% above the threshold. Then the frequency of stimulation was changed to 2 Hz and the experiment proceeded for another 1.5 or 5 h. The constant flow of DMEM bubbled with 95% O_2/5% CO_2 was stopped every 20 min and a recirculation system providing DMEM containing 5.0 μCi/ml L-[ring-4-^{3}H]-phenylalanine (Amersham) was switched on for 10 min. Then the constant perfusion with unlabeled medium was again resumed.

For measuring protein synthesis each muscle was minced and homogenized using a motor-driven glass/glass homogenizer containing 1.0 ml ice-cold 5% perchloric acid. The samples were centrifugated for 20 min at 19,000 × g, the supernatants discarded and the pellets washed four times with ice-cold 5% perchloric acid, heated in perchloric acid for 20 min at 80°C to remove transfer RNA-bound [^{3}H]phenylalanine, cooled, and then washed with perchloric acid twice more. The remain-

*Corresponding author. Fax: (49) (6421) 28 6966.

SSDI 0014-5793(94)00464-7

ing pellet was dissolved in 0.75 ml of 0.2 N NaOH. Hundred microliters were used for protein determinations and 0.3 ml portions for liquid scintillation counting.

For the determination of ANP gene expression papillary muscles were prepared and incubated as described above. RNA was isolated by the guanidinium–thiocyanate–phenol–chloroform method [10]. Aliquots (30 μg per lane) were size-fractionated by agarose gel electrophoresis, and transferred to nylon membranes. Probes for rat pre-pro-ANP (kindly provided by C. Seidman, Department of Genetics, Harvard University, Boston) and human actin (kindly provided by M. Moos, Department of Neurobiology, University of Heidelberg) were labeled with ^{32}P using SP6-transcription. Blots were washed for 2 × 5 min in 2 × SSC/0.1% SDS at room temperature and 2 × 15 min in 0.1 × SSC/0.1% SDS at 68°C. Blots were exposed to XAR-2 film (Eastman Kodak) for identifying the position of the bands. For quantification, the areas on the filters corresponding to the bands were excised and counted in a β-counter (Beckmann).

3. Results and discussion

The incorporation of [^{3}H]phenylalanine into muscle protein increased between 1.5 and 3 h of incubation in slack papillary muscles and showed a decrease at 5 h (Fig. 1). In stretched muscles the incorporation was linear over the entire incubation period. After 5 h 3–4 times more radioactivity had accumulated in the protein of stretched as compared with that of slack papillary muscles (Fig. 1).

Atrial natriuretic peptide mRNA was clearly detectable by Northern blot analysis in extracts from single papillary muscles. After 1.5 h of incubation the ANP mRNA levels of stretched and unstretched muscles had slightly decreased, but there was no significant difference in the ANP mRNA/β-actin mRNA ratios between both groups (Fig. 2). A dramatic fall in the mRNA levels for ANP and α-actin was noticed in unstretched muscles after 5 h of incubation. The decrease in α-actin mRNA levels was much less pronounced in stretched muscles. The most striking effect of stretch at 5 h was a more than

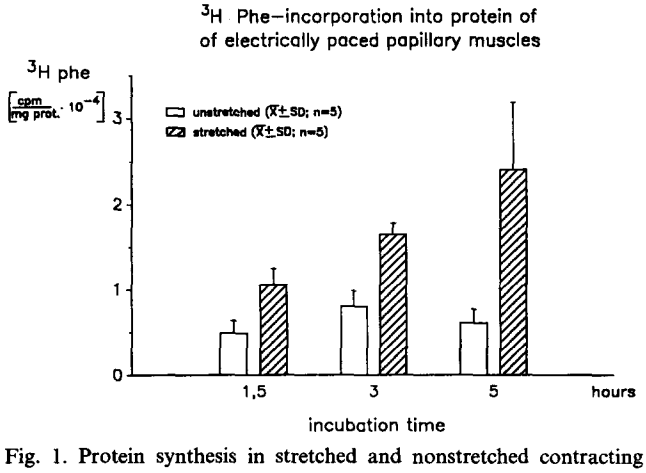


Fig. 1. Protein synthesis in stretched and nonstretched contracting papillary muscles. Protein synthesis is expressed on the ordinate as cpm of [^{3}H]phenylalanine incorporated into mg protein. Incubation periods were 1, 5, 3 and 5 h. Hatched columns, stretched muscles; open columns, unstretched muscles.

threefold increase in the ANP mRNA levels as compared to the levels measured in freshly excised muscles.

These results demonstrate for the first time that myocytes of the papillary muscle similar to muscle cells of the ventricular wall possess the ability to express the ANP gene in response to mechanical stress. This property appears to be intrinsic to the muscle cells and does not depend on the presence of circulating factors or on cardiac nerve activity.

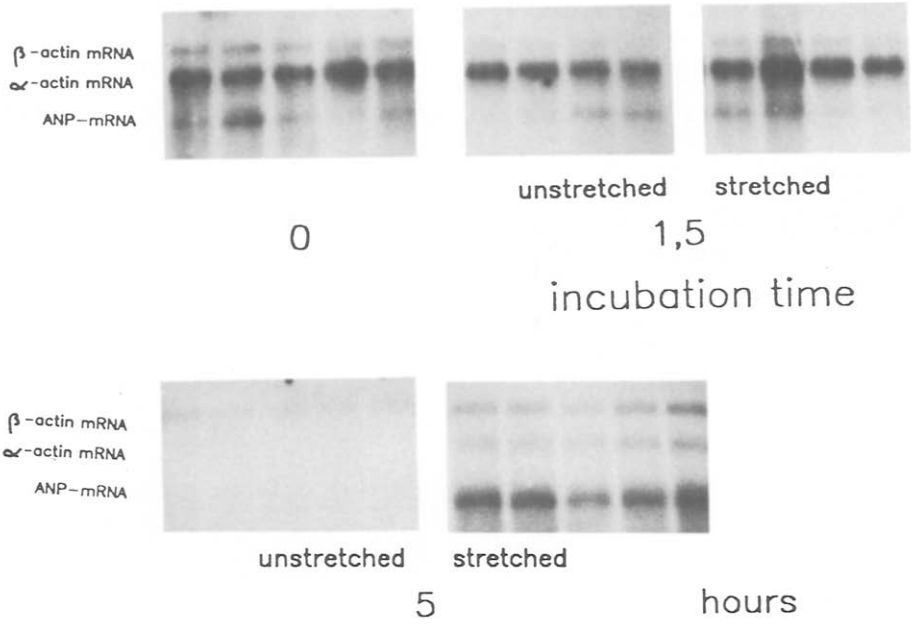
The induction of ANP gene expression occurs much more rapidly than suggested by previous studies performed in vivo. In rats ventricular ANP mRNA levels have been shown not to rise before more than 18 h after coarctation of the aorta [4]. A similar time course of expression has been reported for the genes of skeletal α-actin or β-isomyosin heavy chain [2,3]. The early activation of papillary ANP gene expression indicates that the peptide plays an important part in the adaptation of the heart to sudden changes in working conditions, long before hypertrophy has developed. Ventricular ANP has been proposed to serve the reduction of cardiac load by acting on blood vessels and the kidney, similar to the atrial hormone. However, expression in such a small part of the heart as the papillary muscle which with respect to its mass may deliver only insignificant amounts of the hormone to the circulation, raises some doubts about solely endocrine functions of ventricular ANP. The peptide may in addition exert some local effects at the site of the heart itself.

One possible function could be the modulation of the electrical properties of cardiac cells. Such a role is suggested by histochemical studies demonstrating the presence of high amounts of ANP in the impulse conducting system [5].

A para- or autocrine route of secretion within the myocardium could complement the peripheral effects of ANP in the adaptational response to volume expansion. Evidence supporting this view comes from experiments in isolated ventricular myocytes which were shown to relax upon administration of ANP [11]. Such an effect should result in an improvement of cardia compliance associated with a reduction in end-diastolic pressure.

Finally, ANP could play a role in the control of cardiac growth. The peptide has been reported to antagonize the proliferative effects of growth factors on vascular smooth muscle and endothelial cells [12]. One may therefore speculate that ANP is also involved in the fine-tuning of the hypertrophic response of the myocardium to an increased load.

The mechanism by which myocardial stretch activates ANP gene expression is currently not understood. Sequence analysis of the gene encoding ANP reveals the presence of binding sites for the c-*fos*/c-*jun* protooncogene heterodimer and other nuclear proteins in the 5′-region [13]. Protooncogenes may indeed constitute a link in the cascade of events resulting in ANP gene expres-



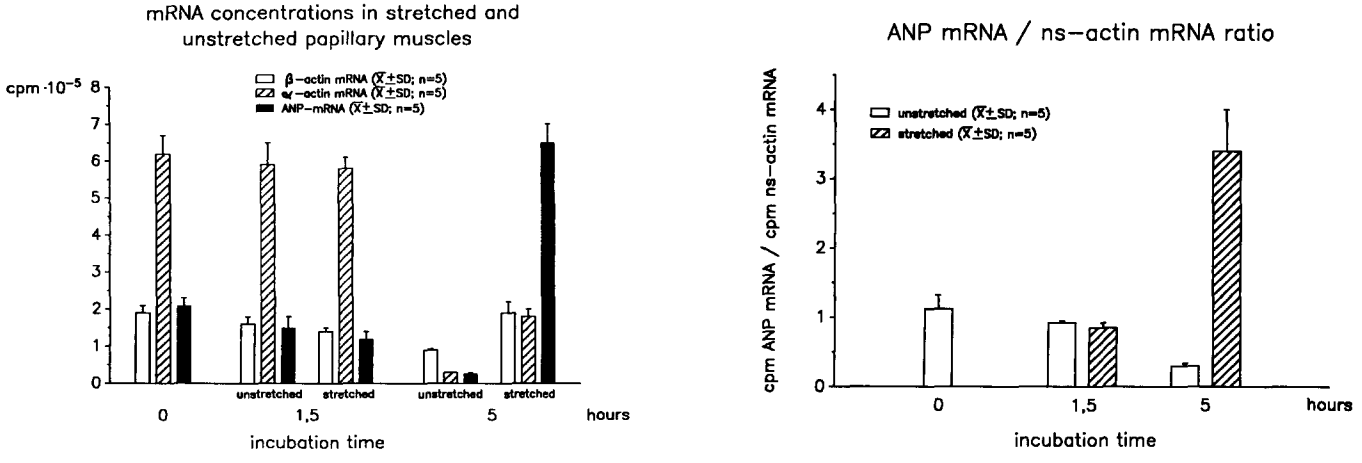


Fig. 2. The effect of stretch on atrial natriuretic peptide and actin gene expression in contracting papillary muscles. Top: Northern blot analysis of mRNA extracts (30 μg per lane) from papillary muscles after various periods of electrostimulation. Bottom: levels of actin and atrial natriuretic peptide mRNA in stretched and nonstretched papillary muscles. After hybridization with the ^{32}P-labeled probes the individual bands were localized autoradiographically, excised and counted. Left: absolute counts for β-actin (open columns), α-actin (hatched columns) mRNA, ANP mRNA (black columns). Right: effect of stretch on the natriuretic peptide/β-actin mRNA ratio.

sion, since their induction has been shown to preceed the appearance of ventricular ANP mRNA after imposition of cardiac overload [14]. However, the mechanisms by which myocardial stress is transmitted to protooncogene expression remain to be identified. The isolated papillary muscle preparation may be an excellent experimental model for investigations in this field.

Acknowledgements: This word was supported by the Deutsche Forschungsgemein-schaft (La-501/4-1). C.J. was a guest investigator sponsored by the Deutsche Forschungsgemeinschaft within the framework of scientific cooperation between the FRG and the Academy of Sciences, Russia.

References

[1] Nadal-Ginard, B. and Mahdavi, V. (1989) J. Clin. Invest. 84, 1693–1700.
[2] Schwartz, K., de la Bastie, D., Bouveret, P., Oliviero, P., Alonso, S. and Buckingham, M. (1986) Circulat. Res. 59, 551–555.
[3] Izumo, S., Lompre, A.M., Matsuoka, R., Koren, G., Schwartz, K., Nadal-Ginard, B. and Mahdavi, V. (1987) J. Clin. Invest. 79, 970–977.
[4] Mercadier, J.J., Samuel, J.L., Michel, J.B., Zongaro, M.A., de la Bastie, D., Lompre, A.M., Wisnewsky, C., Rappaport, L., Levy, B. and Schwartz, K. (1989) Am. J. Physiol. 257, H979–H987.
[5] Bach, H., Stumpf, W.E., Ando, E., Nokihara, K. and Forssmann, W.G. (1986) Anat. Embryol. 175, 223–226.

[6] Thompson, R.P., Simson, J.A. and Currie, M.G. (1986) Anat. Embryol. 175, 227–233.
[7] Lattion, A., Michel, J.B., Arnauld, E., Corvol, P. and Soubrier, F. (1986) J. Physiol. 251, H890–H896.
[8] Day, M.L., Schwartz, D., Wiegand, R.C., Stockmann, P.T., Brunnert, S.R., Tolunay, H.E., Currie, M.G., Standaert, D.G. and Needleman, P. (1987) Hypertension 9, 485–491.
[9] Lee, T.R., Bloch, K.D., Pfeffer, J.M., Pfeffer, M.A., Neer, E.J. and Seidmann, C.E. (1988) J. Clin. Invest. 81, 431–434.
[10] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[11] Neyses, L. and Vetter, H. (1989) Biochem. Biophys. Res. Commun. 163, 1435–1443.
[12] Cahill, P.A. and Hassid, A. (1991) Biochem. Biophys. Res. Commun. 179, 1606–1613.
[13] Knowlton, K.U., Baracchini, E., Ross, R.S., Harris, A.N., Henderson, S.A., Evans, S.M., Glembotski, C.C. and Chien, K.R. (1991) J. Biol. Chem. 266, 7759–7768.
[14] Izumo, S., Nadal-Ginard, B. and Mahdavi, V. (1988) Proc. Natl. Acad. Sci. USA 85, 339–343.