Vol. 155, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1988 Pages 449-454

INCREASE IN THE RELATIVE ABUNDANCE OF PREPROENKEPHALIN A MESSENGER RNA IN THE VENTRICLES OF CARDIOMYOPATHIC HAMSTERS

Michel Ouellette and Léa Brakier-Gingras

Département de Biochimie, Université de Montréal,
Montréal, Québec, H3C 3J7, Canada

Received July 22, 1988

SUMMARY: Preproenkephalin A messenger RNA was detected in hamster heart by Northern blot analysis using a human preproenkephalin A cDNA probe. Ventricular levels of this messenger were one order of magnitude lower than atrial levels, which were equivalent to brain levels. Furthermore, in the heart of cardiomyopathic hamsters, an animal model of cardiac hypertrophy and congestive heart failure, the relative abundance of the preproenkephalin A messenger RNA was found to increase three- to four-fold in ventricles while no change was seen in atria. These results support the hypothesis that the heart has the potential for locally synthesizing enkephalins and provide evidence that alterations in preproenkephalin A messenger RNA levels are associated with the development of cardiac hypertrophy and failure. © 1988 Academic Press, Inc.

Preproenkephalin A is a common precursor for the enkephalin opioid peptides (1,2). The preproenkephalin A gene is expressed in the central nervous system (3) and in various peripheral tissues, including the adrenal medulla (1,2,4) and the reproductive tissues (5). Howells *et al.* (6) have recently observed that preproenkephalin A messenger RNA is also present in rat heart. These observations and the fact that enkephalins have been detected in guinea-pig heart (7-9) lead to the hypothesis that the heart may synthesize and secrete enkephalins. In this study, we have investigated the expression of the preproenkephalin A gene in hamster heart under normal and pathological conditions, using cardiomyopathic hamsters (10,11) as an animal model of cardiac hypertrophy and congestive heart failure. We have observed that preproenkephalin A messenger RNA is present in both ventricles and atria of hamster heart and that its relative abundance is increased in the ventricles but not in the atria of cardiomyopathic hamsters.

MATERIALS AND METHODS

Animals: Male Golden Syrian hamsters (Lakeview strain) were obtained from a commercial farm (Canadian Breeding Farm and Laboratories, Saint-Constant, Québec, Canada). Male cardiomyopathic hamsters (CHF 14.6) were purchased from the Canadian Hybrid Farm (Halls Harbour, Nova Scotia, Canada). Fed animals were killed by decapitation at 60 days. Atria, ventricles, adrenal glands, brain and liver were removed and immediately frozen in liquid nitrogen. The tissues were stored at -70°C until use.

0006-291X/88 $1.50
Copyright © 1988 by Academic Press, Inc.
All rights of reproduction in any form reserved.

Isolation of messenger RNAs (poly(A)$^+$-RNAs): RNAs were prepared from individual tissues, consisting of pooled samples from 3-20 animals. Total RNA was extracted in guanidium thiocyanate from frozen tissues and pelleted by centrifugation through a CsCl gradient, following the method of Chirgwin *et al.* (12). Poly(A)$^+$-RNAs were isolated by oligo(dT) affinity chromatography (13) and were quantified by UV absorption ($1A_{260}$ = 45 μg).

RNA blot analysis: Poly(A)$^+$-RNA samples were denatured by heating at 70°C for 10 min in 1 mM EDTA, 50 mM 3-(4-morpholino)propanesulfonic acid, pH 7.5, 7% formaldehyde, 50% formamide. They were serially diluted, fractionated by electrophoresis on a 1.2% agarose/7% formaldehyde gel and transferred to a nylon membrane (Hybond-N, Amersham) by electroblotting. The blots were first prehybridized in 6 x SET (1 x SET is 150 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA, pH 7.5), 50% formamide, 0.5% SDS, 5 x Denhardt's (1 x Denhardt's = 0.02% each of bovine serum albumin, Ficoll-400 and polyvinylpyrrolidone), 250 μg/ml denaturated herring sperm DNA and 250 μg/ml calf liver tRNA. They were hybridized for 40 h at 37°C in hybridization buffer (same as prehybridization buffer except for 2 x Denhardt's, 50 μg/ml herring sperm DNA and 50 μg/ml calf liver tRNA). The probe (500 000 cpm/ml) was a 918 base-pair *Hinc*II fragment of human preproenkephalin A cDNA from plasmid pHPE-9 (14), a gift from Dr. E. Herbert. This fragment contains the coding sequence of the preproenkephalin A messenger RNA which is extremely conserved among species (15). It had been labeled with [α-^{32}P]dCTP (Amersham, 3000 Ci/mmol) to a specific radioactivity of 1-3 x 10^8 cpm/μg by random priming (16). The blots were successively washed at 55°C in 6 x SET, 2 x SET, 1 x SET and 0.1 x SET, with 0.5% SDS, and exposed to Fuji X-ray films with an intensifying screen for 1-3 days at -70°C. The cDNA probe was stripped from the blots, and [5'-^{32}P]-labeled oligo(dT)$_{18}$ was hybridized to the poly(A) tails of the membrane-bound mRNAs in order to normalize signal strength (17). Oligo(dT)$_{18}$ (Pharmacia) was 5'-labeled with [γ-P^{32}]ATP (Amersham, 3000 Ci/mmol) to a specific radioactivity of 7-9 x 10^6 cpm/μg, using an exchange reaction (18). Quantification of the autoradiograms was carried out using a soft laser scanning densitometer (LKB). The relative amount of preproenkephalin A mRNA in the different tissues was assessed by dividing the absorbance corresponding to the cDNA probe by the absorbance corresponding to the oligo(dT)$_{18}$ probe. The size of preproenkephalin A mRNA was determined from the position of migration of coelectrophoresed size markers.

RESULTS AND DISCUSSION

We have used a cDNA probe for human preproenkephalin A to address the following questions: can preproenkephalin A messenger RNA be detected in hamster heart and, in the affirmative, is its relative abundance altered under pathological heart conditions? A single mRNA band was detected on the autoradiograms of the blots containing poly(A)$^+$-RNAs from hamster brain, adrenal glands, atria and ventricles, hybridized to the [^{32}P]-labeled human preproenkephalin A cDNA probe (Figure 1). This band had a mobility corresponding to a RNA of about 1400 nucleotides, similar to human, bovine and rat preproenkephalin A mRNA (3-6). The relative abundance of preproenkephalin A mRNA was the highest in the adrenal glands, equivalent in brain and atria and the lowest in ventricles. Liver poly(A)$^+$-RNA never gave a positive signal (data not shown). Densitometric scanning of the autoradiograms was used to quantify the differences in preproenkephalin A mRNA levels in various hamster tissues. It was found that the relative

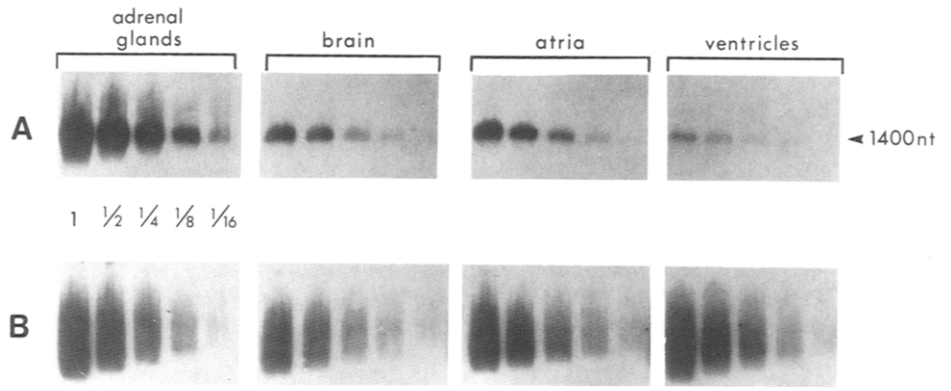

Figure 1. Identification of preproenkephalin A mRNA in various hamster tissues by Northern blot analysis. Poly(A)$^+$-RNAs were isolated from the brain, adrenal glands, atria and ventricles of 60-day-old hamsters, fractionated by electrophoresis on a 1.2% denaturing agarose gel, blotted onto a nylon sheet and hybridized to a [^{32}P]-labeled *Hinc*II fragment derived from a human preproenkephalin cDNA probe (A). The probe was then removed and the filter was hybridized to [^{32}P]-labeled oligo(dT)$_{18}$ (B). Each sample was serially diluted as indicated, the highest amount of RNA loaded per lane being about 3 μg. The size of the preproenkephalin A mRNA in each tissue was about 1400 nucleotides (nt), estimated from the position of migration of coelectrophoresed size standards.

abundance of preproenkephalin A mRNA in atria represents about 20% of that in adrenal glands and is about seven-fold higher in atria than in ventricles (Table 1).

Northern blot analysis of poly(A)$^+$-RNAs from hamster heart demonstrates that transcription of the preproenkephalin A gene occurs in both

TABLE 1

Preproenkephalin A mRNA in various hamster tissues

Tissue	Relative abundance of preproenkephalin A mRNA
Brain	7.6
Adrenal glands	33.4
Ventricles	1.0
Atria	7.3

The relative abundance of preproenkephalin A mRNA was quantified by densitometric scanning of the autoradiograms resulting from Northern blot analysis of the mRNA population in various hamster tissues (Figure 1). It is expressed in arbitrary units (area under the peak corresponding to the preproenkephalin A mRNA in the autoradiograms divided by the area under the peak corresponding to the poly(A)$^+$-RNAs), a value of 1.0 being ascribed to the ventricles. Results are the means of three independent experiments. Standard deviations on the means is equal to or less than 15%.

hamster atria and ventricles. This supports the hypothesis that there is a local synthesis of enkephalins in hamster heart, in agreement with the previous observations of Howells *et al.* (6) in rat heart. However, the situation differs from that encountered in rat heart where preproenkephalin A mRNA is present predominantly in the ventricles. In hamster heart, in contrast, the relative abundance of this messenger is about one order of magnitude higher in atria than in ventricles. Enkephalins synthesized by the heart could be released by the same process of secretion as the atrial natriuretic factor, a peptide hormone known to be synthesized and secreted by the heart (19). Cardiac atrial myocytes contain secretory granules involved in the storage and regulated secretion of the atrial natriuretic factor whereas ventricular myocytes are poor in secretory granules and release this factor constitutively (20,21). We suggest that enkephalins synthesized in the heart could affect the function of the cardiac muscle. Indeed, there are specific opioid receptors in the heart (22-24). Moreover, it has been demonstrated that enkephalins reduce the release of noradrenaline from sympathetic nerve terminals in the heart (25) and, in addition to these presynaptic effects, that they exert postsynaptic effects which modulate the response of the heart to noradrenaline (26,27). These postsynaptic effects appear, however, to be species dependent since they antagonize the action of noradrenaline in rats but stimulate it in guinea-pigs.

Figures 2 and 3 are autoradiograms of blots containing poly(A)$^+$-RNAs from the ventricles and the atria of 60-day-old cardiomyopathic and control hamsters. The relative abundance of preproenkephalin A mRNA was increased in the ventricles of cardiomyopathic hamsters, when compared to controls.

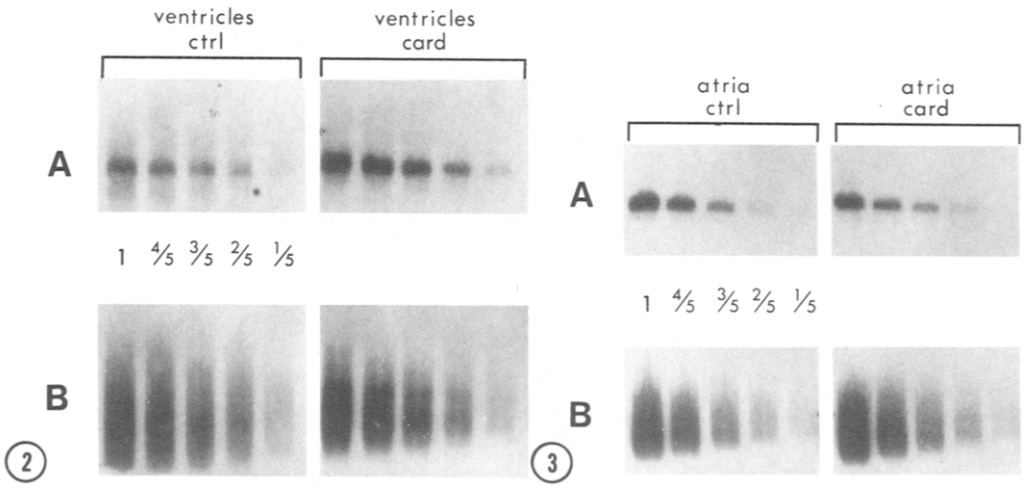

Figure 2. Example of Northern blot analysis of preproenkephalin A mRNA in the ventricles of 60-day-old control (ctrl) and cardiomyopathic (card) hamsters. Details as in Figure 1.

Figure 3. Example of Northern blot analysis of preproenkephalin A mRNA in the atria of 60-day-old control (ctrl) and cardiomyopathic (card) hamsters. Details as in Figure 1.

This increase was reproducibly observed with three independent RNA preparations. Densitometric scanning of the autoradiograms indicated that cardiomyopathic hamsters had three to four times more ventricular preproenkephalin A mRNA than controls. No significant change in the relative abundance of preproenkephalin A mRNA could be detected in the atria. The increase in the relative abundance of preproenkephalin A mRNA in the ventricles but not in the atria of cardiomyopathic hamsters is reminiscent of what is observed with the atrial natriuretic factor. In the heart of cardiomyopathic hamsters, the abundance of the mRNA coding for this factor was also increased in ventricles but not in atria (28). The increase in preproenkephalin A mRNA content in the ventricles of cardiomyopathic hamsters could be due to either its increased synthesis or increased stability. Increased preproenkephalin A mRNA synthesis might result from intracellular calcium overload in the heart, a factor which greatly contributes to the pathogenesis of the disease (29). Indeed, in adrenal medulla, an increased influx in calcium has been associated with the induction of preproenkephalin A gene expression (30). Furthermore, the preproenkephalin A gene is regulated by cAMP (31) and increased preproenkephalin A mRNA synthesis could also be due to the increase in cardiac cAMP level (32), which is related to an enhanced sympathetic activity in the heart of cardiomyopathic hamsters (33).

It is important to ask how the increase in preproenkephalin A gene expression in the heart affects the progression of the cardiomyopathy in hamsters. It is interesting that this increase is observed in an early phase of the disease. At 60 days, the hearts of cardiomyopathic hamsters are in the necrotic stage, characterized by multiple foci of necrosis, which precedes the compensatory hypertrophy leading to progressive heart failure. The enhanced expression of preproenkephalin A gene in the heart of cardiomyopathic hamsters could be directly associated with the progression of the disease. Whether it contributes to worsen the situation or is part of an adaptative process counteracting the progression of the disease is presently a matter of speculation. More light on that question could be provided by examining different models of cardiac hypertrophy and failure and determining whether an increase in the expression of preproenkephalin A gene is a common characteristic of these pathologies.

ACKNOWLEDGEMENTS

We wish to thank Drs. Guy Boileau, Luc DesGroseillers and Vicenzo De Luca for helpful discussions and comments. The skilful secretarial assistance of Ms Lorraine Charette is greatly appreciated and we thank Ms Christine Ostiguy for the photographs of the autoradiograms. This work was supported by the Fondation Blanche Cloutier. Michel Ouellette is a recipient of a studentship from the Medical Research Council of Canada.

REFERENCES

1. Noda, M., Furutani, Y., Takahashi, H., Toyosato, M., Hirose, T., Imayana, S., Nakanishi, S. and Numa, S. (1982) Nature 295, 202-206.
2. Gubler, U., Seeburg, P.H., Hoffman, B.J., Gage, L.P. and Udenfriend, S. (1982) Nature 295, 206-208.
3. Yoshikawa, Y., Williams, C. and Sabol, S.L. (1984) J. Biol. Chem. 259, 14301-14308.
4. Comb. M., Seeburg, P.H., Adelman, J., Eiden, L. and Herbert, E. (1982) Nature 295, 663-666.
5. Kilpatrick, D.L., Howells, R.D., Noe, M., Bailey, L.C. and Udenfriend, S. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 7467-7469.
6. Howells, R.D., Kilpatrick, D.L., Bailey, L.C., Noe, M. and Udenfriend, S. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 1960-1963.
7. Lang, R.E., Hermann, K., Dietz, R., Gaida, W., Ganten, D., Kraft, K. and Unger, T. (1983) Life Sci. 32, 399-406.
8. Weihe, E., Mc Knight, A.T., Corbett, A.D., Hartschuh, W., Reinecke, M. and Kosterlitz, H.W. (1983) Life Sci. 33, suppl. 1, 711-714.
9. Weihe, E., Mc Knight, A.T., Corbett, A.D., and Kosterlitz, H.W. (1985) Neuropeptides 5, 453-456.
10. Jasmin, G. and Eu, H.Y. (1979) Ann. New-York Acad. Sci. 317, 46-58.
11. Jasmin, G. and Proschek, L. (1982) Muscle and Nerve 5, 20-25.
12. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294-5299.
13. Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. U.S.A., 69, 1408-1412.
14. Comb, M., Rosen, H., Seeburg, P.H., Adelman, J. and Herbert, E. (1983) DNA 2, 213-229.
15. Rosen, H., Douglass, J. and Herbert, E. (1984) J. Biol. Chem. 259, 14309-14313.
16. Feinberg, A.P. and Vogelstein, B. (1983) Anal. Biochem. 132, 6-13.
17. Harley, C.B. (1987) Gene Anal. Techn. 4, 17-22.
18. Arrand, J.E. (1985) in: Nucleic Acid Hybridization. A Practical Approach (B.D. Hames and S.J. Higgins eds.), pp. 17-45, IRL Press, Oxford-Washington.
19. de Bold, A.J. (1985) Science 230, 767-770.
20. Bloch, K.D., Seidman, J.G., Naftilan, J.D., Fallon, J.T. and Seidman, C.E. (1986) Cell 47, 695-702.
21. Cantin, M., Thibault, G., Ding, J., Gutkowska, J., Garcia, R., Jasmin, G., Hamet, P. and Genest, J. (1988) Am. J. Pathol. 130, 552-568.
22. Gautret, B. and Schmitt, H. (1984) Eur. J. Pharmacol. 102, 159-163.
23. Krumins, S.A., Faden, A.I. and Feuerstein, G. (1985) Biochem. Biophys. Res. Comm. 127, 120-128.
24. Mantelli, L., Corti, V. and Ledda, F. (1987) Gen. Pharmacol. 18, 309-313.
25. Fuder, H., Buder, M., Riers, H.D. and Rothacher, G. (1986) Naunyn-Schmiedeberg's Arch. Pharmacol. 332, 148-155.
26. Ruth, J.A., and Eiden, L.E. (1984) Neuropeptides 4, 101-108.
27. Ruth, J.A., Cuizon, J.V. and Eiden, L.E. (1984) Neuropeptides 4, 185-191.
28. Franch, H.A., Dixon, R.A.F., Blaine, E.H. and Siegl, P.K.S. (1988) Circ. Res. 62, 31-36.
29. Proschek, L. and Jasmin, G. (1982) Muscle and Nerve 5, 26-32.
30. Kley, N., Loeffler, J.P., Pittius, C.W. and Höllt, V. (1987) J. Biol. Chem. 262, 4083-4089.
31. Comb, M., Birnberg, N.C., Seasholtz, A., Herbert, E. and Goodman, H.M. (1986) Nature 323, 353-356.
32. Harrow, J.A.C., Singh, J.N., Jasmin, G. and Dhalla, N.S. (1975) Can. J. Biochem. 53, 1122-1127.
33. Sole M.J. (1984) in: Catecholamines: Basic and Peripheral Mechanisms (Alan R. Liss ed.), pp. 309-317, New-York.