Vol. 95, No. 1, 1980
July 16, 1980

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 367-373

INCREASED MYOCARDIAL PYRIMIDINE NUCLEOTIDE SYNTHESIS IN ISOPROTERENOL-INDUCED CARDIAC HYPERTROPHY IN RATS

J. Olivares, A. Ray, J. Aussedat, M. Verdys & A. Rossi

Laboratoire de Physiologie Animale, Université Scientifique et Médicale de Grenoble, B.P. 53 X, 38041 Grenoble Cédex
FRANCE

Received April 15,1980

SUMMARY

In a first phase (up to 12h) after the first injection of isoproterenol (5mg.kg^{-1} b.w.) the pyrimidine nucleotide pools were increased and the rates of incorporation of inorganic phosphate into the α-phosphate groups of nucleotides were raised from 16 to 58 nmol.g^{-1}.h^{-1} for uracil nucleotides and from 11 to 32 nmol.g^{-1}.h^{-1} for cytosine nucleotides. At a later stage, while the pool sizes decreased slowly toward control levels, these rates of labelling also decreased though still remaining above control values. A similar pattern of changes was induced by the eighth daily isoproterenol injection, but the alterations were attenuated.

The cardiac muscle cell is able to respond to work overload by an increase in the synthesis of cellular components which leads to cardiac hypertrophy. During this process, a rapid increase of RNA synthesis occurs very early on (1,2,3,4). The myocardial free nucleotides, which provide energized precursors for the synthesis of RNA, probably play a key role in the early phase of cardiac hypertrophy. Changes in adenine nucleotide metabolism are now well documented (5,6) , but less attention has been given to the pyrimidine nucleotides of the heart. In an earlier paper we showed that the myocardial content of uracil and cytosine nucleotides was increased during isoproterenol-induced cardiac hypertrophy in rats (7). Since an expansion of the uracil nucleotide pool was also reported in cardiac hypertrophy produced by aortic stenosis (8), it is most likely that this alteration should be considered as a feature of the cardiac hypertrophic process. Although an increase in uridine labelling has been previously noted (8), little information is now available on the changes in turnover rates of these nucleotides in cardiac hypertrophy. This lack of quantitative data is due to methodological

0006-291X/80/130367-07$01.00/0

difficulties. The possibility that the [^{32}P]phosphate labelling of the α-phosphate groups of nucleotides could be used to quantify the rate of synthesis of nucleotides in the myocardium has been checked by our group in previous studies (9,10). This method was applied in this study for the examination of the changes in the synthesis of pyrimidine nucleotides during isoproterenol-induced cardiac hypertrophy in rats.

MATERIALS AND METHODS

Animals. 150 female Wistar rats (220 to 280g) were used. Cardiac hypertrophy was induced by daily subcutaneous injections of isoproterenol (5mg.kg^{-1},b.w.).

Labelling procedure. [^{32}P]phosphate, in the form of sodium salt dissolved in a saline solution (CEA, Saclay), was injected into the femoral vein of animals slightly anesthetized by ether (dose 0.5 mCi.100g^{-1}). The animals were killed 2,4,6,8 or 10 hours later. The hearts were excised, and the ventricles rapidly rinsed and wiped on filter paper before chilling in liquid nitrogen. Labelling kinetics were followed in control rats and in four conditions of isoproterenol treatment. On the first day of treatment, two kinetics were commenced by injecting the tracer 5 or 16 hours after the administration of isoproterenol. Similar conditions were chosen to investigate the changes occuring after the eighth daily injection of the drug.

Analytical procedure. Since large amounts of tissue were needed for obtaining purified cytosine nucleotides, the ventricles from 5 rats were pooled in each experiment. The method used in this study was described in detail in previous papers (9,10). In brief, the nucleotides, extracted from the tissue by cold perchloric acid, were alkaline-hydrolyzed into 5'-nucleoside monophosphates, UMP and CMP being separated by Dowex 1X8 column chromatography and purified by paper chromatography. The pool sizes of uracil and cytosine nucleotides were calculated from UV absorbance measurements of column chromatography eluates. The specific activities of the α-phosphate groups were measured for UMP and CMP eluted from the paper chromatogram. The specific activity of inorganic phosphate was obtained on a nucleotide-free aliquot of the initial acid extract : the phosphate content being measured according to Bartlett (11) and the radioactivity by Cerenkov counting.

Mathematical analysis of labelling kinetics. Mathematical analysis was based on the assumptions that intracellular nucleotide pools were not compartmented and that the inorganic phosphate may be considered as the proximate precursor of the α-phosphate groups of nucleotides. The changes in the radioactivity of the nucleotides are given by the difference between inputs and outputs of radioactivity.
inputs = $\rho_s . P^* / P$, where ρ_s is the rate of incorporation, and P^*/P the specific activity of inorganic phosphate.
outputs = $\rho_u . N^* / N$, where ρ_u is the rate of utilization, and N^*/N^* the specific activity of nucleotides.

Changes in radioactivity within the nucleotide pool are given by :
$\frac{\Delta N^*}{\Delta t} = \rho_s \cdot \overline{P}^* - \rho_u \cdot \overline{N}^*$ (equation 1). Relation between ρ_s and ρ_u is given by

$\rho_u = \rho_s - \frac{\Delta N}{\Delta t}$ (equation 2).

Equations 1 and 2 give the formula : $\rho_s = \frac{N^{**} - \Delta N.\frac{N^{**}}{N}}{\left(\frac{P^{**}}{P} - \frac{N^{**}}{N}\right)\Delta t}$

RESULTS

Changes in pool sizes. As shown by the figures 1 and 2, each injection of isoproterenol induced an increase in the myocardial content of pyrimidine nucleotides. This enlargment reached a maximal value about 12 to 15 hours after the injection of the drug, then the pool sizes returned slowly toward control values. However the increases were less pronounced after 8 days of treatment.

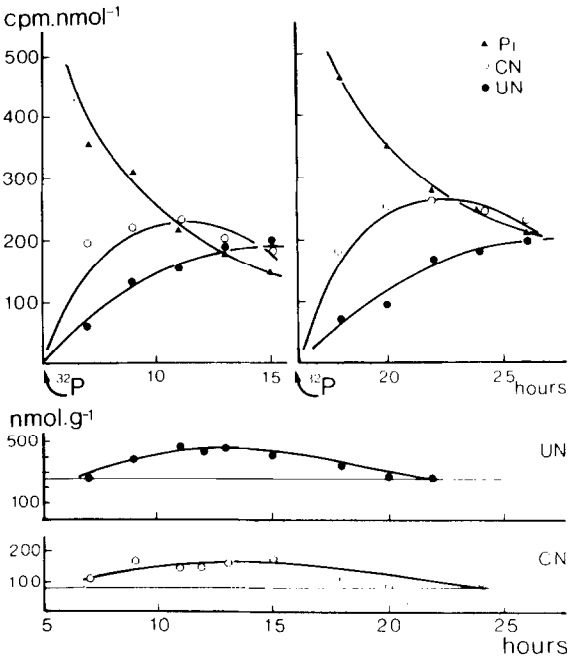

FIGURE 1 - CHANGES IN POOL SIZES AND [^{32}P] LABELLING OF CARDIAC PYRIMIDINE NUCLEOTIDES INDUCED BY A SINGLE ISOPROTERENOL INJECTION INTO RATS.

Isoproterenol (5mg.kg^{-1} b.w.,s.c.) was injected at time zero; [^{32}P]phosphate was administered intravenuously 5 or 16 hours after isoproterenol injection. Rats were killed 2,4,6,8 or 10 hours later ; for each period of time the ventricles from 5 animals were pooled.
Pool sizes (lower panel) were estimated by U.V. absorption.
Specific activities (upper panel) were measured on α-phosphate groups of nucleotides and on inorganic phosphate.
Pi = inorganic phosphate
CN = cytosine nucleotides UN = uracil nucleotides

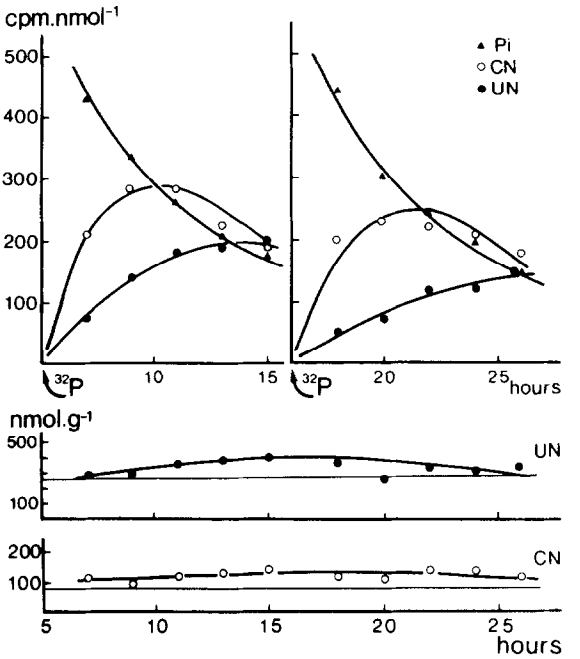

FIGURE 2 - CHANGES IN POOL SIZES AND [^{32}P] LABELLING OF CARDIAC PYRIMIDINE NUCLEOTIDES INDUCED BY THE EIGHTH DAILY INJECTION OF ISOPROTERENOL INTO RATS.

Same legend as figure 1.

Time-course of labelling. A visual analysis of the time-course labelling patterns (fig.1) reveals that, when compared with controls (fig.3), the incorporation of [^{32}P]phosphate into the α-phosphate groups of uracil nucleotides was considerably accelerated during the period of time 5 to 15 hours after a single isoproterenol injection. Indeed intersections of the curves of time-course specific activities of uracil nucleotides and inorganic phosphate are reached earlier. Although this stimulating effect of isoproterenol was diminished later (16 to 26 hours post injection), an increase in labelling was still present. The labelling of cytosine nucleotides was much less modified by the treatment. On the other hand, on the eighth day of isoproterenol treatment (fig.2), the stimulating effect of the drug was still present but slightly reduced. Calculation of the rates of incorporation of labelled phosphate into the α-phosphate groups of nucleotides corroborates these observations (table 1).

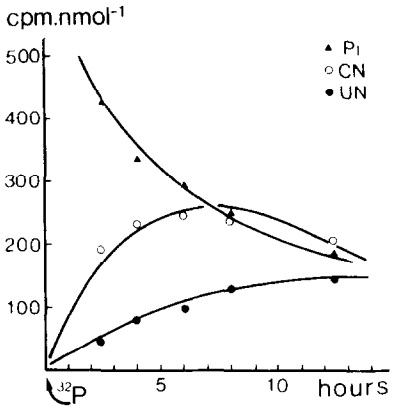

FIGURE 3 - TIME-COURSE LABELLING OF INORGANIC PHOSPHATE (Pi) AND OF ∝PHOSPHATE GROUPS OF CYTOSINE AND URACIL NUCLEOTIDES (CN and UN) IN CONTROL RATS.

[^{32}P]phosphate was administered intravenuously at time zero. Each measurement of specific activity was performed on batches of pooled ventricles from 5 rats.

DISCUSSION

The validity of the method used in this study was discussed in previous papers (9,10) ; in the following, we will consider the values obtained as estimates of the rates of synthesis of nucleotides. Although the mechanisms responsible for the development of cardiomegaly may be complex when isoproterenol is used, this experimental model was chosen because it offered two main advantages. Since cardiac hypertrophy could be quantitatively reproduced, several hearts could be pooled for conveniently studying pyrimidine nucleo-

TABLE 1 - RATES OF INCORPORATION OF INORGANIC PHOSPHATE INTO THE ∝PHOSPHATE GROUPS OF PYRIMIDINE NUCLEOTIDES IN RAT HEARTS.

		Uracil nucleotides		cytosine nucleotides
Control rats		16		11
Isoproterenol treated rats				
first day	(8-13h)	58	(6-8h)	32
	(19-24h)	24	(17-19h)	18
eighth day	(8-13h)	40	(6-8h)	33
	(19-24h)	24	(17-19h)	16

The incorporation rates were estimated, during the period of time indicated into bracket, according to the formula given in the section methods.
Values are given in nmol.h^{-1}.g^{-1} (tissue wet weight).

tides. On the other hand, the dry weight of ventricles and the myocardial concentration of RNA were raised rapidly -respectively 43 and 39 per cent increase in five days (7)- so that a large and fast increase in the total RNA content of the heart occured. Thus, some features of the biochemical changes which accompanied hypertrophy were perhaps revealed in this way.

It has been previously suggested, based on the observation that the cardiac uracil nucleotide pool was enlarged after aortic constriction, that the synthesis of these nucleotides may, in part, control the rate of RNA synthesis (8). Indeed, the cardiac concentration of uracil nucleotides is much lower than in tissues with high synthetic activity. Futhermore, we may assume that the availability of cytosine nucleotides may be a limiting factor in this synthesis. The requirement of these precursors for RNA metabolism can be estimated : in physiological conditions, most of the myocardial RNA (about $2mg.g^{-1}$) turns over with an half-life of about 6 days (12) ; thus $9\ nmol.g^{-1}$ are taken from the free nucleotide pool every hour. This calculated value is in the same range as the measured rate of cytosine nucleotide synthesis. Therefore, the significant and rapid increase of the rates of pyrimidine nucleotide synthesis revealed in these experiments is of considerable interest, since it demonstrates the potential adaptation of the myocardial cell to a radical change in its metabolism.

Further experiments are now needed to precisely state the pyrimidine synthesis pathways in the hypertrophying heart, the mechanisms of control of this metabolism, and the alterations occuring in other models of experimental hypertrophy. The possible role of pyrimidine nucleotides as a triggering or controlling factor in the development of cardiac hypertrophy must also be considered in further investigations.

ACKNOWLEDGMENT

This research was supported by a grant from the DGRST (n°77-7-1023). The authors expressed their gratitude to prof. M.DUC-JACQUET and J.DELLA-DORA for their invaluable help and advice for the mathematical treatment of experimental data.

REFERENCES

1. Schreiber, S.S., Oratz, M., EVANS, C., SILVER, E. and ROTSCHILD, M.A. (1968) Am.J.Physiol. 215, 1250-1259.
2. Posner, B.I. and Fanburg, B.L. (1968) Circ.Res. 23, 137-145.
3. Mayfield, E.D., Chidoni, J.J., Bresnick, E. and Stanton, H.C. (1968) Proc.Soc.Exp.Biol.Med. 129, 91-96.
4. Wood, W.G., Lindenmayer, G.E. and Schwartz, A. (1971) J.Mol.Cell.Cardiol. 3, 127-138.
5. Zimmer, H.G. and Gerlach, E. (1974) Circ.Res. 35, 536-543.
6. Zimmer, H.G. and Gerlach, E. (1978) Pflügers Arch. 376, 223-227.
7. Rossi, A., Olivares, J., Aussedat, J. and Ray, A. (1979) J.Mol.Cell. Cardiol. 11 (suppl.2) 50.
8. Koide, T. and Rabinowitz, M. (1969) Circ.Res. 24, 9-18.
9. Rossi, A., Mandel, P. and Dessaux, G. (1972) Arch.Int.Physiol.Bioch. 80, 59-77.
10. Rossi, A. (1975) J.Mol.Cell.Cardiol. 7, 891-906
11. Bartlett, G.R. (1959) J.Biol.Chem. 234, 466-468.
12. Erdos, T. and Bessada, R. (1966) Biochim.Biophys.Acta 129, 628-631.