Glycogen metabolism: a ^{13}C-NMR study on the isolated perfused rat heart

N. Lavanchy, J. Martin* and A. Rossi

Laboratoire de Physiopathologie du Métabolisme Cardiaque, CNRS UA 632 USMG, BP 68, 38402 St Martin d'Heres Cédex, and **Centre d'Etudes Nucléaires de Grenoble, Grenoble, France*

Received 7 August 1984; revised version received 2 October 1984

Glycogen synthesis from D-[1-^{13}C]glucose was observed in the perfused rat heart by ^{13}C-NMR spectroscopy at 62.9 MHz. The glycogenogenesis was stimulated by pretreatment of the animals with isoprenaline. Whereas in hearts from control rats the incorporation of D-[1-^{13}C]glucose into the glycogen remained below the detection threshold, 5 min proton-decoupled ^{13}C-NMR spectra revealed, in hearts from treated rats, a significant labelling of the glycogen within the first minutes of the perfusion and a further linear increase of the glycogen resonance for up to 25 min. This model was used to monitor the appearance of ^{13}C-labelled lactate during ischemia.

Perfused rat heart *^{13}C-NMR* *D-[1-^{13}C]Glucose* *Glycogen metabolism*

1. INTRODUCTION

Much attention has been focused, over the past few years, on ^{13}C-NMR spectroscopy in metabolism studies 'in vivo'. Using this technique, experiments into the metabolism of cardiac glycogen have mainly been performed 'in situ' [1,2]. Authors in [1,2] were able to follow glycogen synthesis from exogenous D-[1-^{13}C]glucose in the guinea-pig heart 'in situ' and the degradation of the ^{13}C-labelled glycogen during anoxia. Other studies revealed that, in the perfused heart, in spite of the presence of insulin, the identification of the typical glycogen resonance required a long perfusion time [3]. Here, we have tried to describe appropriate conditions both physiological and as regards the ^{13}C-NMR spectroscopy, thus enabling the observation of the synthesis of glycogen from exogenous D-[1-^{13}C]glucose to a significant extent and of the glycogenolysis during a subsequent ischemia: only a short perfusion time was required (~1 h or even less), thus maintaining the perfused heart in a good physiological state. To this end, the myocardial glycogen-synthase was activated 'in situ' before perfusion of the isolated heart: a single isoprenaline injection of the rats (5 mg · kg^{-1} s.c.) induced a marked depletion of glycogen stores (80%), which in turn vigorously activated the glycogen-synthase (submitted). When the heart was isolated and perfused, the glycogen-synthase remained in an activated state, whilst the glycogen-phosphorylase activation decreased to near basal values (unpublished). Thus, we made use of this procedure to obtain a resynthesis of cardiac glycogen from exogenous D-[1-^{13}C]-glucose and to observe the kinetics of glycogen synthesis and the time-course changes induced by a short period of total global ischemia.

2. MATERIAL AND METHODS

2.1. *NMR measurements*

^{13}C-NMR spectra of the isolated perfused rat heart were recorded at 62.9 MHz on a Brucker WM 250 spectrometer (probe diameter 15 mm) with the use of a deuterium field/frequency lock. 10% dioxane in D_2O placed in a capillary tube was used as an external standard and assigned a value of 67.40 ppm.

Published by Elsevier Science Publishers B.V.
00145793/85/$3.00

Bilevel proton decoupling was employed with ~0.5 W during the delay and 3 W during data acquisition. The temperature of the heart was maintained at 37°C. The 50° pulse width (40 μs) was used and each ^{13}C-NMR spectrum was acquired over a period of 5 min with a spectral width of 13 kHz, an acquisition time of 0.15 s and a recycle time of 0.41 s. Before Fourier-transform, a line-broadening of 10 Hz was applied to the free-induction decay.

2.2. *Preparation of the rats*

Wistar rats (230–240 g body wt) were submitted to a stimulation of the beta-adrenergic receptors by a single isoprenaline injection (5 mg · kg^{-1} s.c.) 1 h before isolation and perfusion of the hearts.

2.3. *Experimental protocol*

The rat hearts were perfused via the aorta under a 100 cmH_2O pressure with a bicarbonate buffer adjusted after gassing (95% O_2/5% CO_2) at pH 7.4 at heart level. The glucose of the perfusion solution (5.5 mM) was in the form of D-[1-^{13}C]-glucose with 99% ^{13}C atoms (C.E.A. Saclay, France) during the first 30 min of the perfusion, enabling the recording of 5 ^{13}C-NMR spectra. The perfusate was partially recycled after filtration (Millipore filter, 0.22 μm diameter).

The heart was then perfused with a ^{13}C natural abundance glucose-containing solution. A new ^{13}C-NMR spectrum was recorded after a 3 min period, before submitting the myocardium to a sever ischemic event: a 15 min total global ischemia with proton-decoupling maintained. Finally, the reperfusion of the heart was monitored for 10 min.

2.4. *Biochemical analysis*

The glycogen content of the hearts was measured for both control and isoprenaline-treated rats, (i) in situ and (ii) at the end of a 30 min perfusion. After protein solubilization using 40% KOH (100°C, 30 min) and an overnight precipitation at 4°C by ethanol, the glycogen pellet was dissolved in 2 N HCl and hydrolyzed to glucose (100°C, 3 h). The glucose content was then enzymically determined. The glycogen content was expressed in mg glucose per g wet wt.

3. RESULTS AND DISCUSSION

For an individual ^{13}C 5 min NMR spectrum, which represents the signal averaging over 720 accumulations, no detectable resonance could be assigned to the glycogen in control hearts either for ^{13}C natural abundance exogenous glucose (fig.1A), or for D-[1-^{13}C]-glucose in the fluid (99% ^{13}C atoms) in spite of the presence of the 2 peaks corresponding to the Carbone-1 of the labelled glucose (fig.1B). When the same experimental protocol was applied to hearts from isoprenaline-treated rats, the successive spectra acquired during a 30 min. perfusion showed a regular increase in heart glycogen, at least over the first 25 min. The ^{13}C-labelled glycogen appeared within the first 5 or 10 min, and quite obviously an accumulation time of less than 5 min could have been used.

A spectrum recorded after an additional 3 min of perfusion with ^{13}C natural abundance exogenous glucose showed that the peaks arising from the ^{13}C-labelled glucose practically disappeared. Moreover, it revealed no significant ^{13}C-labelled lactate accumulation, allowing us to assert that no anaerobic metabolism occurred. In this respect, our results are in disagreement with those of [3] which showed a production of ^{13}C-labelled lactate throughout experimentation with a maximally oxygenated perfusion solution. These discrepancies can probably be explained by the length of the perfusion period (117 min) needed to label glycogen from D-[1-^{13}C]glucose.

Fig.2 shows that glycogenogenesis from D-[1-^{13}C]glucose can be expressed in a relative quantitative way. Note that with our NMR recycling-time conditions (0.41 s), no saturation of the glycogen peak occurs [1]. Moreover, authors in [4] suggested that every [^{13}C]-glucose incorporated into the glycogen molecule was seen by ^{13}C-NMR spectroscopy. The apparent rate of synthesis was constant over the first 25 min, while a slight decrease subsequently occurred. Biochemical measurements of the glycogen content of the heart 'in situ' (before perfusion) and at the end of a 25 min perfusion revealed an amount of 2.46 mg · g^{-1} wet wt freshly synthesized glycogen (table 1). In fact, further experiments are required in order to measure quantitatively the exact proportion of glycogen synthesized from exogenous glucose and that from other substrates.

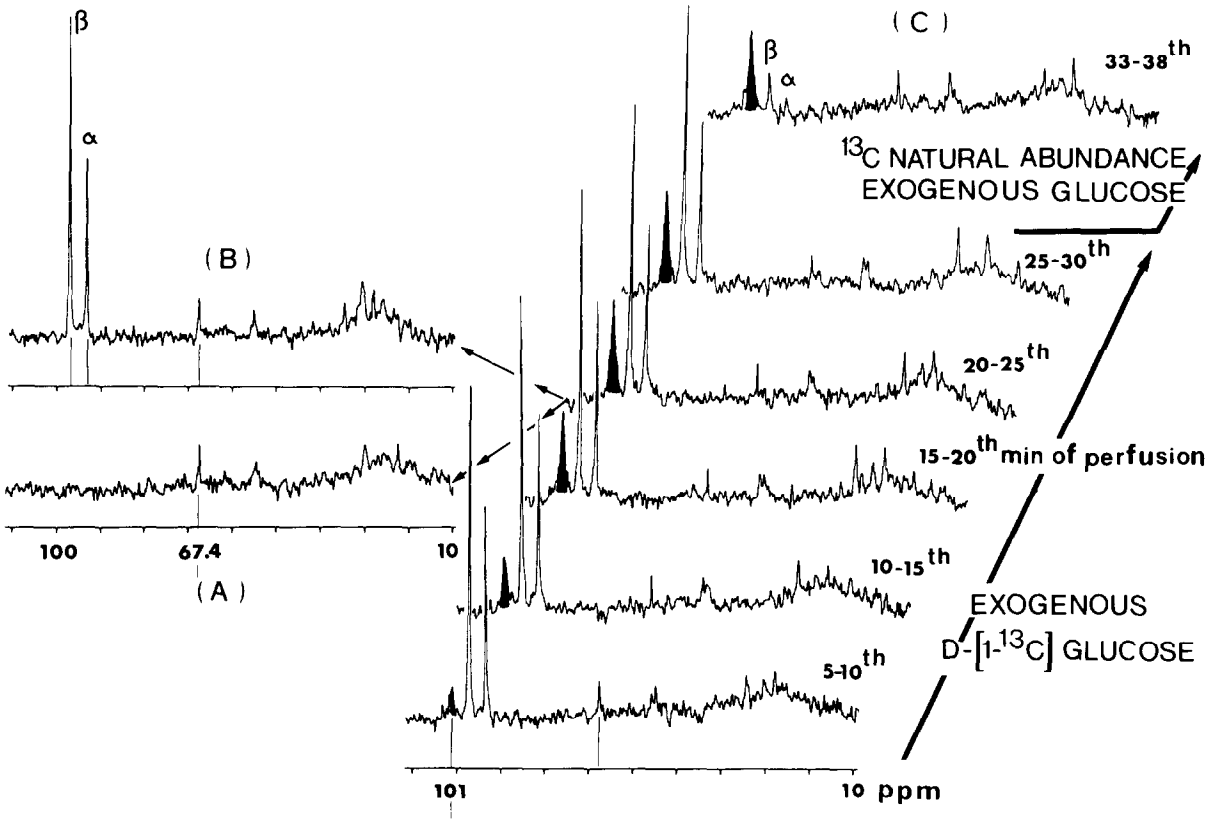

Fig.1. 62.9 MHz proton-decoupled ^{13}C 5 min NMR spectra of the isolated perfused rat heart. The perfusion solution with 5.5. mM glucose immersed the heart. Each spectrum is the Fourier-transform average of 720 transients, acquired with 13 kHz spectral width, 0.15 s acquisition time, 50° pulse angle, 0.41 s recycle time. A 10 Hz line-broadening was applied to the free induction decay before Fourier-transform. (A) ^{13}C natural abundance glucose-perfused heart (20–25th min perfusion). (B) D-[1-^{13}C]Glucose (99% ^{13}C atoms) perfused heart (20–25th min perfusion). (C) Heart from isoprenaline-treated rat (5 mg·kg^{-1}-sc, 1 h before isolation) perfused in (B) during the first 30 min and in (A) afterwards. Resonance: glycogen 101 ppm; C-1 glucose β 96.8 ppm, α 92.9 ppm; dioxane 67.4 ppm.

When a significant amount of the glycogen stores was thus visualized by ^{13}C-NMR spectroscopy, the hearts were submitted to a 15 min total global ischemia. Fig.3 illustrates the changes occurring during this period and during the subsequent 10 min reperfusion. The mobilization of the glycogen took place from about the 5th min of ischemia onwards and resulted in an almost complete depletion of the labelled glycogen reserves (figs.3,2B). The apparition and increase of a peak at 21 ppm during ischemia illustrated the production of lactate as a consequence of the setting in of anaerobic metabolism. On reperfusion, in spite of the washout of the lactate in the perfusion fluid, a significant proportion of the ^{13}C-labelled lactate remained within the tissue (fig.3). In the hearts from control rats, ischemia and reperfusion ^{13}C-NMR spectra did not allow the detection of significant amounts of lactate. Note that the lactate resonance was submitted to a probably marked saturation phenomenon: the very few measurements of tissue-lactate T_1-time were in fact performed on frog muscle at 29°C by authors in [5] (T_1 = 3.6 s ± 20%). Nevertheless, the lactate-Noe value measured by these authors seemed to be higher than that of glycogen measured on the liver by authors in [4]: 1.9 vs 1.2.

In conclusion, this work demonstrates that it is possible to obtain high quality proton-decoupled ^{13}C-NMR spectra showing myocardial glycogen

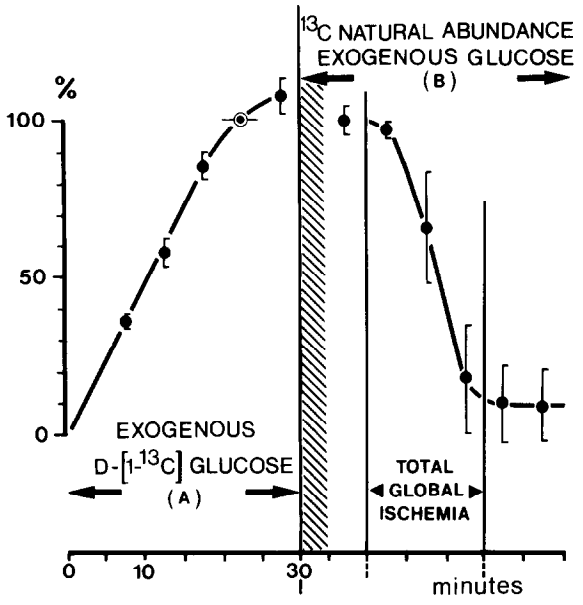

Fig.2. Relative quantititative time-course of glycogen synthesis occurring from D-[1-^{13}C]glucose (5.5 mM) in the perfused heart of isoprenaline-treated rat (5 mg · kg^{-1} -sc) (A), and of the glycogenolysis induced by ischemia (B). Each point is the average of the 101 ppm signal intensities measured on the ^{13}C-proton-decoupled 5 min NMR spectra from 3 hearts. The value 100% is taken at 20–25th min perfusion. Mean ± SD.

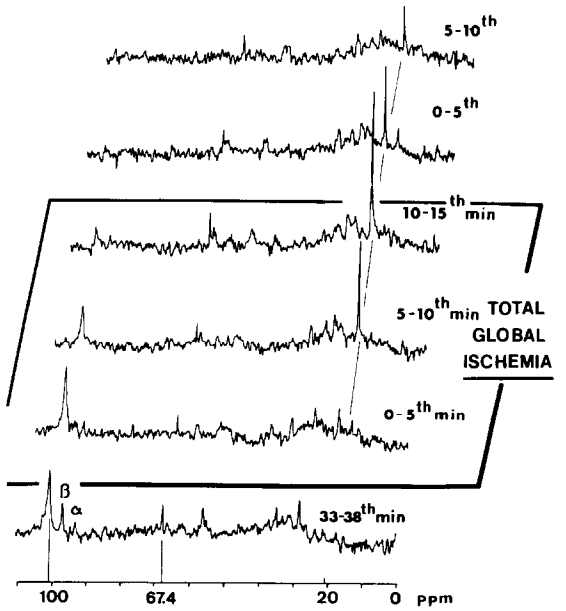

Table 1

Glycogen content of hearts 'in situ' and at the end of a 25 min perfusion from control and isoprenaline-pretreated rats

	In situ	25th min of perfusion
Controls	4.05 ± 0.15 (35)	3.92 ± 0.22 (14)
Isoprenaline-treated (5 mg · kg^{-1} s.c.)	0.71 ± 0.13 (10)	3.17 ± 0.27 (9)

Values are expressed as means ± SE *(n)*. Glycogen content is in mg glucose · g^{-1} wet wt

from isolated perfused rat heart with excellent signal-to-noise ratio and resolution in a short data accumulation time. This was made possible by the choice of particularly favourable physiological conditions which permit the synthesis of cardiac glycogen at high rates from exogenous D-[1-^{13}C]-glucose without the addition of insulin. This model can be used for the investigation and quantitation of the metabolic fluxes of cardiac glycogen metabolism in the isolated perfused rat heart. These conditions are particularly suitable for the measurement of glycogenesis rates related to the activity of key enzymes involved in the glycogen metabolism and for studying the effects of hormonal or non-hormonal factors. It is also possible to study, in such an experimental situation, the rate of mobilization of glycogen under hypoxic conditions, and possibly even to correlate the rate of lactate accumulation with data concerning changes in intracellular pH and in high-energy phosphate compound contents obtained by ^{31}P-NMR spectroscopy. The main relevant physiological conclusion to be drawn from these experiments is that,

Fig.3. 62.9 MHz proton-decoupled ^{13}C 5 min NMR spectra of isolated rat heart submitted to ischemia and reperfused. Hearts from isoprenaline-treated rats (5 mg · kg^{-1}-sc) were perfused with buffer containing 5.5 mM D-[1-^{13}C]glucose for the first 30 min, 5.5 mM ^{13}C natural abundance glucose afterwards. Spectra were acquired as described for fig.1. Resonance assignments see fig.1. Lactate 21 ppm.

contrary to several previous assertions, a marked glycogenesis can occur in the isolated perfused rat heart. The insulin does not seem to be the main contributing factor, i.e., if the glycogen-synthase has already been activated, the rate of penetration of the glucose into the myocardial cell is sufficiently high to provide precursors for the glycogen synthesis in the perfused heart.

ACKNOWLEDGEMENT

This work was supported by INSERM (P.R.C. No. 130068).

REFERENCES

[1] Neurohr, K.J., Barrett, E.J. and Shulman, R.G. (1983) Proc. Natl. Acad. Sci. USA 80, 1603–1607.
[2] Neurohr, K.J., Gollin, G., Barrett, E.J., Rothman, D.L. and Shulman, R.G. (1984) Magn. Res. Med. 2, 214–215.
[3] Hoekenga, D.E., Brainard, J.R., Hutson, J.Y. and Matwiyoff, N.A. (1984) Magn. Res. Med. 2, 167–168.
[4] Sillerud, L.O. and Shulman, R.G. (1983) Biochemistry 22, 1087–1094.
[5] Doyle, D.D. and Barany, M. (1982) FEBS Lett. 140, 237–240.