Differential rates of protein synthesis *in vitro* and RNA contents in rat heart ventricular and atrial muscle

David M. SMITH* and Peter H. SUGDEN

Department of Cardiac Medicine, Cardiothoracic Institute, 2 Beaumont Street, London W1N 2DX, U.K.

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The rates of protein synthesis in perfused rat heart ventricular or atrial muscle were measured by incorporation of [U-¹⁴C]phenylalanine in the presence of the remaining plasma amino acids. Atrial protein-synthesis rates were about twice the ventricular rates. Atrial RNA contents were also about twice the ventricular contents. Thus the efficiencies of protein synthesis (protein-synthesis rate/RNA) in the two compartments were similar. There were marked differences in ventricular and atrial RNA contents during the course of rat growth. Atrial RNA content was always greater than ventricular content and declined more slowly during growth, producing a 2-fold change in atrial/ventricular RNA-content ratio between the 88 g and 370 g rat groups.

Protein synthesis in the whole heart has been studied extensively (for a review, see Morgan *et al.*, 1979). Little attention has been paid to comparisons between the ventricles and atria in this regard. Ultrastructurally, common atrial fibres appear to be quite similar to common ventricular fibres (Sommer & Johnson, 1979). However, ventricles and atria do differ in some other respects. For example, differences have been observed in collagen content (Caspari *et al.*, 1975), in post-infarction DNA synthesis (Rumyantsev & Mirakjan, 1968) and in the light and/or heavy chains of myosin (for a review, see Cummins, 1983). Atrial muscle contracts and relaxes with a greater velocity and a shorter time course than for ventricular muscle (Korecky & Michael, 1972; Cummins, 1983). We have now investigated the rates of protein synthesis and RNA content in rat heart ventricular and atrial muscle.

Experimental

Materials

Chemicals were from BDH Chemicals, Dagenham, Essex RM8 1RZ, U.K. Biochemicals (including amino acids) were from Sigma (London) Chemical Co., Poole, Dorset BH17 4NN, U.K., except for tRNA (from *Escherichia coli* M.R.E. 600), which was from BCL, Lewes, East Sussex BN7 1LG, U.K. [U-¹⁴C]Phenylalanine was from Amersham International, Amersham, Bucks. HP7 9LL,

U.K. Male Sprague–Dawley rats were from Bantin and Kingman, Hull, Humberside HU11 4QE, U.K. They were kept for at least 5 days before use, during which time they had free access to food and water.

Heart perfusions

Hearts were perfused as working preparations by the method of Taegtmeyer *et al.* (1980) as described in detail previously (Sugden & Smith, 1982b). Hearts were initially perfused as non-recirculating Langendorff (1895) preparations with Krebs & Henseleit (1932) bicarbonate-buffered saline solution supplemented with 5 mM-glucose and equilibrated with O₂/CO₂ (19:1). The common pulmonary vein was cannulated as quickly as possible and the preparation was switched to a recirculating working perfusion (100 ml of perfusate). The filling pressure was 5 cm of water and the aortic pressure was 70 cm of water. The perfusate was the same as for the initial Langendorff perfusion, except that it was supplemented with various concentrations of plasma amino acids (see the Results section) including [U-¹⁴C]phenylalanine (sp. radioactivity about 0.1 Ci/mol). Cardiac output over the course of the perfusion was measured as described previously (Sugden & Smith, 1982b).

Incorporation of [U-¹⁴C]phenylalanine into cardiac protein

After 15–120 min of perfusion as working preparations, hearts were removed from the cannulae. The right and left atria were carefully removed and frozen in liquid N₂. The ventricles were diced and

* To whom reprint requests should be addressed.

homogenized in 5% (w/v) trichloroacetic acid (15 ml) with a Polytron homogenizer (Northern Media Supply, North Cave, Humberside HU15 2PG, U.K.). Atria were ground to a fine powder in ground-glass homogenizers cooled in liquid N₂, and then homogenized in 5% trichloroacetic acid (5 ml). The precipitated protein was separated by centrifugation in a bench centrifuge and washed with 5% trichloroacetic acid (10 ml each wash; three washes for ventricular protein and two for atrial protein). The precipitated protein was resuspended in water (8–12 ml for ventricular protein, 4–8 ml for atrial protein) and digested by the addition of 0.25 vol. of 0.5 M-NaOH at 37°C for 1 h. Samples of this digest were used to measure [U-¹⁴C]phenylalanine incorporation into protein or RNA content. For the former, protein was precipitated by addition of 0.05 vol. of 100% trichloroacetic acid and removed by centrifugation in a bench centrifuge. Trichloroacetic acid was removed by shaking with diethyl ether (5 ml). Ether was evaporated under a hair-dryer and the protein was dissolved in 3 ml of NCS tissue solubilizer, with heating at 50°C if necessary. Radioactivity incorporated was measured by liquid-scintillation spectrometry with a toluene-based fluor (15 ml) containing 5 g of 2,5-diphenyloxazole/litre and 0.1 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene/litre. Quenching was determined by the external-standard method.

Analytical methods

[U-¹⁴C]Phenylalanine specific radioactivity in all perfusions was measured by liquid-scintillation spectrometry of samples (1 ml) of perfusate with 10 ml of PCS Phase Combining System as fluor and by measurement of the phenylalanine content of suitably diluted perfusate samples by the method of Rubin & Goldstein (1970) as described previously (Sugden & Smith, 1982a). Protein content of the NaOH digests was measured by the method of Gornall *et al.* (1949). Rat ventricular and atrial protein were purified by trichloroacetic acid precipitation of NaOH digests and trichloroacetic acid washing followed by ethanol and diethyl ether extraction and drying over P₂O₅ *in vacuo*. It was found that rat heart ventricular or atrial protein developed 0.93 of the A₅₄₀ that was developed by an equal weight of crystalline bovine serum albumin (dried over P₂O₅ *in vacuo*). Total RNA in NaOH digests was measured by the method of Munro & Fleck (1969). A protein-content correction was made by the method of Lowry *et al.* (1951). A conversion factor of one A₂₆₀ unit being equivalent to 32 µg of RNA/ml was used. In the present paper the term 'total' means that all RNA species are measured and does not refer to the total content of RNA of the whole tissue.

Phenylalanine content of ventricular protein

Ventricular protein (15 mg, purified as above) was hydrolysed under N₂ in 6 M-HCl containing 2 mg of phenol/ml at 110°C for 24, 48 or 72 h. After drying over solid NaOH and P₂O₅ *in vacuo*, the residues were taken up in 50 mM-Tris/HCl, pH 8.5, and assayed for phenylalanine as described above. Phenylalanine release was maximal after 48 h hydrolysis, declining thereafter. The phenylalanine content was 0.141 ± 0.014 mmol/g of ventricular protein (three observations).

Statistical methods

Results are presented as means $\pm$ S.E.M., with the numbers of observations in parentheses. Statistically significant difference was taken as being established at $P < 0.05$ by a two-tailed Student's *t* test.

Results

Time course of incorporation of [U-¹⁴C]phenylalanine into ventricular or atrial protein

The incorporation of [U-¹⁴C]phenylalanine (0.4 mM) into ventricular or atrial protein in the presence of five times the normal plasma concentration of the remaining amino acids was linear with time for at least 2 h of perfusion (Fig. 1). Plasma amino acid concentrations in the normal fed rat were taken from Williams *et al.* (1980). The rate of [U-¹⁴C]phenylalanine incorporation into atrial protein was about 1.7 times that into ventricular protein. [U-¹⁴C]Phenylalanine incorporation into atrial protein was statistically significantly greater than into ventricular protein at all time points, at $P < 0.05$ or less. Rates of ventricular and atrial protein synthesis were linear with time for up to 2 h when hearts were perfused with 0.4 mM-[U-¹⁴C]-phenylalanine and other plasma amino acids at a concentration of 0.2 mM (results not shown). The rate of ventricular protein synthesis was similarly linear with time when hearts were perfused with 0.4 mM-[U-¹⁴C]phenylalanine and other plasma amino acids at 0.05 mM (results not shown).

Effects of amino acid concentrations on ventricular and atrial protein synthesis

In order to establish that the observed differences between ventricular and atrial rates of protein synthesis were not caused by differential effects of amino acid concentration on protein-synthesis rates, and to establish that maximal protein-synthesis rates were being measured, the effects of amino acid concentrations on protein-synthesis rates were investigated (Table 1). [U-¹⁴C]Phenylalanine (0.4–1.6 mM) was chosen as radiolabel because (i) its intracellular concentration and specific radioactivity

Fig. 1. Time course of rat heart ventricular and atrial protein synthesis

Hearts were perfused as described in the Experimental section with 0.4 mM-[U-¹⁴C]phenylalanine and five times the normal fed-rat plasma concentrations of the other amino acids (Williams *et al.*, 1980). Statistically significant difference of atrial (●) from ventricular (▲) protein synthesis: * $P < 0.05$; ** $P < 0.001$. The numbers of observations were three at 15 and 67.5 min, and seven at 120 min. Rat weights for the 15 min point were 313 ± 15 g, for the 67.5 min point, 321 ± 9 g, and for the 120 min point, 292 ± 9 g. There is no significant difference between the weights of the rat groups.

equilibrate rapidly (less than 5 min) with the perfusate (McKee *et al.*, 1978), (ii) the specific radioactivity of [U-¹⁴C]phenylalanyl-tRNA similarly rapidly equilibrates with that of the perfusate [U-¹⁴C]phenylalanine (McKee *et al.*, 1978), and (iii) [U-¹⁴C]phenylalanine is not significantly metabolized by the heart (Williams *et al.*, 1981). At the concentrations of [U-¹⁴C]phenylalanine used (0.4–1.6 mM), dilution of specific radioactivity by endogenous cardiac phenylalanine produced by cardiac protein degradation is negligible (about 1–2%). As shown in Table 1, there were no statistically significant differences in the rates of ventricular or atrial protein synthesis at any of the amino acid concentrations used. An increase in [U-¹⁴C]phenylalanine concentration and/or an increase in the concentration of the remaining amino acids did not affect protein-synthesis rates. We are thus satisfied that, in perfusions containing 5 mM-glucose and unsupplemented by hormones such as insulin, the measured rates of protein synthesis were maximal as far as provision of amino acids was concerned (but of course less than the maximal rates that could be achieved in the presence of insulin; see the Discussion section). Rates of atrial protein synthesis were consistently about twice the rates of ventricular protein synthesis (Tables 1 and 2). Rats used in this study had similar body and heart weights, and the falls in cardiac output over the course of the perfusions were similar (Table 1). Cardiac outputs after 70 min of perfusion were also similar, with the exception of group (a).

Total RNA content of ventricles and atria

Total RNA concentrations were measured at the end of perfusions. There was no detectable decrease

Table 1. Effects of amino acid concentration on the rates of protein synthesis in ventricles and atria

Hearts were perfused and protein synthesis was measured as described in the Experimental section. The rat body weights, heart wet weights, cardiac outputs after 70 min of perfusion and declines in cardiac output, i.e. $100 \times [1 - (\text{cardiac output after 110 min} / \text{cardiac output after 10 min})]$, were respectively: group (a), 320 ± 14 g, 0.998 ± 0.046 g, 48.6 ± 4.4 ml/min per g wet wt. of heart, -2 ± 6 ; group (b), 309 ± 7 g, 0.941 ± 0.026 g, 57.0 ± 4.4 ml/min per g wet wt. of heart, 0 ± 4 ; group (c), 299 ± 16 g, 0.919 ± 0.052 g, 57.6 ± 3.7 ml/min per g wet wt. of heart, 3 ± 6 ; group (d), 292 ± 9 g, 0.904 ± 0.049 g, 58.9 ± 5.5 ml/min per g wet wt., 3 ± 3 . Plasma concentrations of amino acids for normal fed rats were taken from Williams *et al.* (1980). Statistical significance for difference from identically perfused ventricular samples: * $P < 0.001$.

Perfusate amino acid concn.	Protein synthesis (nmol of phenylalanine incorporated/2 h per mg of protein)	
	Ventricles	Atria
(a) 0.4 mM-[U- ¹⁴ C]phenylalanine, other amino acids 0.05 mM	1.124 ± 0.051 (5)	—
(b) 0.4 mM-[U- ¹⁴ C]phenylalanine, other amino acids 0.2 mM	1.131 ± 0.061 (8)	2.245 ± 0.135 (8)*
(c) 1.6 mM-[U- ¹⁴ C]phenylalanine, other amino acids 0.2 mM	1.186 ± 0.074 (8)	—
(d) 0.4 mM-[U- ¹⁴ C]phenylalanine, other amino acids at 5 times plasma concentration	1.184 ± 0.101 (7)	2.023 ± 0.161 (4)*

Table 2. Comparison of the RNA content, protein-synthesis rates and efficiency of protein synthesis for ventricles and atria. RNA was measured as described in the Experimental section in hearts (0.942 ± 0.017 g) from 303 ± 5 g rats. The atrial/ventricular protein-synthesis-rate ratio and the efficiency of protein synthesis were calculated for perfusions at 0.4 mM-[U- 14 C]phenylalanine and other amino acids at 0.2 mM (Table 1). Statistical significance for difference from identically perfused ventricular samples: * $P < 0.001$.

	No. of observations	Ventricles	Atria	Atrial/ventricular
RNA content (μ g/mg of protein)	39	10.49 ± 0.40	$20.96 \pm 0.43^*$	2.07 ± 0.07
Atrial/ventricular protein-synthesis rate	8	—	—	1.99 ± 0.07
Efficiency of protein synthesis (pmol of phenylalanine incorporated/2 h per μ g of RNA)	8	93 ± 9	95 ± 6	1.12 ± 0.15

Fig. 2. Developmental changes in ventricular and atrial total RNA contents

The atrial (●) and ventricular (▲) RNA contents are shown in Fig. 2(a). Hearts were removed as quickly

in ventricular or atrial RNA content over the course of the perfusion (results not shown). There was no difference in atrial or ventricular RNA contents between hearts perfused in the morning or in the afternoon (results not shown). Total RNA content of the atria was about twice that of the ventricles (Table 2). The efficiency of protein synthesis in terms of phenylalanine incorporation/2 h per μ g of RNA was thus the same for ventricles and atria (Table 2).

Developmental aspects of total RNA content of ventricles and atria

Although it is known that heart total RNA content falls with age in the rat (Waterlow *et al.*, 1978), we know of no comparative study between ventricles and atria. Atrial total RNA content was always significantly greater ($P < 0.01$ or less) than ventricular total RNA content (Fig. 2a). Although both ventricular and atrial total RNA contents declined with age, the fall in ventricular content was proportionally greater than that of atrial content. The RNA contents for 370 g rats, expressed as a percentage of those in 88 g rats, were 28% for the

as possible (starting at 08:30 h), from animals under pentobarbital anaesthesia (60 mg/kg) and rinsed in 0.9% NaCl before dissection. Ventricular weights were: for 88 g rats, 0.361 ± 0.005 g; for 187 g rats, 0.632 ± 0.014 g; for 228 g rats, 0.739 ± 0.032 g; for 272 g rats, 0.816 ± 0.018 g; and for 370 g rats, 0.957 ± 0.020 g; with four observations for each rat group (three for the 370 g group). Atrial RNA content was statistically significantly different from the ventricular RNA content for a given rat weight group at $P < 0.01$ or < 0.001 . For the ventricular group each point was statistically significantly different from its neighbours at $P < 0.01$ or < 0.001 . For the atrial group, all points are statistically significantly different from the 88 g-rat group at $P < 0.05$. Fig. 2(b) shows the atrial/ventricular RNA-content ratio during development. With the exception of the 187 g-rat group, all points were statistically significantly different from the 88 g-rat group at $P < 0.01$ or < 0.001 .

ventricles and 66% for atria. Thus during development there was a large increase in the atrial/ventricular total-RNA ratio (Fig. 2b).

Discussion

We have shown that there are differences in protein-synthesis rates and total RNA contents between discrete parts of the same organ. Since most of the tissue RNA is ribosomal (for a review, see Waterlow *et al.*, 1978), there is presumably a difference between the ribosomal contents of ventricular and atrial muscle. Our findings also support the idea that protein-synthesis rate for tissues is a function of RNA content, in the absence of other perturbing factors such as insulin (for a review, see Waterlow *et al.*, 1978). We do not know whether our findings reflect differences between ventricular and atrial cardiac myocytes themselves or whether the findings are merely a function of intra-organ cellular heterogeneity. Although myocytes constitute about 75% of the cell protein, they represent about 25% of the cell number. The remaining cellular content includes vascular cells and fibroblasts. Although the collagen content of the atrium is greater than that of the ventricle (Caspari *et al.*, 1975), it seems unlikely that the differences in protein-synthesis rate and RNA content could be attributed to a higher fibroblast content in the atrium, since these cells are poor in ribosomes. We cannot exclude the possibility of a larger content of some non-myocytic cells in the atrium which exhibit a high rate of protein turnover.

In this study, we pooled the left and right atria and ventricles. Both atria and ventricles are perfused by the coronary circulation, with atria possibly receiving some of their oxygen supply by diffusion through their thin walls. It is unlikely that differences in protein-synthesis rates between areas of the heart could be caused by poor oxygenation (which inhibits protein synthesis; see Jefferson *et al.*, 1971; Kao *et al.*, 1976).

In general, the ventricular rate of protein synthesis and the ventricular total RNA content compare reasonably well with values given by others (Morgan *et al.*, 1980; Williams *et al.*, 1980) for working heart preparations. A calculation of the rate of phenylalanine incorporation *in vivo* into rat ventricular protein can be made on the basis of the data in the present paper and knowledge that the fractional rate of protein synthesis in the hearts of 130g rats *in vivo* is 20%/day (Garlick *et al.*, 1980). Because the ventricles constitute about 90% of the heart wet weight and because we do not know whether Garlick *et al.* (1980) included the atria in their measurements, the following calculation is based on our data for ventricles alone. The phenyl-

alanine content of Sprague-Dawley rat ventricular protein is 0.141mmol/g; we assume that the phenylalanine content is similar in the 130g Wistar rats used by Garlick *et al.* (1980). For 130g Wistar rats, the phenylalanine incorporation rate into heart protein *in vivo* is 2.35 nmol/2h per mg of protein. By assuming that the protein-synthesis rate in a given tissue is roughly proportional to its RNA content (Waterlow *et al.*, 1978), this value of the rate of protein synthesis can be corrected for the decreased RNA content of the rat hearts used in our experiments by using data from Table 2 and Fig. 2(a). This calculation makes the assumption that the 130g Wistar rats used by Garlick *et al.* (1980) have cardiac RNA contents similar to the Sprague-Dawley rats used in our experiments. The rate of protein synthesis *in vivo* of 2.35 nmol/2h per mg of protein can therefore be corrected to 1.45 nmol/2h per mg of protein for the approx. 300g rats used in our experiments. This agrees reasonably with data in Table 1. In the whole fed animal it is likely that insulin (which can stimulate both atrial and ventricular protein synthesis in the working perfused heart by 50–60%; P. H. Sugden & D. M. Smith, unpublished work) and/or other factors may increase the protein synthesis rate above that observed *in vitro*. In the 1-year-old 700–800g CFY rat, which has a cardiac RNA concentration of 9.1 µg/mg of protein (Table 16.8 of Waterlow *et al.*, 1978), which is similar to that of our 300g rats (Table 2), the fractional rate of protein synthesis *in vivo* is 10.8%/day (Crie *et al.*, 1981). Again, assuming that the phenylalanine content of ventricular protein is the same between strains of rats, a fractional synthesis rate of 10.8%/day is equivalent to a rate of phenylalanine incorporation of 1.27 nmol/2h per mg of protein. This value should be increased by 16% to take into account the slightly higher concentration of RNA in the hearts of rats used in our experiments, when it becomes 1.47 nmol of phenylalanine/2h per mg of protein. A further calculation from data in Table 2 shows that the rate of protein synthesis corresponds to 8.5g of protein/day per g of RNA. This is slightly less than rates *in vivo* given in Table 16.8 of Waterlow *et al.* (1978), but is not unreasonable considering the absence of insulin and/or other factors from perfusions. Although calculations of this nature are fraught with difficulties, we believe them to be a useful exercise in that they show a surprising correspondence between rates of cardiac protein synthesis *in vivo* and *in vitro*.

An implication of our finding of greater protein-synthesis rates in the atria than in ventricles is that, if heart protein mass is relatively stable, rates of protein degradation in the atria must be greater than in the ventricles. This possibility has been largely ignored previously (see, e.g., Sugden & Smith,

1982a). The results also imply a more rapid turnover of atrial protein than of ventricular protein.

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