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Regional variation and differential sensitivity of rat heart protein synthesis *in vivo* and *in vitro*

Victor R. PREEDY, David M. SMITH, Nessa F. KEARNEY and Peter H. SUGDEN
Department of Cardiac Medicine, Cardiothoracic Institute, 2 Beaumont Street, London W1N 2DX, U.K.

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In vivo, fractional rates of protein synthesis in atrial muscle of hearts taken from fed rats were 70% greater than in ventricular muscle. After 3 days starvation, atrial protein synthesis is inhibited, but the inhibition is less than in ventricles. A crude subcellular fractionation of the aqueous homogenates by centrifugation at 32000*g* showed that the supernatant and precipitate proteins were synthesized at the same rate in the ventricles. The fractional rates of protein synthesis and RNA/protein ratios in the right ventricle were 10% greater than in the left ventricle. Protein synthesis in both of these regions was inhibited equally by starvation. *In vitro*, rates of protein synthesis in atria and ventricles of anterogradely perfused rat hearts were stimulated by saturating insulin concentrations and were inhibited by starvation, but the effects in atria were smaller than in ventricles. Rates of protein synthesis in atria *in vitro* were 80–95% of rates *in vivo*. The heart therefore shows considerable regional variation in rates of protein synthesis *in vivo* and *in vitro*, and the sensitivity of protein synthesis in the various regions to interventions such as insulin and starvation differs.

We have shown that, in the heart perfused *in vitro*, the rate of protein synthesis and RNA/protein ratios in atria of hearts from approx. 300g rats were about twice the ventricular values (Smith & Sugden, 1983*a*). The efficiencies of protein synthesis (protein-synthesis rates expressed relative to RNA) were thus similar in the two compartments. Because the heart perfused *in vitro* is removed from the humoral milieu *in vivo*, and because the trauma involved in the removal of the heart from the animal may cause biochemical derangements, it was important to establish that differences between atria and ventricles could be observed *in vivo*. We also sought to investigate whether there was any differential sensitivity of atria and ventricles towards interventions (insulin, starvation) known to alter protein-synthesis rates, and whether differences exist between protein-synthesis rates in the right and left ventricle and between subcellular fractions. We have shown that the rates of protein synthesis in ventricles of the rat heart anterogradely perfused *in vitro* as in Taegtmeyer *et al.* (1980) correspond closely to rates *in vivo* (Preedy *et al.*, 1984). We investigated whether the same was true for atria.

Abbreviations used: k_s, fractional rate of protein synthesis; S_p, S_i and S_B, plasma (free), tissue (free) and protein (bound) specific radioactivity of [4-^{3}H]phenylalanine respectively.

Experimental

Sources of materials and rats, and subsequent handling of rats, were described in Preedy *et al.* (1984). Measurement of protein-synthesis rates *in vivo* used the method of Garlick *et al.* (1980) as described previously (Preedy *et al.*, 1984). The specific radioactivity of the 150mM-[4-^{3}H]phenylalanine injected was 0.17 or 0.7Ci/mol (see the Tables). At 10min after injection of the radiolabel, rats were decapitated and the hearts removed rapidly into an ice/water mixture for subsequent dissection. The right and left atria were dissected away and combined for each heart [we were unable to detect any difference between these two regions *in vitro* at normal left-atrial filling pressures (Smith & Sugden, 1983*b*)]. In some cases, the right-ventricular free wall was dissected away from the left-ventricular wall and interventricular septum (the septum being functionally a part of the left ventricle). Samples were stored and processed as described previously (Preedy *et al.*, 1984). A crude subcellular fractionation was carried out by adding potassium phosphate buffer (pH6.8) to the aqueous homogenates to give a final concentration

of 10mM. The homogenate was centrifuged at 32000*g* for 10min. The supernatants and precipitates (the latter containing myofibrils and other particulate matter) were processed as above.

Protein-synthesis rates *in vitro* were measured in the anterogradely perfused heart preparation of Taegtmeyer *et al.* (1980) as described previously (Preedy *et al.*, 1984). Right and left atria and ventricles from single hearts were combined and processed. Values of k_s for atria were calculated from a knowledge of the incorporation of [4-^{3}H]phenylalanine into protein in terms of pmol/mg of protein per day [see Smith & Sugden (1983*a*,*b*) and Preedy *et al.* (1984)] and the phenylalanine content of atrial protein (0.250 ± 0.005pmol/mg of protein; mean ± S.E.M., four observations) determined as in Preedy *et al.* (1984).

RNA was measured by the method of Munro & Fleck (1969) and protein by the method of Gornall *et al.* (1949), by using the standardization procedure described by Smith & Sugden (1983*a*).

Results are expressed as means ± S.E.M. Statistical significance was determined by a two-tailed unpaired Student's *t* test in most cases, except for results in Table 3, when a paired test was used. Values of $P < 0.05$ were taken as being statistically significant.

Results

Cardiac protein synthesis rates in vivo

Values of k_s in atrial muscle were 65–75% greater than in ventricular muscle in fed rats *in vivo* (Table 1). The atrial/ventricular ratio of k_s in fed rats appeared to be less *in vivo* (Table 1) than in glucose-perfused hearts from fed rats *in vitro* (Table 2; see also Smith & Sugden, 1983*a*). In Expt. 1 (Table 1), the atrial/ventricular ratio of k_s was significantly less ($P < 0.05$) than the atrial/ventricular ratio of RNA/protein, and the efficiency of protein synthesis in the ventricle was greater than in the atrium. *In vitro*, atrial/ventricular ratios of k_s and RNA/protein ratio in glucose-perfused hearts from fed rats are the same (Table 2; see also Smith & Sugden, 1983*a*). These results suggest that, although the rates of protein synthesis in atria *in vivo* are greater than ventricular rates, the differences are not as large as we have previously reported *in vitro* (Smith & Sugden, 1983*a*). It should be noted that the observed differences in k_s and RNA/protein ratios between atria and ventricles cannot be ascribed to any large differences in non-collagenous protein content between blood-free atria and ventricles (148 ± 3 and 159 ± 2mg/g wet wt. respectively, for 13 observations). The slight (but significant at $P < 0.05$) difference in protein content is probably related to the greater collagen content of atria (Caspari *et al.*, 1975).

We carried out a crude subcellular fractionation of hearts from experiments *in vivo* (Table 1). The ratio k_s in the 32000*g* supernatant/k_s in the 32000*g* precipitate was unity. The results were consistent in that the k_s values in the three fractions (whole homogenate mixed protein, and 32000*g* supernatant and precipitate) were the same. Using prolonged (6h) infusions of [U-^{14}C]tyrosine, Bates & Millward (1983) suggested that k_s ratios of sarcoplasmic to myofibrillar proteins *in vivo* were about 2 in several skeletal muscles from adult (400–450g body wt.) rats. In younger (less than 100g body wt.) rats, the ratio was less, but was always greater than 1 (but heart was not studied). The ratio was unity in the perfused rat heart (Sender & Garlick, 1973) or hemicorpus (Preedy & Garlick, 1983). Other workers, using skeletal muscles from other animals (man, fowl, rabbit), have sometimes shown *in vivo* k_s ratios of sarcoplasmic to myofibrillar proteins greater than unity (Halliday & McKeran, 1975) or of unity (Laurent *et al.*, 1978; Lobley & Lovie, 1979). In the fowl heart the ratio was about 1.4 (Laurent *et al.*, 1978). All previous work *in vivo* has used constant-infusion techniques for administration of labelled amino acids. There are problems of interpretation when this technique is used, since S_i rarely equilibrates with S_p and protein-synthesis rates may be decreased during infusion (Preedy & Garlick, 1984). Although these factors should not affect results in theory (provided that all amino acid pools used in the synthesis of protein have a common specific radioactivity), the 'flooding dose' method of Garlick *et al.* (1980) is more reliable, and this is the first time its use has been reported in fractionation studies. Alternatively, since 82% of ventricular protein is precipitated at 32000*g*, and since myofibrillar protein constitutes about 31% of ventricular protein (D. M. Smith & P. H. Sugden, unpublished work), the discrepancy between our results and those of Bates & Millward (1983) could be explained if the non-myofibrillar protein precipitated at 32000*g* turned over at a rate of about 2.5–3 times greater than the myofibrillar protein. This point requires further investigation.

In Table 1, Expt. 2, we show that, in the 3-day-starved rat *in vivo*, the k_s values in atria are decreased compared with the fed rat, but are still greater than in ventricles. However, the decreases in k_s (27%) and efficiency of protein synthesis (21%) in atria are not as large as in ventricles (40% and 39% respectively). In this experiment, the efficiency of atrial protein synthesis in starved rats was not significantly different from that in fed rats. There is a small decrease (9%) in atrial RNA/protein ratio in starved rats. We have previously shown small decreases in the ventricular RNA/protein ratios on starvation (Preedy *et al.*, 1984; see

Table 1. *Protein-synthesis rates in atria and ventricles in vivo*

Hearts were taken from fed or 3-day-starved rats and processed as descibed in the Experimental section. Seven animals were used in each group, except that for the subcellular fractionation, five hearts were processed. Body weight of rats in Expt. 1 was 269±3g. In Expt. 2, body weight of fed rats was 304±5g. For the starved rats, body weight was initially 288±5g and was 229±4g after 3 days of starvation. In Expt. 1, S_p at 10min was 333±2d.p.m./nmol. In Expt. 2, S_p at 10min after injection was 1335±18d.p.m./nmol in fed rats and 1327±6d.p.m./nmol in starved rats. The finding that the ventricular S_i is some 12% higher than S_p for starved animals in Expt. 2 is probably an artifact and arises from cumulative errors during processing of samples. For subcellular fractionation the S_i used was obviously the same for all fractions from a given heart. In Expts. 1 and 2, statistical significance of ventricular versus atrial value was $*P<0.05$; $**P<0.01$; $***P<0.001$. In Expt. 2, statistical significance of values in atria of starved rats versus atria of fed rats was $\dagger P<0.05$ (for statistical significance of starved versus fed ventricular values, see Preedy *et al.*, 1984).

Nutritional state	Compartment	S_i (d.p.m./nmol)	S_B (d.p.m./nmol)	k_s (%/day)	RNA/protein ratio (μg/mg)	Efficiency of protein synthesis (g of protein synthesized/g of RNA per day)
Expt. 1						
Fed	Atrium	325±3	0.477±0.033	18.9±1.3	12.4±0.5	15.3±0.9
	Ventricle	339±4*	0.284±0.011***	10.8±0.3***	6.0±0.1***	18.1±0.5*
	Atrial/ventricular ratio	0.96±0.04	1.69±0.12	1.76±0.12	2.08±0.08	0.85±0.06
	Ventricular 32000*g* supernatant	–	0.297±0.017	11.3±0.5	–	–
	Ventricular 32000*g* precipitate	–	0.288±0.011	11.0±0.3	–	–
	Supernatant/precipitate ratio	–	1.014±0.039	1.027±0.028	–	–
	Mixed ventricular protein	336±4	0.290±0.014	11.0±0.3	–	–
Expt. 2						
Fed	Atrium	1236±26	1.304±0.153	14.8±1.3	10.7±0.2	14.5±1.5
	Ventricle	1358±25**	0.908±0.071	9.0±0.7**	6.2±0.2***	14.5±1.0
	Atrial/ventricular ratio	0.91±0.03	1.53±0.23	1.64±0.24	1.75±0.05	0.95±0.15
Starved	Atrium	1285±9	1.060±0.099	10.8±1.0†	9.7±0.4†	11.4±1.2
	Ventricle	1487±6***	0.605±0.087	5.4±0.8***	6.1±0.3***	8.9±1.3
	Atrial/ventricular ratio	0.87±0.01	1.98±0.34	2.28±0.38	1.62±0.10	1.26±0.21

Table 2. *Protein-synthesis rates in atria in vitro*

Hearts were taken from fed or 3-day-starved rats and were perfused and processed as described in the Experimental section. Rats were from the same batch as those used in Expt. 2 of Table 1, and results may be compared directly. Insulin, when present in perfusates, was at a concentration of 50munits/ml. Body weight of the fed group was 287 ± 3g (eight rats), of the starved group it was 283 ± 3g (six rats) initially and 222 ± 3g after starvation for 3 days, and of the insulin-perfused group it was 286 ± 3g (five rats). Statistical significance of values versus values for fed-rat hearts (perfused in the absence of insulin) was: $*P<0.05$; $**P<0.01$; $***P<0.001$. For values of atrial/ventricular ratios in starved rat hearts versus fed+insulin-perfused rat hearts, statistical significance was: $\dagger P<0.05$; $\dagger\dagger P<0.001$.

	Atrium			Atrial/ventricular ratio		
	Fed	Starved	Fed+ insulin	Fed	Starved	Fed+ insulin
k_s (%/day)	11.4±0.3	6.8±0.2***	13.7±0.6**	1.96±0.06	2.21±0.11††	1.55±0.05***
RNA/protein ratio (μg/mg)	12.34±0.33	10.78±0.51*	11.93±0.44	1.82±0.05	1.84±0.08	1.73±0.07
Efficiency of protein synthesis (g of protein synthesized/g of RNA per day)	9.3±0.3	6.3±0.3***	11.6±0.7***	1.09±0.05	1.21±0.09†	0.90±0.3*

also Table 3 of the present paper). There is thus a differential sensitivity of protein synthesis in atria and ventricles towards starvation, which is reflected by changes in the atrial/ventricular ratios of k_s and efficiency for fed and starved rats.

We measured protein-synthesis rates in the right-ventricular free wall and the left-ventricular wall plus interventricular septum (Table 3). The k_s values and RNA/protein ratios were about 10% higher in the right ventricle than in the left. After 3 days starvation, there were significant decreases ($P<0.01$) in k_s, RNA/protein ratios and efficiencies in both compartments compared with fed animals. The RNA/protein ratio in the right ventricle of starved rats was still significantly greater than in the left. Thus both compartments are affected by starvation, which decreases both RNA/protein ratio and efficiency of protein synthesis.

In general, Tables 1 and 3 show that the greater the rate of protein synthesis the less was the value of S_i. Thus S_i values are greater in atria or ventricles of starved rats than in fed rats, and greater in ventricles than in atria. Similar results were obtained in prolonged constant-infusion experiments (Garlick *et al.*, 1975).

Cardiac protein-synthesis rates in vitro

Compared with glucose-perfused hearts of fed rats, k_s values in the atrium are decreased by 40% after 3 days starvation and increased by 20% by including saturating concentrations of insulin (50munits/ml) in perfusates (Table 2). (All hearts were perfused with 5mM-glucose as fuel.) Comparable changes in ventricles were −47% and +52% (results not shown). Changes in atrial efficiency of protein synthesis were −32% and +24% for 3-day-starved and plus-insulin perfusions respectively (compared with −38% and +50% for ventricles, results not shown). The differential sensitivity of atrial and ventricular protein synthesis to starvation and insulin *in vitro* produced significant alterations in the atrial/ventricular ratios of k_s and efficiency after these interventions (Table 2). In hearts of starved rats, the atrial/ventricular ratio of k_s was significantly different ($P<0.05$) from that for RNA/protein (Table 2). Differential sensitivity of atrial and ventricular protein synthesis to insulin may be the reason why in the fed rat *in vivo* (when plasma insulin concentrations will be high), the atrial/ventricular k_s ratio is significantly ($P<0.05$) less than the atrial/ventricular RNA/protein ratio (Table 1), whereas in hearts of fed rats perfused with glucose in the absence of insulin there is no difference (Table 2; see also Smith & Sugden, 1983*a*). It is not known whether the synthesis of any subcellular fraction is disproportionately affected by starvation or insulin. In the perfused heart, however, Sender & Garlick (1973) showed that insulin stimulated synthesis of both the sarcoplasmic and myofibrillar proteins equally.

A comparison of the rates of atrial protein synthesis *in vivo* and *in vitro* shows that *in vitro* k_s values and efficiencies in glucose/insulin-perfused hearts from fed rats are about 93% and 80% respectively of values *in vivo* for the fed rat (Table 1, Expt. 2, and Table 2, for which the same batch of rats was used). The k_s values and efficiencies for atria of starved rats *in vivo* are similar to the values for glucose-perfused fed-rat heart atria *in vitro*, suggesting the importance of insulin in the

Table 3. *Protein-synthesis rates in left and right ventricles in vivo*

Hearts were taken from fed or 3-day-starved rats as described in the Experimental section. Seven fed rats and four starved rats were used. Body weight was 325±6g. After 3 days of starvation, rats had lost about 20% of their initial body weight. The right-ventricular free wall constituted 20±1% of the total ventricular wet weight. For right-ventricular values versus values for left ventricle+septum, statistical significance was $*P<0.05$ and $**P<0.01$ by a paired t test. Data for S_i versus S_B and for the fed-to-starved transition were not analysed statistically [results were similar to those in Preedy *et al.* (1984) for whole ventricles].

Nutritional state	Compartment	S_i (d.p.m./nmol)	S_B (d.p.m./nmol)	k_s (%/day)	RNA/protein ratio (μg/mg)	Efficiency of protein synthesis (g of protein synthesized/g of RNA per day)
Fed	Left-ventricular free wall + interventricular septum	349±4	0.296±0.007	11.22±0.29	6.47±0.08	17.34±0.45
	Right-ventricular free wall	358±7	0.335±0.007**	12.66±0.34**	6.95±0.15**	18.20±0.18
Starved	Left-ventricular free wall + interventricular septum	373±5	0.216±0.009	7.69±0.41	5.23±0.19	14.76±0.98
	Right-ventricular free wall	371±14	0.226±0.016	8.05±0.32	5.57±0.25*	14.46±0.69

regulation of atrial protein synthesis *in vivo*. The conclusions drawn from the comparison of rates of protein synthesis in atria *in vivo* and *in vitro* are the same as those we have previously made for the ventricles (Preedy *et al.*, 1984).

Discussion

There is considerable regional variation in rates of protein synthesis in the heart *in vivo* and *in vitro*. In this paper, we have shown that protein-synthesis rates in atria and ventricles *in vivo* differ as we have shown *in vitro* (Smith & Sugden, 1983*a*). Thus differences *in vitro* cannot be ascribed entirely to the removal of humoral factors. Furthermore, right and left ventricles show differences in k_s values and RNA/protein ratios *in vivo*. However, the atrial/ventricular ratios of k_s *in vivo* were not as great as in the glucose-perfused fed-rat heart *in vitro*, and this difference may be the result of differential sensitivity of atrial and ventricular protein synthesis to insulin. The stimulation of atrial protein synthesis by insulin *in vitro* was less than in the ventricle. Similarly the inhibition of atrial protein synthesis by starvation was greater in the ventricle than in the atrium both *in vivo* and *in vitro*. Atrial protein synthesis is thus less sensitive than ventricular synthesis to these interventions. It is interesting that, even within a single organ, protein synthesis shows not only a difference in terms of absolute rate but also a differential sensitivity to interventions. Differential sensitivity of protein synthesis in different muscles towards saturating concentrations of insulin *in vitro* has been shown previously. Preedy & Garlick (1983) showed in the perfused rat hemicorpus that protein synthesis in the soleus (a slow, aerobic, muscle) was not stimulated by insulin, whereas in the gastrocnemius or plantaris (fast, anaerobic, muscles) insulin stimulated by about 40–45%. However, we believe that this is the first time regional differential sensitivity has been shown within the same organ.

The biological reasons for differences in k_s between atria and ventricles are obscure. In many ways (e.g., in its time to peak tension) atrial muscle resembles fast skeletal muscle, and ventricular muscle resembles slow skeletal muscle (see Cummins, 1983, for a review). However, k_s values in slow skeletal muscles are greater than in fast skeletal muscles (see, e.g., Laurent *et al.*, 1978; Odedra *et al.*, 1982). It has been suggested that muscular activity may be of prime physiological importance in determining skeletal muscle k_s since muscle hypertrophy can still be induced by mechanical overloading in diabetic or starved rats (Goldberg *et al.*, 1975; Goldberg, 1979). Other work (e.g. Garlick *et al.*, 1983) has implicated

insulin and possibly other humoral factors as being important in regulation of protein synthesis. Probably both factors are important. For the heart, the wet weight declines at about the same rate as the body weight during starvation, and thus the cardiac output/g of heart should not vary. Blood pressure should be relatively unchanged. Pressure–volume work done/g of heart thus should not change dramatically during starvation, and *in vivo* this type of work accounts for more than 90% of heart work. Protein synthesis is inhibited during starvation, and thus mechanical activity cannot be the sole determinant of cardiac k_s. Furthermore, *in vitro*, when cardiac output/g and afterloads are identical in hearts from fed and starved rats, differences in k_s are still observed.

The values for k_s and efficiency of protein synthesis for atria in hearts perfused with media containing glucose/insulin *in vitro* are similar to those *in vivo*. Since fed rats were used, this comparison is valid because it is likely that plasma insulin concentrations in the experiments *in vivo* will be high (see Preedy *et al.*, 1984, for a discussion). Of relevance are the experiments by Garlick *et al.* (1983), who showed that infusion of insulin into fed rats to very high plasma concentrations did not increase skeletal-muscle protein-synthesis rates, showing that, in the fed rat, endogenous insulin concentrations are maximally effective. The finding that k_s *in vitro* is closer to k_s *in vivo* than are the efficiencies *in vitro* and *in vitro* reflects the presence of blood in the hearts *in vivo*. The finding that efficiency *in vitro* is about 80% of the value *in vivo* may reflect oxidation of cysteine in the perfusate (Chua *et al.*, 1983). It is also known that hypertensive pressures will stimulate atrial and ventricular protein synthesis *in vitro* (Schreiber *et al.*, 1981; Smith & Sugden, 1983*b*, Kira *et al.*, 1984), but presumably the rats used in our experiments *in vivo* were not hypertensive, nor were the perfusion pressures *in vitro*. [Afterload *in vitro* was in fact hypotensive. Raising the afterload to normotensive values (14kPa) may increase ventricular protein-synthesis rates by about 10% (D. M. Smith & P. H. Sugden, unpublished work).] Thus, as with ventricular protein synthesis (Preedy *et al.*, 1984), atrial synthesis rats *in vitro* correspond closely to rates *in vivo*.

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