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Stimulation of left-atrial protein-synthesis rates by increased left-atrial filling pressures in the perfused working rat heart *in vitro*

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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We investigated the effect of an increase in the left-atrial filling pressure on the rate of left-atrial protein synthesis in the left-side-perfused working rat heart preparation of Taegtmeyer, Hems & Krebs [(1980) *Biochem. J.* **186**, 701–711]. An increase in filling pressure (preload) at a constant aortic pressure (afterload) increased both the intra-atrial pressure and the atrial stroke volume. The aortic pressure (afterload) was held constant. An increase in filling pressure from 5 to 20 cmH_2O at an aortic pressure of 70 cmH_2O, or an increase in filling pressure of 7.5 to 20 cmH_2O at an aortic pressure of 100 cmH_2O, significantly stimulated the rates of left-atrial protein synthesis by 30–40%. The stimulation was observed when the rates of protein synthesis were expressed relative to either protein or RNA content. Since perfusate entering the right atrium from the coronary circulation left that atrium passively, the rate of protein synthesis in this compartment can be used as an internal control. Rates of right-atrial protein synthesis were similar to those in the left atria exposed to the lower filling pressures and were unaffected by the increases in left-atrial filling pressure. We suggest that the acute effects of increased left-atrial filling pressure on protein synthesis in that compartment may be important in the development of left-atrial hypertrophy. This condition is seen in patients who have raised pulmonary venous pressures in, for example, mitral stenosis.

The response of the heart to an increased workload is to increase its protein mass. The process of ventricular hypertrophy, which occurs in response to a number of experimental manipulations, has been extensively investigated, since it is of considerable clinical importance (for reviews, see Rabinowitz & Zak, 1972; Zak & Rabinowitz, 1979). For example, aortic coarctation *in vivo* (which induces cardiac pressure overload and subsequent cardiac hypertrophy) causes early changes in RNA and protein synthesis (Florini & Dankberg, 1971) and in the activity of RNA polymerases (Cutilletta *et al.*, 1978). However, the means by which an increased workload causes an increase in protein accretion has remained elusive. In order to resolve the responses in the heart to increased workload, many investigators have turned to perfusions *in vitro*. Several reports have suggested that increases in pressure workload in the anterogradely perfused (working) heart induced by increases in aortic pressure (afterload) caused a stimulation of ventricular protein synthesis (for a review, see Schreiber *et al.*, 1981). However, such investigations are bedevilled by difficulties in interpretation. For example, the time course of protein synthesis has been non-linear during experiments (Hjalmarson & Isaksson, 1972*a*), working hearts have been compared with Langendorff (1895)-perfused hearts (Morgan *et al.*, 1980), and increases in aortic pressure proportionally increase the rates of coronary flow, which may itself stimulate protein synthesis by, for example, improving substrate delivery and opening previously constricted capillaries. The experiments of Schreiber *et al.* (1975) have suggested a lack of effect of coronary flow on protein synthesis, provided that the rate of coronary flow is sufficient to meet the oxygen and substrate supply to the heart. Furthermore, *in vivo*, hearts exposed to a raised aortic pressure, but with a relatively constant stroke volume (increased pressure workload), hypertrophy by thickening their left-ventricular wall widths but keeping their chamber volumes relatively constant (concentric hypertrophy). Hearts induced to pump larger volumes of fluid at a constant aortic pressure (increased volume workload) respond by increasing their left-ventricular chamber volumes but keeping their wall widths relatively constant (eccentric hypertrophy). Both processes involve increases in protein accretion, but the deposition of protein within the heart differs. Although some investigators have suggested that there is a stimulation of protein synthesis by increased volume workload *in*

vitro (Schreiber *et al.*, 1966; Tomita, 1966), most investigators agree that there is no such effect (Hjalmarson & Isaksson, 1972*a*; Schreiber *et al.*, 1975, 1981).

Atrial protein synthesis has been largely ignored. There are several pathological examples of left- or right-atrial hypertrophy in conditions such as mitral stenosis or regurgitation (left atrium) or analogous tricuspid-valve problems (right atrium). In valvular stenosis, a raised intra-atrial pressure is observed. We have compared atrial and ventricular protein-synthesis rates and RNA contents (Smith & Sugden, 1983*b*). It seemed to us that the anterogradely perfused heart presented an ideal system in which the effects of raised filling pressures on atrial protein-synthetic rates could be investigated in the absence of perturbing factors which have dogged investigations of ventricular protein synthesis.

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-^{14}C]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 in the Departmental animal house for at least 5 days before use, during which time they had free access to food and water.

Heart perfusions

Hearts were perfused as anterograde (working) preparations by the method of Taegtmeyer *et al.* (1980) as described in detail previously (Sugden & Smith, 1982*b*). Hearts were initially perfused as non-recirculating Langendorff (1895) preparations with Krebs & Henseleit (1932) bicarbonate-buffered saline solution supplemented with 5mM-glucose and equilibrated with O_2/CO_2 (19:1). The pulmonary vein was cannulated as quickly as possible and the preparation was switched to a recirculating, anterogradely perfused, preparation. The perfusate (100ml) was the same in content as for the Langendorff preperfusion, except that it was supplemented with 0.4mM-[U-^{14}C]phenylalanine (sp. radioactivity 0.17Ci/mol) and the other plasma amino acids at a concentration of 0.2mM. Left-atrial filling pressures and aortic pressures are described in the Tables. Coronary and aortic flows were measured as described previously (Sugden & Smith, 1982*b*). Heart rate was measured by a pressure transducer attached to the side arm of the aortic cannula and a suitable recorder. Lactate release into the perfusate was measured by the method of Hohorst (1963).

Incorporation of [U-^{14}C]phenylalanine into atrial or ventricular protein

After 120min of perfusion, the tubing leading to the left-atrial and aortic cannulae was closed and the left and right atria were dissected free from the heart. They were frozen separately in liquid N_2. Atria were ground to a fine powder in ground-glass homogenizers cooled in liquid N_2 and were homogenized in 5% (w/v) trichloroacetic acid (5ml). Precipitated protein was separated by centrifugation in a bench centrifuge, washed once with 10ml of 5% trichloroacetic acid and twice with 10ml of 0.5M-$HClO_4$. The precipitated protein was resuspended in 4ml of water and digested by addition of 1ml of 0.5M-NaOH and incubation at 37°C for 1h. After this time, the digests were centrifuged in a bench centrifuge to remove undigested collagen, and the protein in the supernatant (4ml) was precipitated by the addition of 0.2ml of 12.5M-$HClO_4$. The protein was separated by centrifugation in a bench centrifuge and the supernatants were used for RNA determinations by the method of Munro & Fleck (1969) as described previously (Smith & Sugden, 1983*b*). [U-^{14}C]Phenylalanine incorporation into the washed precipitated atrial protein was measured by dissolving the protein in 3ml of NCS tissue solubilizer (with heating at 50°C if necessary), followed by liquid-scintillation spectrometry in a toluene-based fluor (15ml) containing 5g of 2,5-diphenyloxazole/litre and 0.1g of 1.4-bis-(5-phenyloxazol-2-yl)benzene/litre. Quenching was determined by the external-standard method. Protein in the 0.1M-NaOH digests was determined by the method of Lowry *et al.* (1951), with rat heart atrial protein (Smith & Sugden, 1983*b*) as a standard. Rates of ventricular protein synthesis were measured as described previously (Smith & Sugden, 1983*b*). In this instance, protein was measured by the method of Gornall *et al.* (1949).

Measurement of [U-^{14}C]phenylalanine specific radioactivity in perfusates

[U-^{14}C]Phenylalanine specific radioactivity in perfusates was measured as described previously (Smith & Sugden, 1983*b*). Radioactivity in perfusate samples was measured by liquid-scintillation spectrometry (Smith & Sugden, 1983*b*), with quenching estimated by the external-standard method. Phenylalanine in suitably diluted perfusates was measured by the method of Rubin & Goldstein (1970) as modified by Sugden & Smith (1982*a*). Concentrations and specific radioactivities of [U-^{14}C]phenylalanine were constant over the course of the perfusions.

Statistical methods

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

Results

General considerations

In the perfusion system used in this investigation, perfusate enters the left atrium. It passes into the left ventricle and is expelled via the aortic cannula. A proportion enters the coronary circulation, from which it passes into the right atrium. It then either exits passively from the cut vena cava or passes into the right ventricle, from which it is expelled against no applied resistance through the pulmonary artery (which is also cut). There are no valves between the pulmonary vein and the left atrium. Thus the applied left-atrial filling pressure is exerted against the left-atrial wall. The effects of increasing left-atrial filling pressure are shown in Table 1. At an aortic pressure of 70 cmH_2O, raising the filling pressure increases the aortic flow, the cardiac output and the stroke volume. Coronary flow is also increased. The probable reason for this is that, although coronary flow is normally proportional to the aortic pressure, in this case the coronary flow is insufficient to support the oxygen requirement for the increased external work done, and the heart becomes hypoxic (Sugden & Smith, 1982*b*). This is borne out by the finding that at a filling pressure of 20 cmH_2O lactate release is stimulated (Table 1; see also Sugden & Smith, 1982*b*). The coronary vessels therefore dilate to increase coronary flow to a maximum. In contrast, increasing the filling pressure at an aortic pressure of 100 cmH_2O increases aortic flow, cardiac output and stroke volume, but does not increase coronary flow, which is now sufficient to satisfy the oxygen demands. This is borne out by the finding that lactate release is not stimulated by raising the filling pressure (Table 1). There were no significant differences in heart rates between the various preparations.

Table 1. *Cardiac performance data*

Hearts were perfused as described in the Experimental section. Aortic and coronary flows, cardiac output, heart rate and stroke volume refer to values determined after 70 min of perfusion. Lactate production was measured over the 10–40 min perfusion period. Values for the increased filling pressure perfusions were statistically significantly different from values at the lower filling pressure but identical aortic pressure at: $*P<0.01$, $**P<0.001$.

Filling pressure (cmH_2O)	Aortic pressure (cmH_2O)	No. of observations	Rat body wt. (g)	Heart wet wt. (g)	Aortic flow (ml/min per g wet wt. of heart)	Coronary flow (ml/min per g wet wt. of heart)	Cardiac output (ml/min per g wet wt. of heart)	Cardiac output at 110 min/ cardiac output at 10 min	Heart rate (beats/min)	Stroke volume (μl)	Lactate production (μmol/30 min per g wet wt.)
5	70	10	329 ± 4	0.995 ± 0.037	37.8 ± 1.3	13.6 ± 0.8	51.4 ± 1.5	0.94 ± 0.06	252 ± 12	212 ± 9	6.6 ± 1.8
20	70	8	322 ± 8	1.000 ± 0.020	74.2 ± 2.5**	17.8 ± 0.8*	92.0 ± 2.8*	0.89 ± 0.03	283 ± 14	333 ± 11**	17.9 ± 2.6*
7.5	100	10	318 ± 8	0.964 ± 0.021	42.4 ± 2.3	20.9 ± 1.6	62.2 ± 3.7	0.97 ± 0.05	250 ± 16	254 ± 16	5.9 ± 1.0
20	100	10	317 ± 8	0.992 ± 0.041	58.7 ± 4.5*	23.0 ± 1.6	81.7 ± 5.1*	0.92 ± 0.06	244 ± 11	388 ± 22*	6.0 ± 1.3

Stimulation of left-atrial protein synthesis by increases in left-atrial filling pressure

We have shown previously that rates of atrial protein synthesis are linear with time (Smith & Sugden, 1983*b*). Amino acid concentrations used in the present study are saturating for protein synthesis (Smith & Sugden, 1983*b*). We found that left-atrial protein-synthesis rates at two aortic pressures were stimulated by 30–40% by increasing the left atrial pressure 3–4-fold (Table 2). The stimulation was apparent when protein-synthesis rates were expressed relative to either protein or

Table 2. *Comparison of the rates of atrial protein synthesis at various left-atrial filling pressures*

Hearts were perfused and protein synthesis was measured as described in the Experimental section. Statistical significance is $*P < 0.01$, or $**P < 0.001$, for rates of protein synthesis in left atria for hearts perfused at 20 cmH_2O filling pressure/70 cmH_2O aortic pressure compared with perfusions at 5 cmH_2O filling pressure/70 cmH_2O aortic pressure, or for hearts perfused at 20 cmH_2O filling pressure/100 cmH_2O aortic pressure compared with perfusions at 7.5 cmH_2O filling pressure/100 cmH_2O aortic pressure. For rates of protein synthesis in right atria, statistical significance, compared with rates of protein synthesis in the left atria in the same perfusions, is: $\dagger P < 0.05$; $\dagger\dagger P < 0.01$; $\dagger\dagger\dagger P < 0.001$.

Filling pressure (cmH_2O)	Aortic pressure (cmH_2O)	No. of observations	Protein synthesis in: Left atrium: pmol of phenylalanine/2 h per mg of protein	Left atrium: pmol of phenylalanine/2 h per μg of RNA	Right atrium: pmol of phenylalanine/2 h per mg of protein	Right atrium: pmol of phenylalanine/2 h per μg of RNA
5	70	10	1648 ± 78	89 ± 5	1732 ± 88	95 ± 9
20	70	8	2280 ± 56**	124 ± 7**	1710 ± 88†††	96 ± 9†
7.5	100	10	1799 ± 108	76 ± 5	1927 ± 114	84 ± 6
20	100	10	2324 ± 110*	102 ± 5*	1852 ± 113††	81 ± 5††

RNA. Because the right atrium is vented into the perfusion chamber and because (at least for the perfusions at 100 cmH_2O aortic pressure) delivery of perfusate into the right atrium from the coronary sinus and perfusion of the heart by the coronary circulation are the same at both filling pressures used (Table 1), the rate of protein synthesis in the right atrium can be used as an internal control for left-atrial protein-synthesis rates. Thus there is no difference between right-atrial protein-synthesis rates for any of the four preparations studied (Table 2). However, in the preparations with raised left-atrial filling pressure, there are significant increases in left-atrial protein-synthesis rates compared with right-atrial protein-synthesis rates in the same hearts (Table 2). These differences can be seen when protein-synthesis rates are expressed relative to protein or to RNA. We were unable to detect any significant stimulation in the rates of ventricular protein synthesis by increasing the left-atrial filling pressure at a constant aortic pressure, thereby increasing volume workload (Sugden & Smith, 1982*b*; Table 1). For the four filling/aortic pressures used (given as cmH_2O), rates of ventricular protein synthesis (given as pmol of phenylalanine incorporated/2 h per mg of protein) in pooled ventricular protein were respectively: 5/70, 1052 ± 57 (10); 20/70, 1103 ± 77 (8); 7.5/100, 1113 ± 79 (10); 20/100, 1183 ± 76 (10). This result agrees with the conclusions of other workers (Hjalmarson & Isaksson, 1972*a*; Schreiber *et al.*, 1975). Furthermore, there was no significant stimulation of ventricular protein-synthesis rates by increasing the aortic pressure from 70 to 100 cmH_2O at a filling pressure of 20 cmH_2O. The increase in aortic pressure in the latter experiments is, however, less than that imposed by workers who have observed stimulation of protein-synthesis rates (Hjalmarson & Isaksson, 1972*a*).

An increase in filling pressure at a given aortic pressure increases the external work done by the heart. This increases the demand for fuels (glucose in our experiments) and oxygen from the coronary circulation. If oxygen supply is insufficient, this is reflected in an increased release of lactate. We observed stimulation of lactate release in perfusions at 20 cmH_2O filling pressure and 70 cmH_2O aortic pressure (Table 1; see also Sugden & Smith, 1982*b*), indicating cardiac hypoxia. However, there was no difference between the rates of protein synthesis in this preparation and the 5 cmH_2O-filling-pressure preparation when either the right atria or pooled ventricles were investigated. This is interesting, since it has been suggested that in both heart and diaphragm provision of lactate (admittedly with adequate oxygenation) can stimulate protein synthesis (Rannels *et al.*, 1974; Hedden & Buse, 1982). Secondly, it has been suggested that, although cardiac anoxia inhibits protein synthesis, protein synthesis is much less sensitive to hypoxia than is protein degradation (Jefferson *et al.*, 1971; Chua *et al.*, 1979). Hearts perfused at 20 cmH_2O filling pressure and 70 cmH_2O aortic pressure are quite severely hypoxic and resemble hearts perfused with medium equilibrated with $O_2/N_2/CO_2$ (Sugden & Smith, 1982*b*; Smith & Sugden, 1983*a*). It therefore seems unlikely that endogenously derived lactate can alter protein-synthesis rates unless lactate production and hypoxia are acting against one another. *In vivo*, in the heart, it is always likely that stimulation of endogenous lactate production and hypoxia are contemporaneous. Finally, it should be mentioned that there is no significant difference in RNA content between right and left atria (results not shown).

Discussion

The use of the anterogradely perfused heart to investigate effects of intra-atrial pressure/stretch on atrial protein synthesis has not been described previously. It offers a useful model system for investigating cardiac protein synthesis which is free of many of the variables (e.g. in coronary flow) which have detracted from investigations of the effects of preload and afterload (i.e. workload) on ventricular protein synthesis.

Because there are not any valves regulating the entry of perfusate to the atrium, an increased filling pressure must increase the atrial wall tension. However, the volume of the atrium must also presumably increase with an increased filling pressure, since stroke volume increases (Table 1). This assumes that changes in atrial stroke volume are not brought about by changes in atrial ejection fraction. Because raised filling pressure increases atrial volume (this can be seen during the perfusions), atrial myofibrillar length must also increase. We do not know the relative importance of atrial wall tension and passive stretch in the stimulation of atrial protein synthesis that we have observed. Certainly both active tension development and passive stretch stimulate protein synthesis in the isolated rabbit heart papillary-muscle preparation (Peterson & Lesch, 1972). In skeletal muscle there are changes in the rates of protein synthesis and degradation in response to passive stretch, with rates of synthesis being stimulated (for a review, see Goldspink, 1981; see also Goldspink *et al.*, 1983).

We have discussed previously whether rates of ventricular protein synthesis in hearts perfused *in vitro* are comparable with rates *in vivo* (Smith & Sugden, 1983*b*). We concluded that the rates we measured *in vitro* were about 70% of those for fed rats *in vivo* after making allowances for different heart RNA contents. We suggested that this discrepancy might be the result of the presence of stimulators of protein synthesis such as insulin in the plasma of fed animals. We do not have any information about the rates of atrial protein synthesis *in vivo*, but, extrapolating from the ventricular data, they might be expected to be about 50% higher than rates *in vitro* when fed rats are used in both instances. The rates of protein synthesis described here are maximal as far as provision of amino acids is concerned (Smith & Sugden, 1983*b*). However, at a filling pressure of 5 cmH_2O and an aortic pressure of 70 cmH_2O, atrial protein synthesis is stimulated by 50–60% by addition of 50 munits of insulin/ml compared with experiments in the absence of insulin at saturating amino acid concentrations (P. H. Sugden & D. M. Smith, unpublished work). We do not know whether the stimulatory effects of maximally effective concentrations of insulin (and possibly as yet unknown humoral factors) and the effects of filling pressure on the rates of left-atrial protein synthesis are additive, or whether filling pressure affects the insulin-concentration dependence of protein synthesis, or whether effects of filling pressure on left-atrial protein synthesis would no longer be observed at maximally effective concentrations of insulin. This is clearly an important point, but we do not have any pertinent results as yet. It is, however, known that cardiac pressure development *in vitro* affects both the concentration-dependence of inhibition and maximal inhibition of protein degradation by insulin (Rannels *et al.*, 1975; Sugden & Smith, 1982*a*; Smith & Sugden, 1983*a*). Cardiac pressure development *in vitro* also affects the insulin-concentration dependence and maximal rates (in the presence of maximally effective insulin concentrations) of carbohydrate uptake (Neely *et al.*, 1967; Sugden & Smith, 1982*b*). Such findings suggest that cardiac workload may have effects on insulin-affected processes which are additional to the effects of maximally or submaximally effective concentrations of insulin. It should be noted that the effect of raising left-atrial filling pressure is to increase left-atrial workload by increasing left-atrial wall tension and by increasing stroke volume.

It has been suggested that increased cardiac workload increases amino acid uptake by the left ventricle (Ahrén *et al.*, 1972). This effect was only observed when afterload (aortic pressure) was increased and not when filling pressure was increased. It is possible that the stimulation of left-atrial protein synthesis by increased filling pressure could be caused by increased intracellular amino acid concentration induced by the stimulation of amino acid transport by increased atrial workload. [It should be noted that Ahrén *et al.* (1972) investigated only left-ventricular amino acid uptake.] We consider this unlikely in view of the saturating amino acid concentrations used in the present experiments (Smith & Sugden, 1983*b*). Furthermore, Morgan *et al.* (1980) have observed decreases in intracellular amino acid concentration with increased workload when rates of protein synthesis were increased. It has been suggested that the stimulation of protein synthesis by workload is exerted at the level of ribosomal initiation (Hjalmarson & Isaksson, 1972*b*; Morgan *et al.*, 1980). The mechanism by which this is brought about is obscure. Many of these experiments (and those on amino acid uptake) are open to objections regarding stimulation of coronary flow by afterload (see above).

We suggest that the acute effects of raised intra-atrial pressure/wall stretch may be important in the development of atrial hypertrophy. In mitral stenosis, the left-atrial pressure is frequently raised from a normal value of 4–11 cmH_2O (average

7 cmH_2O) to values of 25–30 cmH_2O or more. This condition is often characterized by an enlarged left atrium with a thickened wall (Ross, 1982). In mitral regurgitation, left-atrial hypertrophy may also be observed. This condition resembles a volume overload on the left atrium. On the right side of the heart, the much rarer conditions of tricuspid stenosis and regurgitation may cause right-atrial hypertrophy (Ross, 1982). Pulmonary arterial hypertension causes right-ventricular and atrial hypertrophy. Pulmonary-venous hypertension induced by conditions other than mitral stenosis (see above) also results in left-atrial hypertrophy (Lukas, 1982). Thus conditions that raise intra-atrial pressure (and to a lesser extent, volume pumped by the atrium) often cause atrial hypertrophy. The acute effects that we describe in this paper may be of importance in mediating this response.

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