Effects of pressure overload and insulin on protein turnover in the perfused rat heart

Prostaglandins are not involved although their synthesis is stimulated by insulin

David M. SMITH and Peter H. SUGDEN

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

A modified anterogradely perfused rat heart preparation is described in which all the cardiac output passes through the coronary circulation. Such a preparation develops hypertensive aortic pressures. Hypertensive aortic pressures or insulin stimulate the rate of cardiac protein synthesis and inhibit the rate of protein degradation. Aortic pressure and insulin may be important in the regulation of cardiac nitrogen balance *in vivo*. By abolishing cardiac prostaglandin synthesis with 4-biphenylacetate, we were able to investigate the possible involvement of prostaglandins in the modulation of protein turnover by pressure overload or insulin. There was no evidence of any involvement. However, insulin stimulated and cycloheximide inhibited cardiac prostaglandin synthesis. These findings are consonant with an enzyme involved in prostaglandin synthesis being short-lived and prostaglandin synthesis being rapidly influenced by activators and inhibitors of protein synthesis and degradation.

INTRODUCTION

Passive stretch stimulates k_s in skeletal muscle (reviewed in [1]; see also [2]) and in right-ventricular papillary muscle [3]. In the perfused heart, increased aortic or pulmonary arterial pressure stimulates k_s and possibly inhibits k_d *in vitro* [4–10]. *In vivo*, hypertension (i.e. pressure overload) induces cardiac hypertrophy (reviewed in [11] and [12]). However, the relevance of the acute effects of pressure overload on protein turnover *in vitro* to the development of cardiac hypertrophy remains obscure.

Using isolated muscle preparations, much recent work has been concentrated on the involvement of prostaglandins in protein turnover [13,14]. In brief, prostaglandin precursors (such as arachidonate or prostaglandin H_2) stimulated both k_s and k_d in soleus and diaphragm, an effect which was blocked by indomethacin. Stimulation of k_s was attributed to prostaglandin $F_{2\alpha}$, and stimulation of k_d to prostaglandin E_2 . There was some heterogeneity of response between muscles: both k_s and k_d were stimulated by arachidonate or prostaglandin H_2 in soleus or diaphragm, whereas only k_d was stimulated in extensor digitorum longus or atrial strips. With respect to protein synthesis, similar results were obtained with rabbit forelimb muscles [15]. Further studies with these muscles showed that stimulation of k_s by intermittent stretching and inhibition of k_s by dexamethasone were correlated with prostaglandin $F_{2\alpha}$ synthesis [16,17]. Furthermore, stimulation of muscle k_s by insulin was prevented by indomethacin *in vitro* and *in vivo*, and was correlated with prostaglandin concentrations [18,19].

In this study, we used the anterogradely perfused heart in which perfusion is via left atrial inflow [20,21]. This preparation more closely simulates the situation *in vivo* than does retrograde perfusion. We also describe a

modified anterograde preparation in which all the cardiac output passes through the coronary circulation and the heart develops the maximum aortic pressure of which it is capable. We have attempted to determine the relevance of stimulation of k_s by acute changes in aortic pressure to the situation *in vivo*, and we have investigated whether prostaglandins might be involved in the regulation of cardiac protein turnover.

EXPERIMENTAL

Materials and animals

Sources have been given previously [22]. Male Sprague–Dawley rats were housed and fed as described previously [22]. Additionally, prostaglandin assay kits were from Amersham International, Amersham, Bucks., U.K., and 4-biphenylacetic acid was from Aldrich, Gillingham, Dorset, U.K.

Heart perfusions

Hearts from fed rats (about 300 g body wt.) were perfused anterogradely with bicarbonate-buffered saline [23] as described previously [21,24]. Concentrations of substrates or hormones added to perfusates are described in the Tables. When the sodium salts of lactate and acetate (prepared as in [25]) were present, perfusate NaCl concentrations were decreased accordingly. When present, insulin and/or 4-biphenylacetic acid (100 μ l of 20 mg/ml in ethanol per 100 ml of perfusate) were included in both the retrograde preperfusion medium (which was discarded) and the anterograde recirculated perfusion medium. At the concentration present in the perfusions, ethanol did not affect k_s or k_d . When k_s was measured, the anterograde perfusate alone contained 0.4 mM-[U- 14 C]phenylalanine (sp. radioactivity about

Abbreviations used: k_s , rate of protein synthesis relative to tissue protein content; k_{RNA} , rate of protein synthesis relative to tissue RNA content; k_d , rate of protein degradation.

0.1 Ci/mol) and the remaining amino acids necessary for protein synthesis at the concentration of 0.2 mM as described previously [26]. After 2 h of anterograde perfusion, [U-¹⁴C]phenylalanine incorporation into ventricular protein and the specific radioactivity of [U-¹⁴C]phenylalanine in the perfusates were measured as described in [27,28]. In all protein-synthesis experiments, k_{RNA} was also measured. However, for the sake of brevity, we have not presented these results, since changes in k_s could not be ascribed to differences in RNA/protein ratios between hearts. When k_d was measured, amino acids were omitted from perfusates and 20 μM -cycloheximide was included in both the retrograde-perfusion and the anterograde-perfusion media. Release of phenylalanine was measured as described in [25] by the method of Rubin & Goldstein [29] and was converted into units of pmol of phenylalanine released/2 h per mg of protein by using a protein/dry-weight ratio of 0.8 [22]. When prostaglandin release was measured, after switching from retrograde to anterograde perfusion, the initial 200 ml of perfusate pumped by the heart were discarded. After this, 100–120 ml of perfusate was recirculated and samples (0.5 ml) were withdrawn every 20 min. Perfusate volumes were measured after 2 h of perfusion by the addition of [U-¹⁴C]sucrose [25].

In the anterograde perfusions (during which k_s or k_d was measured), hearts were perfused at three different combinations of preload (left-atrial filling pressure) and afterload (aortic pressure) which were imposed immediately on switching from the retrograde preperfusion to the anterograde perfusion.

(i) Hypotensive, in which the preload was 0.5 kPa (3.7 mmHg) and afterload was 7 kPa (51.5 mmHg), i.e. as in the 'low workload' preparations described in [21,24]. Perfusion pressures are below those *in vivo* (Table 1).

(ii) Normotensive, in which the preload was 1.5 kPa (11.0 mmHg) and afterload was 14 kPa (103 mmHg), i.e. as in the 'high workload' preparations described [21,24]. Perfusion pressures approximate to those *in vivo*. In such preparations, the mean aortic pressure will be approx. 14 kPa (103 mmHg).

(iii) Hypertensive, in which the preload was 2 kPa (14.7 mmHg) and the tubing leading to the aortic overflow was clamped off. All the cardiac output thus passed through the coronary circulation and the heart developed the maximum aortic pressure of which it was capable. Aortic pressures are above those *in vivo* (Table 1).

Protein synthesis in subcellular fractions of the heart

The subcellular fractionation procedure has been described in detail [30] and will only be outlined. Ventricles were homogenized and centrifuged at 33000 g for 10 min. The supernatant (sarcoplasmic) fraction was retained. The precipitate was washed and myofibrillar proteins were extracted into buffer containing 0.3 M-KCl and 5 mM-ATP. After centrifugation and further re-extraction of the precipitate, the solubilized myofibrillar proteins were re-precipitated by dilution with water. Sarcoplasmic protein was precipitated by addition of HClO_4 (final concn. 0.5 M) and prepared for radioactivity counting as described previously [27,28]. The myofibrillar fraction was prepared for radioactivity counting as in [30]. The precipitate remaining after extraction of myofibrils (the stromal fraction) was

dissolved in 0.1 M-NaOH, insoluble material (collagen etc.) was removed by centrifugation (bench centrifuge), protein was precipitated in 0.3 M-trichloroacetic acid and was counted for radioactivity as in [27,28].

Other methods

Protein was measured as in [31] or [32] as appropriate, with bovine serum albumin as a standard. RNA was measured as in [33]. Lactate was measured as in [34], and glucose was measured by the glucose oxidase method with *o*-dianisidine as chromogen [35]. Results are presented as means $\pm$ S.E.M. with the numbers of observations (*n*) in parentheses. Significance of differences between groups was tested by a two-tailed unpaired Student's *t* test, with values of *P* < 0.05 taken as being statistically significant.

RESULTS AND DISCUSSION

General comments on the heart preparations

The two preparations used mostly were the hypotensive and hypertensive preparations (see the Experimental section for details). Selected performance data are shown in Table 1. The coronary flow in the hypertensive preparation was about twice that in the hypotensive preparation. The coronary and aortic flows did not decrease over the course of the perfusions. The peak systolic and mean aortic pressures were 2–3-fold greater in the hypertensive preparation. Aortic pressures decreased slightly over the course of the perfusions, but the hypertensive preparation maintained pressures greater than those encountered *in vivo* in normotensive animals. Hydraulic power (the product of the cardiac output and mean aortic pressure) was about 15% greater in the hypertensive preparation. Heart rate was about 10% less (*P* < 0.05) in the hypotensive preparation. Glucose uptake was about 35% greater (*P* < 0.01) in the hypertensive preparation, but lactate output was similar in both preparations, accounting for less than 10% of the glucose uptake. This suggests that oxygenation in both preparations was adequate.

Although we did not measure heart ATP and phosphocreatine contents, we did measure cardiac outputs and developed pressures throughout our perfusions (Table 1). The mechanical performance of the heart is a sensitive criterion of its 'physiological integrity'. Although phosphocreatine concentrations and mechanical performance decline rapidly during ischaemia, ATP concentrations decline more slowly (reviewed in [36]). Lactate output also increases dramatically. Since our hearts were functionally stable even at hypertensive pressures and released only small amounts of lactate, we consider it unlikely that there are gross disturbances in phosphocreatine or ATP contents during perfusion.

Protein synthesis in the heart perfused anterogradely at hypotensive and hypertensive pressures

We first carried out a series of experiments to determine whether the stimulation of protein synthesis by increased aortic pressure might be of physiological significance (Table 2). In Expt. 1, we confirmed that the ventricular k_s in the hypertensive preparation was greater (by 36%) than in the hypotensive preparation. The ventricular k_s in the normotensive preparation (perfused with 10 mM-glucose) was 1456 ± 66 pmol of

Table 1. Performance data for hypotensive and hypertensive heart preparations

Hearts were perfused as described in the Experimental section, with 10 mM-glucose as fuel. Coronary flow, aortic flows and cardiac output were measured after 70 min of antegrade perfusion as previously described [24]. Aortic pressure and heart rate were recorded from a pressure transducer and chart recorder attached to a sidearm in the aortic cannula 8 cm above the heart. The initial reading was taken after 10 min of antegrade perfusion, and the final reading after 110 min, and 0.8 kPa or 5.9 mmHg was added to this. Mean aortic pressure was calculated from the formula: mean aortic pressure = diastolic aortic pressure + 0.33 (peak systolic pressure – diastolic pressure).

	Hypotensive	Hypertensive
Coronary flow (ml/min per g wet wt.)	14.7 ± 0.8 (6)	28.6 ± 2.8 (6)
Aortic flow (ml/min per g wet wt.)	52.0 ± 4.8 (6)	0
Cardiac output (ml/min per g wet wt.)	66.7 ± 5.2 (6)	28.6 ± 2.8 (6)
Initial peak systolic pressure (kPa)	11.0 ± 0.3 (6)	22.3 ± 1.0 (6)
Initial peak systolic pressure (mmHg)	81.9 ± 2.2 (6)	164.0 ± 7.4 (6)
Initial diastolic aortic pressure (kPa)	5.2 ± 0.3 (6)	18.5 ± 1.1 (6)
Initial diastolic aortic pressure (mmHg)	38.2 ± 2.2 (6)	136.0 ± 8.1 (6)
Initial mean aortic pressure (kPa)	7.2 ± 0.3 (6)	19.7 ± 1.0 (6)
Initial mean aortic pressure (mmHg)	52.9 ± 2.2 (6)	144.9 ± 7.4 (6)
Final peak systolic pressure (kPa)	10.5 ± 0.3 (6)	19.7 ± 1.5 (6)
Final peak systolic pressure (mmHg)	77.2 ± 2.2 (6)	144.9 ± 11.0 (6)
Final diastolic aortic pressure (kPa)	5.4 ± 0.3 (6)	16.2 ± 1.3 (6)
Final diastolic aortic pressure (mmHg)	39.7 ± 2.2 (6)	119.1 ± 9.6 (6)
Final mean aortic pressure (kPa)	7.1 ± 0.4 (6)	17.3 ± 0.5 (6)
Final mean aortic pressure (mmHg)	52.2 ± 2.9 (6)	127.2 ± 3.7 (6)
Heart rate (beats/min)	270 ± 9 (12)	300 ± 8 (12)
Glucose uptake (μmol/h per g wet wt.)	71 ± 4 (12)	95 ± 5 (12)
Lactate output (μmol/h per g wet wt.)	12 ± 2 (12)	12 ± 1 (12)

Table 2. Effects of hypertensive aortic pressures on cardiac protein synthesis

Hearts were perfused as described in the Experimental section. Statistical significance: ^a*P* < 0.05, ^b*P* < 0.01, ^c*P* < 0.001 for hypertensive versus hypotensive preparations in the same Expt.; ^d*P* < 0.05, ^e*P* < 0.01, ^f*P* < 0.001 for the effects of insulin in Expt. 4.

Expt. no.	Perfusate additions	Tissue or fraction	<i>k_s</i> (pmol of phenylalanine incorporated/2 h per mg of protein)	
			Hypotensive	Hypertensive
1	10 mM-glucose	Ventricles	1220 ± 37 (5)	1661 ± 27 (5) ^c
2	5 mM-glucose/ 10 mM-lactate/ 10 mM-acetate/ insulin (50 munits/ml)	Ventricles	1745 ± 54 (8)	1946 ± 50 (8) ^a
3	10 mM-glucose	Right ventricle Left ventricle + septum	1151 ± 37 (4)	1634 ± 70 (4) ^c
4	10 mM-glucose	Sarcoplasmic	1058 ± 43 (4)	1500 ± 22 (4) ^c
		Myofibrillar	2309 ± 43 (6)	2616 ± 64 (6) ^b
		Stromal	775 ± 20 (6)	1058 ± 24 (6) ^c
	10 mM-glucose/ insulin (50 munits/ml)	Sarcoplasmic	1047 ± 46 (6)	1774 ± 75 (6) ^c
		Myofibrillar	2624 ± 24 (4) ^e	2578 ± 55 (4)
		Stromal	1113 ± 28 (4) ^f	1088 ± 19 (4)
			1555 ± 34 (4) ^f	2174 ± 135 (4) ^{bd}

phenylalanine incorporated/2 h per mg of protein (*n* = 6, *P* < 0.05 versus *k_s* in either hypotensive or hypertensive preparations). Because differences in *k_s* between preparations were relatively small, subsequent experiments compared the hypertensive with the hypotensive preparation exclusively. As stated in the Experimental section, left-atrial filling pressure was lowest in

the hypotensive and was increased progressively in the normotensive and hypertensive preparations. Although it could be argued that the increase in filling pressure caused the observed stimulation of *k_s* (Table 2), we have shown that an increase in filling pressure with afterload held constant does not alter *k_s* [28]. We thus suggest that the stimulation of *k_s* in Table 2 is an effect of afterload.

In Expt. 2, insulin and non-carbohydrate fuels were included in the perfusate, thus raising k_s in the hypotensive preparation to that observed in the fed rat *in vivo* [22,26]. The stimulation of k_s by hypertensive perfusion pressures was still statistically significant, but was less than in the glucose-perfused hearts. In Expt. 3, we show that hypertensive pressures stimulate k_s by 42% in both the right-ventricular free wall and in the left-ventricular free wall + interventricular septum, confirming the results of Takala [8], who used the retrogradely perfused heart. This result is important because, *in vivo*, raised aortic or pulmonary arterial pressures ('pressure overload') produce concentric hypertrophy of the left or right ventricle respectively. Since in Expt. 3 there was equal stimulation of k_s in both compartments, it seems to us unlikely that this phenomenon is related to development of cardiac hypertrophy, unless the left-ventricular k_d is decreased compared with the right-ventricular k_d . This postulate cannot be tested in our preparation. The conclusion that acute hypertensive stress is not of immediate relevance to stimulation of k_s observed during development of concentric hypertrophy (reviewed in [7,11,12]) is in agreement with the finding that, *in vitro*, acute volume overloading of the heart (which, *in vivo*, induces eccentric ventricular hypertrophy) does not stimulate ventricular k_s [4,6,28]. We do not mean to suggest that stimulation of k_s by perfusion pressure is of no physiological significance. It may assist in maintaining cardiac nitrogen balance *in vivo* and may be one factor in explaining why cardiac k_s is proportionately less inhibited by protein deprivation or starvation than is the gastrocnemius k_s [37].

The mechanism by which hypertensive aortic pressures stimulate k_s *in vitro* has been discussed [10]. It has been suggested that stretching of the ventricular walls is the most important factor. Increases in aortic pressure increase the coronary vessel volume and sarcomere length, decrease compliance and stretch and thicken the ventricular wall [38–41].

In Expt. 4, we showed that three subcellular fractions incorporated phenylalanine at different rates. Comparison of results between different fractions could be biased in the unlikely event of the fractions having different phenylalanine contents, but comparison between identical fractions would be valid. However, the phenylalanine contents of the myofibrillar fraction and heart protein at least are the same [30]. Hypertensive pressures stimulated k_s in all fractions in the glucose-perfused heart. In the hypotensive preparation, insulin stimulated k_s in all fractions, whereas, in the hypertensive preparation, only the stromal fraction showed stimulation of k_s by insulin. If, on the basis of protein recovered in the various fractions relative to total protein recovered, assuming similar recoveries of protein in all fractions (sarcolemmal fraction = 47%, myofibrillar fraction = 33%, stromal fraction = 20%), a mean k_s is calculated, the results are (in pmol of phenylalanine incorporated/2 h per mg of protein): glucose/hypotensive, 1554; glucose/hypertensive, 1936; glucose + insulin/hypotensive, 1914; glucose + insulin/hypertensive, 2007. These findings extend previous observations in retrogradely or anterogradely perfused hearts [42,43], where myosin synthesis was shown to be stimulated *in vitro* by pressure overload or insulin.

It could be argued that the various experimental

manipulations to which the hearts are exposed might affect the specific radioactivity of the phenylalanyl-tRNA precursor. However, McKee *et al.* [44; see also 45] showed that, at a perfusate [^{14}C]phenylalanine concentration of 0.4 mM, the specific radioactivities of [^{14}C]phenylalanyl-tRNA and perfusate [^{14}C]phenylalanine equilibrated within 3–4 min. Values of k_s were similar whether calculated from either perfusate [^{14}C]phenylalanine or [^{14}C]phenylalanyl-tRNA specific radioactivities, indicating rapid equilibration of the phenylalanine pools. In the anterogradely perfused heart, increasing the perfusate [^{14}C]phenylalanine concentrations to 1.6 mM did not increase k_s compared with 0.4 mM [27]. Thus it is unlikely that effects of aortic pressure or insulin could be explained in this way, since [^{14}C]phenylalanyl-tRNA specific radioactivity rapidly attains its theoretical maximum even under 'control' conditions. We recognize that equilibration of aminoacyl-tRNA specific radioactivity may be a problem in some isolated skeletal-muscle preparations [46] (a point ignored by many workers, e.g. [13–18,47]) but, unlike the heart, such preparations are not perfused via an intact circulatory system.

Cardiac protein turnover and prostaglandin production

In the rat heart, prostaglandin I_2 (prostacyclin) is the major prostaglandin synthesized, with prostaglandin E_2 or prostaglandin $\text{F}_{2\alpha}$ being synthesized at 30–40% or 10–20% of the rate of prostaglandin I_2 respectively [48]. Although some prostaglandin I_2 may be synthesized by the endothelial cells of the coronary circulation [49], rat cardiac myocytes release equal amounts of 6-oxo-prostaglandin $\text{F}_{1\alpha}$ (the stable

Fig. 1. Time course of prostaglandin synthesis in the perfused heart

All hearts were perfused with 10 mM-glucose as described in the Experimental section. Appearance of 6-oxo-prostaglandin $\text{F}_{1\alpha}$ in the perfusate was measured in a hypotensive heart (●), in a heart perfused with 100 μunits of insulin/ml (▲) and in a heart perfused with 20 μg of 4-biphenylacetic acid/ml (■).

breakdown product of prostaglandin I_2) and prostaglandin $F_{2\alpha}$, with the rate of prostaglandin E_2 release being about twice that of prostaglandin $F_{2\alpha}$ or 6-oxo-prostaglandin $F_{1\alpha}$ [50]. We measured k_s in hypotensive and hypertensive preparations and in insulin-perfused preparations in the presence and absence of 4-biphenylacetic acid, which essentially completely inhibits prostaglandin synthase (Fig. 1). As a measure of prostaglandin synthesis, we measured production of 6-oxo-prostaglandin $F_{1\alpha}$. Although the prostaglandins putatively involved in protein turnover are prostaglandins $F_{2\alpha}$ and E_2 , they are synthesized from the common endoperoxide intermediate prostaglandin H_2 (the prostaglandin synthase product), as is prostaglandin I_2 . In some experiments, we measured prostaglandin $F_{2\alpha}$ release. We used radioimmunoassay kits from Amersham International that are based on ^{125}I -6-oxo-prostaglandin $F_{1\alpha}$ or ^3H prostaglandin $F_{2\alpha}$. We followed the suppliers' protocol, except that the volumes of reagents used were halved. Standard curves were set up in bicarbonate-buffered saline [23] containing the relevant additions (glucose, insulin, 4-biphenylacetic acid, cycloheximide, amino acids). However, at the concentrations used in our experiments, there was no interference of the additions with the standard curve.

Release of 6-oxo-prostaglandin $F_{1\alpha}$ into the perfusate was linear with time (Fig. 1) and was abolished by 4-biphenylacetic acid. From the linear regression line of 6-oxo-prostaglandin $F_{1\alpha}$ release against time, a rate of 6-oxo-prostaglandin $F_{1\alpha}$ release was obtained for each perfusion. Results comparing k_s with 6-oxo-prostaglandin $F_{1\alpha}$ release are shown in Table 3. Perfusion at hypertensive pressures stimulated ventricular k_s , but not 6-oxo-prostaglandin $F_{1\alpha}$ release. Stimulation of k_s by hypertension was still observed when prostaglandin synthesis was abolished by 4-biphenylacetic acid. Basal (hypotensive) k_s values were unaffected by 4-biphenylacetic acid. Physiological concentrations of insulin stimulated both k_s and 6-oxo-prostaglandin $F_{1\alpha}$ release. However, stimulation of k_s by insulin was still observed when prostaglandin synthesis was abolished by

4-biphenylacetic acid. Although prostaglandin $F_{2\alpha}$ release was only about 10% of 6-oxo-prostaglandin $F_{1\alpha}$ release, its release was inhibited completely by 4-biphenylacetic acid (results are not shown). Indomethacin (20 μM ; 100 μl of a 20 mM solution in ethanol per 100 ml of perfusate) did not prevent stimulation of k_s by insulin (results not shown). There was no effect of 4-biphenylacetic acid on cardiac outputs or aortic pressures (results not shown).

In experiments which examine the involvement of prostaglandins in protein turnover, the order of addition of insulin and prostaglandin synthase inhibitor can affect results [47]. If skeletal muscle was pre-exposed to insulin, subsequent addition of indomethacin did not prevent the stimulation of k_s by insulin during the test incubation. If insulin and indomethacin were present during both the preincubation and the test incubation, indomethacin prevented the stimulation of k_s by insulin. The latter condition prevails in our experiments, i.e. hearts were exposed simultaneously to insulin and 4-biphenylacetic acid.

As mentioned above, prostaglandin production by the heart is stimulated by physiological concentrations of insulin and is also inhibited by cycloheximide (Table 3). Insulin stimulation of prostaglandin production was abolished by cycloheximide. Very recently, two groups have described inhibition of prostaglandin release by cycloheximide in skeletal muscles [51,52]. Inhibition of prostaglandin production by cycloheximide has been ascribed variously to a decrease in arachidonate supply [51] or to translational inhibition of the synthesis of prostaglandin synthase [52]. To our knowledge, insulin has not been shown previously to stimulate cardiac prostaglandin production *in vitro*, although it has been shown to raise prostaglandin synthesis in skeletal muscle *in vitro* [18,47] and to raise prostaglandin concentrations *in vivo* [19]. The effects of insulin and/or cycloheximide on cardiac prostaglandin synthesis are consonant with either translational modulation of the synthesis of prostaglandin synthase (an enzyme which may have a very short half-life [52]), or inhibition of prostaglandin

Table 3. Effects of hypertensive aortic pressure and insulin on protein and prostaglandin synthesis in the presence and absence of 4-biphenylacetic acid

Hearts were perfused as described in the Experimental section with 10 mM-glucose. Statistical significance: ^a $P < 0.01$, ^b $P < 0.001$ versus hypotensive preparations; ^c $P < 0.01$ versus hypotensive preparations in the presence of insulin.

	4-Biphenylacetic acid absent		+ 4-Biphenylacetic acid (20 $\mu\text{g}/\text{ml}$)	
	k_s (pmol of phenylalanine incorporated/2 h per mg of protein)	6-Oxo-prostaglandin $F_{1\alpha}$ release (ng/h per g dry wt.)	k_s (pmol of phenylalanine incorporated/2 h per mg of protein)	6-Oxo-prostaglandin $F_{1\alpha}$ release (ng/h per g dry wt.)
Hypotensive	1187 $\pm$ 20 (14)	467 $\pm$ 34 (8)	1214 $\pm$ 34 (9)	18 $\pm$ 6 (3)
Hypertensive	1430 $\pm$ 38 (10) ^b	519 $\pm$ 60 (6)	1463 $\pm$ 34 (10) ^b	58 $\pm$ 24 (3)
Hypotensive + insulin (100 $\mu\text{units}/\text{ml}$)	1697 $\pm$ 50 (5) ^b	757 $\pm$ 46 (8) ^b	1625 $\pm$ 46 (5) ^b	46 $\pm$ 14 (8)
Hypotensive + 20 μM -cycloheximide	—	314 $\pm$ 26 (7) ^a	—	—
Hypotensive + insulin (100 $\mu\text{units}/\text{ml}$) + 20 μM -cycloheximide	—	444 $\pm$ 81 (4) ^c	—	—

Table 4. Effects of hypertensive aortic pressures and insulin on protein degradation in the presence and absence of 4-biphenylacetic acid

Hearts were perfused with 10 mM-glucose as described in the Experimental section. Statistical significance: ^a*P* < 0.05, ^b*P* < 0.01 versus hypotensive preparations (no insulin).

	<i>k_d</i> (pmol of phenylalanine released/2 h per mg of protein)	
	4-Biphenylacetic acid absent	+ 4-Biphenylacetic acid (20 µg/ml)
Expt. 1		
Hypotensive	3200 ± 125 (5)	3225 ± 150 (5)
Hypertensive	2250 ± 225 (4) ^b	2200 ± 100 (4)
Expt. 2		
Hypotensive	3500 ± 175 (4)	—
Hypotensive + insulin (200 µunits/ml)	2800 ± 125 (3) ^a	2550 ± 100 (4) ^b

synthase degradation, or an effect on arachidonate supply [51]. However, since prostaglandin synthase is supposed to be the rate-limiting step for prostaglandin synthesis [53], this enzyme must be the most favoured candidate for the locus of modulation. Further investigation is necessary to clarify the situation.

We also examined the effects of 4-biphenylacetic acid on *k_d* (Table 4). 4-Biphenylacetic acid did not affect basal (hypotensive) *k_d* values, and the inhibition of *k_d* by hypertension or insulin could still be observed in the presence of 4-biphenylacetic acid. The effects of 4-biphenylacetic acid on protein degradation are complicated by the presence of cycloheximide (which itself inhibits prostaglandin synthesis; Table 3). The only way to establish whether 4-biphenylacetic acid affects basal *k_d* values would be to omit cycloheximide and use the method of Tischler *et al.* [54] or of Palmer *et al.* [55] to measure *k_s* and *k_d*. Here, tyrosine is omitted from or is present only at low concentrations in the medium and *k_s* is measured by [¹⁴C]phenylalanine incorporation. Net tyrosine release into the medium is measured. From the tissue protein phenylalanine/tyrosine ratio, *k_d* can be calculated after expression of *k_s* in tyrosine equivalents and addition of the net rate of tyrosine release. Unfortunately, this method is reliable only when tissue preparations are in gross negative nitrogen balance (e.g. rabbit forelimb muscles [47] or the incubated diaphragm [56]). When we attempted to apply the method of Tischler *et al.* [54] to the perfused heart (which is closer to nitrogen equilibrium than are skeletal-muscle preparations [22]), we could not obtain any stimulation of *k_s* by insulin, presumably because there was insufficient intracellular tyrosine to support protein synthesis (results not shown). We recognize that, under control conditions, our heart preparations are in negative nitrogen balance. However, in the presence of insulin, our preparations are approximately in nitrogen balance [22]. It should be noted that all preparations in which effects of prostaglandins have been demonstrated are in gross negative nitrogen balance, even in the presence of insulin (e.g. [50]).

Notwithstanding the effects of cycloheximide on

prostaglandin synthesis, *k_d* was still inhibited by hypertension or insulin when prostaglandin synthesis is abolished by 4-biphenylacetic acid (Table 4), militating against involvement of prostaglandins. Indeed, it would be surprising if insulin inhibition of *k_d* operated via prostaglandins, since prostaglandin E₂ stimulates *k_d* [13], and insulin raises prostaglandin concentrations (see [19] and Table 3). It should be noted that effects of aortic pressure on *k_d* are only observed at hypertensive values. When we compared normotensive with hypotensive preparations, *k_d* values were the same [57], although inhibition of *k_d* by insulin was greater in the normotensive preparations.

After these studies were completed, Gordon *et al.* [58] reported that effects of aortic pressure on *k_s* or *k_d* in the retrogradely perfused heart could not be attributed to an effect on prostaglandin synthesis. In those studies, high concentrations (100 µM) of indomethacin or meclofenamate were used to decrease prostaglandin synthesis. However, these inhibitors decreased prostaglandin synthesis by only about 50%, and the problem of inhibition of prostaglandin synthesis by cycloheximide was not recognized.

General conclusions

Aortic pressure may have a role *in vivo* in maintaining cardiac nitrogen balance. The acute stimulation of cardiac *k_s* by hypertensive aortic pressures seen *in vitro* may not be directly relevant to the development of pressure-overload hypertrophy *in vivo*, which probably involves alterations at the genomic level [59]. We were unable to establish any role for prostaglandins in the acute stimulation of *k_s* or the acute inhibition of *k_d* by hypertensive aortic pressures or insulin *in vitro*.

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