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Effects of the administration of angiotensin II on cardiac glycogen metabolism in the rat

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Abstract. Changes in glycogen metabolism after an intravenous injection of angiotensin II were investigated in the left and right ventricles of the rat heart, as a function of location within the ventricular wall. Hearts were cut into 100-μm thin sections, all of which were analysed for glycogen content, glucose incorporation into glycogen and 2-deoxyglucose uptake and phosphorylation after the intravenous injection of ^{14}C-labelled sugar. In control hearts, glycogen levels were uniform across the wall in both ventricles, while the rate of sugar uptake and phosphorylation, and that of glucose incorporation into glycogen, were significantly higher in the subendocardial myocardium of the left ventricular wall. After angiotensin II administration, heart glycogen levels decreased slightly in the left, but not in the right ventricle, while 2-deoxyglucose uptake and phosphorylation, and glucose incorporation into glycogen, increased 2,5- and 5-fold, respectively. With regard to the distribution across the wall of the left ventricle after angiotensin administration, glycogen levels and glucose incorporation into glycogen were uniformly distributed, whereas sugar phosphorylation was still higher in the subendocardium.

Key words: Angiotensin – Cardiac metabolism – Glycogen – Glycogen metabolism – Sugar uptake and phosphorylation – Hypertension

Introduction

Muscle glycogen is always broken down during contraction – even when glycogen stores do not undergo any alteration – and the rate of glycogen turnover changes dramatically with function, both in fast – and slow-twitch fibres (Villa Moruzzi et al. 1981)

In cardiac muscle, where all fibres contract with the same frequency, glycogen may be degraded and resynthesized at a turnover rate which is not uniform across the wall of the left ventricle. In the resting animal, higher levels of glucose incorporation into glycogen are found in the subendocardium (where intramyocardial pressure is highest during systole) and the subendocardial-to-subepicardial (endo/epi) glycogen turnover ratio is much greater than unity (De Tata et al. 1983). Hence, it has been suggested that as in the case of skeletal muscle, the rate of glycogen turnover in the heart might be an index of cardiac work (De Tata et al. 1983). Here, we show that the intravenous injection of angiotensin II, at a dose which causes acute hypertension, dramatically increases the rate of glycogen turnover in both heart ventricles and changes its regional distribution across the left ventricular wall.

Materials and methods

Animals. Young adult [180–200 g body wt.] male albino rats of the Sprague-Dawley strain were used. Different groups of animals were used for each parameter measured. In all cases, a 12-h fast preceded the experiment. Anaesthesia was induced by injecting sodium pentobarbital (Nembutal; 5 mg 100^{-1}g body wt., intraperitoneally). U-^{14}C-labelled glucose (specific activity 3 mCi/mmol) was dissolved in saline and injected intravenously into the tail (10 μCi 100 g^{-1} body wt.) 10 min before sacrifice. 1-^{3}H-labelled 2-deoxyglucose was diluted in saline and injected at the same dosage and time, by the same route. Angiotensin II (4 μg/rat, Johansson and Siesjo 1977) was dissolved immediately before use and injected together with the labelled sugar. Treatment resulted in a significant increase in the systolic and diastolic blood pressure values, which were measured by a LETICA 5000-5100 electronic pressure meter [Tecniplast gazzada, buggiate (VA), Italy].

The animals were killed by causing cardiac arrest with 1.0 ml of 150 mM KCl injected intravenously. The arrested heart was rapidly removed and dropped into ice, and the left and right ventricle free-walls were excised, frozen and cut into 100-μm-thick sections in a cryostat; four (or three, in the case of the right ventricle) adjoining sections were combined as described elsewhere (De Tata et al. 1983).

Determination of glycogen. Heart sections were dropped into centrifuge tubes containing 1.0 ml of 4N NaOH, which were kept in boiling water for 30 min. Glycogen was purified following the method described by Hassid and Abraham (1957) and assayed spectrophoto-

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metrically by the anthrone procedure. Results are given as $mg^{-1}g$ wet weight.

Incorporation of U-^{14}C-glucose into glycogen. The heart sections were processed as described above, but carrier glycogen was added to the hydrolysate, and glycogen was then purified as described elsewhere (Bergamini et al. 1969). The final sediment was dissolved in 1.0 ml distilled water and assayed for radioactivity in a Triton-Toluene scintillation fluid to an error of less than 3% (Turner 1969). The specific activity of the glucose moiety of uridine diphosphoglucose (UDPG), which is the ultimate precursor for the synthesis of glycogen, was assessed. The left ventricular wall of KCl-arrested hearts was freeze-clamped in liquid nitrogen and cut into two transmural parts; aliquots of the perchloric acid extracts (see below under other assays) were used to assay UDPG concentration. To another aliquot, a large amount of "cold" UDPG was added and UDPG was purified from any contaminating radioactivity by absorption on charcoal columns and subsequent elution with an ethanol:ammonia:water (50:2:48) mixture (Mura et al. 1978). Recovery was assessed by assaying UDPG concentration in the eluate.

2-deoxyglucose uptake and phosphorylation. Heart sections were dropped into centrifuge tubes containing 1.0 ml distilled water. Sugar was then extracted at 100 °C for 10 min. Radioactivity was assessed in aliquots of this extract, to determine the total content of 2-deoxyglucose. Another aliquot of the extract was treated following the Somogyi procedure, as in Tietz (1976), to remove sugar phosphate and then centrifuged. The amount of radioactivity present in the unphosphorylated 2-deoxyglucose was assessed in a liquid scintillation counter, using a counting time sufficiently long to achieve a counting error of less than 3%. Phosphorylated 2-deoxyglucose calculated by subtracting the radioactivity attributable to free 2-deoxyglucose from the total radioactivity in muscle. Results are given as counts mg^{-1} wet weight. The values obtained for free sugar were corrected by subtracting the radioactivity in the extracellular space and are expressed as a ratio of the distribution of radioactivity between intracellular and plasma water. The extracellular space was determined in age-matched control and rats given angiotensin II, on the basis of the distribution of intravenously injected ^{14}C-labelled inulin.

Other assays. Sections from KCl-arrested hearts taken from non-ventilated animals without freeze-clamping in situ we assayed for glucose-6-phosphate, lactate and UDPG levels. Perchloric acid extracts of four heart sections were used after neutralization with solid $KHCO_3$; the assays were performed by enzymatic techniques (Bergmeyer 1974), using a Perkin Elmer Spectrofluorometer 650-10S (The Perkin-Elmer corporation, oak brook, IL, USA). On average, results obtained with this technique were 25% (in control rats) or 15% (in rats given angiotensin II) higher than those obtained with myocardial tissue from KCl-arrested, fully relaxed hearts, quickly frozen in situ, using Wollenberger tongs precooled in liquid nitrogen. Blood glucose was assayed by a glucose oxidase/peroxidase method using commercially available kits (Glucinet, Sclavo ISVT, Siena, Italy).

Chemicals. Oyster glycogen, glucose-6-phosphate, UDPG, nicotinamide-adenine dinucleotide and its phosphorylated form, and angiotensin II were products of Sigma (St. Louis, Mo., USA). Crystalline glucose-6-phosphate dehydrogenase and UDP-glucose dehydrogenase were purchased from Boehringer (Mannheim, FRG). U-^{14}C-labelled glucose, inulin and 2-deoxy-1-^{3}H-glucose were obtained from the Radiochemical Centre (Amersham, UK). All products used were of analytical grade.

Statistical analysis. Data are given as means ± SEM. P values are given in the legends to figures and tables. Statistical significance was evaluated by using Student's *t*-test and one-way analysis of variance.

Results

Effects of the administration of angiotensin II on mean aortic pressure and heart rate

In control rats, mean aortic pressure and heart rate remained constant throughout the experimental procedure. The intravenous administration of angiotensin II (4 μg) had a significant effect on mean aortic pressure (+68%, +52% and +35% respectively, 1, 5 and 9 min after the injection), while heart rate was unchanged. Therefore, the product of heart rate and mean aortic pressure, a better index of the cardiac workload, increased significantly (+59%, +44% and +26% respectively, 1, 5 and 9 min after the injection of angiotensin II).

Effects of angiotensin II administration on glycogen metabolism in heart ventricles

Table 1 shows that the intravenous administration of angiotensin II (4 μg/rat) had significant effects on heart sugar metabolism, which were similar in the left and right ventricles. In the cardiac tissues of control rats, the 2-deoxyglu-

Table 1. Effect of the intravenous administration of angiotensin II (4 μg/rat) on the sugar metabolism of the left and the right ventricles of the rat heart

	Glycogen ($mg\ g^{-1}$)	Glycogenesis	Glucose uptake	Glucose phosphorylation
			$\mu mol/min^{-1}\ g^{-1}$ wet tissue	
Treatment	Left ventricle			
Controls	3.12 ± 0.07	0.12 ± 0.003	0.48 ± 0.04	0.48 ± 0.04
Angiotensin II	2.92 ± 0.04	0.53 ± 0.004	1.56 ± 0.03	1.10 ± 0.05
	Right ventricle			
Controls	2.86 ± 0.06	0.06 ± 0.001	0.49 ± 0.03	0.47 ± 0.008
Angiotensin II	2.91 ± 0.08	0.34 ± 0.006	1.39 ± 0.04	1.09 ± 0.04

The values represent the mean of at least 5 cases ± SEM. The fluxes were calculated by using the operational equation described by Takala et al. (1981). Angiotensin II administration had no significant effect on glycogen levels, while the incorporation of ^{14}C-glucose into glycogen (glycogenesis) increased significantly ($P < 0.01$). Both glucose uptake ($P < 0.01$) and glucose phosphorylation ($P < 0.01$) increased significantly after angiotensin II administration

cose distribution never exceeded the inulin distribution space, in agreement with Morgan et al. (1961). In angiotensin-II-treated rats, there was a significant intracellular accumulation of free 2-deoxyglucose and the rate of its phosphorylation increased considerably (+130%). The rate of glucose incorporation into glycogen exhibited the largest and most significant increase (+340%).

Effects of angiotensin II administration on the regional distribution of glucose metabolism across the heart walls

Glycogen distribution in the layers of the myocardium. In all the figures presented, the horizontal axis represents a progression of values for successive serial sections. The first and last values are always those of the subepicardial and subendocardial layers, respectively. It should be mentioned here that these two layers contain larger amounts of connective tissue.

The results of the determination of glycogen distribution throughout the walls of the left and right ventricles in control rats, and 10 min after the administration of angiotensin II are summarized in Fig. 1. The levels of glycogen are very close to those previously reported (De Tata et al. 1983) and are similar in all myocardial layers of both ventricles, irrespective of angiotensin II administration [the slight (6%) negative effect of the hypertensive hormone is not significant]. Incidentally, a 12% decrease in heart glycogen levels was reported by Takala et al. (1984) in rats exercised for 20 min; this decrease was evenly distributed across the left ventricle wall.

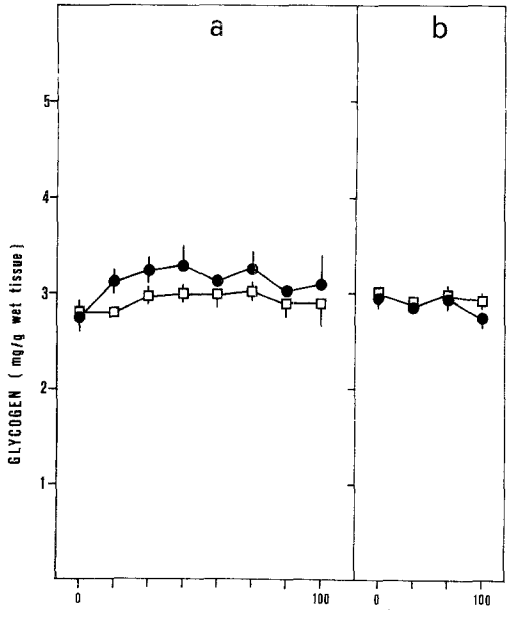


Fig. 1. Distribution of glycogen across the wall of the left (**a**) and the right (**b**) ventricle in control (●) and angiotensin-II-treated rats (□). *Abscissa*: ventricular wall thickness, from epicardium (0) to endocardium (100) is presented. Points are given as means of five cases. *Vertical bars* represent 2 SEM (they have been deleted when smaller than the symbol used). No significant difference in glycogen content can be observed among the different myocardial layers and angiotensin II administration has no significant effect on glycogen levels or distribution

Distribution of labelled glycogen after the intravenous administration of U-^{14}C-labelled glucose. In control rats, a highly significant ($P < 0.01$) gradient of glucose incorporation was found in the walls of the left, but no of the right ventricle (higher values were found in the subendocardial layers), as reported by De Tata et al. (1983) (Fig. 2). In angiotensin-II-treated rats, the incorporation increased significantly in both ventricles ($P < 0.01$) and tended to be higher in the subepicardial layers; as a result, the endo/epi turnover ratio decreased to 0.9 from a value of 1.5 in the resting rat. It should also be mentioned that the incorporation of glucose into glycogen was always significantly different in the two ventricles, with lower levels being found in the right ventricle ($P < 0.01$).

There is no significant difference in the distribution of the specific activity of ^{14}C-labelled UPDG across the left ventricular wall (Table 2). Angiotensin II administration caused a significant ($P < 0.01$) 100% increase in the specific activity of ^{14}C-labelled UPDG both in the inner and in the outer part of the wall.

2-Deoxyglucose uptake and phosphorylation. In rat heart, the rate of glucose uptake may be the factor limiting the rate of sugar metabolism (Morgan et al. 1961). The phosphorylation by hexokinase and the transfer into glycogen of the glucose units, by glycogen synthase, are other limiting steps along the metabolic path leading to glycogen synthesis

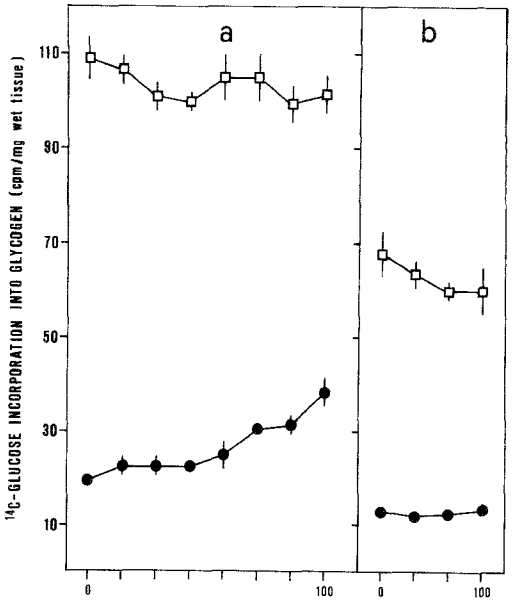
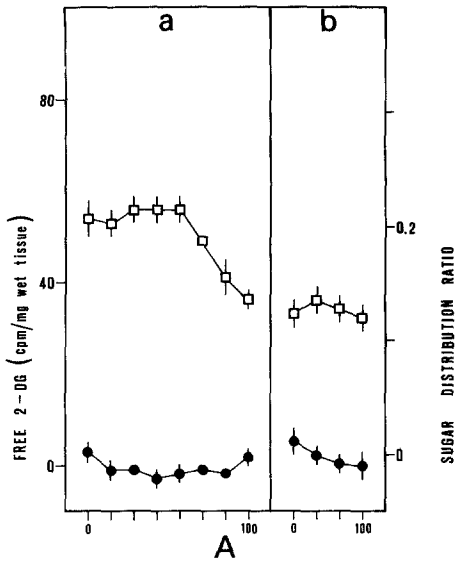


Fig. 2. Glucose incorporation into glycogen in vivo in different myocardial layers of the left (**a**) and the right (**b**) ventricle. Ten microcuries of ^{14}C-labelled glucose 100 g^{-1} body weight was injected into the tail vein of anaesthetized rats in 0.2 ml saline (controls, ●) or in 0.2 ml saline containing 4 μg angiotensin II (□) 10 min before sacrifice. Glycogen was purified and the radioactivity was assessed as described in the text. Results are given as counts mg^{-1} wet cardiac tissue. Means of five cases are given. *Vertical bars* represent 2 SEM (they have been deleted when smaller than the symbol used). *Abscissa*: ventricular wall thickness from epicardium (0) to endocardium (100) is presented. Radioactivity is significantly affected by angiotensin II administration ($P < 0.01$) and it is not distributed uniformly across the wall in control rats ($P < 0.01$), unlike in those given angiotensin II.

Table 1. Effects of the intravenous administration of angiotensin II (4 μg/rat) on the uridine diphosphoglucose (UDPG) concentration and on the specific activity of the glucose moiety in UDPG, in subepicardial (Epi) and in subendocardial (Endo) layers of the left ventricular wall

	Epi	Endo
UDPG concentration		
Controls	0.112 ± 0.010	0.110 ± 0.008
Angiotensin II	0.107 ± 0.008	0.096 ± 0.007
UDP-^{14}C-glucose specific activity		
Controls	18.77 ± 2.17	19.58 ± 2.05
Angiotensin II	39.21 ± 4.71	34.97 ± 4.69

Values are the means of 6 cases ± SEM. Results are expressed as nmol/ mg^{-1} wet tissue (UDPG concentration) and cpm nmol^{-1} UDPG (specific activity), respectively.
No significant transmural differences were detected either in controls or in angiotensin-II-treated rats regarding UDPG levels and UDP-^{14}C-glucose specific activity. The specific activity of UDP-^{14}C-glucose rose significantly ($P < 0.01$) in both the ventricular layers after angiotensin II administration



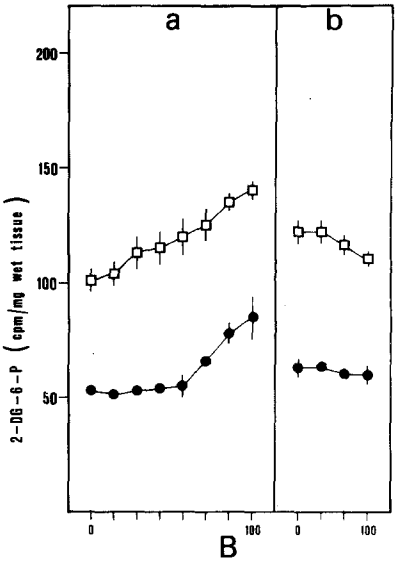


Fig. 3. Effects of angiotensin II administration (□) on the accumulation of 2-deoxyglucose (A) and of 2-deoxyglucose-6-phosphate in different myocardial layers of the left (**A***a*,**B***a*) and of the right (**A***b*, **B***b*) ventricular wall of the rat heart. The experiment was performed as in Fig. 2. Results are given as counts/ mg^{-1} wet cardiac tissue and as the distribution ratio of radioactivity between intracellular water and plasma (free 2-deoxyglucose). Means of five cases are given. *Vertical bars* represent 2 SEM (they have been deleted when smaller than the symbol used). *Abscissa*: Ventricular wall thickness from epicardium (0) to endocardium (100) is presented. The concentration of free sugar in cardiac cells is very low in controls, and is significantly increased by angiotensin II administration, the levels attained being higher in the subepicardial tissue ($P < 0.01$). The accumulation of 2-deoxyglucose is increased considerably by angiotensin II adminstration ($P < 0.01$) and is always higher in the subendocardial layers ($P < 0.01$)

(see Bergamini et al. 1969). The rates of 2-deoxyglucose uptake and phosphorylation are enhaced to a different extent by various stimuli (such as insulin, work, anoxia: see, for example, Bergamini 1969). An evaluation of the accumulation of 2-deoxyglucose-6-phosphate after the injection of trace amounts of the labelled sugar (which is transported and phosphorylated like glucose but is not metabolized further; Kipnis and Cori 1959) may provide a good index of hexokinase activity in the intact tissue (Karpatkin et al. 1966) and help to evaluate the contribution of the first two metabolic steps to increased glucose incorporation.

The ratio of the distribution of 2-deoxyglucose in intracellular water and in circulating blood in the different layers of the left (Fig. 3Aa) and right (Fig. 3Ab) ventricles, together with the radioactivity accumulated (as counts mg^{-1} wet weight) after exposure to a dose of radioactivity similar to that used in the glucose incorporation studies for a comparable period of time. In control rats, the levels of free 2-deoxyglucose were very low in all myocardial layers across the wall of both the right and the left ventricles. After the administration of angiotensin II, there was a significant increase in the distribution of 2-deoxyglucose in the intracellular water; levels appeared to be highest in the midmyocardial and in the subepicardial layers of the left ventricular wall. In control rats, the accumulation of 2-deoxyglucose phosphate is uniform across the wall in the right ventricle (Fig. 3Bb), but is significantly different in the different layers of the left ventricular myocardium (Fig. 3Ba). Angiotensin II administration caused a 2-fold increase in the rate of accumulation of sugar phosphate, the transmural gradient of the left ventricle being preserved because of the significantly higher subendocardial values.

Glucose-6-phosphate, lactate and UDPG assays. These data are likely to be strongly influenced by the freezing procedure we used in order to separate myocardial layers (Materials and methods). Here again, glucose-6-phosphate levels were higher in the outer part of the left ventricular wall (0.33 ± 0.007 and 0.28 ± 0.007 μmol g^{-1} wet tissue, respectively). After the administration of angiotensin II, sugar phosphate levels decreased significantly down to 0.17 ± 0.003 (outer part) and 0.18 ± 0.002 μmol g^{-1} wet tissue (inner part; changes were confirmed with quick-freezing techniques), with the disappearance of the transmural differences in glu-

cose-6-phosphate distribution. With regard to lactate, the levels were similar to plasma lactate concentration in controls (1.30 ± 0.05 μmol g^{-1} wet tissue and 1.00 ± 0.12 μmol ml^{-1}, respectively) and increased significantly in angiotensin II-treated rats (heart lactate 3.38 ± 0.16 μmol g^{-1}; plasma lactate 1.86 ± 0.38 μmol ml^{-1}, respectively). There was always a uniform distribution across the wall of the left ventricle. Hence, on the basis of lactate concentration it may be suggested that glucose (and glycogen) breakdown after cardiac arrest was probably very limited, or even negligible in control rats and lower than 1 μmol^{-1} g wet tissue (or 0.16 mg glycogen g^{-1} wet tissue) after angiotensin II administration. The levels of UDPG were not affected by the administration of angiotensin II and were similar in all myocardial layers.

Discussion

Our data clearly show that the administration of angiotensin II causes a sudden, dramatic increase in arterial blood pressure and a simultaneous dramatic increase in sugar uptake and phosphorylation, as well as in the rate of glucose incorporation into glycogen, in both ventricles of rat heart. The degree of hypertension induced by angiotension II was similar to that obtained by Johanson and Siesyo (1977); however, it slowly decreased. There was a parellel increase in the product of heart rate and mean aortic pressure. In many respects, the changes in sugar metabolism are very similar to those in rat skeletal muscle following exercise (Villa Moruzzi et al. (1981).

Increased incorporation of labelled glucose into glycogen during increased heart work was shown to occur in the perfused working heart preparation many years ago by Chain et al. (1969). From a quantitative point of view, after the administration of angiotensin II, the rate of sugar phosphorylation and the specific activity of UDPG after ^{14}C-glucose injection are doubled; and glucose incorporation into glycogen exhibits a 4-to 5-fold increase. Hence, our data appear to indicate that the administration of angiotensin causes a true increase in the rate of glycogen synthesis in cardiac muscle. However, caution should be taken in making a quantitative interpretation: it appears from our experiments that there are hormone-dependent differences in the dilution of labelled sugar in the glucose phosphate pools, which could not be assessed properly due to the technique employed. These differences may cause an overestimation of the effects of angiotensin on the rate of glucose incorporation into glycogen. On the other hand, the administration of angiotensin appears to increase the rate of glycogen breakdown, which might lead to the underestimation of the effects of the hormone. The latter may not be secondary, since we cannot exclude the possibility that the outer, probably the most heavily labelled, glucose units of the polysaccharide chains might be broken down at a higher rate.

In this respect, it is noteworthy that glycogen levels do not increase under our experimental conditions. Rather they tend to decrease after the administration of angiotensin. This implies that the increase in the rate of glycogen synthesis after the administration of the hormone is (over) compensated by a simultaneous change in glycogen breakdown, and that in rat cardiac muscle an apparent steady-state of glycogen levels may result from very different dynamics of glycogen metabolism. In conclusion, it appears that the rate of cardiac glycogen turnover may be rapidly adjusted in response to the workload imposed, and that there is an interesting mechanism involved in the glycogen turnover in the heart related to function. Similar conclusions were reached with fast- and slow-twitch skeletal muscles at changing workloads (Bergamini et al. 1980; Villa Moruzzi et al. 1981). If this is the case, the differences in glucose incorporation into glycogen observed across the left ventricular wall, and the changes after angiotensin adminstration, might provide information about the effects of the hormone on the regional distribution of workload in the myocardium.

As an additional comment, differences in metabolism across the heart wall may indicate that the transmural distribution of workload is not uniform and may change with the workload itself. The present data further reveal that this non-uniformity is a peculiar feature of the left ventricle. In this respect, it should be mentioned that differences have also been found in the transmural distribution of enzyme activities across the left but not the right ventricular wall of different mammalian species. These differences might reflect a specific metabolic adaptation of muscle cells to long-lasting differences in the transmural distribution of workload (De Tata et al. 1986) and have been found to change with chronic hypertension (De Tata et al. 1988a) and ageing (De Tata et al. 1988b).

Experiments with 2-deoxyglucose show that angiotensin causes an increase in the tissue content of both free and phosphorylated sugar. In cardiac and skeletal muscle tissues, any increase in workload should result in an increase in the accumulation of 2-deoxyglucose-phosphate, whereas the levels of unphosphorylated sugar inside the cells may not change (see e.g. Morgan et al. 1961). On the other hand, there are stimuli such as insulin and anoxia which can cause simultaneous accumulation of both free and phosphorylated 2-deoxyglucose in muscle cells (Bergamini 1969; Karpatkin et al. 1966). Hence, we cannot exclude that another factor may be operating after angiotensin administration, by adding its own contribution to the effects of the increase in workload. At a first glance, angiotensin-induced cardiac hypoxia (Fowler and Holmes 1964; Douglas 1985) may appear to be a good candidate. However, our data about transmural distribution do not provide any evidence that the accumulation of free 2-deoxyglucose after angiotensin administration is greater in the subendocardial layers, which may be more deeply involved than epicardial layers in regional ischaemia (Ichihara and Abiko 1982). As an additional comment, hypoxia should cause considerable glycogen breakdown (e.g. Crass and Pieper 1975), but no significant decrease in glycogen levels was detected during the acute hypertensive attack in the present experiments.

As a final comment, the finding that the processes of sugar phosphorylation and of glucose incorporation into glycogen are not uniformly distributed across the heart wall of the resting rat is in agreement with previous data (Takala and Hassinen 1981; De Tata et al. 1983). The observation that the administration of angiotensin increases the rate of

glycogen turnover, glucose uptake and causes a significant change in the profile of its distribution across the left ventricular wall, is in agreement with recent data (Takala et al. 1983; 1984). These investigators showed that exercise, or an increase in aortic pressure result in an increase in the total uptake of glucose and the abolition of the transmural gradient of glucose uptake in vivo, or in the beating perfused heart. It should be noted that the change in glucose distribution across the left ventricular wall is at variance with the process of sugar phosphorylation, which exhibits a similar increase but remains higher in the subendocardial regions.

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