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Prostaglandin-E_2 and cyclic adenosine 3'-5' monophosphate levels in the hypertrophied rat heart

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To assess whether prostaglandin-E_2 (PGE_2) and cyclic adenosine 3'-5'-monophosphate (cAMP) are involved in the cardiac response to chronic pressure overload, we measured by specific radioimmunoassay method the cardiac tissue and plasma concentrations of PGE_2 and cAMP in an animal model of left ventricular hypertrophy. The cardiac hypertrophy was accompanied by a significant increase in PGE_2 content, and a significant decrease in cAMP content, in the heart. In addition, we found elevated PGE_2 and cAMP levels in arterial plasma samples from the rats with hypertrophied hearts compared to normal rats. These findings suggest a link between cardiac and vascular PGE_2 and cAMP generation and the hemodynamic stresses of advanced cardiac overload.

INTRODUCTION

Recent investigations have demonstrated the protective effect of prostanoids in pathophysiological changes or toxic damage of the heart (11). It has also been demonstrated that augmentation of endogenous synthesized prostaglandin (PGs) may be an important factor in the adaptation of myocardium to acute cardiac overload (5, 10, 18). Furthermore, we demonstrated in a previous study that PGE_2 and $PGF_2\alpha$ were present in fresh atrial tissue slices of patients with heart valve disease (7), and we observed a greater synthesis of these PGs by chronic overloaded rat hearts (19). These findings suggest a relationship between the increase of cardiac PGs synthesis and elevated work load. Despite much research on the possible metabolic and biochemical factors underlying cardiac overload, a complete understanding of this condition has not been achieved. The adenylate cyclase system has been implicated in the regulation of protein synthesis by the acutely overloaded myocardium (16). In addition, a significant increase in cardiac cyclic AMP levels, associated with activation of myocardial prostaglandin synthetase, was found as early as five minutes after aortic constriction (10). The present study was performed to examine a possible interaction between cardiac loading and heart tissue and arterial plasma levels of PGE_2 and cAMP one month after surgical production of aortic stenosis.

METHODS AND MATERIALS

Animals and surgery

Female Sprague-Dawley rats of uniform age were purchased from Santiago Breeding Laboratories Inc. The selected rats (n=36), with initial similar weight (177 ± 2.44 g body wt) and normal systolic arterial pressure (105 ± 0.87 mm Hg), were kept under the same conditions of water access, food (commercial rat chow), humidity and light (12/12, light/dark). The rats were divided into two groups: a) Sham-operated Normotensive Rats (NTR, n = 18), which were subjected to the simulated surgical procedures; and b) Experimental Hypertensive Rats (EHR, n = 18), which

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were subjected to a subdiaphragmatic aortic constriction, according to the method of Grimm et al. (9), to develop a chronic pressure overload.

Thirty days after surgery, EHR and NTR were anesthetized with sodium pentobarbital (40 mg/kg i.p.). Heparin (Organon, 200 IU/Kg, iv) was administered, and arterial blood pressure was recorded from the left common carotid artery. The arterial cannula (PE-50; 9.5 cm) was connected to a Statham P23Db transducer which was attached to a Gilson ICM-B Polygraph. Blood was withdrawn (7 ml) from the carotid artery in a plastic syringe containing EDTA (1 mM) and indomethacin (0.1 mM), and immediately centrifuged at 2500 rpm for 20 min in a refrigerated Sorvall RC-2B centrifuge. The plasma samples were decanted at once and maintained frozen at -30° C until analyzed. The chest of the rat was opened, the heart was quickly excised and trimmed free of large vessels, adhering fat and connective tissue, and rinsed well in a preoxygenated ice-cold physiological solution (4° C) to remove blood. After dissection of atria and ventricles, and weighing each chamber separately, slices of right and left atria (RA, 31.5 ± 1.00 mg, and LA, 30.8 ± 0.73 mg, respectively), and slices of right and left ventricles (RV, 133 ± 0.71 mg, and LV, 133 ± 1.03 mg, respectively), were homogenized with a Polytron tissue disintegrator (Model SDT-080 EN Tissumizer, Tekmar Co.). Homogenates were filtered through two thicknesses of surgical gauze and immediately centrifuged at 2500 g for 10 min. The resulting supernatants were stored at –30° C until required for PGE_2 and cAMP analyses. Conditions of blood and tissue collection, and preparation of homogenates were the same for NTR and EHR, and indomethacin (0.1 mM) was added after excision of the heart and before homogenization of slices saved for PG assays, to prevent the generation of PGs.

Determination of prostaglandin-E_2

The PGE_2 concentrations in plasma and heart tissue samples were measured by the radioimmunoassay method (RIA), as reported previously (6, 20). Briefly, each sample acidified to pH 3.0 with 98% formic acid was extracted twice with three times the sample volume of a mixture of cyclohexane/ethylacetate (1:1, v/v), and then purified by chromatography on silicic acid columns (100 mesh, Mallinckrodt Chem. Works) to separate PGE from PGF series and from other PGs, and especially from nonspecific lipids. The radioactivity recovery of labelled-PGE_2 added (1000 cpm/0.1 ml) to the samples before extraction was 95 ± 7.5%. The RIA of immunoreactive-PGE_2 (i-PGE_2) from the corresponding chromatographic fraction was performed in parallel to the standard unlabelled-PGE_2 (Upjohn Co., Kalamazoo, MI), using tritiated-PGE_2 (New England Nuclear Corp., Boston, MA) as the radioligand, and using the appropriately diluted antisera to this PG (PGE_2-BSA, Seragen Inc., Boston).

The radioactivity was determined in a liquid scintillation counter (△Packard 1600TR automatic liquid scintillation analyzer, efficiency 60%).

Determination of cyclic 3'-5' monophosphate (cAMP)

The cAMP cardiac levels were assayed by the protein binding method (3, 8). Each cardiac tissue supernatant was extracted three times with two volumes of ethyl ether. The extracts were dried under 100% N_2 and redissolved in 20 mM K_2HPO_4 (pH 6.0). The binding reaction was carried out at 4° C for 90 min. The cAMP plasma concentration was measured by the method of Nistrup Madsen et al (14), a procedure which allows measurement of cAMP in unextracted plasma samples. A standard curve was obtained by simultaneous determination with unlabelled cAMP (Sigma Chemical Co.).

Reagents used in this work were of analytical grade (Sigma Chemical Co.) and the glass material used was siliconized (Prosil-28, PCR, Inc., Florida).

Statistical analysis

Results are expressed as means ± SEMs. Differences between means were assessed with Student's t-test, significance being accepted with values of probability less than 0.05.

RESULTS

Content of i-PGE_2 and cAMP in the hypertrophied rat heart

Thirty days after coarctation of the aorta, arterial pressure increases of 41% (from 104 ± 1 to 147 ± 2 mm Hg) were associated with significant increases (44%) in left ventricular weight (from 540 ± 3 to 780 ± 9 mg wet weight). The chronic increase in cardiac work load was accompanied by simultaneous changes in PGE_2 and cAMP contents in atria and ventricles. Table I shows the values of i-PGE_2 and cAMP concentrations in atria and ventricles of NTR and EHR. We found higher levels of i-PGE_2 in the homogenates of atria and ventricles of the hypertrophied rat heart compared to the respective control values ($p < 0.001$). In contrast to these very high PGE_2 heart tissue contents, significant decreases of cAMP concentrations were observed in the same cardiac samples ($p < 0.001$). In addition, we determined that i-PGE_2 and cAMP concentrations were higher in the atria than in the ventricles.

Changes in PGE_2 and cAMP arterial plasma levels

Figure 1 shows that both i-PGE_2 and cAMP concentrations were significantly increased in the arterial plasma of the aortic constricted rats compared to sham-operated ones (222% and 40%, respectively).

DISCUSSION

This study demonstrates that rat chronic pressure overload is accompanied by markedly higher PGE_2 concentration in cardiac and in arterial plasma samples. It has been reported that neurohumoral mechanisms are activated during acute decompensation, but normalize during the chronic cardiac compensated phase (4). Even though this study was performed one month after surgical production of aortic stenosis, the PGE_2 plasma and tissue content remained elevated during sustained pressure overload. In addition, we observed higher levels of PGE_2 in both atria and in both ventricles of the hypertrophied rat hearts. Our finding of a higher PG content in atria than in ventricles is in agreement with that of Mentz et al (12), who demonstrated that atria show higher PGI_2-formation than ventricles in guinea pigs and rabbits. Increases of PGs in a tissue could result from changes in either the processes of biosynthesis and/or degradation, but the latter alternative was shown to be unlikely because isolated rabbit hearts did not appreciably metabolize exogenous PGs (1). Furthermore, it is possible that increased PGE_2

TABLE I

Contents of immunoreactive prostaglandin-E_2 (i-PGE_2) and cyclic adenosine 3'-5' monophosphate (cAMP) in rat heart tissue

HEART CHAMBERS	i-PGE_2 (ng/g wet weight of tissue)				cAMP (pmol/g wet weight of tissue)			
	RA	RV	LA	LV	RA	RV	LA	LV
NTR (n = 10)	55.8 ±4.3	39.0 ±3.2	61.8 ±2.6	41.5 ±3.2	263 ±20.0	76.0 ±8.0	315.0 ±30.0	21.0 ±3.0
EHR (n = 10)	125.0* ±4.1	56.7** ±4.0	226.7* ±8.1	136.3* ±11.3	176.0* ±19.0	38.0* ±7.0	142.0* ±24.0	7.0* ±1.0

RA = right atrium; LA = left atrium; RV = right ventricle; LV = left ventricle.
NTR = normotensive rats; EHR = experimental hypertensive rats
n = number of animals.
Means ± SEMs. *$p < 0.001$, **$p < 0.005$.

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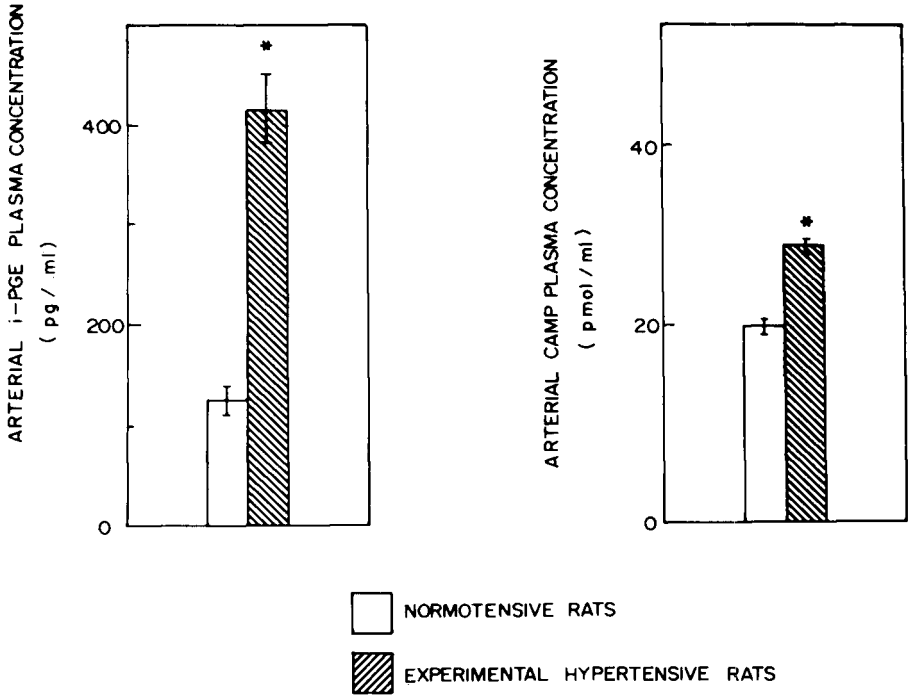

Fig. 1: Arterial plasma concentrations of immunoreactive prostaglandin-E_2 (i-PGE, left side) and cyclic AMP (cAMP, right side) in sham-operated normotensive rats (open bars) and experimental hypertensive rats (hatched bars). Means ± SEMs. * $p < 0.001$. n = 10 in each group.

levels in arterial plasma of EHR represent both active secretion of this PG by the left side of the heart and increased PGE_2 synthesis by the arterial vascular tissue.

The underlying mechanism for the cardiac responses to PGs has not been fully elucidated. We have not yet explained the finding of decreased cardiac cAMP levels in EHR, but the rate of extrusion into the extracellular space or increased degradation may be important factors regulating the cardiac intracellular level of cAMP under chronic overloaded conditions. It has been reported that α_1-stimulation, usually coupled to a positive inotropic effect of cardiomyocytes, stimulates cAMP degradation (2), and that the increased concentration of PGs in the pericardial fluid modulates cardiac neural regulation (13). It is possible that in the first stage of the acute cardiac decompensation, the relation between PGE and cAMP could play a critical part in preserving cardiac function to compensate the sudden increase in heart work (10), while in the chronic compensated phase, another mechanism mediated by PGs independently of cAMP should be considered in the hemodynamic compensatory events. Moreover, it has been demonstrated that the cardiac responses to PGE_1 in sheep are not mediated by cAMP (17). Furthermore, it has been reported that PGs might mediate changes in protein turnover induced by muscle tension (15). It is possible that in the state of so-called stable compensatory hypertrophy, active growth and stimulation of protein synthesis persist within myocardial cells for longer times than usually assumed. That phenomenon could be associated with local PGs increase. Our findings support the suggestion that increased myocardial and vascular PGs production may represent an homeostatic adaptation or a pathophysiological response of the chronically overloaded heart.

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