Subcellular distribution of prostaglandin-E_2 and prostaglandin-$F_{2\alpha}$ in atrial tissue from patients with mitral valve disease

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The distribution of prostaglandin-E_2 (PGE_2) and prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) was studied in subcellular fractions isolated from homogenates of human atrial fresh tissue by differential centrifugation. Right and left atrial samples were excised from the same heart of six patients with mitral valve disease at the time of open heart surgery. The atrial fractions investigated were mitochondrial (8,500 g pellet), microsomal (100,000 g pellet) and cytosol soluble (100,000 g supernatant) fractions. After extraction of prostaglandins from the three atrial fractions and separation of PGE from PGF series by chromatography on silicic acid column, these prostaglandins were measured by radioimmunoassay. The results showed that PGE_2 and $PGF_{2\alpha}$ were located mainly in the soluble cytosolic fraction of right and left atrial tissue ($p < 0.001$). Furthermore, the prostaglandins levels were higher in left than in right atria of these patients ($p < 0.001$). The relation between prostaglandins heart generation in response to elevated work load of mitral valve disease is discussed.

Key-terms: *prostaglandins, left atrium, right atrium, subcellular fractions, mitral valve disease*

INTRODUCTION

Experimental and clinical studies have documented that heart tissue and isolated myocytes have the capacity to synthesize prostaglandins (1, 3, 4, 8). In addition, Berger *et al* (4) demonstrated that dog heart tissue converts arachidonic acid mainly into PGE_2, $PGF_{2\alpha}$ and PGI_2.

A significant increase in cardiac cAMP levels, associated with activation of myocardial prostaglandin synthase, was found 5 min after aortic constriction in the cat (25), suggesting involvement of prostaglandins and adenylyl cyclase system in the early response of myocardium to acute pressure overload. In addition, Chazov *et al* (6) reported that augmentation of prostaglandins may be an important local factor for the adaptation of rabbit myocardium to acute cardiac overload, and we (27) found elevated PGE_2 atrial levels in an animal model of left ventricular hypertrophy. These findings suggest that enhanced capacity of prostaglandin production by the chronically overloaded heart represents a compensatory event. In accordance with this hypothesis, the presence of prostaglandins in blood samples of the coronary sinus in patients

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with coronary artery disease has been reported (5), and we (8) demonstrated that PGE_2 and $PGF_{2\alpha}$ are present in atrial tissue of patients with heart diseases.

It has also been shown (12, 22) that cyclooxygenase (COX), the enzyme responsible for the conversion of arachidonic acid into prostaglandins, exists in two forms, named COX-1 and COX-2, which are encoded by different genes (16). COX-1 is expressed constitutively, and COX-2 may be induced by a series of pro-inflammatory stimuli (23). Recent studies have shown that COX-2 induction and increased formation of prostaglandins may contribute to the pathophysiology of congestive heart failure (24).

The present study was performed to define the distribution of PGE_2 and $PGF_{2\alpha}$ in subcellular fractions isolated from homogenates of atrial tissues from patients with mitral valve disease (MVD). The mitochondrial (MitF, 8,500 g pellet), microsomal (MicF, 100,000 g pellet), and cytosol soluble (CysF, 100,000 g supernatant) fractions were analyzed. In addition, we determined whether differences do exist in these prostaglandins levels between right and left atrial subcellular fractions obtained from the same hearts of patients with MVD.

MATERIALS AND METHODS

Materials

The reagents were of analytical grade (Merck and Sigma Chemical Co). Silicic acid (100 mesh) was from Mallinckrodt Inc. Standard unlabelled prostaglandins were obtained from Upjohn Co, Kalamazoo, MI. Tritiated prostaglandins with specific activity 165 Ci/mmol (NET-428) and 150 Ci/mmol (NET-433) for [^{3}H]-PGE_2 and [^{3}H]-$PGF_{2\alpha}$, respectively were purchased from New England Nuclear Co, Boston, MA. The antisera of high affinity and specificity to these prostaglandins (PGE_2-BSA, and $PGF_{2\alpha}$-BSA) were obtained from Diagnostics Pasteur (France). Indomethacin was from Sigma Chemical Co, St Louis, MO. All glass material used in this work was siliconized (Prosil-28, PCR Research Chemical Inc).

Patients

We studied six patients with chronic MVD, three women and three men, aged 37 ± 5 (mean ± SEM). Five had pure mitral stenosis and one had mitral regurgitation without other heart disease. The patients recruited were in New York Heart Association Functional Class II or III, and were selected at the Cardiology Section of the Salvador Hospital in Santiago, Chile. The study conformed to the Ethics Committee requirements of the Salvador Hospital.

Preparation of homogenates and subcellular fractions

Atrial samples obtained during open heart surgery were rapidly washed in ice-cold physiological solution and weighed. The average weights of the right (n = 6) and left (n = 6) atrial samples were 245.8 ± 9.7 and 256.8 ±13.5 mg, respectively. Each atrial sample was processed individually as reported previously (26).

The fresh atrial tissue was minced fine with scissors and was homogenized with a polytron tissue disintegrator (Model SDT-080 EN Tissumizer, Teckmar Co), along with 5.0 ml of a cold homogenizing solution (mM/l): saccharose, 250.4; HEPES, 2.5; and EGTA, 0.5; containing indomethacin 0.1 mM, to prevent the generation of prostaglandins during this and the following steps. Homogenates were filtered through two layers of surgical gauze and immediately centrifuged at 600 g for 10 min, using a Sorvall RC-2B centrifuge, to remove nuclei and coarse cell debris. In the following differential fractionation, a Hitachi model 55-P ultracentrifuge with a type RP 40 rotor was used. The supernatant solution obtained at 600 g was further centrifuged at 8,500 g for 10 min to sediment a crude MitF, leaving a supernatant containing other particles together with the cytosol. The resulting supernatant was centrifuged at 100,000 g for 90 min to sediment a MicF, leaving a clear CysF as the final supernatant. The precipitated MitF and MicF pellets were then homogenized in 0.2 ml of deionized

water (neutral pH) and dissolved in a 96% aqueous ethanol mixture (2/1, v/v) for prostaglandins analysis.

Determination of prostaglandins

The PGE_2 and $PGF_{2\alpha}$ concentrations in each subcellular fraction were measured by radioimmunoassay (RIA), as reported previously (7, 26). Each subcellular fraction (MitF, MicF and CysF) was adjusted to pH 3 with 98% formic acid and extracted twice for acid lipids 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 to separate PGE from PGF series and from other prostaglandins, and specially from nonspecific lipids. The radioactivity recovery of labelled prostaglandins added (1,000 cpm/0.1 ml) to the samples before extraction was about 95%.

The RIA of immunoreactive PGE_2 (i-PGE_2) and immunoreactive $PGF_{2\alpha}$ (i-$PGF_{2\alpha}$) from the corresponding chromatographic fraction was performed in parallel to the standard unlabelled prostaglandins using tritiated prostaglandins as the radioligand and using the appropriately diluted antisera to these prostaglandins. The antibody cross reaction for i-PGE_2 and i-$PGF_{2\alpha}$ was estimated with an error of 0.01. Separation of antibody-antigen complexes from free antigen was achieved by adsorption of the free tracer onto activated dextran-charcoal suspension and 10 min later centrifuged for 10 min at 2,000 rpm. The supernatant containing the antigen-antibody was decanted into a counting scintillation vial and 10 ml of scintillation cocktail was added. The reproducibility of the RIA was investigated by testing the standard samples in triplicate and the unknown samples in duplicate in the same RIA and repeated the analysis of the samples already analyzed in another RIA during the same week.

The variation coefficients of intra-assay and inter-assay were 5.3%, and 7.1%, respectively. The radioactivity was determined in a liquid scintillation counter (Packard, Model 1600TR automatic liquid scintillation analyzer, efficiency 60%).

Statistical analysis

Results are expressed as means ± standard errors of the means (SEM). Differences between means were assessed by Student's *t*-tests, significance being accepted for $p < 0.05$.

RESULTS

Subcellular distribution of i-PGE_2 and i-$PGF_{2\alpha}$ in right and left atria of patients with MVD

The three subcellular fractions obtained from fresh atrial samples of patients with MVD were found to contain PGE_2 and $PGF_{2\alpha}$. Figure 1 shows the distribution of these prostaglandins in mitochondrial fraction (MitF), microsomal fraction (MicF) and cytosol soluble fraction (CysF) of right and left atrial tissues. These results demonstrate that i-PGE_2 and i-$PGF_{2\alpha}$ are located mainly in the CysF of both atrial tissues, since the final supernatant after centrifuging at 100,000 g contained between 71.7 ± 2.0 and 74.9 ± 7.0 per cent of the total prostaglandin content in the right and left atria, respectively. In addition, the subcellular distribution of i-PGE_2 and i-$PGF_{2\alpha}$ in MitF, MicF and CysF of right and left atria were remarkably similar.

Comparison of prostaglandins concentrations between right and left atria of patients with MVD

Figure 2 illustrates the i-PGE_2 and i-$PGF_{2\alpha}$ subcellular fractions values of right and left atrial samples removed from the same hearts of six patients with MVD. The prostaglandins atrial concentrations are expressed as pg/mg wet weight of tissue ± SEM. The highest i-PGE_2 and i-$PGF_{2\alpha}$ concentrations were in subcellular fractions obtained from the left atrium. Prostaglandins levels of the MicF and CysF of left atria were markedly elevated compared to those observed in these subcellular fractions of right atria.

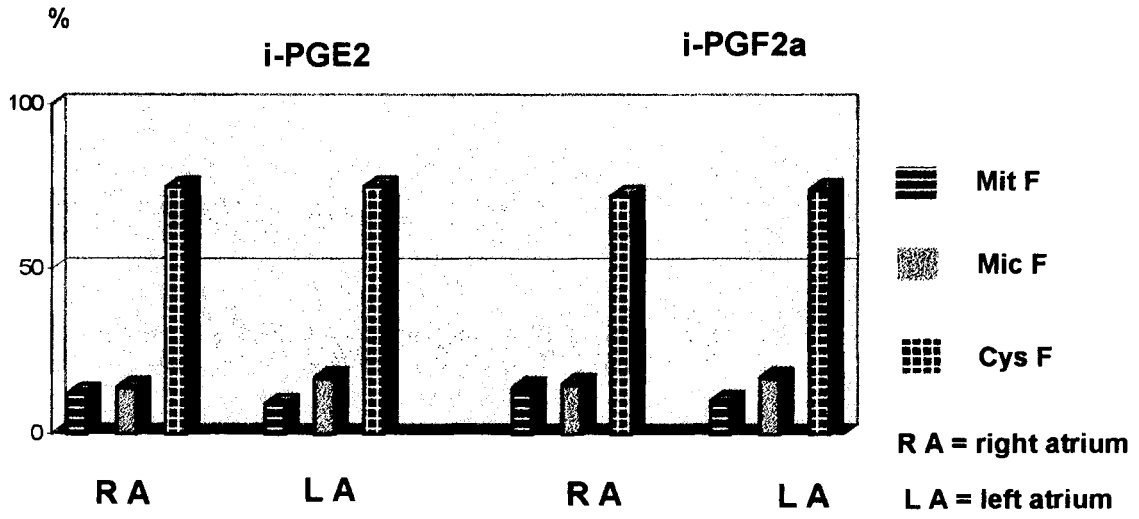

Fig 1: Subcellular distribution of i-PGE_2 and i-$PG_{2\alpha}$ among mitochondrial (MitF), Microsomal (MicF) and cytosol soluble (CysF) fractions. Means ± SEMs as percentages of total prostaglandins contents in samples of right atrium (RA) and left atrium (LA) of patients with mitral valve disease. * $p < 0.001$ when comparing CysF with either MitF or MicF.

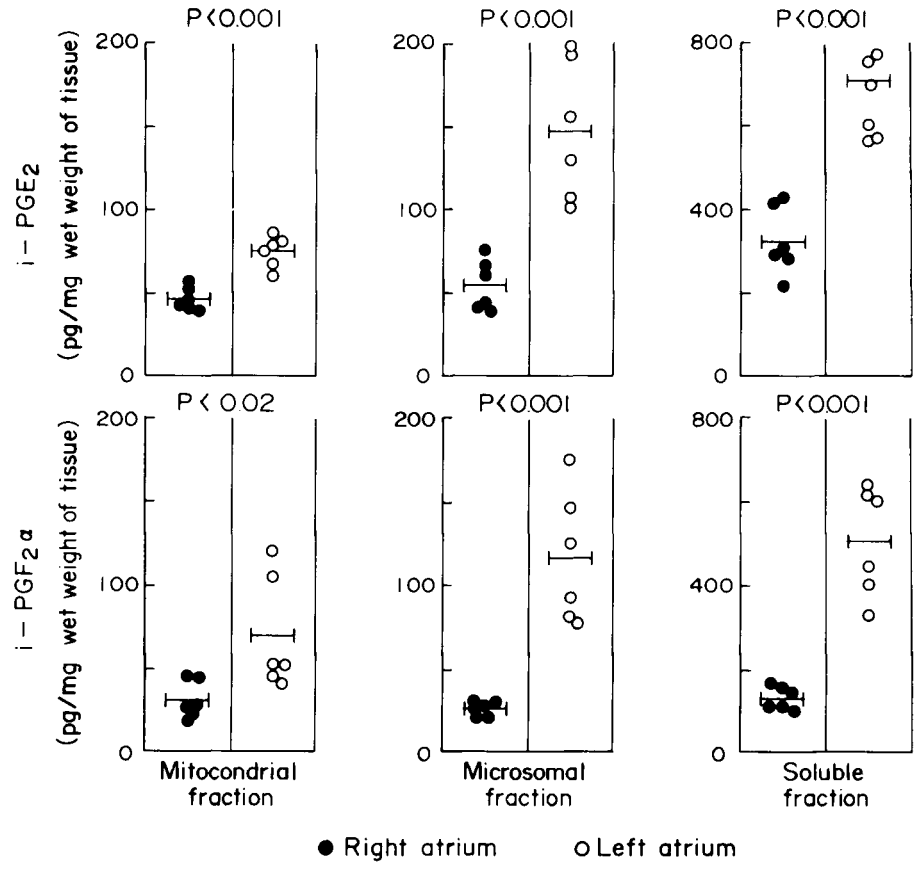

Fig 2: Immunoreactive PGE_2 and $PGF_{2\alpha}$ contents in subcellular fractions of atrial samples from patients with MVD (n = 6 in each atrium). Horizontal lines, means in each subcellular fraction. $p < 0.02$ when comparing mitochondrial fraction between left and right atria; $p < 0.001$ when comparing microsomal and soluble fractions of left and right atria.

$PGE_2/PGF_{2\alpha}$ ratio in subcellular fractions of atrial tissue

The $PGE_2/PGF_{2\alpha}$ ratios in each subcellular fraction of right and left atria are presented in Table I. Ratios are expressed as means ± SEM. No significant differences in these ratios among the MitF and MicF of right and left atria were observed. However, we found higher i-PGE_2 than i-$PGF_{2\alpha}$ levels in the CysF from right atrial samples. These results argue for a preferential PGE_2 synthesis in the right atrium compared to the left atrium of patients with MVD.

DISCUSSION

The present study showed different subcellular PGE_2 and $PGF_{2\alpha}$ distribution in atrial tissue of patients with MVD. The demonstration that synthesized prostaglandins were recovered mainly in the CysF of the human atrial homogenates is consistent with the generally accepted view that prostaglandins are neither stored nor concentrated within specific subcellular particles, but are rapidly released from the site of synthesis into the cytoplasm of the cell to pass to the extracellular compartment without undergoing cell metabolic inactivation (9, 21).

Anggård *et al* (2) showed that whereas PGE_2 is mainly present in the CysF, prostaglandin-synthase occurred exclusively in the microsomal fraction of rabbit kidney homogenates. Also, Parkers *et al* (19) reported that prostaglandin-synthase was located in the microsomal fraction of the Guinea-pig lung. However, these findings differ from those of Kataoka *et al* (10), who reported a wide distribution of PGE and PGF material throughout subcellular compartments in cerebral cortex tissue. These disparities indicate that prostaglandins synthesis might vary from tissue to tissue and their distribution in subcellular particles may be species dependent.

Furthermore, the different enzymes can also contribute to explain the intracellular distribution of prostaglandins. Since both COX-1 and COX-2 can generate prostaglandins and they have different subcellular distribution (22), this could also explain the subcellular distribution of prostaglandins. In addition, we demonstrated in this work, that MicF and CysF of left atrial tissue have markedly higher PGE_2 and $PGF_{2\alpha}$ concentrations that those subcellular fractions of right atrial samples obtained from the same hearts of patients with MVD under similar conditions and assay procedures.

The above results raise the possibility that heart tissue of patients with MVD increases PGE_2 and $PGF_{2\alpha}$ synthesis in response to a higher workload. Furthermore, Lai *et al* (13) have reported that $PGF_{2\alpha}$ induces cardiac myocyte hypertrophy *in vitro* and cardiac growth *in vivo*. It has been reported (18) that infusion of prostaglandins ameliorates severe chronic heart failure. In contrast, administration of inhibitors of prostaglandins synthesis contributes to heart failure decompensation (11, 15). Recently, pericardial levels of 8-iso-$PGF_{2\alpha}$ have been found to increase in patients with heart

Table I

$PGE_2/PGF_{2\alpha}$ ratios in each subcellular fraction of atrial samples from patients with mitral valve disease. (Means ± SEMs; n = 6).

Subcellular fractions	Right atrium	Left atrium	P
MitF (8,500 g pellet)	1.7 ± 0.29	1.3 ± 0.26	NS
MicF (100,000 g pellet)	2.2 ± 0.41	1.5 ± 0.35	NS
CysF (100,000 g supernatant)	2.6 ± 0.38	1.4 ± 0.26	<0.01

MitF, mitochondrial fraction; **MicF**, microsomal fraction; **CysF**, cytosol soluble fraction.
NS, not significant difference.

failure (14). Those prostaglandin levels were correlated with functional severity of heart failure and were associated with ventricular dilatation.

Wong *et al* (24) demonstrated an increased COX-2 expression in the myocardia of patients with congestive heart failure. The pathophysiological role that COX-2 plays may depend on a number of factors, among which the cell types and their inherent prostanoid synthetic pathways appear to be the key determinants (17, 20). It is possible that increased induced-COX-2 expression in the left atria of patients with MVD results in increased synthesis of PGE_2 and $PGF_{2\alpha}$, and contributes to the pathophysiology of congestive heart failure. However, further studies are needed to clarify the significance of the enhanced capacity of the heart prostaglandins synthesis and their relationship with myocardial performance.

ACKNOWLEDGMENTS

This work was supported by Grant M-1871-8413, Department for Research Development, University of Chile, Santiago, Chile.

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