

PGE₂ and 6-Keto-PGF_{1α} Generation and Cyclic AMP in the Atria of Rats during Normoxia and Hypoxia

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Abstract

Circulating antibodies can be detected in chronic periodontitis patients given the presence of serum auto antibodies against β_1 -adrenoceptor (β_1 -AR) in periodontitis patients by using cardiac membranes or a synthetic β_1 -AR peptide corresponding to the second extracellular loop of human β_1 -AR as antigens. Periodontitis patients also exhibit enhanced atria contractility in normoxia and tonic contraction in hypoxia. Atenolol, synthetic β_1 peptide and nifedipine abrogate the action of both Isoproterenol and β_1 IgG on atria contractility in both experimental conditions. In turn, β_1 IgG display a partial agonist-like activity and modifies the contractility of isolated atria, accompanied by an accumulation of cyclic AMP nucleotide in normoxia and hypoxia. The autoantibody is able to provoke an increment in the concentrations of PGE₂ and 6-keto-PGF_{1α}, which is abrogated by preincubating atria with adrenergic antagonist, synthetic β_1 peptide and prostanoid receptor antagonists. On this basis this study seeks to correlate the periodontitis infection to an increased risk of cardiac disease highlighting the role of β_1 IgG in the alteration of myocardial contractility and the subsequent increment of prostaglandins (PGE₂ and 6-keto-PGF_{1α}) accompanied by an increment in the production of cyclic AMP, resulting in an effective adaptation of myocardium function in acute ischemia.

Keywords: Myocardium; Hypoxia; Periodontitis; Prostanoids; cAMP; Contractility

Abbreviations: AR: Adreno Receptors; COX-2: Cyclooxygenase-2; PKC: Protein Kinase C; PTKs: Protein Tyrosine Kinases; ROS: Reactive Oxygen Species; NO: Nitric Oxide; CAL: Clinical Attachment Loss; cAMP: Cyclic Adenosine Monophosphate; ANOVA: Analysis of Variance; ISO: Isoproterenol; KRB: Krebs Ringer bicarbonate; HRV: Heart Rate Variability

Introduction

Intermittent hypoxia has been demonstrated to have powerful cardiovascular protective capabilities [1]. These effects can be grouped into three major categories: 1: adaptation of organs and tissues responsible for oxygen uptake and transport [2], 2: proliferation and increased density of vascular networks [3], and 3: increased mitochondrial density in the brain, the liver and the heart [4]. Consequently, adaptation to intermittent hypoxia has been used to treat patients with ischemic heart disease [5] and with post myocardial infarction heart failure [6]. Periodontitis is on its part characterized by a gingival inflammation in which periodontopathic bacteria generate immunological inflammatory responses and is seen as a key risk factor for the onset of cardiovascular disease [7-9]. In this connection it is important to mention, that autoantibodies against atria cardiac β_1 -adrenoreceptors (AR) are able to mimic the effect of an authentic β_1 -AR agonist acting on atria β_1 -AR [10] in the sera of patients with periodontitis. The joined release of

host-derived inflammatory mediators (such as cytokines) and serum anti- β_1 -AR IgG into the circulation of chronically-inflamed periodontal tissues may thus provide a link between periodontal disease and cardiovascular disease [8,9]. Other studies state that the exposure of the heart to a sub lethal ischemic stress induces a phenotypic change that renders the myocardium relatively resistant to a subsequent ischemic insult occurring 24 to 72 hours later, corresponding to late phase of ischemic preconditioning [11,12]. Late phase of ischemic preconditioning is itself associated with the up regulation of cyclooxygenase-2 (COX-2) expression; the COX-2-dependent synthesis of prostanoids (ie, prostaglandin E₂ and prostaglandin I₂) is viewed as essential for the observed cardio protective effects [13]. Prostanoids have been shown to alleviate myocardial ischemia/reperfusion injury [14]. What remains unknown on the one hand is which are the signaling pathways whereby a sub lethal ischemic stress leads to an increased expression of prostanoids in the heart; on the other, how the modulation of COX-2 in adult myocardium takes place.

And yet, COX-2 has been shown to be unregulated by herbal ester and oxidative stress in isolated neonatal myocyte [15]. The expression of COX-2 has been reported to be controlled, among other factor(s), by reactive oxygen species (ROS) [16], protein kinase C (PKC) [17], protein tyrosine kinases (PTKs) [18] in macrophage and platelet cells and to be accompanied by an increment of PGE₂ and PGI₂. In turn, these signaling elements are acknowledged to be implicated in late phase of ischemic preconditioning and in ischemic heart by demonstrating that the development of delayed cardio protection is triggered by the formation of nitric oxide (NO) and ROS during the initial phase of ischemic preconditioning stimulus and by the subsequent early activation of an araquidonic acid cascade that involves an increase of PGE₂ and PGI₂ with the sequential recruitment of PKC [19,20]. Consequently, in the present study, we test the hypothesis that β₁ IgG participate in the induction of COX-2 in response to sub lethal myocardial ischemia, whereas the cardiac synthesis of prostanoids such as PGE₂ and 6-keto-PGF_{1α} are able to protect the myocardium in the course of ischemia under normoxia and hypoxia conditions. We also study the contractile pattern of the atrium in these experimental conditions without addition of (control), and in the presence of isoproterenol and β₁ IgG. Our results suggest that the enhancement of PGE₂ and 6-keto-PGF_{1α} with the subsequent increment on cyclic AMP may be involved in the pathological process during hypoxia condition in isolated rat atria myocardium.

Materials and Methods

Patients

The study group consisted of 16 male adult patients with periodontitis who were attending the Periodontology Clinic from the metropolitan area of Buenos Aires. These men were aged 32 to 50 years old. Healthy subjects aged 30 to 46 years old were used as control (14 male subjects). The assessment of clinical parameters was carried out by a calibrated periodontist following on the basis of clinical parameters, attending to the severity of the disease and the periodontal tissue destruction [21]. The characteristic clinical signs of periodontitis were the following: loss of clinical attachment; horizontal or/and angular alveolar bone loss; periodontal pocket formation; and gingival inflammation. At least six sites with ongoing periodontal disease were required to be considered in the study. Clinical measurements on patients with periodontitis included sites with alveolar bone loss >2 mm and a pocket depth > 5mm with bleeding and attachment loss >3 mm. In the healthy subjects (control group), the probing depth was < 3 mm and the attachment loss was < 2 mm. The probing pocket depth and the clinical attachment level were also assessed at six sites per tooth and the bleeding on probing was assessed at four sites per tooth. None of the subjects (patients in group I and controls in group II) had either systemic illnesses or were smokers. The patients with periodontitis had not received periodontal treatment or antibiotics within the preceding 5 months or any anti-inflammatory drugs 3 weeks prior to the study. The pocket

probing depth (PPD: ≥ 5 mm), the clinical attachment loss (CAL: ≥ 4 mm) and bleeding on probing: positive, are also determined. All of the patients consented to participate in the study and the investigation was conducted according to the tenets of the Declaration of Helsinki of 1975 as revised in 2000.

Human sera and IgG purification

Sera and the corresponding IgG were obtained from patients with periodontitis and healthy individuals. Six ml of blood was obtained by venipuncture and allowed to clot at room temperature, and the serum was separated by centrifugation at 2000 g and stored at -20°C until used in assays. The IgG was obtained by precipitation with 50% ammonium sulphate and followed by 3 washes and re-precipitation with 33% ammonium sulphate. The resulting precipitate was submitted to chromatography on DEAE-cellulose, equilibrated with 10 mM phosphate buffer (pH 8). The eluted peaks were concentrated by ultra filtration to 10 mg protein/ml. Control immune electrophoresis with goat anti-human total serum and goat non-specific anti-human IgG showed only one precipitin line.

Animals

Adult male Wistar strain rats (250-300 g) were used. The animals were housed in standard environmental conditions and fed with a commercial pellet diet and water ad libitum. The experimental protocol followed the Guide to The Care and Use of Experimental Animals (DHEW Publication, NIH 80-23). Rats were anesthetized with a mixture of ketamine and xylazine (50 and 5 mg kg⁻¹ respectively) and killed by decapitation.

Contractile study

Rats were decapitated and atria were removed quickly and placed in a glass chamber containing a Krebs Ringer bicarbonate (KRB) solution (pH 7.4) gassed with 5% CO₂ in oxygen at 37°C. After a stabilization period of 30 min, spontaneous tension and frequency were recorded using a force transducer coupled to an ink-writing oscillograph, as previously described [22]. Then, the preparations were paced by means of a bipolar electrode using a SK4 Grass stimulator, with stimuli duration of 2 msec and a voltage that was 10% above threshold. The constant resting tension applied to the atria (preload tension) was 750 mg. The contractility (dF/dt) was assessed by recording the maximum rate of isometric force development above the externally applied resting tension. To obtain the maximum IgG effect, different concentrations of IgG were added to normal rat atria every 10 min. The average control (basal) force of tonic contraction in equilibrium before the addition of drugs or β₁ IgG was 205 ± 18 mg, n= 10. Control values (equal to 100%) referred to the dF/dt before the addition of different IgG concentrations.

Assay for cyclic adenosine monophosphate (cAMP)

Rat atria (10 mg) were incubated in 1 ml KRB for 30 min and the β₁-IgG added in the last 15 min in normoxia and hypoxia. When blockers were used, they were added 25 min before the

addition of the antibody. After incubation, atria was homogenized in 2 ml of absolute ethanol and centrifuged at $6000 \times g$ for 15 min at $4^{\circ}C$. Pellets were then re-homogenized in ethanol-water (2:1). Supernatants were collected and evaporated to dryness. Cyclic AMP in the residue was dissolved in a $400 \mu l$ of 0.05 M sodium acetate buffer (pH 6.2). The determination of the nucleotide was done using ELISA according to the protocol of production of cAMP from Amersham Biosciences (Piscataway, NJ, USA). Results were expressed in Pico moles per milliliters (pmol/ml).

PGE₂ and 6-keto-PGF_{1 α} assays

Atria were incubated in 0.50 ml KRB gassed with 5% CO₂ in oxygen at $37^{\circ}C$ in normoxia and hypoxia. Concentration-response curve of β_1 IgG and isoproterenol were added to the isolated atria before the end of the incubation period. Blockers were added 10 min before the addition of different concentrations of β_1 IgG (maximal effect $1 \times 10^{-8} M$) and isoproterenol (maximal effect $1 \times 10^{-7} M$). Atria were then homogenized and transferred into 1.5 ml polypropylene micro-centrifuge tubes. Subsequently, all samples were processed according to the protocol supplied with the PGE₂ and 6-keto-PGF_{1 α} Biotrak Enzyme Immunoassay (ELISA) System (Cayman Chemical, Ann Arbor, MI). The results are expressed as nanograms per milliliters (ng/ml).

Experimental Protocol

The isolated atria underwent a 50 min stabilization under basal conditions (normoxia), during which they were equilibrated in a buffer gassed with 95% O₂, 5% CO₂ ($37^{\circ}C$, pH 7.4; $pO_2 > 600$ mmHg). In hypoxia condition atria were equilibrated in a buffer gassed with 95% N₂, 5% CO₂ ($37^{\circ}C$, pH 7.4; $pO_2 > 100$ mmHg) for 50 min. The adrenergic agonist (isoproterenol, $1 \times 10^{-7} M$) and the antibody (β_1 IgG $1 \times 10^{-8} M$) were added in the last 10 min before the beginning the hypoxic period. The total duration of this experimental design was 120 min. Contractility was then registered and processed for the biochemical analysis of the production of PGE₂, 6-keo-PGF_{1 α} or cAMP. All this protocol was done in atria subjected to normoxia and hypoxia. In the blocking experiments, atenolol ($1 \times 10^{-7} M$, a β_1 -specific adrenergic antagonist) and synthetic β_1 adrenergic peptide ($5 \times 10^{-5} M$) were added 20 min before agonists. Normal β_1 IgG was used as control (basal values). The contractility of atria (dF/dt g/s) and the determination of tonic contraction (mg) were done in both experimental conditions (normoxia and hypoxia).

Drugs

Stock solutions of isoproterenol (ISO), atenolol, PGE₂, 6-keto-PGF_{1 α} , nifedipine, (Sigma Chemical Company, St. Louis, MO, USA) were freshly prepared before each experiment in their respective specific buffers. The β_1 synthetic peptide sequence corresponding to the second extracellular loop of the human β_1 -AR was HWWRA ESDEA RRCYN DPKCC DFVTN RC.

Statistical Analysis

A Student's t-test for unpaired values was used to determine the levels of significance. Analysis of variance (ANOVA) and a post hoc test (Dunnett's method and Student-Newman-Keuls test) were employed when pair-wise multiple comparison procedures were necessary. The differences between means were considered significant at a $P < 0.05$.

Results

Contractility studies

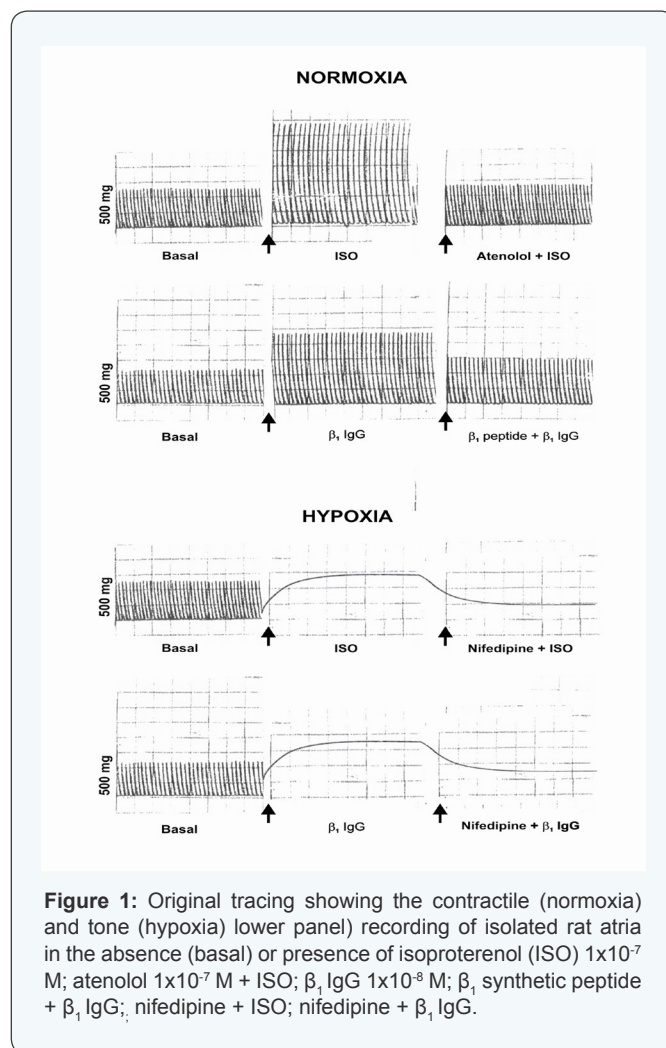


Figure 1: Original tracing showing the contractile (normoxia) and tone (hypoxia) lower panel) recording of isolated rat atria in the absence (basal) or presence of isoproterenol (ISO) $1 \times 10^{-7} M$; atenolol $1 \times 10^{-7} M$ + ISO; β_1 IgG $1 \times 10^{-8} M$; β_1 synthetic peptide + β_1 IgG; nifedipine + ISO; nifedipine + β_1 IgG.

Figure 1 shows that iso proterenol (ISO) $1 \times 10^{-7} M$ (maximal stimulation) enhances atria contractility through the activation of atria β_1 adrenoreceptor in normoxia, given that in the presence of the β_1 specific antagonist atenolol $1 \times 10^{-7} M$, this stimulatory action of ISO was blocked. Furthermore, the β_1 IgG ($1 \times 10^{-8} M$) from the serum of patients with chronic periodontitis also causes a stimulation of atria contractility, which is abolished when the β_1 IgG is previously incubated with the β_1 synthetic peptide ($1 \times 10^{-5} M$). On the other hand when the preparation was in hypoxia, the presence of ISO at $1 \times 10^{-7} M$ resulted in an increase in tone. This effect was mimicked by the autoantibody β_1 IgG at $1 \times 10^{-8} M$. The increment in tone, produced by both ISO

and β_1 IgG, were blunted by nifedipine (calcium blocker agent) at $5 \times 10^{-5} \text{M}$. The heart is a major site of β_1 adrenoreceptors' expression and cardiac β_1 adrenoreceptors mediate the organ's response to changes in circulating nor epinephrine levels and increased myocyte contractility, resulting in positive inotropic and chronotropic effects on the heart. Figure 2A shows that in a concentration-response curve ISO enhance cardiac contractility (dF/dt) through the activation of β_1 adrenoreceptors during normoxia whereas atenolol ($1 \times 10^{-7} \text{M}$) blocks this action. Atenolol alone at this concentration is without effect in the system. Figure 1B shows that increasing concentrations of β_1

IgG were applied to beating isolated rat atria during normoxia, increased atria contractility, which itself reaches the maximal effect at $1 \times 10^{-8} \text{M}$. This effect is almost blocked by pretreating atria with atenolol and with the β_1 synthetic peptide. Normal IgG from healthy individuals used as control did not have any effect on our preparations. During hypoxia, both ISO (1×10^{-7}) and β_1 IgG ($1 \times 10^{-8} \text{M}$) resulted in a time-dependent increase in tone (Figure 1C). Both actions were abolished by preincubating atria with nifedipine ($5 \times 10^{-5} \text{M}$). Normal IgG was ineffective in the system.

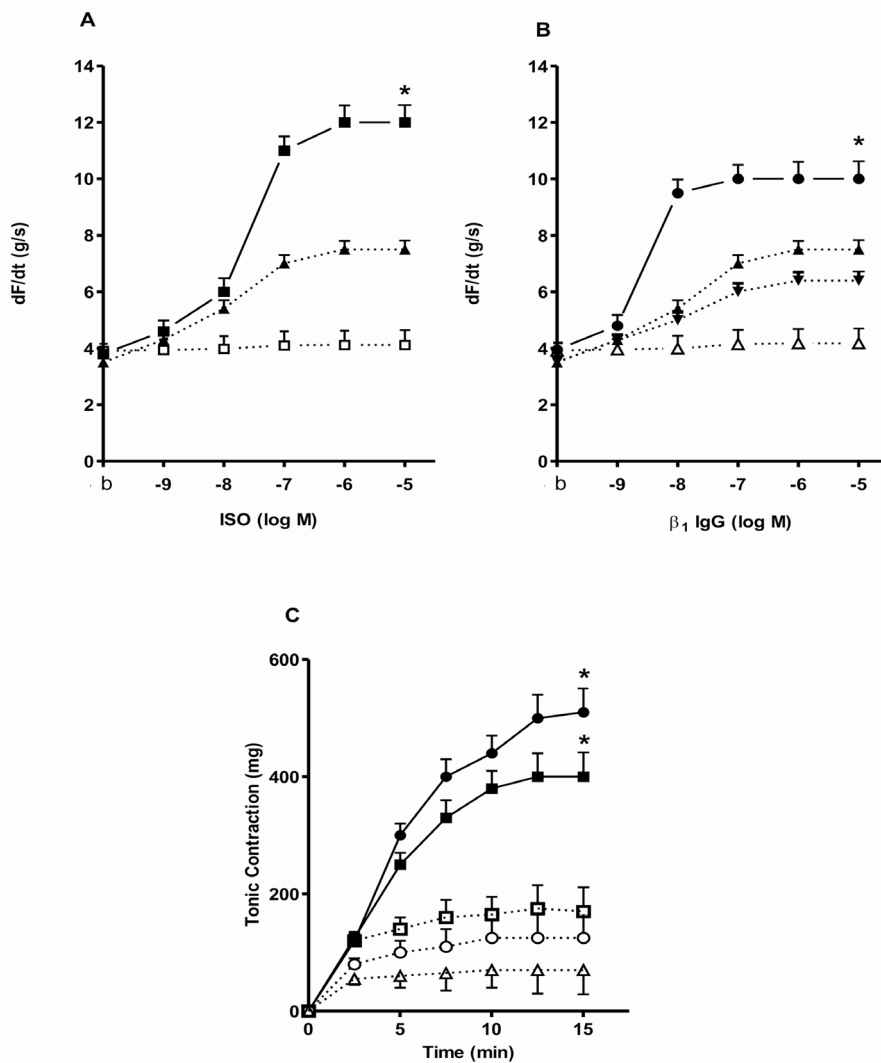


Figure 2: Effects of isoproterenol and β_1 IgG on the contractility of rat atria.

Figure 2A: Contractility recordings of isolated rat atria treated with increasing concentrations of isoproterenol (ISO) (■) or atenolol $1 \times 10^{-7} \text{M}$ + ISO (▲). Atenolol alone at this concentration (□) and basal values (b) are also shown.

Figure 2B: Contractility recordings of isolated rat atria treated with increasing concentrations of β_1 IgG (●) or atenolol $1 \times 10^{-7} \text{M}$ + β_1 IgG (▲) or β_1 synthetic peptide $1 \times 10^{-5} \text{M}$ + β_1 IgG (▼). Normal IgG (Δ) and basal values (b) are also shown. Values are the mean \pm SEM of eight experiments in each case.

Figure 2C: Time course of the action of isoproterenol alone (ISO) (■) or in the presence of nifedipine ($5 \times 10^{-5} \text{M}$) (□) and β_1 IgG alone (●) or in the presence of nifedipine ($5 \times 10^{-5} \text{M}$) (○) on isolated rat atria tonic contractions. Normal IgG (Δ) was also shown. Values are mean \pm SEM of six experiments in each case. * $P < 0.001$ β_1 IgG and ISO vs. antagonist agents.

Biochemical determinations

It can be seen in (Figure 3) that different concentrations of β_1 IgG are able to provoke an increased production of PGE₂ (upper panel, normoxia) and 6-keto-PGF_{1 α} (lower panel, normoxia) in rat isolated atria. This action is inhibited by specific β_1 receptor antagonist atenolol 1x10⁻⁷ M and β_1 synthetic peptide 1x10⁻⁵M respectively. The same (Figure 3) shows that in the course of experimental hypoxia (see upper and lower panel), the β_1 IgG significantly enhances both prostanoids and this action is blunted by atenolol 1x10⁻⁷ M and β_1 synthetic peptide 1x10⁻⁵ M as described above. It is interesting to note, that the increment of

PGE₂ is significantly higher in hypoxia as compared to normoxia, whereas 6-keto-PGF_{1 α} values are not significantly different. On the contrary, ISO 1x10⁻⁷ upon rat isolated atria is able to increase the generation of PGE₂ and 6-keto-PGF_{1 α} in both experimental conditions (normoxia versus hypoxia), and atenolol blunted this action. Normal IgG is unable to modify the generation of both prostanoids in our system (Table 1). As shown in (Table 2), β_1 IgG and ISO trigger an increase in the levels of endogenous cAMP in comparison with basal values of this nucleotide in rat isolated atria during normoxia and hypoxia. It is important to note, that the increment of cAMP observed in hypoxia condition, are significant lower than those observed in normoxia conditions.

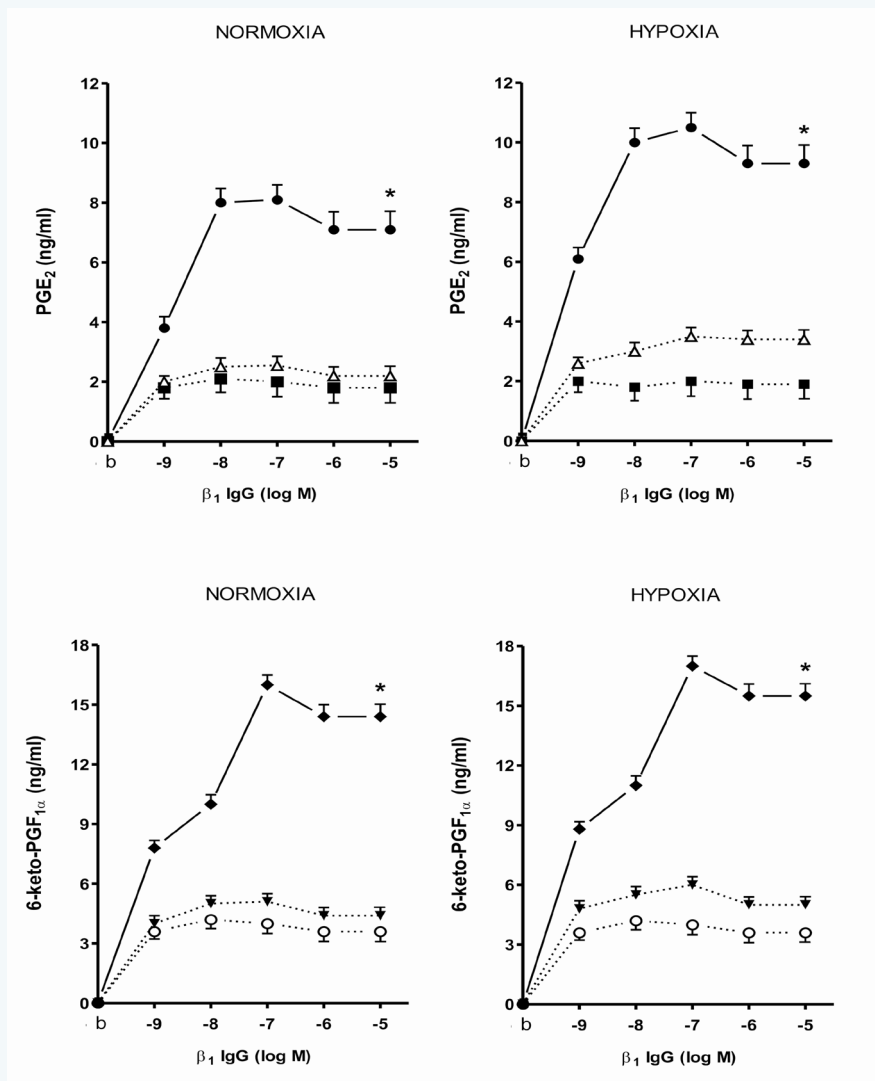


Figure 3: Production of PGE₂ and 6-keto-PGF_{1 α} in isolated rat atria. **Normoxia:** upper panel: concentration-response curve of β_1 IgG on the PGE₂ (●) production by the isolated rat atria alone or in the presence of β_1 synthetic peptide (Δ) or atenolol (■); lower panel: concentration-response curve of β_1 IgG on the 6-keto-PGF_{1 α} (◆) production by the isolated rat atria alone or in the presence of β_1 synthetic peptide (▼) or atenolol (○). **Hypoxia:** : upper panel: concentration-response curve of β_1 IgG on the PGE₂ (●) production by the isolated rat atria alone or in the presence of β_1 synthetic peptide (Δ) or atenolol (■); lower panel: concentration-response curve of β_1 IgG on the 6-keto-PGF_{1 α} (◆) production by the isolated rat atria alone or in the presence of β_1 synthetic peptide (▼) or atenolol (○). Values are mean ± SEM of five experiments in each case performed by duplicate. *P<0.0001 β_1 IgG vs. antagonist agents.

Table 1: Influence of ISO and normal IgG atria on prostaglandins production in rat isolated atria. Values are mean ± SEM of six experiments in each case performed by duplicate. * P < 0.001 ISO vs. Basal; ** P < 0.01 ISO vs. ISO + atenolol.

ADDITIONS	Concentration of Prostaglandins (ng/ml)			
	Normoxia		Hypoxia	
	PGE ₂	6-Keto- PGF _{1α}	PGE ₂	6-Keto- PGF _{1α}
Basal	4.7 ± 0.3	5.1 ± 0.4	5.8 ± 0.5	5.9 ± 0.5
ISO (1x10 ⁻⁷ M)	9.7 ± 0.6*	16.7 ± 1.3*	14.6 ± 1.3*	16.9 ± 1.5*
ISO + atenolol (1x10 ⁻⁷ M)	3.8 ± 0.4**	5.7 ± 0.6**	7.3 ± 0.6**	5.4 ± 0.5**
Normal IgG (1x10 ⁻⁸ M)	4.8 ± 0.4	5.3 ± 0.7	5.9 ± 0.6	5.9 ± 0.7

Table 2: Cycle AMP accumulation induced by β₁ IgG and ISO in rat isolated atria.

Additions	Normoxia (cAMP pmol/ml)	Hypoxia (cAMP pmol/ml)
Basal	0.8 ± 0.02	0.7 ± 0.01
ISO (1x10 ⁻⁷ M)	2.2 ± 0.18*	1.7 ± 0.11*
ISO + atenolol (1x10 ⁻⁷ M)	0.7 ± 0.05	0.9 ± 0.03
β ₁ IgG (1x10 ⁻⁸ M)	2.2 ± 0.31*	1.4 ± 0.09*
β ₁ IgG + atenolol (1x10 ⁻⁷ M)	0.6 ± 0.02	0.6 ± 0.05
β ₁ IgG + β ₁ synthetic peptide (5x10 ⁻⁵ M)	0.9 ± 0.08	0.58 ± 0.04
Normal IgG (1x10 ⁻⁸ M)	1.1 ± 0.09	0.61 ± 0.05

Values are mean ± SEM of six experiments in each case performed by duplicate. * P < 0.001 β₁ IgG and ISO vs. Basal; ** P < 0.001 β₁ IgG and ISO in hypoxia vs. in normoxia.

Discussion

An increased prevalence of cardiovascular and autoimmune diseases in periodontitis has been reported [23,24]. Herein, we demonstrate an association between periodontal infections and an increased risk of cardiovascular disease, pointing to the role of anti β₁-adrenoreceptor (AR) antibodies (β₁ IgG) in the serum of patients with periodontitis. The results provide evidence that components of the serum IgG fraction from patients with chronic periodontitis recognize cardiac membranes. Most of the sera that react positively against the surface of rat cardiac membranes show a positive immune reactivity to the human β₁-AR peptide. In this sense, we establish that the β₁-AR is the target for the anti-rat cardiac auto-antibodies described in patients with periodontitis [25] using a synthetic peptide with an identical amino acid sequence of the second extracellular loop of cardiac human β₁-AR. Knowing that the amino acid sequence of rodent

and human β₁-AR peptides have strong homology [26], we study the β₁ IgG mediated effect of auto-antibodies from patients with periodontitis on rat atria tissue contractility. It is generally accepted that the heart is a major site of β₁-AR expression when it mediates the positive chronotropic effect on the heart. Given that the serum of patients with periodontitis contains auto-antibodies against the cardiac and human gingival fibroblast membrane β₁-AR, we tested the atria contractility in the presence of an authentic β₁ adrenergic agonist isoproterenol and β₁ IgG from patients with periodontitis. The results in normoxia condition provide evidence that components of the serum β₁ IgG fraction from patients with periodontal disease enhance atria dF/dt in that way ISO does, i.e., by recognizing and activating the atria β₁-AR. This is the case since atenolol and β₁ synthetic peptide inhibited the stimulatory action of the antibody upon dF/dt on rat atria contractility, we study the β₁-AR-mediated

effect of auto-antibodies (β_1 IgG) from patients with periodontitis on rat cardiac tissue. Now when rat atria is exposed to hypoxia, ISO and β_1 IgG are only able to provoke a tonic contraction without rhythmic activity, which is in turn depressed by nifedipine, an calcium channel inhibitor. It may be that there is an alteration in the kinetic of myocardium intracellular calcium concentration handling in hypoxia condition and this is what provokes an altered calcium flux as nifedipine abrogates the tonic contraction. A similar phenomenon was described in infarcted heart [27]. Nifedipine was also considered more potent blocking calcium channel agents as an inhibitor of depolarization-induced contractions of arteries [28]. We also demonstrated in previous works that those patients from whom sera contain anti-cardiac and anti β_1 IgG have a decrease in heart rate variability (HRV). This is an important finding with respect to the *in vivo* effects of the auto-antibodies, since the aberrant β_1 sympathetic receptor's expression may result in a decrease in HRV and it is a risk factor for the development of cardiovascular diseases, including heart failure, myocardial infarction, and hypertension [29]. Experimental hypoxia is associated with the changes of the PGE₂/6-keto-PGF_{1 α} systems. Considering that PGE₂ and PGI₂ are mainly located in the cytosolic supernatant fraction of the heart atria homogenates and arteries, and their concentrations (ng/mg protein) are significantly higher in atria tissue than in ventricles, this association supports the idea of a regional variability in the araquidonic acid cascade and its products derived from the cyclooxygenase(s) system during hypoxia. Alterations in the arachidonic acid's metabolism are often involved in the myocardial disturbances associated with hypoxia and ischemia [30]. Additionally [31] demonstrated that the prostanoid production pattern changes in hypoxic rat atria, generating a cardioprotective action involving the prevention of the increases of lactate and cAMP during ischemia [32]. If so, then PGE₂ and PGI₂ would be expected to have an important physiological role in the hypoxic rat heart. In this sense, a possible mechanism could be that just after the hypoxic stimulus, COX-2-derived PGE₂ and 6-keto-PGF_{1 α} generation rises in our study preparations exerting a vasodilatory effect in order to regulate and/or modulate the maintenance of blood flow through the hypoxic tissues. Thus, the maintained levels observed in PGE_{2s} and 6-keto-PGF_{1 α s} systems during hypoxia could be involved in the adaptation of the heart to such situations. Hypoxia seems to directly stimulate the expression of atria cox-2 enzyme, provoking a specific enhancement in the synthesis of PGE₂ and 6-keto PGF_{1 α} . The result is vasodilatation and a decreased vascular resistance of the ischemic bed and the subsequent improvement of the blood flow that in turn, facilitates cardiac function [33]. Atenolol abrogates the effects of both ISO and the β_1 IgG but the synthetic β_1 peptide only abrogates the effect of the autoantibodies. This demonstrates that these autoantibodies recognize the cardiac β_1 adrenoceptor from sarcolemma of rat atria and its capacity to interact with atria β_1 -AR. Moreover, acting as inducers of cox-2 mRNA levels,

in turn, provoked an increase in the cardiac proinflammatory substances such as PGE₂ and PGI₂. The major new finding of this work is the demonstration that an anti- β_1 adrenoceptor IgG behaving as a partial adrenergic agonist, has the capacity to alter the rate of transcription of specific proinflammatory target genes, triggering the cardiac production of PGE₂ and 6-keto PGF_{1 α} in response to receptor-mediated signaling events at the receptor cell cardiac atrium. The coupling of the β_1 IgG and ISO on β_1 -AR in atria permits the transduction of one signalling pathway as cAMP elevation. This effect was abrogated by an antagonistic β_1 -AR agent (atenolol) and by the synthetic β_1 peptide. It is interesting that the partial agonist activity of the auto-antibodies observed *in vitro* mimicked the action on HRV of a partial β_1 adrenergic agonist (celiprolol) described *in vivo* [34]. In our work, ISO and β_1 IgG have the capacity to increase the levels of cAMP at similar values, whereas in hypoxia condition, both the levels of cAMP and the contractile force (tonic contraction) were lower than in normoxia. These results indicate changes in the prostanoids' generation associated with changes in the adenylate cyclase activity and the physiological contractile function of the rat atria. On the basis of our results we suggest the possibility that the alterations in the contractile pattern of atria in the presence of ISO and β_1 IgG be caused by antibody fixation and activation of the cardiac β_1 -AR. If so, then, β_1 IgG could exacerbate the course of ischemic heart diseases by altering the focus of immune function with up-regulation of its own production and suppression of cell mediated immunity. It is important to note, that patients with periodontitis might develop the anti β_1 -AR autoantibodies (β_1 IgG) as a result of molecular mimicry by bacterial pathogens acting on cardiac β_1 -AR as a result of receptor alteration and/or degradation during the inflammatory response. The microbial challenge and inflammatory response in the periodontium via a similar mechanism could be causing increased risk for myocardium diseases. This being the case, neo-antigens from cardiac β_1 -AR tissue may be the key to generation of these autoantibodies (β_1 IgG) coupled to an increased generation of prostanoids and cyclic AMP accumulation, i.e. a clinical manifestation of autoimmunity. The pathogenic properties of anti β_1 IgG antibodies have been ascribed to their potency to continuously stimulate the sympathetic system. Thus, β_1 IgG triggers the following two important mechanisms: the generation of auto-antibodies by targeting cardiac β_1 -AR altering the physiologic behaviour of the myocardium; the exacerbating or maintaining of a chronic inflammatory process in periodontitis disease through the induction of a suppressed immune response [35,36] and an enhancement of PGE₂'s and 6-keto PGF_{1 α} 's production. Cardiac eicosanoid's release in chronic hypoxic conditions is primarily beneficial and results in an effective adaptation of the heart at its function. In acute ischemia it is however primarily deleterious, since it enhances inflammatory reactions with an increased generation of prostanoids, different cytokines and ROS molecules.

Conclusion

On the other hand, the autoantibody provokes an increment in the concentrations of PGE₂ and 6-keto-PGF_{1α} that is abrogated by the pre incubation of atria with the adrenergic antagonist, and the synthetic β₁ peptide respectively. We demonstrate in this study an association between periodontitis infection and an increased risk of cardiac disease, highlighting the role of β₁ IgG in the alteration of myocardial contractility with an increment of prostaglandins (E₂/I₂) turnover together with an increment in the production of cyclic AMP, resulting in an effective adaptation of the myocardium's function in acute ischemia.

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Conflict of interest

There are no conflicts of interest.

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