JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2010, 61, 1, 29-36
www.jpp.krakow.pl

F.M. PECANHA[1,4*], G.A. WIGGERS[1,4*], A.M. BRIONES[1], J.V. PEREZ-GIRON[2], M. MIGUEL[1], A.B. GARCIA-REDONDO[1], D.V. VASSALLO[3], M.J. ALONSO[2], M. SALAICES[1]

THE ROLE OF CYCLOOXYGENASE (COX)-2 DERIVED PROSTANOIDS ON VASOCONSTRICTOR RESPONSES TO PHENYLEPHRINE IS INCREASED BY EXPOSURE TO LOW MERCURY CONCENTRATION

[1]Departamento de Farmacologia, Universidad Autonoma de Madrid, Spain; [2]Departamento de Ciencias de la Salud III, Universidad Rey Juan Carlos, Alcorcon, Spain; [3]Departamento de Ciencias Fisiologicas, Universidade Federal do Espirito Santo, Brasil; [4]Departamento de Ciencias Fisiologicas, Universidade Federal do Pampa, Brasil

We have previously demonstrated that chronic exposure to low-dose of mercury induced endothelial dysfunction and increased vasoconstrictor responses. The aim of this work was to investigate if mercury exposure alters contractile prostanoids production from cyclooxygenase-2 (COX-2) and its contribution to phenylephrine responses. For this, aortic segments from 3-month old Wistar rats daily treated with $HgCl_2$ (1st dose 4.6 μg/kg, subsequent dose 0.07 μg/kg/day, i.m.) or vehicle for 30 days were used. Mercury treatment did not affect systolic blood pressure but increased phenylephrine-induced vasoconstriction. The non selective COX inhibitor, indomethacin (10 μmol/l) reduced the response to phenylephrine more in aortic segments from mercury-treated than control rats. The selective COX-2 inhibitor NS 398 (1 μmol/l), the thromboxane A_2/prostaglandin H_2 receptor (TP) antagonist SQ 29,548 (1 μmol/l), the TXA_2 synthase inhibitor furegrelate (1 μmol/l), the EP_1 receptor antagonist SC 19220 (1 μmol/l) and the AT_1 receptor antagonist losartan (10 μmol/l) reduced phenylephrine response only in vessels from mercury-treated rats. TXA_2 and PGE_2 levels were greater in the incubation medium of vessels from treated than untreated rats; NS 398 decreased these levels only in the mercury group. COX-2 protein was localized in adventitial and endothelial cells. Aortic COX-2 mRNA expression and plasma angiotensin converting enzyme activity were greater in mercury-treated rats. These results suggest that treatment with low doses of mercury increases the release of COX-2-derived vasoconstrictor prostanoids and its participation in phenylephrine responses. The increased activation of the renin-angiotensin system after mercury treatment might be associated to this increased COX-2 activity.

Key words: *mercury, rat aorta, cyclooxygenase-2, vasoconstrictor prostanoids, angiotensin II, angiotensin converting enzyme, thromboxane A_2*

INTRODUCTION

Mercury has historically been used in a wide variety of human activities that significantly increased its emission into the atmosphere. After the disasters in Minamata (1) and Iraq (2) numerous studies highlighted the toxic effects of mercury in different organs and systems (3-5). In addition, during the last years adverse effects of mercury on the heart and vessels have been outlined (6-10) and it has been shown that mercury exposure increases the risk of hypertension, carotid atherosclerosis and coronary heart disease (11-13). It was demonstrated in animal and human studies that mercury increases free radical production and oxidative stress (10, 14-17), affects antioxidant defences (18, 19) and increases lipid peroxidation (20), among others. Recently, we have demonstrated that both acute and chronic administration of low doses of mercury increased phenylephrine-induced contraction in different vascular beds (9, 10) and elicited endothelial dysfunction (10) as a result of the decreased NO bioavailability induced by oxidative stress increase.

Cyclooxygenase (COX) catalyzes the conversion of arachidonic acid to prostanoids, known as important inflammatory response mediators. In addition, endothelial prostanoids production contributes to the regulation of vascular tone. In healthy blood vessels, most prostanoids are formed by the constitutive isoform of COX (COX-1). However, these mediators may also be synthesized by the inducible isoform, COX-2, that can be induced by inflammatory agents such as lipopolysaccharide or cytokines (21-23). In fact, increased vascular COX-2 expression has been reported in pathological conditions associated with inflammatory processes, such as atherosclerosis or hypertension (24-26). Moreover, some authors have suggested a role for contractile COX-2-derived products in both the endothelial dysfunction (27-

*These authors equally contributed to this study

29) and in the vascular contractile responses (24, 25, 30) observed with hypertension or ageing. Acute mercury exposure resulted on angiotensin converting enzyme (ACE) activation (9). In addition, we have recently demonstrated the participation of angiotensin II *via* AT_1 receptors in the increased participation of COX-2-derived contractile mediators in phenylephrine responses in aorta from hypertensive rats. Interestingly, angiotensin II induces COX-2 expression and prostanoid production in vascular smooth muscle cells (31, 32) and adventitial fibroblasts (33).

Different chemical forms of mercury have demonstrated contradictory effects on prostanoids production in different cell types. Thus, methyl mercury reduces PGE_2 and $PGF_{2\alpha}$ production in rabbit kidney medulla slices (34), while $HgCl_2$ increases PGE_2 production in rat glomeruli and glomerular cells (35). Phospholipase A_2 activation has been reported to be involved in the effect of methyl mercury on vascular endothelial cells cytotoxicity (36) as well as in increased platelet aggregation (37). Moreover, the perfusion of the rat tail vascular bed with $HgCl_2$ produces vasoconstriction mediated by COX-pathway products (17). The purpose of the present study was to investigate whether the chronic exposure to low doses of mercury, which approaches the human exposed mercury levels in blood (38, 39), alters contractile prostanoids production from COX-2 and its contribution to phenylephrine responses in aortic rings. In addition, the role of ACE activation in the increased phenylephrine responses observed after low-doses mercury exposure was also investigated.

MATERIALS AND METHODS

Animals

Three-month-old male normotensive Wistar rats were obtained from colonies maintained at the Animal Quarters of the Universidad Autonoma de Madrid. During treatment, rats were housed at a constant room temperature, humidity, and light cycle (12:12-h light-dark). Rats had free access to tap water and were fed with standard chow *ad libitum*. All experiments comply with the current Spanish and European laws (RD 233/88 Ministerio de Agricultura, Pesca y Alimentacion and 609/86). Rats were divided into two groups: control (vehicle - saline solution, i.m.) and rats treated with mercury chloride for 30 days (1st dose 4.6 µg/kg, subsequent dose 0.07 µg/kg/day, i.m. to cover daily loss). The mercury levels in acid-digested samples of whole blood at 30 days of treatment were measured in duplicate by atomic fluorescence spectrometry (PSA Analytical, Model 1025 Millenium System) in the Centro de Espectrometria Atomica (Universidad Complutense de Madrid), as described (10).

Rats were euthanized by decapitation and thoracic aorta was then carefully dissected out and cleaned of connective tissue. For reactivity experiments the thoracic aorta was divided into segments of 2 mm in length. For analysis of mRNA expression, arteries were rapidly frozen in liquid nitrogen and kept at -80°C until the day of analysis.

Reactivity experiments

For isometric tension recording, each aortic segment was set up in an organ bath containing 5 ml of Krebs-Henseleit solution (KHS, in mM: 115 NaCl, 25 $NaHCO_3$, 4.7 KCl, 1.2 $MgSO_4$ $7H_2O$, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 11.1 glucose and 0.01 Na_2EDTA) at 37°C continuously gassed with a 95% O_2-5% CO_2 mixture (pH=7.4). Two horizontally arranged stainless steel pins (75 µm in diameter) were passed through the lumen: one was fixed to the organ bath wall and the other one was vertically connected to a force-displacement transducer (LETICA TRI 011) and to a recorder (MacLab/8e ADI instruments; Cast Hill, Australia). Aortic segments were subjected to a tension of 1.5 g which was readjusted every 15 min during a 60 min equilibration period before drug administration.

Vessels were initially exposed to 75 mM KCl to check their functional integrity and the presence of endothelium was confirmed by the ability of 10 µM acetylcholine (Sigma Chemical Co., St. Louis, Missouri, USA) to relax segments contracted with phenylephrine (Sigma Chemical) at a concentration that produces close to 50% of the contraction induced by 75 mM KCl. After 60 min of washout, a single concentration-response curve to phenylephrine was performed. The effects of losartan (Merck & Co., Inc, Rahway, NY, USA), indomethacin (Sigma Chemical), NS398 (Calbiochem-Novabiochem GmbH, Gad Soden Germany), SQ 29,548 (ICN Iberica, Barcelona, Spain), SC 19,220 (Cayman Chemical, Ann Arbor, Mitchigan, USA) and furegrelate (Sigma Chemical) were investigated by their addition 30 min before phenylephrine.

Measurements of thromboxane A_2 and PGE_2 production

The measurements of PGE_2 and the metabolite of thromboxane A_2 (thromboxane B_2) were determined in the incubation medium after phenylephrine concentration response curves were performed, using enzyme immunoassay commercial kits (Cayman Chemical). The medium was frozen in liquid nitrogen, kept at -80°C until analysis and processed following the manufacturer's instruction.

RT-PCR real time assay

COX-2 mRNA was determined in aortic segments obtained immediately upon removal from the animal. Segments were frozen in liquid nitrogen and stored at -80°C until analysis. Total RNA was obtained by using TRIzol (Invitrogen Life Technologies, Carlsbad, CA, USA). A total of 1 µg of DNAse I treated RNA was reverse-transcribed into cDNA using the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA) in a 20 µl reaction. PCR was performed in duplicate for each sample using 0.5 µl of cDNA as template for COX-2, 1 x TaqMan Universal PCR Master Mix (Applied Biosystems) and 10 x of Taqman Gene Expression Assays (Applied Biosystems, Rn00568225_m1) in a 20 µl reaction. For quantification, quantitative RT-PCR was carried out in an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, from the CAT of Universidad Rey Juan Carlos) using the following conditions: 2 min 50°C, 10 min 95°C and 40 cycles: 15 s 95 °C, 1 min 60°. As a normalizing internal control we amplified β_2 microglobulin (Rn00560865_m1). To calculate the relative index of gene expression, we employed the $2^{-\Delta\Delta Ct}$ method (40) using the untreated samples as calibrator.

Immunofluorescence

Aortic segments were fixed with 4% phosphate-buffered paraformaldehyde (pH=7.6) for 2 h and washed in three changes of phosphate-buffered saline solution (PBS, pH=7.4). After cleaning, arterial segments were placed in PBS containing 30% sucrose (as a cryoprotectant) for ~2 h. Segments were then transferred to a cryomold containing Tissue Tek OCT embedding medium for 20 min (Sakura Finetek Europe, The Netherlands) and frozen in a beaker of isopentane that had been cooled in liquid nitrogen. Tissues were kept at -70°C until the day of the experiments. Frozen transverse sections (14 µm) were cut onto gelatine-coated slides and air-dried for at least 60 min. After blockade, sections were incubated with a polyclonal antibody against COX-2 (1:200, Cayman Chemical) in PBS

containing 2% bovine serum albumin for 1 h at 37°C in a humid box. After washing, rings were incubated with the secondary antibody donkey anti-rabbit IgG conjugated to Alexa 594 (Molecular Probes, Leiden, The Netherlands) at a dilution 1:200 for a further 1 h at 37°C in a humid box. After washing, immunofluorescent signals were viewed using an inverted Leica TCS SP5 confocal laser scanning microscope with oil immersion lens (x40). Alexa 594 labelled antibody was visualized by excitation at 543 nm and detection at 600-700 nm and Hoechst-labelled nuclei were visualized by excitation at 351 and 364 nm and detection at 400-500 nm.

The specificity of the immunostaining was evaluated by omission of the primary antibody and processing as above. Under these conditions, no staining was observed in the vessel wall of either control or mercury treated rats. To assess the presence of the three vascular layers, the sections were stained with the nuclear dye, Hoechst 33342 (0.01 mg/ml, Sigma Chemical).

Measurement of plasma ACE activity

ACE activity was measured by a fluorimetric method adapted from Friedland and Silverstein (41). Briefly, triplicate plasma samples (3 µl) were incubated for 15 minutes at 37°C with 40 µl of assay buffer containing the ACE substrate 5 mM Hip-His-Leu (Sigma Chemical). The reaction was stopped by addition of 190 µl of 0.35N HCl. The generated product His-Leu, was measured fluorimetrically following 10 minutes incubation with 100 µl of 2% o-phatal-dialdehyde in methanol. Fluorescence measurements were carried out at 37°C in a Fluostar Optima plate reader (BMG Labtech, Offenburg, Germany) with 350 nm excitation and 520 nm emission filters. The fluorescence plate reader was controlled by the Fluostar Optima Software. Black 96-Well polystyrene microplates (Biogen Cientifica, Madrid, Spain) were used. A calibration curve with ACE from the rabbit lung (Sigma Chemical) was included in each plate.

Data analysis and statistics

All values are expressed as mean±SEM of the number animals used in each experiment. To normalize forces obtained from each preparation, vasoconstrictor responses were expressed as the % of contraction induced by 75 mM KCl. To compare the effect of indomethacin on the response to phenylephrine in segments from both treatments groups, some results were expressed as 'differences of area under the concentration-response curves' (dAUC) in control and experimental situations. AUCs were calculated from the individual concentration-response curve plots; the differences were expressed as a percentage of the AUC of the corresponding control situation. Results were analyzed using either Student's t-test, two-way ANOVA for comparison between groups or Mann-Whitney non-parametric test. When ANOVA showed a significant treatment effect, Bonferroni´s *post hoc* test was used to compare individual means. Differences were considered statistically significant at $P<0.05$.

RESULTS

Rats exposed to mercury chloride show increased blood mercury concentration, attaining blood mercury level at 30 days of ≈8 ng/ml, as described (10). Rats had similar body weight and systolic blood pressure, as previously described (10).

Effect of mercury treatment on vasoconstrictor response

Vasoconstrictor responses to 75 mM KCl, were similar in aorta from mercury-treated rats compared to controls (mercury: 2.72±0.5, control 2.9±0.39 g, n=28 and 36, respectively, $P>0.05$).

Treatment with mercury for 30 days increased the vasoconstrictor responses induced by phenylephrine in

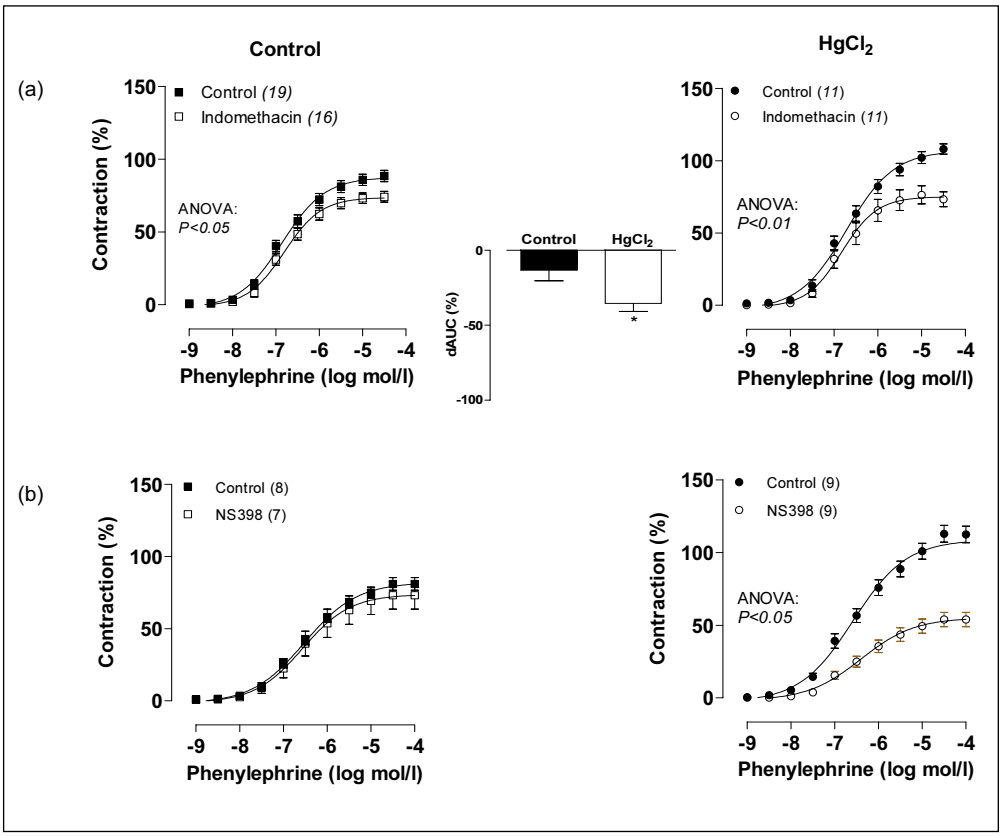

Fig. 1. Effect of (a) indomethacin (10 µmol/l) and (b) NS398 (1 µmol/l) on the concentration-response curve to phenylephrine in aortic segments from control and $HgCl_2$ treated rats. Results (mean±SEM) are expressed as a percentage of the response to 75 mmol/l KCl. Number of animals used is indicated in parentheses. Insert graph in (a) shows differences in area under the concentration-response curve (dAUC) in the presence and the absence of indomethacin. *$P<0.01$ *vs.* control.

endothelium-intact aortic rings, as previously described (10). Thus, mercury treatment increased E_{max} (Control: 87.8±2.4 %, n=36 *vs*. $HgCl_2$: 115±3.9 % of vasoconstrictor responses to KCl, n=28; P<0.001) but did not modify pD_2 (Control: 6.68±0.06 *vs*. $HgCl_2$: 6.64±0.07, P>0.05).

Effect of mercury treatment on COX pathway

Previous findings of our group suggest that COX-2-derived products are involved in phenylephrine vasoconstrictor responses in aorta (25). Indomethacin (10 μmol/l), a non selective COX inhibitor, reduced the response to phenylephrine in aortic segments from both groups (*Fig. 1a)*; however, this reduction was greater in segments from mercury-treated than control rats, as shown by the comparison of the dAUC values (*Fig. 1a* insert). The selective COX-2 inhibitor NS 398 (1 μmol/l) diminished phenylephrine contractile responses in vessels from mercury-treated but not from control rats (*Fig. 1b*). These results suggest the participation of COX-2 derived contractile prostanoids on phenylephrine responses only in vessels from mercury-treated rats.

To identify the contractile prostanoid involved in phenylephrine responses, aortic rings were incubated with the TP receptor antagonist SQ 29,548 (1 μmol/l), the TXA_2 synthase inhibitor furegrelate (1 μmol/l) and the EP_1 receptor antagonist SC 19,220 (1 μmol/l). As shown in *Fig. 2*, all treatments reduced the response to phenylephrine only in aortic rings from mercury-treated rats, suggesting that TXA_2 and PGE_2 participate in phenylephrine responses only in mercury-treated rats.

Effects of mercury treatment on COX-2 activity and expression

The levels of both TXA_2 and PGE_2 in the incubation medium of aortic segments were significantly higher in samples from treated than untreated rats (*Fig. 3*). When vessels were incubated with NS 398 (1 μmol/l), a reduction of the production of the two prostanoids was observed only in samples from the mercury

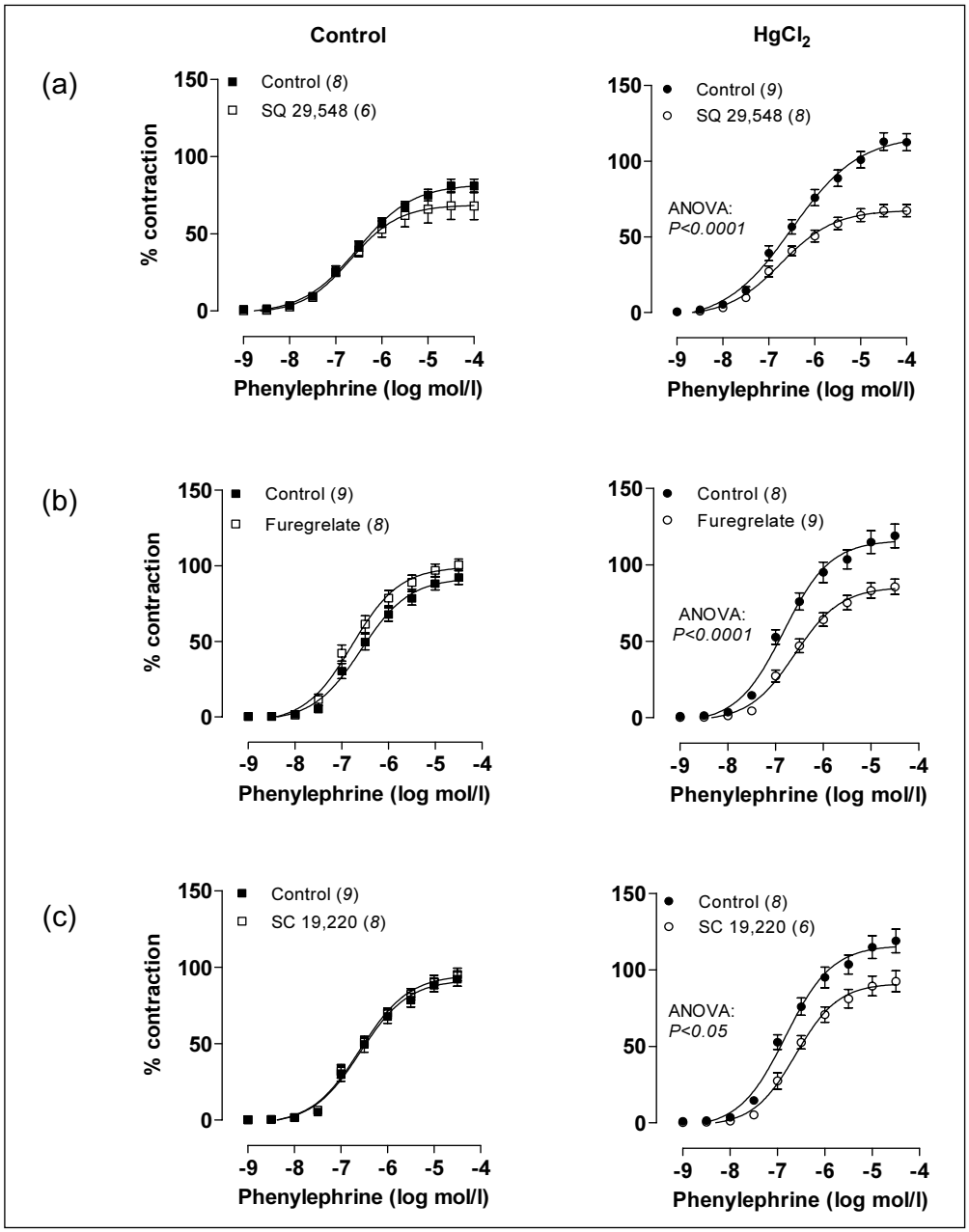

Fig. 2. Effect of (a) SQ 29,548 (1 μmol/l), (b) Furegrelate (1 μmol/l) and (c) SC 19,220 (1 μmol/l) on the concentration-response curve to phenylephrine in control and $HgCl_2$ treated rats. Results (mean±SEM) are expressed as a percentage of the response to 75 mmol/l KCl. Number of animals used is indicated in parentheses.

group. In these conditions, PGE_2 and TXA_2 release reached similar levels in both groups (*Fig. 3*).

COX-2 protein was essentially localized in adventitial and endothelial cells of aorta from treated (*Fig. 4a*) and untreated (results not shown) rats. COX-2 mRNA expression was greater in vessels from mercury-treated rats than control rats (*Fig. 4b*).

Effect of mercury treatment on renin angiotensin system activity

We have previously demonstrated that acute mercury administration induces serum ACE activation (9). Then, we tested whether mercury induces similar effects in chronically-treated rats. Plasma ACE levels were greater in mercury treated than control rats (*Fig. 5a*). To check whether this increased ACE activity has a role on the increased vasoconstrictor responses induced by phenylephrine after mercury treatment, vessels were incubated with losartan (10 µmol/l). This drug had no effect on the vasoconstrictor responses induced by phenylephrine in aortic rings from control rats. However, in mercury-treated rats, a reduction of phenylephrine vasoconstrictor responses was observed (*Fig. 5b*).

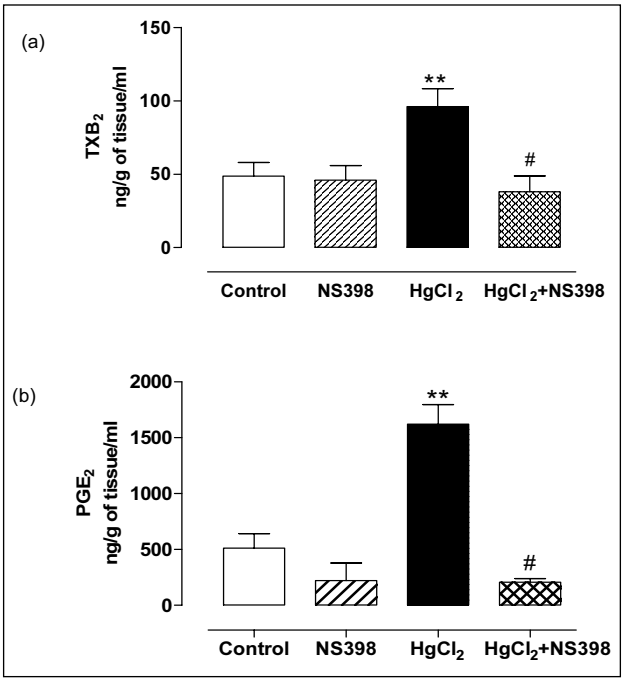

Fig. 3. Effect of NS 398 (1 µmol/l) on the (a) TXB_2 and (b) PGE_2 levels measured in the incubation medium of aortic segments from control (n=5-7) and $HgCl_2$-treated (n=7-9) rats. Results are expressed as mean±SEM **P<0.01 *vs.* control, # P<0.01 *vs.* $HgCl_2$.

DISCUSSION

Results presented here demonstrate that exposure to low dose of mercury chloride increases the participation of COX-2-derived TXA_2 and PGE_2 on vasoconstrictor responses to phenylephrine. This participation might help to explain the greater phenylephrine responses observed in aorta from $HgCl_2$ treated animals. A possible role for angiotensin II is also suggested.

Mercury has been identified as a hazard and a risk factor for cardiovascular diseases in humans (13). The US Enviromental Protection Agency's recommended the reference blood concentration of mercury of 5.8 ng/ml. Below this concentration, mercury exposure is considered to be without adverse effect (42-44). The dose of mercury chloride used in the present study attained a blood mercury concentration of around 8 ng/ml, close to the levels of exposed humans (38, 39, 45). During the last years, adverse effects of mercury on the heart and vessels have been described (6-10). Specifically, an association between mercury exposure and hypertension in humans has been shown (14, 46-48). Moreover, in rats chronically treated with a high dose of mercury (0.5 mg/kg body wt/day or 200 µg/ml of $HgCl_2$ in drinking water for 180 days) an increase in blood pressure has been reported (49, 50). However, we have previously described that chronic (30 days) exposure to low $HgCl_2$ concentrations does not induce changes in blood pressure despite the endothelial dysfunction and augmented vasoconstrictor responses found in both aorta and mesenteric resistance arteries (10), suggesting the existence of compensatory autonomic reflexes, as has been previously shown (8).

In a previous report we have shown that $HgCl_2$ treatment increases oxidative stress that reduces NO bioavailability, leading to endothelial dysfunction and increased phenylephrine responses (10). Herein, we evaluated the participation of prostanoids, known regulators of vascular tone, in the increased vascular contractile responses to phenylephrine observed in rat aorta after mercury treatment. *In vitro* exposure to mercury chloride or methyl mercury induces activation of arachidonate cycloperoxidation (51) and the production of PGE_2 in rat glomeruli and glomerular cells (35). The perfusion of the rat tail vascular bed with $HgCl_2$ produces vasoconstriction mediated by COX-pathway products (17). In addition, mercury induces the release of arachidonate and its metabolites from human platelets (52), and increases the biosynthesis of TXA_2 and PGI_2 in the heart (37). Those effects were described to occur after acute exposure to mercury compounds and in the micromolar range. However, to our

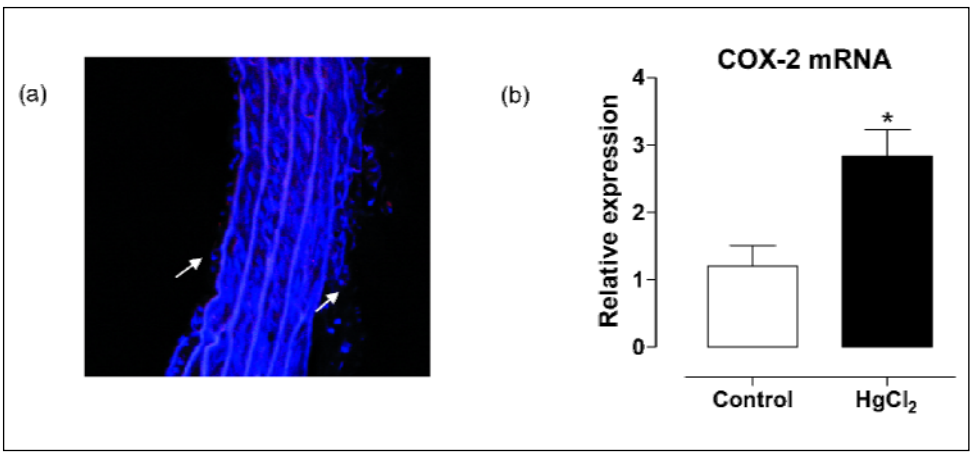

Fig. 4. (a) Representative photomicrographs of COX-2 immunofluorescence (arrow) in aortic rings from $HgCl_2$ treated rats. Image size 387x387 µm. n=3 animals. (B) Quantitative RT-PCR assessment of COX-2 mRNA expression in aorta from control (n=7) and $HgCl_2$-treated (n=9) rats. Results (mean±SEM) are expressed as the relative expression of mRNA in $HgCl_2$-treated compared to control rats. Mann-Whitney test: *P<0.05.

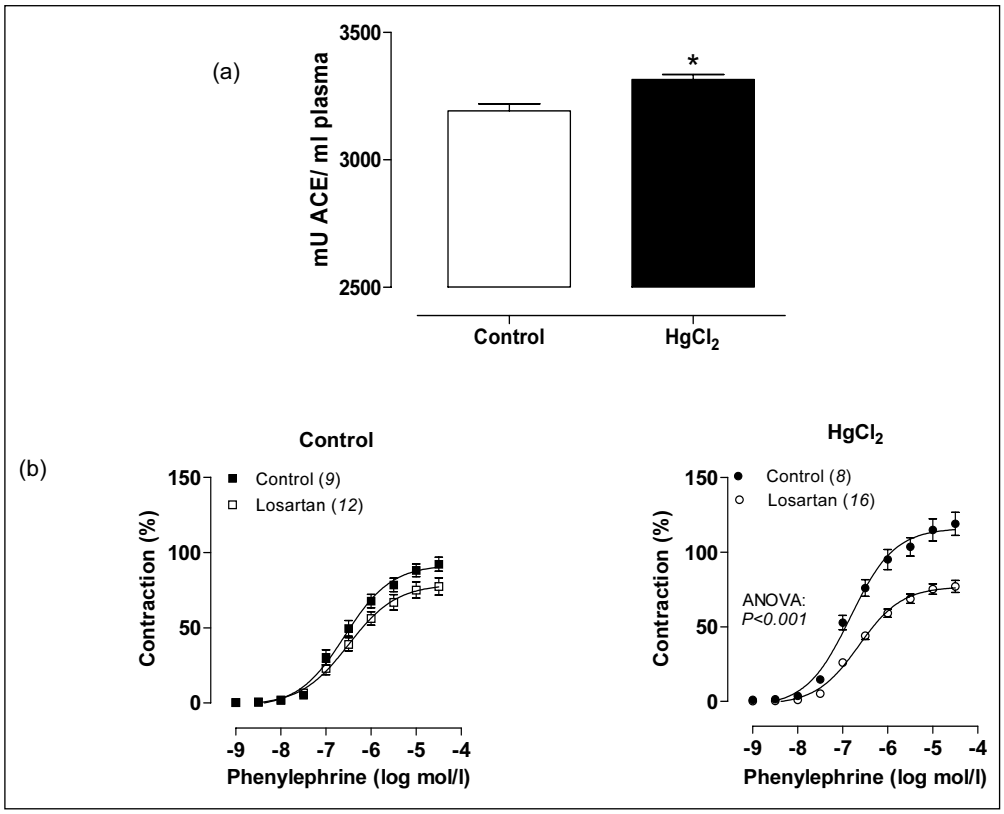

Fig. 5. (a) Angiotensin converting enzyme (ACE) activity (mU/ml) in plasma of Control and $HgCl_2$ treated rats *P<0.01 *vs.* control. (b) Effect of losartan (10 μmol/l) on the concentration-response curve to phenylephrine in endothelium-intact aortic segments from Control and $HgCl_2$ treated rats. Results (mean±SEM) are expressed as a percentage of the response to 75 mmol/l KCl. Number of animals used is indicated in parentheses.

knowledge, there are no studies analyzing the participation of COX-derived products in a rat model of chronic exposure to low doses of mercury chloride. The larger reduction of the increased vasoconstrictor response to phenylephrine by the COX blockade with indomethacin in mercury-treated rats suggests that arachidonate vasoconstrictor metabolites were playing a role on the mercury actions. This effect was specific for conductance arteries, since no differences in indomethacin effects were observed in mesenteric resistance arteries (unpublished observations).

Vascular COX-2 expression has been reported in pathological conditions associated with inflammatory processes, such as atherosclerosis or hypertension (24-26). COX-2- derived prostanoids contribute to the endothelial dysfunction observed in hypertensive or aged animals (27-29). In addition, these prostanoids greatly contribute to the vasoconstrictor responses observed in hypertensive rats (24, 25, 30). In the last years, it has become evident that prostanoids production by COX-2 is also involved in the modulation of vascular and endocrine responses in physiological conditions (24-26, 53). We and others have described COX-2 expression in endothelial and adventitial cells of unstimulated arteries (23, 25, 54). In agreement, in the present study we found that COX-2 was localized essentially in adventitia and endothelial cells. To investigate the participation of COX-2 derived products on phenylephrine we used the COX-2 inhibitor NS 398. This drug reduced the vasoconstrictor response to phenylephrine in the chronic mercury-treated animals and not in controls, suggesting the participation of contractile prostanoids from COX-2 only in that group. The greater participation of COX-2 derived products that occurs after mercury treatment could be associated to the upregulation of this isoform. Thus, we found greater mRNA levels of COX-2 protein expression in segments from mercury treated than untreated rats. We then investigated the participation of the COX-derived vasoconstrictor metabolites PGE_2 and TXA_2 on phenylephrine responses. The TP receptor antagonist SQ 29,548, the thromboxane A_2 inhibitor furegrelate and the EP_1 receptor antagonist SC 19,220 diminished phenylephrine responses only in mercury treated animals, thus demonstrating the participation of both TXA_2 and PGE_2 on mercury actions. Moreover, PGE_2 and TXA_2 production was larger in aortas from chronic $HgCl_2$-treated rats; these prostanoids were derived from COX-2 since selective COX-2 blockade abolished prostanoids production. All together these results suggest that chronic mercury exposure induces the release of vasoconstrictor prostanoids from COX-2 that affects vascular contractile responses to phenylephrine, although we can not discard an effect of Hg treatment reducing the involvement of vasodilator prostanoids, such as PGI_2, on phenylephrine responses.

The renin-angiotensin system is a central component of the physiological and pathological responses of cardiovascular system (55, 56). Angiotensin II modulates prostaglandin production by activating phospholipase A_2 or regulating COX-2 expression, although contradictory results have been reported depending on the cell type or stimulus (31-33, 57, 58). In cultured rat vascular smooth muscle cells (31, 32) and in adventitial fibroblasts (33) angiotensin II induces COX-2 expression. In aortic segments we have shown that the incubation of aortic segments with angiotensin II increased the inhibitory effect of NS 398 in phenylephrine responses (32). Moreover, losartan treatment decreased the increased production of COX-2-derived products and their participation in contractile responses in hypertension (32). However, it also has been described that COX-2 products were not involved in the angiotensin II-dependent endothelial dysfunction in hypertensive rats (59). We have previously described that small concentrations of mercury induces serum ACE activation (9). Herein, we observed that after chronic low dose mercury exposure the plasma ACE activity were increased. Moreover, losartan decreased phenylephrine responses only in aorta from mercury-treated rats suggesting increased local angiotensin II effect after mercury exposure. Since endothelial cells are a major source of ACE (60, 61) it is

possible the participation of the endothelial layer in the production of angiotensin II in vessels from mercury treated rats. Based on our findings and the extensive reports showing that angiotensin II increases COX-2 expression in different cell types, we might hypothesize that the increased local angiotensin II levels might be responsible, at least in part, for the increased COX-2 activity after chronic mercury exposure. Additional studies will be needed to address this possibility.

In conclusion, we demonstrated for the first time, that treatment with small doses of mercury increases the release of COX-2 derived vasoconstrictor prostanoids and its participation in vasoconstrictor responses. The increased activation of the renin-angiotensin system after mercury treatment would be associated with this increased COX-2 activity. These mechanisms would contribute to explain the endothelial dysfunction and the increased vasoconstrictor responses induced by mercury exposure (10). These observations also offer further evidence that chronic mercury exposure, even at small concentration, is hazardous and it is an environmental risk factor for cardiovascular disease.

Acknowledgements: This study was supported by Ministerio de Ciencia e Innovacion (SAF 2006-02376, 2009-07201 and PHB2005-0008-PC), Instituto de Salud Carlos III (Red RECAVA, RD06/0014/0011), and Fundacion Mutua Madrilena, Giulia Wiggers and Franck Peçanha were granted with scholarships from CAPES and FAPES (Brazil).

Conflict of interests: None declared.

REFERENCES

1. Eto K. Minamata disease. *Neuropathology* 2000; 20(Suppl): S14-S19.
2. Bakir F, Damluji SF, Amin-Zaqi L, *et al.* Methylmercury poisoning in Iraq. *Science* 1973; 181: 230-241.
3. Harada M. Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. *Crit Rev Toxicol* 1995; 25: 1-24.
4. Mutter J, Naumann J, Sadaghiani C, Schneider R, Walach H. Alzheimer disease: mercury as pathogenetic factor and apolipoprotein E as a moderator. *Neuro Endocrinol Lett* 2004; 25: 331-339.
5. Hodgson S, Nieuwenhuijsen MJ, Elliott P, Jarup L. Kidney disease mortality and environmental exposure to mercury. *Am J Epidemiol* 2007; 165: 72-77.
6. Vassallo DV, Moreira CM, Oliveira EM, Bertollo DM, Veloso TC. Effects of mercury on the isolated heart muscle are prevented by DTT and cysteine. *Toxicol Appl Pharmacol* 1999; 156: 113-118.
7. Moreira CM, Oliveira EM, Bonan CD, Sarkis JJ, Vassallo DV. Effects of mercury on myosin ATPase in the ventricular myocardium of the rat. *Comp Biochem Physiol C Toxicol Pharmacol* 2003; 135: 269-275.
8. Machado AC, Padilha AS, Wiggers GA, Siman FDM, Stefanon I, Vassallo DV. Small doses of mercury increase arterial pressure reactivity to phenylephrine in rats. *Env Tox Pharmacol* 2007; 24: 92-97.
9. Wiggers GA, Stefanon I, Padilha AS, Peçanha FM, Vassallo DV, Oliveira EM. Low nanomolar concentration of mercury chloride increases vascular reactivity to phenylephrine and local angiotensin production in rats. *Comp Biochem Physiol C Toxicol Pharmacol* 2008; 147: 252-260.
10. Wiggers GA, Peçanha FM, Briones AM, *et al.* Low mercury concentrations cause oxidative stress and endothelial dysfunction in conductance and resistance arteries. *Am J Physiol Heart Circ Physiol* 2008; 295: H1033-H1043.
11. Salonen JT, Seppanen K, Lakka TA, Salonen R, Kaplan GA. Mercury accumulation and accelerated progression of carotid atherosclerosis: a population-based prospective 4-year follow-up study in men in eastern Finland. *Atherosclerosis* 2000; 148: 265-273.
12. Virtanen JK, Voutilainen S, Rissanen TH. Mercury, fish oils, and risk of acute coronary events and cardiovascular disease, coronary heart disease, and all-cause mortality in men in eastern Finland. *Arterioscler Thromb Vasc Biol* 2005; 25: 228-233.
13. Houston MC. The role of mercury and cadmium heavy metals in vascular disease, hypertension, coronary heart disease, and myocardial infarction. *Altern Ther Health Med* 2007; 13: S128-S133.
14. Kobal AB, Horvat M, Prezelj M, *et al*. The impact of long-term past exposure to elemental mercury on antioxidative capacity and lipid peroxidation in mercury miners. *J Trace Elem Med Biol* 2004; 17: 261-274.
15. Huang YL, Cheng SL, Lin TH. Lipid peroxidation in rats administrated with mercuric chloride. *Biol Trace Elem Res* 1996; 52: 193-206.
16. Wolf MB, Baynes JW. Cadmium and mercury cause an oxidative stress-induced endothelial dysfunction. *Biometals* 2007; 20: 73-81.
17. da Cunha V, Souza HP, Rossoni LV, França AS, Vassallo DV. Effects of mercury on the isolated perfused rat tail vascular bed are endothelium-dependent. *Arch Environ Contam Toxicol* 2000; 39: 124-130.
18. Mahboob M, Shireen KF, Atkinson A, Khan AT. Lipid peroxidation and antioxidant enzyme activity in different organs of mice exposed to low level of mercury. *J Environ Sci Health B* 2001; 36: 687-697.
19. Gstraunthaler G, Pfaller W, Kotanko P. Glutathione depletion and in vitro lipid peroxidation in mercury or maleate induced acute renal failure. *Biochem Pharmacol* 1983; 32: 2969-2972.
20. Stacey NH, Kappus H. Cellular toxicity and lipid peroxidation in response to mercury. *Toxicol Appl Pharmacol* 1982; 63: 29-35.
21. Vagnoni KE, Christiansen ND, Holyoak GR, Janowiak MA, Martin PH. Cellular source in ewes of prostaglandin-endoperoxide synthase-2 in uterine arteries following stimulation with lipopolysaccharide. *Biol Reprod* 1999; 61: 563-568.
22. Hernanz R, Alonso MJ, Briones AM, Vila E, Simonsen U, Salaices M. Mechanisms involved in the early increase of serotonin contraction evoked by endotoxin in rat middle cerebral arteries. *Br J Pharmacol* 2003; 140: 671-680.
23. Briones AM, Salaices M, Vila E. Ageing alters the production of nitric oxide and prostanoids after IL-1beta exposure in mesenteric resistance arteries. *Mech Ageing Dev* 2005; 126: 710-721.
24. Adeagbo AS, Zhang X, Patel D, *et al.* Cyclo-oxygenase-2, endothelium and aortic reactivity during deoxycorticosterone acetate salt-induced hypertension. *J Hypertens* 2005; 23: 1025-1036.
25. Alvarez Y, Briones AM, Balfagon G, Alonso MJ, Salaices M. Hypertension increases the participation of vasoconstrictor prostanoids from cyclooxygenase-2 in phenylephrine responses. *J Hypertens* 2005; 23: 767-777.
26. Antman EM, DeMets D, Loscalzo J. Cyclooxygenase inhibition and cardiovascular risk. *Circulation* 2005; 112: 759-770.
27. Widlansky ME, Gokce N, Keaney JF Jr, Vita JA. The clinical implications of endothelial dysfunction. *J Am Coll Cardiol* 2003; 42: 1149-1160.
28. Blanco-Rivero J, Cachofeiro V, Lahera V, *et al.* Participation of prostacyclin in endothelial dysfunction induced by

aldosterone in normotensive and hypertensive rats. *Hypertension* 2005; 46: 107-112.
29. Wong SL, Leung FP, Lau CW, *et al.* Cyclooxygenase-2-derived prostaglandin F2alpha mediates endothelium-dependent contractions in the aortae of hamsters with increased impact during aging. *Circ Res* 2009; 104: 228-235.
30. Zerrouk A, Auguet M, Chabrier PE. Augmented endothelium-dependent contraction to angiotensin II in the SHR aorta: role of an inducible cyclooxygenase metabolite. *J Cardiovasc Pharmacol* 1998; 31: 525-533.
31. Ohnaka K, Numaguchi K, Yamakawa T, Inagami T. Induction of cyclooxygenase-2 by angiotensin II in cultured rat vascular smooth muscle cells. *Hypertension* 2000; 35: 68-75.
32. Alvarez Y, Perez-Giron JV, Hernanz R, *et al*. Losartan reduces the increased participation of cyclooxygenase-2-derived products in vascular responses of hypertensive rats. *J Pharmacol Exp Ther* 2007; 321: 381-388.
33. Beltran AE, Briones AM, Garcia-Redondo AB, *et al.* p38 MAPK contributes to angiotensin II-induced COX-2 expression in aortic fibroblasts from normotensive and hypertensive rats. *J Hypertens* 2009; 27: 142-154.
34. Fujimoto Y, Yabuno T, Kato M, Nishioka K, Fujita T. Effect of methyl mercury on prostaglandin synthesis in rabbit kidney medulla slices. *Res Commun Chem Pathol Pharmacol* 1986; 54: 115-119.
35. Sraer JD, Baud L, Sraer J, Delarue F, Ardaillou R. Stimulation of PGE2 synthesis by mercuric chloride in rat glomeruli and glomerular cells in vitro. *Kidney Int Suppl* 1982; 11: S63-S68.
36. Mazerik JN, Hagele T, Sherwani S, *et al*. Phospholipase A2 activation regulates cytotoxicity of methylmercury in vascular endothelial cells. *Int J Toxicol* 2007; 26: 553-569.
37. Ally A, Buist R, Mills P, Reuhl K. Effects of methylmercury and trimethyltin on cardiac, platelet, and aorta eicosanoid biosynthesis and platelet serotonin release. *Pharmacol Biochem Behav* 1993; 44: 555-563.
38. Gupta M, Bansal JK, Khanna CM. Blood mercury in workers exposed to the preparation of mercury cadmium telluride layers on cadmium telluride base. *Ind Health* 1996; 34: 421-425.
39. Chen C, Qu L, Li B, *et al*. Increased oxidative DNA damage, as assessed by urinary 8-hydroxy-2'-deoxyguanosine concentrations, and serum redox status in persons exposed to mercury. *Clin Chem* 2005; 51: 759-767.
40. Livak KJ, Schmittgen TD. Analysis of relative gene expression data real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 2001; 25: 402-408.
41. Friedland J, Silverstein E. A sensitive fluorimetric assay for serum angiotensin-converting enzyme. *Am J Clin Pathol* 1976; 66: 416-424.
42. National Academy of Sciences. Toxicological Effects of Methylmercury. National Research Council, Washington, DC, USA, 2000.
43. Rice DC. The US EPA reference dose for methylmercury: sources of uncertainity. *Environ Res* 2004; 95: 406-413.
44. Stern AH. Balancing the risks and benefits of fish consumption. *Ann Intern Med* 2005; 142: 949.
45. McKelvey W, Gwynn RC, Jeffry N, *et al.* A biomonitoring study of lead, cadmium, and mercury in the blood of New York City adults. *Envirom Health Perspect* 2007; 115: 1435-1441.
46. Torres AD, Rai AN, Hardiek ML. Mercury intoxication and arterial hypertension: report of two patients and review of the literature. *Pediatrics* 2000; 105: E34.
47. Bofetta P, Sallsten G, Garcia-Gomez M, *et al*. Mortality from cardiovascular diseases and exposure to inorganic mercury. *Occup Environ Med* 2001; 58: 461-466.
48. Garcia-Gomez M, Boffetta P, Caballero Klink JD, Espanol S, Gomez-Quintana J. Cardiovascular mortality in mercury miners. *Med Clin* (*Barc*) 2007; 128: 766-771.
49. Wakita Y. Hypertension induced by methyl mercury in rats. *Toxicol Appl Pharmacol* 1987; 89: 144-147.
50. Carmignani M, Boscolo P, Artese L, *et al.* Renal mechanisms in the cardiovascular effects of chronic exposure to inorganic mercury in rats. *Br J Ind Med* 1992; 49: 3226-3232.
51. MacFarlane DE. The effects of methyl mercury on platelets. Induction of aggregation and release via activation of the prostaglandin synthesis pathway. *Mol Pharmacol* 1980; 19: 470-476.
52. Hornberger W, Patscheke H. Hydrogen peroxide and methyl mercury are primary stimuli of eicosanoid release in human platelets. *J Chem Clin Biochem* 1989; 27: 567-575.
53. Gadek-Michalska A, Bugajski AJ, Bugajski J. Prostaglandins and interleukin-1beta in the hypothalamic-pituitary-adrenal response to systemic phenylephrine under basal and stress conditions. *J Physiol Pharmacol* 2008; 59(3): 563-575.
54. Tabernero A, Schneider F, Potenza MA, *et al.* Cycloxygenase-2 and inducible nitric oxide synthase in omental arteries harvested from patients with severe liver diseases: immuno-localization and influence on vascular tone. *Intensive Care Med* 2003; 29: 262-270.
55. Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol* 2007; 292(1): C82-C97.
56. Szczepanska-Sadowska E. Role of neuropeptides in central control of cardiovascular responses to stress. *J Physiol Pharmacol* 2008; 59(Suppl 8): 61-89.
57. Harris RC, Zhang MZ, Cheng HF. Cyclooxygenase-2 and the renal renin-angiotensin system. *Acta Physiol Scand* 2004; 181: 543-547.
58. Slice LW, Chiu T, Rozengurt E. Angiotensin II and epidermal growth factor induce cyclooxygenase-2 expression in intestinal epithelial cells through small GTPases using distinct signaling pathways. *J Biol Chem* 2005; 280: 1582-1593.
59. Cheng ZJ, Tikkanen I, Vapaatalo H, Mervaala EM. Vascular effects of COX inhibition and AT1 receptor blockade in transgenic rats harboring mouse renin-2 gene. *J Physiol Pharmacol* 2002; 53(4 Pt 1): 597-613.
60. Rush JW, Aultman CD. Vascular biology of angiotensin and the impact of physical activity. *Appl Physiol Nutr Metab* 2008; 33: 162-172.
61. Paul M, Poyan MA, Kreutz R. Physiology of local renin-angiotensin systems. *Physiol Rev* 2006; 86: 747-803.

Received: April 21, 2009
Accepted: January 12, 2010

Author's address: Dr. Mercedes Salaices, Departamento de Farmacología. Facultad de Medicina. Universidad Autónoma de Madrid, Arzobispo Morcillo 4, 28029 Madrid, Spain; Phone: 00-34-91-4975378; Fax: 00-34-91-4975302;
E-mail: mercedes.salaices@uam.es