Endogenous testosterone increases leukocyte–endothelial cell interaction in spontaneously hypertensive rats


A R T I C L E   I N F O

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A B S T R A C T

Aims: Inflammation may have an important role in the beginning and in the progress of cardiovascular diseases. Testosterone exerts important effects on vascular function, which is altered in arterial hypertension. Thus, the aim of this study was to evaluate the influence of endogenous testosterone on leukocyte behavior in post-capillary venules of the mesenteric bed of spontaneously hypertensive rats (SHR). Main methods: 18 week-old intact SHR, castrated SHR and normotensive rats (intact Wistar) were used. Blood pressure was measured by tail plethysmography and serum testosterone levels by ELISA. Leukocyte rolling, adhesion and migration were evaluated in vivo in situ by intravital microscopy. Key findings: Castration significantly reduced blood pressure and reversed the increased leukocyte rolling and adhesion observed in SHRs. Leukocyte counts and other hemodynamic parameters did not differ among groups. SHRs displayed increased protein expression of P-selectin and ICAM-1 in mesenteric venules when compared to intact Wistar. Castration of SHRs restored the protein expression of the cell adhesion molecules. Significance: The findings of the present study demonstrate the critical role of endogenous testosterone mediating the effects of hypertension increasing leukocyte–endothelial cell interaction. Increased expression of cell adhesion molecules contribute to the effects of endogenous testosterone promoting increased leukocyte rolling and adhesion in SHRs.

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Introduction

Previous studies have indicated that there may be sex differences in the development of cardiovascular diseases (Nigro et al., 1997; Nikiforov and Mamaev, 1998). These differences have been attributed to the sex hormones estrogen and testosterone. We have previously demonstrated gender-associated differences in the vascular dysfunction displayed by experimental models of hypertension, such as spontaneously hypertensive rats (SHR) (Dantas et al., 2004) and deoxycorticosterone-salt hypertensive rats (Montezano et al., 2005). The protective role of ovarian hormones, especially estrogen, was also reported in these studies. Surprisingly, little information exists regarding the effects of testosterone on vascular function, and the few studies on the effects of testosterone have produced conflicting results. The role of androgens on vascular function is receiving considerable attention since observations that endogenous testosterone may contribute to the development of endothelial dysfunction in hypertension (Singh et al., 2007; Vasudevan et al., 2006). Endothelial dysfunction was initially identified as impaired vasodilation, but the term also includes proinflammatory and prothrombic alterations (Endemann and Schiffrin, 2004). The low-grade inflammation state present in the vasculature in hypertension has gained widespread attention. Studies with human and different models of hypertension have demonstrated increased expression of cell adhesion molecules in the vessel media (Callera et al., 2004; De Ciuceis et al., 2005; Preston et al., 2002), augmented plasma levels of inflammatory cytokines and C-reactive protein (Blake et al., 2003; Preston et al., 2002).

The vascular endothelium has an important role in the control of leukocyte traffic to the interstitium. Leukocyte extravasation, which is essential for the inflammatory response, involves margination and capturing of free-flowing leukocytes to the endothelium, followed by leukocyte rolling, leukocyte activation with firm adhesion to endothelial cells and subsequent transmigration through the vascular endothelium (Walzog and Gaehgens, 2000). These steps depend on the activation of the endothelial cell through induction of endothelial adhesion molecule expression and secretion of inflammatory mediators by these cells. This multistep process is considered a key factor in the pathogenesis of vascular dysfunction and tissue injury (Krieglstein and Granger, 2001).
Considering that alterations in endothelial cells, which are involved in the inflammatory response, can contribute to hypertension (Ofosu-Appiah et al., 1997) and that androgens act on these cells, the present study was conducted in order to investigate the effects of endogenous testosterone (revealed by castration) on leukocyte–endothelial cell interaction in hypertension using intravital microscopy. For this, we used male SHR, which display hypertension and vascular dysfunction, providing a suitable model to investigate the effects of endogenous testosterone on inflammatory response in hypertension.

Methods

Animals

The investigation was approved by the Ethical Committee for Animal Research of the Institute of Biomedical Sciences, University of Sao Paulo (Protocol no. 145/10), conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85–23, revised 1996). The experiments were performed with 18-week-old male SHR and normotensive control Wistar rats from our breeding stock maintained at the Institute of Biomedical Sciences (University of Sao Paulo). The breeding conditions were followed as previously described (Lobato et al., 2011). Rats were divided into 3 groups: intact Wistar rats (intact Wistar, n=25), intact SHR (intact SHR, n=25) and SHR that were castrated at 12 weeks of age and studied at 18 weeks of age (castrated SHR, n=25). Blood pressure (BP) was measured in unanesthetized rats by an indirect tail-cuff method (PowerLab 4/S, AD Instruments Pty Ltd) (Lobato et al., 2011). Plasma testosterone level was determined by enzyme immunoassay (Cayman Chemical Co).

Leukocyte–endothelial cell interaction – intravital microscopy

Surgical procedure

Rats were anesthetized with chloral hydrate (450 mg/kg, SC), and the mesentery was exteriorized for microscopic observation in vivo situ. The tissue was placed on a thermostatically controlled board (37 °C) with a transparent platform. The preparation was superfused (2 mL/min) with Ringer Locke's solution (37 °C, pH 7.4), bubbled with 95% N2–5% CO2 (2 mL/min) with Ringer Locke's solution (37 °C) with a transparent platform. The preparation was superfused with Ringer Locke's solution (37 °C, pH 7.4), bubbled with 95% N2–5% CO2 (2 mL/min) with Ringer Locke's solution (37 °C, pH 7.4), bubbled with

Centerline velocity of red blood cells (V_{RBC}) was measured using an optical Doppler velocimeter (Microcirculation Research Institute, Texas A & M University, College Station, USA). Measurements were made after the stabilization period (unstimulated condition). For microvessels <25 μm, V_{RBC} = V_{mean}. Venular shear rates (γ) were calculated from the Newtonian definition: \( \gamma = 8 \left( \frac{V_{mean}}{D_{v}} \right) \), where \( D_{v} = \text{venule diameter (mm)} \). The leukocyte rolling velocity (V_{WBC}) was determined from the time required for a leukocyte to move along a 100-μm length of the venule. A mean of 10 estimates of transit time was used to calculate V_{WBC}.

Leukocyte counts

Blood samples were collected from the tail of unanesthetized rats. Rats were placed in a hold chamber, and their tails were warmed at 40 °C for about 30 s. Total leukocytes were counted with a Neubauer chamber. Differential leukocyte counts were measured on blood-smeared slides stained with May-Grünwald and Giemsy dyes. A total of 100 cells were counted and classified on the basis of normal morphological criteria.

Immunohistochemistry analysis for detection of P-selectin and ICAM-1

Under anesthesia, the abdominal cavity was opened and a polyethylene cannula was inserted into the superior mesenteric artery. The whole mesenteric bed was perfused (2 mL/min) with physiological solution (0.9%, 3 min) followed by perfusion with paraformaldehyde 4% (PFA, 15 min). The mesentery was removed and mesenteric venules were dissected and fixed in PFA 4% for 6 h. Samples were soaked with 30% sucrose for cryoprotection (overnight) and embedded in freezing medium and stored at −80 °C.

Serial cross sections (14 μm) were prepared and collected on glass slides previously coated with poly-L-lysine solution (Sigma, St Louis, MO). Venules sections were incubated for 30 min in a 0.3% hydrogen peroxidase solution to block the endogenous peroxidase and for 1 h in phosphate-buffered saline containing 1% normal horse serum (for ICAM-1) or normal goat serum (for P-selectin) to block nonspecific sites. After that, sections were incubated with mouse anti-rat ICAM-1 (CD54) monoclonal antibody (50.0 mg/mL; BD PharmingenTM, San Diego, CA, USA) or rabbit anti-human P-selectin (CD62P) polyclonal antibody, which cross react with P-selectin from mouse, rat, and dog (50.0 mg/mL; BD PharmingenTM, San Diego, CA, USA), overnight at 4 °C. In negative control sections primary antibodies were replaced with phosphate-buffered saline. Venules sections were then incubated with horse anti-mouse (for ICAM-1) or goat anti-rabbit (for P-selectin) antibody conjugated with biotin (1.5 mg/L, Vectastain® Elite ABC kit, Vector Laboratories, Burlingame, CA, USA) for 1 h. The sections were subsequently incubated with avidin–biotin–peroxidase complex for 1 h to amplify the reaction, which was visualized by using 3,3′-diaminobenzidine. The staining for ICAM-1 and P-selectin in mesenteric venules was measured as mean optical density by a computer system (KS-300 Software, Zeiss, Germany).
Venule (μm) was determined after 4 h of the injection of LTB4 (0.1 μM) into the abdominal cavity. Results are mean±SEM. * P<0.05 vs. intact Wistar. SHR: 303±6; castrated SHR: 317±7; n=10. On the other hand, BP in castrated SHR (171±2 mm Hg) was lower than that observed in intact SHRs (195±3 mm Hg), but it was still higher when compared to the values observed in normotensive rats (122±1 mm Hg) (Fig. 1A). Testosterone levels in intact SHR (512±26 pg/mL) were increased when compared to intact Wistar (270±27 pg/mL). The hormone levels decreased after castration (17±2 pg/mL) (Fig. 1B).

Leukocyte–endothelial cell interaction

Under baseline conditions, increased number of rolling leukocytes was observed in mesenteric venules from intact SHRs (136±6 cells/10 min) when compared to Wistar rats (94±7 cells/10 min). Castration decreased this parameter (111±3 cells/10 min) (Fig. 2A). At basal condition (without any stimulus) the number of adherent and emigrated leukocytes was not different among the groups (data not shown). The superfusion with LTB4 increased the leukocyte adherence and emigration in mesenteric venules from all experimental groups. However, the leukocyte adhesion induced by LTB4 in intact SHR (11±0.4 cells/100 μm length) was high when compared to intact Wistar rats (8±0.3 cells/100 μm length). Castration restored the leukocyte adhesion induced by LTB4 in mesenteric venules of SHRs (9±0.2 cells/100 μm length) to the levels observed in Wistar rats (Fig. 2B). After LTB4 stimulus the number of emigrated leukocytes was similar among the groups (in cells/2500 μm², intact Wistar: 10±0.8; intact SHR: 9±0.9; castrated SHR: 10±0.8, Fig. 2C).

Venular diameter, venular blood flow velocity, venular shear rate, leukocyte velocity and blood leukocyte count

Tables 1 and 2 provide comparisons of hemodynamic parameters among the experimental groups. Compared to intact Wistar rats, both intact and castrated SHRs did not show changes in mean venular diameter, venular blood flow velocity, venular shear rate and leukocyte velocity (Table 1). The total and differential leukocyte count did not differ among the groups, indicating that the differences observed in our study were not related to changes in the number of circulating cells (Table 2).

Immunohistochemistry analysis for detection of P-selectin and ICAM-1

Immunohistochemistry analysis of P-selectin and ICAM-1 in venular endothelial cells revealed that the basal expression of these cell adhesion molecules was higher in SHRs when compared to Wistar rats. Castration of SHR decreased the protein expression of these molecules in venular endothelial cells to levels similar to those observed in Wistar rats (Fig. 3A and B).

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**Results**

**General characteristics of castrated SHR**

Six weeks after surgery, there was no significant difference in body weight among the groups (in grams, intact Wistar: 325±9; intact SHR: 303±6; castrated SHR: 317±7; n=10). On the other hand, BP in castrated SHR (171±2 mm Hg) was lower than that observed in intact SHRs (195±3 mm Hg), but it was still higher when compared to the values observed in normotensive rats (122±1 mm Hg) (Fig. 1A). Testosterone levels in intact SHR (512±26 pg/mL) were increased when compared to intact Wistar (270±27 pg/mL). The hormone levels decreased after castration (17±2 pg/mL) (Fig. 1B).

**Real-time PCR of P-selectin and ICAM-1 mRNA**

Under anesthesia, the abdominal cavity was opened and the mesenteric venules were dissected, frozen and stored at −80 °C. Total cellular RNA was isolated from the mesenteric venules using TRizol® Reagent according to the manufacturer’s instructions. Total RNA (2 μg) was reverse transcribed. Quantitative real-time reverse-transcriptase polymerase chain reaction (qPCR) reactions were performed as previously described in detail (Carneiro et al., 2008). Diluted RT samples (1:10) were submitted to real-time PCR amplification using specific oligonucleotides for P-selectin (forward, TAATCCCCCGACTG-TAAAG; reverse, AGGTGCAATGCTCCTCTC; 199 bp) and ICAM-1 (forward, CCTCTTGCCAAAGACGAAAC; reverse, ACTCGCTCTGGGAAC-TAAAG; reverse, AGGTTGGCAATGGTTCACTC; 198 bp). Beta-actin was used as an internal control (forward, CCTCTTGCGAAGACGAGAAC; reverse, ACTCGCTCTGGGAAC-TAAAG; reverse, AGGTTGGCAATGGTTCACTC; 199 bp) and ICAM-1 (forward, CCTCTTGCCAAAGACGAAAC; reverse, ACTCGCTCTGGGAAC-TAAAG; reverse, AGGTTGGCAATGGTTCACTC; 198 bp). Real-time PCR reactions were performed using the Corbett Research system (Corbett Life Sciences, Sydney, Australia). Data were calculated from the cycle threshold (Ct) value using the ΔCt method for quantification (Pfaf, 2001). All oligonucleotides and reagents used in this protocol were purchased from Invitrogen Co., San Diego, CA.

**Statistical analysis**

Data were compared using One-way analysis of variance (ANOVA) followed by Tukey Multiple Comparison Test. Data are expressed as mean±standard error of the mean (S.E.M.) with n indicating the number of experiments. P values less than 0.05 were considered significant.
Real-time PCR for detection of P-selectin and ICAM-1 mRNA

Venules from intact SHR displayed similar P-selectin and ICAM-1 mRNA levels when compared to normotensive rats. Castration of SHR did not alter the mRNA expression of these molecules (Fig. 4A and B).

Discussion

The inflammatory response involves a complex sequence of interactions between circulating neutrophils and the venular endothelium. It is well known that this process involves a range of adhesion molecules on leukocytes and endothelial cells, as well as extensive intracellular signaling that drives adhesion and chemotaxis on the one hand and controls a transitory modulation of endothelial integrity on the other (Kluger, 2004; Muller, 2009). Although there has been general agreement on the role of leukocytes that allow the rolling and tethering to take place, endothelial cells play an active and essential role during the inflammatory responses (Cepinskas et al., 1997; Cook-Mills and Deem, 2005). Considering that endothelial cell-associated adhesion molecules are critical participants in the vascular dysfunction and tissue injury associated with a wide variety of inflammatory and cardiovascular diseases, we sought to investigate whether the absence of endogenous testosterone alters leukocyte-endothelial cell interactions and the expression of adhesion molecules on endothelial cells.

Postcapillary venules were chosen since this vessel segment has been considered as the most important site for leukocyte adhesion to the vascular endothelium in response to noxious stimuli (Rodrigues et al., 2008). The major finding of the present study is that endogenous testosterone increases leukocyte–endothelial cell interaction in hypertension, by interfering with two of the main steps of the inflammatory response, which include leukocytes rolling and adherence. Augmented P-selectin and ICAM-1 expression in venular endothelial cells seems to be involved in this process.

Male sex is an independent risk factor for cardiovascular diseases. Although the influence of androgens acting on endothelial cells has been previously demonstrated (Singh et al., 2007; Vasudevan et al., 2006), the role of endogenous testosterone on leukocyte–endothelial cell interaction has been scarcely investigated, and in vivo works in animal models are limited (Littleton-Kearney and Hurn, 2004). We demonstrated that testosterone levels were increased in SHRs and castration promoted decrease in blood pressure, indicating that androgens contribute to both hypertension and altered leukocyte behavior in this condition. It’s important to consider that the effects promoted by very low testosterone levels on leukocyte behavior seem to be more relevant than the effects of blood pressure. This is supported by the observations that changes in rolling and adherence could be observed after castration, even with the blood pressure remaining elevated. The role of testosterone exacerbating hypertension in male adult SHRs has been previously demonstrated (Reckelhoff et al., 1998),

### Table 1

<table>
<thead>
<tr>
<th>Venular diameter (in μm)</th>
<th>Intact Wistar</th>
<th>Intact SHR</th>
<th>Castrated SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venular blood flow velocity (in mm s⁻¹)</td>
<td>16.9 ± 0.5</td>
<td>17.2 ± 0.3</td>
<td>16.5 ± 0.4</td>
</tr>
<tr>
<td>Venular shear rate (in s⁻¹)</td>
<td>1.7 ± 0.1</td>
<td>1.8 ± 0.3</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Leukocyte velocity (in μm s⁻¹)</td>
<td>721 ± 22</td>
<td>793 ± 28</td>
<td>773 ± 26</td>
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<td>Values are means ± S.E.M for 10 animals. P&gt;0.05.</td>
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### Table 2

| Blood leukocyte counts (per μL of blood) in intact Wistar, intact SHR and castrated SHR. |
|-----------------|--------------|------------|---------------|
| Cell type       | Intact Wistar | Intact SHR | Castrated SHR |
| Total number    | 11,110 ± 616 | 9850 ± 817 | 11,930 ± 1239 |
| Lymphocyte      | 8053 ± 470   | 7679 ± 727 | 9295 ± 921    |
| Neutrophil      | 2249 ± 298   | 1548 ± 129 | 2136 ± 342    |
| Monocyte        | 652 ± 189    | 513 ± 79   | 717 ± 151     |
| Eosinophil      | 154 ± 42     | 109 ± 44   | 180 ± 47      |
| | | | |
| Values are means ± S.E.M for 10 animals. P>0.05. |

Fig. 3. Effect of castration on adhesion molecule protein expression in mesenteric venules. A and B represent bar graphs of the protein expression of P-selectin (A) and ICAM-1 (B), in non-stimulated venules of the mesenteric bed from intact Wistar, intact SHR and castrated SHR. C and D represent microscopic images of P-selectin (C) and ICAM-1 (D) protein expression. As negative controls, preparations not treated with the primary antibody were also tested. Results were obtained from analysis using the software KS300 3.0. Results are mean ± SEM. * P<0.05 vs. intact Wistar and castrated SHR. n=5/group.
but, for the first time, we showed the influence of testosterone on important pathways of the inflammatory cascade.

Intravital microscopy allows evaluating leukocyte behavior into the 3 main steps: rolling, adherence and transmigration. Rolling behavior was evaluated in this study without any stimulus except that induced by exposure trauma. The augmented number of rolling leukocytes in SHRs and the reduction promoted by castration, in addition to the fact that the levels of testosterone are increased in this model, indicate the potential role of endogenous testosterone mediating rolling behavior.

A great number of mediators are released during the inflammatory response, including LTβ4, which has been identified as a key mediator of leukocyte–endothelial cell interaction and as a potent chemotactant responsible for the recruitment of neutrophils to the site of inflammation (Martinez et al., 2005). Although multiple mediators work together in inflammation, LTβ4 has been implicated in the initiation of leukocyte–endothelium interaction during inflammation (Schafer et al., 2005). In our study, the enhanced LTβ4-induced leukocyte adhesion observed in hypertensive rats was attenuated by castration, which suggests that endogenous testosterone exerts a proinflammatory effect contributing not only for the increased leukocyte rolling but also for the increase in leukocyte adherence in SHRs. Although increased leukocyte adherence in hypertension has already been demonstrated in clinical and experimental studies (Blank et al., 1990; Schmid-Schonbein et al., 1991), our study is the first to demonstrate the role of endogenous testosterone mediating this process.

Migration through the venular wall is the final step of the inflammatory process. To determine whether the augmented rolling and adhesion in response to LTβ4 was associated to increased leukocyte emigration, we turned to intravital microscopy, monitoring the LTβ4-induced response in the venular mesenteric bed. Interestingly, an important finding in the present study was that endogenous testosterone did not alter leukocyte migration. This is supported by the fact that, in contrast to the abnormal leukocyte rolling and adhering behavior, no changes in the extent of emigrated leukocytes could be observed among groups after castration. This result indicates that endogenous testosterone increases the initial steps of an inflammatory response but not the formation of an inflammatory focus. Of relevance, it is important to consider that although castration did not change leukocyte migration to the interstitial space, endogenous testosterone seems to play a role promoting leukocyte activation as demonstrated by the increased adhesion of these cells to the endothelium. Activated leukocytes may increase the release of important inflammatory mediators that could ultimately influence the release of cytokines, reactive oxygen species and also the blood pressure levels (Dokken, 2008; Elneihoum et al., 1997; Hokama et al., 2000). Considering this, although the results obtained in the present study do not provide support to a role of androgens promoting inflammation in hypertension, our data indicate that endogenous testosterone increases leukocyte–endothelial cell interaction and leukocyte activation in this condition.

Hemodynamic forces such as flow and resistance may influence the leukocyte–endothelial cell interactions (Martinez et al., 2005). Considering this, the venular diameter and blood flow velocity were measured to calculate the wall shear rate. It has been demonstrated that leukocyte adhesion depends on shear rate. Low shear rates promote leukocyte adherence to the endothelium in postcapillary venules (Martinez et al., 2005), which would explain the increase in leukocyte adherence observed in SHRs as well as the effects of castration. However, castration did not alter venular blood flow velocity and venular shear rate. Therefore, we can exclude interference of these parameters on the alterations observed in our study.

Leukocyte rolling is a prerequisite for firm adhesion. Slow rolling is necessary for efficient conversion from rolling to firm adhesion (Forlow et al., 2000). It was demonstrated that leukocyte recruitment is reduced by increased rolling velocity (Sperandio et al., 2001). However, in the present study, no difference in Vwbc was observed among groups. Therefore, Vwbc is not involved on the effects promoted by castration on inflammatory responses of SHRs.

The leukocyte count is also a rigorous predictor of rolling and adhesion similar to blood flow velocity and wall shear rate (Ley, 2001). However, we did not detect any statistically significant difference in the number of circulating leukocytes among the groups. Thus, an influence of this parameter on the increased rolling and adherence could be also excluded.

Leukocyte–endothelium interactions are affected not only by hemodynamic forces but also by alterations in endothelial and leukocyte expression of cell adhesion molecules. The expression of cell adhesion molecules on both the surface of leukocytes and on endothelial cells is a well known factor that determines the level of leukocyte–endothelium interaction in the microcirculation (Komatsu et al., 1997). In the present study, we investigated the protein expression of P-selectin and ICAM-1 in endothelial cells by immunohistochemistry. The mRNA expression of these molecules was evaluated by real time PCR technique. Considering the important role of adhesion molecules on leukocyte behavior, we hypothesized that they could mediate the effects promoted by the decreased level of androgens in the inflammatory response in vivo.

Since P-selectin is a determinant molecule of leukocyte rolling in the early phase of an inflammatory response, we investigated whether an altered expression of this adhesion molecule would contribute to the effects promoted by castration in SHRs. Quantification of P-selectin protein expression in venules showed that the basal expression of this molecule, increased in SHRs, was reversed after castration. Reduction in CAMs on endothelial cells could also be involved in the correction of leukocyte adherence after castration. In fact, we demonstrated that castration reversed the increased ICAM-1 expression observed in venules of SHRs. Considering that selectins and members of the immunoglobulins super gene family, such as ICAM-1, play an important role on rolling and adhesion behavior, the increased protein expression of these molecules during the inflammatory process could explain, at least in part, the alterations of the leukocyte behavior in SHRs.

Of relevance, it is important to consider that according to the common theory of steroids action, these hormones typically act at the level of gene transcription by interaction with intracellular, nuclear receptors, which act as ligand-dependent transcription factors (Falkenstein et al., 2000). However, we did not observe an increase in mRNA of either P-selectin or ICAM-1 despite the increased protein expression of these molecules in SHRs. In addition to the regulation of gene expression at the transcriptional levels, gene expression may also be modulated by the interaction of nuclear receptors with sequence-specific transcription factors, evolving effects mediated by direct protein–protein interactions, which are termed nontranscriptional activities (Pietras, 2005). Furthermore, testosterone may induce phosphorylation events and Ca²⁺-induced signaling (Vicenzo et al., 2006). These observations might explain the fact that we did not
observe an increase in mRNA of either P-selectin or ICAM-1 despite the increased protein expression of these molecules.

An additional aspect of the biological actions of testosterone is its ability to increase the levels of reactive oxygen species and inflammatory mediators such as NFκB (Falkenstein et al., 2000). Considering that oxidative stress has been described to enhance the expression of adhesion molecules via either transcriptional or post transcriptional regulatory factors, the latter could constitute an additional mechanism by which testosterone increases the protein expression of the adhesion molecules analyzed in the present study, without changing the mRNA levels of these molecules. Further studies are needed to investigate the role of these mechanisms mediating the effects of endogenous testosterone on the expression of adhesion molecules.

Conclusions

Taken together, the results of the present study demonstrate the critical role of endogenous testosterone mediating the effects of hypertension increasing leukocyte–endothelial cell interaction. Low levels of endogenous testosterone induced by castration restored the alterations of the leukocyte behavior by modulating the expression of the cell adhesion molecules such as P-selectin and ICAM-1.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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