Chronic Ethanol Consumption Enhances Phenylephrine-Induced Contraction in the Isolated Rat Aorta

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ABSTRACT

Changes in reactivity to phenylephrine in aortas isolated from 2-, 6-, and 10-week ethanol-treated rats and their age-matched control and isocaloric rats were investigated. Chronic ethanol consumption enhances the contractile response of endothelium-intact and -denuded rat aortic rings to phenylephrine, a response that is time-independent. Pretreatment with indomethacin reduced $E_{\text{max}}$ for phenylephrine in denuded aortas from ethanol-treated rats but not control or isocaloric rats. After indomethacin treatment, no differences in $E_{\text{max}}$ from phenylephrine were observed among the groups. SQ29548 ([1S-[1α-2α(Z)3α,4α]-7-[3-[[phenylamino]carbonyl]hydrazino)methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid), an antagonist of prostaglandin H$_2$/thromboxane A$_2$ (TXA$_2$) receptors, did not alter phenylephrine-induced contraction in control or isocaloric aortas. However, in ethanol-treated aortas, $E_{\text{max}}$ was reduced to control level. Moreover, phenylephrine-stimulated release of thromboxane B$_2$, a stable metabolite of TXA$_2$, was higher in tissues from ethanol-treated rats. Simultaneous measurement of the changes in [Ca$^{2+}$], and contraction induced by phenylephrine showed that both parameters are higher in the rat aorta from ethanol-treated rats. CaCl$_2$-induced contraction in free Ca$^{2+}$ solution containing phenylephrine was increased in ethanol-treated aortas. Additionally, the enhancement in CaCl$_2$-induced contraction was prevented by SQ29548. The major contribution of the present study is that it demonstrates a detailed description of the mechanisms involved in the enhancement of phenylephrine-induced contraction in rat aorta from ethanol-treated rats. We provided evidence that this response was not different among the three periods of treatment employed in this study and that it is maintained by two mechanisms: an increased release of vascular smooth muscle-derived vasoconstrictor prostanoids (probably TXA$_2$) and an enhanced extracellular Ca$^{2+}$ influx.

Chronic ethanol consumption is associated with cardiovascular dysfunctions independent of other known risk factors (Altura and Altura, 1982). Much of the research investigating the chronic effects of ethanol on the cardiovascular system has dealt with vascular responsiveness to vasoconstrictor agents (Strickland and Wooles, 1988; Hatton et al., 1992). Previous reports suggest that enhanced vascular reactivity to vasoconstrictor agents (Pinardi et al., 1992) or impairment of the vascular relaxation (Kahonen et al., 1999) contribute to the cardiovascular complications associated with chronic ethanol consumption.

Enhanced vascular reactivity to $\alpha_1$-adrenoceptor agonists was demonstrated in different arteries from ethanol-treated rats. In this line, Pinardi et al. (1992) found that chronic ethanol consumption significantly enhanced the contractile response induced by phenylephrine in aortic rings with intact endothelium. The contractile response of superior mesenteric artery to noradrenaline was shown to be greater in the rings from ethanol-treated rats (Hatton et al., 1992). Likewise, Stewart and Kennedy (1999) demonstrated an ethanol-associated increase in the maximum response to phenylephrine in endothelium-denuded aortic rings. Recently, Ladipo et al. (2002) observed that chronic ethanol consumption increased the sensitivity of rat aortic rings to noradrenaline. Although the chronic effects of ethanol on the cardiovascular system has led to vascular responsiveness to vasoconstrictor agents (Strickland and Wooles, 1988; Hatton et al., 1992). Previous reports suggest that enhanced vascular reactivity to vasoconstrictor agents (Pinardi et al., 1992) or impairment of the vascular relaxation (Kahonen et al., 1999) contribute to the cardiovascular complications associated with chronic ethanol consumption.
it has been described that chronic ethanol consumption enhances α₁-induced contraction, the mechanisms underlying this response are poorly understood.

On the other hand, unchanged (Chan and Sutter, 1983; Utkan et al., 2001) or attenuated (Strickland and Woolf, 1988) responses to α₁-adrenoceptor agonists have also been described. The reason for these differences is not entirely clear, but contributing factors may be different experimental designs, different protocols of ethanol administration, or the duration of chronic treatment. The periods of chronic ethanol treatment differ among the several studies published (from 18 days to 24 weeks) (Pinardi et al., 1992; Sahna et al., 2000). Moreover, the majority of the experiments designed to study the vascular effects of chronic ethanol consumption on α₁-receptor-induced contraction use only one period of treatment (Chan and Sutter, 1983; Utkan et al., 2001; Brown et al., 2002). Thus, little is known about the time course for the development of enhanced contraction induced by α₁-agonists in ethanol-treated rats.

Based on the above-mentioned studies, we can conclude that detailed influences of long-term ethanol consumption on the control of arterial tone remain largely unknown. Furthermore, contradictory results on the effects of ethanol intake on vascular function have been published. Thus, the purpose of this study was to determine whether there are any changes in vascular reactivity to phenylephrine in aortas from chronically ethanol-treated rats after three different periods of treatment. A second purpose was to evaluate in detail the mechanisms underlying the effects of long-term ethanol consumption on phenylephrine-induced contraction.

**Materials and Methods**

**Experimental Design**

Male Wistar rats were housed under standard laboratory conditions with free access to food and water. The housing conditions and experimental protocols are in accordance with the Ethical Animal Committee from the Campus of Ribeirão Preto (University of São Paulo, Brazil).

The rats, initially weighing 300 to 350 g (80–100 days old), were randomly divided into three groups: control, isocaloric, and ethanol. Control rats received tap water ad libitum. Rats from the isocaloric group received a solution containing an isocaloric amount of sucrose (290.50 g/liter) instead of ethanol. Rats from the ethanol group received 20% (v/v) ethanol in their drinking water (adapted from Chan and Sutter, 1983). To avoid a considerable loss of animals, the ethanol-treated group was submitted to a brief and gradual adaptation period. The animals received 5% ethanol in their drinking water in the 1st week, 10% in the 2nd week, and 20% in the 3rd week. At the end of the 3rd week, the experimental stage began. The same procedure was adopted for the isocaloric group. In these groups, the caloric content of the liquid diet was adjusted to match that of the ethanol-exposed groups. The isocaloric groups were included in the study protocol to evaluate whether alterations in caloric intake following ethanol consumption would explain the possible influences of ethanol on arterial responses. The rats were treated for 2, 6, and 10 weeks and weighed weekly.

**Blood Ethanol and Serum Glucose Measurements**

Blood was collected from the aorta of anesthetized rats using heparinized syringes. The samples were placed in 10-ml head space vials by adding 1.0 g of sodium chloride, 1.0 ml of water, 100 μl of the internal standard (acetonitrile, 1 ml/l) solution, and 1.0 ml of blood. Ethanol analysis was carried out using a CG-17A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and an HSS-4A head space sampler (Shimadzu). Calibration standards were prepared in the same head space vials (0.10–3.16 mg/ml). The results are expressed as milligrams of ethanol per milliliter of blood.

For glucose measurements, the blood was centrifuged at 8000g to 10,000g for 10 min at room temperature. The serum was analyzed for glucose content using available commercial kits (Labtest Diagnóstica, São Paulo, Brazil) and the Abbott AutoAnalyzer (model ABAA VP; Abbott Laboratories, Abbott Park, IL). The results are expressed as milligrams per deciliter.

**Vessel Ring Preparation**

The thoracic aorta was quickly removed, cleaned of adherent connective tissues, and cut into rings (5–6 mm in length). Two stainless steel stirrups were passed through the lumen of each ring. One stirrup was connected to an isometric force transducer (Leica, Wetzlar, Germany) to measure tension in the vessels. The rings were placed in a 5-ml organ chamber containing Krebs’ solution gassed with 95% O₂/5% CO₂ and maintained at 37°C. The composition of Krebs’ solution was as follows: 118.0 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 15.0 mM NaHCO₃, 5.5 mM glucose, and 2.5 mM CaCl₂. The rings were stretched until an optimal basal tension of 1.50 g, which was determined by length-tension relationship experiments, and then were allowed to equilibrate for 60 min with the bath fluid being changed every 15 to 20 min. In some rings, the endothelium was removed mechanically by gently rolling the lumen of the vessel on a thin wire. Endothelial integrity was assessed qualitatively by the degree of relaxation caused by acetylcholine (1 μM) in the presence of contractile tone induced by phenylephrine (0.1 μM). For studies of endothelium-intact vessels, the ring was discarded if relaxation with acetylcholine was not 80% or greater. For studies of endothelium-denuded vessels, the rings were discarded if there was any degree of relaxation.

**Experimental Protocols**

**Concentration-Response Curves for Phenylephrine and Endothelin-1.** After 60 min of equilibration, each aortic ring was exposed three times to phenylephrine (0.1 μM) to attain its maximum contractility. Each ring was sequentially washed and re-equilibrated and was allowed to relax to baseline. After 30 min, cumulative concentration-response curves for phenylephrine (10⁻³–10⁻⁵ M) or endothelin-1 (10⁻¹–10⁻⁷ M) were determined in intact and denuded rings. In another set of experiments, the rings were stimulated with 90 mM KCl. The vascular responsiveness to the agonists was studied in aortic rings from control, isocaloric, and ethanol-treated rats after treatment for 2, 6, and 10 weeks.

**Contribution of NO and Endothelial Cyclooxygenase Arachidonic Acid Metabolites in Modulating the Response to Phenylephrine.** Cumulative concentration-response curves for phenylephrine were obtained in endothelium-intact aortic rings from control, isocaloric, and ethanol-treated rats in the absence or in the presence of the NOS inhibitor L-NAME (100 μM). The participation of cyclooxygenase-arachidonic acid metabolites was investigated by obtaining cumulative concentration-response curves for phenylephrine, in endothelium-intact aortic rings, in the presence of the cyclooxygenase inhibitor indomethacin (10 μM).

**Contribution of Nonendothelial Cyclooxygenase-Arachidonic Acid Metabolites in the Enhanced Reactivity of the Rat Aorta to Phenylephrine.** Endothelium-denuded rings were preincubated with indomethacin (10 μM), AH6809 (an antagonist of PGF₂α receptors, 10 μM), or SQ29548 [an antagonist of PGH₂/thromboxane A₂ (TXA₂) receptors, 3 μM] for 30 min, before obtaining concentration-response curves for phenylephrine.

**Measurement of TXA₂.** Rat aortas (5–6 mm in length) were isolated, and the endothelium was gently removed with a humid cotton swab. The tissues were transferred to an organ bath contain-
ing 2.5 ml of Krebs’ solution at 37°C continuously bubbled with a mixture of 95% O₂ and 5% CO₂. The arteries were placed in the Krebs’ solution for 30 min. The 30-min medium samples were systematically collected and stored (at −80°C) for posterior determination of \( \Delta \text{TB} \), the stable metabolite of \( \text{TXA}_2 \), by enzyme immunoassay (using commercially available kits from GE Healthcare, Little Chalfont, Buckinghamshire, UK). For each experimental group, control samples (for estimation of basal release) were collected. In another set of experiments, the tissues from control, isocaloric, and ethanol-treated rats were exposed to phenylephrine (1 \( \mu \)M) for 30 min.

**Simultaneous Measurement of Tension and Variations in Cytosolic-Free Ca²⁺**. The thoracic aorta was cut helicoidally and placed in physiological saline solution containing 123 mM NaCl, 4.67 mM KCl, 1.2 mM MgCl₂, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 15.5 mM NaHCO₃, 11.5 mM glucose, and 10 mM HEPES. The endothelium of the arteries was gently removed with a humid cotton swab. The loading of the vascular smooth muscle with Fura-2 AM was done using standard technique (Benchekroun et al., 1995). Briefly, the vessels were tied to one end with a metallic support and placed in a quartz cuvette containing the loading solution (10 \( \mu \)M Fura-2/AM in physiological saline solution containing 0.3% bovine serum albumin in the presence of 0.2% Cremophor EL and 1 mM probenecid) at room temperature for 2 h while being oxygenated. The initial tension of the thoracic aorta was 2 g. Experiments were then performed with a double-wavelength excitation fluorometer (Photon Technology International, Lawrenceville, NJ) (Claing et al., 1994; Benchekroun et al., 1995). The responses were expressed as increase in vessel tension (grams), and the variation of ratio of Ca²⁺ (nonbound) to Fura-2 (F340/F380) reflected the total cytoplasmatic-free Ca²⁺ level (Benchekroun et al., 1995). The tension of the vessels was measured by an isometric transducer and recorded on a grass physiograph. The total intracellular-free Ca²⁺ (\( \text{Ca}^{2+}_{\text{i}} \)) increase responses to phenylephrine (0.01–10 \( \mu \)M) were analyzed once both \( \text{Ca}^{2+}_{\text{i}} \) increase and contractions had reached a steady state. Agonist-induced changes in \( \text{Ca}^{2+}_{\text{i}} \) are represented by the percentage change in Ca²⁺ fluorescence ratio (F340/F380). At the end of the experiments, the preparations were also challenged with extracellular Ca²⁺ chelator EGTA (30 mM).

**Contribution of Intracellular and Extracellular Ca²⁺ in the Enhanced Reactivity of the Rat Aorta to Phenylephrine**. To further analyze the relative contribution of the release of intracellular Ca²⁺ on the enhanced reactivity to phenylephrine, contractile response to this agonist was obtained in calcium-free medium. With this purpose, the normal Krebs’ solution was replaced by a Ca²⁺-free solution (approximately 90 mg/ml in the 6th week (control, 108.30 ± 8.21, n = 9), isocaloric (104.50 ± 7.35, n = 11) and 100.7 ± 6.56 mg/dl, respectively. No significant differences were found in serum glucose levels after 6 (control, 108.83 ± 5.46, n = 11; isocaloric, 104.56 ± 10.86, n = 9; ethanol, 104.50 ± 7.35, n = 11) and 10 (control, 108.30 ± 7.78, n = 6; isocaloric, 112.61 ± 5.02, n = 7; ethanol, 115.40 ± 8.75, n = 6) weeks of treatment (ANOVA/Bonferroni’s comparison test). These data indicates that the experimental protocol employed did not induce an enhancement of glucose levels.

**Concentration-Response Curves for Phenylephrine and Endothelin-1**. The \( E_{\text{max}} \) of the concentration-response curve was significantly higher in arteries from ethanol-treated rats than in arteries from control or isocaloric animals, without there being significant differences among pD₂ values. The \( E_{\text{max}} \) or pD₂ values for phenylephrine-induced contraction did not significantly differ between control and isocaloric groups (Fig. 1; Table 1). In arteries from control, isocaloric, or ethanol-treated rats, mechanical removal of the endothelium significantly increased both the maximal contraction and the pD₂ values for phenylephrine (Table 1).

**Blood ethanol levels in the ethanol-treated rats** averaged 1.83 ± 0.22 mg/ml in the 2nd week (n = 10), 1.71 ± 0.23 mg/ml in the 6th week (n = 9), and 1.81 ± 0.20 mg/ml in the 10th week (n = 11). No ethanol was detectable in the blood of control and isocaloric animals. The blood ethanol levels were not significantly different among the three different periods of treatment (ANOVA/Bonferroni’s comparison test). In the 2-week-treated rats, serum glucose levels in the control (n = 9), isocaloric (n = 7), and ethanol (n = 10) groups averaged 108.92 ± 8.21, 105.01 ± 4.47, and 101.70 ± 6.56 mg/dl, respectively. No significant differences were found in serum glucose levels after 6 (control, 108.83 ± 5.46, n = 11; isocaloric, 104.56 ± 10.86, n = 9; ethanol, 104.50 ± 7.35, n = 11) and 10 (control, 108.30 ± 7.78, n = 6; isocaloric, 112.61 ± 5.02, n = 7; ethanol, 115.40 ± 8.75, n = 6) weeks of treatment (ANOVA/Bonferroni’s comparison test). These data indicates that the experimental protocol employed did not induce an enhancement of glucose levels.
ditionally, we observed that 90 mM KCl-induced contraction of endothelium-intact rings did not differ among the groups after 2, 6, or 10 weeks of treatment (data not shown). Since the period of treatment did not influence the effect induced by ethanol treatment on phenylephrine-induced contraction, the following experiments designed to investigate the mechanisms underlying this response were obtained in aortic rings from 2-week-treated rats and its respective age-matched control and isocaloric animals.

**Contribution of NO and Endothelial Cyclooxygenase Arachidonic Acid Metabolites in Modulating the Response to Phenylephrine.** Incubation with L-NAME significantly enhanced the maximal contraction induced by phenylephrine in arterial segments from control, isocaloric, and ethanol-treated rats when compared with the responses obtained in the absence of L-NAME. Furthermore, there was a significant increase in the $E_{\text{max}}$ values for phenylephrine in L-NAME-treated rings from ethanol-treated rats compared with control or isocaloric groups (Table 1).

**TABLE 1**
Effect of chronic ethanol consumption on the $E_{\text{max}}$ (in grams) (A) and $pD_2$ values (B) for phenylephrine in endothelium-intact (Endo+) or endothelium-denuded (Endo−) aortic rings

<table>
<thead>
<tr>
<th>A period (in weeks)</th>
<th>Control (Endo+)</th>
<th>Isocaloric (Endo+)</th>
<th>Ethanol (Endo−)</th>
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<tr>
<td>2</td>
<td>1.61 ± 0.08 (13)</td>
<td>2.16 ± 0.11a (10)</td>
<td>2.25 ± 0.14a (8)</td>
</tr>
<tr>
<td>6</td>
<td>1.71 ± 0.10 (12)</td>
<td>2.15 ± 0.15a (6)</td>
<td>2.26 ± 0.19a (7)</td>
</tr>
<tr>
<td>10</td>
<td>1.50 ± 0.11 (8)</td>
<td>1.96 ± 0.09a (7)</td>
<td>2.28 ± 0.19a (10)</td>
</tr>
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<table>
<thead>
<tr>
<th>B period (in weeks)</th>
<th>Control (Endo+)</th>
<th>Isocaloric (Endo+)</th>
<th>Ethanol (Endo−)</th>
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<tr>
<td></td>
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<tr>
<td>2</td>
<td>6.90 ± 0.06 (13)</td>
<td>7.44 ± 0.06a (10)</td>
<td>7.04 ± 0.08 (8)</td>
</tr>
<tr>
<td>6</td>
<td>6.96 ± 0.06 (12)</td>
<td>7.46 ± 0.05a (6)</td>
<td>7.19 ± 0.15 (7)</td>
</tr>
<tr>
<td>10</td>
<td>7.02 ± 0.07 (8)</td>
<td>7.50 ± 0.04a (8)</td>
<td>7.02 ± 0.08 (10)</td>
</tr>
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$^a$ Compared with control and isocaloric groups.

$^b$ Compared with the respective groups with endothelium ($p < 0.05$; ANOVA).

**Fig. 1.** Effect of chronic ethanol consumption on phenylephrine-induced contractile response in rat aortic rings. Concentration-response curves for phenylephrine were determined in endothelium-intact (Endo+, left) or endothelium-denuded (Endo−, right) aortic rings from control, isocaloric, and ethanol-treated rats.
the absence (A) or after a 30-min period of incubation with 100 
μM indomethacin, isometric, and ethanol-treated rats. The curves were determined in
endothelium-intact aortic rings from control and isocaloric rats. The curves were determined in endothelium-intact aortic rings from control and isocaloric rats. No changes in pD2 values were detected (data not shown). On the other hand, preincubation with AH6809 did not produce changes in the concentration-response curves for phenylephrine in either of the groups. The association of SQ29548 and indomethacin or SQ29548 and AH6809 did not show further suppression than that observed when the inhibitors were added alone (Table 3).

**Measurement of TXB2.** Basal release of TXB2 did not differ among control (189.3 ± 30.8 pg/ml, n = 4), isocaloric (184.0 ± 18.2 pg/ml, n = 4), or ethanol-treated aortas (170.7 ± 28.5 pg/ml, n = 4). On the other hand, the phenylephrine-stimulated release of TXB2 in the medium bath was significantly higher in tissues from ethanol-treated rats (258.0 ± 12.3 pg/ml, n = 4) when compared with control (181.5 ± 20.2 pg/ml, n = 4) or isocaloric tissues (173.5 ± 11.3 pg/ml, n = 4) (ANOVA/Bonferroni’s comparison test). No significant differences were found between basal and stimulated levels of TXB2 in control or isocaloric tissues (ANOVA/Bonferroni’s comparison test).

**Simultaneous Measurement of Tension and Variations in Cytosolic-Free Ca2++.** The contractile and [Ca2+]i-increasing effects of phenylephrine at 1 and 10 μM were higher in aortas from ethanol-treated rats compared with control and isocaloric rats (Fig. 4). Once the contractile and [Ca2+]i-increasing effects of 10 μM phenylephrine had fully developed, and still in the presence of the agonist, exposure of the rat aorta to EGTA (30 mM) promoted reduction in the magnitudes of contraction and Fura-2 fluorescence (data not shown).

**Contribution of Intracellular and Extracellular Ca2+ in the Enhanced Reactivity of the Rat Aorta to Phenylephrine.** In Ca2+-free medium, phenylephrine (1 μM) induced a rapid phasic contraction that reached a peak and then returned close to baseline levels. The magnitude of the peak phasic response was similar in control (0.70 ± 0.09 g; n = 9), isocaloric (0.72 ± 0.12 g; n = 9), and ethanol-treated rats (0.65 ± 0.13 g; n = 6) (ANOVA/Bonferroni’s comparison test).

CaCl2-induced contraction was greater in aortic rings from ethanol-treated rats (E\text{max} = 2.96 ± 0.20 g; n = 10) when compared with control (E\text{max} = 2.09 ± 0.20 g; n = 7) and isocaloric (E\text{max} = 1.93 ± 0.12 g; n = 8) aortas. On the other hand, no differences in pD2 values were found among control, isocaloric, and ethanol aortas (3.33 ± 0.16, 3.42 ± 0.10, and 3.29 ± 0.09, respectively). After treatment of the rings with SQ29548, no differences in the E\text{max} or pD2 values for CaCl2 on aortic rings from control (E\text{max} = 1.94 ± 0.11 g; pD2 = 3.39 ± 0.06; n = 8), isocaloric (E\text{max} = 2.01 ± 0.17 g; pD2 = 3.40 ± 0.10; n = 8), and ethanol-treated rats (E\text{max} = 2.19 ±
The present findings demonstrate that chronic ethanol consumption produces an enhanced responsiveness to phenylephrine in rat aortas. On the other hand, unchanged (Chan and Sutter, 1983; Utkan et al., 2001) or attenuated (Strickland and Woolles, 1988) responses to α1-adrenoceptor agonists have also been described. Reasons for these differences are not clear, although it was suggested that they could be explained by different experimental conditions, including the type of artery or agonist examined, the method used for vascular studies, different concentration of ethanol used, different experimental model, or the duration of chronic ethanol treatment (Pinardi et al., 1992; Sahna et al., 2000).

Some reports suggest that the period of exposure to ethanol is the major factor in the development of cardiovascular abnormalities (Abdel-Rahman and Woolles, 1987; Strickland and Woolles, 1988). Results presented here demonstrate that chronic ethanol consumption produced an enhanced responsiveness to phenylephrine in aortas, although evidencing no relation between the period of treatment and the magnitude in the enhancement of phenylephrine-induced contraction. However, our data do not rule out the possibility that ethanol displays a time-dependent effect at periods of treatment shorter or longer than that employed in the present work. It is important to note that the increased vascular responsiveness to phenylephrine was already observed in 2-week ethanol-treated animals. Thus, our results support the notion that the altered vascular responsiveness associated with ethanol intake can be already apparent in early stages of ethanol consumption.

The enhanced responsiveness to phenylephrine was also observed after endothelium denudation, further corroborating previous findings that increased sensitivity to α-adrenergic agonists is not dependent on the presence of endothelium in the rat aorta (Stewart and Kennedy, 1999). It is important to note that the enhanced response to phenylephrine is not the result of a nonspecific increase in the reactivity of the rat aorta induced by chronic ethanol consumption since the contractile responses to endothelin-1 or KCl were similar in ethanol-treated and control rats. Furthermore, no changes were detected in phenylephrine-induced contraction among the three experimental groups. The contribution of endothelial prostanoids derived from the arachidonic acid-cyclooxygenase pathway are also produced by the vascular smooth muscle of rat aortic rings (Stanke-Labesque et al., 1999). It is important to note that in addition to the endothelial cells, the prostanoids derived from the arachidonic acid-cyclooxygenase pathway are also produced by the vascular smooth muscle of rat aortic rings (Stanke-Labesque et al., 2004). Interestingly, we found that indomethacin reduced the maximum contraction evoked by phenylephrine in denuded aortic rings from ethanol-treated rats but not in control and isocaloric aortas. Together with our previous finding that indomethacin reduces phenylephrine-induced contraction of

### Discussion

The possibility that the endothelium may also contribute to the enhanced reactivity was also studied. In control, isocaloric, and ethanol aortas, incubation of the rings with L-NAME significantly enhanced the maximal contraction induced by phenylephrine, suggesting that the enhanced responsiveness of ethanol-treated aortas to phenylephrine was not due to an impaired release of NO. Our results also show that maximum contraction induced by phenylephrine was significantly diminished by indomethacin in endothelium-intact aortic rings from control, isocaloric, and ethanol-treated rats. However, no changes were detected in phenylephrine-induced contraction among the three experimental groups. The contribution of endothelial prostanoids derived from the arachidonic acid-cyclooxygenase pathway modulating the contraction induced by α-adrenergic agonist has been reported previously (Tabernero et al., 1999; Xavier et al., 2003). However, this modulation, as shown in the present study, seems to be greater in the aorta from ethanol-treated rats.

It is important to note that in addition to the endothelial cells, the prostanoids derived from the arachidonic acid-cyclooxygenase pathway are also produced by the vascular smooth muscle of rat aortic rings (Stanke-Labesque et al., 2004). Interestingly, we found that indomethacin reduced the maximum contraction evoked by phenylephrine in denuded aortic rings from ethanol-treated rats but not in control and isocaloric aortas. Together with our previous finding that indomethacin reduces phenylephrine-induced contraction of
endothelium-intact rat aorta, these data indicate that the vasoconstrictor prostanoid(s) that modulate the response of control and isocaloric aortas to phenylephrine have an endothelial origin. However, after preincubation of denuded rat aorta with indomethacin, no changes could be detected in the $E_{\text{max}}$ values to phenylephrine among the three experimental groups. Similar results were obtained when endothelium-intact aortas were exposed to indomethacin, further indicating that the prostanoid(s) that modulated the vascular responses to phenylephrine after treatment with ethanol are produced by the vascular smooth muscle. Thus, our results indicate the greater modulatory role of smooth muscle-derived prostanoids on aortas from ethanol-treated rats could contribute to the enhanced reactivity of the rat aorta to phenylephrine.

In the process of analyzing the possible prostanoid(s) involved in the enhanced reactivity to phenylephrine, we found that AH6809, an antagonist of PGF$_2\alpha$ receptors, did not modify the contractile response induced by phenylephrine in ethanol-treated, control, or isocaloric denuded aortas. This result suggests that PGF$_2\alpha$ does not modulate the action of phenylephrine and that the enhancement in phenylephrine-induced contraction after chronic ethanol consumption is not related to an increased production of this prostanoid. On the other hand, a competitive antagonist of PGH$_2$/TXA$_2$ receptors, SQ29548, reduced the maximum response evoked by phenylephrine in aortic rings from ethanol-treated rats, but not in those from control or isocaloric rats. The lack of effect of SQ29548 in control aortas corroborates previous findings by Xavier et al. (2003). When SQ29548 and indomethacin or SQ29548 and AH6809 were simultaneously added, no further additional inhibitory effect on phenylephrine-induced contraction was observed. The measurement of smooth muscle-derived TXB$_2$, a stable metabolite of TXA$_2$, showed that the basal release of TXB$_2$ did not differ among the three groups, whereas the phenylephrine-stimulated release of TXB$_2$ was significantly higher in tissues from ethanol-treated rats. The enhanced TXB$_2$ release is consistent with the functional effect of the antagonist SQ29548 on phenylephrine-induced contraction and suggests that TXA$_2$ accounts for the enhanced reactivity to phenylephrine after treatment with ethanol. Our data corroborate previous findings, namely that chronic ethanol consumption enhances the production of TXA$_2$ (Karanian et al., 1985; Nanji et al., 1997a,b).

The current findings also show that contractions of the rat

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**Fig. 3.** Effect of indomethacin, SQ29548, and AH6809 on phenylephrine-induced contraction of rat aortic rings. Concentration-response curves for phenylephrine were determined in endothelium-denuded aortic rings from control, isocaloric, and ethanol-treated rats. The curves were determined in the absence (control) or after a 30-min period of incubation with indomethacin (10 $\mu$M), SQ29548 (3 $\mu$M), AH6809 (10 $\mu$M), or combination of these drugs.
aorta induced by phenylephrine are correlated with slowly developing and sustained simultaneous changes in \([\text{Ca}^{2+}]_i\), which are concentration-dependent. However, the relationship between the increase in \([\text{Ca}^{2+}]_i\), and the contractile response was not linear as previously reported (Claing et al., 2002). Both the changes in \([\text{Ca}^{2+}]_i\), and contraction induced by phenylephrine in the rat aorta from ethanol-treated rats are higher than those found for control and isocaloric aortas. We found that the contractile response to phenylephrine (1 \(\mu\text{M}\)) in \([\text{Ca}^{2+}]_i\)-free medium, which is used to verify the contribution of intracellular \(\text{Ca}^{2+}\) release after stimulation with phenylephrine (for review, see Horowitz et al., 1996) did not contribute to the enhanced contractile response of ethanol-treated aortas. On the other hand, we observed that the enhanced responsiveness to phenylephrine (1 \(\mu\text{M}\)) is modulated by PGH$_2$/TXA$_2$. Based on these results, we can suggest that these prostanoids mediate the enhanced reactivity to phenylephrine by mechanisms that alter the mobilization or sensitivity to extracellular \(\text{Ca}^{2+}\).

In summary, the major new finding of the present work is that the enhanced vascular response to phenylephrine observed in the aorta of ethanol-treated rats is maintained by two mechanisms: an increased release of vascular smooth muscle-derived vasoconstrictor prostanoids (probably TXA$_2$) and an enhanced extracellular \(\text{Ca}^{2+}\) influx. Also, we verified no relation between the period of treatment with ethanol, employed in the present work, and the magnitude in the enhancement of phenylephrine-induced contraction.

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**References**


Karanian JW, Slejtanov M, and Salem N Jr (1985) Effect of ethanol on prostacyclin...


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