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# Inhibition of nitric oxide-guanylate cyclase-dependent and -independent signaling contributes to impairment of β-adrenergic vasorelaxations by cyclosporine

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ABSTRACT

This study investigated the role of endothelium- and smooth muscle-dependent mechanisms in the interaction of cyclosporine (CyA), an immunosuppressant drug, with β-adrenoceptor (isoprenaline)-mediated relaxations in isolated rat aortas precontracted with phenylephrine. CyA effects were assessed in the absence and presence of $N^G$-nitro-L-arginine methyl ester (L-NAME, nitric oxide synthase inhibitor), methylene blue (guanylate cyclase inhibitor), or propranolol (β-adrenoceptor antagonist). In aortas with intact endothelium (E+), pretreatment with L-NAME or methylene blue significantly reduced isoprenaline ($1 \times 10^{-9}$ to $1 \times 10^{-7}$ M) relaxations in contrast to no effect for tetraethylammonium ($K^+$ channel blocker), or diclophenac (cyclooxygenase inhibitor), suggesting a major role for the nitric oxide-guanylate cyclase (NO-GC) pathway, but not endothelial hyperpolarizing factor or vasodilator prostanoids, in isoprenaline responses. Isoprenaline relaxations were still evident, though significantly attenuated, in endothelium-denuded aortas (E−) and were resistant to L-NAME or methylene blue. Acute exposure to CyA (2 μM) caused propranolol-sensitive reductions in isoprenaline responses in E+ and E− aortas. The CyA-induced attenuation of isoprenaline responses in E+ aortas largely disappeared in L-NAME-treated aortas and after supplementation with L-arginine, the substrate of nitric oxide. CyA also reduced the endothelium-independent, GC-dependent aortic relaxations evoked by sodium nitroprusside, an effect that was virtually abolished by methylene blue. We conclude that: (i) endothelial and smooth muscle mechanisms contribute to aortic β-adrenoceptor relaxations and both components are negatively influenced by CyA, and (ii) NO-GC signaling plays an integral role in the vascular CyA-β-adrenoceptor interaction. The clinical relevance of the present study is warranted given the established role of impaired vascular function in CyA toxicity.



## 1. Introduction

Calcineurin inhibitors are clinically important immunosuppressive agents. Since its first appearance in the 1980s as the prototypic calcineurin inhibitor, CyA continues to constitute a valuable component of the immunosuppressive protocols used by solid organ transplant recipients and by patients with autoimmune diseases [1]. Nevertheless, one critical adverse effect for CyA is vascular toxicity. Among several other pathophysiological factors, impairment of vascular reactivity

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to vasodilator stimuli represents a principal cause of the vasculotoxic effects of CyA and related diseases such as hypertension [2], microvascular thromboses [3], and renal vasoconstriction and subsequent nephrotoxicity [4,5]. Accumulated evidence from previous studies including our own has shown that vascular relaxations elicited by acetylcholine [6,7], bradykinin [8], substance P [9], calcium ionophore A23187 [8], prostaglandin $E_1$ [10], SKF38393 [11], and adenosine analogues [12] are remarkably compromised by CyA. A recent study from our laboratory has shown that chronic CyA administration impairs endothelium-dependent vasodilations via, at least partly, reducing the availability of testosterone and inhibiting its facilitatory effect on cholinergically mediated renal vasodilations [13].

The vasorelaxant response to β-adrenoceptor activation by selective drugs such as isoprenaline is widely believed to involve stimulation of adenylyl cyclase and subsequent increase in intracellular cAMP concentration and activation of cAMP-dependent protein kinase in smooth muscle cells [14]. This produces vasorelaxation via decreasing intracellular $Ca^{2+}$ and reducing the sensitivity of the contractile apparatus to $Ca^{2+}$ [14]. Although this effect of isoprenaline is believed to be endothelium independent, conflicting reports are available regarding the role of vascular endothelium in vascular responses to β-adrenoceptor activation. For example, in support of an endothelium-independent effect of isoprenaline, removal of the endothelium was found to have no inhibitory effect on isoprenaline relaxations [15–17]. In contrast, other studies demonstrated attenuation of isoprenaline relaxations in endothelium denuded vascular preparations [18,19]. Additional evidence for the endothelium involvement was manifest from the observations that inhibition of guanylate cyclase or nitric oxide synthase reduces responses to isoprenaline [18,20,21]. Possible explanations for these inconsistent findings include differences in precontraction levels [17], animal age [20], and technical problems due to incomplete removal of endothelial cells [22].

Although the negative vascular impact of CyA has been the subject of extensive research, little is known about the interaction of CyA with the β-adrenoceptor-mediated vascular control and the identity of the underlying cellular mechanisms. For instance, CyA was found to diminish vasodilatation induced by isoprenaline [10] and to reduce β-adrenergic receptor density [23]. CyA also inhibits the adenylyl cyclase pathway and cAMP accumulation [24], intracellular events that follow the activation of β-adrenoceptors. The present study was undertaken to further characterize the vascular CyA–β-adrenoceptor interaction in rat isolated aortas precontracted with phenylephrine. Three main issues are addressed here: (i) the endothelium dependence of isoprenaline vasorelaxation, (ii) the influence of CyA on endothelium-dependent and independent components of the isoprenaline response, and (iii) role of the NO/GC pathway in the CyA–isoprenaline interaction.

## 2. Materials and methods

Male Wistar rats (230–280 g; High Institute of Public Health, Alexandria, Egypt) were used in the present study.

### 2.1. Rat isolated aortic ring preparations

Isolation of the rat aorta and recording of isometric contraction were performed as described in our previous studies [25–27]. Rats were killed by decapitation and thoracic aortas were removed, trimmed free of connective tissues and cut into ring segments 3 mm in length. In experiments involving endothelium-denuded rings, the endothelium was removed mechanically by gentle rubbing of the intimal surface of the aorta with a fine forceps. The forceps was inserted into the lumen of the ring and rolled back and forth on a filter paper moistened with the physiological solution [26,28]. Proper removal of the endothelium was tested by the absence of vasorelaxant responses to acetylcholine in rings precontracted with phenylephrine [26,28]. Aortic rings with and without endothelium were mounted in 10 ml organ baths containing physiological solution at 37 °C and aerated with 95% $O_2$ and 5% $CO_2$. The physiological solution was composed of the following (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $KH_2PO_4$ 1.2, $NaHCO_3$ 25, and glucose 11.1. Aortic rings were mounted were mounted in the organ baths by means of two stainless steel wire hooks inserted through the lumen of the ring. One of the hooks was anchored to a stationary pin at the bottom of the organ bath and the other was connected to an isometric force–displacement transducer (Grass FT-03C), which was connected to a Grass polygraph (Model 7D) for recording isometric contractions of the aorta. An optimum resting tension of 1 g was placed on the tissue and an equilibration period of 2 h was allowed before the start of the experiment, with the bath fluid being replaced every 20 min.

### 2.2. Protocols and experimental groups

After the 2 h equilibration period, cumulative concentration–response curves to stepwise cumulative addition of isoprenaline ($1 \times 10^{-9}$ to $1 \times 10^{-7}$ M) were established in phenylephrine (10 μM)-precontracted tissues as described in our previous studies [12,28,29]. Each new addition of isoprenaline was made after the response to the previous addition had attained a steady state. As detailed below, two dose–response curves of isoprenaline were established in each preparation before and after exposure to a particular drug treatment. A wash period of 60 min was allowed after the first curve to help the muscle relax to baseline tension. The aorta was then re-contracted with phenylephrine (10 μM) for the establishment of the second isoprenaline dose–response curve.

#### 2.2.1. Role of endothelium- and smooth muscle-dependent mechanisms in isoprenaline relaxations

This experiment evaluated the vasorelaxant effect of isoprenaline in aortic rings with or without endothelium. The roles of the endothelium-derived relaxing factors and β-adrenergic receptors in the isoprenaline response were also investigated. Isoprenaline dose–response curves were established in rings with intact endothelium before and 60 min after treatment with L-NAME 100 μM, tetraethylammonium 3 mM, diclophenac 7 μM, methylene blue 0.1 μM, or propranolol 0.3 μM. Isoprenaline relaxations were also determined in endothelium-denuded rings in the absence and presence of L-NAME, methylene blue, or propranolol. In preliminary

control experiments, two consecutive dose–response curves of isoprenaline were constructed in four aortic ring preparations and exhibited comparable vasorelaxant responses, thus eliminating time as a factor that may alter isoprenaline responses.

#### 2.2.2. *Effect of CyA on isoprenaline vasorelaxations*

This experiment evaluated the effect of acute exposure to cyclosporine (2 μM) on isoprenaline vasorelaxations in aortas with intact or denuded endothelium. The effect of CyA on isoprenaline relaxations was determined in the absence and presence of propranolol (0.3 μM), L-NAME (100 μM), or methylene blue (0.1 μM). The percentage reductions in the response to the highest isoprenaline concentration and slopes of isoprenaline curves evoked by CyA in the absence and presence of each of the abovementioned inhibitors were computed. The slopes were determined by regression analyses of the individual dose–response curves of isoprenaline as in our previous studies [12,30–32]. The effect of L-arginine (100 μM) supplementation on CyA-induced attenuation of isoprenaline vasorelaxations was also investigated.

#### 2.2.3. *Effect of CyA on sodium nitroprusside vasorelaxations*

This experiment determined the influence of CyA on aortic vasorelaxations evoked by sodium nitroprusside ($1 \times 10^{-9}$ to $1 \times 10^{-5}$ M), a nitrovasodilator that acts by generating nitric oxide and stimulating smooth muscle guanylate cyclase resulting in the accumulation of cGMP and subsequent vasodilatation [33]. The CyA–sodium nitroprusside interaction was evaluated in the absence and presence of methylene blue (0.1 μM). To verify the specificity of the interaction of CyA with the NO/GC pathway, we assessed in a separate experiment ($n = 6$) the effect of CyA (2 μM) on the aortic vasorelaxant response to papaverine (10 μM).

### 2.3. *Drugs*

Phenylephrine hydrochloride, isoprenaline hydrochloride, $N^G$-nitro-L-arginine methyl ester, L-arginine, tetraethylammonium, sodium nitroprusside (Sigma Chemical Co., St. Louis, MO, USA), papaverine (Recordati, Milano, Italy), and diclophenac (Novartis AG, Basel, Switzerland) were purchased from commercial vendors. Cyclosporine was a gift from Novartis Pharma, AG (Basel, Switzerland). Cyclosporine was dissolved in a mixture of ethanol/distilled water (1:1) and a volume of 0.2 ml was mixed with the Krebs' solution as in our previous studies [7,11,12].

### 2.4. *Statistical analysis*

Values are expressed as mean ± S.E.M. Vasorelaxant responses to isoprenaline or sodium nitroprusside were calculated as the percentage decrease in the steady state contraction obtained with phenylephrine as in our previous studies [12,28,29]. The analysis of variance (ANOVA) followed by a Newman–Keuls post hoc analysis was used for multiple comparisons with the level of significance set at $P < 0.05$.

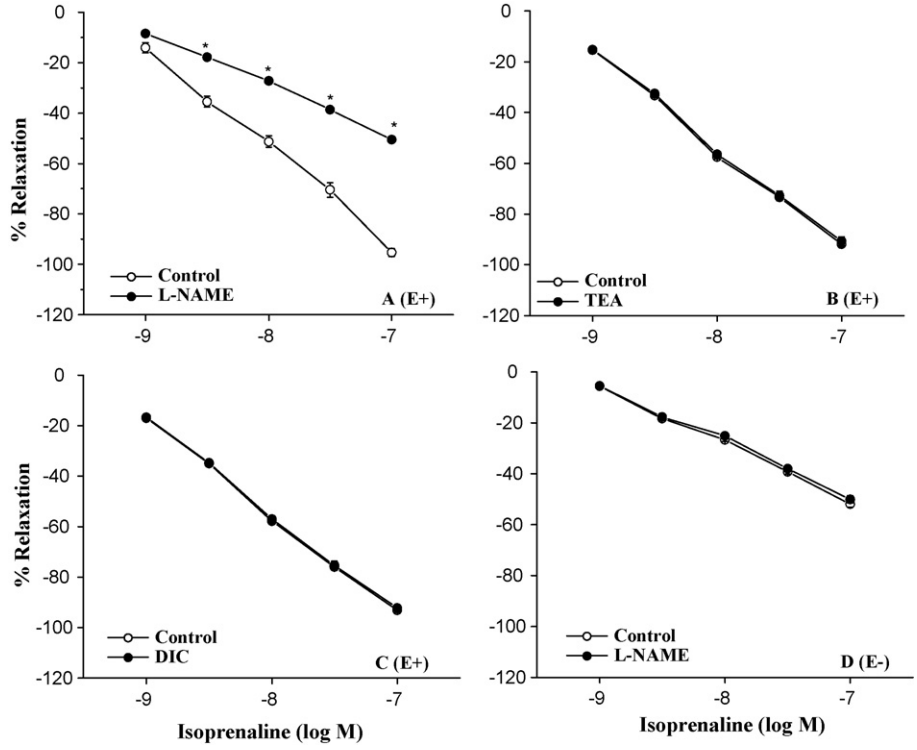


**Fig. 1 – Vasorelaxant effects of isoprenaline in phenylephrine (10 μM)-precontracted aortic rings in the absence (control) and presence of $N^G$-nitro-L-arginine methyl ester 100 μM (L-NAME, nitric oxide synthase inhibitor, panel A), tetraethylammonium 3 mM (TEA, $K^+$ channel blocker, panel B), or diclophenac 7 μM (DIC, cyclooxygenase inhibitor, panel C). Panel D shows the effect of L-NAME on isoprenaline responses in endothelium-denuded aortas (E−). Values are means ± S.E.M. of six to eight observations. *$P < 0.05$ compared with control values.**

## 3. Results

### 3.1. *Role of endothelium- and smooth muscle-dependent mechanisms in isoprenaline relaxations*

The effects of inhibition of nitric oxide synthase, $K^+$ channels, or cyclooxygenase produced by L-NAME, tetraethylammonium, and diclophenac, respectively, on the vasorelaxant responses to isoprenaline in the phenylephrine-precontracted rat isolated aortas with intact endothelium are shown in Fig. 1. Isoprenaline ($1 \times 10^{-9}$ to $1 \times 10^{-7}$ M) elicited dose-dependent vasorelaxations (Fig. 1). Exposure to L-NAME (100 μM, Fig. 1A), but not tetraethylammonium (3 mM, Fig. 1B) or diclophenac (7 μM, Fig. 1C), caused significant ($P < 0.05$) reductions in isoprenaline relaxations. In endothelium-denuded aortas, isoprenaline produced concentration-dependent relaxations (Fig. 1D); however, the responses were reduced by approximately 50% compared with those seen in aortas with intact endothelium. For example, the relaxant response produced by the highest concentration of isoprenaline ($1 \times 10^{-7}$ M) in aortas with intact or denuded endothelium amounted to 95.3 ± 1.5% and 51.9 ± 1.3%, respectively. In E− preparations, prior treatment with L-NAME had no effect on isoprenaline relaxations (Fig. 1D). The inhibition of guanylate cyclase activity by methylene blue (0.1 μM) significantly reduced isoprenaline relaxations in aortas with intact endothelium (Fig. 2A) in contrast to no effect in endothelium denuded preparations (Fig. 2B). Blockade of β-adrenergic receptors by propranolol (0.3 μM) caused significant reductions in isoprenaline relaxations in both E+ and E− aortas (Fig. 3).

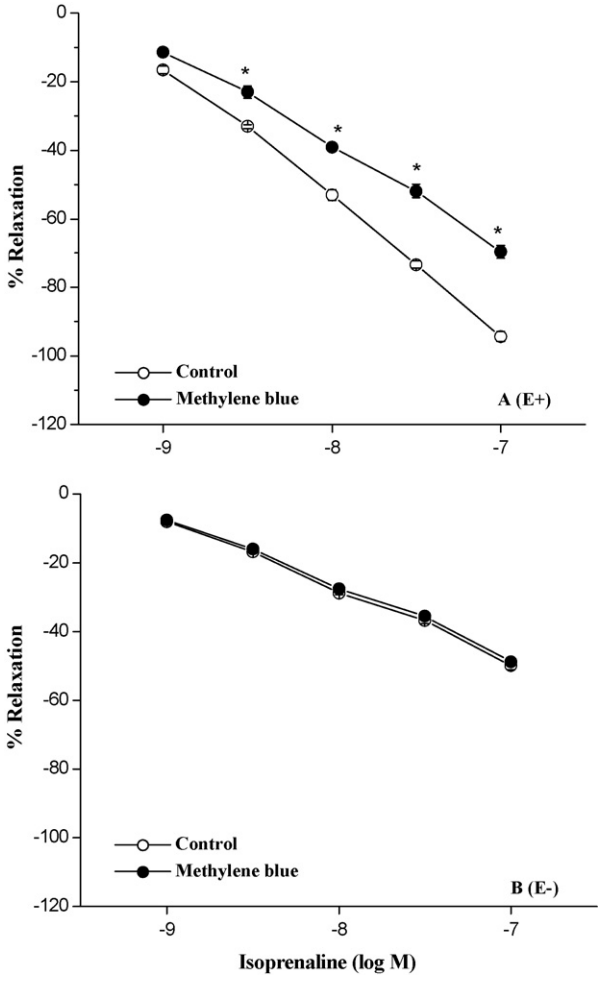


**Fig. 2 – Effect of inhibition of soluble guanylate cyclase (methylene blue, 0.1 μM) on isoprenaline vasorelaxations in phenylephrine (10 μM)-precontracted aortas with intact (E+, panel A) or denuded (E−, Panel B) endothelium. Values are means ± S.E.M. of six observations. *P < 0.05 compared with control values.**

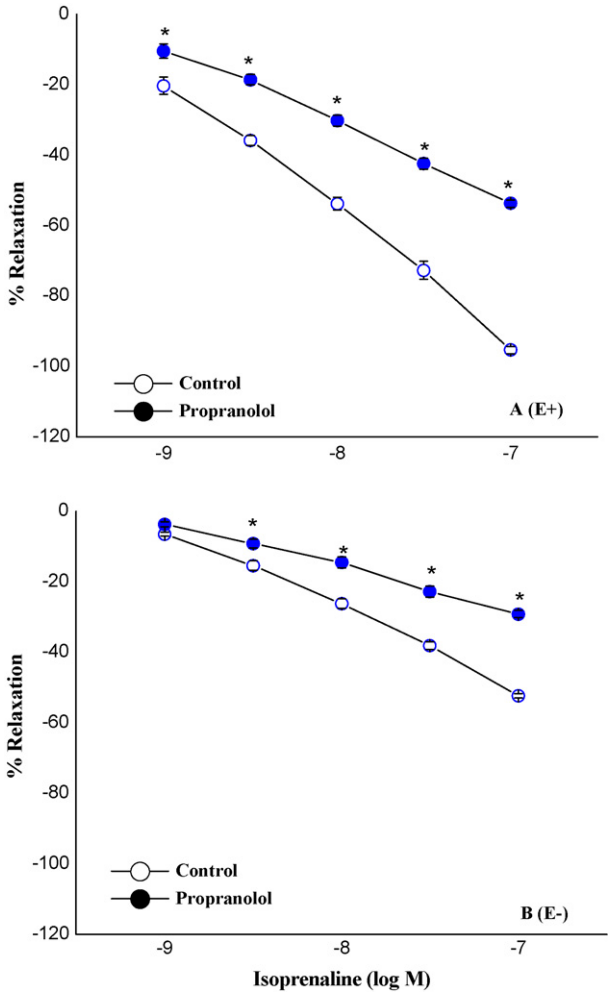


**Fig. 3 – Effect of blockade of β-adrenoceptors (propranolol, 0.3 μM) on isoprenaline vasorelaxations in phenylephrine (10 μM)-precontracted aortas with intact (E+, panel A) or denuded (E−, panel B) endothelium. Values are means ± S.E.M. of six to seven observations. *P < 0.05 compared with control values.**

### 3.2. *Effect of CyA on aortic vasorelaxant responses to isoprenaline and sodium nitroprusside*

Figs. 4–6 depict the influence of CyA on β-adrenergic-mediated vasorelaxations in aortas with intact or denuded endothelium. Compared with control (vehicle-treated) values, the infusion of CyA (2 μM) caused significant reductions in the relaxant

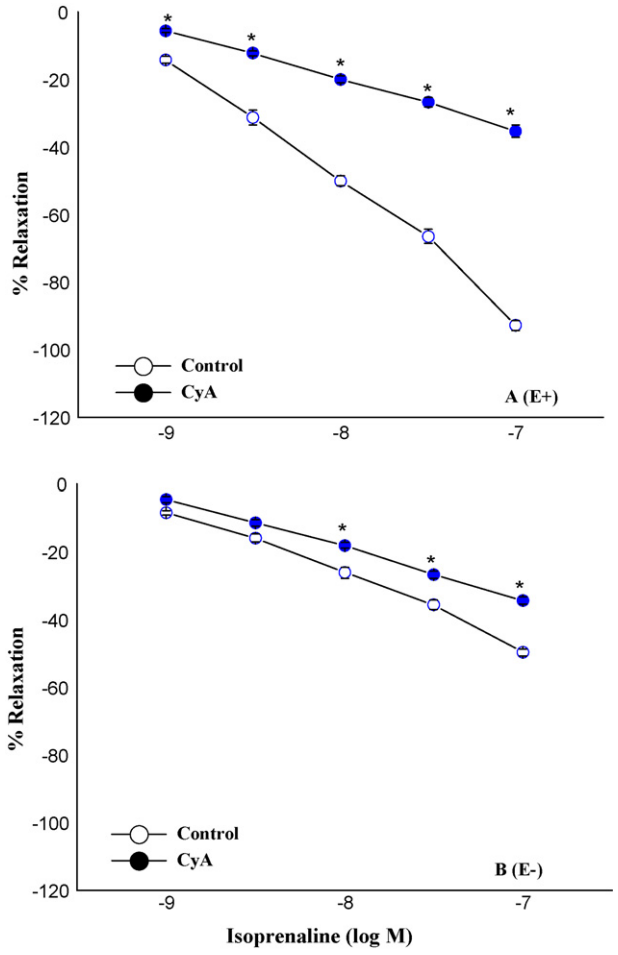


**Fig. 4 – Effect of cyclosporine (CyA, 2 μM) on isoprenaline vasorelaxations in phenylephrine (10 μM)-precontracted aortas with intact (E+, panel A) or denuded (E−, panel B) endothelium. Values are means ± S.E.M. of seven observations. *P < 0.05 compared with control values.**

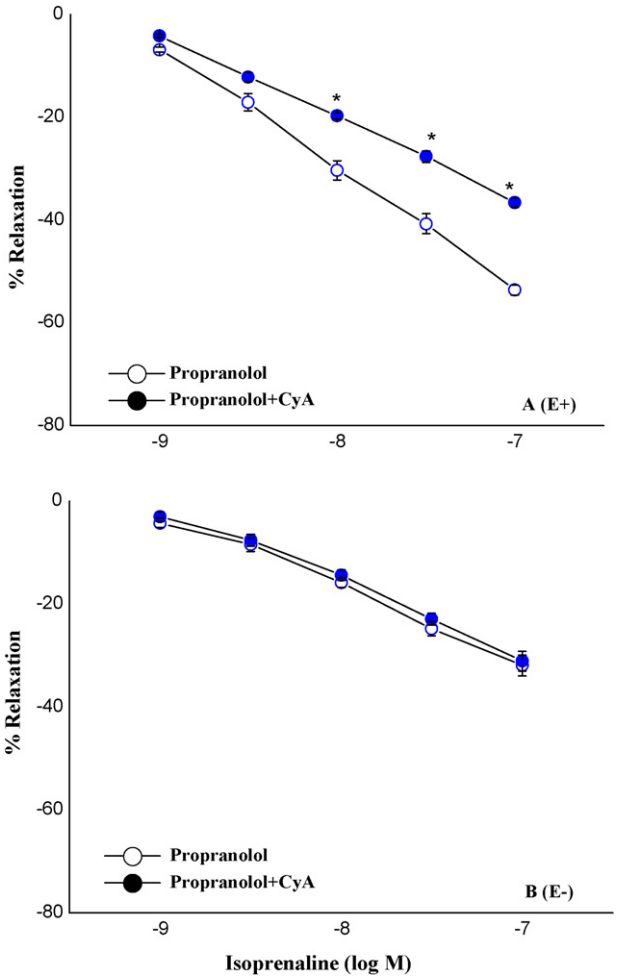


**Fig. 5 – Effect of blockade of β-adrenoceptors (propranolol, 0.3 μM) on cyclosporine (CyA, 2 μM)-induced attenuation of isoprenaline vasorelaxations in phenylephrine (10 μM)-precontracted aortas with intact (E+, panel A) or denuded (E−, panel B) endothelium. Values are means ± S.E.M. of six to seven observations. *P < 0.05 compared with corresponding propranolol values.**

effects of isoprenaline in E+ aortas (Fig. 4A). Relaxations produced by isoprenaline, especially at the high concentration range, were also reduced by CyA in E− aortas (Fig. 4B). In tissues treated with propranolol (0.3 μM) to block β-adrenergic receptors, the attenuating effect of CyA on isoprenaline relaxations in E+ aortas was still evident (Fig. 5A) but the attenuation was much less than the CyA effect seen in the absence of propranolol (Fig. 4A). For example, as shown in Table 1, the percentage reductions caused by CyA in the slope of the isoprenaline curve and in the response to the highest concentration of isoprenaline ($1 \times 10^{-7}$ M) were significantly

**Table 1 – The percentage reductions evoked by cyclosporine (CyA, 2 μM) in the relaxant responses to isoprenaline ($10^{-7}$ M) and in the slopes of the isoprenaline dose–response curves in the absence and presence of propranolol (0.3 μM), $N^G$-nitro-L-arginine methyl ester (L-NAME, 100 μM), or methylene blue (0.1 μM) in aortic rings with intact (E+) or denuded (E−) endothelium**

| Treatment | E+ aortas | | E− aortas | |
|---|---|---|---|---|
| | Isoprenaline ($10^{-7}$ M) | Slope | Isoprenaline ($10^{-7}$ M) | Slope |
| CyA | 62.3 ± 2.2 | 62.1 ± 2.6 | 30.9 ± 3.5 | 29.8 ± 2.4 |
| CyA (propranolol) | 32.0 ± 1.1* | 31.6 ± 1.5* | 2.8 ± 2.3* | 2.3 ± 1.1* |
| CyA (L-NAME) | 30.5 ± 3.1* | 30.1 ± 2.1* | – | – |
| CyA (methylene blue) | 44.7 ± 3.1* | 44.3 ± 3.2* | – | – |

Values are means ± S.E.M. *P < 0.05 compared with corresponding CyA values in the absence of the inhibitors.

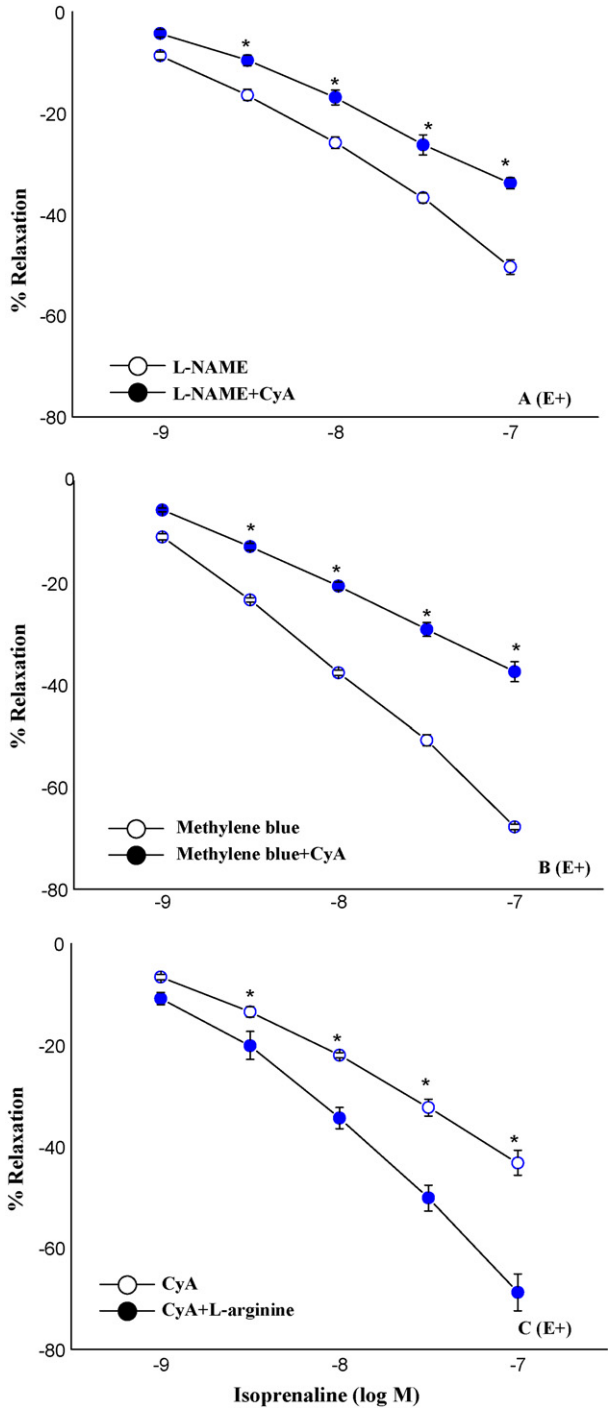


**Fig. 6 – Effect of prior treatment with L-NAME (100 μM, panel A) or methylene blue (0.1 μM, panel B), or L-arginine supplementation (100 μM, panel C) on cyclosporine (CyA, 2 μM)-induced attenuation of isoprenaline vasorelaxations in phenylephrine (10 μM)-precontracted aortas. Values are means ± S.E.M. of six to seven observations. *P < 0.05 compared with corresponding L-NAME (panel A), methylene blue (panel B) or CyA values (panel C).**

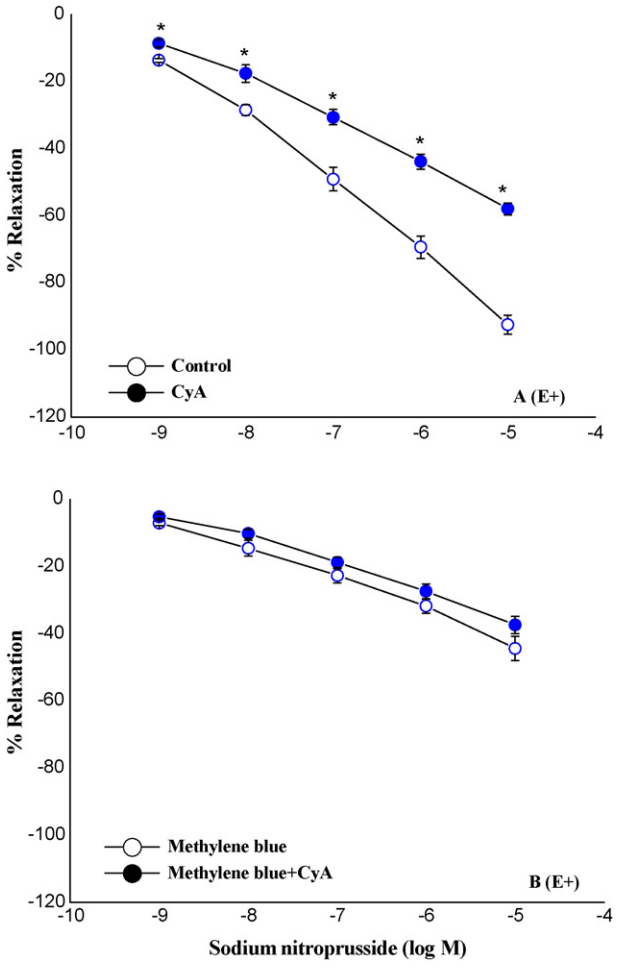


**Fig. 7 – Effect of cyclosporine (CyA, 2 μM) on vasorelaxant responses to sodium nitroprusside in phenylephrine (10 μM)-precontracted aortas in the absence (panel A) and presence of methylene blue (0.1 μM, panel B). Values are means ± S.E.M. of five to seven observations. *P < 0.05 compared with control (panel A) or methylene blue (panel B) values.**

reduced in the presence of propranolol. In contrast, the ability of CyA to attenuate isoprenaline relaxation was lost in propranolol-treated E− preparations (Fig. 5B, Table 1).

In aortas treated with L-NAME or methylene blue (Table 1, Fig. 6A and B), CyA decreased isoprenaline relaxations but its effect was significantly less compared with the effects of CyA when tested alone. For example, the percentage reduction by CyA of the response to $10^{-7}$ M isoprenaline in the presence of L-NAME was 62.3 ± 2.2% versus 30.5 ± 3.1% in L-NAME-untreated tissues. The reduction evoked by CyA in the slope of the isoprenaline curve was also less evident in the presence of L-NAME or methylene blue (Table 1), suggesting the involvement of nitric oxide-guanylate cyclase signaling in CyA–isoprenaline interaction. In CyA-pretreated tissues, the exposure to L-arginine (100 μM) elicited significant increases in isoprenaline relaxations (Fig. 6C). Similar to its effect on isoprenaline relaxations, the aortic vasorelaxant responses to sodium nitroprusside ($1 \times 10^{-9}$ to $1 \times 10^{-5}$ M) were significantly reduced by CyA (Fig. 7A) and this effect was abolished in

the presence of methylene blue (Fig. 7B). In contrast, the aortic vasorelaxant response to papaverine (10 μM) was not altered in the presence of CyA (42.1 ± 2.2% versus 38.9 ± 2.5%).

## 4. Discussion

Vasodilation induced via activation of β-adrenoceptors is believed to play an integral role in the regulation of vascular tone. Here we report on the vascular interaction of the immunosuppressant drug CyA with β-adrenoceptor-mediated relaxations and related cellular mechanisms in isolated rat aortas. The results showed that aortic isoprenaline relaxations involve both endothelium-dependent and independent mechanisms as suggested by interventions such as endothelium removal and pharmacological, inhibition of nitric oxide synthase and guanylate cyclase activity. CyA suppressed isoprenaline relaxations and its effects were attenuated (E+ aortas) and abolished (E− aortas), respectively, in the presence of propranolol, highlighting a role for vascular β-adrenoceptors in the interaction. The attenuation of the vasodilatory effect of the endotheliam-derived NO appears to be pivotal for the interaction since the suppressant effect of CyA on isoprenaline vasorelaxations was reversed by L-arginine supplementation and reduced after inhibition of NOS or guanylate cyclase. The involvement of guanylate cyclase in the vasculotoxic effect of CyA is also supported by the ability of CyA to elicit methylene blue-sensitive inhibition of vasorelaxant responses to the NO donor sodium nitroprusside.

Reported findings regarding the endothelium dependency of β-adrenoceptors-mediated vasorelaxations are not uniform [14,15,17–20]. The present demonstration that isoprenaline relaxations were evident in E+ and E− preparations is consistent with the view that both endothelium and smooth muscle components are engaged in the relaxant response to β-adrenoceptor activation [18,19]. Notably, the ability of isoprenaline to relax E− aortas cannot be interpreted, as indicated by others [22], to suggest incomplete removal of the endothelium. In effect, the possibility that incomplete removal of the endothelium accounts for the remaining response to isoprenaline in E− aortas seems unlikely because: (i) endothelium removal completely abolished the relaxant response to acetylcholine, (ii) L-NAME produced a similar inhibition of isoprenaline responses to endothelium removal, and (iii) treatment of endothelium-denuded preparations with L-NAME produced no further attenuation of the isoprenaline response. The observation that endothelium removal or L-NAME treatment produced similar attenuation of isoprenaline relaxations may also highlight that the endothelium-dependent fraction, which corresponded to about 50% of the isoprenaline response, is entirely due to the endothelium-derived NO. This conclusion receives more support from the observation that blockade of potassium channels or inhibition of cyclooxygenase activity by tetraethylammonium and diclophenac, respectively, were without effect on isoprenaline responses, thus arguing against any role for other endothelial-derived factors such as the hyperpolarizing factor or vasodilator prostanoids in isoprenaline responses.

One important objective of the present study was to study the interaction of CyA with β-adrenoceptor-mediated vasorelaxations and to establish pharmacological evidence of the underlying signaling mechanisms. The results showed that, regardless of the endothelium status (i.e. intact or denuded), acute exposure to CyA caused significant reductions in the aortic vasorelaxant responses to isoprenaline. Two important features of the CyA-isoprenaline are presented here. First, the suppressant effect of CyA on isoprenaline vasorelaxations appears to be mediated via β-adrenoceptors because it was remarkably attenuated in tissues pretreated with propranolol. Second, the interruption of the NO-GC pathway is critical to the detrimental effect of CyA on the reactivity of vascular β-adrenoceptors. This latter conclusion is supported by the observation that the inhibition of NOS or guanylate cyclase activity by L-NAME or methylene blue, respectively, attenuated the suppressant effect of CyA on the aortic responsiveness to isoprenaline. As shown in Table 1, the percentage reductions in the relaxant response to the highest of isoprenaline and slopes of the dose–vasorelxant response curves evoked by CyA were reduced in the presence of L-NAME or methylene blue. Further, the ability of L-arginine to enhance isoprenaline relaxations in CyA-treated tissues and to restore isoprenaline responses to near-control values provides more support to the fundamental role of NO deficiency in the CyA–isoprenaline interaction.

To further characterize the role of GC in the vascular effect of CyA and to determine whether the CyA–NO interaction is due to a primary effect of CyA on the endothelial NO pathway or is secondary to the inhibition by CyA of the NO-dependent GC in vascular smooth muscle, we tested the effect of CyA on sodium nitroprusside-induced aortic relaxations in the absence and presence of the guanylate cyclase inhibitor methylene blue. Sodium nitroprusside is a nitrovasodilator that acts by generating nitric oxide, which activates the soluble fraction of smooth muscle guanylate cyclase and elevates tissue cGMP content [33]. The latter activates cGMP-dependent protein kinase, thus decreasing phosphorylation of myosin light chain and initiating muscle relaxation [33]. The results showed that nitroprusside vasorelaxations were clearly blunted by CyA in isolated aortas and this effect was completely abolished in tissues pretreated with methylene blue, thereby implicating GC in the vasculotoxic effect of CyA. Pertinent to this finding are the obervations that CyA diminishes the elevated cGMP level that follows sodium nitroprusside treatment [8] and the inhibition of GC by methylene blue protects against CyA-induced vascular toxicity [34]. The possibility, however, that CyA disrupts the generation of NO of endothelial origin cannot be ruled out because: (i) reduced nitric oxide plasma levels associate cyclosporine therapy [4], (ii) endothelial dysfunction and suppression of eNOS expression are established effects for CyA [7,12,13,35,36], and (iii) supplementation with L-arginine, the nitric oxide precursor, prevents and/or reverses cyclosporine-induced endothelial dysfunction in this study and others [9]. It is tempting, therefore, to conclude that the deleterious effect of CyA on vascular endothelium-related responses may involve not only direct interaction with endothelial cells but also interruption of the post-endothelium smooth muscle GC pathway. Notably, the present

observation that CyA failed to modify the aortic vasorelaxant action of papaverine conceivably rules out the possibility of a non-specific interaction of CyA with aortic vascular reactivity.

The current study presented interesting findings regarding the role of vascular β-adrenoceptors in isoprenaline relaxations on the one hand, and in the CyA–isoprenaline interaction on the other. Remarkably, propranolol caused significant but only partial attenuation of isoprenaline vasorelaxations in aortas with intact or denuded endothelium. It cannot be argued that the concentration of propranolol (0.3 μM) employed in the present study might have not been adequate for β-adrenoceptor blockade because a similar concentration of propranolol [19] or even less [37] has been used for effective blockade of β-adrenoceptors [19,37]. Alternatively, the failure of propranolol to completely eliminate isoprenaline responses might be likely attributed to the presence of atypical propranolol-insensitive β-adrenoceptors in vascular tissues. Indeed, both classical ($\beta_1/\beta_2$) and atypical ($\beta_3/\beta_4$) adrenoceptors have been identified in the rat aorta and implicated in the modulation (lowering) of vascular tone [19,34]. Similar findings of a propranolol-resistant component in the response to isoprenaline have been reported in other vascular preparations such as the rat common carotid [37] and mesenteric [38] arteries. With this idea in mind, it is possible also that the endothelium status (intact or denuded) determines the contribution of the two populations of β-adrenoceptors (classical and atypical) to isoprenaline relaxations and, therefore, its interaction with CyA. The ability of propranolol to fully negate CyA-induced attenuation of isoprenaline responses in E− but not in E+ aortas (see Fig. 5) may infer the confinement of atypical β-adrenoceptor to endothelial, rather than smooth muscle, sites where it modulates the endothelium-dependent, propranolol-resistant component of the CyA–isoprenaline interaction. More studies are needed, however, to validate this assumption.

In summary, mechanical (endothelium removal) and pharmacological (β-adrenoceptor blockade and NOS and GC inhibition) procedures employed in the present investigation implicated endothelium-dependent and -independent machinery in the vasorelaxant effect of isoprenaline and its amelioration by CyA. The endothelium component of the CyA–isoprenaline interaction probably involves the NO-sensitive pathway and related smooth muscle GC. The endothelium-independent component of the response, which is dependent on adenylyl cyclase and cAMP accumulation in smooth muscle [14], is also pivotal for the adverse effect of CyA on β-adrenoceptor-mediated vasorelaxations. Notably, the present study describes some important insights of the vasculotoxic effect of CyA, which may help establish a potential therapeutic strategy against the toxicity caused by CyA and related immunosuppressants.

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