THE ROLE OF ENDOTHELION AND ENDOTHELION-DERIVED RELAXATION FACTORS IN NITRIC OXIDE-INDUCED AORTIC RELAXATION

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ABSTRACT

The endothelium plays a key role in the control of vascular patency and tone. Thus, the main objective of the study was to determine the role of endothelium and its derived relaxation factors in mediating relaxation of rat thoracic aorta, in response to nitric oxide (NO) donor “sodium nitroprusside (SNP)” using PowerLab tissue bath system. Endothelial denudation enhanced relaxation responses of SNP(1X10^5 to 3X10^5 M) with IC_{90} of 5.872X10^{-8} as compared to control rings with IC_{90}’s of 2.266X10^{-8}M, as well as the maximum relaxation (E_{max}) for both groups were 106.2±4.95% and 83.13±14.755%, respectively. The relaxation responses to SNP in aortic rings were significantly increased by Indomethacin and Clotrimazole pretreatment with IC_{90}’s of 1.72X10^{-7} M and 3.314 X10^{-8}M, and E_{max} were increased to 121.5±5% and 121.1±8.09%, respectively. Whilst, no changes were observed in aortic rings pretreated with L-nitroargininemethylester (L-NAME) and methylene blue. The results of the current study had shown that endothelium denudation and blocking of endothelium derived-relaxation factors enhanced vasodilator effect of NO; this may be account for the role of endothelium in the vasodilatory effects of NO.

Keywords: Nitric Oxide, Endothelium denudation, Endothelium derived-relaxation factors, Organ bath, PowerLab system, Aorta.

INTRODUCTION

The vascular endothelium is strategically located at the interface between the circulating blood and vessel wall, exists in vivo as a monolayer of cells coupled to one another by gap and tight junctions, providing a permeability barrier to the movement of cell metabolites and nutrients, while allowing the transfer of electrical signals within the intact tissue (Adams and Hill, 2004). The endothelium performs not only a barrier function but also serves as a biomechanical sensor that maintains vascular integrity. Blood flow through a vessel creates fluid shear stress from the friction of blood against the vessel wall. This force, which acts in parallel to the vessel surface, activates the endothelium (Ying and Sanders, 2002).

Maintenance of vascular tone and blood flow by the endothelium is complex, involving many substances and an interplay among numerous cellular mechanisms (Panza, 1997). Endothelial cells synthesize and release various factors that modulate in short terms vascular tone, in long terms atherosclerosis, angiogenesis and inflammation. The vasoactive factors include relaxing substances, prostaglandin I\_2 (PGI\_2), NO, endothelium-derived hyperpolarizing factors (EDHF), C-natriuretic peptide, and contracting substances, such as, thromboxane A\_2 (TXA\_2), endothelin-1, angiotensin II, superoxide anion (Zhang, 2007).

Nitric oxide is a signaling mediator with diverse as well as opposing biological activities. The complexity of the NO response reflects the variety of its chemical reactions and biological properties. Steady-state NO concentration is a key determinant of its biological outcome as precise cellular responses are differentially regulated by specific NO concentrations (Ignarro, 2010). At high concentrations, NO readily reacts with oxygen, especially with superoxide, forming highly reactive, cytotoxic substances, such as peroxynitrite. At lower concentrations, NO serves regulatory roles via activation of soluble guanylate cyclase (sGC), resulting in increased cGMP levels in target cells. In vascular smooth muscle cells, cGMP causes relaxation by reducing intracellular calcium concentration (Hampi and Herget, 2000), activation of cGMP-dependent protein kinase, and opening of potassium channels (Shelkovnikov et al.,2004) and hyperpolarizes membrane potential in many vessels, including rat and rabbit aorta (Si et al.,2002).

Nitric oxide-induced endothelium-dependent relaxation can be pharmacologically inhibited by...
analouges of L-arginine such as L-N-G- monomethyl arginine (L-NMMA) or L-NAME, which compete with the natural precursor L- arginine at the catalytic site of the enzyme (Luscher and Barton, 1997). Here we further examined the endothelium-dependent and independent aortic relaxant action of SNP.

MATERIALS AND METHODS

Adult male Wistar rats (Rattus norvegicus) were used for this study. The animals were kept under standard laboratory conditions. After anaesthetized, the chest cavity was opened, after removal of excess tissue and fat, thoracic aorta was isolated and transferred to beaker containing Krebs solution (composition in mM: NaCl 136.9, KCl 5.4, Glucose 5.5, NaHCO3 23.8, MgCl2 1.0, CaCl2 1.5, and EDTA.Na2 0.003), equilibrated with 95% oxygen and 5% CO2. The beaker was placed in the water bath at 37°C.

The procedure of Aziz and coworkers (2009) with some modifications was followed to study the vascular reactivity in the isolated aorta. Two stainless steel wires were carefully inserted into lumen of the aortic rings. One wire was anchored to the hook at the base of an organ bath (Model 166051, Radnoti, Monrovia Ca, USA) and other wire was connected to force transducer (MLT0201/RAD 5 mg-25 gm, AD instruments, Sydney, Australia) coupled to the transbridge amplifier (ML 224, Quad Bridge Amp, ADinstruments). Data was acquired with a PowerLab Data Acquisition System (ML 870, Power Lab, ADinstruments) using the chart software (Version 7) for measurement of isometric tension. The degree of contraction and relaxation were indicated by the tension development in the recording system and expressed in gram.

Rings were allowed to equilibrate for 60 minutes at a resting tension of 2 grams with changes of buffer every 15 min. When the isometric tension had stabilized, inhibitory concentration-response curves of the NO donor (SNP 1X10^-8 to 3X10^-5 M) were constructed against contractions induced with phenylephrine (1X10^-6 M; PE).

To detect the role of endothelial cells in the relaxant effect of SNP, sandwich preparations were made similar to those described by Dong et al.,(1997), in which the endothelial layer in a long segment of the aorta was removed by gently rubbing the intimal surface of the rings with a syringe needle covered by a piece of cotton. The endothelium-denuded segment was then cut into several pieces; each piece was tested to confirm the removal of the endothelium by the lack of any response to ACh(1X10^-5 M) following the pre-constriction with PE(1X10^-6 M).

The role of endothelium/NO, cGMP, PGI2 and epoxeicosatrienoic acid in association with vasorelaxation induced by SNP were evaluated following incubation of endothelium-intact rings with, L-NAME(3X10^-4 M),methylene blue (3mM), Indomethacin (3X10^-5 M) or Clotrimazole(3X10^-5 M), blockers of above mentioned factors respectively, for 10 minutes prior to application of PE.

The concentration-response curves were fitted with a Hill equation, from which the half maximal inhibitory concentration (IC50) values were given as geometric mean with 95% confidence intervals (95% CI). Maximum contractile responses to SNP were calculated as an Emax produced by PE and were expressed as the means ± standard error of the mean (SEM). The tension produced by PE was defined as 0% relaxation, and the baseline tension before addition of vasoconstrictors were defined as 100% relaxation.

STATISTICAL ANALYSIS

The statistical analysis was performed using two-way analysis of variance (ANOVA) supported by Bonferroni test when carrying out pair wise comparison between the same doses of different groups (Motulsky and Christopolous, 2003). P-value less than 0.05 (P<0.05) were considered as statistically significant. All the graph, calculation and statistical analyses were performed using GraphPad Prism software version 5.0 for Windows (GraphPad Software, San Diego California USA).

RESULTS

From the results we see that cumulative addition of SNP, at the plateau phase of the contraction induced by PE (1X10^-6 M) in rats thoracic aortic rings caused contraction-dependent inhibition of the PE-induced contraction, in both, endothelium-intact and endothelium-denuded preparation (Figure 1).
The IC_{50} (IC_{50} of CI 95%) and \( E_{\text{max}} \) are shown in Table (1). Endothelium denudation significantly (P<0.001) induced relaxation at doses \((3 \times 10^{-7} - 1 \times 10^{-5})\) and (P<0.05) at dose \((3 \times 10^{-5})\) for SNP with IC_{50} 5.872\( \times 10^{-8} \) M (with IC_{50} of CI 95% 4.149\( \times 10^{-9} \) to 8.309\( \times 10^{-7} \)), and 2.266\( \times 10^{-6} \) (with IC_{50} of CI 95% 1.437\( \times 10^{-6} \) to 3.572\( \times 10^{-6} \)) in the denuded and intact endothelial rings, respectively. The \( E_{\text{max}} \) for endothelium denuded and intact rings were 106.2±4.95% and 86.68±2.132%, respectively.

The IC_{50}, (IC_{50} of CI 95%) and \( E_{\text{max}} \) for the relaxant response to SNP following sustained contraction were significantly (P<0.001) higher in rings preincubated with Indomethacin and Clotrimazole than control rings (Figures ), with IC_{50}1.72\( \times 10^{-7} \) M (IC_{50} of CI 95% between 6.995 \( \times 10^{-8} \) to 4.231 \( \times 10^{-7} \)) and 3.314 \( \times 10^{-8} \)M (7.912 \( \times 10^{-11} \) to 1.388 \( \times 10^{-8} \)), and \( E_{\text{max}} \) were 121.5±5% and 121.1±8.09%, respectively. However, L-NNAME and methylene blue pretreatments did not alter the dilation induced by SNP (Table 1).
DISCUSSION

It is well known that the endothelium plays an important role in the regulation of vascular tone by synthesis and release of endothelium-derived relaxing factors, including NO, PGI₂ and EDHF. Thus, it was decided to investigate role of endothelium and EDRFs involved in SNP-induced responses.

In the present study, endothelium removal enhanced the relaxant response to SNP, which may be attributed to the inhibitory effects of the factors released from endothelium. However, this endothelial factor not basal NO, because of L-NAME showed no influence on the relaxant effect of SNP in aortic rings. Although, Macdonald and his group (1989), concluded that endothelium releases ET-1 which abolishes the action of SNP, therefore by removing of endothelium SNP can further relax arteries.

Similar results were obtained to endothelial denudation in aortic rings pretreated with indomethacin or clotrimazole. These observations indicate that the releasing of PGI₂ and products of CYP₄₅₀ from intact endothelium blocks the action of SNP. However, Lugo et al., (1998) explained that prostaglandins may express an inhibitory action on NOS, and Khatsenko et al., (1993) showed that overproduction of NO is involved in the suppression of CYP₄₅₀ activity by lipopolysaccharide. From these results, we can conclude that vasodilatory response of SNP will increase when aortic rings pretreated with either Indomethacin or Clotrimazole.

The results of the present study demonstrated that inhibition of basal NO and sGC had no effects on SNP-induced relaxation, indicating that endothelial derived NO and sGC do not appear to modulate this vasodilator response. These results along with previously described results explain that SNP directly activate different K⁺ channels.

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<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>Endothelium Denudation</th>
<th>L-NAME (3X10⁻⁴ M)</th>
<th>Indomethacin (3X10⁻⁵ M)</th>
<th>Clotrimazole (3X10⁻⁵ M)</th>
<th>Methylene blue (3mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₅₀</td>
<td>2.266X10⁻⁶</td>
<td>5.872X10⁻⁸</td>
<td>7.195X10⁻⁷</td>
<td>1.72X10⁻⁷</td>
<td>3.31X10⁻⁴</td>
<td>1.33X10⁻⁶</td>
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<tr>
<td>95% CI</td>
<td>1.437X10⁻⁶ to 4.149X10⁻⁶</td>
<td>4.878X10⁻⁷ to 1.061X10⁻⁶</td>
<td>6.995X10⁻⁸ to 4.231X10⁻⁷</td>
<td>7.912X10⁻¹ to 1.388X10⁻⁵</td>
<td>9.326X10⁻⁷ to 1.895X10⁻⁶</td>
<td></td>
</tr>
<tr>
<td>IC₅₀</td>
<td>3.572X10⁻⁶</td>
<td>8.309X10⁻⁷</td>
<td>1.061X10⁻⁶</td>
<td>4.231X10⁻⁷</td>
<td>1.388X10⁻⁵</td>
<td>1.895X10⁻⁶</td>
</tr>
<tr>
<td>E_max± SEM</td>
<td>86.68 ± 2.132</td>
<td>106.2± 4.295</td>
<td>93.69 ± 0.762</td>
<td>121.5 ± 5</td>
<td>121.1 ± 8.09</td>
<td>97.00± 3.79</td>
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NO

The objective of this experiment was to investigate the role of the endothelium and EDRFs in the relaxation response to SNP using the PowerLab device. The removal of the endothelium led to a significant increase in the response to SNP at 5.872×10^-8 mol and 2.266×10^-6 mol. The relaxation percentage was 106.2±4.95% and 86.68±2.132% compared to control, respectively. The relaxation of the artery was significantly reduced by the use of NO. In conclusion, the endothelium and EDRFs play a crucial role in the relaxation response to SNP.
المستحدث ب SNP في الحلقات الأبهر تعززت معنويًا بواسطة Indomethacin و Clotrimazole عند الترکیز الفعال7.72X10⁻⁷ مول و 3.314 X10⁻⁸ مول و ان نسبة الأسترخاء ازدادت الى 121.5±5% و 121.1±8.09% على التوالي. في حين، لم يحدث أي تغيير في الأسترخاء المستحدث ب SNP في الحلقات الأبهر بواسطة المعالمة المسبقة بـ L-NAME و مثيلين الازرق. استنتجت النتائج بأن ان تجريد الأندوثيليم و تعطیل EDRFs أدت الى تحفز استجابة الأسترخاء الأبهر ل NO و يوضح دور الأندوثيليم في استجابة الأسترخاء الأبهر ل NO.