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Vasorelaxant response to isoprenaline, nitric oxide donor, calcitonin gene-related peptide and vasoactive intestinal peptide in aortic rings of adult C57BL/6J mice

Siu L. Chan, Ronald R. Fiscus *

Department of Physiology, Faculty of Medicine, Epithelial Cell Biology Research Centre and Centre for Gerontology and Geriatrics, The Chinese University of Hong Kong, BMSB Room 507, Shatin, New Territories, Hong Kong, China

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Abstract

The mouse and tissues from this species are increasingly used as experimental models because of the wide variety of gene deletions and overexpressions available in this species. Yet, very little is known about normal vascular responses in the mouse. We investigated the vasorelaxant responses on thoracic aortic rings from the adult male C57BL/6J mouse. Isoprenaline, acetylcholine, calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP) and sodium nitroprusside all caused concentration-dependent relaxations in aortic rings possessing healthy endothelium and precontracted with phenylephrine. Maximum relaxations were $64.9 \pm 2.6\%$, $66.8 \pm 2.9\%$, $114.3 \pm 4.6\%$, $65.1 \pm 4.2\%$ and $116.2 \pm 5.1\%$ with $-\log EC_{50}$ values of 6.76 ± 0.14, 7.04 ± 0.11, 8.53 ± 0.14, 8.29 ± 0.26 and 8.10 ± 0.20 for isoprenaline, acetylcholine, CGRP, VIP and sodium nitroprusside, respectively. There were significantly smaller responses to isoprenaline, acetylcholine, CGRP and VIP when the endothelium was denuded. The maximum relaxations for isoprenaline, CGRP and acetylcholine were $48.3 \pm 5.1\%$, $99.6 \pm 4.4\%$ and $5.7 \pm 1.6\%$ with $-\log EC_{50}$ values of 6.44 ± 0.40 and 8.23 ± 0.192, respectively, following endothelium removal. The response to VIP was completely dependent to endothelium. Without precontraction, isoprenaline, at the higher doses, caused small contractions. These experiments provide new information about vascular responses of five vasodilators in aortic rings of adult male C57BL/6J mice. © 2001 Published by Elsevier Science B.V.

Keywords: Vasorelaxation; Isoproterenol; Nitric oxide (NO); (CGRP) (calcitonin gene-related peptide); (VIP) (vasoactive intestinal peptide); Acetylcholine

1. Introduction

The mouse and tissues from this species are increasingly used as experimental models because of the availability of wide variety of gene deletions and overexpressions in this species. Yet, little is known about the normal vascular responses in the mouse. Vasodilation responses of isoprenaline, calcitonin-gene related peptide (CGRP), vasoactive intestinal peptide (VIP), sodium nitroprusside and acetylcholine have been investigated in various species and tissues. Different species and tissues showed different responses to these vasodilators and the endothelium dependency of these responses also differed in different species and tissues (Fiscus, 1988).

Isoprenaline generally causes vasodilations in humans (Pan et al., 1986) and other species (Deisher et al., 1989). In isolated rat aorta (Gray and Marshall, 1992; Toyoshima et al., 1998) and porcine coronary artery (Rubanyi and Vanhoutte, 1985), vasorelaxant responses to isoprenaline were partly dependent on endothelium. However, the relaxant responses to isoprenaline were reported to be endothelium independent in canine coronary artery (Krauss et al., 1992; Bea et al., 1994) and rabbit aorta (Furchgott and Zawadzki, 1980). Recently, Russell and Watts (2000) reported that isoprenaline did not cause vasorelaxations, but rather contractions, in mouse aortic rings precontracted with $PGF_{2\alpha}$ of adolescent (5–6 weeks, 16–18 g) mice (Russell and Watts, 2000). Thus, the mouse appears to be unusual among the different species in terms of its response to isoprenaline. One possible difference could be that very young mice were used, rather the adult animals, as in most other studies. In the present study, we investigated whether isoprenaline causes contractions or relaxations in aortic rings of the adult (12–20 weeks, 30–35 g) male C57BL/6J mouse. We also test the endothelium dependency of the isoprenaline-induced responses.

* Corresponding author. Tel.: +852-2609-6780; fax: +852-2603-5022.
E-mail address: ronfiscus@cuhk.edu.hk (R.R. Fiscus).

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CGRP has potent vasorelaxant effects in many blood vessels. In human cerebral, coronary, gastric and radial arteries (Thom et al., 1987) and rat aorta (Brain et al., 1985; Grace et al., 1987), the vasorelaxations induced by CGRP were dependent on endothelium. In rat aortic rings, CGRP-induced vasorelaxations are mediated by cAMP and cGMP responses that depend on nitric oxide (NO) release from the endothelium (Fiscus, 1988; Fiscus et al., 1991; Wang et al., 1991). In contrast to rat aortic relaxations, the vasorelaxations caused by CGRP in pig coronary arteries (Franco-Cereceda et al., 1987), bovine coronary artery (Greenberg et al., 1987a), feline cerebral artery (Edvinsson et al., 1985) and rat caudal artery (Fiscus et al., 1992) are independent of endothelium. Unexpectedly, CGRP-induced vasorelaxations in CD1 mouse aorta were only partly dependent on endothelium (Pomerleau et al., 1997). Whether or not this is true for all strains of mice is currently unknown.

VIP, a 28-amino acid peptide, induces vasorelaxations in various species and tissues. In bovine coronary artery (Greenberg et al., 1987a), bovine and human pulmonary arteries (Greenberg et al., 1987b) and canine coronary artery and saphenous veins (Nakashima et al., 1996), VIP effects were not endothelium dependent. However, endothelial cells were required for VIP to relax rat aorta (Davies and Williams, 1984) and human gastric and transverse cervical arteries (Thom et al., 1987). In aortic rings of the CD1 mouse, VIP-induced vasorelaxations have been reported to be completely dependent on endothelium. As with CGRP, vasorelaxant responses to VIP have not been reported in aortic rings from other strains of mice.

The C57BL/6J mouse is commonly used as a control in experiments using genetically modified mice. Therefore, we have determined the actions of isoprenaline, CGRP and VIP as well as two other agents, acetylcholine and sodium nitroprusside, in aortic rings of C57BL/6J mice. The dependency of these responses on endothelium was also determined.

2. Materials and methods

2.1. Animals and preparations of isolated aortic rings

The protocols for animal use comply with European Community guidelines and were approved by the Animal Research Ethics Committee of The Chinese University of Hong Kong. Male C57/BL6J mice (30–35 g, 12–20 weeks) were an inbred strain imported from Animal Resource Centre of Australia, originating from Jackson Laboratory of the USA. The mice were injected intraperitoneally with heparin (800 USP in 0.2 ml saline of 140 mM). After 15 min, mice were euthanized by 95% CO_2 and decapitated. The thoracic aortas were excised rapidly and placed in Krebs–Ringer–Bicarbonate (KRB) solution (aerated with 95% O_2/5% CO_2). The aortas were dissected free of surrounding fat and connective tissue and cut transversely into rings 3–4-mm wide and mounted on two triangular-shaped stirrups of tungsten wire (0.15 mm). Normally, two rings were taken from one mouse. Extreme care was taken not to damage the endothelium. In some experiments, the endothelial cell layer was removed by rubbing the luminal side of the vessel with an L-shaped stainless steel wire. The aortic rings were placed in 5-ml organ baths containing KRB solution bubbled with 95% O_2/5% CO_2, pH 7.4, at 37 °C. The aortic rings were subjected to an initial loading tension of 0.8 g and allowed to equilibrated for 45–60 min (with changes of the bathing fluid every 10 min) before the experiment began.

2.2. Recording of mechanical responses

Contractile and relaxant responses were measured isometrically using force displacement transducers (FT03; Grass Instruments, Quincy, MA, USA) and recorded using Powerlab system with Chart for Windows program [v3.4.3 (MLS013/W); ADInstruments, Australia].

2.2.1. Determination of optimal resting tension

Mouse aortic rings were allowed to equilibrate for 45 min with changes of bathing fluid every 10 min. They were then placed under different levels of preload (the testing passive tension). Complete dose–response curves of phenylephrine were conducted by adding the phenylephrine to the organ bath in a cumulative manner. The magnitude of force generated by phenylephrine (1×10^{-7} M), which gave 70% of maximum contraction, was used as the tension generated at that particular passive tension. This procedure was repeated multiple times, using passive tensions ranging from 0.45 to 1.15 g, in order to generate a length–tension curve for the determination of the optimal passive tension under which mouse aortas should by placed.

2.2.2. Determination of agonist-induced contractions of mouse aortic rings

Aortic rings were equilibrated for 45–60 min under optimal tension (0.8 g) obtained from the length–tension experiment. Then the aortic rings were contracted by phenylephrine (1×10^{-7} M) for one or two times and washed until the tension returned to the optimal passive tension (0.8 g). Complete dose–response curves of phenylephrine and isoprenaline were conducted by adding the agonist to the organ bath in a cumulative manner.

2.2.3. Determination of agonist-induced relaxations of mouse aortic rings

The equilibration and contraction by phenylephrine were conducted the same as described above. The aortic rings were then contracted with phenylephrine (1×10^{-7} M) and allowed to reach a stable plateau of contraction. In

order to test the presence of a healthy endothelium, all rings were first tested with acetylcholine (1×10^{-11}–1×10^{-5} M). Aortic rings showing relaxation to acetylcholine (1×10^{-5} M) of more than 50% were counted as endothelium-intact and those showing less than 10% were counted as endothelium-denuded. The relaxant agents, including isoprenaline acetylcholine, rCGRP and VIP and sodium nitroprusside, were added to the organ bath in cumulative manner. The percentage of relaxation was presented in the remaining contractile tension as a percentage of initial tension.

2.3. Solutions and chemicals

The ionic composition of the Krebs solution was as follows (mM): NaCl 118.5, KCl 1.74, $MgSO_4 \cdot 7H_2O$ 1.18, KH_2PO_4 1.18, $CaCl_2 \cdot 2H_2O$ 1.25, glucose 10, EDTA 0.03 and $NaHCO_3$ 24.9. The Krebs solution was bubbled with 95% oxygen and 5% carbon dioxide and the pH was 7.4. Chemicals were obtained from the following sources: phenylephrine hydrochloride, acetylcholine, sodium nitroprusside, isoproterenol (Sigma, St. Louis, MO, USA), VIP (Calbiochem, San Diego, CA, USA) and rCGRP (Phoenix Pharmaceuticals, Inc., Mountain View, CA, USA). All chemicals were prepared in nanopure deionized water. The required working solutions were made by dilution in the deionized water immediately before use. All concentrations referred to are final bath concentrations.

2.4. Data analysis

The results are expressed as the means ± standard error of the mean (S.E.M.). Graphs are constructed using GraphPad Prism by Intuitive Software for Science (San Diego, CA, USA, http://www.graphpad.com). Nonlinear regression analysis with equation for sigmoidal dose–response (variable slope) was used. The *N* values represent the number of different mice and *n* values represent the number of rings. Only the length–tension experiment used *n* value and all the other experiments used *N* value. The data were analyzed using ANOVA followed by the Student–Newman–Keuls (S–N–K) test (unpaired). Probabilities less than 5% ($p < 0.05$) were considered significant.

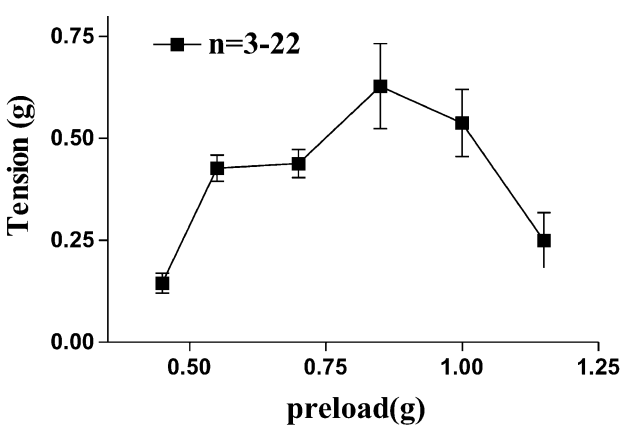


Fig. 1. Length–tension relationship for the endothelium-intact thoracic aortic rings of C57BL/6J male mice. Points represent means and vertical bars the S.E.M. ($n = 3$–22).

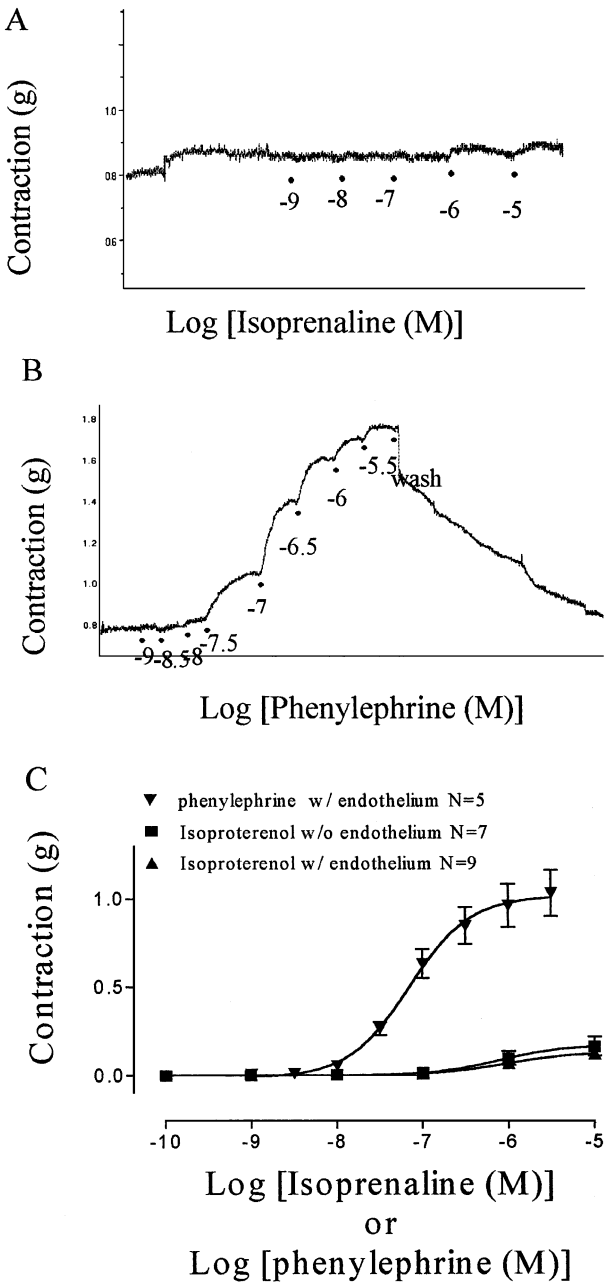


Fig. 2. Isoprenaline- and phenylephrine-induced contractions in the mouse thoracic aorta. (A) Original recording showing contractile response induced by cumulative doses of isoprenaline. (B) Original recording showing contractile response induced by cumulative doses of phenylephrine. (C) Cumulative concentration–response curves for isoproterenol and phenylephrine. Values are expressed in magnitude of contraction above the basal tension. Points represent means and vertical bars the S.E.M.

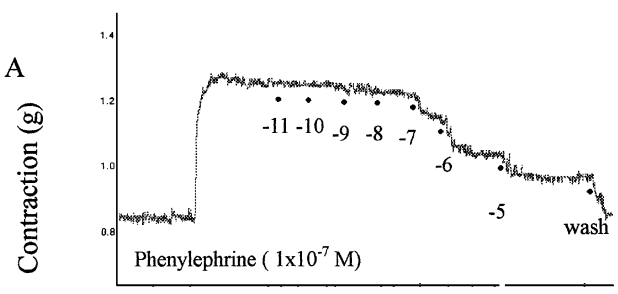
3. Results

3.1. Effects of basal tension on phenylephrine-induced contractions

A length–tension relationship was first performed to determine the passive tension (preload) at which aortic rings from C57BL/6J mouse performed optimally under active stimulus (1×10^{-7} M phenylephrine) (Fig. 1). Fig. 1 depicts the findings that a passive tension of smaller than 0.5 g and greater than 1.1 g resulted in a significantly smaller magnitude of contraction. Passive tension of about 0.8 g results in an optimum and maximal contraction to phenylephrine. Therefore, a passive tension of 0.8 g was used in all of the following experiments.

3.2. Isoprenaline- and phenylephrine-induced contractions

Fig. 2 (Panel A) shows that isoprenaline did not cause any contraction when its concentration was less than 1×10^{-6} M. However, isoprenaline caused a small contraction of less than 0.1 and 0.2 g when its concentrations were 1×10^{-6} and 1×10^{-5} M, respectively. There was no significant difference in contraction to isoprenaline between rings with and without endothelium. Fig. 2 (Panel B) shows that phenylephrine caused a large contraction of about 1.0 g active tension.



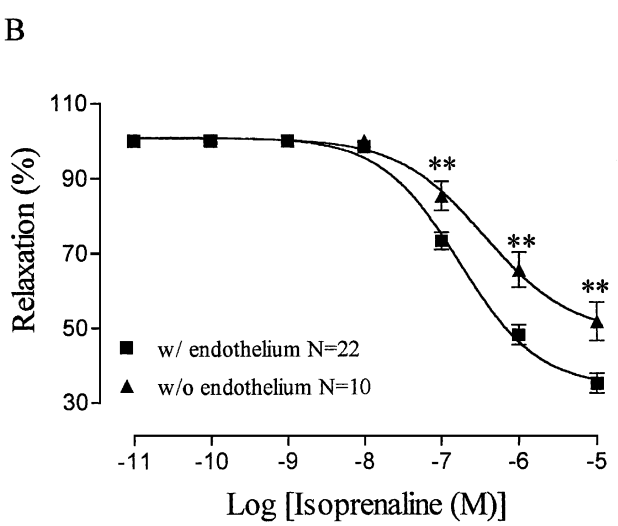
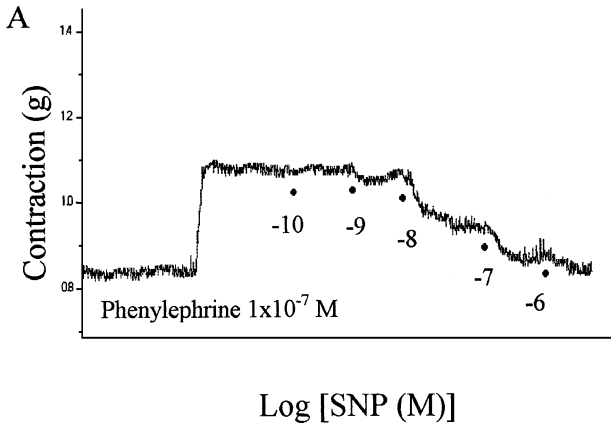


Fig. 3. Isoprenaline-induced relaxations in the endothelium-intact and endothelium-denuded rings of mouse thoracic aorta. (A) Original recording showing relaxant response induced by isoprenaline. (B) Cumulative concentration–response curves for isoprenaline. Points represent means and vertical bars the S.E.M. Response is reported as a percentage of the contraction elicited by 1×10^{-7} M phenylephrine. * * Significantly different ($p < 0.01$) from rings with endothelium.



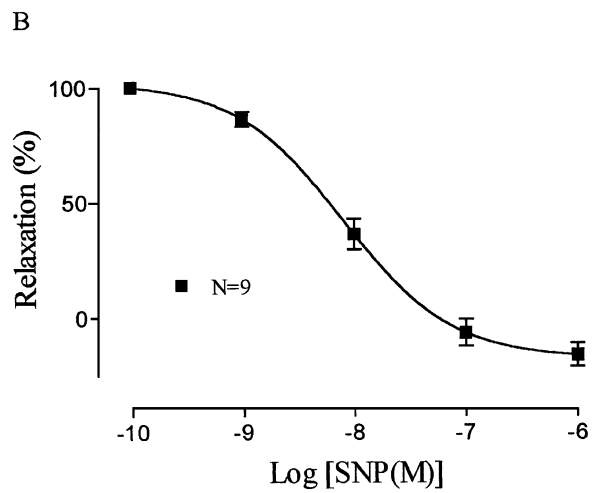


Fig. 4. Sodium nitroprusside (SNP)-induced relaxations in the endothelium-intact rings of mouse thoracic aorta. (A) Original recording showing relaxant response induced by sodium nitroprusside. (B) Cumulative concentration–response curves for sodium nitroprusside. Points represent means and vertical bars the S.E.M. Response is reported as a percentage of the contraction elicited by 1×10^{-7} M phenylephrine.

3.3. Isoprenaline-induced relaxations

Fig. 3 (Panel A) shows the original recording of isoprenaline-induced relaxations. Fig. 3 (Panel B) shows that isoprenaline (1×10^{-11}–1×10^{-5} M) caused concentration-dependent relaxations, reaching a maximum of $64.9 \pm 2.6\%$ and $48.3 \pm 5.1\%$ decrease of the phenylephrine-induced precontractions in endothelium-intact and endothelium-denuded aortic rings, respectively. The $-\log EC_{50}$ values for the isoprenaline-induced relaxations were 6.76 ± 0.14 and 6.44 ± 0.40 in endothelium-intact and endothe-

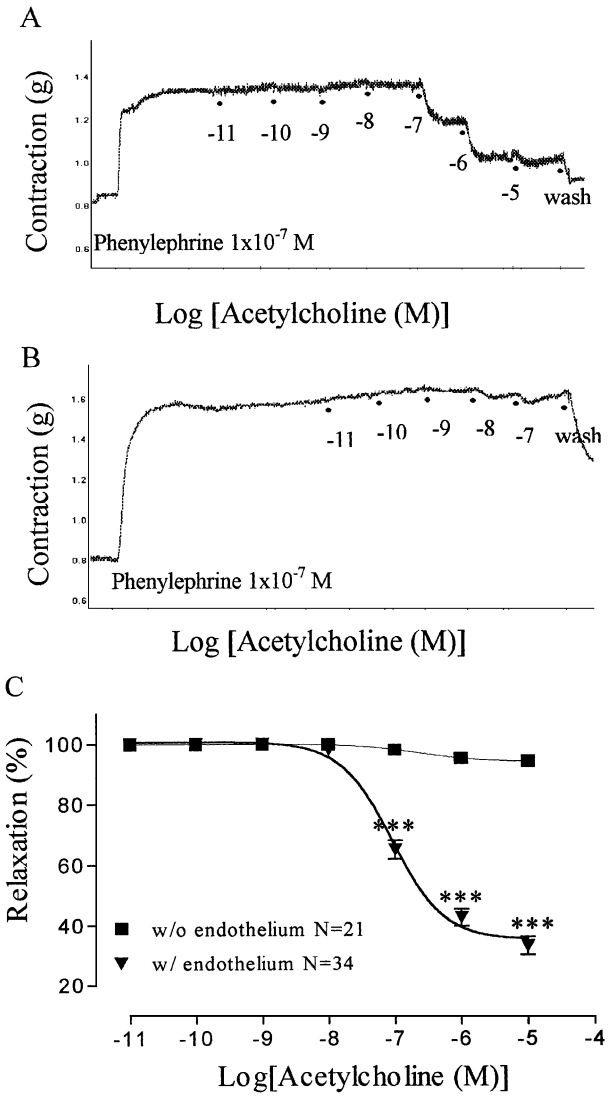


Fig. 5. Acetylcholine-induced relaxations in the endothelium-intact and endothelium-denuded rings of mouse thoracic aorta. (A) Original recording showing relaxant response induced by acetylcholine in a ring with endothelium. (B) Original recordings showing relaxant response induced by acetylcholine in an endothelium-denuded rings. (C) Cumulative concentration–response curves for acetylcholine in aortic rings with and without endothelium. Points represent means and vertical bars the S.E.M. Response is reported as a percentage of the contraction elicited by 1×10^{-7} M phenylephrine. *** Significantly different ($p < 0.0001$) between rings with and without endothelium.

lium-denuded rings, respectively. There were significant differences ($p < 0.01$) in the responses to isoprenaline at 1×10^{-7}, 1×10^{-6} and 1×10^{-5} M between rings with and without endothelium.

3.4. Sodium nitroprusside-induced relaxations

Fig. 4 (Panel A) shows the original recording of sodium nitroprusside-induced relaxations. Fig. 4 (Panel B) shows that sodium nitroprusside (1×10^{-10}–1×10^{-6} M) caused concentration-dependent relaxations, reaching a maximum of $116.1 \pm 5.1\%$ decrease of the phenylephrine-induced precontractions. The $-\log EC_{50}$ value for the sodium nitroprusside-induced relaxation was 8.10 ± 0.20.

3.5. Acetylcholine-induced relaxations

Fig. 5 (Panel A) shows an original recording of acetylcholine-induced relaxation in aortic rings with endothelium. Fig. 5 (Panel B) shows that removal of endothelium caused almost complete loss of acetylcholine-induced relaxation in the aortic rings. Fig. 5 (Panel C) shows that acetylcholine (1×10^{-11}–1×10^{-5} M) caused concentration-dependent relaxations, reaching a maximum of $66.8 \pm 2.9\%$ and $5.7 \pm 1.6\%$ decrease of the phenylephrine-induced precontractions in endothelium-intact and endothelium-denuded aortic rings, respectively. The $-\log EC_{50}$ for the acetylcholine-induced relaxations in endothelium-intact and endothelium-denuded rings were 7.03 ± 0.11 and 6.62 ± 0.78, respectively. There was significant difference ($p < 0.0001$) of the $-\log EC_{50}$ values between rings with and without endothelium. Furthermore, the vasorelaxant responses to acetylcholine at 1×10^{-7}, 1×10^{-6} and

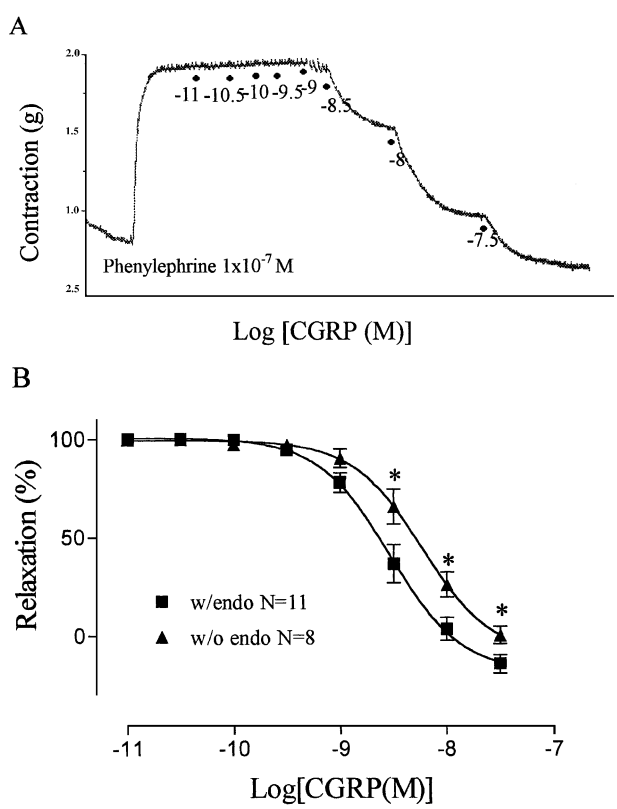


Fig. 6. CGRP-induced relaxations in the endothelium-intact and endothelium-denuded rings of mouse thoracic aorta. (A) Original recording showing relaxant response induced by CGRP in a ring without endothelium. (B) Cumulative concentration–response curves for CGRP. Points represent means and vertical bars the S.E.M. Response is reported as a percentage of the contraction elicited by 1×10^{-7} M phenylephrine. * Significantly different ($p < 0.05$) from rings with endothelium.

1×10^{-5} M were significantly ($p < 0.0001$) different between rings with and without endothelium.

3.6. CGRP-induced relaxations

Fig. 6 (Panel A) shows an original recording of CGRP-induced relaxation in an aortic ring with endothelium. Fig. 6 (Panel B) shows that CGRP (1×10^{-11}–3×10^{-7} M) caused concentration-dependent relaxations, reaching a maximum of $114.3 \pm 4.6\%$ and $99.6 \pm 4.4\%$ decrease of the phenylephrine-induced precontractions in endothelium-intact and endothelium-denuded aortic rings, respectively. The $-\log EC_{50}$ for the CGRP-induced relaxations in endothelium-intact and endothelium-denuded rings were 8.53 ± 0.14 and 8.23 ± 0.19, respectively. Furthermore, the vasorelaxant responses to CGRP at 3×10^{-9}, 1×10^{-8} and 3×10^{-8} M were significantly ($p < 0.05$) different between rings with and without endothelium.

3.7. VIP-induced relaxations

Fig. 7 (Panel A) shows an original recording of VIP-induced relaxation in an aortic ring with endothelium. Fig. 7 (Panel B) shows an original recording of the lack of VIP-induced vasorelaxation in aortic rings without endothelium. VIP (1×10^{-11}–1×10^{-7} M) caused concentration-dependent relaxations reaching a maximum of $65.1 \pm 4.2\%$ and $0 \pm 0\%$ decrease of the phenylephrine-induced precontractions in endothelium-intact and endothelium-denuded aortic rings, respectively. The $-\log EC_{50}$ for the VIP-induced relaxations in the endothelium-intact rings was 8.29 ± 0.26 and the response to VIP was completely dependent on endothelium.

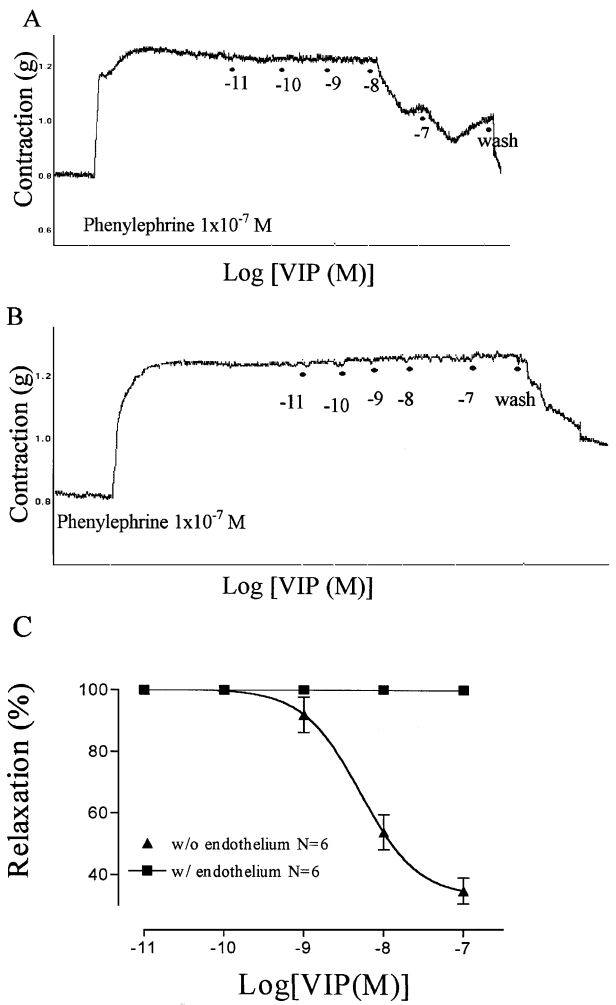


Fig. 7. VIP-induced relaxations in the endothelium-intact and endothelium-denuded rings of mouse thoracic aorta. (A) Original recording showing relaxant response induced by VIP in a ring with endothelium. (B) Original recording showing relaxant response induced by VIP in an endothelium-denuded ring. (C) Cumulative concentration–response curves for VIP in aortic rings with and without endothelium. Points represent means and vertical bars the S.E.M. Response is reported as a percentage of the contraction elicited by 1×10^{-7} M phenylephrine.

4. Discussion

This study was undertaken to characterize the vascular reactivity of the mouse aorta in response to five different vasodilators, including isoprenaline, the NO donor sodium nitroprusside, acetylcholine and two neuropeptides (CGRP and VIP) in adult male C57BL/6J mouse, a strain commonly used as a control for experiments using genetically altered mice. We also determined whether these vasorelaxant responses in mouse aortic rings were dependent on the endothelium.

Russell and Watts (2000) have reported that isoprenaline is a potent contractor in aortic rings of adolescent mice (5–6 weeks, 16–18 g). However, in most other species, isoprenaline is a vasodilator. We, therefore, test whether isoprenaline acts a vasoconstrictor or vasodilator in the adult (12–20 weeks, 30–35 g) C57BL/6J mouse. Our studies showed that isoprenaline caused concentration-dependent relaxation in adult mouse aortic rings. This isoprenaline-induced vasorelaxation is similar to responses in other species. For example, isoprenaline induced vasorelaxation in simian facial veins (Chiba and Tsukada, 1991), rabbit (Ahn et al., 1995) and canine (Evora et al., 1992) coronary arteries, porcine pulmonary arteries (Kukkonen et al., 1997) and rat thoracic aorta (Gray and Marshall, 1992). The data of the present study also show that the isoprenaline-induced vasorelaxations in mouse aorta are partially dependent on endothelium. Endothelium dependency of isoprenaline-induced relaxations varies in different species and tissues (Rubanyi and Vanhoutte, 1985; Gray and Marshall, 1992; Krauss et al., 1992; Bea et al., 1994; Toyoshima et al., 1998).

The vasorelaxant response to isoprenaline obtained in the present study is completely different from the vasoconstrictor response obtained by Russell and Watts (2000). This may be due to the difference in age of the mice used in the two studies. In our studies, isoprenaline causes

vasorelaxation in aortic rings precontracted with phenylephrine and the vasorelaxation was partially dependent on the presence of endothelium. However, Russell and Watts (2000) showed that isoprenaline caused contraction in mouse aortic rings precontracted with $PGF_{2\alpha}$. The difference of the response to isoprenaline may be due to the difference of the ages of the models used. The mice they used were 16–18 g (about 5–6 weeks old), which represents aldolescent mice. In the present study, the mice used were 30–35 g and were about 12–20 weeks of age i.e. adult mice. Alternatively, the difference in response to isoprenaline may be due to the different vasoconstrictor used to precontract the aortic rings.

Russell and Watts (2000) showed that isoprenaline, in the adolescent mice, is a potent vasoconstrictor, causing more than 50% of the contractions induced by phenylephrine. However, in our studies with adult C57BL/6J mice, we found that isoprenaline can only cause a very small magnitude of contraction (0.1–0.2 g) at the highest dose tested (1×10^{-5} M), much smaller than that induced by phenylephrine (1.0 g at 1×10^{-5} M). This difference in contractile response to isoprenaline again may be due to the difference in age of animals.

We also showed that α-CGRP and VIP caused concentration-dependent vasorelaxations in the C57BL/6J adult mouse aorta. This is in agreement with the large number of reports showing the vasorelaxant effect of CGRP in various species and vascular preparation in vitro, as listed in Section 1. In our studies with aortic rings of C57BL/6J mice, we found that CGRP caused concentration-dependent relaxations that reached a maximum of 114.3% and 99.6% decrease of the phenylephrine-induced precontraction in endothelium-intact and endothelium denuded rings, respectively. Furthermore, there were significantly larger vasorelaxant responses to CGRP at 3×10^{-9}, 1×10^{-8} and 3×10^{-8} M in mouse aortic rings with endothelium as compared to rings without endothelium. Therefore, CGRP-induced vasorelaxations in C57BL/6J mouse aorta are partly dependent on the endothelium. Since there is no difference in the contracting force induced by 1×10^{-7} M phenylephrine in aortic rings with and without endothelium (data not shown), it is unlikely that the decrease in potency of CGRP was associated with an increase in contracting force of the rings.

These results are similar to the findings of Pomerleau et al. (1997) using the CD1 strain of mouse. They reported CGRP caused 110.4% and 102.4% of maximum relaxation in endothelium-intact and endothelium-denuded rings, respectively. However, we found that the potency of CGRP in the C57BL/6J mouse aorta of the present study is higher than that in CD1 (Pomerleau et al., 1997). The $-\log EC_{50}$ for rat α-CGRP in our studies were 8.53 and 8.23 in aorta with and without endothelium, respectively. By comparison, Pomerleau's studies showed that CGRP had a $-\log EC_{50}$ of 8.11 and 7.89 for rings with and without endothelium, respectively. The difference of potency may be due to the difference of strain or experimental conditions.

VIP induces vasorelaxation in various arteries. Similar to CGRP, the dependency of VIP on endothelium also differs greatly between species and different vascular beds. In human gastric and transverse cervical arteries (Thom et al., 1987) and CD1 mouse aorta (Pomerleau et al., 1997), the response to VIP is dependent on endothelium. In contrast, in human pulmonary artery (Thom et al., 1987) and cat cerebral artery (Edvinsson et al., 1985), the response is independent of endothelium. We found that the VIP-induced vasorelaxations in C57BL/6J mouse aortic rings were completely dependent on endothelium. Our results are, thus, in agreement with Pomerleau et al. (1997), who had shown VIP-induced vasodilations in CD1 mouse aorta are also completely dependent on endothelium. However, we obtained a maximum response of 65.1% at a concentration of 1×10^{-7} M of VIP, whereas Pomerleau et al. (1997) obtained a maximum relaxation of less than 50% at a 10-fold higher concentration of VIP. The health of the endothelium or the amount of functional endothelial cells remaining in the mouse aortic rings after preparation and equilibration might explain the difference in response to VIP. For comparison, we also found that the relaxation in response to acetylcholine/carbamylcholine in our studies was also much higher than that in the study by Pomerleau et al. (1997).

In mouse aorta, there are several differences between CGRP and VIP on the relaxation response. Firstly, the maximum response of CGRP and VIP are 114.3% and 65.1%, respectively, in aorta with preserved endothelium. Second, the CGRP-induced vasorelaxation is only slightly dependent on endothelium, but VIP-induced vasorelaxation is completely dependent on endothelium. Lastly, the response to CGRP is much slower to develop than that of VIP. For example, CGRP required almost 1 h to reach maximum relaxation, whereas VIP required only 5 min.

In conclusion, we showed that isoprenaline induced vasorelaxations in adult mouse aortic rings precontracted with phenylephrine, but cause only small vasocontractions at very high concentration of isoprenaline in aortic rings without precontraction. These data are very different from the data of a previous study of isoprenaline effects in mouse aorta, in which isoprenaline was shown to cause contraction, but not relaxation. We have also demonstrated that both CGRP and VIP are potent vasodilators in aortic rings of the C57BL/6J strain of mouse. Similar to responses in aortic rings of the CD1 mouse, CGRP-induced vasorelaxations are partly dependent on endothelium, while VIP-induced vasorelaxations are completely dependent on endothelium, in aortic rings of the adult C57BL/6J mouse.

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