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DILATOR ACTIONS OF ENDOTHELIN IN CORONARY RESISTANCE VESSELS AND THE ABDOMINAL AORTA OF THE GUINEA PIG

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Summary

Endothelin has been characterized as a potent constricting factor. The purpose of this study was to investigate possible dilator effects of this peptide and to examine whether dilator responses occur through an endothelium-mediated mechanism in guinea pig coronary resistance vessels and isolated aortic rings. Changes in perfusion pressure after bolus injections of endothelin were measured using a constant-flow modified Langendorff preparation with a transducer between the flow pump and the heart. An immediate fall in perfusion pressure, averaging 6 mmHg, was observed after injection of endothelin (10^{-14}-10^{-12} moles). This effect was maximal at 1 minute and tended to return toward baseline levels within 4 minutes. In response to endothelin (10^{-9} M), isolated aortic rings relaxed 35% after being contracted with prostaglandin $F_{2\alpha}$ (10^{-7} M). In both preparations, dilation was converted to constriction after endothelium damage by oxygen radicals or endothelium removal (mechanical rubbing). Dilator responses to endothelin were blocked by pretreatment for 30 minutes with indomethacin (14 μM) in the presence of an intact endothelium in coronary resistance vessels, whereas in the abdominal aorta they were not. We conclude that endothelin has significant dilator properties and that this effect is opposed by its constrictor action at higher doses. In addition, dilator responses to endothelin require an intact endothelium in both coronary vessels and abdominal aorta. Finally, endothelin-induced dilation in coronary resistance vessels appears to occur through a cyclooxygenase product-mediated mechanism.

Endothelin, a recently discovered 21-residue vasoactive peptide (1), has potent constrictor effects in vitro in both resistance (2,3) and non-resistance vessels (3-8), as well as potent pressor effects in vivo (9). In several animal species including the rat (10), dog (11), and cat (12,13), an initial transient depressor response to endothelin occurs prior to its potent and long lasting pressor effects. Recently, Warner, deNucci, and Vane (14) reported in vitro dilation to endothelin

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in rat mesentery and deNucci et al. (15) blocked the endothelin-induced dilation with oxyhemoglobin. Observations of constrictor as well as dilator responses suggest that endothelin may play a modulating role in the local control of vascular tone.

The goal of the current study was to characterize dilator responses to endothelin in two different regional vascular beds of the guinea pig (coronary resistance vessels and aorta). Whether dilator responses to endothelin require an intact endothelium or occur through a cyclooxygenase product-mediated mechanism was examined.

Methods

Male guinea pigs (Charles Rivers, n=17) weighing approximately 325 grams were used in this study. Animals had free access to food and water and were group-housed in light-cycled (0600-1800) temperature-controlled quarters.

Two types of physiologic measurements were investigated: 1) perfusion pressure in the coronary vasculature using a modified Lagendorff procedure and 2) force generation in isolated abdominal aortic rings. For coronary vascular perfusion experiments, guinea pigs were give 300 units of intraperitoneal heparin and sacrificed by a single blow to the head under ketamine (40 mg/kg) and xylazine (6 mg/kg) anesthesia. The chest was opened and the heart arrested in situ by a 4 ml injection of iced 16mM KCl into the inferior vena cava. The heart was then rapidly excised and the ascending aorta cannulated with polyethylene tubing. The coronary vasculature was perfused at a constant flow rate with a buffer containing the following (mmol/l): NaCl 105.6, KCl 14.8, $MgSO_4 \cdot 7H_2O$ 1.1, KH_2PO_4 1.2, $NaHCO_3$ 25.0, dextrose 11.0, and $CaCl_2 \cdot 2H_2O$ 1.25. The potassium concentration was maintained at a high level in order to keep the heart in the arrested state throughout the experiment. The perfusion medium was maintained at 37°C and aerated with a mixture of 95% O_2 and 5% CO_2 (pH 7.4). The heart was allowed to equilibrate for at least 20 minutes prior to the initiation of the experiment. The flow rate was adjusted to a level necessary to generate an initial perfusion pressure of 25 mmHg. The coronary perfusion rate averaged 4 $\pm$ 0.3 ml/min. Agents were injected 1 cm above the heart in 0.1 ml amounts using a 1 second bolus technique at 4 minute intervals. Changes in perfusion pressure were measured using a transducer via a t-tube between the flow pump and the heart, and recorded on a Grass polygraph.

Following the equilibration period, the coronary vasculature was made to constrict to a bolus injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 10^{-7} moles). Subsequent to this treatment, coronary vascular tone stabilized at a level that was approximately 20 mmHg above the initial perfusion pressure. At this level of perfusion pressure, constrictor responses to bolus injections of prostaglandin $F_{2\alpha}$ (10^{-7} moles) were reproducible for a period of approximately 2 hours. Dilator responses to endothelin (10^{-14}-10^{-9} moles) were characterized on this background of coronary vascular tone. In another set of experiments, disruption of the endothelium was achieved by exposing the vessels to free radicals generated by electrolysis of the buffer solution (16). The perfusate was stimulated by platinum wire electrodes within the

tubing cannulating the ascending aorta 2 cm above the suspended heart. A 9 V stimulus was delivered as a train of square waves of 2 msec pulse duration at a frequency of 4 Hz for 5 minutes. Changes in perfusion pressure were measured before and after endothelium damage. After endothelium damage, dose-response curves to endothelin (10^{-14}-10^{-9} moles) were obtained. In another set of experiments, coronary vascular responsiveness was observed after treatment for 30 minutes in the presence of the cyclooxygenase inhibitor, indomethacin (14 μM).

For isolated vascular ring studies, segments of the abdominal aorta were excised and placed in a physiological salt solution (PSS) of the following composition (mM): NaCl 130, KCl 4.7, $NaHCO_3$ 14.9, KH_2PO_4 1.17, $CaCl_2$ 1.6, $MgSO_4$ 0.7, dextrose 5.5, and $CaNa_2$ EDTA 0.03. The vessel segments were cleaned of excess connective tissue and cut into 3 mm ring segments. Two wires were passed through the lumen taking care not to damage the endothelium. The endothelium was removed from some preparations by gently rubbing the intimal surface. The failure of acetylcholine (1 μM) to relax a contraction induced by $PGF_{2\alpha}$ (0.1 μM) demonstrated the effectiveness of the endothelium removal. After the rings were mounted in the bath, a passive tension of 2 g was applied to each ring and isometric tension was measured with a force transducer. This level of passive tension was determined to be optimal for maximum force development to norepinephrine (10^{-6} M) and prostaglandin $F_{2\alpha}$ (10^{-5} M). The PSS in the tissue bath was bubbled with 95% O_2 and 5% CO_2 and maintained at a temperature of 37°C (pH 7.2).

Initial studies involved quantitating relaxation responses to increasing doses of endothelin (10^{-11}-10^{-8} M). Ring segments were contracted with prostaglandin $F_{2\alpha}$ (10^{-7} M), and following a plateau of the contractile response, endothelin was added to the bath at 4 minute intervals (Fig 3). Relaxation responses to endothelin were also measured in the presence of indomethacin. In these experiments, the rings were treated with indomethacin (14 μM) for 30 minutes.

Synthetic human/porcine endothelin (ET-1; Peptides International, Louisville, KY, USA) was mixed with distilled water to obtain concentrations of 10^{-5} M. Serial dilutions were performed using either PSS or distilled water. Acetylcholine (Miochol, IOLAB Pharmaceuticals, Claremont, CA, USA), nitroprusside (Nipride, Abbott Laboratories, Chicago, IL, USA), and prostaglandin $F_{2\alpha}$ (Sigma, St. Louis, MO, USA) were diluted in PSS and prepared daily. Indomethacin (Sigma) was dissolved in 100 mM $NaCO_3$. Data are reported as means $\pm$ SEM. An unpaired analysis (Student's t-test) was used to compare observations between the control and treated groups. The Bonferroni correction was used when multiple comparisons were made. A p value of 0.05 was considered statistically significant.

Results

Coronary vasodilation was observed in response to endothelin (Figure 1). An immediate fall in perfusion pressure, averaging 6 mmHg, occurred after injection of endothelin (10^{-14}-10^{-12} moles). This effect was maximal at 1 minute and tended to return toward baseline levels within 4 minutes. In 2 out of 5 animals, a

similar dilator response was observed at a dose of 10^{-11} moles. Higher concentrations of endothelin produced only elevations in perfusion pressure. After endothelium damage, endothelin induced only constriction. Dilation to acetylcholine was used to indicate the presence of an intact and functional endothelium. During electrical stimulation of the perfusate, perfusion pressure rose 16 $\pm$ 4 mmHg. After endothelium damage of the coronary circulation, constrictor responses to acetylcholine were observed, indicating endothelium dysfunction.

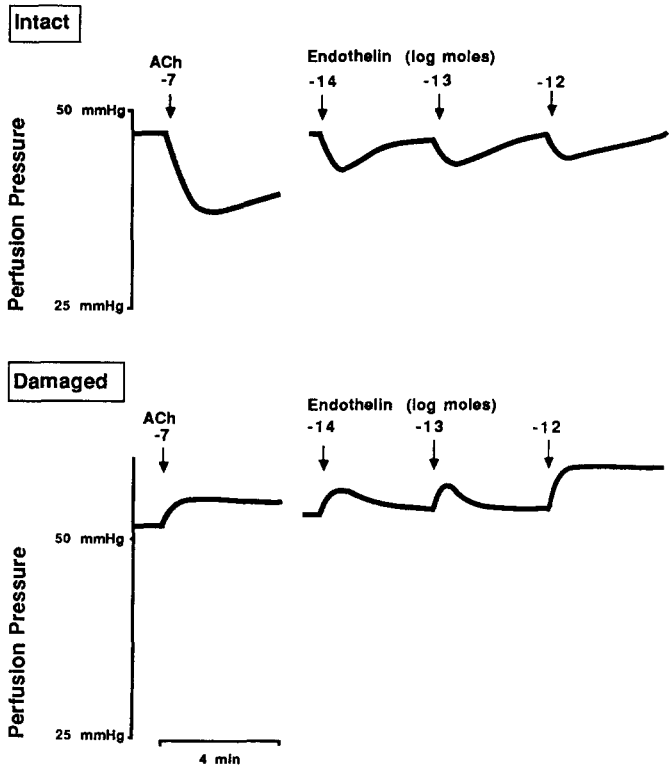

FIG. 1

Representative tracings of coronary vasodilation to acetylcholine and endothelin in the presence of an intact endothelium, and coronary constriction after endothelium removal was induced through electrical stimulation of the coronary perfusate for 5 minutes.

Coronary dilation was observed in response to acetylcholine (10^{-7} moles) and nitroprusside (10^{-5} moles) in the presence of an intact endothelium (Figure 1 and 2A). After endothelium damage, the dilation to acetylcholine was converted to constriction in all experiments, whereas the magnitude of the dilation to nitroprusside was unchanged. Dilation was observed in

response to endothelin (10^{-14}-10^{-12} moles) in the intact preparation (Figure 2B). Endothelin-induced dilation at low doses was converted to constriction after endothelium damage and after treatment for 30 minutes with indomethacin (14 μM) in the presence of an intact endothelium.

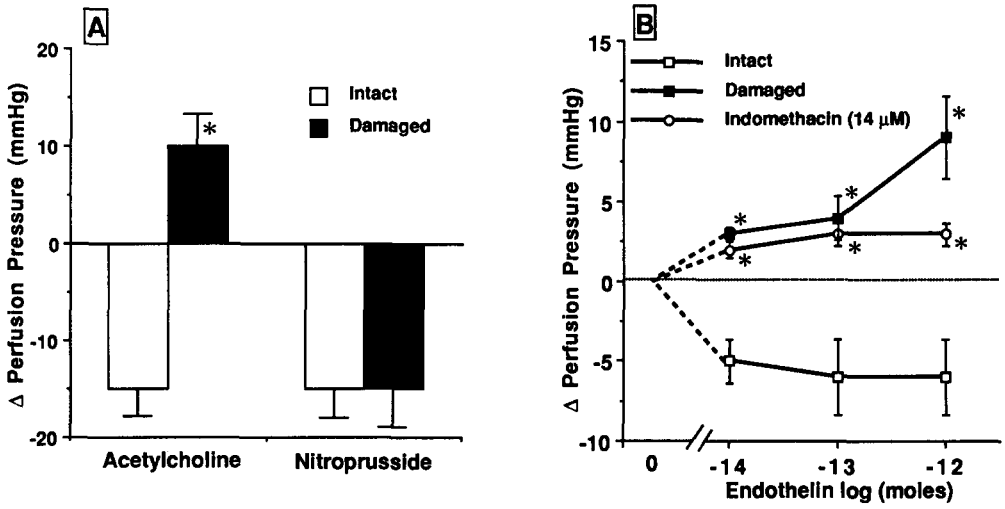

FIG. 2

Panel A: Effect of acetylcholine and nitroprusside on coronary perfusion pressure (mean ± SEM) in the presence of an intact (n = 5) or damaged endothelium (n = 6). After endothelium damage, perfusion pressure was significantly ($p < 0.05$) increased in response to acetylcholine, but unchanged in response to nitroprusside. Panel B: Effect of endothelin on coronary perfusion pressure in the presence of an intact endothelium (n = 5), after endothelium damage (n = 6), and after indomethacin pretreatment (n = 6). Constrictor responses were significantly different compared to the dilator responses ($p < 0.05$; indicated by asterisks).

Figure 3 shows the results of one experiment and demonstrates the protocol employed in examining the relaxing effects of endothelin in isolated aortic segments. Endothelin caused a dose-dependent relaxation of the prostaglandin $F_{2\alpha}$ - contracted abdominal aortic ring with intact endothelium (upper panel). Exposure to 10^{-8} M endothelin consistently caused contraction. Aortic rings which had the endothelium removed (lower panel) did not relax to endothelin and showed a significant contraction. The relaxant property of low concentrations of endothelin was also evident in aortic rings contracted with norepinephrine (10^{-8} M) indicating that the relaxation is not specific to the contractile agonist.

Aortic rings with intact endothelium and contracted with prostaglandin $F_{2\alpha}$ relaxed to acetylcholine (Figure 4). Treatment with indomethacin had no significant effect on the magnitude of the relaxation induced by acetylcholine. Removal of the endothelium, however, completely abolished the relaxation response. Endothelin caused relaxation in intact aortic rings at 10^{-10} and 10^{-9} M concentrations. The relaxation was converted to a contraction with removal of the endothelium. Intact rings treated with indomethacin exhibited relaxation which was similar to that observed in the untreated rings.

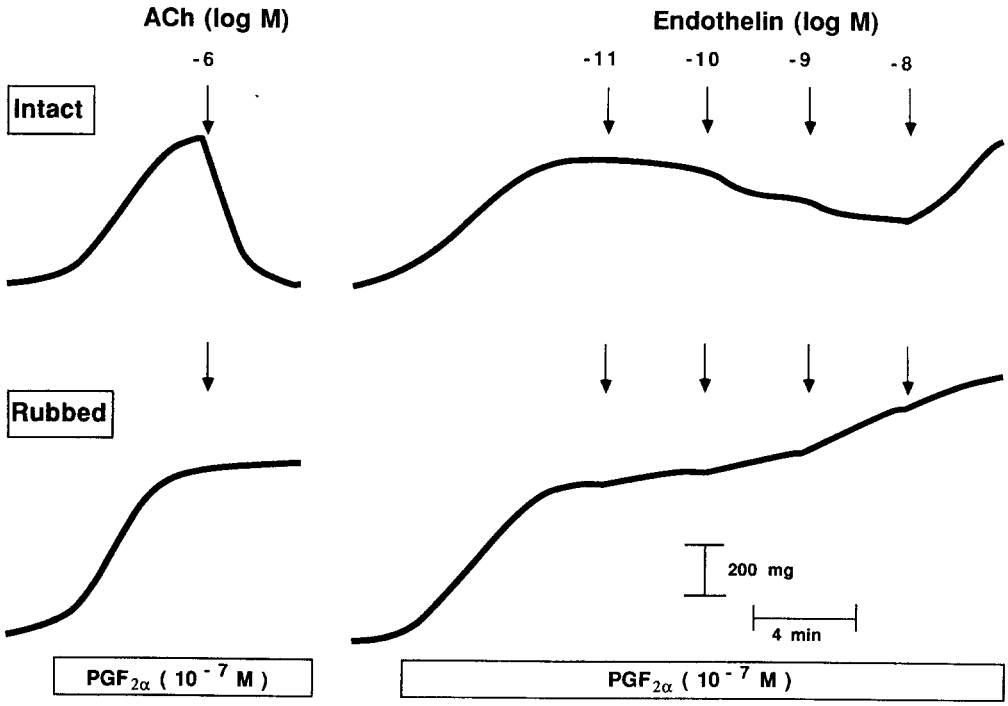

FIG. 3

Representative tracings of the responses of abdominal aortic rings to acetylcholine and endothelin in the presence of an intact endothelium (Intact) and after mechanical removal of the endothelium (Rubbed). Intact vessels relaxed to both acetylcholine and endothelin. After endothelium removal, the relaxation response to acetylcholine was abolished and endothelin induced only contractile responses.

Discussion

The present study demonstrates that endothelin, a potent constrictor peptide, also has dilator actions in the coronary

vessels and relaxant properties in the abdominal aorta of the guinea pig. Endothelin-induced dilation was short-lived, and was present only at low doses; as higher doses produced constriction. These observations are consistent with findings of endothelin-induced dilation in perfused rabbit aorta and isolated perfused rat mesentery (17).

Warner et al. (14) and deNucci et al. (15) found that endothelin-induced dilation required the presence of an intact endothelium, as its removal with sodium deoxycholate abolished dilator responses. Based on this finding, Warner et al. (17) hypothesized that endothelin-induced dilation may occur through the stimulated release of endothelium-derived relaxant factor (EDRF). In support of this hypothesis, we noted that dilator responses to endothelin in both the coronary vessels and abdominal aorta were converted to constrictor responses after damage to the endothelium. This sustained constrictor effect was

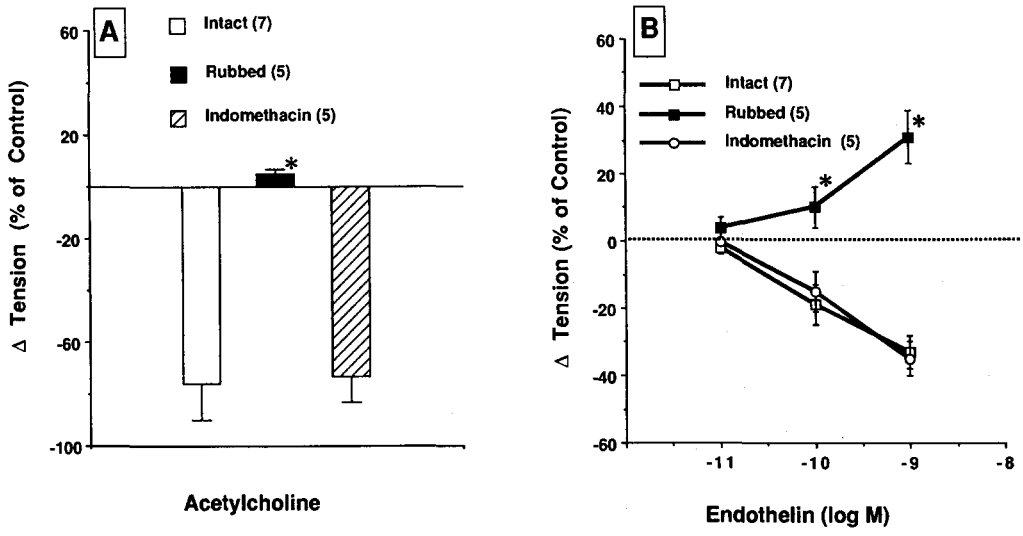

FIG. 4

Response of abdominal aortic rings to acetylcholine and endothelin. Panel A: Relaxation to acetylcholine (10^{-6} M) was observed in the presence of an intact endothelium (Intact) and following a 30 minute exposure to indomethacin (14 μM). After endothelium removal (Rubbed), acetylcholine-induced relaxation was abolished. Panel B: Endothelin-induced dilation in intact rings and those exposed to indomethacin. The relaxation was converted to contraction after endothelium removal. Values are means $\pm$ SEM. Asterisks indicate a statistically significant difference from values for intact segments ($p < 0.05$).

noted after coronary vessels were exposed to free radicals and after the endothelium of the abdominal aorta was removed by mechancial rubbing. As further support of the hypothesis, deNucci et al. (15) abolished endothelin-induced dilation in the presence of oxyhemoglobin (30 μM) in the isolated perfused mesentery. In addition, Warner et al. using the same animal model, inhibited endothelin-induced dilation with oxyhemoglobin and methylene blue (100 μM), inhibitors of EDRF.

Recent reports indicate that endothelin stimulates the release of prostacyclin. Infusion of endothelin (1 or 10 nM) for 3 minutes into the perfused guinea pig lung stimulated an increase in a stable metabolite of prostacyclin, 6-keto-$PGF_{1\alpha}$ (15), and injection of a pressor dose of endothelin (40 pM) stimulated release of 6-keto-$PGF_{1\alpha}$ in the isolated perfused rat mesentery (18). Additionally, prostacyclin inhibited endothelin-induced contraction in a dose-dependent manner in the human internal mammary artery (19). Based on these findings, prostaglandins, such as prostacyclin, may serve to counteract endothelin-induced effects. In support of this hypothesis, endothelin-induced constriction was potentiated after blockade with indomethacin in the isolated perfused rabbit kidney and spleen (20), and indomethacin blocked the prostaglandin-mediated anti-aggregation effect of endothelin on platelets (21). In the present study, we found that endothelin-induced dilation in the coronary vessels was converted to constriction after indomethacin blockade in the presence of an intact endothelium. This finding suggests that endothelin-induced dilation is mediated through the release of a cyclooxygenase product. In support of the role of prostaglandins in mediating coronary responsiveness in the guinea pig, investigators in our laboratory (22) also found that acetylcholine-induced dilation in the coronary vasculature was mediated by a cyclooxygenase product, whereas the acetylcholine-induced relaxation in the abdominal aorta was not.

In conclusion, endothelin has dilator effects at low doses in the coronary vasculature of the guinea pig which are mediated by a cyclooxygenase product. Endothelin-induced relaxation in the abdominal aorta required the presence of an intact endothelium, but was not mediated by a cyclooxygenase product. Our results support the hypothesis that endothelin stimulates the release of a cyclooxygenase product and EDRF, and that these substances counteract the constrictor actions of endothelin.

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