Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor

(lung/guinea pig/rat/icosanoids/cyclooxygenase inhibitors)

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ABSTRACT Endothelin releases prostacyclin and thromboxane A2 from guinea pig or rat isolated lungs and endothelium-derived relaxing factor in the perfused mesentery of the rat. Endothelin is also substantially removed by the pulmonary circulation of the rat in vitro and in vivo and by guinea pig lungs in vitro. In the rat, the effects of endothelin on the blood pressure vary from pressor (in pithed rats) to purely depressor in anesthetized rats where the resting blood pressure is high. It therefore has the characteristics of a local pressor hormone, rather than a circulating one.

The endothelial cell (EC) is known to release vasoactive substances such as prostacyclin (PGI2) (1) and endothelium-derived relaxing factor (EDRF) (2), recently identified as nitric oxide (3). Release of endothelium-dependent vasoconstrictor factors has been observed in response to various chemical and physical stimuli such as norepinephrine (4), thrombin (5), hypoxia (5, 6), increased transmural pressure (7), and mechanical stretch (8).

Masaki and his colleagues (9) have recently characterized from cultures of porcine aortic ECs a 21-amino acid peptide, which they called endothelin (ET). In the chemically denervated rat, porcine ET is the most potent pressor substance yet described, with a long duration of action. They suggested that ET directly activates dihydropridine-sensitive calcium channels.

We report here that apart from its vasoconstrictor activity, ET can release potent vasodilator substances such as PGI2 and EDRF and is also removed by the pulmonary circulation.

MATERIALS AND METHODS

Superfusion Bioassay. Spiral strips of de-endothelialized vascular smooth muscle from the rabbit (mesenteric artery, celiac artery, carotid artery, aorta, jugular vein, mesenteric vein) and other smooth muscle preparations (guinea pig trachea, guinea pig ileum, rat stomach strip, rabbit duodenum) were mounted in a cascade (10) and superfused at 5 ml·min⁻¹ with Krebs–Ringer solution containing indomethacin (5.6 µM). Agonists such as ET (1–50 pmol), bradykinin (1–10 pmol), substance P (1–10 pmol), and angiotensin II (1–10 pmol) were injected over the assay tissues.

Isolated Lungs. Male Dunkin–Hartley guinea pigs (300–400 g) or male Wistar rats (200–300 g) were anesthetized with sodium pentobarbital (Sagatal, 70 µmol·kg⁻¹, i.v.) and a thoracotomy was performed. The pulmonary artery and the trachea were cannulated and the lungs were removed and placed in a warm chamber. The lungs were perfused at 5 ml·min⁻¹ via the pulmonary artery with oxygenated (95% O₂/5% CO₂) and warmed (37°C) Krebs–Ringer solution (11). The lungs were left to stabilize for 30 min and ET was infused for 3 min at a flow rate of 0.1 ml·min⁻¹ to achieve a final concentration of 1 or 10 nM. The effluent from lungs was collected and analyzed by RIA for 6-oxoprostaglandin F₁α (6-oxo-PGF₁α) and thromboxane (TX) B₂ as measures of prostacyclin and TXA₂ release (12). The removal of ET was calculated by comparing the contractions of the assay tissues in response to infusions of ET directly over the tissues with those in response to infusions given through the lungs (13).

Isolated Mesentery. The rat isolated perfused mesentery was prepared from male Wistar rats (200–300 g) pretreated with heparin (1000 units·kg⁻¹, i.p.) (14). The mesenteric bed was perfused at 5 ml·min⁻¹ with warmed (37°C) and gassed (95% O₂/5% CO₂) Krebs–Ringer solution, which contained indomethacin (5.6 µM) and sometimes albumin (0.5% wt/vol). Vascular tone in the bed was increased by an infusion of norepinephrine, U46619, 9,11-dideoxy-9α,11α-methanoepoxyprostaglandin F₂α (0.03–1.5 µM), or methoxamine (30–100 µM) to produce an increase in perfusion pressure from 15–22 to 35–130 mmHg. In experiments in which the ECs were removed from the mesentery by infusing sodium deoxycholate (2.4 mM), the Krebs–Ringer solution contained albumin (0.5%) to minimize subsequent edema (15).

ECs. ECs were isolated by treatment of bovine aortae with 0.02% (wt/vol) collagenase. Cells were grown to confluence in plastic vessels and then removed by treatment with 0.05% (wt/vol) trypsin and seeded onto Cytodex 3 microcarrier beads. The beads were stirred for 3–7 days until the cells became confluent and were then packed into a jacketed column and perfused (5 ml·min⁻¹ at 37°C) with gassed (95% O₂/5% CO₂) Krebs–Ringer solution, which contained superoxide dismutase. EDRF was bioassayed on a cascade of rabbit aortae (16–18).

Blood Pressure. Male Wistar rats (250–300 g) were anesthetized with thiobutabarbital (Inactin) (0.5 mmol·kg⁻¹) or pithed under halothane anesthesia. The right atrium and the left ventricle were cannulated via the right common carotid artery and left jugular vein, respectively. Peripheral blood pressure and heart rate were measured on a Grass model 7D polygraph from a femoral artery cannula. In the anesthetized animals, ET (0.01–1 nmol·kg⁻¹) was injected in volumes of either 1.0 or 0.3 ml·kg⁻¹ alternately into the arterial or venous sides of the circulation. In the pithed rats, cumulative dose–response curves to both intraarterial (i.a.) and i.v. ET were made over a dose range of 0.2–2 nmol·kg⁻¹.

Bradykinin, substance P, angiotensin II, acetylcholine (ACH), norepinephrine, methoxamine, sodium deoxycholate, indomethacin, albumin, piroxicam, methylene blue, and

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RESULTS

ET (1 or 10 nM) added to rabbit or human platelet-rich plasma did not induce aggregation, nor did it affect the aggregation induced by ADP, collagen, or arachidonic acid (n = 3). The activity of ET on rabbit jugular vein was not affected by incubation in rabbit or human platelet-poor plasma (n = 3) or in rabbit whole blood (n = 3) for up to 90 min at 37°C, showing that it was resistant to degradation by plasma peptidases and blood cells.

Isolated Smooth Muscle. Venous strips were more sensitive to ET than arterial strips (Fig. 1) and, in particular, the rabbit jugular vein and mesenteric vein were contracted by as little as 0.5–2.5 pmol of ET in a reproducible and dose-dependent way. The rat stomach strip was also a useful bioassay tissue in that contractions produced by ET (5–15 pmol) returned to baseline faster than those of venous strips and much faster than those of arterial ones.

Release of Icosanoids from Lungs. ET (1 or 10 nM) infused for 3 min through isolated lungs of guinea pigs induced a sustained release of PGII and TXA2 (Fig. 2). In rat lungs, ET (10 nM) induced a much stronger release of PGII than TXA2 (6.6 ± 2.1 ng ml−1 and 0.4 ± 0.2 ng ml−1 for 6-oxo-PGF1α and TXB2, respectively; n = 4).

Removal of ET by Lungs. ET (1 nM) infused through the pulmonary circulation of guinea pig isolated lungs (treated with indomethacin to prevent icosanoid release interfering with the bioassay) was substantially removed, so that only 40% ± 3.6% (n = 4) of the amount infused was detected in the effluent (Fig. 3). The removal of ET was not affected by an angiotensin-converting enzyme inhibitor (captopril) given in sufficient concentrations (10 μM) to inhibit substantially the inactivation of bradykinin (19).

Release of EDRF. ACh provoked dose-dependent vasodilatations of the rat isolated perfused mesentery due to EDRF release, for after removal of the ECs with sodium deoxycholate or in the presence of oxyhemoglobin (30 μM), vasodilation induced by ACh was suppressed (n > 30; Fig. 4). ET (1–10 pmol) also induced dose-dependent vasodilatations through EDRF release (n = 18). In a further 12 experiments,
EDRF release was not observed, probably because the constrictor effects of ET were dominant. At higher doses, ET provoked sustained increases in perfusion pressure (n = 30; Fig. 4). After removal of the ECs (n = 6) or in the presence of oxyhemoglobin (30 μM; n = 4), ET (1–10 pmol) induced only sustained vasoconstriction. In preparations in which no methoxamine was infused to raise the perfusion pressure, ET (1–100 pmol) induced dose-dependent vasoconstrictions, which were substantially potentiated by the removal of the endothelial cells (n = 8) or by the presence of oxyhemoglobin (30 μM; n = 5; Fig. 4) or methylene blue (100 μM; n = 5). Bay K 8644 (1–300 pmol) did not release EDRF in the rat isolated mesentery (n = 3). ET (1–50 pmol) or Bay K 8644 (0.27–27 nmol) did not release EDRF or PGI₂ when injected through columns of bovine aortic ECs (n = 4).

**Fig. 3.** The pulmonary circulation removes ET. The effluent from guinea pig lungs treated with indomethacin (5.6 μM) superfused a rat stomach strip (RSS). ET (1 nM) infused through the lungs (TL) induced a contraction of the RSS smaller than that induced by ET (0.5 nM) infused over the tissues (OT), indicating a removal of >50% in a single passage. Bradykinin (3 nM) was removed by the lungs as described (5), whereas iloprost (ILO) was not. Similar results were obtained in four other experiments.

**Blood Pressure Measurements.** Surprisingly, in anesthetized rats ET produced dose-dependent pressor responses (Fig. 5A). These reductions in peripheral blood pressure were most likely due to vasodilatation, for there were no apparent effects on the heart. However, as shown in Fig. 5, these depressor responses were smaller when ET was given i.v. than when the peptide was injected into the left ventricle. When the basal blood pressure was lower, the depressor responses were followed by dose-dependent and sustained increases in blood pressure (30 min following 1 nmol·kg⁻¹, i.a.; Fig. 5B).

In pithed rats with low resting blood pressure of 48 ± 1.3 mmHg (n = 5), ET produced dose-dependent and prolonged pressor responses (Fig. 5C) and regularly increased dp/dt max, suggesting venoconstriction. Again, i.v. administration of ET caused a smaller pressor response than when it was given into the arterial circulation.

In the pithed rat, a second injection of ET (1 nmol·kg⁻¹) gave a similar effect to the first (41.7 ± 9.8 and 45.3 ± 13.2 mmHg, respectively; n = 5). However, indomethacin (14 μmol·kg⁻¹, i.v.) given before the second injection brought about an enhancement of the pressor response to 78.3 ± 9.2 mmHg (n = 6; Fig. 6). A similar potentiation was seen with the cyclooxygenase inhibitor piroxicam (15 μmol·kg⁻¹). The control pressor response to ET (1 nmol·kg⁻¹) was 31 ± 9.6 mmHg, whereas after piroxicam it was increased to 72.7 ± 9.9 mmHg (n = 4).

**DISCUSSION**

ET is the most potent pressor agent yet described (9). Our results indicate that ET can also release potent vasodilator substances such as PGI₂ [as shown in guinea pig (20) and rat lungs] and EDRF [as shown in the rat isolated perfused mesentery (21)].
ET and Bay K 8644 (22) did not release EDRF or PG\textsubscript{II} from a column of bovine aortic ECs grown on microcarrier beads in culture. Since such cells are known to lose some receptors (such as those for ACh or 5-hydroxytryptamine), it is possible that they also lack functional voltage-dependent calcium channels, or else these channels are not relevant for the release of EDRF. Indeed, the finding that Bay K 8644 did not induce release of EDRF in the rat isolated perfused mesentery, whereas ET does, would reinforce this latter interpretation.

ET is stable in plasma and whole blood, which adds physiological relevance to the disappearance of ET observed in the lung circulation in vitro and in vivo. Such extensive pulmonary removal (>50% in a single passage) indicates that ET would be cleared from the circulation in three to five circulation times, or 1–2 min. Thus, the long-lasting pressor responses must be due to ET binding to, and continuously activating, vascular smooth muscle. From our results, it is not possible to say whether the disappearance is due to metabolic inactivation or to an uptake system. However, the finding that captopril had no action on the disappearance of ET indicates that ET is not a substrate for angiotensin-converting enzyme.

ET induced release of PG\textsubscript{II} and TX\textsubscript{A}2 in guinea pig lungs and mainly PG\textsubscript{II} in rat lungs. Such a difference in profile oficosanoid release between guinea pigs and rats has been seen with other substances (23). The results obtained with the cyclooxygenase inhibitors indomethacin and piroxicam in rats in vivo are consistent with the release of cyclooxygenase products observed in this model. Indomethacin strongly augmented the pressor activity of ET, indicating that a prostaglandin (probably PG\textsubscript{II}) was indeed limiting the pressor activity of ET. Interestingly, the vasodilator effects of ET in anesthetized rats with high blood pressure were not attenuated by indomethacin, suggesting that these were due mainly to release of EDRF.

Thus, ET has the characteristics of a local hormone, which, when released by the EC, will constrict the underlying vascular smooth muscle. Circulating ET can also release vasodilator substances such as PG\textsubscript{II} and EDRF. One interpretation of these results is that ET released abuminally by ECs acts locally on the underlying smooth muscle causing vasoconstriction, whereas the pressor activity of any ET reaching the circulating blood is limited by the release of PG\textsubscript{II} and EDRF and by inactivation in the lungs. It is noteworthy that ET was more potent as a constrictor on venous than arterial smooth muscle. In addition, the increase in $dp/dt_{max}$ suggests vasoconstriction in these animals. ET is thought to be released in hypoxia (5, 6) so these effects on veins could be an important contribution to the overall reactions of the body to hypoxic stimuli.

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