Endothelin-1 is a Risk Factor for Pathogenesis of Hypertension

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Abstract

The purpose of this present study was to investigate the effects of endothelin-1 (ET-1) on the systemic blood pressure, microvascular blood flow velocity and diameter of arterioles and venules of the rat mesentery in vivo. For this purpose, the mesentery was arranged for in situ intravital microscopic observation under transillumination, and cumulative injections of ET-1(30-2000 pmole/kg) were infused intravenously through a catheter inserted into the right jugular vein. Infusion of low doses of ET-1(30-125 pmole/kg) induced a slight increase in the systemic blood pressure, a dose-dependent increase in blood flow velocity of arterioles (20-30 μm) and venules (30-50 μm). Diameters of arterioles and venules exhibited no significant change as compared with the control data. On the contrary, the infusion of high doses of ET-1(250-2000 pmole/kg) induced a long-lasting pressor effect, a dose-dependent decrease in the blood flow velocity of arterioles and venules. Microvascular diameter exhibited a vasoconstrictive effect more prominent in arterioles than in venules. These findings suggest that vasoconstriction produced by ET-1 in rat mesenteric microcirculation may be the causal factor for its potent pressor effect in rats. Moreover, ET-1 may be involved in the regulation of the blood flow velocity distribution of rat mesenteric microcirculation. Finally, ET-1 may be considered as one of the more important risk factors which contribute to the pathogenesis of hypertension.

Keywords: ET-1; rat mesentery; microvascular blood flow velocity, Intravital microscope system

Introduction

Endothelin-1 (ET-1) is an endothelium-derived 21-amino acid peptide, produced a potent and sustained vasoconstrictive effects on isolated vessels of various experimental animals through its own receptors (Yanagisawa and Masaki, 1989; Yanagisawa *et al.,* 1988). It has been reported that increased rate of release of ET-1 from the vascular endothelium may contribute to the pathogenesis of hypertension (Ernesto, 2005; David, 2005; Pinto-Sietsma and Paul, 1998; Kohno et al., 1990; Saito et al., 1990), cardiogenic shock, chronic dialysis and pulmonary arterial hypertension (Nazzareno *et al.,* 2004; Cernacek and Stewart, 1990), myocardial infarction, congestive heart failure (Ergul *et al.,* 2000) and patients that have undergone chronic hemodialysis (Miyauchi *et al.,* 1991).

ET-1 has been shown to be an extremely potent in contracting isolated arterial vessels and causes a long-lasting pressor response when injected intravenously to chemically enervated rats (Yanagisawa *et al.,* 1988). As have been noted previously, a topical application of endothelin produced either dilation or constriction of cerebral arterioles in newborn pigs depending in concentration (Armstead *et al.,* 1989).

ET-1 was more potent in constricting both arterioles and venules of the rat mesentery as compared with norepinephrine (Fortes *et al.,* 1989). However, the effects of ET-1 on systemic blood pressure as well as microvascular blood flow velocity and diameters (arterioles and venules) of the rat mesenteric microcirculation have not well been documented. Thus, the aim of this present study was to characterize the response of the mesentery microvasculature to an intravenously infused ET-1 (30-2000 pmole/kg) by a direct microscopic observation using an intravital microscopic system, a microvascular blood flow velocity measurement using a ten channel´s dual-sensor method and microvascular diameters measurement directly on a

printed image by means of a graphic video printer.

Material and Methods

Animals

Twenty male Wister rats (Charles River, 130-400 g) were used in these experiments. Each animal was anesthetized with sodium pentobarbital (Nembutal, Abbot Laboratories; 50 mg/kg b. w.). The trachea was incubated to ensure a patent airway, and the right carotid artery and right jugular vein were cannulated. The systemic blood pressure was monitored by use of a pressure transducer (Polygraph system, Nihon kohden) through a catheter inserted into the right carotid artery. The rat mesentery was arranged for in situ intravital microscopic observation under transillumination according to the routine procedure. The mesentery was placed over a transparent plate in a saline bath maintained at a controlled temperature of 37 °C.

Experimental Protocol

ET-1(30-2000 pmole/kg) was dissolved in 0.05% albumin solution (peptide Institute, Osaka, Japan) and infused intravenously through a catheter inserted into the right jugular vein. The changes in systemic blood pressure, diameter and blood flow velocity of arterioles (20-40 μm) and venules (45-65 μm) of rat mesentery were measured simultaneously, 4-6 min. before (control) and after administration of ET-1.

Microvascular Measurements

The images of microvessel were displayed on a monitor TV screen at a magnification of about 100-600. The microvascular diameters were measured directly on a printed image of microvessel by means of a graphic video printer (Sony, Tokyo, Japan, and UP-850). The blood flow velocity of arterioles and venules were measured using a ten-channel's dual sensor method (Sato and Ohshima, 1988), by projecting an image of the microvascular field onto a screen of the dual sensor at a magnification of 200-450. The centerline axis of microvessel image to be studied is adjusted to coincide with the direction of paired dual sensors. These sensors detected the brightness changes of sampling points due to the passing of erythrocytes. The electrical signals of brightness detected by photo sensors were amplified and recorded on a cassette data recorder (Teac XR-

50E, Hitachi Digital Memoriscope VC-810). Off-line analysis, the blood flow velocity of arterioles and venules were measured by use of a one bit cross-correlation technique (62-9300, Osaka Nihon Kagako Kogyo Co., Ltd., Japan).

Data Analysis

The results were expressed as mean ± S.E., statistical analysis for significant differences between control and ET-1 infused was done with Student>s paired t-test. The significance was assessed at 5% confidence level. The blood flow velocity and diameter of arterioles and venules were normalized with their control data.

Results and Discussions

A schematic diagram for examining the mesentery microcirculation in response to bolus injections of ET-1 (30-2000 pmole/kg) is shown in Fig. 1.

The intravenous injection of each dose of ET-1 (30-2000 pmole/kg) in an anesthetized rats elicited a rapid depressor, a transient pressor followed by a long-lasting pressor as shown in Fig. 2.

The systemic blood pressure was measured at the phase of long-lasting pressor, 4-6 min. after each infusion of ET-1. The infusion of low doses of ET-1 (30-125 pmole/kg) elicited a slight pressor effect (Fig. 2). Normalized blood flow velocity (% change in blood flow velocity) of arterioles was ($114 \pm 11\%$ mmHg, Mean ± SE, n = 7) and that of venules was ($123 \pm 7\%$ mmHg,
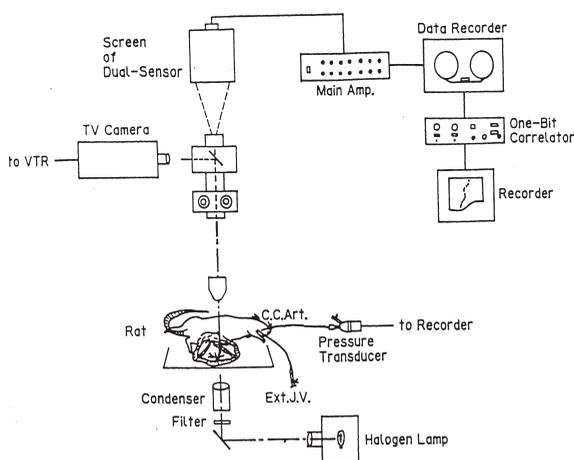

Fig 1. Schematic diagram of the experimental apparatus.

Mean ± SE, n = 7) of the rat mesentery as compared with the control runs (Fig. 3). On the contrary, the infusion of high doses of ET-1 (250-2000 pmole/kg) produced a long-lasting pressor effect (Fig. 2). Systemic blood pressure increased from (128 ± 4 mmHg, n = 4 to 165 ± 5 mmHg, n = 2, at 1000 pmole/kg) (Fig. 2). Normalized blood flow velocity of arterioles was 44 ± 16% mmHg, n = 5, at 1000 pmole/kg, and that of venules was 38 ± 12%, n = 5, at 1000 pmole/kg of ET-1.

Fig. 4 shows that the normalized diameter (% change in diameter) of arterioles was 75 ± 6%, n = 11 and that of venules was 91 ± 4%, n = 11, at 1000 pmole/kg of ET-1 as compared with the control runs. Infusion of 1000 pmole/kg of ET-1 caused impairment stasis for a period of 25 min. in the microvascular blood flow velocity both in arterioles and venules followed by an immediate reversal flow. Whereas, the infusion of 2000 pmole/kg of ET-1 caused a severe damage to the rats, the arterial blood pressure decreased sharply from its baseline level and all the tested rats have died.
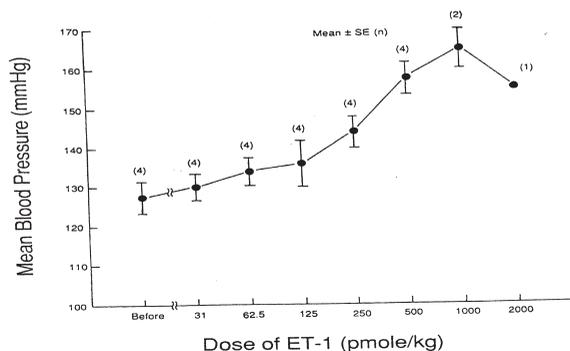

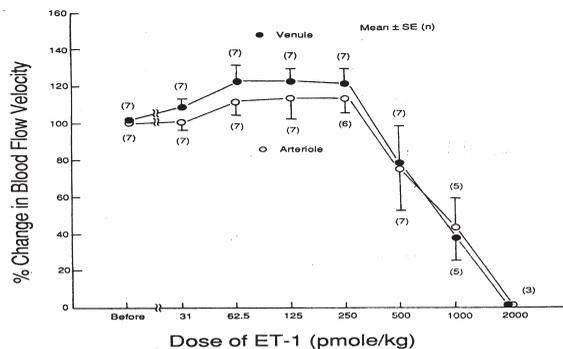**Fig 4.** % Change in diameter versus dose of ET-1 (pmole/kg).

Fig 2. Mean blood pressure (mmHg) versus dose of ET-1 (pmole/kg).

Fig 3. % change in blood flow velocity versus dose of ET-1 (pmole/kg).
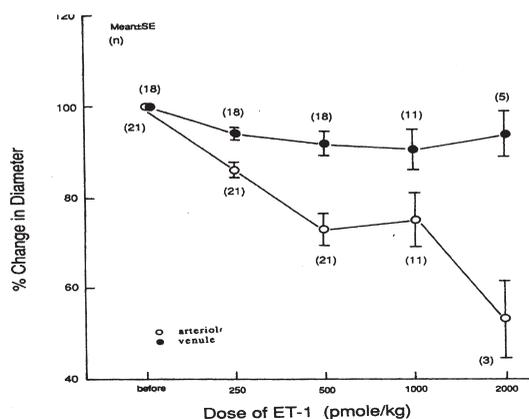
The present study demonstrates that infusion of ET-1 intravenously in anesthetized rats was a dose-dependent response. This response changes from a slight pressor effect, dose-dependent increase in blood flow velocity of arterioles and venules with the infusion of low doses of ET-1 (30-125 pmole/kg) to a long-lasting pressor effect, dose-dependent decrease in microvascular blood flow velocity and vasoconstrictive effect more prominent in arterioles than in venules of the rat mesentery with the infusion of high doses of ET-1 (250-2000 pmole/kg).

These findings seemed to emphasize the more important role of arterioles in regulating the mesenteric microcirculation. Since ET-1 has been reported to exert its action after binding to its specific receptors (Yanagisawa et al., 1988; Yanagisawa and Masaki, 1989), a possible reason for the varying action among arterioles and venules is the distribution of endothelin receptors per unit area in the surface of vascular smooth muscle cells, and it may be higher in arterioles than in venules.

It has been reported that epinephrine and vasopressin are shown to produce a vasoconstriction in large vessels ranging from 100-200 μm in diameter (Saito et al., 1990). Moreover, a topical administration of ET-1 has been produced either a dilation or constriction in piglet cerebral microcirculation, depending on concentration (Armstead et al., 1989). Moreover, ET-1 has been shown to be extremely potent in causing vasoconstriction in the rabbit tenuissimus muscle and hamster cheek pouch (Ohlen et al., 1989). However, no vasoactive agents so far respond, other than ET-1 most potently constricts the small sized microvessel (Homma et al., 1990).

The present findings suggest that ET-1 may play an important role in governing mesentery resistance as well as regulating blood flow velocity distribution of the rat mesenteric microcirculation. Moreover, the infusion of ET-1 constricts small sized arterioles and venules less than 40 μm in diameter i.e., ET-1 most potently constricts the small-sized microvessel. ET-1 may have an important role in governing and regulating the mesenteric microcirculation dimensions and resistance. Moreover, ET-1 may be involved in the regulation of blood flow velocity distribution of rat mesenteric microcirculation. Finally, ET-1 may be considered as one of the more important risk factors which contribute to the pathogenesis of hypertension.

References

Armstead W.M., Mirro R., Leffler C.W. and Busija D.W. 1989. Influence of endothelin on piglet cerebral microcirculation. *A. M. J. Physiol 257 (Heart Circ Physiol* 26: H707-H710.

Cernacek P. and Stewart D.J. 1990. Immunoreactive endothelin in human plasma: Marked elevations in patients in cardiogenic shock. *Biochem and Biophys Res. Community.* 161: 562-567.

Chilian W.M., Layne S.M., Eastham C.L. and Marcus M.L. 1989. Heterogeneous microvascular coronary a-adrenergic vasoconstriction. *Circ Res* 64: 376-388.

Clozel J.P. and Clozel M. 1989. Effects of endothelin on the coronary vascular bed in open-chest dogs. *Circ Res* 65: 1193-1200.

David M.P. 2005. Endothelin, angiotensin, and oxidative stress in hypertension. *Hypertension* 45, 477.

Ergul A., Grubbs A.L., Zhang Y., Spinale F.G. 2000. Selective upregulation of endothelin converting enzyme in the human failing heart. *J. Card. Fail.* 6, 314-320.

Ernesto L.S. 2005. Vascular endothelin in hypertension. *Vascular Pharmacology* 43 (1), 19-29.

Fortes Z.B., de Nucci G. and Garcia-Leme J. 1989. Effect of Endothelin-1 on arterioles and venules in vivo. *J. Cardiovas. Pharmacology* 13: S200-S201.

Homma S., Miyaauchi T., Goto K., Sugishita Y., Sato M., Ohshima N. 1990. Effects of endothelin-1 on coronary microcirculation in isolated beating hearts of rats. *Cardiovas Pharmacology* 17: S276-S278.

Kohno M., Yasunari K., Murakawa K., Yokokawa K., Horio T., Fukui T. and Takeda T. 1990. Plasma Immunoreactive endothelin in essential hypertension. *Am. J. Med.* 88: 614-618.

Koller A. and Kaley G. 1990. Endothelium regulates skeletal muscle microcirculation by a blood flow velocity-sensing mechanism. *Am. J. Physiol.* 258 (Heart Circ Physiol 27): H916-H920.

Masaki T. 1989. the discovery, the present state and the future prospects of endothelin. *J. Cardiovas. Pharmacology.* 13: S1-S4.

Miyauchi T., Ishikawa T., Tomobe Y., Yanagisawa M., Kimura S., Sugishita Y., Ito I., Goto K. and Masaki T. 1989. Characteristics of pressor response to endothelin in spontaneously hypertensive and Wistar-Kyoto rats. *Hypertension* 14: 427-434.

Miyauchi T., Suzuki N., Kurihara T., Yamaguchi I., Sugishita Y., Matsumoto H., Goto K. and Masaki T. 1991. Endothelin-1 and endothelin-3 play different roles in acute and chronic alterations of blood pressure in patients with chronic hemodialysis. *Biochem and Biophys Res Commun* 178: 276-281.

Nazzareno G., Alessandra M. and Angelo B. 2004. The endothelin system in pulmonary arterial hypertension. *Cardiovascular Research* 61 (2), 227-237.

Ohlen A., Raud J., Hedqvist P. and Wiklund N.P. 1989. Microvascular effects of endothelin in the rabbit tenuissimus muscle and hamster cheek pouch. *Microvascular Res* 37: 115-118.

Pinto-Sietsma S.J. and Paul M. 1998. A role for endothelin in the pathogenesis of hypertension. *Kidney Int Suppl* 67, S115-S121.

Rosenblum W.I. 1986. Endothelial dependent relaxation demonstrated in vivo in cerebral arterioles. Stroke 17: 494-497.

SaitoY., Nakao K., Mukoyama M. and Imura H. 1990. Increased Plasma endothelin level in patients with essential hypertension [Letter]. *N. Engl. J. Med.* 322: 205.

Sato M. and Ohshima N. 1988. Velocity profiles of blood flow in microvessel measured by ten channels> dual-sensor method. *Biorheology* 25: 279-288.

Yanagisawa M., Kurihara H., Kimura S., Tomobe Y., Kobayashi M., Mitsui Y., Yazaki Y., Goto K. and Masaki T. 1988: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 332: 411-415.

Yanagisawa M. and Masaki T. 1989. Endothelin, a novel endothelium-derived peptide. Pharmacological activities, regulation and possible roles in cardiovascular control. *Biochemistry Pharmacology* 38: 1877-1883.

يعتبر ET-1 عامل خطر وهام في تولد مرض ضغط الدم المرتفع

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الملخص

الهدف من هذه الدراسة هو فحص تأثيرات الاندوسيلين-١ (ET-1) علي ضغط الدم، وسرعة مرور الدم، وقطر الأوعية الدموية الدقيقة في مساريقا (الأغشية التي تغلف الأمعاء وتربطها بالجدار البطني) الفئران الحية، ولهذا الغرض تم إعداد مساريقا الفئران الحية للملاحظة الميكروسكوبية تحت الفحص الضوئي، ثم الحقن المتراكم بجرعات من الاندوسيلين-١ (30-2000 /pmole kg) وريديا من خلال أنبوبة مطاطية أدخلت في مجري الوريد الو داجي. أحدث حقن الجرعات المنخفضة من 125-30) ET-1 pmole/kg) ذيادة طفيفة في ضغط الدم، وزيادة كافية في سرعة مرور الدم في الشرايين (30-20μm) والأوردة (30-50μm) الدموية الدقيقة، ولم يحدث إي تغيرات كافية في أقطار الشرايين والأوردة الدموية الدقيقة قبل الحقن (قيم التحكم). علي العكس تماما، أحدث حقن الجرعات العالية من 250-2000) ET-1 pmole/kg) وريديا ضغط دم عالي دائم وثابت لمدة طويلة، نقص كاف في سرعة مرور الدم في الشرايين والأوردة الدموية الدقيقة، وانقباض وتقلص هذه الأوعية الدموية الدقيقة، والتي ظهرت بصورة واضحة في الشرايين عن الأوردة الدموية الدقيقة. تقترح هذه النتائج البحثية إن انقباض وتقلص الأوعية الدموية الدقيقة الموجودة في مساريقا الفئران والناتجة عن حقن ET-1 وريديا ربما يكون السبب الأساسي لها هو رفع ضغط الدم. علاوة علي أن ET-1 ربما يقوم بدور هام في تنظيم سرعة مرور الدم في الأوعية الدموية الدقيقة لهذه الفئران، وفي النهاية ربما يعتبر ET-1 احد العوامل الخطرة والهامة والتي تلعب دور هام في نشوء وتولد ضغط الدم المرتفع.