

The Role of Phosphoramidon on the Biological Activity of Big Endothelin-1 in the Rat Mesenteric Microcirculation in Vivo

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Abstract

The goal of the present study was to clarify the role of metalloprotease inhibitor phosphoramidon on the effects induced by big endothelin-1 (big ET-1) in the rat mesenteric microcirculation in vivo, through investigating the systemic blood pressure, diameter and blood flow velocity of arterioles and venules of the rat mesentery. For this purpose, the rat mesentery was arranged for in situ intravital microscopic observation under transillumination and separate cumulative injections of big ET-1 and phosphoramidon were infused into the right jugular vein, respectively. In these experiments twenty-five rats (Charles River, 130 - 140 g) were used. The experiments were divided into two groups. In the first group of experiments, cumulative injections of big ET-1 (1000-8000 pmole/kg) were infused through a catheter inserted into the right jugular vein. Each dose of big ET-1 was infused 25 min prior to the infusion of the following dose. Infusion of big ET-1 (1000-8000 pmole/kg) elicited a long-lasting pressor effect. The infusion of low doses of big ET-1 (1000-2000 pmole/kg) elicited a significant ($p < 0.05$) dose-dependent increase in the microvascular blood flow velocity both in arterioles (20 - 30 μm) and venules (30 - 50 μm), and diameters of arterioles and venules exhibited a slight not significant vasodilator effect. The infusion of high doses of big ET-1 (4000-8000 pmole/kg) elicited significant dose-dependent decrease in the blood flow velocity of arterioles and venules, and diameters returned to the control runs. This may be attributed to the gradual conversion of big ET-1 to ET-1, and ET-1 is a potent vasoconstrictor. In the second group of experiments, cumulative injections of phosphoramidon (30 mg/kg /10 min) were administered 10 min prior to the infusion of big ET-1. These findings suggested that phosphoramidon significantly suppressed long-lasting pressor effect, dose-dependent increase, dose-dependent decrease and slow vasodilator effect produced by big ET-1 in the rat mesenteric microcirculation, i.e., phosphoramidon markedly decreased the activity of endothelin-converting enzymes (ECE), which converts big ET-1 to ET-1 in the rat mesenteric microcirculation.

Key words: Big endothelin-1; phosphoramidon (PR); blood flow velocity; rat mesentery; microcirculation.

Introduction

It has been reported by Yanagisawa and Masaki, 1989 that ET-1 is formed from a 39-amino acid (big endothelin-1) by endothelin-converting enzymes (ECE), which are found whether in the endothelium (McMahon et al., 1991; Hisaki et al., 1991; Fukuroda et al., 1990; Ikegawa et al., 1990; Kohno et al., 1990) or in the vascular smooth muscle cells (VSMC) (McMahon et al., 1989). Plasma ET-1 and big ET-1 were elevated in patients with hypertension (David, 2005; Pinto-Sietsma and Paul, 1998; Kohno et al., 1990; Saito et al., 1990), the endothelin system in pulmonary arterial hypertension (Nazzareno et al., 2004), vascular endothelin in

hypertension (Ernesto, 2005), cardiogenic shock (Cernacek and Stewart, 1990), myocardial infarction and patients undergone chronic hemodialysis (Miyauchi et al., 1991; Homma et al., 1990). Thus, it is speculated that not only ET-1, but rather big ET-1 are involved in the pathophysiology of other diseases. It has been reported that the injection of big ET-1 intravenously into anesthetized ganglion-blocked rats produced a sustained pressor response that presumably was due to the conversion of big ET-1 to ET-1 by ECE (Ergul et al., 2000; McMahon et al., 1991; D'Orleans-Juste et al., 1990). Inhibition of ECE should effectively block the

biological effect of ET-1 (Hisaki et al., 1991; Miyauchi et al., 1991). As have been demonstrated a metalloprotease inhibitor, phosphoramidon-dose dependently originally found in cultured endothelial cells (Hisaki et al., 1991; Ikegawa et al., 1990; McMahon et al., 1989). However, the effects of phosphoramidon on inhibiting the conversion of big ET-1 to ET-1 in the macrocirculation and microcirculation have not been well documented. Moreover, the role of phosphoramidon on the effects produced by big ET-1 in the rat mesenteric microcirculation has not been reported. The primary purpose was to clarify the role of phosphoramidon on the effects produced by big ET-1 in the rat mesenteric microcirculation through investigating the systemic blood pressure, blood flow velocity and diameter of arterioles and venules of the rat mesentery.

Materials and methods

Animals

Twenty-five 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). To ensure a patent airway, the trachea was incubated. 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 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

In the present study, two groups of experiments were used. In each group of experiments, the mesentery was arranged for in situ intravital microscopic observation under transillumination and separate cumulative injections of big ET-1 and phosphoramidon were infused into the right jugular vein, respectively. In the first group of experiments, cumulative injections of big ET-1 (1000-8000 pmole/kg) were infused through a catheter inserted into the right jugular vein, each dose of big ET-1 was infused 25 min. prior to the infusion of the following dose. In the second group of experiments, cumulative injections of phosphoramidon (30 mg/kg/10 min.) were administered 10 min. prior to the infusion of each dose of big ET-1. Synthetic big ET-1

(1000, 2000, 4000 and 8000 pmole/kg) was dissolved in 0.05% albumin solution (peptide Institute, Osaka, Japan), and infused separately and intravenously through a catheter inserted into the right jugular vein. The changes in systemic blood pressure, microvascular diameter (arterioles 20-30 μ m and venules 30-50 μ m) and microvascular blood flow velocity were measured Off-line analysis, simultaneously.

Microvascular measurements

Images of arterioles (20-30 μ m) and venules (30-50 μ m) of the rat mesentery were displayed on a monitor TV screen at a magnification of about 200-600. Diameters of arterioles and venules were measured directly on a printed image 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 200-400. The centerline axis of microvessel image to be studied was adjusted to coincide with the direction of paired dual sensors. These sensors detected the brightness changes of the sampling points due to the passing of red blood cells. The electrical signals of brightness detected by photo sensors were amplified and recorded on a cassette data recorder (Teac XR-50E, and Hitachi Digital Memoriscop VC-810). Off-line analysis, the blood flow velocity of arterioles and venules were measured by use of one bit cross-correlation technique (62-9300, Osaka Nihon Kagako Kogyo Co., Ltd., Japan).

Data analysis

The results were expressed as mean \pm S.E., statistical analysis for significant differences between big ET-1 infused, phosphoramidon infused and non-infused groups (control) were done with student's paired t-test. The significance was assessed at 5% confidence level. The blood flow velocity and diameters of arterioles and venules were normalized (% change) with their control data.

Results

A schematic diagram of the experimental apparatus used for investigating the rat mesentery microcirculation to bolus injections of big ET-1 and phosphoramidon is shown in Fig. 1. Infusion of big ET-1 at doses of 1000, 2000, 4000 and 8000 pmole/kg induced a long-lasting pressor response as shown in Fig. 2. The systemic blood pressure significantly

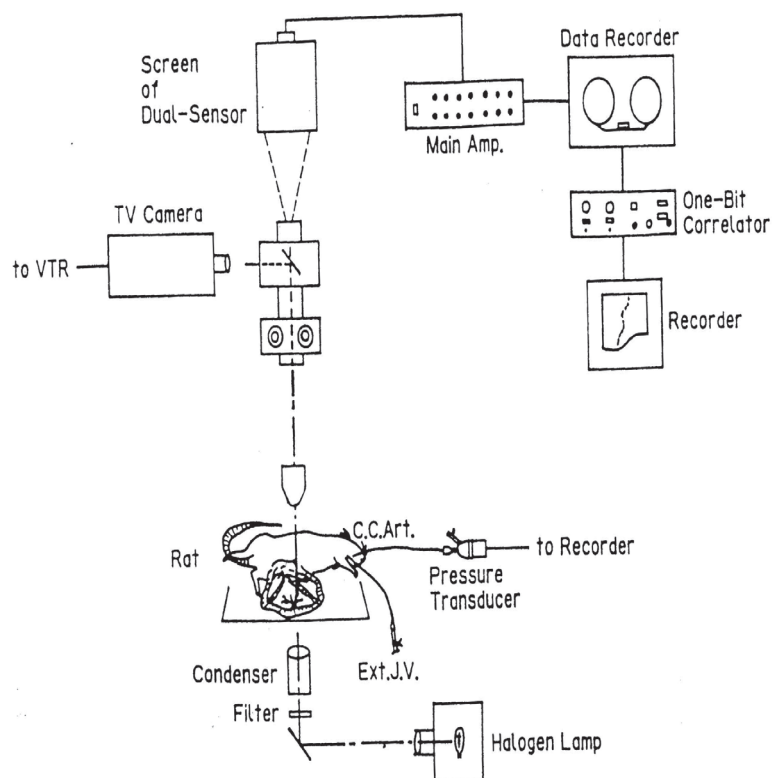


Fig. 1 Schematic diagram of the experimental apparatus.

($p < 0.05$) increased from 123 ± 5 mmHg (mean + SE, $n = 11$) in control groups to 150 ± 5 ($n = 9$) at a maximum dose of 8000 pmole/kg. The administration of phosphoramidon (PR; 30 mg/kg/10 min) just 10 min prior to the infusion of big ET-1, greatly suppressed the pressor effect of big ET-1, the systemic blood pressure slightly and not significantly ($p < 0.05$) increased from 127 ± 10 mmHg in control groups to 136 ± 3 ($n = 5$) at 8000 pmole/kg (Fig. 2). Administration of big ET-1, 1000 and 2000 pmole/kg elicited a significant ($p < 0.05$) increase in blood flow velocity of arterioles ($135 \pm 7\%$ at 1000 pmole/kg, and $113 \pm 9\%$ at 2000 pmole/kg) and venules ($138 \pm 7\%$, at 1000 pmole/kg, $120 \pm 9\%$ at 2000 pmole/kg) as shown in Fig. 3. Slight increase in the microvascular diameters ($9 \pm 4\%$, $n = 9$ at 2000 pmole/kg) as shown in Fig. 4. However, the administration of big ET-1, 4000 and 8000 pmole/kg decreased significantly ($p < 0.05$) the microvascular blood flow velocities as compared with those of the control runs as shown in Fig. 3, and the microvascular diameters returned to the control values as

shown in Fig. 4.

In Fig. 3, the administration of phosphoramidon significantly inhibited the dose-dependent increase and dose-dependent decrease in the blood flow velocity of arterioles and venules produced by big ET-1 in the rat mesentery.

Discussion

The present study demonstrates that cumulative injections of big ET-1 (1000-8000 pmole/kg) infused separately and intravenously through a catheter inserted into the right jugular vein, elicited along-lasting pressor effect. It has been reported that the estimated ED 50 value of big ET-1 was about 2.4-fold less potent than of ET-1, a finding which suggests gradual conversion of big ET-1 to ET-1 may contribute to the long-lasting pressor effect of big ET-1 (McMahon et al., 1991; Hisaki et al., 1991; Fukuroda et al., 1990; D'Orleans-Juste et al., 1990). It has been reported that a bolus injection of human big ET-1 into the left ventricle of anesthetized rabbits produced a marked increase in

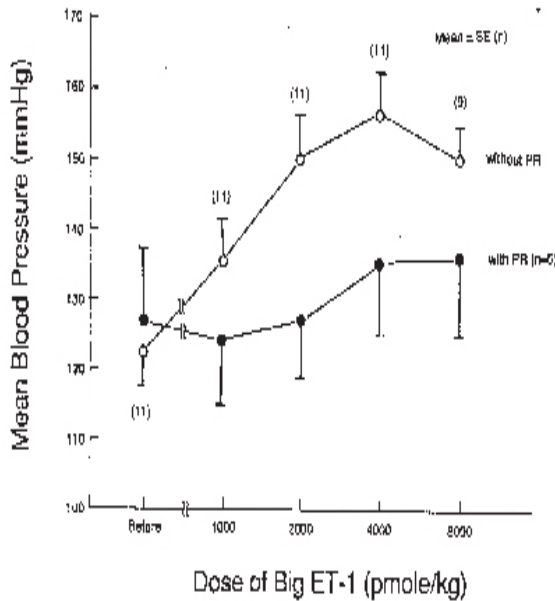


Fig 2. Represents mean blood pressure (mmHg) versus dose of big ET-1 (pmole/kg).

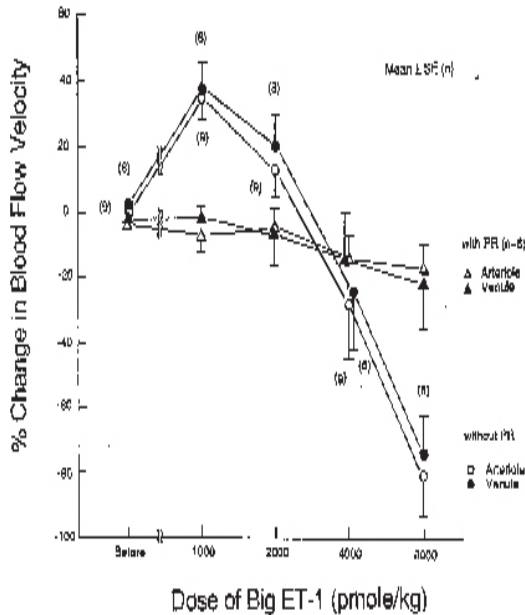


Fig 3. Represents % change in blood flow velocity versus dose of big ET-1 (pmole/kg).

the plasma levels of ET-1, a finding which suggested that a slow conversion of big ET-1 to ET-1 contributes to the long-lasting pressor effect of big ET-1 (Yanagisawa et al., 1988). In the present study, the infusion of big ET-1 (1000-2000 pmole/kg) and big ET-1 (4000-8000 pmole/

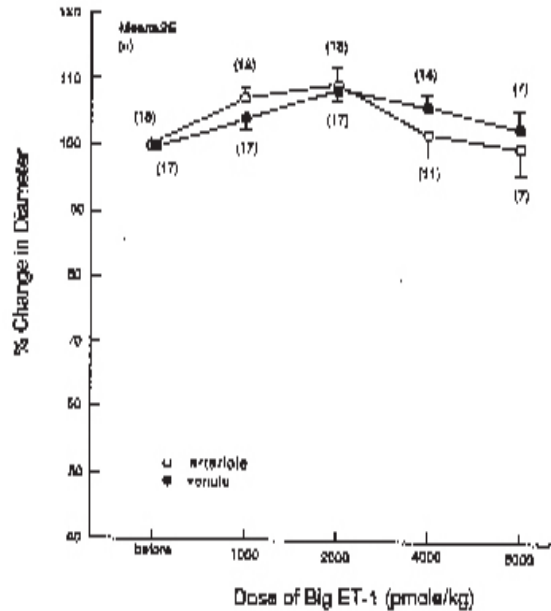


Fig 4. Represents % change in diameter versus dose of big ET-1 (pmole/kg).

kg) elicited significant dose-dependent increase and dose-dependent decrease in the blood flow velocity of arterioles and venules, respectively. The microvascular diameter of arterioles and venules exhibited no significant changes with the infusion of big ET-1 (1000-8000 pmole/kg) as compared with the control data. It becomes conceivable that a gradual conversion of big ET-1 (4000-8000 pmole/kg) to ET-1 (250-2000 pmole/kg) approximately is responsible for the dose-dependent increase and dose-dependent decrease of microvascular blood flow velocity, respectively. It has been reported that big ET-1 has a weak vasoconstrictor activity about two orders of magnitude less than that of ET-1 (Fukuroda et al., 1990). Big ET-1 or ET-1 have been caused contraction of isolated porcine coronary arteries, but the potency of big ET-1 was about 1/150 that of ET-1 (Homma et al., 1990). Thus, it is likely that big ET-1 is less converted to ET-1 in the rat mesentery than in any other organs, due to the absence of some unknown enzymes, promoting the conversion of big ET-1 to ET-1. It has been reported that a metalloproteinase inhibitor may participate in the intracellular processing of big ET-1 in the vascular endothelial cells (Hisaki et al., 1991; Fukuroda et al., 1990). As we have noted previously, big ET-1 caused a very small contraction in the isolated rat aorta, and no

difference in magnitude of the contractile response to big ET-1 in the presence or absence of endothelium, i.e., the processing of big ET-1 must occur in the vascular smooth muscle cells (Miyachi et al., 1991). In the present study, the administration of phosphoramidon 10 min. prior to the infusion of big ET-1 significantly (* $p < 0.05$) suppressed the long-lasting pressor effect, the dose-dependent increase in the microvascular blood flow velocity, and the dose-dependent decrease in the microvascular blood flow velocity produced big ET-1 in the rat mesentery. It seems reasonable to consider that big ET-1 is gradually converted to ET-1 in the microcirculation of rat mesentery, and phosphoramidon markedly decreased the activity of endothelin-converting enzymes (ECE), which converts big ET-1 to ET-1 in the rat mesenteric microcirculation. It becomes also conceivable as demonstrated from the results of the present study that phosphoramidon is a powerful blocker of ET-1.

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دور Phosphoramidon علي التأثيرات الناتجة من Big Endothelin-1 في الأوعية الدموية الدقيقة لمسار ريقا الجرزان الحية

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المخلص

الهدف من هذه الدراسة الحالية هو إيضاح الدور المثبط لل metalloprotease phosphoramidon علي التأثيرات الناتجة من big endothelin-1 في مسار ريقا الجرزان الحية، من خلال دراسة ضغط الدم، قطر وسرعة مرور الدم في الأوعية الدموية الدقيقة لهذه الجرزان. لهذا الغرض، تم أعداد مسار ريقا الجرزان الحية للملاحظة الميكروسكوبية تحت الفحص الضوئي والحقن المنفصل والمتراكم بجرعات من big endothelin-1 و phosphoramidon وريديا من خلال أنبوبة مطاطية مدخلة في مجري الوريد الوداجي، علي التوالي. استخدم خمسة وعشرين جرزا (Charles River, 130-140 g) في هذه التجارب. قسمت التجارب إلي مجموعتين. في المجموعة الأولى من هذه التجارب، تم حقن متراكم بجرعات من big endothelin-1; 1000 -8000 pmole/kg وريديا من خلال الأنبوبة المطاطية المدخلة في مجري الوريد الوداجي. استغرقت كل جرعة من big endothelin-1 فترة خمسة وعشرين دقيقة قبل حقن الجرعة التالية لها. أحدث حقن جرعات من big endothelin-1 من 1000 – 8000 pmole/kg زيادة مستديمة في ضغط الدم. أحدث حقن الجرعات المنخفضة من big endothelin-1 من 1000-2000 pmole/kg زيادة كافية (* $p < 0.05$) في سرعة مرور الدم في الشرايين (20-30 μm) والأوردة (30-50 μm) الدموية الدقيقة، وذادت أقطار الشرايين والأوردة الدموية الدقيقة زيادة طفيفة ولكنها ليست ذات دلالة إحصائية. أحدث حقن الجرعات العالية من big endothelin-1 من 4000 -8000 pmole/kg نقص ذا دلالة إحصائية (* $p < 0.05$) في سرعة مرور الدم في الشرايين والأوردة الدموية الدقيقة، وتراجعت أقطار هذه الشرايين والأوردة الدموية الدقيقة لقيم التحكم. في المجموعة الثانية من هذه التجارب، تم حقن متراكم بجرعات من phosphoramidon (30 mg/kg/10 min) قبل حقن جرعات big endothelin-1 بعشرة دقائق. اقترحت هذه النتائج إن phosphoramidon قد تغلب علي الزيادة المرتفعة والدائمة والممتدة في ضغط الدم بدرجة كافية، والزيادة في سرعة مرور الدم في الشرايين (20-30 μm) والأوردة (30-50 μm) الدموية الدقيقة، والزيادة الطفيفة في أقطار الشرايين والأوردة الدموية الدقيقة في مسار ريقا هذه الجرزان عند حقن الجرعات المنخفضة من big endothelin-1 من 1000 – 2000 pmole/kg ، وتعني هذه النتائج البحثية إن phosphoramidon قد منع تحويل big endothelin-1 إلي endothelin-1 في الأوعية الدموية الدقيقة لمسار ريقا هذه الجرزان، والذي ربما يعزي إلي منع الإنزيم الذي يقوم بتحويل big endothelin-1 إلي endothelin-1.