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Diltiazem Facilitates Endothelin Clearance from the Blood Stream to Reduce Toxic Elevation of Plasma Endothelin Level in Rodents*

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Abstract—Pretreatment with diltiazem at a dose of 2 mg kg^{-1} intravenously protected against sudden death induced by intravenous administration of endothelin-1 (ET-1, 5 nmol kg^{-1}), with an apparent decrease in the plasma immunoreactive-ET-1 (IR-ET-1) in mice. These effects, which were also observed in anaesthetized rats, disappeared in rats with bilateral ligation of the renal arteries. In the latter, the exogenous ET-1-induced elevation of plasma IR-ET-1 tended to be higher than that in the sham-operated controls. Furthermore, in anaesthetized rats, diltiazem inhibited ET-1-induced decreases in renal blood flow and increased renal accumulation of IR-ET-1. These results indicate that part of the clearance of ET-1 from the bloodstream occurs in the kidney, and that diltiazem enhances the elimination of the peptide, presumably by improvement in the renal circulation, this action leading to alleviation of the toxic effects of ET-1.

Endothelin-1 (ET-1) is a 21-amino acid peptide isolated from cultured porcine endothelial cells (Yanagisawa et al 1988). Since ET-1 has been reported to exert various pharmacological actions, including highly potent vasoconstriction, it has been speculated that it may play an important pathophysiological role in several diseases, such as acute renal failure (Shibouta et al 1990; Mino et al 1992), myocardial infarction (Watanabe et al 1991) and pulmonary hypertension (Stewart et al 1991; Yoshibayashi et al 1991).

ET-1 given exogenously, however, is rapidly eliminated from the circulation. In man, besides pulmonary clearance, renal and limb uptake of the peptide is known to contribute to the short half-life ($t_{\frac{1}{2}}$) (Gasic et al 1992; Wagner et al 1992). In rats, intravenously injected ET-1 is immediately cleared mainly through the lung, kidney and liver with $t_{\frac{1}{2}}$ ranging from 40 s to 7 min (Anggard et al 1989; Shiba et al 1989; Sirvio et al 1990; Suzuki et al 1990). In our previous study using mice, ET-1 at a dose of 2·5 nmol kg^{-1} was also completely eliminated within 5 min (Matsuura et al 1992).

Intravenous administration of ET-1 caused sudden death in rats and mice which was prevented by Ca^{2+}-channel blockers (Terashita et al 1989; Matsuura et al 1992), but the preventive mechanism was explained only by their Ca^{2+}-antagonistic activity. In addition, the effects of Ca^{2+}-channel blockers on plasma immunoreactive ET-1 (IR-ET-1) and its clearance remain to be clarified.

In the present study, we examined the effect of diltiazem, a Ca^{2+}-channel blocker, on ET-1-induced sudden death and the elevation of plasma IR-ET-1 in both rats and mice.

Materials and Methods

Chemicals

Human ET-1 and phosphoramidon were purchased from the Peptide Institute Inc. (Osaka, Japan). Rabbit ET-1 antiserum was purchased from Novabiochem AG (Geneva, Switzerland). Diltiazem, verapamil and nifedipine were purchased from Sigma (St Louis, USA).

Animals

Male ICR mice (6–7 weeks old, Charles River Japan, Inc.) and male Sprague-Dawley rats (8–10 weeks old, Charles River Japan, Inc.) were used in this study. For renal blood flow measurement, male Sprague-Dawley rats, 560–670 g, were used. All animals were kept under standard conditions (temperature, 22–24°C; humidity, 50–60%) with free access to standard food and tap water.

ET-1-induced sudden death in mice

Saline, diltiazem (0·125, 0·5 or 2 mg kg^{-1} as the hydrochloride salt), verapamil hydrochloride (2 mg kg^{-1}) or nifedipine (0·5 mg kg^{-1}) was intravenously administered to mice 10 min before injection of ET-1 (5 nmol kg^{-1}, i.v.). The volume of all treatments was 0·05 mL/10 g body weight. Mortality (%) and latency (s) to death, as judged by the cessation of respiration, were measured for 60 min after ET-1 injection as described previously (Matsuura et al 1992).

Measurement of plasma IR-ET-1 in mice and rats

In mice, either saline, diltiazem (2 mg kg^{-1}), nifedipine (0·5 mg kg^{-1}) or phosphoramidon (2 mg kg^{-1}) were intravenously administered 10 min before ET-1 (5 nmol kg^{-1}) injection. Blood samples were collected by cardiac puncture at 0, 1, 3 and 5 min after ET-1 injection as described previously (Matsuura et al 1992). Rats, 200–240 g, were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.). After tracheal intubation, a polyethylene catheter was inserted into the left carotid artery for blood sampling. The right femoral vein was cannulated for drug administration. Diltiazem was given intravenously at doses ranging from 0·125 to 2 mg kg^{-1}, 10 min before ET-1 injection. Thereafter, blood samples (0·1 mL) were collected at 0, 1, 3 and 5 min. The amount of IR-ET-1 in the plasma was measured by enzyme immunoassay (EIA) as described previously (Mat-

*A preliminary account of some of the results has been presented to the Japanese Pharmacological Society (Ashizawa et al 1992).

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suura et al 1992). Competitive EIA for ET-1 was employed using rabbit ET-1 antiserum-coated microtest plates, and after blocking with 10% foetal bovine serum, the mixture of standard ET-1 or 1% plasma and β-galactosidase-labelled ET-1 was incubated. After washing, the bound enzyme activity was measured using 4-methylumbelliferyl-β-D-galactoside as a substrate. Plasma at a concentration of 1% (v/v) did not affect the response in EIA.

Measurement of IR-ET-1 in mouse kidney

Male ICR mice were used. Saline or diltiazem was administered intravenously 10 min before injection of ET-1 (2·5 nmol kg^{-1}, i.v.). At 0, 1, 3 and 5 min after ET-1 injection, the left kidney of each mouse was rapidly removed and frozen on dry ice. IR-ET-1 in the kidney was measured according to the method of Shibouta et al (1990). The tissues were homogenized in 4 vol chloroform:methanol (2:1) at 4°C. After 0·2 vol water had been added to the homogenates, they were centrifuged at 2500 *g* for 25 min. The aqueous phase was then acidified with 3 vol acetic acid (4%). The solution containing 3% acetic acid was pumped at a rate of 1 mL min^{-1}, through a Sep-Pak C18 cartridge (Waters Associates, Milford, MA) and after washing with 10 mL water, the absorbed peptides were eluted with methanol. After evaporation, the dry residue was dissolved in an assay buffer and subjected to IR-ET-1 assay as described previously (Matsuura et al 1992).

Reverse-phase high-performance liquid chromatography (RP-HPLC)

The IR-ET-1 fraction was separated on RP-HPLC as described previously (Matsuura et al 1992). A Capcellpak C18 SG120 column (4·6 × 150 mm, Shiseido, Tokyo, Japan) was used. ET-1 was eluted in a 30-min linear gradient of 0–50% acetonitrile in 0·02% trifluoroacetic acid followed by isocratic elution at 50% acetonitrile in 0·02% trifluoroacetic acid for 20 min at 0·5 mL min^{-1}; the retention time of ET-1 was 28·4 min.

ET-1-induced sudden death and bilateral ligation of renal arteries in rats

In male rats anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.), the left carotid artery and right femoral vein were cannulated for measurement of arterial blood pressure and for the injection of saline, diltiazem or ET-1, respectively. Saline (1 mL kg^{-1}) or diltiazem (2 mg kg^{-1}, i.v.) was given intravenously 10 min before ET-1 injection at a lethal dose of 5 nmol kg^{-1}. Mean arterial blood pressure was recorded on a polygraph (Nihon Kohden, RM-6000) via a pressure transducer (Nihon Kohden, TP-300T) for 60 min after the injection of ET-1. Mortality (%) and latency to death (s) was judged by the fall in mean arterial blood pressure (less than 25 mmHg) according to the method of Terashita et al (1989). Renal arteries of both kidneys were isolated. Renal artery ligation was performed in the experimental group 10 min before saline or diltiazem administration, and the ligation was continued throughout the experiment. ET-1 was administered intravenously 10 min after the injection of saline or diltiazem.

Measurement of renal blood flow in anaesthetized rats

Male Sprague-Dawley rats, 560–670 g, were used. Under sodium pentobarbitone anaesthesia (60 mg kg^{-1}, i.p.), the abdomen was incised and the left renal artery was isolated from the surrounding tissue. An electromagnetic blood flow meter (Nihon Kohden, MFV-2100) was used to measure renal blood flow with a magnetic flow probe (Nihon Koden, model 010T, 1 mm diam.) attached around the left renal artery. Five minutes after the intravenous administration of saline or diltiazem (2 mg kg^{-1}), ET-1 (1 nmol kg^{-1}, i.v.) was injected via the femoral vein, and the changes in renal blood flow were recorded for 10 min after ET-1 injection.

Statistical analysis

In experiments on lethality, if an animal survived over 60 min, its latency was regarded as 3600 s. Statistical analysis was as described previously (Matsuura et al 1992), i.e. mortality was evaluated by the Fisher exact probability test and others were evaluated using an unpaired Student's *t*-test or one-way analysis of variance with the Bonferroni modification. $P < 0.05$ was considered statistically significant.

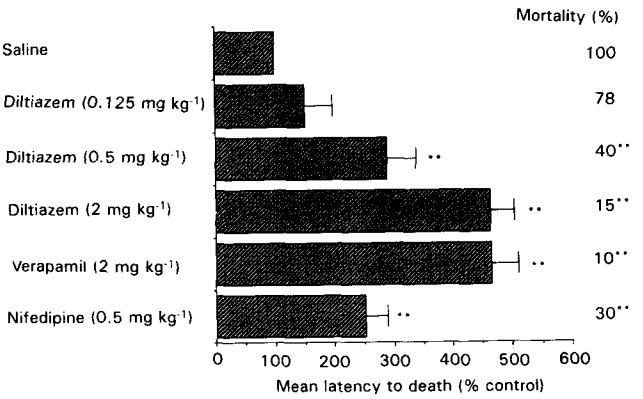

FIG. 1. Effects of Ca^{2+}-channel blockers on ET-1-induced sudden death in mice. Each drug was intravenously administered 10 min before injection of ET-1 (5 nmol kg^{-1}). Latency to death in each group was expressed as percent of control. Nine to thirteen mice were used in each group. Values are means ± s.e.m. $^{**}P < 0.01$ compared with saline control.

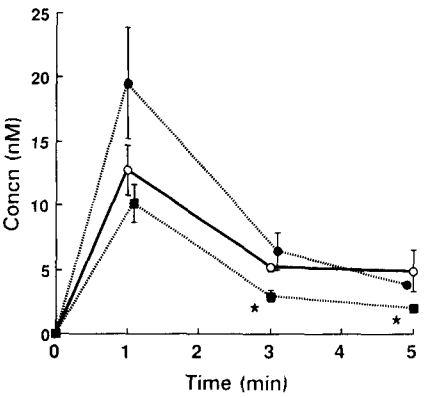

FIG. 2. Plasma IR-ET-1 in mice after the administration of ET-1 (5 nmol kg^{-1}), and the effects of diltiazem (2·0 mg kg^{-1}, i.v.) and phosphoramidon (2·0 mg kg^{-1}, i.v.). Blood samples were collected by cardiac puncture. ○ Saline control; ● phosphoramidon; ■ diltiazem. Seven mice were used in each group. Values are means ± s.e.m. $^{*}P < 0.05$ compared with saline control.

Table 1. Effects of diltiazem and bilateral renal artery ligation on ET-1-induced sudden death in anaesthetized rats.

	Mortality (%)	Latency[a] (min)	Changes[b] (%)
Sham-operated			
Saline	80	24·8 ± 6·4	0
Diltiazem	50#	38·7 ± 7·2*##	+55·8
Renal artery ligation			
Saline	100	18·8 ± 2·2	−24·5
Diltiazem	80	27·1 ± 5·6	+9·2

Bilateral renal artery ligation was performed 10 min before saline (1 mL kg^{-1}) or diltiazem (2 mg kg^{-1}) injection, and the occlusion was continued throughout the experiment. In the sham-operated group, renal arteries of both kidneys were isolated but not ligated. ET-1 was administered intravenously 10 min after the injection of diltiazem or saline. Mortality was assessed 60 min after ET-1 injection. If an animal survived beyond 60 min, its latency was regarded as 60 min. Ten rats were used in each group.

[a] Values are means ± s.e.m.

[b] Changes from sham + saline group as shown in percent.

* $P < 0.05$ compared with corresponding saline-treated animals

# $P < 0.05$, ## $P < 0.01$ compared with saline-treated, renal artery-ligated animals.

Results

ET-1-induced sudden death in mice

The latency to sudden death after the administration of ET-1 (5 nmol kg^{-1}) was 392·1 ± 69·1 s; no mouse survived beyond 10 min after ET-1 administration. Diltiazem at a dose of 0·125 to 2 mg kg^{-1} dose-dependently prolonged the latency to death and suppressed mortality (Fig. 1). Other Ca^{2+}-channel blockers, verapamil (2 mg kg^{-1}) and nifedipine (0·5 mg kg^{-1}), also inhibited ET-1-induced sudden death (Fig. 1).

Plasma IR-ET-1 in mice and rats

When ET-1 (5 nmol kg^{-1}) was administered intravenously to mice, plasma IR-ET-1 rapidly increased with a maximum concentration of 12·3 nM at 1 min after the injection and then rapidly decreased (Fig. 2). RP-HPLC analysis indicated that IR-ET-1 had a retention time corresponding to intact ET-1. Diltiazem, at a dose of 2 mg kg^{-1} intravenously, suppressed the elevation of IR-ET-1 by about 20%, 40% and 60% at 1, 3 and 5 min after ET-1 administration, respectively. In addition, pretreatment with nifedipine (0·5 mg kg^{-1}) significantly decreased plasma IR-ET-1 at 1 min after ET-1 injection (data not shown). Following phosphoramidon (a metalloproteinase inhibitor) administration, there tended to be higher IR-ET-1 levels than in the controls (Fig. 2). In anaesthetized rats, effects of diltiazem (0·125, 0·5 and 2·0 mg kg^{-1}) on plasma IR-ET-1 were also examined. Pretreatment with diltiazem (2 mg kg^{-1}) reduced IR-ET-1, but the effect was not significant.

Effects of diltiazem and bilateral ligation of renal arteries on ET-1-induced sudden death and plasma IR-ET-1 in rats

Table 1 shows the effects of diltiazem and renal artery ligation on mortality and latency to death in ET-1 (5 nmol kg^{-1})-induced sudden death using anaesthetized rats. In sham-operated control rats, a sudden drop in blood pressure, resulting in sudden death, was observed in 8 out of 10 rats within 40 min after the injection of ET-1. Renal artery ligation did not significantly promote ET-1-induced death. Pretreatment with diltiazem at a dose of 2 mg kg^{-1} intravenously, significantly prolonged latency to death by 55·8% ($P < 0.05$) in sham-operated animals, but this protective effect of diltiazem disappeared with renal artery ligation. Therefore, alleviation of the toxic effect of ET-1 by diltiazem is partly attributable to its effects on renal function.

The effects of diltiazem and renal artery ligation on IR-ET-1 in plasma are shown in Fig. 3 and the overall effect as measured by the area under the curve is shown in Table 2.

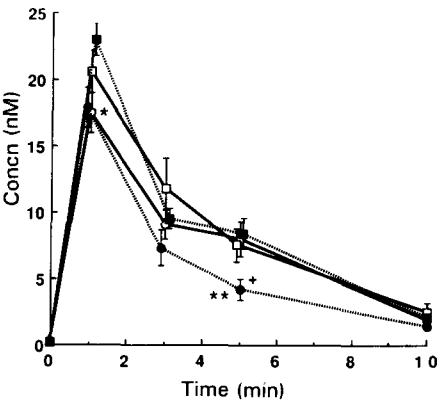

FIG. 3. Plasma IR-ET-1 after the administration of ET-1 (5 nmol kg^{-1}), and the effects of diltiazem and renal artery ligation (RAL). ○ Sham + saline; ● sham + diltiazem; □ RAL + saline; ■ RAL + diltiazem. Six rats were used in each group. Values are means ± s.e.m. † $P < 0.05$ compared with sham-operated animals receiving saline, * $P < 0.05$, ** $P < 0.01$ compared with renal artery-ligated animals receiving saline.

Table 2. Total plasma IR-ET-1 in rats following administration of ET-1.

	Area under the curve from 0 to 10 min (nM min)
Sham-operated	
Saline	86·7 ± 10·5
Diltiazem	62·5 ± 8·7*
Renal artery ligation	
Saline	96·6 ± 14·4
Diltiazem	99·3 ± 8·8

* $P < 0.05$ compared with all other groups.

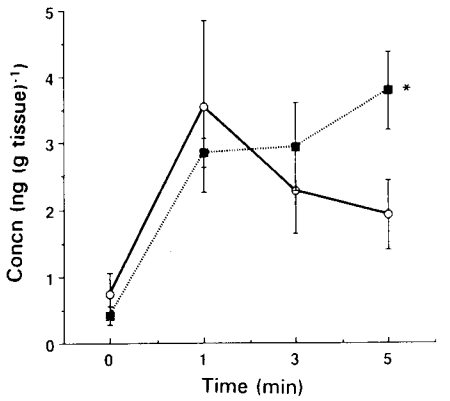

FIG. 4. Effects of diltiazem (2 mg kg^{-1}) on the time course of IR-ET-1 in the mouse kidney. Saline (○) or diltiazem (■) was administered intravenously 10 min before injection of ET-1 (2·5 nmol kg^{-1}, i.v.). Six mice were used in each group. Values are means ± s.e.m. *$P < 0.05$ compared with saline control.

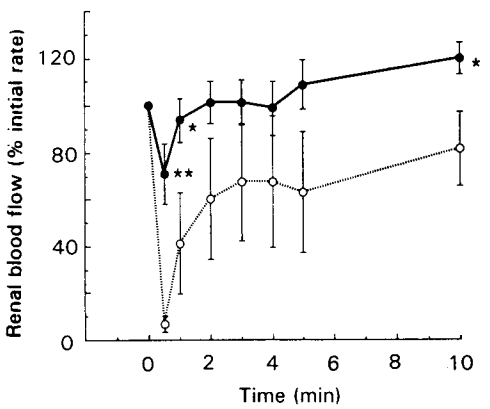

FIG. 5. Effects of pretreatment with diltiazem (2 mg kg^{-1}) on ET-1 (1 nmol kg^{-1}, i.v.)-induced decrease in renal blood flow in anaesthetized rats. The renal blood flow was expressed as percent of the value at 0 min. Values are means ± s.e.m. ○ Saline control; ● diltiazem. *$P < 0.05$, **$P < 0.01$ compared with saline control.

IR-ET-1 in kidneys and the effect of diltiazem in mice

After ET-1 administration to mice, IR-ET-1 in the kidneys was rapidly elevated at 1 min and then gradually decreased. Pretreatment with diltiazem showed the facilitation of IR-ET-1 accumulation in the kidneys with significantly higher levels of IR-ET-1 being present at 5 min after ET-1 administration (Fig. 4). Furthermore, RP-HPLC analysis indicated that intact ET-1 accounted for about 80% of the IR-ET-1 in the kidneys with or without diltiazem (data not shown).

Renal blood flow in anaesthetized rats (Fig. 5)

Intravenous injection of ET-1 at a dose of 1 nmol kg^{-1} reduced blood flow in the left renal artery in rats. The blood flow was reduced to 10% of the initial value at 30 s after ET-1 treatment and then was slowly restored, but it did not completely recover within 10 min. Pretreatment with diltiazem (2 mg kg^{-1}) markedly inhibited the decrease in renal blood flow, showing only a 30% fall at 30 s after ET-1 treatment.

Discussion

We have shown that diltiazem lowered the elevation of IR-ET-1 in plasma in both mice and rats receiving ET-1. The inhibitory effect of diltiazem on sudden death and on elevation of IR-ET-1 disappeared with bilateral ligation of the renal arteries. Furthermore, diltiazem increased renal accumulation of IR-ET-1 in mice after ET-1 administration and inhibited ET-1-induced severe decrements in renal blood flow in rats. Thus, the inhibitory effects of diltiazem on ET-1-induced sudden death and the elevation of plasma IR-ET-1 are at least partly due to its effects on the kidneys. The enhancement of clearance of ET-1 by diltiazem is presumably due to improvement in renal circulation, leading to an increase in the amount of ET-1 reaching the kidneys and hence the accumulation of ET-1 in the kidneys. Since RP-HPLC analysis in the present study indicated that intact ET-1 accounted for about 80% of IR-ET-1 and Abassi et al (1992) also showed that intact ET accounted for about 90%, most of the IR-ET-1 in the kidney probably exists in a form bound to endothelin receptors.

Whether the inhibitory effect of diltiazem on the elevation of plasma IR-ET-1 is the main mechanism preventing ET-1-induced sudden death is still unclear. The results concerning the influence of diltiazem and renal artery ligation in the present study gave some information about the relationship between plasma IR-ET-1 and mortality after ET-1 administration. IR-ET-1 (as AUC for 10 min) in sham-operated rats receiving diltiazem, in which improvement in sudden death was observed, was significantly lower than in the sham-operated rats receiving saline and renal artery ligation with and without diltiazem. This observation suggests that although Ca^{2+}-antagonism by diltiazem to inhibit arrhythmias and vasoconstriction could be important, a decrease in IR-ET-1 by diltiazem may partly contribute to the inhibition of sudden death induced by ET-1. From the results of using other Ca^{2+}-channel blockers, verapamil and nifedipine, the inhibition of ET-1-induced sudden death and the elevation of plasma IR-ET-1 may be a common characteristic of Ca^{2+}-channel blockers.

The clearance of ET-1 by the kidneys has been well documented (Kohno et al 1989; Pernow et al 1989; Sirvio et al 1990). Kohno et al (1989) showed that bilateral nephrectomy delayed the disappearance of ET-1 from plasma and enhanced the elevation of blood pressure after intravenous ET-1 administration, which is in agreement with our results. In our study, we obstructed renal circulation by ligation of the renal arteries and observed enhancement in the ET-1-induced sudden death and the tendency toward a slow decrease in plasma IR-ET-1. ET-1 in the kidney is presumed to be degraded by enzymes, in which phosphoramidon-sensitive neutral endopeptidase 24.11 (Vijayaraghavan et al 1990) is reported to be involved, on the brush border of the renal proximal tubules. This presumption is consistent with our findings that the IR-ET-1 in the plasma of phosphoramidon-treated mice was apparently higher than control, because phosphoramidon could inhibit the degradation of ET-1. Nevertheless, the hydrolysis product of ET-1 was not found in the renal tissue either in our study or in that of Abassi et al (1992). In the former study, degraded ET-1 was not detected by rabbit ET-1 antiserum in either mouse

plasma or kidney, and in the latter, the ^{125}I-containing hydrolysis product did not remain in the kidneys. Although the precise fate of injected ET-1 is unclear, the kidney might be one of the important organs for eliminating ET-1 from the circulation.

In conclusion, diltiazem facilitated ET-1 clearance from the bloodstream, presumably by improvement in the renal circulation, resulting in the accumulation of ET-1 in the kidney. Besides the Ca^{2+}-antagonistic action of diltiazem on the cardiovascular system, the decrease in plasma IR-ET-1 by the drug may contribute to the alleviation of the toxic activity of ET-1.

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References

Abassi, Z. A., Tate, J. E., Golomb, E., Keiser, H. R. (1992) Role of neutral endopeptidase in the metabolism of endothelin. Hypertension 20: 89–95

Anggard, E., Galton, S., Rae, G., Thomas, R., McLoughlin, L., Nucci, G., Vane, J. R. (1989) The fate of radioiodinated endothelin-1 and endothelin-3 in the rat. J. Cardiovasc. Pharmacol. 13 (Suppl. 5): S46–S49

Ashizawa, N., Okumura, H., Matsuura, A., Kobayashi, F. (1992) Effects of diltiazem HCl on exogenous endothelin-induced sudden death and elevation of plasma endothelin level in rodents. Jpn. J. Pharmacol. 58 (Suppl.): 115P

Gasic, S., Wagner, O. F., Vierhapper, H., Nowotny, P., Waldhausl, W. (1992) Regional hemodynamic effects and clearance of endothelin-1 in humans: renal and peripheral tissues may contribute to the overall disposal of the peptide. J. Cardiovasc. Pharmacol. 19: 176–180

Kohno, M., Murakawa, K., Yasunari, K., Yokokawa, K., Horio, T., Kurihara, N., Takeda, T. (1989) Prolonged blood pressure elevation after endothelin administration in bilaterally nephrectomized rats. Metabolism 38: 712–713

Matsuura, A., Okumura, H., Ashizawa, N., Kobayashi, F. (1992) Big endothelin-1-induced sudden death is inhibited by phosphoramidon in mice. Life Sci. 50: 1631–1638

Mino, N., Kobayashi, M., Nakajima, A., Amano, H., Shimamoto, K., Ishikawa, K., Watanabe, K., Nishikibe, M., Yano, M., Ikemoto, F. (1992) Protective effect of a selective endothelin receptor antagonist, BQ-123, in ischemic acute renal failure in rats. Eur. J. Pharmacol. 221: 77–83

Pernow, J., Hemsen, A., Lundberg, J. M. (1989) Tissue specific distribution, clearance and vascular effects of endothelin in the pig. Biochem. Biophys. Res. Commun. 161: 647–653

Shiba, R., Yanagisawa, M., Miyauchi, T., Ishii, Y., Kimura, S., Uchiyama, Y., Masaki, T., Goto, K. (1989) Elimination of intravenously injected endothelin-1 from the circulation of the rat. J. Cardiovasc. Pharmacol. 13 (Suppl. 5): S98–S101

Shibouta, Y., Suzuki, N., Shino, A., Matsumoto, H., Terashita, Z., Kondo, K., Nishikawa, K. (1990) Pathophysiological role of endothelin in acute renal failure. Life Sci. 46: 1611–1618

Sirvio, M. L., Metsarinne, K., Saijonmaa, O., Fyhrquist, F. (1990) Tissue distribution and half-life of ^{125}I-endothelin in the rat: importance of pulmonary clearance. Biochem. Biophys. Res. Commun. 167: 1191–1195

Stewart, D. J., Levy, R. D., Cernacek, P., Langleben, D. (1991) Increased plasma endothelin-1 in pulmonary hypertension: marker or mediator release? Ann. Intern. Med. 114: 464–469

Suzuki, N., Matsumoto, H., Kitada, C., Kimura, S., Miyauchi, T., Fujino, M. (1990) A sandwich-type enzyme immunoassay to detect immunoreactive big-endothelin-1 in plasma. J. Immunol. Methods 127: 165–170

Terashita, Z., Shibouta, Y., Imura, Y., Iwasaki, K., Nishikawa, K. (1989) Endothelin-induced sudden death and the possible involvement of platelet activating factor (PAF). Life Sci. 45: 1911–1918

Vijayaraghavan, J., Scicli, A. G., Carretero, O. A., Slaughter, C., Moomaw, C., Hersh, L. B. (1990) The hydrolysis of endothelin by neutral endopeptidase 24.11 (enkephalinase). J. Biol. Chem. 265: 14150–14155

Wagner, O. F., Vierhapper, H., Gasic, S., Nowotny, P., Waldhausl, W. (1992) Regional effects and clearance of endothelin-1 across pulmonary and splanchnic circulation. Eur. J. Clin. Invest. 22: 277–282

Watanabe, T., Suzuki, N., Shimamoto, N., Fujino, M., Imada, A. (1991) Contribution of endogenous endothelin to the extension of myocardial infarct size in rats. Circ. Res. 69: 370–377

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., Masaki, T. (1988) A novel potent vasoactive peptide produced by vascular endothelial cells. Nature 322: 411–415

Yoshibayashi, M., Nishioka, K., Nakao, K., Saito, Y., Matsumura, M., Ueda, T., Temma, S., Shirakami, G., Imura, H., Mikawa, H. (1991) Plasma endothelin concentration in patients with pulmonary hypertension associated with congenital heart defects. Circulation 84: 2280–2285