Possible Mechanisms of Sudden Death and Hemoconcentration Induced by Endothelin-1 and Big Endothelin-1 in Mice

Hiroshi Okumura,*,(1) Naoki Ashizawa, Tomoji Aotsuka, Rieko Asakura, Fujio Kobayashi, and Akihiro Matsuura

Pharmaceutical Research Laboratories, Sapporo Breweries Limited, 10 Okatohe, Yaizu, Shizuoka 425, Japan.

Received October 18, 1993; accepted January 24, 1994

We investigated the profiles of sudden death and hemoconcentration induced by endothelin-1 (ET-1) and big endothelin-1 (big ET-1) in mice using various compounds as pharmacological tools.

In ET-1-induced sudden death (5 nmol/kg, i.v.), pretreatment with the Ca2+-channel blockers, diltiazem, nifedipine or verapamil at a dose of 2 mg/kg, i.v. significantly inhibited the mortality and prolonged the latency to death. These Ca2+-channel blockers, however, failed to inhibit the rise in hematocrit (Ht), namely hemoconcentration, induced by ET-1 (2.5 nmol/kg). A beta-adrenoceptor agonist, isoproterenol (1 mg/kg) tended to prolong the latency, whereas, a beta-adrenoceptor blocker, propranolol (2 mg/kg), and an alpha- and beta-adrenoceptor blocker, labetalol (5 mg/kg), aggravated the sudden death. Esculetin (10 mg/kg) and fenbuthen (10 mg/kg), which are enzyme inhibitors in the arachidonic cascade, prevented only the hemoconcentration. Anti-arrhythmic drugs, lidocaine (1 mg/kg) and disopyramide (20 mg/kg) did not improve any parameters.

Big ET-1 also caused sudden death (20 and 25 nmol/kg, i.v.) and hemoconcentration (10 nmol/kg, i.v.). Of several proteinase inhibitors, only a metalloproteinase inhibitor, phosphoramidon (2 mg/kg i.v.), prevented the sudden death and the hemoconcentration induced by big ET-1 but not by ET-1. Ca2+-channel blockers exerted their protective effects only when a lower dose of big ET-1 was employed.

These results indicate that the sudden death caused by both peptides is mainly due to myocardial ischemia and respiratory disorder, and that hemoconcentration caused by them is due not to their vasoconstrictor action but to their effects on the vascular permeability via secondary endogenous factors.

Keywords endothelin-1; sudden death; hemoconcentration; calcium-channel blocker; big endothelin-1; mouse

A potent constrictor of vascular smooth muscle, endothelin-1 (ET-1) is produced through cleavage of the 38-amino acid precursor, big endothelin-1 (big ET-1).2,3) ET-1 has been reported to cause various biological actions in coincidence with a rise in intracellular free Ca2+ concentration via voltage-dependent Ca2+-channels; i.e., constriction of various arteries,4-6) hypertension,7-10) chemotaxis of human monocytes11) and so on. When the dose of ET-1 in vivo increased far beyond its physiological concentration, it would provoke sudden death in rats,12) mice13,14) and guinea pigs.13) The lethal effect of ET-1 was blocked by pretreatment with Ca2+-channel blockers or platelet activating factor (PAF) antagonists.12) The mechanism of ET-1-induced sudden death was speculated to be due to arrhythmias by ET-1-induced myocardial ischemia and subsequent hyperkalemia.12,13) However, the authors did not confirm the mechanisms using anti-arrhythmic agents.

A precursor of ET-1, big ET-1, also caused sudden death in mice, which was effectively blocked by phosphoramidon.14) Big ET-1 induced an increase in hematocrit (Ht), namely hemoconcentration, in mice as ET-1 did.15) These big ET-1-induced responses would be caused by ET-1, which is converted from big ET-1 by endothelin converting enzymes (ECE), but these responses have not been evaluated by many of the agonists, antagonist or inhibitors.

In the present study, we further evaluated both ET-1- and big ET-1-induced sudden death and hemoconcentration in mice using various compounds, which have different pharmacological properties, to specify the causative factor(s) underlying these responses.

MATERIALS AND METHODS

Animals Male, ICR mice, 6—7 weeks old, were purchased from Charles River Japan Inc. Animals were housed in polycarbonate cages and maintained on a 12 h light/dark cycle at 23±1°C and 55±10% humidity. Animals were allowed free access to rat chow (CRF-1, Oriental Yeast Co., Tokyo, Japan) and water ad libitum.

Sudden Death Induced by Endothelin-1 and Big Endothelin-1 ET-1 (5 nmol/kg) or big ET-1 (20 or 25 nmol/kg) dissolved in saline solution was intravenously given to mice at a volume of 0.05 ml/10 g body weight as previously reported.13) Mortality (%) and latency (s) to death, as judged by the cessation of respiration, were measured for up to 60 min after the injection. Saline or test compounds were intravenously given to mice 10 min before the ET-1 or big ET-1 injection.

Hemoconcentration Induced by Endothelin-1 and Big Endothelin-1 ET-1 (2.5 nmol/kg) or big ET-1 (20 or 25 nmol/kg) was applied to animals as described above. Blood was collected 10 min after the ET-1 or big ET-1 injection. Blood samples (ca. 40 μl) were collected from the orbital sinus with heparin-coated glass capillaries. Hematocrit was measured by centrifuging blood at 12000 rpm for 5 min and is shown as a percent. Saline or tested compounds were intravenously or intraperitoneally given to mice 10 min before the ET-1 or big ET-1 injection.

Measurement of Endothelin Converting Enzyme (ECE) Activity in Vitro ECE was prepared from bovine aortic endothelial cells as described.15) After preincubation of the inhibitor with ECE (5 μg protein) in 200 mM Tris–HCl buffer (pH 7.0) containing 10 μM actinomycin for 10 min,
100 nm big ET-1 as a substrate was added to the reaction mixture. The mixture was then incubated at 37°C for 4 h. The reaction was stopped by boiling for 5 min. After cooling with ice, the amount of IR-ET-1 in the reaction mixture was measured by enzyme immunoassay (EIA) as described previously. Briefly, sandwich-EIA was employed using a rabbit IgG anti-ET-1(15-21) as the immobilized antibody and rabbit IgG F(ab')2-HRP anti-ET-1 as the labeled antibody. The assay showed no cross reactivity with big ET-1 at a concentration of 100 nm.

**Measurement of Degradation of ET-1 in Vitro** The influence of test compounds on the degradation of ET-1 was measured by sandwich-EIA. The tested compounds were added to the rabbit IgG anti-ET-1(15-21)-coated microtest plate at a concentration of 10 and 100 μM. After incubation for 1 h at 37°C, the amount of IR-ET-1 was measured as described above.

**Materials** Human ET-1, human big ET-1, phosphoramidon, aprotinin, pepstatin A and E-64 were supplied by the Peptide Institute (Osaka, Japan), chlorpromazine HCl, disopropyl fluorophosphate, EDTA, prazosin HCl and Triton-X100 by Wako (Osaka, Japan), rabbit IgG anti-ET-1(15-21) and rabbit IgG F(ab')2-HRP anti ET-1 by International Reagents Corp. (Kobe, Japan), and actinonin, bovine serum albumin (BSA), captopril, diltiazem HCl, verapamil HCl, indomethacin, salbutamol and α-phenylenediamine by Sigma (St. Louis, U.S.A.). CV-3988 (rac-3-(N-n-octadecyl-carbamoyloxy)-2-methoxypropyl-2-thiazolio-ethyl phosphate) was synthesized in the Pharmaceutical Research Laboratories (Sapporo Breweries, Ltd.). All other chemicals were of analytical grade.

**Data Analysis** Data are expressed as means ± S.E. mean from the animals. Where a difference was found across the groups, an unpaired Student’s t-test or one-way analysis of variance with the Barferroni modification was used. p values less than 0.05 were regarded as statistically significant.

**RESULTS**

**Effects of Various Compounds on ET-1-Induced Responses in Vitro and in Vitro** The effects of various compounds on sudden death induced by ET-1 in mice are shown in Table 1. Pretreatment with diltiazem at a dose of 0.5 or 2 mg/kg, i.v. significantly inhibited the mortality and prolonged the latency to death. Intraperitoneal administration of diltiazem at a dose of 20 mg/kg also prevented the ET-1-induced sudden death and other Ca²⁺-channel blockers, nifedipine (0.5 and 2 mg/kg, i.v.) and verapamil (2 mg/kg, i.v.) too, significantly suppressed the sudden death induced by ET-1. The following compounds exerted protective actions in ET-1-induced sudden death. An alpha₁-adenoreceptor antagonist prazosin (1 mg/kg, i.v.), beta-adenoreceptor agonists, isoproterenol (1 mg/kg, i.v.) and salbutamol (10 or 30 mg/kg, i.v.), a major tranquilizer, chlorpromazine (5 mg/kg, i.v.), a specific PAF antagonist, CV-3988 (50 mg/kg, i.p.), and a vasodilator, papaverine (3 mg/kg, i.v.) were effective. A non-selective beta-adenoreceptor antagonist, propranolol (2 mg/kg, i.v.) an alpha- and beta-adenoreceptor antagonist, labetalol (5 mg/kg, i.v.), and a 5-lipoxygenase inhibitor, esculetin (10 mg/kg, i.v.), tended to aggravate sudden death, while anti-arrhythmic drugs, lidocaine (1 mg/kg, i.v.) and disopyramide (20 mg/kg, i.v.), had no effect on sudden death by ET-1.

The effects of various compounds on hemoconcentration induced by intravenous administration of ET-1 to mice are shown in Fig. 1. Diltiazem (2 mg/kg, i.v.) and nifedipine (2 mg/kg, i.v.) failed to affect the ET-1-induced hemoconcentration in mice in spite of showing protective activity against ET-1-induced lethality. Alpha-adrenoceptor antagonists, phenoxbenzamine (20 mg/kg, i.p.) and prazosin (0.1 and 1 mg/kg, i.v.), significantly inhibited the ET-1-induced hemoconcentration, but yohimbine (3 mg/kg, i.v.) had no effect. Chlorpromazine (5 mg/kg, i.v.) significantly suppressed the ET-1-induced rise in Ht. These three effective compounds, however, also decreased normal Ht values by themselves without ET-1. Salbutamol (10 mg/kg, i.v.) significantly suppressed the ET-1-induced hemoconcentration without influence on normal Ht values. Labetalol (5 mg/kg, i.v.) slightly inhibited the hemoconcentration (not significant), but propranolol (5 mg/kg, i.v.) did not show any inhibition. Among compounds related to the arachidonate cascade, esculetin and fensulfon significantly inhibited hemoconcentration, but indomethacin (3 mg/kg, i.v.) and ibuprofen (200 mg/kg, p.o.) did not. Sulpiride (20 mg/kg, i.p.), a specific dopaminergic D₂-antagonist, significantly inhibited hemoconcentration, and dexamethasone (10 mg/kg, i.v.) slightly attenuated the hemoconcentration with a p value of 0.08. On the other hand, a PAF-antagonist etizolam (30 mg/kg, i.v.)...
The effect of various compounds on the degradation of endothelin-1 in vitro are shown in Table II. Nifedipine slightly accelerated the degradation of ET-1 in vitro and phosphoramidon at a higher concentration of 100 μM inhibited ET-1-degradation.

**Effects of Various Compounds on Big ET-1-Induced Responses in Vivo and in Vitro** In our previous study, the LD₉₀ value of big ET-1 in the sudden death experiment in mice was estimated at 22.9 nmol/kg, i.v., but the mortality was rather variable around this LD₉₀ value, therefore, we employed two doses of big ET-1 in the present study. Table III shows the results with the higher dose of big ET-1 (25 nmol/kg, i.v.) and Table IV shows the results with the lower threshold dose of big ET-1 (20 nmol/kg, i.v.). Although diltiazem (2 mg/kg, i.v.) did not improve the sudden death induced by the higher dose of big ET-1 (25 nmol/kg), the drug (0.5 and 2 mg/kg, i.v. and 20 mg/kg, i.p.) surely suppressed the sudden death caused by the threshold dose of big ET-1. Other Ca²⁺-channel blockers, nifedipine (0.5 and 2 mg/kg, i.v.) and verapamil (2 mg/kg, i.v.), also significantly inhibited the mortality and prolonged the latency to big ET-1 (20 nmol/kg)-induced sudden death. Among proteinase inhibitors, phosphoramidon significantly inhibited the sudden death induced both by doses of big ET-1 (Tables III and IV) and ECE activity in vitro (Table V). Of other proteinase inhibitors, antipain (2 mg/kg, i.v.) alone prolonged the latency. Other compounds did not induce any beneficial activities even at the lower dose of big ET-1-induced lethality. It is striking that chlorpromazine (50 μM) inhibited ECE activity by
TABLE IV. Effects of Various Compounds on Sudden Death Induced by Threshold Dose of Big ET-1 (20 nmol/kg, i.v.) in Mice

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>% inhibition</th>
<th>% prolongation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diltiazem</td>
<td>2</td>
<td>i.v.</td>
<td>36</td>
<td>n.s.</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>2</td>
<td>i.v.</td>
<td>41</td>
<td>n.s.</td>
</tr>
<tr>
<td>Verapamil</td>
<td>2</td>
<td>i.v.</td>
<td>61</td>
<td>107</td>
</tr>
<tr>
<td>Captorpli</td>
<td>5</td>
<td>i.v.</td>
<td>0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>5</td>
<td>i.v.</td>
<td>-18</td>
<td>n.s.</td>
</tr>
<tr>
<td>Disopyramide</td>
<td>20</td>
<td>i.v.</td>
<td>-29</td>
<td>n.s.</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>3</td>
<td>i.v.</td>
<td>-18</td>
<td>n.s.</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>1</td>
<td>i.v.</td>
<td>-14</td>
<td>n.s.</td>
</tr>
<tr>
<td>Phosphoramidon</td>
<td>2</td>
<td>i.v.</td>
<td>90</td>
<td>142</td>
</tr>
<tr>
<td>Prazosin</td>
<td>1</td>
<td>i.v.</td>
<td>-18</td>
<td>n.s.</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>30</td>
<td>i.v.</td>
<td>-18</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Test compounds were administered intravenously to mice (n=8–14) 10 min prior to big ET-1 (20 nmol/kg, i.v.) injection. Mortality (%) and latency (s) to death, as judged by the cessation of respiration, were measured up to 60 min after the injection. When the animal survived over a 60-min experimental period, latency of the animal was regarded as 3600 s. a) Significantly different from control (p<0.05), b) p<0.01.

TABLE V. Effects of Various Compounds on Endothelin-Converting Enzyme (ECE) Activity of Cultured Endothelial Cells in Vitro

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (μM)</th>
<th>Inhibition (%)</th>
<th>IC₅₀ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antipain</td>
<td>100</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Aprotinin</td>
<td>30 μg/ml</td>
<td>-18</td>
<td></td>
</tr>
<tr>
<td>Captorpli</td>
<td>300</td>
<td>-11</td>
<td></td>
</tr>
<tr>
<td>Chymostatin</td>
<td>200</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Leupeptin</td>
<td>100</td>
<td>-11</td>
<td></td>
</tr>
<tr>
<td>Pepstatin A</td>
<td>10 μM</td>
<td>-11</td>
<td></td>
</tr>
<tr>
<td>Phosphoramidon</td>
<td>100</td>
<td>79</td>
<td>0.15</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>50 μM</td>
<td>90</td>
<td>2.1</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>50 μM</td>
<td>50</td>
<td>98</td>
</tr>
<tr>
<td>EDTA</td>
<td>100 μM</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>50 μM</td>
<td>-6</td>
<td></td>
</tr>
<tr>
<td>Nifedipine</td>
<td>50 μM</td>
<td>-17</td>
<td></td>
</tr>
<tr>
<td>Prazosin</td>
<td>50 μM</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Salbutamol</td>
<td>50 μM</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Verapamil</td>
<td>20 μM</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

ECE was obtained from cultured endothelial cells of a bovine aorta through the process of homogenization and centrifugation. Test compounds and ECE were added to the rabbit IgG anti ET-1(21-22)-coated microtest plate. The amount of immunoreactive endothelin-1 like activity was measured by sandwich enzyme immunoassay using rabbit IgG Fab/2-HRP anti ET-1. Enzyme activity was measured with o-phenylenediamine as chromogen.

90%, and its IC₅₀ value was estimated to be 2.1 μM. Diltiazem also inhibited ECE activity, but its activity was rather weak (IC₅₀ = 98 μM).

The effects of various compounds on hemoconcentration induced by intravenous administration of big ET-1 to mice are shown in Fig. 2. Compounds which inhibited the ET-1-induced hemoconcentration, chlorpromazine (5 mg/kg), phenoxybenzamine (20 mg/kg), prazosin (0.1 mg/kg), salbutamol (10 mg/kg) and sulpiride (30 mg/kg), also significantly reduced the big ET-1-induced hemoconcentration. Additionally, phosphoramidon and a phytotoxin aspergillomarasine A, significantly inhibited the hemoconcentration.

DISCUSSION

ET-1-Induced Sudden Death in Mice Yorikane and Koike reported that ET-1 had an arrhythmogenic activity so that ET-1 was thought to be one of important causal factors involved in incidence of arrhythmias in myocardial ischemia-reperfusion. Lin et al. also demonstrated that one of the crucial factors in ET-1-induced lethality was determined as arrhythmias by myocardial ischemia followed by hyperkalemia. Additionally, we have recently observed an elevation of plasma K⁺ concentration with ET-1 injection, which was effectively blocked by diltiazem (unpublished data). This hyperkalemia might be a consequence of the suppression of Na⁺/K⁺-ATPase activity by hypoxia. It is well accepted that Ca²⁺-channel blockers, such as diltiazem, attenuated ET-1-induced responses by inhibiting Ca²⁺ influx through voltage-dependent Ca²⁺-channels into target cells. In the present study, ET-1-induced sudden death in mice was also abolished by pretreatment with Ca²⁺-channel blockers, diltiazem, nifedipine and verapamil. These results imply that Ca²⁺-channel blockers reduce ET-1-induced lethality by their Ca²⁺-channel blocking activities on coronary vessels and myocardium.

Although Terashita et al. also claimed a causative role of arrhythmias in ET-1-induced sudden death, anti-arrhythmic drugs, lidocaine and disopyramide, failed to attenuate the sudden death. This indicates that improvement of myocardial ischemia, which is a cause of fatal arrhythmias, is most important for the prevention from ET-1-induced lethality.

Prazosin and chlorpromazine, alpha₁-adrenoceptor antagonists, significantly prolonged the latency to death induced by ET-1. These compounds also significantly reduced Ht in mice by 11.0% and 9.6%, respectively, even in normal mice. Therefore, alleviation of ET-1-induced sudden death by alpha₁-adrenoceptor antagonists is attributable to their potent vasodilating action and blood viscosity lowering effects, which may lead to improved blood circulation.

ET-1 is a potent bronchoconstrictor in vitro and in vivo so that this action may be involved in the pathogenesis of sudden death. Beta-adrenoceptor agonists,
such as salbutamol (beta₂-selective) and isoproterenol (non-selective), are potent bronchodilators, and are used clinically as anti-asthmatic agents. In the present study, these beta-adrenoceptor agonists prolonged the latency to death induced by ET-1, while a beta-adrenoceptor antagonist, propranolol, and an alpha- and beta-adrenoceptor antagonist, labetalol worsened it. Additionally, we observed that artificial ventilation remarkably prolonged the latency to death induced by ET-1 in anesthetized rats (data not shown). These results strongly suggest that respiratory disorder is one of the major causes of ET-1-induced lethality.

Terashita et al. reported that pretreatment with specific PAF antagonists, CV-6209 (2-[N-acetyl-N-(2-methoxy-3-octadecylcarbamoyloxypropoxycarbonyl)aminoethyl]-1-ethylpyridinium chloride) and WEB2086 ((3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo-[4,3-a][1,4]diazepine-2-yl]-1-(4-morpholinyl)-1-propanone), significantly prevented the incidence of sudden death by ET-1 in rats. They speculated that ET-1-induced death was mediated by both the direct vasoconstricting action of ET-1 and the secondary action of endogenously released PAF. In our present study, another PAF antagonist, CV-3988, also tended to inhibit the ET-1-induced sudden death. PAF has been shown to stimulate the release of leukotrienes in vitro. Arachidonate metabolites, particularly leukotrienes formed via the 5-lipoxygenase pathway, have been suggested to be involved in the PAF-induced bronchoconstriction. In our study, however, a lipoxygenase inhibitor, esculetin, shortened the latency to death by 65%, while the cyclooxygenase inhibitor, indomethacin, did not affect the sudden death induced by ET-1. Although no detailed explanation is possible, these results may suggest some relation of PAF and lipoxygenase products to the pathogenesis of the sudden death induced by ET-1.

The present study strongly suggested that myocardial ischemia and bronchoconstriction play major roles in the sudden death induced by ET-1 in mice. Ca²⁺-channel blockers and alpha-adrenoceptor antagonists prevent the myocardial ischemia by inhibition of Ca²⁺ influx followed by coronary vasodilation, finally leading to the prevention of sudden death. Beta-adrenoceptor agonists and PAF antagonist, on the other hand, would dilate bronchial smooth muscles to prevent the fatal bronchospasm induced by ET-1.

Big ET-1-Induced Sudden Death in Mice Since big ET-1-induced sudden death was completely prevented by the pretreatment with phosphoramidon, as shown in this study and by Matsuura et al., it was accepted that big ET-1-induced sudden death must be caused by ET-1 which is produced by the conversion of big ET-1. Accordingly, we expected that the mechanisms of big ET-1-induced sudden death must be identical with those of ET-1-induced sudden death and that big ET-1-induced sudden death should be inhibited by the same compounds effective in ET-1-induced sudden death. However these compounds failed to inhibit the sudden death induced by a higher dose (25 nmol/kg, i.v.) of big ET-1. Sudden death induced by the threshold dose of big ET-1 (20 nmol/kg, i.v.) was blocked only by Ca²⁺-channel blockers. We reported that plasma IR-ET-1 level by 25 nmol/kg i.v. of big ET-1 was almost equivalent to that by 5 nmol/kg i.v. of ET-1. Under that condition, diltiazem failed to inhibit sudden death by big ET-1 without decreases in heart tissue IR-ET-1, while diltiazem decreased both mortality and heart tissue IR-ET-1 in ET-1-induced sudden death (data not shown). Thus, these indicate that the amount of IR-ET-1 not in plasma but in tissue, especially heart tissue, is important in the lethality of ET-1, and it is speculated that increase in tissue IR-ET-1 level induced by 20 nmol/kg of big ET-1 might be higher than that induced by 5 nmol/kg of ET-1.

ET-1-Induced Hemoconcentration in Mice There are two possible mechanisms for ET-1-induced increase in Ht: an increase in vascular permeability via release of endogenous PAF and/or thromboxane A₂ and vasoconstriction of splenic sinus as a hemocyte storage. However, the ET-1-induced hemoconcentration was not affected by the pretreatment with Ca²⁺-channel blockers, indicating that ET-1-induced changes in Ht were probably not due to vasoconstriction of hemocyte storage, such as spleen, but to an increase in vascular permeability. Fenbufen, salbutamol and sulpiride also significantly inhibited the ET-1-induced hemoconcentration without influence on the normal Ht in mice. These results suggest that cyclooxygenase pathways, beta-adrenergic receptors and dopaminergic D₂ receptors would be involved in ET-1-induced hemoconcentration in mice. Chlorpromazine, phenoxybenzamine and prazosin significantly inhibited ET-1-induced hemoconcentration, but these three compounds also significantly reduced the normal Ht value by themselves (data not shown), implying that these compounds may be considered to decrease systemic Ht by enhancement of hemocyte storage to splenic sinus through their alpha-adrenoceptor antagonism.

Big ET-1-Induced Hemoconcentration in Mice Big ET-1 (10 nmol/kg, i.v.) also caused hemoconcentration in mice, which was not inhibited by Ca²⁺-channel blockers. Salbutamol and sulpiride prevented the rise in Ht induced by big ET-1 as well as by ET-1. Additionally a metalloprotease inhibitor, phosphoramidon, and a naturally-occurring phytotoxin, aspergillosarmin A, which was reported to inhibit ECE activity in vitro and in vivo suppressed big ET-1-induced hemoconcentration. These results indicate that big ET-1-induced hemoconcentration must be caused by ET-1 produced by the conversion of big ET-1 as observed in big ET-1-induced sudden death. But esculetin and fenbufen, which were effective in the hemoconcentration by ET-1, failed to inhibit that caused by big ET-1. Although the exact reason is unclear, there are two possible hypotheses. One would be that lipoxygenase and cyclooxygenase pathways may be partly involved in the hemoconcentration induced by ET-1 and big ET-1, and the other would be that big ET-1-induced hemoconcentration was more susceptible to its own hemodynamic effects than that by ET-1. Although ET-1 and big ET-1 were reported to be equipotent for their hemodynamic effects in vivo, we used a higher dose of big ET-1 (10 nmol/kg, i.v.) than that of ET-1 (2.5 nmol/kg, i.v.) in order to obtain an equivalent change in Ht. Vasoconstriction induced by 10 nmol/kg of big ET-1 may
therefore prevent exuded plasma from returning to blood vessels.

**Conclusion** Although Terashita et al.\(^1\) and Lin et al.\(^2\) assumed that ET-1 given exogenously would cause arrhythmias accompanied by myocardial ischemia and result in sudden death, we determined that drugs which belonged to the class I anti-arrhythmic agents failed to inhibit the ET-1-induced sudden death in mice. This suggests that incidence of arrhythmia is one of the diverse circulatory disfunctions induced by ET-1. Additionally, we showed that beta-adrenoceptor agonists prevent sudden death by ET-1, while beta-adrenoceptor antagonists aggravate it. This suggests that bronchoconstriction may play an important role in the pathogenesis of ET-1-induced sudden death in mice. Although the effects of several compounds tested were not always similar in either ET-1- or big ET-1-induced lethality, we suppose that the lethality of the latter is based on the same mechanisms as ET-1-induced sudden death since phosphoramidon inhibits big ET-1-induced sudden death alone. An exact interpretation of the mechanism of big ET-1-induced sudden death requires further information, particularly as to the role of tissue ET-1 level.

ET-1- and big ET-1-induced hemocoagulation was not inhibited by Ca\(^{2+}\)-channel blockers, but was suppressed by salbutamol and sulpiride. This indicates that ET-1-induced hemocoagulation is caused not by its vasconstrictor action but by an increase in vascular permeability via several endogenicous factors. These mechanisms also require further investigation.

**Acknowledgments** We express our special thanks to Ms. Chihoro Hattori-Hosono, Ms. Mayumi Tamada-Takahashi and Ms. Yasuko Aoshima for their technical assistance.

**REFERENCES AND NOTES**


