DIFFERING ACTIONS OF ENDOTHELIN-1 ON CANINE SYSTEMIC RESISTANCE AND CAPACITANCE VESSELS

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In anesthetized open-chest dogs, an intravenous bolus injection of endothelin-1 (ET-1, 400 pmol/kg) caused transient hypotension (initial hypotensive phase; phase 1), followed by a continuous elevation of blood pressure (late hypertensive phase; phase 2). The constriction and dilation of the systemic capacitance and resistance vessels were evaluated from the change in mean circulatory pressure (MCP) and in total peripheral resistance (TPR) in phases 1 and 2. To examine the modification of the action of ET-1 on the blood vessels by the baroreceptor reflex or by the endothelium-derived relaxing factor (EDRF) released by ET-1 in phase 1, we performed experiments in dogs under total spinal anesthesia (TSA group), methylene blue-treated dogs (MB group) as well as in the untreated dogs (control group). ET-1 decreased the TPR significantly, and increased the MCP significantly in phase 1 in the control (n=8) and TSA (n=8) groups; there was no difference between the groups. ET-1 had no significant effect on TPR but increased the MCP significantly in MB group (n=8) during phase 1. The percentage increase of MCP in the MB group significantly exceeded that of the control group. ET-1 increased both the TPR and MCP significantly in phase 2 in the control group (n=8).

This study indicated that the vasocostrictor action of ET-1 on the systemic capacitance vessels in phase 1 did not result from a baroreceptor reflex, and that the vasodilator action of ET-1 on the systemic resistance vessels may be at least in part mediated via EDRF released by ET-1. We suggest that the vasoconstrictor action of ET-1 on the systemic capacitance vessels is strong, but the vasodilator action of EDRF on the systemic capacitance vessels is weak.

(Jpn Circ J 1992; 56: 847–854)

ENDOTHELIN-1 (ET-1) constricts the isolated coronary, femoral and mesenteric arteries of various species. However, in vivo studies have shown that the intravenous injection of ET-1 produced a transient fall, followed by a continuous elevation, of arterial blood pressure. The action of ET-1 on the blood vessels thus differs between the in vitro isolated arteries and those in vivo causing a transient dilation of the latter. ET-1 has been reported to constrict isolated veins. However, it has not been established whether ET-1 constricts capacitance vessels in vivo and whether it would transiently dilate the capacitance vessels as it does the resistance vessels.

In the present study, we examined the dilation or constriction of the systemic

Key words:
Mean circulatory pressure
Systemic capacitance vessels
Systemic resistance vessels
Endothelin-1 (ET-1)
Endothelium-derived relaxing factor (EDRF)

(Received September 21, 1991; accepted December 27, 1991)
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capacitance and resistance vessels in anesthetized dogs during the initial transient hypertensive phase and the late continuous hypertensive phase following the intravenous injection of ET-1. The dilation or constriction of the systemic capacitance vessels was evaluated from the change in mean circulatory pressure (MCP)\(^5,6\) and that of the systemic resistance vessels from the change in total peripheral resistance (TPR). To examine the contribution of the baroreceptor reflex, the experiment was also performed under total spinal anesthesia (TSA). The study was also repeated in the presence of methylene blue (MB), an inhibitor of soluble guanylate cyclase; during the initial hypotensive phase.

MATERIALS AND METHODS

1. Methods

Since the details of the method have been reported elsewhere\(^5,9\) an outline is presented. Mongrel dogs were anesthetized with intravenous pentobarbital (25 mg/kg) and heparinized (300 U/kg). As shown in Fig. 1, following left lateral thoracotomy, a Cournand catheter was introduced into the right atrium through a contralateral jugular vein to measure aortic and right atrial pressures under artificial ventilation using a ventilator (Akoma Ikakogyo Co., Ltd., frequency 20/min, tidal volume 20 ml/kg). Cardiac output (CO) was measured by placing an electromagnetic flow probe (MF-27, Nihon Kohden Co., Ltd.) around the root of the aorta. With electrodes placed on the right auricle and the apex, ventricular fibrillation was initiated by applying an alternating current (20 volts, frequency 10/sec). Arterio-venous (A-V) shunts were created by connecting the bilateral femoral arteries and veins by 2 vinyl tubes; a constant flow pump (C-16, Tokyo Rikakikai Co., Ltd.) was set in the circuit so that arterial blood could be translocated rapidly to the venous side. All parameters together with the electrocardiogram were recorded simultaneously using a polygraph (RMC-1100, Nihon Kohden Co., Ltd.).

2. Techniques

Experiments were performed 20 min after all instrumentation had been terminated. To measure mean circulatory pressure (MCP), ventricular fibrillation was induced with the fibrillator, the A-V shunts were opened at the same time and arterial blood was trans-
located rapidly into the venous side by operating the constant flow pump. As a result, the mean blood pressure (MBP) fell and the right atrial pressure (RAP) rose rapidly, reaching an equilibrium pressure. This equilibrium pressure was MCP. After measuring the MCP, the heart beat was restored to sinus rhythm by using defibrillator (25–30 watt·sec).

According to Guyton, MCP is defined as,

\[ \text{MCP} = \frac{(\text{EVa} + \text{EVv})}{(\text{Ca} + \text{Cv})} \]

where the EVa represents the extra volume of the arterial system, the EVv the extra volume of the venous system. Since arterial compliance (Ca) is negligibly small in respect to venous compliance (Cv), MCP can be rewritten as,

\[ \text{MCP} = \frac{(\text{EVa} + \text{EVv})}{\text{Cv}} \]

when EV is constant, MCP is inversely proportional to Cv; an increase in the tone of the systemic capacitance vessels raises the MCP. A decrease of the tone of the systemic capacitance vessels lowers the MCP. When Cv is constant, a decrease of EV, i.e., dilation of the systemic capacitance vessels due to an increase of the unstressed volume, lowers the MCP. An increase in EV, i.e., a constriction of the systemic capacitance vessels due to a decrease of the unstressed volume, raises the MCP.

In this study, we evaluated the constriction of the systemic capacitance vessels from the rise of the MCP and dilation of the systemic capacitance vessels from the fall of the MCP. The constriction or dilation of the systemic resistance vessels was evaluated from the rise or fall of the total peripheral resistance (TPR).

Various hemodynamic parameters other than MCP and resistance to venous return (RVR) were obtained immediately before the initiation of ventricular fibrillation. The MCP measurements showed a good reproducibility.

3. Experiments

As in the typical case shown in Fig. 2, an intravenous injection of ET-1 (400 pmol/kg) produced a transient fall of MBP 1 to 2 min after injection, followed by a rise of MBP, reaching a plateau 10 to 15 min after injection. MCP and TPR were obtained during (1) the initial hypotensive phase and (2) the late hypertensive phase 15 min after injection of ET-1 in a different series of untreated dogs.

To eliminate the baroreceptor reflex, total spinal anesthesia (TSA) was accomplished by injecting bupivacaine (5 mg/kg) into a subarachnoid space through the suboccipital region. A continuous infusion of epinephrine was started immediately using a micro-infusion pump (STC-502, Terumo Co., Ltd.), thereby maintaining the MBP at about 70 mmHg (TSA group).

Another group of dogs was pretreated with a bolus intravenous injection of methylene blue (MB, 30 mg/kg), a soluble
### TABLE I  EFFECTS OF ENDOTHELIN-1 ON HEMODYNAMIC PARAMETERS

<table>
<thead>
<tr>
<th></th>
<th>MBP (mmHg)</th>
<th>TPR (dyne·sec·cm⁻²)</th>
<th>MCP (mmHg)</th>
<th>CO (ml/min/kg)</th>
<th>RAP (mmHg)</th>
<th>HR (beats/min)</th>
<th>RVR (dyne·sec·cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control group during the initial hypotensive phase (n=8)</strong></td>
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<tr>
<td>Before</td>
<td>ET-1</td>
<td>99.8±3.4</td>
<td>6656±426</td>
<td>10.2±0.4</td>
<td>113.5±5.8</td>
<td>1.6±0.1</td>
<td>151.8±9.6</td>
</tr>
<tr>
<td>After</td>
<td>ET-1</td>
<td>78.3±3.4*</td>
<td>4539±202**</td>
<td>11.7±0.5*</td>
<td>129.3±7.1*</td>
<td>1.8±0.1</td>
<td>152.4±12.0</td>
</tr>
<tr>
<td><strong>Control group during the late hypertensive phase (n=8)</strong></td>
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<tr>
<td>Before</td>
<td>ET-1</td>
<td>94.0±4.5</td>
<td>5266±218</td>
<td>8.9±0.3</td>
<td>121.5±6.4</td>
<td>1.7±0.1</td>
<td>157.4±6.1</td>
</tr>
<tr>
<td>After</td>
<td>ET-1</td>
<td>110.8±5.8**</td>
<td>7374±355**</td>
<td>10.1±0.6*</td>
<td>102.5±6.8**</td>
<td>1.6±0.1</td>
<td>152.9±4.8</td>
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<td><strong>TSA group during the initial hypotensive phase (n=8)</strong></td>
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<tr>
<td>Before</td>
<td>ET-1</td>
<td>70.3±0.6</td>
<td>4926±199</td>
<td>10.1±0.4</td>
<td>101.3±4.2</td>
<td>2.1±0.1</td>
<td>123.9±9.3</td>
</tr>
<tr>
<td>After</td>
<td>ET-1</td>
<td>55.8±2.3**</td>
<td>3495±238**</td>
<td>11.4±0.2*</td>
<td>114.4±5.5*</td>
<td>2.3±0.2</td>
<td>129.8±10.0</td>
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<tr>
<td><strong>TSA group during the late hypertensive phase (n=8)</strong></td>
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<tr>
<td>Before</td>
<td>ET-1</td>
<td>71.1±0.7</td>
<td>5128±654</td>
<td>10.2±0.6</td>
<td>113.8±12.1</td>
<td>1.7±0.1</td>
<td>135.8±5.1</td>
</tr>
<tr>
<td>After</td>
<td>ET-1</td>
<td>91.6±1.9**</td>
<td>7775±1030**</td>
<td>12.1±0.6**</td>
<td>97.9±11.5**</td>
<td>1.5±0.2</td>
<td>131.6±5.1</td>
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<tr>
<td><strong>MB group during the initial hypotensive equivalent phase (n=8)</strong></td>
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<tr>
<td>Before</td>
<td>ET-1</td>
<td>80.1±8.3</td>
<td>5669±478</td>
<td>8.9±0.5</td>
<td>95.1±6.0</td>
<td>2.5±0.3</td>
<td>146.3±7.3</td>
</tr>
<tr>
<td>After</td>
<td>ET-1</td>
<td>86.8±7.5</td>
<td>5196±287</td>
<td>11.5±0.6**</td>
<td>113.4±9.0**</td>
<td>2.6±0.3</td>
<td>144.3±6.6</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SE.

* = p<0.05; ** = p<0.01

In each group, “before ET-1” means baseline values and “after ET-1” means values recorded after the administration of ET-1. For other abbreviations, see list of abbreviations and glossary.
guanylate cyclase inhibitor (MB group). Experiments were performed in the TSA and MB groups as well as in the control group. In the MB group, ET-1 was injected 15 to 20 min after a bolus intravenous injection of MB. Since a transient early fall of MBP did not occur in the MB group, MCP and TPR were obtained 2 min after ET-1 injection when a transient fall of MBP would have occurred if MB was not used.

Experiments were performed in the following 5 groups at these times:

1. Control group
   a. During the initial hypotensive phase (n=8, BW: 10.9±0.5 kg, mean±SE)
   b. During late hypertensive phase (n=8, BW: 12.3±1.3 kg)

2. TSA group
   c. During initial hypotensive phase (n=8, BW: 11.7±0.8 kg)
   d. During late hypertensive phase (n=8, BW: 10.9±0.7 kg)

3. MB group
   e. During initial hypotensive equivalent phase (n=8, BW: 12.0±0.7 kg)

ET-1 (PEPTIDE INSTITUTE INC., 4198-s) was dissolved in distilled water and 400 pmol/kg of ET-1 was injected into a forelimb vein.

The baseline hemodynamic parameters were measured at the following points. In the control group, the baseline hemodynamic parameters were measured at the end of 15 min after the completion of instrumentation. In TSA group, the baseline hemodynamic parameters were measured at the end of about 15 min after the institution of TSA where the MBP became stable at about 70 mmHg. In MB group, the baseline parameters were measured at about 15 min after the injection of MB, i.e., when the MBP, after a surge of hypertension, became stable.

4. Statistical analysis

All values were expressed as mean±SE. Data were analyzed statistically using Student’s t-test, with p<0.05 considered to be statistically significant.

5. Abbreviations and glossary

- BW (kg): body weight
- CO (ml/min/kg): cardiac output per body weight
- HR (beats/min): heart rate
- MBP (mmHg): mean blood pressure
- %ΔMBP: percent change of MBP from the baseline blood pressure of 100
- MCP (mmHg): mean circulatory pressure
- %ΔMCP: percent change of MCP from
Fig. 4. Comparisons of %ΔMBP, %ΔTPR and %ΔMCP in control vs TSA group during the initial hypotensive and late hypertensive phases (mean±SE). initial=initial hypotensive phase, late=late hypertensive phase, □=control groups, ●=TSA group.

For other abbreviations, see list of abbreviations and glossary.

Fig. 5. Comparisons of %ΔMBP, %ΔTPR and %ΔMCP in control vs MB group during the initial hypotensive equivalent phase (mean±SE). □=control group, □=MB group, *p<0.05

For abbreviations, see list of abbreviations and glossary.

RAP (mmHg): mean right atrial pressure
RVR (dyne·sec·cm⁻⁵): resistance to venous return
TPR (dyne·sec·cm⁻⁵): total peripheral resistance
%ΔTPR: percent change of TPR from the baseline TPR of 100
TSA: total spinal anesthesia
TPR and RVR were calculated using the following formulae:

$$TPR = \frac{MBP}{(CO \times BW)} \times 8 \times 10^4$$
$$RVR = \frac{(MCP-RAP)}{(CO \times BW)} \times 8 \times 10^4$$

RESULTS

I. Effects of ET-1 on MBP, MCP and TPR (Table I)

1. Control group
   a: Initial hypotensive phase
      ET-1 lowered the MBP and TPR significantly and raised the MCP significantly.
   b: Late hypertensive phase
      ET-1 raised the MBP, TPR and MCP significantly.

2. TSA group
   c: Initial hypotensive phase
      ET-1 lowered the MBP and TPR significantly and raised the MCP significantly.

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d: Late hypertensive phase
ET-1 raised the MBP, TPR and MCP significantly.

(3) MB group
e: Initial hypotensive equivalent phase
As shown in Fig. 3, an intravenous injection of MB produced a marked transient elevation of MBP from 111.5±7.8 to 180.6±12.6 mmHg and then tended to decline toward the baseline, reaching a steady level of 81.3±10.4 mmHg slightly below baseline about 15 min after the MB injection. In this group, basal MCP before MB was not measured. ET-1 produced no significant change in either the MBP or TPR in the presence of MB. However, the MCP was elevated significantly.

II. Comparisons of %ΔMBP, %ΔTPR and %ΔMCP with ET-1 between groups
(1) Comparison of control vs TSA groups (Fig. 4)
There was no significant difference in %ΔMBP, %ΔTPR and %ΔMCP during the initial hypotensive and late hypertensive phases between the control vs TSA groups.

(2) Comparison of control vs MB groups in the initial hypotensive phase (Fig. 5)
In the control vs the MB group, the %ΔMBP (-22.2±3.4%) and %ΔTPR (-30.5±4.0%) noted in the control group were significantly greater than %ΔMBP (9.9±3.9%) and %ΔTPR (-6.1±5.7%) in the MB group. In the control vs the MB group, the %ΔMCP (16.1±5.3%) in the control group was lower than %ΔMCP (30.7±3.1%) in the MB group.

DISCUSSION
An intravenous injection of ET-1 (0.1–1 nmol/kg) in rats produced transient hypotension within 2 min, followed by hypertension lasting more than 1 h. In the present study, ET-1 produced a similar change in blood pressure (Fig. 2). It has been reported that ET-1 administration releases endothelium-derived relaxing factor (EDRF) or PGl2 from the isolated artery in rats or guinea pigs; it has been speculated that the transient hypotension induced by ET-1 in vivo is caused by these vasodilator substances. The initial transient hypotension induced by ET-1 is not inhibited by indomethacin, a cyclooxygenase inhibitor, while the late hypertension is exaggerated by indomethacin. Therefore, the transient hypotension induced by ET-1 would mainly be caused by EDRF. In the present study, we investigated whether the transient hypotension induced by ET-1 would be inhibited by MB. The experiment in which ET-1 (10 nmol/kg/min) was infused constantly 30 min after a bolus injection of MB, 3 mg/kg, showed that MB did not inhibit the transient hypotension caused by ET-1. In the present study, we used doses of MB higher than those used in previous rat experiments described above. As a result, the fall of MBP and TPR caused by ET-1 disappeared (Fig. 5).

If ET-1 released EDRF which in turn would dilate the systemic resistance vessels transiently, there would be a transient dilation of the systemic capacitance vessels. However, this study showed that the systemic capacitance vessels were constricted (Fig. 5). It seemed possible that this constriction of the systemic capacitance vessels would result from a baroreceptor reflex due to hypotension. However, this possibility was excluded since the constriction of the systemic capacitance vessels by ET-1 was observed even in the presence of TSA (Fig. 4).

ET-1 markedly constricts isolated canine femoral and saphenous veins? In the present study, both the systemic resistance and capacitance were constricted at the same time during the late hypertensive phase in the control and TSA groups. This finding indicates that ET-1 constricts the systemic capacitance vessels in vivo. The present study also showed that, during the initial hypotensive phase, ET-1 dilated the systemic resistance vessels but constricted the systemic capacitance vessels. If it is presumed that the early transient fall in MBP is brought about by EDRF that was released by ET-1 judging from the result of MB group (Fig. 5), the possible explanations for the difference in ET-1 action on the systemic capacitance and resistance vessels between the initial hypotensive vs the late hypertensive phases are as follows: the study performed with isolated arteries and veins (femoral and mesenteric arteries in dogs, femoral and saphenous veins in dogs)
showed that the vasodilator action of nitric oxide (NO) was weaker in the veins than in the arteries? Thus, it is reasonable to conclude that (a) the vasoconstrictor action of ET-1 is stronger in the veins than the arteries and that (b) the vasodilator action of EDRF is stronger in the arteries than in the veins. It seems likely that when the EDRF which may be released by ET-1 dilates the systemic resistance vessels, the vasoconstrictor action of ET-1 on the systemic capacitance vessels is stronger than the vasodilator action of EDRF on the systemic capacitance vessels, thereby constricting the systemic capacitance vessels as a net action. This possibility is supported strongly by the observation that the MCP following intravenous ET-1 in the MB group exceeded that of the control group (Fig. 5). Our study showed that ET-1 caused a biphasic change in the MBP, and that ET-1 constricted both the systemic resistance and capacitance vessels in the late hypertensive phase. However, it dilated the systemic resistance vessels, but constricted the capacitance vessels, in the initial hypotensive phase.

The differing actions of ET-1 on the systemic resistance and capacitance vessels in the initial hypotensive phase was the combined result of the difference of vasodilator action of EDRF released by ET-1 between the systemic resistance and capacitance vessels and the differing vasoconstrictor action of ET-1 itself between the systemic resistance and capacitance vessels.

These observations suggest that the actions of ET-1 per se and EDRF on the systemic resistance and capacitance vessels differ.

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