EFFECTS OF ENDOThELIN AND
VASOPRESSIN ON PORTAL PRESSURE OF RATS

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Summary

Endothelin is a vasoconstrictor peptide which has recently been isolated and sequenced from the vascular endothelial cells. It was reported to increase blood pressure in vivo and produce a prolonged contraction with a slow onset in vitro. The purpose of this study was to investigate whether endothelin can lower the portal pressure as another endogenous vasoconstriction peptide-vasopressin (AVP) can. Heart rate, systemic blood pressure, portal pressure, and portal vein blood flow were measured. Effects of endothelin on these parameters were compared with those of AVP. Endothelin 10^{-10} mol/Kg significantly decreased all of the parameters mentioned. At the higher dose (5x10^{-10} mol/Kg), however, the portal pressure and blood pressure were increased and portal vein blood flow was unchanged. On the other hand, AVP decreased the portal pressure and portal vein blood flow but elevated the systemic blood pressure. In vitro experiments revealed that endothelin contracted both tail artery and portal vein of rat and vasopressin contracted only tail artery. We concluded that although both are endogenous vasoconstricting peptides, endothelin and AVP affect differently on arterial and venous vascular beds as well as on portal pressure.

Endothelin, which has been recently identified from cultured endothelial cells of aorta, is one of the most potent vasoconstrictor known (1). Its vascular actions have been studied intensively since. In general, the vasoconstriction produced by endothelin is long lasting in various tissue preparations (2-4). However, tissue specificity to the vasoconstrictor action has also been demonstrated (5). In studying the mechanisms of action, a multifactorial pathway has been suggested, including opening of ion channels (1, 6), stimulating phosphatidylinositol turnover (7), activating protein kinase C (8), and modulating adenylate cyclase (Yang et al., submitted).

~ 88 ~
In a recent study, Van Renterghem et al. (9) reported that the molecular mechanism of vasoconstriction by endothelin was similar to another endogenous vasoconstricting peptides, vasopressin. Faraci (10) reported a similar vasoconstricting effect of endothelin and vasopressin on cerebral blood vessels in anesthetized rats. Arginine vasopressin (AVP) has long been indicated in the treatment of variceal bleeding (11, 12) because of its ability of lowering the portal pressure due to a vasoconstricting effect on the splanchnic vascular beds and a reduction in the portal venous inflow. Since both endothelin and AVP are endogenous vasoconstricting peptides and have similar molecular mechanism on the vascular smooth muscle, it is of interest to investigate the effect of endothelin on the portal pressure. In the present study, both in vivo and in vitro experiments were conducted to compare the action of endothelin and AVP on the portal pressure of rats.

Methods

In vivo experiment

Male Sprague-Dawley rats (300-350 g) were anesthetized with sodium pentobarbital (50 mg/Kg i.p.). Right common carotid artery and jugular vein were cannulated with PE-50 tubing for systemic blood pressure recording (via Statham pressure transducer) and i.v. injection, respectively. The trachea was also intubated to keep the airway patent. A cannula was placed at the ileocolic vein for portal pressure recording (13). The portal vein was then carefully isolated and an electromagnetic flow probe (1.5 mm in diameter) was applied to measure the blood flow via a flowmeter (Gould SP 2202). Heart rate was computed with a biotachometer triggered by the arterial pressure pulses. All of the parameters were displayed on a Gould physiograph (ES 1000).

After surgery, the animals were not receiving any treatment before all of the recordings were stabilized. For each animal only one injection was given to avoid the interaction between doses and between drugs. Endothelin (rat 1-28) was purchased from Cambridge Research Biochemicals and arginine vasopressin from Sigma Chemical Company.

In vitro tension study.

Experimental technique has been previously reported (14). Male Sprague-Dawley rats (250-300 gm) were anesthetized with sodium pentobarbital 50 mg/Kg. The ventral caudal artery was quickly isolated and put in a Petri-dish containing aerated Krebs-Henseleit (K-H) solution. The composition of K-H solution (in mM) is: NaCl 115; KCl 5; CaCl2 2.1; MgSO4 1.2; NaH2PO4 1.2; NaHCO3 25; and glucose 11. A segment of blood vessel approximately 1.5 cm in length was then cut into helical strip and was suspended in Sawyer-Bartleson tissue chamber. The tissue chamber was continuously bubbled with a gas mixture of 95% O2 and 5% CO2. The isometrically developed forces were recorded on Grass polygraph via Grass FT03 force displacement transducers. The strip was equilibrated in the chamber for at least 1 hr under basal tension of 0.7 gm. Isolated portal vein was prepared with the similar manner when needed. The readiness of the tissue was indicated by the consistent responses by two consecutive tests with KCl 60 mM. Following the equilibration, cumulative concentrations (10-9-3x10-7 M) of endothelin and
AVP (3x10^{-8}-10^{-9} M) were used to initiate the contraction. When the entire concentration study was completed, the tissue was discarded for the effect of endothelin was so long lasting and also to avoid desensitization. Seven rats, two strips from each, were used for studying the dose-response of endothelin and AVP. Another twelve rats, one strip of tail artery and portal vein from each were sacrificed to compare the actions of tail artery and portal vein.

Statistically significant difference in the responses before and after treatment of either endothelin or AVP in in vivo study was determined by Student's paired t-test at p<0.05. Statistically significant difference in the responses to the same concentration of endothelin or AVP (in vitro) was determined by Student's t-test at p<0.05. Data are presented as mean±SEM.

Results

In vivo experiment

Intravenous injection of endothelin at lower dose (10^{-10} mol/Kg) significantly decreased the portal pressure and portal vein blood flow (Figure 1 and Table 1). In addition, these effects were accompanied with decreases in the blood pressure and heart rate. The blood pressure was decreased immediately after the injection and was recovered in two minutes. However, the decrease in heart rate was due to the volume effect of the injection, since it also appeared in the injection of saline at the same volume. It should be noted that the basal level of the portal pressure presented in this study were higher than the actual value because of the application of flow probe.

![Figure 1](image)

Typical responses to endothelin 10^{-10} mol/Kg in heart rate (HR), systemic blood pressure (BP), portal pressure (PP) and portal vein blood flow (FLOW) in anesthetized rat.

Higher dose (5x10^{-10} mol/Kg) of endothelin, however, exhibited different effects (Table 1). It significantly increased the portal pressure at five minutes after the injection but did not alter the portal vein blood flow. Associated with the increase in portal pressure, the blood pressure, albeit decreased initially, was in-
TABLE I

Effect of Endothelin and VP on the Heart Rate (HR), Systemic Blood Pressure (BP), Portal Pressure (PP) and Portal Vein Blood Flow (FLOW) of Anesthetized R

<table>
<thead>
<tr>
<th></th>
<th>Control*</th>
<th>Endothelin 10^-10 mol/Kg</th>
<th>Endothelin 5x10^-10 mol/Kg</th>
<th>AVP 3x10^-10 mol/Kg</th>
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<tbody>
<tr>
<td></td>
<td>30 sec after</td>
<td>5 min after</td>
<td>30 sec after</td>
<td>30 sec after</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>314 ± 23(*)</td>
<td>291 ± 18*</td>
<td>281 ± 11(5)</td>
<td>261 ± 9(6)</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td>133 ± 7(10)</td>
<td>110 ± 8*</td>
<td>116 ± 11(11)</td>
<td>110 ± 3(*)</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>15.3 ± 1.5(10)</td>
<td>14.7 ± 1.4*</td>
<td>18.3 ± 2.4(5)</td>
<td>19.6 ± 2.6(7)</td>
</tr>
<tr>
<td>FLOW (ml/min)</td>
<td>8.1 ± 1.1(8)</td>
<td>6.4 ± 1.0*</td>
<td>8.4 ± 1.8(5)</td>
<td>4.9 ± 0.2(7)</td>
</tr>
<tr>
<td></td>
<td>Control*</td>
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</tr>
</tbody>
</table>

*: Before injection.
*: Significantly different from control at p<0.05.
Number in parenthesis indicates the number of animals used.
HR and Flow in some of the rats were not recorded.

Effect of Endothelin and VP on the Heart Rate (HR), Systemic Blood Pressure (BP), Portal Pressure (PP) and Portal Vein Blood Flow (FLOW) of Anesthetized R

As has been reported, i.v. injection of AVP 3x10^-10 mol/Kg induced significant decrease in the portal pressure and portal vein blood flow accompanied with the decrease in heart rate and increase in the blood pressure (Figure 2 and Table 1).

In vitro experiment

Both endothelin and AVP were similar in their efficacy to produce the vasocontraction on the rat tail artery although AVP was much less sensitive (Figure 3). To compare the effects of endothelin on tail artery and portal vein in the same rat, it was found that the portal vein was less sensitive to endothelin at the lower doses.

~ 91 ~
Typical responses to AVP 3x10^-10 mol/Kg in heart rate (HR), systemic blood pressure (BP), portal pressure (PP), and portal vein blood flow (FLOW) in anesthetized rat.

![Graph showing typical responses to AVP](image)

**Fig. 2**

Concentration-responses curves of endothelin and AVP on rat tail artery (n=7).

![Graph showing concentration-responses curves](image)

**Fig. 3**

Somewhat as predicted, endothelin did decrease the portal pressure and portal vein blood flow. However, these effects could only be induced at lower dose. At higher dose endothelin increased the portal pressure and did not change the portal vein blood flow. In vitro experiment demonstrated that endothelin was able to pro-

(Figure 4). On the other hand, AVP (up to 10^-5 M) produced essentially no response on the portal vein (Data not shown).

**Discussion**

duce con-

[Image 0x0 to 610x842]
duce contraction on the isolated portal vein to a similar extent as on the arterial tissue, although not as sensitive at the lower concentrations.

![Concentration-responses curves of endothelin on rat tail artery and portal vein (n=6). Responses was indicated as percent of response to KCl 60 mM.](image)

**Fig. 4**

Concentration-responses curves of endothelin on rat tail artery and portal vein (n=6). Responses was indicated as percent of response to KCl 60 mM.

The decrease in portal vein blood flow may be due to the sympathetic reflex from the lowering of blood pressure as has been produced with some other hypotensive agents (15, 16). Since lower dose of endothelin expressed only hypotensive action, the decrease in portal pressure by such dose may result from the decrease in portal vein blood flow which overcome the direct effect of endothelin on the venovascular. Although the portal pressure was decreased by the lower dose of endothelin, it could be increased by the higher dose. It is, therefore, to be concluded that as an vasoconstrictor peptide, endothelin should be carefully handled in regard to the effect on portal pressure.

The hypotensive action of endothelin was not only existed at the lower dose, it was also presented in the higher dose of initial phase. This cannot be explained as an injection artifact. Yanagisawa and the colleagues had demonstrated a dose-dependent initial transient decrease in blood pressure, whereas in the treatment of angiotensin II this initial hypotensive effect was not observed (2). Similar finding has also been reported in conscious normotensive (5) and hypertensive rats (17), cats (18), and dogs (19). Knuepfer et al. demonstrated that the skeletal muscle vasculature was sensitive to the vasodilatory action of endothelin (20). Similar finding was observed by Minkes and Kadowitz (18). These authors suggested that the responses to endothelin depend on the vascular beds studied. It was reported that the vasodilatory effect of endothelin may involve the release of endothelium-derived relaxing factor (21). However, in the present in vitro study, we failed to observe a vasodilatory effect in either quiescent or high potassium and norepinephrine-contracted intact tail artery (not shown). Thus,
the nature of this hypotensive action by endothelin remains to be clarified.

In order to investigate the diverse effect on portal pressure by endothelin and AVP, in vitro experiments with artery and vein were carried out. It was interesting to note that AVP contracted only arterial blood vessel, not the venous. Tomita et al. have also demonstrated that while the arterial pressure of the fetal sheep was elevated after AVP infusion, the venous pressure was unchanged (22). These findings are in contrast to the results of Fortes et al., who have reported that the venules of mesentery were constricted by [Lys8]vasopressin (23). The discrepancy may be due to the vascular sensitivity or the intrinsic activities of the peptide (24).

After the higher dose of endothelin, in addition to the arterial vasoconstriction, the venous resistance increased due to the constriction of venous vasculature so that the portal vein blood flow was not significantly altered and resulted in an increase in portal pressure. Whereas in AVP treated rat, the venous resistance was not changed, the arterial vasoconstriction lead to the decrease in portal vein blood flow and, hence, the portal pressure.

The present study was carried out with normal instead of portal hypertensive rats. It is aware that the splanchnic vasculature may be changed and that the responses to drugs may differ in the portal hypertensives. Nevertheless, our results indicated that endothelin and AVP elicited different response on portal pressure, despite the similarity on the vasoconstricting properties on the arterial vasculature. It is unlikely that endothelin could be used for the treatment of variceal bleeding in liver cirrhosis.

Acknowledgments

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