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Effects of endothelin on porcine coronary arterial strips

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ABSTRACT Endothelin, a novel endothelium derived 21-residue vasoconstrictor peptide synthesized by Peninsula Laboratories, provoked a concentration-dependent contraction of porcine coronary arterial strips. EC_{50} value for endothelin was 14±SD 4 nmol/L ($n=6$), and significantly lower than the values for 5-hydroxytryptamine (5-HT, 0.28 ± 0.07 μmol/L, $n = 6$) and 15-methyl-prostaglandin $F_{2\alpha}$ (15-methyl-$PGF_{2\alpha}$, 4 ± 3 μmol/L, $n=7$). The maximal increase in tension caused by endothelin was 5.4 ± 1.1 g, being much greater than that induced by 5-HT (3.7 ± 0.8 g, $P<0.05$) and 15-methyl-$PGF_{2\alpha}$ (3.7 ± 0.6 g, $P<0.01$). The changes in tension provoked by endothelin (2-20 nmol/L) were attenuated significantly after pretreated with tetrodotoxin (TTX, 30 μmol/L, $P<0.05$ or 0.01). The results suggest that endothelin is one of the most potent vasoconstrictive agents, and its action is partially related to voltage-sensitive Na^+ channel in the cell membrane.

KEY WORDS drug dose-response relationship; swine; coronary vessels; vasoconstriction; vascular endothelium; serotonin; prostaglandins F; tetrodotoxin; verapamil; endothelin

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Since the discovery of endothelium-dependent vasodilatation by Furchgott and Zawadzki in 1980[1], it has been recognized that the vascular endothelial cells play an important role in the regulation of vascular smooth muscle tension. In recent years it has been confirmed that in addition to endothelium derived relaxing factor, vascular endothelial cells produce a substance

which possesses a potent vasoconstriction (endothelium-derived contracting factors, EDCF)[2,3]. A novel, endothelium-derived 21-residue vasoconstrictor peptide has been isolated and purified from the culture supernatant of porcine aortic endothelial cells. It has been shown to be a high potent vasoconstrictor, and named endothelin by Yanagisawa in 1988[4], and with an EC_{50} of least one order of magnitude lower than the reported values for angiotensin II[5], vasopressin[6] or neuropeptide Y[7]. Synthetic endothelin was prepared according to the analytically determined structure (Fig 1). This study was designed to compare the vasoconstrictive effect of endothelin synthesized by Peninsula Laboratories, with 5-HT and 15-methyl-$PGF_{2\alpha}$ in porcine coronary artery, and to investigate the probable effect of endothelin on Na^+ channel.

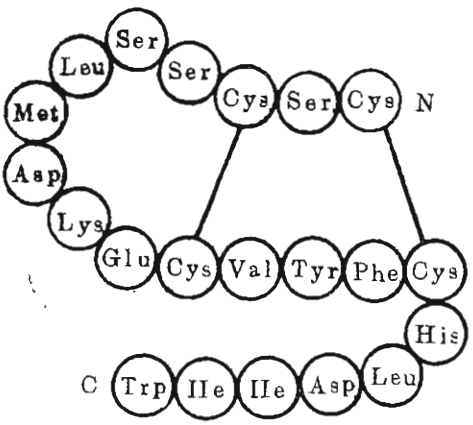

Fig 1. Amino-acid sequence of endothelin.

MATERIALS AND METHODS

Right proximal coronary arteries were isolated from fresh adult porcine hearts, brought from a local slaughter-house 20-30 min after death and kept in 4℃ Krebs-Ringer solution[4] gassed with 95% O_2 + 5% CO_2. Connective tissue was removed. Arterial segments were cut into 2.5 × 15 mm helical strips with the intima denuded by rubbing with a small swab[4](The effectiveness of intimal denudation was assessed by abolition of the vasodilatory response to substance P 0.1 μmol/L). Arterial strips were suspended in 5 ml glass organ chambers filled with Krebs-Ringer solution maintained at 37 ± 0.5℃ and gassed with 95% O_2 + 5% CO_2. Isometric tension was continuously recorded by a recorder (type 3066 pen recorder, Yokogawa Hokushin Electric, Tokyo). Coronary arterial strips were allowed to equilibrate at a resting tension of 3 g for 2 h before the test.

Effects of endothelin, 5-HT and 15-methyl-$PGF_{2\alpha}$ on porcine coronary arterial strips Different concentrations of endothelin were added in a cumulative fashion, and cumulative concentration-response curve (CCRC) to endothelin was obtained. The CCRCs to 5-HT and 15-methyl-$PGF_{2\alpha}$ were obtained in the same fashion mentioned above. Only one CCRC was made per preparation. EC_{50} for each CCRC was calculated by the computer program of linearization of dose-respones curve (Hanes-Woolf method). EC_{50} value for each drug was expressee as $\bar{x} \pm SD$.

Effect of TTX on vasoconstriction induced by endothelin Two helical strips were cut from the same arterial segment, and put simultaneously in 2 muscle chambers. Initially, KCl 30 mmol/L was added into the chambers. After the effect reached the maximum, KCl was washed out for 3 times with Krebs-Ringer solution. After 30 min, TTX 30 μmol/L was added into one chamber, different concentrations of endothelin were added in a cumulative fashion 20 min afterwards. In the other chamber, only endothelin was added. The contractile response to each concentration of endothelin was expressed as % of those induced by KCl 30 mmol/L on each strip. The CCRCs to endothelin in the TTX pretreated and untreated strips were obtained.

Effect of verapamil on vasoconstriction induced by endothelin in strips untreated or pretreated with TTX Two strips

from the same artery were prepared as above. In one chamber, endothelin 25 nmol/L was added. In the other chamber, TTX 3 μmol/L was administered at first, and the same concentration of endothelin was added 20 min afterwards. After a stable tension induced by endothelin was obtained in both strips, verapamil 50 μmol/L was given.

Drugs Drugs used were endothelin (Peninsula Laboratories Inc, Belmont CA 94002, USA), serotonin creatinine sulfate (Swiss), 15-methyl-$PGF_{2\alpha}$ (Injection, 2 mg/ml, Shanghai 9 th Pharmaceutic Factory), TTX (Fisheries research Institute of Hebei Province), verapamil (Injection, 5 mg/ml, Shanghai Tianfeng Pharmaceutic Factory). They were dissolved in distilled water.

Statistics Data were expressed as $\bar{x}\pm$ SD. Differences of mean values with and without TTX were assessed by a pair-*t* test.

RESULTS

Effects of endothelin, 5-HT and 15-methyl-$PGF_{2\alpha}$ on porcine coronary arterial strips The concentration-dependent contraction curves caused by endothelin (2-200 nmol/L, $n=6$), 5-HT (0.03-3 μmol/L, $n=6$) and 15-methyl-$PGF_{2\alpha}$ (0.27-81 μmol/L, $n=7$) were shown in Fig 2. EC_{50} for endothelin, 5-HT and 15-methyl-$PGF_{2\alpha}$ were 14 ± 4 nmol/L, 0.28 ± 0.07 and 4 ± 3 μmol/L, respectively. EC_{50} value for endothelin was 20 and 314 times less than the values for 5-HT and 15-methyl-$PGF_{2\alpha}$ respectively.

Vasoconstriction induced by endothelin developed slowly, 2-5 min after administration and reached a steady-state tension in about 10-20 min. In contrast, vasoconstriction caused by 5-HT and 15-methyl-$PGF_{2\alpha}$ appeared 1-2 min after the drugs, and reached a stable level in 5-7 min.

Maximal increase in tension caused by endothelin 0.2 μmol/L was 5.4 ± 1.1 g ($n=6$), while those induced by 5-HT 3 μmol/L and 15-methyl-$PGF_{2\alpha}$ 81 μmol/L were 3.7±0.8 g ($n=6$) and 3.7±0.6 g ($n=7$) respectively, and significantly less than that by endothelin ($P<0.05$, and 0.01 in turn).

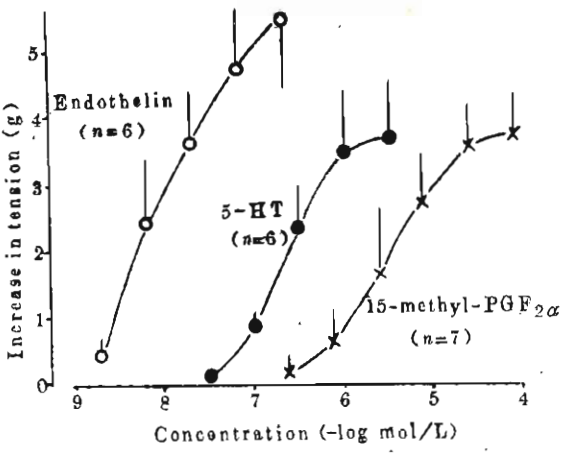

Fig 2. Cumulative concentration-response curves for endothelin, 5-hydroxytryptamine (5-HT) and 15-methyl-prostaglandin $F_{2\alpha}$ (15-methyl-$PGF_{2\alpha}$) in the porcine right coronary arterial strips. $\bar{x}\pm$SD.

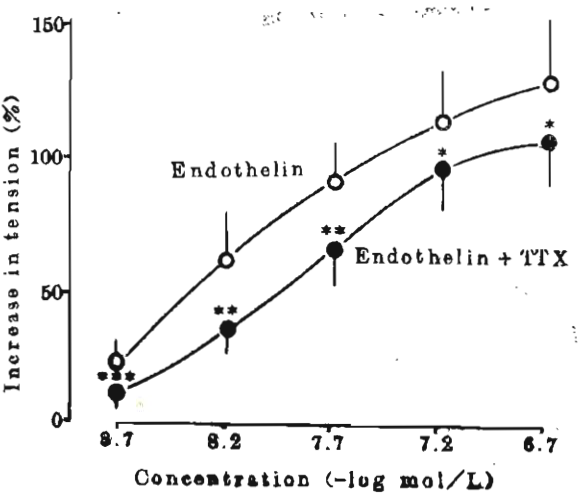

Fig 3. Effects of tetrodotoxin (TTX, 30 μmol/L) on the cumulative concentration-response curves for endothelin in the porcine right coronary arterial strips. Increases in tension induced by endothelin were expressed as % of those induced by KCl 30 mmol/L in each strip, respectively. $n=6$, $\bar{x}\pm$SD. *$P>0.05$, **$P<0.05$, ***$P<0.01$

Effect of TTX on vasoconstriction induced by endothelin Since the vasoconstriction caused by endothelin was long-lasting and difficult to be washed out, a pair test design was used. The CCRCs elicited by endothelin 2–200 nmol/L in the strips with or without TTX 30 μmol/L were shown in Fig 3. In the strips pretreated with TTX, a voltage-dependent Na^+ channel blocker, the responses to endothelin were attenuated significantly.

Effect of verapamil on vasoconstriction induced by endothelin in strips untreated or pretreated with TTX In control group, increase in tension provoked by endothelin 25 nmol/L was 3.4± 1.0 g, while in the group pretreated with TTX, the change in tension induced by the same concentration of endothelin was 2.7 ± 0.9 g ($n=13$), a value significantly less than that of untreated strips ($P<0.05$). Verapamil 50-μmol/L relaxed completely the vasoconstriction provoked by endothelin either in control or in TTX group.

DISCUSSION

In this parallel comparison study, we found that EC_{50} for synthetic endothelin was 20 and 314 times less than the values for 5-HT and 15-methyl-$PGF_{2\alpha}$ respectively, and that the maximal increase in tension induced by endothelin was also much greater than that obtained by 5-HT and 15-methyl-$PGF_{2\alpha}$. These results suggest that the vasoconstriction of endothelin is much stronger than that of 5-HT and 15-methyl-$PGF_{2\alpha}$, and that endothelin is one of the most potent vasoconstrictive agents known to date.

It has been known that TTX binds to voltage-sensitive Na^+ channel located in excitable cell membrane, inhibits sodium ion transport, decreases intracellular sodium activity of nerve endings, and eventually, leads to decrement in transmitter releases. We found that following pretreatment with TTX 30 μmol/L, the vasoconstriction caused by endothelin 2–20 nmol/L was attenuated significantly, but not disappeared. The contraction of coronary artery provoked by EDCF or endothelin was not affected by α-adrenergic, serotonergic, H_1-histaminergic and cholinergic antagonists, and was also unchanged by the addition of cyclooxygenase and lipoxygenase inhibitors[2,3,4]. The contraction of the vascular smooth muscle evoked by endothelin may come from acting directly on the smooth muscle cell. Amino-acid sequence evaluation shows significant regional homologies between endothelin and α-Scorpion toxins[4,8] which bind to TTX-sensitive Na^+ channel and inhibit the inactivation of the activated channel[9], suggesting also that endothelin may act directly on membrane channels. We found further that the vasoconstriction induced by endothelin was relaxed completely by verapamil 50 μmol/L. The finding agrees with the report that the vasoconstriction of endothelin was markedly attenuated in the presence of nicardipine[4], and suggests that the increase in the influx of extracellular Ca^{2+} through calcium channel in the cell membrane of smooth muscle is required for the action of endothelin. Furthermore, the vasoconstriction of endothelin was decreased significantly by TTX, suggesting the possibility that endothelin may also act on TTX-sensitive Na^+ channel, inhibit the inactivation of Na^+ channel, increase intracellular sodium activity, and raise the concentration of free calcium by increasing sodium-calcium exchange. The decrement in contraction of endothelin in the strips pretreated with TTX may come from the interaction between endothelin and TTX on the Na^+ channel.

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血管内皮素对猪冠状动脉条的作用

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提要 血管内皮素，一种从猪主动脉内皮得到的 21个氨基酸组成的新的血管收缩肽，由美国 Peninsula Lab 合成。它引起猪冠状动脉条浓度依赖性收缩，其EC_{50}为 14±SD 4 nmol/L($n=6$)，分别为 5-羟色胺(0.28±0.07 μmol/L, $n=6$)和 15-甲基前列腺素 $F_{2\alpha}$(4±3 μmol/L, $n=7$)的 1/20 和 1/314。内皮素产生的最大张力增加是 5.4±1.1 g，比 5-羟色胺(3.7±0.8 g, $P<0.05$)和 15-甲基前列腺素 $F_{2\alpha}$(3.7±0.6 g, $P<0.01$)为大。先给予河豚毒素 30 μmol/L 明显减弱内皮素 2-20 nmol/L 的作用。结果提示内皮素是一种作用极强的血管收缩物质，它的作用部分地与细胞膜上电压敏感性钠通道有关。

关键词 药物剂量-效应关系；猪；冠状血管；血管收缩；血管内皮；血清素；前列腺素 F类；河豚毒素；维拉帕米；内皮素