

## 토끼 적출 신동맥에 있어서 substance P에 의한 이완작용 기전

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### Mechanism of vasodilatation induced by substance P in isolated rabbit renal artery

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**Abstract** : The effects of removing the endothelium on the vasodilatory response to substance P, calcitonin gene-related peptide (CGRP), and vasoactive intestinal peptide (VIP) was examined in the isolated rabbit renal artery. The vasodilator response to substance P (0.1  $\mu$ M) was completely absent in vessels in which the endothelium had previously been removed. There was no significant difference in the vasodilatation produced in response to CGRP (0.1  $\mu$ M), or VIP (0.1  $\mu$ M) in the intact and removed-endothelium rabbit renal artery segments. L-NAME (100  $\mu$ M) significantly reduced the vasodilatory response to substance P (0.1  $\mu$ M). This inhibition was significantly attenuated when L-arginine (10 mM) was also present in the organ bath along with L-NAME (100  $\mu$ M). Indomethacin (1  $\mu$ M) did not significantly affect the vasodilatation produced in response to substance P (0.1  $\mu$ M). The inhibitory effect of L-NAME (100  $\mu$ M) and indomethacin (1  $\mu$ M) on the vasodilatory response to substance P (0.1  $\mu$ M) was not significantly different from that produced by L-NAME (100  $\mu$ M) alone. This study indicates that substance P induced vasodilatation via an endothelium-dependent mechanism in the isolated rabbit renal artery. It also established that CGRP and VIP induced vasodilatation by an endothelium-independent mechanism and substance P-induced vasodilatation is at least partially via NO.

**Key words** : vasodilatation, nitric oxide, substance P, calcitonin gene-related peptide, vasoactive intestinal peptide, renal artery, rabbit

### Introduction

Endothelial cells play a key role in the control of vascular tone by virtue of their ability to synthesize and release endothelium-derived relaxing factors (EDRF). Various agents, including acetylcholine(Ach), bradykinin, adenosine 5-triphosphate(ATP), substance P and serotonin

elicit vasodilatation in certain vascular beds and isolated blood vessels via an action at receptors located on endothelial cells, leading to the release of these factors [2, 6, 8, 22].

In some blood vessels, the release of EDRF also mediates the vascular relaxation evoked by certain vasodilators including Ach, ATP, substance P and

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bradykinin [6, 9, 10, 12].

EDRF has been identified as the free radical of nitric oxide (NO) [11, 14, 15] and has chemical and pharmacological properties identical to it [11, 14, 23].

In endothelial cells, NO is produced from the conversion of L-arginine into L-citrulline by NO synthase (NOS) [20, 24, 25, 30]. Analogues of L-arginine are, therefore, potentially specific inhibitors of EDRF-mediated effects on vascular tone. In fact, it has been shown that NG-nitro-L-arginine methyl ester (L-NAME) is an inhibitor of enzymatic synthesis of NO from L-arginine [30].

The relaxation induced by Ach has been shown to induce release of prostaglandins and NO [1, 11, 16]. In the kidney, inhibition of cyclooxygenase activity has been shown to reduce autoregulatory responses to changes in blood pressure [13]. EDRF and prostacyclin may be released in parallel and have synergistic thrombin-resistant effects [28].

The purpose of this study is to establish the hypothesis that the some vasodilatory peptides induce vasodilatory mechanism via an endothelium of the isolated rabbit renal artery.

## Material and Methods

### Animals

Male New Zealand White rabbits weighing 2–3.5 Kg were sacrificed by pentobarbital sodium (100 mg/kg) anesthesia and exsanguination. The renal arteries were excised and cleared of surrounding fatty tissue under a dissecting microscope.

### Tissue preparation

The isolated renal artery was cut into approximately 5 mm length segments while still in 4°C Krebs ringer solution. These were mounted horizontally under isometric conditions in a 10 ml organ bath by inserting a tungsten wire through the lumen of the vessel ring, taking care not to damage the endothelium.

### Recording system

The specimen was then attached to a stationary support. Another wire was inserted and connected to the force transducer (FT03, Grass). The responses were recorded on a polygraph (79, Grass ink-writing polygraph). The arterial ring preparations were then placed under an initial resting tension of 1 g and allowed to equilibrate

for at least 1 hour in Krebs ringer solution of the following composition (mM): NaCl, 120; KCl, 4.75; Glucose, 6.4; NaHCO<sub>3</sub>, 25; KH<sub>2</sub>PO<sub>4</sub>, 1.2; MgSO<sub>4</sub>, 1.2; and CaCl<sub>2</sub>, 1.2, pH 7.4. The solution was maintained at 37°C and aerated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The endothelium was removed from one of a pair of adjacent ring segments by drawing a fine silk thread through the lumen. A cumulative concentration-response curve to noradrenaline was established on each segment of the artery in order to fix maximal contractile response and the concentration of agonist required to produce 40% maximal response (EC40). After contractions stabilized, increasing concentration of Ach (0.01–10 µM) were added to organ bath in a cumulative manner.

A submaximal concentration of calcitonin gene-related peptide (CGRP, 0.1 µM), vasoactive intestinal peptide (VIP, 0.1 µM) and substance P (0.1 µM) were applied to the specimen at raised tone. Only one dose of each peptide was applied to the strips due to desensitization of the preparations on repeated exposure of the peptides to the ring segments. The peptides were removed from the Krebs solution once a maximal relaxation was attained, again, to minimize the possibility of desensitization occurring. These experiments were carried out on vessel segments with and without an endothelium.

An inhibitor was then added to the organ bath and the specimen was allowed to equilibrate for 20 min. Four separate experiments were carried out. The effect of L-NAME (100 µM) on relaxant response with the addition of L-arginine (10 mM) was the first experiment to be conducted. The second set of experiments established whether the competitive inhibitory effect of L-NAME could be prevented by addition of substrate. The third set of experiments examined the inhibitory effect of indomethacin (1 µM) on endothelium-dependent vasodilation. The fourth set of experiments was carried out to determine a possible dual role for nitric oxide and prostanoids in endothelium-dependent vasodilatation, therefore both L-NAME (1 µM) and indomethacin (1 µM) were added to the organ bath.

Relaxed response to substance P, CGRP and VIP are expressed as the percent relaxation of the noradrenaline-induced contraction. Data are expressed as mean±SE. The results were analysed using student *t*-test and probability of less than 0.05 was considered significantly.

At least 20 min was allowed between washing one drug from the organ bath and testing it with another. During this time, the Krebs solution was changed approximately every 5 min.

Acetylcholine bromide, noradrenaline bitartrate, sodium nitroprusside, NG-nitro-L-arginine methyl ester, indomethacin, and L-arginine were obtained from Sigma Chemical Company. Calcitonin gene-related peptide, vasoactive intestinal peptide and substance P were obtained from Cambridge Research Biochemicals. All drugs were dissolved in distilled water except noradrenaline, which was dissolved in ascorbic acid (100  $\mu$ M).

## Result

### Effects of removing the endothelium on the relaxant response to various vasodilators

The vasodilatation produced in response to substance P (0.1  $\mu$ M; Fig. 1B) was completely absent in vessels in which the endothelium had been removed.

The vasodilatation produced by CGRP (0.1  $\mu$ M) and VIP (0.1  $\mu$ M) was not significantly affected by the removal of the endothelium from the renal artery (Fig. 1C,D and 2)

### Effect of N<sup>G</sup>-nitro-L-arginine methyl ester and L-Arginine

The effect of L-NAME (100  $\mu$ M) and L-arginine (10 mM) on the vasodilatation produced in response to substance P (0.1  $\mu$ M; Fig. 3) is illustrated.

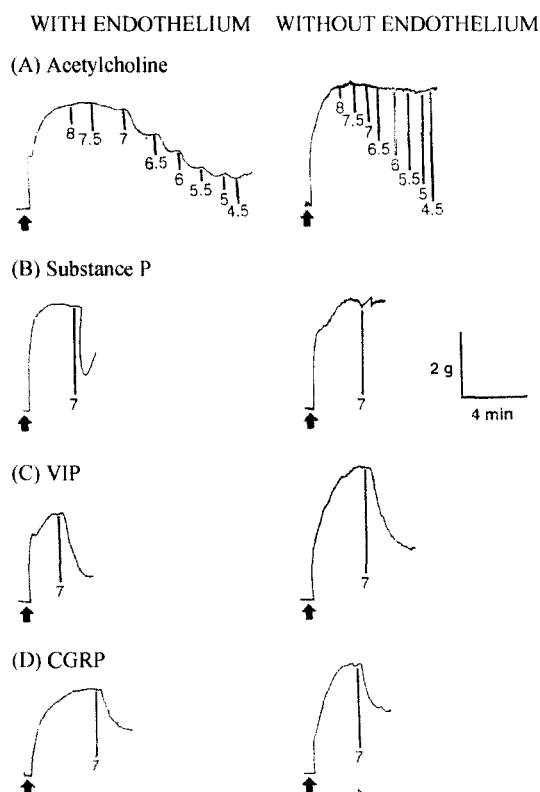
L-NAME (100  $\mu$ M) significantly reduced the vasodilatation produced in response to substance P (0.1  $\mu$ M; Fig. 3). This inhibition by L-NAME (100  $\mu$ M) was significantly attenuated in the presence of L-arginine (10 mM; Fig. 3).

The resetting basal tone of the specimen was unaffected by the addition of L-NAME (100  $\mu$ M) and L-NAME (100  $\mu$ M) with L-arginine (10 mM).

### Effect of the prostanoïd synthesis inhibitor, indomethacin on vasodilatation

The effect of indomethacin (1  $\mu$ M) on the vasodilatation produced by substance P (0.1  $\mu$ M) is illustrated in Fig. 3.

Indomethacin (1  $\mu$ M) did not significantly affect the vasodilatation produced in response to substance P (0.1  $\mu$ M; Fig. 3).

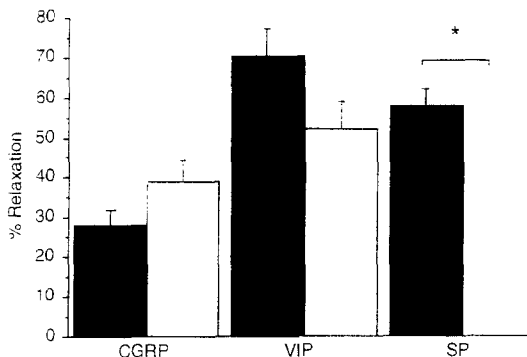


**Fig. 1.** Relaxations produced in the isolated renal artery of the rabbit in response to (A) acetylcholine, (B) substance P, (C) vasoactive intestinal peptide (VIP) and (D) calcitonin gene-related peptide (CGRP). The final concentration of agonist in the organ bath is expressed as  $-\log(\text{agonist})$  M. The ring segments were initially precontracted with noradrenaline (1  $\mu$ M;  $\uparrow$ ). The vasodilatory response were examined on the isolated renal artery segments with and without an intact endothelium and the responses in both cases are illustrated above.

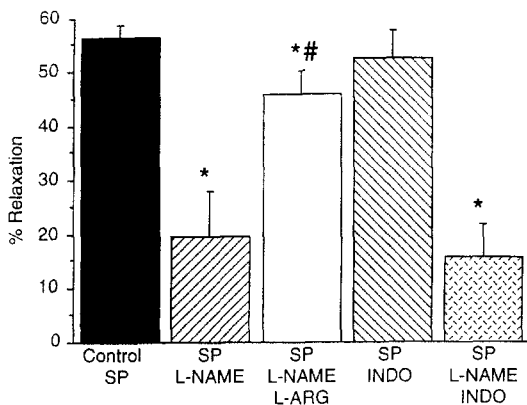
### Effect of L-NAME and Indomethacin

The effect of L-NAME (100  $\mu$ M) and indomethacin (1  $\mu$ M) on the vasodilatation produced in response to substance P (0.1  $\mu$ M; Fig. 3) is illustrated.

The addition of L-NAME (100  $\mu$ M) and indomethacin (1  $\mu$ M) to the organ bath significantly reduced the vasodilatation produced in response to substance P (0.1  $\mu$ M; Fig. 3). However, the relaxation produced in response to substance P (0.1  $\mu$ M) in the presence of L-NAME (100  $\mu$ M) and indomethacin (1  $\mu$ M) was not significantly different from the relaxation produced in the presence of L-NAME (100  $\mu$ M).



**Fig. 2.** A bar chart showing rabbit renal artery vasodilatation in response to calcitonin gene-related peptide(CGRP; 0.1  $\mu$ M), vasoactive intestinal peptide(VIP; 0.1  $\mu$ M) and substance P(SP; 0.1  $\mu$ M) expressed as a percentage of the noradrenaline(1  $\mu$ M;  $\uparrow$ ) induced contraction. The responses are obtained from preparations with(filled bar) and without(empty bar) endothelium. The bar charts show the mean $\pm$ S.E., n>6. \*Indicates significant difference from control(closed bar) P<0.05 taken to be significant.



**Fig. 3.** A bar showing rabbit renal artery vasodilatation in response to substance P(SP; 0.1  $\mu$ M) in the presence of L-NAME(100  $\mu$ M), L-NAME(100  $\mu$ M) with L-arginine(10 mM), indomethacin(1  $\mu$ M) and L-NAME(100  $\mu$ M) with indomethacin(1  $\mu$ M). The results are expressed as a percentage of the noradrenaline(1  $\mu$ M;  $\uparrow$ ) induce contraction. The bar charts show the mean $\pm$ S.E., n>6. \*Indicates significant difference from control (closed bar) and # indicates significant difference from the response to substance P in the presence of L-NAME(100  $\mu$ M) alone. P<0.05 taken to be significant.

## Discussion

In this study, the dependence of the endothelium in

response to the renal artery to several vasoactive agents was assessed by examining their reactivity before and after the initial surface was rubbed to remove the endothelium.

The endothelium has been shown to have an obligatory role in the relaxation of isolated arteries to Ach [5, 7, 12, 26, 27, 33]. This study supports the previous report that Ach-induced vasodilatation of the rabbit renal artery also depends on the presence of an intact endothelium.

In this study, the vasodilatation produced in response to substance P was completely absent in vessels in which the endothelium had been removed. The vasodilatation produced by CGRP and VIP was not significantly affected by the removal of the endothelium from the renal artery. Choline acetyltransferase, the enzyme responsible for the synthesis of substance P, has been shown to be contained within the endothelial cells of a variety of blood vessels [19, 21]. In addition, substance P has been shown to be released from endothelial cells following an increase in flow in the rat hind limb [29] and an increase in flow through perfused columns of human umbilical vein endothelial cells grown on microcarrier beads [21].

So the dilatory responses evoked by substance P was endothelium-dependent in the rabbit renal artery.

In the rabbit renal artery although part of the vasodilator response to Ach was blocked by L-NAME, still was considerable resistance to complete blockage [17, 18]. L-NAME significantly reduced the vasodilator response to substance P in the rabbit renal artery, suggesting that substance P induces relaxation via NO.

The prostanoids only played a relatively small role in the relaxation induced by Ach in rabbit renal artery [1, 16, 32]. The relaxation induced by Ach has been shown to induce release of prostaglandins and NO[1, 12, 16]. In the kidney, inhibition of cyclooxygenase activity has been shown to reduce autoregulatory responses to changes in blood pressure [13]. EDRF and Prostacyclin may be released in parallel and have synergistic thrombin-resistant effects [28].

In this study, the prostanoid synthesis inhibitor, indomethacin [32] did not have any effect on the vasodilator response to substance P either in the absence or presence of L-NAME. Therefore, prostanoids do not appear to play any role in the vasodilator response to substance P. Substance P has been shown to elicit hyperpolarization of the vascular smooth

muscle [3], thus in addition to acting via NO, substance P can also induce a response via the activation of the endothelium-derived hyperpolarizing factor [4].

The inhibition by L-NAME was significantly attenuated in the presence of L-arginine in this study. The fact that L-arginine at least partially prevented the inhibition of response to substance P by L-NAME substantiates the claim that L-NAME was selectively inhibiting the enzyme NOS.

The results show that the dilatory responses evoked by substance P was endothelium-dependent in the rabbit renal artery. It also demonstrated that vasodilatation in response to substance P was at least partly mediated via endothelial production of NO and that the dilator responses to CGRP and VIP were endothelium-independent.

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