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Involvement of nitric oxide and prostanoid on the endothelium-dependent vasodilatation by acetylcholine in the isolated rabbit renal artery

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Abstract : The effect of the nitric oxide synthase(NOS) inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), and the prostanoid synthesis inhibitor, indomethacin, on the vasodilatation produced in response to acetylcholine(Ach) on the isolated rabbit renal artery was examined. The vasodilatory responses to Ach(10^{-8} - 3×10^{-5} M) were completely absent in thevessel which the endothelium had previous been removed. L-NAME (10^{-4} M) significantly reduced the vasodilatory response to the Ach(10^{-8} - 3×10^{-5} M). When L-arginine (10^{-3} M) was also present in the organ bath along with L-NAME(10^{-4} M), this inhibitory effect of L-NAME (10^{-4} M) on the vasodilatory response to Ach (10^{-8} - 3×10^{-5} M) was significantly attenuated, Indomethacin (10^{-6} M) did not significantly affect the vasodilatory responses to Ach (10^{-8} - 3×10^{-5} M). The inhibition by L-NAME (10^{-4} M) and indomethacin (10^{-6} M) on vasodilatory response to Ach was significantly greater than the inhibition due to L-NAME (10^{-4} M) alone. The present study has established that Ach induce relaxation via an endothelium-dependent mechanism, this relaxation to Ach involves both nitric oxide(NO) and prostanoid in the isolated rabbit renal artery.

Key words : endothelium-dependent relaxation, nitric oxide, acetylcholine, indomethacin, 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. Various agents, including acetylcholine(Ach), bradykinin, adenosine 5-triphosphate(ATP), substance P and 5-hydroxytryptamine (5-HT) 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[1,2,3,4,5,6,7,8,9]. These factors include prostacyclin[10,11,12] and endothelium-derived relaxing fator(EDRF)[13].

The endothelium-derived relaxing mediator to arouse widespread interest as a contributor to the thrombo-resistant and vasodilatory properties of the endothelium was prostacyclin[14]. Although they are also produced by smooth muscle cells, prostaglandins are synthesized primarily eithin endothelial cells[15,16]. Prostacyclin can be released from endothelial cells by a variety of stimuli incuding bradykinin, arachidonic acid, thrombin, the inophore A23187, ATP and histamine[17,18].

In some blood vessels, the release of EDRF also mediates the vascular relaxation evokes by certain vasodilators including Ach, ATP, substance P and bradykinin[1,13,19,20,21]. EDRF has been identified as the free radical of nitric oxide(NO)[22] and indeed, EDRF has chemical and pharmcological properties identical to those of NO[23,24,25]. In endothelial cells, NO is produced from the conversion of L-arginine into L-citrulline by NO synthase(NOS)[26,27,28]. Analogues of L-arginine are, therefore, potentially specific inhibitors of EDRF-mediated effects on vascular tone. Infact, it has been shown that N^G-nitro-L-arginine methyl ester(L-NAME) is an inhibitor of enzymatic synthesis of NO from L-arginine[29].

The purpose of this study was to establish whether Ach was inducing relaxation via an endothelial-dependent

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mechanism of the isolated rabbit renal artery, by looking at vessels with and without an intact endothelium. The effects of the NOS inhibitor, L-NAME and the prostanoid synthesis inhibitor, indomethacin[30] on endothelium-dependent vasodilatory response to Ach was studied to establish a more defined role of the endothelium in vasodilatory responses in the isolated rabbit renal artery.

Materials and Methods

Animals

Male New Zealand White rabbit weighting 2-3.5 kg were killed by pentobarbital sodium (100 mg/kg) anaesthesia and exsanguination. The renal arteries were excised and cleared of surrounding fatty tissue under a dissecting microscope.

Tissue preparation

The isolated renal artery cut into rings approximately 5 mm length in 4°C ice cold Krebs ringer solution and these were mounted horizontally under isometric conditions in 10 m*l* organ baths by inserting a tungsten wire through the lumen of the vessel ring taking care not to damage the endothelium.

Recording system

The preparation was then attached to a stationary support. another wire similarly inserted was 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 h in Krebs ringer solution of the followering composition (mM) : NaCl, 120 ; KCl, 4.75 ; Glucose, 6.4 ; $NaHCO_3$, 25 ; KH_2PO_4, 1.2 ; $MgSO_4$, 1.2 and $CaCl_2$, 1.2 pH 7.4. The solution was maintained at 37°C and aerated with 95% O_2 and 5% CO_2 . The endothelium was removed from one of a pair of adjacent ring segments by drawing a fine silk thread through the lumen. A cummulative concentration-response curve to noradrenaline was established on each segment of the artery in order to find maximal contractile response and the concentration of agonist required to produced 40% maximal response(EC_{40}). The vessel segments were contracted to approximately 40% of maximal tension with noradrenaline. After contractions stabilized, increasing concentration of Ach(10^{-8} - 3×10^{-5} M) were added to organ bath in a cummulative manner.

In order to look at the effect of the inhibitors L-NAME and indomethacin, control dose-response relationships to Ach were first determined. An inhibitor was then added to the organ bath and the preparation allowed to equilibrate for 20 min. Dose-response relationships were re-evaluated in the presence of the inhibitor. Four separate experiments were carried out. The first looked at the effect of L-NAME (10^{-4} M) on relaxant responses. With the addition of L-arginine (10^{-3} M) 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 (10^{-6} M) on endothelium -dependent vasodilatation. 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 (10^{-4} M) and indomethacin (10^{-6} M) were added to the organ bath.

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

Statistical analysis were used by student t-test. Results were considered a significant when $p \leq 0.05$

Drugs

The following chemicals were used : acetylcholine bromide(Ach), -noradrenaline bitatrate, N^G-nitro-L-arginine methyl ester(L-NAME), indomethacin, L-arginine. These chemicals were purchased from the Sigma Chemical Co. Other chemicals used were of analytical grade. All drugs were dissolved in distilled water except noradrenaline which was dissolved in ascorbic acid(10^{-4} M).

Results

Effect of removing the endothelium on the relaxant response to Ach

The vasodilatations were produced in dose-dependent responses to acetylcholine(10^{-8} - 3×10^{-5} M). This vasodilatory response was completely absent in vessels in which the endothelium had been removed.

The EC_{40} maximal contractile response to noradrenaline in vessels with and without an intact endothelium was not significantly different being 2.13 ± 0.13 g(n = 78) and 1.96 ± 0.10 g (n = 88) respectively.

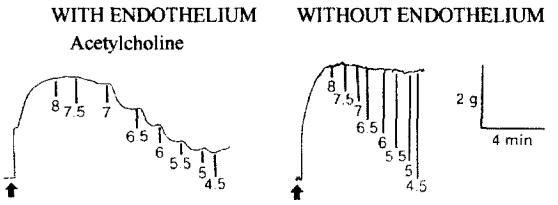

Fig 1. Relaxation produced in the isolated renal artery of the rabbit in response to acetylcholine. The final concentration of agonist in the organ bath is expressed as -log(agonist) M. The ring segments were initially preconstricted with noradrenaline(↑ ; EC_{40} value). The vasodilatory responses were examined on isolated renal artery segments with and without an intact endothelium and responses in both cases are illustrated above.

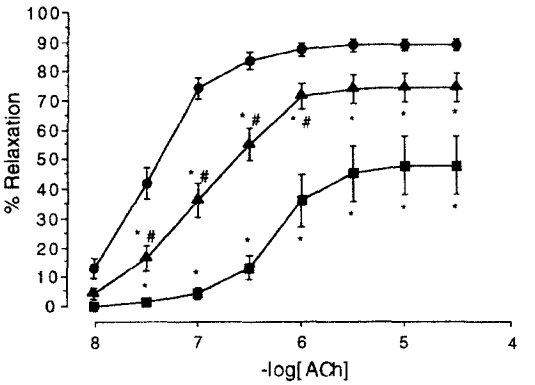

Fig 2. Concentration-response curves of rabbit renal artery in response to acetylcholine in the absence(●) and presence of L-NAME (10^{-4} M ; ■) and L-NAME (10^{-4} M) with L-arginine(10^{-3} M ; ▲). The vasodilatory responses are expressed as a percentage of the noradrenaline (EC_{40} value) induced contraction. Each point represents the mean ± s.e. ($n \geq 6$). ★ indicates significant difference from control (●) and # indicates significant difference from response obtained in the presence of L-NAME (10^{-4} M ; ■).

Effect of N^G-nitro-L-arginine methyl ester(L-NAME) and L-arginine on the relaxant response to Ach

The effect of L-NAME (10^{-4} M) and L-arginine (10^{-3} M) on the vasodilatation produced in response to Ach (10^{-8} - 3×10^{-5} M) is illustrated.

The vasodilatation produced in response to Ach (10^{-8} - 3×10^{-5} M) was significantly reduced in the response of L-NAME (10^{-4} M). When L-arginine (10^{-3} M) was also present in the organ bath along with L-NAME (10^{-4} M), the inhibitory effect was significantly attenuated. The maximum relaxation for Ach were significantly higher than when only L-NAME (10^{-4} M) was in organ bath.

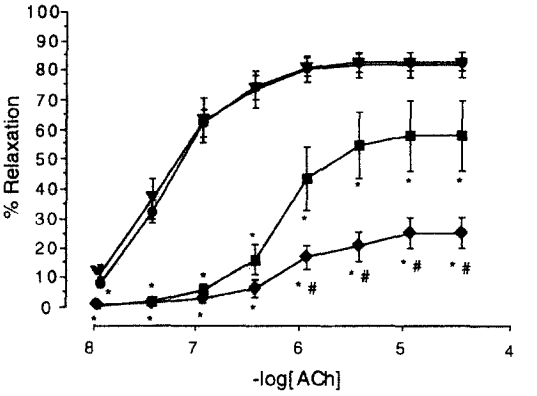

Fig 3. Concentration-response curves of the rabbit renal artery to the acetylcholine in the absence (●) and presence of L-NAME (10^{-4} M ; ■), indomethacin (10^{-6} M ; ▼) and L-NAME (10^{-4} M) with indomethacin (10^{-6} M ; ◆). The vasodilatory responses are expressed as a percentage of the noradrenaline (EC_{40} value) induced contraction. Each point represents the mean ± s.e. ($n \geq 6$). ★ indicates significant difference from control (●) and # indicates significant difference from response obtained in the presence of L-NAME (10^{-4} M ; ■).

The resting basal tone of the preparations was unaffected by the adddition of L-NAME (10^{-4} M) and L-NAME (10^{-4} M) with L-arginine (10^{-3} M)

Effect of indomethacin on the relaxant response to Ach

The effect of indomethacin (10^{-6} M) on the vasodilatation produced by Ach(10^{-8} - 3×10^{-5} M) is illustrated.

The vasodilatation produced in response to Ach (10^{-8} - 3×10^{-5} M) was not significantly affected by the addition of indomathacin(10^{-6} M) to the organ bath.

Indomethacin(10^{-6} M) did not significantly affected the basal tone of the preparations.

Effect of L-NAME and Indomethacin on the relaxant response to Ach

The effect of L-NAME (10^{-4} M) and indomethacin (10^{-6} M) on the vasodilatation produced in response to Ach (10^{-8} - 3×10^{-5} M) is illustrated.

L-NAME (10^{-4} M) and indomethacin (10^{-6} M) significantly reduced the relaxation to Ach (10^{-8} - 3×10^{-5} M)

The maximum vasodilatation produced in response to Ach (10^{-8} - 3×10^{-5} M) in the presence of L-NAME (10^{-4} M) and indomethacin (10^{-6} M) was significantly smaller than the maximum vasodilatation produced in presence of L-NAME (10^{-4} M) alone.

The addition of both L-NAME (10^{-4} M) and indomethacin (10^{-6} M) to the organ bath did not significantly affect the basal tone of the preparartions.

Discussion

In this study, the endothelium dependent of the response of the renal artery of rabbit to Ach was assessed by examining the reactivity before and after the initial surface was rubbed to remove the endothelium. After establishing endothelial dependence of Ach a more detailed study was carried out using the nitric oxide synthesis inhibitor, L-NAME and the prostanoid synthesis inhibitor, indomethacin.

The endothelium has been shown to have an obligatory role in the relaxation of isolated artery to Ach[2,4,13,31,32,33]. This study shows that Ach-induced vasodilatation of the rabbit renal artery also depends on presence of an intact endothelium. This relaxation is due to the production of both NO and prostanoids. Ach has been shown to induce release of prostaglandins[7,11,13] and NO[13], and Ach induced relaxation in the perfused rat kidney was shown to be dependent upon the formation of EDRF[21,34].

Autoregulation of blood flow in several vascular beds has been shown to have a prostaglandin component. In the kidney cyclo-oxygenase inhibition has been shown to reduce autoregulator response to change in blood pressure[35].

Several agonists which enhance prostacyclin production also stimulate EDRF formation in endothelial cells. However, although prostacyclin and EDRF may be released in parallel and have synergistic thrombin-resistant effects[15,36], their production is subject to separate control[12,37,38,39].

Prostanoids only play a relatively small role in the relaxation induced by Ach in the rabbit renal artery. This is suggest by the fact that indomethacin alone did not have any effect on the vasodilatory reponse to Ach. It was only when the NO component of the vasodilatory reponse to Ach had been removed by addition of L-NAME that the inhibitory role of indomethacin was observed.

In the rabbit renal artery although part of the vasodilatory response to Ach was blocked by L-NAME there was considerable resistance to complete blockade[25,40]. The fact that L-arginine at least partially prevented the inhibition of the response to Ach by L-NAME substrates the claim that L-NAME was selectively inhibiting the enzyme NOS.

The study has revealed that in the isolated rabbit renal artery Ach induce relaxation by an endothelium-dependent mechanism. The results show that in the rabbit renal artery, dilator responses evoked by Ach were endothelium-dependent. whereas, vasodilatation in the response to Ach was mediated via endothelial production of both NO and prostanoids.

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토끼 적출 신동맥에 있어서 acetylcholine에 의한 내피세포 의존성 이완작용에 대한 nitric oxide와 prostanoid의 연관성

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국문초록 : 토끼 적출 신동맥에 있어서 acetylcholin(Ach)에 의한 이완작용에 대한 nitric oxide(NO) 합성 억제제인 N^G-nitro-L-arginine methyl ester(L-NAME)과 prostanoid 합성 억제제인 indomethacin의 영향을 관찰하였다. Ach(10^{-8} - 3×10^{-5} M)에 대한 이완작용은 내피세포를 제거시킴으로서 완전히 사라졌다. L-NAME (10^{-4} M)은 Ach (10^{-8} - 3×10^{-5} M)의 이완작용을 현저히 감소시켰으며 L-arginin (10^{-3} M)에 의해 Ach의 이완작용에 대한 L-NAME (10^{-4} M)의 억제효과가 현저히 약하게 나타났다. Indomethacin (10^{-6} M)은 Ach (10^{-8} - 3×10^{-5} M)의 이완작용에 영향을 미치지 못하였다. L-NAME (10^{-4} M)에 indomethacin (10^{-6} M)의 첨가는 Ach (10^{-8} - 3×10^{-5} M)의 이완작용이 L-NAME (10^{-4} M) 단독의 경우보다 더 큰 억제효과를 나타내었다. 이와 같은 결과로 토끼적출 신동맥에서 Ach은 내피세포 의존성 이완작용을 나타내며, NO와 prostanoid가 수반되어 나타나는 것으로 사료되어진다.

Key words : endothelium-dependent relaxation, nitric oxide, acetylcholine, indomethacin, renal artery, rabbit