Mechanisms of NO-Resistant Relaxation Induced by Acetylcholine in Rabbit Renal Arteries

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(Received 28 August 2000/Accepted 12 September 2000)

ABSTRACT. The effects of K^+ channel blockers and P_{2Y} receptor agonist/antagonist on the vasorelaxation mediated by endothelium-derived hyperpolarizing factor (EDHF) were investigated in the rabbit renal artery. Acetylcholine (ACh, 1 nM-10 μ M) induced endothelium-dependent relaxation of arterial rings precontracted with norepinephrine (NE, 1 μ M) in a concentration-dependent manner. N^G -nitro-L-arginine (L-NAME, 0.1 mM), an inhibitor of NO synthase, partially inhibited the ACh-induced endothelium-dependent relaxation. The ACh-induced relaxation was only partially inhibited by L-NAME whereas combined addition of L-NAME and 30 mM KCl completely inhibited the relaxation. The ACh-induced relaxation observed in the presence of L-NAME was significantly reduced by a combination of iberiotoxin (0.1 μ M) and apamin (1 μ M), and almost completely blocked by 4-aminopyridine (5 mM). The ACh-induced relaxation was antagonized by P_{2Y} receptor antagonist, cibacron blue (10 and 100 μ M) in a concentration-dependent manner. Furthermore, ADP β S, a potent P_{2Y} agonist, induced the endothelium-dependent relaxation, and this relaxation was markedly reduced by either the combination of iberiotoxin and apamin or by cibacron blue alone. In conclusion, ACh may activate the release of ATP from endothelial cells which in turn activates a P_{2Y} receptor on the endothelial cells followed by a release of EDHF, resulting in a vasorelaxation via a mechanism that involves activation of both the voltage-gated K^+ channels and the Ca^{2+} -activated K^+ channels.

KEY WORDS: ATP, K^+ channel, rabbit renal artery.

J. Vet. Med. Sci. 63(1): 37-40, 2001

The vascular endothelial cells play an important role in maintaining the vascular homeostasis by liberating several vasodilator substances, including nitric oxide (NO), prostacyclin (PGI₂), and endothelium-derived hyperpolarizing factor (EDHF) [1]. EDHF is released by an increase in the Ca^{2+} concentration within endothelial cells caused by agonists including acetylcholine (ACh), bradykinin, and ATP [7, 10]. EDHF activates K^+ channels of smooth muscle cells, hyperpolarizes the cell membrane and thus causes the relaxation of smooth muscle [2]. There are several types of K^+ channels in vascular smooth muscle cells, and studies with various K^+ channel blockers have led to controversial conclusions on the identity of the K^+ channels involved in the EDHF-dependent vasorelaxation [8].

As it is generally accepted that the P_{2Y} purinoceptors are located in vascular endothelial cells, the P_{2Y} -mediated relaxation is an endothelium dependent phenomenon [13]. Recent studies suggest that activation of the P_{2Y} receptors induces endothelium-dependent vasodilation mediated by EDHF [10, 14, 15, 18]. In the rat mesenteric artery, P_{2Y} receptor-induced vasodilatation is antagonized by a combination of the K^+ channels inhibitors, charybdotoxin and apamin, which prevents hyperpolarization and relaxation mediated by EDHF in different blood vessels [3, 9, 20]. However, no study has provided direct evidence supporting that EDHF is released by activation of the endothelial P_{2Y} receptors.

The aims of this study were to pharmacologically determine (a) whether EDHF contributes to endothelium-dependent vasorelaxation in the rabbit renal artery, and (b) the effects of K^+ channel blockers and P_{2Y} receptor antagonist on the ACh-induced relaxation in order to characterize the mechanism of action of ACh. The effect of a potent P_{2Y} ago-

nist, ADP β S, on NE-contracted tissues was also investigated and the results were compared with that of ACh-induced relaxation.

MATERIALS AND METHODS

Preparation of artery rings: New Zealand white rabbits (2-3 kg) of either sex were killed by exsanguination after anaesthesia with sodium pentobarbital (30 mg/kg iv). The renal artery was quickly excised and placed in a cold physiological salt solution (PSS) of the following composition (in mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 23.8, EDTA 0.01, glucose 5.5. The pH of the solution after saturation with 95% O_2 + 5% CO_2 gas mixture was 7.4. The vessels were cut into 1 mm-wide ring segments and were placed in 20 ml tissue baths between two L-shaped hooks, one of which was attached to a force transducer for isometric measurement. The tissue baths were thermostatically kept at 37°C. A resting tension of 0.5 g was maintained throughout the experiments.

After a 90 min equilibration, tissues contracted by NE (1 μ M). When stable contractions were obtained, ACh was added cumulatively to determine the concentration-response relationship. The function of the endothelium was examined at the beginning of each experiment by observing the effects of ACh (1 μ M) to induce relaxation. In some experiments, endothelium was mechanically removed by gentle rubbing with moistened cotton, functional removal of endothelium was confirmed by the lack of a relaxant response to ACh (1 μ M). Unless otherwise stated, L-NAME (0.1 mM) was added to the PSS.

Chemicals: The chemicals used were acetylcholine,

ADP β S, 4-aminopyridine (4-AP), apamin, cibacron blue, iberiotoxin, indomethacin, N^G-nitro-L-arginine (L-NAME), and norepinephrine (Sigma Chemical).

Statistics: The results of the experiments are expressed as mean $\pm$ S.E.M and the number of preparations taken from separate animals was indicated by n. Statistical significance was examined by Student's paired or unpaired t test and P values less than 0.05 were considered significant.

RESULTS

In the endothelium-intact rabbit renal artery rings precontracted with NE (1 μ M), ACh (1 nM–10 μ M) elicited a concentration-dependent relaxation (Fig. 1). The ACh-induced vasorelaxation was completely abolished by mechanical removal of endothelium (n=8). The relaxant response was markedly reduced by the NOS inhibitor, L-NAME (0.1 mM). In arteries contracted with 30 mM KCl plus 1 μ M NE in the presence of 0.1 mM L-NAME, ACh did not induce relaxation, suggesting that the relaxation was mediated by both the release of NO and an endothelium-dependent hyperpolarization.

The contribution of K⁺ channels to the ACh-induced relaxation was assessed in a renal artery in the presence of L-NAME (0.1 mM). The ACh-induced relaxation was almost completely abolished by an inhibitor of the delayed rectifier K⁺ channels, 4-AP (5 mM) (Fig. 2). The ACh-induced relaxation was also strongly inhibited by an inhibitor of the large conductance Ca²⁺-activated K⁺ channels, iberiotoxin (0.1 μ M) (Fig. 2), and further reduced by the combination of iberiotoxin (0.1 μ M) plus apamin (1 μ M) (Fig. 2). On the other hand, an inhibitor of the ATP-sensitive K⁺ channels, glibenclamide (10 μ M), did not affect the ACh-induced relaxation (n=5, data not shown).

The possible involvement of the P_{2Y} purinoceptor to ACh-induced relaxation was assessed with cibacron blue, a P_{2Y} receptor antagonist, in the rabbit renal artery pretreated with L-NAME (0.1 mM). The ACh-induced relaxation was significantly reduced by a cibacron blue (10 and 100 μ M) in a concentration-dependent manner (Fig. 3).

In the endothelium-intact ring segments precontracted with NE (1 μ M), ADP β S also produced concentration-dependent relaxations. The relaxant response was markedly reduced either by the combination of iberiotoxin (0.1 μ M) plus apamin (1 μ M) or by cibacron blue (100 μ M) (Fig. 4). The ADP β S-induced vasorelaxation was completely abolished by the mechanical removal of endothelium (n=6).

DISCUSSION

The muscarinic agonist, ACh, produced endothelium-dependent relaxations in the segments of rabbit renal arteries contracted with NE. Inhibition of NO release with L-NAME reduced but did not abolish the ACh-induced relaxation. These results indicate that the endothelium-dependent relaxant response to ACh in the rabbit renal artery is mediated by at least two different factors, namely NO and EDHF. More-

Fig. 1. Average concentration-response curves for the relaxant effects of ACh in the absence (●) and presence of L-NAME (100 μ M,) or L-NAME plus 30 mM KCl (▼) in the rabbit renal artery precontracted by NE (1 μ M). (▽) Endothelium was removed. Responses are expressed as percentage of contraction elicited by NE (1 μ M) before the addition of ACh. Each point represents mean of 6–10 rings and S.E.M. is shown by vertical bar.

Fig. 2. Effects of K⁺ channel blockers on ACh-induced relaxation in the endothelium-intact, L-NAME (0.1 mM)-pretreated segments of the rabbit renal artery precontracted with NE (1 μ M). ●, Control; ○, in the presence of iberiotoxin (0.1 μ M); ▼, in the presence of iberiotoxin (0.1 μ M) and apamin (1 μ M); ▽, in the presence of 4-AP (5 mM). Responses are expressed as the percentage of contraction elicited by NE before the addition of ACh. Each point represents mean of 5–8 rings and S.E.M. is shown by vertical bar.

over, the ACh-induced relaxation was completely prevented by L-NAME in the presence of high extracellular K⁺, suggesting that EDHF is released from endothelial cells and contributes to the endothelium-dependent relaxation. Similar observations have been made with the rabbit carotid artery

Fig. 3. Effects of P_{2Y} purinoceptor antagonist, cibacron blue, on ACh-induced relaxation in the endothelium-intact, L-NAME (0.1 mM)-pretreated rabbit renal artery precontracted with NE (1 μ M). ●, Control; ○, in the presence of 10 μ M cibacron blue; ▼, in the presence of 100 μ M cibacron blue. Responses are expressed as percentage of the contraction elicited by NE before the addition of ACh. Each point represents mean of 6 rings and S.E.M. is shown by vertical bar.

Fig. 4. Average concentration-response curves for the relaxant effects of ADP β S, a potent P_{2Y} agonist, in the endothelium-intact rabbit renal artery precontracted with NE (1 μ M). ●, Control; ○, in the presence of iberiotoxin (1 μ M) plus apamin (1 μ M); ▼, in the presence of 100 μ M cibacron blue. () Endothelium was removed. Responses are expressed as percentage of the contraction elicited by NE before the addition of ACh. Each point represents mean of 5–6 rings and S.E.M. is shown by vertical bar.

and the rat renal artery [4, 7].

The cellular mechanism(s) underlying the EDHF-mediated smooth muscle relaxation has not been fully elucidated, especially with respect to the subtype of K^+ channels. In the present study, ACh-induced relaxation was abolished by 4-AP, a specific inhibitor of delayed rectifier K^+ (K_v) channels, suggesting that K_v channels are involved in the ACh-mediated relaxation of the rabbit renal artery. The EDHF-mediated relaxation in the rat hepatic artery has been shown to be inhibited by 4-AP and charybdotoxin [19, 20]. Since charybdotoxin inhibits not only Ca^{2+} -activated K^+ channels (BK_{Ca}), but also K_v channels, this leads to the conclusion that the K_v channels are a target for EDHF. Iberiotoxin, the selective inhibitor of BK_{Ca} channels, also significantly reduced ACh-induced relaxation, suggesting that the BK_{Ca} channels also play a role in the ACh-induced relaxation. Application of apamin in the presence of iberiotoxin further inhibited the ACh-induced relaxation, suggesting that small conductance Ca^{2+} channel (SK_{Ca}) channel is also involved in the ACh-induced relaxation [1]. Taken together, these data clearly indicate that in the rabbit renal artery, both the K_v and BK_{Ca} are involved in the ACh-mediated, NO-resistant relaxation.

In the present study, the ACh-induced relaxation was inhibited by an antagonist of purinoceptor, cibacron blue, in a concentration-dependent manner. These results indicate that the response may be evoked by the activation of the P_{2Y} purinoceptors. The significant inhibition of the ACh-induced relaxation is likely to be due to a specific action,

because cibacron blue has been demonstrated to be a selective P_{2Y} receptor antagonist in vascular smooth muscles [6]. A number of studies reported that the P_{2Y} purinoceptors are typically located on the endothelial cells, and the activation of endothelial P_{2Y} induced the release of EDHF [10, 12, 13]. Vasoactive substances such as noradrenaline, bradykinin, ACh and serotonin are known to release ATP from endothelial cells [16, 17]. These results indicated that ATP derived from endothelial cells activates the P_{2Y} purinoceptors on the endothelial cells to induce the EDHF mediated responses.

In the present study, the selective P_{2Y} receptor agonist, ADP β S, induced relaxation of the NE-precontracted rabbit renal artery as ACh did. This relaxation may be due to activation of P_{2Y} purinoceptors located on endothelial cells with subsequent release of EDHF, since the vasorelaxation induced by ADP β S was antagonized by iberiotoxin plus apamin, cibacron blue or by endothelial denudation. Similarly, in rat mesenteric arteries, it has been reported that stimulation of the P_{2Y} receptors triggers endothelium-dependent relaxation, which was inhibited by the combination of iberiotoxin and apamin in the presence of L-NOARG and indomethacin [9]. Recent studies also suggests that activation of the P_{2Y} receptors induces endothelium-dependent vasodilation mediated by EDHF [10, 11, 14, 15, 18]. Therefore, it is suggested that ADP β S activates the P_{2Y} receptors to release EDHF from endothelial cells of the rabbit renal arteries.

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