Full Paper

Potassium Channel Opener Pinacidil Induces Relaxation of the Isolated Human Radial Artery

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Abstract. Taking into consideration that the search for drugs capable of modifying blood flow through human radial artery (RA) is warranted, the present study was designed to examine the vasodilatory effects of the potassium channel opener, pinacidil on the RA and to define the contribution of different K⁺-channel subtypes in the endothelium-independent pinacidil action on this blood vessel. Pinacidil relaxed the RA rings with endothelium and without endothelium with comparable potency. *N*-nitro-L-arginine methyl ester (L-NAME) and methylene blue did not affect the pinacidil-induced vasorelaxation in rings with endothelium. In the rings without endothelium, the K⁺-channel blockers glibenclamide and tetraethylammonium (TEA) moderately antagonized the pinacidil-induced relaxation, while charybdotoxin and 4-aminopyridine did not. In endothelium-denuded rings, precontracted with 100 mM K⁺, the relaxant responses to pinacidil were highly significantly shifted to the right compared to those obtained in RA precontracted with phenylephrine, but pinacidil-induced maximal relaxation was not affected. Addition of nifedipine did not but addition of nifedipine and nickel (Na⁺-Ca²⁺ exchanger inhibitor) did cause a statistically significant rightward shift of the pinacidil concentration-relaxation curve, although the effect 0.1 mM pinacidil was preserved. Thus, pinacidil induces relaxation of the human RA in endothelium-independent manner, and glibenclamide- and TEA-sensitive vascular smooth muscle K⁺ channels are probably involved. Its ability to completely relax the RA precontracted with K⁺-rich solution suggests that pinacidil has additional K⁺ channel-independent mechanism(s) of action. It seems that stimulation of the forward mode of the Na⁺-Ca²⁺ exchanger plays a part in this K⁺ channel-independent effect of pinacidil.

Keywords: human radial artery, pinacidil, K⁺ channel, Na⁺-Ca²⁺ exchanger

Introduction

Radial artery (RA) is frequently used for revascularization in coronary artery bypass grafting (CABG) because the short- and medium-term patencies of this graft are improved compared to those of the saphenous vein graft (1). However, it remains a concern that RA has a greater reactivity and propensity to spasm when

compared to the internal thoracic artery (ITA). The mechanism triggering vascular spasm is currently unclear, although different factors or events seem to be involved. Circulating levels of many potential spasmogens (norepinephrine, thromboxane A₂, endothelin-1, serotonin, angiotensin II) are elevated during and after cardiac surgery and may contribute to the development of graft's spasm (2, 3). Hypersensitivity of vascular smooth muscle, a cause of arterial spasm in vivo, may also be induced by a decrease in K⁺ conductance, which leads to increased Ca²⁺ entry and availability (4).

Various vasodilators have been studied in RA, but an

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ideal agent for prevention and treatment of spasm has not yet been found. Calcium antagonists, due to their mechanism of action, are not very potent in reversing spasm evoked by endothelin-1 and thromboxane A₂ (5). Diltiazem shows the least potency of all, but still this drug is routinely prescribed to patients with RA graft. Nitrates are very potent vasodilators, but the problem of tolerance to these drugs is present and systemic administration may lead to hypotension and decreased flow through the arterial graft (5). Thus, the search for a better vasorelaxant agent is of a great interest.

Recently it has been suggested that hyperpolarizing cardioplegia with K⁺-channel openers (KCOs) is superior to depolarizing cardioplegia in protecting the endothelial function in the coronary microcirculation (6). Hyperpolarizing cardioplegia preserves, whereas depolarizing cardioplegia reduces the endothelium-derived hyperpolarizing factor-mediated relaxation of coronary microcirculation. Effects of various KCOs have been studied in the ITA, but only two studies were conducted on the RA (7, 8). They suggested that the KCO nicorandil relaxes RA precontracted with phenylephrine, endothelin-1, and thromboxane A₂ analogue and relaxes part of the diltiazem-resistant contraction (7).

Pinacidil, a cyanoguanidine, is an antihypertensive agent that may relax vascular smooth muscle. The mechanism of action has not yet been fully established, but it has previously been suggested that vascular relaxant response to pinacidil is associated with the opening of smooth muscle K⁺ channels (9). Among the KCOs, the mechanism of action of pinacidil is not as well defined as those for levromakalim and nicorandil, and events additional to hyperpolarization of the vascular smooth muscle have been proposed (10). Pinacidil has not been previously investigated on the RA.

Taking into consideration that the search for drugs capable of modifying blood flow through RA is warranted, the present study was designed to examine the vasodilatory effects of pinacidil on the RA and to define the contribution of different K⁺-channel subtypes in the endothelium-independent pinacidil action on this blood vessel. The experiments were performed on arterial segments with denuded or with intact endothelium in order to determine whether the effect of pinacidil is endothelium-dependent.

Materials and Methods

The remaining segments of left RA (n = 70) were taken from 45 male patients during CABG. The mean age $\pm$ S.E.M. was 62 ± 2 years. Therapy with calcium antagonists and long-acting nitrates was ceased in all

patients 48 h prior to surgical intervention. No operative mortality and cardiac morbidity was revealed in these patients. Only arteries without macroscopic evidence of atherosclerosis were used. All patients gave their informed consent for excision of remaining tissue and the experimental protocol was approved by The Ethical Committee of Institute for Cardiovascular Diseases "Dedinje". Research has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The vessel segments were taken within 10 min after clamping blood flow, placed in cold (4°C) Krebs-Ringer-bicarbonate solution (120 mM NaCl, 5 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 25 mM NaHCO₃, 1.2 mM KH₂PO₄, 11 mM glucose, and 0.032 mM Na₂EDTA) and taken to the lab immediately.

Artery segment preparations

The artery segments were dissected from connective tissue and cut into 3-mm rings. When necessary, endothelium was removed mechanically by rubbing with a steel wire. Vessel rings were mounted on two stainless steel wires in a 10-ml organ bath with Krebs-Ringer-bicarbonate solution (37°C, pH 7.4) aerated with 95% O₂ and 5% CO₂. One of the wire hooks was connected to a transducer (F30; Hugo Sachs, Freiburg, Germany), amplifier (model 301, Hugo Sachs) and recording system (R60; Rikadenki, Tokyo) that recorded changes in isometric tension. The other wire hook was attached to a displacement unit allowing fine adjustments of passive tension.

The preparations were equilibrated for 60 min and washed every 15 min with fresh buffer during this period. All vessels were then gradually passively stretched to an optimal resting tension, determined in a way previously described by our group (3, 11).

Experimental procedure

After 15 min of equilibration, the presence of functional endothelium was assessed. Rings were precontracted with phenylephrine (10 μ M) and acetylcholine (20 μ M) was added into the organ bath. If the maximal relaxant effect was more than 80% of the initial contraction, we considered functional endothelium to be present.

In order to study the vasorelaxant properties of pinacidil in the RA with and without endothelium, the segments were initially contracted with phenylephrine (10 μ M), as it was previously shown to elicit 80% of maximal contraction (3). When the contraction reached a steady state, cumulative concentration-response curves to pinacidil were obtained by adding increasing half-logarithmic molar concentrations (0.01–100 μ M). A

subsequent concentration was added to the organ bath after the previous concentration had produced its equilibrium response or after 10 min if no response was obtained. Relaxation produced by each concentration of pinacidil was measured and expressed as a percentage of the maximum possible relaxation (i.e., relaxation back to the baseline tension). Experiments followed a multiple curve design.

Therefore, the following protocol was used: 1) contraction to phenylephrine and concentration-response curve to pinacidil (used as tissue control, uncorrected), followed by three washes, addition of the enzyme inhibitor or K^+ -channel inhibitor and a 20 min equilibration period; 2) contraction to phenylephrine and the concentration-response curve to pinacidil.

In a separate series of experiments, vascular rings were first contracted with $10 \mu\text{M}$ phenylephrine to obtain the control contraction. The second contraction was produced by $100 \text{ mM } K^+$ (to annul the effect of K^+ -channel activation). In a final set of experiments, the second contractions was produced by $100 \text{ mM } K^+$ in the presence of $1 \mu\text{M}$ nifedipine (to block the effect of K^+ and Ca^{2+} -channels activation), and the third contractions was induced by $100 \text{ mM } K^+$ in the presence of $1 \mu\text{M}$ nifedipine and $300 \mu\text{M}$ nickel (Na^+ - Ca^{2+} exchanger inhibitor). Contractions produced by phenylephrine and $100 \text{ mM } K^+$, $100 \text{ mM } K^+$ + nifedipine, or $100 \text{ mM } K^+$ + nifedipine + nickel were of comparable amplitude. K^+ -rich Krebs-Ringer bicarbonate solution was prepared by equimolar replacement of 100 mM NaCl with 100 mM KCl .

Data and statistical analysis

Relaxation produced by each concentration of pinacidil was measured and expressed as a percentage of the maximum possible relaxation (i.e., relaxation back to the baseline tension). The concentration of pinacidil producing 50% of its own maximum response (EC_{50}) was determined for each curve by using a non-linear least square fitting procedure of the individual experimental data. The results are expressed as the means $\pm$ S.E.M.; n refers to the number of experiments. All calculations were done by using the computer program Graph Pad Prism (Graph Pad Software, Inc., San Diego, CA, USA).

“Sensitivity” is used throughout this article to describe the location of the concentration-contraction curve and is measured by the EC_{50} value. “Reactivity” (E_{max}) describes the range of responses and is measured as the maximal relaxation.

Drugs

The following drugs were used: (*R*)-(-)-phenylephrine

hydrochloride, pinacidil, *N*-nitro-L-arginine-methyl ester hydrochloride (L-NAME), methylene blue (MB), glibenclamide, charybdotoxin, 4-aminopyridine (4-AP), tetraethylammonium chloride (TEA), nifedipine, and nickel (Sigma-Aldrich, Inc., St. Louis, MO, USA). Glibenclamide was dissolved in polyethylene glycol, pinacidil was dissolved in 0.01 N HCl , and all other drugs were dissolved in distilled water. Previous experiments have shown that the solvents used had no effect on preparations at the concentrations applied. All drugs were added directly into the bath and the concentrations given are the calculated final concentrations in the bath solution.

Results

Vasorelaxant effects of pinacidil on RA precontracted with phenylephrine

Original recordings of the vasorelaxant effect of pinacidil in RA with (A) and without (B) endothelium are shown in the Fig. 1. Pinacidil ($0.01 - 100 \mu\text{M}$) was found to induce a concentration-dependent relaxation of the RA (EC_{50} values: $0.80 \pm 0.06 \mu\text{M}$ in the presence of endothelium and $1.22 \pm 0.10 \mu\text{M}$ in the absence of endothelium, $n = 15$ both, $P > 0.05$) (Fig. 1C). E_{max} to pinacidil in both experimental series was $100 \pm 0\%$.

Effects of enzyme inhibitors (L-NAME, MB) on pinacidil-induced relaxation of RA with intact endothelium

L-NAME ($100 \mu\text{M}$) and MB ($30 \mu\text{M}$) were not found to affect basal tension of the RA with endothelium nor the contraction induced by phenylephrine (data not shown, $n = 5$, both).

L-NAME ($100 \mu\text{M}$, $n = 5$) did not affect relaxation of the RA (EC_{50} values: $0.78 \pm 0.09 \mu\text{M}$ in the absence and $0.68 \pm 0.08 \mu\text{M}$ in the presence of L-NAME, $P > 0.05$) produced by pinacidil (Fig. 2A). E_{max} to pinacidil was not suppressed ($100 \pm 0\%$, $P > 0.05$).

MB ($30 \mu\text{M}$) did not antagonize the vasorelaxant effect of pinacidil on the RA (EC_{50} values: $0.74 \pm 0.08 \mu\text{M}$ in the absence and $0.70 \pm 0.09 \mu\text{M}$ in the presence of MB, $n = 5$, $P > 0.05$; E_{max} to pinacidil was $100 \pm 0\%$ for each, $P > 0.05$) (Fig. 2B).

Effects of K^+ -channel inhibitors on the pinacidil-induced relaxation of RA without endothelium

Glibenclamide ($10 \mu\text{M}$), TEA (6 mM), charybdotoxin (10 nM), and 4-AP (3 mM) neither affected the basal tension of RA without endothelium nor the contraction induced by phenylephrine (data not shown, $n = 4 - 6$).

Glibenclamide used in concentration of $10 \mu\text{M}$ ($n = 6$) significantly modified the relaxation of RA

Fig. 1. Original recording of vasorelaxant effect of pinacidil in the radial artery with (A) and without (B) endothelium. To assess the endothelial integrity of the preparation, we used acetylcholine (ACh, 20 μ M). Failure of arteries to relax to acetylcholine was considered to indicate a state of endothelial denudation. After it was determined that functional endothelium was present/absent, phenylephrine (10 μ M) was used to induce sustained tonic contraction of the radial artery. Cumulative concentrations of pinacidil (0.01–100 μ M) were then added to the organ bath, until return to baseline tension. C: Cumulative concentration-response curves to pinacidil in the radial artery with endothelium (black circle) and without endothelium (white circle) precontracted with phenylephrine (10 μ M). Responses are expressed as a percentage of the maximum possible relaxation (i.e., return to baseline tension). Each point represents the mean $\pm$ S.E.M. ($n = 15$).

Fig. 2. Effects of enzyme inhibitors on the pinacidil-induced relaxation of RA with intact endothelium. Contractions were provoked by phenylephrine (10 μ M). Concentration-response curve for pinacidil in the absence (circle) and presence (triangle) of 100 μ M L-NAME (A) or 30 μ M methylene blue (B). Responses are expressed as a percentage of the maximum possible relaxation (i.e., return to baseline tension). Each point represents the mean $\pm$ S.E.M. ($n = 4-6$). E+ (with endothelium), MB = methylene blue.

induced by pinacidil (EC_{50} values: $1.32 \pm 0.12 \mu$ M control vs $4.72 \pm 0.10 \mu$ M in the presence of glibenclamide, $P < 0.05$) without significant change in E_{max}

($100 \pm 0\%$ vs $95 \pm 3\%$, $P > 0.05$) (Fig. 3A).

Incubation of the RA with 6 mM TEA ($n = 5$) produced a shift to the right of concentration-response

Fig. 3. Effects of K^+ -channel inhibitors on the pinacidil-induced relaxation of RA without endothelium. Contractions were provoked by phenylephrine ($10 \mu\text{M}$). Concentration-response curve for pinacidil in the absence (circle) and presence (triangle) of glibenclamide $10 \mu\text{M}$ (A), tetraethylammonium 6 mM (B), charybdotoxin 10 nM (C), and 3 mM 4-aminopyridine (D). Responses are expressed as a percentage of the maximum possible relaxation (i.e., return to baseline tension). Each point represents the mean $\pm$ S.E.M. ($n = 4 - 6$). $*P < 0.05$. E- (without endothelium), Glb = glibenclamide, TEA = tetraethylammonium, Cbtx = charybdotoxin, 4-AP = 4-aminopyridine.

curve to pinacidil that was statistically significant (EC_{50} values: $1.30 \pm 0.11 \mu\text{M}$ in the absence of TEA and $4.06 \pm 0.09 \mu\text{M}$ in the presence of TEA, $P < 0.05$) without change in E_{max} ($100 \pm 0\%$ vs $97 \pm 3\%$, $P > 0.05$) (Fig. 3B).

Charybdotoxin (10 nM , $n = 4$) did not antagonize the vasorelaxant effect of pinacidil on RA (EC_{50} : $1.10 \pm 0.09 \mu\text{M}$ in the absence of charybdotoxin and $1.04 \pm 0.12 \mu\text{M}$ in the presence of charybdotoxin, $P > 0.05$; E_{max} was $100 \pm 0\%$ for each, $P > 0.05$) (Fig. 3C).

4-AP (3 mM , $n = 5$) was not found to significantly modify the relaxant effect of pinacidil on RA without endothelium (EC_{50} values: $1.38 \pm 0.11 \mu\text{M}$, control vs $0.97 \pm 0.12 \mu\text{M}$, for 4-AP, $P > 0.05$; E_{max} : $100 \pm 0\%$, $P > 0.05$) (Fig. 3D).

Effects of pinacidil in the presence of high K^+ , nifedipine, and nickel

In the RA precontracted with $100 \text{ mM } K^+$, the relaxant response to pinacidil ($0.01 - 100 \mu\text{M}$) was highly significantly ($P < 0.01$) shifted to the right (EC_{50} value was $19.81 \pm 0.11 \mu\text{M}$ and $E_{\text{max}} = 98 \pm 2\%$, $n = 5$) compared to those obtained in the RA precontracted with phenylephrine (EC_{50} value was $1.26 \pm 0.07 \mu\text{M}$ and $E_{\text{max}} = 100 \pm 0\%$, $n = 5$) (Fig. 4). The difference between the E_{max} was not significant ($P > 0.05$).

In order to minimize the influence of K^+ channels and voltage-gated Ca^{2+} channels (VGCCs) in vascular smooth muscle, the second contraction was made by $100 \text{ mM } K^+$ in the presence of $1 \mu\text{M}$ nifedipine. Relaxant response to pinacidil ($0.01 - 100 \mu\text{M}$) was highly significantly ($P < 0.01$) shifted to the right (EC_{50} value

Fig. 4. Effects of K^+ -rich Krebs-Ringer bicarbonate solution, without and with nifedipine and nickel, on the relaxant effect of pinacidil in the radial artery without endothelium. Concentration-response curves for pinacidil ($0.01-100 \mu\text{M}$) were obtained in preparations precontracted with $10 \mu\text{M}$ phenylephrine (circles), K^+ -rich ($100 \text{ mM } K^+$) Krebs-Ringer bicarbonate solution (triangles), K^+ -rich ($100 \text{ mM } K^+$) Krebs-Ringer bicarbonate solution with addition of nifedipine ($1 \mu\text{M}$) (square), and K^+ -rich ($100 \text{ mM } K^+$) Krebs-Ringer bicarbonate solution with addition of nifedipine ($1 \mu\text{M}$) and nickel ($300 \mu\text{M}$) (diamond). Responses are expressed as a percentage of the maximum possible relaxation (i.e., return to baseline tension). Each point represents the mean $\pm$ S.E.M. ($n = 5$). * $P < 0.05$, ** $P < 0.01$. E- (without endothelium), Nif = nifedipine, Ni^{2+} = nickel.

was $21.23 \pm 0.13 \mu\text{M}$ and $E_{\text{max}} = 97 \pm 3\%$, $n = 5$) compared to those obtained in the RA precontracted with phenylephrine (EC_{50} value was $1.26 \pm 0.07 \mu\text{M}$ and $E_{\text{max}} = 100 \pm 0\%$, $n = 5$), but was not different to the experimental situation when contraction was elicited with $100 \text{ mM } K^+$ (Fig. 4). Pretreatment with Ni^{2+} ($300 \mu\text{M}$), a putative Na^+-Ca^{2+} exchanger inhibitor, significantly ($P < 0.05$) inhibited the pinacidil induced relaxation following inhibition of K^+ channels and VGCCs (EC_{50} value was $61.42 \pm 0.22 \mu\text{M}$ and $E_{\text{max}} = 96 \pm 3\%$, $n = 5$) (Fig. 4).

Discussion

The present results provide the first direct comparison of the effects of pinacidil on the isolated human RA in rings with intact and with removed endothelium. Studies of the vasorelaxant effect of levcromakalim and nicorandil have shown that these agents have greater sensitivity and reactivity in the RA when compared to ITA (7, 8). This indicates tissue selectivity for different KCOs. However, pinacidil had equal potency in inhibiting contractions of the RA ($EC_{50} = 1.22 \mu\text{M}$, reported here) and contractions of the ITA ($EC_{50} = 1.67 \mu\text{M}$) elicited by phenylephrine (11). A number of studies obtained on different experimental models have revealed levcromakalim to be a more potent

KCO than pinacidil. Indeed, it has been previously shown by our group that levcromakalim relaxed more potently the ITA than pinacidil (11). Interestingly, the sensitivity of RA to pinacidil reported here is higher than the sensitivity of RA to levcromakalim reported by Hamilton et al. (8).

It is not well defined how the presence of intact endothelium influences the vasorelaxation produced by pinacidil. In conscious dogs, Ghaleh et al. have shown that vasodilatation of coronary arteries produced by pinacidil is endothelium-dependent, since removal of endothelium reduced this effect to 76% (12). The results from our study support the observation of Gollasch et al. obtained on the human coronary artery that pinacidil has an endothelium-independent mechanism of action (13). Moreover, our results show that L-NAME, an inhibitor of NO synthetase, and MB, an inhibitor of guanylyl cyclase, did not antagonize the effect of pinacidil, so we can assume that NO and cGMP are not involved in the mechanism of action of pinacidil in the human RA with intact endothelium. This finding is consistent with the results obtained in rat aorta and pulmonary artery (14).

In order to analyze the contribution of different types of K^+ channels to the pinacidil-induced endothelium-independent relaxation in the RA, we used agents that are known to possess a K^+ channel-blocking activity.

Numerous investigators have suggested that the effect of pinacidil on the vascular smooth muscle is mediated by ATP-sensitive K^+ (K_{ATP}) channels (10). In order to analyze the contribution of these channels to the pinacidil-induced relaxation of RA, glibenclamide was used. Glibenclamide is known to be one of the most selective blockers of K_{ATP} channels, but it can also block voltage-sensitive K^+ (K_{V}) channels in concentrations higher than $10 \mu\text{M}$ (15). In the present study, glibenclamide used in a high concentration caused slight, but significant antagonism (EC_{50} ratio = 3.6), suggesting the involvement of the K_{ATP} channels. Previously, we have shown that glibenclamide in high concentrations (over $10 \mu\text{M}$) non-competitively antagonized the response to pinacidil on the ITA (11). In contrast, the effect of levcromakalim on the ITA was competitively antagonized with significantly lower concentrations of glibenclamide ($0.1 \mu\text{M}$). Comparable results were obtained in the rat aortic rings where, in contrast to other KCOs, the vasorelaxant action of pinacidil was relatively resistant to glibenclamide (11). Also, there are evident differences in sensitivity to glibenclamide among various tissues and species. In the dog coronary artery, the presence of a low concentration of glibenclamide ($0.3 \mu\text{M}$) caused a significant shift to the right of concentration-relaxation curve to pinacidil (16). In our

results, we have shown that the vasorelaxant effect of pinacidil in the human radial artery is less sensitive to glibenclamide, which can be important in diabetic patients treated with oral antidiabetic drugs.

There are very few reports showing that pinacidil induces vasorelaxation by opening vascular smooth muscle big calcium-sensitive K^+ (BK_{Ca}) channels, including the study performed on the human coronary arteries and rabbit renal artery (17, 18). However, in the present study, we did not confirm this result. TEA was found to selectively block the BK_{Ca} channels in concentrations lower than 1 mM ($K_D = 0.29$ mM) (19), while in higher concentrations, up to 10 mM, it can block K_{ATP} channels as well (20). Here, TEA (6 mM) in the concentration clearly sufficient to block BK_{Ca} channels produced incomplete antagonism of the pinacidil effect on the RA; for example, shifted the concentration-response curve of pinacidil only 3-fold. To further test the hypothesis that BK_{Ca} channels might be involved in pinacidil-induced relaxation of the RA, charybdotoxin was used. Charybdotoxin was originally described as a selective inhibitor of BK_{Ca} channels in a variety of tissues (21). However, charybdotoxin is not a specific blocker of the BK_{Ca} channels; it also blocks intermediate conductance Ca^{2+} -activated K^+ channels and K_V channels (20, 22). In each case, channel inhibition occurs with similar potency, in the low nanomolar range ($K_D = 0.3$ nM). The concentration of charybdotoxin used in our study (10 nM) was sufficient to block BK_{Ca} channels, but did not alter relaxation of the RA induced by pinacidil. These results do not support participation of BK_{Ca} channels in the relaxation of RA produced by pinacidil. Thus, it is suggested that high concentration of TEA in this study probably blocked K_{ATP} channels. This observation needs further evaluation.

In order to analyze the contribution of the K_V channels to the pinacidil-induced relaxation in the RA, we used 4-AP. This compound is the most widely used blocker in the identification of potassium channel types. Used at low millimolar concentration, 4-AP achieved some selectivity for K_V channels (20, 23). In the present study, 4-AP used in concentration of 3 mM did not antagonize the pinacidil-induced relaxation of the RA, which excludes involvement of K_V channels.

Our data suggest the involvement of K_{ATP} channels in the relaxant effect of pinacidil, but according to the relative resistance of this effect to glibenclamide, it seems that pinacidil exerts endothelium-independent relaxation of the RA by acting on multiple sites. The results of our experiments performed in K^+ -rich solution also confirm the involvement of K^+ channels in pinacidil-induced endothelium-independent relaxation

of the RA. However, the ability of pinacidil (100 μ M) to completely relax RA precontracted with K^+ -rich solution support the notion that pinacidil, in RA, acts at least in part, through K^+ channel-independent mechanism(s). K^+ channel-independent vascular effects have also been reported for pinacidil when it was used at concentrations higher than 1 μ M (11, 24, 25). The mechanism of this pinacidil action is not well defined. Previously it has been shown that pinacidil neither stimulated Ca^{2+} uptake into the endoplasmic reticulum nor inhibited the store-operated Ca^{2+} entry as the other possible K^+ channel-independent mechanisms (24). Although pinacidil was described to inhibit intracellular Ca^{2+} mobilization, this action was thought to be indirect, resulting from a decrease in IP_3 production and was hyperpolarization-dependent and glibenclamide-sensitive (26–28). Here we have shown that Ni^{2+} significantly inhibited the pinacidil-induced relaxation following inhibition of K^+ channel and VGCCs. In the applied concentration, Ni^{2+} (300 μ M) could inhibit Ca^{2+} efflux via the Na^+ - Ca^{2+} -exchanger (29) and blocked the VGCCs (30). However, the inhibition of pinacidil-induced relaxation could not be due to the VGCCs-blocking effect of Ni^{2+} in the presence of nifedipine, a selective blocker of the L-type of VGCCs. Our results are in agreement with data obtained by Tsang et al. and Schweda et al. (24, 31). They have shown that stimulation of the forward mode Na^+ - Ca^{2+} -exchange pathway is in part responsible for pinacidil produced K^+ channel-independent vasorelaxation. Similar results were obtained by Meisheri et al. (32).

The present data show that pinacidil exhibits potent vasorelaxant properties in the human RA and therefore should be considered as a possible agent for the treatment of vasospasm. The effect of pinacidil is endothelium-independent, and it does not involve NO and cGMP as a second messenger. The mechanism of action of pinacidil on the vascular smooth muscle probably involves activation of K_{ATP} channels. Its ability to completely relax the RA precontracted with K^+ -rich solution suggests that K^+ channel-independent mechanism(s) also take part in its vasorelaxant effect. It seems that stimulation of the forward mode of the Na^+ - Ca^{2+} exchanger plays a part in this K^+ channel-independent effect of pinacidil. However, we need further evaluations to understand the mechanism of action of pinacidil used in high (≥ 100 μ M) micromolar concentrations.

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