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Full paper

Nicorandil directly and cyclic GMP-dependently opens K^+ channels in human bypass grafts

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ABSTRACT

As we previously demonstrated the role of different K^+ channels in the action of nicorandil on human saphenous vein (HSV) and human internal mammary artery (HIMA), this study aimed to analyse the contribution of the cGMP pathway in nicorandil-induced vasorelaxation and to determine the involvement of cGMP in the K^+ channel-activating effect of nicorandil. An inhibitor of soluble guanylate cyclase (GC), ODQ, significantly inhibited nicorandil-induced relaxation, while ODQ plus glibenclamide, a selective ATP-sensitive K^+ (K_{ATP}) channel inhibitor, produced a further inhibition of both vessels. In HSV, ODQ in combination with 4-aminopyridine, a blocker of voltage-gated K^+ (K_V) channels, did not modify the concentration-response to nicorandil compared with ODQ, whereas in HIMA, ODQ plus iberiotoxin, a selective blocker of large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels, produced greater inhibition than ODQ alone. We showed that the cGMP pathway plays a significant role in the vasorelaxant effect of nicorandil on HSV and HIMA. It seems that nicorandil directly opens K_{ATP} channels in both vessels and BK_{Ca} channels in HIMA, although it is possible that stimulation of GC contributes to K_{ATP} channels activation in HIMA. Contrary, the activation of K_V channels in HSV is probably due to GC activation and increased levels of cGMP.

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1. Introduction

Nicorandil (N-[2-hydroxyethyl])-nicotinamide nitrate) is an antianginal agent with a distinctive dual mechanism of action. The drug exerts its pharmacodynamic effect through the opening of K^+ channels and by increasing cyclic guanosine monophosphate (cGMP) levels by activating guanylate cyclase (GC) via its nitrate (1). The opening of K^+ channels in vascular smooth muscle cells leads to K^+ efflux and membrane hyperpolarisation, which inhibits calcium influx and promotes relaxation (2,3). Cyclic GMP activates the cGMP-dependent protein kinase (PKG), which initiates phosphorylation events that lead to vascular relaxation (4).

There is some evidence that plasmalemmal K^+ channels play a significant role in PKG-induced vasorelaxation (5). For example, large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels and voltage-gated K^+ (K_V) channels have been found to be activated via the cGMP pathway in human umbilical (6) and human pulmonary (7) arteries. The properties of nicorandil as a hybrid compound raise the possibility that activation of GC and the resulting increase in cGMP might contribute to K^+ channel activation (8).

Recently, we have demonstrated that different K^+ channel subtypes are involved in the nicorandil-induced relaxation of human bypass grafts. In particular, we have shown that: i) nicorandil endothelium-independently relaxed human saphenous vein (HSV)

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and human internal mammary artery (HIMA), the most commonly used conduit vessels for coronary artery bypass surgery; ii) ATP-sensitive K^+ (K_{ATP}) channels and 4-aminopyridine (4-AP)-sensitive K^+ channels located in the smooth muscle of HSV mediated the relaxation induced by nicorandil and iii) K_{ATP} and BK_{Ca} channels are probably involved in the action of nicorandil on HIMA (9). Until now, it has not been clear whether the nicorandil-induced activation of these K^+ channels in HSV and HIMA occurs directly or dependently of cGMP. Previous studies have demonstrated that the K^+ channel-dependent effect of nicorandil is independent of cGMP, acting on the channel (10,11). In contrast, some authors suggested the possible involvement of cGMP on nicorandil-induced activation of K_{ATP} (8) and BK_{Ca} (12) channels. Thus, the principal aim of the present study was to analyse the contribution of the cGMP pathway to the nicorandil-induced vasorelaxation of HSV and HIMA and to determine the role of cGMP as a second messenger in the K^+ channel-activating effect of nicorandil.

2. Materials and methods

2.1. Tissue preparation

Discarded segments of HSV (n = 32) and HIMA (n = 39) were obtained from 53 male patients (mean age ± S.E.; 63 ± 6 years) undergoing coronary artery bypass surgery. All patients were informed in detail about the aims of investigation and gave their written consent for the excision of remaining tissue. The experiments involving human vessels were approved by the Ethics Committee of Institute for Cardiovascular Diseases "Dedinje" and carried out in accordance with the Declaration of Helsinki and guidelines on Good Clinical Practice. After excision, the vessel segments were immediately placed in cold (4 °C) Krebs-Ringer-bicarbonate solution, and transported to the laboratory for study.

Excess fat and connective tissue were dissected and the HSV and HIMA segments were cut into 3-mm rings. One to two rings were obtained from each vessel segment. The endothelium was removed mechanically by gently rubbing the intimal surface with a stainless steel wire. Denudation of endothelium was verified by the inability of veins and arteries to relax after treatment with acetylcholine (1 μM) (13).

The rings were mounted between two stainless-steel triangles in a 10 ml organ bath filled with Krebs-Ringer-bicarbonate solution, maintained at 37 °C and aerated with 95% O_2–5% CO_2. The preparation was stretched to a resting force of 2 g (14,15) and equilibrated for 60 min, with frequent washing and force adjustment.

2.2. Experimental protocol

After equilibration, HSV and HIMA rings were contracted with phenylephrine (10 μM). The concentration of phenylephrine was elected on the basis of previous publications (16,17). When the contractile response by this vasoconstrictor agent reached a stable plateau, increasing cumulative concentrations of nicorandil (0.001 μM–300 μM) were added to the bath. Increasing concentrations of nicorandil were added after the relaxation evoked by the previous concentration reached its plateau or after 20 min if no response was obtained. To determine the role of the cGMP pathway in nicorandil-induced relaxation, the rings were treated with (1) 1H-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one (ODQ), an inhibitor of soluble GC, and (2) different K^+ channel blockers for minimum 20 min before the next concentration-response curve to nicorandil was assessed. Control rings had no blocking drugs added before titration with nicorandil.

2.3. Treatment of data and statistics

The relaxation produced by each concentration of nicorandil was measured and expressed as a percentage of the maximum possible relaxation (i.e., relaxation back to the baseline tension). The concentration of nicorandil producing 50% of the maximum response (EC_{50}) was calculated from each concentration–relaxation curve using a non-linear least squares fit with a logistic function and presented as pD_2 ($pD_2 = -\log EC_{50}$).

The results are expressed as the mean ± standard deviation (SD). The value of *n* indicates the number of experiments. Significant difference between means of different groups was determined by the unpaired Student's *t*-test, and a *P* value <0.05 was considered statistically significant. All calculations were performed using the computer program Origin (version 8; OriginLab Corporation, Northampton, MA, USA).

2.4. Drugs

The following drugs were used: nicorandil, phenylephrine hydrochloride, acetylcholine iodide, ODQ, glibenclamide, 4-AP and iberiotoxin (Sigma–Aldrich Inc., St. Louis, MO, USA). Nicorandil was dissolved in distilled water prior to being used. Glibenclamide and ODQ were dissolved in dimethyl sulfoxide. Previous experiments established that the solvents used had no effects on the preparations at the concentrations applied (data not shown). The drugs were added directly to the bath, and the concentrations given are the calculated final concentrations in the bath solution.

Krebs-Ringer-bicarbonate solution had the following composition (in mM): 118.3 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 25 $NaHCO_3$, 1.2 KH_2PO_4 and 11.1 glucose; pH 7.4.

3. Results

3.1. Effects of nicorandil on HSV and HIMA without endothelium pre-contracted by phenylephrine

Nicorandil (0.001 μM–300 μM) induced a concentration-dependent relaxation of denuded HSV and HIMA rings with pD_2 values of 5.87 ± 0.10 (maximal response 100 ± 3%, n = 8) (Fig. 1A) and 5.60 ± 0.09 (maximal response 100 ± 4%, n = 8) (Fig. 1B), respectively. The difference between the pD_2 values was not statistically significant ($P > 0.05$).

A time-matched control was used throughout the experiments, and any observed changes in tension were used for the subsequent adjustment of the drug-induced relaxation. The following method was used: the percentage relaxation in control conditions was compared with that in the presence of nicorandil at the same time-point after phenylephrine application.

3.2. Effects of ODQ and the combination of ODQ and K^+ channel blockers on the relaxation response of HSV and HIMA to nicorandil

In control rings, to which ODQ or a combination of ODQ plus K^+ channel blockers were not added, the relaxation induced by nicorandil was similar in initial and subsequent concentration-response curves. No significant differences with respect to either pD_2 or maximal response were found between the relaxation curves.

To examine the role of soluble GC in nicorandil-induced relaxation, the effects of ODQ (10 μM), an inhibitor of soluble GC, were investigated. Pre-incubation with ODQ caused a significant inhibition of the nicorandil-stimulated relaxation of HSV (Table 1 and Fig. 1A) and partially antagonised the nicorandil-induced relaxation of HIMA (Table 2 and Fig. 1B).

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