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The Effects of Potassium Channels in Human Internal Mammary Artery

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Abstract
Background: Structural and functional changes in potassium channels of vascular smooth muscle cells may contribute to the development of diseases such as hypertension. We aim to investigate the vascular effects of potassium channel openers and blockers in human internal mammary artery (HIMA). ***Methods:*** Remaining segments of HIMA from 18 consecutive patients undergoing coronary artery bypass surgery were obtained to examine the vascular effects of various potassium channel openers (staurosporine, hydrochlorothiazide and cromakalim) and potassium channel blockers (4-aminopyridin [4-AP], charybdotoxin [CTX] and glibenclamide [GLBC]). ***Results:*** Noradrenaline (NA)-induced maximal contractions were inhibited by all 3 K^+-channel blockers but only fully inhibited by 4-AP (95.6%). Only NA-induced contractions were reversed by CTX. Only K^+-induced maximal contractions were significantly inhibited by 4-AP (95.6%, $p < 0.05$). Only acetylcholine-induced relaxation was fully inhibited by CTX. Only sodium nitroprusside-induced relaxations in potassium chloride-precontracted strips could be reversed by GLBC. ***Conclusions:*** Drugs affecting potassium channels may be useful in the treatment of hypertension and management of perioperative vasospasm during the coronary artery bypass surgery.

Introduction

The vascular endothelium and smooth muscle layer have a great significance in relation to both pathogenesis of hypertension and also hypertension-related end-organ injury. Increase in the vascular tonus and the sensitivity to vasoactive mediators are important in the pathogenesis of hypertension. The potassium channels have important roles in the regulation of vascular tone but their functions have not yet been clearly understood. They organize the membrane potential and ionic movements of smooth muscle cells, so that the membrane potential determines the activity of voltage-gated calcium channels. The main ionic flow responsible for the resting membrane potential of smooth muscle cells is the potassium flow toward the

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Table 1. General characteristics of patients

	HIMA (n = 18)	
	male	female
Sex, n	14	4
Age, years, mean	62.1	60
Blood pressure, mm Hg, mean	140.7/85.7	142.5/92.5
Hypertension	14	4
Myocardial infarction	8	3
Hyperlipidemia	4	2
Smoking	14	4
Statines	4	2
Beta-blockers	4	4
Calcium antagonists	3	2
Angiotensin converting enzyme inhibitors	5	2
Diuretics	3	2
Acetylsalicylicacid	2	2
Nitrates	7	1

outside of the cell. The inhibition of this flow, that is, blocking the potassium channels causes membrane depolarization, opening of the voltage-gated calcium channels, increase in the intracellular Ca^{2+} and vasoconstriction, in that order. The opening of potassium channels causes membrane hyperpolarization, the closure of the voltage-gated calcium channels and reduction of the intracellular Ca^{2+} and vasodilation, in that order [1, 2].

Despite more than 16 groups of potassium channels having been identified up to now, their physiological functions are still not well-known. The 3 most common potassium channels in the vascular smooth muscle cells are voltage-sensitive retarded rectifying potassium channels (K_V), voltage-sensitive highly conductive potassium channels (BK_{Ca}) and the less frequently encountered ATP-sensitive K^+ channels (K_{ATP}). Several openers such as staurosporine (STS) (K_V), hydrochlorothiazide (HCT) (BK_{Ca}) and cromakalim (CRK) (K_{ATP}) and blockers such as 4-aminopyridin (4-AP) (K_V), charybdotoxin (CTX) (BK_{Ca}) and glibenclamide (GLBC) (K_{ATP}) affect the functions of these channels [2].

The aim of this study was to compare the vascular effects of potassium channels (K_V, BK_{Ca} and K_{ATP}), which play an important role in the receptor-mediated (noradrenaline [NA]) and depolarization potassium chloride (KCl) contractions and endothelium-dependent (acetylcholine [Ach]) and endothelium-independent (sodium nitroprusside [NSP]) relaxation responses in human internal mammary artery (HIMA) strips of hypertensive patients.

Materials and Methods

HIMA segments from 18 consecutive patients undergoing coronary artery bypass surgery, according to AHA/ACC (American Heart Association/American College of Cardiology) criteria, were obtained without regard to preoperative drug therapy [3]. General characteristics of the patients are shown in table 1.

Organ-Bath Experiments

HIMAs were intraluminally washed with Krebs solution and were placed in oxygenated physiological Krebs solution at 4°C and then immediately transferred to the laboratory. The mean time between harvesting and experimentation was 30 min. The arterial segments were carefully cleaned of surrounding fatty, connective and nonvascular tissue. The arteries were cut into rings of 3–4 mm length and then all the rings were longitudinally cut, and strips were suspended in 20 ml organ baths containing Krebs solution. The solution was gassed with 95% O_2 and 5% CO_2 at 37°C. The strips were attached to a force displacement transducer, and changes in isometric tension were recorded continuously on a multichannel recorder polygraph by using a computer software (COMMAT Ltd., MAY, FDT 10A, Ankara, Turkey). The strips were equilibrated for at least 45 min under the resting tension of 2 g. After equilibration for 45 min, strips were exposed to 40 mmol/l KCl until the response became stable, and then washed several times with fresh buffer and allowed to equilibrate for an additional 45 min. Each strip was exposed to a single vasoconstrictor agent.

Effects of K^+-Channel Blockers on the NA- and KCl-Induced Contractions

After equilibration of HIMA strips, 10^{-5} mol/l NA (submaximal dose) or 40 mmol/l KCl (submaximal dose) was added into the organ bath, and the contractions were recorded as control. To show whether or not the pretreatment with K^+-channel blockers (GLBC, CTX and 4-AP) would have altered the contraction response to the vasoconstrictors (NA and KCl), the strips were preincubated for 30 min with GLBC (10 μmol/l, K_{ATP}-channel antagonist), CTX (10 nmol/l, BK^+_{Ca}-channel antagonist) and 4-AP (10 μmol/l, K_V-channel antagonist) and then were contracted with NA (10^{-5} mol/l) or KCl (40 mmol/l). The effect of each dose of vasoconstrictors was continuously recorded for a fixed time (5 min).

Effect of K^+-Channel Openers on the NA- and KCl-Induced Contractions

After equilibration of HIMA strips, 10^{-5} mol/l NA or 40 mmol/l KCl was added into the organ bath, and the contraction force was recorded for a fixed time (5 min); then, CRK (10 μmol/l, K_{ATP}-channel agonists), HCT (30 μmol/l, BK^+_{Ca}-channel agonists) and STS (0.1 μmol/l, K_V-channel agonists) were added into the organ bath, and the effects of each dose of K^+-channel agonists were recorded for a fixed time (10 min).

In the second series of experiments, after preincubation for 30 min with GLBC (10 μmol/l), CTX (10 nmol/l) and 4-AP (10 μmol/l), HIMA strips were contracted for 5 min with NA (10^{-5} mol/l) or KCl (40 mmol/l), and then, CRK (10 μmol/l), HCT (30 μmol/l) and STS (0.1 μmol/l) were added into the organ bath and the effect of each dose of K^+-channel agonists was recorded for a fixed time (10 min).

Effect of K^+-Channel Blockers on the Ach- and SNP-Induced Vasodilation in Precontracted HIMA

To show whether or not the pretreatment with K^+-channel blockers would have altered the relaxation response to the vasodilators after preincubation for 30 min with GLBC (10 μmol/l), CTX (10 nmol/l) and 4-AP (10 μmol/l), strips were contracted for 5 min with NA (10^{-5} mol/l) or KCl (40 mmol/l), and then, Ach (10^{-4} mol/l) or SNP (10^{-4} mol/l) was added into the organ bath. The effect of each dose of vasodilators was recorded for a fixed time (10 min).

Statistical Analysis

Data were analyzed with the SPSS software version 20.0 for Windows (SPSS Inc., Chicago, Ill., USA). The reactivity of HIMA strips was expressed as a maximal contraction or maximal relaxation (E_{max}). Results were expressed as mean ± SEM. Student's t tests and one-way analysis of variance following the Tukey–Kramer tests were used to compare differences between the groups. A value of $p < 0.05$ was considered statistically significant.

Results

Effects of K^+-Channel Blockers on the NA- and KCl-Induced Contractions

GLBC and CTX significantly inhibited the submaximal single-dose NA-induced contractions of HIMA strips. But, only 4-AP completely abolished the submaximal single-dose NA-induced contractions of HIMA strips, while single-dose KCl-induced contractions of HIMA strips were significantly inhibited with 4-AP; GLBC and CTX showed no effect on it (table 2).

Effects of K^+-Channel Openers on the NA- and KCl-Induced Contractions

CRK and HCT significantly relaxed the contractions induced by a submaximal single-dose NA in HIMA strips. GLBC significantly suppressed the relaxant response to CRK but only CTX completely suppressed the relaxant response to HCT (table 3). HCT significantly increased the contractions induced by a submaximal single-dose KCl in HIMA strips. In KCl-precontracted HIMA strips, CTX reversed the contractile responses to HCT but 4-AP significantly increased the responses to KCl and STS (table 4).

Effects of Ach in KCl-Precontracted HIMA Which Was Under K^+-Channel Blockage

In HIMA strips precontracted with single-dose NA, Ach produced a significant vasodilation and CTX reversed the relaxant responses to Ach (fig. 1). Neither GLBC nor CTX reversed the relaxant responses to SNP in HIMA strips. Because 4-AP completely reversed the effect of NA, the effect of SNP could not be measured (fig. 2). KCl precontractions were significantly increased with Ach, and this increment was inhibited by both CTX and 4-AP whereas GLBC had no significant effect on it (fig. 3). Neither 4-AP nor CTX reversed the relaxant responses to SNP, whereas GLBC reversed the responses induced by SNP (fig. 4).

Table 2. The effect of K^+-channel blockers on NA- and KCl-induced contractions

E_{max} mg tension/mg ww	C	GLBC+	CTX+	4-AP+
NA	n = 28 227±14	n = 10 124±17**	n = 10 116±17**	n = 10 10±3**, †, ‡
KCl	n = 34 158±15	n = 12 107±9	n = 10 159±23	n = 10 74±14*

* 4-AP vs. C-KCl, $p < 0.05$; ** GLBC vs. C-NA, 4-AP vs. C-NA, $p < 0.001$; † 4-AP vs. GLBC + NA, $p < 0.001$; ‡ 4-AP vs. CTX + NA, $p < 0.001$.

C = Control.

Table 3. The effect of K^+-channel openers on NA-induced contractions

K^+-channels	NA + K^+-channel opener	K^+-channel blocker + NA + K^+-channel opener
K_{ATP}	NA + CRM n = 12 43±9**	GLBC + NA + CRM n = 12 75±5*, ††, ‡
BK_{Ca}	NA + HCT n = 12 67±5**, †, ‡	CTX + NA + HCT n = 12 113±3‡
K_V	NA + STS n = 10 104±3†††	4-AP + NA + STS n = 10 Full block
C-NA		100±00

* GLBC + NA + CRM vs. C-NA, $p < 0.01$; ** NA + CRM vs. C-NA, $p < 0.001$; ‡ NA + HCT vs. C-NA, $p < 0.001$; † NA + HCT vs. NA + CRM, $p < 0.05$; †† GLBC + NA + CRM vs. NA + CRM, $p < 0.01$; ††† NA + STS vs. NA + CRM, $p < 0.001$; GLBC + NA + CRM vs. CTX + NA + HCT, $p < 0.001$.

C-NA = NA contraction response.

Table 4. The effect of K^+-channel openers on KCl-induced contractions

K^+-channels	KCl + K^+-channel opener	K^+-channel blocker + KCl + K^+-channel opener
K_{ATP}	KCl + CRM n = 15 88±2**	GLBC + KCl + CRM n = 14 113±3††
BK_{Ca}	KCl + HCT n = 10 116±4*, ††	CTX + KCl + HCT n = 10 105±1
K_V	KCl + STS n = 11 109±1†	4-AP + KCl + STS n = 10 128±8**, ‡, ¥¥
C-KCl		100±0.00

* KCl + HCT vs. C-KCl, $p < 0.05$; ** 4-AP + KCl + STS vs. C-KCl, $p < 0.001$; ‡ 4-AP + KCl + STS vs. KCl + STS, $p < 0.05$; † KCl + STS vs. KCl + CRM, $p < 0.01$; †† KCl + HCT vs. KCl + CRM, $p < 0.001$; ¥¥ 4-AP + KCl + STS vs. CTX + KCl + HCT, $p < 0.01$.

C-KCl = KCl contraction response.

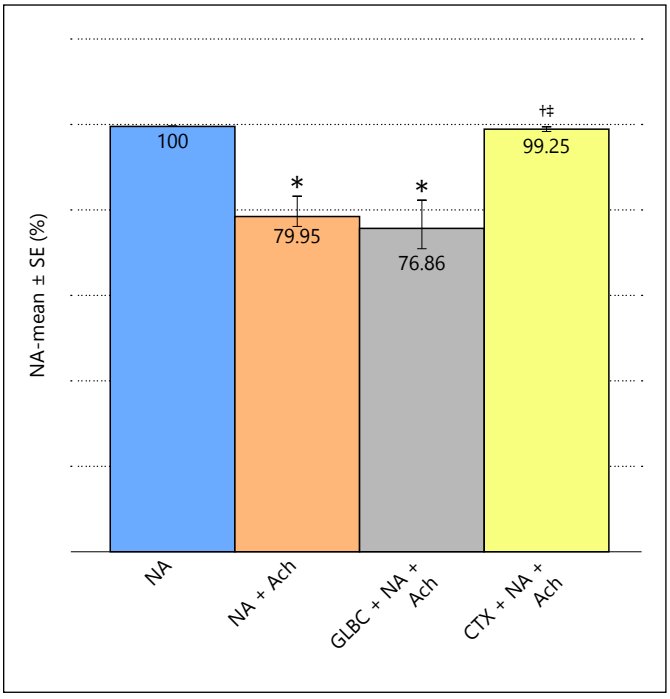

Fig. 1. The effect of K^+-channel blockers on Ach-induced vasodilation in NA-precontracted HIMA (* $p < 0.001$ vs. control, † $p < 0.001$ vs. NA + Ach, ‡ $p < 0.005$ vs. GLBC + NA + Ach).

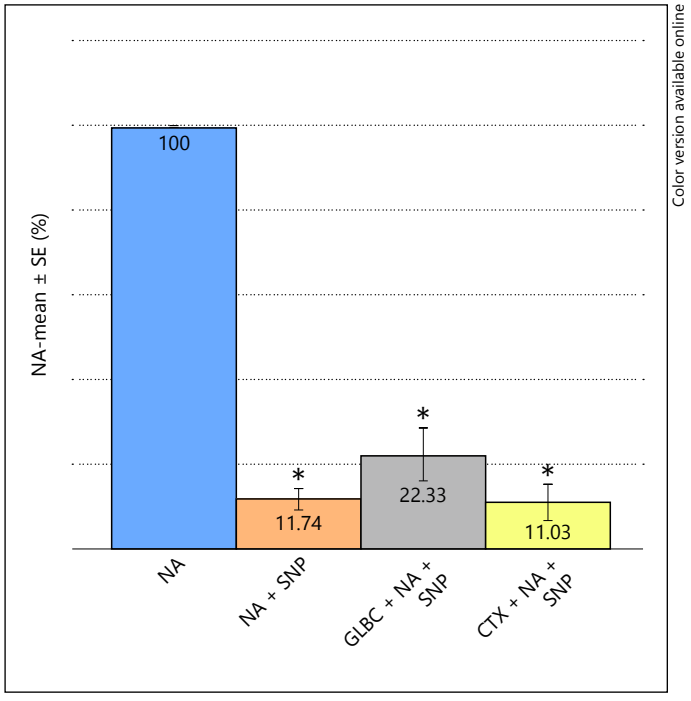

Fig. 2. The effect of K^+-channel blockers on SNP-induced vasodilatation in NA-precontracted HIMA (* $p < 0.001$ vs. control).

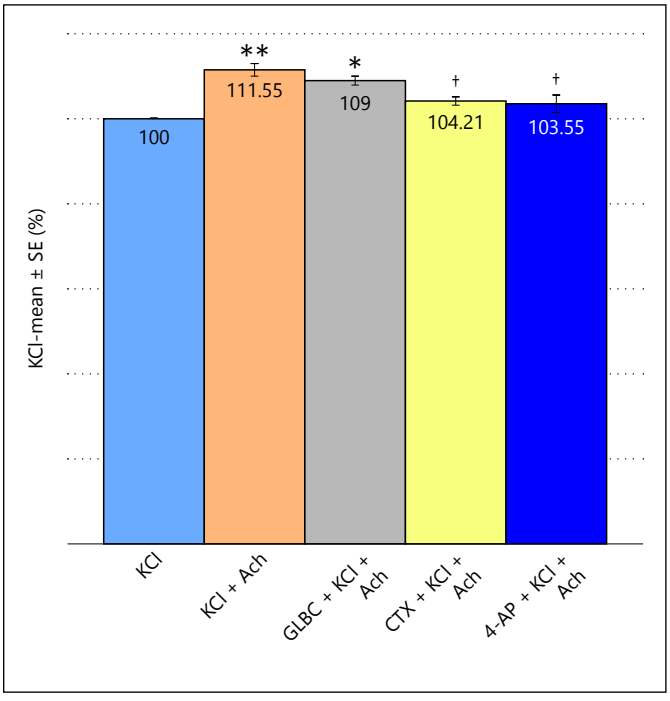

Fig. 3. The effect of Ach in KCl-precontracted HIMA under K^+-channels blockage (* $p < 0.05$ vs. control, ** $p < 0.001$ vs. control, † $p < 0.05$ vs. KCl + Ach).

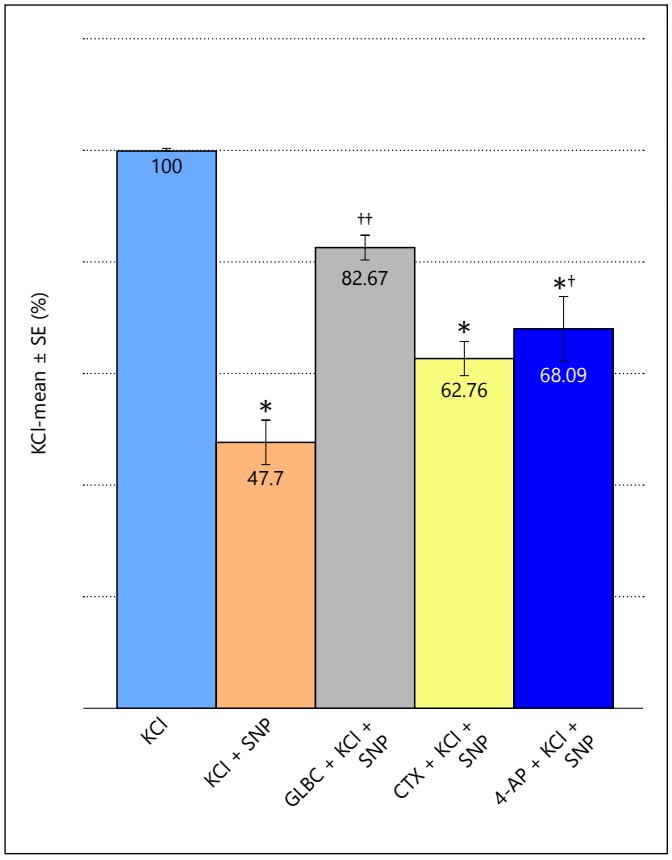

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Fig. 4. The effect of K^+-channel blockers on SNP-induced vasodilation in KCl-precontracted HIMA (* $p < 0.001$ vs. control, † $p < 0.05$ vs. KCl + SNP, †† $p < 0.001$ vs. KCl + SNP).

Discussion

Potassium channels play an important role in the regulation of vascular tone, and they organize the membrane potential of smooth muscle cells. Therefore, potassium channels are the potential targets of many new drugs for several cardiovascular diseases [1, 2].

According to our results; the submaximal NA contractions in HIMA were completely blocked with 4-AP. Activation of vascular smooth muscle by agonists such as NA not only causes an increase in calcium release from intracellular stores, but also depolarizes the membrane potential as well. However, there are studies suggesting that 4-AP may cause intracellular alkalinization and/or calcium release from the sarcoplasmic reticulum in different vascular segments, and by muscarinic receptor activation it may indirectly activate the BK_{Ca}-sensitive potassium flow and it causes membrane hyperpolarization and results in relaxation [4]. This indirect hyperpolarizing effect of 4-AP has correlation with our results.

The relaxation caused by CRK and its significant inhibition with GLBC was found to be consistent with other studies. The effects of levocromakalim, aprikalim and GLBC were shown to be in an endothelium-independent fashion and related with the opening of postsynaptic K_{ATP}-channels in vascular smooth muscle cells and their blockage [5].

The relaxation in HIMA caused by HCT, and the complete inhibition of this relaxation with CTX, is supported with previous experimental studies [6]. It was shown that HCT increased the K^+ efflux by opening the BK_{Ca} channels, and it caused the hyperpolarization, which consequently results in relaxation. Besides, it has been reported that intracellular acidosis inhibits the opening of BK_{Ca} channels, and on the contrary, intracellular alkalinization increases the channel opening and causes relaxation as a result of hyperpolarization [7].

Despite being shown that STS inhibits the contraction of vascular smooth muscle by opening the K_V-channels, on the contrary we found that STS did not affect these contractions [8]. Another group of researchers showed that the increase in intracellular calcium produced by agonists inhibits the K_V flow in smooth muscle cell [9]. In our study, the agonists showed their effects by increasing the level of intracellular calcium, too. Therefore, this increment may have probably abolished the effects of STS as a result of K_V channel blockage. In addition, high extracellular K^+ levels may also non-selectively inhibit potassium channels in the vascular smooth muscle membrane. In our study, the ineffectiveness of STS for KCl precontractions may also be partially explained by this blockage [8].

SNP-mediated relaxation could be reversed with neither GLBC nor CTX. While Ach relaxation was not affected from GLBC, it was completely inhibited with CTX. This effect of CTX is consistent with literature [10]. Nitrous oxide (NO) and NO donors (like SNP) increase the level of intracellular cGMP and also directly activate the Ca^{2+}-dependent K^+-channels in the cell membrane and cause hyperpolarization. As a result, they cause relaxation by decreasing the level of calcium via the closure of intracellular voltage-dependent Ca^{2+}-channels. Therefore, the blockage of BK_{Ca}-channels by CTX may completely inhibit the effect of Ach. NO is released from the endothelium, and it is a mediator in the effect of Ach. Thus, BK_{Ca}-channels had a partial effect on relaxation caused by NO [11].

KCl-precontracted HIMAs were inhibited mainly (approximately 53%) by SNP, and this inhibition could be

reversed completely with GLBC. In experimental hypertensive animal models, it was shown that Ach relaxation responses completely disappeared in KCl pre-contracted carotid arteries, while SNP responses were not affected with CTX and 4-AP. These findings are found to be correlated with our results [12, 13].

As it is known, NO donors such as SNP increase the level of intracellular cGMP by activating the guanylate cyclase enzyme in the vascular smooth muscle. Elevated cGMP levels cause the opening of K_{ATP} channels, membrane hyperpolarization and smooth muscle relaxations, in that order, due to the decrease in the level of intracellular calcium. The effect of GLBC on SNP-mediated relaxation responses in KCl pre-contracted HIMA strips may be explained with the blockage of this mechanism by GLBC [14].

Conclusion

According to our knowledge, this study is one of the most comprehensive studies on K^+-channels blockers and openers in HIMA. There are intense efforts in developing new targets and new drugs in the treatment of diseases such as hypertension. K^+-channels play a central role in the homeostasis of vascular tones. As it was found in our and similar studies, K channels may affect both the contraction and relaxation responses. These results, taken together, can be used in the treatment of several cardiovascular diseases.

Limitations of the Study

The most important limitations of this study are the small number of patients and the use of only HIMA vessels.

Disclosure Statement

The authors declare that they have no conflict of interest.

Financial Disclosure

The authors declared that this study received no financial support. The use of remaining HIMA segments was approved by the Human Ethics Committee of the Istanbul University. Informed consent was obtained from all of the patients who participated in the study.

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