The comparison of the responsiveness of human isolated internal mammary and gastroepiploic arteries to levcromakalim: an alternative approach to the management of graft spasm

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Aims We studied the effectiveness of levcromakalim, a potassium channel opener (KCO), in the prevention and reversal of spasm in arterial grafts used in coronary artery bypass operations, namely, internal mammary artery (IMA) and gastroepiploic artery (GEA).

Methods Spasm was mimicked *in vitro* in arterial rings from 109 patients by increasing the vascular tension with noradrenaline, the thromboxane analogue U46619, endothelin-1 and K^+ .

Results GEA displayed considerably higher contractile force to these agents than IMA. Pretreatment with levcromakalim depressed significantly the maximal contractile responses (either absolute or relative) to noradrenaline and U46619 but did not affect those of endothelin-1 and K^+ in both of the arteries. Sensitivities (to all agents, except to endothelin-1) decreased significantly after levcromakalim. In experiments evaluating the antispasmodic activity of levcromakalim, a higher relaxant capacity was observed in GEA than IMA (for K^+ contraction; IMA: $31.32 \pm 3.83\%$, $n = 13$ vs GEA: $98.01 \pm 0.71\%$, $n = 7$, $P < 0.05$). This different activity of levcromakalim between two arterial grafts was apparent even when GEA rings were contracted to higher force (g) than that of IMA (for K^+ contraction; GEA: $72.56 \pm 4.96\%$, $n = 7$). Responses to levcromakalim were similar in IMA and GEA when endothelin-1 was used as the spasmogenic agent (IMA: $80.98 \pm 4.85\%$, $n = 10$ vs GEA: $91.93 \pm 3.17\%$, $n = 7$, $P > 0.05$).

Conclusions Our results provide evidence that levcromakalim may have a therapeutic value in the treatment of spasm of coronary artery bypass grafts, especially GEA.

Keywords: internal mammary artery, gastroepiploic artery, spasm, levcromakalim

Introduction

IMA and GEA are frequently used as arterial grafts for coronary revascularization [1]. Graft spasm during the perioperative or postoperative period limits the arterial graft longevity by reducing luminal blood flow [2, 3]. Endogenous spasmogenic agents such as noradrenaline, endothelin, thromboxane A_2 or depolarization of the cell membrane contribute to this effect [1, 4]. At present, nitrovasodilators, Ca^{++} channel blockers and papaverine are used in the prevention and treatment of graft spasm. However, in some cases, these vasodilators remain ineffective [5–8]. Thus, the search for new, more effective agents to manage graft spasm is appropriate.

K^+ channels are involved in the regulation of electrophysiology of the cell membrane. Their activation results in cellular hyperpolarization due to K^+ efflux which in turn causes the inhibition of Ca^{++} influx through voltage operated Ca^{++} channels (VOC) and Ca^{++} release from

intracellular stores through IP_3 [9]. ATP-sensitive K^+ channels have been identified in vascular, cardiac and skeletal muscle cells, which are activated by drugs referred to as K^+ channel openers (KCOs, e.g., cromakalim, pinacidil, nicorandil) and inhibited by sulphonylurea drugs such as glibenclamide [9–11]. Increased K^+ channel activity in spontaneous hypertensive rats and under hypoxic conditions suggests that K^+ channel function might be changed in some cardiovascular diseases [12–14]. These channels have also been shown to be involved in the responses of various endogenous vasodilators including adenosine, prostacyclin, nitric oxide and endothelium derived hyperpolarizing factor (EDHF) [11]. Thus, KCOs may possess beneficial therapeutic values in vasospastic disorders.

The purpose of the present study was to examine the responsiveness of IMA and GEA rings to levcromakalim, a K_{ATP} channel opener, in order to determine the role of K^+ channels in the regulation of vascular tone of these arteries.

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Methods

Sampling and preparation of the vessels

Internal mammary artery grafts were supplied from patients undergoing coronary artery revascularization whereas gastroepiploic arteries were supplied from patients undergoing gastrectomy. Experiments were performed in vessels obtained from patients who were not exposed to sulphonylurea type oral antidiabetic agents. Some clinical characteristics of the patients and their drug therapies are given in Table 1. Use of the vessels was approved by the Institutional Review Boards of Baskent University and Oncology Hospital. All patients gave informed consent. Care was taken during harvesting of the vessels so as not to stretch and touch the endothelial surface. The vessel species were placed immediately into cold (4 °C) Krebs Ringer-bicarbonate solution of the following composition (mM): NaCl 118, KCl 4.7, KH₂PO₄ 1.2, NaHCO₃ 25, MgSO₄·7H₂O 1.2, CaCl₂ 2.5, glucose 11.1, and disodium EDTA 0.026.

The vessels were cleaned of adherent connective tissues and cut into rings of 3–4 mm in length. Rings were suspended between two stainless steel L-shaped hooks in a 10 ml jacketed organ bath containing Krebs Ringer-bicarbonate solution at 37 °C and aerated with 95% O₂ and 5% CO₂. One hook was fixed to a micrometric manipulator allowing adjustments in resting tension of the rings and the other was connected to a force displacement transducer (Grass model FT 03 and May Com model FDT 10-A) for the measurement of isometric force. The optimal point of length-tension relation had been determined previously by repeated exposure to noradrenaline (5×10^{-6} M) at different levels of resting tension [15, 16]. In both of the arteries, 2 g was determined to be optimal for maximal noradrenaline responsiveness.

Experimental protocol

At the end of equilibration period, the viabilities of the vessel segments were checked by noradrenaline (5×10^{-6} M) and preparations which developed a tension of less than 1 g were discarded. The experiments were performed on endothelium intact rings of the vessels and the presence of endothelium was checked by functional relaxation to

acetylcholine (10^{-6} M). Two to five rings were obtained from each artery specimen.

The experiments were performed to evaluate the preventive and antispasmodic effects of levcromakalim against various spasmogenic agents. Firstly, the cumulative dose-response relations to noradrenaline (10^{-9} – 10^{-4} M), the thromboxane analogue U 46619 (10^{-9} – 10^{-6} M), endothelin-1 (10^{-10} – 10^{-7} M) and KCl (K⁺, 15–100 mM) were constructed in both of the vessel segments. To determine whether pretreatment of IMA and GEA with levcromakalim would alter the responsiveness to the above spasmogens, the cumulative dose-response curves of these agents were repeated in the presence of levcromakalim in both of the vessel rings with the exception of endothelin-1. Endothelin-1 induced contractions could not be reproduced in the same preparation and thus the cumulative dose-response curve in the presence of levcromakalim was performed in the adjacent ring of the same vessel. Levcromakalim was applied at concentrations corresponding to 50% of its maximum relaxant response (EC₅₀) in both of the arteries (see EC₅₀ values in Table 3) and added 30 min before the second cumulative dose-response curve of contractile agent.

To determine the antispasmodic activity of levcromakalim, the concentration of each spasmogenic agent required to produce approximately the same contractile force (g) in both IMA and GEA was determined and used to develop submaximal contraction. This contractile force corresponds to 70–90% of the maximal contraction in IMA and 30–40% in GEA (low force). In an additional group of studies, GEA rings were contracted to 70–90% of the maximal contraction (high force) by increasing the concentrations of the contractile agents. To realize these experiments, the vessel rings were contracted with noradrenaline (5×10^{-6} M for IMA and 5×10^{-7} M or 5×10^{-6} M for GEA), U 46619 (5×10^{-8} M for IMA and 10^{-8} M or 5×10^{-8} M for GEA), endothelin-1 (3×10^{-8} M for IMA and 5×10^{-9} M or 3×10^{-8} M for GEA) and K⁺ (40 mM for IMA and 20 mM or 40 mM for GEA). Afterwards, increasing concentrations of levcromakalim (BRL 38227, 10^{-9} – 10^{-4} M) were assessed on IMA and GEA rings precontracted with either of the contractile agents. Relaxation capacities of the arteries were tested with sodium nitroprusside (SNP) which was applied at maximal concentration (10^{-4} M) at the end of experiments. In time match control experiments, we determined that precontractions induced by the spasmogenic agents were stable enough for the period required to construct the cumulative-relaxation curve of levcromakalim.

In some experiments, IMA and GEA rings were incubated for 30 min with indomethacin (10^{-5} M), L^G-nitro-L-arginine (L-NOARG, 10^{-4} M) and glibenclamide (10^{-4} M) in order to evaluate the role of prostaglandins, nitric oxide and K_{ATP}-channels, respectively in the relaxant responses of levcromakalim.

Drugs

All drugs used were purchased from Sigma Chemical Co. (St Louis, MO) except endothelin-1 (Peninsula Labs.). U 46619 (9,11 dideoxy-11 alpha, 9 alpha-epoxymethano-prostaglandin F_{2α}) and levcromakalim (BRL 38227) were

Table 1 Clinical characteristics of patients undergoing coronary artery bypass and gastrectomy operations.

Parameters	IMA	GEA
Number of patients, <i>n</i>	73	36
Sex, <i>n</i>		
Female	8	10
Male	65	26
Age (years)	56.3 ± 1.2	59.1 ± 1.8
Drug therapy, <i>n</i>		
nitrovasodilators	40	0
Ca ⁺⁺ antagonists	57	5
β-adrenoceptor antagonists	3	1
diuretics	0	1
ACE inhibitors	0	1

ACE; angiotensin converting enzyme.

generous gifts of Upjohn Company (Kalamazoo, MI) and SmithKline Beecham Pharmaceuticals (Welwyn, Herts), respectively. A stock solution of noradrenaline was prepared in 0.001N HCl and ascorbic acid was added to prevent oxidation. Levromakalim and glibenclamide were dissolved in DMSO, and indomethacin in 5% (wt/vol) sodium bicarbonate. Other drugs were prepared in distilled water. DMSO was determined to have no effect on either of the contractile agent induced responses.

Statistical analysis

The results are given as mean $\pm$ s.e.mean. The maximal contractile response ($E_{\max}$) to each contractile agent was expressed according to K^+ (40 mM) induced contraction in that vessel ring. The maximal relaxations to levromakalim ($E_{\max}$) were expressed as the percent decreases of the precontractions. The sensitivities of IMA and GEA to spasmogenic agents and levromakalim are expressed as the effective concentration that elicited 50% of the maximal response (either contraction or relaxation, EC_{50}) and calculated separately for each dose-response curve by probit analysis. EC_{50} values were expressed as negative log M. In all experiments n is the number of patients from whom the vessels were obtained. Statistical analysis was determined by Student's paired or unpaired t -test and a P value less than 0.05 was considered significant.

Results

Contraction

The changes in contractile forces (g) induced by noradrenaline, the thromboxane analogue U 46619, endothelin-1 and K^+ in IMA and GEA rings are shown in Figure 1. The maximal absolute contractions (g) induced by each spasmogenic agent were considerably higher in GEA than IMA. However, the maximal relative contractions ($E_{\max}$, expressed as percent of contraction to K^+ , 40 mM) were identical in both of the arteries with the exception of noradrenaline (Table 2). On the other hand, the effective concentrations

of the spasmogens that causes 50% of the maximal contraction (EC_{50}) are similar in IMA and GEA (Table 2). Both of the arterial grafts are more sensitive to endothelin-1 and U 46619 than the other two contractile agents.

Effects of pretreatment with levromakalim The pretreatment of IMA and GEA rings with levromakalim, at concentrations corresponding to EC_{50} values, significantly depressed the maximal contractions (relative or absolute) to noradrenaline and U 46619 but not to endothelin-1 and K^+ (Figure 2 and Table 2).

In regard to sensitivity, the EC_{50} values for noradrenaline, U 46619 and K^+ were significantly ($P < 0.05$) increased after pretreatment with levromakalim indicating a shift of their dose-response curves to the right in both vessels. However, no significant change was observed in the sensitivity to endothelin-1 (Table 2).

In the absence of levromakalim no change in contractile properties or in sensitivity was observed in either vessel. Furthermore, the addition of levromakalim did not alter the resting tension of the arteries (data not shown).

Relaxation

Levromakalim induced dose-dependent relaxations in IMA and GEA (low and high force) rings (Figure 3 and Table 3). The maximal relaxations to levromakalim ($E_{\max}$) were markedly higher in GEA (either low or high force) than IMA with the exception of relaxation against endothelin-1 induced precontraction (Table 3). Besides, high force GEA rings displayed partially reduced relaxations to levromakalim compared with low force rings (Table 3). On the other hand, SNP fully relaxed IMA and GEA rings precontracted with either of the spasmogens (Table 3).

In IMA donors, preoperative therapy with Ca^{++} channel blockers did not influence the responsiveness of the artery to levromakalim. For instance in the contraction of noradrenaline, the maximal relaxation to levromakalim was $63.09 \pm 3.45\%$ ($n = 10$) in IMA rings from donors that took Ca^{++} channel blockers and was $63.31 \pm 6.02\%$ ($n = 5$) in rings from donors that did not take such therapy ($P > 0.05$).

Figure 1 Changes in contractile forces (g) to noradrenaline (●), U 46619 (▲), endothelin-1 (◆) and K^+ (■) in internal mammary artery (a) and gastroepiploic artery (b) ($n = 6-8$). Values are mean $\pm$ s.e.mean.

Parameter	Control		+ levromakalim	
	IMA	GEA	IMA	GEA
$E_{\max}$				
Noradrenaline	97.71 ± 6.62	156.09 ± 10.55#	70.58 ± 11.73*	99.68 ± 9.09*
U 46619	116.62 ± 4.81	133.80 ± 8.41	92.13 ± 6.46*	109.80 ± 8.17*
Endothelin-1	93.39 ± 9.54	117.14 ± 13.94	77.94 ± 6.93	91.94 ± 10.79
K ⁺	130.07 ± 9.95	125.51 ± 7.34	124.91 ± 11.90	122.59 ± 6.91
EC_{50}				
Noradrenaline	6.10 ± 0.14	6.00 ± 0.09	5.74 ± 0.12*	5.49 ± 0.18*
U46619	7.78 ± 0.10	7.74 ± 0.10	7.09 ± 0.14*	7.18 ± 0.04*
Endothelin-1	8.18 ± 0.06	8.15 ± 0.10	8.16 ± 0.12	8.25 ± 0.11
K ⁺	31.36 ± 2.89	23.99 ± 2.37	42.51 ± 2.83*	33.38 ± 4.26*
Force (g)				
Noradrenaline	1.78 ± 0.20	7.84 ± 1.19#	1.23 ± 0.16*	5.09 ± 0.93*
U 46619	2.55 ± 0.14	6.98 ± 1.17#	2.00 ± 0.13*	5.75 ± 1.11*
Endothelin-1	1.79 ± 0.17	6.35 ± 1.24#	1.51 ± 0.22	6.26 ± 1.16
K ⁺	3.12 ± 0.31	6.67 ± 1.23#	2.94 ± 0.25	6.65 ± 1.29

Data are presented as mean ± s.e.mean; $n=6-8$ experiments. $E_{\max}$ values are expressed according to K⁺ (40 mM) induced contractions (see *Methods* for the doses of contractile agents). EC_{50} values are expressed as $-\log M$ except for K⁺ (mM).

*Shows statistically significant difference ($P<0.05$) between the responses in the absence (control) and presence of levromakalim.

#Shows statistically significant difference ($P<0.05$) between the responses in IMA and GEA.

In regard to sensitivity, the effective concentrations of levromakalim that causes 50% of the maximal relaxation (EC_{50}) against noradrenaline, U 46619 and K⁺ contractions were significantly higher in IMA than in GEA rings ($P<0.05$). However, it was similar in both of the arteries against endothelin-1 contraction (Table 3). These data indicate that levromakalim produced higher antispasmodic activity in GEA compared with IMA.

On the other hand, the time required for levromakalim to cause maximal relaxation was similar in the two arteries (i.e. for endothelin-1 contraction; IMA: 8.5 ± 0.76 min, $n=15$ vs GEA: 9.56 ± 0.81 min, $n=8$, $P>0.05$).

Effects of indomethacin, L-NOARG and glibenclamide The incubation of the arterial rings with the combination of indomethacin (10^{-5} M), used to inhibit prostaglandin synthesis, and L-NOARG (10^{-4} M), used to inhibit nitric oxide synthesis, did not affect the relaxations to levromakalim ($P>0.05$, Table 4). However, the responses to levromakalim were completely abolished in both IMA and GEA rings in the presence of glibenclamide (10^{-4} M), the K_{ATP} channel blocker (Table 4). Furthermore, addition of glibenclamide did not modify the resting tension of the arteries (data not shown).

Discussion

Graft spasm limits graft function by reducing luminal blood flow. Spasm can result from endothelial injury during surgical preparation which initiates the abnormal platelet-endothelium interaction [17, 18]. Endothelial reactivity is accepted as a crucial factor in graft longevity [15, 18–20]. The mechanism of graft spasm is not clear. However, elevated plasma levels of noradrenaline, thromboxane A₂ and endothelin-1 have been measured during or after

coronary artery bypass grafting [1, 4]. These above factors, either alone or in combination, may have implications in the genesis of graft spasm.

Currently, graft spasm is controlled by nitrovasodilators, nifedipine and papaverine used either systemically or topically [5–8]. We and other researchers have previously shown that these drugs are effective vasodilators against in vitro graft spasm [16, 21, 22]. However, in some cases, the above vasodilators may not provide the expected benefit in native graft spasm [6–8]. This may be related to the complexity of spasm. Moreover, some unwanted side effects have been associated with these drugs. For instance, the negative inotropic effect of nifedipine may aggravate congestive heart disease. The lack of tissue specificities of the available vasodilators can cause coronary steal [23, 24]. In this respect, levromakalim may offer some advantages over classical vasodilators because of its relative selectivity for coronary blood flow and large dose range between its vasodilator and myocardial effects [24, 25].

In the present study, we aimed to test the effectiveness of levromakalim in the reversal and prevention of arterial graft spasm *in vitro*. For this purpose, we selected four spasmogenic agents, namely, noradrenaline, endothelin-1, the thromboxane A₂ analogue U 46619 and K⁺ to mimic the native graft spasm. These agents produce contractions depending on Ca⁺⁺ influx through VOC and/or Ca⁺⁺ mobilization from intracellular stores by interfering with the synthesis of IP₃ and consequently lead to an increase in intracellular free Ca⁺⁺ level. Conversely, levromakalim, by causing hyperpolarization, prevents Ca⁺⁺ entry through VOC or inhibits agonist stimulated IP₃ synthesis and thus, reverses the contraction due to a decrease in intracellular free Ca⁺⁺ concentration [9, 11]. We first investigated the efficacies and potencies of the above spasmogens in IMA and GEA in the absence and presence of levromakalim.

Table 2 Maximal contractions (relative, $E_{\max}$; absolute, g) and EC_{50} values to spasmogens in the absence and presence of levromakalim in IMA and GEA rings.

Figure 2 Dose-response curves to noradrenaline (a), U 46619 (b), endothelin-1 (c) and K⁺ (d) in the absence (—) and presence (---) of levromakalim in internal mammary artery (▲) and gastroepiploic artery (■). Levromakalim was added at concentrations corresponding to EC₅₀ values before the second dose-response curves of the contractile agents (*n*=6–8). Values are mean ± s.e.mean.

Figure 3 Dose-response curves showing relaxations induced by levromakalim against noradrenaline (●), U 46619 (▲), endothelin-1 (◆) and K⁺ (■) induced contractions in internal mammary artery (a, *n*=10–15) and gastroepiploic artery (b, *n*=7–8). Values are mean ± s.e.mean.

		Levcromakalim		SNP
Contractile agent	n	EC ₅₀	E _{max}	E _{max}
IMA				
Noradrenaline	15	6.18 ± 0.11	63.16 ± 2.92	105.14 ± 1.93
U 46619	10	5.72 ± 0.17	56.88 ± 4.10	100.40 ± 1.81
Endothelin-1	10	6.75 ± 0.19	80.98 ± 4.85	102.97 ± 2.70
K ⁺	13	5.31 ± 0.15	31.32 ± 3.83	94.53 ± 2.79
GEA (low forced)				
Noradrenaline	8	6.83 ± 0.12*	94.33 ± 1.45*	104.66 ± 1.94
U 46619	8	6.92 ± 0.16*	92.41 ± 2.76*	101.10 ± 1.33
Endothelin-1	7	6.95 ± 0.23	91.93 ± 3.17	102.35 ± 1.72
K ⁺	7	7.23 ± 0.13*	98.01 ± 0.71*	102.81 ± 1.95*
GEA (high forced)				
Noradrenaline	8	6.66 ± 0.16*	81.68 ± 3.53*#	99.05 ± 1.96*
U 46619	6	6.61 ± 0.12*	81.92 ± 3.96*	98.05 ± 1.61
Endothelin-1	7	6.78 ± 0.28	82.84 ± 4.66	98.35 ± 1.36*
K ⁺	7	6.75 ± 0.24*	72.56 ± 4.96*#	95.95 ± 2.16

Data are presented as mean ± s.e.mean EC₅₀ values are expressed as $-\log M$. Low forced refers the contractile force corresponding to 30–40% of the maximal contraction whereas high forced refers the contractile force corresponding to 70–90% of the maximal contraction in GEA rings (see *Methods* for the doses of contractile agents)

*Shows statistically significant difference ($P < 0.05$) between the responses in IMA and GEA.

#Shows statistically significant difference ($P < 0.05$) between the responses in low and high forced GEA.

Table 4 The effects of indomethacin (Indo, 10^{-5} M) plus L^G-nitro-L-arginine (L-NOARG, 10^{-4} M) or glibenclamide (Gli, 10^{-4} M) administrations on maximal relaxations to levcromakalim in precontracted IMA and GEA rings.

		Levcromakalim		
Pretreatment	n	IMA	n	GEA
In contraction of noradrenaline				
Control	5	57.09 ± 6.57	5	91.92 ± 3.69
Indo plus L-NOARG	5	54.27 ± 7.11	5	89.06 ± 4.65
Gli	5	5.36 ± 2.37	4	5.08 ± 0.89
In contraction of U 46619				
Control	4	53.51 ± 7.33	6	92.92 ± 3.32
Indo plus L-NOARG	4	60.14 ± 5.42	6	87.62 ± 4.37
Gli	4	5.64 ± 2.10	4	2.35 ± 1.38
In contraction of endothelin-1				
Control	5	78.28 ± 3.87	5	90.06 ± 4.80
Indo plus L-NOARG	5	77.21 ± 5.43	5	91.14 ± 6.63
Gli	4	4.81 ± 1.18	4	4.60 ± 1.31
In contraction of K ⁺				
Control	6	30.53 ± 6.95	6	95.77 ± 1.88
Indo plus L-NOARG	6	29.02 ± 5.39	6	89.17 ± 4.38
Gli	5	3.45 ± 1.51	5	2.47 ± 0.78

Data are presented as mean ± s.e.mean. For the doses of contractile agents see *Methods*.

GEA exhibited markedly higher responsiveness to these spasmogens than IMA when their contractile responses were evaluated in terms of gram tension (absolute contraction). However, both arteries appeared so similar when contractile responses were compared in terms of K⁺ induced contraction (relative contraction), except for noradrenaline. The spasmogens displayed similar potency order in the arteries

Table 3 Maximal relaxations (E_{max}) and EC₅₀ values of levcromakalim and E_{max} values for sodium nitroprusside (SNP, 10^{-4} M) in precontracted IMA and GEA rings.

in which U 46619 and endothelin-1 were at equal sensitivity, consistent with a relevant study [1].

The incubation of the vessel rings with levcromakalim significantly decreased the sensitivity to noradrenaline, U 46619 and K⁺, and also depressed the maximal contractile responses (both absolute and relative contractions) to noradrenaline and U 46619 in both of the arteries. The effectiveness of levcromakalim at low K⁺ concentration (EC₅₀ value) and its ineffectiveness at high K⁺ (maximal response) are in accordance with previous studies [9, 11]. The ineffectiveness of levcromakalim on endothelin-1 induced contractions is difficult to interpret because contraction to this agent could not be reproduced in the same preparation.

Secondly, we examined the relaxation capacity of contracted rings of the arteries to levcromakalim. The EC₅₀ values of levcromakalim exhibited diversity in IMA but uniformity in GEA implying a different relaxant profile between these arteries. Levcromakalim was a weak vasodilator in contracted IMA, except for endothelin-1 contraction. As GEA produced much greater contractile force than IMA in response to spasmogens, we studied GEA rings at two different contractile forces (low and high). Levcromakalim fully reversed the contractions at low force GEA, comparably with nitrovasodilators and nifedipine [16]. At high force GEA, relaxations to levcromakalim were incomplete. This finding is consistent with the observation that increasing tone by using increasing concentrations of contractile agents decreased the responsiveness to vasodilators including KCOs [22, 26, 27]. The contraction of each vessel to equigram force was considered as a valid technique in the comparison of their relaxation capacities *in vitro* [20, 28], although it is accepted that such conditions may not be directly applicable *in vivo*.

In both of the arteries, the relaxations to levcromakalim were completed approximately in 10 min. A previous study in IMA which compared the time to reach maximal relaxations of glyceryl trinitrate and nifedipine, reported that glyceryl trinitrate took less time than nifedipine to achieve this [29]. Our results showed that levcromakalim is a rapidly acting relaxant agent resembling glyceryl trinitrate. This property may have a clinical value in the treatment of the graft spasm. Levcromakalim induced relaxations are related solely with the activation of K_{ATP} channels because these responses were almost completely abolished by glibenclamide but not modified by indomethacin and L-NOARG.

The most important finding of the present study was almost complete reversal by levcromakalim of the contractions in GEA compared with IMA. The moderate effect of levcromakalim in IMA was not due to differences between the groups of patients. SNP, a directly acting vasodilator, fully reversed the contractions to either spasmogen in IMA and GEA rings. Furthermore, preoperative Ca^{++} channel blocker therapy in IMA donors did not influence the responsiveness to levcromakalim. Reversing contractions in GEA by increasing concentrations of levcromakalim revealed that K^+ channels are more functional in GEA than IMA. This interpretation conflicts with the finding that levcromakalim was very effective against endothelin-1 contraction in IMA as well as in GEA. Recent studies also reported that KCOs can fully reverse endothelin-1 induced contractions in the isolated vessels and also the venoconstriction in human subjects *in vivo* [27, 30]. The almost complete reversal of endothelin-1 contraction by KCOs can be related to the depolarizing activity of endothelin-1 by blocking K_{ATP} channels specifically [31]. Therefore, it can be proposed that the number of K^+ channels or their functionality show diversity between two arterial beds.

In recent studies, it has been reported that K_{ATP} channels play an important role in the maintenance of vascular tone in whole organ or *in vivo* studies [9, 11, 32]. K_{Ca} channels were reported to be activated in the resting state of the arteries from SHR but not WKY suggesting a compensatory mechanism against the high level of resting tone in SHR [12]. In the present study, addition of glibenclamide did not alter the resting tone of both IMA and GEA suggesting that K_{ATP} channels are not activated in the resting state of these arteries. The results of other *in vitro* studies generally support this finding [9, 11]. The effectiveness of glibenclamide solely *in vivo* conditions is possibly related to the presence of endogenous K_{ATP} agonists as suggested previously [32]. Furthermore, in the present study, the ineffectiveness of levcromakalim on resting tensions of IMA and GEA shows the absence of myogenic tone in these arteries.

Taken together, our overall findings revealed that levcromakalim could be beneficial in the treatment of spasm in GEA.

The results of present study showed that GEA is more responsive to contractile agents and more reactive to the K^+ channel opener levcromakalim than IMA. These properties may be useful in the management of the graft spasm. Our findings suggest that KCOs can be included in the list of vasodilator agents used especially in GEA spasm.

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