Actions of Cromakalim in Isolated Human Saphenous Vein

Roberto Soares de Moura and Waldir Jasbik

The present study was performed to evaluate the effect of cromakalim on human vein in vitro. Branches of human saphenous vein (leftovers), obtained from patients undergoing heart revascularization surgery, were cut into rings and suspended in an organ chamber filled with Krebs-Ringer solution for the measurement of isometric contractile force. Concentration-response curves to norepinephrine and serotonin were constructed before and after pretreatment with cromakalim. The concentration-response curves to norepinephrine and serotonin were displaced to the right, and the maximal responses to both agonists were significantly inhibited by cromakalim in a concentration-dependent manner. Following sustained contraction induced by prostaglandin F2α or 20 mM KCl, the cumulative addition of cromakalim to the organ chamber produced a concentration-dependent relaxation. However, in veins precontracted with 60 mM KCl the addition of cromakalim in concentrations of up to 10⁻⁵ M did not induce relaxation. The relaxation induced by cromakalim in veins precontracted with prostaglandin F2α was significantly inhibited by glibenclamide. These results indicate that cromakalim has a dilator effect in human vein that may play a helpful role in the treatment of angina. The venodilator effect of cromakalim in human saphenous vein probably involves activation of adenosine triphosphate-regulated potassium channels. (Hypertension 1992;19[suppl II]:II-121-II-124)

Potassium channel activators are a new class of pharmacological substances with different chemical and pharmacological characteristics. Cromakalim, a benzopyran derivative, is a new vasodilator substance with antihypertensive properties. Independent of the endothelium, this drug appears to relax directly the vascular smooth muscle by activation of the sarcolemma potassium channels, leading to hyperpolarization of the cell membrane. The vascular dilator effect of cromakalim has been shown to occur in arteries and veins of experimental animals. However, because arterioselectivity of cromakalim has been suggested to occur in humans, we decided to study the effect of cromakalim in isolated human saphenous vein.

The increase in K⁺ conductance induced by cromakalim in animal vascular smooth muscle seems to involve activation of adenosine triphosphate (ATP)-regulated potassium channels. Therefore, we also decided to study the effect of glibenclamide, a selective antagonist of this potassium channel, on cromakalim-induced venodilation to determine if an ATP-regulated potassium channel occurs in the human saphenous vein.

Methods

Preparation of Blood Vessels

Segments of branches of the long saphenous vein (leftovers) were obtained from patients undergoing heart revascularization surgery. The vessel segments, once removed from the patient's leg, were immediately placed in a modified Krebs-Ringer bicarbonate solution (millimolar composition 118.3 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25 NaHCO₃, 0.026 EDTA, and 11.1 glucose). The segments were carefully cleaned of perivascular tissues and cut into rings approximately 3 mm long. All experiments were performed on the day of surgery.

Organ Chamber Experiments

Rings of human saphenous vein were suspended in organ chambers filled with 30 ml modified Krebs-Ringer bicarbonate solution and aerated with 95% O₂-5% CO₂ (pH 7.4, 37°C). Each ring was suspended by two stainless steel stirrups passed through its lumen. One stirrup was anchored inside the organ chamber. The other stirrup was connected to a force transducer (FTA 10; Hewlett-Packard Co., Palo Alto, Calif.) for the measurement of isometric force on a Hewlett-Packard 7754A recorder. All rings were progressively
stretched to the optimal point of the length-tension curve as determined by the response to 60 mM KCl and allowed to equilibrate for 60 minutes.

**Protocols**

Concentration–response curves to norepinephrine or serotonin were constructed by increasing by half-log increments the concentration in the organ chamber. After the construction of an initial concentration–response curve, the rings were washed every 15 minutes with Krebs-Ringer bicarbonate solution and were allowed to relax to baseline. One hour after the vessels were completely relaxed from the contraction induced by norepinephrine or serotonin, they were incubated with cromakalim. Thirty minutes later and in the presence of cromakalim, the concentration–response curves to norepinephrine or serotonin were reconstructed. In eight rings from four patients, the concentration–response curves to norepinephrine (n=4) and serotonin (n=4) were constructed before and after pretreatment with cromakalim vehicle (time control).

Saphenous vein rings were contracted with prostaglandin F\textsubscript{2a} and with KCl. Once the contraction was stabilized, concentration–response curves for cromakalim were constructed by increasing the concentration in the organ chamber by half-log increments. The effect of cromakalim was also studied in saphenous vein rings contracted with prostaglandin F\textsubscript{2a} and pretreated with glibenclamide.

**Drugs**

Serotonin, creatinine sulfate, and l-norepinephrine bitartrate were purchased from Sigma Chemical Co., St. Louis, Mo. and prostaglandin F\textsubscript{2a} (Lutalyse) from the Upjohn Co., Kalamazoo, Mich. Glibenclamide was obtained gratis from Hoechst do Brasil Quimica e Farmaceutica S.A., Rio de Janeiro, and cromakalim was a gift from Beechman Pharmaceuticals Research Division, Betchworth, UK. Stock solutions of 10\textsuperscript{-2} M cromakalim were made up in ethanol and stock solutions of 10\textsuperscript{-2} M glibenclamide in 50% ethanol/dimethyl sulfoxide. Norepinephrine, serotonin, and prostaglandin F\textsubscript{2a} were prepared daily in Krebs-Ringer bicarbonate solution and kept on ice until used.

**Data Analysis**

Contraction induced by norepinephrine or serotonin is expressed as a percent of the maximal response to norepinephrine or serotonin obtained during the first concentration–response curve. Relaxation induced by cromakalim is expressed as a reduction of the contraction to prostaglandin F\textsubscript{2a} or KCl. Results are expressed as mean±SEM; n represents the number of patients from whom vessels were taken. The data were statistically evaluated using Student’s t test for paired or unpaired observations. Values were considered to differ significantly when the probability value was less than 0.05.

![Figure 1](http://hyper.ahajournals.org/lookup/doi/10.1161/01.HYP.19.2.111) Line graph shows effects of cromakalim on contractile responses of human saphenous vein elicited by cumulative additions of norepinephrine. \(\bigcirc\), Control responses; ■, responses in presence of 3×10\textsuperscript{-7} M cromakalim; □, responses in presence of 10\textsuperscript{-7} M cromakalim; \(\blacktriangledown\), responses in presence of 10\textsuperscript{-5} M cromakalim. Ordinate scale, % of initial control maximal response (5.23±0.7 g). Each point is mean from six experiments; vertical lines show SEM.

**Results**

**Action of cromakalim on venoconstrictor effects of norepinephrine and serotonin.** Norepinephrine and serotonin induced concentration-dependent increases in tension in isolated rings of human saphenous vein. Cromakalim (3×10\textsuperscript{-7} to 10\textsuperscript{-5} M) significantly inhibited the maximal response of saphenous vein to norepinephrine (n=6) and to serotonin (n=6). The concentration–response curves to norepinephrine and to serotonin were significantly displaced to the right by cromakalim in a concentration-dependent manner (Figures 1 and 2). The concentration–response curves to norepinephrine

![Figure 2](http://hyper.ahajournals.org/lookup/doi/10.1161/01.HYP.19.2.111) Line graph shows effects of cromakalim on contractile responses of human saphenous vein elicited by cumulative additions of serotonin. \(\bigcirc\), Control responses; Δ, responses in presence of 3×10\textsuperscript{-7} M cromakalim; □, responses in presence of 10\textsuperscript{-7} M cromakalim; \(\blacktriangledown\), responses in presence of 10\textsuperscript{-5} M cromakalim. Ordinate scale, % of initial control maximal response (5.9±0.9 g). Each point is mean from six experiments; vertical lines show SEM.
and to serotonin were not significantly changed by cromakalim vehicle (n=4, data not shown).

**Action of cromakalim on venoconstrictor effect of KCl.** During sustained contraction induced by 60 mM KCl, addition of 10^{-7} to 10^{-5} M cromakalim (n=6) did not relax human saphenous vein (Figure 3). However, during sustained contraction induced by 20 mM KCl, cromakalim (n=6) produced a concentration-dependent relaxation (Figure 3).

**Relaxant effect of cromakalim in rings contracted with prostaglandin F_{2a} and antagonism of relaxation by glibenclamide.** In isolated human saphenous vein, 10^{-6} M prostaglandin F_{2a} produced sustained contraction in all rings. During sustained contraction induced by prostaglandin F_{2a} cromakalim (n=6) induced a concentration-dependent relaxation (Figure 4). At 3 \times 10^{-6} M (n=6) and 10^{-5} M (n=6) glibenclamide produced a significant rightward displacement of the concentration-response curve to cromakalim in human saphenous vein contracted with prostaglandin F_{2a} (Figure 4). At the concentrations studied, glibenclamide did not change the basal tone of human saphenous vein.

**Discussion**

Our data on the isolated human saphenous vein corroborate the inhibitory property of cromakalim on the vasoconstrictor effect of norepinephrine, serotonin, and low concentrations of K^+ (20 mM); nevertheless, cromakalim has not shown an antagonist effect for the response induced by 60 mM K^+. The ability of cromakalim to relax rings of human saphenous vein contracted with 20 mM K^+ but not 60 mM K^+ agrees with previous results. Probably, during contraction induced by 20 mM K^+, the membrane potential reaches a value less negative than the threshold for activation of the calcium channels. Addition of cromakalim changes the membrane potential to a value close to the new potassium equilibrium, which is more negative than the threshold for activation of the voltage-operated calcium channels.

Furthermore, our study also points out that cromakalim is an antagonist of the venoconstrictor effect of prostaglandin F_{2a}. The inhibitory effect of cromakalim was greater when the saphenous vein was contracted with prostaglandin F_{2a}, suggesting that the effect of cromakalim varies according to the source of Ca^{2+} used by the constrictor agent.

The cromakalim vasodilator effect mechanism is not yet entirely understood. It has been postulated that the relaxant effect of cromakalim in the rat portal vein is probably due to its hyperpolarization action indirectly closing voltage-sensitive calcium channels. The venodilator effect of cromakalim may also involve an endothelium-derived hyperpolarizing factor-dependent mechanism, although in rat aorta and in rat mesenteric artery, the vasodilator effect of cromakalim is endothelium-independent.

Contrary to results obtained in healthy volunteers that suggest an arterioselective dilator effect of cromakalim, our results show that in vitro, cromakalim is able to produce a venous dilator effect even at 10^{-7} M, a concentration similar to the plasma levels observed in patients treated with a single oral dose of 2 mg cromakalim. When injected intravenously, drugs that have arterioselective actions, such as diazoxide, usually induce a large increase in cardiac output. Since the intravenous injection of cromakalim was not related to an increase in cardiac output in either dogs or rats, it may be that this drug is not arterioselective.

Thomas et al., studying the acute hemodynamic effect of cromakalim in patients with angina pectoris, found a significant decrease in left ventricular end-
diastolic pressure between the cromakalim-treated and placebo-treated groups. These data are in accordance with ours and suggest that cromakalim may have a mild venous dilator effect.

The type of potassium channel involved in the effect of cromakalim is not completely established. ATP-regulated potassium channels may play a role in the hyperpolarization induced by cromakalim. Many reports in the literature have shown that glibenclamide, a potent antagonist of ATP-regulated potassium channels, significantly blocks the vasodilator effect,6-16-20 the hyperpolarization,21 and the stimulation of rubidium-86 ion efflux20 induced by cromakalim.

Our results show that glibenclamide significantly antagonizes the vasodilator effect of cromakalim in human saphenous vein contracted with prostaglandin F2α. Therefore, ATP-regulated potassium channel may occur in the human saphenous vein, and the vasodilator effect of cromakalim probably involves activation of this type of channel.

The results of this study suggest that cromakalim is not an arterioselective vasodilator in humans. Its venodilator effect in human saphenous vein probably involves the opening of an ATP-regulated potassium channel. This venodilator action of cromakalim may play a role on its beneficial effect in patients with angina.

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