

Vasorelaxant effects of the potassium channel opener SR 47063 on the isolated human saphenous vein and rat aorta

D.N. Criddle¹ and
R.S. de Moura²

¹Departamento de Ciências Fisiológicas, Centro de Ciências da Saúde, Universidade Estadual do Ceará, Fortaleza, CE, Brasil

²Departamento de Farmacologia, Instituto de Biologia, Centro Biomédico, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brasil

Abstract

The vasorelaxant effects of SR 47063 (4-(2-cyanimino-1,2-dihydropyrid-1-yl)-2,2-dimethyl-6-nitrochromene), a new K⁺-channel opener structurally related to levcromakalim, were examined in isolated human saphenous vein (HSV) and rat aorta (RA). HSV or RA rings were precontracted with either KCl or noradrenaline and cumulative relaxant concentration-response curves were obtained for SR 47063 (0.1 nM to 1 μM) in the presence or absence of 3 μM glibenclamide. SR 47063 potently relaxed HSV and RA precontracted with 20 mM (but not 60 mM) KCl or 10 μM noradrenaline in a concentration-dependent manner, showing slightly greater activity in the aorta. The potency of the effect of SR 47063 on HSV and RA was 12- and 58-fold greater, respectively, than that reported for the structurally related K⁺-channel opener levcromakalim. The vasorelaxant action of SR 47063 in both blood vessels was strongly inhibited by 3 μM glibenclamide, consistent with a mechanism of action involving ATP-dependent K⁺-channels.

Key words

- SR 47063
- K⁺-channel
- Human saphenous vein
- K_{ATP}
- Rat aorta
- Levcromakalim
- Glibenclamide

Correspondence

D.N. Criddle
Departamento de Ciências Fisiológicas
Centro de Ciências da Saúde
Universidade Estadual do Ceará
Av. Paranjana, 1700
60740-000 Fortaleza, CE
Brasil
Fax: + 55-85-299-2700
E-mail: criddle@uece.br

Research supported by CNPq,
FAPERJ and FUNCAP.

Received July 28, 1999
Accepted May 3, 2000

Introduction

SR 47063 (4-(2-cyanimino-1,2-dihydropyrid-1-yl)-2,2-dimethyl-6-nitrochromene) is a potassium channel opener (K⁺-channel opener) currently in clinical trials that has been shown to exert hypotensive effects on rats *in vivo* (1) and protective effects on ventricular function in cardioplegia (2). Several studies have demonstrated that SR 47063 exhibits a profile of action consistent with that of a K⁺-channel opener in rat vascular (3,4) and human airway smooth muscle (5). Current electrophysiological studies suggest

that this structurally diverse group of drugs may act via the opening of relatively small conductance, glibenclamide-sensitive K⁺-channels in both arterial and venous smooth muscle (6-9).

Existing data suggest that SR 47063 is more potent than structurally related drugs such as cromakalim and bimakalim in guinea pig heart (3,10). However, comparatively little data exist regarding the effects of SR 47063 on isolated blood vessels, with no published reports about the human vasculature. We have previously described the actions of levcromakalim and P1060, a struc-

turally unrelated K⁺-channel opener, on the isolated human saphenous vein (11). Therefore we decided to assess the potency of SR 47063 in vascular tissue using the isolated human saphenous vein and the rat aorta, and to examine whether the relaxant action of this drug was consistent with that of a K⁺-channel opener. Preliminary data from this study have previously been presented to the British Pharmacological Society (12).

Material and Methods

Preparation of blood vessels

Segments of branches of the long saphenous vein (leftovers) were obtained from patients undergoing heart revascularization surgery at the Pedro Ernesto University Hospital, Universidade do Estado do Rio de Janeiro (UERJ). Institutional approval for use of this tissue was obtained. On removal from the patient, the vessel segments were immediately placed in Krebs-Henseleit solution of the following composition: 118.3 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, 0.026 mM EDTA, and 11.1 mM glucose, carefully cleaned of perivascular tissue and cut into approximately 0.3-cm long rings. All experiments were performed on the day of surgery.

Male Wistar rats (250-350 g) were killed by stunning and cervical dislocation. The thoracic aorta was removed by careful dissection, placed in Krebs-Henseleit solution and cut into approximately 0.3-cm long rings.

Organ chamber experiments

Rings of saphenous vein and aorta were suspended in organ chambers filled with 30 ml of Krebs-Henseleit solution bubbled with 95% O₂ and 5% CO₂ at 37°C. Each ring was suspended by two stainless steel stirrups passed through its lumen. One stirrup was

anchored inside the organ chamber and the other was connected to a force transducer (FTA 10; Hewlett-Packard Co., Palo Alto, CA, USA) for the measurement of isometric force with 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 (approximately 4 applications of KCl) and allowed to equilibrate for 60 min.

Experimental protocols

Following equilibration, blood vessel rings were exposed to either 3 μM glibenclamide or appropriate vehicle for 15 min before spasmogen addition. Either KCl (20 mM or 60 mM) or noradrenaline (10 μM) was used to precontract the tissues. Following the development of a stable plateau contraction in response to the spasmogen, SR 47063 (0.1 nM to 1 μM) was applied cumulatively to the organ bath in log increments and concentration-response curves were constructed.

Drugs

SR 47063 (Sanofi Recherche, Montpellier, France) and noradrenaline (Sigma Chemical Co., St. Louis, MO, USA) were prepared as stock solutions (3 and 10 mM, respectively) in absolute ethanol and diluted in distilled water on the day of the experiment. Glibenclamide (Sigma) was prepared as a stock solution (10 mM) in dimethyl sulfoxide and diluted in distilled water.

Statistical analysis

Data are reported as the mean ± SEM of N observations. IC₅₀ values were calculated for each experimental concentration-effect curve and expressed as the mean of N experiments together with the appropriate SEM. Data were analyzed statistically by the unpaired Student *t*-test.

Results

Relaxant effects of SR 47063 on KCl- and noradrenaline-induced contraction of the human saphenous vein

KCl (20 mM) induced contraction of the human saphenous vein with a plateau response of 1.34 ± 0.10 g (N = 20, 9 patients). Cumulative application of SR 47063 (0.1 nM to 1 μ M) elicited a concentration-dependent relaxation of KCl-induced tone, an effect that was maximal at 0.1 μ M (Figure 1A). However, in the presence of 3 μ M glibenclamide the relaxant concentration-response curve was shifted to the right by approximately 50-fold (Table 1; Figure 1A). In saphenous vein rings precontracted with 60 mM KCl, SR 47063 at concentrations up to 1 μ M did not induce relaxation (N = 5). Noradrenaline (10 μ M) elicited a greater contraction of the saphenous vein than 20 mM KCl, with a stable plateau response of 2.34 ± 0.32 g (N = 14, 6 patients). SR 47063 induced a concentration-dependent inhibition of this developed tone with a reduction of 87% at a concentration of 1 μ M (Figure 1B). SR 47063 was slightly less potent against noradrenaline-induced compared with KCl-induced contraction (Table 1). The relaxation of noradrenaline-precontracted veins was also inhibited by glibenclamide; at the highest concentration of SR 47063 tested (1 μ M) approximately 75% of the contraction

induced by noradrenaline remained in the presence of glibenclamide and thus precluded calculation of the IC₅₀ value.

Relaxant effects of SR 47063 on KCl- and noradrenaline-induced contraction of the rat aorta

Application of 20 mM KCl to the aorta induced a biphasic contraction with a sustained plateau response of 0.81 ± 0.12 g (N = 16). SR 47063 relaxed the precontracted aorta in concentration-dependent manner,

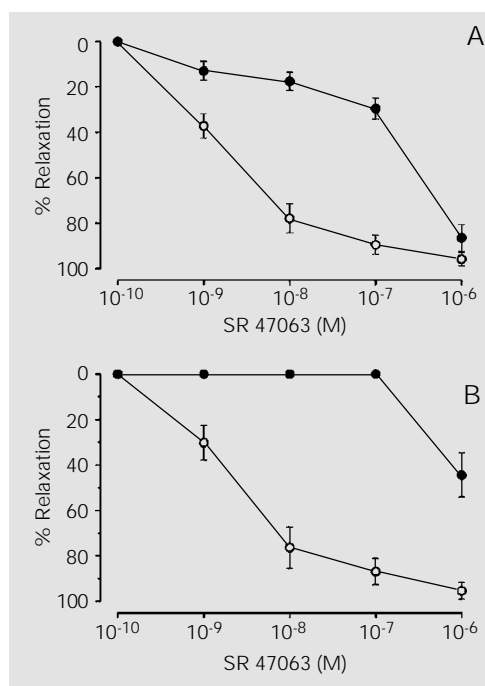


Figure 1 - Relaxant effects of SR 47063 on human saphenous vein precontracted with 20 mM KCl (A) and 10 μ M noradrenaline (B) in the presence (filled circles) and absence (open circles) of 3 μ M glibenclamide. Data are reported as means \pm SEM (N = 6-11).

Table 1 - IC₅₀ values for SR 47063-induced relaxation of human saphenous vein and rat aorta.

Values are reported as means, with 95% confidence limits and number of observations in parentheses. *P < 0.01 for control vs glibenclamide (glib) (Student t-test). DR^A = + glib IC₅₀/control IC₅₀.

| Spasmogen | Human saphenous vein | | | Rat thoracic aorta | | |
|-------------------------------|------------------------------|-------------------------------------|-----------------|-----------------------------|-------------------------------------|-----------------|
| | Control IC ₅₀ | + glib IC ₅₀ | DR ^A | Control IC ₅₀ | + glib IC ₅₀ | DR ^A |
| KCl (20 mM) | 8.1 nM (7.48-8.72) (11) | 0.412 μ M (0.272-0.552) (9)* | 50.9 | 3.86 nM (2.07-5.55) (8) | 0.219 μ M (0.151-0.287) (8)* | 56.7 |
| Noradrenaline (10 μ M) | 30.2 nM (20.64-34.00) (6) | - | - | 8.82 nM (7.50-10.14) (8) | - | - |

with a complete reduction of induced tone at a concentration of 1 μM (Figure 2A). This action was inhibited significantly in the presence of glibenclamide (3 μM) with an approximate 57-fold shift of the IC_{50} value (Figure 2A; Table 1). Noradrenaline (10 μM) induced a plateau contraction of 0.91 ± 0.13 g (N = 14) which was fully relaxed by cumulative application of SR 47063 (Figure 2B). In the presence of 3 μM glibenclamide this effect was greatly inhibited, with approximately 56% of the contraction remaining at a concentration of 1 μM SR 47063.

Discussion

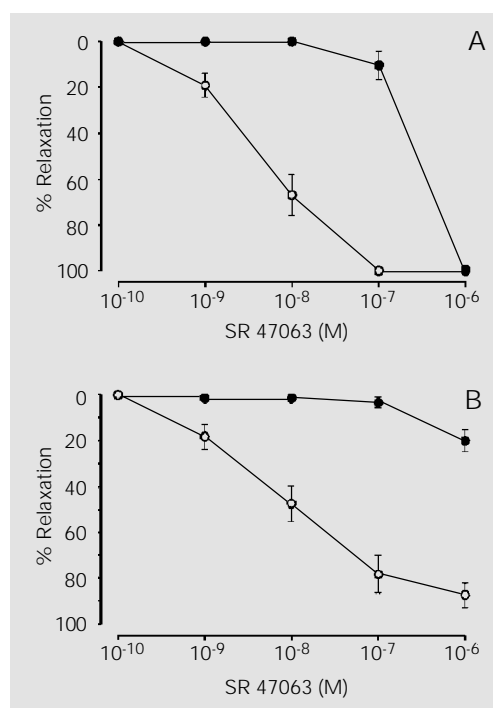
The present study is the first description of the effects of SR 47063 on isolated human vasculature, extending previous pre-clinical studies performed on animal tissues. We found that SR 47063 has a potent relaxant action on 20 mM KCl- and noradrenaline-precontracted human saphenous vein. However, this relaxant effect was not observed in the presence of a high concentration of potassium (60 mM). Thus, SR 47063 exhibits a

profile of pharmacological activity characteristic of K^+ -channel openers in a variety of vascular tissues including rabbit aorta, dog coronary and middle cerebral artery (13-15), and the current results are in agreement with previous studies with this drug on rat vascular (3,4), human bronchial (5) and frog skeletal muscle preparations (16,17).

The relaxant effects of SR 47063 on both saphenous vein and aorta were very sensitive to inhibition by glibenclamide, an inhibitor of ATP-dependent K^+ -channels (K_{ATP}) in vascular smooth muscle (7), consistent with an action of SR 47063 on this type of K^+ -channel. A recent electrophysiological investigation has shown that submicromolar concentrations of SR 47063 potentially activate glibenclamide-sensitive K^+ -currents in guinea pig cardiac muscle cells (18), whilst higher concentrations also activate ATP-sensitive channels in skeletal muscle (16). However, we are unaware of any published data regarding the single-cell electrophysiology of SR 47063 in vascular smooth muscle, although the bulk of evidence appears to suggest that SR 47063, in common with its structural analogue levcromakalim, exerts its vascular relaxant effects via the opening of K_{ATP} .

In the guinea pig heart SR 47063 is 25-30-fold more potent in decreasing coronary perfusion pressure than the structurally related benzopyran cromakalim (3). We have previously reported that levcromakalim relaxes 20 mM KCl-induced contractions of the human saphenous vein with an IC_{50} value of 97 nM (10) and thus our present results indicate that SR 47063 is approximately 12-fold more active than levcromakalim in this tissue. This is in agreement with previous data indicating that SR 47063 is approximately 14-fold more potent than levcromakalim in inhibiting the spontaneous contractile activity of another blood vessel, the rat portal vein, with respective IC_{50} values of 5.6 nM (3) and 80 nM (19). Interestingly, comparison of the present study with a re-

Figure 2 - Relaxant effects of SR 47063 on rat aorta precontracted with 20 mM KCl (A) and 10 μM noradrenaline (B) in the presence (filled circles) and absence (open circles) of 3 μM glibenclamide. Data are reported as means \pm SEM (N = 8).



port on the rat aorta (20) also indicates that SR 47063 is approximately 58-fold more potent against 20 mM KCl-induced contractions than its structural analogue levcromakalim. Indeed this potency difference extends to *in vivo* decreases in mean arterial blood pressure, with SR 47063 being about 6-fold and 15-fold more potent than cromakalim in spontaneously hypertensive and normotensive anesthetized rats, respectively (1). At present no published comparative clinical data on humans are available to confirm or refute this putative potency advantage of SR 47063 over levcromakalim.

In conclusion, we have shown that SR 47063 possesses a potent vasorelaxant activity on both human and animal blood vessels,

exhibiting effects consistent with the opening of K_{ATP} channels. This compound appears to possess a degree of selectivity for vascular smooth muscle since higher concentrations of SR 47063 are required to elicit comparable relaxations of human bronchial (5) and bladder smooth muscle (21), and guinea pig cardiac muscle (10). Thus, our present results support the possibility of a future therapeutic use for SR 47063 in the treatment of hypertension.

Acknowledgments

We wish to thank Sanofi Recherche for the generous gift of SR 47063 and Dr. W. Jazbik for providing the saphenous vein.

References

- Lacour C, Canals F, Galindo G & Gaultier P (1991). Hypotensive effects of a novel potassium channel opener, SR 47063, in rats. *Fundamental and Clinical Pharmacology*, 5: 829 (Abstract).
- Dorman BH, Hebbar L, Zellner JL, New RB, Houck WV, Acsell J, Nettles C, Hendrick JW, Sampson AP, Mukherjee R & Spinale FG (1998). ATP-sensitive potassium channel activation before cardioplegia. Effects on ventricular and myocyte function. *Circulation*, 98: II-176-II-183.
- Guiraudou P, Rizzoli G, Gavraud R, Nisato D & Gaultier P (1991). New potassium channel opener, SR 47063, effects on rat portal vein and on isolated perfused guinea pig heart. *Fundamental and Clinical Pharmacology*, 5: 829 (Abstract).
- Cristophe B, Chatelain P & Manning AS (1992). SR 47063, a new K^+ channel opener: effect on ^{86}Rb efflux from rat aorta. *British Journal of Pharmacology*, 106: 304P (Abstract).
- Martin CAE, Naline E & Advenier C (1991). Comparison of the effects of SR 47063 and cromakalim on airway smooth muscle. *Fundamental and Clinical Pharmacology*, 5: 836 (Abstract).
- Kajioka S, Oike M & Kitamura K (1990). Nicorandil opens a calcium-dependent potassium channel in smooth muscle cells of the rat portal vein. *Journal of Pharmacology and Experimental Therapeutics*, 254: 905-913.
- Noack Th, Deitmer P, Edwards G & Weston AH (1992). Characterization of potassium currents modulated by BRL 38227 in rat portal vein. *British Journal of Pharmacology*, 106: 717-726.
- Beech DJ, Zhang H, Nakao K & Bolton TB (1993). Single channel and whole-cell currents evoked by levcromakalim in smooth muscle cells from rabbit portal vein. *British Journal of Pharmacology*, 110: 583-590.
- Criddle DN, Greenwood IA & Weston AH (1994). Levcromakalim-induced modulation of membrane potassium currents, intracellular Ca^{2+} and mechanical activity in rat mesenteric artery. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 349: 422-430.
- Gaultier P & Bertrand JP (1991). Study of a new potassium channel opener, SR 47063, on action potential and contraction of guinea pig papillary muscle. *Fundamental and Clinical Pharmacology*, 5: 828 (Abstract).
- Criddle DN, Jazbik W & Soares de Moura R (1995). Differential vasorelaxant effects of levcromakalim and P1060 in the isolated KCl- and RbCl-precontracted human saphenous vein: possible involvement of intracellular calcium stores. *European Journal of Pharmacology*, 286: 123-130.
- Criddle DN, Soares De Moura R, Domingos LR & De Lima Maia E (1995). Vasorelaxant effects of SR 47063 in human saphenous vein and rat aorta. *British Journal of Pharmacology*, 117: 263P (Abstract).
- Cook NS, Weir SW & Danzeisen MC (1988). Anti-vasoconstrictor effects of the K^+ channel opener cromakalim on the rabbit aorta - comparison with the Ca^{2+} -antagonist isradipine. *British Journal of Pharmacology*, 95: 741-752.
- Masuzawa K, Asano M, Matsuda T, Imaizumi Y & Watanabe M (1990). Comparison of effects of cromakalim and pinacidil on mechanical activity and $^{86}Rb^+$ efflux in dog coronary arteries. *Journal of Pharmacology and Experimental Therapeutics*, 253: 586-593.
- Masuzawa K, Asano M, Matsuda T, Imaizumi Y & Watanabe M (1990). Possible involvement of ATP-sensitive K^+ channels in the relaxant response of dog middle cerebral artery to cromakalim. *Journal of Pharmacology and Experimental Therapeutics*, 255: 818-825.
- Forestier C, Depresle Y & Vivaudou M (1993). Intracellular protons control the affinity of skeletal muscle ATP-sensitive K^+ channels for potassium-channel-openers. *FEBS Letters*, 325: 276-280.
- Forestier C, Pierrard J & Vivaudou M (1996). Mechanism of action of K channel openers on skeletal muscle K_{ATP} chan-

- nels. Interactions with nucleotides and protons. *Journal of General Physiology*, 107: 489-502.
18. Tourneur Y, Marion A & Gaultier P (1994). SR 47063, a potent channel opener, activates K_{ATP} and a time-dependent current likely due to potassium accumulation. *Journal of Membrane Biology*, 142: 337-347.
19. Edwards G, Henshaw M, Miller M & Weston AH (1991). Comparison of the effects of several potassium-channel openers on rat bladder and rat portal vein in vitro. *British Journal of Pharmacology*, 102: 679-686.
20. Greenwood IA & Weston AH (1993). Effects of Rb on responses to potassium channel openers in rat isolated aorta. *British Journal of Pharmacology*, 109: 925-932.
21. Gonçalves de Mello R, Soares de Moura R & Damião R (1994). Actions of SR 47063 in human isolated detrusor. *Canadian Journal of Physiology and Pharmacology*, 72 (Suppl 1): P21-1.25 (Abstract).