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Cardiovascular effects of SCA40, a novel potassium channel opener, in rats

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1 Experiments have been performed to investigate the cardiovascular actions in the rat of SCA40, a novel potassium channel opener which is a potent relaxant of guinea-pig airway smooth muscle *in vivo* and *in vitro*.

2 SCA40 (0.01–30 μM) caused a complete and concentration-dependent relaxation of rat isolated thoracic aorta contracted with 20 mM KCl but failed to inhibit completely the spasmogenic effects of 80 mM KCl.

3 The ATP-sensitive K^+-channel blocker, glibenclamide (3 μM), failed to antagonize the relaxant action of SCA40 on 20 mM KCl-contracted rat isolated thoracic aorta.

4 SCA40 (0.001–100 μM) had dual effects on rat isolated atria. At low concentrations, SCA40 produced a concentration-dependent decrease in the rate and force of contractions. At higher concentrations (greater than 1 μM) SCA40 induced concentration-dependent increases of atrial rate and force.

5 *In vivo*, in normotensive Wistar rats, SCA40 elicited a dose-dependent (1–100 μg kg^{-1}) decrease in mean arterial pressure which was accompanied by a moderate dose-dependent increase in heart rate. SCA40 (100 μg kg^{-1}) had a slightly greater hypotensive effect than cromakalim (100 μg kg^{-1}) but the duration of the hypotension was longer with cromakalim than with SCA40.

6 The hypotensive effect of SCA40 was not reduced by propranolol, atropine, N^G-nitro-L-arginine methyl ester (L-NAME) or glibenclamide.

7 It is concluded that the mechanism by which SCA40 relaxes vascular smooth muscle *in vitro* and *in vivo* involves activation of K^+-channels distinct from glibenclamide-sensitive ATP-sensitive K^+-channels.

Keywords: Rat thoracic aorta; smooth muscle relaxation; SCA40; potassium channels; hypotensive activity

Introduction

SCA40 (6-bromo-8-methylaminoimidazo[1,2-a]pyrazine-2-carbonitrile) is a newly synthesized imidazopyrazine derivative possessing potent smooth muscle relaxant activity *in vitro* in guinea-pig isolated trachealis and potent anti-bronchospastic activity *in vivo*. Its weak cyclic AMP phosphodiesterase inhibitory activity only partially explains these relaxant properties (Bonnet *et al.*, 1992). Since SCA40 failed to inhibit completely the spasmogenic effects of 80 mM KCl in guinea-pig isolated trachealis, potassium channel opening properties have been proposed for it (Laurent *et al.*, 1993). SCA40 relaxant activity in guinea-pig isolated trachealis was not blocked by the ATP-sensitive K^+-channel blocker glibenclamide but was antagonized by charybdotoxin (ChTX), a purified peptide toxin present in *Leiurus quinquestriatus* venom, which has been found to block large-conductance Ca^{2+}-dependent K^+-channels in a variety of cells (Castle *et al.*, 1989). As opposed to potassium channel openers such as cromakalim, the relaxant activity of SCA40 does not involve ATP-sensitive K^+-channels, rather it appears to activate ChTX-sensitive K^+-channels such as large-conductance Ca^{2+}-activated K^+-channels.

ATP-sensitive K^+-channel openers, such as cromakalim, pinacidil and nicorandil have been shown to possess vascular smooth muscle relaxant and antihypertensive properties (Richer *et al.*, 1990). It has been proposed that potassium channel openers induce hyperpolarization of the smooth muscle cell membrane, which in turn reduces entry through voltage-sensitive channels of cytosolic calcium leading to vasorelaxation (Quast & Cook, 1989).

Recently evidence has been obtained for the involvement of Ca^{2+}-activated K^+-channels in the regulation of arterial tone. Small and large-conductance Ca^{2+}-activated K^+-channels have been identified in vascular smooth muscle cells from different species: bovine (Vazquez *et al.*, 1989); rabbit (Inoue *et al.*, 1986); guinea-pig (Benham *et al.*, 1986); and, rat (Van Renterghem & Lazdunski, 1992). Brayden & Nelson (1992) reported that TEA and ChTX were able to depolarize and constrict pressurized rabbit cerebral arteries. They concluded that the activation of Ca^{2+}-activated K^+-channels could lead to vasodilatation. Rusch *et al.* (1992) showed that a Ca^{2+}-activated K^+-current was enhanced in arterial membranes from genetic and experimental models of hypertensive rats. Asano *et al.* (1993) showed that ChTX-sensitive Ca^{2+}-activated K^+-channels were highly activated in arteries from spontaneously hypertensive rats (SHR) as compared to normotensive rats. All these findings suggest that activation of ChTX-sensitive Ca^{2+}-activated K^+-channels may be an important mechanism that regulates the myogenic tone, particularly in SHR arteries.

ATP-sensitive K^+-channel openers reduce the duration of the myocardial action potential in ventricular and atrial cells leading to negative inotropic activity (Shigenobu *et al.*, 1991). *In vivo*, ATP-sensitive K^+-channel openers lowered blood pressure and caused reflex tachycardia (Richer *et al.*, 1990). *In vitro*, ATP-sensitive K^+-channel openers have been shown to suppress spontaneous and oscillatory activities in isolated cardiac Purkinje fibres (Steinberg *et al.*, 1988) and to produce a negative chronotropic response in a dog heart preparation (Murakami *et al.*, 1992). On the other hand, an arrhythmogenic effect of ATP-sensitive K^+-channel openers has been postulated (Steinberg *et al.*, 1988).

The aim of the present study was to examine the effects of SCA40 *in vitro* in rat thoracic aorta and atria and to examine

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the cardiovascular properties of SCA40 in normotensive rats *in vivo*.

Methods

Effects of SCA40 against tone induced by KCl in rat thoracic aorta

Male Wistar rats (Iffa Credo, Lyon, France) weighing 300–350 g, were killed by a blow to the head and the thoracic aorta rapidly removed. Each aorta was cut into 4 rings, each 3–4 mm in length. Two stainless steel wire hooks were passed through the lumen of each ring. One wire was attached to the base of a 40 ml tissue bath and the other one to an isometric myograph transducer connected to a Physiograph Narco Bio-system. Tissues were suspended in a Chenoweth Koelle buffer. At the outset of each experiment, tissues were subjected to an applied tension of 0.5 g and allowed to equilibrate for 30 min during which time they were washed every 5 min. KCl (20 mM or 80 mM) induced contractions which reached stable maxima within 5 min. Cumulative log concentration-response curves to SCA40 were determined for aortic rings contracted with KCl (20 or 80 mM) taking the intensities of the initial contractions as 100%. Then, cumulative log concentration-response curves were determined for the relaxant action of SCA40 in aortic rings contracted with KCl (20 mM) in the absence (control) or in the presence of glibenclamide (3 μM). Relaxant responses were expressed as the percentage reduction in KCl-induced contraction. Relaxant potency was expressed as the negative log EC_{50}, where EC_{50} is the concentration producing 50% inhibition of the contraction. The EC_{50} values were calculated by linear regression analysis applied to the linear portion of each dose-response curve.

Rat isolated atria studies

Male Wistar rats were killed by a blow on the head and the heart was rapidly removed and placed in a beaker containing oxygenated Chenoweth-Koelle solution. Right and left atria were then removed and mounted in 40 ml organ baths filled with Chenoweth-Koelle solution. Changes in tension were measured isometrically with a myograph transducer connected to a Physiograph Narco Bio-system. The right atria were allowed to beat spontaneously, while left atria were paced at a frequency of 1.6 Hz (pulse duration of 5 ms and a voltage twice the threshold). After a 45 min equilibration period, the basal tension was adjusted to 1 g, right atria were used to measure the effects of drugs on rate, and left atria to measure the effects on tension. Cumulative concentration-response curves to SCA40 were determined. SCA40 effects were measured as differences in developed tension or rate from basal activity. Results are expressed as percentage variation from basal values.

Blood pressure studies in rats

Normotensive male Wistar rats weighing 300–350 g, fed with UAR A04 diet and fasted 18 h prior to the experiment, were used. Rats were anaesthetized with ethylurethane (1.2 g kg^{-1}, i.p.) and were maintained at a body temperature of 37°C. The left common artery and the tail vein were cannulated for the measurement of blood pressurre and the intravenous administration of drugs respectively. A Narco Bio-system pressure transducer was used to record the mean arterial pressure (MAP) and heart rate (HR) was derived from the arterial pulse signal. Following a stabilization period of 30 min, MAP and HR were recorded. SCA40 was injected intravenously in increasing doses (1, 3, 10, 30, 100 μg kg^{-1}). Blood pressure and heart rate were allowed to return to baseline between each SCA40 dose.

In a second set of experiments, the time-course of the blood pressure responses to SCA40 and cromakalim were evaluated after i.v. administration (doses of 100 μg kg^{-1} for each drug).

In another series of experiments, the effects of SCA40 (10 μg kg^{-1}) on rat MAP were evaluated before and after i.v. administration of specific drugs: propranolol (1 mg kg^{-1}); atropine (1 mg kg^{-1}); N^G-nitro-L-arginine methyl ester (L-NAME, 20 mg kg^{-1}); and, glibenclamide (20 mg kg^{-1}). These drugs were injected 15 min prior to the second administration of SCA40. β-Adrenoceptor and muscarinic cholinoceptor receptor blockade was assessed by i.v. administration of isoprenaline (1 μg kg^{-1}) and acetylcholine (2 μg kg^{-1}), respectively. ATP-sensitive potassium channel blockade was assessed by i.v. administration of cromakalim (75 μg kg^{-1}).

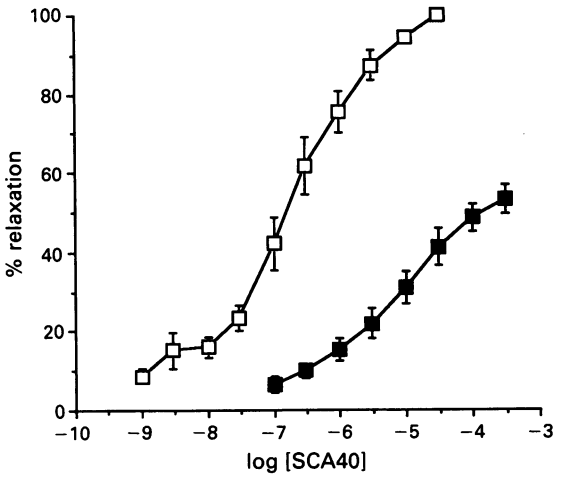

Figure 1 Rat isolated thoracic aorta: relaxant activity of SCA40 against established contraction to KCl 20 mM (□) and KCl 80 mM (■). Abscissa scale: – log molar concentration of SCA40. Ordinate scale: percentage reduction in responses to KCl. Each point is the mean ± s.e.mean derived from at least 6 experiments.

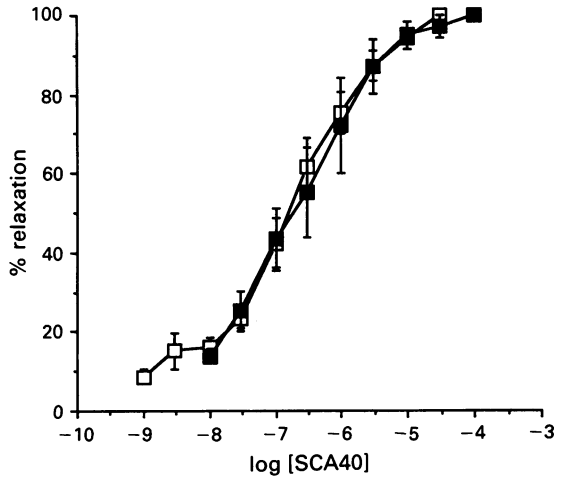

Figure 2 Rat isolated thoracic aorta: relaxant activity of SCA40 against established contraction to KCl 20 mM in absence (□) or in presence of glibenclamide 3 μM (■). Abscissa scale: – log molar concentration of SCA40. Ordinate scale: percentage reduction in responses to KCl 20 mM. Each point is the mean ± s.e.mean derived from at least 6 experiments.

Statistical evaluation of results

Statistical evaluation of the results was assessed by use of a two-tailed, unpaired *t* test. The null hypothesis was rejected when $P<0.05$.

Drugs and solutions

The substances used were obtained from the following sources: SCA40 was synthesized as already described (Bonnet *et al.*, 1992). (±)-Isoprenaline, propranolol, N^G-nitro-L-arginine methyl ester (L-NAME), acetylcholine, atropine and glibenclamide: (Sigma Chemicals (U.S.A.); cromakalim was a gift from Sanofi Laboratories (France); KCl, ethyl-carbamate (urethane) were from Prolabo (France).

For *in vivo* experiments, isoprenaline, L-NAME, propranolol, acetylcholine, atropine were dissolved in isotonic saline. SCA40, cromakalim and glibenclamide were dissolved in ethanol. Further dilutions of SCA40 and cromakalim were made in isotonic saline.

For *in vitro* experiments, 20 mM stock solution of SCA40 and glibenclamide were made up in ethanol. Further dilutions were made up in distilled water.

The Chenoweth-Koelle solution used in the tissue bath experiments had the following composition (mM): NaCl 120, KCl 5.6, $CaCl_2$ 2.4, $MgCl_2$ 2.2, $NaHCO_3$ 15 and glucose 10. This solution was maintained at 37°C and gassed continuously with a mixture of 95% O_2, 5% CO_2.

Results

Rat thoracic aorta studies

Cumulative concentration-response curves to SCA40 on rat isolated thoracic aorta precontracted with 20 and 80 mM KCl are shown in Figure 1. SCA40 produced concentration-dependent inhibition of the response to 20 mM KCl, full relaxation of the KCl contraction being produced by 30 μM SCA40. When aortic preparations were contracted with 80 mM KCl, the maximum relaxation produced by SCA40 corresponded to approximately 50% of the maximum relaxation that could be achieved against 20 mM KCl-induced contraction. Moreover, the relaxation concentration-response curve to SCA40 against 80 mM KCl-induced contraction was shifted to the right approximately 1 000 fold compared with SCA40 relaxant activity against 20 mM KCl ($-\log EC_{50} = 6.86 \pm 0.10$ and 3.77 ± 0.09 respectively). In the presence of glibenclamide 3 μM (Figure 2), the relaxation concentration-response curve to SCA40 against 20 mM KCl-induced contraction was not modified with respect to the maximum response or location ($-\log EC_{50} = 6.86 \pm 0.10$ and 6.68 ± 0.12, in absence and presence of 3 μM glibenclamide respectively).

In vitro *chronotropic and inotropic activity of SCA40*

Cumulative concentration-responses curves on tension and rate of beating of rat isolated atria are shown in Figure 3. SCA40 produced dual effects in rat isolated atria. At low concentrations (0.001–1 μM), SCA40 exhibited a dose-dependent decrease in the rate of contraction (up to 28%). Similarly, SCA40, at low concentrations, produced a dose-dependent decrease in the force of contraction (up to 34%). At higher doses (>1 μM) SCA40 induced a dose-dependent increase of the beating frequency and contractile force such that 100 μM SCA40 exhibited positive chronotropic and inotropic activities.

Blood pressure studies in rats

Following the stabilization period of 30 min, the baseline mean arterial pressure (MAP) was between 95 and 120 mmHg and the baseline heart rate (HR) between 300 and 400 beats min^{-1}. The effects of SCA40 (1–100 μg kg^{-1}, i.v.) in anaesthetized normotensive rats are shown in Figure 4. SCA40 elicited a potent dose-dependent (1–100 μg kg^{-1}) decrease in MAP. The reduction in MAP was accompanied by a moderate dose-dependent increase in HR (less than 30 beats min^{-1} at 100 μg kg^{-1}). At lower doses (1 to 10 μg kg^{-1}), the reduction of MAP was maximal 10 s after administration of SCA40 and then returned to basal value within 1 min. At higher doses (30 and 100 μg kg^{-1}) MAP returned slowly, over 20 to 30 min, to the baseline level.

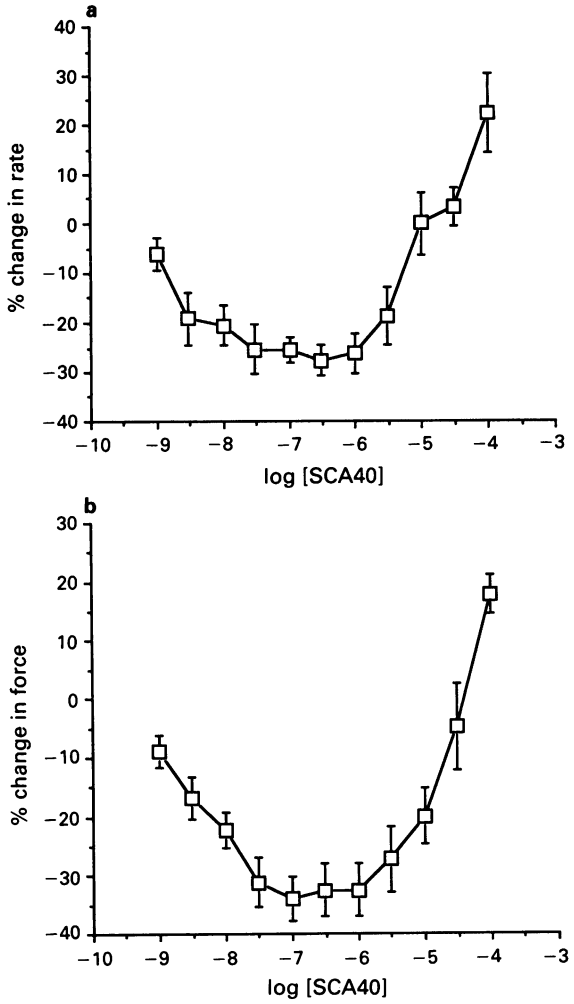

Figure 3 Concentration-response curves for the effects of SCA40 on: (a) rate and (b) contractile force of rat isolated atria. Abscissae scale: – log molar concentration of SCA40. Ordinate scales: change in rate and force expressed as a percentage of baseline values. Each point is the mean ± s.e.mean derived from 4 to 6 experiments.

Figure 5 shows the time course of the effects of i.v. administration of 100 μg kg^{-1} SCA40 and cromakalim on MAP. The fall in MAP produced by SCA40 was slightly greater than that induced by cromakalim but the hypotension lasted longer with cromakalim than with SCA40.

The effects of specific drugs on the decrease in blood pressure produced by SCA40 (10 μg kg^{-1}) are presented in Table 1. Intravenous injection of the β-adrenoceptor antagonist propranolol (1 mg kg^{-1}) had no significant effect on the SCA40 pressure response but caused a significant reduction of the isoprenaline-induced (1 μg kg^{-1}) blood pressure decrease. Atropine (1 mg kg^{-1}) also caused no attenuation of the SCA40 pressure response although muscarinic cholinoceptors were blocked as assessed by the significant decrease of the acetylcholine-evoked (2 μg kg^{-1}) pressor response. L-NAME (10 mg kg^{-1}) elicited an increase in MAP from 102.8 ± 10.4 to 153.5 ± 11.2 mmHg. After L-NAME the decreases in MAP induced by both acetylcholine (2 μg kg^{-1}) or SCA40 were enhanced. The fall in blood pressure due to SCA40 was not affected by glibenclamide (20 mg kg^{-1}) while the cromakalim-induced (75 mg kg^{-1}) decrease in blood pressure was significantly reduced by glibenclamide.

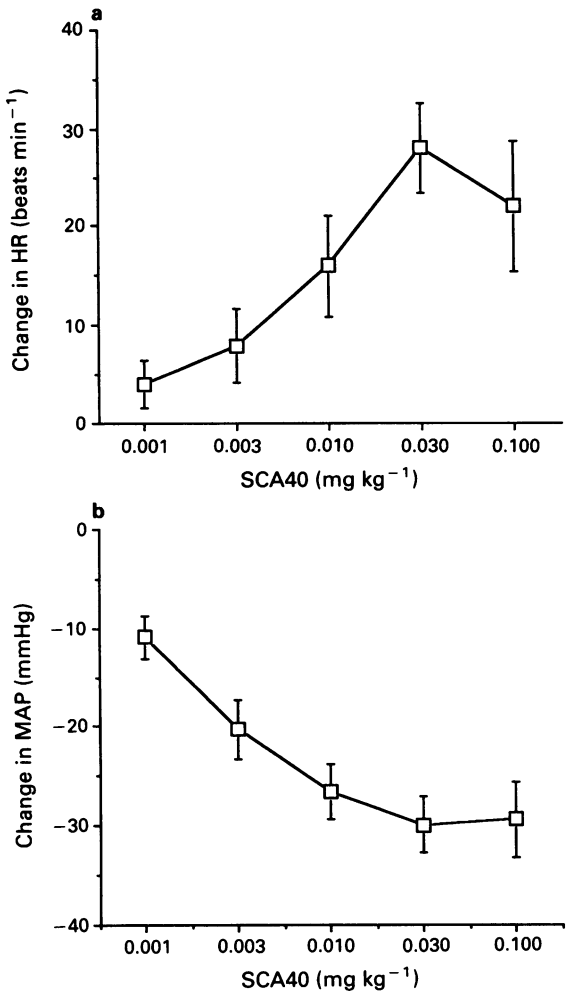

Figure 4 Dose-response curves for the effects of SCA40 on: (a) heart rate (HR) and (b) mean arterial pressure (MAP) in normotensive anaesthetized Wistar rats. Abscissa scale: i.v. doses of SCA40 (mg kg^{-1}, log scale). Ordinate scales: (a) change from baseline in HR (beats min^{-1}); (b) change from baseline in MAP (mmHg). Each point is the mean ± s.e.mean derived from 4 to 6 experiments.

Discussion

SCA40 is a newly synthesized imidazo[1,2-a]pyrazine derivative which exhibits potent smooth muscle relaxant properties *in vitro*, potent anti-bronchospasmic activity *in vivo* and moderate cyclic AMP phosphodiesterase inhibitory activity (Bonnet *et al.*, 1992). However, increased cyclic AMP formation due to the inhibition of cyclic AMP phosphodiesterase cannot totally explain the potent SCA40 smooth muscle relaxant activity (Bonnet *et al.*, 1992). In guinea-pig isolated trachea, SCA40 was able to inhibit completely the contractions induced by a low concentration of KCl (20 mM); in contrast, contractions induced by 80 mM KCl were only partially inhibited by SCA40 (Laurent *et al.*, 1993). Such a pharmacological profile has been described for K^+-channel openers (Hamilton *et al.*, 1986; Robertson & Steinberg, 1990). With high K^+ concentrations the potassium equilibrium potential is such that the hyperpolarization induced by K^+-channel openers is too weak to close voltage-operated Ca^{2+}-channels. The relaxant activity of SCA40 in guinea-pig

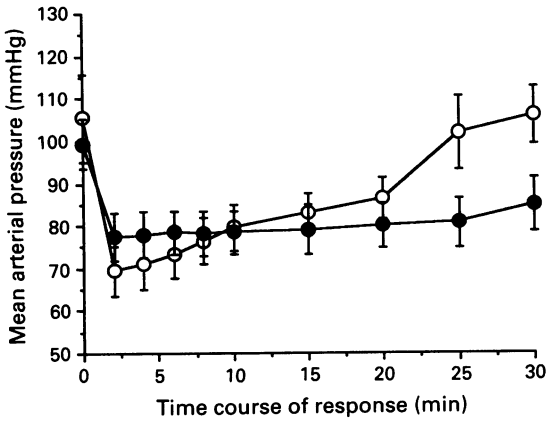

Figure 5 Time course of the effects of SCA40 100 μg kg^{-1} (○) and cromakalim 100 μg kg^{-1} (●) on mean arterial blood pressure (MAP) measured in anaesthetized normotensive Wistar rats after i.v. administration. Each point is the mean ± s.e.mean derived from at least 4 experiments.

Table 1 Effects of various antagonists (propranolol, 10 mg kg^{-1}; atropine, 1 mg kg^{-1}; N^G-nitro-L-arginine methyl ester (L-NAME) 10 mg kg^{-1}; glibenclamide, 20 mg kg^{-1}) on mean arterial pressor (MAP) responses evoked by i.v. administration of SCA40 (10 μg kg^{-1}), isoprenaline (1 μg kg^{-1}), acetylcholine (2 μg kg^{-1}) and cromakalim (75 μg kg^{-1}) in anaesthetized, normotensive rats

Pretreatment	*Agonist*	*Initial MAP* (mmHg)	*MAP change* (mmHg)	*% change*
Group 1				
Vehicle	SCA40	112.9 ± 7.0	− 30.1 ± 2.9	− 26.8 ± 2.6
Group 2				
Vehicle	Isoprenaline	92.8 ± 5.5	− 35.9 ± 3.8	− 38.7 ± 3.6
Propranolol	Isoprenaline	112.5 ± 6.3	− 8.2 ± 1.2[b]	− 7.3 ± 1.2[b]
	SCA40	95.8 ± 7.9	− 30.8 ± 5.9	− 31.5 ± 4.4
Group 3				
Vehicle	Acetylcholine	106.4 ± 10.5	− 34.7 ± 4.5	− 33.1 ± 1.8
Atropine	Acetylcholine	109.1 ± 16.6	− 10.7 ± 1.8[b]	− 9.8 ± 0.3[b]
	SCA40	100.7 ± 9.8	− 28.9 ± 5.4	− 28.1 ± 2.5
Group 4				
Vehicle	Acetylcholine	102.8 ± 10.4	− 37.7 ± 4.4	− 36.7 ± 1.8
L-NAME	Acetylcholine	153.4 ± 11.2	− 75.4 ± 6.4[b]	− 49.3 ± 3.3[b]
	SCA40	145.0 ± 13.3	− 62.2 ± 9.1[a]	− 42.1 ± 3.9[a]
Group 5				
Vehicle	Cromakalim	88.6 ± 7.9	− 25.6 ± 4.3	− 28.2 ± 2.7
Glibenclamide	Cromakalim	98.0 ± 8.4	− 8.7 ± 1.7[b]	− 8.7 ± 1.5[b]
	SCA40	109.6 ± 6.6	− 27.4 ± 2.8	− 25.2 ± 2.5

Rats were divided into 5 groups and each antagonist was injected i.v. to each rat 15 min before SCA40 injection. Acetylcholine, isoprenaline and cromakalim were injected 5 min before and 15 min after the antagonist administration. Each value represents the mean ± s.e.mean of four to six animals.

[a]Indicates a significant difference from the value in group 1, SCA40 alone (two-tailed unpaired *t* test); [b]indicates a significant difference from the corresponding values in the absence of each antagonist (two-tailed unpaired *t* test).

trachea was antagonized by charybdotoxin (ChTX) but not by glibenclamide, which suggested that the relaxant activity of SCA40 does not involve ATP-sensitive K^+-channels but rather large-conductance Ca^{2+}-activated K^+-channels or other ChTX-sensitive K^+-channels (Laurent *et al.*, 1993). In rat isolated thoracic aorta, SCA40 exhibited a similar profile. SCA40 was able to inhibit completely the contractions induced by low concentrations of KCl (20 mM) as opposed to high concentrations (80 mM) of KCl. As in guinea-pig isolated trachealis tissue, SCA40, at high concentrations (10–100 μM), retained some relaxant activity against the spasm induced by 80 mM KCl (50% of the maximum relaxation that could be achieved against 20 mM KCl-induced contraction). This relaxant activity of SCA40 at high concentration might be attributed to its cyclic AMP phosphodiesterase inhibitory properties. The relaxant activity of SCA40 in thoracic aorta was not antagonized by glibenclamide which suggests that the relaxant activity of SCA40 in vascular tissue, as in trachealis tissue, does not involve ATP-sensitive K^+-channels.

ATP-sensitive K^+-channel openers directly induce negative chronotropic and inotropic responses in heart preparations (Yanagisawa *et al.*, 1988; 1989; Murakami *et al.*, 1992) but little is known about the role of the large-conductance Ca^{2+}-activated K^+-channels or other ChTX-sensitive K^+-channels in heart, since no activators of these channels have yet been developed. In the present study, SCA40 produced dual effects on rat isolated atria. At low concentrations, SCA40 induced dose-dependent negative chronotropic and inotropic responses. ATP-sensitive K^+-channel openers have been shown to shorten the action potential in cardiac muscle and thereby produce negative inotropic responses (Shinegobu *et al.*, 1991). Due to its K^+-channel opener properties, SCA40 might increase outward potassium currents in cardiac cells, which might explain negative inotropic effects. In our experiments, SCA40 did not reduce the force of atrial contractions below 35% of the basal force. Thus, the maximal negative inotropic effects of SCA40 appeared to be less than those of ATP-sensitive K^+-channel openers since these compounds have been shown to reduce contractile force in cardiac muscle from guinea-pig and dog by 70 to 90% (Yanagisawa *et al.*, 1988; 1989; Shinegobu *et al.*, 1991; Murakami *et al.*, 1992). If the negative inotropic and chronotropic activities of SCA40 can be attributed to activation of ChTX-sensitive K^+-channels, these results suggest that ChTX-sensitive K^+-channels are present in sinoatrial pacemaker cells and in atrial cells and the channels might be involved in the pacemaker and contractile activities, but to a smaller extent than ATP-sensitive K^+-channels. At higher concentrations (> 1 μM) SCA40 induced a dose-dependent increase of the sinus rate and atrial contractility. The positive chronotropic and inotropic activities observed at high concentrations might be due to cyclic AMP phosphodiesterase inhibitory activity of SCA40.

In normotensive male Wistar rats, SCA40 displayed a dose-dependent (1–100 $\mu g\,kg^{-1}$) decrease in MAP after i.v. administration. The hypotensive action of SCA40 is consistent with the smooth muscle relaxant activity exhibited *in vitro* by this new potassium channel activator. The hypotensive effect of 100 $\mu g\,kg^{-1}$ SCA40 was slightly greater but shorter in duration than that of 100 $\mu g\,kg^{-1}$ cromakalim.

The hypotensive action of SCA40 was accompanied by a moderate dose-dependent increase in HR. The tachycardia induced by i.v. administration of SCA40 was abolished by prior administration of the β-adrenoceptor blocker, propranolol (data not shown) without affecting the hypotensive response, suggesting this to be a reflex effect rather than a direct action of SCA40 on the heart. Similar results have been reported for ATP-sensitive K^+-channel openers (Cook & Hof, 1988; Pacioreck *et al.*, 1990).

The hypotensive response induced by SCA40 (10 $\mu g\,kg^{-1}$) was not abolished by prior administration of the muscarinic cholinoceptor blocker, atropine (1 $mg\,kg^{-1}$) or the β-adrenoceptor antagonist, propranolol (1 $mg\,kg^{-1}$). These results suggest that the hypotensive response induced by SCA40 is not mediated by muscarinic cholinoceptor or β-adrenoceptor activation.

L-NAME (10 $mg\,kg^{-1}$) induced a large increase in MAP. Such a result has already been reported in rats (Van Gelderen *et al.*, 1991). Following administration of L-NAME, the fall in MAP induced by acetylcholine was increased. Van Gelderen *et al.* (1991) reported similar results in anaesthetized rats and concluded that the hypotensive response to acetylcholine in rat is largely independent of the arginine-NO pathway. The hypotensive response to SCA40 was also increased in the presence of L-NAME, indicating that the hypotensive response to SCA40 is also largely independent of the arginine-NO pathway. The hypotensive effects of SCA40 were not modified by prior i.v. administration of glibenclamide (20 $mg\,kg^{-1}$), whereas, in the same dose, glibenclamide significantly inhibited the fall in blood pressure induced by cromakalim. These results suggest that the hypotensive activity of SCA40 is not mediated by the same mechanisms as that of cromakalim and consequently, does not involve ATP-sensitive potassium channels.

The present study shows that SCA40, a novel potassium channel opener which has been shown to be a potent relaxant of guinea-pig airway smooth muscle *in vitro* and *in vivo*, is also a potent vascular smooth muscle relaxant *in vitro* and *in vivo*. As in tracheal tissue, the vascular smooth muscle relaxant activity of SCA40 does not involve ATP-sensitive K^+-channels.

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