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Effects of the two enantiomers, S-16257-2 and S-16260-2, of a new bradycardic agent on guinea-pig isolated cardiac preparations

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1 The electromechanical effects of two enantiomers, S-16257-2 (S57) and S-16260-2 (R60), were studied and compared in guinea-pig isolated atria and ventricular papillary muscles. The possible stereoselectivity of the interaction on the cardiac Na^+ channel was analysed by comparing the effects of the two enantiomers on the onset and recovery kinetics of the frequency-dependent V_{max} block.

2 In spontaneously beating right atria, S57 and R60 (10^{-8} M - 10^{-4} M) exerted a negative chronotropic effect ($pIC_{50} = 5.07 \pm 0.19$ and 4.76 ± 0.18, respectively) and prolonged the sinus node recovery time, this effect being more marked with S57. In electrically driven left atria, S57 decreased ($P < 0.05$) contractile force only at 10^{-4} M and R60 at concentrations $\geq 5 \times 10^{-5}$ M, whereas in papillary muscles the negative inotropic effect appeared at concentrations $> 10^{-5}$ M.

3 In papillary muscles driven at 1 Hz, S57 and R60 at concentrations higher than 5×10^{-6} M produced a concentration-dependent decrease in the maximum upstroke velocity (V_{max}) and amplitude of the cardiac action potential without altering the resting membrane potential or the action potential duration. S57 and R60 had no effect on the characteristics of the slow action potentials elicited by isoprenaline in ventricular muscle fibres depolarized in high K^+ (27 mM) solution.

4 At 5×10^{-5} M, S57 and R60 produced a small tonic V_{max} block. However, in muscles driven at rates between 0.5 and 3 Hz both enantiomers produced an exponential decline in V_{max} (frequency-dependent V_{max} block) which augmented at higher rates of stimulation. The onset and offset rates of the frequency-dependent V_{max} block were similar for both drugs. Both S57 and R60 prolonged the recovery time constant from the frequency-dependent block from 20.1 ± 2.9 ms to 2-3 s.

5 At 5×10^{-5} M, S57 and R60 shifted the membrane responsiveness curve in a hyperpolarizing direction.

6 It can be concluded that S57 and R60, the two enantiomers of the new bradycardic agent, produced a similar frequency-dependent V_{max} block which indicated that the interaction with the Na^+ channel was not stereospecific. The analysis of the onset and offset kinetics of the frequency-dependent V_{max} block suggested that both enantiomers can be considered as Na^+ channel blockers with intermediate kinetics, e.g., class IA antiarrhythmic drugs.

Keywords: S 16257; contractile force; action potential; frequency-dependent V_{max} block; bradycardic agents

Introduction

Sinus tachycardia is a common physiological response that may help to maintain homeostasis by increasing cardiac output but also increases myocardial oxygen demands and decreases time for myocardial relaxation and diastolic ventricular filling (Sonnenblick *et al.*, 1968). In the presence of a flow-limiting coronary artery stenosis a decrease in diastolic perfusion time, secondary to exercise-induced tachycardia, may be especially deleterious by further reducing subendocardial myocardial perfusion (Boudoulas *et al.*, 1979; Hoffman, 1984). Under these circumstances, a reduction of heart rate prolongs the diastolic perfusion time and reduces myocardial oxygen demands, thus leading to an improvement in ischaemic zone perfusion and function. Bradycardic agents, i.e. β-adrenoceptor blocking agents and calcium channel blockers are frequently used in the treatment of effort-induced angina pectoris (Cruickshank & Prichard, 1987; Opie, 1989). However, these drugs also exhibit negative inotropic and hypotensive effects which may antagonize the beneficial effects of the bradycardia on myocardial blood flow by unmasking α-adrenoceptor vasoconstrictor mechanisms or increasing the extracellular component of coronary resistance (via an increase in left ventricular end-diastolic pressure) and reducing coronary artery perfusion pressure, respectively.

Specific bradycardic agents represent a new approach in the management of angina pectoris with depressed left ventricular function (Kobinger & Lillie, 1987; Guth, 1991). They block sinus tachycardia and markedly attenuate exercise-induced increases in heart rate at concentrations at which they have no direct effects on the inotropic state or vascular tone (Kobinger & Lillie, 1987; Guth, 1991; O'Brien *et al.*, 1992). The precise mechanism of action of these agents is still uncertain even when in rabbit sinoatrial node cells the bradycardic effect of zatebradine has been attributed to a frequency-dependent inhibition of the hyperpolarizing-activated current (I_f) (Goethals *et al.*, 1993).

S-16257-2 (7,8-dimethoxy 3-{3{[1S)-(4,5-dimethoxybenzocyclobutan-1-yl) methyl] methylamino}propyl} 1,3,4,5-tetrahydro-2H-benzazepine 2-one) is a new bradycardic agent that slows the spontaneous rate in rabbit isolated sino-atrial node and sheep Purkinje fibres and prolongs the action potential duration (APD), this effect being quite marked in Purkinje fibres, but very weak in guinea-pig ventricular papillary muscles (Thollon *et al.*, 1994). However, the effects of the drug on phase 0 characteristics of the cardiac action potential have not yet been reported. Furthermore, S-16257-2 is the (S)-enantiomer of a chiral molecule (Figure 1) and the (R)-enantiomer, S-16260-2, has been recently synthesized. Thus, the present work was undertaken to compare the effects of S-16257-2 (S57) and S-16260-2 (R60) on: (1) rate and contractile

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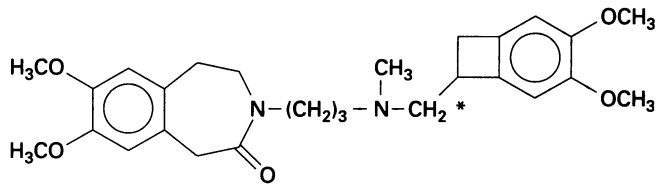

Figure 1 Chemical structure of 7,8-dimethoxy 3-{3-{[(4,5-dimethoxy benzocyclobutan-1-yl)methyl]methylamino}propyl}1,3,4,5-tetrahydro-2H-benzazepine 2-one. The asterisk shows the asymmetric carbon that differentiates S-16257-2 (S57) from S-16260-2 (R60).

force in guinea-pig isolated atria and papillary muscles, and (2) ventricular action potential characteristics. Moreover, (3) the possible stereoselectivity of the interaction on the cardiac Na^+ channel was analysed by comparing the effects of the two enantiomers on the onset and recovery kinetics of the frequency-dependent V_{max} block.

Methods

General procedure

Guinea-pigs of either sex weighing 350–450 g were killed by cervical dislocation and their hearts were rapidly removed. Right and left atria and left ventricular papillary muscles were dissected and mounted vertically in 10 ml organ baths containing Tyrode solution of the following composition (mM): NaCl 125, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 24, NaH_2PO_4 0.42 and glucose 11. The solution was bubbled with 95% O_2: 5% CO_2 and maintained at 34°C. Under these conditions right atria beat spontaneously, while left atria and papillary muscles were electrically driven at a basal rate of 1 Hz through bipolar platinum electrodes with rectangular pulses (1 ms duration, twice threshold strength) delivered from a multipurpose programmable stimulator (Cibertec CS 220, Madrid, Spain). Rate and amplitude of contractions were measured isometrically by a force-displacement transducer and recorded on a Letica 2000 (Cibertec S.A., Madrid, Spain) polygraph. Resting tension was adjusted to 1 g (left atria) and 0.5 g (papillary muscles) and a 30 min equilibration period was allowed to elapse before control measurements were taken. The sinus node recovery time was determined as described elsewhere (Tamargo, 1980). After control values for each parameter were obtained, incremental concentrations of each drug were added every 30 min to the bath to obtain a complete concentration-response curve. The values for the different parameters obtained in the absence of each drug were used as controls and compared with those obtained after each increment in drug concentration. Only one drug was tested in each experiment.

Electrophysiological studies

Guinea-pigs of either sex (250–350 g) were killed by cervical dislocation and hearts were rapidly removed and brought into a dissection chamber where papillary muscles of 2–3 mm in length and less than 1 mm in diameter were excised from the right ventricle. The muscles were pinned to the bottom of a Lucite chamber and superfused continuously at a constant rate of 7 ml min^{-1} with Tyrode solution bubbled with 95% O_2 and 5% CO_2 and maintained at 34 ± 0.5°C (pH = 7.4). The preparations were initially driven at 1 Hz and a period of 1 h was allowed for equilibration during which a stable impalement was obtained. Driving stimuli were rectangular pulses (1 ms duration, twice threshold strength) delivered to the preparation from a multipurpose programmable stimulator (Cibertec CS-220). Electrical stimulation was applied to the surface of the preparation through Teflon-coated bipolar electrodes of silver wire.

Transmembrane action potentials were conventionally recorded through glass microelectrodes filled with 3 M KCl (tip resistance of 8–15 MΩ). The microelectrode was connected via Ag-AgCl wire to high impedance, capacity neutralizing amplifiers (WPI model 701, New Haven, CT, U.S.A.). The maximal rate of depolarization (V_{max}) of the action potential was obtained by electronic differentiation (Valenzuela *et al.*, 1988). The amplifier output was linear between 10–1000 V s^{-1} and had variable input filters (3 Hz-260 KHz, E. Ehler, Homburg/Saar, Germany). A suitable frequency filter for minimizing noise without reducing the V_{max} was selected for each individual experiment. In order to avoid latency-induced alterations of V_{max}, stimulus intensity and duration were adjusted throughout each experiment to maintain a constant latency (1–2 ms) between stimulus and upstroke of the action potential (Valenzuela *et al.*, 1988). Both action potential and V_{max} were displayed on a storage oscilloscope (model 4104N, Tektronix Inc., Beaverton, OR, U.S.A.) and the oscilloscope traces were photographed with a kymographic camera (Grass C4, Grass Instrument Co., Quincy, MA, U.S.A.). The following action potential characteristics were determined from

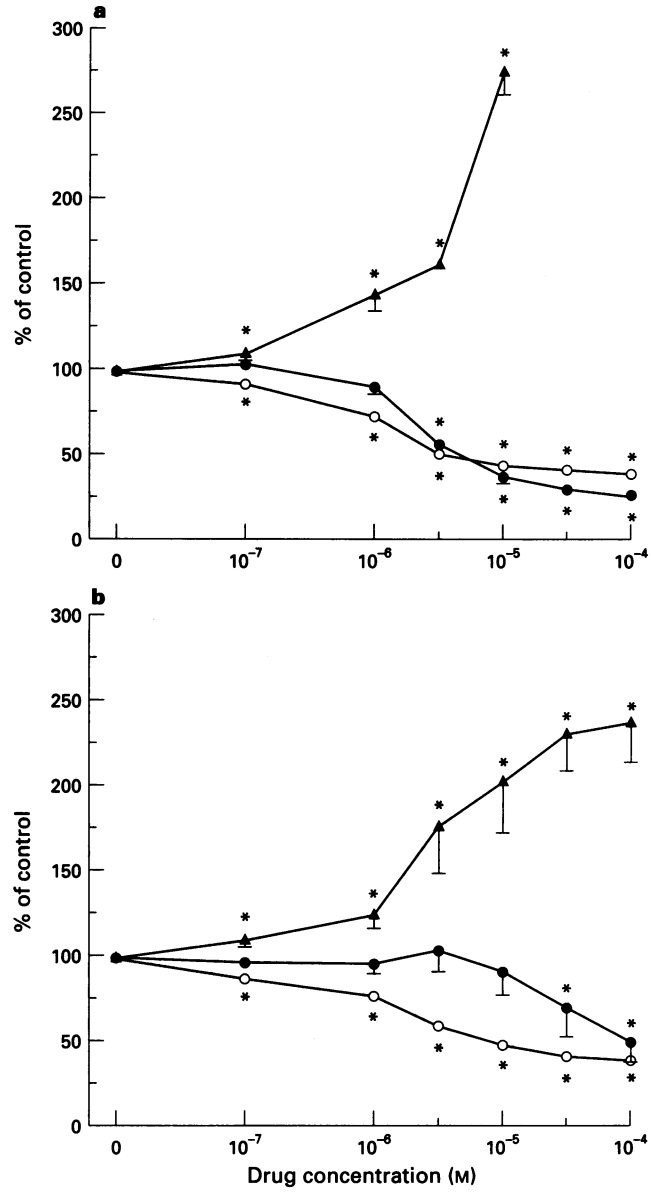

Figure 2 Concentration-response effects of (a) S57 and (b) R60 on (○) sinus rate, (●) peak contractile force and (▲) sinus node recovery time in spontaneously beating guinea-pig isolated right atria. Ordinate scale: % of control values. Abscissa scale: drug concentration (M). Each point represents the mean ± s.e.mean of at least 6 experiments. $^*P < 0.05$ vs control.

recordings: resting membrane potential, amplitude, V_{max} and action potential duration at the 50% (APD_{50}) and 90% (APD_{90}) level of repolarization. All experimental results were obtained from a single continuous impalement throughout the whole experiment.

To study rate-dependent effects on V_{max} block produced by S57 and R60, muscles were initially driven at a basal rate of 0.02 Hz. Following the equilibration period, the preparations were driven by trains of stimuli at varying rates for 40 s (0.5, 1 and 2 Hz) and 16 s (3 Hz). Rest periods of 5 min, which were sufficient to ensure full recovery from frequency-dependent decreases in V_{max}, were interpolated between the trains of stimuli (Valenzuela *et al.*, 1988; Delpón *et al.*, 1990). A similar experimental protocol was followed after exposure to S57 and R60. Under these circumstances two types of V_{max} inhibition were detected, i.e. Tonic and frequency (use)-dependent V_{max} block. Tonic blockade is the decrease of V_{max} of the first action potential preceded by a rest period, whereas frequency-dependent blockade was defined by the decrease of V_{max} during a train from the value of the first action potential to a new steady-state. Recovery from frequency-dependent V_{max} block was studied by applying a single test stimulus at various coupling intervals after a stimulation train for 8 s at 3 Hz. The strength of the test stimuli was adjusted so that the latency of the conditioning and the test stimuli were identical and constant (1–2 ms). For each drug, the onset of and recovery from frequency-dependent V_{max} block were fitted by exponential functions for calculations of the respective rate constants.

The effective refractory period (ERP), defined as the period in which no propagated action potentials can be obtained, was measured by introducing premature test-stimuli (S_2) of twice threshold strength at different intervals from the preceding basic action potential. S_2 was delivered every eighth basic stimulus. Premature test-stimuli were initially introduced late in the diastole and then progressively earlier in the cycle. The relationship between V_{max} and the resting membrane potential, i.e. membrane responsiveness, was studied in papillary muscles driven at 0.1 Hz and the resting membrane potential was de-

Table 1 Electrophysiological effects of S57 on transmembrane action potentials in guinea-pig papillary muscles driven at 1.0 Hz

Concentration (M)	*RMP* (mV)	*APA* (mV)	V_{max} (Vs^{-1})	APD_{50} (ms)	APD_{90} (ms)	ERP/APD_{90}
Control	−84.1 ± 0.8	122.8 ± 1.0	206.4 ± 9.1	179.3 ± 9.4	208.2 ± 8.9	1.03 ± 0.01
10^{-7}	−84.0 ± 0.9	123.1 ± 0.9	206.5 ± 10.3	178.2 ± 7.8	208.6 ± 7.6	1.03 ± 0.01
10^{-6}	−83.1 ± 1.0	122.7 ± 0.7	207.1 ± 11.6	184.3 ± 8.2	211.4 ± 8.9	1.03 ± 0.01
5×10^{-6}	−83.4 ± 1.4	123.4 ± 0.9	193.1 ± 16.2	181.0 ± 12.8	213.0 ± 11.5	1.02 ± 0.01
10^{-5}	−82.0 ± 1.4	122.7 ± 0.8	185.4 ± 12.3*	188.2 ± 9.1	219.6 ± 10.0	1.03 ± 0.01
5×10^{-5}	−82.7 ± 2.2	120.5 ± 1.8*	141.2 ± 16.1**	154.4 ± 3.6	194.4 ± 5.2	1.04 ± 0.01

Mean ± s.e.mean, $n = 7$. RMP, resting membrane potential; APA, amplitude of the action potential; V_{max}, maximal upstroke of the action potential; APD_{50} and APD_{90}, action potential duration at the 50% and 90% level of repolarization; ERP, effective refractory period. *$P < 0.05$; **$P < 0.01$.

Table 2 Electrophysiological effects of R60 on transmembrane action potentials in guinea-pig papillary muscles driven at 1.0 Hz

Concentration (M)	*RMP* (mV)	*APA* (mV)	V_{max} (Vs^{-1})	APD_{50} (ms)	APD_{90} (ms)	ERP/APD_{90}
Control	−86.2 ± 1.3	120.0 ± 0.8	211.9 ± 13.6	171.0 ± 10.3	201.5 ± 9.7	1.04 ± 0.00
10^{-7}	−86.0 ± 1.4	121.2 ± 0.8	213.0 ± 13.3	174.5 ± 9.2	206.0 ± 8.0	1.03 ± 0.00
10^{-6}	−85.6 ± 1.5	121.4 ± 0.9	207.5 ± 11.7	177.0 ± 10.4	210.5 ± 9.8	1.03 ± 0.00
5×10^{-6}	−85.5 ± 1.8	121.0 ± 0.7	201.4 ± 12.5	182.5 ± 9.5	218.7 ± 8.1	1.02 ± 0.01
10^{-5}	−85.5 ± 1.8	119.8 ± 0.5	191.0 ± 7.1*	173.5 ± 6.3	212.5 ± 7.0	1.02 ± 0.00
5×10^{-5}	−85.2 ± 1.7	117.0 ± 1.1*	152.0 ± 8.6*	154.4 ± 8.4	198.1 ± 10.1	1.03 ± 0.00

Mean ± s.e.mean, $n = 7$. *$P < 0.05$; **$P < 0.01$. For abbreviations, see Table 1.

Table 3 Effects of S57 (A) and R60 (B) on slow action potentials elicited by 10^{-6} M isoprenaline in guinea-pig papillary muscles depolarized with KCl (27 mM) driven at a basal rate of 0.1 Hz

Concentration (M)	*RMP* (mV)	*APA* (ms)	V_{max} (Vs^{-1})	APD_{50} (ms)	APD_{50} (ms)
A					
Control	−46.3 ± 2.3	84.0 ± 1.9	18.4 ± 1.9	219.0 ± 13.7	234.5 ± 15.4
10^{-7}	−46.5 ± 2.3	83.8 ± 1.3	20.1 ± 1.6	228.5 ± 14.6	243.7 ± 13.4
10^{-6}	−46.5 ± 2.3	85.0 ± 1.6	19.8 ± 1.7	223.0 ± 12.9	238.5 ± 12.1
10^{-5}	−46.5 ± 2.3	82.8 ± 1.5	19.2 ± 1.8	224.0 ± 10.8	240.5 ± 10.8
10^{-4}	−45.5 ± 1.8	81.0 ± 2.1	16.1 ± 1.6	232.0 ± 12.8	256.0 ± 12.5
B					
Control	−43.4 ± 0.9	83.6 ± 1.0	16.9 ± 1.1	272.5 ± 19.3	291.0 ± 20.7
10^{-7}	−43.4 ± 0.9	83.6 ± 1.1	18.5 ± 1.3	263.5 ± 15.0	288.0 ± 16.6
10^{-6}	−43.8 ± 1.1	83.0 ± 1.2	18.6 ± 2.0	247.5 ± 14.4	271.5 ± 16.3
10^{-5}	−43.6 ± 1.3	82.6 ± 0.7	18.2 ± 1.9	264.0 ± 18.5	289.5 ± 21.1
10^{-4}	−43.2 ± 1.1	82.0 ± 1.1	15.5 ± 1.8	240.0 ± 20.1	273.5 ± 21.4

Mean ± s.e.mean, $n = 6$. *$P < 0.05$; **$P < 0.01$. For abbreviations, see Table 1.

Table 4 Frequency-dependent V_{max} block induced by S57 and R60 in guinea-pig papillary muscles

	Frequency-dependent V_{max} *block (%)*			
	0.5 Hz	*1 Hz*	*2 Hz*	*3 Hz*
Control	2.2±0.3	4.2±0.5	8.4±1.0	10.1±1.3
S57 (5×10^{-5} M)	16.4±0.8**	30.5±1.3***	46.2±1.9***	56.1±1.7***
Control	1.6±0.7	3.4±0.5	7.2±1.1	10.8±1.9
R60 (5×10^{-5} M)	11.8±0.4**	22.5±0.8***	37.7±1.2***	49.3±1.8***

Mean ± s.e.mean, $n=7$; **$P<0.01$; ***$P<0.001$.

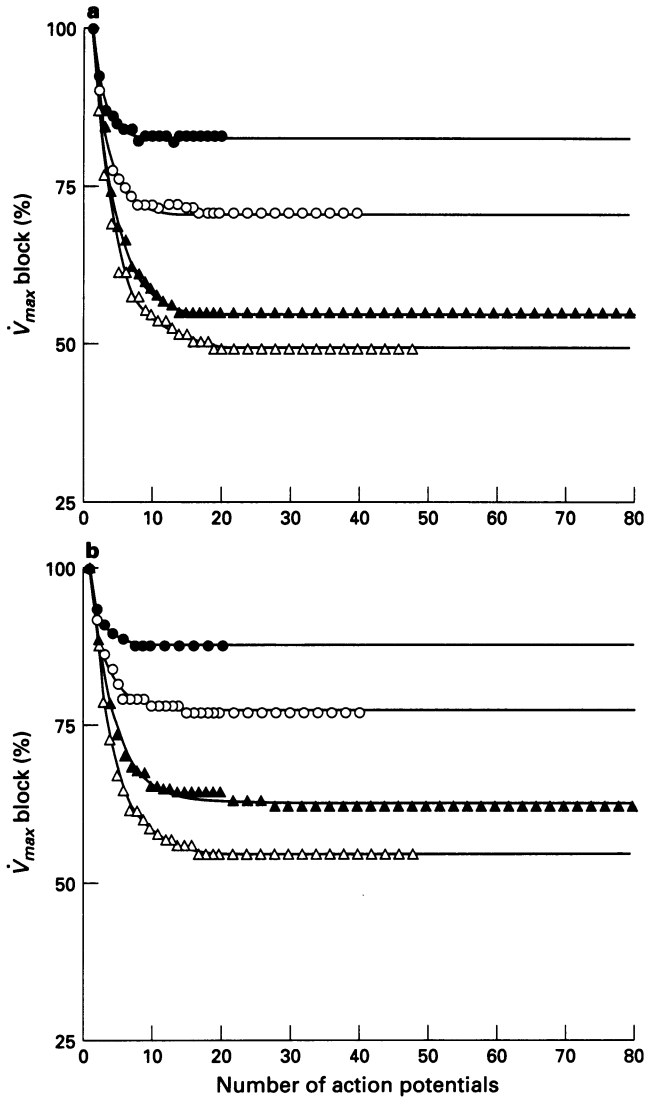

Figure 3 Onset of frequency-dependent depression of V_{max} induced by (a) S57 and (b) R60, 5×10^{-5} M, in guinea-pig papillary muscles driven by trains of stimuli at various rates (0.5–3 Hz). Ordinate scale: percentage of V_{max} block. Frequency-dependent V_{max} block (%) results from [1-V_{max}(ss,drug)/V_{max}(first beat, drug)], where V_{max}(ss) is the steady-state value attained during continuous stimulation and V_{max}(first beat) the value of the first beat of each train of stimuli. Abscissa scale: number of action potentials. (●) 0.5 Hz, (○) 1 Hz, (▲) 2 Hz and (△) 3 Hz.

polarized by increasing the extracellular K^+ concentration, $[K^+]_o$, from 2 to 16 mM (Delpón *et al.*, 1990). The V_{max} was measured each time after an equilibration period of 8 min. Curves were obtained in the absence and after 30 min exposure to the desired concentration of S57 and R60.

Slow action potentials were elicited in papillary muscles rendered unexcitable by depolarizing with 27 mM K^+ Tyrode solution. Under these conditions, the fast inward Na^+ current was voltage-inactivated and excitability, i.e. slow action potentials, was restored in depolarized muscles driven at 0.1 Hz by adding isoprenaline (10^{-6} M) to the perfusate (Delpón *et al.*, 1989).

Table 5 Rate constant of development of frequency-dependent V_{max} block [$K(AP^{-1})$] in the presence of S57 or R60 (5×10^{-5} M)

Frequency of stimulation (Hz)	*S57*	*R60*
0.5	0.70±0.04	0.59±0.07
1.0	0.38±0.02	0.41±0.04
2.0	0.28±0.02	0.30±0.04
3.0	0.27±0.02	0.28±0.03

Mean ± s.e.mean, $n=6$.

After control values for each parameter were obtained, incremental concentrations of each drug were added to the bath to obtain a complete concentration-response curve. The interval between concentrations of each drug was 30 min, since preliminary time-response studies indicated that their effects had stabilized within 30 min. The values for the different parameters obtained in the absence of each drug were used as a control and compared with those obtained after each increment in drug concentration. Only one drug was tested in each experiment.

Drugs used

S57 and R60 were synthesized and kindly provided by Servier (IRIS, Courbevoie, France). Drugs, as a powder, were initially dissolved in distilled deionized water as a 10^{-2} M stock solution. Further dilutions were carried out in Tyrode solution. Ascorbic acid (10^{-4} M) was added to prevent oxidation of isoprenaline. Throughout the paper data are given as the means ± s.e. mean and Student's paired t test was used to estimate the significance of differences from control values. The negative logarithm of the concentration of S57 or R60 producing 50% inhibition of the maximal inotropic or chronotropic response (pIC_{50}) was calculated as the mean ± s.e. mean of the individual pIC_{50}s using non-linear regression analysis. For statistical comparison of more than two groups, a one-way analysis of variance was performed. A P value of less than 0.05 was considered as significant. More details on each procedure are given under Results.

Results

Effects on atrial rate and cardiac contractility

The effects of S57 and R60, 10^{-8} M–10^{-4} M, were compared on rate and amplitude of spontaneous contractions in 12 right atria. Control values of both parameters were 158±5 beats min^{-1} and 321±24 mg, respectively. Figure 2 shows that both

drugs produced a concentration-dependent decrease in both rate and amplitude of spontaneous contractions. For S57 and R60 the negative chronotropic effect reached significant values at all concentrations tested (pIC_{50} values being 5.07 ± 0.19 and 4.76 ± 0.18, respectively), while the negative inotropic effect reached significant values at concentrations higher than 10^{-6} M for S57 ($pIC_{50} = 5.15 \pm 0.22$) and higher than 10^{-4} M for R60 ($pIC_{50} = 4.13 \pm 0.07$. $P < 0.05$). The negative inotropic effect was reversed by increasing the Ca^{2+} concentration in the bathing media from 1.8 to 6 mM or by adding 10^{-6} M isoprenaline. In contrast, the negative chronotropic effect was only slightly reversed by adding 10^{-6} M isoprenaline to the bathing media. The control values for the sinus node recovery time averaged 380 ± 27 ms ($n = 12$). S57 and R60 produced a concentration-dependent prolongation of the sinus node recovery time ($P < 0.05$), but at concentrations higher than 5×10^{-6} M this effect was more marked for S57 than for R60 ($P < 0.05$). At these high concentrations the sinus node recovery time could not be measured in 2 out of 8 experiments exposed to S57 because atria recovered their spontaneous activity 10 s after being electrically paced. This is the reason why these data were not included in Figure 2.

In isolated left atria and papillary muscles driven at a basal rate of 1 Hz the control values for contractile force were 560 ± 30 mg ($n = 19$) and 75 ± 14 mg ($n = 12$) respectively. At concentrations up to 10^{-5} M, S57 and R60 had no significant effects on atrial contractile force, but at higher concentrations R60 produced a significant ($P < 0.05$) negative inotropic effect which was also observed in the presence of 10^{-4} M S57. At concentrations $> 10^{-5}$ M, S57 and R60 also produced a significant ($P < 0.05$) negative inotropic effect in papillary muscles. Thus, at 10^{-4} M, S57 decreased atrial and ventricular contractile force by $13 \pm 6\%$ and $60 \pm 7\%$ and R60 by $33 \pm 7\%$ and $37 \pm 5\%$, respectively.

Effects of S57 and R60 on transmembrane action potentials

The electrophysiological effects of S57 and R60 (10^{-7} M to 5×10^{-5} M) on the action potential characteristics were studied in guinea-pig papillary muscles driven at the basal rate of 1 Hz. Results obtained under control conditions and 30 min after

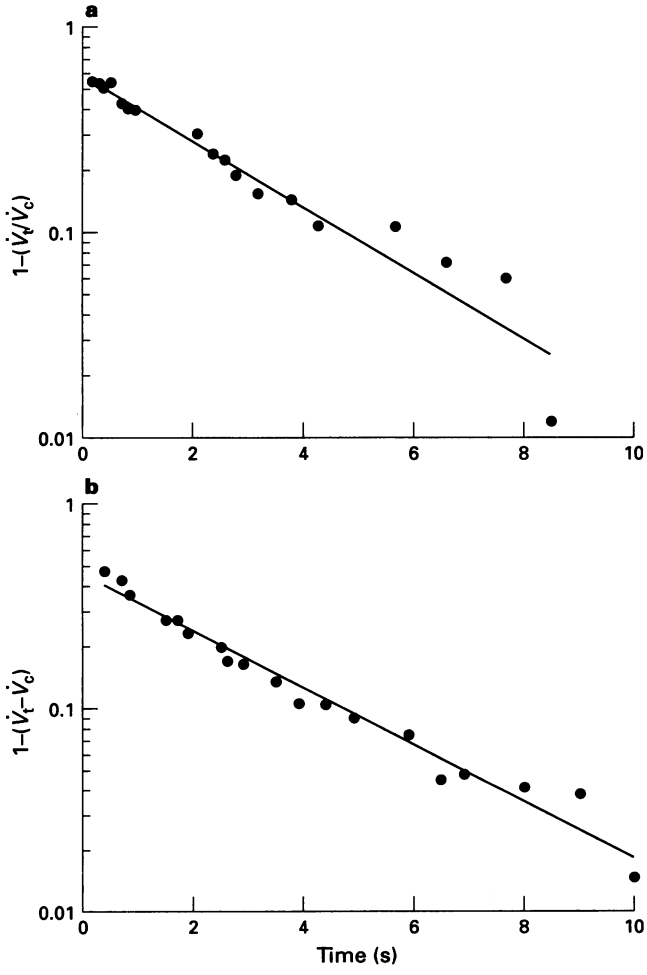

Figure 4 Effects of (a) S57 and (b) R60 on the recovery process of V_{max} block. Ordinate scale: normalized V_{max} values (V_t/V_c), where V_t is the V_{max} of the test action potential and V_c is the V_{max} of the first action potential of the train. Abscissa scale: test interval defined as the interval between the V_{max} of the last action potential of the train and the V_{max} of the test action potential.

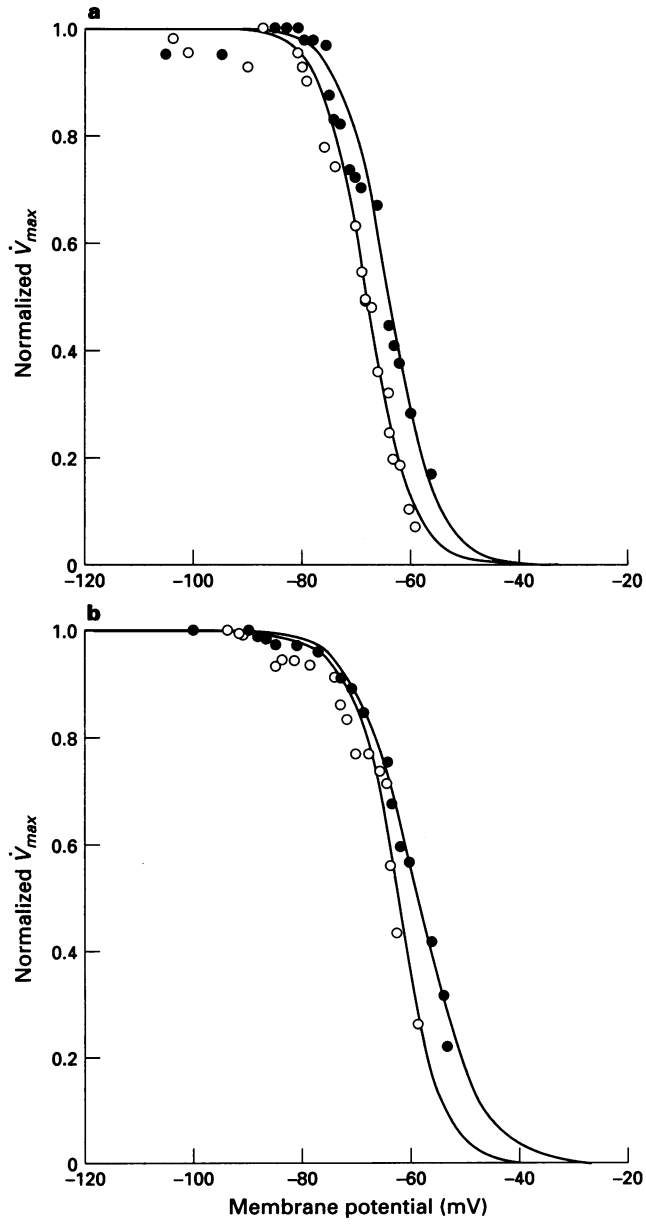

Figure 5 Effects of (a) S57 and (b) R60 on the relationship between V_{max} and the resting membrane potential from which the action potential arises. Ordinate scale: normalized V_{max} values. Abscissa scale: membrane potential. (●) Controls; (○) S57 and R60, 5×10^{-5} M.

each increment in drug concentration are shown in Tables 1 and 2. At concentrations between 10^{-7} and 5×10^{-6} M, S57 and R60 had no effect on transmembrane action potential characteristics. At higher concentrations and even when they had no effect on resting membrane potential or APD_{50} and APD_{90} values, both enantiomers significantly ($P<0.05$) decreased the amplitude and V_{max} of the action potential. S57 and R60 did not modify the ERP and thus, the ERP/APD_{90} ratio remained unaltered.

The effects of S57 and R60 were also studied on the slow, Ca^{2+}-dependent, action potentials elicited by isoprenaline, 10^{-6} M, in ventricular muscles depolarized in high K^+ (27 mM) Tyrode solution and driven at 0.1 Hz. Results obtained in 12 papillary muscles following perfusion with increasing drug concentrations, between 10^{-7} M and 10^{-4} M, are summarized in Table 3. It can be observed that neither S57 nor R60 produced changes in the characteristics of the slow action potentials and, therefore, did not exhibit Ca^{2+} channel blocking properties.

Frequency-dependent effects of S57 and R60

The influence of stimulation frequency on the inhibitory effect of S57 and R60 on V_{max} was studied in papillary muscles by applying trains of pulses at different rates (0.5–3 Hz), separated from one another by a rest period of 5 min. Following the perfusion with 5×10^{-5} M S57 and R60, the V_{max} of the first action potential in each train preceded by a rest period was reduced (e.g. tonic block) by $8.8 \pm 0.4\%$ ($n=7$) and $6.2 \pm 1.1\%$ ($n=7$), respectively. These data indicated that even at this high concentration these drugs exhibited a low affinity for the resting state of the Na^+ channels. When applying a train of pulses in the presence of S57 and R60 there was a gradual decrease of V_{max} from beat to beat to a new steady-state, which depended on the stimulation frequency. Table 4 summarizes the percentage decrease in V_{max} from the first action potential of the train to a new steady-state level in the absence and in the presence of 5×10^{-5} M S57 and R60 in muscles driven at 0.5–3 Hz. Under control conditions an increase in driving rate progressively decreased V_{max} by approximately 10%. In the presence of S57 and R60, the degree of frequency-dependent V_{max} block significantly increased with the driving rate ($P<0.01$), this increase being more marked at fast (2 and 3 Hz, $P<0.001$) than at slow (0.5 and 1 Hz, $P<0.01$) stimulation frequencies. However, the maximum frequency-dependent V_{max} block produced at 3 Hz was similar for both enantiomers. The frequency-dependent V_{max} block produced by 5×10^{-5}M S57 and R60 in two different papillary muscles driven at 0.5–3 Hz is shown in Figure 3.

Onset kinetics of frequency-dependent V_{max} block can be defined in terms of a rate-dependent process. In muscles driven at 0.5–3 Hz, the onset kinetics of S57 and R60 were best fitted by a single exponential curve, from which the onset rate constant per action potential [K, (AP^{-1})] was calculated. The value of K depends on stimulation frequency, decreasing to a similar extent at faster driving rates in the presence of S57 and R60 (Table 5).

Recovery kinetics of frequency-dependent V_{max} *block*

To study the effects of S57 and R60 on the recovery kinetics of frequency-dependent V_{max} block, papillary muscles were driven every 5 min by a train of stimuli at a frequency of 3 Hz for 8 s and a test-stimulus was applied at variable coupling intervals from 250 ms to 10 s. Under control conditions, the recovery from inactivation occurred as a monoexponential process with a time constant (τ_{re}) which averaged 21.7 ± 2.9 ms ($n=12$). In the presence of S57 and R60, 5×10^{-5} M, the recovery from V_{max} block was also fitted by a monoexponential function and the values of the τ_{re} averaged 2.3 ± 0.4 s ($n=6$) and 2.9 ± 0.2 s ($n=6$) (Figure 4), respectively. The y-intercept of the exponential fit can be taken as the fraction of Na^+ channels blocked, which rose to values of $56.1 \pm 2.2\%$ and $52.6 \pm 2.8\%$ for S57 and R60, respectively ($P>0.05$). These data suggested that the block of Na^+ channels induced by both enantiomers was not stereoselective.

Effect on membrane responsiveness

The relationship between V_{max} and the membrane resting potential from which the action potential arises, i.e. membrane responsiveness, was analysed in 8 papillary muscles driven at a basal rate of 0.1 Hz. The resting membrane potential was depolarized stepwise by increasing the $[K^+]_o$ in the bathing media from 2 to 16 mM and the V_{max} values were measured when the resting membrane potential reached steady-state at each $[K^+]_o$. Figure 5 shows that at 5×10^{-5} M, S57 and R60 shifted the membrane responsiveness curve in a hyperpolarizing direction, so that the membrane potential at which V_{max} was reduced to half of its maximum value was shifted by 3.2 ± 0.5 mV ($n=4$) and 4.3 ± 0.4 mV ($n=4$). These results indicated that depressant effects on V_{max} were slightly more pronounced at depolarized than at normal membrane potentials.

Discussion

This study compared the electromechanical properties of two enantiomers, S57 and R60, in guinea-pig isolated atrial and ventricular muscle fibres. The present results indicated that S57 and R60: (a) reduced atrial rate and prolonged the sinus node recovery time at concentrations which induced no negative inotropic effects; (b) produced a similar voltage- and frequency-dependent V_{max} block of the ventricular action potential, which suggested that their interaction with the Na^+ channel was not stereospecific; and (c) the onset and recovery kinetics of frequency-dependent V_{max} block are compatible with those of intermediate kinetics Na^+ channel blockers, e.g., class IA antiarrhythmic drugs. Both S57 and R60 produced a similar decrease in atrial rate and prolonged the sinus node recovery time, a more sensitive test for sinus function, but this prolongation was more marked with S57. Since the negative chronotropic effect was observed at concentrations ($<5 \times 10^{-6}$ M) at which both drugs had no effect on action potential characteristics, it is unlikely that it may be associated with an inhibition of the I_{Na} or with changes in action potential duration. However, at higher concentrations the bradycardic effect produced by both enantiomers can be related, at least partly, to their inhibitory effect on the I_{Na}. Both enantiomers also decreased contractile force in spontaneous right atria, S57 being more potent than R60. Because in guinea-pig atria a decrease in rate may produce parallel changes in contractile force (Koch-Weser & Blinks, 1963), their inotropic effects were also studied in electrically driven left atria and papillary muscles. Under these conditions, S57 and R60 decreased contractile force only at the highest concentrations tested ($>10^{-5}$ M). Therefore, S57 and R60 depress sinoatrial function at concentrations two to three orders of magnitude lower than those that decrease cardiac contractile force and suggested that the negative inotropic effects observed in right atria may be related to their negative chronotropic effect.

Na^+ channel blockers are characterized by their ability to depress the V_{max} of the cardiac action potentials (Hondeghem & Katzung, 1984; Tamargo *et al.*, 1992). In this study, V_{max} values were used as an indirect index of the magnitude of the I_{Na}. The V_{max} is a monotonic but non-linear index of peak I_{Na}, but there is little doubt that V_{max} is mainly generated by this current (Hondeghem, 1978; Sheets *et al.*, 1988). Whether or not possible non-linearities between V_{max} and Na^+ conductance affect the present results remains to be seen in reliable patch-clamp experiments performed under physiological conditions of both temperature and external Na^+ concentration.

S57 and R60 inhibited the V_{max} of the ventricular action potentials without altering the resting membrane potential and

thus, they exhibied Na^+ channel blocking (class I antiarrhythmic) actions. According to the modulated receptor hypothesis (Hondeghem & Katzung, 1984), Na^+ channel blockers bind to a specific binding site located within or functionally associated with the Na^+ channel and its affinity increases with the transition from the rested to the activated/inactivated state of the channel. Like other Na^+ channel blockers (Campbell, 1983a,b; Hondeghem & Katzung, 1984; Tamargo *et al.*, 1992), high concentrations of S57 and R60 caused little tonic V_{max} block in normally polarized ventricular fibres, which indicated that they exhibit little affinity for the resting state of the channel. Frequency-dependent V_{max} block can be explained by a preferential binding of the drugs to the activated and/or inactivated state of the Na^+ channel during a train of action potentials when the diastolic interval between pulses is too short to allow complete recovery of Na^+ availability (Hondeghem & Katzung, 1984; Tamargo *et al.*, 1992). In the presence of S57 and R60, the onset rate of frequency-dependent V_{max} block was faster at higher drug concentrations and at the lower stimulation frequencies. At 3 Hz the K values (0.27 ± 0.02 AP^{-1} and 0.28 ± 0.03 AP^{-1}, respectively) were quite similar to those reported for tocainide, a fast kinetics Na^+ channel blocker (Campbell, 1983a,b). The rate of onset of frequency-dependent V_{max} block, however, is affected by changes in drug concentration and stimulation rate (Hondeghem & Katzung, 1984; Grant *et al.*, 1984; Tamargo *et al.*, 1992). The τ_{re}, which represents the rate of unbinding of the drugs from the inactivated Na^+ channels, is independent of changes in drug concentration or the stimulation rate and, therefore, is considered one of the most reliable parameters to subdivide Na^+ channel blockers (Campbell, 1983a,b; Hondeghem & Katzung, 1984; Tamargo *et al.*, 1992). S57 and R60 prolonged the τ_{re} to 2.3–2.9 s. These values are similar to those previously described for procainamide (2.3 s, Courtney, 1980), imipramine (2.5 s, Delpón *et al.*, 1993) and quinidine (3.7, Sánchez-Chapula, 1985), e.g. intermediate kinetics Na^+ channel blockers (class IA antiarrhythmic drugs). The similar prolongation of the τ_{re} explains why the diastolic interval during the trains was short enough to avoid a complete recovery of V_{max} and thus, S57 and R60 produced a similar frequency-dependent V_{max} block at all driving rates. Therefore, since no differences in the onset and offset kinetics of the V_{max} block were observed in the presence of S57 and R60, their interaction with the Na^+ channel was not stereospecific. Furthermore, in muscles driven at a basic rate much greater than the τ_{re}, S57 and R60 shifted the relationship between V_{max} and resting membrane potential in the hyperpolarizing direction, causing a greater depression of V_{max} at less negative membrane potentials. This result suggested that both enantiomers exhibited a somewhat enhanced affinity for the inactivated state of the Na^+ channel. However, our experiments do not allow us to estimate the relative contribution of activated vs. inactivated block of Na^+ channels that causes the voltage- and frequency-dependent V_{max} block.

The repolarization of the cardiac action potential is the result of a delicate balance between inward (Na^+ and Ca^{2+}) and outward K^+ currents. S57 and R60 did not modify the V_{max} of the slow action potentials, a fairly good approximation of the slow inward Ca^{2+} current (I_{Ca}) (Malecot & Trautwein, 1987), so that it is unlikely that they had an inhibitory effect on this ionic current. Because S57 and R60 inhibited the I_{Na}, an acceleration of the repolarization and a shortening of the ventricular APD would be expected, particularly at high drug concentrations. In fact, a blockade of the Na^+ 'window' current has been suggested to operate for lignocaine and quinidine (Carmeliet & Saikawa, 1982). However, this APD shortening might be counteracted if S57 and R60 inhibited outward K^+ currents. Very recently, we have demonstrated that S57 and R60 inhibited the human cloned K^+ channel (hKv1.5) expressed in a stable mouse cell line (Delpón *et al.*, 1994).

The clinical relevance of the use-dependent V_{max} block produced by S57 and R60 is at present unknown. Since both enantiomers reduce heart rate and may suppress sinus tachycardia, it would be expected that the frequency-dependent decrease in intracardiac conduction velocity secondary to I_{Na} inhibition might be progressively relieved as the driving rate was slowed. The frequency-dependent V_{max} block, however, may be an interesting property that can lead to the suppression of cardiac arrhythmias in an ischaemic cardiac substrate. In addition, the I_{Na} plays a role in the final phase of diastolic depolarization. Whether the inhibition of I_{Na} could contribute, at least partly, to their bradycardic effect must be clarified in further patch-clamp studies.

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