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Stereospecific in vitro and in vivo effects of the new sinus node inhibitor (+)-S 16257

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Abstract

The effects of the two isomers, (+)-S 16257 and (−)-S 16260, of a new bradycardic agent, (±)-S 15544 (7,8-dimethoxy 3-{3-{[(4,5-dimethoxybenzocyclobutan-1-yl) methyl] methylamino}propyl}1,3,4,5-tetrahydro-2*H*-3-benzazepin-2-one), were compared in vitro and in vivo on cardiac spontaneous rate and repolarization time. In the isolated rabbit sino-atrial node, the three compounds (3 μM) were equi-effective to reduce the action potential firing rate. In anesthetized pigs, both isomers (0.03, 0.1, 0.3 and 1 mg kg^{-1} i.v.) were equipotent to reduce heart rate. For all compounds, the negative chronotropic effect resulted from a reduction in the slope of diastolic depolarization of pacemaker cells. In sino-atrial node cells, (−)-S 16260 (3 μM) increased action potential duration while (+)-S 16257 had a smaller effect. In driven guinea-pig papillary muscles exposed to increasing concentrations of compounds (0.1 to 10 μM) a small prolongation of action potential duration was observed. This prolongation was more marked in rabbit Purkinje fibers stimulated at a low rate. In all cardiac preparations the highest prolongation was observed with (−)-S 16260. In vivo, (−)-S 16260 prolonged QT_c at the two highest doses tested while (+)-S 16257 had no effect. In conclusion, resolution of (±)-S 15544 into its two enantiomers yielded compounds with the same bradycardic effects. Of the isomers, (+)-S 16257 has an increased specificity with minimal direct effect on action potential repolarization. © 1997 Elsevier Science B.V.

Keywords: S 16257; Bradycardic agent; Stereospecificity; Transmembrane action potential; Heart rate; QT interval

1. Introduction

Reduction of sinus heart rate is of major interest in the treatment of cardiac ischemia. Myocardial oxygen balance can be improved by both reducing metabolic demand and improving diastolic perfusion, especially in the subendocardial layers, the most vulnerable area of the myocardium (Harron et al., 1982; Guth et al., 1987a,b; Indolfi et al., 1989). Therefore, specific bradycardic agents, compounds able to reduce sinus rate at concentrations devoid of any negative inotropic and hypotensive effects (Kobinger and Lillie, 1984, 1987), may represent an interesting approach for the treatment of myocardial ischemia, especially in patients with left ventricular dysfunction. The fundamental basis for the automaticity of pacemaker cells is the slow diastolic depolarization which brings the membrane potential from the most hyperpolarized level up to the threshold for action potential. The control of this slope of diastolic depolarization is a key mechanism by which spontaneous rhythm is physiologically adjusted by moderate autonomic activity (Di Francesco, 1995a). Therefore, the diastolic depolarization rate of pacemaker cells is an interesting target for specific bradycardic agents. Two different subclasses of such compounds have been described: (i) drugs derived from clonidine, such as alinidine or ZD-7288 (BoSmith et al., 1993) and (ii) compounds with a chemical structure related to verapamil, such as zatebradine (Goethals et al., 1993). (+)-S 16257 (7,8-dimethoxy 3-{3-{[(1*S*)-(4,5-dimethoxybenzocyclobutan-1-yl) methyl] methylamino}propyl}1,3,4,5-tetrahydro-2*H*-3-benzazepin-2-one) is a new sinus node inhibitor, presently undergoing clinical trial, that belongs to the latter group. In vitro, the bradycardic effect of (+)-S 16257 was quite similar to that observed with zatebradine (Thollon et al., 1994). Reduc-

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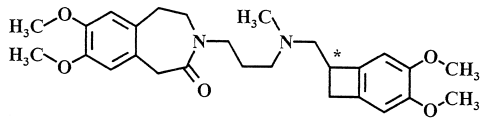

Fig. 1. Chemical structure of (±)-S 15544. The chiral center is denoted by an asterisk.

tion of spontaneous firing rate in pacemaker cells by (+)-S 16257 resulted from a block of the hyperpolarization-activated I_f current (Bois et al., 1996), thus decreasing the diastolic depolarization rate of phase 4 of the action potential. The major improvement with this compound was that it had only a weak effect on action potential repolarization, contrary to zatebradine that markedly prolonged the action potential within the bradycardic concentration range (Thollon et al., 1994). Zatebradine was described not only as a I_f inhibitor, but also as a blocker of $I_{Ca,T}$, $I_{Ca,L}$ or I_{Kr} (Doerr and Trautwein, 1990; BoSmith et al., 1993; Bois et al., 1996). Furthermore, it is well known that the effects of compounds prolonging action potential duration, i.e. class III antiarrhythmic agents, are increased if the heart rate is slowed (Surawicz, 1992). Under these conditions, these compounds could become proarrhythmic, leading to the development of tachyarrhythmias and even 'torsades de pointes' (Carlsson et al., 1990). Thus, a really specific bradycardic agent, devoid of direct effect on action potential repolarization, should be safer in use.

Since (+)-S 16257 is the (+)-enantiomer (*S*-stereoisomer) of the racemate compound, (±)-S 15544 (chemical structure shown in Fig. 1), the electropharmacological profile of (+)-S 16257 and (−)-S 16260, the other enantiomer (*R*-stereoisomer), were compared. The bradycardic effects of both isomers were analyzed in vitro with isolated sino-atrial node preparations and in vivo in anesthetized swine. The effects of each compound on cardiac repolarization were examined in vitro on the action potential of spontaneously beating rabbit sino-atrial node or driven cardiac preparations, guinea-pig papillary muscles and rabbit Purkinje fibers, and finally in vivo on the swine QT_c interval.

2. Materials and methods

2.1. In vitro experiments

Governmental and institutional guidelines for the care and use of animals were followed at all times. Male New Zealand white rabbits and Hartley guinea-pigs (Ch. River, France) were stunned by a blow on the neck. After exsanguination, the hearts were rapidly excised and placed in an oxygenated Tyrode's solution at 4°C. The cardiac preparations were removed from the right ventricle (papillary muscle or Purkinje fibre) or right atria (sino-atrial node tissue) and subsequently mounted in a 3.5 ml tissue bath. The preparations were continuously superfused at a constant flow rate (5 ml min^{-1}) with Tyrode's solution of the following composition (in mmol l^{-1}): NaCl 130, KCl 5.6, $CaCl_2$ 2.15 or 1.80 (for guinea-pig or rabbit preparations, respectively), NaH_2PO_4 0.6, $NaHCO_3$ 20, $MgCl_2$ 1.1 and glucose 11. The temperature was kept constant at 36 ± 0.5°C and the pH was maintained at 7.4 by bubbling with a mixture of 95% O_2 and 5% CO_2. Papillary muscles and Purkinje fibers were driven at a basal frequency of 1 Hz (twice threshold) with a Grass S88 stimulator. For guinea-pig papillary muscles, the tendinous end was connected to a Gould UC2 force transducer to record isometric tension. The muscles were carefully stretched until the peak of the length–tension relationship was obtained. All preparations were left to stabilize for at least two hours before electrical measurements were initiated.

Glass microelectrodes filled with 3 M KCl (resistance 18–24 MΩ), connected via an Ag/AgCl junction to a high-input impedance amplifier (Biologic VF 180A), were used to record transmembrane potentials. Membrane potential was displayed on a Tektronix digital storage oscilloscope. Action potential and isometric force (for papillary muscles) were continuously recorded with a Gould pen chart recorder. Both signals were stored and analyzed using a specific software (Clovis, CLOD Sarl, France), installed on a personal computer equipped with a 12-bit analog–digital Metrabyte DAS50 Converter.

Rabbit preparations, i.e. spontaneous beating sinus node tissue or 0.25 Hz driven Purkinje fibers, were exposed to racemate ((±)-S15544) or its enantiomers [(−)-S16260 and (+)-S 16257] at 3 μM for 30 or 40 min, respectively. For Purkinje fibers, after wash-out of the drug (minimum of one hour), the preparations were exposed to a higher concentration (10 μM) for 40 min. Guinea-pig papillary muscles were exposed to increasing concentrations of compounds, applied cumulatively (0.1–10 μM, 30 min exposure for each concentration).

2.2. In vivo experiments

The electrocardiographic and hemodynamic effects of (+)-S 16257 were evaluated versus those of (−)-S 16260 in anesthetized pigs. Animals were cared for in accordance with the principles of the *Guide to the Care and Use of Experimental Animals*. All procedures used in this study were approved by the local committee on animal care. These experiments were performed on 25 anesthetized pigs of either sex weighing 18–27 kg (Large–White, de Frenelles E.A.R.L, 8–10 weeks old). General anesthesia was induced by intramuscular injection of an anesthetic mixture of tiletamine and zolazepam (15 mg kg^{-1}) and maintained with a sodium thiopental intravenous perfusion

(8 mg kg^{-1} h^{-1}). The pigs were intubated and ventilated with a respirator Mark 8 (Bird). The respiratory rate and tidal volume were adjusted to maintain PCO_2 at 35–45 mm Hg, and pH at 7.3–7.4. The oxygen partial pressure (PO_2) always exceeded 80 mm Hg. A saline-filled catheter was implanted into the left saphenous vein for drug infusion. A surface electrocardiogram ECG (DII) was continuously recorded to measure heart rate, QT and PR intervals. The corrected QT interval [QT_c = (QT interval in ms)/(*R–R* interval in s)$^{1/2}$] was determined according to the formula of Bazett (1920).

After a stabilizing period of at least 30 min, four consecutives doses of drugs (0.03, 0.1, 0.3 and 1 mg kg^{-1}) or vehicle (0.2 ml kg^{-1}) were given at 30 min intervals. The duration of injection was 1 min. Heart rate, QT and PR intervals were recorded before injection, at the end of the injection (1 min) and after 3 , 5 , 10 , 20 and 30 min exposure to each dose.

2.3. Statistical analysis

Values are expressed as means ± S.E.M. for *n* experiments performed.

Statistical significance was evaluated by two-way analysis of variance with repeated measures. In the case of significant interaction, a one-way complementary analysis, followed by a Newman–Keuls test, was performed. A *P* value of less than 0.05 was considered statistically significant.

2.4. Solutions and drugs

The three molecules tested were synthesized in the Servier Research Institute (IdRS). All stock solutions were prepared freshly every day: the racemate was initially dissolved in a HCl 0.01 N solution and both enantiomers, as the hydrochloride salt, were dissolved in distilled water to obtain a 0.1 mM solution. Further dilutions were carried out in Tyrode's solution or sodium chloride solution (0.9%) for in vitro and in vivo experiments, respectively.

3. Results

3.1. Bradycardic effect in isolated sino-atrial node preparations

The effects of (±)-S 15544 and its enantiomers, (+)-S 16257 and (−)-S 16260, were examined on pacemaker activity, in rabbit sino-atrial node preparations, at 3 μM. As illustrated in Fig. 2, the three compounds reduced the spontaneous action potential firing rate of pacemaker cells. The three compounds were equally effective (Fig. 3, open columns) to reduce action potential frequency: −19.6 ± 2.5%, −16.9 ± 1.4% and −16.3 ± 2.1%, for (+)-S 16257, (−)-S 16260 and (±)-S 15544, respectively, after 30 min.

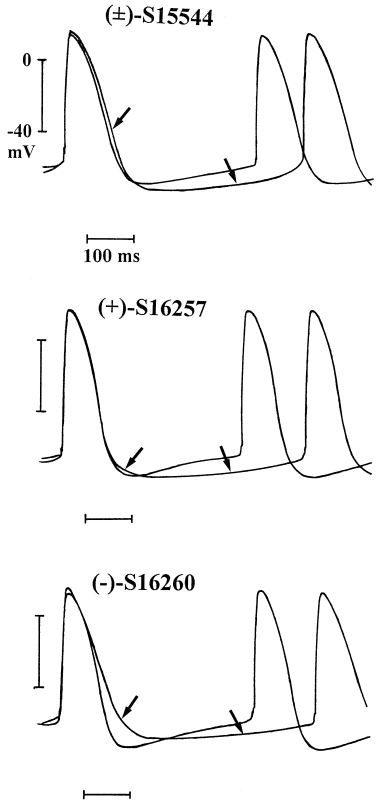

Fig. 2. Representative recordings of spontaneous action potentials from rabbit sino-atrial node preparations before and 30 min after exposure (arrows) to 3 μM of (±)-S 15544 or its enantiomers, (+)-S 16257 and (−)-S 16260.

3.2. Mechanism of action in pacemaker cells

For the three compounds, the bradycardic effect resulted from a reduction in the diastolic depolarization slope in pacemaker cells (Fig. 2), without significant change in the maximal diastolic potential or the threshold potential (Table 1), thus prolonging the diastolic depolarization time. The cycle length was significantly ($P \leq 0.01$) prolonged, by 73.3 ± 12.1, 97.5 ± 20.1 and 78.8 ± 10.9 ms, for (±)-S 15544, (+)-S 16257 and (−)-S 16260, respectively (7.9 ± 4.5 ms reduction for drug-free experiments). No significant difference was observed between the effects of the three compounds on cycle length.

3.3. Effect on action potential duration in isolated sino-atrial node preparations

In spontaneously beating rabbit sino-atrial node preparations a weak increase in action potential duration was noted during exposure to 3 μM (±)-S 15544 and its enantiomers (Figs. 2 and 3, filled columns). The prolongation of action potential duration at 50% repolarization (APD_{50}) was significantly more marked with (−)-S 16260

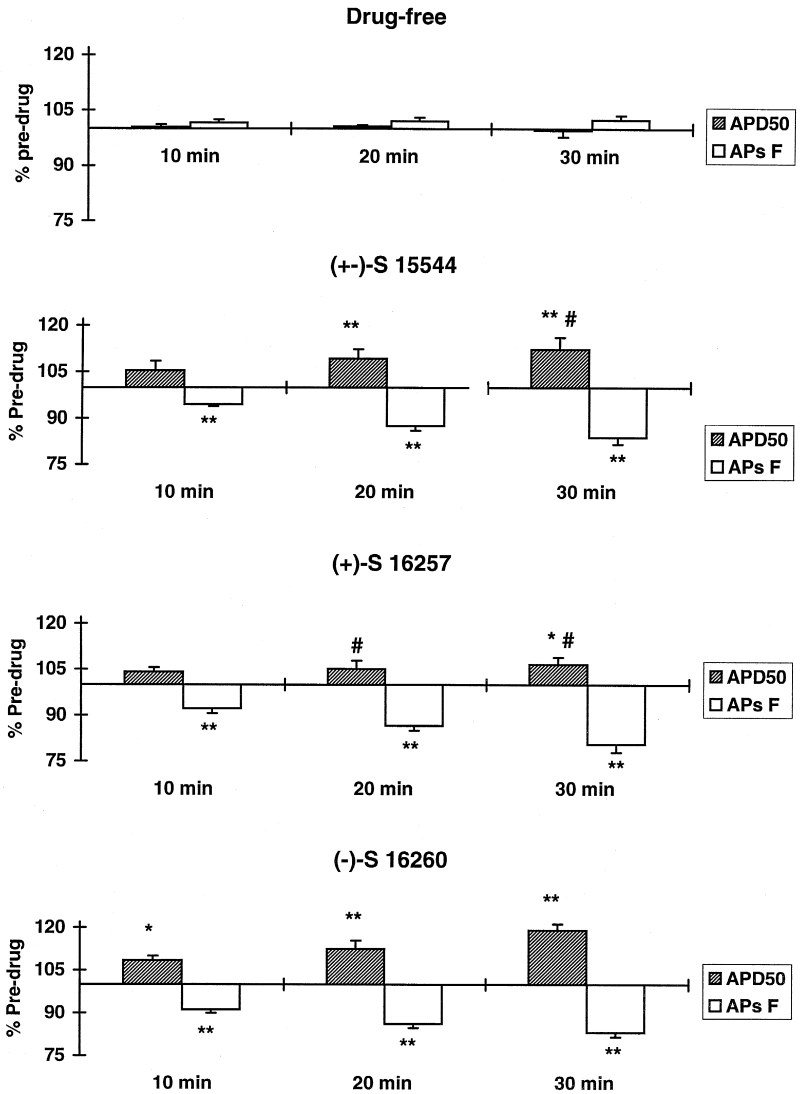

Fig. 3. Effects of 3 μM (±)-S 15544 ($n = 6$) and its enantiomers, (+)-S 16257 ($n = 6$) and (−)-S 16260 ($n = 6$) on spontaneous action potential firing rate (APs F) and action potential duration at 50% repolarization (APD_{50}), in rabbit sinus node pacemaker cells. Values are expressed as means ± s.e.mean, as percent of pre-drug value, for n experiments, after 10, 20 and 30 min of drug application. Open columns and filled columns represent the bradycardic effect and the prolongation of action potential, respectively. $^{*}P \leq 0.05$; $^{**}P \leq 0.01$: significance of differences from drug-free experiments ($n = 12$). $^{\#}P \leq 0.05$; $^{\#\#}P \leq 0.01$: significance of differences from the (−)-S 16260 treated group.

than with (+)-S 16257 ($P \leq 0.01$). The least effect was obtained during exposure to (+)-S 16257 (Fig. 3). The prolongation induced by (+)-S 16257 reached statistical significance after 30 min. At this time, APD_{50} was prolonged by 12.2 ± 3.9% ($P \leq 0.01$), 6.7 ± 2.2% ($P \leq 0.05$) and 19.2 ± 2.2% ($P \leq 0.01$), with (±)-S 15544, (+)-S 16257 and, (−)-S 16260, respectively.

3.4. Effect on action potential duration in driven cardiac preparations

The effects of (±)-S 15544 and its enantiomers on ventricular action potentials were studied in guinea-pig papillary muscles driven at 1 Hz. The preparations were exposed to increasing concentrations of compounds (from 0.1 to 10 μM, 30 min exposure for each concentration). The pre-drug values for the control group ($n = 16$) for action potential amplitude, resting potential and dV/dt_{max} (V_{max}) were 122.4 ± 0.8 mV, −84.1 ± 0.3 mV and 214.2 ± 10.1 V s^{-1}, respectively. No significant changes in these action potentials parameters were observed with the compounds. The only alteration observed was a weak lengthening of action potential, significant from 0.3 μM onward. The prolongation was maximal at 3 μM for all compounds: 9.0 ± 0.9% and 11.6 ± 1.4% increase in APD_{90} with (+)-S 16257 and (−)-S 16260, respectively. At any concentration, the prolongation was more marked with (−)-S 16260 (Fig. 4), but the statistical analysis including all compounds and concentrations showed no significant differences between the effects of the 3

Table 1
Effects of racemate, (±)-S 15544, and its enantiomers, (+)-S 16257 and (−)-S 16260, on spontaneous action potential parameters of rabbit sino-atrial node tissue

Compound (3 μM)	APA (mV)	MDP (mV)	Th. P (mV)
Drug-free ($n=12$)			
T0	71.8 ± 2.6	−70.3 ± 1.2	−60.2 ± 1.9
T30 min	71.9 ± 2.4	−68.8 ± 1.4	−59.2 ± 2.3
(±)-S 15544 ($n=6$)			
pre-drug	71.8 ± 1.9	−68.7 ± 1.2	−57.2 ± 1.7
T30 min	75.2 ± 2.9	−69.4 ± 1.5	−60.4 ± 1.2
(+)-S 16257 ($n=6$)			
pre-drug	74.4 ± 3.1	−71.5 ± 2.6	−60.0 ± 3.5
T30 min	83.1 ± 2.8	−72.8 ± 1.9	−66.0 ± 2.8
(−)-S 16260 ($n=6$)			
pre-drug	70.6 ± 3.6	−67.3 ± 1.6	−53.2 ± 3.2
T30 min	70.8 ± 3.8	−67.3 ± 0.7	−55.8 ± 2.9

Data are expressed as means ± s.e.mean, for n experiments. APA, amplitude of the action potential; MDP, maximal diastolic potential; Th. P, threshold potential.
No difference from drug-free experiments and no difference between the three treated groups.

molecules. Recordings obtained at the maximal effect (3 μM) are shown in Fig. 5 (top panel).

The effects of the three agents on action potential duration of rabbit Purkinje fibers driven at a low rate (0.25 Hz) were studied during a 40 min exposure to 3 μM and 10 μM. The prolongation of APD_{50} and APD_{90} (Fig. 6A and B, respectively) was more marked at the higher concentration. At any concentration tested, the effects were more pronounced during exposure to (−)-S 16260 than to other compounds: 14.1 ± 5.0% and 69.2 ± 34.3% prolongation in APD_{50}, at 3 μM, for (+)-S 16257 and (−)-S 16260, respectively. However, due to the variability of the response to (−)-S 16260, the differences between effects of the three compounds failed to reach statistical significance ($0.05 \leq P \leq 0.1$). The effects of these compounds at 10 μM are illustrated in Fig. 5 (bottom panel).

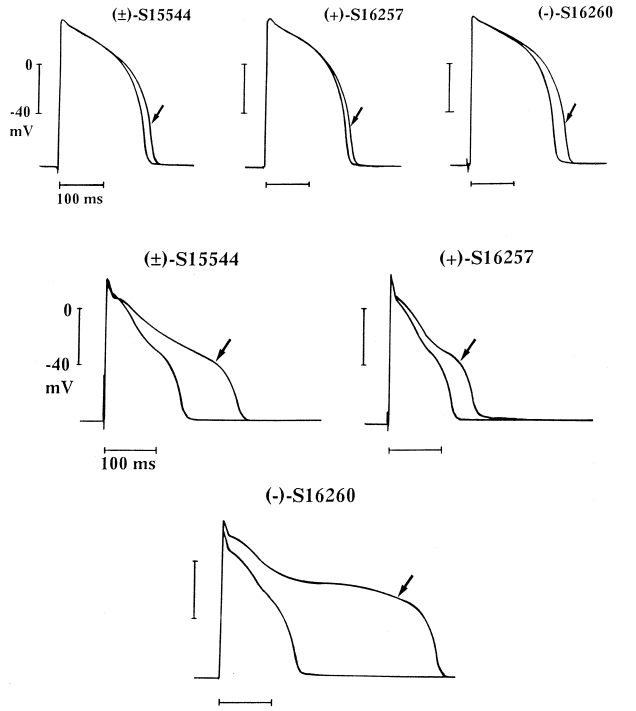

Fig. 5. Top panel: representative recordings of maximal effects of (±)-S 15544 and its enantiomers (+)-S 16257 and (−)-S 16260, on action potentials of guinea-pig papillary muscles. Recordings obtained after 30 min exposure to 3 μM compounds (arrows) are superimposed to pre-drug control action potentials. The preparations were driven at 1 Hz. Bottom panel: representative recordings of action potentials in rabbit Purkinje fibers before and after (arrows) 40 min exposure to 10 μM (±)-S 15544 and its enantiomers (+)-S 16257 and (−)-S 16260. The preparations were driven at 0.25 Hz.

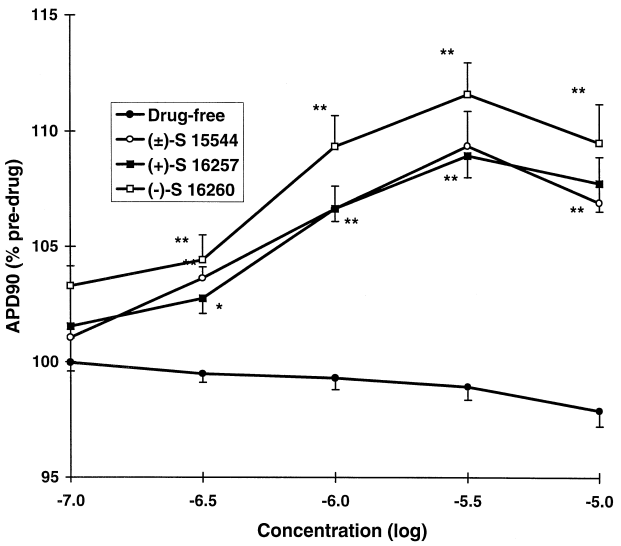

Fig. 4. Effects of (±)-S 15544 ($n=6$) and its enantiomers, (+)-S 16257 ($n=11$) and (−)-S 16260 ($n=8$) on action potential duration at 90% repolarization (APD_{90}), in ventricular preparations. Comparison with control drug-free experiments ($n=16$). The guinea-pig papillary muscles were driven at 1 Hz and exposed to cumulative concentrations of compounds (from 0.1 to 10 μM, 30 min exposure for each concentration). Values are expressed as means ± s.e.mean, as percent of pre-drug value, for n experiments. $^{*}P \leq 0.05$; $^{**}P \leq 0.01$: significance of differences from drug-free experiments.

3.5. Bradycardic effect in anesthetized pigs

The bradycardic effects of (+)-S 16257 or (−)-S 16260 were compared in anesthetized pigs during the administration of increasing doses of both compounds (from 0.03 to 1 mg kg^{-1} i.v.). All results were calculated as the percent variation from the control value recorded before the first dose. No statistical difference was observed between these control pre-drug values of heart rate: 99.9 ± 3.8, 104.0 ± 4.4 and 116.7 ± 5.1 beats min^{-1}, for vehicle, (+)-S 16257

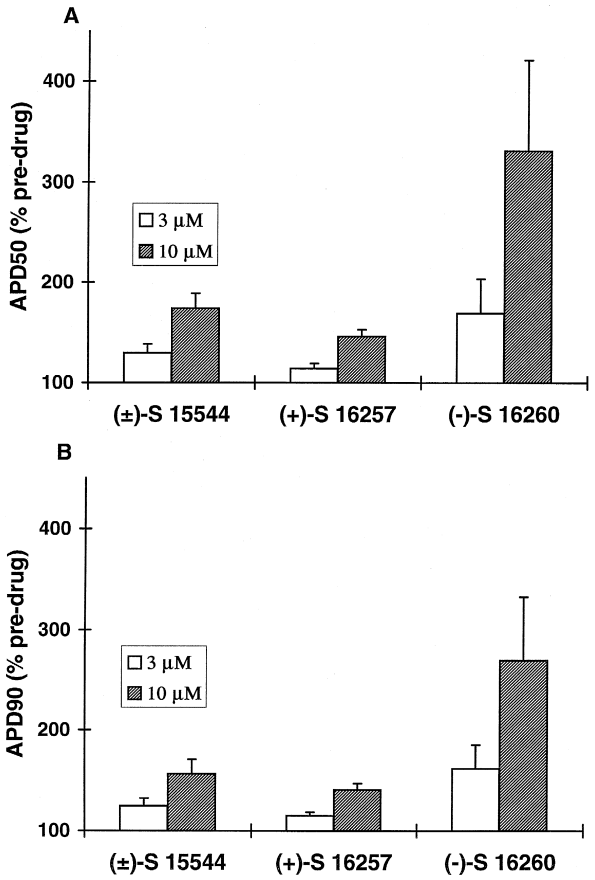

Fig. 6. Effects of (±)-S 15544 ($n = 5$) and its enantiomers, (+)-S 16257 ($n = 5$) and (−)-S 16260 ($n = 6$) on action potential duration at 50% (A) and 90% (B) repolarization (APD_{50} and APD_{90}, respectively) in rabbit Purkinje fibers driven at 0.25 Hz. Preparations were exposed for 40 min to 3 μM of compounds. Then, after washout for a minimum of one hour, a concentration of 10 μM was applied for 40 min. Values are expressed as means ± s.e.mean, as percent of pre-drug value, for n experiments. Significance of the differences between the effects in the treated groups at 10 μM (variance analysis): $P = 0.089$ and $P = 0.088$ for APD_{50} and APD_{90}, respectively.

and (−)-S 16260, respectively. As shown in Table 2 for both compounds, the threshold dose for a significant effect was 0.1 mg kg^{-1}: −15.7 ± 3.0% and −20.3 ± 3.6% reductions in heart rate for (+)-S 16257 and (−)-S 16260, respectively. At this dose the maximal bradycardia occurred at the mean time of 25.0 ± 1.7 and 11.5 ± 4.6 min, for (+)-S 16257 and (−)-S 16260, respectively. For higher concentrations, the maximal bradycardia occurred more rapidly (after 12.1 ± 3.4 and 5.5 ± 1.0 min at 0.3 mg kg^{-1}, for (+)-S 16257 and (−)-S 16260, respectively) and was increased in a dose-dependent way (Table 2). These effects were different from pre-drug values at the end of injection (1 min, Fig. 7) and remained significant ($P \leq 0.01$) thirty minutes post injection (14.5 ± 3.1% and 17.5 ± 4.9% at 0.1 mg kg^{-1} for (+)-S 16257 and (−)-S 16260, respectively). As shown in Table 2, both compounds induced quite similar bradycardia except for the higher dose (1 mg kg^{-1}) which induced a more marked reduction with (−)-S 16260.

3.6. Effect on corrected QT and PR interval in anesthetized pigs

The effects of (+)-S 16257 and (−)-S 16260 on QT_c and PR intervals were compared in anesthetized pigs. No statistical difference was observed between the control pre-drug values of QT_c intervals: 508.8 ± 26.2, 485.4 ± 27.1 and 512.7 ± 19.2 ms, for vehicle, (+)-S 16257 and (−)-S 16260, respectively. Similarly, no statistical difference was observed between the control pre-drug values of PR intervals: 101.0 ± 3.9, 99.6 ± 2.9 and 91.7 ± 6.0 ms, for vehicle, (+)-S 16257 and (−)-S 16260, respectively.

As shown in Fig. 7 and Table 2, administration of (+)-S 16257 did not change significantly the QT_c duration at any dose tested. In contrast, in the same range of doses, (−)-S 16260 induced a prolongation of the QT interval which remained significant after correction for heart rate: 11.9 ± 4.8% ($P \leq 0.05$) and 14.3 ± 8.2% ($P \leq 0.01$) at the doses of 0.3 and 1 mg kg^{-1}, respectively. This prolongation was maximal within the first min post-injection (Fig. 7) and did not remain significant at the time of maximal bradycardia (Table 2). No significant alteration of PR

Table 2
Effects of (+)-S 16257 and (−)-S 16260 on the electrocardiographic parameters of anesthetized pigs, at the time of maximal bradycardia

Doses	ΔHR (%)	ΔQT_c (%)	ΔPR (%)
Vehicle (ml kg^{-1}, $n = 10$)			
0.2	−2.38 ± 0.94	−0.21 ± 1.82	0.61 ± 1.15
0.2	−2.61 ± 1.81	1.96 ± 2.04	0.14 ± 1.55
0.2	1.08 ± 3.41	0.50 ± 1.13	−0.38 ± 1.16
0.2	0.86 ± 3.65	1.46 ± 1.27	−1.80 ± 1.52
(+)-S 16257 (mg kg^{-1}, $n = 10$)			
0.03	−8.03 ± 1.53	−1.04 ± 1.04	0.44 ± 1.32
0.1	−15.66 ± 3.00[a]	−0.02 ± 1.31	−2.34 ± 1.74
0.3	−27.14 ± 3.61[a]	2.39 ± 2.08	−0.67 ± 2.54
1	−36.82 ± 4.06[a]	4.75 ± 4.26	6.76 ± 3.35
(−)-S 16260 (mg kg^{-1}, $n = 6$)			
0.03	−7.00 ± 1.28	−0.23 ± 0.98	1.15 ± 2.45
0.1	−20.35 ± 3.62[a]	−0.75 ± 2.27	2.07 ± 3.25
0.3	−34.97 ± 5.24[a]	8.17 ± 4.23	3.92 ± 4.55
1	−48.63 ± 5.94[a,b]	9.48 ± 6.35	6.48 ± 6.44

Values are expressed as means ± s.e.mean as percent change from control value before the first dose or vehicle administration. ΔHR, maximal bradycardic effect; ΔQT_c, variation of QT_c interval; ΔPR, variation of PR interval. The parameters were evaluated at the time of maximal bradycardia for each dose.

[a] $P \leq 0.01$: significance of differences from vehicle.

[b] $P \leq 0.05$: significance of differences from the (−)-S 16257-treated group.

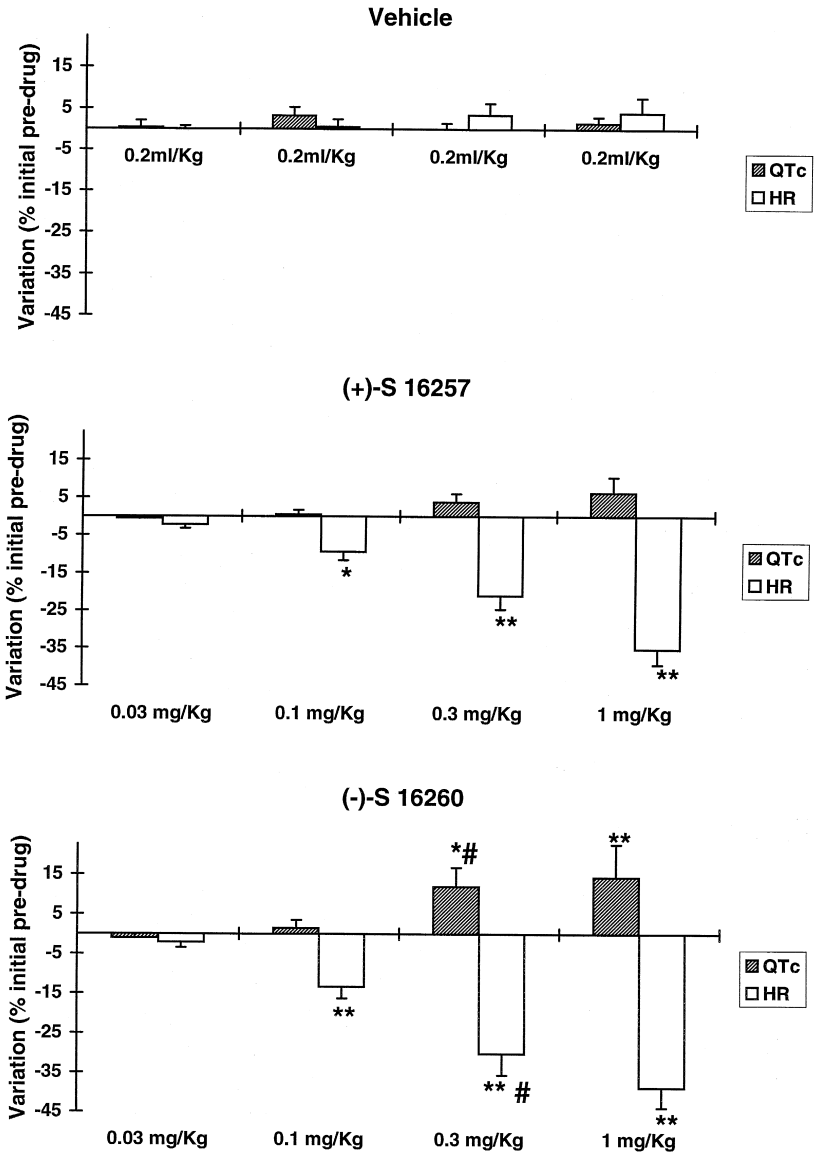

Fig. 7. Effects of (+)-S 16257 ($n = 10$) and (−)-S 16260 ($n = 6$) on QT_c interval and heart rate (HR) in anesthetized pigs. Values are expressed as means ± s.e.mean, as percent change from pre-drug value before the first administration, for n experiments. Variations of QT_c and HR, at 0.03, 0.1, 0.3 and 1 mg kg^{-1}, were compared with vehicle application ($n = 10$). Both parameters were evaluated 1 min after each dose (maximal QT_c prolongation). $^{*}P \leq 0.05$; $^{**}P \leq 0.01$: significance of differences from vehicle experiments. $^{\#}P \leq 0.05$: significance of differences from (−)-S 16257-treated group.

values was observed at the time of maximal effects on either the QT_c (1 min) or heart rate (Table 2). In Fig. 7, the reduction of heart rate at the time of maximal effect on QT interval (1 min) is shown simultaneously with QT_c prolongation.

4. Discussion

The electropharmacological profile of (+)-S 16257, a new sino-atrial node inhibitor, was compared with those of (±)-S 15544, the racemate compound, and (−)-S 16260, the other enantiomer, both in vitro and in vivo. Our results show that, in vitro, the three compounds had the same inhibiting effects on spontaneous action potential firing rate of rabbit sino-atrial node. These results are consistent with those of Perez et al. (1995) showing, in guinea-pig isolated right atria, a similar reduction of the spontaneous beating rate with both enantiomers. In the present study, the bradycardic effect was clearly induced by a reduction of the diastolic depolarization rate of pacemaker cells, with no change in maximal diastolic potential or threshold potential. At least five ionic currents are thought to be involved in the net inward current underlying spontaneous diastolic depolarization in the sino-atrial node (Irisawa et al., 1993): the decay of the outward potassium current (I_{Kr}, delayed rectifier), the slow activation of the inward hyperpolarizing-activated I_f current (mixed Na^+ and K^+),

the activation of two types of inward calcium currents, $I_{Ca,T}$, (transient) and $I_{Ca,L}$ (long-lasting) and activation of the time-independent background current, $I_{b,Na}$. The relative contribution of these currents to pacemaker depolarization is still being debated (Di Francesco, 1995b,c; Verheijck et al., 1995; Vassale, 1995). It is accepted, however, that one of the most important of these currents for the regulation of diastolic depolarization is the I_f pacemaker current (Irisawa et al., 1993; Di Francesco, 1995a; Di Francesco and Mangoni, 1994; Zaza et al., 1996). In the present study, reduction of the diastolic depolarization rate was probably the result of a block of the I_f pacemaker current. It was recently shown that (+)-S 16257 inhibits specifically the I_f current in rabbit sino-atrial node cells (Bois et al., 1996), in a use-dependent way, with no effect on other time-dependent currents involved in pacemaking (I_{Kr}, $I_{Ca,T}$, $I_{Ca,L}$) in the bradycardic concentration range. As expected for I_f blockers, the two enantiomers induced in vivo a marked bradycardia in anesthetized pigs. Both compounds reduced the heart rate to the same extent in a dose-dependent way, with a threshold dose of 0.1 mg kg^{-1} to obtain a significant effect. These results are in accordance with those of Simon et al. (1995) showing that (+)-S 16257 induced a dose-dependent bradycardia in conscious dogs, over the same range of doses. In humans, the results of the first intravenous phase I study of (+)-S 16257 have also shown that acute i.v. injection of this compound was accompanied by a deep, dose-dependent and exclusive decrease of maximal heart rate during exercise (Carre et al., 1995). In vitro, despite quite similar bradycardic effects on sino-atrial node preparations, the two isomers of (±)-S 15544 acted differently on action potential repolarization, with the smallest effect for (+)-S 16257. Since action potential duration is markedly dependent on rate (Surawicz, 1992), we have compared the effects of both isomers in driven preparations. In all preparations tested, while (+)-S 16257 had a weak effect on action potential repolarization, (−)-S 16260 prolonged more markedly the action potential duration. In guinea-pig papillary muscles, Perez et al. (1995) have shown small prolongations of APD_{50} and APD_{90} with both compounds (4.8 ms and 17.2 ms prolongations of APD_{90} at 5 μM, for (+)-S 16257 and (−)-S 16260, respectively) that did not reach statistical significance. The only significant effect of both compounds in this latter study, was inhibition of the maximal upstroke of the action potential (V_{max}) at very high concentrations (10 and 50 μM) and no alteration within the range of bradycardic concentrations. In our present study the prolongation of the repolarization time was more pronounced in Purkinje fibers driven at a slow rate, suggesting possible inhibition of the delayed rectifier (I_k) current (Surawicz, 1992) with (−)-S 16260, and a poor effect of (+)-S 16257 on this current. As recently shown by Bois et al. (1996), within the bradycardic concentration range, (+)-S 16257 has no effect on $I_{Ca,T}$, $I_{Ca,L}$ or I_{kr}. At higher concentrations (+)-S 16257 weakly inhibited the rapid component of the delayed rectifier current (I_{kr}). These results are well consistent with the small prolongation of action potential duration we now obtained with this compound. As described for class III antiarrhythmic agents, prolongation of action potential would lead to an increase of the QT interval (Carlsson et al., 1990; Rasmussen et al., 1992). In our in vivo study in anesthetized pigs, while both enantiomers had quite similar bradycardic effects i.e. about 30% reduction of heart rate at 0.3 mg kg^{-1} i.v., prolongation of the corrected QT interval was observed only with (−)-S 16260. On the contrary, (+)-S 16257 had no effect on QT_c, at any of the doses tested. The results obtained both in vitro and in vivo suggested a stereoselective alteration of the repolarization time. The effects of (±)-S 15544 on action potential duration were between those of its enantiomers, probably as a result of the mixture of both their effects. The optical property of ionic channels modulators could be determinant for their activity, as illustrated with the opposite effects on calcium channels described for (−)-R-202-791, a Ca^{2+} channel blocker, and (+)-S-202-791, the other enantiomer (Dolphin and Scott, 1989; Wei et al., 1989). Such opposite effects of isomers are quite unusual for agents that prolong action potential duration, as class III antiarrhythmic compounds; for example, no difference of effects on repolarization was observed with (D) and (L)-sotalol (Carmeliet, 1985; Singh, 1992). Argentieri et al. (1993) have shown that in isolated canine Purkinje fibers with action potentials shortened by isoproterenol, the class III activity of CK-4000 (the (*S*)-enantiomer of CK-3579) was significantly greater (approximatively two fold) than that of CK-4001 (the (*R*)-enantiomer). Under normal conditions all agents prolonged action potential duration with equal potency. Escande et al. (1992) also demonstrated that RP58866, a class III antiarrhythmic agent, and RP 62719 (Terikalant), its eutomer ((*S*)-(−) isomer), lengthened the action potential in a comparable manner. In contrast, the distomer, RP 62718 ((*R*)-(+) isomer), produced a three-fold lower effect at the same concentration. It would thus be of interest to study the effects of optical isomers of such compounds in order to increase or to prevent this activity on cardiac repolarization.

In conclusion, (±)-S 15544 is a potent sinus node inhibitor acting by inhibition of the pacemaker I_f current. Resolution of this agent into its two enantiomers yields compounds with similar bradycardic efficiency in vitro and in vivo, suggesting no stereoselectivity for the inhibition of I_f by the two optical isomers. These results also emphasize the important stereospecificity for action potential prolongation. Of the two isomers, (−)-S 16260 induced lengthening of the action potential in all cardiac preparations tested while (+)-S 16257 had a weak effect on action potential repolarization time. Moreover, in vivo, only (−)-S 16260 increased the QT_c interval. The results showed that the novel, specific bradycardic agent, (+)-S 16257, has a higher electrophysiological specificity than (−)-S

16260. Since the I_f current plays a key role under conditions of increased adrenergic tone, the high specificity of (+)-S 16257 for this current would be of interest for clinical use. Furthermore, its very weak effects on other ionic channels would be in favor of increased safety.

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