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Electrophysiological effects of S 16257, a novel sino-atrial node modulator, on rabbit and guinea-pig cardiac preparations: comparison with UL-FS 49

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1 S 16257 is a new bradycardic agent. Its electropharmacological profile has been compared to that of the known bradycardic compound UL-FS 49 (Zatebradine). Intracellular recordings of action potentials (APs) were performed with conventional glass microelectrodes.

2 In the rabbit isolated sino-atrial node (SAN) tissue, S 16257 and UL-FS 49 (1 μM, 3 μM and 10 μM) were equipotent in slowing spontaneous APs firing predominantly by decreasing the rate of diastolic depolarization (at 3 μM, $-23.8 \pm 3.9\%$ and $-27.9 \pm 2.6\%$, respectively). For the two compounds a maximal effect was obtained at 3 μM. In these preparations, action potential duration at 50% of total repolarization (APD_{50}) was more affected by UL-FS 49 than S 16257 at any concentration tested (at 3 μM, $+8.9 \pm 2.9\%$ and $+29.1 \pm 3.7\%$ for S 16257 and UL-FS 49, respectively; $P \leqslant 0.01$).

3 To estimate the direct effects on AP duration, driven cardiac preparations were exposed to these agents. In guinea-pig papillary muscles, paced at a frequency of 1 Hz, increasing concentrations of S 16257 or UL-FS 49 (0.1 to 10 μM, 30 min exposure for each concentration) slightly prolonged AP repolarization. This prolongation was more marked for UL-FS 49 (at 1 μM, $+6.1 \pm 0.6\%$ and $+11.2 \pm 1.3\%$ elevation of APD_{50}, for S 16257 and UL-FS 49, respectively).

4 Application of UL-FS 49 (3 μM) to rabbit Purkinje fibres, triggered at a frequency of 0.25 Hz, induced a marked prolongation of APD_{50} and APD_{90} ($+149.4 \pm 51.2\%$ and $+86.0 \pm 15.4\%$, respectively). S 16257 (3 μM) induced only a weak prolongation of AP ($+14.1 \pm 5.0\%$ and $+14.8 \pm 3.3\%$ for APD_{50} and APD_{90}, respectively) significantly smaller than in the case of UL-FS 49.

5 These results show that S 16257 slows the rate of spontaneous AP firing in isolated SAN mainly by a reduction of the diastolic depolarization of the cells, which suggests an inhibition of the pace-maker current (I_f). S 16257 and UL-FS 49 are equipotent in their bradycardic effect but S 16257 is more specific as it induces less increase in myocardial repolarization time.

Keywords: S 16257; UL-FS 49; specific bradycardic agent; action potential; sino-atrial node; Purkinje fibres; papillary muscles

Introduction

Myocardial ischaemia, whatever may be its clinical expression, always results from an imbalance between oxygen supply and demand. This imbalance may lead to irreversible myocardial damage. As heart rate is one of the major determinants of myocardial oxygen consumption (Laurent *et al.*, 1956; Sonnenblick *et al.*, 1968), agents able to reduce sinus heart rate are of major interest for the treatment of ischaemic heart diseases. This can be achieved with β-adrenoceptor antagonists or some calcium channel blockers; however, these agents may exert concomitant negative inotropic and hypotensive effects (Opie, 1989; Kern *et al.*, 1989), potentially deleterious during ischaemia. Recently the pharmacological properties of a novel class of substances, specific bradycardic agents (SBAs), have been described (Kobinger & Lillie, 1987). Such agents induce sinus bradycardia at concentrations that are devoid of additional haemodynamic effects (Krumpl *et al.*, 1986; 1988; Franke *et al.*, 1987; Raberger *et al.*, 1987a; Van Woerkens *et al.*, 1992). SBAs have been shown to act by reducing the oxygen demand of the heart and by increasing the diastolic period which induces an elevation of the subendocardial blood flow (Harron *et al.*, 1982; Raberger *et al.*, 1987b; Indolfi *et al.*, 1989). One of the most potent SBAs described so far, UL-FS 49 (Zatebradine), has been reported to slow the action potentials (APs) firing of the pacemaker cells, via an inhibition of the hyperpolarization activated I_f current (Van Bogaert & Gothals, 1987; 1992; Van Bogaert *et al.*, 1990).

In the present paper, we describe the electropharmacological profile, in isolated cardiac preparations, of a new sinus node inhibitor, S 16257 (7,8-dimethoxy 3-{3-{[(1*S*)-(4,5-dimethoxybenzocyclobutan-1-yl) methyl] methylamino}propyl} 1,3,4, 5-tetrahydro-2*H*-benzazepin 2-one). The effects of S 16257 are compared to those of UL-FS 49, on sinoatrial node cells, papillary muscles and Purkinje fibres.

Methods

Isolated cardiac preparations

Governmental and institutional guidelines for the care and the use of animals were followed at all times.

Male New Zealand White rabbits and Hartley guinea-pigs were stunned by a blow on the head. After exanguination, hearts were rapidly removed and placed in an oxygenated Tyrode solution at 4°C. The cardiac preparations were excised from the right ventricle (papillary muscle or Purkinje fibre) or right atrium (sinus node tissue) and mounted in a tissue bath (3.5 ml). The isolated preparations were superfused with oxygenated Tyrode solution at a constant flow rate (5 ml min^{-1}). The temperature was kept at 36°C ± 0.5.

For guinea-pig papillary muscle, the mural end of the preparation was pinned to the base of the experimental chamber and the tendinous end was connected to a Gould UC2 force transducer via a fine silk thread to record the isometric tension. The muscles were carefully stretched until the peak of the length-tension relationship was reached. Rab-

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bit sino-atrial node tissue and Purkinje fibre were carefully pinned to the base of the experimental chamber.

Electrical recordings

The papillary muscles and the Purkinje fibres were stimulated at a basal frequency of 1 Hz with rectangular pulses of 2 ms duration, twice threshold intensity, applied through two platinum electrodes. The stimuli were delivered to the stimulating electrodes from a Grass S88 stimulator through a Digitimer Ds2 stimulus isolation unit. All the preparations were equilibrated with the solution for at least 2 h before starting the study.

Transmembrane potentials were recorded with conventional glass microelectrodes filled with 3 M KCl (tip resistance of 18–22 MΩ) and connected to a high input impedance Biologic VF180A amplifier. The membrane potential was displayed on a Tektronix 2230 digital storage oscilloscope. Membrane potential and isometric force (for papillary muscles) were continuously recorded on a Gould RS3400 pen chart recorder. Storage and analysis of both signals were also performed with specific software (Clovis, CLOD Sarl), installed on a personal computer, equipped with a 12 bit analog-digital DAS50 converter. All experimental results were obtained from continuous impalements of single cells throughout the whole experiment.

Experimental protocol

Each sino-atrial node preparation was exposed to S 16257 or UL-FS 49 at one fixed concentration, for 40 min. Then a 60 min wash out of the drug was performed. The concentrations tested for both substances were 1 μM, 3 μM and 10 μM.

Papillary muscles were exposed to cumulative concentrations of S 16257 or UL-FS 49 in the following sequence: 0.1 μM, 0.3 μM, 1 μM, 3 μM and 10 μM, 30 min exposure for each concentration.

After reducing the rate of pacing from 1 Hz to 0.25 Hz, Purkinje fibres were exposed to S 16257 or UL-FS 49, at 3 μM, for 40 min. Then a wash-out of the drug was performed (minimum of 1 h), but in some preparations no reversibility was obtained, even after 2 h of wash-out. Then the preparations were exposed to S 16257 or UL-FS 49 at 10 μM for 40 min.

Drugs and solutions

The control Tyrode solution contained the following (in mM): NaCl 130, KCl 5.6, $CaCl_2$ 2.15 and 1.8 for guinea-pig and rabbit preparations, respectively, NaH_2PO_4 0.6, $NaHCO_3$ 20, $MgCl_2$ 1.1 and glucose 11. The pH of the Tyrode solution was 7.4 after bubbling with O_2/CO_2 (95:5, v/v).

UL-FS 49 (7,8-dimethoxy 3-{3-{[2-(3,4-dimethoxyphenyl)-ethyl]methylamino}-propyl}1,3,4,5-tetrahydro-2*H*-benzazepin 2-one dihydrochloride) and S 16257 (7,8-dimethoxy 3-{3-{[(1*S*)-(4, 5-dimethoxybenzocyclobutan-1-yl) methyl] methylamino} propyl}1,3,4,5-tetrahydro-2*H*-benzazepin 2-one hydrochloride) were synthesized in the Institute. Both molecules as a powder were initially dissolved in distilled water (0.1 mM). Further dilutions were carried out in Tyrode solution.

Statistical analysis

Values are expressed as means ± s.e.mean. Statistical significance was evaluated by a two-way analysis of variance with repeated measures. One way complementary analysis followed by a Newman-Keuls test was performed at fixed times of superfusion (or at fixed concentrations for papillary muscles). Differences were considered significant for $P \leqslant 0.05$. Five to 11 experiments were performed for each group.

Results

Bradycardic effects of S 16257 and UL-FS 49 on rabbit SAN preparations

Superfusion of rabbit isolated sino atrial node preparations with S 16257 induced a reduction of spontaneous action potentials (APs) firing. A maximal bradycardic effect was obtained after a 40 min exposure to 3 μM, for both agents (− 23.8 ± 3.9% and − 27.9 ± 2.6%, for S 16257 and UL-FS 49, respectively). As shown in Figure 1, during application of S 16257, the reduction of APs firing was of same order of magnitude as that induced by UL-FS 49, at any concentration tested (for example, − 12.3 ± 5.2% and − 8.6 ± 1.3% after a 40 min exposure to 1 μM S 16257 and UL-FS 49, respectively).

Reduction of the diastolic depolarization rate by S 16257 and UL-FS 49, in rabbit SAN preparations

As shown in Table 1 and Figure 2, the bradycardic effect of S 16257 was predominantly mediated by a reduction in the rate of diastolic depolarization, thus increasing the cycle

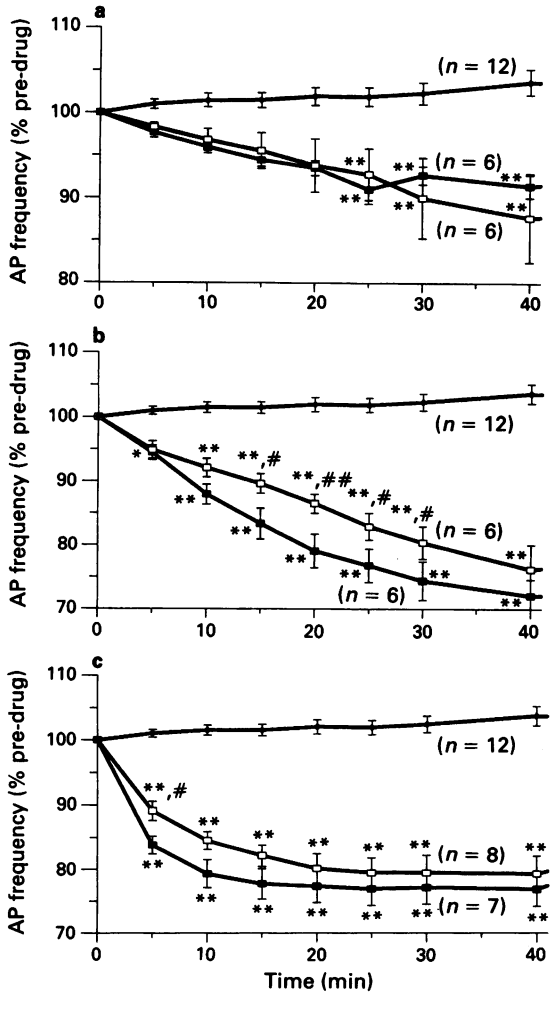

Figure 1 Reduction of spontaneous action potentials (APs) firing in rabbit sino-atrial node during a 40 min exposure to S 16257 (□) or UL-FS 49 (■). Bradycardic agents were applied at 1 μM (a), 3 μM (b) and 10 μM (c). Data are expressed as means ± s.e.means for *n* preparations. Values are expressed as a percentage of the initial rate just before application of the drugs. (◆) Drug-free experiments (same control group for a, b and c). $^{*}P \leqslant 0.05$; $^{**}P \leqslant 0.01$: significance of differences from drug-free superfusate. $^{\#}P \leqslant 0.05$; $^{\#\#}P \leqslant 0.01$: significance of differences between the two treated-groups.

Table 1 Comparative effects of S 16257 and UL-FS 49 on action potential parameters of rabbit pace-maker cells

	DDR (mV s^{-1})		*MDP* (− mV)		*Thr Pot* (− mV)	
	Pre-drug	Δ 40 min	Pre-drug	Δ 40 min	Pre-drug	Δ 40 min
Drug-free						
($n = 12$)	54.5 ± 8.6	0.3 ± 0.8	70.3 ± 1.2	− 0.3 ± 1.6	60.2 ± 1.9	0.1 ± 1.4
S 16257						
1 μM ($n = 6$)	67.9 ± 9.1	− 21.2 ± 3.6**	68.0 ± 2.7	− 1.3 ± 4.0	55.3 ± 3.5	0.8 ± 4.0
3 μM ($n = 6$)	53.8 ± 5.0	− 36.1 ± 1.8**	71.5 ± 2.6	1.0 ± 1.1	60.2 ± 3.5	6.5 ± 2.1*
10 μM ($n = 8$)	83.0 ± 11.0	− 42.7 ± 5.1**	66.3 ± 1.4	− 1.5 ± 1.6	57.0 ± 1.3	1.6 ± 1.8
UL-FS 49						
1 μM ($n = 6$)	76.0 ± 11.6	− 32.1 ± 3.9**††	70.8 ± 1.1	0.8 ± 2.3	56.2 ± 3.8	4.2 ± 3.3
3 μM ($n = 6$)	72.2 ± 15.9	− 46.5 ± 7.3**†	69.3 ± 2.2	1.0 ± 2.3	55.7 ± 2.4	7.5 ± 1.4*
10 μM ($n = 7$)	78.0 ± 13.5	− 10.7 ± 12.7††	67.4 ± 2.0	− 5.0 ± 1.5	57.7 ± 2.2	− 3.1 ± 2.3

Bradycardic agents were applied at 1 μM, 3 μM or 10 μM, for 40 min. Data are expressed as means ± s.e.mean for n preparations. DDR: diastolic depolarization rate; MDP: maximal diastolic potential; Thr Pot: threshold potential.
*$P<0.05$; **$P<0.01$: significance of differences from drug-free superfusate.
†$P<0.05$; ††$P<0.01$: significance of differences between the two treated groups.

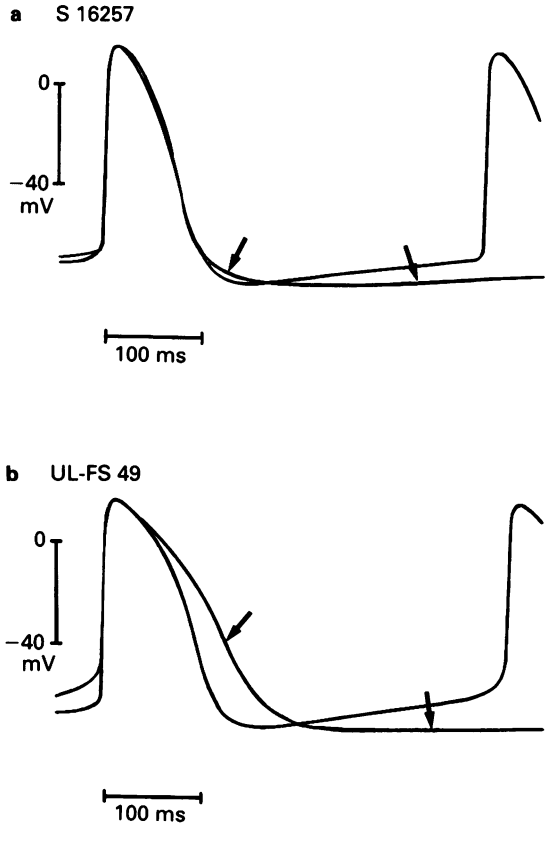

Figure 2 Representative recordings of spontaneous action potentials (APs) during a 40 min exposure of rabbit sinus node preparations to S 16257 (a) and UL-FS 49 (b) at 3 μM. Arrows show APs after the 40 min exposure to bradycardic agents. The other traces are the respective baseline APs before drug application.

length of the beating preparations. The slope of the diastolic depolarization phase was reduced from 67.9 ± 9.1 to 46.7 ± 5.6 mV s^{-1} and from 76.0 ± 11.6 to 43.9 ± 11.2 mV s^{-1} for S 16257 (1 μM) and UL-FS 49 (1 μM), respectively. At the higher concentration, an increase of diastolic depolarization rate (DDR) was observed with UL-FS 49. This reduction in DDR occurred without any significant change in maximal diastolic potential. Threshold potential was slightly modified, as shown in Table 1: 6.5 ± 2.1 mV and 7.5 ± 1.4 mV more negative after a 40 min exposure to S 16257 (3 μM) and UL-FS 49 (3 μM), respectively.

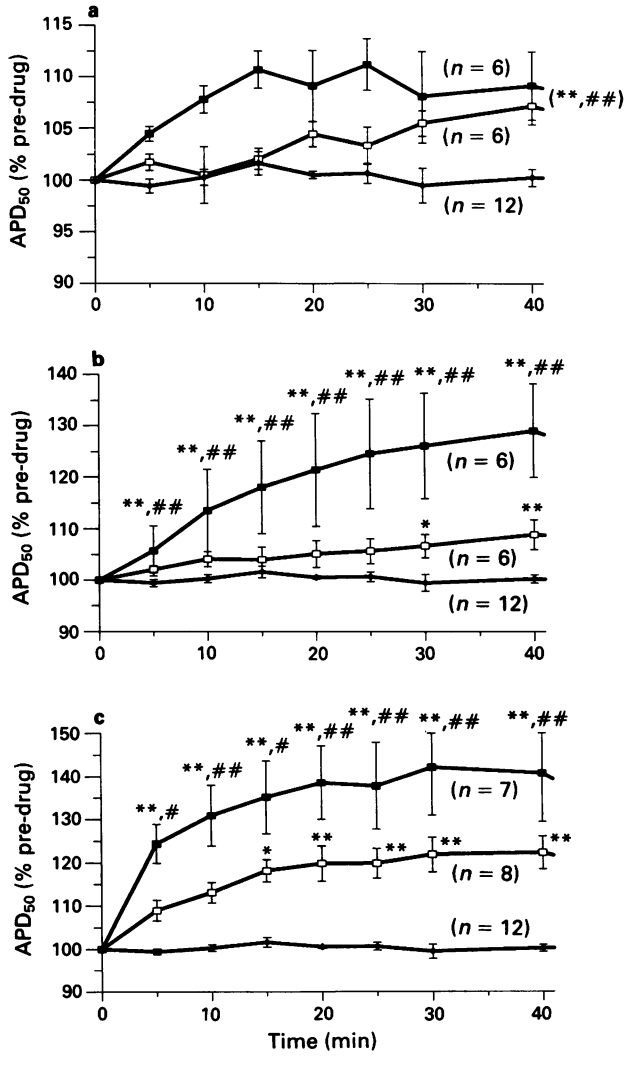

Figure 3 Prolongation of spontaneous action potential duration at 50% of repolarization (APD_{50}) in rabbit sino-atrial node, during a 40 min exposure to S 16257 (□) or UL-FS 49 (■). Bradycardic agents were applied at 1 μM (a), 3 μM (b) and 10 μM (c). Data are expressed as mean ± s.e.mean for n preparations. Data are expressed as the percentage of the initial value just before application of the drugs. (◆) Drug-free experiments (same control group for a, b and c). (**,**)$P \leqslant 0.01$: significance of differences from drug-free superfusate profile and between the two treated-groups profile. *$P \leqslant 0.05$; **$P \leqslant 0.01$: significance of differences from drug-free superfusate at fixed times. *$P \leqslant 0.05$; **$P \leqslant 0.01$: significance of differences between the two treated-groups at fixed times.

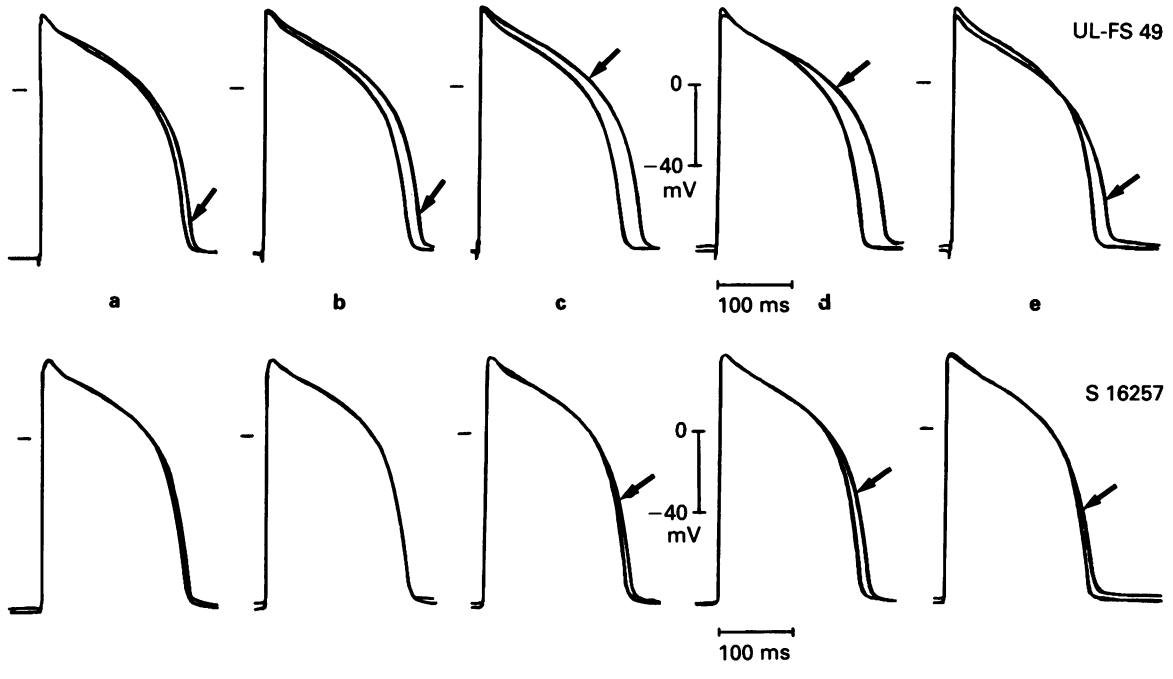

Figure 4 Comparative effects of S 16257 and UL-FS 49 on action potentials (APs) of guinea-pig papillary muscles. Concentrations of bradycardic agents were applied cumulatively (from 0.1 μM to 10 μM, 30 min exposure for each concentration). Representative recordings of APs after a 30 min exposure to UL-FS 49 (top panels) and S 16257 (bottom panels) at 0.1 μM (a), 0.3 μM (b), 1 μM (c), 3 μM (d) and 10 μM (e). Arrows show APs after the 30 min exposure to bradycardic agents. The other traces are the respective baseline APs before drug application.

Prolongation of AP repolarization by S 16257 and UL-FS 49, in rabbit SAN preparations

As shown in Figures 2 and 3, AP duration measured at 50% repolarization (APD_{50}) was augmented dose-dependently during application of the bradycardic agents. This AP prolongation was more pronounced with UL-FS 49 at any concentration tested. For example, during superfusion with 3 μM S 16257 and UL-FS 49, increases of APD_{50} of 8.9 ± 2.9% and 29.1 ± 3.7% respectively, were observed.

Prolongation of AP duration by S 16257 and UL-FS 49, in guinea-pig papillary muscles

To eliminate the contribution of changes in frequency in AP prolongation, increasing concentrations of S 16257 and UL-FS 49 were applied to driven guinea-pig papillary muscles (from 0.1 to 10 μM, 30 min exposure for each concentration). During the application of these compounds, no modification of AP amplitude, resting potential and dV/dt_{max}, in comparison with drug-free experiments, were noted (control values for drug-free experiments: 122.4 ± 0.8 mV, − 84.1 ± 0.3 mV and 214.2 ± 10.1 V s^{-1}, for the three parameters, respectively). All AP duration parameters were increased significantly. As illustrated in Figure 4, this augmentation of the repolarization time was maximal during exposure to 3 μM (elevations of APD_{90} of + 9.0 ± 0.9% and + 13.0 ± 1.1% for S 16257 and UL-FS 49, respectively). From 0.3 to 3 μM, this effect was more pronounced during UL-FS 49 exposure ($P \leqslant 0.01$). In control conditions, during hours of perifusion a run down of the isometric tension was observed (from 187.7 ± 27.0 mg to 128.4 ± 20.0 mg after 150 min perifusion). This reduction was less pronounced during application of UL-FS 49 (0.3 and 1 μM) and 1 μM S 16257 (no difference between the two treated groups).

Prolongation of AP duration by S 16257 and UL-FS 49, in rabbit Purkinje fibres

Rabbit Purkinje fibres, paced at a low rate (0.25 Hz), were exposed to 3 μM S 16257 or UL-FS 49 solutions, for closer examination of the effect of the bradycardic agents on AP duration. As shown in Figure 5, APD_{50} and APD_{90} of these preparations were markedly augmented by UL-FS 49 (3 μM). S 16257 had only a weak effect on AP repolarization time. The time needed for recovery when the drug was washed out was related to the effect on APD observed during application. In some preparations exposed to UL-FS 49, no reversibility was observed, even after 2 h of wash out. Application of an increased concentration (10 μM), after wash out of the drug (3 μM), induced a more marked prolongation of AP, as shown in Figure 6.

Discussion

The present paper describes the electropharmacological profile of a new specific bradycardic agent, S 16257. The results show that S 16257 reduced the spontaneous action potentials (APs) firing of the pacemaker cells to the same extent as the well known SBA, UL-FS 49. There are four ways of reducing the heart sinus rate: (a) by prolonging AP repolarization time; (b) by reducing maximal diastolic potential (more electronegative potential); (c) by shifting the threshold potential to a more positive level; and (d) by slowing the rate of diastolic depolarization. Experiments performed in rabbit sinoatrial node (SAN) preparations showed that S 16257, as UL-FS 49, acted mainly by reducing the slope of diastolic depolarization, without significant change in maximal diastolic potential. Although the threshold potential was slightly modified by both drugs (potential more electronegative in some preparations), this variation could not account for the

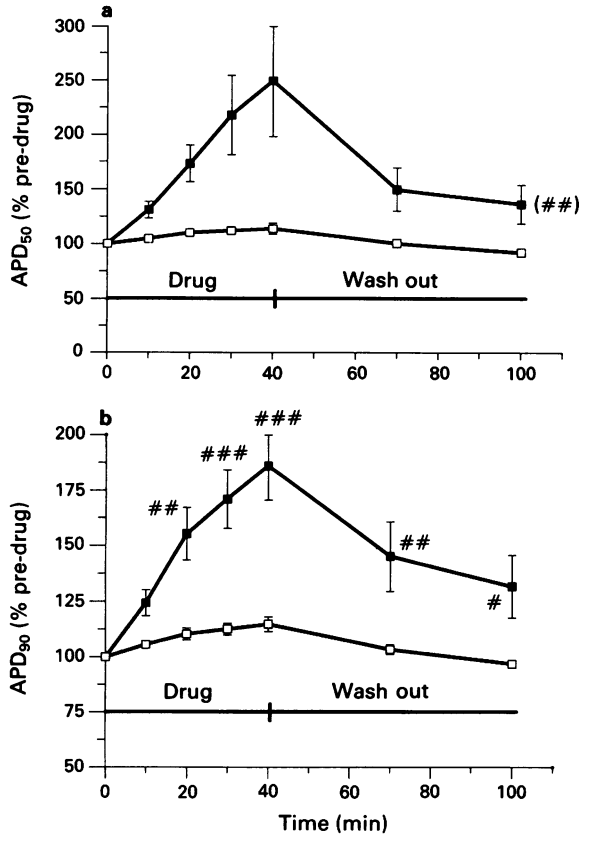

Figure 5 Prolongation of action potential duration at 50% (a) and 90% (b) of repolarization (APD_{50} and APD_{90}, respectively) in rabbit Purkinje fibres, during a 40 min exposure to S 16257 (□) or UL-FS 49 (■). Bradycardic agents were applied at 3 μM. Data are expressed as mean ± s.e.mean for *n* preparations. Data are expressed as the percentage of the initial value just before application of the drugs. (**)$P \leq 0.01$: significance of differences between the two treated-groups profiles. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$: significance of differences between the two treated-groups at fixed times.

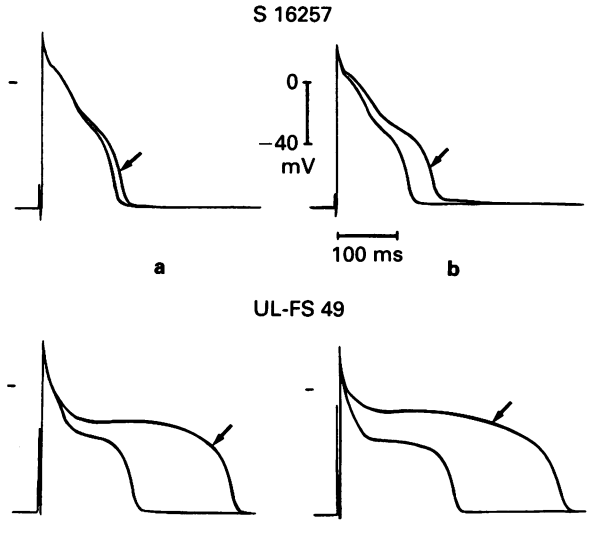

Figure 6 Comparative effects of S 16257 and UL-FS 49 on action potentials (APs) of rabbit Purkinje fibres. Representative recordings of APs after a 40 min exposure to S 16257 (top panels) and UL-FS 49 (bottom panels) at 3 μM (a, left panels) and at 10 μM (b, right panels), in two preparations. Arrows show APs after the 40 min exposure to bradycardic agents. The other traces are the respective baseline APs just before drug application (control and wash-out of 3 μM for (a) and (b), respectively).

bradycardic effect because it did not move in the way of reducing spontaneous APs frequency. Furthermore, this phenomenon could limit bradycardia to physiological values (about 25–30% reduction). However, we must point out that the threshold potential was not easy to determine, since in some cells the AP onset lacked a precise inflection point between diastolic depolarization and upstroke of the AP, and was characterized by a progressive acceleration of the rate of depolarization. The observed modification in threshold may be the result of a change in the way that recorded cells were driven by the other nodal cells. The bradycardic action obtained with UL-FS 49 on rabbit SAN preparations agreed with the results of experiments performed in other laboratories (Kobinger & Lillie, 1984; Lillie & Kobinger, 1986; Doerr & Trautwein, 1990).

S 16257, in rabbit SAN preparations, had less effect than UL-FS 49 on AP repolarization at any concentration tested, despite a quite similar bradycardic action. The marked increase in AP duration during application of UL-FS 49 could contribute to some extent to the bradycardia induced by this agent.

Because the duration of the AP in cardiac cells is very sensitive to the rate, the increase in APD_{50} during exposure to UL-FS 49, could be the consequence of the increase in cycle length, although this AP prolongation was very limited with S 16257, for a similar bradycardia. Therefore, we exposed driven guinea-pig papillary muscles to these agents with the aim of evaluating their direct effects on cardiac AP repolarization. These preparations are not the most sensitive for AP prolongation but are the most well known cardiac preparations. In these cardiac muscles, S 16257 and UL-FS 49 caused a concentration-dependent increase in AP duration from 0.1 μM to 1 μM. We found significantly more pronounced effect with UL-FS 49, at any concentration tested.

The cardiac AP duration may be enhanced by an increase of inward currents, e.g. Ca^{2+} current, and/or a decrease of outward currents, e.g. K^+ current. Among ionic currents implicated in AP repolarization, the potassium delayed rectifier current (I_K) is one of the major determinants of AP duration (Anumonwo *et al.*, 1991). Therefore a moderate class III antiarrhythmic effect of UL-FS 49 could not be excluded. Carmeliet (1985) have described prolongation of AP in guinea-pig papillary muscles with a moderate class III agent, sotalol, to a similar extent to that induced by UL-FS 49 in our study. Although this alteration is small in these preparations, prolongation of AP was demonstrated with sotalol in man (Echt *et al.*, 1982). Generally I_K is more prevalent in Purkinje fibres than in ventricular cells (Surawicz, 1992) and effects of inhibiting agents are more marked in these preparations (Carmeliet, 1985; Li *et al.*, 1990; Gwilt *et al.*, 1991; Abrahamsson *et al.*, 1993). Therefore we have exposed rabbit Purkinje fibres, driven at a slow rate, to the bradycardic agents. Our results indicate that UL-FS 49 markedly prolonged the duration of phase 2 (APD_{50}) and phase 3 (APD_{90}) of AP repolarization, with a more pronounced effect on phase 2. Because block of I_K prolongs the duration of AP predominantly by lengthening phase 2 and this effect is more pronounced in Purkinje fibres and at longer cycles (Surawicz, 1992), inhibition of I_K would be expected. The exact ionic mechanism(s) underlying this effect of UL-FS 49 could be clarified by using the patch clamp technique.

In conclusion, S 16257 is a novel potent SBA, acting by reducing AP firing of pacemaker cells. Reduction of the diastolic rate of depolarization suggests a similar mechanism of action to that of UL-FS 49 (Van Bogaert & Goethals, 1987; 1992; Van Bogaert *et al.*, 1990): inhibition of the inward hyperpolarization-activated current which is one of the most important currents in pacemaking (Di Francesco, 1991; Irisawa *et al.*, 1993). Studies of inhibition of the I_f current by S 16257 in isolated cells, by use of the patch clamp technique, are in progress.

In comparison with UL-FS 49, S 16257 has less effect on AP repolarization of all cardiac preparations used. This

difference of action on AP should mean that it is safer to use S 16257 than UL-FS 49. Indeed, early after depolarizations (EADs) and EADs-triggered activity have been suggested as a possible cause of polymorphic ventricular tachyarrhythmias, better known as 'Torsades de pointes' (El-Sherif *et al.*, 1988; Wit, 1990; Libersa *et al.*, 1992) and are more likely to occur when action potential duration is prolonged and when the heart rate is slow (Damiano & Rosen, 1984; Levy, 1989).

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