Pharmacological profile of SL 59.1227, a novel inhibitor of the sodium/hydrogen exchanger

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1 The NHE1 isoform of the Na⁺/H⁺ exchanger plays an important role in the regulation of intracellular pH and in cardiac cell injury caused by ischaemia and reperfusion. SL 59.1227 is a novel imidazolylopyridine Na⁺/H⁺ antiporter inhibitor which is structurally unrelated to previously described acylguanidine inhibitors such as cariporide.

2 Recovery of pH_i following an intracellular acid load was measured in CCL39-derived PS120 variant cells, selectively expressing either NHE1 or NHE2 isoforms of the Na⁺/H⁺ exchanger. pH_i recovery was potently and selectively slowed by SL 59.1227 in NHE1-expressing cells (IC₅₀ 3.3 ± 1.3 nM) *versus* NHE2-expressing cells (2.3 ± 1.0 μM). The respective IC₅₀ values for cariporide were 103 ± 28 nM (NHE1) and 73 ± 46 μM (NHE2).

3 In anaesthetized rats following left coronary artery occlusion (7 min) and reperfusion (10 min) SL 59.1227 (10–100 μg kg⁻¹ min⁻¹ i.v.) inhibited ischaemia-mediated ventricular tachycardia (71–100%) and reperfusion-induced ventricular fibrillation (75–87%) and prevented mortality. Bolus i.v. administration of SL 59.1227 (1 mg kg⁻¹) produced anti-arrhythmic effects when administered either before or during ischaemia.

4 Cardiac infarct size was determined in anaesthetized rabbits following left coronary artery occlusion (30 min) and reperfusion (120 min). Infarct size measured as a percentage of the area at risk was 36.2 ± 3.4% (control group) *versus* 15.3 ± 3.9% (SL 59.1227 0.6 mg kg⁻¹ i.v.).

5 SL 59.1227 is the first example of a potent and NHE1-selective non-acylguanidine Na⁺/H⁺ exchanger inhibitor. It possesses marked cardioprotective properties.

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Abbreviations: AAR, area at risk; BCECF, 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester; ECG, electrocardiogram; HR, heart rate; MAP, mean arterial pressure; NHE, sodium/hydrogen exchanger; NMDG, N-methyl-D-glucamine; TTC, 2,3,5-triphenyltetrazolium chloride

Introduction

During acute myocardial ischaemia, ATP degradation and accumulation of end products of anaerobic metabolism lead to a profound intracellular acidosis (Dennis *et al.*, 1991). The ubiquitous sodium-hydrogen antiporter (NHE1), which is largely quiescent under physiological conditions, is therefore stimulated, resulting in proton extrusion and concomitant sodium influx (Frelin *et al.*, 1984). The subsequent rise in intracellular sodium may stimulate the Na⁺/K⁺ ATPase, thus accelerating degradation of ATP stores in ischaemia. This process will generate more protons and create a vicious circle as long as substantial amounts of ATP are available, contributing to intracellular accumulation of Na⁺ during ischaemia and to a coupled increase in Ca²⁺ *via* Na⁺/Ca²⁺ exchange. Restoration of flow during reperfusion generates a large transsarcolemmal pH gradient and induces further NHE1 activation, H⁺ extrusion, and Na⁺ entrance. In the face of Na⁺/K⁺ ATPase inhibition caused by ischaemia, Na⁺_i increases strongly which results in intracellular Ca²⁺ overload through reversal or inhibition of Na⁺/Ca²⁺ exchange. The combined accumulation of

Na⁺_i and Ca²⁺_i is thought to induce cardiac arrhythmia, myocardial stunning and irreversible cell injury (Ladzunski *et al.*, 1985; Karmazyn, 1988; Tani & Neely, 1989; Meng & Pierce 1990; Murphy *et al.*, 1991; Pike *et al.*, 1993; Liu *et al.*, 1997; Park *et al.*, 1999). Therefore, the activation of NHE1, while essential for restoration of normal intracellular pH, will result in a deleterious cation overload. Experimental evidence indicates that pharmacological NHE1 inhibition by agents including the diuretic amiloride, its N-5 substituted analogues and more recently acyl guanidine derivatives such as cariporide (Scholz *et al.*, 1995) mediates myocardial protection during ischaemic and reperfusion injury (see for review Fröhlich & Karmazyn, 1997; Avkiran, 1999). The most recently described NHE1 inhibitors possess better selectivity towards NHE1 compared with other isoforms of the exchanger or ion transporting systems such as Na⁺/Ca²⁺ exchanger, Ca²⁺ or K⁺ channels. However, they share the same monocyclic acylguanidine structure found in the amiloride-based exchanger inhibitors (Duff *et al.*, 1991; Ruiz-Petrich *et al.*, 1996; Scholz *et al.*, 1993, 1995). In this study we report the pharmacological characterization of 3-[(cyclopropylcarbonyl)amino]-N-[2-(dimethylamino)ethyl]-4-[4-(5-methyl-1H-imidazol-4-yl)]piperidin-1-yl]benzamide, SL 59.1227, a novel NHE1

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inhibitor, which is structurally unrelated to the acylguanidine derivatives (Figure 1).

Methods

The animals used in these experiments were treated in accordance with ethical guidelines edited by the European community (EC directive 86/609), the council of Europe (Convention ETS 123) and the French government (decree of 19.10.87).

Effects on pHi in stably transfected hamster fibroblast cell lines expressing NHE1 and NHE2 isoforms

CCL39-derived PS120 variant cells selectively expressing either the cardiac major isoform NHE1 or the epithelial isoform NHE2 of the Na^+/H^+ antiporter were cultured in small Petri dishes and seeded at a density of 1 to 3.10^6 cells/dish in culture medium containing 10% foetal bovine serum. Penicillin-streptomycin (100 u ml^{-1} and $100 \mu\text{g ml}^{-1}$, respectively) and glutamine were added between days 1 and 3 of culture. Two hours before each experiment, the culture medium was discarded and replaced by NaCl-containing assay buffer at pH 7.4 (Standard HEPES-buffered tyrode solution).

The pH_i of cultured cells was measured with the pH sensitive fluorescent dye BCECF by means of a spectrofluorimeter (Photon Technology International, PTI). Cells cultured on glass coverslips were loaded for 30 min with 5–10 μM of BCECF-AM. The coverslip was fixed on a customized holder specially adapted to a quartz cuvette allowing perfusion of the different assay buffers. BCECF fluorescence was monitored continuously by switching between the two excitation wavelengths (440 and 490 nm) with an emission of 530 nm. The signal was recorded at the frequency of 3 points per second and converted into pH units using calibration by the nigericin technique. Briefly, at the end of each experiment, cells were exposed to three nigericin calibration solutions of known pH (pH_o). This operation clamped pH_i to the values of pH_o in the calibration solutions and the fluorescent ratio was then converted to a pH value using PTI 'Felix 1.1' software.

Under our experimental conditions, the activity of the $\text{Na}^+/\text{HCO}_3^-$ symport is abolished by the use of HEPES-buffered, (HCO_3^- free) solutions. Standard HEPES-buffered tyrode solution contains (in mM): NaCl 140, KCl 5.4, MgCl_2 1.2, CaCl_2 1, glucose 11, HEPES 10 and the pH is adjusted to 7.4 using TRIS-HCl. Na^+ -free buffer is obtained by isotonic substitution of NaCl with N-methyl-D-glucamine (NMDG) and the pH is adjusted to 7.4 with HCl. Acid loading solution is composed of (mM): NH_4Cl 10, NMDG 130, KCl 5.4, MgCl_2 1.2, CaCl_2 1, glucose 11 and HEPES 10 and the pH is adjusted to 7.4 with HCl.

Calibration solutions contain (in mM): KCl 100, MgCl_2 1, CaCl_2 2, HEPES 50 and nigericin 10 μM and are adjusted to three different pH values in the range of 6.8–7.8.

For each coverslip tested, two control NH_4Cl prepulses were performed to measure the rate of pH_i recovery in the absence of Na^+/H^+ blockade. Preparations which showed a difference in the rate of recovery in the two control NH_4Cl prepulses were discarded. Vehicle (H_2O), SL 59.1227 or cariporide were then incorporated in the Na^+ -free-buffer during both the establishment of acidosis and the recovery period, and 1–3 concentrations of compound were tested per coverslip.

The rate of Na^+ -dependent pH_i recovery ($\text{dpH}_i \text{ dt}^{-1}$) is calculated from a linear fit of the initial phase (30 s) of pH recovery in the presence of Na^+ -containing perfusate. The percentage of reduction of the slope of the linear fit (control NH_4Cl prepulse) is calculated for each concentration of compound. Half-maximal inhibitory concentrations (IC_{50}) are determined for each compound on the two NHE isoforms by the 'Allfit' programme according to the extra sum of squares principle (De Lean *et al.*, 1978).

Ischaemia/reperfusion-induced arrhythmia studies in the anaesthetized rat

Male Sprague-Dawley rats, weighing 250–300 g were anaesthetized with pentobarbitone sodium (60 mg kg^{-1} , i.p.), placed on a heated jacket to control body temperature and artificially ventilated with room air *via* a tracheal cannula (Hugo Basile pump, Apelex, Massy, France). The femoral veins were cannulated for intravenous infusion. The electrocardiogram (ECG) was recorded from limb leads, systemic blood pressure (MAP) was monitored from the carotid artery by a Statham P23 XL transducer (Gould, Longjumeau, France) and heart rate (HR) was measured with a cardiometer triggered by the pulse pressure signals. All parameters were displayed on a Graphtec polygraph (Bioseb, Chaville, France).

The chest was opened by a left thoracotomy to expose the heart. After incising the pericardium, the heart was exteriorized by gentle pressure on the rib. A silk suture was placed around the left coronary artery, near its origin to form a snare using a small length of vinyl tubing. The heart was replaced in the chest and the animal was allowed to recover for about 15 min, during which an intravenous infusion of pentobarbitone sodium ($30 \text{ mg kg}^{-1} \text{ h}^{-1}$) was initiated.

The coronary artery was occluded by applying tension to the ligature for 7 min then reperused by releasing the tension for a further 10 min, before the experiment was terminated. ECG changes, MAP and HR were measured before and during the occlusion/reperfusion period. Ventricular ectopic activity was assessed according to the criteria advocated in the Lambeth Convention (Walker *et al.*, 1988). The incidence of ventricular tachycardia (VT), fibrillation (VF) and mortality were determined, together with the duration of ventricular tachycardia. Successful occlusion was confirmed by the observation of ST elevation during the first few seconds of occlusion and reperfusion by a clear colour change from cyanosis to hyperaemic appearance in the reperused region. No effort was made to re-animate or to defibrillate any animal which exhibited fatal arrhythmia.

In a first series of experiments, SL 59.1227 (10, 30 or $100 \mu\text{g kg}^{-1} \text{ min}^{-1}$) or the vehicle (methanesulphonic acid 0.05% in saline $0.2 \text{ ml min}^{-1} \text{ kg}^{-1}$) was infused starting

Figure 1 Molecular structures of Cariporide and SL 59.1227.

8 min before the coronary occlusion. In a second series of experiments, SL 59.1227 (1 mg kg⁻¹, i.v., in 2 min) was administered 32 min or 7 min before ischaemia or 5 min before reperfusion.

Cardiac infarct size studies in anaesthetized rabbits

New Zealand rabbits (ESD, France), weighing 2.3 to 3.2 kg were anaesthetized with ketamine (50 mg kg⁻¹, i.m.) and xylazine (5 mg kg⁻¹, i.m.) placed on a heated jacket to control body temperature and artificially ventilated with room air *via* a tracheal cannula (Ugo Basile pump, Apelex, Massy, France). The jugular veins were cannulated for intravenous infusion. ECG was recorded from limb leads (DII derivation), MAP was monitored from the carotid artery by a Statham P23 XL transducer (Gould, Longjumeau, France) and HR was measured with a cardi tachometer triggered by the pulse pressure signals. All parameters were displayed on a Grass polygraph (Franchiseville, France).

An intravenous infusion of ketamine (30 mg kg⁻¹ h⁻¹) and xylazine (70 mg kg⁻¹ h⁻¹) was initiated and a left thoracotomy performed to expose the heart. After incising the pericardium, a silk suture was placed around the circumflex coronary artery to form a snare using a small length of vinyl tubing. The animal was allowed to recover for about 30 min and was submitted to an occlusion of the coronary artery during 30 min followed by a 120 min period of reperfusion. Rabbits were divided in two groups which were infused for 5 min with either vehicle (0.5 ml kg⁻¹, i.v.), or SL 59.1227 (0.6 mg kg⁻¹, i.v.) 10 min before the occlusion of the artery.

At the end of the experiment, the heart was excised and rinsed throughout the coronary artery vasculature using saline. Infusion of 1 ml Indian ink was performed after re-occlusion of the coronary artery to differentiate the zone at risk (tissue with no coloration). The left ventricle was dissected and cut into several transverse sections of 1.5 mm wide which were then incubated at 37°C for 15 min in 1% TTC in 0.1 M phosphate buffer adjusted to pH 7.4. The TTC stained the noninfarcted myocardium, developing a deep red colour, whereas the infarcted area remained pale. The slices were immersed in 10% formalin for at least 2 days to enhance the contrast of the stain. Slices were weighed and pressed between two glass plates before determination of the areas of the infarct and the area at risk (AAR) using planimetry software (Visioloab, Biocom, France).

Animals showing arrhythmia or a sustained decrease in MAP (<70 mmHg) during the stabilization period were eliminated from the study.

Drugs and materials used

CCL39-derived PS120 variants cells were obtained from Dr J. Pouyssegur's laboratory (CNRS UMR 135; Nice, France). Tissue-culture reagents were obtained from GIBCO (France). BCECF was purchased from Molecular Probes (Eugene, U.S.A.). Chemicals, formalin were purchased from Prolabo (Paris, France). Ammonium chloride, NMDG, TTC, nigericin were obtained from Sigma (Paris, France). Ketamine hydrochloride from UVA laboratories (Ivry sur Seine, France). Xylazine from Bayer Pharma (Puteaux, France). Sodium pentobarbitone from Sanofi (Paris, France). SL 59.1227 and cariporide were synthesized by the Cardiovascular Chemistry Department of Synthelabo Recherche (Chilly Mazarin, France).

Statistical analysis

All values are expressed as mean ± s.e.mean. Differences between groups in haemodynamics were compared by use of a two-way analysis of variance with repeated measures followed by a Dunnett test. Kruskal–Wallis test was used to analyse the differences in the duration of ventricular tachycardia. Fisher's test with Bonferroni correction was used to analyse the differences between the incidences of arrhythmias and mortality. Student *t*-test was used to analyse the differences between the area at risk and infarct size in the control and drug-treated group. Differences between groups were considered significant if *P* < 0.05.

Results

Effects on p*H*_i in stably transfected hamster fibroblast cell lines expressing NHE1 and NHE2 isoforms

No significant modification of the speed of recovery of pH from acidosis was observed in the control group (not shown). SL 59.1227 was studied in the concentration range of 0.1 nM to 10 μM for the NHE1 isoform and 0.1–100 μM for the NHE2 isoform. SL 59.1227 reduced the speed of recovery of pH from acidosis in a concentration-dependent manner, complete blockage being reached at 1 μM for NHE1 and 100 μM for NHE2 (Figures 2 and 3). IC₅₀ values were 3.3 ± 1.3 nM (*n* = 5) and 2.3 ± 1.0 μM (*n* = 4) respectively for the NHE1 and NHE2 isoforms. Thus, SL 59.1227 is 700 times more potent against the NHE1 than the NHE2 isoform. Under the same experimental conditions, cariporide was also active giving IC₅₀ values of 103 ± 28 nM (*n* = 5) and 73 ± 46 μM (*n* = 4) for the NHE1 and NHE2 isoforms, respectively (Figure 3).

Ischaemia/reperfusion induced arrhythmia studies in the anaesthetized rat

There were no significant differences between the experimental groups in the HR and MAP values obtained at the different time points of the protocol, except in the group treated with high dose SL 59.1227 which showed a mild bradycardia (e.g. Table 1 for the infusion series). In the

Figure 2 Inhibitory effects of SL 59.1227 on the rate of p*H*_i recovery in NHE1-transfected fibroblasts after NH₄Cl prepulses (representative case). At the highest concentration of SL 59.1227 tested (10⁻⁷ M), the inhibitory effect was immediately reversed (↓) by washout of SL 59.1227-containing solution with standard Na⁺-containing solution.

Table 1 Effects of SL 59.1227 on mean arterial blood pressure (MAP) and heart rate (HR) in the anaesthetized rat

	Dose ($\mu\text{g kg}^{-1} \text{ min}^{-1}$)	Baseline	2 min	5 min	8 min
HR (b min^{-1})					
Vehicle		468 $\pm$ 10	468 $\pm$ 10	466 $\pm$ 11	462 $\pm$ 11
SL 59.1227	10	461 $\pm$ 11	452 $\pm$ 10	443 $\pm$ 11	445 $\pm$ 11
SL 59.1227	30	476 $\pm$ 10	468 $\pm$ 11	466 $\pm$ 13	463 $\pm$ 11
SL 59.1227	100	452 $\pm$ 19	429 $\pm$ 13	425 $\pm$ 13*	420 $\pm$ 14*
MAP (mmHg)					
Vehicle		105 $\pm$ 5	108 $\pm$ 5	107 $\pm$ 4	106 $\pm$ 4
SL 59.1227	10	105 $\pm$ 6	113 $\pm$ 8	104 $\pm$ 7	105 $\pm$ 8
SL 59.1227	30	106 $\pm$ 5	110 $\pm$ 7	103 $\pm$ 8	102 $\pm$ 6
SL 59.1227	100	105 $\pm$ 5	109 $\pm$ 7	107 $\pm$ 8	102 $\pm$ 7

Haemodynamic values were assessed before (baseline), 2, 5 and 8 min after SL 59.1227 (10, 30 or 100 $\mu\text{g kg}^{-1} \text{ min}^{-1}$) or vehicle (methanesulphonic acid 0.05% in saline 0.2 ml $\text{min}^{-1} \text{ kg}^{-1}$) infusion initiation. * $P < 0.05$ compared to the Vehicle group.

Figure 3 Inhibitory effects of SL 59.1227 and cariporide on the rate of pH_i recovery in NHE1 and NHE2-transfected fibroblasts after NH_4Cl prepulses.

control group of the infusion experiments, the 7 min-period occlusion of the coronary artery induced ventricular tachycardia (21 ± 4 s duration) in all animals ($n = 19$) and ventricular fibrillation in 37% of them. Reperfusion led to ventricular tachycardia (28 ± 4 s duration), reversible or irreversible ventricular fibrillation (79% animals) and mortality (42% animals).

SL 59.1227 infusion induced a dose-dependent decrease in duration and incidence of ventricular tachycardia during the ischaemic period and decreased or abolished fibrillation and mortality during the reperfusion period (Figure 4). In the control group of the bolus experiments, the 7 min-period occlusion of the coronary artery induced ventricular tachycardia (23 ± 8 s duration) in 90% animals ($n = 10$) and ventricular fibrillation in 50% of them. Reperfusion led to ventricular tachycardia (30 ± 5 s duration), reversible or irreversible ventricular fibrillation (100% animals) and mortality (80% animals). Bolus administration of SL 59.1227 induced a similar decrease in fibrillation or mortality during the reperfusion period whether the administration occurred 32 or 7 min before ischaemia or 5 min before reperfusion. A similar protective effect of SL 59.1227 was observed during the ischaemic period when the compound was given either 32 or 7 min before the occlusion. SL 59.1227 administration before reperfusion failed to inhibit ischaemia-induced ventricular tachycardia but induced 100% protection against fibrillation (Figure 5).

Cardiac infarct size studies in anaesthetized rabbits

There were no significant differences in haemodynamic parameters between groups throughout the experiment (Table 2). The AAR were the same in the control ($n = 10$) and the treated group ($n = 8$) (63.8 ± 3.8 , and $66.4 \pm 4.3\%$ of the left ventricular mass, respectively). Coronary occlusion plus reperfusion produced an infarct which was $36.2 \pm 3.4\%$ of the AAR in the control group. SL 59.1227 reduced infarct size significantly by 54% (Figure 6).

Discussion

We have presented data showing that SL 59.1227 is a novel Na^+/H^+ exchange inhibitor with a potent and selective effect on the NHE1 isoform (*versus* the NHE2 isoform). To demonstrate this, we used cultural NHE1- and NHE2-transfected fibroblasts exposed to brief periods of intracellular acidosis; the recovery of pH_i to neutral values was slowed by SL 59.1227 and cariporide. SL 59.1227 was a highly potent inhibitor of pH_i recovery in NHE1-expressing cells, approximately 30 times more potent than cariporide. Both SL 59.1227 and cariporide showed high selectivity between NHE1 and NHE2 isoforms (approximately 700 fold). Scholz *et al.* (1995) report K_i values in transfected fibroblasts for cariporide of 0.05 μM against NHE1 and 3 μM against NHE2. In our study, this compound was somewhat less potent, notably for NHE2. This divergence may be due to the difference in the techniques used to measure exchanger activity (i.e. $^{22}\text{Na}^+$ -uptake in Scholz *et al.*, 1995 *versus* pH_i measurements in our study) and the corresponding experimental conditions. Cariporide has also been reported to show high selectivity with respect to the kidney epithelial NHE3 isoform of the exchanger (Scholz *et al.*, 1995), for which no data are available for SL 59.1227. Although mechanisms other than NHE inhibition could contribute to changes in pH_i recovery we can exclude an inhibitory effect of SL 59.1227 on Na^+/K^+ ATPase, since this compound was inactive against the Na^+/K^+ ATPase in A7r5 cells up to 10 μM (unpublished observation). Furthermore, since we eliminated the activity of the $\text{Na}^+/\text{HCO}_3^-$ symport, another mechanism involved in the regulation of pH_i by performing the experiments in HCO_3^- free solution, it can be assumed that the effect of SL 59.1227 on pH_i recovery is due to inhibition of the Na^+/H^+ antiporter.

Compounds reported to be inhibitors of the Na^+/H^+ antiporter, such as cariporide, HOE 694, EMD 96785, or EIPA (5-(*N*-ethyl-*N*-isopropyl)amiloride), are characterized

Figure 4 Effects of i.v. infusion of SL 59.1227 10, 30, 100 $\mu\text{g kg}^{-1} \text{min}^{-1}$ (SL) on the arrhythmias induced by a 7 min-ischaemia period followed by a 10 min-reperfusion period in the anaesthetized rat. SL 59.1227 was infused starting 8 min before ischaemia. * $P < 0.05$, compared to the control group, Kruskal–Wallis or Fisher–Bonferroni test.

Figure 5 Effects of i.v. bolus SL 59.1227 (SL: 1 mg kg^{-1} in 2 min) on the arrhythmias induced by a 7 min-ischaemia period followed by a 10 min-reperfusion period in the anaesthetized rat. * $P < 0.05$, compared to the control group, Kruskal–Wallis or Fisher–Bonferroni test.

by the presence of an acylguanidine function in their structure (Baumgarth *et al.*, 1998). SL 59.1227, an imidazopyperidine derivative, represents the first example of a potent inhibitor of the Na^+/H^+ exchanger which does not contain the acylguanidine function. Although the imidazole

motif can be regarded as a guanidine bioisostere, it cannot be assumed that SL 59.1227 on the one hand, and cariporide and related acylguanidines on the other hand, have the same mode of interaction with NHE1. Indeed, since the crystal structure of the Na^+/H^+ exchanger has not yet been defined

Table 2 Effects of SL 59.1227 on mean arterial blood pressure (MAP) and heart rate (HR) in the anaesthetized rabbit.

	Baseline	5 min	Pre-Isc	30-Isc	120-Rep
HR (b min⁻¹)					
Vehicle	164 ± 10	163 ± 10	163 ± 10	184 ± 13	196 ± 11
SL 59.1227	167 ± 8	165 ± 7	168 ± 7	185 ± 5	197 ± 7
MAP (mmHg)					
Vehicle	84 ± 4	82 ± 3	82 ± 3	66 ± 4	58 ± 4
SL 59.1227	73 ± 5	80 ± 5	73 ± 3	60 ± 3	52 ± 2

Measurements were assessed at baseline, at the end of SL 59.1227 (0.6 mg kg⁻¹) or vehicle (methanesulphonic acid 0.05% in saline 0.5 ml min⁻¹ kg⁻¹) infusion (5 min), before ischaemia (pre-Isc), at the end of ischaemia (30-Isc) and following reperfusion. (120-Rep)

Figure 6 Effects of SL 59.1227 (0.6 mg kg⁻¹ in 5 min, 15 min before ischaemia i.v.) on the infarct size (as a % of AAR) induced by a 30 min left coronary occlusion followed by a 120 min reperfusion period in the anaesthetized rabbit ($n=10/8$). * $P<0.05$, compared to the control group, Student *t*-test.

it will not be easy to establish the precise modes of interaction of imidazolypiperidine and acylguanidine inhibitors with this target.

The sarcolemmal myocardial sodium/hydrogen exchanger (NHE1) has been shown to be involved in the cellular damage induced by ischaemia and reperfusion. Such evidence was previously provided by relatively non-specific inhibitors such as amiloride and its 5-amino-substituted derivatives and more recently by acylguanidine compounds such as HOE694, cariporide (Scholz *et al.*, 1993, 1995; Linz *et al.*, 1998). EMD 85131 (Gumina *et al.*, 1998), KB-R9032 (Hashimoto *et al.*, 1999) and MS-31-038 (Banno *et al.*, 1999). The latter agents exhibit improved selectivity for the NHE1 isoform and protect against fatal arrhythmias, contractile dysfunction and infarct

size. Our results confirm the cardioprotective potential of this pharmacological class because we have demonstrated that SL 59.1227 inhibits the consequences of cardiac ischaemia and reperfusion *in vivo*, i.e., it reduces infarct size in a rabbit model and suppresses ventricular arrhythmias in the rat. We consider that the anti-arrhythmic and cardioprotective effects of SL 59.1227 can be attributed to NHE1 inhibition. We are not aware of other pharmacological activities of SL 59.1227 which could explain these effects. For example, we can exclude a contribution from inhibition of electrogenic cardiac ion currents (e.g. Na⁺) since increasing concentrations of SL 59.1227 (up to 10 μ M) did not modify the action potential (maximal upstroke velocity and action potential duration) in either guinea-pig papillary muscle or piglet Purkinje fibre and did not inhibit batrachotoxin binding at Na⁺ channels (unpublished observations).

The results of the present study indicate that SL 59.1227 effectively suppressed ischemia/reperfusion arrhythmias in anaesthetized rats, even when it is administered after the onset of occlusion. These effects are consistent with those reported for HOE694 in isolated rat hearts (Yasutake *et al.*, 1994). The cardioprotective benefit associated with administration at reperfusion may be dependent upon the duration of ischaemia since, in general, in infarct studies (which involve prolonged ischaemia) NHE1-inhibitors have been reported to demonstrate better cardioprotective effects when administered before the ischaemic period rather than only at reflow (reviewed by Avkiran, 1999). For example, HOE694 was shown to be either ineffective or weakly effective as an inhibitor of myocardial ischaemia-reperfusion injury if administered before reperfusion using a porcine model (Klein *et al.*, 1995; Rohmann *et al.*, 1995). This would appear to be inconsistent with the original hypothesis proposed by Lazdunski *et al.* (1985) that activation of NHE1 at the time of reperfusion represents a major component of reperfusion-associated dysfunction and cell injury. The inability of NHE1 inhibitors to fully exhibit cardioprotective effects when administered at the time of reflow may also be related to the model used. In this respect, EMD 85131 showed significant myocardial protection at 0.5–0.75 mg kg⁻¹ i.v. when administered either before ischaemia or reperfusion in a canine infarct model (Gumina *et al.*, 1998). The authors suggested that these discrepancies probably reflected the differences in collateral blood flows between pigs and dogs, allowing, in the latter species, improved delivery of the drug to the ischaemic tissue. This issue is particularly important for this therapeutic class of compounds because of their possible use during clinical intervention after an ischaemic period, for example in patients with acute myocardial infarction undergoing thrombolysis.

We have demonstrated that SL 59.1227, a novel imidazolypiperidine derivative, structurally unrelated to the acylguanidine series, is a potent inhibitor of the NHE1 isoform and shows cardioprotective properties. As such, it represents the first member of a new chemical family of Na⁺/H⁺ exchange inhibitors.

References

- AVKIRAN, M. (1999). Rational basis for use of sodium-hydrogen exchange inhibitors in myocardial ischemia. *Am. J. Cardiol.*, **83**, 10G–18G.
- BANNO, H., FUJIWARA, J., HOSOYA, J., KITAMORI, T., MORI, H., YAMASHITA, H. & IKEDA, F. (1999). Effects of MS-31-038, a novel Na⁺/H⁺ exchange inhibitor, on the myocardial infarct size in rats after postischemic administration. *Arzneimittelforschung*, **49**, 304–310.
- BAUMGARTH, M., BEIER, N. & GERCKE, R. (1998). Bicyclic acylguanidine Na⁺/H⁺ antiporter inhibitors. *J. Med. Chem.*, **41**, 3736–3747.
- DE LEAN, A., MUNSON, P.J. & ROBBARD, D. (1978). Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am. J. Physiol.*, **235**, E97–E102.

- DENNIS, S.C., GEVERS, W. & OPIE, L.H. (1991). Protons in ischaemia: where do they come from: where do they go to? *J. Mol. Cell. Cardiol.*, **23**, 1077–1086.
- DUFF, H.J., BROWN, C.E., CRAGOE, E.S. & RAHMBERG, M. (1991). Antiarrhythmic activity of amiloride: mechanisms. *J. Cardiovasc. Pharmacol.*, **17**, 879–888.
- FRELIN, C., VIGNE, P. & LAZDUNSKI, M. (1984). The role of Na^+/H^+ exchange system in cardiac cells in relation to the control of the internal Na^+ concentration. *J. Biol. Chem.*, **259**, 8880–8885.
- FRÖHLICH, O. & KARMAZYN, M. (1997). The Na-H exchanger revisited: an update on Na-H exchange regulation and the role of the exchanger in hypertension and cardiac function in health and disease. *Cardiovasc. Res.*, **36**, 138–148.
- GUMINA, R.J., MIZUMURA, T., BEIER, N., SCHELLING, P., SCHULTZ, J.J. & GROSS, G.J. (1998). A new sodium/hydrogen inhibitor, EMD 85131, limits infarct size in dogs when administered before or after coronary artery occlusion. *J. Pharmacol. Exp. Ther.*, **286**, 175–183.
- HASHIMOTO, K., SUGIYAMA, A., XUE, Y.X., AYE, N.N. & CHINO, D. (1999). Antiarrhythmic effects of Na^+/H^+ exchange inhibitors. *Eur. Heart. J.*, K31–K37.
- KARMAZYN, M. (1988). Amiloride enhances postischemic ventricular recovery: possible role of Na^+/H^+ exchange. *Am. J. Physiol.*, **255**, H608–H615.
- KLEIN, H.A., PICH, S., BOHLE, R.M., WOLLENWEBER, J. & NEBENDAHL, K. (1995). Myocardial protection by Na^+/H^+ exchange inhibition in ischemic reperfused porcine hearts. *Circulation*, **92**, 912–917.
- LAZDUNSKI, M., FRELIN, C. & VIGNE, P. (1985). The sodium-hydrogen exchange system in cardiac cells: its biochemical and pharmacological properties and its role in regulating internal concentrations of sodium and internal pH. *J. Mol. Cell. Cardiol.*, **17**, 1029–1042.
- LINZ, W., ALBUS, U., CRAUSE, P., JUNG, W., WEICHERT, A., SCHÖLKENS, B.A. & SCHOLZ, W. (1998). Dose-dependent reduction of myocardial infarct mass in rabbits by the NHE1 inhibitor cariporide (HOE 642). *Clin. Exp. Hypertens.*, **20**, 733–749.
- LIU, H., CALA, P.M. & ANDERSON, S.E. (1997). Ethylisopropylamiloride diminishes changes in intracellular Na, Ca and pH in ischemic newborn myocardium. *J. Mol. Cell. Cardiol.*, **29**, 2077–2086.
- MENG, H.P. & PIERCE, G.N. (1990). Protective effects of 5-(N,N-dimethyl)amiloride on ischemia-reperfusion injury in hearts. *Am. J. Physiol.*, **258**, H1615–H1619.
- MURPHY, E., PERLMAN, M., LONDON, R.E. & STEENBERGEN, C. (1991). Amiloride delays the ischemia-induced rise in cytosolic free calcium. *Circ. Res.*, **68**, 1250–1258.
- PARK, C.O., XIAO, X.H. & ALLEN, D.G. (1999). Changes in intracellular Na^+ and pH in rat heart during ischemia: role of Na^+/H^+ exchanger. *Am. J. Physiol.*, **276**, H1581–H1590.
- PIKE, M.M., LUO, C.S., CLARK, M.D., KIRK, K.A., KITAKAZE, M., MADDEN, M.C., CRAGOE, E.J. & POHOST, G.M. (1993). NMR measurements of Na^+ and cellular energy in ischemic rat heart: role of Na^+/H^+ exchange. *Am. J. Physiol.*, **265**, H2017–H2026.
- RUIZ-PETRICH, E.R., PONCE-ZUMINO, A.P., MOFFAT, M.P., RIOUX, Y. & SCHANNE, O.F. (1996). Modulation of the electrophysiological effects of ischemia reperfusion by methylisobutyl amiloride. *J. Mol. Cell. Cardiol.*, **28**, 1129–1141.
- ROHMANN, S., WEYGANDT, H. & MINCK, K.O. (1995). Preischemic as well as postischemic application of a Na^+/H^+ exchange inhibitor reduces infarct size in pigs. *Cardiovasc. Res.*, **30**, 945–951.
- SCHOLZ, W., ALBUS, U., COUNILLON, L., GÖGELEIN, H., LANG, H.J., LINZ, W., WEICHERT, A. & SCHÖLKENS, B.A. (1995). Protective effects of HOE-642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. *Cardiovasc. Res.*, **29**, 260–268.
- SCHÖLZ, W., ALBUS, U., LANG, H.J., LINZ, W., MARTORANA, P.A., ENGLERT, H.C. & SCHÖLKENS, B.A. (1993). HOE694, a new Na^+/H^+ exchange inhibitor and its effects in cardiac ischaemia. *Br. J. Pharmacol.*, **109**, 562–568.
- TANI, M. & NEELY, J.R. (1989). Role of intracellular Na^+ in Ca^{2+} overload and depressed recovery of ventricular function of reperfused ischemic rat hearts. Possible involvement of H^+/Na^+ and $\text{Na}^+/\text{Ca}^{2+}$ exchange. *Circ. Res.*, **65**, 1045–1056.
- WALKER, M.J., CURTIS, M.J., HEARSE, D.J., CAMPBELL, R.W., JANSE, M.J., YELLON, D.M., COBBE, S.M., COKER, S.J., HARNES, J.B., HARRON, D.W., HIGGINS, A.J., JULIAN, D.G., LAB, M.J., MANNING, A.S., NORTHOVER, B.J., PARRATT, J.R., RIEMERSMA, R.A., RIVA, E., RUSSELL, D.C., SHERIDAN, D.J., WINSLOW, E.R. & WOODWARD, B. (1988). The Lambeth Conventions: guidelines for the study of arrhythmias in ischemia, infarction, and reperfusion. *Cardiovasc. Res.*, **22**, 447–455.
- YASUTAKE, M., IBUKI, C., HEARSE, D.J. & AVKIRAN, M. (1994). Na^+/H^+ exchange and reperfusion arrhythmias: protection by intracoronary infusion of a novel inhibitor. *Am. J. Physiol.*, **267**, H2430–H2440.

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