ABSTRACT
The present study investigates whether 3-(R)-[3-(2-Methoxyphenylthio-2-(S)-methylpropyl]amino-3,4-dihydro-2H-1,5-benzoaxathiepine Bromhydrate (F 15845) prevents ischemia-induced heart remodeling by reduction of the intracellular Na⁺ overload.

Ischemia and subsequent reperfusion induce functional impairment and structural alterations of the myocardium. Key factors in this process are disturbances of myocardial energy metabolism and ion homeostasis (Tani, 1990; Silverman and Stern, 1994; Pierce and Czubryt, 1995). Thus, ischemia leads to a rapid cessation of oxidative phosphorylation and contractile function, Na⁺/K⁺-ATPase activity is reduced, and persistent entry of Na⁺ leads to hypercontracture and possible cell rupture. However, it was more marked and maintained upon reperfusion. The cardioprotective properties of myocardial infarction associated with short- (24-h) and long-term (14-day) reperfusion were measured in anesthetized rats. After 24-h reperfusion, F 15845 (5 mg/kg) significantly reduced infarct size (32.4 ± 1.7% with vehicle and 24.2 ± 3.4% with F 15845; P < 0.05) and decrease of troponin I levels (524 ± 93 μg/l with vehicle versus 271 ± 63 μg/l with F 15845; P < 0.05). It is important that F 15845 limits the long-term expansion of infarct size (35.2 ± 2.6%, n = 19 versus 46.7 ± 1.6%, n = 27 in the vehicle group; P < 0.001). Overall, F 15845 attenuates [Na⁺] in heart, and prevents (or reverses) contractile and biochemical dysfunction in ischemic and remodeling heart. F 15845 constitutes a new generation of cardioprotective agent.

3-(R)-[3-(2-Methoxyphenylthio-2-(S)-methylpropyl]amino-3,4-dihydro-2H-1,5-benzoaxathiepine Bromhydrate (F 15845) Prevents Ischemia-Induced Heart Remodeling by Reduction of the Intracellular Na⁺ Overload

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ABBRévIATIoNS: F 15845, 3-(R)-[3-(2-Methoxyphenylthio-2-(S)-methylpropyl]amino-3,4-dihydro-2H-1,5-benzoaxathiepine Bromhydrate; NMR, nuclear magnetic resonance; LPC, lysophosphatidylcholine; TrDOTP, thulium(III)-1,4,7,10-tetraazacyclododecane-N,N,N′,N″-tetra-(methylene phosphonate); dP/dt max, maximum positive and negative first derivative of left ventricular pressure; PEG, polyethylene glycol.
posed as a target for pharmacological intervention (Hammarström and Gage, 2002; Makielski and Valdivia, 2006; Hale et al., 2008; Shroyck and Belardinelli, 2008). This current also has been referred to as late, sustained, noninactivating, or persistent (Wasserstrom and Salata, 1988; Hammarström and Gage, 2002; Makielski and Valdivia, 2006; Saint, 2008). A known inhibitor of these slowly inactivating Na channels is ranolazine (Belardinelli et al., 2004; Hasenfuss and Maier, 2008). Electrophysiological studies demonstrate that ranolazine is effective at concentrations that are far below those necessary for the blockade of Na channels in control conditions (Belardinelli et al., 2004; Hasenfuss and Maier, 2008). Ranolazine is only weakly potent against the persistent sodium current and is also poorly selective versus several potassium channels (Antzelevitch et al., 2004). Thus, it remains unknown whether the cardioprotective activity of ranolazine is attributable to blockade of the persistent sodium current or whether it is due to a combination of effects. We recently described a new, selective, and potent persistent sodium current blocker, F 15845 (Le Grand et al., 2008; Vacher et al., 2009), which is currently entering in phase II clinical trials for the treatment of refractory angina. It is unknown, however, whether blockade of persistent sodium current by F 15845 can influence the ischemic Na accumulation of the heart. Here, we addressed the long-term cardioprotection and prevention of ischemia-induced heart remodeling. We also determined the effects of F 15845 on the intracellular Na of isolated perfused hearts using 23Na NMR spectroscopy and compared the cardioprotection properties obtained in myocardial infarction after short- (24-h) and long-term (14-day) reperfusion.

Materials and Methods

Animals were housed and tested in an Association for the Assessment and Accreditation of Laboratory Animal Care-accredited facility in strict compliance with all applicable regulations, and the protocol was carried out in compliance with French regulations and local Ethical Committee guidelines for animal research.

Veratrine-Induced Diastolic Contracture in Rat Isolated Left Atria. Male Wistar rats (400–450 g, OFA strain; Ifa Credo, L’Arbresele, France) were euthanized with an overdose of pentobarbital sodium (120 mg/kg i.p.). The left atrium was rapidly excised and mounted vertically in an organ bath containing Krebs’ solution of the following composition: 119 mM NaCl, 5.6 mM KCl, 1.17 mM MgSO4, 2.1 mM CaCl2, 1.0 mM NaH2PO4, 25.0 mM NaHCO3, and 10.0 mM glucose, pH 7.4; maintained at 34°C and gassed with 95% O2, 5% CO2 (impulse duration, 1 ms; 2 × threshold current) via two electrodes (Campden stimulator 915; Phymep, Paris, France). The force of isometric contraction was measured with a UC2 transducer (Statham Inc., Oxnard, CA). The amplifier was connected to an MP 100 interface (Biopac Systems, Goleta, CA), and the analog signal was analyzed by a Vectra VL18 computer (Hewlett Packard, Palo Alto, CA) by means of Acknowledge III interactive software (Biopac Systems).

After a 30-min equilibration period, a concentration of drug or vehicle was injected into the bath. Fifteen minutes later, veratrine (100 μg/ml) was added. Systolic isometric tension development was measured before drug or solvent injection and just before the addition of veratrine. After 15-min incubation of the vehicle, addition of veratrine (100 μg/ml) induced a transient initial increase in systolic tension followed by increases in diastolic tension (diastolic contraction). The maximal amplitude of veratrine-induced contraction was measured irrespective of time.

Lysophosphatidylcholine Intoxication. Ventricular myocytes were isolated from male Hartley guinea pig hearts (specific pathogen-free microbial status, 250–400 g; Charles River Laboratories, Les Oncins, France) by the technique described previously (Mitra and Morad, 1985). The cell suspension obtained with protease (0.3 mg/ml; Sigma-Aldrich, St. Louis, MO) and collagenase (1 mg/ml, type IA; Sigma-Aldrich) was then placed in a Petri dish and used 1 to 4 h after preparation. Only cells exhibiting a rod-shaped morphology and no signs of sarcolemmal blebbing were used for the experiments. The extracellular medium was a Tyrode’s solution containing 125 mM NaCl, 2.6 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgSO4, 5 mM CaCl2, 10 mM HEPES, and 5.5 mM d- (+)-glucose, pH 7.3 with NaOH. All experiments were performed at 34 ± 1°C on the stage of a vertical microscope (Optophot-2; Nikon, Tokyo, Japan). Cell images were continuously video-recorded at 400× magnification (3CCD color videocamera, DXC-930P; Sony, Tokyo, Japan). After a 15-min equilibration period, a concentration of drug was microinjected into the cells studied. LPC (10 μM) was added 20 min later. The amplitude of LPC-induced hypercontraction was recorded and analyzed 10 times over a 30-min period.

NMR Spectroscopy. Male C57BL/KsJ-leprdb/leprdb diabetic (db/db) mice (males 12–15 weeks of age) and their C57BL/KsJ-lepr+ (db/+ ) nondiabetic control littersmates were purchased from Janvier (Le Genest St-Isle, France). Mice, fed dietary status, were anesthetized with sodium pentobarbital (60 mg/kg) and heparinized (100 IU) intraperitoneally. The heart was quickly removed and placed in ice-cold Krebs-Henseleit bicarbonate buffer. The heart underwent a Langendorff perfusion (80 mm Hg perfusion pressure) with modified Krebs-Henseleit bicarbonate perfusate: 118 mM NaCl, 5.9 mM KCl, 1.2 mM MgSO4, 25 mM NaHCO3, 1.75 mM CaCl2, 0.5 mM EDTA, and 11 mM glucose, gassed with 95% O2, 5% CO2, pH 7.4 (37°C) (20-min stabilization period) and was then immersed in a set volume of warmed perfusate (37°C). Isovolumic left ventricular developed pressure was monitored using a fluid-filled polyvinylchloride film balloon inserted into the left ventricle via the mitral valve and was connected with a 23 ID pressure transducer (Statham Inc.). For 23Na NMR measurements, we used the shift agent thulium(III)–1,4,7,10-tetraazacyclododecane–N,N,N,N,N,NII-tetra(methylene phosphonate) (TmDOTP5–) (Macroyclics, Dallas, TX), as described previously (El Banani et al., 2000). TmDOTP5– was added to the perfusate to reach a concentration of 3.5 mM. It was present before ischemia (10 min), during ischemia, and for the first 20 min of reperfusion. Because TmDOTP5– is a sodium salt that binds Ca2+ to a significant extent, both [Na+] and [Ca2+] were corrected. NaCl was adjusted to keep the total Na content unchanged, and the total Ca2+ content was increased to 3.42 mM, resulting in a free ionized Ca2+ of 0.85 to 0.95 mM, measured by using a Ca2+ ion-selective electrode (Thermo Fisher Scientific, Waltham, MA).

The 23Na spectra were recorded on an AM 400WB spectrometer (Bruker, Newark, DE) at 105.84 MHz, using a multinuclear NMR probe (Anzawa et al., 2006). Each 23Na NMR spectrum was obtained by accumulation of 280 consecutive free induction decays using 90° pulses and a 205-ms interpulse delay with a 2500-Hz spectral width and a 1-K data point time domain. 23Na NMR spectra were processed for the quantitative analysis of the intracellular component ([Na+]i).

First, removal of the overlapping spectral extracellular component was carried out by the Hankel-Lanczos singular value decomposition method (Pijnappel et al., 1992; Vanhamme et al., 1997; Anzawa et al., 2006). Each 23Na NMR spectrum was obtained by accumulation of 280 consecutive free induction decays using 90° pulses and a 205-ms interpulse delay with a 2500-Hz spectral width and a 1-K data point time domain. 23Na NMR spectra were processed for the quantitative analysis of the extracellular component ([Na+]o).

The experimental protocol consisted of 15 min of control perfusion followed by 16 min of no-flow ischemia and 40 min of reperfusion. F 15845 (0.5 μM) was added to the perfusion solution 10 min before inducing ischemia and was present throughout in each group (diabetic or nondiabetic hearts).

Myocardial Infarction and 24-h Reperfusion in the Anesthetized Rat. The caudal vein of male Sprague-Dawley rats (220–
300 g, OFA strain; Iffa Credo) was cannulated for anesthesia (60 mg/kg pentobarbital sodium) and for intravenous administration of compounds. Then, the animals were intubated and ventilated at 60 respirations/min (2.5 ml/respiration) (ventilator model 683; Harvard Apparatus Inc., Holliston, MA) while anesthesia was maintained. The temperature of a heating pad (Homeothermic blanket control unit; Harvard Apparatus Inc.) was adjusted to 37°C. A left thoractomy was performed, and a silk suture (4.0) was placed around the left coronary artery—1 mm from its origin. Both ends of the silk thread were passed through a polyethylene tube. The left coronary artery was occluded by pressing the polyethylene tube against another polyethylene tube placed on the heart. After 15 min of ischemia, the polyethylene tube was removed to initiate the reperfusion. Then, the thorax was closed and the animals were awakened 1 h after the end of the ischemia. Finally, the reperfusion was performed for 24 h in conscious animals. The compound or vehicle was administrated intravenously over 5 min using a single bolus 10 min before the initiation of the myocardial infarction. The half-life (2.9 h) of the compound in rat plasma suggests that the compound was present in sufficient concentration during the ischemic phase and at the least during the first hour of reperfusion. At the end of the experiment, the animals were euthanized and the hearts were stained by using the 2,3,5-triphenyltetrazolium chloride (0.15%) infusion technique. The heart was cut into five slices of approximately 2 mm. Individual slices were photographed in color, and the extent of myocardial necrosis and the area at risk were quantified by image analysis software (Leica Microsystems Imaging Solutions, Cambridge, UK) in each slice. The quantitative determination of cardiac troponin I in rat plasma was performed by AxSYM System (Abbott Laboratories, Abbott Park, IL).

Ischemia-Induced Myocardial Remodeling. The technique of myocardial infarction was similar to that described above (see Myocardial Infarction and 2-h Reperfusion in the Anesthetized Rat). After 30 min of ischemia, the polyethylene tube was removed to initiate the reperfusion. Then, the thorax was closed and the animals awakened up 1 h after the end of the ischemia. Finally, the reperfusion was performed for 14 days in conscious animals. The compound or vehicle was administrated intravenously over 5 min using a single bolus 10 min before the initiation of the myocardial infarction. Fourteen days after myocardial infarction or sham operation, animals were anesthetized with 2% isoflurane. Then, the left carotid artery was cannulated to continuously measure arterial pressure via a pressure sensor (DTX PLUS; Becton Dickinson, Le Pont de Chaix, France). The analog arterial pressure signal was amplified (Gould Transducer; Gould Instrument Systems Inc., Cleveland, OH), digitized, and simultaneously recorded by means of data acquisition software (AcqKnowledge; Biopac Systems). Systolic, diastolic, and mean arterial pressures were continuously recorded. The right carotid artery was cannulated with a Millar probe (MIKROTIPS pressure sensor; Millar Instruments Inc., Houston, TX) that was retrogradely passed across the aortic valve and advanced into the left ventricle. Left ventricular function was measured via a pressure transducer (Gould Transducer; Gould Instrument Systems Inc.). The analog ventricular pressure signal was digitized and simultaneously recorded by means of data acquisition software (AcqKnowledge; Biopac Systems). Heart rate, maximum positive and negative first derivative of left ventricular pressure (dP/dt \(_{\text{max}}\) and \\(dP/dt\) \(_{\text{min}}\)), and systolic and left end-diastolic pressures were continuously recorded. The pudenidal vein was cannulated for intravenous administration of vehicle or dobutamine. After a 5- to 15-min stabilization period, animals received cumulative incremental doses of dobutamine (0.25–63 \(\mu\)g/kg i.v. bolus), inducing increases in left ventricular function and heart rate. Immediately after the sacrifice, the heart was rapidly excised and fixed with alcohol, formaldehyde, and acetate, and then the ventricles were cut into five cross-sectional samples of 2 mm each. The five regions were then processed into paraffin with an automated tissue processor. A color videocamera (DXC-390P color videocamera; Sony, Paris, France) relayed the image to a computer through analysis software applications (Leica Microsystems Imaging Solutions). The following equation was used to calculate infarct size (i.e., the modified zone): percentage of infarct of left ventricle = \((\text{epicardial infarct (in millimeters)} + \text{endocardial infarct (in millimeters)})/\text{left ventricle epicardial circumference (in millimeters)} + \text{left ventricle endocardial circumference (in millimeters)}) \times 100\) (Sandmann et al., 2001).

Drugs. F 15845 was synthesized by the Division of Medicinal Chemistry I, Centre de Recherche Pierre Fabre (Castres Cedex, France). F 15845 was dissolved in dimethyl sulfoxide as a 10 mM stock solution prepared freshly for each in vitro experiment. The highest final concentration of dimethyl sulfoxide was 0.1% (F 15845, 10 \(\mu\)mol). Veratrine and LPC were purchased from Sigma-Aldrich. F 15845 (salt-to-base ratio, 1.31) was dissolved in polyethylene glycol (PEG) 300 for each in vivo experiments. After dissolution, sterile 0.9% saline was added to obtain a final solution containing 40% PEG in 0.9% sterile saline.

Statistical Analysis. All values are expressed as means ± S.E.M. Intragroup statistical analysis of results (drug versus baseline) was performed by the paired t test after testing for homogeneity of variance with analysis of variance with repeated measures. Intergroup statistical analyses of results (drug versus vehicle) were performed using one-way analysis of variance followed by Dunnett’s t test when analysis of variance was significant. Any P value <0.05 was considered significant (SigmaStat 2.03; Systat Software, Inc., San Jose, CA).

Results

Veratrine-Induced Diastolic Contracture. F 15845 was investigated for its effects on basal tension and diastolic contracture in rat isolated left atria (Le Grand et al., 1993) elicited by veratrine, a potent activator of the persistent sodium current (Sunami et al., 1993). Veratrine-induced diastolic contracture mimics that evoked by ischemia (Fig. 1A) (Le Grand et al., 1993). F 15845 concentration-dependently reduced veratrine-induced diastolic contracture from 0.1 \(\mu\)M (IC\(_{50}\) = 0.14 \(\mu\)M), with complete inhibition obtained at 10 \(\mu\)M (Fig. 1B). Weak negative inotropic effects occurred only at 10 \(\mu\)M F 15845, indicating direct cardioprotection. The calcium channel blocker diltiazem only weakly inhibits diastolic contracture at the highest concentration studied (Fig. 1B), whereas the \(\beta_1\)-adrenoceptor antagonist atenolol, the Na+/H+ exchanger inhibitor cariporide, and the class I antiarrhythmic lidocaine are all inactive (Fig. 1B), illustrating the resistance of diastolic contracture to inhibition by these agents.

Lysophosphatidylcholine Intoxication of Cardiomyocytes. Lysophosphatidylcholine is a potent activator of the persistent sodium current (Undrovina et al., 1992). It promotes sodium loading, which results in calcium overload (Haigney et al., 1994; Piper, 2000), hypercontracture, and death in cardiomyocytes. The cardioprotective effects of F 15845 were therefore investigated in guinea pig isolated ventricular cardiomyocytes subjected to LPC intoxication. LPC (10 \(\mu\)M) induced a time-dependent reduction in cardiomyocyte length, loss of rod shape, hypercontracture, and death of all cells. F 15845, from 1 \(\mu\)M, preserved viability in 54.2 ± 12.5% of quiescent cardiomyocytes exposed to LPC (Fig. 2). Under similar experimental conditions, atenolol, diltiazem, and cariporide, anti-ischemic agents operating through other mechanisms, are less active toward LPC intoxication (Fig. 2).

Ischemia-Induced Increase in Intracellular Na\(^+\) in Isolated Hearts from Control and Diabetic (db/db) Mice. Persistent sodium current increases [Na\(^+\)], during ischemia and early reperfusion, leading in turn to calcium

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overload and enhanced myocardial injury (Baetz et al., 2003). Accordingly, the effects of F 15845 on [Na\(^+\)]\(_i\) were investigated during global no-flow ischemia in normal and diabetic db/db mouse hearts (Anzawa et al., 2006). Isolated mouse hearts were subjected to 16 min of no-flow ischemia, and [Na\(^+\)]\(_i\) was monitored using \(^{23}\)Na nuclear magnetic resonance spectroscopy. During ischemia a rapid increase in [Na\(^+\)]\(_i\) was observed in both normal and diabetic hearts (Fig. 3, A and B). Upon reperfusion, [Na\(^+\)]\(_i\) declined slowly in nondiabetic hearts, whereas it remained elevated in diabetic hearts (approximately 50% above basal levels after 30 min of reperfusion; Fig. 3A). At the concentration of 0.3 \(\mu\)M, the effects of F 15845 were clearly different between control and diabetic hearts. F 15845 significantly reduced [Na\(^+\)]\(_i\) increase during ischemia in control db/+ hearts, although that reduction became significant only at the end ischemia, whereas this reduction was more marked (approximately \(-40\%) in diabetic db/db rats. In the latter hearts, the drug was not only effective in slowing the rise in [Na\(^+\)]\(_i\), but also in decreasing its maximal amplitude of the increase. Moreover, [Na\(^+\)]\(_i\), was lower during the first 10 min of reperfusion when F 15845 was present. The smaller rise in [Na\(^+\)]\(_i\), during ischemia in db/+ hearts was associated with a delayed increase in diastolic pressure (Fig. 4B). In parallel with the reduction in [Na\(^+\)]\(_i\), increase, both at end ischemia and upon reperfusion in diabetic hearts, there was a trend toward a less elevated diastolic pressure (Fig. 4A).

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**Fig. 1.** A, typical recordings of veratrine-induced diastolic contracture in presence of F 15845 or its vehicle. B, concentration-dependent inhibition of diastolic contracture by F 15845 (filled circles; 10 nM–10 \(\mu\)M), weak inhibition by diltiazem (filled triangles; 0.1–10 \(\mu\)M); and inactivity of atenolol (open circles; 10 \(\mu\)M), cariporide (open triangles; 10 \(\mu\)M), and lidocaine (filled squares; 10 \(\mu\)M). ***, \(P < 0.001\) compared with corresponding vehicle.

**Fig. 2.** Protective effects of F 15845, atenolol, diltiazem, and cariporide in guinea pig cardiomyocytes subjected to LPC intoxication, assessed by the percentage of rod-shaped (i.e., viable) cells remaining. *, \(P < 0.05\) and **, \(P < 0.01\) compared with vehicle with LPC.

**Fig. 3.** Effects of F 15845 (open symbols) on [Na\(^+\)]\(_i\), during 16 min of no-flow ischemia and 40 min of reperfusion in hearts of diabetic (A) and control mice (B). Both received vehicle (black symbols) or compound at the concentration of 0.3 \(\mu\)M. Data are means \(\pm\) S.E.M. *, \(P < 0.05\) compared with vehicle group.
Myocardial Infarction in Anesthetized Rat. The cardioprotective effects of F 15845 in reducing myocardial damage in the acute phase of myocardial infarction were addressed in anesthetized rats subjected to a 15-min occlusion of left coronary artery followed by 24-h reperfusion. Compounds were administered intravenously over 5 min, 10 min before the occlusion. F 15845 limits myocardial infarct size from 5 mg/kg (percentage of infarct size, 32.4 ± 1.7%, n = 13 in the vehicle group and 24.2 ± 3.4%, n = 8; P < 0.05; F 15845, 5 mg/kg). Furthermore, F 15845-induced reduction of infarct size is associated with a decrease in troponin I plasma levels measured 1 h after reperfusion (524 ± 93 µg/l, n = 9 in the vehicle group versus 271 ± 63 µg/l, n = 11; P < 0.05 in the F 15845 group).

Myocardial Infarction-Induced Late Infarct Expansion and Left Ventricular Dysfunction in the Rat after 14-Day Reperfusion: Effect of F 15845 on Heart Remodeling. The long-term cardioprotective effects of F 15845 against heart remodeling were addressed in the rat subjected to 14-day reperfusion. Left ventricular function was evaluated under baseline conditions and with infusion of incremental doses of dobutamine. F 15845 (2.5 mg/kg) was devoid of effect on the left ventricular dysfunction (Fig. 5). At 5 mg/kg, F 15845 significantly improved the positive dP/dt max (7366 ± 547 mm Hg/s, n = 9 versus 4278 ± 615 mm Hg/s, n = 12 in the vehicle group; P < 0.05) in the baseline conditions and in the presence of dobutamine, suggesting a partial restoration of the left ventricular function (Fig. 5).

The infarct size was also assessed by histological analysis. F 15845 (2.5 mg/kg) was devoid of significant effect on infarct size (Fig. 6), but 5 mg/kg F 15845 significantly reduced the expansion of infarct size (35.2 ± 2.6%, n = 19 versus 46.7 ± 1.6%, n = 27 in the vehicle group; P < 0.001) (Fig. 6). In conclusion, in the above-mentioned myocardial ischemia-reperfusion models in the rat, cardioprotective effects of F 15845 against heart remodeling were clearly demonstrated at the 5 mg/kg i.v. dose.

Discussion

The present results show that F 15845, a highly effective blocker of persistent cardiac sodium current, exerts anti-ischemic activity against veratrine-induced diastolic contracture without any significant effects on the baseline cardiac function. In addition, it prevents the LPC-induced cell hyper-

![Fig. 4. Effects of F 15845 (open symbols) on left diastolic pressure during 16 min of no-flow ischemia and 40 min of reperfusion in hearts of diabetic (A) and control mice (B). Both received vehicle (black symbols) or compound at the concentration of 0.3 µM. Data are means ± S.E.M. *P < 0.05 compared with vehicle group.](image)

![Fig. 5. Variations of cardiac contractility (A; positive dP/dt max) and cardiac relaxation (B; negative dP/dt max) after 30-min left descending coronary occlusion and 14-day reperfusion in the sham-operated group (n = 7), in vehicle-treated rats (40% PEG in sterile saline; n = 11), and in F 15845-treated rats (2.5 mg/kg, n = 9 and 5 mg/kg, n = 9). Data are means ± S.E.M. versus vehicle. +, P < 0.05 and **, P < 0.01 compared with vehicle.](image)
cause an accumulation of intracellular sodium, and the concentration of Na$^+$ during ischemia. Activation of the persistent sodium current (Tamareille et al., 2002; Pinet et al., 2008; Shryock and Belardinelli, 2008). Sodium is subsequently exchanged for calcium through the Na$^+$/Ca$^{2+}$ exchanger (Murphy et al., 1999), which in turn leads to toxic or even lethal calcium overload. If this calcium overload and downstream events can be prevented either by blockade of Na$^+$ channels or cardiac specific blockade of Na$^+$ channel current, the link between increased Na$^+$ entry into cells and calcium overload is established (Belardinelli et al., 2006). In contrast, hypoxia is known to impair inactivation of Na$^+$ channels and to increase persistent sodium current (Saint, 2008). Tissue hypoxia and reperfusion generate metabolites (palmitoyl-l-carnitine and lysophosphatidylethanolamine, thrombin) and reactive oxygen/nitrogen species (hydrogen peroxide and nitric oxide) that increase persistent sodium current in ventricular myocyte (Tamareille et al., 2002; Pinet et al., 2008; Shryock and Belardinelli, 2008). Moreover, LPC, a second messenger involved in thrombin signaling, is known to maintain sodium channel in bursting activity, giving rise to noninactivating sodium current (Haigney et al., 1994). Thus, LPC-modified Na$^+$ channel function causes Ca$^{2+}$ loading and contractile dysfunction against which the protective effects of F 15845 were tested (Tamareille et al., 2002). Our results show that F 15845, a new, persistent sodium channel blocker (Le Grand et al., 2008; Vacher et al., 2009), counteracted LPC-induced hypercontracture, suggesting that reduction of Na$^+$ channel dysfunction induced Ca$^{2+}$ loading in isolated cardiomyocytes exerts cardioprotective activities. Because LPC accumulates quickly during ischemia and reperfusion and is proarrhythmic, it is conceivable that it contributes to the pathogenesis of ischemic cell death (Sedlis et al., 1997). F 15845 also reduced veratrine-induced diastolic contracture in a concentration-dependent manner. Veratrine intoxication of isolated cardiac tissues is a well established model of modified Na$^+$ channel function-induced Ca$^{2+}$ loading and contractile failure (Haigney et al., 1994). Veratrine binds to the open state of the Na$^+$ channel and increases its probability of opening (Suzuki et al., 1993). The ensuing increase in Na$^+$ influx is accompanied by Ca$^{2+}$ loading and diastolic contracture (Suzuki et al., 1993; Haigney et al., 1994). Therefore, F 15845, which prevented irreversible cell injury mediated both by LPC and veratrine, is endowed with potent, direct cytoprotective properties and its activity proceeds through blockade of the persistent sodium current.

During global ischemia, [Na$^+$]i increases rapidly (Fig. 3) and ends up in calcium overload, which triggers the diastolic contracture (Haigney et al., 1994; Le Grand et al., 1995). From Fig. 3B, there seemed to be a prolonged rise in [Na$^+$], very early in reperfusion (during approximately 2 min) with F 15845-treated hearts. However, this nonsignificant rise was probably related to the reperfusion switch itself rather than having any physiological significance. The smaller gain in [Na$^+$]i in the presence of F 15845 during ischemia in control hearts was associated with a delayed increase in diastolic pressure. In diabetic hearts, the reduction in [Na$^+$]i became particularly pronounced at the end of ischemia and such an effect was maintained upon reperfusion. It was also associated with a trend toward a less elevated diastolic pressure. It should be noted that the period of ischemia here was short (16 min). Given the kinetics of [Na$^+$]i increase, one would expect [Na$^+$]i to increase even more with a longer duration of ischemia, which would then probably lead to a more marked difference in diastolic pressure between untreated and treated diabetic hearts. Diabetes is known to affect various ionic transporters (Shimoni et al., 2004; Anzawa et al., 2006), and this may well be the case for slowly
inactivating Na\(^+\) channels (Feuvray and Darmellah, 2008). It has been shown that long-chain fatty acyl derivatives, such as acyl carnitine, are present in excess in the diabetic heart (Feuvray et al., 1979); the latter also accumulates in the sarcolemma during ischemia and increases persistent sodium current (Wu and Corr, 1994). Alternatively or together, because in diabetic db/db mouse hearts cardiomyocyte have a deficiency in Ca\(^{2+}\) handling (Belke et al., 2004), this may contribute to enhancing persistent Na\(^+\) current (Wagner et al., 2006; Maltsev and Undrovinas, 2008). Whatever the changes in slowly inactivating Na\(^+\) channels in hearts from type 2 diabetic db/db mice, they lead to increased sensitivity to F 15845. This observation seems of particular relevance for diabetic patients with impaired myocardial perfusion.

F 15845 (0.3 \(\mu\)M) greatly attenuates [Na\(^+\)] during ischemia. However, the protective effect of F 15845 against ischemia-induced [Na\(^+\)] elevation was different in nondiabetic and diabetic mice of which the susceptibility to ischemia has been already demonstrated (Anzawa et al., 2006). F 15845 (0.3 \(\mu\)M) lowered [Na\(^+\)] increase during ischemia in control hearts, whereas that in diabetic hearts was significant only at end ischemia, being then more marked than in normal hearts. The lesser gain in [Na\(^+\)], during ischemia in control hearts was also associated with a delayed increase in diastolic pressure. In contrast, in diabetic hearts, which [Na\(^+\)] was affected late, in ischemia and in early reperfusion, we observed only a tendency toward a reduction in the diastolic pressure. It should be noted that the duration of ischemia was short (16 min). Given the kinetics of [Na\(^+\)], one would expect [Na\(^+\)] to grow with the duration of ischemia, which allowed to anticipate a greater difference in diastolic pressure between untreated and F 15845-treated diabetic hearts. The observation of a significant reduction in [Na\(^+\)] gain by F 15845 after a short period of ischemia that is maintained upon reperfusion may be of particular relevance for diabetic patients with impaired myocardial perfusion.

The cardioprotective properties of F 15845 were demonstrated in anesthetized rats upon 15-min ligation of coronary artery. Under these conditions, F 15845 (5 mg/kg) reduced infarct size and plasma troponin I levels. Furthermore, F 15845 at this dose was devoid of significant effects on blood pressure, heart rate, and myocardial oxygen consumption (Vacher et al., 2009), revealing a direct cardioprotective action. The importance of troponin I for the diagnosis of myocardial infarction originates from its cardiac specificity. Here, troponin I levels were reduced by F 15845 after 1-h reperfusion. This result was correlated with a reduction of infarct size by 25.3\% after 24-h reperfusion. Therefore, blockade of the persistent sodium current by F 15845 causes a direct and potent cardioprotection in this model of infarction. During reperfusion, the excessive accumulation of intracellular calcium is known to be responsible for the ischemic and postischemic dysfunction and myocardial cell injury (Murphy et al., 1999). As discussed above, calcium overload originates at least in part from enhanced persistent sodium current (Belardinelli et al., 2006). Hence, inhibition by F 15845 of persistent sodium should attenuate ventricular mechanical dysfunction, cell death (increase of infarct size), and the release of troponin I, which characterize severe ischemic conditions.

In addition, we demonstrate that F 15845 dose-dependently reduced the ventricular infarct size 14 days after myocardial infarction. The cardioprotection provided by F 15845 also involved a partial restoration of the cardiac contraction, suggesting that it could affect the remodeling of heart consecutive to myocardial infarction (Weiss et al., 1981). It is interesting that the extent of protection afforded by F 15845 against late infarct is in the range of that obtained with Na\(^+\)/H\(^+\) exchanger inhibitors (Grosjean et al., 2007), whose efficacy has been proven in clinical trials. The precise role of persistent sodium current in cardiac remodeling or heart failure, however, is still unclear, although it has been shown that remodeling and/or heart failure alters persistent sodium current through factors modulating Na\(^+\) channel gating, Ca\(^{2+}\)/calmodulin/calmodulin-dependent protein kinase II, cytoskeleton, membrane phospholipid composition, and other scaffolding proteins such as caveolin (Maltsev and Undrovinas, 2008). The importance of persistent sodium current contribution to heart failure mechanisms has been demonstrated in experiments designed to “correct” persistent sodium current. Thus, partial persistent sodium current inhibition and/or acceleration of persistent current decay rescues normal repolarization, decreases beat-to-beat action potential duration variability, and improves Ca\(^{2+}\) handling and contractility of failing cells (Goldman and Balke, 2002; Maltsev and Undrovinas, 2008). Undoubtedly, blockade of persistent sodium current positions itself as a novel target for the treatment of the failing heart (Noble and Noble, 2006; Maltsev and Undrovinas, 2008; Shryock and Belardinelli, 2008), a status that is supported by our results of the improvement of ventricular dysfunction and systolic tension during late infarct expansion.

The protocol used here involved a single bolus administration of the product before ischemia. It was designed for candidate selection rather than to reflect any clinical strategies. The protection provided by F 15845 against remodeling after 14 days of reperfusion results from the pharmacodynamic actions it triggers during the first few hours of the experiment. Regardless, these data support that the first minutes of ischemia and/or reperfusion play a crucial role in the long-term outcomes. Thus, administration of the product before ischemia constitutes a limitation only if extrapolated to first episode myocardial infarction. At this early stage, we think F 15845 may be more adapted for the management of many forms of chronic heart diseases initiated by recurrent ischemic, hypoxic, metabolic events. Further studies are underway to elucidate the mechanism of cardioprotection and better define the true potential afforded by F 15845.

In conclusion, results of in vitro and in vivo studies show that F 15845 can reduce [Na\(^+\)], prevent or reverse contractile and biochemical dysfunction in ischemic and remodeling heart as well as in hearts exposed to the ischemic metabolites (e.g., LPC). These cardioprotective activities of F 15845 are potentiated in pathological states such as diabetes in complete agreement with the specific interaction of the compound with ischemic state of the channel. Finally, F 15845 constitutes a new generation of persistent sodium current inhibitor that prevents heart dysfunction in acute and late phase of postmyocardial infarction beside its antianginal activity.

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