

Restoration of Cardiomyocyte Functional Properties by Angiotensin II Receptor Blockade in Diabetic Rats

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Recent evidence suggests that blockade of the renin-angiotensin system ameliorates diabetes-induced cardiac dysfunction, but the mechanisms involved in this process remain elusive. We investigated the effect of treatment with an angiotensin II receptor blocker, losartan, on the metabolic and electrophysiological properties of cardiomyocytes isolated from streptozotocin-induced diabetic (STZ) rats. Glucose uptake and electrophysiological properties were measured in ventricular cardiomyocytes from normoglycemic and STZ-induced diabetic rats given vehicle or 20 mg · kg⁻¹ · day⁻¹ losartan for 8 weeks. Insulin and β -adrenergic stimulation failed to increase the glucose uptake rate in STZ cardiomyocytes, whereas the α -adrenergic effect persisted. Concurrently, a typical prolongation of action potential duration (APD) and a decrease of transient outward current (I_{to}) were recorded in patch-clamped STZ myocytes. Treatment with losartan did not affect body weight or glycemia of diabetic or control animals. However, in losartan-treated STZ-induced diabetic rats, β -adrenergic-mediated enhancement of glucose uptake was completely recovered. APD and I_{to} were similar to those measured in losartan-treated control rats. A significant ($P < 0.0001$) correlation between metabolic and electrophysiological parameters was found in control, diabetic, and losartan-treated diabetic rats. Thus, angiotensin receptor blockade protects the heart from the development of cellular alterations typically associated with diabetes. These data suggest that angiotensin receptor blockers may represent a new therapeutic strategy for diabetic cardiomyopathy. *Diabetes* 53:1927–1933, 2004

Diabetes is known to be associated with cardiac dysfunction that is only partly accounted for by ischemic heart disease (1). Several experimental data show that hyperglycemia and/or hypoinsulinemia could directly affect myocardial function. For

example, in rats made hypoinsulinemic with an injection of streptozotocin (STZ), a widely used animal model for hyperglycemia and diabetes, fatty acid plasma concentrations were increased and glucose transport and oxidation were severely depressed in most insulin-sensitive tissues (2). Because of insulin deficiency, the rat myocardial energy supply is forced essentially toward fatty acid metabolism (3). This alteration in myocardial energy substrate utilization has detrimental consequences that may contribute to the development of cardiac dysfunction (4). Furthermore, hyperglycemia is associated with several myocardial electrophysiological and metabolic alterations both in vitro and in vivo (5–7). Indeed, recent findings (2) suggest that important links may exist between cardiac metabolism, maladaptive remodeling, and altered electrical features, although a clear correlation is still lacking.

Activation of the renin-angiotensin system and subsequent signaling through the type 1 angiotensin II receptors (AT₁s) appear to contribute to the development of cardiovascular diseases in diabetes, including cardiomyopathy (8). ACE inhibitors are known to improve the outcome of heart failure in diabetic patients in a similar manner to, or even to a greater extent than, that seen in nondiabetic individuals (9); however, their therapeutic efficacy could be largely mediated through AT₁-independent mechanisms, such as modulation of nitric oxide (NO) synthesis (10). A variety of data indicate a direct effect of angiotensin II (ANG II) on cardiomyocytes in diabetes and suggest that angiotensin receptor blockers (ARBs) may represent a novel therapeutic approach for the treatment of diabetic cardiomyopathy and prevention of sudden cardiac death (1,11). Tissue concentrations of ANG II and AT₁ density increase in the diabetic rat myocardium (12). ARBs hinder cardiomyocyte apoptosis (a feature of diabetic cardiomyopathy) (13) by reducing autocrine ANG II production by cells in culture (14). In vitro exposure to a hyperglycemic medium alters the contractile properties of isolated ventricular myocytes, an effect that is prevented by ARBs (15). Finally, incubation of cardiac diabetic myocytes with an ARB or ACE inhibitor restores their electrical properties, particularly the expression of transient outward current (I_{to}), which is typically depressed in this setting (16). Despite these observations, experimental data supporting the benefits of in vivo treatment with ARBs on diabetes-induced cardiac derangement are still lacking. Treatment of STZ-induced diabetic rats with an ARB prevents the decline of GLUT4 expression in the heart, which is typical of hypoinsulinemic diabetic animals (17), but it is still unknown whether this phenomenon is associated with a

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Received for publication 9 December 2003 and accepted in revised form 14 April 2004.

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ANG II, angiotensin II; AP, action potential; APD, action potential duration; ARB, angiotensin receptor blocker; AT₁, type 1 angiotensin II receptor; 2-DG, 2-deoxy-D-glucose; HP, holding potential; I_{to} , transient outward current; STZ, streptozotocin.

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TABLE 1
 Characteristics of normoglycemic and STZ-induced diabetic rats, with and without losartan treatment

	Ctrl	Ctrl _{Los}	STZ	STZ _{Los}
Rats (<i>n</i>)	8	8	12	9
Daily water consumed (ml)	22.3 ± 1.4	22.0 ± 1.0	89.9 ± 10.3*	115.2 ± 19.7*
Plasma glycemia (mmol/l)	2.7 ± 0.4	3.0 ± 0.2	32.8 ± 6.2*	35 ± 6.06*
Body weight (g)	343 ± 22.9	316 ± 15.6	229 ± 10.8*	242 ± 10.9*
Heart weight (g)	1.31 ± 0.07	1.14 ± 0.05	1.05 ± 0.06†	1.05 ± 0.07
Heart weight-to-body weight ratio (mg/g)	3.88 ± 0.18	3.65 ± 0.20	4.64 ± 0.26	4.30 ± 0.26
Cells (<i>n</i>)	52	55	45	73
Cell membrane capacitance (pF)	133.9 ± 7.4	115.8 ± 4.2	136.8 ± 5.7	122.0 ± 5.5

Data are means ± SE. **P* < 0.01, †*P* < 0.05 vs. respective controls.

concomitant recovery of single cardiomyocyte functionality, similar to that reported for in vitro exposure to ARBs (15,16).

An essential feature of both type 1 and type 2 diabetes is the impairment of the cardiomyocyte glucose supply. Under normal physiological conditions, this supply is ensured by specialized transporters, namely the GLUT4 isoform, which is highly expressed in the heart. In cardiomyocytes, GLUT4 recruitment (i.e., trafficking toward plasma membrane and recycling) is mainly controlled by insulin (18), although other stimuli, such as the Gq-linked receptor agonists, norepinephrine, and ANG II, can produce GLUT4 translocation (19).

The aim of our study was to assess the effect of in vivo treatment with losartan, an ARB, on some of the metabolic and electrical parameters known to be profoundly altered in diabetic ventricular myocytes (5,7), such as glucose disposition, action potential duration, and *I*_{to}, the main current controlling repolarization in the rat ventricle. Preliminary data from this study have appeared in abstract form (20).

RESEARCH DESIGN AND METHODS

All of the experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) for experimental animal care. Diabetes was induced by a single injection of STZ (55 mg/kg in a sterile citrate solution; pH 4.5) in the tail vein of male Wistar rats (Charles River, Calco [LC], Italy). Rats were then placed in single cages, and their water consumption was monitored every day. Free access to food and water was guaranteed to each rat for the duration of the experiment. Losartan was dissolved into the drinking water, and its concentration was adjusted according to water consumption to maintain a daily dosage of 20 mg/kg.

Rats were divided into four groups: untreated normoglycemic rats (Ctrl), normoglycemic rats treated with losartan added to drinking water for 3 weeks (Ctrl_{Los}), untreated diabetic rats made diabetic by a single injection of STZ (STZ), and diabetic rats treated with losartan added to tap water 1 week before diabetes induction and continued for 2 weeks afterward (STZ_{Los}). At the end of treatment, rats were fasted overnight, weighed, and killed. Single left ventricular myocytes were isolated from the left ventricle of control or diabetic rats using a protocol based on previously described procedures (21,22) and used within the day. Blood samples were taken from each rat.

2-Deoxy-D-glucose uptake. Cardiomyocytes resuspended in buffer A (see SOLUTIONS) were adapted to Ca²⁺ by stepwise adding of CaCl₂ up to 1 mmol/l. The cardiomyocytes were then washed with buffer E to eliminate glucose and counted using a hemocytometer. Cardiomyocytes were distributed in flat-bottomed plastic vials (30,000 cells/vial in 1 ml of buffer E) and incubated in a shaking water bath at 37°C. To evaluate adrenergic-dependent stimulation of glucose uptake, cardiomyocytes were preincubated with the adrenergic antagonists prazosin or propranolol (1 μmol/l) for 15 min before adding isoprenaline (100 μmol/l) or phenylephrine (10 nmol/l to 1 mmol/l), respectively, for an additional 10 min. Insulin-dependent stimulation was evaluated after 30 min of exposure to insulin (10 nmol/l, Humulin; Ely Lilly, Indianapolis, IN). At the end of the incubation period, sugar uptake was started by adding [³H]2-deoxy-D-glucose (2-DG, 16.5 μmol/l [3 μCi/ml]; ICN Pharmaceuticals,

Costa Mesa, CA) and stopped after 10 min by adding phloretin (400 μmol/l). Specific glucose uptake was assessed as described by Fischer et al. (23). Cells were then filtered on nitrocellulose filters (8 μmol/l; Sartorius, Edgewood, NY) under a light vacuum and washed twice with cold buffer A. The filters were dried at room temperature for 5 min, and the radioactivity present on filters was measured using a β-counter machine (Packard Instrument, Meriden, CT). Each experiment was run in triplicate. Glucose uptake was expressed as pmol · 10⁶ cells⁻¹ · 10 min⁻¹ of the amount of [³H]2-DG recovered inside cells.

Electrophysiological recordings. The experimental setup for patch-clamp (whole cell) recording and data acquisition was similar to that previously described (24,25). The patch-clamped cell was superfused by means of a temperature-controlled microsuperfusor that allows rapid changes of the solution bathing the cell with normal or modified Tyrode's solutions (see SOLUTIONS). Temperature was maintained at 36–37°C. Patch-clamp pipettes had a resistance of 1.5–2.5 MΩ when filled with the internal solution (see SOLUTIONS). Cell membrane capacitance was measured by applying a ±10 mV pulse starting from a holding potential (HP) of -70 mV. The current transient after this clamp protocol was then fitted with a monoexponential model to compute series resistance, peak current, and the time constant of current decay (24). Action potentials (APs) were elicited at a rate of 0.2 or 1 Hz and sampled at 2 kHz. The following parameters were measured: maximum diastolic potential, overshoot, AP amplitude, and AP duration (APD) at -20 mV, -50 mV, and 50 and 90% repolarization (APD₋₂₀, APD₋₅₀, APD₅₀, and APD₉₀, respectively). The *I*_{to} was evoked by steps in the range of -40 to 70 mV from an HP of -70 mV (sampling rate, 5 kHz); a prestep to -40 mV was used to inactivate sodium current. Series resistance and membrane capacitance were compensated by ~80% to minimize the capacitive transient. *I*_{to} was measured as the peak outward current at the beginning of the depolarizing step and normalized with respect to the membrane capacitance value. Recovery from inactivation was evaluated by applying double pulses to 60 mV, separated by intervals of 5–300 ms.

Solutions. The composition of the low-calcium solution for cell isolation was as follows (in mmol/l): 120 NaCl, 10 KCl, 1.2 KH₂PO₄, 1.2 MgCl₂, 10 D⁺-glucose, 20 taurine, and 10 HEPES-NaOH, pH 7.0. The solutions for measuring glucose uptake (in mmol/l) were buffer A (6 KCl, 1 Na₂HPO₄, 0.2 NaH₂PO₄, 1.4 MgSO₄, 128 NaCl, 10 NaHEPES, and 0.1 CaCl₂, pH 7.4) and buffer E (buffer A containing 2% fatty acid-free BSA and CaCl₂). Solutions for electrophysiological measurements (in mmol/l) were normal Tyrode's solution (140 NaCl, 5.4 KCl, 1.5 CaCl₂, 1.2 MgCl₂, and 10 glucose, and 5 HEPES-NaOH, pH 7.35), Tyrode's solution for *I*_{to} (normal solution plus 0.5 mmol/l CdCl₂, used to block calcium current), and pipette solution (130 K-aspartate, 5 Na₂-ATP, 2 MgCl₂, 5 CaCl₂, 11 EGTA, and 10 HEPES-KOH, pH 7.2, pCa 7.0).

Data analysis and statistics. Analysis and fitting of electrophysiological measurements were performed as previously described (24). Concentration-effect curves were analyzed using the GraphPad Prism program (version 3.00; GraphPad Software, San Diego, CA). All data are expressed as means ± SE. Statistical analysis was performed by means of the GraphPad Prism and GraphPad Instat program (version 3.05, GraphPad Software), using the Student's *t* test or one-way ANOVA followed by the Student-Newman-Keuls test. *P* < 0.05 was considered significant.

RESULTS

Characterization of animal groups. After 3 weeks, STZ rats showed a significant increase in water consumption and plasma glycemia, as expected (Table 1). Diabetes also caused a marked reduction in body weight and heart weight; as a result, the heart weight-to-body weight ratio, an index of cardiac hypertrophy, was not signifi-

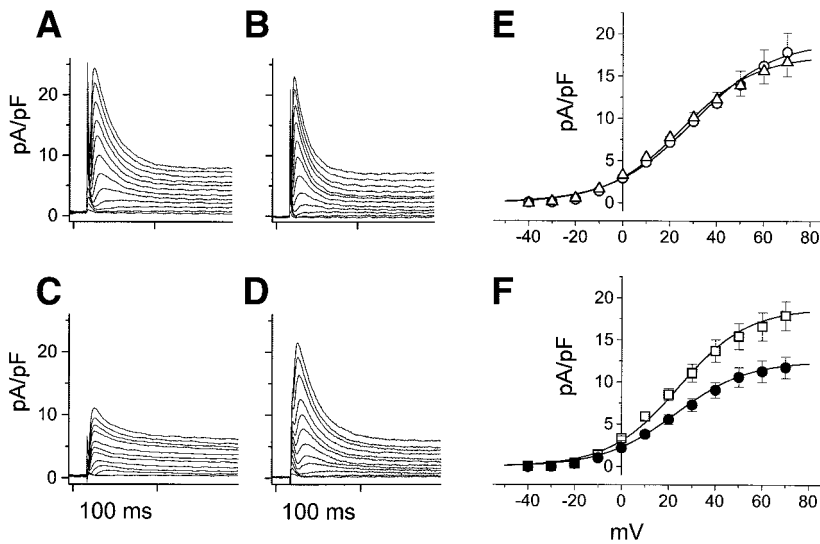


FIG. 1. Effect of diabetes and losartan treatment on I_{to} . Typical recording obtained in myocytes from Ctr (A), Ctr_{Los} (B), STZ (C), and STZ_{Los} (D) rats. E and F: Current-voltage relation. Each point is the mean \pm SE of values obtained in 23–36 different cells from Ctr (\circ), Ctr_{Los} (Δ), STZ (\bullet), and STZ_{Los} (\square) rats. Lines represent fitting of data points with a Boltzmann function. Number of rats per group is as indicated in Table 2.

cantly changed (Table 1). Concurrently, membrane capacitance, an electrophysiological index of cell size and hypertrophy, was similar in Ctr and STZ rats. Treatment with losartan did not modify any of these parameters; in particular, the concentration of plasma glucose was unchanged.

Effect of ARBs on I_{to} . The repolarizing potassium current I_{to} density was markedly decreased in STZ rats in comparison with Ctr rats at each voltage. Average values for maximal I_{to} density were reduced from 19.7 ± 2.5 pA/pF in Ctr rats ($n = 23$) to 12.6 ± 1.4 pA/pF in STZ rats ($n = 34$; $P < 0.05$). Treatment with losartan fully reversed I_{to} depression in STZ animals, but did not affect this parameter in losartan-treated normoglycemic rats; maximal I_{to} densities in normoglycemic and diabetic rats were similar (17.3 ± 1.8 pA/pF [Ctr_{Los}, $n = 23$] vs. 18.7 ± 1.8 pA/pF [STZ_{Los}, $n = 36$]; $P < 0.05$ STZ_{Los} vs. STZ). The midpoint and slope of the current-voltage relation were not affected by diabetes or losartan pretreatment (Fig. 1E and F).

Recovery from inactivation, a feature that may indicate changes in the expression of different isoforms of the I_{to} channel, was evaluated by applying double pulses to 60 mV, separated by intervals of 5–300 ms. The results are shown in Fig. 2, where current amplitude, normalized with respect to the amplitude of the first pulse, is reported as a function of recovery time (fractional recovery). Although

the difference in current density was evident by comparing traces obtained in STZ rats (Fig. 2C) with those from Ctr, Ctr_{Los}, and STZ_{Los} rats (Fig. 2A, B, and D, respectively), fitting data with a monoexponential function gave similar time constants in all groups (Fig. 2E and F).

The α -adrenergic agonist phenylephrine (30 μ mol/l) in the presence of the β -blocker propranolol (0.1 μ mol/l) decreased I_{to} in normoglycemic rats (from 20.8 ± 3.0 to 16.3 ± 3.0 pA/pF; $n = 15$; $P < 0.02$), STZ-induced diabetic rats (from 13.0 ± 1.7 to 10.6 ± 1.6 pA/pF; $n = 21$; $P < 0.02$), and STZ_{Los} rats (from 18.9 ± 1.9 to 16.1 ± 1.7 pA/pF; $n = 23$; $P < 0.02$), although current density was significantly smaller in the untreated diabetic group ($P < 0.05$ vs. control rats) and recovered in the losartan-treated animals ($P < 0.05$ vs. STZ rats) (data not shown).

Effect of ARBs on myocyte AP. The AP characteristics in untreated and losartan-treated rats are summarized in Table 2; typical APs measured during stimulation at 0.2 Hz are shown in Fig. 3. The APD was markedly prolonged in myocytes from STZ rats compared with those from Ctr rats. Although treatment with losartan did not modify the AP profile in normoglycemic rats, it completely reversed APD prolongation in STZ_{Los} rats. AP amplitude and diastolic potential were not affected by either diabetes or AT₁ blockade.

When similar measurements were repeated in myocytes paced at a higher and somewhat more physiological rate

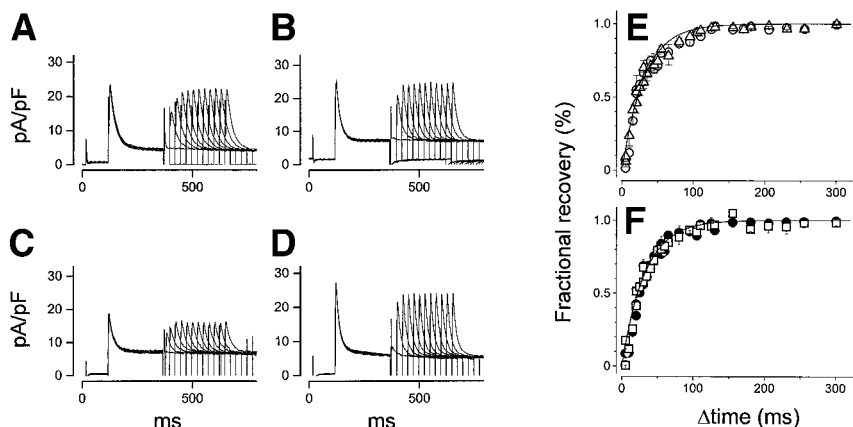


FIG. 2. I_{to} recovery from inactivation in myocytes from Ctr (A), Ctr_{Los} (B), STZ (C), and STZ_{Los} (D) rats. E and F: Fractional current recovery plotted as a function of recovery time (Δ time). Each point is the mean \pm SE of 10–16 cells from Ctr (\circ), Ctr_{Los} (Δ), STZ (\bullet), and STZ_{Los} (\square) rats. Lines represent fitting of data points with a mono-exponential function, giving the following time constants (in ms): Ctr, 30.5 ± 5.8 ; Ctr_{Los}, 31.3 ± 2.3 ; STZ, 31.7 ± 4.7 ; and STZ_{Los}, 30.2 ± 6.8 . Number of rats per group is as indicated in Table 2.

TABLE 2

Characteristics of APs in normoglycemic and diabetic rats with or without losartan treatment

Group	<i>n</i> (cells/rats)	MDP (mV)	OS (mV)	AP (mV)	APD ₋₂₀ (ms)	APD ₋₅₀ (ms)	APD ₉₀ (ms)
Ctr	28/7	-75.9 ± 0.9	26.4 ± 1.9	102.3 ± 2.6	43.9 ± 9.0	64.6 ± 10.2	105.8 ± 10.5
Ctrl _{Los}	20/5	-73.9 ± 1.1	23.2 ± 1.7	97.0 ± 2.5	31.5 ± 3.9	62.8 ± 4.5	91.3 ± 5.0
STZ	28/8	-72.8 ± 0.7	23.7 ± 1.5	96.5 ± 1.7	59.9 ± 9.5	108.7 ± 12.7*	144.2 ± 13.4*
STZ _{Los}	20/7	-73.9 ± 1.0	24.9 ± 2.3	98.6 ± 3.1	39.0 ± 7.4	71.1 ± 10.5 [†]	100.2 ± 11.4 [†]

Data are means ± SE. Driving rate was 0.2 Hz. * $P < 0.05$ STZ vs. Ctr and Ctrl_{Los}; [†] $P < 0.05$ STZ_{Los} vs. STZ. MDP, maximum diastolic potential; OS, overshoot.

(1 Hz), APD measured at 50 or 90% repolarization was significantly prolonged in STZ compared with Ctr or Ctrl_{Los} rats. Again, pretreatment with losartan completely reversed APD prolongation to values similar to those measured in Ctr or Ctrl_{Los} rats (Fig. 4A). Interestingly, when the stimulation rate was increased from 0.2 to 1.0 Hz, the rate-dependent changes of APD were more pronounced in the diabetic conditions (Fig. 4B); in fact, the ratio between APD₅₀ measured at low (0.2 Hz) and high (1 Hz) driving rates was significantly larger in STZ cells compared with Ctr or Ctrl_{Los}. Again, this parameter was restored by losartan, thus suggesting that treatment with the ARB might also act by normalizing the rate-dependent adaptation of APD.

Glucose uptake in cardiomyocytes from normoglycemic and diabetic rats. We next tested whether treatment with losartan could also influence the perturbation of glucose homeostasis. To achieve this, basal glucose uptake and its modulation by hormonal (insulin) and neuro-humoral signals (α - and β -adrenoceptor agonists) were measured in the same pool of cardiomyocytes used for electrophysiological measurements. In cells prepared from Ctr rats, all signals significantly stimulated glucose uptake over basal (Fig. 5A–C). The same extent of stimulation was measured in cells from Ctrl_{Los} rats; thus data from Ctr and Ctrl_{Los} were pooled and referred to as Ctr. Insulin and isoprenaline failed to significantly enhance the glucose uptake in cardiomyocytes from STZ rats

(Fig. 5A and C), whereas the phenylephrine concentration-response curve shifted toward the left (half-maximal effective concentration [EC₅₀]: 26.7 ± 1.5 [Ctr] vs. 0.95 ± 0.19 μ mol/l [STZ]; $P < 0.01$) (Fig. 5B).

In STZ_{Los} cells, insulin was still unable to induce a significant increase in glucose uptake (Fig. 5A). However, the effect of isoprenaline was completely restored in STZ_{Los} cells (Fig. 5C), and the EC₅₀ for the concentration-response curve obtained with phenylephrine returned to a value not significantly different from that measured in Ctr cells (10.1 ± 1.4 μ mol/l; $P > 0.05$) and significantly different from that measured in STZ cells ($P < 0.05$) (Fig. 5B).

Correlation between cellular metabolic and electrophysiological properties. Finally, we compared the effects of the ARB on metabolic and electrophysiological characteristics measured in parallel in myocytic pools from the same rat hearts. We chose two parameters, β -adrenergic stimulation of glucose uptake and I_{to} density, which were most affected by diabetes. In Fig. 6, the average I_{to} density measured in myocytes from a single heart was plotted as a function of β -adrenergic stimulation of glucose uptake in cells from the same heart. In Fig. 6, it is evident that all data from diabetic rat hearts are clustered in the lower left side of the plot, whereas those from Ctr and STZ_{Los} diabetic rats are distributed toward the upper right side. Linear regression demonstrated a significant correlation between the two parameters ($r = 0.80513$; $P < 0.0001$), suggesting that metabolic and electrophysiological cellular properties vary in accordance in these settings.

DISCUSSION

The primary novel result of our study was that the ARB losartan exerts a protective action against diabetes-induced cardiomyocyte dysfunction independent of normalization of the hyperglycemic condition. In fact, treatment with losartan modified several metabolic and electrophysiological parameters measured in isolated cardiomyocytes without affecting glycemia.

In our study, we used a well-characterized model for type 1 diabetes, the STZ-injected rat (7). In this animal, we found that not only was the insulin-dependent activation of glucose uptake blunted, as already described (4), but also that the adrenergic stimulation was remarkably changed. In STZ cardiomyocytes, only phenylephrine stimulated glucose uptake. Moreover, the concentration-response curve for phenylephrine was significantly shifted leftward. This latter effect might represent the result of tissue adaptation for finding alternative mechanisms to supply fuel when insulin and β -adrenergic systems are both blunted, as reported (26) for other cellular mecha-

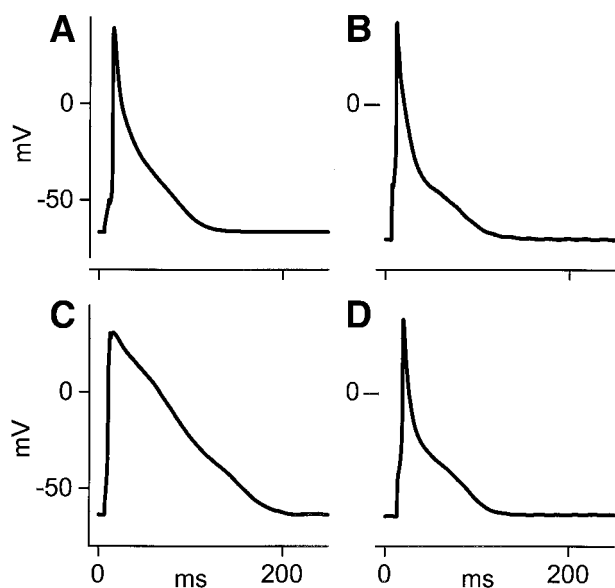


FIG. 3. Typical action potentials from myocytes of Ctr (A), Ctrl_{Los} (B), STZ (C), and STZ_{Los} (D) rats. The driving rate was 0.2 Hz. Number of rats per group is as indicated in Table 2.

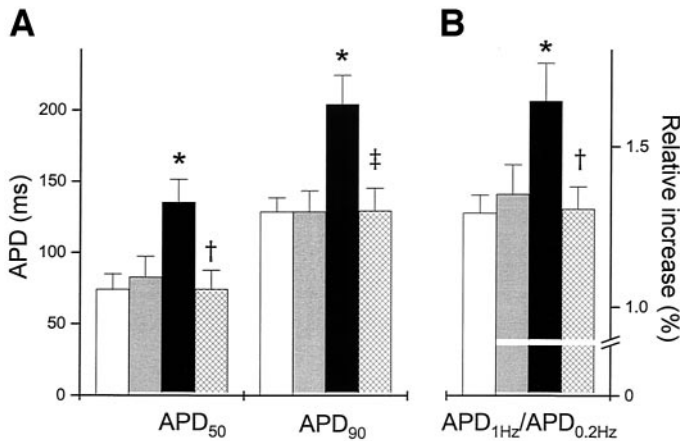


FIG. 4. Restoration of the rate dependence of APD by losartan. **A:** APD measured at 50 (APD₅₀) or 90% (APD₉₀) repolarization during stimulation at 1 Hz. **B:** Ratio of the APD measured at 50% repolarization at 0.2 (APD_{0.2Hz}) or 1 Hz (APD_{1Hz}) driving rates. Each column represents the mean \pm SE of 15–20 cells. \square , Ctr; \blacksquare , Ctr_{Los}; \blacksquare , STZ; \boxtimes , STZ_{Los}. * $P < 0.05$ STZ vs. Ctr and Ctr_{Los}; † $P < 0.05$ STZ_{Los} vs. STZ; ‡ $P < 0.01$ STZ_{Los} vs. STZ. Number of rats per group is as indicated in Table 2.

nisms. Our results showed for the first time that losartan treatment fully restored the β -adrenergic-dependent activation of glucose uptake. In contrast, the same treatment failed to restore the insulin-dependent activation of the transporters (27). Further investigation is necessary to characterize changes in adrenoceptor function and/or expression in ARB-treated diabetic rats.

Our results demonstrated that this ARB can largely prevent the electrophysiological abnormalities associated with diabetes-induced cardiac remodeling. This observation is based on several findings. The major electrophysiological alteration documented in diabetic ventricular myocytes is a depression of I_{to} (5). In this study, the I_{to} density was completely restored without modifying its voltage-dependent and kinetic properties. Because changes in I_{to} density are attributable to a depression of channel expression (28), at least for the main isoform (6), it is conceivable that an ARB acts by counteracting this phenomenon. In parallel, prolongation of APD, a major alteration in diabetic myocytes (29) attributable to a depressed I_{to} (5,30), was also completely counteracted by losartan. Reversal of the AP prolongation might also have favorable effects on

contractile function, especially relaxation, which is also severely prolonged in STZ-induced diabetic rats (31). Changing the driving rate from 0.2 to 1 Hz produced a statistically greater prolongation of APD in diabetic than in control cells, in agreement with previous experimental data (5) and a recent mathematical model of diabetes-induced electrophysiological alterations (30). Increased dispersion of the QT interval, which is a function of APD, has been described in diabetic patients (32,33) and is known to predict cardiac arrhythmic death (32,34). Alterations of the QT interval are only partly explained by autonomic neuropathy (35), existing myocardial ischemia (36), or left ventricular hypertrophy (36). In our experimental model, the duration of hyperglycemia was likely insufficient to determine autonomic denervation; furthermore, the lack of variations in the heart weight-to-body weight ratio allowed us to rule out the possibility of a relevant ventricular hypertrophy in STZ-induced diabetic animals. The observed increase of APD in diabetic cardiomyocytes can therefore be attributed, directly or indirectly, to hypoinsulinemia and/or hyperglycemia. It should also be considered that STZ treatment determines a rele-

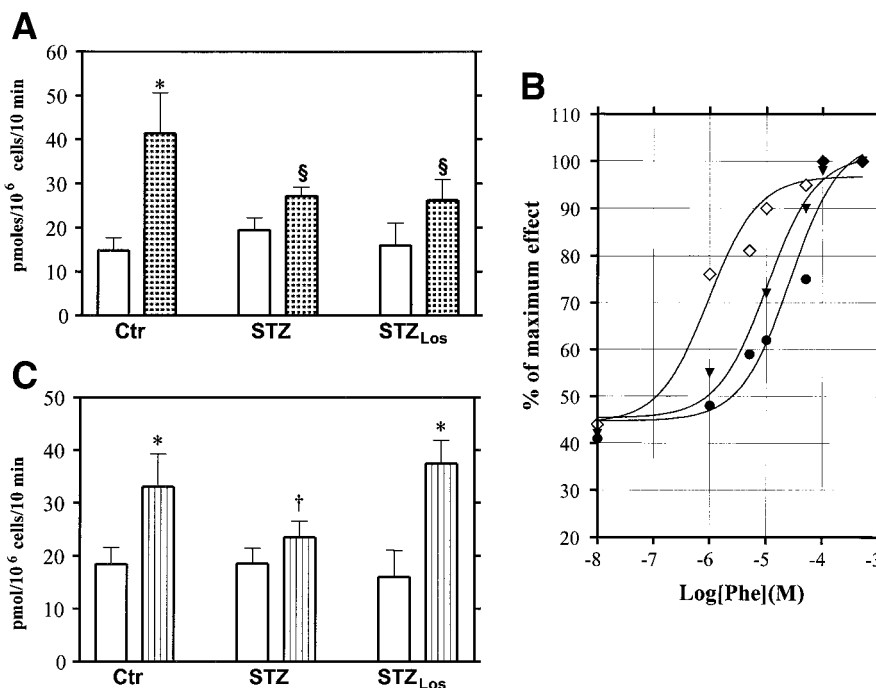


FIG. 5. Insulin- and adrenergic-dependent modulation of glucose uptake in cardiomyocytes isolated from normoglycemic (Ctr), untreated (STZ), and losartan-treated (STZ_{Los}) diabetic rats. **A:** Effect of a 30-min preincubation with 10 nmol/l insulin (\blacksquare) on basal glucose uptake (\square). Results are means \pm SE of eight experiments run in duplicate. **B:** Concentration-response curves obtained in myocytes preincubated with phenylephrine (Phe) (10 nmol/l to 1 mmol/l) in the presence of 1 μ mol/l propranolol. Each point is the mean of 6–8 experiments run in triplicate and represents the percentage of maximum phenylephrine effect obtained in Ctr (●), STZ (◇), and STZ_{Los} (▲) rats. Lines represent fitting of data points. **C:** Isoprenaline-dependent (100 μ mol/l, \blacksquare) stimulation of glucose uptake in cells pretreated with prazosin (1 μ mol/l). Results are means \pm SE of eight experiments run in duplicate. * $P < 0.05$ stimulated vs. basal values; § $P < 0.05$ STZ and STZ_{Los} vs. Ctr; † $P < 0.05$ STZ vs. Ctr and STZ_{Los}. Number of rats per group is as indicated in Table 2.

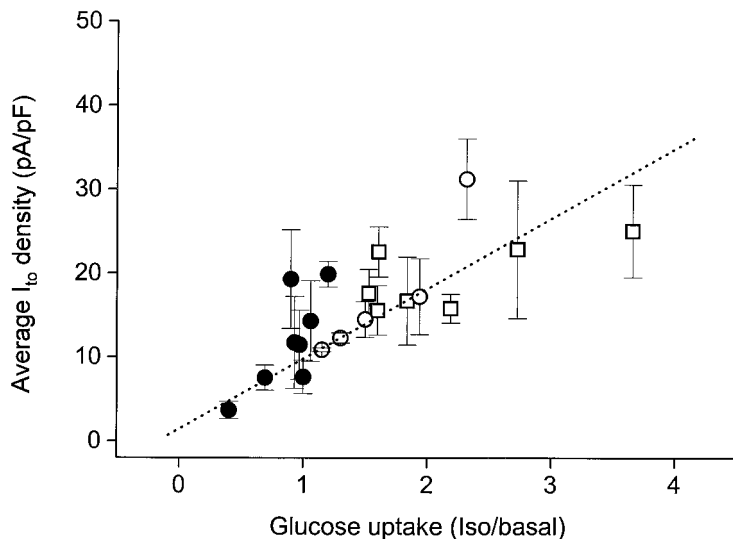


FIG. 6. Correlation between cellular metabolic and electrophysiological properties. Average I_{to} density, measured in myocytes from a single heart, was plotted as a function of the percentage increase of glucose uptake induced by β -AR stimulation (Iso/basal) in cells from the same heart. \circ , normoglycemic rats; \bullet , STZ rats; \square , STZ_{Los} rats. Line represents linear fitting of data points ($r = 0.80513$; $P < 0.0001$). Number of rats per group is as indicated in Table 2.

vant weight loss that could contribute to some of the electrophysiological disturbances observed in STZ-induced diabetic rats.

We have shown for the first time that an ARB normalized the rate-dependence of APD toward control values, thus suggesting that drug treatment could act by restoring the mechanistic linkage that controls APD and its rate-dependent accommodation. This suggests that hyperglycemia-induced alterations of the QT interval in diabetic patients could, at least partly, be reversed by treatment with ARBs. However, to our knowledge, no clinical evidence exists of a reduction in QT duration or dispersion in diabetic patients treated with an ARB, although losartan has been shown (37) to reduce sudden death in diabetic patients, possibly through an effect on myocardial repolarization.

In agreement with previous data (6), changes in myocytic properties were not associated with the development or regression of ventricular hypertrophy. These results differ from those obtained in hypertension-induced electrophysiological remodeling (24,25).

Another novel finding of our study was the significant correlation between the metabolic marker (isoprenaline-induced stimulation of glucose uptake) and electrophysiological marker (I_{to} density) of diabetes-induced cardiomyocyte dysfunction. Thus, although losartan did not restore the insulin-dependent effect on glucose uptake, the drug might improve alternative pathways, such as the β -adrenergic pathway, and be able to reduce the imbalance between glucose uptake and energy request.

Our findings agree with previous data suggesting that the autocrine/paracrine action of ANG II may contribute to diabetes-induced cardiac remodeling. ARBs attenuate the apoptotic signaling in diabetic myocytes by interfering with the AT_1 -activated intracellular cascade (38). Incubation of myocytes from STZ rats with ARBs or ACE inhibitors restored their electrophysiological profile (16). Finally, diabetes promotes a persistent alteration in subcellular localization of the ϵ -isoform of protein kinase C (39), a myocyte-specific isoform possibly implicated in the regulation of gene expression and strongly involved in the control of glucose uptake. Such an alteration is mediated by autocrine stimulation of AT_1 s and fully antagonized by

ARBs, also in the presence of persisting hyperglycemia (39). Altogether, the present results and data in the literature indicate that ARBs work by counteracting the detrimental effect of local overproduction of ANG II. The lack of recovery of insulin-dependent stimulation of glucose uptake may suggest that insulin and β -agonists act by stimulating the trafficking of different intracellular pools of transporters (18,19).

In conclusion, the present experimental data suggest that angiotensin receptor blockade could be useful in the treatment of cardiac dysfunction in diabetic patients, although the insulinopenic animal model used here more closely resembles type 1 than type 2 diabetes. This hypothesis needs to be explored further in appropriate clinical trials.

ACKNOWLEDGMENTS

This study was supported by a grant from Italian Ministry of University and Research (National projects entitled "Molecular mechanisms of ischemic myocardial damage in hyperglycemia and diabetes" and "Mechanisms of progression toward heart failure"). We wish to thank Merck Sharp & Dohme for providing the losartan.

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