Long-term treatment of spontaneously hypertensive rats with losartan and electrophysiological remodeling of cardiac myocytes

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Abstract

Objective: Cardiac hypertrophy due to pressure overload is associated with several cellular electrophysiological alterations such as prolongation of action potential duration (APD), decrease in transient outward current (\(I_{\text{to}}\)) and occurrence of the pacemaker current \(I_{r}\). These alterations may play a role in sudden arrhythmic death, which is a major risk factor in myocardial hypertrophy and failure. Since angiotensin II is a key signal for myocyte hypertrophy, we tested if an 8-week treatment of old spontaneously hypertensive rats (SHR) with the antagonist of type-1 angiotensin II receptor (AT\(_1\)), losartan (10 mg/kg/day), was able to influence the cellular electrophysiological remodeling associated with cardiac hypertrophy. Methods: Left ventricular myocytes were isolated from control (CTR) or losartan-treated (LOS) 18-month old SHR. Patch-clamped LVM were superfused with a normal Tyrode’s solution (to measure action potential) or appropriately modified Tyrode’s solution (to measure \(I_{\text{to}}\) and \(I_{r}\)). Results: Heart weight to body weight ratio (HW/BW) was significantly smaller in LOS (5.69±0.25 mg/g) than in CTR rats (6.67±0.37 mg/g; \(P<0.05\)). Membrane capacitance, an index of cell size, was significantly reduced in LOS (342±12, \(n=92\)) vs. CTR (422±14 pF, \(n=96\), \(P<0.001\)). APD was significantly shorter in LOS than in CTR (at \(-60\) mV: 197±23 vs. 277±19 ms, \(n=28\), \(P<0.001\)); this effect was paralleled by a larger maximum \(I_{\text{to}}\) density in the LOS group (LOS: 15.1±1.4 pA/pF, CTR: 10.0±0.8 pA/pF (\(n=27\), \(P<0.02\)). \(I_{r}\), elicited by hyperpolarizing steps (range: \(-60\) to \(-130\) mV), was consistently recorded in SHR cells; however, its maximal specific conductance was significantly lower in LOS than in CTR rats (28.6±3.6 vs. 54.2±8.0 pS/pF, \(n=55\), \(P<0.001\)). Voltage of half-maximal activation \(V_{1/2}\) of both \(I_{\text{to}}\) and \(I_{r}\) was unchanged by the treatment. Conclusions: AT\(_1\) receptor blockade with losartan prevents the development of myocyte hypertrophy and associated electrophysiological alterations in old SHR. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Angiotensin; Hypertrophy; Membrane currents; Myocytes; Remodeling

1. Introduction

Sudden death due to ventricular arrhythmias is a major risk factor in patients with myocardial hypertrophy and failure [1–3]. Despite optimal medical treatment, mortality in heart failure patients remains high. Recently, the ELITE study reported that treatment of elderly heart failure patients with the type-1 angiotensin II receptor (AT\(_1\)) antagonist losartan was unexpectedly able to reduce mortality by lowering the incidence of sudden deaths [4]. In order to gain insight into the mechanism by which losartan exerts its favorable effect, we planned to study the effect of chronic treatment with losartan on the electrophysiological alterations occurring in SHR with severe cardiac hypertrophy.

Spontaneously hypertensive rats (SHR), a widely used...
ACE inhibitors affect both the angiotensin and bradykinin pathways. The availability of AT$_1$ antagonists will disclose the importance of angiotensin II in the progression of these alterations. For all these reasons, we thought it would be interesting to assess the effect of long-term treatment with losartan, a selective AT$_1$ antagonist, on the electrophysiological alterations occurring in SHR during the development of severe cardiac hypertrophy. To this aim, we studied cell capacitance (an index of cell size), action potential, and membrane currents ($I_{\text{so}}$ and $I_{\text{f}}$) from left ventricular myocytes isolated from the heart of 18-month old SHR after 8-week of treatment with saline or losartan. Preliminary data have been published in abstract form [22].

2. Methods

2.1. Experimental design

Twenty-four spontaneously hypertensive (SHR) male rats of 16 months (Charles River, Italy) were kept in our animal facility; 12 of them were given 10 mg/kg/day losartan (losartan-treated group) dissolved in tap water for 8 weeks; a control group of 12 rats received only tap water during the same period of time, that is, until the age of 18 months.

The water consumption by each animal was measured twice a week, and the amount of drug dissolved in drinking water adjusted, in order to keep the daily dosage at 10 mg/kg/day. At the end of treatment, systolic blood pressure was measured using a tail-cuff system (Basile, Italy). Despite the fact that systolic blood pressure was measured in only five animals for each group, and not routinely, due to the high risk that old SHR may die when submitted to the stress of manipulation, previous studies have clearly documented that losartan 10 mg/kg/day has a modest antihypertensive effect in SHR [23, 24]; this is the case in our study as well.

2.2. Cell isolation

This investigation conforms to the rules for the care and use of laboratory animals of the European Community (86/609/CEE) and with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Single left ventricular myocytes were isolated from control or losartan treated SHR using a protocol based on previously described procedures [14, 17]. Rats were injected with 500 IU heparin i.p., anesthetized with ether and decapitated. After thoracotomy, the heart was rapidly excised, weighed, mounted in a Langendorff apparatus and perfused for 20 min with a low-calcium solution (LCS, see Solutions) prewarmed to 37°C and equilibrated with 100%
O2. The solution was then quickly changed to LCS plus 1 mg/ml collagenase (Type I, Sigma, Italy), 0.03 mg/ml dispase (Boehringer, Italy), 1 mg/ml albumin (Fatty Acid Free Fraction V, Serva, Italy) for 25–30 min. The left ventricle and the septum were removed with fine scissors, minced and the pieces were stirred in the LCS. Cardiomyocytes that appeared in the supernatant were purified by gravity sedimentation, collected and stored in LCS at room temperature. The isolated cells were kept at room temperature in LCS, supplemented with 1 mM CaCl2 and 4% penicillin/streptomycin (Gibco BRL, Italy) and used within the day.

2.3. Electrophysiological recordings

Cells were placed in an experimental bath on the platform of an inverted microscope (Nikon Diaphot TMD, Japan). The patch-clamp technique (whole cell recording) was used to measure electrophysiological properties of the isolated myocytes. Experiments were performed using a patch amplifier (Axopatch-1B, Axon Instruments, CA, USA) interfaced to a 486 personal computer by means of a DAC /ADC interface (Labmaster Tekmar, Scientific Solutions, USA). Data were viewed on-line on an analogic oscilloscope and on a computer screen. Experimental control, data acquisition and preliminary analysis were performed by means of the integrated software package pClamp (Axon Instruments, CA). Cells were superfused with normal Tyrode’s solution or with Tyrode’s solutions properly modified to measure the transient outward current (Ito) and the hyperpolarization-activated inward current (Ih) (see Solutions). Temperature was maintained in the range of 36–37°C. Patch-clamp pipettes, prepared from glass capillary tubes (Garner Glass Co., CA, USA) by means of a two-stage vertical puller (Hans Otchoski, Homburg, Germany), had a resistance of 1.5–2.5 MΩ when filled with the internal solution (see Solutions). The patch-clamped cell was superfused by means of a temperature-controlled micro-superfusor, which allowed rapid changes in the solution bathing the cell.

2.4. Solutions

The composition of the solutions employed was as follows (in mM): Low Calcium Solution (LCS): NaCl 120, KCl 10, KHPO4 1.2, MgCl2 1.2, n(+)-glucose 10, taurine 20, HEPES–NaOH, 10 (pH 7.0). Normal Tyrode’s solution: NaCl 140, KCl 5.4, CaCl2 1.5, MgCl2 1.2, glucose 10, HEPES–NaOH 5 (pH 7.35); Tyrode’s solution for transient outward current: Normal solution plus 0.5 mM CdCl2 used to block calcium current. Tyrode’s solution for hyperpolarization-activated inward current measurements: NaCl 140, KCl 25, CaCl2 1.5, MgCl2 1.2, BaCl2 5, MnCl2 2, 4-aminopyridine 0.5, glucose 10, HEPES–NaOH 5 (pH 7.35); this solution allowed for the reduction of interference from other currents, i.e. L-type calcium current, inward rectifier-like current and transient outward potassium current. Pipette solution: K-aspartate 130; Na2-ATP 5, MgCl2 2, CaCl2 5, EGTA 11, HEPES–KOH 10 (pH 7.2; pCa 7.0).

2.5. Data analysis and statistics

Data analysis and fitting were performed by using the program Origin 4.1 (MicroCal Software Inc., MA, USA) running on a Pentium personal computer. For fitting functions, non-linear models of convergence to solutions were used.

Action potentials were elicited at a rate of 0.2 Hz and sampled at 2 kHz. Depolarizing steps (700 ms) to positive potentials activated a 4-aminopyridine-sensitive outward current, which was composed of transient and steady-state component, as already described in rat ventricular myocytes [14]. Outward currents were evoked by steps in the range of −40 to +70 mV from a HP of −70 mV (sampling rate: 5 kHz); a pre-step to −40 mV was used to inactivate sodium current. Series resistance and membrane capacitance were compensated in order to minimize the capacitive transient. Ito was measured as peak outward current at the beginning of the depolarizing step, and normalized with respect to the membrane capacitance value (see below) [14]. Steady-state currents were measured at the end of the depolarizing steps.

Ih was defined as a time-dependent, hyperpolarization-activated inward current completely and reversibly blocked by adding 4 mM CsCl to the extracellular solution [17]. Ih was evoked by hyperpolarizing steps in the range of −60 to −140 mV from a HP of −40 mV (sampling rate: 0.5 kHz); the duration of the step was decreased (from 3200 to 1600 ms) as the activation became progressively faster. To evaluate steady-state values of the hyperpolarization-activated current, data were fitted to an exponential decay. Current amplitudes were measured as the difference between the value at steady state and that at the beginning of the test pulse, and normalized with respect to the membrane capacitance value (see below). Specific conductance was determined as a function of membrane potential according to the following equation:

\[
g_{\text{f}} = \frac{I}{V_{\text{in}} - V_{\text{rev}}} 
\]

where \(g_{\text{f}}\) is the conductance (in pS/pF) calculated at the membrane potential \(V_{\text{in}}, I\) the current density (in pA/pF) and \(V_{\text{rev}}\) the reversal potential of the fully activated current [17].

For both \(I_{\text{to}}\) and \(I_{\text{h}}\), the Boltzmann function:

\[ y = \frac{1}{1 + e^{(V-V_{1/2})/k}} \]

was used to fit the activation data, where \(V\) (mV) is the test membrane potential, \(V_{1/2}\) (mV) is the fitted potential for
half-maximal activation and \( k \) (mV) is related to the slope of the activation curve.

Cell membrane capacitance (\( C_m \)) was measured by applying a \( \pm 10 \)-mV pulse starting from a holding potential of \(-70\) mV. The current transient following this clamp protocol was fitted with a mono-exponential model to compute series resistance (\( R_s \)) and then \( C_m \) using the two equations given below:

\[
R_s = \frac{\Delta V}{I_{\text{peak}}}
\]

and

\[
C_m = \frac{\tau}{R_s}
\]

where \( I_{\text{peak}} \) is the maximum level of current (relative to the holding current) following the depolarization and \( \tau \) is the time constant of the exponential current decay. The membrane capacitance values obtained have been used to compute ionic current densities (\( I_{\text{no}} \) and \( I_i \) density in \( \text{pA/pF} \)).

All data are expressed as mean±standard error of the mean (S.E.M.). Statistical analysis was performed by means of the Graph Pad Instat program, using the Mann–Whitney test (non-parametric test) for comparing membrane capacitance values, and Student’s \( t \)-test for grouped data (two-sided \( P \) value) for all other values. A probability value of less than 0.05 was considered significant.

3. Results

The dose of losartan (10 mg/kg/day) was chosen on the basis of literature data, showing that this dose causes a modest reduction in blood pressure, but significantly reduces cardiac hypertrophy [23,24]. The data have been confirmed in our study: in five rats for each group we measured blood pressure, which was only slightly reduced by losartan treatment (225±30 vs. 234±24 mmHg in control; not significant). Blood pressure measurements were not performed routinely, because old SHR may die suddenly when manipulated by the operator.

Despite the lack of a statistically significant reduction in blood pressure, 8 weeks of treatment with losartan was able to significantly reduce the degree of cardiac hypertrophy as measured by the HW/BW ratio. HW/BW was 6.67±0.37 mg/g in control rats and 5.69±0.25 mg/g in losartan-treated rats (\( P<0.05 \)). At least part of the reduction in cardiac hypertrophy appears to be due to a decreased cell size. This is shown in Fig. 1, where the distribution of membrane capacitance in the control and treated groups is reported. Cell membrane capacitance is a good index of cell dimension; it can be appreciated that the two cell populations (96 cells from control and 92 from losartan-treated) have a different distribution. The cells isolated from the heart of rats treated with losartan show a distribution toward the smaller dimensions, the mean value being 342±11.6 vs. 422±13.9 pF in the control group. These data are consistent with our previous results in SHR and normotensive WKY rats, in which the cell capacitance measurement nicely reflected the degree of cell hypertrophy.

Fig. 2 shows representative action potentials recorded
from ventricular myocytes isolated from losartan-treated or saline-treated SHR. It is apparent that action potential duration is shorter in the losartan-treated myocyte. This was a consistent finding, as shown in Fig. 3, which summarizes the results obtained in 28 myocytes. It is apparent that the only change found was on the action potential duration, which was significantly shortened either at $-20 \text{ mV}$ ($92\pm15 \text{ vs. } 141\pm14 \text{ ms}$) or at $-60 \text{ mV}$ ($197\pm23 \text{ vs. } 277\pm19 \text{ ms}$). The other action potential characteristics (measured in losartan vs. control) were completely unaffected: amplitude ($104.9\pm2.1 \text{ vs. } 102.4\pm1.7 \text{ mV}$) maximum diastolic potential ($-73.5\pm1.0 \text{ vs. } -74.2\pm1.0 \text{ mV}$) and overshoot ($31.4\pm1.5 \text{ vs. } 28.2\pm1.1 \text{ mV}$). The repolarizing current $I_{to}$ largely controls action potential duration in the rat. A decrease in $I_{to}$ is responsible for the prolongation of action potential duration in hypertrophied myocytes in SHR [14]. In order to assess the ionic basis of the effect of losartan on action potential duration, we studied the characteristics of $I_{to}$.

As shown in Fig. 4, the density of $I_{to}$ was larger in myocytes from losartan-treated animals. The peak outward currents were larger following the protocol (see inset) used to elicit $I_{to}$. This finding was confirmed by the current-voltage relationship obtained in 27 cells from both control or losartan-treated rats: maximum current density was $15.1\pm1.4 \text{ pA/pF}$ in losartan-treated rats and $10.0\pm0.8 \text{ pA/pF}$ in control rats ($P<0.02$); the midpoint and slope of the activation curve were similar in control ($32.8\pm1.7$ and $16.1\pm1.6 \text{ mV}$) and in losartan-treated rats ($34.7\pm1.2$ and $16.4\pm0.6 \text{ mV}$). The steady-state currents, which likely represent $I_{k}$ [14], were slightly but significantly increased in losartan-treated rats (at $+60 \text{ mV}: 6.7\pm0.6 \text{ vs. } 5.2\pm0.4 \text{ pA/pF}, P<0.05$), and might contribute to APD shortening.

Fig. 5 also shows that the pacemaker current $I_{f}$ was affected by losartan treatment. However, the effect was in the opposite direction, in that $I_{f}$ density was clearly reduced in myocytes from the losartan-treated group $g_{max}$, i.e., the maximal current specific conductance calculated by fitting the activation curve with a Boltzmann function, was $54.2\pm8.0 \text{ pS/pF}$ in control and $28.6\pm3.6 \text{ pS/pF}$ in

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**Fig. 3.** Summary of action potential parameters measured in single left ventricular myocytes of control (open columns) and losartan-treated (black columns) SHR. MDP, maximum diastolic potential; OS, overshoot; AP, action potential amplitude; APD, action potential duration at $-20$ and $-60 \text{ mV}$, respectively. * $P<0.05$; ** $P<0.01$.

**Fig. 4.** Effect of losartan treatment on transient outward current density. Left panels: typical recordings of $I_{to}$ evoked during depolarization from $-40$ to $70 \text{ mV}$, in cells from control (a) and losartan-treated SHR (b). Panel c: Average $I-V$ relationships of $I_{to}$ density (in pA/pF) as a function of step potential (in mV), obtained in control (open circles) and losartan-treated (closed circles) SHR. Lines are fitted using a Boltzmann function. The voltage clamp protocol is shown in inset.
losartan-treated rats ($P<0.02$). The representative recordings show that the current is clearly reduced to all voltages; this is even more evident from the mean current–voltage relationship. The activation of the current was not modified by the treatment, $V_{1/2}$ and $k$ being, respectively, $-84.7\pm1.1$ and $8.3\pm2.8$ mV in control and $-87.8\pm1.3$ and $7.5\pm2.4$ mV in losartan-treated rats. In fact the relationship between the time constant of activation reciprocal ($1/\tau$) and voltage is not different in the control and losartan groups.

4. Discussion

In the present study we evaluated the effect of chronic treatment with losartan on the electrophysiological alterations occurring in old SHR, i.e. during a phase of their life during which the development of severe cardiac hypertrophy occurs. SHR is a widely used model for left ventricular hypertrophy; the old SHR may better mimic the clinical course of untreated or poorly controlled essential hypertension in humans [17,25]. The main and novel finding of this study is that blockade of $AT_1$ not only affects development of cardiac and cellular hypertrophy, but also affects the electrophysiological alterations, which characteristically occur in the hypertrophied myocyte. To our knowledge, this is the first demonstration that chronic blockade of $AT_1$ receptors affects cardiac ionic currents. It is already known that chronic treatment with ACE-inhibitors or an $AT_1$ antagonist is able to prevent action potential prolongation [21,26]; at least for captopril the effect is associated with restoration of the repolarizing current $I_{\text{rep}}$ [21], which in the rat is the main current controlling the repolarization phase. Those experiments have been, however, performed in quite young animals, i.e. 18-weeks old at the end of treatment, in which the development of hypertrophy is at its early stages and during which the development of severe cardiac hypertrophy occurs. SHR is a widely used model for left ventricular hypertrophy; the old SHR may better mimic the clinical course of untreated or poorly controlled essential hypertension in humans [17,25]. The main and novel finding of this study is that blockade of $AT_1$ not only affects development of cardiac and cellular hypertrophy, but also affects the electrophysiological alterations, which characteristically occur in the hypertrophied myocyte. To our knowledge, this is the first demonstration that chronic blockade of $AT_1$ receptors affects cardiac ionic currents. It is already known that chronic treatment with ACE-inhibitors or an $AT_1$ antagonist is able to prevent action potential prolongation [21,26]; at least for captopril the effect is associated with restoration of the repolarizing current $I_{\text{rep}}$ [21], which in the rat is the main current controlling the repolarization phase. Those experiments have been, however, performed in quite young animals, i.e. 18-weeks old at the end of treatment, in which the development of hypertrophy is at its early stages and during which the development of severe cardiac hypertrophy occurs. SHR is a widely used model for left ventricular hypertrophy; the old SHR may better mimic the clinical course of untreated or poorly controlled essential hypertension in humans [17,25].
found in myocytes isolated from the old SHR have been found also in myocytes isolated from the failing human heart [19,20]. Taken together, our data suggest that an 8-week treatment with losartan is able to counteract all these electrophysiological alterations when they are already established, and that this could occur also in human with obvious and important therapeutic implications. The present data, however, do not permit to definitely conclude that losartan causes a true regression, i.e. at the level of age-matched normotensive rats, of the electrophysiological remodeling associated with hypertrophy, rather than blocking the progression of the phenomenon. In fact, we lack experimental data in SHR at 16 month of age and historical data show a large variability in many parameters. This is especially true for cell capacitance, APD and consequently $I_{\text{f}}$, which vary continuously both in normal and in hypertrophied myocytes. $I_{\text{f}}$ expression does not depend on cell size [18,27]. It is not present in normal myocytes and its density is directly related to the severity of hypertrophy [18]. 18 month-old SHR show a mean value of $I_{\text{f}}$ density of 48 pS/pF (range: 35.3–54.4 pS/pF [17,18,28]), being 54.2 pS/pF in the present study. Losartan-treated 18-month old SHR have an $I_{\text{f}}$ density of 28.6 pS/pF, a value which is lower than that found in all SHR of the same age we have used, and similar to that found in historical age-matched WKY (20–21 pS/pF [18,28]). $I_{\text{f}}$ current, being expressed only in hypertrophy, can be considered a better index than cell capacitance, APD and $I_{\text{to}}$, to assess the effect of losartan on electrophysiological remodeling. In so doing, a regression of electrophysiological alterations toward age-matched control by chronic treatment with losartan can be hypothesized. Studies in progress will clarify this point.

Interestingly, the effect of losartan was observed for a daily dosage, which does not significantly affect systolic blood pressure. This is not surprising since regression of cardiac hypertrophy in the absence of significant lowering of blood pressure has been reported for both ACE-inhibitors and AT$_1$ antagonists [23,26,29,30]. However, many other studies failed to show a regression in cardiac hypertrophy in the absence of lowered blood pressure [31–34]. The reasons for such discrepancies are not obvious, but are possibly the consequences of the different models of cardiac hypertrophy used; in fact cardiac hypertrophy appears to encompass more than one phenotype, even at the cellular level, and it is likely that multiple switches exist in the process of hypertrophy [35]. Recent evidence suggests that the AT$_1$-mediated angiotensin II signaling is not essential for the development of pressure overload-induced cardiac hypertrophy [36]. In fact, knock-out mice for the A subtype of the AT$_1$ receptor, exhibit the same degree of cardiac hypertrophy of the wild type in response to aortic constriction. Thus the myocardium has alternative signaling mechanisms for the development of hypertrophy; in this line, recent evidence suggests that load, independent of the renin–angiotensin system (RAS) is capable of stimulating cardiac growth [37] and that component of the cardiac RAS are independently and differentially regulated by mechanical stretch and angiotensin II in neonatal cardiac myocytes [38]. On the whole, the activation of the RAS is certainly playing an important role, which, however, requires better understanding [39].

The present results add novel information on the effect of pharmacological treatment on electrophysiological remodeling, showing for the first time that AT$_1$ blockade with losartan is able to influence the electrophysiological alterations associated with cardiac and cellular hypertrophy; they also suggests an important role of angiotensin II and of the stimulation of AT$_1$ receptors in their development. Recent data demonstrating that long-term exposure of canine isolated myocytes to angiotensin II causes changes in the properties of $I_{\text{to}}$ [40] which are responsible for altered in action potential configuration, support such a view. Our results indeed clearly demonstrate that action potential duration is shorter in losartan-treated myocytes, and that this effect is due to a greater $I_{\text{to}}$ and possibly $I_{\text{K}}$. We [14] have previously demonstrated that the main ionic alteration responsible for action potential prolongation caused by chronic hypertension is a specific decrease in $I_{\text{to}}$, the other currents influencing repolarization ($I_{\text{Ca}}$ and $I_{\text{K}}$) being unmodified. The present results are consistent with data obtained in SHR treated with captopril [21] and strongly support the idea that changes in $I_{\text{to}}$ are major determinants in influencing action potential duration in cardiac hypertrophy [13]. Thus, it may be speculated that chronic exposure of ventricular myocytes to angiotensin II, as probably occurs in hypertension, may cause a decrease in $I_{\text{to}}$ channel expression, and that blockade of the RAS system by ACE-inhibitors or blockers of AT$_1$ receptors may antagonize this phenomenon. The electrophysiological changes in action potential duration, $I_{\text{to}}$ and $I_{\text{to}}$ observed in the present study are likely independent of direct electrophysiological effects of losartan or its active metabolite (E3174), taking into account the recently reported actions of these compounds on potassium currents [41].

Losartan-treated myocytes also show an $I_{\text{f}}$ current of smaller amplitude than saline-treated rats. The pacemaker current $I_{\text{f}}$ is physiologically present in pacemaker cells [42]; in rat ventricular myocytes it is expressed during the early neonatal phase [27], is absent in adult ventricular myocytes [17], and is re-expressed during hypertrophy [18]. Its density is directly related to the severity of cardiac hypertrophy [18] and we have hypothesized that it may play a role in the genesis of ventricular arrhythmias in the hypertrophied heart. $I_{\text{f}}$ is also present in ventricular myocytes isolated from the failing human heart [19,20] where its expression seems to depend on the etiology of the underlying disease [43]. Whether its re-expression in ventricular myocytes during cardiac hypertrophy and failure is due to the continuous stimulation of the AT$_1$
receptors is an appealing possibility, which requires further experimentation.

On the whole the overexpression of \( I_f \) and the decrease in \( I_{\omega} \) in ventricular myocytes in disease states associated with an increased propensity for ventricular arrhythmias strongly suggest their role in arrhythmogenesis. A decrease in \( I_{\omega} \) is the ionic mechanism likely responsible for the prolongation of action potential duration; being dishomogeneous, it may cause a dispersion of repolarization, which is per se an arrhythmogenic mechanism [16]. The overexpression of the depolarizing current \( I_f \) may increase the likelihood that a subthreshold delayed after depolarization (a frequent event in hypertrophied or failing myocyte, \([44,45]\)) may reach threshold and trigger an arrhythmia in a heart where the substrate for reentry is certainly present.

The observation that a sub-antihypertensive dose of losartan is able to influence the cellular electrophysiological remodeling associated with cardiac and cellular hypertrophy, suggests that this phenomenon may be the electrophysiological basis for the reduced incidence of sudden deaths described in the ELITE study [4].

5. Limitations

One limitation of the study is that patch-clamp experiments were performed at a low stimulation rate (0.2 Hz); under these conditions the contribution of \( I_{\omega} \) to action potential duration is much higher due to its frequency-dependent inactivation. Furthermore, the role of \( I_{\omega} \) in controlling repolarization in large mammals is disputed. Despite the reported reduction in \( I_{\omega} \) in human failing hearts [46], simulation studies of human ventricular action potential showed a small contribution of this current to APD [47]. Both the fast and slow component of the delayed rectifier \( I_K \) resulted of utmost importance [47]. However, electrophysiological and molecular evidence for the presence of these currents in human ventricle and of their alterations in heart failure are still preliminary. The presence of \( I_f \) in both human and rat myocytes is clearly documented, but direct proof of its role in ventricular arrhythmogenesis is still lacking. Finally, many other cellular electrophysiological changes occurring in the hypertrophied and failing heart may contribute to the phenomenon of electrophysiological remodeling [48].

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