Mineralocorticoid Receptor Antagonism Prevents the Electrical Remodeling That Precedes Cellular Hypertrophy After Myocardial Infarction

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Background—Cardiac hypertrophy underlies arrhythmias and sudden death, for which mineralocorticoid receptor (MR) activity has recently been implicated. We sought to establish the sequence of ionic events that link the initiating insult and MR to hypertrophy development.

Methods and Results—Using whole-cell, patch-clamp and quantitative reverse transcription–polymerase chain reaction techniques on right ventricular myocytes of a myocardial infarction (MI) rat model, we examined the cellular response over time. One week after MI, no sign of cellular hypertrophy was found, but action potential duration (APD) was lengthened. Both an increase in Ca\(^{2+}\) current (\(I_{Ca}\)) and a decrease in K\(^{-}\) transient outward current (\(I_{to}\)) underlay this effect. Consistently, the relative expression of mRNA coding for the Ca\(^{2+}\) channel \(\alpha_{1C}\) subunit (Ca\(_v\) 1.2) increased, and that of the K\(^{-}\) channel K\(_v\) 4.2 subunit decreased. Three weeks after MI, AP prolongation endured, whereas cellular hypertrophy developed. \(I_{Ca}\) density, Ca\(_v\) 1.2, and K\(_v\) 4.2 mRNA levels regained control levels, but \(I_{to}\) density remained reduced. Long-term treatment with RU28318, an MR antagonist, prevented this electrical remodeling. In a different etiologic model of abdominal aortic constriction, we confirmed that APD prolongation and modifications of ionic currents precede cellular hypertrophy.

Conclusions—Electrical remodeling, which is triggered at least in part by MR activation, is an initial, early cellular response to hypertrophic insults. (Circulation. 2004;110:776-783.)

Key Words: hypertrophy ■ action potentials ■ ion channels ■ remodeling ■ hormones

Hypertrophy represents the common heart response to a variety of intrinsic or extrinsic physiological or pathological stimuli. Hypertrophy precedes and is part of overt heart failure and is a key factor in primary heart diseases. The hypertrophied heart shows remodeling of its metabolic, biochemical, and electrophysiological properties and is an independent risk factor for ventricular arrhythmias and sudden death. In this regard, recent clinical studies have shown that mineralocorticoid receptor (MR) antagonists reduce mortality and morbidity in patients with ventricular hypertrophy. The rationale for those studies came from the so-called aldosterone breakthrough in long-term angiotensin-converting-enzyme inhibition therapy. The presence of cardiac MRs suggests that they have local regulatory roles. The mechanisms of this action in cardiac remodeling are not fully understood. The antiarrhythmic benefits of MR antagonists might be linked to their role in ameliorating reactive fibrosis and/or, alternatively, to their electrophysiological effects. Recently, we showed that aldosterone treatment of isolated myocytes mimics the electrophysiological changes that occur during cardiac remodeling. Irrespective of etiology, the common electrophysiological feature of the hypertrophied cardiomyocyte is action potential duration (APD) prolongation, which is involved in a higher propensity to arrhythmias. The change in cardiac repolarization stems from an imbalance of repolarizing and depolarizing ionic currents. However, the downregulation of the outward K\(^{-}\) current, especially the transient one (\(I_{to}\)), and/or the upregulation of the L-type Ca\(^{2+}\) current (\(I_{Ca}\)) are still controversial. Changes may arise not only from the specific etiology and animal models used but also from the stage of the disease. Moreover, alteration of ion channels is thought to be an end-organ response that accompanies structural hypertrophy, resulting from global protein synthesis stimulation or fetal gene reprogramming. However, temporal dissociation of electrical remodeling and structural hypertrophy
has been reported. Moreover, ionic remodeling might be a primary factor in triggering cardiac hypertrophy. Accordingly, electrical remodeling could be independent of mechanical overload, and a specific signal must initiate the remodeling.

In the present study, we analyzed the electrical characteristics of rat myocytes at different stages after myocardial infarction (MI). We show that electrical remodeling precedes cellular hypertrophy. APD lengthened significantly, $I_{Ca}$ was upregulated, and $I_{to}$ was downregulated already at 1 week after MI. Changes in current were matched by changes in mRNA levels. Cellular hypertrophy was evident only 3 weeks after surgery. Moreover, we observed that treatment of post-MI rats with RU28318 prevented early electrical remodeling. In a different model, left ventricular (LV) compensated hypertrophy induced by abdominal constriction, we confirmed that AP prolongation and modifications of ionic currents precede cellular hypertrophy.
Methods

Animal Models
Myocardial Infarction
MI was induced in 250- to 280-g male Wistar rats (Janvier, Le Genest Saint Isle, France) by left coronary artery ligation. Only post-MI hearts that showed a transmural scar were used (always ≥4: number of parts occupied by the scar from a total of 6, as assessed by visual division of the LV free wall).

Abdominal Aortic Constriction
LV hypertrophy was induced in 150- to 180-g male Wistar rats by abdominal aorta constriction (AC). Age-matched, sham-operated animals were used as controls.

RU28318 Treatment
An osmotic minipump (Alzet 2ML4) was implanted subcutaneously at the time of surgery to deliver 50 \( \mu \)g/h RU28318, provided by Aventis (France).

Cell Isolation and Recording Techniques
Animals were euthanized 1 and 3 weeks after surgery with intraperitoneal injection of sodium pentobarbital (50 mg/kg). No apparent signs of failure (pulmonary congestion or chamber dilatation) were observed. Ventricular myocytes were isolated enzymatically. This investigation conformed to the European Community guide for the care and use of laboratory animals (French decree no. 87/848 of October 19, 1987).

Cellular hypertrophy was monitored by 2-photon microscopy (Zeiss LSM 510 NLO) to calculate cell volume (\( V_C \)), and the whole-cell patch-clamp technique was used to measure membrane capacitance (\( C_m \)). An electrical index of membrane surface area. To measure \( V_C \), the voltage-sensitive dye 1-(3-sulfonatopropyl)-8-[b-[2-(di-n-butylamino)-6-naphthyl]vinyl]pyridium betaine (di-4-ANEPPS)–loaded cells were illuminated at 840 nm with a mode-locked Ti:sapphire laser (Mira 900, Coherent) and recorded in 3D. Images of spherical beads (Molecular Probes) were recorded under the same conditions to calculate the point spread function. Deconvolution was performed with Huygens (Bitplane AG). \( V_C \) was estimated by the myocyte cross-sectional area at the center multiplied by the deconvoluted image thickness.

AP and whole-cell current were monitored at 0.1 Hz with an Axopatch 1D amplifier and recorded with pCLAMP-7 (Axon Instruments) at 23°C to 25°C. Series resistance was electronically compensated (40% to 60%). \( I_C \) and \( I_o \) were measured with protocols and solutions previously described. RT-PCR and Real-Time PCR

Total RNA was extracted from isolated cardiomyocytes by a Trizol method (Invitrogen), and its integrity was analyzed by electrophoresis with a chip-based RNA analysis system (Agilent Technologies). To obtain cDNA, 200 ng total RNA was reverse-transcribed with use of the Taqman Gold reverse transcription–polymerase chain reaction (RT-PCR) kit (Applied Biosystems). Real-time PCR analysis was done with an iCycler iQ detection system (Bio-Rad) in a master mix that contained specific primers, as described elsewhere (400 nmol/L for \( C_{a} \), 1.25; and cyclophilin; 500 nmol/L for \( K_{o4} \), 2.25). AmpliTag

Figure 2. Ca\(^{2+}\) currents after MI. A, Current density–voltage relations for \( I_C \) in sham (open circles, \( n=11 \)) and post-MI (closed circles, \( n=15 \)) myocytes 1 week after MI. Insets: Sample records of \( I_C \) generated by 1-week-old sham (gray) and post-MI (black) myocytes. B, Availability of \( I_C \) as determined by applying conventional 2-pulse protocol. Discontinuous lines represent fit to Boltzmann equation. C, Activation kinetics of \( I_C \) (\( \tau_{peak} \)) plotted vs voltage. D, Voltage dependence of inactivation time course. E, F, G, H, Same as in A–D, respectively, but 3 weeks after surgery in 23 post-MI vs 19 sham myocytes. *P<0.05, **P<0.005, ***P<0.0005. Abbreviations are as defined in text.
Gold DNA polymerase, and Taqman probes (100 nmol/L) tagged at the 5' end with the fluorescent molecule 6-carboxyfluorescein (5'-FAM) containing the fluorescent quencher moiety 6-carboxytetramethylrhodamine (3'-TAMRA) in 3'. Each measurement was performed in triplicate.

Statistics
Data are presented as mean±SEM and were analyzed with either an unpaired t test or ANOVA. P<0.05 was considered significant.

Results
Ionic Channel Remodeling Precedes Cellular Hypertrophy After MI
To understand the sequence of events that link the initial insult to hypertrophy, we examined the cellular response over time in post-MI hearts (12 versus 7 animals for 1 week and 12 versus 12 animals for 3 weeks; post-MI versus sham, respectively). To ensure that electrophysiological alteration was assessed in noninfarcted tissue exclusively, only myocytes isolated from the right ventricles (RVs) were used. This procedure eliminates artifacts due to ischemia or apoptosis and thus, deals with reactive hypertrophy. After LV infarction, RV hypertrophy, an important determinant in the prognosis of heart failure, is also observed and might be independent of mechanical factors.

One week after surgery, Vc (Figure 1A) and Cm (see below) were similar in myocytes isolated from post-MI and sham-operated rats, indicating that cellular hypertrophy had not occurred at this stage. Three weeks after MI, myocytes were hypertrophied: Vc (Figure 1D) and Cm (see below) were increased significantly. In contrast, the AP was prolonged already after 1 week (Figure 1B), and this lengthening was maintained with hypertrophy development (Figure 1E). APDs were consistently lengthened from 20% of repolarization 1 and 3 weeks after MI (Figure 1C and 1F, respectively). The zero-current potential (ER) and AP amplitude were similar among all groups (data not shown).

To determine the ionic basis involved in this APD prolongation, we examined Ica and Ito. After 1 week, Ica amplitudes were substantially larger in post-MI than in sham myocytes (inserts in Figure 2A). Normalized to Cm, the increase of Ica densities was statistically significant in the −10- to +20-mV voltage range (Figure 2A). No changes in voltage-dependent availability were observed (Figure 2B). Neither the potential of half-inactivation (V0.5: −25.4±1.7 versus −24.8±1.4 mV for 5 post-MI versus 5 sham, respectively) nor the slope factor (k: −7.8±1.4 versus −8.5±1.6 for post-MI versus sham, respectively) was changed. However, a significant acceleration of activation rates was observed in 1-week...
post-MI myocytes (Figure 2C). Over the −10- to +20-mV range, the time to peak values (tpeak), determined as the time from onset of depolarization to the time of maximal current amplitude, were significantly reduced. The inactivation kinetics were determined by fitting the decay phase of the current traces to a biexponential function. The fast time constants from onset of depolarization to the time of maximal current amplitude were significantly reduced. The inactivation kinetics were not altered 3 weeks after MI (Figure 3D and 3H). The time courses of activation or inactivation were not altered at week 3 (Figure 3C and 3G).

To investigate the molecular mechanisms of the changes in Ica and Ikr densities after MI, the expression level of mRNA corresponding to the α-subunits of Ca1.2 and K4.2, respectively, were quantitatively assessed by real-time RT-PCR. Data were normalized to the amount of mRNA coding for cyclophilin present in the same cell extracts. When expressed as a percentage of the mean amount of mRNA found in sham-operated cells in each experiment, we observed after 1 week a 24% increase in Ca1.2 mRNA (Figure 4A) and a 39% decrease in K4.2 mRNA (Figure 4B). By 3 weeks, molecular levels of both channels returned to their respective control levels (Figure 4C and 4D).

**Figure 4.** Quantitative real-time RT-PCR analysis of mRNA coding for Ca2+ and K+ channel α-subunits after MI. Amplitude histograms show relative expression of Ca1.2 (top) and K4.2 (bottom) mRNAs in isolated myocytes from sham-operated (open bars) and post-MI (closed bars) rats 1 (A and B) and 3 (C and D) weeks after surgery. Columns represent mean values of n experiments. *P<0.05. Abbreviations are as defined in text.

**MR Activation Contributes to Electrical Remodeling in Post-MI Myocytes**

To examine whether inappropriate activation of the MR plays a role during the early response to hypertrophy, we investigated the effect of the MR antagonist RU28318 on animals subjected to MI or sham operation (in 6 versus 5 animals at 1 week and in 4 versus 4 animals at 3 weeks, MI versus sham, respectively). Although it interacts in vitro with the glucocorticoid receptor, RU28318 has been used as a selective MR antagonist because it is rapidly converted in vivo into its lactone form, which is highly selective for MR. Infarct size was similar in the post-MI treated groups compared with untreated groups. RU28318 treatment did not have any effect on Cm after 1 week (Figure 2A), whereas it blunted the increase in Cm in post-MI myocytes 3 weeks after surgery. RU28318 also prevented lengthening of the APD (Figure 5B). AP area, evaluated by integration of voltage variation over time, increased significantly, reflecting APD lengthening in post-MI myocytes, because neither AP amplitude nor Eap was modified. At both weeks 1 and 3, RU28318 treatment blunted the AP prolongation. The Ica density-voltage relations were fitted with a function to estimate the maximal conduc-
Hypertrophy Develops AC Alters Ionic Currents Before Cellular Remodeling

To investigate whether early electrical remodeling is specific to the post-MI model, we examined the well-established AC model by using approaches similar to those in the post-MI model. To minimize electrophysiological heterogeneity, we selected cells from the LV apex. Hypertrophy was not evident until 3 weeks after surgery (heart weight–to–body weight ratios [in mg/g] of AC versus sham-operated rats, respectively, were 4.67±0.1 [n=8] versus 4.61±0.1 [n=10] at week 1 and 5.10±0.1 [n=12] versus 4.51±0.1 [n=11] at week 3; P<0.005). Whereas there was no change in Cm or Vc at week 1 (Figure 6A), significant increases were observed 3 weeks after AC (Figure 6D). As in the post-MI model, AC myocytes also showed AP prolongation after 1 week, whereas no change in Eq or AP amplitude was detected. At week 1, an increase in Is and a decrease in Ic (Figure 6A through 6C) might explain the AP prolongation. No changes in Ica or In activation or inactivation were observed (data not shown). This could indicate an increase in the number of Ca2+ channels and a decrease in the number of Ic channels. Consistently, the increase in Ica induced by β-adrenergic stimulation was similar in sham and AC animals. At 0 mV, 1 μmol/L isoproterenol increased Ica by 175±20% (n=5) versus 174±10% (n=11) in AC versus sham myocytes, respectively.

Three weeks after AC, cellular hypertrophy had normalized the elevated Ica values to control levels, whereas In density remained reduced.

**Discussion**

Cardiac hypertrophy develops as an adaptation to chronic insults. Here we show that electrical remodeling takes place before cellular hypertrophy develops, indicating that these alterations may be independent of mechanical overload. We demonstrate that the mineralocorticoid system, an important component of the neurohormonal reaction, plays an important role in electrical remodeling after MI.

Our results are consistent with published work on electrical remodeling studied at different periods during the development of hypertrophy. Most importantly, our data show that electrical remodeling occurs before cellular hypertrophy development. AP prolongation at the early stages of cardiac hypertrophy may be linked to the upregulation of Ica and the downregulation of In. Later, once cellular hypertrophy is established, only reduced Ica persists, whereas Ic values regain control levels. After 3 weeks, the decrease in repolarizing In would result in AP prolongation. This repolarization rate slowing would enhance Ica activation, which in turn modulates APD. This hypothesis does not exclude that concomitant changes in other currents may also be involved in AP prolongation. The reported changes in Ica and In are rather controversial. A downregulation in In is generally reported, whereas an increased, a decreased, or an unchanged Ica have been described. These discrepancies may reflect differences in etiology, species, nature of analyses, experimental conditions, and even more important, the stage of disease. Our data support the last hypothesis, because they show that Ica increases at early stages and returns to control values once cellular hypertrophy becomes established. Whereas we observed a downregulation of K,4.2 genes and currents before manifest hypertrophy occurred, only the In density decrease persisted with cellular hypertrophy. We suggest that this might reflect a Ca2+ dependence on Ica regulation, as has been noted for aldosterone regulation. After 3 weeks, when Ica regained control levels, the absence of a net regulation of the K,4.2 gene and current magnitude rendered a decrease in density related to the increase in cell size. However, we cannot exclude the possibility that other processes, such as alterations of protein trafficking, come into play.
Taken together, our results show that electrical remodeling precedes cellular hypertrophy, independently of the so-called mechanoconversion, and might be one of the primary factors. In this regard, calcineurin, which triggers the hypertrophic response, is a Ca\(^{2+}\)-dependent phosphatase. Hence, an increase in Ca\(^{2+}\) signaling must precede calcineurin activation to initiate hypertrophy. In the present work, using 2 different models of cardiac hypertrophy, we have shown that AP prolongation, which enhances Ca\(^{2+}\) influx, precedes cardiac hypertrophy. This early enhancement may be responsible for initiating the hypertrophic response. For example, cardiac-specific overexpression of L-Type Ca\(^{2+}\) channels resulted in an increased Ca\(^{2+}\) influx and enhanced basal contractility in mice at 8 weeks of age and progressed to cardiomyopathy by 8 months of age. The signal that induces the increase in L-type Ca\(^{2+}\) channel gene transcription might be at the origin of hypertrophy. We thus suggest that early after an insult, there are neurohormonal signals that induce the Ca\(^{2+}\) influx increase and activate gene transcription, thereby triggering cardiac hypertrophy.

The role of inappropriate MR activation, as an independent contributor to cardiovascular injury, has been suggested by numerous studies. Collectively, different animal and clinical studies have emphasized the pathophysiological cardiac role of MRs. One of the more striking results of the resurgent use of the aldosterone-blocking agent spironolactone or its analogue eplerenone to treat patients with systolic LV dysfunction has been a reduction in the incidence of arrhythmias, which have been mainly related to fibrosis. However, other protective mechanisms have been underscored. Aldosterone is the major regulator of body ion homeostasis. In this regard, we recently established in vitro that aldosterone decreases I\(_{\text{Na}}\) secondary to enhanced Ca\(^{2+}\) signaling, which probably arises from the aldosterone-induced upregulation of I\(_{\text{Ca}}\). In the present study, the MR antagonist RU28318 blunted early electrical remodeling. Moreover, spironolac-
tone is also a powerful blocker of $I_{ca}$ on vascular smooth muscle cells.\textsuperscript{30} We suggest that, besides fibrinogenesis, MR activation has a crucial role in electrical remodeling early after myocardial insult and before morphological remodeling. These early changes in ionic currents might open new therapeutic perspectives.

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