

Aldosterone Upregulates Ca^{2+} Current in Adult Rat Cardiomyocytes

Jean-Pierre Bénitah, Guy Vassort

Abstract—Aldosterone is associated with the pathogenesis and progression of left ventricular hypertrophy and heart failure, independent of its relation with arterial blood pressure. However, little information exists about the possible influence of this mineralocorticoid on cardiomyocyte electrical activity. The present study was designed to determine the role of aldosterone on whole-cell Ca^{2+} current (I_{Ca}) in isolated adult rat ventricular myocytes using the patch-clamp technique. We found that incubation of cells with 1 $\mu\text{mol/L}$ aldosterone for 24 hours increases the density of I_{Ca} significantly. This “long-term” aldosterone treatment had no significant effects on the kinetics and voltage dependence of I_{Ca} inactivation. Moreover, no demonstrable influence of aldosterone on I_{Ca} could be detected during short-term exposure (up to 6 hours), under our experimental conditions. The classical aldosterone intracellular receptor antagonist spironolactone (250-fold excess) was able to blunt the aldosterone-induced increase in I_{Ca} density. These effects were also observed with lower concentrations of aldosterone (10 and 100 nmol/L). Moreover, inhibitors of transcription (actinomycin D, 5 $\mu\text{g/mL}$) and protein synthesis (cycloheximide, 20 $\mu\text{g/mL}$) prevented the aldosterone-dependent increase in I_{Ca} . Therefore, the long latency I_{Ca} stimulation effect of aldosterone might result from an increased channel expression. We suggest that this genomic action contributes to the increased I_{Ca} observed during cardiac remodeling. (*Circ Res.* 1999;85:1139-1145).

Key Words: aldosterone ■ Ca^{2+} current ■ heart

To fully understand the syndrome of heart failure, the interrelationship of underlying causes and regulatory systems must be considered. The activation of several neurohormonal systems has been shown to be an important mechanism contributing to the development of congestive heart failure.¹ In this regard, clinical and experimental data have suggested that the renin-angiotensin-aldosterone system might be one of the primary causes, rather than only a secondary cofactor, in the pathogenesis of left ventricular hypertrophy and heart failure.² Aldosterone is an important determinant of human left ventricular hypertrophy, independent of blood pressure alterations.³ Moreover, exogenous aldosterone has been shown to be arrhythmogenic in dogs after coronary ligation.⁴ The ability of aldosterone to stimulate myocardial fibrosis is likely to be important.^{5,6} However, several cellular electrophysiological changes have been observed in myocardial hypertrophy and heart failure that predispose to cardiac arrhythmias.⁷ A direct effect of aldosterone on myocyte membrane currents has not yet been investigated.

In addition to the classical actions of aldosterone in regulating the membrane ionic movements not only in kidney but also in nonepithelial cells,⁸ several studies argue in favor of an effect of this hormone on cardiovascular functions

through direct actions on myocytes. In fact, aldosterone can bind with high affinity to rat heart.⁹ An agonist-specific mineralocorticoid receptor has been evidenced in cardiomyocytes.^{10,11} In addition to the classical adrenal biosynthetic pathway, production of aldosterone has been demonstrated within cardiac tissue.¹² Moreover, a recent study provides evidence that chronic myocardial infarction is associated with an increase in myocardial aldosterone production.¹³ This raises further questions about how aldosterone might affect the heart directly.

Little information exists about the possible influence of aldosterone on myocyte ionic homeostasis. De Mello and Motta¹⁴ reported a depolarizing action of aldosterone on atria muscle and pacemaker fibers of rabbit. More recently, it has been established that aldosterone can regulate membrane Na^+ transport in cardiac cells. Experimental data demonstrate that aldosterone directly stimulates Na^+ , K^+ -ATPase α_1 subunit mRNA synthesis and protein accumulation in cardiac cells.¹⁵ Moreover, it has been shown in vitro that the exposure of cardiac myocytes to aldosterone rapidly activates the Na^+ - K^+ - 2Cl^- cotransporter to enhance Na^+ influx and stimulate the Na^+ - K^+ pump.¹⁶ By affecting the activity of the ionic transporter, aldosterone modulates acid-base balance in cardiac cells.¹⁷ These data suggest that aldosterone plays a role in the regulation of cardiac function.

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Evidence to support this has accumulated since the late 1950s, suggesting that corticosteroids exert a positive inotropic effect on the heart somewhat similar to digitalis. For instance, aldosterone has been shown to exert a cardiotoxic effect in a rat heart-lung preparation¹⁸ and in cat papillary muscle.¹⁹ Despite this, some investigators have failed to obtain inotropic effects of corticosteroids in isolated heart preparations.²⁰ These data suggest that aldosterone might have an effect on Ca^{2+} influx. Hence, it has been proposed that the Na^+/Ca^+ exchanger could be a target for steroids in heart.²¹ Moreover, it has been shown that aldosterone increased free $[\text{Ca}^{2+}]_i$ in vascular smooth muscle and endothelial cells.^{22,23}

Considering the above data, the aim of the present study was to investigate whether aldosterone has an effect on Ca^{2+} current (I_{Ca}) of cardiac myocytes. We report that treatment of rat ventricular myocytes with aldosterone for 24 hours increased I_{Ca} density, whereas no rapid aldosterone effect (<6 hours) could be detected. Moreover, this "long-term" effect of aldosterone, which did not modify voltage dependence and kinetics of the current, was prevented by a mineralocorticoid receptor antagonist and by inhibitors of transcription and protein synthesis.

Materials and Methods

This investigation conforms to the *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85-23, revised 1996). Isolated cardiac ventricular myocytes from adult male Wistar rats (250 to 350 g; Elevage JANVIER, France) were prepared using a collagenase (Worthington type II) perfusion method as previously described.²⁴ This procedure yielded quiescent rod-shaped myocytes, viable for up to 2 days when incubated at 37°C in Tyrode's solution containing (in mmol/L) NaCl 130, NaH_2PO_4 0.4, NaHCO_3 5.8, MgCl_2 0.5, CaCl_2 1, KCl 5.4, glucose 22, HEPES 25, and insulin 10^{-3} supplemented with 1 mg/mL BSA, 100 IU/mL penicillin and 0.1 $\mu\text{g}/\text{mL}$ streptomycin (titrated to pH 7.4 with NaOH).

Electrophysiological Procedure

The whole-cell patch-clamp method²⁵ was used (Axopatch 1D amplifier, Axon Instruments). Micropipettes had tip resistances ranging from 1 to 1.5 M Ω . The capacitive current was determined as previously described.²⁶ Electronic compensation (40% to 60%) was then used to reduce the series resistance. Membrane currents sampled at 10 kHz, were filtered at 2 kHz, and digitized using pClamp6 software (Axon Instruments). I_{Ca} was measured at room temperature (23°C to 25°C) while cells were bathed in the external solution containing (in mmol/L) NaCl 140, MgCl_2 1.1, CaCl_2 1.8, CsCl 4, glucose 10, and HEPES 10 (pH adjusted to 7.4 with LiOH). The internal pipette solution contained (in mmol/L) CsCl 135, MgCl_2 4, EGTA 5, glucose 10, HEPES 10, Na_2ATP 5, and Na_2 creatine phosphate 3 (pH adjusted to 7.2 with LiOH). The currents were elicited at 0.1-Hz frequency from a holding potential of -80 mV. Before each pulse, a rapid voltage ramp (80 mV/s) to -40 mV was used to inactivate Na^+ current and T-type Ca^{2+} channels.

Drugs

D-Aldosterone and spironolactone (Sigma) were first dissolved in 100% EtOH. Then, stock solutions of 0.1 mmol/L for aldosterone and 25 mmol/L for spironolactone were prepared in H_2O . This gave 0.1% EtOH in the final solutions, which had no effect on the currents measured (data not shown). Actinomycin D mannitol (AmD; Sigma) and cycloheximide (CHX, Sigma) were both directly dissolved in H_2O .

Statistics

Data are expressed as mean \pm SD in the text and as mean \pm SEM in the graphs. Statistical changes induced by aldosterone or other treatments were analyzed by Student *t* test for unpaired samples.

Results

I_{Ca} Increase in Rat Ventricular Cells After 24 Hours of Aldosterone Treatment

The basic hormonal effect of aldosterone, ie, stimulation of Na^+ transport, involves a time-dependent process, in which the mineralocorticoid binds to the intracellular receptor and stimulates protein synthesis.²⁷ In the present study, we have investigated the effects of a long-term exposure of myocytes to aldosterone on the functional expression of high voltage-activated I_{Ca} . Isolated adult rat ventricular myocytes were incubated for at least 24 hours at 37°C in Tyrode's solution supplemented with antibiotics (see Materials and Methods), to which 1 $\mu\text{mol}/\text{L}$ D-aldosterone was added. Control recordings were made from cells maintained in Tyrode's storage solution, in the absence of aldosterone.

Figure 1A, left, shows typical examples of the voltage- and time-dependent inward Ca^{2+} currents recorded in myocytes incubated for 24 hours in the absence (control, left panel) or presence of aldosterone (right panel). Membrane currents were elicited by 300-ms steps from -40 to the -50 to $+30$ -mV range in increments of 10 mV applied at 0.1-Hz frequency. In the two groups, the currents peaked within about 10 ms after the onset of depolarization and gradually declined, with maximal peak current at a potential of -10 mV. Exposure to aldosterone induced an increase in the magnitude of peak currents compared with control.

Because variations in cell size might account for this difference, I_{Ca} amplitudes, measured as the difference between the peak current and the steady-state current at the end of the voltage steps, were normalized to the membrane capacitance (C_m). Before electronic compensation for C_m and series resistance, the mean C_m (in pF, \pm SD) was 172.9 ± 65.8 for control cells ($n=13$) and 170.5 ± 50.4 ($n=10$) for 24-hour aldosterone-treated cells. There was no significant statistical difference between the two groups ($P<0.5$). On average, the current density-voltage (*I-V*) relationships for whole-cell I_{Ca} in control and aldosterone-treated myocytes show a similar characteristic "bell shape" and voltage dependence (Figure 1A, right). There was a significant increase ($P<0.05$) in current densities in the -30 to $+30$ -mV voltage range after aldosterone treatment. The peak I_{Ca} density at -10 mV was larger in aldosterone-treated cells (-20.0 ± 3.7 pA/pF, $n=10$) than in control cells (-13.2 ± 1.6 pA/pF, $n=13$).

I_{Ca} Voltage Dependence and Kinetics Are Unaltered by Long-Term Exposure to Aldosterone

To further compare the voltage-dependent properties of I_{Ca} in the presence or absence of aldosterone treatment, the steady-state inactivation was determined in 8 cells of each group. A conventional 2-pulse protocol was used to establish steady-state inactivation relationships. Prepulses (in the -50 - to $+60$ -mV range, from -40 mV) of 1-second duration were used to inactivate the current. The degree of inactivation was determined by applying a second pulse (test pulse) to 0 mV.

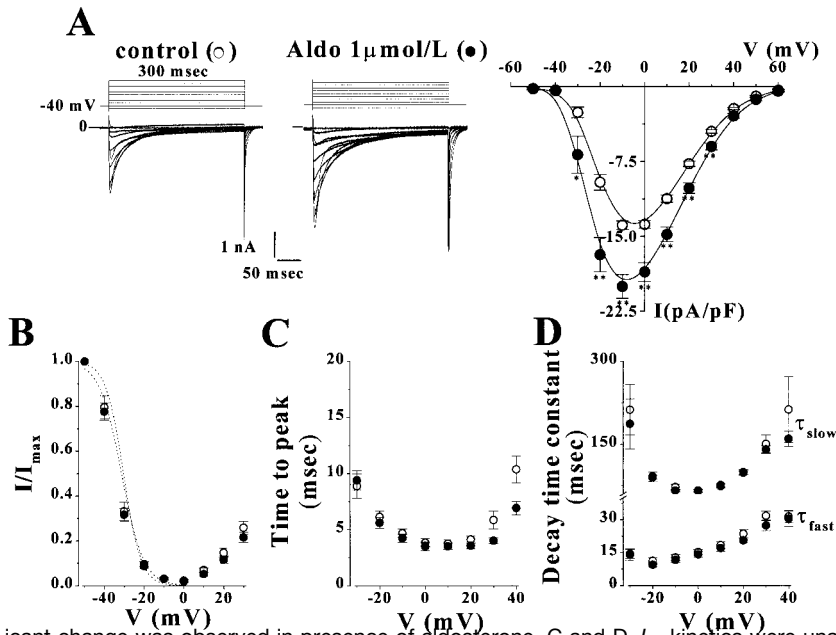


Figure 1. Long-term effect of aldosterone treatment on cardiac I_{Ca} . A, I_{Ca} increase in rat ventricular cells after 24 hours of aldosterone treatment. Left panels, Representative examples of I_{Ca} family recorded in rat ventricular myocytes incubated 24 hours in control conditions (left column, $C_m=171.7$ pF) or in the presence of 1 $\mu\text{mol/L}$ aldosterone (right column, $C_m=170.7$ pF). Right panel, $I-V$ relationships for whole-cell I_{Ca} measured after 24 hours of incubation at 37°C without (\circ , $n=13$) or with (\bullet , $n=10$) 1 $\mu\text{mol/L}$ aldosterone. Sequential comparisons of individual values using Student unpaired t tests show a statistical significant increase in I_{Ca} density with aldosterone treatment in the -30 - to $+30$ -mV voltage range ($*P<0.05$, $**P<0.001$). B, Availability of I_{Ca} was not modified by aldosterone. The degree of inactivation was determined by applying a conventional 2-pulse protocol for cells incubated for 24 hours without (\circ , $n=8$) or with 10 $\mu\text{mol/L}$ aldosterone (\bullet , $n=8$). Discontinuous lines represent the average fits of individual experimental points to the Boltzmann equation. No significant change was observed in presence of aldosterone. C and D, I_{Ca} kinetics were unaltered by aldosterone. C, Activation kinetics of I_{Ca} expressed as the time from onset of voltage step to the peak of current amplitude are plotted versus voltage potential. Aldosterone did not significantly affect the activation kinetics of I_{Ca} . D, Voltage dependence of time course of inactivation. Decay phases of the inward current were fitted to a biexponential function. None of the fast (τ_{fast}) or slow (τ_{slow}) components were significantly affected by aldosterone.

The test current amplitude (I) normalized to the maximum test current (I_{max}) was plotted against the prepulse potential in Figure 1B. There were only minor differences in steady-state inactivation between cells incubated for 24 hours without (\circ , $n=8$) or with 10 $\mu\text{mol/L}$ aldosterone (\bullet , $n=8$). Individual experimental points were fitted to the Boltzmann equation: $I/I_{\text{max}}=1/(1+\exp[(V-V_{50}/k])$, where I/I_{max} is the normalized test-pulse current amplitude, V is the prepulse potential, V_{50} is the prepulse potential of half-maximal inactivation, and k is the slope factor. Because of relief of inactivation for voltages, positive to 0 mV, only data negative to 0 mV were fit. Resulting V_{50} and k values were (in mV, \pm SD) -33.4 ± 2.2 and 4.9 ± 0.5 mV in the presence of aldosterone and -32.9 ± 2.7 and 4.6 ± 0.3 mV in the absence of aldosterone, respectively. The lack of effect of aldosterone on Ca^{2+} channel availability was also observed with Na^+ -free solution (choline chloride substituting NaCl in the external solution) and longer prepulses (5 seconds). Under this experimental condition, after aldosterone treatment, the peak I_{Ca} density at 0 mV was significantly larger (-17.9 ± 2.0 pA/pF, $n=6$) than in control cells (-11.5 ± 3.9 pA/pF, $n=5$). Moreover, the availability of the Ca^{2+} channel was unaltered. The V_{50} and k values were (in mV, \pm SD) -36.6 ± 2.2 and 4.9 ± 0.9 mV in the presence of aldosterone and -34.5 ± 2.3 mV and 3.8 ± 1.5 mV in control, respectively.

A change in the kinetics of I_{Ca} could result in an alteration of Ca^{2+} influx. Visual inspection of the current traces, as those presented in Figure 1A, does not suggest that the time course of activation or inactivation of I_{Ca} was modified by aldosterone treatment. To further quantify this observation, the kinetics of I_{Ca} for each cell were analyzed.

The activation kinetic of I_{Ca} was measured for every depolarizing step as the time from the onset of the voltage step to the peak of current. Time-to-peak values are plotted

against voltage in Figure 1C. Over the whole voltage range, the time-to-peak values were not significantly different between myocytes maintained for 24 hours in the presence or absence of aldosterone.

The time course of inactivation of I_{Ca} was determined by analysis of the decay phase of current traces in response to voltage steps. Best fits were obtained with an equation including a sum of two exponentials plus a constant expressed as $A_{\text{fast}}\exp(-t/\tau_{\text{fast}})+A_{\text{slow}}\exp(-t/\tau_{\text{slow}})+A_0$, where τ and A are the time constant and the initial amplitude of the two components subscribed fast and slow, respectively, and A_0 is the amplitude of the time-independent component. The pooled mean values for the fast (τ_{fast}) and slow (τ_{slow}) time constants of inactivation as a function of voltage are shown in Figure 1D. Both the slow and fast I_{Ca} inactivation at all voltages studied were similar in the presence or absence of aldosterone. At -10 mV (in $\text{ms}\pm$ SD), τ_{fast} was 12.8 ± 4.1 versus 11.8 ± 3.4 and τ_{slow} was 72.2 ± 16.1 versus 68.0 ± 8.5 , in 13 control myocytes versus 10 aldosterone-treated myocytes, respectively.

Lack of Short Latency Effects of Aldosterone on I_{Ca} of Rat Ventricular Myocytes

In contrast to the classical long latency effect of aldosterone, a short-term effect on cells has been proposed.²⁸ Among the data suggesting that aldosterone has a nongenomic, short latency effect is the increased free $[\text{Ca}^{2+}]$, observed in vascular smooth muscle cells²³ and porcine aortic endothelial cells.²² The role of the phosphoinositide and cAMP as important second messengers in aldosterone effects has been documented.^{29,30} Because cardiac I_{Ca} is enhanced by these intracellular messengers, we examined the possible rapid actions of aldosterone on I_{Ca} . Figure 2A shows an example of

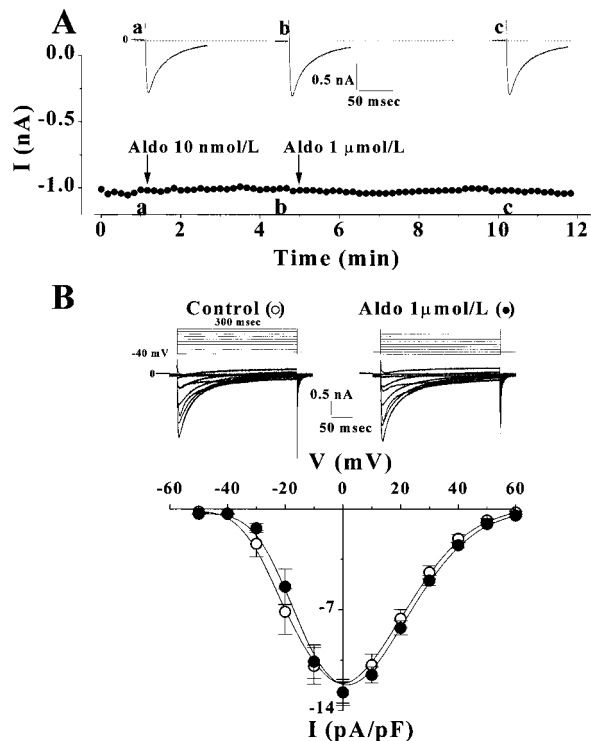


Figure 2. Lack of short-latency effects of aldosterone on I_{Ca} . A, I_{Ca} was unaltered by direct external exposure to aldosterone. Upper panels, Selected I_{Ca} traces (for sake of clarity, only the first 100-ms recording traces are shown) taken before (a) and after external application of aldosterone at the concentrations of 10 nmol/L (b) and 1 μ mol/L (c). The dashed horizontal line indicates zero current level. Bottom panel, Time course of aldosterone effect on I_{Ca} amplitude for the entire experiment, of which the top records were a part. No current changes were observed either at 0 mV as described here or in the whole of potentials (data not shown). B, Short-term incubation in aldosterone had no effect on I_{Ca} . Upper panels, Representative examples of original recordings demonstrating typical whole-cell I_{Ca} family in control (left, $C_m=166.3$ pF) and 6 hours of aldosterone-treated cells (right, $C_m=169.1$ pF). Bottom panel, I - V relationships for the pooled amplitude I_{Ca} density (pA/pF) recorded from rat ventricular myocytes incubated in control solution (\circ , $n=9$) or after at least 3 hours of treatment with 1 μ mol/L aldosterone (\bullet , $n=10$). No significant difference was found at any voltage.

the effect of direct perfusion of aldosterone on I_{Ca} . The bottom panel shows representative Ca^{2+} current amplitude in response to consecutive 300-ms depolarizing pulses (from -40 to 0 mV, 0.1 Hz) in freshly dissociated rat ventricular myocytes. I_{Ca} was insensitive to 10 nmol/L and then 1 μ mol/L aldosterone for >7 minutes. The upper panel shows current traces before (a) and after exposure to aldosterone (b and c); the kinetics of these currents did not change, suggesting that neither the amplitude nor the gating was affected. Similar results were consistently observed in 10 cells. To further check the lack of rapid effect of aldosterone, we examined the effect of a short incubation (3 to 6 hours) of the myocytes with aldosterone at 37°C . Figure 2B, top, shows that I_{Ca} recorded in a cell treated for 6 hours with 1 μ mol/L aldosterone (right) was similar when compared with I_{Ca} recorded in a cell in control conditions (left). To avoid error in pooling data from different-sized myocytes, we normalized the I_{Ca} amplitude by the cell capacitance to obtain I_{Ca} density.

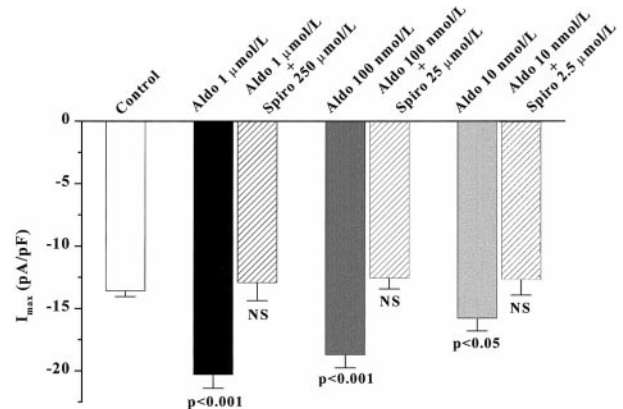


Figure 3. Specificity of the long-term aldosterone effect on I_{Ca} . Bar graph compares maximum I_{Ca} peak densities in myocytes incubated for 24 hours in the absence of drugs (white column, $n=13$) or in the presence of aldosterone: 1 μ mol/L (black column, $n=10$), 100 nmol/L (gray column, $n=11$), 10 nmol/L (light gray column, $n=11$), and with both aldosterone and excess of 250-fold spironolactone (hatched columns, $n=7$ for each concentration). Mean I_{Ca} peak density is significantly increased by aldosterone treatment to that measured in control cells. This effect was prevented by coincubation with spironolactone.

Membrane capacitance was of similar magnitude in control cells and in cells that were incubated with aldosterone (162.4 ± 50.9 [$n=9$] versus 170.4 ± 57.1 pF [$n=10$] in control versus aldosterone). The I - V relationships of I_{Ca} are shown in Figure 2B, bottom. The current density of I_{Ca} was not significantly different after a short-term incubation with aldosterone ($P>0.5$). This observation rules out a rapid nongenomic action of aldosterone on cardiac I_{Ca} .

Specificity of the Aldosterone-Induced Increase in I_{Ca}

To confirm that the I_{Ca} increase after 24 hours of exposure of myocytes to aldosterone occurs via classical intracellular mineralocorticoid receptor (MR), the effect of the classical MR antagonist spironolactone was examined. Because spironolactone exhibits a low affinity for the MR receptor,²⁷ a 250-fold concentration was used to antagonize the aldosterone treatment. Figure 3 compares the effect of a mixture of aldosterone and spironolactone to 24 hours of incubation of myocytes without or with aldosterone alone. In the presence of spironolactone, the aldosterone-induced increase of I_{Ca} was prevented. Aldosterone stimulated I_{Ca} density at -10 mV by $\approx 144\%$ ($P<0.001$), whereas in the presence of spironolactone, I_{Ca} density was not significantly different from control (-12.7 ± 1.5 [$n=8$] versus -13.9 ± 1.3 pA/pF [$n=13$], for cells incubated with aldosterone and spironolactone versus control, respectively [$P>0.5$]).

Spironolactone alone was without effect on I_{Ca} density (data not shown). This inhibition by a specific antagonist demonstrates the involvement of classical intracellular MR in the aldosterone effect.

Aldosterone is known to possess glucocorticoid-like action that has been reported to upregulate cardiac I_{Ca} .^{31,32} With the high aldosterone concentration used (1 μ mol/L), it is thus dangerous to form a conclusion about the specific effect of aldosterone. Therefore, we repeated the experiments with

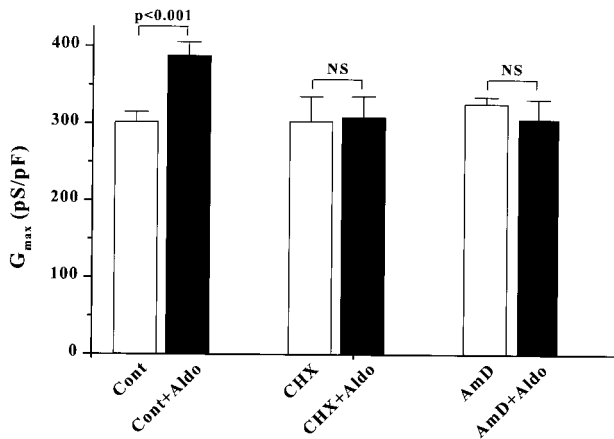


Figure 4. Inhibitors of transcription and protein synthesis inhibitors prevented the aldosterone-induced increase of cardiac I_{Ca} . Amplitude histograms of pooled maximal I_{Ca} conductances of cardiac cells incubated for 24 hours with no drug (Cont, $n=13$), with 1 $\mu\text{mol/L}$ aldosterone (Aldo, $n=10$), with 20 $\mu\text{g/mL}$ cycloheximide (CHX, $n=5$), with both cycloheximide and aldosterone (CHX+Aldo, $n=8$), with 5 $\mu\text{g/mL}$ actinomycin D (AmD, $n=5$), or both actinomycin D and aldosterone (AmD+Aldo, $n=7$). The mean G_{max} showed a significant increase after 24 hours of aldosterone treatment. Actinomycin D and cycloheximide, which did not have any effect by themselves, blunted this effect.

lower, more physiological concentrations of aldosterone and spironolactone.

The right part of Figure 3 summarizes the results of 24 hours of treatment with either 100 or 10 nmol/L aldosterone. Both lower aldosterone concentrations stimulated significantly maximum I_{Ca} peak density, and these effects were prevented in the presence of 250-fold excess of spironolactone.

Inhibitors of Transcription and Protein Synthesis Prevent Aldosterone-Induced Increase in I_{Ca}

Classical mineralocorticoid action is achieved through the interaction of intracellular MR proteins and promoters of target genes that enhance the transcription and protein synthesis. Thus, aldosterone modulation of cardiac I_{Ca} could involve an increase in channel number through a stimulation of mRNA and protein synthesis. To test this hypothesis, we analyzed the effects on I_{Ca} density of cell incubation with both aldosterone (1 $\mu\text{mol/L}$) and either the inhibitor of transcription actinomycin D (5 $\mu\text{g/mL}$) or the protein synthesis inhibitor cycloheximide (20 $\mu\text{g/mL}$) for 24 hours. These data are summarized in Figure 4. The I - V relationships in the different conditions were fitted with a function combining the Goldman-Hodgkin-Katz equation and the Boltzmann relationship to estimate maximal conductance: $I = (V - V_{rev})G_{max} \{ [1 + \exp[(V - V_{0.5})/\alpha]] + 1 \}$, where V is the voltage and the parameters estimated by the fit, where V_{rev} is the reversal potential; G_{max} , the maximal conductance; $V_{0.5}$, the half point of the relationship; and α the slope factor. On average, cell incubation with aldosterone alone augmented the G_{max} of Ca^{2+} channel ≈ 1.3 -fold in a voltage-independent manner compared with control. Both inhibitors did completely blunt aldosterone-induced increase in G_{max} . It is worth noting that in both the cycloheximide-treated and actinomycin D-treated groups, no significant changes in G_{max} or in any of the parameters estimated by the fit (data not shown) were denoted compared with control

($P > 0.5$). Thus, the increase in cardiac I_{Ca} after incubation of cells with aldosterone for 24 hours seems to involve a gene regulation.

Discussion

The present study establishes for the first time that the mineralocorticoid hormone aldosterone upregulates I_{Ca} in rat ventricular myocytes through a specific genomic regulation. This conclusion is supported by the finding that incubation of cells with aldosterone for 24 hours increases I_{Ca} density without any modification of the kinetics and the voltage dependence of the current. Even though no rapid aldosterone electrophysiological effect (< 6 hours) could be detected, this increase was prevented by the MR antagonist spironolactone or by inhibitors of transcription and protein synthesis. These results are of potential interest at a number of levels: cardiac role for MR per se and possible pathophysiological and clinical implications.

Genomic Regulation of Cardiac I_{Ca} by Aldosterone

Aldosterone, like other steroid hormones, initiates its effects by binding to intracellular receptors; these receptors are then able to control the transcription of several genes. For instance, it has been shown that aldosterone regulates the expression of amiloride-sensitive Na^+ channels in colon, lung, and kidney³³ and of ATP-regulated K^+ channels in kidney.³⁴ In both neonatal and adult cardiomyocytes, aldosterone can regulate the expression of the major cardiac Na^+ , K^+ -ATPase isoform gene.¹⁵ Moreover, it has been shown that long-term treatment (24 hours) of cultured neonatal rat cardiac cells with aldosterone stimulates the activities of Na^+/H^+ antiporter and $\text{Cl}^-/\text{HCO}_3^-$ exchanger.¹⁷ To our knowledge, this is the first time that an aldosterone-induced functional expression of Ca^{2+} current is reported. However, it is noteworthy that cardiac MR binds minerals and glucocorticoids with equal affinity, in contrast with classical target tissues such as kidney and colon,³⁵ and that expression of Ca^{2+} channel subunit α_{1C} mRNA is upregulated by glucocorticoids,^{31,32} including in atria and ventricles of rat heart.³⁶ In addition, it has been shown that MR-hormone complex can activate transcription of a target gene by binding an upstream transcription regulatory element that contains an interacting glucocorticoid-responsive element.³⁷ Thus, the aldosterone-induced increase in I_{Ca} might be due to the upregulated expression of the α_{1C} gene in cardiomyocytes. However, if a control of transcription is involved in the effect of aldosterone on I_{Ca} (Figure 4), it cannot be concluded whether this genomic effect is direct or indirect. In any case, it is plausible that aldosterone may induce the transcription of gene(s) encoding regulatory protein(s) and hence increases Ca^{2+} channel activity.

Absence of Rapid, Nongenomic Effect of Aldosterone

Over the past 10 years, there has been an increased amount of evidence for rapid, nongenomic effect of aldosterone in various tissues.^{8,22,23,28-30} Concerning cardiac myocytes, it has been reported that activation of sarcolemmal Na^+ transport can occur within the first 30 minutes of aldosterone

exposure.¹⁶ In the present study, there was no evidence of rapid action of aldosterone on I_{Ca} (see Figure 2). However, aldosterone application causes a rapid increase (within <1 hour) in free $[Ca^{2+}]_i$ level in endothelial cells and vascular smooth muscle cells.^{22,23} The mechanisms underlying this rapid effect are not completely understood; however, they involve phosphoinositide hydrolysis²⁹ and cAMP,³⁰ which might induce Ca^{2+} channel phosphorylation. Under our experimental conditions, $[Ca^{2+}]_i$ is expected to be clamped at a low level by EGTA in the pipette filling solution. This may have prevented detection because release of Ca^{2+} from intracellular stores and changes in cytosolic levels of Ca^{2+} are thought to be involved in the second messenger cascade for short-latency, nongenomic effects of aldosterone.³⁸ Thus, we could not exclude this possibility. Nevertheless, the absence of alterations of the voltage dependence and kinetics of I_{Ca} (see Figure 1), even after short-term incubation with aldosterone (data not shown), suggests that none of the potential phosphorylation systems that have been involved in the rapid nongenomic effect of aldosterone^{28–30} is implicated in the present study.

Pathophysiological Implications

The presence of MRs in heart^{9–11} and in the steroid hormone biosynthetic pathway¹² suggests that aldosterone may play a role in the regulation of cardiac function. An increase in myocardial aldosterone production in the rat model of chronic myocardial infarction, with a slight cardiac hypertrophy,¹³ has been reported recently. This might be in relation to cardiac fibrosis and/or to Ca^{2+} channel adaptation during the hypertrophic process. Indeed, cardiac hypertrophy results from a selective activation of the expression of various genes and protein synthesis.³⁹ The electrophysiological alteration pattern observed during cardiac hypertrophy and failure, crucial for arrhythmia risk, has recently been reviewed.⁷ Notably, it has been shown that I_{Ca} density is unchanged in both rat hypertrophy and cardiac failure.^{24,26} The unchanged Ca^{2+} channel number per surface area with increased cell size implies that the total number of channels increases proportionally with the degree of hypertrophy. Therefore, a genomic regulation of the Ca^{2+} channel has been invoked.²⁶ These authors suggested that during the hypertrophic process, a synthesis of Ca^{2+} channels was first induced before an increase in cell size. We therefore suggest that the aldosterone genomic action reported in the present study contributes to the increased I_{Ca} amplitude observed during cardiac remodeling.

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