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Rapid communication

Aldosterone increases T-type calcium channel expression and in vitro beating frequency in neonatal rat cardiomyocytes

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Abstract

Objective: Although aldosterone has been implicated in the pathogenesis of cardiac hypertrophy and heart failure, its cellular mechanism of action on cardiomyocyte function is not yet completely elucidated. This study was designed to investigate the effect of aldosterone on calcium channel expression and cardiomyocyte contraction frequency.
Methods: Cultured neonatal rat ventricular cardiomyocytes were stimulated in vitro with 1 μmol/L aldosterone for 24 h. Calcium currents were then measured with the patch clamp technique, while calcium channel expression was assessed by real-time RT-PCR.
Results: In the present study, we show that aldosterone increases Ca^{2+} currents by inducing channel expression. Indeed, aldosterone led to a substantial increase of L- and T-type Ca^{2+} current amplitudes, and we found a concomitant 55% increase of the mRNA coding for α_{1C} and β_2 subunits of cardiac L channels. Although T-type currents were relatively small under control conditions, they increased 4-fold and T channel α_{1H} isoform expression rose in the same proportion after aldosterone treatment. Because T channels have been implicated in the modulation of membrane electrical activity, we investigated whether aldosterone affects the beating frequency of isolated cardiomyocytes. In fact, aldosterone dose-dependently increased the spontaneous beating frequency more than 4-fold. This effect of aldosterone was prevented by actinomycin D and spironolactone and reduced by RU486, suggesting a mixed mineralocorticoid/glucocorticoid receptor-dependent transcriptional mechanism. Moreover, inhibition of T currents with Ni^{2+} or mibefradil significantly reduced beating frequency towards control values, while conditions affecting L-type currents completely blocked contractions.
Conclusion: Aldosterone modulates the expression of cardiac voltage-operated Ca^{2+} channels and accelerates beating in cultured neonatal rat ventricular myocytes. This chronotropic action of aldosterone appears to be linked to increased T channel activity and could contribute to the deleterious effect of an excess of this steroid in vivo on cardiac function.
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Keywords: Renin angiotensin system; Ca-channel; Gene expression; Contractile function; Ventricular arrhythmias

1. Introduction

Since the publication of the RALES clinical trial [1] demonstrating the beneficial effect on the cardiac function of spironolactone, an aldosterone antagonist, this hormone has been rapidly considered as a major cardiovascular risk factor. Aldosterone is clearly involved in the development of the cardiac hypertrophy and fibrosis associated with congestive heart failure, and its action appears independent of a rise of the blood pressure [2–4]. However, the pathophysiological mechanisms of aldosterone action remain largely unknown.

The high mortality associated with congestive heart failure is due to higher incidence of ventricular arrhythmias.

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A drastic reduction of the frequency of arrhythmic events has been recently observed in patients treated with spironolactone, strongly suggesting that aldosterone may contribute to the incidence of ventricular arrhythmia [5]. Among the various electrical mechanisms leading to arrhythmia, T-type calcium channel dysregulation could be important because of the function of these channels in heart pacemaker [6–8].

Low-threshold, voltage-operated T-type Ca^{2+} channels appear as particularly interesting candidates for playing a causal role in various pathologies [9]. Their recent cloning and molecular characterization revealed that these channels have evolved apart from L-type and other neuronal Ca^{2+} channels, suggesting a particular role in the cell, and therefore specific consequences or "channelopathies" in case of dysfunction. Three genes code for the various T channel isoforms (α_{1G}, α_{1H} and α_{1I}) that are apparently all composed of a single, large α_1 subunit [10]. Currents generated by α_{1G} and α_{1H} channels are nearly identical, but the higher sensitivity of α_{1H} to nickel (IC_{50}=10 μmol/L vs. 300 μmol/L for α_{1G}) represents a practical mean for discriminating between these channels [11].

One of the first physiological functions proposed for T channels was a support for a pacemaker current [12], because they activate at very negative voltages and resulting calcium entry leads to membrane depolarization. This role is well recognized in the heart, where nickel exerts a negative chronotropic effect [6,7].

Changes in their expression levels have been observed during the development of various organs, suggesting an important role for T channels at specific stages of the fetal life [13,14]. Particularly, in most mammalian species, T currents are robustly expressed in embryonic heart, in both atrial and ventricular myocytes, but are absent or much reduced in postnatal ventricular myocytes [15,16]. A quantitative analysis of the rat α_1 subunit transcripts revealed that, whereas α_{1C} (L-type channel) does not vary in expression during the development and remains 10–100 times more abundant than other channels, the levels of α_{1H} mRNA are high in embryonic tissue and at 3 weeks postnatal, but become undetectable at 5 weeks [17]. In contrast, the levels of α_{1G}, already present in fetal mouse myocardium [18], are maintained in rats during the postnatal period, as well as in adults [17]. Similar observations were made in human heart, where mRNA coding for both α_{1G} [19] and α_{1H} [20] have been identified but with significant decreases of α_{1H} during development [21]. No T currents were detected in human adult ventricular or atrial cells [22,23].

In the present study, we hypothesized that aldosterone excess induces a re-expression of T-type calcium channels in the heart. This hypothesis was supported by several arguments: (1) aldosterone has been shown in vitro to increase the expression of T-type channels in adrenal cells [24] and of L-type channels in cardiomyocytes [25], but the induction of T channels in cardiomyocytes had not been evaluated yet; (2) like other "fetal genes" re-expressed upon ventricular remodeling, T channels decrease during development but re-appear in ventricular cells from hypertrophied rat hearts or after infarction [26,27]; (3) mibefradil, an inhibitor of T channels, exerts a beneficial action on the cardiac function [28,29] and T channel blockade results in lower pacemaker activity of the sinus node [30]; and finally (4) mineralocorticoid antagonists improve tachycardia, arrhythmias and ventricular fibrillation in congestive heart failure [5,31], while mineralocorticoid receptor over-expression leads to tachycardia [32].

2. Experimental procedures

2.1. Cell culture

Neonatal cardiac cells were isolated from 1- to 2-day-old Wistar rats ventricles by digestion with low trypsin–EDTA and type 2 collagenase, as previously described [33]. Animals were killed in conformity with the Guide for the Care and Use of Laboratory Animals published by the NIH (Publication No. 85-23). Importantly, using high trypsin concentrations during cell preparation appeared deleterious for T currents, possibly explaining earlier negative results [34]. Freshly isolated cells were seeded in 90-mm Petri dishes to allow selective adhesion of cardiac fibroblasts [35]. Thereafter, cardiomyocytes were decanted from the plates and seeded in Petri dishes or in six-well culture plates.

2.2. Cell contraction frequency

Cardiomyocytes were seeded in laminin-coated Petri dishes. Spontaneously contracting cell monolayers were incubated for the indicated times with the appropriate concentration of agonist or vehicle in serum-free DMEM. Cell beating frequency was determined by counting the number of monolayer contractions per time unit under microscope.

In a few preparations, images of contracting cell monolayers were recorded on an Axiovert S100TV microscope, using an ×100 1.3 NA oil immersion objective (Carl Zeiss AG). Image acquisition (30 images/s) and analysis were performed with the Metamorph/Metafluor 4.1.2 software. Contractions were followed in real time by analyzing the variations of the mean light intensity within a small region arbitrarily fixed at the edge of single cells.

2.3. Electrophysiological recordings

Cardiomyocytes were plated for electrophysiological recordings after enrichment on a discontinuous (40.5% and 58.5% layers) Percoll (Amersham) density gradient.

Patch-clamp recordings were performed in the whole-cell configuration using an Axopatch 1D amplifier (Axon Instruments). Currents were filtered at 1–2 kHz, digitized and sampled at 5 kHz using a TL-1-125 interface and pClamp V.6 software (Axon Instruments). The bath solution

contained (in mmol/L) 125 *N*-methyl-glucamine, 5 4-aminopyridine, 20 tetraethylammonium chloride, 2 $CaCl_2$, 2 $MgCl_2$ and 10 D-glucose and was buffered to pH 7.4 with 10 HEPES. The patch pipettes were filled with solution containing (in mmol/L) 130 CsCl, 10 EGTA, 3 Mg-ATP, 0.4 Li-GTP; pH was adjusted to 7.2 with 25 HEPES.

In order to discriminate between T- and L-type currents, a 2-pulse voltage protocol was employed [15].

2.4. Total RNA isolation and mRNA quantification

Total RNA from cardiomyocytes was extracted using the NucleoSpin® RNA II kit (Macherey-Nagel) and its integrity was analyzed by electrophoresis with a chip-based RNA analysis system (Agilent Technologies).

Total RNA (400 ng) was reverse-transcribed using the Taqman Gold RT-PCR kit (Applied Biosystems) and random hexamers. The relative abundance of channel subunit mRNAs was assessed by Taqman qPCR and cyclophilin A was used to normalize data. Reactions in Taqman Universal Master Mix (Applied Biosystems) were performed using an iCycler iQ detection system (Bio-Rad). The sequence and concentration of specific primers and Taqman probes are indicated in Table 1 and were the same as those previously described elsewhere [36].

2.5. Drugs

Aldosterone, corticosterone, spironolactone, RU486, actinomycin D, $NiCl_2$, nitrendipine and laminin were purchased from Sigma. Mibefradil was from Roche.

2.6. Statistics

Results are expressed as the means ± S.E.M. unless stated otherwise. The statistical significance of changes was analyzed by 2-tail paired or unpaired Student's *t*-tests.

3. Results

3.1. Aldosterone increases T-type and L-type calcium currents in neonatal rat cardiomyocytes

Calcium currents were elicited in cultured neonatal rat ventricular cardiomyocytes by 200-ms step depolarization of the cells from a holding potential of −100 mV. As shown in Fig. 1A, exposure of the cells for 24 h to 1 μmol/L aldosterone markedly increased the size of the inward currents. Although aldosterone had no effect on cell capacitance (25.7 ± 3.8 pF after aldosterone treatment vs. 28.3 ± 2.8 pF in control cells, mean ± S.D., $n = 14$), current amplitudes were normalized by the cell capacitance (in pA/pF) to allow comparisons independently of the individual cell size variability. Aldosterone did not affect the kinetics of the current activation and inactivation, nor their steady-state inactivation (data not shown). The analysis of the current–voltage relationship revealed that the amplitude of the peak current was increased by aldosterone at any voltage between − 50 and + 50 mV but aldosterone response appeared proportionally more important at negative potentials (Fig. 1B). This shift of the IV-curve towards negative voltages was obvious when expressing the aldosterone-induced current as the current ratio between treated and untreated cells (Fig. 1C). A peak of aldosterone response was clearly revealed around − 40 mV, where low-threshold T-type currents have the highest amplitude.

Low Ni^{2+} concentrations are known to preferentially inhibit T-type channels (α_{1H} isoform), while higher concentrations, within the millimolar range, affect both T- and L-type currents. We found that aldosterone-treated cells responded to low Ni^{2+} more extensively at negative voltages than at more depolarized voltages (Fig. 1D,E). Indeed, approximately 70% of the current elicited at − 30 mV was suppressed by 50 μmol/L Ni^{2+}, while less than 25% of the

Table 1
Primers and probes used in real-time RT-PCR assays

Gene	Oligonucleotide sequence	Conc. (nmol/L)	Ref.
α_{1C}	fw: 5′-AGCAACTTCCCTCAGACGTTTG	400	[36]
	rev: 5′-GCTTCTCATGGGACGGTGAT	400	
	probe: 5′-CAACAAGACAGGGAACAACCAAGCGG	100	
β_2	fw: 5′-CCGACTATCTGGAGGCGTACTG	500	[36]
	rev: 5′-GTGGCTAAAGTCCGGCTAAGG	500	
	probe: 5′-ACCCACCCTCCCAGCAGTAACCTCC	100	
α_{1G}	fw: 5′-ACGCTGAGTCTCTCTGGTTTGTC	900	[36]
	rev: 5′-TGCTTACGTGGGACTTTTCAGA	900	
	probe: 5′-CGGGTGCAGATCCTACGTCCGC	100	
α_{1H}	fw: 5′-GGCGAAGAAGGCAAAGATGA	900	[36]
	rev: 5′-GCGTGACACTGGGCATGTT	900	
	probe: 5′-AGGCCTCCAAGATGCTGCAACGTTC	100	
cyclo	fw: 5′-TGTGCCAGGGTGGTGACTT	400	[49]
	rev: 5′-TCAAATTTCTCTCCGTAGATGGACTT	400	
	probe: 5′-ACACGCCATAATGGCACTGGTGG	100	

Abbreviations: fw, forward primer; rev, reverse primer; probe, 6-carboxy-fluorescein- and 6-carboxy-tetramethylrhodamine-modified Taqman probe; cyclo, cyclophilin A. Sequences of oligonucleotides were obtained from the indicated reference.

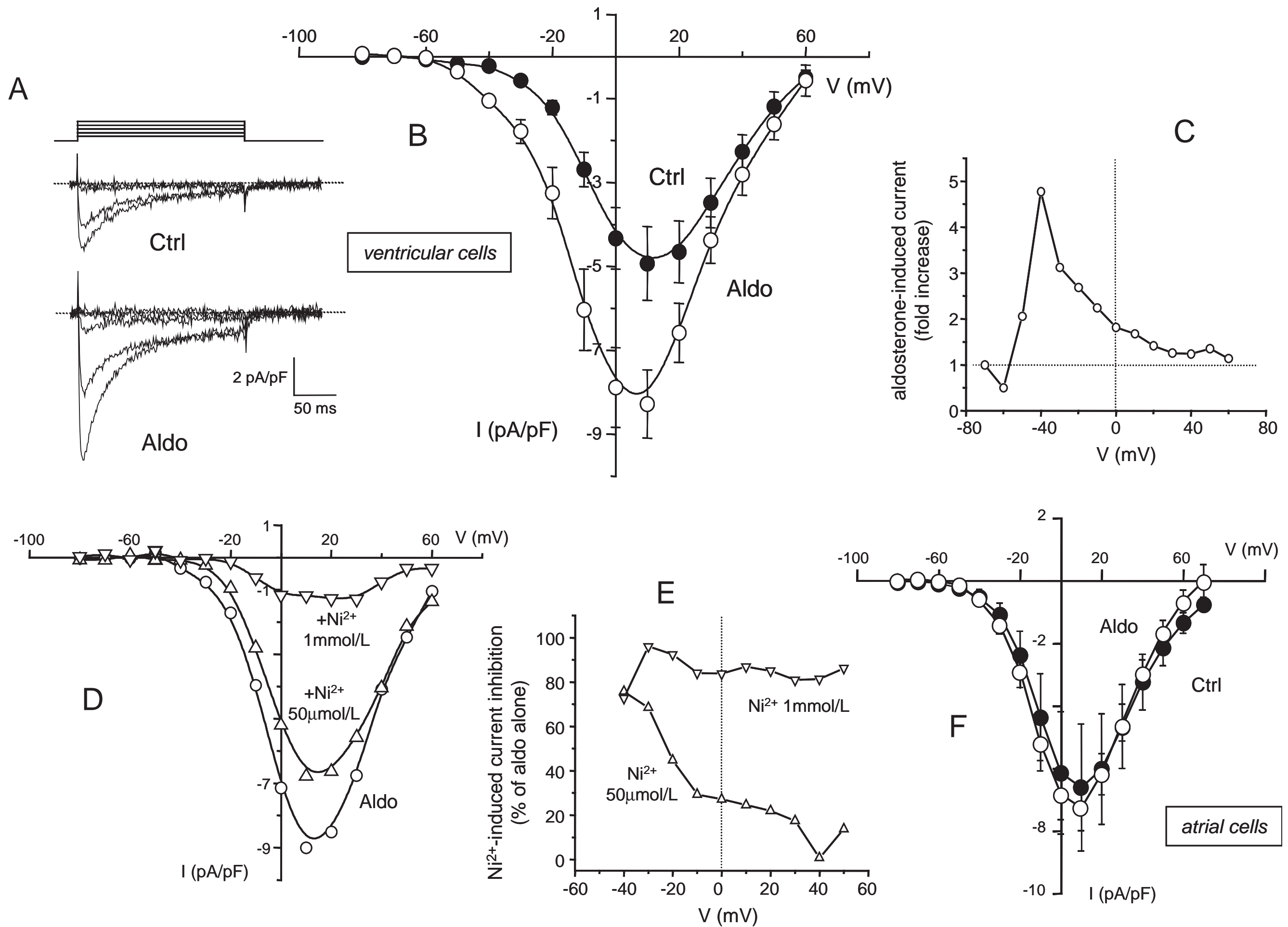

Fig. 1. Aldosterone increases low-threshold calcium currents in neonate rat ventricular cardiomyocytes. (A) Typical Ca^{2+} currents elicited by step depolarization from a holding potential of − 100 mV were recorded in an untreated cell (Ctrl) and in a cell exposed for 24 h to 1 μmol/L aldosterone (Aldo). (B) Voltage–current relationship in control and aldosterone-treated cells. Data are the mean ± S.E.M. from five and seven independent recordings for control and treated cells, respectively. (C) The aldosterone-induced current, determined at each voltage from data illustrated in panel B, was expressed in ratio of the corresponding control current. (D) Effect of low (50 μmol/L) and high (1 mmol/L) Ni^{2+} concentrations on calcium currents in an aldosterone-treated cell (1 μmol/L for 24 h). Currents were recorded before (Aldo) and a few minutes after the addition of Ni^{2+}. (E) Same data as in panel D, but expressed as the percentage of current inhibition induced by Ni^{2+}. (F) The same protocol as described for panel B has been applied to cardiomyocytes isolated from heart atria ($n = 5$ and 7 for Aldo and Ctrl conditions, respectively).

current recorded at positive potentials was affected, confirming that low Ni^{2+} concentrations can discriminate between low- and high-threshold currents. At 1 mmol/L, Ni^{2+} inhibited most of the current at any tested voltage.

Interestingly, when cardiomyocytes were prepared from the heart atrium instead of the ventricle, no effect of aldosterone was observed (Fig. 1F).

To determine the relative effect of aldosterone on T- and L-type channels, we have used a 2-pulse activation protocol. As previously published [15], the current elicited at − 30 mV can be considered as almost exclusively due to T channel activation, while high-threshold L channels are mainly responsible for the current recorded during the second pulse at + 10 mV (Fig. 2A). In untreated cells, the amplitude of the low-threshold T-type current was much lower than that of L current (Fig. 2B,C). Nevertheless, after 24 h treatment with 1 μmol/L aldosterone, T current amplitude increased to 420%, while L current only rose to 190%.

3.2. Modulation of calcium channel expression by aldosterone

Because the aldosterone-induced increase of current amplitudes occurred without modification of other electrophysiological properties, we suspected that this change resulted from a modulation of channel expression. The levels of mRNA coding for the various calcium channel isoforms expressed in cardiomyocytes have been therefore assessed by real-time RT-PCR (Fig. 3). Aldosterone (1 μmol/L for 24 h) significantly raised by approximately 60% the levels of mRNA coding for α_{1C} and β_2, the major L channel isoform in the heart and its principal ancillary subunit, respectively. The T channel response to aldosterone

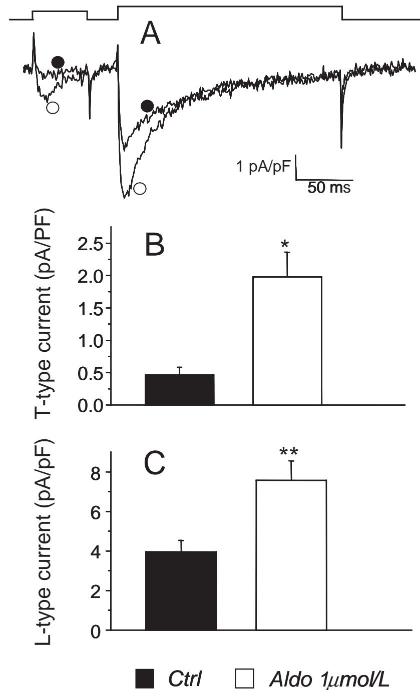

Fig. 2. Differential effect of aldosterone treatment on low- and high-threshold calcium currents. (A) Low-threshold (T-type) and high-threshold (L-type) Ca^{2+} channels were separately activated with a two-pulse protocol. Holding potential was −100 mV, the low step to −30 mV and the high step to +10 mV. The black dot shows the current recorded from a representative control cell and the white dot from a cell treated for 24 h with 1 μmol/L aldosterone. (B, C) Low-threshold (B) and high-threshold (C) current amplitudes determined in cells treated as indicated below (n=7).

was clearly isoform-dependent, with a much larger increase (up to 410%) in the expression of α_{1H}, while the small rise of α_{1G} (to 130%) did not reach statistical significance. These inductions of channel expression were in the same order of magnitude as the increase observed for the corresponding currents.

Spironolactone, a mineralocorticoid receptor (MR) antagonist, at 10 μmol/L, tended to prevent in each case aldosterone action. The effect of spironolactone was only significant for α_{1H}, for which approximately 70% of the raise was prevented. This observation suggests that the transcriptional response to aldosterone is mediated, at least in part, by the MR.

3.3. Acceleration of cardiomyocyte beatings by aldosterone

Neonatal rat cardiomyocytes maintained in primary culture have the property to spontaneously and repeatedly contract in Petri dishes. Untreated control cells contracted at a slow basal frequency, with occasional transient accelerations (Fig. 4A, left panel), while treatment of the same cells for 24 h with 1 μmol/L aldosterone markedly accelerated these in vitro beatings (Fig. 4A, right panel). The reproducibility of the basal contractions from one preparation to the other was markedly improved in the presence of laminin, which was systematically used as a substrate for the cell culture in the following experiments. Under these conditions, the mean basal beating frequency in control cells was 61±9 events/min (n=18 preparations).

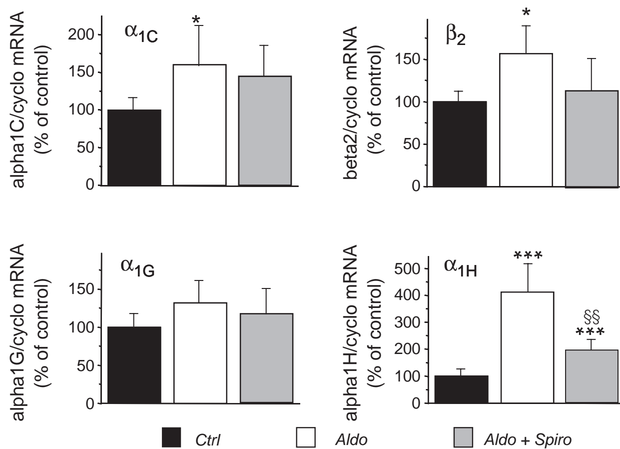

Fig. 3. Aldosterone induces the expression of calcium channel subunits in ventricular cardiomyocytes. Cells were stimulated for 24 h with 1 μmol/L aldosterone (Aldo) in the absence or in the presence of spironolactone (Spiro, 10 μmol/L). α_{1C}, β_2, α_{1G} and α_{1H} mRNA levels were determined by quantitative RT-PCR and normalized to cyclophilin A (cyclo) mRNA. By comparing real-time curves (C_T values), α_{1G} mRNA levels were estimated to be approximately twice those of α_{1H} in control cells, while the levels of α_{1C} and β_2 mRNA amounted to 30 times those of α_{1H}. Experiments were performed 4–7 times and results are expressed as a percentage of the control (Ctrl). *P<0.05 and ***P<0.001 compared to control values. §§P<0.01 compared to aldosterone values.

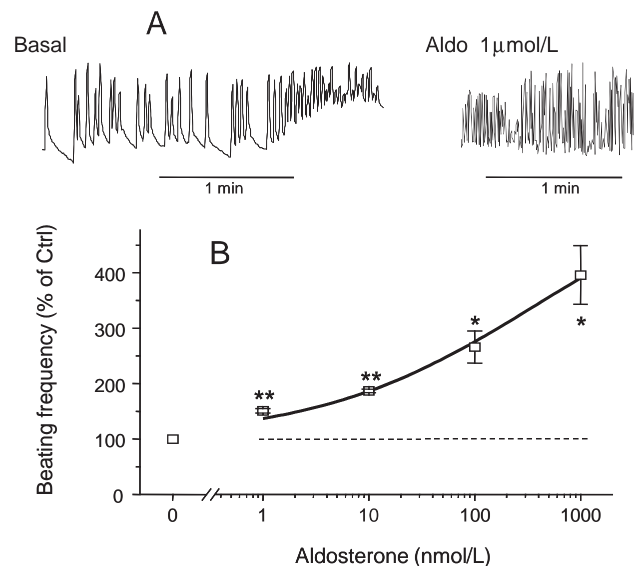

Fig. 4. Aldosterone increases the contraction frequency of cardiomyocytes in a concentration-dependent manner. (A) Contraction frequency was followed in real time by analyzing images of cells treated (Aldo) or not (Ctrl) with 1 μmol/L aldosterone for 24 h. (B) Monolayer contractions were counted under a microscope and expressed as a percentage of the beating frequency of non-treated cells. Experiments were performed 3 times. **P<0.01 and *P<0.05 compared to control values.

As shown in Fig. 4B, aldosterone treatment for 24 h induced a concentration-dependent increase in the beating frequency. A significant 50% raise of the frequency was already observed at 1 nmol/L aldosterone, a concentration close to the MR EC_{50} (0.1 nmol/L); however, the fact that further increase persists at much higher concentrations (1 μmol/L) suggests that additional receptors could mediate the aldosterone effect, including the glucocorticoid receptor (GR), which is known to be activated by aldosterone with an $EC_{50} > 1$ μmol/L [37]. In order to determine the mechanism of the aldosterone chronotropic action, the effect of several drugs was tested.

As shown in Fig. 5, 24 h stimulation with 1 μmol/L aldosterone significantly increased the beating frequency of cardiomyocytes to 475% of the basal level ($n = 18$). This response was prevented when actinomycin D was added simultaneously with aldosterone ($n = 3$), demonstrating that a transcriptional mechanism is indeed necessary, and excluding a nongenomic action of the hormone [38]. Concerning the specificity of the receptor involved, we found that spironolactone completely abolished the hormone-induced increase in beating frequency, while a 50% reduction of the response was observed in the presence of the GR antagonist RU486. However, the basal frequency was also markedly affected by this drug (by 36%, $p < 0.005$, $n = 5$), and a significant response to aldosterone, corresponding to a 4-fold increase in beating rate, was maintained even in the absence of a functional GR. Moreover, corticosterone, the main glucocorticoid in rats,

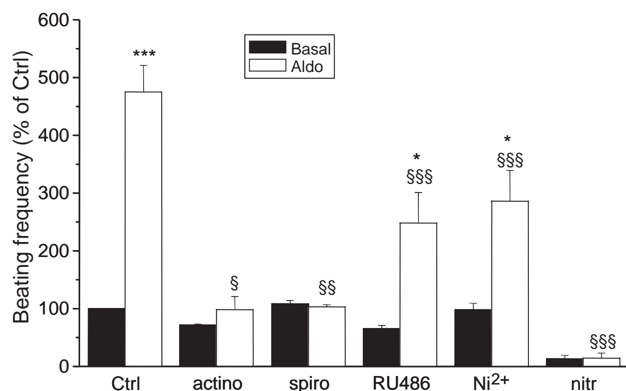

Fig. 5. Mechanism of aldosterone action on the cardiomyocyte beating frequency. Cells were cultured in laminin-coated Petri dishes and stimulated for 24 h in the absence (basal, filled bars) or presence of 1 μmol/L aldosterone (Aldo, open bars). Transcription, MR activity, GR activity and T-type or L-type calcium channel activity were inhibited with actinomycin D (actino, 2.5 μg/ml, 24 h), spironolactone (spiro, 10 μmol/L, 24 h), RU486 (1 μmol/L, 24 h), nickel (Ni^{2+}, 100 μmol/L, 30 min) or nitrendipine (nitr, 1 μmol/L, 30 min), respectively. Monolayer contractions were counted under a microscope and expressed as a percentage of the basal beating frequency of untreated (Ctrl) cells. Aldosterone alone was tested in each of the 18 cell preparations, while other drugs were included in 3–7 independent experiments. *$P < 0.05$ and ***$P < 0.001$ when compared to corresponding basal values; §$P < 0.05$, §§$P < 0.01$ and §§§$P < 0.001$ when compared to values obtained in control, aldosterone-stimulated cells (Ctrl/Aldo).

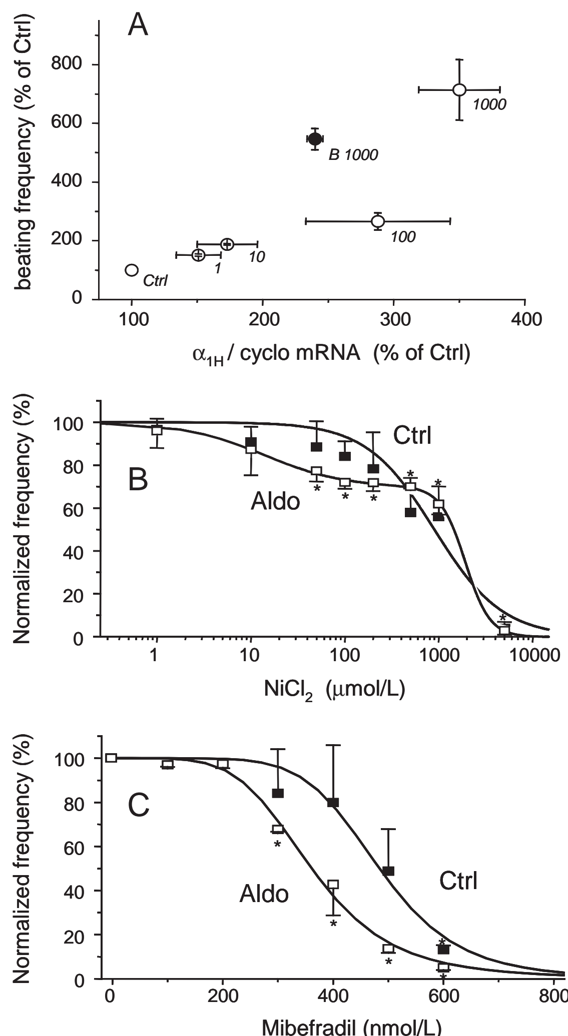

Fig. 6. T-type calcium channel activity regulates the beating frequency of cardiomyocytes. (A) Correlation between aldosterone-induced α_{1H} channel expression and beating frequency was established by comparing both responses to various aldosterone concentrations (indicated in nmol/L next to symbols). The responses to 1000 nmol/L corticosterone (Kendall's compound B) are also illustrated (filled symbol). (B, C) Calcium channel activity was inhibited with increasing concentrations of nickel (B) or mibefradil (C) in aldosterone-treated (Aldo, 1μmol/L) or untreated (Ctrl) cells. Cell monolayer contractions were counted under a microscope 30 min after the addition of the calcium channel inhibitor and results were expressed as a percentage of the beating frequency determined just before inhibitor addition. Experiments were performed 3 times and IC_{50} values were obtained after curve fitting according to a 4-parameter logistic model (or the addition of 2 such models in the case of Ni^{2+} inhibition in aldosterone-treated cells), using the non-linear curve fit option of Origin™ V 6.0. *$P < 0.05$ compared to the corresponding values in the absence of inhibitor.

at 1 μmol/L partially mimicked the response to aldosterone on both α_{1H} expression and contraction frequency (Fig. 6A), indicating that GR activation can also induce the same responses.

Our results therefore indicate that aldosterone increases the beating frequency in cardiomyocytes probably via a mixed MR/GR-dependent mechanism.

3.4. Role of T-type calcium channels in the modulation of the beating frequency

We hypothesized that, because of their particular electrophysiological properties, low-threshold T-type calcium channels could be responsible for the modulation of the beating frequency. In agreement with our hypothesis, the expression of α_{1H} in response to aldosterone was also concentration-dependent and correlated with the beating rate (Fig. 6A). To formally demonstrate the involvement of T channels, we reduced pharmacologically their activity after aldosterone has induced their expression. As shown in Fig. 5, low nickel concentrations (100 μmol/L), which preferentially affect α_{1H} channels, reduced by 50% the response to aldosterone, but, importantly, without affecting the basal frequency (measured in untreated cells). Because L channels are expressed at much higher levels than T channels in neonate rat cardiomyocytes, we also tested the consequence of specifically inhibiting L currents. In contrast to nickel, the L-type channel antagonist nitrendipine (1 μmol/L) markedly reduced the beating frequency in both aldosterone-treated and untreated cells, and even completely stopped the contractions in many cases.

The role of T channels on the beating frequency was further investigated using increasing concentrations of Ni^{2+} and mibefradil, two inhibitors of these channels (Fig. 6). A concentration–inhibition curve by Ni^{2+} is shown in Fig. 6B, where the measured frequencies were normalized to the frequency determined in corresponding cells without inhibitors. In naïve (Ctrl) cells, expressing low levels of T channels, a significant reduction of the beating frequency was observed only at concentrations of Ni^{2+} above 200 μmol/L, concentrations known to also affect α_{1G} and L-type channels [11,9]. In contrast, after aldosterone treatment (and T channel expression) Ni^{2+} action appeared clearly biphasic, with a first step between 10 and 100 μmol/L, where α_{1H} are preferentially inhibited, followed by a second phase leading finally to complete contraction arrest.

A change of cell sensitivity to mibefradil was also observed after cardiomyocyte treatment with aldosterone (Fig. 6C). Indeed, the IC_{50} for the mibefradil inhibition was shifted from 480 nmol/L in control cells down to 360 nmol/L in aldosterone-treated cells. In contrast to Ni^{2+} action, no formal biphasic action of mibefradil could be observed probably because of the poorer ability of mibefradil for discriminating between T and L channels, and its tendency of blocking α_{1G} somewhat better than α_{1H} [9].

4. Discussion

In the present study, we have demonstrated that, in cultured neonate rat ventricular cardiomyocytes, aldosterone induces the functional expression of α_{1H}, a T-type calcium channel, resulting in a marked acceleration of spontaneous cell contractions. Importantly, these effects of aldosterone appeared within 24 h, before any sign of cell hypertrophy.

A modulation of the activity of calcium channels through their expression has been demonstrated in various cell types. A change in the expression of a specific channel isoform, systematically linked to a given pathology, is suggestive of a role for this channel in the pathophysiological mechanism of the disease. For example, in adult rat cardiomyocytes, α_{1C} L-type calcium channel over-expression has been proposed to directly participate to the electrical remodeling occurring before the development of cardiac hypertrophy and resulting in the increase of the action potential duration, reflected by a longer QT interval on the electrocardiogram [39].

T-type channels have been also proposed to be involved in cardiac pathologies. Macroscopic T-type currents, that are almost undetectable in control adult animals, are re-expressed in left ventricular cardiomyocytes of both cats [40] and rats [27] when cardiac hypertrophy is experimentally induced by aortic stenosis. Similarly, re-expression of T-type channels after myocardial infarction or exposure to growth hormone-secreting tumors has been clearly demonstrated in remodeled rat left ventricle, even before the appearance of hypertrophy [41,42].

Left ventricular hypertrophy is an adaptive response that enables the heart to maintain cardiac function. However, cardiac hypertrophy is associated with sudden death due to arrhythmias or other events of cardiac origin [43]. The unique properties of T-type channels, particularly their low threshold of activation, makes them particularly prone to modify electrophysiological characteristics of the cells in which they are expressed. Thus, not surprisingly, a causative role for T channels has been proposed for explaining calcium overload and arrhythmias occurring in a genetically determined cardiomyopathic Syrian hamster strain expressing higher T currents in their myocytes [44].

In our study, the rate of "beating" correlated to α_{1H} expression and the acceleration induced by aldosterone was reduced by inhibition of T-type Ca^{2+} channels. In contrast, affecting L-type channel activity led to severe contractile dysfunctions, confirming the crucial role of these channels in triggering the excitation–contraction coupling. T channels, on the other hand, affect the frequency of action potentials. Indeed, because of their low threshold of activation, T channels activate early during the phase between two contractions, before the onset of the next action potential. The resulting inward Ca^{2+} current is too small for triggering Ca^{2+} release from the stores [8], but sufficient for efficiently depolarizing the cell. The voltage threshold for the next action potential is then reached more rapidly under these conditions.

In the whole heart, pacemaker cells from the sino-atrial node normally control the rate of cardiac contractions by imposing their own frequency to ventricular cardiomyocytes. This control is profoundly disturbed if ventricular cells activate too early, before receiving the signal from the

"conductor", this situation favoring the occurrence of arrhythmias.

Although much caution is necessary when extrapolating observations performed in vitro, with a particular cell model, to in vivo pathological situations, our results nevertheless suggest a new possible molecular mechanism for explaining the high incidence of ventricular arrhythmias associated with hyperaldosteronism. As previously mentioned, T channel over-expression and beating acceleration occurred relatively early, after only 24 h exposure to aldosterone and without any change in the cell size. This fast response suggests that calcium channel over-expression could be an early event in the development of cardiac pathologies involving mineralocorticoids and could orchestrate secondary modifications occurring later within the cells.

Interestingly, no response to aldosterone was observed in atrial cells. The lower sensitivity to aldosterone of atrial cells as compared to ventricular cells requires further investigation to discriminate between several possible explanations, such as differential expression of steroid receptors, cellular modulators or channel isoforms; however, based on our results, we would predict that mineralocorticoid antagonists should be more efficient to prevent ventricular arrhythmias than atrial fibrillation, even if T channels appear to be clearly involved in the latter pathology [45].

Both α_{1G} and α_{1H} isoforms could a priori be responsible for modulating the cell beating in vitro, because these two T channel subtypes share very similar electrophysiological properties. A role for α_{1G} is indeed supported by the relatively steeper reduction of beating frequency observed in control cells (where α_{1G} probably predominates) when increasing Ni^{2+} concentration from 200 to 500 μmol/L, as compared to the inhibition observed at the same Ni^{2+} concentrations in aldosterone-treated cells (Fig. 6B). However, only the α_{1H} isoform is induced by the hormone and selective inhibition of this isoform with low Ni^{2+} concentrations [11] were sufficient to normalize the beating rate. It is therefore conceivable that, although both α_{1G} and α_{1H} modulate the beating rate under basal conditions, α_{1H} is principally responsible for the chronotropic response to aldosterone.

A possible action of glucocorticoids on the expression of T channels and the modulation of beating frequency has been also addressed in the present study. This was particularly relevant in these cells, because glucocorticoids are classically considered as exerting a protective action on the cardiac function, but can mimic mineralocorticoids under particular conditions [46]. We observed a marked reduction of the aldosterone-induced acceleration of beatings in the presence of RU486 and we found that corticosterone could mimic aldosterone on both beating frequency and α_{1H} channel expression. Therefore, we cannot exclude that aldosterone, at least at micromolar concentrations, also exerts its action through the glucocorticoid receptor. Because of the partial overlap between the mineralocorticoid and the glucocorticoid signaling, further investigation is required for clearly determining the relative contribution of MR and GR to the control of T channel expression, and therefore for understanding how glucocorticoids can exert in vivo their protective action on the heart [46].

Because T channel knock out mice are available [47], these animals will represent a valuable model for demonstrating in vivo the exquisite relationship existing between aldosterone and these channels. However, it is already intriguing to realize that T channels appear highly re-expressed under various pathological situations. This observation is reminiscent of the re-expression of fetal genes observed during the evolution of several diseases, like cardiac hypertrophy or cancer [48]. The apparent association between T channel expression and the beating frequency of isolated cardiomyocytes suggests that aldosterone-induced ventricular arrhythmias could be improved by pharmacologically decreasing the activity of T-type channels. Hopefully, new findings on molecular properties and pathophysiological functions of this particular class of calcium channels will help to target them with specific pharmacological drugs in order to improve disorders directly linked to a dysregulation of these channels.

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