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Impaired modulation of the cardiac L-type Ca^{2+} channel activity by *ahnak-1* after myocardial infarction

La modulación de la actividad del canal de Ca^{2+} tipo L cardiaco por la ahnak-1 se modifica después del infarto del miocardio

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RESUMEN

Introducción El canal de Ca^{2+} tipo L cardiaco (Ca_v 1.2) es factor importante en la repolarización cardiaca y la fuente principal de Ca^{2+} activador durante el acoplamiento excitación-contracción en las células cardiacas. Las fallas en su regulación son causa mayor de arritmias y disfunción contráctil. Recientemente demostramos que la proteína del citoesqueleto *ahnak-1* modula la corriente de Ca^{2+} a través de los canales Ca_v 1.2 (I_{CaL}) al interactuar con la subunidad-β, reguladora, del canal Ca_v 1.2 y que la variante genética de *ahnak-1* I5483T (previamente Ile5236Thr), interfiere la estimulación β-adrenérgica de I_{CaL}.

Objetivo Estudiar la variante I5483T en cardiomiocitos ventriculares disociados de corazones de rata remodelados después de un infarto (PMI)..

Método Se utilizó la técnica de "*patch-clamp*" para registrar I_{CaL} en miocitos ventriculares, disociados enzimáticamente, de ratas jóvenes (2 meses) y de ratas "sham" y PMI de seis meses.

Resultados La I_{CaL} basal se incrementó de 11±0,5 A/F en cardiomiocitos jóvenes a 14,6±1,1 A/F y 15,7±1 A/F en cardiomiocitos "*sham*" y PMI, respectivamente, mientras que el isoproterenol (ISO, 1 μmol/L) incrementó I_{CaL} en 101±6%, 109±10% y 104±12%, respectivamente, por encima de los valores basales. Cuando las células se perfundieron intracelularmente con un péptido de *ahnak-1* que contenía la secuencia mutada (10 μmol/L) la I_{CaL} basal se incrementó a 20±1 A/F, 22±2 A/F y 21±2 A/F en cardiomiocitos jóvenes, "*sham*" y PMI, respectivamente. En esas células el ISO aumentó I_{CaL} en 11±4%, 33±6% y 79±12% respectivamente.

Conclusiones La modulación de I_{CaL} por la *ahnak-1* está afectada por la isquemia miocárdica y la remodelación. Como la *ahnak-1* y los canales Ca_v 1.2 están co-localizados en el sistema T-tubular transverso, la remodelación pudiera afectar la interacción de la *ahnak-1* con la subunidad reguladora β de esos canales.

Palabras Clave: *ahnak-1*, canales de calcio, patch-clamp, corazón, cardiomiocitos, infarto del miocardio.

ABSTRACT

Introduction The L-type cardiac Ca^{2+} channel (Ca_v 1.2) is an important determinant of cardiac repolarization and the main source of activator Ca^{2+} during excitation-contraction coupling in cardiac cells. Its defective regulation is a major cause of arrhythmias and contractile dysfunction. We have recently shown that the cytoskeletal protein *ahnak-1* modulates Ca^{2+} current through Ca_v 1.2 channels (I_{CaL}) by interacting with the regulatory β-subunit of the Ca_v 1.2 channel and that the genetic variant of *ahnak-1* I5483T (previously Ile5236Thr), interferes with the β-adrenergic stimulation of I_{CaL}.

Objective To extend our study of the I5483T variant to ventricular cardiomyocytes dissociated from remodelled infarcted rat hearts (PMI).

Method The patch-clamp technique was used to record I_{CaL} from enzymatically dissociated ventricular cardiomyocytes from young (2-month-old) and six-month-old sham-operated and PMI rats.

Results Basal I_{CaL} was increased from 11±0,5 A/F in young cardiomyocytes to 14.6±1.1 A/F and 15.7 ± 1 A/F in sham and PMI cardiomyocytes respectively, while isoprenaline (ISO, 1 μmol/L) further increased I_{CaL} by 101±6%, 109±10% and 104±12% respectively. When cells were intracellularly perfused with a peptide containing the mutated *ahnak-1* sequence (10 μmol/L) basal I_{CaL} was increased to 20±1 A/F, 22±2 *A/F and 21±2 A/F in young, sham and PMI cardiomyocytes respectively. In these cells ISO increased I_{CaL} by 11±4%, 33±6% and 79±12%* respectively.

Conclusions Modulation of I_{CaL} by *ahnak-1* is impaired by myocardial ischemia and remodelling. Since *ahnak-1* and Ca_v 1.2 channels co-localize in the transverse T-tubule system, remodelling of T-tubules could affect the interaction of *ahnak-1* with the regulatory β-subunit of these channels.

Key words: *ahnak-1*, calcium channels, patch-clamp, heart, cardiomyocytes, myocardial infarction

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INTRODUCTION

The $Ca_v1.2$ L-type Ca^{2+} channel (LTCC) of cardiomyocytes is a complex multimeric molecular sarcolemmal ensemble[1] and is mostly localized in the transverse tubular system of cardiomyocytes.[2,3] Activation of LTCC generates a Ca^{2+} current (I_{CaL}) through the sarcolemma large enough to be involved in the control of action potential duration[4] and serves as a trigger for Ca^{2+} release from the sarcoplasmic reticulum during the excitation-contraction coupling (the "calcium-induced calcium release").[1,4-6]

The typical structure of LTCC in ventricular cardiomyocytes is a macromolecular multimeric complex consisting of a pore-forming unit α_1C, an intracellular β (mostly β_2) subunit and the dimer $\alpha_2\delta$ ($\square_2\square$-1) subunit in a 1:1:1 ratio[1, 7]. The α_1C subunit contains all the necessary structures to allow the channel to gate and confers the Ca^{2+} selectivity as well as the electrophysiological and pharmacological properties of the LTCC.[7-10] The β and $\alpha_2\delta$ subunits seem to be involved in membrane targeting of α_1C and influence LTCC inactivation.[9-12]

It has been consistently reported that L-type Ca^{2+} channel activity can be modulated by the cytoskeleton.[13] Recently, the giant cytoskeletal protein *ahnak-1* (5890 amino acids) has emerged as an important modulator of β-adrenergic regulation of the cardiac L-type Ca^{2+} channel (for a recent review see 14). Haase et al.,[15] and Hohaus et al.,[16] provided evidence that *ahnak-1* could have a physiological role in cardiac β-adrenergic signalling via its interaction with the regulatory β-subunit of the L-type Ca^{2+} channel. Later on, we showed that intracellular perfusion of rat ventricular cardiomyocytes with small *ahnak-1* fragments involved in high affinity ($K_D \sim 50$ nM) interaction of the *ahnak-1* distal C-terminus (C2) with the β_2-subunit induced an increase in I_{CaL} density and slowed down its inactivation.[17] That *ahnak-1* C1 terminus-derived fragments also modulate I_{CaL} was further confirmed by Haase et al.,[18] who demonstrated that the *ahnak*-1 polymorphism, I5483T (previously Ile5236Thr) interferes with β-adrenergic stimulation of I_{CaL}. The proximal *ahnak-1* C terminus (C1) contains multiple interaction sites with the β-subunit. Intracellular application of this mutated peptide to rat ventricular cardiomyocytes increased I_{CaL} by ~60% and slowed down its fast inactivation time constant together with a leftward shift of its availability curve. These effects were similar to those observed after β-adrenergic stimulation in control cardiomyocytes. The response of I_{CaL} of rat ventricular cardiomyocytes intracellularly perfused with the I5483T-*ahnak-1* fragment to β-adrenergic stimulation was greatly diminished. Interpretation of these data was that in rat ventricular cardiomyocytes *ahnak-1* could serve as a "physiological brake" on I_{CaL} when normally attached to the β-subunit. Relief of this inhibition during β-adrenergic stimulation or when *ahnak-1*-derived peptides are intracellularly applied increases I_{CaL} and changes its inactivation time course.[18] This interpretation has been recently challenged by our results with cardiomyocytes dissociated from mice that do not express *ahnak-1*(KO) in which I_{CaL} density was not increased as expected for an autoinhibitor.[19,20] However, the lack of increase in I_{CaL} density in KO cardiomyocytes could be also related to a disruption of the cytoskeleton integrity[13] and/or to a decrease in plasma membrane expression of L-type Ca^{2+} channels.[21] The role of *ahnak-1* as a modulator of I_{CaL} is reinforced by experiments demonstrating that perfusion of KO ventricular cardiomyocytes with small fragments encompassing the amino acids of *ahnak-1* C terminus involved in I_{CaL} regulation had no effect on this ionic current.[19,20]

However, it is not known whether the *ahnak-1* modulator role is altered or not in pathological states. It has been reported that in remodelled post myocardial infarcted (PMI) hearts, I_{CaL} density is decreased or not affected[7]. More consistently its inactivation time course is reported to be slower in PMI cardiomyocytes.[7,22] It has been also reported that the transverse tubular system (T-system) is remodelled in failing hearts[23-25] a fact that could partially account for the observed changes in I_{CaL} because most of the Ca_v 1.2 channels are expressed in the T-system. Because *ahnak-1* and the Ca_v 1.2 channels co-localize in the T-system,[15-16] the modulator activity of *ahnak-1* on Ca_v 1.2 channels could be altered in remodelled PMI cardiomyocytes. It was thus the aim of the present investigation, to characterize the well-documented modulator activity of the I5483T polymorphism[18] on Ca_v 1.2 channels of cardiomyocytes isolated from PMI rat ventricles.[22] Since the genetic variant I5483T of *ahnak-1* is functional and may cause individual differences in I_{CaL} response upon physiological challenges or therapeutic interventions, it is important to address the issue whether the interaction of *ahnak-1* with the cardiac Ca^{2+} channel could be altered in pathological states.

METHOD

I5483T polymorphism

The synthetic *ahnak-1* peptides, either wild-type (*GGLPG**I**GVQGLE*; from here on GIG) or mutated (*GGLPG**T**GVQGLE*, from here on GTG), corresponding to amino acid positions 5478-5489

of *ahnak-1* were purchased from Biosyntan GmbH (Berlin-Buch, Germany; see 18 for details).

Myocardial infarction model in rats

Male Wistar rats weighing 180-230 g were submitted to left anterior coronary ligation according to Aimond et al.[22] In brief, rats were anaesthetized with an intraperitoneal mixture of ketamine (150 mg/kg) and chlorpromazine (15 mg/kg) before being intubated and ventilated. After median-left thoracotomy and opening of the pericardium, the left coronary artery was occluded with a 7-0 silk suture at the apex. Successful occlusion was recognized by pallor of anterior left ventricular free wall and by the occurrence of immediate regional dyskinesia. Sham-operated rats were submitted to the same treatment except the coronary artery ligation. Data concerning heart and haemodynamic status of sham-operated and PMI Wistar rats used in this study have been previously published.[22,26] Typically in PMI rats, left ventricles were markedly dilated under M-mode echocardiography and heart weight/body weight was increased by ~ 40% despite large infarcted area while end-diastolic pressure demonstrated a significant increase.

Isolation of adult ventricular cardiomyocytes

Single ventricular cells from young (2-month-old), sham-operated and PMI rat hearts (4 months after surgery, 6-month-old) were dispersed by an enzymatic method similar to that previously described.[17] Isolated myocytes were kept in this physiological solution (Ca^{2+}=1 mmol/l) at room temperature (21E - 23EC) and used within 6-8 hours.

Patch-clamp recordings

For recording the L-type Ca-current (I_{CaL}), the whole cell variant of the patch-clamp method was used. K^+-currents were blocked by Cs (intracellular and extracellular; see below), respectively. The fast inward Na^+ current was blocked with tetrodotoxin (TTX) at a concentration of 50 μmol/L. The composition of the standard extracellular solution was (mmol/L): NaCl, 117; CsCl, 20; $CaCl_2$, 2; $MgCl_2$,1.8; glucose, 10; HEPES, 10; pH was adjusted to 7.4 at 21EC. The pipette ("intracellular") solution contained (mmol/l): CsCl, 130; Na_2-GTP, 0.4; Na_2-ATP, 5; Na_2-creatinphosphate, 5;ethyleneglycol-bis-(β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), 11; $CaCl_2$ 4.7 (free Ca^{2+} ~ 120 nmol/l); HEPES, 10; pH was adjusted to 7.2 with CsOH.

For routine monitoring of currents, cells were clamped by 300-ms voltage-clamp pulses to 0 mV from a holding potential of -80 mV at a frequency of 0.25 Hz. Current amplitude was estimated as the difference between peak inward current and the current level at the end of the 200-ms pulse. Cells intracellularly perfused with GIG or GTG (10 μmol/L) were let to stabilize for at least 5 minutes after patch rupture before beginning the experiment. Current-to-voltage relationships (I/V) and availability curves (f_4 vs V_m) were determined by standard double-pulse protocols[17]. Availability curves of I_{CaL} were fitted from -80 to 0 mV by a Bolztmann distribution of the type: $f_4 = 1/1 + \exp[(V_m-V_f)/s]$, where V_f is the potential for half inactivation and s the slope factor. Pulse generation, data acquisition and on-line analysis were done, using computer facilities and ACQUIS1 software (version 2.0, CNRS License, France).

Statistical evaluation

Results were analysed by the Students' *"t"*-test and are expressed as means and standard errors of means. The criterion for significance was $p<0.05$.

RESULTS

Characteristics of L-type Ca^{2+} currents from control young, sham and PMI cardiomyocytes are shown in Table 1. As can be seen I_{CaL} density was significantly ($p<0.05$) increased in sham and PMI cardiomyocytes. Inactivation time course of I_{CaL} was barely affected except for the slow inactivation time constant of I_{CaL} in PMI cardiomyocytes in which it was significantly greater. Potentials for half inactivation ($V_{0.5}$) and slope factors of availability curves were not significantly different. Under control condition, isoproterenol (ISO, 1 μmol/L) increased I_{CaL} by about 100% in the three populations of cardiomyocytes together with a ~5 mV leftward shift of $V_{0.5}$ (Table 1). In each case, a small but significant increase in the fast inactivation time constant of I_{CaL} was observed.

Intracellular perfusion of cardiomyocytes from young (N=8), sham (N=7) and PMI (N=9) hearts with 10 μmol/L GIG (the wild-type peptide) had no effect on basal I_{CaL} nor on its response to 1 μmol/L isoproterenol (data not shown). However, when cardiomyocytes were intracellularly perfused with 10 μmol/L GTG, basal I_{CaL} was significantly increased in the three cell populations (Table 1). Nevertheless, it is to note that while in young cardiomyocytes basal I_{CaL} was increased by ~81% (compare with 18), in sham and PMI cardiomyocytes I_{CaL} was increased by 52% and 32% respectively (Table 1). As previously reported[18] the fast

Tabla1. I_{CaL} characteristics of cardiomyocytes dissociated from young, sham and PMI hearts. *Control* refers to the values obtained with standard extracellular solution. *GTG* refers to cardiomyocytes intracellularly perfused with the *ahnak1* peptide GGLPGTGVQGLE (10 μmol/L). ISO: isoproterenol 1 μmol/L

	dI_{CaL} (A/F)	τ_{fast} (ms)	τ_{slow} (ms)	$V_{0.5}$ (mV)	s (mV)
YOUNG (N = 8)					
Control	11.1 ± 0.5	5.1 ± 0.3	51.0 ± 2.8	-32.5 ± 1.4	5.1 ± 0.5
+ ISO	22.4 ± 1.3*	6.7 ± 0.2*	50.2 ± 1.9	-37.6 ± 1.5*	5.2 ± 0.4
% increase by ISO	101 ± 6				
GTG - YOUNG (N = 8)					
Control	20.1 ± 0.8**	6.6 ± 0.3**	52.8 ± 2.3	-36.7 ± 2.0**	5.3 ± 0.5
ISO	22.3 ± 1.2	6.9 ± 0.4	49.7 ± 1.8	-37.1 ± 2.1	5.2 ± 0.5
% increase by ISO	11 ± 4**				
SHAMS (N = 9)					
Control	14.6 ± 1.1***	5.4 ± 0.6	54.2 ± 2.6	-32.3 ± 1.4	5.2 ± 0.7
+ ISO	29.7 ± 1.6*	6.7 ± 0.6*	50.1 ± 3.2	-37.6 ± 2.0*	5.1 ± 0.6
% increase by ISO	109.0 ± 10.4				
GTG - SHAMS (N = 9)					
Control	22.1 ± 2.1**	5.3 ± 0.3	57.1 ± 4.7	-37.1 ± 1.9**	5.3 ± 0.5
+ ISO	29.3 ± 3.0*	7.1 ± 1.0*	53.3 ± 2.6	-38.0 ± 2.0	5.2 ± 0.5
% increase by ISO	33.3 ± 5.5***				
PMI (N = 15)					
Control	15.7 ± 0.9***	5.1 ± 0.4	74.4 ± 7.8**	-33.2 ± 2.2	5.4 ± 0.4
+ ISO	32.1 ± 2.6*	6.9 ± 0.3*	58.7 ± 4.2	-37.8 ± 2.1*	5.3 ± 0.5
% increase by ISO	104.0 ± 11.7				
GTG - PMI (N = 18)					
Control	20.7 ± 1.5**	5.3 ± 0.4	69.3 ± 6.5**	-36.4 ± 2.3**	5.3 ± 0.4
+ ISO	36.1 ± 3.0*	6.4 ± 0.4*	61.8 ± 4.2**	-37.8 ± 2.2	5.4 ± 0.6
% increase by ISO	78.7 ± 12.3***				

*: p<0.05 with respect to the previous control condition. **: p<0.05 with respect to cardiomyoctes intracellulary perfused with control solution. ***: p<0.05 with respect to young cardiomyocytes. dI_{CaL} : L-type Ca^{2+} current density. τ_{fast} : Fast inactivation time constant. τ_{slow} : Slow inactivation time constant. $V_{0.5}$: Potential for half availability. s: slope factor.

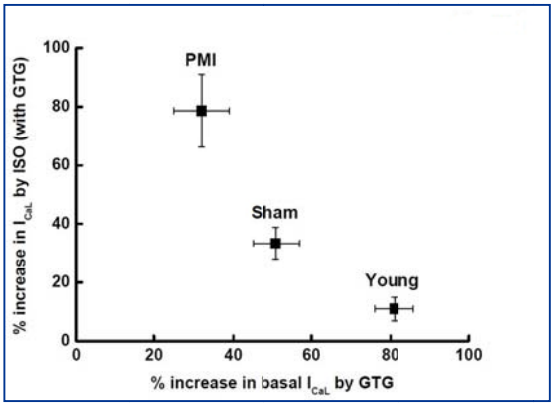

Figure 1. Effects of intracellular perfusion with GTG on basal I_{CaL} and on its response to β-adrenergic stimulation. The graph summarizes the main finding from Table 1 and represents the percent increase of I_{CaL} (mean ± SEM) by ISO (1 μmol/L) as a function of GTG (10 μmol/L) effect on basal I_{CaL} (percent increase of basal I_{CaL} expressed as mean ± SEM) in Young, Sham and PMI cardiomyocytes. As can be seen, the lower the effect of GTG perfusion on basal I_{CaL} (PMI cardiomyocytes), the higher the increase in I_{CaL} by ISO.

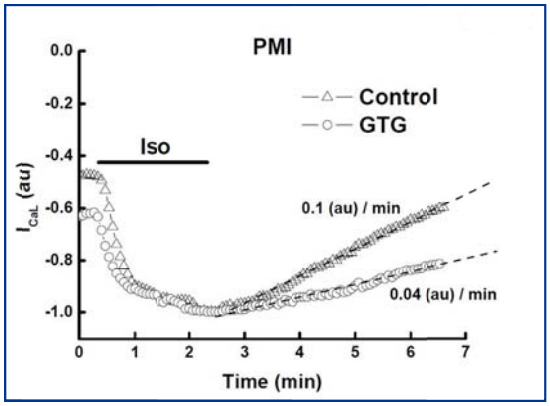

Figure 2. Effect of intracellular perfusion with GTG on the washout of ISO effect on I_{CaL} in PMI cardiomyocytes. I_{CaL} was recorded from two different PMI cardiomyocytes, one perfused with control intracellular solution (□, Control) and the other with an intracellular solution containing GTG (10 μmol/L; O, GTG). The horizontal line indicates extracellular perfusion with ISO (1 μmol/L; ~ 2 min). Currents were normalized to the maximal ISO effect and are expressed in arbitrary units (au). The speed of washout of ISO effect in each PMI cardiomyocyte is presented as the slope of the decay of I_{CaL} in au / min. As can be seen, washout of ISO effect is much slower in the cardiomyocyte intracellularly perfused with GTG.

inactivation time constant of I_{CaL} in young cardiomyocytes was slightly increased; however, there were no significant effects of GTG on the inactivation time course of I_{CaL} of sham and PMI cardioyocytes. In agreement with our previous results[18], ISO (1 μmol/L) barely (~ 11%) increased I_{CaL} in cardiomyocytes from young hearts (Table 1).

However, in sham and PMI cardiomyocytes ISO was able to increase I_{CaL} by ~33% and 78% respectively (Table 1). Figure 1 summarizes these results. In these cells, the behaviour of I_{CaL} inactivation time course under β-adrenergic stimulation was not different from control cells.

Full washout of ISO effects on I_{CaL} usually required 4 to 5 min regardless of cardiomyocytes condition, i.e. whether they come from young, sham or PMI hearts. The same was true for GTG (intracellularly) -perfused cardiomyocytes fromyoung and sham hearts. However, in PMI cardiomyocytes intracellularly perfused with GTG, more than 10 min were needed for a full washout of ISO effects on I_{CaL}. Figure 2 illustrates an example of ISO effects and recovery in two PMI cardiomyocytes, one in control condition and the other intracellularly perfused with GTG.

DISCUSSION

The present study confirms and extends our previous results[18] on the role of *ahnak-1* C1-terminus in β-adrenergic regulation of cardiac L-type Ca^{2+} channels and suggest that cardiac remodelling after infarction could modify this modulator role of *ahnak-1* on the Ca_v 1.2 channel.

Ahnak-1 C-terminus interacts with the β_2-subunit of the cardiac Ca_v 1.2 Ca^{2+} channel via multipoint attachment sites.[16] The roles of some of these sites in the modulation of Ca_v 1.2 Ca^{2+} channel have been demonstrated. We have shown that targeting the high affinity interaction sites located in the *ahnak-1* C2-terminus (aa 5535-5890) increased I_{CaL} amplitude and slowed down its inactivation time course.[17,19] Our group also demonstrated that the *ahnak-1* C1-terminus (aa 4889-5535) contains several amino acid sequences able to modulate I_{CaL} inactivation.[20] Interestingly, the genetic variant of a short amino acid sequence in the C1-terminus (I5483T) is able to mimic the β-adrenergic response of I_{CaL}. Cardiomyocytes from young rat hearts intracellularly perfused with the peptide fragment corresponding to this polymorphism (GTG) show an increased I_{CaL} density that was barely further increased by the well-known β-adrenergic agonist isoproterenol.[18] The increase in I_{CaL} by the intracellular GTG fragment was not affected by acetylcholine clearly indicating that it had no action on the β-adrenergic intracellular signalling cascade. The present results confirm these previous findings of the GTG action in cardiomyocytes from young rat hearts: in GTG-perfused cardiomyocytes basal I_{CaL} density was increased by ~ 81% with respect to control cells and ISO was hardly effective (~ 11%) in increasing further I_{CaL}.

A different picture emerged in the experiments using cardiomyocytes from sham and PMI rat hearts. Both sham and PMI cardiomyocytes showed, under control conditions, I_{CaL} densities that were significantly greater than I_{CaL} from young cardiomyocytes a fact that is indicative of the myocardial remodelling that occurs with age and especially after ischemia.[7,22] Interestingly, the response of I_{CaL} to β-adrenergic stimulation of these cardiomyocytes was not changed. However, the response of I_{CaL} to intracellular GTG in sham and PMI cardiomyocytes was different from young cardiomyocytes. In sham and PMI cardiomyocytes basal I_{CaL} was increased by intracellular GTG by 51% and 32%, respectively. More "strikingly", ISO was able to further increase I_{CaL} by 33% and 78% in sham and PMI cardiomyocytes, respectively in clear contrast to the almost lack of effect of β-adrenergic stimulation in young cardiomyocytes in the presence of GTG. One possible explanation for this finding is that remodelling affects the interaction between *ahnak-1* and the β_2-subunit of the Ca_v 1.2 Ca^{2+} channel. It has been shown that *ahnak-1* is mainly expressed in the sarcolemma including the transverse tubular system.[15,16] The Ca_v 1.2 Ca^{2+} channel complex is mainly localized in the T-system.[3] Partial loss of the T-system, T-tubule disorganization and alterations in the crosstalk between Ca_v 1.2 Ca^{2+} channels and ryanodine receptors of the sarcoplasmic reticulum due to T-tubule remodelling have been reported in failing hearts.[23-25,27,28] It is then conceivable that in failing hearts (such as PMI hearts) interaction of *ahnak-1* with the β subunit of the Ca_v 1.2 channel is modified thus accounting for the differential results we found in young, sham and PMI cardiomyocytes in which a lower effect of GTG on basal I_{CaL} (PMI cardiomyocytes) corresponds to a higher response of I_{CaL} to β-adrenergic stimulation (see Figure 1). It is to note here that GTG-perfused PMI cardiomyocytes also showed longer washout times of ISO effect. However, at this moment, the precise mechanism of how T-tubule remodelling could affect *ahnak-1* interaction with the β subunit of the Ca_v 1.2 Ca^{2+} channel remains to be elucidated. This proposal does not exclude other speculative explanations

such as "internalization" of *ahnak-1* during ischemia (Morano et al., unpublished results) and/or re-expression of species-specific isoforms of β subunits (and/or α_1C subunit) that could occur upon myocardial remodelling thus modifying the interactions with *ahnak-1*.

Taken together, our data highlight the importance of *ahnak-1* for cardiac Ca^{2+} channel function. Although it is still difficult to extrapolate the present findings to the clinical setting, we should emphasize two important things demonstrated by our results. First, disruption of *ahnak-1* - Ca^{2+} channel interaction (by the intracellularly-perfused *ahnak-1* fragments) results in an increase in Ca^{2+} current and a slowing down of its inactivation, *i.e.* a "gain of function". Second, the action of these peptides mimics the situation in remodelled hearts in which the interaction between the two molecular partners (*ahnak-1* and Ca^{2+} channels) is disrupted due to remodelling of the T-tubular system.[23-25] The resulting "gain of function" causes a delayed cardiomyocyte repolarization (long QT) and, more important, intracellular Ca^{2+} overload, two factors that increase the risk of arrhythmias. That lethal arrhythmias could occur by a slower Ca^{2+} channel inactivation has recently been shown for Timothy's syndrome[29]. Besides contributing to a better understanding of the extremely complex process of ventricular remodelling after ischemia (an important clinical condition), our study identifies new players (and potential new therapeutic targets) contributing to the ultimate cause of ventricular remodelling, the intracellular Ca^{2+} overload.

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