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ORIGINAL ARTICLE

L-type Ca^{2+} channels' involvement in IFN-γ-induced signaling in rat ventricular cardiomyocytes

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Abstract

The purpose of this study was to examine the effects of interferon-γ (IFN-γ) on calcium movement in rat ventricular myocytes. *L-type* Ca^{2+} currents ($I_{Ca,L}$) were recorded with the whole-cell configuration of the patch-clamp techniques. IFN-γ induces current density reduction at the test potential of 0 mV by 47.6 ± 7.4%. Heparin, a selective inhibitor of inositol-1,4,5-triphosphate (IP_3)–induced Ca^{2+} release, applied via a patch pipette, induced an $I_{Ca,L}$ amplitude decrease of about 46 ± 5.6%. The addition of IFN-γ to heparin-treated cells has no effect on $I_{Ca,L}$. Ryanodine induced an $I_{Ca,L}$ current amplitude decrease of 35.1 ± 6.2%. The addition of IFN-γ to ryanodine-treated cells caused an additional $I_{Ca,L}$ inhibiting of 17.6 ± 4.8%. Both cyclopiazonic acid (CPA), a specific SERCA inhibitor, and a combination of CPA and ryanodine caused a significant reduction of the $I_{Ca,L}$ amplitudes. Subsequent addition of IFN-γ inhibited $I_{Ca,L}$ for an additional 16.3 ± 4.4%. The employment of chelerythrine in this study prevented IFN-γ-induced *L-type* Ca^{2+} channel inhibition in only 10 min from the start of perfusion. Proposed mechanisms of regulation involved IFN-γ-induced IP3-sensitive Ca^{2+} release probably by a Ca^{2+}-dependent translocation of PKC from the cytoplasm to the cell membrane as the obligatory first step of the IFN-γ-induced PKC-dependent *L-type* Ca^{2+} channel inhibition.

Keywords Cytokine · Interferon-γ · *L-type* Ca^{2+} channels · Store-operated Ca^{2+} entry · Ventricular cardiomyocytes · Rat

Introduction

Despite many studies examining the role of different cytokines [2, 14, 18, 19, 22], only few studies have addressed the role of interferon-γ (IFN-γ) in adverse myocardial remodeling in animals [5]. Studies using isolated rat atrial preparation showed that concentrations of IFN-γ ranging between 2 and 10 U/mL have an inhibitory effect on contraction [5]. In addition to the effects on contractility, the family of serine/threonine kinases was another system involved in the IFN-γ-induced contractile changes [23]. Also, there is evidence for the involvement of IFN-γ signals through protein kinase C (PKC) isozymes in different cell types through mechanisms involving other signaling complexes [24]. Actually, this study has shown that IFN-γ (100 U/mL) incubation of murine bone marrow–derived mast cells and human mast cell lines is followed by increased phosphorylation of PKC in addition to mitogen-activated protein (MAP) kinases, Janus kinase (Jak1/2) kinases, and signal transducers and activators of transcription 1 (Stat1). It was further confirmed that phosphorylation of Stat1 and IFN-γ-induced gene expression required both PKC-α and phosphatidylinositol 3 kinase (PI3K), suggesting the existence of two distinct steps (activation and targeting) in the regulation of PKC-α-mediated gene expression [24]. Studies targeting the mechanisms involved in the activation of PKC isozymes in response to IFN-γ, occurring through integrin-mediated interactions, indicated another level of complexity [15]. The illustrated data above show cell-

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based PKC-mediated effects of IFN-γ, with multiple mechanisms associated with the activation and targeting of different isozymes [15]. Taking into account the general Ca^{2+} dependence of PKC-mediated effects and the complexity of IFN-γ-induced signaling networks, attention has turned to the convergence of these pathways at the level of intracellular Ca^{2+} signaling and its role in the propagation of the effects of IFN-γ in isolated rat ventricular cardiomyocytes. Ca^{2+} entry through *L-type* Ca^{2+} channels plays a key role in the induction of cardiac action potential and the development of myocardial contraction, and *L-type* Ca^{2+} channels are important targets for modulation by different enzymes and cardiotonic drugs [3, 9]. To the best of our knowledge, little is known about the role of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in cardiomyocytes exposed to IFN-γ. In this direction, recent findings indicated that in cardiomyocytes, ryanodine receptors type 2 (RyR2) (the predominant RyR isoform in cardiac muscle) is an essential protein for the excitation-contraction (EC) coupling in cardiac muscle through its mediation of Ca^{2+} release and altered intracellular Ca^{2+} load [21, 25]. Based on this, it will be important to examine the functional communication between the *L-type* Ca^{2+} channel and RyR2 in the cardiac myocytes exposed to IFN-γ. This kind of communication could indicate a new signaling pathway for *L-type* Ca^{2+} channels by RyRs in condition of increased IFN-γ.

Building on the abovementioned works, the present study examined whether IFN-γ influences cardiomyocyte's *L-type* Ca^{2+} channels, and if so, whether the effect involves changes in the intracellular $[Ca^{2+}]$ content, subsequently followed by changes in the endoplasmic reticulum's Ca^{2+} concentration.

Materials and methods

Animals

All animal experiments were carried out in accordance with the Guide for Care and Use of Laboratory Animals published by the US National Institute of Health (8th edition, 2011). The experimental protocol was approved by the Bioethics Committee of the Moscow State University. Outbred Wister rats weighing 220–250 g ($n = 60$) were kept in the animal house under a 12:12-h light:dark period in standard T4 cages before the experiment and fed ad libitum.

Cell isolation

The hearts were isolated as described in the previous works [7, 20]. The hearts were attached to the Langendorff apparatus for retrograde perfusion with Ca^{2+}-free solution containing (in mmol/L) NaCl 120, KCl 5.4, $MgSO_4x7H_2O$ 5, Na-pyruvate 5, glucose 20, taurine 20, and Hepes 10 at pH of 7.4 adjusted with NaOH. After an initial perfusion period of 5 min with Ca^{2+}-free solution, the hearts were perfused for 18–20 min with the same solution and supplemented with type II collagenase (0.5 mg/mL), type XIV protease (0.08 mg/mL), and 20 μmol/L $CaCl_2$. The perfusion solution was continuously bubbled with carbogen, and the temperature was equilibrated at 37 °C. Finally, the ventricles were separated, chopped, and gently triturated to release the cells into a standard Kraftbrühe medium [13].

Electrophysiology

Voltage clamp experiments were performed on a single cell. Cardiomyocytes after isolation (in small aliquots of the Kraftbrühe medium) were placed in an open perfusion chamber and allowed to adhere to the bottom of the chamber for 10–15 min. Initially, the cells were continuously perfused with the Kraftbrühe medium used while obtaining the whole-cell configuration and contained (in mmol/L) 150 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.2 $MgCl_2$, 10 glucose, and 10 Hepes, with pH adjusted to 7.4 at 20 °C with NaOH. The Cs^+-based solution was used for the measurement of *L-type* Ca^{2+} currents ($I_{Ca.L}$) and had the same composition, excluding the KCl, which was replaced by equimolar CsCl. 4-aminopyridine (4-AP) (2 mmol/L) was added to the solution to eliminate K^+ currents.

Patch pipettes with a mean (± SEM) resistance of 2.09 ± 0.19 MΩ were pulled from borosilicate glass (Sutter Instrument, CA, USA) using a PIP-6 puller (HEKA, Germany). The pipettes were filled with a Cs^+-based electrode solution containing (in mmol/L) CsCl 130, TEA 15, $MgCl_2$ 1, oxaloacetate 5, EGTA 5, Mg-ATP 5, Mg-GTP 0.03, and HEPES 10, with pH adjusted to 7.2 with CsOH. The pipette capacitance was compensated after obtaining a seal with a resistance > 2 GΩ. The entire cell capacitance and access resistance were completely compensated after gaining access to the cell interior. The mean cell capacitance was 82 ± 10.4 pF, while the mean access resistance was 10.6 ± 3.4 MΩ. To obtain current densities, the peak currents were normalized by cell capacitance.

Steady-state activation or inactivation was obtained by conventional protocols, and the corresponding curves of $I_{Ca,L}$ were fitted with the following Boltzmann equation: $I/I_{max} = 1/\{1 + Exp\ [(V - V_{1/2})/k]\}$. I is the Ca^{2+} current, I_{max} is the maximum amplitude of the Ca^{2+} current, V is the voltage of the conditioning pulse, $V_{1/2}$ is the potential of half activation or inactivation, and k is the slope factor. For each separate cell, the data were fitted to the Boltzmann distribution of the form: $V_{1/2}$ and the slope were compared and used to generate a continuous curve that fitted the average normalized data.

Calcium imaging

The cardiomyocytes were loaded with the acetoxy-methyl ester form of Fura-2 (Fura-2 AM; 1 L mol/L) for 20–30 min at

room temperature. After loading, the cells were incubated in a dye-free solution for 30 min to allow conversion of the color to its Ca^{2+}-sensitive form. The cells were plated on glass-bottom dishes and mounted on the stage of an inverted Olympus IX81 microscope with a ×20 objective. Only well-attached cells (as assessed by whether a brief test pulse of shear fluid would move or blow the cell away) were used. Fura-2 fluorescence was excited at 340 nm and focused on the cells via a ×20 objective (LUC Plan FLN 20x/0.45 Ph1, Olympus, Tokyo, Japan). The 510 nm emitted fluorescence was collected by a high-speed cooled CCD camera (Olympus, Tokyo, Japan) and recorded with cellM&cellR software. A quantitative analysis of fluorescent images was performed using Fiji/Image J software.

Drugs

NaCl, NaOH, Na_2ATP, KCl, KOH, $CaCl_2$, $MgCl_2$, $GdCl_3$, TEA, EGTA, 4-AP, HEPES, and glucose were purchased from Sigma-Aldrich (Natick, MA, USA). Interferon-γ was purchased from Gibco (Carlsbad, USA); ryanodine, cyclopiazonic acid (CPA), heparin, and chelerythrine (Chel) were from Tocris Bioscience (Minneapolis, USA). Collagenase type II and protease type XIV were purchased from Worthington, Lakewood, NJ. Fluorescent dye, Fura-2 AM, was purchased from Molecular Probes (Eugene, OR). Carbogen (95% O_2 and 5% CO_2) was obtained from Technical Gases-Moscow (Russian Federation).

Statistical methods

Values are expressed as mean ± SD. In order to obtain data suitable for statistical analysis, the currents were plotted as functions of the applied potential. IFN-γ-induced $I_{Ca.L}$ changes were taken from the recordings from the same cell before and after perfusion with the corresponding compound. A Bonferroni multiple comparison test was used to evaluate the significance of the differences between means, while $p < 0.05$ was considered statistically significant. All the analyses were performed with Graph Pad Prism 4.0 (San Diego, CA).

Results

Effect of IFN-γ on $I_{Ca,L}$

The membrane capacitance was 146 pF ± 24 pF ($n = 21$). The threshold for activation of *L-type* Ca^{2+} channels and the potential of peak currents were − 40 and 0 mV, at the holding potential of − 40 mV (Fig. 1a). Obtained current was identified as $I_{Ca.L}$ current and was sensitive to nifedipine (10 μmol/L) (see Fig. 1 *i*) inset). IFN-γ (2 ng/mL) induces current density reduction at the test potential of 0 mV by 47.6 ± 7.4% (Fig. 1b, c). At the same time, IFN-γ did not change the threshold of activation of $I_{Ca,L}$ and the potential of peak current (− 40 and 0 mV, respectively). In addition, the half potential of activation ($V_{1/2}$) and the slope factor (k) were − 24.6 ± 0.3 mV and 1.9 ± 0.4 under control conditions and − 24.5 ± 0.4 mV and 1.9 ± 0.4 in the presence of IFN-γ (Fig. 2a,

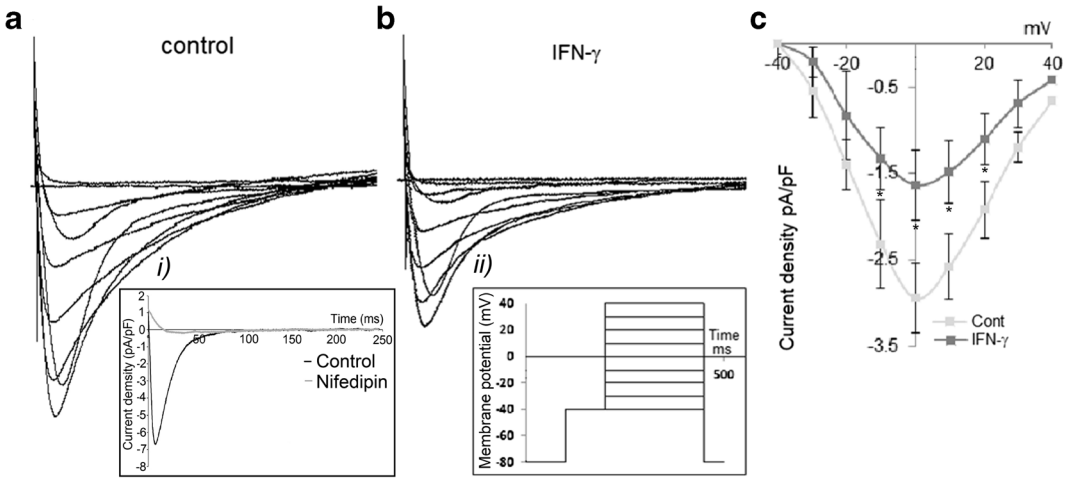

Fig. 1 Changes in the $I_{Ca.L}$, induced by short-term incubation with IFN-γ (2 ng/mL) in rat ventricular cardiomyocytes. **a**, **b** The original traces of $I_{Ca.L}$ were recorded in control (**a**) and after 14–16 min of perfusion in IFN-γ (2 ng/mL) containing Kraftbrühe medium (**b**) (**a** and **b** recordings are from one representative cell). The $I_{Ca.L}$ was elicited by 250 ms depolarizing test pulses for nine different potentials by 10 mV steps between − 40 and + 40 mV, which was preceded by the depolarizing prepulse to − 40 mV from the holding potential of − 80 mV (see inset - *ii*). Inset *i)* refers to current density at 0 mV, in control condition and in the presence of nifedipine. **c** Current voltage characterization of the $I_{Ca.L}$ in control (empty circles, $n = 6$) and IFN-γ perfused (filled circles, $n = 7$). The cells were obtained from five rat hearts. The asterisk symbol indicates significant differences between the two groups, unpaired t test, $p < 0.05$

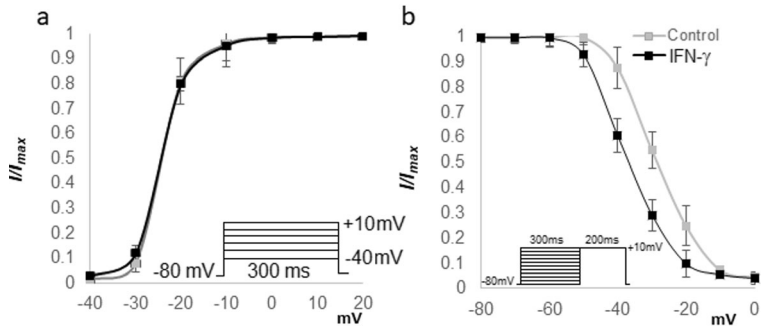

Fig. 2 Steady-state activation and inactivation of $I_{Ca,L}$ in the absence and presence of IFN-γ (2 ng/mL). Protocols are given in the insets. **a** Half activation potential ($V_{1/2}$) and slope factor (k) were − 24.6 ± 0.3 mV and 1.9 ± 0.4 under control conditions, and − 24.5 ± 0.4 mV and 1.9 ± 0.4 after 14–16 min in IFN-γ ($n = 6$ cells from four hearts, $p > 0.05$). **b** Steady-state inactivation was determined by a double-pulse protocol. Half inactivation potential ($V_{1/2}$) and slope factor (k) were − 27.9 ± 1.0 mV and 5.2 ± 0.4 under control conditions, and − 36.8 ± 1.2 mV and 5.3 ± 1.1 after 14–16 min in IFN-γ ($n = 6$ cells from four hearts, $p < 0.05$)

$p > 0.05$). The half potential of inactivation ($V_{1/2}$) in the presence of IFN-γ was shifted from − 27.9 ± 1.0 to − 36.8 ± 1.2 mV, while k was not affected (5.2 ± 0.4 vs 5.3 ± 1.1; Fig. 2b, $p < 0.05$).

SR's role in the IFN-γ-induced reduction of $I_{Ca,L}$

Heparin (10 μmol/L), a selective inhibitor of inositol-1,4,5-triphosphate (IP3)–induced Ca^{2+} release, applied via a patch pipette, induced an $I_{Ca,L}$ amplitude decrease of about 46 ± 5.6% ($n = 9$, $p < 0.001$). The addition of IFN-γ (2 ng/mL) to heparin-treated cells had no effect on $I_{Ca,L}$ ($n = 9$; $p < 0.891$) (Fig. 3a). Ryanodine (Ry) at (20 μmol/L)—i.e., at an inhibitory concentration for Ca^{2+}-induced Ca^{2+}-release channels of SR (Ry receptors)—decreased $I_{Ca,L}$ current amplitudes by 35.1 ± 6.2% ($n = 6$; $p < 0.001$). The addition of IFN-γ (2 ng/mL) to Ry-treated cells induced an $I_{Ca,L}$ inhibition of 17.6 ± 4.8% ($n = 6$; $p < 0.05$) (Fig. 3b). CPA (10 μmol/L), a specific SERCA inhibitor, and a combination of CPA (10 μmol/L) and Ry (20 μmol/L) significantly reduced $I_{Ca,L}$ amplitudes ($n = 6$; $p < 0.001$; Fig. 3c). Subsequent addition of IFN-γ (2 ng/mL) induced an $I_{Ca,L}$ inhibition of an additional 16.3 ± 4.4% in the presence of CPA or 11.2 ± 3.1% in the presence of both, CPA and Ry.

Effect of IFN-γ on the intracellular Ca^{2+} concentration

The results from the Fura-2 intensity of stained cardiomyocytes are presented in Fig. 4. The fluorescence intensity was taken from the manually chosen region of interest along the cell membrane. The changes in the intracellular Ca^{2+} concentration assayed by means of Fura-2 fluorescence were evaluated after incubation in IFN-γ (2 ng/mL) in the presence of 1.8 mmol/L extracellular Ca^{2+} concentration (Fig. 4). The stable level of the intracellular Ca^{2+} concentration in the absence of IFN-γ is demonstrated by a horizontal character of the curve (empty circles). Obtained results indicated that IFN-γ induced intracellular Ca^{2+} sequestration somewhere between the 16th and 22nd minutes of incubation (shown as decreased fluorescence intensity in Fig. 4, black circles). On the same figure, it is clearly shown that after the 22nd minute from incubation in IFN-γ, fluorescence intensity begins to rise until the end of the experiment (50 min). The whole process after the 22nd minute is characterized by a slight rising in the intracellular Ca^{2+} concentration up to the level that it was at the beginning of the experiment. This gradual rising in cytosolic Ca^{2+} is undoubtedly associated with the primed mechanisms for restoration of the intracellular Ca^{2+} homeostasis [16].

PKC involvement in the IFN-γ-induced reduction of $I_{Ca,L}$

Despite the induced current decrease, the Chel (2 μmol/L) employment (as a highly specific PKC antagonist) did not change the threshold of activation of $I_{Ca,L}$ and the potential of the peak current (− 40 and 0 mV, respectively). Furthermore, Chel (2 μmol/L) in this study caused the prevention of IFN-γ-induced *L-type* Ca^{2+} channel inhibition in only 12–14 min from the start of perfusion (Fig. 5).

Discussion

IFN-γ has the potential to regulate a wide range of functions in the heart due to its transcriptional control over many genes associated with the production of cytokines [15]. The classical IFN-γ signaling pathway passes through Janus tyrosine kinase (Jak)1 and the signal transducer and activator of transcription (Stat1) [23], which takes hours to be completed.

At present, the effects of IFN-γ on cardiomyocyte function are unclear due to discrepant findings through different signaling pathways induced by IFN-γ. In this

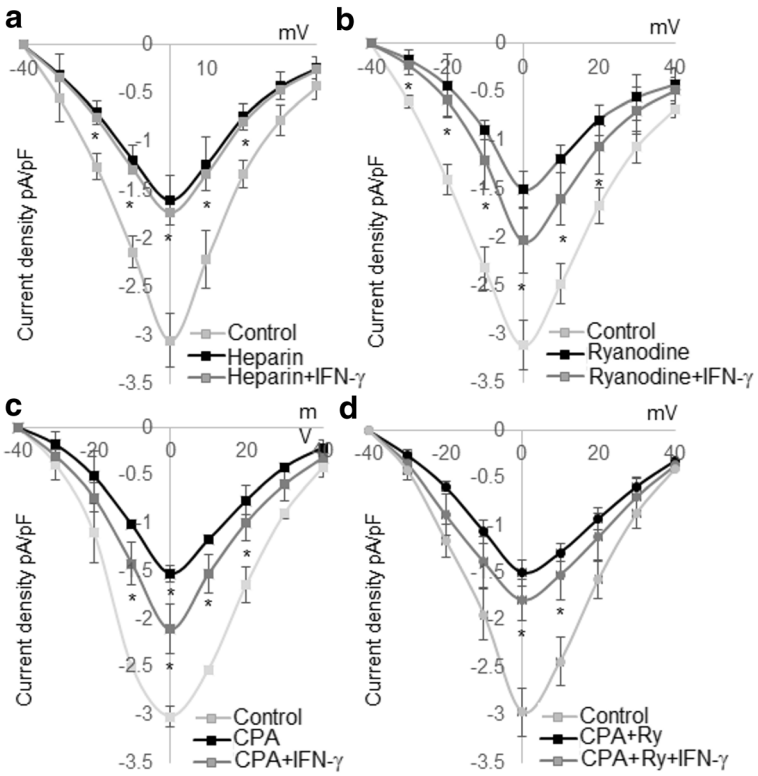

Fig. 3 IFN-γ inhibits the current through *L-type* Ca^{2+} channels ($I_{Ca,L}$). I–V relationships of peak $I_{Ca,L}$ under control conditions (circles), 14–16 min after the application of 10 μmol/L heparin (Hep) (**a**), 20 μmol/L ryanodine (Ry) (**b**), 10 μmol/L cyclopiazonic acid (CPA) (**c**), and combination of 20 μmol/L Ry and 10 μmol/L CPA (**d**), and after subsequent addition of IFN-γ (2 ng/mL) in the presence of Hep, Ry, CPA, and combination of CPA and Ry, respectively (squares) ($n = 9$). Currents were evoked by 250 ms depolarizing test pulses by 10 mV steps between − 40 and + 40 mV, which was preceded by the depolarizing prepulse to − 40 mV from the holding potential of − 80 mV. *$p < 0.05$

study, we reported for the first time that IFN-γ evoked a sustained inhibitory effect on cardiac *L-type* Ca^{2+} channels after 12–14 min of permanent perfusion at a concentration of 2 ng/mL. Therefore, the effects of IFN-γ on intracellular Ca^{2+} probably mediate some cellular functions [1, 6], but until this study, there were no categorical and uniform conclusions of IFN-γ's effect on Ca^{2+} movement in cardiomyocytes. Sarcoplasmic reticulum-Ca^{2+}-stores are important for the regulation of *L-type* Ca^{2+} channel activity, in rat ventricular cardiomyocytes [10]. In rat ventricular cardiomyocytes, Ry-sensitive Ca^{2+} release affects *L-type* Ca^{2+} channels, most likely due to a close contact between the cytoplasmic mouth of these Ca^{2+} release channels and *L-type* Ca^{2+} channels [25]. In this experiment, we received $I_{Ca,L}$ inhibition by application of Ry (20 μmol/L) which was expected to block them completely. Instead, we received partial inhibition that is attributed to Ry's slow diffusion rate through the plasma membrane, resulting with partial or slight activation of *Ca^{2+}*-

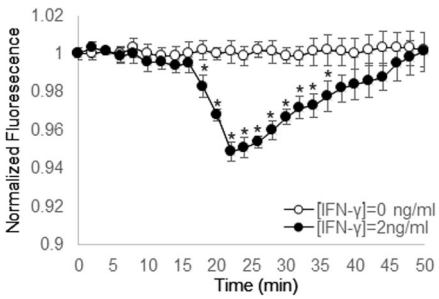

Fig. 4 Fura-signal dynamics. Normalized fluorescence in conditions of 2 ng/mL (IFN-γ), $n = 17$ (black circles); empty circles show the baseline level of fluorescence recorded for 50 min without IFN-γ application ($n = 15$; control conditions). The asterisk symbol indicates significant differences (unpaired *t* test, $p < 0.05$)

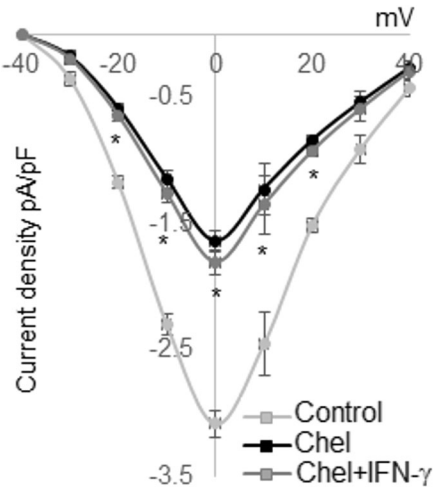

Fig. 5 The effect of the PKC inhibitor chelerythrine on the $I_{Ca,L}$. Current density obtained in the presence of chelerythrine (1 μmol/L), in the cardiomyocytes after the 12–14 min incubation in IFN-γ (2 ng/mL). The cells were obtained from four rat hearts ($n = 6$). The asterisk symbol indicates significant differences (unpaired *t* test, $p < 0.05$)

induced Ca^{2+}-release channels. We also believe that obtained *L-type* Ca^{2+} channel inhibition by IFN-γ in the presence of Ry involves other intracellular players associated with intracellular [Ca^{2+}] modulation [21]. IFN-γ-induced attenuation of the *L-type* Ca^{2+} channel activity could be taken as a negative feedback process that participates in the regulation of the intracellular [Ca^{2+}] during inflammation, which is additionally proven by the obtained confocal decrement of the intracellular [Ca^{2+}]. In addition, IFN-γ-induced left shift of the half potential of inactivation and accelerated steady-state inactivation of *L-type* Ca^{2+} channels indicate of an IFN-γ-induced signalization inevitably associated with the intracellular [Ca^{2+}] regulation in rat ventricular cardiomyocytes. The preliminary data obtained by Chel suggests that the effects of IFN-γ on Ca^{2+} movement appear to involve a modulatory effect on PKC-mediated signal transduction [6]. The mechanism of IFN-γ-induced IP3-sensitive Ca^{2+} release could thus be explained by an IP3-induced Ca^{2+}-dependent translocation of PKC from the cytoplasm to the cell membrane as the obligatory first step of the IFN-γ-induced PKC-dependent *L-type* Ca^{2+} channel inhibition [6, 26]. The last hypothesis was confirmed by the application of a specific PKC inhibitor, which completely abolished the effect of IFN-γ on *L-type* Ca^{2+} currents. This is similar to a described mechanism in murine bone marrow–derived mast cells and human mast cell lines [23], where IFN-γ requires activation of PKC to reduce $I_{Ca,L}$. However, if we take that IP3-buffered IP3-receptors (IP3Rs) are degraded over time [8, 26], then, the subcellular localizations of IP3Rs and the site of IP3 generation, represent an important factor in the induction of highly localized Ca^{2+} signals important for control of the Ca^{2+}-dependent PKC expression [4, 12, 24, 25]. The positioning of IP3 production and IP3Rs is important because differences in the kinetics of local intracellular Ca^{2+} can lead to altered activation of different intracellular players [11, 12], included in the proposed signalization induced by IFN-γ. Taking that IP3 is generated by the PI3 kinases (PI3Ks), further, we speculated about their involvement in the IFN-γ-induced attenuation of the *L-type* Ca^{2+} channels. This is in relation to the finding of Ghigo and cow. [8], who reported that attenuation of the *L-type* Ca^{2+} channels in cardiomyocytes is associated with the decreased signaling of the PI3Kα. In the same direction, Lu with cow. [17], suggested that in PI3Kα-null myocytes, $I_{Ca,L}$ density can be rescued by the infusion of PI (3,4,5) P3, recombinant p110α/p85 PI3K, indicating that PI3Kα mediates PKB activation in microdomains containing *L-type* Ca^{2+} channels. In addition, the same group has confirmed that this event is central in the process of subcellular distribution of these channels from internal membrane compartments to the plasma membrane [17]. This relationship between internal plasma membrane compartments and plasma membrane could be crucial for the action of IFN-γ. Based on this, we assume that acute IFN-γ-induced decrease in intracellular Ca^{2+} could be a result of the PI3Kα-attenuated signaling which is probably the reason for attenuated subcellular distribution of *L-type* Ca^{2+} channels from internal membrane compartments to the plasma membrane. The last undoubtedly lead to decreased *L-type* Ca^{2+} channels activity and further attenuation in intracellular [Ca^{2+}] concentration. Actually, this is in line with the data obtained from confocal microscopy where in the first phase, we found a decrease in intracellular Ca^{2+} concentration. In the second phase, after the 22nd minute, gradual rising in cytosolic Ca^{2+} can be explained by IFN-γ primed mechanisms of store-operated Ca^{2+} entry [1] for the restoration of the intracellular Ca^{2+} homeostasis [16]. The last was partially proven by the application of 2-APB (data not shown).

Conclusion

In conclusion, the present observations disclose a novel mechanism of IFN-γ-induced regulation of *L-type* Ca^{2+} channels in rat ventricular cardiomyocytes. Proposed mechanisms of regulation include IFN-γ-induced IP3-sensitive Ca^{2+} release probably by an IP3-induced Ca^{2+}-dependent translocation of PKC from the cytoplasm to the cell membrane as the obligatory first step of the IFN-γ-induced PKC-dependent *L-type* Ca^{2+} channel inhibition. The effects of IFN-γ on the intracellular Ca^{2+} concentration at the second phase (after 22nd minute), means that IFN-γ fosters mechanisms of slight Ca^{2+} releasing, without affecting the loading capacity.

Based on all above, it seems that the effect of IFN-γ on the rat ventricular Ca^{2+} metabolism is very complex, and we need different experimental approaches to understand it in details. The complex interaction of the Ca^{2+} stores with the intracellular Ca^{2+} concentration and *L-type* Ca^{2+} channels, and the possibility that these may be influenced in different ways by IFN-γ complicate the picture even more.

Author contributions All authors contributed toward data analysis, drafting, and revising the paper and agree to be accountable for all aspects of the work.

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Compliance with ethical standards

All procedures were approved by the Local Bioethical Committee for Animal Care (permission no. 3/2014).

Conflict of interest The authors declare that they have no conflict of interest.

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