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Brain cholesterol metabolite 24-hydroxycholesterol modulates inotropic responses to β-adrenoceptor stimulation: The role of NO and phosphodiesterase

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ABSTRACT

Aims: 24-Hydroxycholesterol (24HC) is the main brain cholesterol metabolite, which level in the circulation is significantly changed under physiological and pathological conditions. Here, we have studied the effect of 24HC on the inotropic responses to β-adrenoceptor (AR) stimulation.

Main methods: Electrical stimulation-evoked contractions were recorded in isolated atria from mice. Fluorescent dyes, Fluo-4 and DAF-FM, were used for estimation of Ca^{2+} transient and NO production, respectively.

Key findings: We revealed that 24HC in the submicromolar range attenuated β-AR-induced positive inotropy in isolated atria. This was accompanied by a decrease in Ca^{2+} transient and unchanged nitric oxide (NO) production. However, β1-AR-induced positive inotropy and enhancement of Ca^{2+} transient were increased by 24HC due to suppression of NO production. Only β2-AR-dependent inotropy and enhancement of Ca^{2+} transient were decreased by 24HC in a NO-independent manner. Inhibition of phosphodiesterase (PDE) suppressed effect of 24HC on β2-AR-dependent contractility as well as on non-subtype specific β-AR activation. Moreover, 24HC counteracted positive inopropic action of PDE inhibitors, IBMX and rolipam. Thus, 24HC modulates the effects of β1- and β2-AR stimulation via different mechanisms linked with change in activity of NO synthase or PDE, respectively. Under conditions of non-selective activation of β-ARs, the depressant effect of 24HC related with β2-AR-dependent signaling dominates.

Significance: We suggest that 24HC could serve as a modulator of atrial β-AR signaling, contributing to regulation of contractility.

1. Introduction

β-Adrenergic signaling is a central mechanism responsible for regulation of heart function. β-Adrenoceptors (β-ARs) contain high-affinity cholesterol binding sites important for stabilization of β-AR conformation, interaction with membrane and agonists [1–3]. Cholesterol supplementation and depletion, cholesterol-like compounds can modify effects of β-AR stimulation [4–8]. Also, spatial organization of cardiac β-AR signaling is dependent on cholesterol- and sphingolipid-rich microdomains termed lipid raft/caveolae [6,9–12].

β1- and β2-ARs are predominant in the heart. Both β1 and β2-ARs activate G_s proteins, which stimulate adenylyl cyclase and, thus, enhance cAMP production. cAMP activates protein kinase A, which phosphorylates certain Ca^{2+} handling proteins, leading to increase in Ca^{2+} transient and contractile response. Hydrolysis of cAMP by phosphodiesterases (PDEs) limits the increase in cAMP and, thus, in Ca^{2+} transient and contraction. β2-ARs can also stimulate G_i proteins, which inhibit adenylyl cyclase, thereby attenuating cAMP production and Ca^{2+} signaling [12–14]. Additionally, β-AR stimulation may increase NO synthase activity in Ca^{2+}-dependent and G_i protein/AKT-dependent

Abbreviations: β-ARs, beta-adrenoceptors; CGP, CGP20712; 24HC, 24-hydroxycholesterol; Fen, fenoterol; Dob, dobutamine; ICI, ICI-118.551; ISO, isoproterenol; NO, nitric oxide; PDE, phosphodiesterase

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manners. Produced NO via S-nitrosylation and activation of cGMP/protein kinase G pathway may decrease Ca^{2+} transient and (or) myofilament responsiveness to Ca^{2+}, thereby suppressing contractility [7,8,11]. Thus, cAMP signaling is a main determinant of positive inotropic response to β-AR stimulation, while activity of PDEs and NO synthases could 'fine-tune' this response.

Under stress conditions, an activation of β-ARs increases the heart rate and contractility, enhancing cardiac output and consequently supplying organs with a greater amount of blood. Chronic hyperactivity of sympathoadrenal system causes cardiac β-AR overstimulation which leads to pathological heart remodeling, contributing to initiation and progression of heart failure, arrhythmia burden and sudden cardiac death [15]. Spatially extended neuronal network of cortical and subcortical regions controls sympathetic activity [16]. Enhanced neuronal activity is associated with increased synthesis of 24-hydroxycholesterol (24HC) [17] which passes through blood–brain barrier to the circulation [18–20]. In rats, ~0.02% of the brain cholesterol was metabolized into 24HC per hour [21]. Intracardiac neurons and axons [22] may be potential source of 24HC. Production of 24HC by cardiomyocytes can take place under pathological conditions, for example in response to doxorubicin [23].

Many neurodegenerative diseases and dyslipidemia markedly increase risk of heart dysfunctions, frequently affect an activity of the sympathetic system and may be associated with changes in 24HC plasma levels [24–29]. However, there are no data about effects of 24HC on cardiac function and β-adrenergic signaling.

In the present work we studied the influence of 24HC on the inotropic responses to β-AR stimulation in atria, with consideration of effects on the β1 and β2-dependent responses. Additionally, we measured $[Ca^{2+}]_{in}$ and NO levels as well as tested involvement of NO synthases and PDEs in the effects of 24HC. Although ventricular contractions mainly determine inotropic function of heart, we consider atria as appropriate tissue for the present study due to: high sympathetic innervation, greater amount of lipid rafts and caveolar-located β2-ARs in atria [30–32]. These facts predict a high sensitivity of atria to cholesterol manipulation and cholesterol derivatives.

2. Methods

2.1. Animals and ethical approval

The study conforms to the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 1996) and European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe No 123; 1985). The isolated atria of B6J stain mice (only males; body weight 22–25 g) were used. Animals had free access to food/water under a 12-h light/12-h dark cycle. After intraperitoneal injection of sodium pentobarbital (40 mg/kg), mice were decapitated with a guillotine, and the atria were excised. The protocol of the experiments met the requirements of the EU Directive 2010/63/EU and was approved by the Bioethics Committee of Kazan State Medical University.

2.2. Bathing solutions and stimulation

The atria were perfused with the carbogen-oxygenated physiological solution (pH – 7.4) containing; in mM: NaCl 137; KCl 5; MgSO4 1; CaCl2 2; NaH2PO4 1.8; NaHCO3 13; D-glucose 11. pH was maintained at 7.4. Temperature of the bathing medium was kept at 24–25 °C which allows the cardiac myocytes to preserve the contractile function [33] and to decrease a rate of fluorescent dye loss [34]. During experiments the atria were stimulated continuously by 5-ms pulses (~10–15% above threshold) at 2 Hz via pair of Pt-electrodes using DS3 simulator (Digitimer Ltd).

As 24HC is a ligand for liver X receptors [35], we performed our experiments in relatively short time scale to avoid possible genomic effects of 24HC. Exposure to 24HC (Enzo Life Sciences) lasted 15 min following 10-min washing period, after which a β-AR agonist was added. 24HC was dissolved in DMSO (Tocris) and the concentration of DMSO in the bathing medium did not exceed 0.001%. At a concentration of 0.001% DMSO did not change the parameters of contraction or Ca^{2+} transient, or DAF-FM fluorescence, consistent with our previous work [8]. We therefore pooled data from the DMSO controls with DMSO-free controls into one group. To stimulate β-ARs, isoproterenol hydrochloride (10, 50 and 100 μM ISO, Sigma), fenoterol (10, 25 and 50 μM, Sigma) and dobutamine (1, 10 and 30 μM, Tocris) were applied. Application of the agonists lasted 15 min for each concentration; the concentration was increased stepwise up to a maximal.

For other pharmacological manipulations we used 0.1 μM ICI-118.551 (a selective blocker of β2-ARs, Sigma), 0.3 μM CGP20712 dihydrochloride (a selective inhibitor of β1-AR, Tocris), 100 μM L-NAME (a NO synthase inhibitor, Sigma), 100 μM IBMX/isobutylmethylxanthine (a non-specific inhibitor of phosphodiesterases, Tocris), 1 and 10 μM rolipram (a selective inhibitor of phosphodiesterase 4,Tocris), 10 μM forskolin (diterpenoid with adenylyl cyclase activating properties, Sigma), 60 μM TRIM/1-(2-trifluoromethylphenyl) imidazole (an inhibitor of neuronal and inducible NO synthases, Tocris), 10 μM cavtratin/caveolin-1 scaffolding domain peptide (an endothelial NO synthase blocker, Enzo Life Science). ICI-118.551, CGP20712, L-NAME, IBMX, rolipram, TRIM or cavtratin were added to the bath solution 45–50 min before the agonists of β-ARs and remained in perfusion throughout the experiment.

2.3. Recording of isometric contractions

The experiments were performed as described previously [7,36]. Briefly, only left entire atria were suspended in a bath (5 ml; 5 ml·min^{-1} perfusion rate) under resting tension of 0.25 g and equilibrated for 60 min before onset of the drug applications (Power-Lab installation, AD Instruments). One end of the atria was tied to a fixed nail, and other end was linked to a force transducer (MLT0420, AD Instruments). The constant passive tension was kept. The signals were recorded and analyzed using LabChart Pro software. The amplitude of contraction was initially detected in grams and then was rendered in percent.

2.4. Fluorescence microscopy: intracellular Ca^{2+} and NO detection

Fluorescence was detected using an Olympus up-right motorized BX51WI microscope with a confocal attachment Disk Speed Unit and objectives (LMPlanFl 20×, UPLANSapo 60×). Images were recorded with CCD camera Orca R2 (Hamamatsu). The analysis was performed in regions of interest in arbitrary units (a.u.), which were converted into percentages (ImagePro software, Media Cybernetics).

The experimental protocols were described previously [7]. Briefly, fluorescent dyes, Fluo4-AM and DAF-FM-diacetate (Thermo Fisher), were dissolved in DMSO. Pluronic F-127 (Thermo Fisher) was added into the dye aliquots to facilitate dissolving Fluo4-AM (1 μM) or DAF-FM-diacetate (4 μM) in physiological medium. The concentrations of DMSO and Pluronic F-127 were < 0.001%. The atria pinned to a chamber (5 ml) were incubated with the dyes for 20 min and were then washed (at 5 ml·min^{-1}) with a physiological solution for 50 min.

DAF-FM-diacetate, a dye for NO oxidation products, was excited by 488 ± 10 nM nm; for the recording a 515 nm long pass filter was used. The high-affinity Ca^{2+} indicator Fluo-4 was excited by 488 ± 10 nM and the emission was recorded using a band-pass 505–550 nm filter at 115 Hz sampling rates. The contraction-relaxation cycle of atria are accompanied by the transient changes in the Fluo-4 signal. The minimal fluorescence (during diastole) was subtracted from the maximal fluorescence (during systole). The Ca^{2+}-transient amplitude and DAF-FM fluorescence before drug application were taken as 1.0.

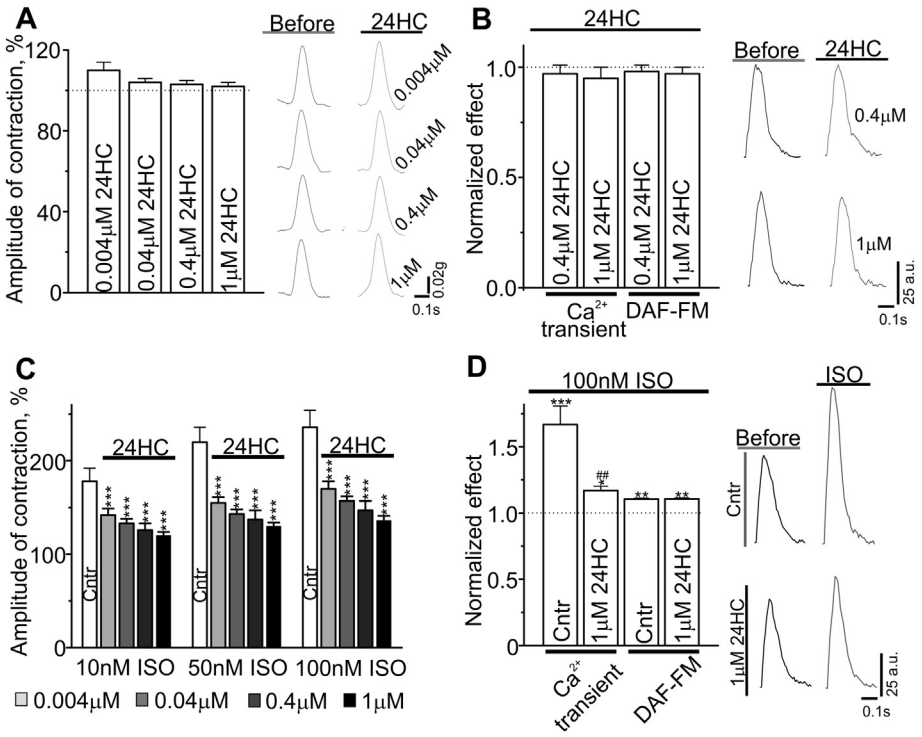

Fig. 1. Influence of 24HC on contractions, Ca^{2+} transient, NO production and responses to ISO. A, Atrial contractions. $N = 10$ for each group. B, Histograms showing the normalized effects of 24HC (0.4 and 4 μM) on peak amplitude of the Ca^{2+} transient and on DAF-FM fluorescence. $N = 14$ atria for each group. C, Positive inotropic effects of ISO in control and 24HC-pretrated atria. N = 14 for each group. D, Effect of ISO on amplitude of Ca^{2+} transient and on DAF-FM fluorescence in control and 24HC-preterated atria. $N = 12$ atria per group. A, B, D, right, representative contraction (A) or Ca^{2+} transient (B, D) traces. A, C; Y-axis shows percentage changes, relative to the value before onset of 24HC (A) or ISO (C) administration. B, D; Y-axis shows the normalized effect of 24HC (B) or ISO (D); the initial value before the 24HC or ISO addition was taken as 1.0. Asterisks denote significant differences $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to the action of ISO in the control (C) or pre-ISO values (D) and $^{\#\#}P < 0.01$ between 24HC-untreated and treated group (D).

2.5. Statistics

Data are shown as mean ± SEM; n is the number of independent experiments on different mice. Statistical significance of the difference was assessed by the unpaired Student's *t*-test or repeated analysis of variance (ANOVA) followed by the Bonferroni correction (Origin Pro 9.2). The sample sizes achieved a statistical power > 80%.

3. Results

3.1. 24HC modulates effects of β-adrenergic stimulation

24HC itself had no effect on amplitude of both contraction and Ca^{2+} transient, and on DAF-FM fluorescence (Fig. 1A, B). β-AR agonist isoproterenol (ISO) increased atrial contractility in a concentration-dependent manner. Pretreatment with 24HC at different concentrations (from 4 nM to 1 μM) attenuated ISO-mediated positive inotropy (Fig. 1C). Note that the depressant effect of 24HC was clearly expressed even at the lowest concentration used (4 nM). This suggests a high sensitivity of β-adrenergic responses to 24HC. Given that the depressant effects of the low (4 and 40 nM) and higher (0.4 and 1 μM) concentrations of 24HC had a comparable magnitude, the oxysterol-dependent modulation may be linked to high-affinity mechanism.

ISO-mediated activation of β-ARs led to increase in both amplitude of Ca^{2+} transient and DAF-FM fluorescence (Fig. 1D). These two events regulate atrial inotropic responses to β-AR stimulation [7,37]. Pretreatment with 24HC markedly attenuated an enhancement of Ca^{2+} transient amplitude induced by ISO (100 nM). At the same time, an increase of DAF-FM fluorescence in response to ISO was virtually unchanged in 24HC-treated atria (Fig. 1D). These data suggest that a decrease in ISO-induced inotropic response may be linked to the depressed amplitude of Ca^{2+} transient after pre-exposure to 24HC.

3.2. 24HC regulates β1- and β2-AR dependent component of ISO-driven responses in different ways

ISO activates all three subtypes of βARs. In the heart β1- and β2-ARs are predominant and may couple with different signaling pathways which regulate cardiac contractility in specific manners [12,13,36]. Additionally, there is crosstalk between β1- and β2-ARs at different levels. To estimate the contribution of the β1- or β2-ARs, ISO is usually applied in the presence of specific β2- or β1-AR antagonist, respectively. Note that β3-ARs have a low expression and limited contribution to regulation of contractions in wild-type mice [8,38].

3.2.1. 24HC potentiates ISO/β1AR-dependent positive inotropy and Ca^{2+} transient, and attenuates NO production

Antagonist of β2-ARs ICI-118.551 (ICI) itself and in co-application with 24HC had no influence on the contractility (Fig. 2A). This is in consistent with our previous data that ICI does not exert inverse agonist properties in mice atria [8]. ICI partially suppressed the inotropic response to ISO (10, 50, 100 nM). Unexpectedly, inotropic response to selective stimulation of β1-ARs (ISO + ICI) was increased by pretreatment with 24HC (Fig. 2B). Thus, 24HC potentiates positive inotropic response to β1-AR stimulation with ISO, despite 24HC depresses an enhancement of contractility induced by non-subtype-specific stimulation of β-ARs with ISO.

An increase in Ca^{2+} transient amplitude in response to ISO (100 nM) was partially suppressed by ICI (Fig. 2C). 24HC potentiated β1-AR (ISO + ICI)-induced enhancement of the Ca^{2+} transient amplitude and under these conditions the maximal amplitude was even higher than that in response to ISO in control (Fig. 2C). This suggests that 24HC increases enhancement of Ca^{2+} transient evoked by activation of β1-ARs.

Selective stimulation of β1-ARs (100 nM ISO + ICI) caused a more profound increase in DAF-FM fluorescence compared to control action of ISO, indicating that β2-AR activation may restrain NO synthesis linked with β1-AR activation (Fig. 2D). In contrast, 24HC markedly suppressed this upregulation of DAF-FM fluorescence (Fig. 2D) induced by selective stimulation of β1-ARs (ISO + ICI). Note that ICI alone did not change both Ca^{2+} transient amplitude and DAF-FM fluorescence (Fig. 2C, D). Thus, 24HC attenuates NO signaling in response to β1-AR stimulation with ISO.

To test the role of the decrease in NO production, the atria were perfused with L-NAME, a NO synthase inhibitor, which effectively suppressed an increase in DAF-FM fluoresce in response to ISO in

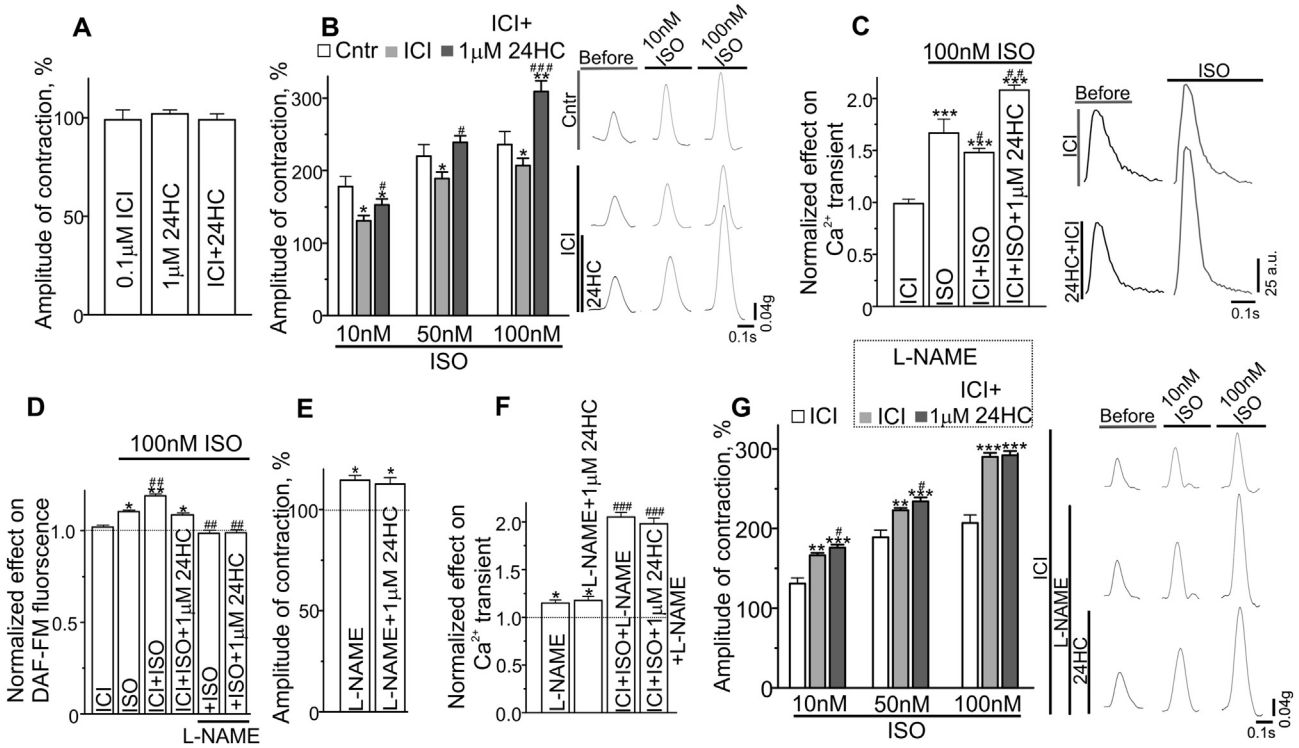

Fig. 2. Effects of 24HC on responses to selective stimulation of β1-ARs (ISO + ICI). Role of NO synthesis. A, Influence of β2-AR antagonist ICI-118.511 (ICI) on contractions. *N* = 8 and 12 for ICI and ICI + 24HC group, respectively. B, Positive inotropic response to selective stimulation of β1-ARs (ISO + ICI). N = 12 and 14 for ICI and ICI + 24HC group, respectively. C, Effect on peak amplitude of the Ca^{2+} transient. N = 8, 12 and 14 for ICI, ICI + ISO and ICI + ISO + 24HC group, respectively. D, Effect on DAF-FM fluorescence. N = 8, 12, 14, 8 and 8 for ICI, ICI + ISO, ICI + ISO + 24HC, L-NAME + ISO and L-NAME + ISO + 24HC group, respectively. E, Influence of NO synthase inhibitor (L-NAME) on contractions. N = 8 per each group. F, Effect of L-NAME on amplitude of Ca^{2+} transient. N = 10, 9, 8 and 9 for L-NAME, L-NAME + 24HC, ICI + ISO + L-NAME and ICI + ISO + L-NAME + 24HC group, respectively. G, Influence of L-NAME on β1-AR (ISO + ICI)-induced inotropy. N = 12 and 14 for L-NAME + ISO + ICI and L-NAME + ISO + ICI + 24HC group, respectively. B, C, G, right, representative contraction (B, G) and Ca^{2+} transient (C) traces. A, B, E, G; Y-axis - percentage changes, relative to the value before onset of drug administration. C, D, F; Y-axis - the normalized effect of drugs; the initial value before the drug addition was taken as 1.0. *P < 0.05, ***P < 0.001 compared to the control effect of ISO and $^{\#}$P < 0.05,$^{\#\#\#}$P < 0.001 between effect of ISO in the presence of ICI in 24HC-untreated and treated atria (B); ***P < 0.001 compared to pre-ISO baseline, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01 compared to the control effect of ISO (C, D); *P < 0.05 versus pre-LNAME baseline (E, F), $^{\#\#\#}$P < 0.001 versus pre-ISO values (F); **P < 0.01, ***P < 0.001 versus the action of ISO in the presence of ICI; $^{\#}$P < 0.05 between effects of ISO (in the presence of L-NAME) in 24HC-untreated and treated atria (G).

control and 24HC-treated atria (Fig. 2D). L-NAME alone slightly increased amplitude of contraction and Ca^{2+} transient. These effects of L-NAME were not modified by 24HC (Fig. 2E, F). Under conditions of NO synthase inhibition, the ability of 24HC to additionally facilitate β1-AR (ISO + ICI)-induced inotropy and enhancement of Ca^{2+} transient was markedly decreased. L-NAME itself mimicked the effects of 24HC (Fig. 2F, G), increasing positive inotropic and Ca^{2+} transient responses to selective stimulation of β1ARs (ISO + ICI). Probably, 24HC may enhance β1AR-mediated contractile response by suppressing NO-production.

Taken together these data suggest that the 24HC-induced potentiation of β1-AR-dependent contractility is associated with upregulation of Ca^{2+} transient and downregulation of NO synthesis, and the latter can be responsible for an increase in positive inotropic response. The discrepancy between the actions of 24HC on ISO-mediated inotropy, enhancement of Ca^{2+} transient and dynamics of DAF-FM fluorescence in the presence and absence of ICI might be linked with different effects of 24HC on β1- and β2-AR signaling.

3.2.2. 24HC suppresses ISO/β2-AR-dependent positive inotropy without changing NO production

The main subtype of β-ARs in the atria is β1-AR [39]. Antagonist of β1-AR itself and in combination with 1 μM 24HC did not affect amplitude of contractions (Fig. 3A). Inhibition of β1-ARs with CGP-20712(CGP) dramatically reduced both positive inotropic effect of ISO and enhancement of Ca^{2+} transient in response to ISO. Under these conditions, pretreatment with 24HC suppressed an increase in both contractility and Ca^{2+} transient (Fig. 3B, C) in response to selective stimulation of β2-ARs (ISO + CGP).

In contrast to β2-AR inhibitor, antagonist of β1-ARs decreased an enhancement of DAF-FM fluorescence induced by ISO (Fig. 3D). This may reflect an involvement of β1-ARs in the enhancement of NO synthesis. 24HC did not affect DAF-FM fluorescence (Fig. 3D) under conditions of selective stimulation of β2-ARs (ISO + CGP). Note that CGP alone had no effect on DAF-FM fluorescence. Taken together these data suggest that 24HC suppresses β2-AR mediated positive inotropy in a NO-independent manner.

3.3. 24HC affects responses to stimulation β1- or β2-AR with selective agonists in different ways

To additionally test the possibility that 24HC affects β1- and β2-AR-dependent responses by different mechanisms, selective β1- and β2-AR agonists were used. Dobutamine is a β-AR agonist with high affinity for β1-ARs and fenoterol primary acts via stimulation of β2-ARs in mice atria.

Dobutamine concentration-dependently induced positive inotropic effect. 24HC augmented the contractile response to dobutamine. The enhancing effect of 24HC was more profound in response to the low concentration (1 μM) of dobutamine (Fig. 4A). Also, 24HC markedly enhanced an increase in Ca^{2+} transient amplitude in response to 1 μM dobutamine (Fig. 4B). Given that at this concentration dobutamine

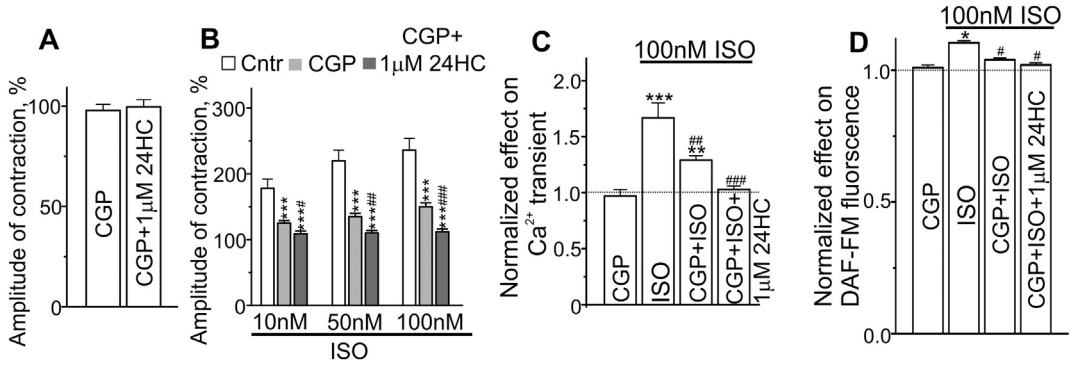

Fig. 3. Effect of 24HC on responses to selective stimulation of β2-ARs (ISO + CGP). A, Influence of β1-AR inhibitor CGP20712 (CGP) on contractions. *N* = 7 and 8 for CGP and CGP + 24HC group, respectively. B, Positive inotropic responses to β2-AR stimulation with ISO + CGP. N = 12 and 14 for CGP + ISO and CGP + ISO + 24HC, respectively. C, Effect on amplitude of Ca^{2+} transient. N = 7, 7 and 8 for CGP, CGP + ISO and CGP + ISO + 24HC group, respectively. D, Effect on DAF-FM fluorescence. N = 8, 12 and 14 for CGP, CGP + ISO and CGP + ISO + 24HC group, respectively. A, B; Y-axis - percentage changes, relative to the value before onset of CGP (A) or ISO (B) administration. C, D; Y-axis - the normalized effect of drugs; the initial value before the application was taken as 1.0. ***P < 0.001 compared to the control effect of ISO, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001 between effect of ISO (in presence CGP) in 24HC-untreated and treated atria (B); ***P < 0.001 compared to pre-ISO baseline, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.01 compared to the control effect of ISO (C, D).

exerts a high selectivity to β1-ARs [40], 24HC may serve as a sensitizer of β1-ARs to pharmacological stimulation.

The action of 24HC on β1-AR-dependent contractile responses could be linked to change in NO signaling (see Section 3.2.2). Dobutamine (1 μM) potentiated DAF-FM fluorescence, indicating on an increase in NO synthesis due to β1-AR stimulation. Pretreatment with 24HC attenuated the dobutamine-evoked NO production (Fig. 4C). This is consistent with a sensitivity of ISO/β1AR-mediated change in DAF-FM fluorescence to 24HC pretreatment. Accordingly, 24HC suppressed NO production induced by selective β1-AR stimulation, which may modulate β1AR-dependent contractility.

Fenoterol concentration-dependently increased atrial contractility, but the effects of fenoterol were less expressed than dobutamine at comparable concentrations. 24HC suppressed fenoterol-induced positive inotropy at all concentrations used (Fig. 4D). At this concentration range, specific β2-AR antagonist completely inhibited the effect of

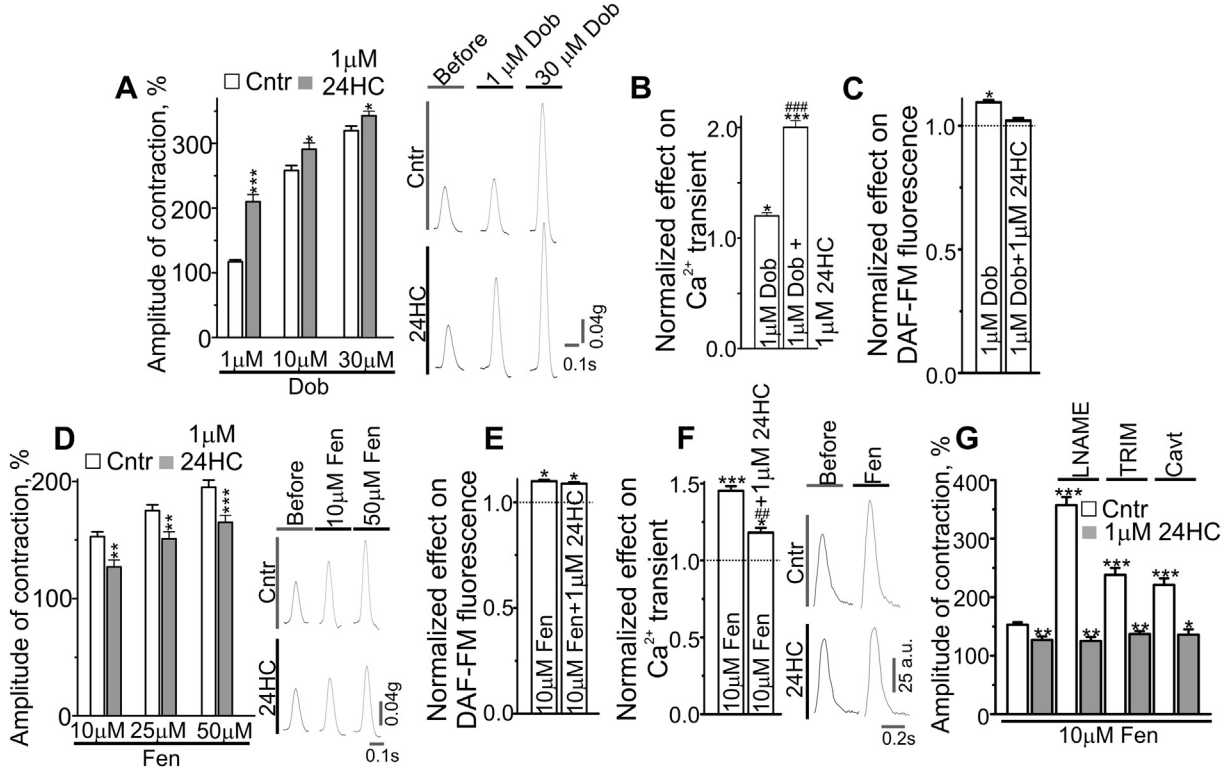

Fig. 4. 24HC modulates responses to β1- and β2-AR agonists. A, Inotropic effect of β1AR agonist dobutamine (Dob). *N* = 9 and 10 for Dob and Dob + 24HC group, respectively. B, Effect of dobutamine on Ca^{2+} amplitude transient. N = 9 and 8 for Dob and Dob + 24HC group, respectively. C, Effect of dobutamine on DAF-FM fluorescence. N = 12 and 11 for Dob and Dob + 24HC group, respectively. D, Inotropic effect of fenoterol (Fen). N = 14 and 16 for Fen and Fen + 24HC group, respectively. E, Effect of fenoterol on DAF-FM fluorescence. *N* = 11 and 12 for Fen and Fen + 24HC group, respectively. F, Influence of fenoterol on Ca^{2+} amplitude transient. N = 14 for each group. G, Influence of NO synthase inhibitors on inotropy of fenoterol. N = 8 for each group. A, D, F; Right, typical contraction (A, D) and Ca^{2+} transient (F) traces. A, D, G; Y-axis - percentage changes, relative to the value before onset of β-AR agonist administration. B, C, E, F; Y-axis - the normalized effect of β-AR agonist; the initial value before the application was taken as 1.0. **P < 0.01, **P < 0.01, ***P < 0.001, *P < 0.05 versus control effect of Dob/Fen (A, D, G); *P < 0.05, ***P < 0.001 versus pre-Dob/Fen baseline, $^{\#\#\#}$P < 0.01 between 24HC untreated and treated group (B, C, E, F).

fenoterol on atrial contractions in the mice [7,8]. This suggests that 24HC depresses a stimulatory action of β2-ARs on atrial contraction.

In contrast to stimulation of β2-ARs with ISO + CGP, exposure to fenoterol increased DAF-FM fluorescence, suggesting an increase in NO level and specific action of fenoterol, known as biased β2-AR agonist [41]. However, 24HC administration did not modify the fenoterol-evoked NO upregulation (Fig. 4E). Importantly, 24HC was able to markedly attenuate an enhancement of Ca^{2+} transient in response to fenoterol (Fig. 4F). NO synthase blockers (L-NAME, TRIM and cavtratin) by themselves enhanced the inotropic effect of fenoterol, and under these conditions, 24HC still significantly attenuated the positive inotropy of fenoterol (Fig. 4G). These data indicate that β2-AR-mediated contractility is suppressed by 24HC in Ca^{2+}-dependent and NO-independent manner.

3.4. Role of PDEs in the effects of 24HC

24HC increased β1-AR (ISO + ICI)-mediated contractility but decreased β2-AR (ISO + CGP)-dependent positive inotropy. However, inotropic response to ISO was suppressed by 24HC, despite β1-ARs are predominant in both atria and ventricles. This means that crosstalk of β1- and β2-ARs could be implicated in the effects of 24HC. PDEs are one of the main elements at a crossroad between β1- and β2-ARs in cardiomyocytes [12].

3.4.1. IBMX decreases the effect of 24HC on inotropic response to ISO

IBMX, a non-selective inhibitor of PDEs, increased amplitude of contractions by 40–50% (a steady-state level) during 20–25 min of the application (Fig. 5A). 24HC significantly suppressed the inotropic action of IBMX (Fig. 5A). In the presence of IBMX, application of ISO induced positive inotropic effect, similar to those in the control (Fig. 5B). 24HC depressed ISO-mediated inotropic response in a much lesser degree when PDEs were inhibited with IBMX (Fig. 5B). These data suggest that 24HC could potentiate an activity of PDEs and the antagonist of PDEs may counteract the inhibitory effect of 24HC on ISO-induced inotropy. In the presence of IBMX, dynamics of DAF-FM fluorescence induced by ISO did not differ between 24HC-treated and untreated atria (Fig. 5C).

IBMX increased positive inotropic response to stimulation of β2-AR (ISO + CGP). Under these conditions (Fig. 5D), 24HC lost the ability to attenuate the inotropic effect of selective stimulation of β2-AR stimulation (ISO + CGP). Thus, inhibition of PDEs suppresses negative effect of 24HC on β2-AR-dependent contractility.

3.4.2. Rolipram attenuates 24HC-mediated decrease in positive inotropy of ISO

PDE4 is a key isoform that controls signaling through β-ARs in atria [12,42,43]. Rolipram, a selective inhibitor of PDE4, increased amplitude of contractions by 25–30% (a steady-state level) during 20-min perfusion. 24HC markedly suppressed this effect of rolipram (Fig. 5E). As in the case of IBMX, rolipram did not change or slightly attenuated ISO-evoked positive inotropic responses. The depressant effect of 24HC on positive inotropy of ISO was significantly reduced in the presence of rolipram (Fig. 5F). Probably, 24HC could potentiate PDE4 activity and this may be a pathway for downregulation of ISO-induced contractility.

Forskolin, a cAMP-accumulation stimulating compound, increased contractions to a stable level and this effect was not affected by 24HC. Rolipram potentiated the positive inotropic action of forskolin and 24HC attenuated this effect of rolipram (Fig. 5G). Probably, under conditions of forskolin treatment the activity of PDEs is relatively high and it could not be additional stimulated by 24HC; only when PDE4 activity was decreased, the effect of 24HC was revealed.

4. Discussion

The main findings of the present study are: 24HC at low concentrations attenuates β-AR-dependent positive inotropic response to ISO which is associated with a decrease in Ca^{2+} transient; at the same time, 24HC changes β1- and β2-AR-driven contractility in different ways; mechanisms of 24HC effects may be related to changes in PDE activity and coupling of β1-ARs with NO signaling (Fig. 6).

Oxysterols are produced not only during pathological conditions (atherosclerosis, hypercholesterolemia, diabetes) but also by healthy cells. Among numerous oxysterols, 24HC is the major brain cholesterol metabolite which plasma level is determined by balance between brain production and hepatic clearance [18,44]. In healthy adult (age 24–47 years) volunteers the plasma level of 24HC was about 0.2 μM (or 80 ng/ml) [45]. Higher plasma levels of 24HC were found in infant (1–5 years; 385 ng/ml), children (6–9 years; 258 ng/ml,) and teenagers (10–18 years; 192 ng/ml) [46]. In the nervous system, 24HC can have genomic action (via cytosolic liver X receptor) and rapid effects linked with change in activity of glutamate NMDA-receptors [35,47,48]. Increased brain production of 24HC was observed during early stage of some neurodegenerative disease and may be caused by high neuronal activity as well as excess cholesterol in brain [17,19,20,25]. In one study, 24HC production was found in HL-1 murine cardiomyocytes in response to doxorubicin, carditoxic agent [23]. Additionally, plasma levels of 24HC were increased during hypercholesterolemia, associated with atherosclerosis, and in offspring from hypercholesterolemic pregnancies [49].

One of the main targets for 24HC in heart may be β-ARs due to their high abundance in heart and dependence of β-AR signaling on cholesterol availability. The latter relies on direct cholesterol binding and location of the cardiac β-ARs and downstream signaling molecules in cholesterol-rich membrane microdomains, planar and caveolar lipid rafts [1–6,10]. Here, we revealed that 24HC (from 4 nM to 1 μM) suppressed the positive inotropic effect of β-AR stimulation with ISO. At similar concentrations 24HC was detected in mice and human plasma [20,45,50,51]. The depressant action of 24HC on ISO-induced inotropy was associated with a decrease in Ca^{2+} transient, without changing NO production. It is known that β1-ARs mainly contribute to positive inotropic action of ISO in atria [39]. Consistent with this, β1-AR selective antagonist (CGP) suppressed the positive inotropic effect of ISO significantly stronger compared to β2-AR inhibitor (ICI). Unexpectedly, 24HC augmented inotropic effect of β1-AR stimulation (ISO + ICI or dobutamine). Moreover, 24HC potentiated a rise in Ca^{2+} transient in response to ISO/β1-AR stimulation. The stimulatory effect of 24HC on the β1-AR-mediated inotropy may be linked with attenuation of NO production. Indeed, 24HC decreased the β1-AR-evoked enhancement of NO synthesis and inhibition of NO synthase significantly masked the potentiating effect of 24HC on the ISO/β1-AR-dependent contractility and Ca^{2+} transient. It is known that NO can decrease positive inotropic effect of ISO and Ca^{2+} transient via stimulation of guanylate cyclase, which produces cGMP, leading to protein kinase G activation. Protein kinase G might inhibit L-type Ca^{2+} channels, essential for Ca^{2+} transient formation. Also, NO could directly suppress L-type Ca^{2+} channels via S-nitrosylation [11,37]. Thus, 24HC can enhance β1-AR-mediated increase in contractility via downregulation of NO signaling. Accordingly, the depressant action of 24HC on the ISO-induced inotropy was not directly related to β1-ARs.

Mechanism by which 24HC may selectively attenuate β1-AR (but not β2-AR)-induced NO production is elusive. Previously, we found that 24HC can decrease NO synthesis during synaptic activity and this could be depended on increase in lipid raft integrity at mice neuromuscular junctions [48,52]. NO synthase activity in cardiomyocytes also could be suppressed in a lipid raft/cholesterol-dependent manner [7,53]. Our data indicate that 24HC increases staining of the atrial membranes with subunit B of cholera toxin, a marker of lipid rafts (Suppl. Fig. 1). Speculatively, β1-ARs could be preferentially incorporated into newly formed lipid rafts in response to 24HC treatment, because atrial β2-ARs are already residents of caveolar lipid rafts [9,10,54]. The lack of influence of 24HC on NO synthesis in response to simultaneous activation

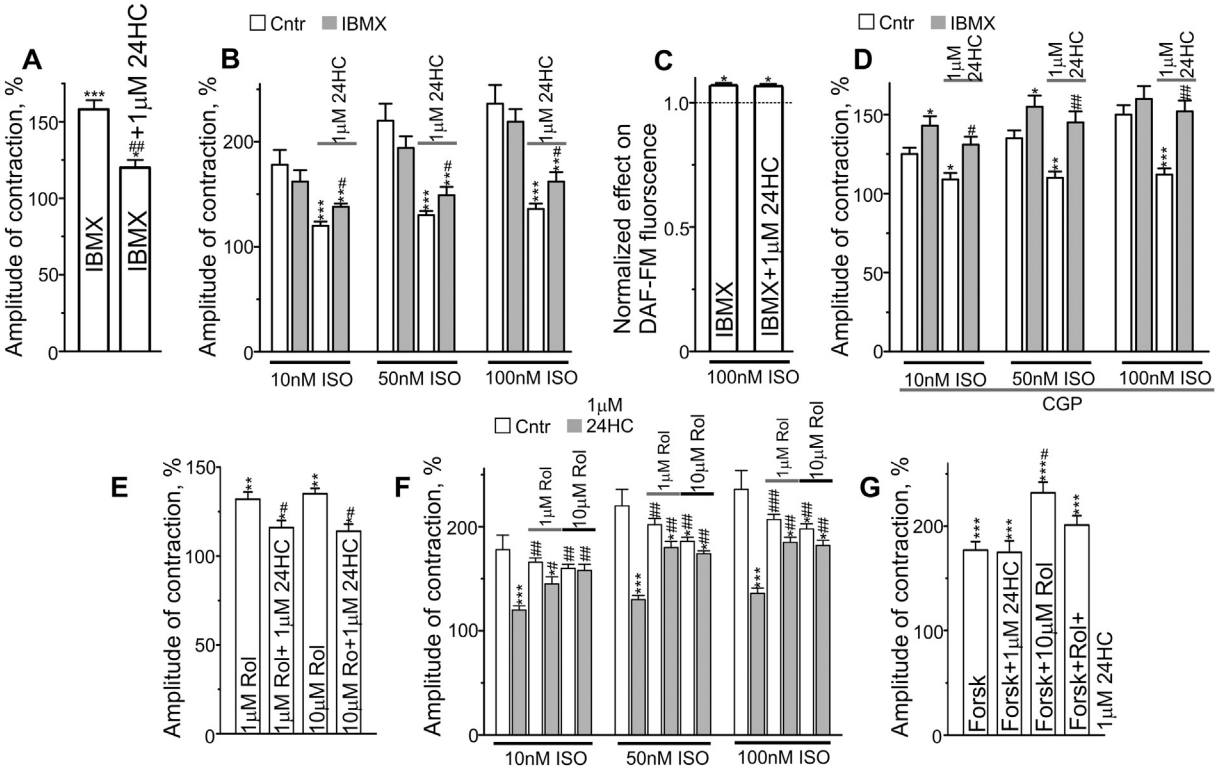

Fig. 5. Role of PDE in the effects of 24HC. A, Influence PDE inhibitor IBMX on contractions. N = 10 and 12 for IBMX and IBMX + 24HC group, respectively. B, Inotropic effects of ISO in the presence of IBMX. N = 10 and 12 for IBMX and IBMX + 24HC group, respectively. C, ISO-induced changes in DAF-FM fluorescence in the presence of IBMX. N = 8 for each group. D, Influence of IBMX on positive inotropic response to selective stimulation of β2-ARs (ISO + CGP). N = 12 and 14 for IBMX + ISO + CGP and IBMX + ISO + CGP + 24HC group, respectively. E, Effect of rolipram (Rol) on contractions. N = 8 for each group. F, Influence of rolipram on positive inotropy of ISO. N = 10, 12, 14 and 14 for 1 μM Rol, 10 μM Rol, 1 μM Rol + 24HC, 10 μM Rol + 24HC group, respectively. G, Effect of adenylyl cyclase-stimulating compound forskolin (Forsk) on contractions. N = 8, 8, 10 and 12 for Forsk, Forsk + 24HC, Forsk + Rol, Forsk + Rol + 24HC. A, B, D–G; Y-axis - percentage changes, relative to the value before onset of drug application. C; Y-axis - the normalized effect of ISO; the initial value before the application was taken as 1.0. *P < 0.05, ***P < 0.001 versus pre-IBMX/ISO/Rol value, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01 between 24HC-untreated and treated group (A, C, E); *P < 0.05, **P < 0.01, ***P < 0.001, versus the action of ISO in the control (B, D, F), $^{\#}$P < 0.05 between 24HC-treated atria in the absence and presence of IBMX (B, D); $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001 between ISO action in 24HC-treated atria and ISO effects in the presence of Rol (F); ***P < 0.001 versus pre-Forsk baseline; $^{\#}$P < 0.05 versus control action of Forsk (G).

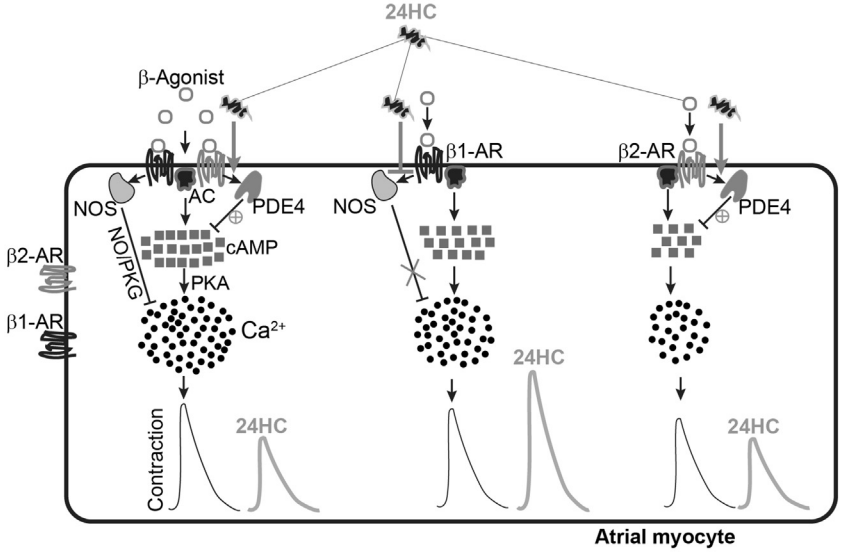

Fig. 6. Putative mechanism of 24HC effects in atrial myocytes. Pharmacological stimulation of β-ARs leads to activation Gs proteins (not shown), which activate adenylate cyclase (AC), producing cAMP. cAMP stimulates protein kinase A (PKA), causing an increase in Ca^{2+} transient and, in turn, contraction. At the same time, several mechanisms which are activated by downstream of β-ARs could limit the increase in $[Ca^{2+}]_{in}$ and contraction. There are increases in NO production by NOS (nitric oxide synthase) and in cAMP hydrolysis by PDE4. NO could decrease Ca^{2+} transient acting directly through S-nitrosylation of Ca^{2+} channels or indirectly through activation of guanylate cyclase/protein kinase G (PKG) signaling. Oxysterol, 24HC, may modulate effects of β-AR-stimulation in atrial myocytes. 24HC increases activation of PDE4, thereby suppressing increase in both $[Ca^{2+}]_{in}$ and contraction induced by co-activation of β1- and β2-ARs. However, under conditions of selective stimulation of β1-ARs, 24HC decreases NO production, causing increase in $[Ca^{2+}]_{in}$ and contraction. In the case of selective stimulation of β2-ARs, 24HC facilitates an activity of PDE4, leading to decrease in $[Ca^{2+}]_{in}$ and contraction. Although, β1-ARs mainly contribute to enhancement of $[Ca^{2+}]_{in}$ and contraction, but the depressant effect of 24HC linked with β2-ARs/PDE4 dominates under conditions of β1-and β2-AR co-stimulation.

of β1- and β2-ARs could be linked with blocking effect of β2-AR activation on coupling between β1-ARs and NO signaling.

In contrast to β1-AR responses, positive inotropic effects of β2-AR stimulation (ISO + CGP or fenoterol) were attenuated by 24HC. 24HC also suppressed β2-AR-induced enhancement of Ca^{2+} transient. However, these effects of 24HC were NO-independent. 24HC did not modify NO production in response to β2-AR stimulation. Additionally, inhibitors of NO synthases (L-NAME, TRIM or cavtratin), which itself potentiated positive inotropy of fenoterol, did not counteract the depressant effect of 24HC. One of the important "breaks" for β-AR signaling is cAMP-hydrolyzing enzymes (PDEs), which limit cAMP pool and stimulatory action of β-ARs [12,14,55,56]. Inhibition of PDEs with IBMX prevented the depressant effect of 24HC on positive inotropy of β2-AR activation with ISO + CGP. Moreover, 24HC-induced suppression of ISO inotropy (related to co-activation of β1- and β2-AR) was significantly less expressed when PDEs were inhibited with IBMX. Similarly, a specific inhibitor of PDE4 (rolipram) counteracted 24HC-mediated suppression of positive inotropic response to ISO. These results indicate that 24HC could attenuate β-AR-dependent inotropy via potentiating PDE activity, in particular PDE4. Consistent with this is that 24HC decreased an enhancement of contractility in response to IBMX or rolipram administration. Note that 24HC suppressed potentiating effect of rolipram on inotropy of adenylyl cyclase-stimulating compound forskolin.

Thus, 24HC may facilitate or suppress positive inotropy linked with β1-ARs or β2-ARs, respectively. Under condition of simultaneous β1- and β2-AR activation, the depressant action of 24HC dominates. This phenomenon may be linked with specific properties of β1- and β2-ARs. β1-ARs are distributed throughout the plasmalemma (in raft and non-raft microdomains), while β2-ARs are mainly located into caveolae of adult cardiomyocytes [9,12,54]. β1-AR stimulation leads to a global increase in cAMP pool, whereas β2-AR activation triggers formation of local cAMP signals, spreading of which can be strongly limited by PDEs [12]. A greater abundance of caveolar system [32] and high density of β2-ARs in atria [30] suggest that β2-ARs may contribute more to β-AR-dependent contractility in atria [12]. Additionally, PDE4 is highly expressed in atria and in lipid raft fraction, and PDE4 function is specifically modulated by β-AR stimulation [12,42,43,57]. It is conceivable that β2AR-dependent activation of PDE4 may be enhanced by 24HC and PDE4 could limit positive inotropic action of selective β2-AR stimulation as well as non-specific β-AR stimulation. More close association of PDE4 with β2-ARs was found to be implicated in heterologous desensitization in response to neurohormonal stimuli [58]. β-AR activation could lead to dissociation of PDE4 from complex with activated β1-ARs and association of PDE4 to internalized β2-AR/arrestin complex (located in endosomes), which, in turn, facilitates cAMP accumulation [14]. It may be a reason why IBMX and rolipram did not markedly modify the inotropy of ISO in control, but potentiated it, when β1-ARs were blocked. In this scenario, 24HC could inhibit the internalization of β2-AR-PDE4 complex that may facilitate cAMP-hydrolyzing function of PDE4 and prevent propagation of cAMP signal into the organelles.

The physiological relevance of the 24HC effects on β-AR-dependent regulation is an open question. 24HC in the circulation mainly originates from neuronal cholesterol metabolism in response to high neuronal activation [17–19]. Probably, plasma 24HC could limit effect of β-AR overstimulation, thereby protecting cardiomyocytes. The enhanced PDE4 activity may limit cAMP-depended nuclear activity of protein kinase A, that stimulates protein kinase A target genes and pro-apoptotic factor, inducible cAMP element repressor, in cardiomyocytes [57]. Changes in the plasma levels of 24HC were found at preclinical stages of neurodegenerative diseases [20,25]. Therefore, 24HC can be further studied as a possible element in connection of some neurodegenerative disorders with alteration of heart regulation [24,27,29].

5. Conclusions

In sum, present study suggests that 24HC, the main brain-derived cholesterol metabolite, could affect β-AR-dependent regulation of atrial contractions. Herein, 24HC specifically modulate β1- and β2-AR signaling, thereby increasing or decreasing the inotropic effects of the β1- and β2-AR activation, respectively. The underlying mechanism of 24HC action may be related to the changes in NO production, PDE activity and crosstalk between β1- and β2-ARs. The results of the our study are subject to the limitations of pharmacological approaches and further molecular and genetic studies are required to discover the precise molecular mechanism of cardiac action of 24HC. Also, in vivo 24HC is present in plasma in free and esterified forms [59], but in our experiments 24HC (in vehicle DMSO) was added in physiological solution. This limits a direct translation of our results to physiological situation.

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Competing interests

We declare no competing interests.

References

[1] X. Cang, L. Yang, J. Yang, C. Luo, M. Zheng, K. Yu, H. Yang, H. Jiang, Cholesterol-β1 AR interaction versus cholesterol-β2 AR interaction, Proteins 82 (5) (2014) 760–770, https://doi.org/10.1002/prot.24456.

[2] M. Manna, M. Niemelä, J. Tynkkynen, M. Javanainen, W. Kulig, D.J. Müller, T. Rog, I. Vattulainen, Mechanism of allosteric regulation of β2-adrenergic receptor by cholesterol, elife 29 (2016) 5, https://doi.org/10.7554/eLife.18432 (pii: e18432).

[3] S. Genheden, J.W. Essex, A.G. Lee, G protein coupled receptor interactions with cholesterol deep in the membrane, Biochim. Biophys. Acta Biomembr. 1859 (2) (2017) 268–281, https://doi.org/10.1016/j.bbamem.2016.12.001.

[4] H. Tsujikawa, Y. Song, M. Watanabe, H. Masumiya, S.A. Gupte, R. Ochi, T. Okada, Cholesterol depletion modulates basal L-type Ca2+ current and abolishes its -adrenergic enhancement in ventricular myocytes, Am. J. Physiol. Heart Circ. Physiol. 294 (1) (2008) H285–H292.

[5] Y.D. Paila, E. Jindal, S.K. Goswami, A. Chattopadhyay, Cholesterol depletion enhances adrenergic signaling in cardiac myocytes, Biochim. Biophys. Acta 1808 (1) (2011) 461–465, https://doi.org/10.1016/j.bbamem.2010.09.006.

[6] D.A. Macdougall, S.R. Agarwal, E.A. Stopford, H. Chu, J.A. Collins, A.L. Longster, J. Colyer, R.D. Harvey, S. Calaghan, Caveolae compartmentalise β2-adrenoceptor signals by curtailing cAMP production and maintaining phosphatase activity in the sarcoplasmic reticulum of the adult ventricular myocyte, J. Mol. Cell. Cardiol. 52 (2) (2012) 388–400, https://doi.org/10.1016/j.yjmcc.2011.06.014.

[7] Y.G. Odnoshivkina, V.I. Sytchev, A.M. Petrov, Cholesterol regulates contractility and inotropic response to β2-adrenoceptor agonist in the mouse atria: involvement of Gi-protein-Akt-NO-pathway, J. Mol. Cell. Cardiol. 107 (2017) 27–40, https://doi.org/10.1016/j.yjmcc.2016.05.001.

[8] V.I. Sytchev, Y.G. Odnoshivkina, R.V. Ursan, A.M. Petrov, Oxysterol, 5α-cholestan-3-one, modulates a contractile response to β2-adrenoceptor stimulation in the mouse atria: involvement of NO signaling, Life Sci. 188 (2017) 131–140, https://doi.org/10.1016/j.lfs.2017.09.006.

[9] V.O. Rybin, X. Xu, M.P. Lisanti, S.F. Steinberg, Differential targeting of beta -adrenergic receptor subtypes and adenylyl cyclase to cardiomyocyte caveolae. A mechanism to functionally regulate the cAMP signaling pathway, J. Biol. Chem. 275 (52) (2000) 41447–41457.

[10] S.D. Pugh, D.A. MacDougall, S.R. Agarwal, R.D. Harvey, K.E. Porter, S. Calaghan, Caveolin contributes to the modulation of basal and β-adrenoceptor stimulated function of the adult rat ventricular myocyte by simvastatin: a novel pleiotropic effect, PLoS One 9 (9) (2014) e106905, , https://doi.org/10.1371/journal.pone.

0106905.
[11] C.E. Sears, E.A. Ashley, B. Casadei, Nitric oxide control of cardiac function: is neuronal nitric oxide synthase a key component? Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 359 (1446) (2004) 1021–1044.
[12] N.K. Bhogal, A. Hasan, J. Gorelik, The development of compartmentation of cAMP signaling in cardiomyocytes: the role of T-tubules and caveolae microdomains, J. Cardiovasc. Dev. Dis. 5 (2) (2018), https://doi.org/10.3390/jcdd5020025 (pii: E25).
[13] R.P. Xiao, P. Avdonin, Y.Y. Zhou, H. Cheng, S.A. Akhter, T. Eschenhagen, R.J. Lefkowitz, W.J. Koch, E.G. Lakatta, Coupling of beta2-adrenoceptor to Gi proteins and its physiological relevance in murine cardiac myocytes, Circ. Res. 84 (1) (1999) 43–52.
[14] Y.K. Xiang, Compartmentalization of beta-adrenergic signals in cardiomyocytes, Circ. Res. 109 (2) (2011) 231–244, https://doi.org/10.1161/CIRCRESAHA.110.231340.
[15] A. Najafi, V. Sequeira, D.W. Kuster, J. van der Velden, β-Adrenergic receptor signalling and its functional consequences in the diseased heart, Eur. J. Clin. Investig. 46 (4) (2016) 362–374, https://doi.org/10.1111/eci.12598.
[16] P. Tahsili-Fahadan, R.G. Geocadin, Heart-brain axis: effects of neurologic injury on cardiovascular function, Circ. Res. 120 (3) (2017) 559–572, https://doi.org/10.1161/CIRCRESAHA.116.308446.
[17] A.O. Sodero, C. Weissmann, M.D. Ledesma, C.G. Dotti, Cellular stress from excitatory neurotransmission contributes to cholesterol loss in hippocampal neurons aging in vitro, Neurobiol. Aging 32 (6) (2011) 1043–1053, https://doi.org/10.1016/j.neurobiolaging.2010.06.001.
[18] D. Lütjohann, O. Breuer, G. Ahlborg, I. Nennesmo, A. Sidén, U. Diczfalusy, I. Björkhem, Cholesterol homeostasis in human brain: evidence for an age-dependent flux of 24S-hydroxycholesterol from the brain into the circulation, Proc. Natl. Acad. Sci. U. S. A. 93 (18) (1996) 9799–9804.
[19] J. Koschack, D. Lütjohann, C. Schmidt-Samoa, E. Irle, Serum 24S-hydroxycholesterol and hippocampal size in middle-aged normal individuals, Neurobiol. Aging 30 (6) (2009) 898–902.
[20] V. Leoni, C. Caccia, 24S-hydroxycholesterol in plasma: a marker of cholesterol turnover in neurodegenerative diseases, Biochimie 95 (3) (2013) 595–612, https://doi.org/10.1016/j.biochi.2012.09.025.
[21] I. Björkhem, D. Lütjohann, O. Breuer, A. Sakinis, A. Wennmalm, Importance of a novel oxidative mechanism for elimination of brain cholesterol. Turnover of cholesterol and 24(*S*)-hydroxycholesterol in rat brain as measured with 18O2 techniques in vivo and in vitro, J. Biol. Chem. 272 (48) (1997) 30178–30184.
[22] I. Durães Campos, V. Pinto, N. Sousa, V.H. Pereira, A brain within the heart: a review on the intracardiac nervous system, J. Mol. Cell. Cardiol. 119 (2018) 1–9, https://doi.org/10.1016/j.yjmcc.2018.04.005.
[23] J.V. Monzel, T. Budde, H.E. Meyer Zu Schwabedissen, M. Schwebe, S. Bien-Möller, D. Lütjohann, H.K. Kroemer, G. Jedlitschky, M. Grube, Doxorubicin enhances oxysterol levels resulting in a LXR-mediated upregulation of cardiac cholesterol transporters, Biochem. Pharmacol. 144 (2017) 108–119, https://doi.org/10.1016/j.bcp.2017.08.008.
[24] A. Silvani, G. Calandra-Buonaura, R.A. Dampney, P. Cortelli, Brain-heart interactions: physiology and clinical implications, Philos. Transact. A Math. Phys. Eng. Sci. 374 (2067) (2016), https://doi.org/10.1098/rsta.2015.0181 (pii: 20150181).
[25] A.M. Petrov, M.R. Kasimov, A.L. Zefirov, Cholesterol in the pathogenesis of Alzheimer's, Parkinson's diseases and autism: link to synaptic dysfunction, Acta Nat. 9 (1) (2017) 26–37.
[26] L. Baila-Rueda, A. Cenarro, I. Lamiquiz-Moneo, R. Mateo-Gallego, A.M. Bea, S. Perez-Calahorra, V. Marco-Benedi, F. Civeira, Bile acid synthesis precursors in subjects with genetic hypercholesterolemia negative for LDLR/APOB/PCSK9/APOE mutations. Association with lipids and carotid atherosclerosis, J. Steroid Biochem. Mol. Biol. 169 (2017) 226–233, https://doi.org/10.1016/j.jsbmb.2016.10.003 (Epub 2016 Oct 18).
[27] D. Ariza, L. Sisdeli, C.C. Crestani, R. Fazan, M.C. Martins-Pinge, Dysautonomias in Parkinson's disease: cardiovascular changes and autonomic modulation in conscious rats after infusion of bilateral 6-OHDA in substantia nigra, Am. J. Physiol. Heart Circ. Physiol. 308 (3) (2015) H250–H257, https://doi.org/10.1152/ajpheart.00406.2014.
[28] M. Reimann, H. Rüdiger, N. Weiss, T. Ziemssen, Acute hyperlipidemia but not hyperhomocysteinemia impairs reflex regulation of the cardiovascular system, Atheroscler. Suppl. 18 (2015) 8–15, https://doi.org/10.1016/j.atherosclerosissup.2015.02.004.
[29] A.M. Schroeder, H.B. Wang, S. Park, M.C. Jordan, F. Gao, G. Coppola, M.C. Fishbein, K.P. Roos, C.A. Ghiani, C.S. Colwell, Cardiac dysfunction in the BACHD mouse model of Huntington's disease, PLoS One 11 (1) (2016) e0147269, , https://doi.org/10.1371/journal.pone.0147269.
[30] B.F. Buxton, C.R. Jones, P. Molenaar, R.J. Summers, Characterization and autoradiographic localization of beta-adrenoceptor subtypes in human cardiac tissues, Br. J. Pharmacol. 92 (2) (1987) 299–310.
[31] E.A. Wehrwein, L.M. Parker, A.A. Wright, J.M. Spitsbergen, M. Novotny, D. Babankova, G.M. Swain, B.A. Habecker, D.L. Kreulen, Cardiac norepinephrine transporter protein expression is inversely correlated to chamber norepinephrine content, Am. J. Phys. Regul. Integr. Comp. Phys. 295 (3) (2008) R857–R863, https://doi.org/10.1152/ajpregu.00190.2008 (Epub 2008 Jun 18).
[32] A.V. Glukhov, M. Balycheva, J.L. Sanchez-Alonso, Z. Ilkan, A. Alvarez-Laviada, N. Bhogal, I. Diakonov, S. Schobesberger, M.B. Sikkel, A. Bhargava, G. Faggian, P.P. Punjabi, S.R. Houser, J. Gorelik, Direct evidence for microdomain-specific localization and remodeling of functional L-type calcium channels in rat and human atrial myocytes, Circulation 132 (25) (2015) 2372–2384, https://doi.org/10.1161/CIRCULATIONAHA.115.018131.
[33] J.R. Blinks, J. Koch-Weser, Physical factors in the analysis of the actions of drugs on myocardial contractility, Pharmacol. Rev. 15 (1963) 531–599 14064357.
[34] V.A. Miriel, J.R. Mauban, M.P. Blaustein, W.G. Wier, Local and cellular Ca2+ transients in smooth muscle of pressurized rat resistance arteries during myogenic and agonist stimulation, J. Physiol. 518 (Pt 3) (1999) 815–824.
[35] J.M. Lehmann, S.A. Kliewer, L.B. Moore, T.A. Smith-Oliver, B.B. Oliver, J.L. Su, S.S. Sundseth, D.A. Winegar, D.E. Blanchard, T.A. Spencer, T.M. Willson, Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway, J. Biol. Chem. 272 (6) (1997) 3137–3140.
[36] U.G. Odnoshivkina, V.I. Sytchev, L.F. Nurullin, A.R. Giniatullin, A.L. Zefirov, A.M. Petrov, β2-Adrenoceptor agonist-evoked reactive oxygen species generation in mouse atria: implication in delayed inotropic effect, Eur. J. Pharmacol. 765 (2015) 140–153, https://doi.org/10.1016/j.ejphar.2015.08.020.
[37] L. Sterin-Borda, A. Genaro, C. Perez Leiros, G. Cremaschi, A. Vila Echagüe, E. Borda, Role of nitric oxide in cardiac beta-adrenoceptor-inotropic response, Cell. Signal. 10 (4) (1998) 253–257.
[38] C. Belge, J. Hammond, E. Dubois-Deruy, B. Manoury, J. Hamelet, C. Beauloye, A. Markl, A.C. Pouleur, L. Bertrand, H. Esfahani, K. Jnaoui, K.R. Götz, V.O. Nikolaev, A. Vanderper, P. Herijgers, I. Lobysheva, G. Iaccarino, D. Hilfiker-Kleiner, G. Tavernier, D. Langin, C. Dessy, J.L. Balligand, Enhanced expression of β3-adrenoceptors in cardiac myocytes attenuates neurohormone-induced hypertrophic remodeling through nitric oxide synthase, Circulation 129 (2013) 451–462, https://doi.org/10.1161/CIRCULATIONAHA.113.004940.
[39] B.T. Liang, P.B. Molinoff, Beta adrenergic receptor subtypes in the atria: evidence for close coupling of beta-1 and beta-2 adrenergic receptors to adenylate cyclase, J. Pharmacol. Exp. Ther. 238 (3) (1986) 886–892.
[40] W. Wang, H. Zhang, H. Gao, H. Kubo, R.M. Berretta, X. Chen, S.R. Houser, Beta1-Adrenergic receptor activation induces mouse cardiac myocyte death through both L-type calcium channel-dependent and -independent pathways, Am. J. Physiol. Heart Circ. Physiol. 299 (2) (2010) H322–H331, https://doi.org/10.1152/ajpheart.00392.2010.
[41] S. Reiner, M. Ambrosio, C. Hoffmann, M.J. Lohse, Differential signaling of the endogenous agonists at the beta2-adrenergic receptor, J. Biol. Chem. 285 (46) (2010) 36188–36198, https://doi.org/10.1074/jbc.M110.175604 (Epub 2010 Sep 13).
[42] R. Hua, A. Adamczyk, C. Robbins, G. Ray, R.A. Rose, Distinct patterns of constitutive phosphodiesterase activity in mouse sinoatrial node and atrial myocardium, PLoS One 7 (10) (2012) e47652.
[43] C.E. Molina, J. Leroy, W. Richter, M. Xie, C. Scheitrum, I.O. Lee, C. Maack, C. Rucker-Martin, P. Donzeau-Gouge, I. Verde, A. Llach, L. Hove-Madsen, M. Conti, G. Vandecasteele, R. Fischmeister, Cyclic adenosine monophosphate phosphodiesterase type 4 protects against atrial arrhythmias, J. Am. Coll. Cardiol. 59 (24) (2012) 2182–2190.
[44] V. Mutemberezi, O. Guillemot-Legris, G.G. Muccioli, Oxysterols: from cholesterol metabolites to key mediators, Prog. Lipid Res. 64 (2016) 152–169, https://doi.org/10.1016/j.plipres.2016.09.002.
[45] A. Babiker, U. Diczfalusy, Transport of side-chain oxidized oxysterols in the human circulation, Biochim. Biophys. Acta 1392 (2–3) (1998) 333–339.
[46] L. Bretillon, D. Lütjohann, L. Ståhle, T. Widhe, L. Bindl, G. Eggertsen, U. Diczfalusy, I. Björkhem, Plasma levels of 24S-hydroxycholesterol reflect the balance between cerebral production and hepatic metabolism and are inversely related to body surface, J. Lipid Res. 41 (2000) 840–845 10787445.
[47] S.M. Paul, J.J. Doherty, A.J. Robichaud, G.M. Belfort, B.Y. Chow, R.S. Hammond, D.C. Crawford, A.J. Linsenbardt, H.J. Shu, Y. Izumi, S.J. Mennerick, C.F. Zorumski, The major brain cholesterol metabolite 24(*S*)-hydroxycholesterol is a potent allosteric modulator of *N*-methyl-D-aspartate receptors, J. Neurosci. 33 (44) (2013) 17290–17300, https://doi.org/10.1523/JNEUROSCI.2619-13.2013.
[48] M.R. Kasimov, M.R. Fatkhrakhmanova, K.A. Mukhutdinova, A.M. Petrov, 24S-Hydroxycholesterol enhances synaptic vesicle cycling in the mouse neuromuscular junction: implication of glutamate NMDA receptors and nitric oxide, Neuropharmacology 117 (2017) 61–73, https://doi.org/10.1016/j.neuropharm.2017.01.030.
[49] J.H. Dumolt, S.K. Radhakrishnan, M.H. Moghadasian, K. Le, M.S. Patel, R.W. Browne, T.C. Rideout, Maternal hypercholesterolemia enhances oxysterol concentration in mothers and newly weaned offspring but is attenuated by maternal phytosterol supplementation, J. Nutr. Biochem. 52 (2018) 10–17, https://doi.org/10.1016/j.jnutbio.2017.09.013.
[50] M. Shafaati, N. Mast, O. Beck, R. Nayef, G.Y. Heo, L. Björkhem-Bergman, D. Lütjohann, I. Björkhem, I.A. Pikuleva, The antifungal drug voriconazole is an efficient inhibitor of brain cholesterol 24S-hydroxylase in vitro and in vivo, J. Lipid Res. 51 (2) (2010) 318–323, https://doi.org/10.1194/jlr.M900174-JLR200.
[51] P.J. Crick, L. Beckers, M. Baes, P.P. Van Veldhoven, Y. Wang, W.J. Griffiths, The oxysterol and cholestenoic acid profile of mouse cerebrospinal fluid, Steroids 99 (Pt B) (2015) 172–177, https://doi.org/10.1016/j.steroids.2015.02.021.
[52] K.A. Mukhutdinova, M.R. Kasimov, A.R. Giniatullin, G.F. Zakyrjanova, A.M. Petrov, 24S-hydroxycholesterol suppresses neuromuscular transmission in SOD1(G93A) mice: a possible role of NO and lipid rafts, Mol. Cell. Neurosci. 88 (2018) 308–318, https://doi.org/10.1016/j.mcn.2018.03.006.
[53] R.S. Ostrom, R.A. Bundey, P.A. Insel, Nitric oxide inhibition of adenylyl cyclase type 6 activity is dependent upon lipid rafts and caveolin signaling complexes, J. Biol. Chem. 279 (19) (2004) 19846–19853.
[54] S.R. Agarwal, D.A. MacDougall, R. Tyser, S.D. Pugh, S.C. Calaghan, R.D. Harvey, Effects of cholesterol depletion on compartmentalized cAMP responses in adult cardiac myocytes, J. Mol. Cell. Cardiol. 50 (3) (2011) 500–509.
[55] T. Christ, A. Galindo-Tovar, M. Thoms, U. Ravens, A.J. Kaumann, Inotropy and L-type Ca2+ current, activated by beta1- and beta2-adrenoceptors, are differently controlled by phosphodiesterases 3 and 4 in rat heart, Br. J. Pharmacol. 156 (1)

(2009) 62–83, https://doi.org/10.1111/j.1476-5381.2008.00015.x.
[56] I. Bedioune, F. Lefebvre, P. Lechêne, A. Varin, V. Domergue, M.S. Kapiloff, R. Fischmeister, G. Vandecasteele, PDE4 and mAKAPβ are nodal organizers of β2-ARs nuclear PKA signaling in cardiac myocytes, Cardiovasc. Res. (2018), https://doi.org/10.1093/cvr/cvy110.
[57] J. Leroy, A. Abi-Gerges, V.O. Nikolaev, W. Richter, P. Lechêne, J.L. Mazet, M. Conti, R. Fischmeister, G. Vandecasteele, Spatiotemporal dynamics of beta-adrenergic cAMP signals and L-type Ca2+ channel regulation in adult rat ventricular myocytes: role of phosphodiesterases, Circ. Res. 102 (9) (2008) 1091–1100, https://doi.org/10.1161/CIRCRESAHA.107.167817.
[58] Q. Shi, M. Li, D. Mika, Q. Fu, S. Kim, J. Phan, A. Shen, G. Vandecasteele, Y.K. Xiang, Heterologous desensitization of cardiac β-adrenergic signal via hormone-induced βAR/arrestin/PDE4 complexes, Cardiovasc. Res. 113 (6) (2017) 656–670, https://doi.org/10.1093/cvr/cvx036.
[59] I. Burkard, K.M. Rentsch, A. von Eckardstein, Determination of 24S- and 27-hydroxycholesterol in plasma by high-performance liquid chromatography-mass spectrometry, J. Lipid Res. 45 (4) (2004) 776–781 (Epub 2004 Jan 16).