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Cholesterol depletion enhances adrenergic signaling in cardiac myocytes

Yamuna Devi Paila [a,1], Ekta Jindal [b,1], Shyamal K. Goswami [b], Amitabha Chattopadhyay [a,*]

[a] Centre for Cellular and Molecular Biology, Council of Scientific and Industrial Research Uppal Road, Hyderabad 500 007, India
[b] School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India

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ABSTRACT

Cardiac myocytes endogenously express α and β adrenergic receptors, prototypes of the G-protein coupled receptor superfamily. Depending upon the dose of norepinephrine (agonist) exposure, hypertrophy and apoptosis are initiated by differential induction of two discrete constituents of the transcription factor AP-1, *i.e.*, FosB and Fra-1. We explored differential adrenergic signaling as a paradigm for understanding how cholesterol dictates cells to choose hypertrophy or apoptosis. For this, we used *fosB* and *fra-1* promoter–reporter constructs for monitoring adrenergic signaling. We show that cholesterol depletion enhances norepinephrine-mediated signaling in cardiac myocytes. Importantly, this increased signaling is reduced to original level upon cholesterol replenishment. We used specific ligands for α and β adrenergic receptors and show that the enhanced signaling upon cholesterol depletion is a combined effect of both α and β adrenergic receptors. These results constitute the first report demonstrating the effect of cholesterol on adrenergic signaling using a direct end-point gene expression.

1. Introduction

Cholesterol is an essential and representative lipid in higher eukaryotic cellular membranes and is crucial in membrane organization, dynamics, function, and sorting [1,2]. Cholesterol is often found distributed nonrandomly in domains in biological membranes [1,3]. Current understanding of the organization of biological membranes involves the concept of lateral heterogeneities in the membrane, collectively termed as membrane domains. Many of these domains (sometimes termed as 'lipid rafts') are thought to be important for the maintenance of membrane structure and function, although characterizing the spatiotemporal resolution of these domains has proven to be challenging [3–6]. The idea of such specialized membrane domains assumes significance in cell biology since physiologically important functions such as membrane sorting and trafficking, signal transduction processes, and the entry of pathogens [3,7–9] have been attributed to these domains. Importantly, cholesterol plays a vital role in the function and organization of membrane proteins and receptors such as G-protein coupled receptors (GPCRs) [10–12].

The GPCR superfamily is the largest and most diverse protein family in mammals, involved in signal transduction across membranes [13,14]. GPCRs are prototypical members of the family of seven transmembrane domain proteins and include >800 members which together constitute ~5% of the human genome [15]. GPCRs regulate physiological responses to a diverse array of stimuli, and mediate multiple physiological processes. For this reason, GPCRs have emerged as major targets for the development of novel drug candidates in all clinical areas [16]. It is estimated that up to 50% of clinically prescribed drugs act as either agonists or antagonists of GPCRs [17]. Adrenergic receptors are important members of the GPCR superfamily and are endogenously expressed in cardiac myocytes. In adult heart, terminally differentiated myocytes are susceptible to discrete pathological consequences like hypertrophy, apoptosis and autophagy, each with immense clinical implications [18,19]. It has been reported that agonists such as angiotensin II and norepinephrine (NE) may cause hypertrophy and apoptosis, depending upon the concentration, thereby providing a framework for understanding the crosstalk between these two pathways [20–22]. NE released from the sympathetic nervous system and its cognate receptors (α and β with several subtypes) play a critical role in cardiac performance and homeostasis [23,24]. In addition, *ex vivo* cardiac myocytes elicit hypertrophic response upon treatment with lower dose (≤10 μM), while higher dose (≥50 μM) of NE induces apoptosis [20,21]. However, in spite of significant enhancement of our knowledge of adrenergic signaling, the role of membrane components such as cholesterol in these processes is not understood yet. Such knowledge is crucial in a better understanding of cardiovascular pathobiology, particularly keeping in mind the close relationship between cholesterol, adrenergic signaling and heart failure [25].

Abbreviations: GPCR, G-protein coupled receptor; MβCD, methyl-β-cyclodextrin; NE, norepinephrine

* Corresponding author. Tel.: +91 40 2719 2578; fax: +91 40 2716 0311.
E-mail address: amit@ccmb.res.in (A. Chattopadhyay).

[1] These authors contributed equally to the work.

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It has previously been demonstrated that cholesterol is required for the function of GPCRs such as the serotonin$_{1A}$ receptor [11,12,26] and the oxytocin receptor [27]. In this paper, we have explored differential adrenergic signaling in cardiac myocytes as a paradigm for understanding how cellular cholesterol dictates cells to choose hypertrophic or apoptotic responses. We earlier demonstrated that both *ex vivo* and *in vivo* myoblasts and myocytes elicit hypertrophic and apoptotic responses through respective pathways, demarcated by the induction of FosB and Fra-1, two distinct members of the AP-1 family of transcription factors [21]. These results have unequivocally established a gene-specific framework for delineating differential signaling leading to hypertrophic and apoptotic responses. In the present study, we used *fosB* and *fra-1* genes as the end-point targets for analyzing respective signaling pathways, and monitored the effect of cellular cholesterol on these pathways. Our results show that cholesterol depletion enhances NE-mediated adrenergic signaling in cardiac myocytes. Importantly, we used specific ligands for α and β adrenergic receptors and our results show that signaling, as monitored by activities of *fosB* and *fra-1* promoters, is enhanced in both types of receptors upon cholesterol depletion.

2. Materials and methods

2.1. Materials

All reagents used in this study were from Sigma Aldrich (St. Louis, MO) unless mentioned otherwise. Fetal bovine serum was purchased from Life Technologies (Carlsbad, CA). Luciferase assay reagents were purchased from Promega (Madison, WI). Amplex Red cholesterol assay kit was from Molecular Probes (Eugene, OR). Bradford reagent was purchased from Bio-Rad (Hercules, CA). Water was purified through a Millipore (Bedford, MA) Milli-Q system and used throughout.

2.2. Methods

2.2.1. Cell culture

H9c2 cells (embryonic rat cardiac myoblasts) were cultured and maintained as monolayer in Dulbecco's Modified Eagle's Medium (DMEM) containing high glucose, supplemented with 10% fetal bovine serum (heat inactivated), 90 U/ml penicillin, 90 μg/ml streptomycin and 5 μg/ml amphotericin B at 37 °C in humidified incubator with 5% CO_2 [21].

2.2.2. Generation of promoter–reporter constructs

Fragments (1493 base pair and 2689 base pair) of 5′ upstream regions of *fosB* and *fra-1* genes were isolated by PCR amplification from rat genomic DNA and cloned in pGL3 luciferase basic vector (Promega) using standard recombinant DNA methods [21]. Genomic DNA isolated from H9c2 cells was PCR amplified using primers (forward 5′ GGAATGGCAGGCTTTCAACAC 3′ and reverse 5′ CTGTGACCACGCTGAGGTCTT 3′) spanning −1033 base pair to +460 base pair of the 5′ upstream region of *fosB* gene. This 1493 bp PCR amplified product was further cloned in pGL3 luciferase basic vector (Promega) at SmaI site. Similarly, 2689 base pair PCR amplified product using *fra1* specific primers (forward 5′ GTGCTAGCCCATGCGTGCTTGTGCGTGT 3′ and reverse 5′ CGAGATCTGCTGGATGT TCGGTA 3′) spanning −2474 base pair to +215 base pair region was cloned in pGL3 vector at Nhe I/Bgl II sites.

2.2.3. Transient transfection

H9c2 cardiac myoblasts were grown in 6-well plate up to 60–70% confluency and transiently transfected with reporter plasmids using Escort IV transfection reagent. Briefly, 2 μg of plasmid DNA and 4 μl of transfection reagent (Sigma) were used per well. Cells were incubated with the transfection reagent in serum- and antibiotic-free medium for 10–12 h, followed by incubation in medium containing 20% serum and antibiotics for 6 h. Cells were then kept in 10% serum containing medium for 6 h. Finally, cells were kept in serum-free medium for 12 h, treated with the agonists for 2 and 6 h (~36–40 h post-transfection). The agonists used were NE, phenylephrine (α-adrenergic receptor agonist) and isoproterenol (β-adrenergic receptor agonist). Cells were harvested after 2 and 6 h of treatment for reporter assay.

2.2.4. Luciferase reporter assays

Cells were lysed in reporter lysis buffer. Cell lysates were analyzed for luciferase activity using the Luciferase Reagent Assay Kit and the corresponding luminescence was measured with a luminometer (Turner Scientific, CA). Normalization of transfection efficiency was done by the estimation of total protein used for the luciferase assay [28].

2.2.5. Cholesterol depletion of cells in culture

Cells were grown in 6-well plate and transfected with reporter constructs. Cholesterol depletion was carried out by treating cells with 5 mM MβCD in serum-free medium for 30 min at 37 °C, followed by wash with serum-free medium [29,30].

2.2.6. Replenishment of cholesterol in cholesterol-depleted cells

Cholesterol-depleted H9c2 cells were replenished with cholesterol using a water-soluble cholesterol–MβCD complex. Cholesterol replenishment was carried out by incubating cholesterol-depleted cells with the cholesterol–MβCD complex for 5 min in a humidified atmosphere with 5% CO_2 at 37 °C [31]. The complex was prepared by dissolving required amounts of cholesterol and MβCD in a ratio of 1:10 (mol/mol) in DMEM medium by constant vortexing at room temperature (~23 °C) [26]. Cholesterol–MβCD complex was freshly prepared and filter sterilized before each experiment.

2.2.7. Analysis of cholesterol content

Cholesterol content in cell lysates was estimated using the Amplex Red cholesterol assay kit [32].

3. Results

The water-soluble compound MβCD has earlier been shown to selectively and efficiently extract cholesterol from cellular membranes by including it in a central nonpolar cavity [33]. Fig. 1 shows the cholesterol content in cholesterol-depleted and cholesterol-replenished H9c2 cardiac myocytes. Upon treatment of cells with 5 mM MβCD, the cholesterol content was reduced to ~48% of that of control (without MβCD or agonist treatment) in case of cells transfected with *fosB* plasmid (Fig. 1a). In case of cardiac myocytes that were transfected with *fra-1*, the corresponding reduction in cholesterol content was comparable (~50%; see Fig. 1b). Cholesterol replenishment of cholesterol-depleted cells was carried out by incubating the cholesterol-depleted cells with cholesterol–MβCD complex, as described in Materials and methods. As shown in Fig.1, replenishment of cholesterol with this complex resulted in recovery of cholesterol. Similar results were obtained when cells were stimulated by 2 μM (Fig. 1a) and 100 μM (Fig. 1b) NE.

Low and high doses (2 and 100 μM) of NE selectively induce the transcription of *fosB* and *fra-1* genes (Jindal and Goswami, manuscript in preparation). This is based on the earlier observation that hypertrophic and apoptotic doses of NE (2 and 100 μM, respectively) differentially induce FosB and Fra-1 [21]. As shown in Fig. 2, *fosB* promoter was induced (~1.5 fold) by 2 μM NE in 2 h and *fra-1* promoter was induced (~2.5 fold) by 100 μM NE in 6 h in control (without MβCD or agonist treatment) cells. It should be noted that promoter activity appears to be modulated with cholesterol content, even in the absence of agonist treatment. Interestingly, cholesterol depletion enhances the induction of *fosB* promoter to ~2.4 fold upon

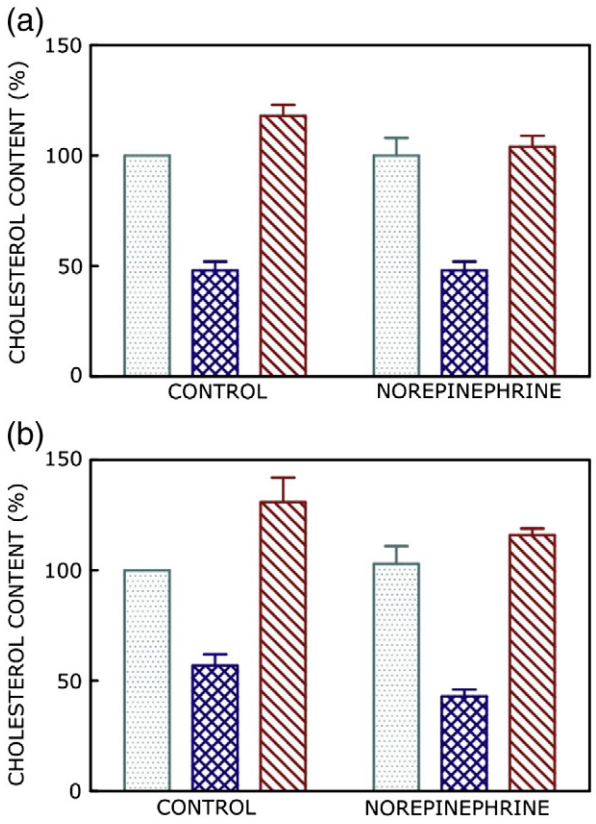

Fig. 1. Cholesterol content of H9c2 cells upon cholesterol depletion and replenishment. H9c2 cells were transfected with (a) *fosB* and (b) *fra-1* promoter–reporter constructs. Cholesterol contents in control (without MβCD or agonist treatment; dotted bar), cholesterol-depleted (crisscrossed bar), and cholesterol-replenished (hatched bar) cells are shown. Corresponding cholesterol contents upon stimulation by 2 μM (panel a) and 100 μM (panel b) NE are also shown. Values are expressed as percentages of cholesterol content of control (without MβCD or agonist treatment) cells. Data represent means ± S.E. of at least four independent measurements. See Materials and methods for other details.

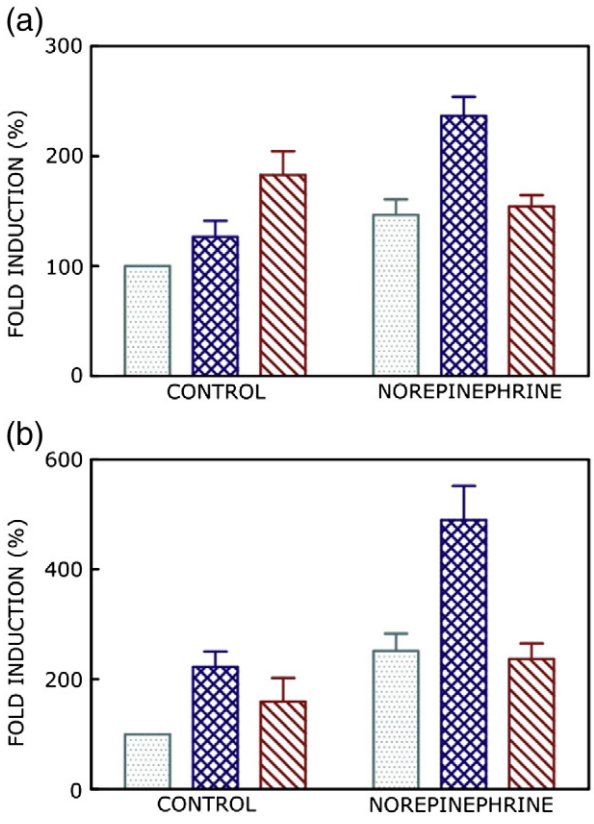

Fig. 2. Effect of cholesterol depletion and replenishment of H9c2 cells on *fosB* and *fra-1* promoter activity. H9c2 cells were transfected with (a) *fosB* and (b) *fra-1* promoter–reporter constructs. Control (without MβCD or agonist treatment; dotted bar), cholesterol-depleted (crisscrossed bar), and cholesterol-replenished (hatched bar) cells were stimulated with 2 and 100 μM NE for *fosB* (2 h) and *fra-1* (6 h) promoter, respectively. Cell lysates were assayed for luciferase activity. Fold induction represents luciferase activities of respective constructs, normalized to control (without MβCD or agonist treatment) cells. Data represent means ± S.E. of at least four independent measurements. See Materials and methods for other details.

stimulation with 2 μM NE (Fig. 2a). Fig. 2a also shows that this induction comes down to original level (~1.5 fold) upon cholesterol replenishment. Similarly, cholesterol depletion enhances the induction of *fra-1* promoter to ~4.9 fold by 100 μM NE and the enhanced induction is reduced to more or less original level (~2.5 fold) upon replenishment with cholesterol (Fig. 2b).

As mentioned earlier, H9c2 cardiac myocytes possess multiple adrenergic receptors (α and β with several subtypes). In order to explore whether the enhanced induction in *fosB* and *fra-1* promoters upon cholesterol depletion is due to specific receptor subtypes (α/β) or due to a combination of these subtypes, we monitored the induction of these promoters upon stimulation with subtype-specific ligands. We used phenylephrine and isoproterenol as specific agonists for α and β adrenergic receptors, respectively. Fig. 3a shows that cholesterol depletion enhances the induction of *fosB* promoter to ~1.5 fold upon stimulation with 10 μM isoproterenol or phenylephrine. Similarly, cholesterol depletion enhances the induction of *fra-1* promoter by ~2.6 and 3.7 folds upon treatment with 100 μM isoproterenol or 10 μM phenylephrine, respectively (Fig. 3b). Overall, our results demonstrate that adrenergic signaling is enhanced upon cholesterol depletion in cardiac myocytes, irrespective of subtype specificity. These results are similar to our previous results in which we demonstrated that cholesterol depletion from cells using MβCD resulted in enhanced function of the serotonin$_{1A}$ receptor [29,30]. Taken together, our results represent the first report describing the effect of cholesterol on the function of adrenergic receptors, using a direct end-point gene expression assay.

4. Discussion

GPCRs are the most ubiquitous among mammalian cell surface receptors and mediate numerous cellular events [34]. Adrenergic receptors are important members of the GPCR superfamily and play crucial role in cardiovascular biology and homeostasis [21,23,24,35,36]. However, mechanisms by which adrenergic stimulation results in activation/inactivation of cognate genes is still emerging [35,36], particularly in the context of membrane lipids. The molecular mechanism underlying the effect of membrane lipids such as cholesterol on the structure and function of integral membrane proteins and receptors is poorly understood and is an active area of research [10,11,37,38]. It has been proposed that such effects could occur either due to a specific molecular interaction with membrane proteins leading to a conformational change in the receptor [27], or due to alterations in the membrane physical properties induced by the presence of cholesterol [39], or due to a combination of both [37]. There is another mechanism by which cholesterol could affect structure and function of membrane proteins. This mechanism invokes the concept of 'nonannular' binding sites of membrane lipids. Interestingly, we recently proposed that cholesterol binding sites in GPCRs could represent nonannular binding sites [38].

Our earlier results showed that hypertrophic and apoptotic responses elicited by NE are marked by the differential induction of FosB and Fra-1 [21]. We confirmed these observations using 2 and 100 μM doses of NE which selectively induce the transcription of *fosB* and *fra-1* genes, respectively (Jindal and Goswami, manuscript in

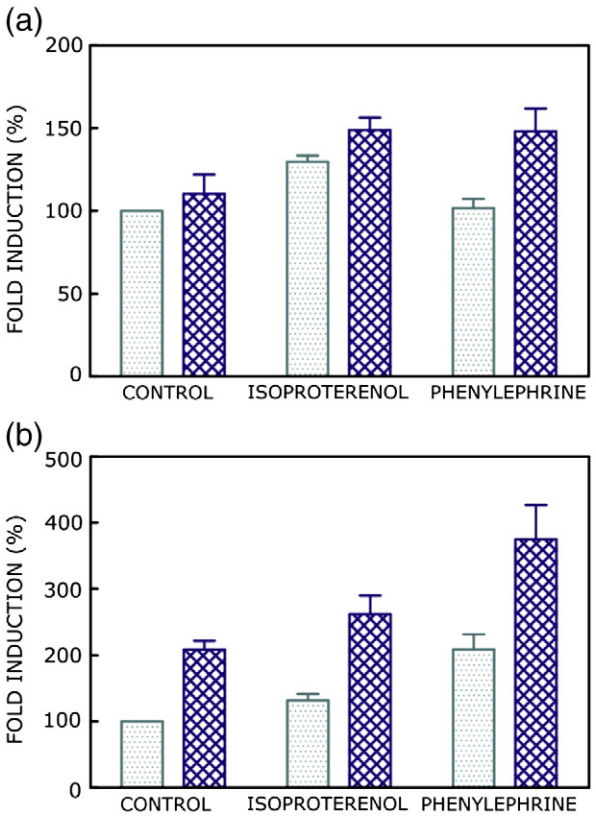

Fig. 3. Effect of cholesterol depletion of H9c2 cells on *fosB* and *fra-1* promoter activity upon stimulation with specific agonists of α- and β-adrenergic receptors. H9c2 cells were transfected with (a) *fosB* and (b) *fra-1* promoter–reporter constructs. Control (without MβCD or agonist treatment; dotted bar) and cholesterol-depleted (criss-crossed bar) cells were treated with 10 μM isoproterenol or phenylephrine for *fosB*, and 100 μM isoproterenol or 10 μM phenylephrine for *fra-1* promoter. Cell lysates were assayed for luciferase activity. Fold induction represents luciferase activities of respective constructs, normalized to control (without MβCD or agonist treatment) cells. Data represent means ± S.E. of at least four independent measurements. See Materials and methods for other details.

preparation). Our present results illustrate that cholesterol depletion enhances the induction of *fosB* and *fra-1* promoters upon stimulation with NE and this increased induction is restored upon cholesterol replenishment. H9c2 cardiac myocytes endogenously express several adrenergic receptors (α and β with many subtypes). We used specific ligands (phenylephrine and isoproterenol) against α and β adrenergic receptors to delineate the enhanced induction in *fosB* and *fra-1* promoters. Our results demonstrate that the enhanced induction in *fosB* and *fra-1* promoters upon cholesterol depletion is a combined effect of both α and β subtypes of adrenergic receptors. Taken together, these novel results demonstrate the effect of cholesterol depletion on the function of adrenergic receptors, using a direct end-point gene expression assay. These results are in agreement with our earlier results with a closely related GPCR, the serotonin$_{1A}$ receptor [29,30]. Interestingly, the β_2-adrenergic receptor and the serotonin$_{1A}$ receptor enjoy ~43% amino acid similarity in the transmembrane region. In fact, it is due to this close similarity, the intronless genomic clone (G-21) encoding the serotonin$_{1A}$ receptor showed cross-hybridization with a full length β-adrenergic receptor probe at reduced stringency [40].

Interestingly, the function of α and β adrenergic receptors is reported to be modulated by cholesterol. It has been recently reported that cholesterol depletion using MβCD increases the α_{1A}-adrenergic receptor-dependent signaling upon ligand stimulation [41]. In addition, cholesterol depletion is reported to enhance β_2-adrenergic receptor-associated signaling, while increased cholesterol content inhibited the signaling [42]. Cholesterol has also been shown to improve the stability of the β_2-adrenergic receptor [43], and appears to be necessary for crystallization of the receptor [44]. The recent crystal structure of the β_2-adrenergic receptor has revealed structural evidence of a specific cholesterol binding site [45]. Importantly, our present results constitute one of the first reports showing the effect of cholesterol on the end-point signaling of the adrenergic receptor.

As mentioned earlier, ~50% of clinically prescribed drugs act as either agonists or antagonists of GPCRs with several ligands of GPCRs among the top 100 globally selling drugs, which points out their immense therapeutic potential [17]. Interestingly, although GPCRs represent major drug targets, only a small fraction of all GPCRs are presently targeted by drugs [46]. This points out the exciting possibility that the receptors which are not recognized yet could be potential drug targets for diseases that are difficult to treat by currently available drugs. GPCRs such as adrenergic receptors are involved in a number of physiological functions and represent important drug targets [47]. Although the pharmacological and signaling features of these receptors have been extensively studied, aspects related to their interaction with membrane lipids have been addressed in very few cases. Progress in deciphering molecular details on the nature of the interaction of adrenergic receptors with cholesterol would enhance our ability to design better therapeutic strategies to combat diseases related to malfunctioning of these receptors.

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References

[1] K. Simons, E. Ikonen, How cells handle cholesterol, Science 290 (2000) 1721–1725.
[2] O.G. Mouritsen, M.J. Zuckermann, What's so special about cholesterol? Lipids 39 (2004) 1101–1113.
[3] S. Mukherjee, F.R. Maxfield, Membrane domains, Annu. Rev. Cell Dev. Biol. 20 (2004) 839–866.
[4] M. Edidin, Shrinking patches and slippery rafts: scales of domains in the plasma membrane, Trends Cell Biol. 11 (2001) 492–496.
[5] S. Munro, Lipid rafts: elusive or illusive? Cell 115 (2003) 377–388.
[6] K. Jacobson, O.G. Mouritsen, R.G.W. Anderson, Lipid rafts: at a crossroad between cell biology and physics, Nat. Cell Biol. 9 (2007) 7–14.
[7] D. Lingwood, K. Simons, Lipid rafts as a membrane-organizing principle, Science 327 (2010) 46–50.
[8] J. Riethmüller, A. Riehle, H. Grassmé, E. Gulbins, Membrane rafts in host–pathogen interactions, Biochim. Biophys. Acta 1758 (2006) 2139–2147.
[9] T.J. Pucadyil, A. Chattopadhyay, Cholesterol: a potential therapeutic target in *Leishmania* infection? Trends Parasitol. 23 (2007) 49–53.
[10] K. Burger, G. Gimpl, F. Fahrenholz, Regulation of receptor function by cholesterol, Cell. Mol. Life Sci. 57 (2000) 1577–1592.
[11] T.J. Pucadyil, A. Chattopadhyay, Role of cholesterol in the function and organization of G-protein coupled receptors, Prog. Lipid Res. 45 (2006) 295–333.
[12] Y.D. Paila, A. Chattopadhyay, Membrane cholesterol in the function and organization of G-protein coupled receptors, Subcell. Biochem. 51 (2010) 439–466.
[13] K.L. Pierce, R.T. Premont, R.J. Lefkowitz, Seven-transmembrane receptors, Nat. Rev. Mol. Cell Biol. 3 (2002) 639–650.
[14] D.M. Rosenbaum, S.G.F. Rasmussen, B.K. Kobilka, The structure and function of G-protein-coupled receptors, Nature 459 (2009) 356–363.

[15] Y. Zhang, M.E. DeVries, J. Skolnick, Structure modeling of all identified G protein-coupled receptors in the human genome, PLoS Comput. Biol. 2 (2006) 88–99.
[16] R. Heilker, M. Wolff, C.S. Tautermann, M. Bieler, G-protein-coupled receptor-focused drug discovery using a target class platform approach, Drug Discov. Today 14 (2009) 231–240.
[17] S. Schlyer, R. Horuk, I want a new drug: G-protein-coupled receptors in drug development, Drug Discov. Today 11 (2006) 481–493.
[18] S. Gupta, B. Das, S. Sen, Cardiac hypertrophy: mechanisms and therapeutic opportunities, Antioxid. Redox Signal. 9 (2007) 623–652.
[19] Y. Lee, A.B. Gustafsson, Role of apoptosis in cardiovascular disease, Apoptosis 14 (2009) 536–548.
[20] C. Communal, K. Singh, D.R. Pimentel, W.S. Colucci, Norepinephrine stimulates apoptosis in adult rat ventricular myocytes by activation of the β-adrenergic pathway, Circulation 98 (1998) 1329–1334.
[21] M.K. Gupta, T.V. Neelakantan, M. Sanghamitra, R.K. Tyagi, A. Dinda, S. Maulik, C.K. Mukhopadhyay, S.K. Goswami, An assessment of the role of reactive oxygen species and redox signaling in norepinephrine-induced apoptosis and hypertrophy of H9c2 cardiac myoblasts, Antioxid. Redox Signal. 8 (2006) 1081–1092.
[22] J. Palomeque, L. Delbridge, M.V. Petroff, Angiotensin II: a regulator of cardiomyocyte function and survival, Front. Biosci. 14 (2009) 5118–5133.
[23] G.W. Dorn, S.B. Liggett, Mechanisms of pharmacogenomic effects of genetic variation within the cardiac adrenergic network in heart failure, Mol. Pharmacol. 76 (2009) 466–480.
[24] J.S. Floras, Sympathetic nervous system activation in human heart failure: clinical implications of an updated model, J. Am. Coll. Cardiol. 54 (2009) 375–385.
[25] M. Bacaner, J. Brietenbucher, J. LaBree, Prevention of ventricular fibrillation, acute myocardial infarction (myocardial necrosis), heart failure, and mortality by bretylium: is ischemic heart disease primarily adrenergic cardiovascular disease? Am. J. Ther. 11 (2004) 366–411.
[26] T.J. Pucadyil, A. Chattopadhyay, Cholesterol modulates the ligand binding and G-protein coupling to serotonin$_{1A}$ receptors from bovine hippocampus, Biochim. Biophys. Acta 1663 (2004) 188–200.
[27] G. Gimpl, V. Wiegand, K. Burger, F. Fahrenholz, Cholesterol and steroid hormones: modulators of oxytocin receptor function, Prog. Brain Res. 139 (2002) 43–55.
[28] P.M. Burch, Z. Yuan, A. Loonen, N.H. Heintz, An extracellular signal-regulated kinase 1- and 2-dependent program of chromatin trafficking of c-Fos and Fra-1 is required for cyclin D1 expression during cell cycle reentry, Mol. Cell. Biol. 24 (2004) 4696–4709.
[29] T.J. Pucadyil, A. Chattopadhyay, Cholesterol depletion induces dynamic confinement of the G-protein coupled serotonin$_{1A}$ receptor in the plasma membrane of living cells, Biochim. Biophys. Acta 1768 (2007) 655–668.
[30] R. Prasad, Y.D. Paila, Md. Jafurulla, A. Chattopadhyay, Membrane cholesterol depletion from live cells enhances the function of human serotonin$_{1A}$ receptors, Biochem. Biophys. Res. Commun. 389 (2009) 333–337.
[31] S. Shrivastava, T.J. Pucadyil, Y.D. Paila, S. Ganguly, A. Chattopadhyay, Chronic cholesterol depletion using statin impairs the function and dynamics of human serotonin$_{1A}$ receptors, Biochemistry 49 (2010) 5426–5435.
[32] D.M. Amundson, M. Zhou, Fluorometric method for the enzymatic determination of cholesterol, J. Biochem. Biophys. Methods 38 (1999) 43–52.
[33] R. Zidovetzki, I. Levitan, Use of cyclodextrins to manipulate plasma membrane cholesterol content: evidence, misconceptions and control strategies, Biochim. Biophys. Acta 1768 (2007) 1311–1324.
[34] A. Woehler, E.G. Ponimaskin, G protein-mediated signaling: same receptor, multiple effectors, Curr. Mol. Pharmacol. 2 (2009) 237–248.
[35] G.M. Kuster, D.R. Pimentel, T. Adachi, Y. Ido, D.A. Brenner, R.A. Cohen, R. Liao, D.A. Siwik, W.S. Colucci, Alpha-adrenergic receptor-stimulated hypertrophy in adult rat ventricular myocytes is mediated via thioredoxin-1-sensitive oxidative modification of thiols on Ras, Circulation 111 (2005) 1192–1198.
[36] Y. Wang, V. De Arcangelis, X. Gao, B. Ramani, Y.S. Jung, Y. Xiang, Norepinephrine- and epinephrine-induced distinct β_2-adrenoceptor signaling is dictated by GRK2 phosphorylation in cardiomyocytes, J. Biol. Chem. 283 (2008) 1799–1807.
[37] Y.D. Paila, A. Chattopadhyay, The function of G-protein coupled receptors and membrane cholesterol: specific or general interaction ? Glycoconj. J. 26 (2009) 711–720.
[38] Y.D. Paila, S. Tiwari, A. Chattopadhyay, Are specific nonannular cholesterol binding sites present in G-protein coupled receptors ? Biochim. Biophys. Acta 1788 (2009) 295–302.
[39] H. Ohvo-Rekila, B. Ramstedt, P. Leppimaki, J.P. Slotte, Cholesterol interactions with phospholipids in membranes, Prog. Lipid Res. 41 (2002) 66–97.
[40] T.J. Pucadyil, S. Kalipatnapu, A. Chattopadhyay, The serotonin$_{1A}$ receptor: a representative member of the serotonin receptor family, Cell. Mol. Neurobiol. 25 (2005) 553–580.
[41] B. Lei, D.P. Morris, M.P. Smith, D.A. Schwinn, Lipid rafts constrain basal alpha(1A)-adrenergic receptor signaling by maintaining receptor in an inactive conformation, Cell. Signal. 21 (2009) 1532–1539.
[42] S.M. Pontier, Y. Percherancier, S. Galandrin, A. Breit, C. Galés, M. Bouvier, Cholesterol-dependent separation of the β_2-adrenergic receptor from its partners determines signaling efficacy: insight into nanoscale organization of signal transduction, J. Biol. Chem. 283 (2008) 24659–24672.
[43] Z. Yao, B. Kobilka, Using synthetic lipids to stabilize purified β_2 adrenoceptor in detergent micelles, Anal. Biochem. 343 (2005) 344–346.
[44] V. Cherezov, D.M. Rosenbaum, M.A. Hanson, S.G.F. Rasmussen, F.S. Thian, T.S. Kobilka, H.-J. Choi, P. Kuhn, W.I. Weis, B.K. Kobilka, R.C. Stevens, High-resolution crystal structure of an engineered human β_2-adrenergic G protein-coupled receptor, Science 318 (2007) 1258–1265.
[45] M.A. Hanson, V. Cherezov, M.T. Griffith, C.B. Roth, V.-P. Jaakola, E.Y.T. Chien, J. Velasquez, P. Kuhn, R.C. Stevens, A specific cholesterol binding site is established by the 2.8 Å structure of the human β_2-adrenergic receptor, Structure 16 (2008) 897–905.
[46] S.H. Lin, O. Civelli, Orphan G protein-coupled receptors: targets for new therapeutic interventions, Ann. Med. 36 (2004) 204–214.
[47] C.A. Taira, A. Carranza, M. Mayer, C. Di Verniero, J.A. Opezzo, C. Höcht, Therapeutic implications of β-adrenergic receptor pharmacodynamic properties, Curr. Clin. Pharmacol. 3 (2008) 174–184.