Biochemical and Biophysical Research Communications 458 (2015) 531–535

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Coupling of β_1-adrenergic receptor to type 5 adenylyl cyclase and its physiological relevance in cardiac myocytes

Takashi Tsunematsu [a], Satoshi Okumura [a, b, *], Yasumasa Mototani [b], Yoshiki Ohnuki [b], Huiling Jin [a], Wenqian Cai [a], Kenji Suita [a], Itaru Sato [a], Masanari Umemura [a], Utako Yokoyama [a], Motohiko Sato [a, c], Takayuki Fujita [a], Yoshihiro Ishikawa [a, **]

[a] Cardiovascular Research Institute, Yokohama City University Graduate School of Medicine, Yokohama 236-0004, Japan
[b] Department of Physiology, Tsurumi University School of Dental Medicine, Yokohama 230-8501, Japan
[c] Department of Physiology, Aichi Medical University, Nagakute, 480-1195, Japan

ARTICLE INFO

Article history:
Received 27 December 2014
Available online 9 February 2015

Keywords:
β-receptor
Adenylyl cyclase
Heart
Cyclic AMP
Apoptosis

ABSTRACT

Myocardial β-adrenergic receptor (β-AR) β_1- and β_2-subtypes are highly homologous, but play opposite roles in cardiac apoptosis and heart failure, as do cardiac adenylyl cyclase (AC) subtypes 5 (AC5) and 6 (AC6): β_1-AR and AC5 promote cardiac remodeling, while β_2-AR and AC6 activate cell survival pathways. However, the mechanisms involved remain poorly understood. We hypothesized that AC5 is coupled preferentially to β_1-AR rather than β_2-AR, and we examined this idea by means of pharmacological and genetic approaches. We found that selective inhibition of AC5 with 2′5′-dideoxyadenosine significantly suppressed cAMP accumulation and cardiac apoptosis induced by selective β_1-AR stimulation, but had no effect on cAMP accumulation and cardiac apoptosis in response to selective β_2-AR stimulation. The results of selective stimulation of β_1-AR and β_2-AR in neonatal cardiac myocytes prepared from wild-type and AC5-knockout mice were also consistent with the idea that β_1-AR selectively couples with AC5. We believe these results are helpful for understanding the mechanisms underlying the different roles of AR subtypes in healthy and diseased hearts.

1. Introduction

Myocardial β-adrenergic receptors (β-AR) show developmentally specific subtype expression: for example, in rats, β_1-AR is the predominant adult isoforms (β_1 vs. β_2 59% vs. 41%), whereas β_2-AR is more highly expressed in the neonate (β_1 vs. β_2 36% vs. 64%) [1]. Thus, in adults under normal physiological conditions, catecholamine actions are mediated predominantly by β_1-AR acting via the Gsα-adenylyl cyclase (AC) pathway; on the other hand, the contribution of β_2-AR to catecholamine responsiveness is most prominent in neonatal ventricle, which lacks sympathetic innervation, and in failing/aged hearts, in which β_1-AR is selectively down-regulated. More importantly, chronic stimulation of β_1-AR and β_2-AR elicits opposing effects on cardiac myocytes [2]. Chronic β_1-AR stimulation by elevated plasma catecholamines and subsequent activation of the Gsα-AC-cyclic AMP (cAMP)-dependent signal transduction pathway play a crucial role in the development of heart failure [1,3]. Conversely, β_2-AR couples concurrently to Gsα and Giα, and activates cell survival pathways. Over the past decade, compelling evidence has accumulated that β_2-AR-Giα mediates a powerful cell survival pathway through activation of phosphatidylinositol 3-kinase (PI3-K)/Akt signaling in the heart [2,4–6]. However, the role of β_2-AR-Gsα in cell survival remains poorly understood.

AC is a membrane-bound enzyme that catalyzes the conversion of ATP to cAMP [7,8]. At least 10 isoforms are known [7,9,10], of which 7 are expressed in the heart, although type 5 (AC5) and type 6 (AC6) are the major AC isoforms in the heart [8,11]. Both are calcium (Ca^{2+})- and Gi-inhibitable and share most, if not all, of their biochemical properties [7,9,12]. AC5 was shown to be an adult isoform, whereas AC6 is more highly expressed in the neonate in

* Corresponding author. Department of Physiology, Tsurumi University School of Dental Medicine, 2-1-2 Tsurumi, Tsurumi-ku, Yokohama 230-8501, Japan. Fax: +81 45 585 2889.
** Corresponding author. Cardiovascular Research Institute, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan. Fax: +81 45 788 1470.
E-mail addresses: okumura-s@tsuruni-u.ac.jp (S. Okumura), yishikaw@yokohama-cu.ac.jp (Y. Ishikawa).

http://dx.doi.org/10.1016/j.bbrc.2015.01.149
0006-291X/

rats [1,8,13,14]. We have previously demonstrated that disruption of AC5 did not alter the expression of β-AR/Gsα/AC/protein kinase A, but significantly inhibited both myocyte apoptosis and development of heart failure in response to chronic catecholamine or pressure-overload stress [11,15]. Conversely, disruption of AC6 promoted the development of myocyte apoptosis and heart failure in response to chronic catecholamine or pressure-overload stress [16–18].

Considering these findings, together with the facts that chronic β_1-AR stimulation plays an important role in the pathogenesis of heart failure, while chronic β_2 stimulation promotes cell survival, in addition to activating PI3-K/Akt signaling via Gi [5,6,19], we hypothesized that β_1-AR couples preferentially to AC5. Here, we examined this idea by means of both pharmacological and genetic studies in cardiac myocytes.

2. Materials and methods

2.1. Reagents

All chemicals were purchased from Sigma–Aldrich, except trypsin 1:250 (Difco), ITS (insulin-transferrin-selenium; GIBCO), [^{3}H]adenine (GE Healthcare), trichloroacetic acid (Wako) and 4′,6-diamidino-2-phenylindole,dihydrochloride (DAPI; Molecular Probes).

2.2. Myocyte preparation

Primary cultures of neonatal mouse cardiomyocytes were prepared from the heart of a 1-day-old mouse, as described previously with some modifications [20,21]. Briefly, cardiomyocytes were obtained by trypsinization and collagenization, and maintained at 37 °C in humidified air containing with 5% CO_2. To reduce the number of contaminating non-myocytes, dissociated cells were preplated on 100-mm culture dishes in minimum essential medium with 10% fetal bovine serum (FBS) containing 1% penicillin-streptomycin for 2 h. The nonattached cardiomyocyte-rich fraction was plated on plastic dishes. The culture medium was changed 24 h after seeding to minimum essential medium containing ITS with 1% penicillin-streptomycin.

2.3. [^{3}H]adenine labeling and cAMP accumulation assay

Assay of cAMP accumulation assays in neonatal myocytes was performed with [^{3}H]adenine as described previously with some modifications [21,22]. Briefly, the cells were incubated with [^{3}H]adenine (3 μCi/well) for 24 h in humidified air containing 5% CO_2. Cells were washed three times with 20 mM HEPES-balanced serum-free minimum essential medium and incubated for 20 min at 37 °C, then pretreated with the same medium containing 0.5 mM IBMX with/without 10^{-7} M ICI18.551/10^{-7} M CGP20712A for 15 min at room temperature (RT). Reactions were started by the addition of isoproterenol (ISO) with/without dd-Ado (5 μM) for 5 min at RT and terminated by the addition of 12% (w/v) trichloroacetic acid, 0.25 mM ATP, and 0.25 mM cAMP. [^{3}H]ATP and [^{3}H]cAMP were separated on acidic alumina as described previously [23]. The cAMP production was calculated as [^{3}H]cAMP/([^{3}H]cAMP+[^{3}H]ATP) × 10^4.

2.4. Terminal transferase dUTP nick endlabeling (TUNEL) staining

In situ labeling of fragmented DNA in cardiomyocytes was performed with a TACS 2-Tdt Blue Apotosis Detection kit (Trevigen, Inc.) according to the manufacturer's instructions, as described previously by us and other groups [21,24,25].

2.5. Statistical analysis

Data were expressed as means ± SEM. The statistical significances of differences in cAMP accumulation in cardiac myocytes (Figs. 1 and 3A) and TUNNEL-positive cardiac myocytes (Figs. 2 and 3B) was determined by one-way ANOVA with Tukey's test. The criterion of significance was taken as $P < 0.05$.

3. Results

3.1. Effect of ICI118.551 or CGP20712A on ISO-promoted cAMP accumulation

We first examined the effects of ISO (10^{-8} to 10^{-6} M) on cAMP accumulation in neonatal cardiac myocytes in the presence and absence of 2′5′-dideoxyadenosine (dd-Ado; 5 μM), a specific AC5 inhibitor [21]. cAMP accumulation was significantly increased from baseline by ISO (10^{-7} or 10^{-6} M), but the magnitude of the increase was significantly suppressed (by approximately 29%) by dd-Ado (Fig. 1A).

We next examined the effect of pretreatment with ICI118.551 (10^{-7} M), a β_2-selective antagonist, on the ISO-promoted cAMP accumulations in neonatal cardiac myocytes (Fig. 1B). Under these conditions (β_1-selective stimulation), cAMP accumulation was significantly increased from baseline by ISO, and after ICI118.551 pretreatment the increase was approximately two-thirds of that in the absence of ICI118.551. The magnitude of the increase was significantly smaller (by approximately 39%) in the presence of dd-Ado.

We also examined the effect of pretreatment of CGP20712A (10^{-7} M), a β_1-selective antagonist, on the ISO-promote cAMP accumulation (Fig. 1C). Under these conditions (β_2-selective stimulation), cAMP accumulation was significantly increased from baseline by ISO (10^{-6} M), but the increase after CGP20712A pretreatment was only approximately one-third of that in the absence of CGP20712A (compare Fig. 1A). In this case, dd-Ado had no effect on the magnitude of the increase.

These data indicate that ISO-promoted cAMP accumulation is predominantly mediated by β_1-AR.

3.2. Effect of ICI118.551 or CGP20712A on ISO-mediated apoptosis of cardiac myocytes

ISO is known to induce cardiac apoptosis through the activation of β-AR, but the downstream regulatory mechanisms remains poorly understood [5,8,11,21,25]. We thus examined the mechanism of ISO-mediated cardiac apoptosis.

We first examined the effect of subtype-specific stimulation of β-AR on ISO-mediated cardiac apoptosis (Fig. 2). Cardiac apoptosis was significantly increased by approximately 1.5-fold from baseline by ISO (Baseline vs. ISO 14 ± 0.9 vs. 23 ± 0.2%, $P < 0.01$, $n = 4$). Pretreatment with ICI118.551 (10^{-7} M) did not significantly reduce ISO-mediated cardiac apoptosis (21 ± 0.8%, $n = 4$), whereas pretreatment with CGP20712A (10^{-7} M) completely suppressed it (11 ± 1.3%, $P < 0.01$ vs. ISO, $n = 4$). These data indicated that ISO-mediated cardiac apoptosis is mediated by activation of β_1-AR.

3.3. β_1-AR preferentially associates with AC5

We previously demonstrated that knockout of AC5 (AC5KO) decreased total AC activity by 30–40% in the mouse heart, but AC5KO was less susceptible to stresses, such as chronic ISO infusion or chronic pressure overload [8,11,12,21]. To confirm the results of pharmacological inhibition of AC5 with dd-Ado in cardiac myocytes (Fig. 1), we employed cardiac myocytes prepared from AC5KO.

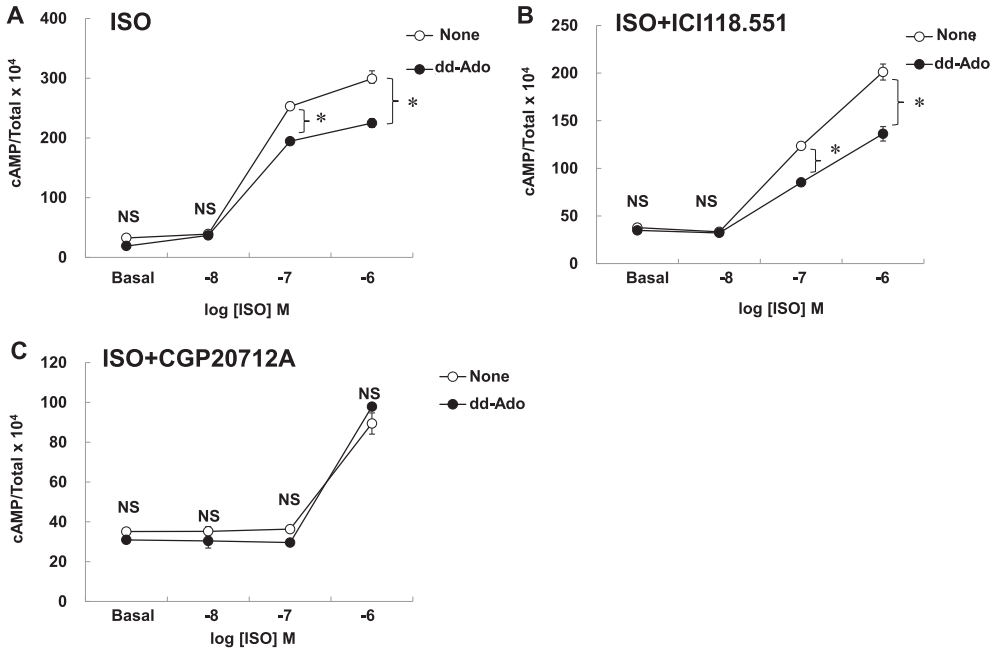

Fig. 1. Effects of AC5 inhibitor dd-Ado on cAMP accumulation in response to subtype-selective β-AR stimulation in cardiomyocytes. cAMP accumulation in neonatal cardiac myocytes treated with ISO (non-selective β-AR subtype stimulation) (A), ISO + ICI118.551 (10^{-7} M) (β_1-AR subtype selective stimulation) (B), or ISO + CGP20712A (10^{-7} M) (β_2-AR subtype selective stimulation) (C) was evaluated in the presence and absence of dd-Ado (5 μM), a selective AC5 inhibitor. cAMP accumulation induced by ISO (10^{-7} or 10^{-6} M) or ISO (10^{-7} or 10^{-6}) + ICI118.551 (10^{-7} M) was significantly decreased by dd-Ado, but no inhibitory effect was observed in the case of ISO + CGP20712A (*$P < 0.01$, n = 5-6).

We first examined the effects of ISO (10^{-6} M) on cAMP accumulation in neonatal cardiac myocytes prepared from WT and AC5KO. cAMP accumulation was increased from baseline by ISO in both WT and AC5KO myocytes, but the increase was significantly suppressed in AC5KO myocytes (by approximately 26% versus WT, $P < 0.01$).

We next examined the effects of ICI118.551 (10^{-7} M) and CGP20712A (10^{-7} M) on ISO (10^{-6} M)-promoted cAMP accumulation in cardiac myocytes. cAMP accumulation was reduced by approximately 26% and 43% by ICI118.551 pretreatment in WT and AC5KO myocytes, respectively. However, CGP20712A pretreatment reduced cAMP accumulation to the baseline level (Fig. 3A).

We also examined the effects of ISO on cardiac apoptosis in WT and AC5KO myocytes by TUNEL staining. The number of TUNEL-positive cardiac myocytes was increased by approximately 1.6-fold from baseline in WT myocytes, but this increase was blocked in AC5KO myocytes, in the presence or absence of ICI118.551 or CGP20712A (Fig. 3B).

Since AC5 is known to play an important role in cardiac apoptosis [8,11,21], these data support the idea that β_1-AR is coupled preferentially to AC5.

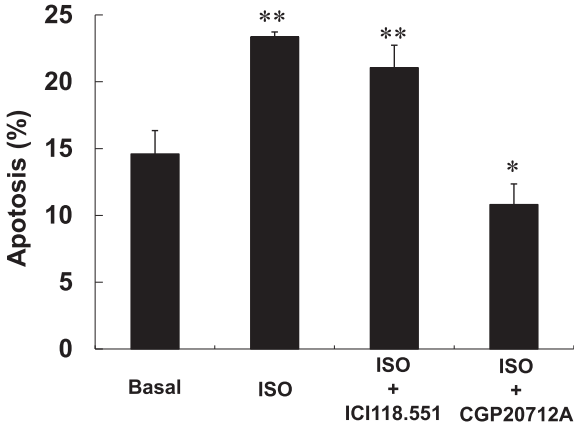

Fig. 2. Apoptosis induced by subtype-selective β-AR stimulation. Induction of TUNEL-positive cardiomyocytes in response to subtype-selective β-AR stimulation: ISO (non-selective β-AR subtype stimulation), ISO (10^{-6} M) + ICI118.551 (10^{-7} M) (β_1-AR subtype selective stimulation) or ISO (10^{-6} M) + CGP20712A (10^{-7} M) (β_2-AR subtype selective stimulation) (*$P < 0.05$, **$P < 0.01$, $n = 4$).

4. Discussion

In order to examine the mechanism underlying the opposing effects of β_1-AR and β_2-AR on cardiac myocytes [2], we adopted two approaches. Firstly, we examined the effects of dd-Ado, a specific AC5 inhibitor [21] on cAMP accumulation and cardiac apoptosis induced by ISO under conditions of selective β_1-AR or β_2-AR stimulation. Secondly, we examined cAMP accumulation and cardiac apoptosis using cardiac myocytes from AC5KO under conditions of selective β_1-AR or β_2-AR stimulation. Both approaches indicated that cAMP accumulation and cardiac apoptosis in response to selective β_1-AR stimulation were significantly suppressed by inhibition or knockout of AC5, whereas no decrease was observed in the case of selective β_2-AR stimulation. These results are consistent with our hypothesis that β_1-AR associates preferentially with AC5.

Caveolin-3, a major subtype in the heart, acts as a scaffolding protein by direct interaction with and modulation of the activity of G-protein-coupled receptor signaling components [26]. We and other groups have reported colocalization of caveolin-3 with G-protein-coupled receptor signaling components including β_1-AR, β_2-AR, and AC5/6 in cardiac myocytes [27–29]. However, caveolin-3 is distributed in both surface sarcolemma and long membrane invaginations known as transverse tubules (t-tubules) in cardiac myocytes [30]. More recently, subtype-specific subcellular distribution of β-AR and cardiac AC isoforms (AC5/6) within the plasma membrane was demonstrated by means of electrophysiological techniques: AC5 is localized mainly at t-tubules and AC6 is localized

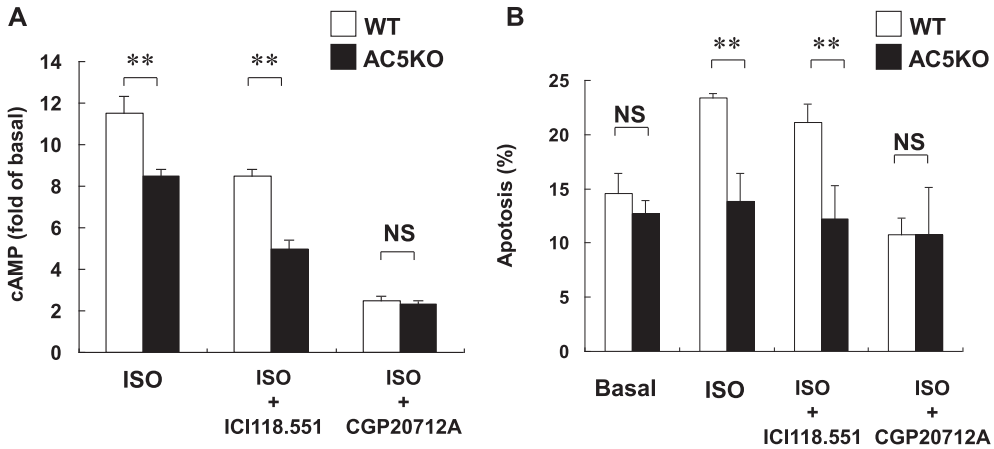

Fig. 3. cAMP accumulation and apoptosis in AC5KO myocytes in response to subtype-selective β-AR stimulation. (A) cAMP accumulation in neonatal cardiac myocytes was compared between AC5KO and WT myocytes treated with ISO (10^{-6} M) (non-selective β-AR subtype stimulation), ISO (10^{-6} M) + ICI118.551 (10^{-7} M) (β_1-AR subtype selective stimulation), or ISO (10^{-6} M) + CGP20712A (10^{-7} M) (β_2-AR subtype selective stimulation). $^{**}P < 0.01$, $n = 4$. (B) Induction of apoptosis in AC5KO and WT myocytes was evaluated by TUNEL staining. The number of TUNEL-positive cardiac myocytes was increased 1.6-fold from baseline in ISO-treated WT myocytes, but the increase was suppressed in AC5KO myocytes, independently of the presence or absence of ICI118.551 or CGP20712A. $^{**}P < 0.01$, *NS*, not significant, $n = 4$–6.

at surface sarcolemma, whereas β_1-AR is localized at t-tubules and surface sarcolemma and β_2-AR is localized only at surface sarcolemma [31,32]. These findings, together with the data obtained in the present study, may suggest that β_1-AR preferentially couples with AC5 at t-tubules, while β_2-AR might preferentially couple with AC6 at surface sarcolemma, and these subtype-specific couplings of β-AR and cardiac AC isoforms might account for opposing effects of β_1/β_2-AR and AC5/6 on cardiac myocytes [2]. Our present findings represent the first evidence in support of this explanation of the differential regulation and functionality of β-AR subtypes in healthy and diseased hearts.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This study was supported in part by grants from the Ministry of Health, Labor and Welfare (Dr. Ishikawa), a Grant-in-Aid for Scientific Research on Innovative Areas (22136009), and grants from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (Drs. Ishikawa (24390200), Okumura (23591087), Fujita (25460296), Sato M (24590280), Yokoyama (25293236), Mototani (22791147)), National Cerebral and Cardiovascular Center 22-2-3 (Dr. Ishikawa), Takeda Science Foundation (Dr. Okumura), Yokohama Foundation for Advancement of Medical Science (Dr. Okumura), Yokohama Academic Foundation (Dr. Ohnuki), Research Foundation for Community Medicine (Dr. Okumura), and Suzuken Memorial Foundation 14-014 (Dr. Okumura).

References

[1] C. Morisco, D.C. Zebrowski, D.E. Vatner, S.F. Vatner, J. Sadoshima, β-adrenergic cardiac hypertrophy is mediated primarily by the β_1-subtype in the rat heart, J. Mol. Cell. Cardiol. 33 (2001) 561–573.
[2] M. Zheng, W. Zhu, Q. Han, R.P. Xiao, Emerging concepts and therapeutic implications of β-adrenergic receptor subtype signaling, Pharmacol. Ther. 108 (2005) 257–268.
[3] G. Lewin, M. Matus, A. Basu, K. Frebel, S.P. Rohsbach, A. Safronenko, M.D. Seidl, F. Stumpel, I. Buchwalow, S. Konig, S. Engelhardt, M.J. Lohse, W. Schmitz, F.U. Muller, Critical role of transcription factor cyclic AMP response element modulator in β_1-adrenoceptor-mediated cardiac dysfunction, Circulation 119 (2009) 79–88.
[4] G. Fajardo, M. Zhao, T. Urashima, S. Farahani, D.Q. Hu, S. Reddy, D. Bernstein, Deletion of the β_2-adrenergic receptor prevents the development of cardiomyopathy in mice, J. Mol. Cell. Cardiol. 63 (2013) 155–164.
[5] Y. Ohnuki, D. Umeki, Y. Mototani, H. Jin, W. Cai, K. Shiozawa, K. Suita, Y. Saeki, T. Fujita, Y. Ishikawa, S. Okumura, Role of cyclic amp sensor Epac1 in masseter muscle hypertrophy and myosin heavy chain transition induced by β_2-adrenoceptor stimulation, J. Physiol. 592 (2014) 5461–5475.
[6] D. Umeki, Y. Ohnuki, Y. Mototani, K. Shiozawa, T. Fujita, Y. Nakamura, Y. Saeki, S. Okumura, Effects of chronic Akt/mTOR inhibition by rapamycin on mechanical overload-induced hypertrophy and myosin heavy chain transition in masseter muscle, J. Pharmacol. Sci. 122 (2013) 278–288.
[7] Y. Ishikawa, C.J. Homcy, The adenylyl cyclases as integrators of transmembrane signal transduction, Circ. Res. 80 (1997) 297–304.
[8] S. Okumura, S. Suzuki, Y. Ishikawa, New aspects for the treatment of cardiac diseases based on the diversity of functional controls on cardiac muscles: effects of targeted disruption of the type 5 adenylyl cyclase gene, J. Pharmacol. Sci. 109 (2009) 354–359.
[9] J. Hanoune, N. Defer, Regulation and role of adenylyl cyclase isoforms, Annu Rev. Pharmacol. Toxicol. 41 (2001) 145–174.
[10] C. Steegborn, Structure, mechanism, and regulation of soluble adenylyl cyclases - similarities and differences to transmembrane adenylyl cyclases, Biochim. Biophys. Acta 1842 (2014) 2535–2547.
[11] S. Okumura, G. Takagi, J. Kawabe, G. Yang, M.C. Lee, C. Hong, J. Liu, D.E. Vatner, J. Sadoshima, S.F. Vatner, Y. Ishikawa, Disruption of type 5 adenylyl cyclase gene preserves cardiac function against pressure overload, Proc. Natl. Acad. Sci. U S A 100 (2003) 9986–9990.
[12] S. Okumura, J. Kawabe, A. Yatani, G. Takagi, M.C. Lee, C. Hong, J. Liu, I. Takagi, J. Sadoshima, D.E. Vatner, S.F. Vatner, Y. Ishikawa, Type 5 adenylyl cyclase disruption alters not only sympathetic but also parasympathetic and calcium-mediated cardiac regulation, Circ. Res. 93 (2003) 364–371.
[13] K. Tobise, Y. Ishikawa, S.R. Holmer, M.J. Im, J.B. Newell, H. Yoshie, M. Fujita, E.E. Susannie, C.J. Homcy, Changes in type VI adenylyl cyclase isoform expression correlate with a decreased capacity for cAMP generation in the aging ventricle, Circ. Res. 74 (1994) 596–603.
[14] I. Espinasse, V. Iourgenko, N. Defer, F. Samson, J. Hanoune, J.J. Mercadier, Type V, but not type VI, adenylyl cyclase mRNA accumulates in the rat heart during ontogenic development. Correlation with increased global adenylyl cyclase activity, J. Mol. Cell. Cardiol. 27 (1995) 1789–1795.
[15] S. Okumura, D.E. Vatner, R. Kurotani, Y. Bai, S. Gao, Z. Yuan, K. Iwatsubo, C. Ulucan, J. Kawabe, K. Ghosh, S.F. Vatner, Y. Ishikawa, Disruption of type 5 adenylyl cyclase enhances desensitization of cyclic adenosine monophosphate signal and increases Akt signal with chronic catecholamine stress, Circulation 116 (2007) 1776–1783.
[16] T. Tang, M.H. Gao, N.C. Lai, A.L. Firth, T. Takahashi, T. Guo, J.X. Yuan, D.M. Roth, H.K. Hammond, Adenylyl cyclase type 6 deletion decreases left ventricular function via impaired calcium handling, Circulation 117 (2008) 61–69.
[17] T. Tang, N.C. Lai, H.K. Hammond, D.M. Roth, Y. Yang, T. Guo, M.H. Gao, Adenylyl cyclase 6 deletion reduces left ventricular hypertrophy, dilation, dysfunction, and fibrosis in pressure-overloaded female mice, J. Am. Coll. Cardiol. 55 (2010) 1476–1486.
[18] T. Tang, N.C. Lai, A.T. Wright, M.H. Gao, P. Lee, T. Guo, R. Tang, A.D. McCulloch, H.K. Hammond, Adenylyl cyclase 6 deletion increases mortality during sustained β-adrenergic receptor stimulation, J. Mol. Cell. Cardiol. 60 (2013) 60–67.

[19] Y. Ohnuki, D. Umeki, W. Cai, N. Kawai, Y. Mototani, K. Shiozawa, H.L. Jin, T. Fujita, E. Tanaka, Y. Saeki, S. Okumura, Role of masseter muscle β_2-adrenergic signaling in regulation of muscle activity, myosin heavy chain transition, and hypertrophy, J. Pharmacol. Sci. 123 (2013) 36–46.
[20] N. Nyui, K. Tamura, K. Mizuno, T. Ishigami, K. Hibi, M. Yabana, M. Kihara, A. Fukamizu, H. Ochiai, S. Umemura, K. Murakami, S. Ohno, M. Ishii, Stretch-induced MAP kinase activation in cardiomyocytes of angiotensinogen-deficient mice, Biochem. Biophys. Res. Commun. 235 (1997) 36–41.
[21] K. Iwatsubo, S. Minamisawa, T. Tsunematsu, M. Nakagome, Y. Toya, J.E. Tomlinson, S. Umemura, R.M. Scarborough, D.E. Levy, Y. Ishikawa, Direct inhibition of type 5 adenylyl cyclase prevents myocardial apoptosis without functional deterioration, J. Biol. Chem. 279 (2004) 40938–40945.
[22] Y. Toya, C. Schwencke, Y. Ishikawa, Forskolin derivatives with increased selectivity for cardiac adenylyl cyclase, J. Mol. Cell. Cardiol. 30 (1998) 97–108.
[23] T. Onda, Y. Hashimoto, M. Nagai, H. Kuramochi, S. Saito, H. Yamazaki, Y. Toya, I. Sakai, C.J. Homcy, K. Nishikawa, Y. Ishikawa, Type-specific regulation of adenylyl cyclase. Selective pharmacological stimulation and inhibition of adenylyl cyclase isoforms, J. Biol. Chem. 276 (2001) 47785–47793.
[24] X. Long, M.T. Crow, S.J. Sollott, L. O'Neill, D.S. Menees, M. de Lourdes Hipolito, M.O. Boluyt, T. Asai, E.G. Lakatta, Enhanced expression of p53 and apoptosis induced by blockade of the vacuolar proton ATPase in cardiomyocytes, J. Clin. Invest. 101 (1998) 1453–1461.
[25] S. Okumura, T. Fujita, W. Cai, M. Jin, I. Namekata, Y. Mototani, H. Jin, Y. Ohnuki, Y. Tsuneoka, R. Kurotani, K. Suita, Y. Kawakami, S. Hamaguchi, T. Abe, H. Kiyonari, T. Tsunematsu, Y. Bai, S. Suzuki, Y. Hidaka, M. Umemura, Y. Ichikawa, U. Yokoyama, M. Sato, F. Ishikawa, H. Izumi-Nakaseko, S. Adachi-Akahane, H. Tanaka, Y. Ishikawa, *Epac1*-dependent phospholamban phosphorylation mediates the cardiac response to stresses, J. Clin. Invest. 124 (2014) 2785–2801.
[26] C. Schwencke, R.C. Braun-Dullaeus, C. Wunderlich, R.H. Strasser, Caveolae and caveolin in transmembrane signaling: implications for human disease, Cardiovasc Res. 70 (2006) 42–49.
[27] C. Schwencke, S. Okumura, M. Yamamoto, Y.J. Geng, Y. Ishikawa, Colocalization of β-adrenergic receptors and caveolin within the plasma membrane, J. Cell. Biochem. 75 (1999) 64–72.
[28] C. Schwencke, M. Yamamoto, S. Okumura, Y. Toya, S.J. Kim, Y. Ishikawa, Compartmentation of cyclic adenosine 3′,5′-monophosphate signaling in caveolae, Mol. Endocrinol. 13 (1999) 1061–1070.
[29] R.S. Ostrom, J.D. Violin, S. Coleman, P.A. Insel, Selective enhancement of β-adrenergic receptor signaling by overexpression of adenylyl cyclase type 6: colocalization of receptor and adenylyl cyclase in caveolae of cardiac myocytes, Mol. Pharmacol. 57 (2000) 1075–1079.
[30] B.P. Head, H.H. Patel, D.M. Roth, N.C. Lai, I.R. Niesman, M.G. Farquhar, P.A. Insel, G-protein-coupled receptor signaling components localize in both sarcolemmal and intracellular caveolin-3-associated microdomains in adult cardiac myocytes, J. Biol. Chem. 280 (2005) 31036–31044.
[31] C. Cros, F. Brette, Functional subcellular distribution of β_1- and β_2-adrenergic receptors in rat ventricular cardiac myocytes, Physiol. Rep. (2013) e00038.
[32] V. Timofeyev, R.E. Myers, H.J. Kim, R.L. Woltz, P. Sirish, J.P. Heiserman, N. Li, A. Singapuri, T. Tang, V. Yarov-Yarovoy, E.N. Yamoah, H.K. Hammond, N. Chiamvimonvat, Adenylyl cyclase subtype-specific compartmentalization: differential regulation of L-type Ca^{2+} current in ventricular myocytes, Circ. Res. 112 (2013) 1567–1576.