Communication

Cardiac-specific Overexpression of the \( \alpha_1 \) Subunit of the L-type Voltage-dependent \( \text{Ca}^{2+} \) Channel in Transgenic Mice

LOSS OF ISOPROTERENOL-INDUCED CONTRACTION*

(Received for publication, June 8, 1999)

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The L-type voltage-dependent calcium channel (L-VDCC) regulates calcium influx in cardiac myocytes. Activation of the \( \beta \)-adrenergic receptor (\( \beta \)AR) pathway causes phosphorylation of the L-VDCC and that in turn increases \( \text{Ca}^{2+} \) influx. Targeted expression of the L-VDCC \( \alpha_1 \) subunit in transgenic (Tg) mouse ventricles resulted in marked blunting of the \( \beta \)AR pathway. Inotropic and lusitropic responses to isoproterenol and forskolin in Tg hearts were significantly reduced. Likewise, \( \text{Ca}^{2+} \) current augmentation induced by isoproterenol and forskolin was markedly depressed in Tg cardiomycocytes. Despite no change in \( \beta \)AR number, isoproterenol-stimulated adenylyl cyclase activity was absent in Tg membranes and NaF- and forskolin responses were reduced. We postulate an important pathway for regulation of the \( \beta \)AR by \( \text{Ca}^{2+} \) channels.

The cardiac L-type voltage-dependent \( \text{Ca}^{2+} \) channel (L-VDCC) is a large glycoprotein complex consisting of \( \alpha_1, \beta, \) and \( \alpha_2/\beta \) subunits. The \( \alpha_1 \) subunit serves as the \( \text{Ca}^{2+} \) conducting pore, while the \( \beta \) and \( \alpha_2/\beta \) subunits are auxiliary and modulate the activity of the \( \alpha_1 \) subunit (1, 2). The level of intracellular \( \text{Ca}^{2+} \) is a determinant of cardiac function. Calcium is not essential for contraction but also for various enzymatic reactions including activation of proteases, phosphatases, kinases, signal transduction cascades, and regulation of gene transcription (3–6).

\( \beta \)-Adrenergic receptor (\( \beta_1 \)AR, \( \beta_2 \)AR) activation regulates the L-VDCC by phosphorylating the \( \alpha_1 \) subunit, thereby causing an increase in \( \text{Ca}^{2+} \) influx (7). This forward signal is via \( \text{CaM} \)-dependent protein kinase A phosphorylation of the channel (7). Other non-cAMP-dependent protein kinase-dependent mechanisms have also been proposed (8). However, there is little or no information regarding possible \( \text{Ca}^{2+} \)-dependent regulation of the \( \beta \)AR signal transduction pathway via the L-VDCC.

In order to investigate possible reciprocal regulation between these pathways in \textit{vivo}, we overexpressed the \( \alpha_1 \) subunit of the \( \text{Ca}^{2+} \) channel in hearts of Tg mice and studied channel activity and the \( \beta \)AR-G protein cascade by physiological and biochemical techniques. We found a major change in cardiac function regulated by the \( \beta \)AR pathway and conclude that \( \text{Ca}^{2+} \) is derived from channel influx modules \( \beta \)AR activity.

EXPERIMENTAL PROCEDURES

Generation of Transgenic Mice—The overexpression construct was generated by ligating the full-length human L-VDCC \( \alpha_1c \) subunit (9) coding sequence to the \( \alpha \)-myosin heavy chain (\( \alpha \)-MHC) promoter (clone 26) (10) and completed with a bovine growth hormone poly(A)-adenylation signal (Fig. 1A). The \( \alpha \)-MHC-L-VDCC construct was cleaved with NotI and the \( \alpha \)-MHC-L-VDCC fusion cDNA fragment was purified and eluted in octoy injection buffer (5 mM Tris-HCl, 0.2 mM EDTA, pH 7.4). This construct (20 \( \mu \)g) was then microinjected into the male pronucleus of fertilized zygotes from superovulated FVB/n mice and the surviving zygotes implanted into pseudopregnant foster mothers. Transgenic founder mice were identified with genomic DNA utilizing polymerase chain reaction and confirmed by restricted Southern blotting. Polymerase chain reaction was carried out with a sense primer (5'-catcattag-cacaaacctcagg-3') specific for the \( \alpha \)-MHC gene (bp 859) and an antisense primer (5'-caagttgcatcctcaacctgc-3') located at bp 1530 of the human \( \alpha_1 \) subunit yielding a 375-bp product from mice expressing the transgene. For Southern blot, genomic DNA was extracted from tail clips of 18-day-old pups, digested with EcoRI, and separated on a 0.7% agarose gel. The \( \alpha \)-MHC-L-VDCC construct (100 pg, 500 pg, and 1 ng) was cleaved with EcoRI and loaded on the gel for quantitation. Following transfer to support nitrocellulose (Hybond-C extra, Amersham Pharmacia Biotech), the DNA was probed with a 2295-bp EcoRI fragment from the fusion construct (Fig. 1A). The fragment contained ~1.5 kilobase pairs of the \( \alpha \)-MHC promoter and 0.75 kilobase pair of the L-VDCC \( \alpha_1c \) cDNA. Radioactive bands were quantitated using PhosphorImager and ImageQuant software (Molecular Dynamics).

RNA Dot Blots of L-VDCC Subunits and Hypertrophic Markers—Total RNA was isolated from frozen heart samples of Tg and nontransgenic (Ntg) littermate controls using TriZol (Life Technologies, Inc.) as described previously (11). All probes were32P-labeled by the random priming technique. Radiolabeled dot blots were quantitated using a PhosphorImager and ImageQuant software (Molecular Dynamics) and normalized to the signal from glyceraldehyde-3-phosphate dehydrogenase (GAPDH), since evidence from other mouse models has demonstrated no change in gene expression of GAPDH in Tg and Ntg litters (11, 12).

This paper is available on line at http://www.jbc.org

Vol. 274, No. 31, Issue of July 30, pp. 21503–21506, 1999
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Printed in U.S.A.

*This work was supported by National Institutes of Health Grants P01 HL22619 (to A. S.), RO1 R37HL 43231, and T32 HL 07382 (to J. M. and A. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: L-VDCC, L-type voltage-dependent calcium channel; \( \beta \)AR, \( \beta \)-adrenergic receptor; Tg, transgenic; Ntg, nontransgenic; AC, adenylyl cyclase; MHC, myosin heavy chain; bp, base pair(s); GAPDH, glyceraldehyde-3-phosphate dehydrogenase; EDD, end diastolic dimension; ESD, end systolic dimension; F, farad.

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Calcium Channels and β-Adrenergic Regulation

Work Performing Hearts—Eight- to ten-week-old Tg and Ntg littermates of either sex were anesthetized with sodium pentobarbital (intraprteroneal 30 mg/kg), protected with heparin, and placed in an isolated working heart mode as described previously (12). Sedated, spontaneously breathing, mice were studied using two-dimensional-guided M-mode echocardiography to estimate left ventricular dimensions and wall thickness. Fractional shortening was calculated as (EDD-ESD)/EDD, where EDD is end diastolic dimension and ESD is end systolic dimension. Echocardiographic measurements were recorded and analyzed blinded.

Isolation of Single Cardiomyocytes and Electrophysiological Recording—Single ventricular cells were isolated from the hearts of 8–10-week-old Tg and Ntg mice by enzymatic dissociation protocol as described previously (14) using Type I collagenase (Worthington). L-type Ca2+ current channels were recorded using the whole-cell mode of the patch clamp method (15).

β-Adrenergic Receptor Density, Adenylyl Cyclase Assays, and cAMP—Measurement of adenylyl cyclase activity using a cAMP 125I radioactiveimmunoassay kit (NEN Life Science Products) and radioligand binding were carried out as described (16). Powdered tissue for cAMP measurements was prepared by homogenizing in 20 mM Tris-HCl, pH 7.5, 250 mM sucrose, 1 mM EDTA, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 0.1 mM phenylmethylsulfonyl fluoride in a glass homogenizer. Membranes were pelleted by centrifugation at 100,000 × g for 1 h at 4 °C, resuspended in 10 mM Tris-HCl, pH 7.5, and stored at −70 °C. The particulate fraction (50 μg/ml of protein) was incubated for 10 min at 30 °C with reagents as described (17), in a final volume of 0.5 ml of cAMP reaction buffer. cAMP measurements were performed using a competitive protein-binding cAMP 125I assay kit (Amersham Pharmacia Biotech) according to the manufacturer’s recommendations. Proteins were solubilized by adding 0.5 ml of 1 N NaOH to the trichloroacetic acid-extracted tubes and quantified using Bio-Rad assay.

Statistical Analysis—Data are reported as means ± S.E. n values are equivalent to the number of mice tested except for the patch clamp experiments, which indicates the number of cardiomyocytes used. A Student’s t test was used for statistical comparisons between Tg and Ntg hearts with a two-tail p value of <0.05 considered significant.

RESULTS

Using the α-MHC promoter, the human L-VDCC α1C subunit was overexpressed in mouse ventricles (Fig. 1A). The α-MHC promoter becomes active within a few days after birth and reaches steady state maximal activity around day 60 (18). We therefore used 8–10-week-old mice for our studies. A total of five founder lines were identified, classified as M1–M5. Two of these transgenic lines died within 8 weeks of age. Pathological examination of these two lines revealed severe myocardial hypertrophy and dilatation. Other transgenic lines were maintained at heterozygosity and compared with Ntg littermates for controls. The M1 and M5 lines had similar α1 subunit protein levels. Experiments were predominantly carried out in the M1 line except as noted.

Transgenic mice had no overt phenotype by observation. Additionally, litter sizes and pup survivals were similar to Ntg littermates. Loading known amounts of plasmid construct, the M1 line was determined to carry eight copies of the transgene (Fig. 1B). A quantitative RNA dot blot showed a 2.8-fold increase in the α1 subunit and no change in the β and α2/β subunits (Fig. 1C, Table I). In order to determine whether the increased gene product was translated into corresponding α1 subunit protein, homogenates were probed with specific antisera that does not distinguish between endogenous and Tg α1 protein. Western analysis revealed a clear increase in α1 subunit protein (data not shown). Assays of 8-week-old ventricular RNA for potentially regulated cardiac genes showed a significant 6.6-fold increase in ANF mRNA levels, but no significant differences in α-MHC, β-MHC, cardiac α-actin, skeletal α-actin, sarco-endoplasmic reticulum ATPase, and phospholamban gene expression (Table I).

We hypothesized that the increased α1 subunit in the transgenics should result in augmented contractility. Indeed, basal contractility and relaxation (expressed as +dP/dt and −dP/dt) were significantly higher for Tg hearts (4762 ± 46 mmHg/s and −3935 ± 56 mmHg/s, n = 5) compared with Ntg hearts (4094 ± 44 and −3160 ± 56, n = 4, p < 0.05). Infusion of the β-adrenergic receptor agonist, isoproterenol, did not elicit the expected inotropic and lusitropic (relaxation, diastole) increases observed in Ntg animals (Fig. 2). Heart rate increases were, however, comparable and normal for isoproterenol effects in both sets of mice (data not shown). Additionally, increases in contractility induced by forskolin (direct AC activator) were significantly increased in Tg mice (4762 ± 46 mmHg/s and −3935 ± 56 mmHg/s, n = 5) compared with Ntg hearts (4094 ± 44 and −3160 ± 56, n = 4, p < 0.05). Infusion of the βAR signaling pathway is not limited to an “uncoupling” of the receptor (Table II).

Echocardiographic measurements indicated that left ventricular end-diastolic dimension and posterior wall thickness were minimally increased in Tg as compared with Ntg mice (Table III). Left ventricular mass in the transgenic hearts was clearly increased (23%) compared with Ntg hearts. However, no significant differences were found in left ventricular end-systolic dimension, septal wall thickness, or fractional shortening percentage (Table III). Heart-to-body weight differences were significantly increased in Tg animals compared with Ntg littermates (Table III). These findings are consistent with a mild hypertrophy without ventricular dysfunction. On the other hand, ex vivo studies of these hearts revealed increases in basal contractility and a dramatic loss of βAR responsiveness.

To determine possible functional changes in the L-type Ca2+ channels, whole-cell voltage clamp recordings were carried out on isolated ventricular cardiomyocytes. Average cell capacitance was significantly larger in the Tg myocytes compared with Ntg myocytes (206.6 ± 16.5 pF, n = 9 versus 161.1 ± 7.1 pF, n = 7, p < 0.05), which indicates cellular hypertrophy. L-VDCC current amplitude was larger in the Tg myocytes com-

FIG. 1. Molecular characterization of α1 subunit overexpression mice. A, schematic representation of the α-MHC-L-VDCC fusion construct used for the generation of the Tg mice. Also included are important EcoRI sites used for generating Southern (2295 bp) and dot blot (1550 bp) probes. αE1 and αE2 represent the first two noncoding exons of the α-MHC gene. B, Southern blot of two Ntg and two M1 Tg mice. 20 μg of genomic DNA and the indicated amount of standard fusion construct were cleaved, blotted, and hybridized. C, total RNA was isolated from cardiac homogenates using Trizol reagent (Life Technologies, Inc.). 2 μg of total RNA was transferred to Hybond N+ (Amersham Pharmacia Biotech) using a dot blot apparatus and hybridized. The mRNA levels of L-VDCC subunits were estimated using the probes: α1 subunit, a 1350-bp EcoRI fragment; β subunit, full-length β2 cDNA; αβ subunit, a 1550-bp HincII fragment. Values were normalized to the hybridization signal of a GAPDH probe. Bars show the mRNA expression level of the L-VDCC subunits.
pared with Ntg myocytes (Fig. 3, A and B). Average peak current amplitude was 1.8 ± 0.2 nA (n = 7) for Tg myocytes and 1.3 ± 0.1 nA (n = 9) for Ntg myocytes (p < 0.05, Fig. 3C). When the current amplitude was normalized for cell capacitance, there was no significant difference between Tg and Ntg cardiomyocytes (8.8 ± 0.6 pA/pF (n = 9) and 8.3 ± 0.6 pA/pF (n = 7)). There were no significant differences in the voltage dependence of activation or the activation/inactivation kinetics of the channels (data not shown).

The L-VDCC has been identified as a downstream phosphorylation target of the β-adrenergic receptor cascade (7). To determine whether this cascade remained intact in the Tg mice, isolated cells were stimulated with isoproterenol. When cells isolated from Ntg mice were superfused with 10⁻⁷ M isoproterenol, peak Ca²⁺ channel currents were augmented by 72.9 ± 21.6% (n = 6), but only 19.4 ± 11.4% (n = 5) for the transgenics (p < 0.05) (Fig. 3E). Consistent with the results in the intact heart experiments, forskolin produced only a small increase in the peak Ca²⁺ current in the Tg hearts compared with the robust increases observed in the Ntgs (Table II).

**TABLE I**

mRNA transcripts for hypertrophic markers and L-VDCC subunits

Abbreviations: du, densitometric units; α SK actin, α skeletal actin; SERCA2, sarco-endoplasmic reticulum ATPase 2; PLB, phospholamban.

| Transcript   | Nontransgenic | Transgenic | Fold increase |
|--------------|---------------|------------|--------------|
| ANF          | 0.2 ± 0.2 (n = 4) | 1.2 ± 0.3 (n = 4) | 6.6*         |
| α-MHC        | 3.5 ± 2.3 (n = 4) | 5.4 ± 1.7 (n = 4) | 1.3          |
| β-MHC        | 0.5 ± 0.2 (n = 4) | 0.6 ± 0.2 (n = 4) | 1.3          |
| α SK actin   | 0.4 ± 0.1 (n = 4) | 0.5 ± 0.1 (n = 4) | 1.3          |
| SERCA2       | 1.8 ± 0.6 (n = 4) | 1.7 ± 0.4 (n = 4) | 0.9          |
| PLB          | 3.1 ± 1.0 (n = 4) | 3.1 ± 0.9 (n = 4) | 1.0          |
| Cardiac actin| 4.9 ± 2.2 (n = 4) | 3.5 ± 0.9 (n = 4) | 0.7          |
| α, subunit   | 10.6 ± 1.1 (n = 7) | 29.4 ± 8.2 (n = 7) | 2.8*         |
| α/β subunit  | 2.5 ± 0.1 (n = 3) | 2.0 ± 0.1 (n = 5) | 0.9          |
| β subunit    | 3.2 ± 0.1 (n = 3) | 3.0 ± 0.2 (n = 5) | 0.9          |

* p < 0.05 versus Ntg.

**TABLE III**

Morphometric and echocardiographic measurements on transgenic and nontransgenic mice

Abbreviations: BW, body weight; HW, heart weight; LVEDD, left ventricular end diastolic dimension; LVESD, left ventricular end systolic dimension; PWT, posterior wall thickness; SWT, septal wall thickness; FS, fractional shortening; LV, left ventricular. Data are presented as means ± S.E.

| Parameter          | Nontransgenic | Transgenic | Fold increase |
|--------------------|---------------|------------|--------------|
| Morphometric       | n = 14        | n = 14     |              |
| Body weight, g     | 23.8 ± 0.8    | 24.6 ± 0.9 |              |
| Heart weight, mg    | 132.2 ± 7.5   | 152.2 ± 6.7|              |
| HW/BW, mg/g        | 5.6 ± 0.3     | 6.2 ± 0.2* |              |
| Echocardiographic   | n = 5         | n = 5      |              |
| LVEDD, mm           | 3.5 ± 0.1     | 3.8 ± 0.04*|              |
| LVESD, mm           | 2.0 ± 0.1     | 2.3 ± 0.08  |              |
| SWT, mm             | 0.55 ± 0.02   | 0.56 ± 0.02 |              |
| FS, %               | 44 ± 2        | 41 ± 2     |              |
| LV mass, mg         | 48.6 ± 2.2    | 59.6 ± 2.6*|              |

* p < 0.05 versus nontransgenic.

**FIG. 2.** Effects of isoproterenol on transgenic and nontransgenic isolated perfused hearts. Whole hearts were antegradey perfused with increasing concentrations of isoproterenol. Increases in contractility (+dP/dt) and relaxation (−dP/dt) were observed at concentrations from 4 × 10⁻⁷ M and continued to increase until reaching toxicity at 8 × 10⁻⁷ M in Ntg hearts; Tg hearts did not respond to isoproterenol at any concentration. [M], molar concentration; R, resistance.

**FIG. 3.** Whole-cell calcium current, isoproterenol responses, and adenylyl cyclase activity in transgenic and nontransgenic hearts. A and B, representative calcium current traces recorded with 1.8 mM Ca²⁺ as a charge carrier from Ntg (A) and Tg (B) mouse ventricular myocytes. Currents were elicited by a 500-ms depolarizing pulse from a holding potential of −60 mV to −30, −10, +10, and +30 mV. C and D, averaged current-voltage relationship (C) and current density-voltage relationships from Ntg and Tg mice. D, data points represent the means of 7 (Ntg) and 9 (Tg) experiments. E, increase in peak current amplitude in response to 10⁻⁷ M isoproterenol. Data are means of six (Ntg) and five (Tg) experiments. F, membrane adenylyl cyclase activity was carried out in the presence of vehicle, 10⁻⁵ M isoproterenol, 10⁻² M NaF, and 10⁻⁴ M forskolin.
To examine the blunted isoproterenol and forskolin responses found in whole heart and single cells, the βAR signaling pathway was investigated using isolated membranes. No change in total βAR receptor density was found (Tg, 19.2 ± 1.2 fmol/mg; Ntg, 19.6 ± 0.6 fmol/mg; n = 3). Isoproterenol-stimulated activities were 150% of basal in Ntg mice (i.e. a ~50% increase over basal). In marked contrast, isoproterenol failed to stimulate AC activity in membranes from the Tg mice (indeed levels were slightly below basal) (Fig. 3F). Basal AC levels were 44.8 ± 17 pmol/min/mg for Ntg and 52.7 ± 11 pmol/min/mg for Tg extracts. As predicted NaF and forskolin-stimulated activities were depressed compared with Ntg, but the values did not reach statistical significance (Fig. 3F). Direct cAMP measurements further supported the loss of the βAR responsiveness. As observed in the AC assays, Tg membranes did not respond to isoproterenol nor was forskolin able to restore cAMP levels to the Ntg levels (Table II).

DISCUSSION

We constructed a transgenic mouse in which the L-type voltage-dependent Ca²⁺ channel was overexpressed. The transgene is defined as an increased VDCC by four criteria, Southern analysis, RNA analysis, electrophysiological identification of an increased VDCC by four criteria, Southern analysis, and an indirect up-regulation of G protein signaling by an increased influx of Ca²⁺ in which a small but sustained Ca²⁺ influx in Tg myocytes that explains the significant increase in basal contraction and relaxation we observed. Accompanying the latter, we found a surprising and striking loss of the usual effects of a well known β₁,₂ adrenergic agonist, isoproterenol, on myocardial contraction. In contrast, the heart rate increases secondary to isoproterenol administration were normal, which defines an interesting separation of β-agonist actions on contractility and heart rate. This differentiation implies that downstream effectors and/or intermediates in the G protein pathway for β-agonist action are different for contractility and heart rate. It seems possible that for heart rate regulation Ca²⁺ derived from the L-VDCC may not be as limiting as it is for contraction. Consistent with the loss of contractile action was a blunting of isoproterenol and forskolin stimulation of Ca²⁺ current in isolated myocytes. Thus, the effects found on the whole heart were for the most part duplicated on single cells for both isoproterenol and forskolin.

We refer to this loss of β-agonist contraction effect as a defect or a “loose coupling” in the βAR signaling pathways. The present data provide convincing evidence for a novel “reciprocal regulation” of βAR signaling by an increased influx of Ca²⁺ provided by the L-VDCC. A possible mechanism to consider is protein kinase C activation by Ca²⁺ (19) that in turn activates βAR kinase leading to phosphorylation of the βAR resulting in a decrease in signaling activity (20). Other mechanisms possibly responsible for the loss of βAR signaling include an inhibition of AC activity by Ca²⁺ (21–23) and an indirect up-regulated phosphatase activity via calcium-calmodulin-dependent protein kinase (24). The latter is supported by recent data implicating calcineurin in cardiac hypertrophy (5). Further possibilities include up-regulation of G, changes in the ratio of βAR;β₂AR isoforms, etc. (20). Consistent with the suggestion of a Ca²⁺-dependent “cascade” involving a phosphatase is the slow development of hypertrophy and subsequent cardiac failure in these animals.

Our results suggest an interesting “cross-talk” between the L-VDCC and the βAR in vivo. A modest increase in the α₁ subunit of the L-VDCC in cardiomyocytes results in a remarkable decrease in β-adrenergic signaling that affects contractility but interestingly does not alter the usual responses of heart rate to βAR agonists. The Ca²⁺ channel α₃ overexpression also produces a setting in which late stage ventricular remodeling occurs. This is surprising, since the cardiac cell has a remarkable network of sarcoplastic reticulum and mitochondria that one would think would be poised to sequester any increase in calcium. In all models of hypertrophy in which “Ca²⁺ overload” was produced in no case was a paradigm used in which a small but sustained Ca²⁺ increase through the L-VDCC was provided. Clearly the present experiments show that the L-VDCC in heart is a highly sensitive conduit for Ca²⁺ as the link between excitation and contraction. Furthermore, it is likely that the increased calcium is sequestered in a pool that is linked to a growth program. A very recent publication has revealed a pathway to gene expression and growth that requires a very low concentration of calcium (25). These Tg mice represent the first animal model with an increase in voltage-dependent Ca²⁺ channels specifically in the heart that provides a paradigm for studies of the role of Ca²⁺ in growth adaptation (25), maladaptation, and receptor/channel regulation.

Acknowledgments—We thank Dr. J. Robbins and J. Gulick for providing Tg mice containing the murine α-MHC promoter. We are very grateful to Dr. G. Dorn for echocardiography and hypertrophic marker experiments and to Dr. S. Liggett and N. Tepe for AC assays. A special thanks to Drs. R.-P. Xiao and D.-J. Wang for cAMP assays. We also thank G. Newman, T. Jackson, and M. Neyland for technical assistance and J. C. Neumann in the Transgenic Core Facility of the University of Cincinnati, College of Medicine for the pronuclear injections.

REFERENCES

1. Mori, Y., Mikala, G., Varadi, G., Kobayashi, T., Koch, S., Wakamori, M., and Schwartz, A. (1996) Jpn. J. Pharmacol. 72, 83–109
2. Garnett, C. A., and Campbell, K. P. (1996) J. Biol. Chem. 271, 27975–27978
3. Fumeau, M., and Vassort, G. (1996) Mol. Cell. Biochem. 157, 65–72
4. Satake, P. V., and Yu, X. T. (1995) Mol. Cell. Biochem. 149, 103–126
5. Molkentin, J. D., Lu, J.-R., Antos, C. L., Markham, B., Richardson, J., Robbins, J., Grant, S. R., and Olson, E. (1998) Cell 93, 215–228
6. Chawla, S., Hardingham, G. E., Quinn, D. R., and Bading, H. (1998) Science 281, 1505–1509
7. McDonald, T. F., Pelzer, S., Trautwein, W., and Pelzer, D. J. (1994) Physiol. Rev. 74, 365–507
8. Dastak, V., Lottrrel, L. M., and Leffkowitz, R. J. (1997) Nature 380, 88–91
9. Schultz, D., Mikala, G., Yatani, A., Engle, D. B., Iles, D. E., Segers, B., Sinke, R. J., Weghuis, D. O., Klockner, U., Wakamori, M., Wang, J.-J., Melvin, D., Varadi, G., and Schwartz, A. (1995) Proc. Natl. Acad. Sci. U. S. A. 90, 6228–6232
10. Gulick, J., Subramaniam, A., Neumann, J., and Robbins, J. (1991) J. Biol. Chem. 266, 9180–9185
11. Jones, W. K., Grupp, I. L., Doetschman, T., Grupp, G., Osisaka, H., Hewett, T. E., Boivin, G. P., Gulick, J., Ng, W. A., and Robbins, J. (1996) J. Clin. Invest. 98, 1906–1917
12. D’Angelo, D. D., Sakata, Y., Lorenz, J. N., Boivin, G. P.,拉萨, R. A., Liggert, S. B., and Dorn, G. W., II. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 8121–8126
13. Grupp, I. L., Grupp, G., and Slyfis, G. (1998) Cardiacoatomic Physiologys in the Genetically Engineered Mouse (Hoit, D. B., and Walsh, R. A., eds) Kluwer Academic Publishers, Norwell, MA
14. Tsuch, N., Nakaya, H., and Kanno, M. (1992) Circ. Res. 71, 1441–1446
15. Hamilt, O. P., Marty, A., Neher, E., Sakman, B., and Sigworth, F. J. (1981) Pfluegers Arch. 391, 85–100
16. Green, S. A., and Liggert, S. B. (1994) J. Biol. Chem. 269, 26215–26219
17. Xu, R.-P., Tomhaye, E. B., Wang, X., Ji, X., Roleyt, M. O., Cheng, H., and Lakatta, E. G. (1996) J. Clin. Invest. 101, 1273–1282
18. Subramaniam, A., Jones, W. K., Gulick, J., Wert, S., Neumann, J., and Robbins, J. (1991) J. Biol. Chem. 266, 24613–24620
19. Akhdar, S., Milano, C. A., Shotwell, K. F., Cho, M., Rockman, H. A., Leffkowitz, R. J., and Koch, W. (1997) J. Biol. Chem. 272, 21253–21259
20. Bristow, M. R. (1997) Am. J. Cardiol. 80, 26L–40L
21. Colvin, R. A., Oibo, J. A., and Allen, R. A. (1991) Cell Calcium 12, 19–27
22. Yu, H. J., Ma, H., and Green, R. D. (1993) Mol. Pharmacol. 44, 689–693
23. Fagan, K., Moss, N., and Cooper, D. M. F. (1998) J. Biol. Chem. 273, 9097–9095
24. Maltos, V. A., Ji, G. J., Wobus, A. M., Fleischmann, B. K., and Hescheler, J. (1999) Circ. Res. 85, 136–145
25. Cattini, A. M., Link, W. A., Ledo, F., Meilstrum, B., and Narahari, J. R. (1999) Nature 398, 80–84