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# Enhanced Expression of β-Adrenergic Receptor Kinase 1 in the Hearts of Cardiomyopathic Syrian Hamsters, BIO53.58

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We cloned an entire coding sequence of β-adrenergic receptor kinase 1 (βARK1) cDNA from the hearts of Syrian hamsters through reverse transcription and subsequent polymerase chain reaction. The cloned cDNA contained 2067 nucleotides coding 689 amino acids. The sequence had 95% homology to rat βARK1 and 90% homology to human homologue. Cardiomyopathic Syrian hamster, BIO53.58, has been used as a model animal of congestive heart failure. M-mode echocardiography confirmed that left ventricular contractility of 20-week-old BIO53.58 was markedly reduced. The expression of βARK1 mRNA in the hearts of BIO53.58 was significantly increased compared to control hamsters, F1b, suggesting that the enhanced βARK1 expression is acting as a negative feedback mechanism in order to maintain intracellular homeostasis against accelerated stimulation by catecholamines *via* phosphorylation of β-adrenergic receptor. 

β-Adrenergic receptor-adenylyl cyclase system is one of the thoroughly investigated membrane signaling systems. Extracellular neurohumoral stimulation, such as norepinephrine from sympathetic nerve endings and epinephrine from adrenal glands, is recognized by β-adrenergic receptors (βAR), then receptor-oriented signal is transferred to catalytic subunit of adenylyl cyclase (AC) through stimulatory GTP binding protein (Gs) [1]. It is well known that sustained stimulation by catecholamines leads to reduced responsiveness of entire system, and such phenomenon is generally called 'desensitization'. Recent investigations revealed that the desensitization process is composed of several distinct molecular events including phosphorylation of βAR (uncoupling) [2], decrease of cell surface βAR (down-regulation) [3], reduced AC activity [4], and enhanced expression of inhibitory GTP binding protein (Gi) [5]. Recently, Ungerer *et al.* reported an enhanced expression of βARK1 in the hearts of congestive heart failure (CHF) patients [6]. Although precise physiological role of enhanced expression of βARK1 in failing hearts has not been fully evaluated, it is highly possible that the elevated βARK1 activity is involved in the desensitization process of β-adrenergic receptor-adenylyl cyclase system in the setting of CHF. So far, βARK1 cDNA was cloned only from rat, bovine and human. In this study, we reported the cDNA sequence of hamster βARK1, then investigated the alteration of βARK1 mRNA expression in the hearts of a genetically inherited CHF model, cardiomyopathic Syrian hamsters, BIO53.58.

## MATERIALS AND METHODS

Cardiomyopathic Syrian hamsters (BIO53.58) and healthy control hamsters (F1b) (7, 15 and 20 weeks old) were obtained from BIO BREEDERS (Fitchburg, USA). Taq DNA polymerase, deoxynucleotides used for the polymerase chain reaction (PCR) and reverse transcription, Molony Murine Leukemia Virus (MMLV)-reverse transcriptase, restriction endonucleases and other modifying enzymes were purchased from Life Technologies (Tokyo, Japan). [$^{32}$P]-dCTP was from DuPont NEN. All other reagents were purchased from Sigma (St. Louis, USA).

*M-mode echocardiography.* Hamsters were anesthetized using urethane and α-chloralose, then laid on a plastic table equipped with a silicon acoustic coupler filled by water, through which ultrasound probe was attached to the chest wall of hamsters. Left ventricular wall motion was evaluated on M-mode echocardiogram obtained by Hitachi EUB565A (Japan) with 7.5MHz probe.

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*Extraction of total RNA from hamster hearts.* Total RNA was extracted from hamster ventricles using RNAzol B (Biotecx Laboratories, Houston, USA) [7]. The final RNA pellets were suspended in appropriate volume of diethylpyrocarbonate-treated water so as to obtain an appropriate RNA concentration, 1 ∼ 2 $\mu$g/$\mu$l.

*Cloning and sequencing.* To obtain complementary DNA (cDNA), 1$\mu$g of total RNA was incubated with 200 unit of MMLV-reverse transcriptase and 23$\mu$M of random hexamers at 37°C for 30min. Three forward primers (S1 ∼ 3) and three reverse primers (A1 ∼ 3) used for PCR were synthesized on a 8909 Expedite Nucleic Acid Synthesis System (Millipore, USA). The positions of these PCR primers within $\beta$ARK1 cDNA sequence are shown in Fig. 1. PCR primers, S1, S2, A2 and A3, were complementary to the rat $\beta$ARK1 cDNA. S3 and A1 were complementary to the hamster $\beta$ARK1 cDNA sequence which was obtained from the first PCR with A2 and S2 primer. Partial cDNA fragments were amplified in 25$\mu$l of PCR buffer (20mM Tris-HCl, pH 8.4, 50mM KCl, 3mM $MgCl_2$), 2$\mu$M of each S and A primers, 200$\mu$M dNTPs, 2.5 units of *Thermus acquaticus* DNA polymerase. PCR was performed for 40cycles, with 30sec of denaturation at 94°C, annealing for 30sec at 5°C below the average Tm of each primer set, and 1 min extension at 72°C. Amplified DNA fragments were subcloned into pBluescript II SK+ plasmid (Stratagene, USA) and sequenced using Sequanase Version 2.0 DNA sequencing kit (Amersham Life Science, USA).

*Semi-quantitative measurement of $\beta$ARK1 mRNA by RT-PCR.* One microgram of total RNA was reverse transcribed into cDNA as described at the previous section. Three different amounts of cDNA and a primer set S2/A2 were used for PCR. PCR was performed with 30 seconds of denaturation at 94°C, annealing for 30 seconds at two different temperature (48°C for first 4 cycles and 50°C for the following 23 cycles), and 45 seconds of extension at 72°C. These conditions were determined by preparatory experiments so as to obtain linearity on the amount of PCR products between 24 to 30 PCR cycle (data not shown). PCR products were separated through 1% agarose gel electrophoresis, then stained by 0.5$\mu$g/ml ethidium bromide and photographed on UV transilluminator. The intensities of DNA bands were assessed by densitmetric scanning of photographs, and used to calculate the relative levels of $\beta$ARK1 mRNA expression (AU, arbitrary unit) using an image analyzing software, NIH image, as shown in Fig. 2.

*Data analysis.* Data are expressed as mean ± SD. The values were compared using unpaired Student's *t*-test. Difference was accepted as significant when p value was less than 0.05.

## RESULTS AND DISCUSSION

Three sets of PCR primers (S1-A1, S2-A2 and S3-A3 as underlined in Fig. 1) were synthesized and used to amplify partial $\beta$ARK1 cDNAs from the hearts of Syrian hamsters. Three amplified fragments overlap each other so as to correct nucleotide misreading within the PCR primers. The amplified fragments were sequenced using standard dideoxy chain termination method. The re-constructed cDNA contained an entire coding sequence of hamster $\beta$ARK1 with 2067 nucleotides corresponding to 689 amino acids same as those of rat, bovine and human [8, 9, 10]. The nucleotide sequence had 95% homology to rat $\beta$ARK1 and 90% homology to human homologue. Since most of nucleotide diversities were located at the third position of codon, the homology between hamster and rat $\beta$ARK1 on amino acid level was reached to 98.7%. Because there was no information of non-coding region of hamster $\beta$ARK1 cDNA, primer S1 and A3 had to be prepared at the both ends of the open reading frame of rat $\beta$ARK1 cDNA. Therefore, nucleotide sequences corresponding to primer S1 and A3 might contain incorrect information. Nevertheless, such high sequence homology of $\beta$ARK1 cDNA between hamster and other mammals suggests that the structure and function of this enzyme have been strictly conserved throughout an evolutional tree of mammals.

Cardiomyopathic Syrian hamsters, BIO53.58, has been used as an animal model of dilated cardiomyopathy and heart failure [11]. As shown at Table 1, M-mode echocardiogram revealed that left ventricular percent fractional shortening (%FS) of this model was progressively damaged with

TABLE 1
Data from M-Mode Echocardiography of Hamsters

| Age (week) | n | %FS (%) F1b | %FS (%) BIO53.58 | p | LVDd (mm) F1b | LVDd (mm) BIO53.58 | p |
|---|---|---|---|---|---|---|---|
| 7 | 6 | 66.5 ± 2.4 | 57.3 ± 3.2 | <0.05 | 5.5 ± 0.3 | 6.1 ± 0.1 | NS |
| 15 | 6 | 67.7 ± 2.2 | 39.9 ± 2.8 | <0.0001 | 6.7 ± 0.2 | 7.5 ± 0.3 | <0.05 |
| 20 | 6 | 63.3 ± 2.2 | 20.8 ± 1.5 | <0.0001 | 7.3 ± 0.2 | 10.3 ± 0.2 | <0.0001 |

%FS, left ventricular percent fractional shortening; LVDd, left ventricular end-diastolic dimension.

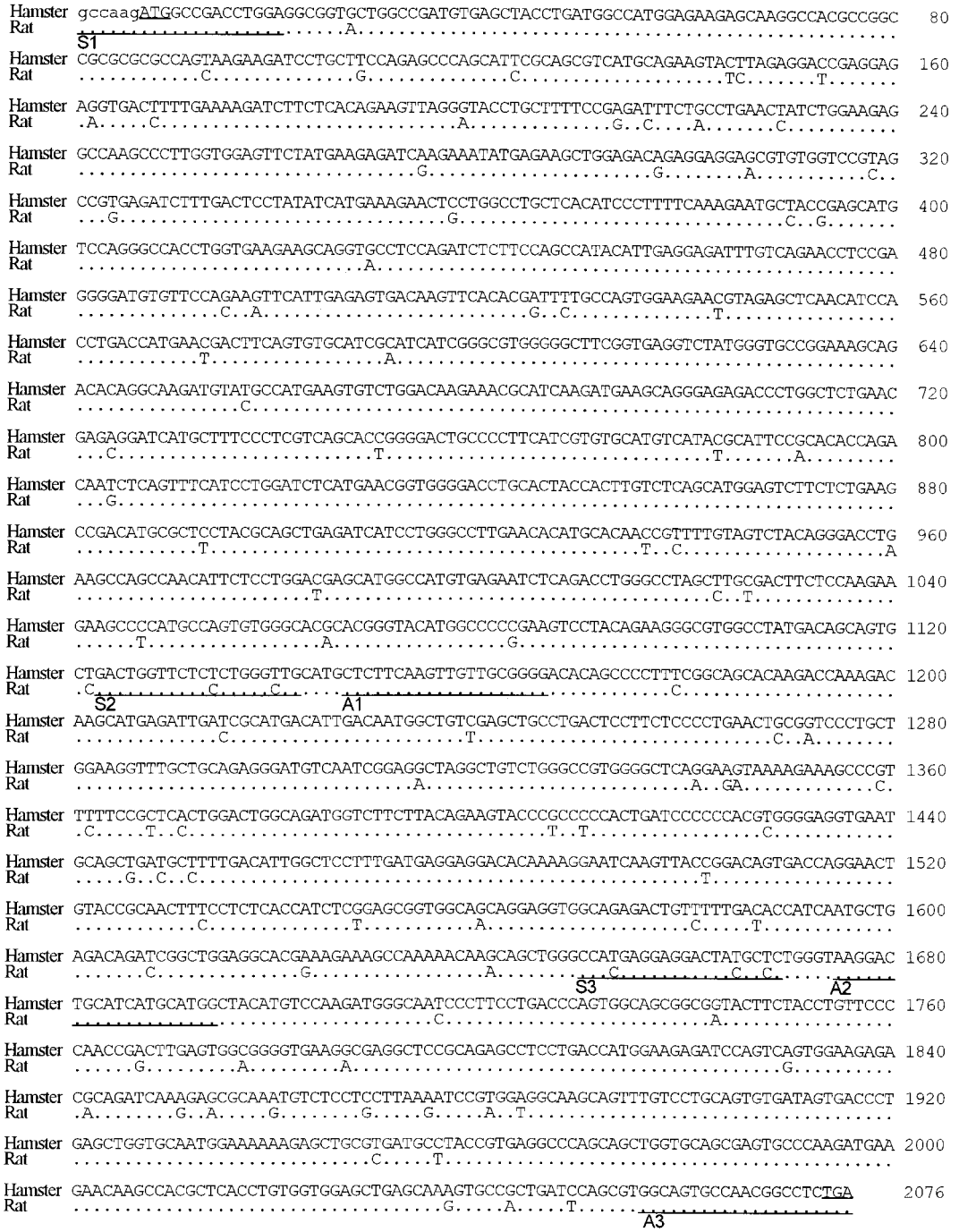


**FIG. 1.** Deduced nucleotide sequence of hamster βARK1 cDNA. Nucleotide sequence of reconstructed hamster βARK1 cDNA is shown with previously reported rat βARK1 cDNA [8]. Nucleotides are numbered on the right side. Identical nucleotides are shown by dots in rat sequence. Locations of the primers used for PCR (sense primers: S1, S2, S3; antisense primer: A1, A2, A3) are underlined. ⩛ATG, translation initiation codon; ⩛TGA, translation termination codon.

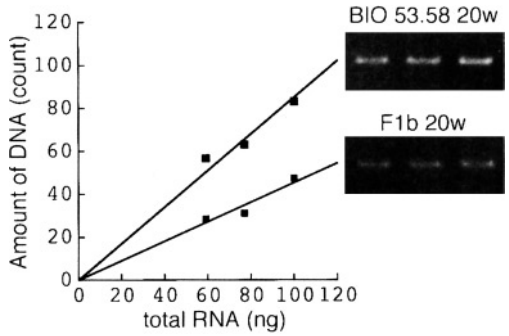


**FIG. 2.** Quantitative analysis of βARK1 mRNA by reverse transcription-polymerase chain reaction in representative animals. Total RNA (1μg) of hamster ventricles were reverse transcribed to cDNA, then βARK1 cDNA was amplified from three different amounts of cDNA pools (equivalent to 60, 80, and 100ng of total RNA) using the specific PCR primers (S2 and A2). The amplified products were separated through 1% agarose gel electrophoresis, and intensity of each DNA band was assessed by densitometric scanning. The slope of the regression lines give the concentration of βARK1 mRNA in arbitrary units as summarized in Fig. 3.

aging, and significantly lower than that of control F1b hamsters at any age we tested. Diameter of left ventricular cavity at end-diastolic phase (LVDd) in BIO53.58 was progressively enlarged with aging, as reported in a previous pathological examination [12]. These data suggest that BIO53.58 is suitable animal model to investigate the alterations of membrane signaling machinery in the setting of CHF. In fact, we have already reported increase of inhibitory GTP binding protein and reduced activity of adenylyl cyclase in this animal model [5], which were consistent with the findings obtained in failing human hearts [13, 4].

As indicated in Fig. 3, semi-quantitative RT-PCR revealed that the expression of βARK1 mRNA was significantly higher in BIO53.58 than that of F1b (0.73 ± 0.24 and 0.37 ± 0.12 for BIO53.58 and F1b, respectively; $p < 0.05$). Enhanced expression of βARK1 was reported by Ungerer *et al.* in the hearts of CHF patients [6]. The results obtained in our study suggested that the enhanced expression of βARK1 might be an ubiquitous phenomenon in the setting of CHF. According to the genomic sequence of human βARK1 gene [14], five prime non-coding region contains several known cis-elements including multiple AP2 sites, which is responsible for PKA dependent gene expression [15]. Although no information of hamster βARK1 gene is available at this moment,

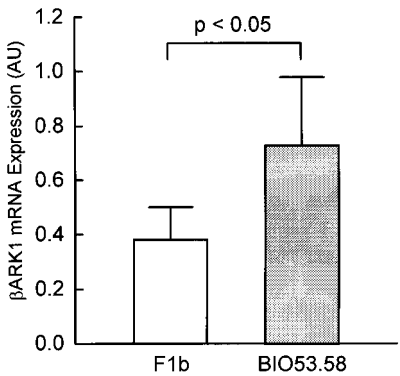


**FIG. 3.** Expression of βARK1 mRNA in BIO53.58 and F1b hamsters. Bar graph showing quantitation of βARK1 mRNA in the ventricles of BIO53.58 and F1b hamsters (5 animals for each group). Semi-quantitative RT-PCR was performed as described in the legend to Fig. 2 and 'Materials and Methods.' Statistical significance was detected between BIO53.58 and F1b hamsters.

there might be the same kind of nucleotide sequences which respond to the transcription factors activated by βAR-adenylyl cyclase pathway.

To date, βARK1 has been thought to be responsible for agonist-dependent phosphorylation of $\beta_2$-AR, and not to be involved in the down-regulation of $\beta_1$-AR [16]. However, Koch *et al.* reported recently that the transgenic mouse overexpressing βARK1 in their hearts showed reduced responsiveness to $\beta_1$-AR stimulation [17]. Their data suggest that βARK1 is involved in the functional modification of both $\beta_1$- and $\beta_2$-AR in hearts. Taken together, our data indicated that accelerated expression of βARK1 might be involved in uncoupling and down-regulation of βAR (presumably both $\beta_1$- and $\beta_2$-AR) in failing hearts *via* receptor phosphorylation. Further investigation of BIO53.58 hamsters would clarify the precise role of enhanced βARK1 expression in CHF, and provide us a better understanding of the molecular events behind catecholamine tolerance.

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